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Biobased Chemistry and Technology: Derivatives of Aldaric acids as Key Intermediates for Sustainable Products and Materials

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To my family

Abstract

Considering the fact that humanity has put a significant global impact on our planet, concerning both the geology and environment, national governments and industries have reached a consensus of developing a sustainable n economy a resource-efficient, green, and competitive low-carbon. Among all the strategies applied, green chemistry should be and must be a driving force for mitigating pollution and reducing resource consumption, and then reach the longer-term decarbonisation target to fulfil the final goals of the Paris Agreement. Like oil refinery, the second-generation biorefinery was treated as one of the important base for global bio-economy by developing integrated sustainable value chains to producing food/feed ingredients, chemicals, materials, fuels, power and/or heat from sustainably sourced biomass. In order to achieve this goal, the modern biorefinery need to deeply investigate first the chemistry, particularly the organic chemistry and biochemistry, involved in the synthesis of platform molecules and then the alternative strategies to convert them into value-added sustainable products and materials by a combination of biotechnological and chemical methods.

Different C5 and C6 sugar building blocks and structural polysaccharides containing repeating units of D-glucose, D-galactose, D-mannose, D-xylose, D-arabinose, etc., constitute the major structure of biomass. The hexoses as six-carbon carbohydrates are the most abundant naturally occurring monosaccharides. Among these, D-fructose and D-glucose are economically suitable for use as chemical raw materials, in particular for the production of dicarboxylic acids. Aldaric acids are a group of sugar acids, where the terminal hydroxyl and carbonyl groups of the sugars have been replaced by terminal carboxylic acids, and are characterised by the formula HOOC-$(CHOH)_n$-COOH. They are key intermediates to useful chemical products and biodegradable polymers.

In order to avoid the loss of carbon during the biorefinery process, direct utilization of six-carbon matrix as platform chemicals has attracted significant attention from academic and industrial viewpoint. D-glucaric acid, one of the more representatives aldaric acids has been deeply investigated regarding its preparation and reactivity, was identified as one of the top 12 platform molecules by the US Department of Energy (DoE) in 2004. Owing the similar structure, other members of aldaric acid family should still have a huge potential to be exploited. This thesis has addressed representative information from literature on the main aldaric acids (glucaric acid, mannaric acid, and galactaric acid) as an introduction on their synthesis, reactivities, application and its potential as platform molecule.

Among these acids, galactaric acid (also known as mucic acid) is of particular interest, as it has been reported as potential intermediate for the production of other C6 dicarboxylic acids of industrial importance such as adipic acid, 2,5-furandicarboxylic acid and terephthalic acid. Moreover, despite the multi-chiral center nature of aldaric acids, its achiral meso form with no optical activity would be

beneficial for its preparation and further synthesis as well as working-up procedure. At moment the possible industrial scale production of biobased galactaric acid has been reported in both chemical and biological methods, which could further contribute to decrease the price of this compound to make it economically feasible as a platform chemicals. Our focus on galactaric acid was on attempt to improve the knowledge on the chemistry of aldaric acids (in particular glucaric acid, the more commercial available representative of the family now available at the level of 30.000 tons/y) by using a compound supplied directly in acid form, through well identified hydrolytic-oxidative bio processes from natural pectin, which are themselves amenable to further upgrading from wasted biomasses. The aim of the galactaric acid project is to exploit the reactivity of this biobased platform molecule in order to develop cost compatible processes for commodity/fine chemicals and novel functional materials, i.e. polymers based on the pyrrole, amide, and ester functional groups. The reactivity of galactaric acid has been sporadically investigated with specific interest for the conversion to adipic acid, 2,5-furandicarboxylic acid and some other heterocyclic compounds, as well as new potential monomer for polymer synthesis, but normally with low yields.

All the facts, along with the limited knowledge of the chemistry and biochemistry of these compounds, have oriented the choice of aldaric acids as research subject for this thesis. The study was centered mainly on galactaric acid as the more representative aldaric acid, with minor attention also to glucaric acid and mannaric acid. This thesis mainly deals with the selected reactivities of galactaric acid as follows, dehydration of aldaric acids, identifying lactones and unsaturated intermediates involved; reduction of aldaric acid and their unsaturated intermediates; acylation of hydroxy groups of aldaric acids and role of esters in the elimination of acyl groups; role of aldarate salts (both inorganic and organic) in the above mentioned processes; possible efficient transformations into value-added products and materials, including five membered aromatic derivatives of furan and pyrrole, six membered derivatives of pyrenes, amide salts, diamides and polyamides. The new insights gained in this work inspired the development of novel biorefinery pathways and processes to convert galactaric acid into a new group of platform chemicals/intermediates, enabling sustainable biofuel production from carbohydrate biomass.

The study of the possibility of dehydrating galactaric acid to give its corresponding unsaturated derivatives was carried out according to a two-step acylation and dehydroelimination approach in presence of acid/base catalyst, providing access to pyrone derivatives. The 2,5-dihydroxyadipic acid and small amount of mono-reduced product were further synthesized by the hydrogenation reaction of the pyronecarboxylic acid. Protected and unprotected 1,4-dicarbonyl compounds (2,5-dihydroxymuconic acid and pyrones) are proved to be relevant intermediates in the dehydration of galactaric acids. They are efficiently converted under mild conditions by amines to pyrrolecarboxylic acid derivatives and unsubstituted pyrroles by decarboxylation under moderate temperatures. A better

understanding of the mechanistic details of the formation of pyrroles from mucic acid and amines is provided.

Galactaro-1,4-lactone is prepared by a simple thermal method with dimethyl sulfoxide as solvent in quantitative yields, opening the possibility to become a potential platform molecule. Inorganic and organic salts of galactaric acid 1,4-lactone are easily prepared and isolated under mild conditions. Mono salt mono amides of mucic acid and diamides were synthesized in high yields by treatment of galactaric acid 1,4-lactone with primary amines, which is a good start point for synthesis of homo- and co-polymers by further thermal polycondensation. These results can rationalize some literature data on reactions of mucic acid with bases. Galactaro-1,4-lactone is selectively mono-, di- and tri-formylated. This last compound and the corresponding acetylated derivative undergo selective de-acylation by a base to mono-unsaturated lactone. Similar process is observed from other aldaric acid mono and dilactones. These mono-unsaturated lactones are key intermediates for further elimination to di-unsaturated derivatives furan-2,5-dicarboxylic acid under acid catalysis.

Thesis organization

The thesis is organised as follows:

Chapter 1

Introduces the general background of this research focusing on challenges of global climate changes environment pollution, highlighting modern biorefinery as coping strategy. Selected candidates of the most widely accepted portfolio of platform molecules identified internationally is introduced to give an overview of the state of art.

Chapter 2

Summarises the current state of aldaric acids as platform molecules and their researches on properties, preparation, applications, chemical reactivity, and known/potential uses. References are made to D-glucaric acid, D-mannaric acid and galactaric acid as representatives of aldaric acids.

Chapter 3

Reports the main topic concerning the discussions of our research on the chemistry of galactaric acid and its new potentialities as platform molecule for chemicals and materials.

Chapter 4

Provides the experimental campaign on the study of galactaric acid reactivity with the details of characterization of all isolated products, including galactaro-1,4-lactone, acyclic salt and amide derivatives of galactarate and di-eliminated products (pyrones, pyrroles and furandicarboxylic acid). Preliminary evaluation of materials coming from polymerization of amido galactarate monomers.

Chapter 5

Reports the main conclusions of the work and the perspectives for future development.

N.B. References are made to the cited literature at the end of each chapter.

Riassunto

Le recenti stime sull'impatto globale prodotto dalle attività umane sul pianeta terra, sia per quanto riguarda la geologia che l'ambiente, hanno indotto governi e industrie a convergere sulla necessità di creare un'economia efficiente sotto il profilo delle risorse, verde, competitiva, e a basse emissioni di carbonio. Tra tutte le strategie applicabili in questa direzione, la chimica verde deve (o dovrebbe) essere la forza trainante per mitigare l'inquinamento e ridurre il consumo di risorse, per poi raggiungere l'obiettivo di ridurre a lungo termine la dipendenza dal carbonio, secondo le linee guida del recente accordo di Parigi sul clima. In questo ambito, un ruolo di rilievo è giocato dalle bioraffinerie di seconda generazione, individuate come basi fondanti per la bioeconomia globale, in grado, come le comuni raffinerie di petrolio, di sviluppare catene di valore integrate per la produzione di alimenti umani e animali, sostanze chimiche, materiali, combustibili, energia e/o calore a partire da produzioni sostenibili di biomasse. Nella moderna bioraffineria questo obiettivo è raggiungibile mediante studi approfonditi interdisciplinari di chimica, chimica organica e biochimica, in grado di individuare strategie di ottenimento e di trasformazioni di molecole semplici di origine biologica ("bio-derived platform chemicals") da cui recuperare prodotti e materiali sostenibili a valore aggiunto. Si prevede che i risultati migliori saranno ottenibili dalla combinazione di approcci integrati di biotecnologia, chimica e ingegneria.

Poiché i componenti principali della biomassa vegetale sono zuccheri a cinque e sei atomi di carbonio (C5 e C6), comuni soprattutto nelle forme condensate dei polisaccaridi strutturali e di riserva, costituiti da unità ripetitive di D-glucosio, D-galattosio, D-mannosio, D-xilosio, L-arabinosio, ecc., l'attenzione di ricercatori e industrie si è concentrata su queste molecole, in particolare sugli esosi (C6) perché più abbondanti e, più specificamente, sui componenti più vicini alla fotosintesi D-glucosio e D-fruttosio. L'ambiente ossidante in cui questi prodotti si generano in natura rende altrettanto facilmente disponibili vie biosintetiche in grado di accedere ad altre piccole molecole, più ossidate degli zuccheri, contenenti tipicamente funzionalità acide. Tra queste si colloca la classe di prodotti studiati in questa tesi, gli acidi aldarici. Questi sono un gruppo di molecole derivate da zuccheri C6 in cui i gruppi terminali idrossimetile e aldeidico sono sostituiti da due gruppi carbossilici e sono quindi caratterizzati dalla formula generale HOOC-$(CHOH)_n$-COOH. Essi si distinguono per la diversa chiralità dei quattro carboni recanti le funzioni alcoliche e sono già noti per specifici usi o quali intermedi per prodotti di chimica fine e per polimeri biodegradabili.

Nell'intento di ottenere processi ad elevata economia atomica inseribili in bioraffinerie, il mondo accademico e industriale ha sviluppato in anni recenti strategie di impiego diretto di queste matrici a sei atomi di carbonio come piattaforma di base incorporabile in prodotti e materiali mediante

funzionalizzazioni ai gruppi carbossilici ed alcolici. L'acido D-glucarico (uno dei più rappresentativi acidi aldarici e tra le 12 migliori molecole di base indicate dal DoE americano) ha attratto la maggior parte delle attenzioni, con approfondimenti sugli aspetti preparativi e di reattività nella direzione della complessazione di cationi inorganici, dell'interazione con molecole biologiche (con ricadute in ambito farmacologo e medico) e dei materiali polimerici. È sentore comune che gli altri i membri della famiglia degli acidi aldarici, avendo una struttura simile, abbiano un potenziale altrettanto elevato dell'acido glucarico, ma le verifiche in tale direzione sono scarse, cosicché questi prodotti costituiscono ancora, ma in modo immotivato, molecole costose e rivolte ad applicazioni di nicchia. Nella tesi sono raccolte ed utilizzate le informazioni rappresentative desunte dalla letteratura scientifica sui principali acidi aldarici (acido glucarico, acido mannarico e acido galattarico) come introduzione alla loro sintesi, reattività, applicazione e loro potenziale come molecole di base.

La tesi è centrata specificamente sull'acido galattarico (noto anche come acido mucico) come modello dell'intera classe. Questo acido, infatti, nonostante la natura multi-chirale dei quattro carboni centrali, esiste in forma *meso* achirale senza attività ottica, molto conveniente in fase di ottenimento e purificazione, oltre che agevolante le ulteriori modifiche sintetiche. Esso, inoltre, è un potenziale intermedio per la produzione di altri acidi dicarbossilici C6 di importanza industriale, quali l'acido adipico, l'acido 2,5-furandicarbossilico e l'acido tereftalico. Lo studio affrontato in questa tesi rappresenta un tentativo di migliorare le conoscenze sulla chimica degli acidi aldarici, di estendere la scala produttiva di questa classe di composti (oltre le 30,000 ton/anno tipica dell'acido glucarico ed oltre le 15,000 ton/anno per l'acido galattarico) e di integrare i processi bio-idrolitici ossidativi a partire da polisaccaridi purificati e, in prospettiva, da scarti di lavorazioni agricole.

Lo studio ha preso in esame la reattività dell'acido galattarico in processi ionici di sostituzione, eliminazione, condensazione e riduzione allo scopo di delineare e sviluppare processi di trasformazione in prodotti della chimica fine, nuovi materiali funzionali e polimeri basati sui monomeri pirrolici, ammidici e esterei. Gli ambiti più promettenti sono stati individuati nelle seguenti trasformazioni: a) disidratazione degli acidi aldarici e loro esteri, b) identificazione dei lattoni e degli intermedi insaturi coinvolti nella disidratazione; c) acilazione dei gruppi ossidrilici e ruolo degli esteri nell'eliminazione dei gruppi acilici; d) riduzione di intermedi insaturi di acidi aldarici; e) preparazione e ruolo dei sali (sia inorganici che organici) nei processi sopra menzionati; f) mono-aldarammidi e loro sali, di-aldardiamidi e polialdarammidi; g) possibili trasformazioni efficienti in prodotti e monomeri aromatici a base di furano, pirrolo e alfa-pirone.

Le nuove conoscenze acquisite hanno ispirato lo sviluppo di nuovi percorsi e processi di bioraffineria per convertire l'acido galattarico in un nuovo gruppo di prodotti chimici/intermedi di base, migliorando così anche la sostenibilità della produzione di biocarburanti da biomasse.

Una parte importante dello studio ha riguardato il galattaro-1,4-lattone, di cui si è sviluppato un metodo preparativo semplice in rese quantitative per riscaldamento in dimetilsolfossido e si è indagata la

versatile chimica, chiarendone le potenzialità come nuova molecola di base. Il lattone infatti è quantitativamente convertito nei suoi sali inorganici e organici in condizioni blande e nei sali dell'acido mucico, superando le difficoltà di solubilità tipiche di questo composto. Inoltre, per trattamento con ammine primarie questo lattone è convertibile in sali di ammonio di galattarammidi e/o in galattarodiammidi in alte rese. Tali derivati si sono dimostrati un buon punto di partenza per la sintesi di omo- e co-polimeri per policondensazione termica. L'insieme dei risultati sul galattaro-1,4-lattone hanno consentito di razionalizzare i dati sporadici di letteratura sulle reazioni tra l'acido mucico e varie tipologie di basi. Interessanti conclusioni sono state anche ottenute sui processi di esterificazione delle funzioni alcoliche del galactaro-1,4-lattone, per i quali si è dimostrato che l'origine della selettiva introduzione delle funzioni aciliche è indotta dalla formazione intermedia di anidride miste tra la funzione acida di questo prodotto e le anidridi alifatiche utilizzate, anidride acetica e formica-acetica. I derivati triacetilati, e soprattutto il triformilato, subiscono de-acilazione selettiva da parte di basi a lattone mono-insaturo, dimostrando peraltro che il processo è comune anche a mono e dilattoni di altri acidi aldarici. Questi lattoni mono-insaturi sono intermedi chiave per l'ulteriore eliminazione ai derivati di-insaturi e all'acido furan-2,5-dicarbossilico sotto catalisi acida. Gli approfondimenti sulla disidratazione dell'acido galattarico sono stati condotti mediante un approccio di acilazione e deidroeliminazione in due fasi, utilizzando catalizzatori misti acido/base, e sono confluiti in un nuovo metodo di sintesi di derivati pironici a partire da carboidrati. L'indagine ha chiarito la rilevanza e versatilità dei derivati dell'acido 2,5-diidrossimuconico quali intermedi chiave nella doppia disidratazione e in successivi processi di aromatizzazione e riduzione. La disponibilità di questi prodotti ha infatti consentito di sviluppare un nuovo metodo di accesso a derivati dell'acido 2,5-diidrossiadipico per idrogenazione catalitica, e, in presenza di ammine primarie, un nuovo metodo di sintesi dell'acido pirrol-2,5-dicarbossilico, (ma anche per blanda decarbossilazione, dell'acido 2-pirrolcarbossilico e pirroli N-sostituiti) nonché semplificare l'accesso all'acido 2,5-furandicarbossilico. L'insieme dei dati raccolti ha tra l'altro fornito una migliore comprensione dei dettagli meccanicistici della trasformazione dell'acido mucico ed ammine in pirroli o ammidi, portando queste reazioni, precedentemente poco indagate, ad interesse preparativo.

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I am deeply grateful for all the help and support I have received throughout my Ph.D. This thesis would not have been possible without the guidance, support, and encouragement of so many people during my time at Politecnico. First of all, I would like to express my sincere gratitude to my thesis advisor Prof. Attilio Citterio for the opportunity to work with him. It has been a great experience. Citterio is always encouraging and supportive. I remembered the time when he was solving my problems faced during the experiments, discussing and interpreting the results of analysis, our long conversation guiding me how to properly plan my research and thesis, and our conversations about career development. I have seen and also really appreciated his constant efforts to equip us with the continuous supports and best resources to do research. I would also like to thank my thesis committee members: Prof. Gaetano Guerra, and Prof. Daniele Dondi. Their insightful comments and suggestion prompted me to optimise my thesis and broaden my research from various perspectives.

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Chapter 1

1.1 - Climate Change as Background

1.1.1 - Climate change as background

From the time of the Industrial Revolution onwards, the population on earth increased more than 10-fold to 7 billion. Industrial production increased more than 40-fold during the "Great Acceleration".[1] Meanwhile the humanity has put a significant global impact on our planet, concerning both the geology and environment. Furthermore, human influence on the climate system is also clear, which has had widespread influences on human and natural systems. Warming of the climate system is unequivocal, and since the 1950s, many of the observed changes are unprecedented over decades to millennia. The atmosphere and ocean have both warmed, the amounts of snow and ice have diminished, and sea level has risen. To be exact, the globally overall surface temperature-as calculated by a linear trend model shows an average increase of 0.85 °C over the period 1880 to 2012, and almost the entire earth has experienced surface warming, as shown Figure 1.1. [2]

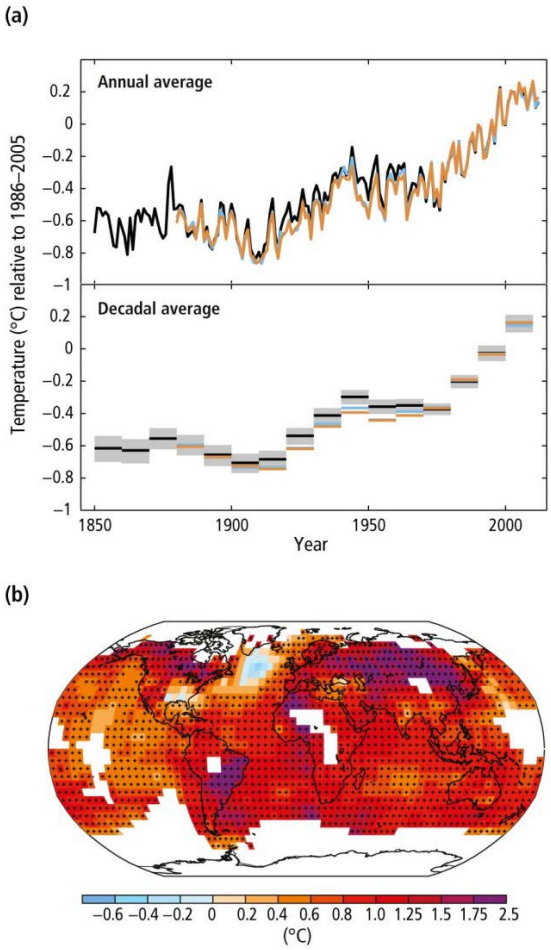


Figure 1.1 – (a) Observed globally averaged combined land and ocean surface temperature anomaly 1850-2012 (relative to the mean of 1986 to 2005 period) with an estimate of decadal mean uncertainty included for one data set (grey shading); (b) Map of the observed surface temperature change from 1901 to 2012.[2]

From preindustrial times until now, global sea level cumulatively roses by 19 cm (Figure 1.2). This rise rate since the mid-19th century has been the largest among the mean rate during the previous 2000 years.[2]

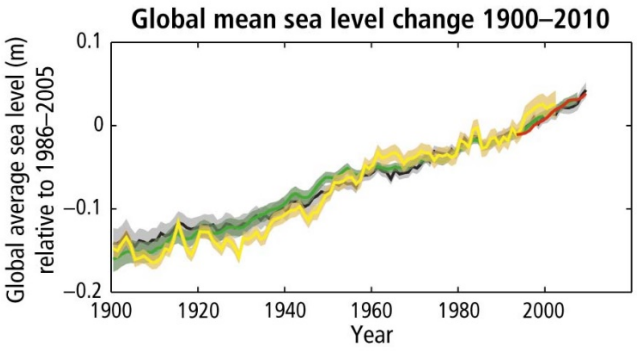


Figure 1.2 - Global mean sea level relative to the mean of 1986–2005.[2]

The climate changes above are mainly caused by natural and anthropogenic substances and processes that alter the energy budget of the Earth. One of the most important drivers is the anthropogenic greenhouse gas (GHG) emission, which has increased since the preindustrial era due to fossil fuel consumption, agriculture, land use changes, and reached the highest level in history during the first decade of 2000s. Historical emissions have driven atmospheric concentrations of CO_2, N_2O and CH_4 to levels that are unparalleled in history, which can trap heat in the atmosphere leading to an uptake of energy by the climate system. Considering only the emission of CO_2 by CO_2eq, which indicates the total weights of all greenhouse gases, it roses from 280 to 430 ppm (part per million).[3] Among all the sources of CO_2 emissions, fossil fuel combustion and industrial processes play the most important role, which contributed respectively 47% and 30% to the total GHG emission increase from 1970 to 2010, as shown in Figure 1.3. The percentage keeps the same for the last 10 years. Moreover, the global average temperature can further increases 2 degrees by 2050 and 4 degrees by the end of this century under the scenarios of CO_2 emissions keep increase by 70% and 250% in industrialized countries and developing countries, respectively.

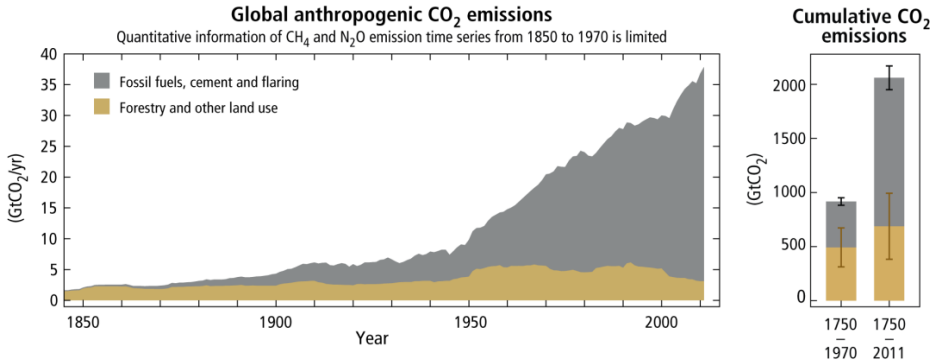


Figure 1.3 - Annual global anthropogenic carbon dioxide (CO_2) emissions (gigatonne of CO_2-equivalent per year, GtCO2/yr) from fossil fuel combustion, cement production and flaring, and forestry and other land use, 1750–2011.[2]

As a long tradition, the European Union (EU) has the world's highest environmental standards, which aims to make green the Europe economy, protect nature, and safeguard the health and life quality of people living in the EU. With the broad range of environmental legislation, air, water and soil pollution has significantly been reduced. In the new century, recognizing all these challenges of global climate change and environment deterioration caused by human activities, the EU has reacted by putting in place a broad range of environmental legislation. The European Climate Change Programmes I and II (EPPC) have been introduced in succession as a Programme to identify and develop all the necessary elements to implement the Kyoto Protocol launched in 1997. Now facing the fact that challenges persist, the 7th Environment Action Programme (EAP) was introduced in January 2014 to guide European environment policy until 2020 in order to tackle together the problems in a structured way.[4] Here, the EU live vision in year 2050 set by the direction was so mentioned:

"In 2050, we live well, within the planet's ecological limits. Our prosperity and healthy environment stem from an innovative, circular economy where nothing is wasted and where natural resources are managed sustainably, and biodiversity is protected, valued and restored in ways that enhance our society's resilience. Our low-carbon growth has long been decoupled from resource use, setting the pace for a safe and sustainable global society." [4]

The main objectives will be:

- to protect, conserve and enhance the Union's natural capital
- to turn the Union into a resource-efficient, green, and competitive low-carbon economy
- to prevent the Union's citizens from environment-related pressures and risks to health and wellbeing[4]

Furthermore, the Paris Agreement set another multinational objective to fight against the climate change, which is to strengthen the global response to the threat of climate change by keeping a global

temperature rise this century well below 2 degrees Celsius above pre-industrial levels and to pursue efforts to limit the temperature increase even further to 1.5 degrees Celsius.[5] In order to reach all these goals, at least a reduction of global Greenhouse gases emission of 40% by 2030 is required. Based on the integrated climate and environment policies, still a huge effort of multidisciplinary research and technology development are essential for the target of emission reduction. In our case, it is important to know what role chemistry plays among all the driving forces of greenhouse gases emission and to what extend it affects the emission structure, in order to fit chemistry in this grand context and help achieve goals. Therefore, a deep-in analysis of the greenhouse gases emission drivers is necessary.

In 2014, the industry sector accounted for around 36% of global final energy consumption. The generated industrial CO_2 emissions are larger than the emissions from either the buildings or transport sectors and just reached 8.3 Gt of CO_2, over 24% of global CO_2 emissions in 2014. For chemicals and petrochemicals sector, it is the largest industrial energy user accounted for 28% industrial final energy consumption in 2014, with 13% of total industrial CO_2 emissions (Figure 1.4).[6] In consideration of the wide product categories and large gap of diverse production scale of chemicals industry, the big difficulty of collecting emission data is obvious. Even though, some valuable conclusions were still reached by the analysis carried out by Intergovernmental Panel on Climate Change (IPCC). For instance, limited key products contribute more than 50% of CO_2 emissions in chemical and petrochemical industry: ethylene, ammonia, nitric acid, adipic acid and caprolactam used in producing plastics, fertilizer, and synthetic fibres. Among all the non-CO_2 greenhouse gases, fluorinated gases and N_2O were the most important emissions in all the manufacturing sectors from 1990 to 2010. Moreover, most of the N_2O emissions from the industrial sector are contributed by the chemical industry, particularly emitted from the production of nitric and adipic acids.[7]

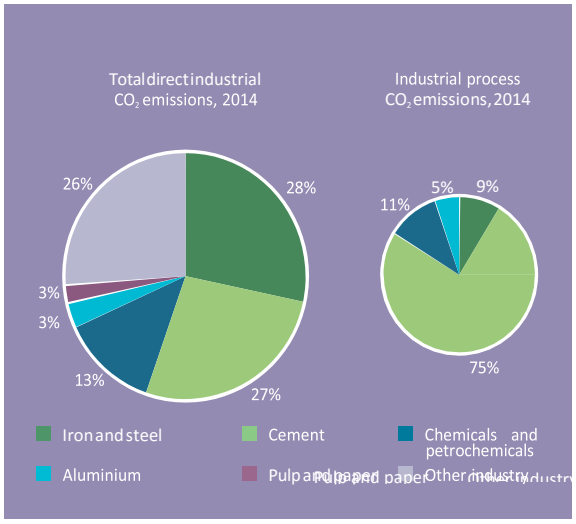


Figure 1.4 – Direct industrial CO_2 emission.[6]

Concerning its proportion in the total emission, any improvement of chemistry in this direction would hence bring significant benefits. Therefore, at moment some mitigation options have already been

discussed in order to reduce the greenhouse gas emissions generated by chemical industry, among which green chemistry should play an essential role in helping to mitigate climate change through innovations.

Taking example of galactaric acid as my research object, oxidation by nitric acid as a traditional chemical route for preparing this chemical leads to relevant issues of NO_x gases formation. Thus, some improved catalytic route and alternative biosynthetic methods were proposed in literature, as discussed in Chapter 2 of this thesis, but were never applied on industrial scale. The study developed in this thesis on the chemistry of galactaric acid provides a further contribution in the direction to devise better the potentiality of natural resources to substitute petroleum as raw material for adipic acid and other key intermediates.

Green chemistry can facilitate the development of integrated life cycle assessment for products to minimize total energy consumption in production by the application of highly integrated value chains for industrial products and the improved valorisation of coal, oil and biomass as feedstocks in chemical industry through new catalytic (chemical and biological) pathways. For example the development of biomass-based route to avoid steam cracking could reduce CO_2 intensity.[8] Another strategy is trying to reuse primary greenhouse gas CO_2, CO as raw material for plastics and coatings used in electronics and food packaging, which can save 50% of petroleum.[9] Eventually, green chemistry should be and must be a driving force for the transition toward less carbon-intensive production processes and innovative bio-based process routes, and then reach the longer-term decarbonisation target to fulfil the final goals of the Paris Agreement.

1.1.2 - Green Chemistry

Green chemistry is the design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances.[10] The concept of green chemistry was first conceived in 1991, and the original intention was to study the development of environmentally friendly processes due to the fact that chemicals, chemistry and chemists have been widely considered as the cause of problems by government and public instead of its positive role in sustainable development.[11] Italy is one of the very early participants and promoters of green chemistry, with the first interuniversity consortium featured in research on green chemistry, Consorzio Interuniversitario “La Chimica per l’Ambiente” (Interuniversity Consortium Chemistry for the Environment), or INCA established in 1993 aiming to uniting academic groups concerned with chemistry and the environment.[12]

One of the most important aspects of green chemistry targets is to design the next generation environmentally benign products and processes, which are profitable while being good for both the human health and the environment. Furthermore the term “hazardous” in the green chemistry definition is also essential to elaborate the aim of green chemistry, which is used in the broadest context including physical (e.g., flammability, explosion), toxicological (e.g., carcinogenic, mutagenic), and global

environmental challenges (e.g., climate change, ozone depletion). In consequence, advances in green chemistry have already addressed both obvious chemical hazards and those associated with such global issues as mentioned above like climate change, energy production, availability of a safe and adequate water supply, food safety, and the presence of toxic substances in the environment.[13] This field has drawn a great deal of attention in the last 20 years because it harnesses chemical innovation to satisfy economic and environmental targets at the same time by collaborating research laboratory with industrial company, government, and generic public.

In order to design environmentally benign products and processes as indicated in the green chemistry and green engineering definitions, the Twelve Principles should be clearly considered and respected as one cohesive set. Introduced in 1998 by Paul Anastas and John Warner, they represent relevant design criteria or guiding framework to sustainability. A principle construct is built for the design of benign chemicals and chemical transformations, which consider all aspects of the process from the raw materials to the transformation safety and efficiency, the toxicity and biodegradability of products and reagents used in order to reduce hazards across all the stages of the life-cycle. The twelve principles of green chemistry states:

I. **Prevention.** It is better to prevent waste than to treat or clean up waste after it has been created.
II. **Atom Economy.** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
III. **Less Hazardous Chemical Syntheses.** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
IV. **Designing Safer Chemicals.** Chemical products should be designed to affect their desired function while minimizing their toxicity.
V. **Safer Solvents and Auxiliaries.** The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
VI. **Design for Energy Efficiency.** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
VII. **Use of Renewable Feedstocks.** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
VIII. **Reduce Derivatives.** Unnecessary derivatization (use of blocking groups, protection / deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
IX. **Catalysis.** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
X. **Design for Degradation.** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

XI. **Real-time analysis for Pollution Prevention.** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

XII. **Inherently Safer Chemistry for Accident Prevention.** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.[10]

They are now also summarized into one much simpler acronym, PRODUCTIVELY, for the convenience of memory.[14]

Since its establishment in 1991, green chemistry as a new framework for technology development has grown very fast and lead to a significant amount of major research, education, and outreach advances around the world. For instance, the development of green solvents has been one of the most active research areas of Green Chemistry with great advances in aqueous (biphasic) catalysis, the use of supercritical fluids in chemical reactions, the application of biobased ionic liquids and fluorous media as environmental friendly solvents, the compact "solvent-free" approaches, and the application of microsystems in synthesis and separations. Great effort was also addressed to improve the synthetic methodologies toward an "ideal synthesis" (Figure 1.5) with the goal to reach atom and step economy to improve efficiency, safety and environmental impacts on industrial scale. A large number of green metric indicators were introduced in the attempt to better evaluate and compare different alternative approaches. Meanwhile, some technologies like the use of microwave and ultrasonic energy sources in synthesis as well as the applications of new catalytic processes and microreactors have continued to obtain advance for green chemistry.[15] Although an impressive amount of successful work has been realized within the green chemistry community all over the globe, the potential of the area is still huge since some of the most important questions of green chemistry are just started to be identified and solved.

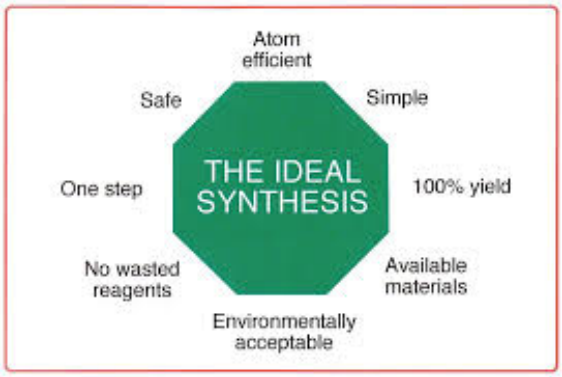


Figure 1.5 – The ideal synthesis.[16]

Since the structure of the feedstock portfolio was always changing along the human history, we can anticipate that more dramatic shifting will occur in the next years due to supply, performance, technical

improvement, economics, public perception and policies. In turn, the future research trends and challenges would inevitably be affected by this shift toward more diverse feedstock, among which benign and renewables materials are one of the most promising component for solving the problem of world resource exhaustion. But the reality is that more than 99% of all organic chemicals are derived from petroleum by 2000.[17] In order to create a more sustainable chemical industry is urgent to devise economically and technologically sustainable approaches for the production of carbon-based chemicals from finite fossil fuel sources to renewable feedstocks. Some significant breakthroughs in the development of renewable feedstock have already been made in approving how to use bio-based materials, such as sugars and starch, for basic chemical building blocks. For example levulinic acid,[18] alcohols, ketones and carboxylic acids as useful chemical intermediates have been produced from agricultural wastes. Alternative feedstocks will not be limited to plant-based sources, chitosan as a potential biopolymer has been isolated and processed from shells of fishery wastes.

As IEA Bioenergy Task 42, biorefinery was treated as one of the important base for global Bio-Economy by developing integrated sustainable value chains to producing food/feed ingredients, chemicals, materials, fuels, power and/or heat from sustainably sourced biomass.[19] Although biorefinery for fuel production is still the mainstream of development. Until now, the economically competitive production of biofuel is always a challenging task. Therefore, the co-production of chemicals can create benefit to compensate the less economic biofuel production. Concerning the main features of biorefinery defined by IEA Biorefinery Task 42 "Biorefinery", platform chemicals are key intermediates between biomass and final products and would address the strategies of integrated biorefinery applications leading to bio-based chemicals.[20] By this way, the majority of currently fossil-derived chemicals can be substituted by the bio-based chemicals with minor environmental impact. All this just meets the global trend of sustainable economy (or circular economy) based on renewable resources.

As a specific example of this trend, several efforts has been made to convert glucose, derived from biomass, into adipic acid (Figure 1.6) using genetically engineered Escherichia coli,[21] but alternative routes were proposed starting from other carbohydrates derivatives (e.g., glucaric acid, galactaric acid, in part discussed further in Chapter 2 of this thesis), but also from lipids and amino acids. This trend is typical of the present uncertainty on the selection of best bio-feedstock for specific chemical and evidence the general gift that should be further acquired in order to develop efficient, economical, environmentally friendly process for industrial scale transformation of biomass into diverse products. In general, it is expected that to meet the principles of green chemistry in the production of large-scale products, e.g. bulk chemicals, pharmaceuticals, and polymers, the best approaches will involve feedstock that need less modification of the original structural framework of the components of the biomass.

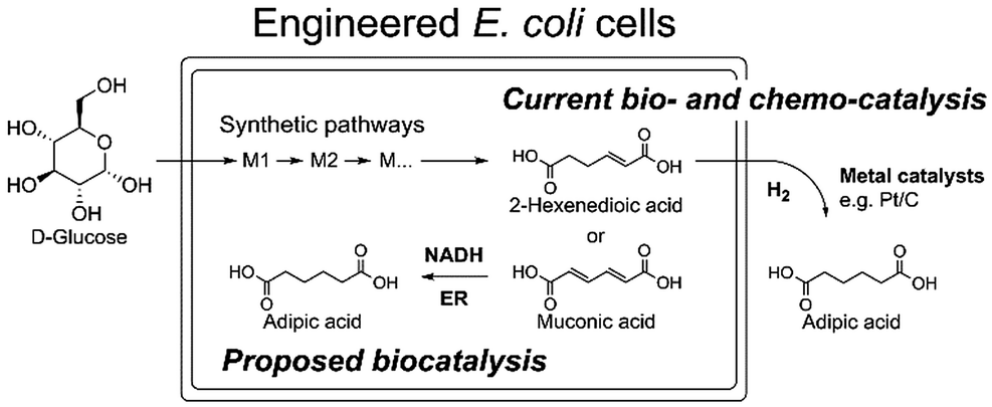


Figure 1.6 – Bio- and chemo-catalytic synthesis of adipic acid from glucose.[21]

1.2 - Introduction of Biomass

1.2.1 - Biomass as feedstock for energy and chemicals

The main global problem of climate change is mainly caused by the emission of carbon dioxide (CO_2) as main greenhouse gases and the excess of CO_2 emitted is essentially related to the increased dependence on fossil fuels, which include coal, petroleum crude oil, and natural gas. In the past century this has provided the energetic foundation for the human economy. Furthermore fossil fuel production and consumption not only are the leading cause of global warming, but also lead to significant pollutions to water, air, and soil, which in turn impose a great threat to human health and environment. With the depletion of fossil fuels supply, the uncertainty of fossil fuel price and the environmental challenges discussed above, it is imperative to develop clean and sustainable alternatives for the sustainable production of fuels and chemicals. In this context biomass has attracted considerable attention and efforts as a sustainable power source.

First of all, biomass stands as the third-largest energy resources around the world.[22] The total live biomass on Earth is estimated about 1899 billion tons, equivalent to 550 - 560 billion tons of carbon, and the total annual primary production of biomass is just over 100 billion tons carbon/year.[23] Biomass is also a carrier of energy, which receives solar energy and store it in form of chemical energy through the photosynthetic process of plants, algae and some types of bacteria. Approximately 3,000 EJ/year of energy are captured in biomass by photosynthesis. To be more specific, the total energy reserved in the biomass on the ground is about 33000 EJ, which corresponds to more than 60 times of annual global energy consumption, 500 EJ/year.[24] What's more, biomass is renewable and CO_2 neutral, which generates lower greenhouse gases during transformation, making it a clean development mechanism (CDM) for reducing greenhouse gas emissions.[22] Biomass is treated as renewable feedstock because it needs only relatively short time to replace the consumed amount. Meanwhile, the CO_2 released from

the biomass transformation can be consumed and stored again in new biomass material, which can be regarded as an overall CO_2 neutral process. At moment, many efforts have been done in many developed and industrialized countries in order to search more efficient way to use biomass for energy market. Sweden, for instance, has already be obtained about 13% of their total energy from biomass, with reducing of fossil fuel usage. Biomass and waste fuels generated 71.4 billion kW·h of electricity in 2016,[25] or 2% of total generation in the United States, according to EIA's recently released annual electric power data (Figure 1.7).

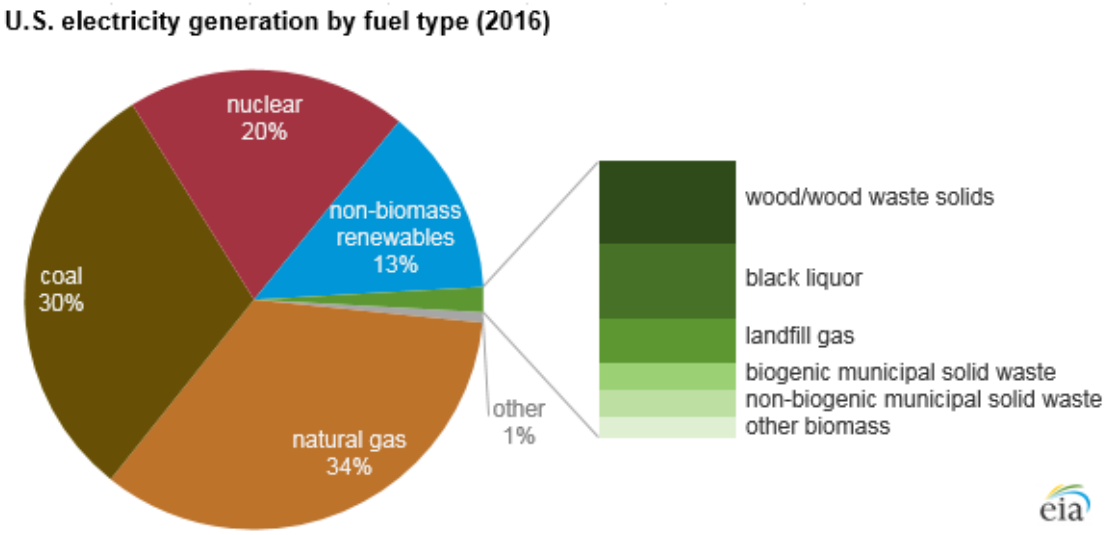


Figure 1.7 - U.S. electricity generation by fuel type in 2016.[25]

The global biomass-based electricity production can be processed by solid, liquid or gaseous biofuels, and the solid form biofuel accounts for the largest portion of bio-power. In order to fulfill the target of greenhouse gas emission reduction, many technologies were introduced to apply different types of biomass fuels, among which co-firing of fossil fuel together with biomass is one of the most successful technology for industrial application. Biomass co-firing has been built and run in over 150 installations worldwide for most combinations of fuels and boiler types. There are already more than hundred in Europe. To overcome the biomass influence on coal-fired plants efficiency, boiler technology have been designed and introduced into co-firing system. Various technologies have been developed to enable co-firing biomass with coal in pulverized coal (PC) boilers. The vast capacity of existing PC boilers offers great potential for increasing biomass utilization and economic benefits compared to new stand-alone biomass power plants, which also are usually significantly smaller than PC plants, as shown in Figure 1.8.

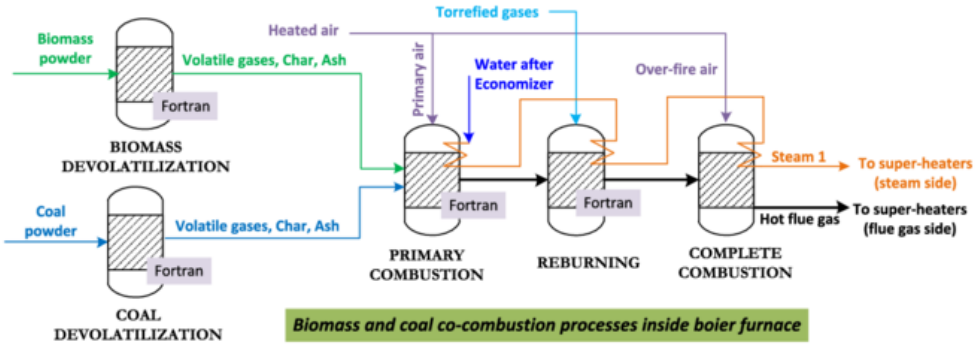


Figure 1.8 - Torrefaction based biomass co-firing power plant.[26]

There are also several commercial experiences in biomass co-firing applications to identify the opportunities, and technical barriers, associated with co-firing coal and biomass. For example, in the past decade KEMA has built co-firing power plant in both direct and indirect processes using different biomass. They have tried to co-firing coal and biomass percentage up to 25 %. More than 50 small and full scale biomass trials have been put into commercial operation.[22]

Apart from the high dependence of fossil fuel energy, the ever-growing pollution of non-biodegradable waste, like plastics, is another grand challenge for sustainable development. Most of the carbon-based compounds currently manufactured by the chemical industry originates from petroleum. About 6% of the global oil consumption is used for manufacturing plastics, whose production each year covers 260 million tons and continues to rise. Plastics accounts for 15-25% of European total waste amount and more than 91% of plastic wastes haven't been recycled. As a result, every year 8 million metric tons of plastic end up in our oceans. Plastic debris has now become the most serious problem affecting the marine environment. Not only the coastal areas of developing countries that lack appropriate waste management infrastructures are concerned, but also the world's oceans as a whole because slowly degrading large plastic items generate microplastic (particles smaller than 1 to 5 mm) particles, which spread over long distances by wind-driven circulation of ocean surface layer. Microplastics can act as carriers for the transfer of persistent organic pollutants from the environment to organisms thanks to their ability of transporting synthetic organic compounds by adsorption.[27] Furthermore, the ban on imports of millions of tonnes of plastic waste by the Chinese government from January 2018 will pose big challenges to the Europe's efforts to recycle more plastic. Since by 2030 the ban might leave 111 million metric tons of plastic trash with nowhere to go based to the amount of plastic waste China imported from 1988 to 2016.[28] Concerning the big difficulty of recycling mineral oil based polymers due to its non-biodegradability, the best option will be to avoid using single-use items, like plastic straws and cups, in the first place. Opting for reusable bottles and bags can reduce the amount of plastic trash produced every year. It's demonstrated that both natural polymers isolated from biomass, like polysaccharides, proteins, and synthetic polymers derived from biomass-based monomer (e.g., lactones, polyols, dicarboxylic acids, amino acids, etc.) containing bonds that are hydrolytically and/or

enzymatically sensitive are potentially well sustainable materials, being intrinsically biodegradable.[29, 30] Therefore, renewable polymers from biomass are now in development worldwide to be used for the production of biodegradable materials similar to that derived from oil-based polymers. Evidences have, however, emerged on the incompatibility of these materials with the traditional plastic recycling processes, which face on new challenges in the immediate future related to the coexistence of two types of plastic.

In this context, biomass has received considerable attention as a sustainable feedstock not only for production of low value biofuels but also high value-added biobased chemicals, which now are still derived mainly from fossil-based feedstock. From Table 1.1, one can preliminarily quantify the value of different "biomass valorization" strategies.[31] This can reduce nonrenewable fuel consumption while simultaneously providing the necessary financial incentive to stimulate expansion of the biomass industry.[32] Because biomass is the only one among all the renewable energy feedstock which can provide organic carbon to chemical industry in sustainable yield including bulk and fine chemicals, pharmaceuticals, food additives, etc. Therefore, there is huge potential for bio-based chemicals to share markets with petro-based products.

Table 1.1 - Approximate valorization of biomass waste for different uses.[33, 34]

	Value ($/t biomass)
Average bulk chemical	1000
Transportation fuel	200-400
Cattle feed	70-200
Generating electricity	60-150
Landfill	-400 (cost)

Thanks to the continuous development of advanced technologies in chemical processing, in the recent years, significant progresses have been achieved in biomass industrial application with diverse bulk chemical products, for instance bioethanol, biodiesel, lactic acid and polylactide (PLA), etc. However, relevant obstacles to the integration of biomass-derived molecules into petroleum industry to get new platform molecules have been evidenced, between which the complex chemical structure of bio-compounds play a crucial role. This means that the huge amount of work on chemistry and process technology, developed to convert reduced molecules of petroleum into useful chemicals and materials, can now be only marginally used when bio-molecules and bio-materials are concerned. The higher oxidation state of bio-molecules requires the development of new strategies to modify selectively the functional groups present, for instance altering the oxygen content by depolymerization, hydrolysis,

dehydration, deoxygenation, oxidation, etc. The value of particular type of biomass depends on its chemical and physical properties. For example, lignin is challenging to break down into chemically useful fragments. Meanwhile, pretreatment of polysaccharides, triglycerides, and proteins can lead more easily to their constituent building blocks: monosaccharides, fatty acids plus glycerol, and amino acids, respectively.[31] Therefore, investigating biomass in a profitable way will be a complicated task involving careful analysis of residual biomass constituents, new efficient separation technologies, and optimized selective functional group modifications, along with strategies in biomass growth, collection and transport. The difficulty stays in the development of well-integrated systems that can provide economical and technical success on industrial scale. This task can originate local solutions but also relevant issues in competition between nations and regions, to be solved hopefully at political supranational level.

1.2.2 - Biomass Classification and Composition

By definition biomass is "Any organic material both aboveground and belowground, and both living and dead, e.g., trees, crops, grasses, tree litter, roots etc. Biomass includes the pool definition for above - and below - ground biomass. Biomass can be mainly split into two distinct categories, waste biomass and energy crops."[35] The main object of our study will be the waste biomass in the direction to meet the requirement of sustainable development. The reason will be further discussed in section 1.3 of this chapter. The commonly used classification scenario of biomass as a feedstock for energy and chemicals purpose include oil seeds, grains, sugar crops, agricultural residues, trees, grasses, and algae. In order to differentiate the biomass raw material based on their origin and source, the European committee for standardization (CEN) has published the classification of biomass integrated in the 27 technical specifications (pre-standards) for solid biofuels during 2003-2006. The solid biofuels are classified by the following sub-categories: a) Woody biomass; b) Herbaceous biomass; c) Fruit biomass; d) Blends and mixtures. This will help us to trace the fuel and chemicals production chain. According to CEN standards, woody biomass includes trees, bushes, and shrubs while herbaceous biomass includes plants that have non-woody stem and which die back at the end of the growing season, like grains or seeds crops from food processing industry and their by-products such as cereal straw. One of the classification of Biomass is reported in Figure 1.10.[33]

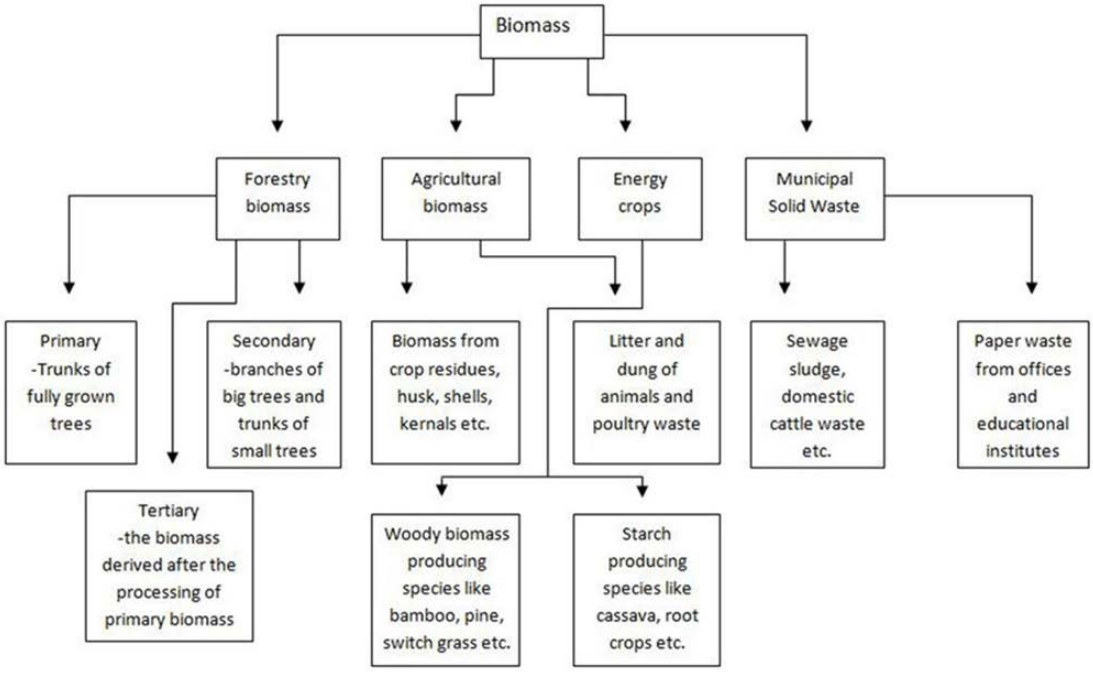


Figure 1.9 – Classification of biomass according to sources.[36]

The composition of biomass varies significantly due to the specificity of individual living species. In many contexts, the term "biomass" refers in particular to plants or plant-based materials, which is also consistent with our object of research. There are mainly six components in plant cell wall: (i) cellulose, (ii) hemicellulose and pectin, (iii) lignin, (iv) starch, (v) proteins, and (iv) ether and alcohol-soluble constituents (e.g. fats, oils, waxes, resin and many pigments, collectively indicated as secondary metabolites). Cellulose, hemicelluloses and lignin are recognized as primary contents inside natural lignocellulosic biomass. Linear cellulose molecules determine the framework of plant cell, whereas hemicellulose works as linker of cell wall cellulose microfibrils via its short-branched hetero-polysaccharide structure, and lignin has the role of a polymeric filler based on its three dimensional amorphous propyl-phenol structure bounded to hemicellulose and cellulose. In addition, cellulosic materials contain abundant cell wall protein and other secondary metabolites. The variety and proportion of these constituents will decide the different types of biomass. For example, lignocellulose biomass contains normally 35-50% of cellulose, 20-35% of hemicellulose and 5-30% of lignin, respectively. Since cellulose, hemicellulose and pectin are all specific polysaccharide (macromolecular carbohydrates consisting of a large number of monosaccharides connected to each other by glycosidic bonds), strong effort has been devoted in the past to elucidate the structure, biosynthesis and biological function of polysaccharides.[37, 38] More recently, the focus was addressed to develop technologies for conversion of unutilized polysaccharides and sugars into recognized platform molecules (such as glycerol, 5-hydroxymethylfurfural, glucaric acid, lactic acid, levulinic acid, etc.) and related

conversion technologies towards high value materials and chemicals. The potential of carbohydrates derivatives biorefinery will be further discussed in the afterward chapters.

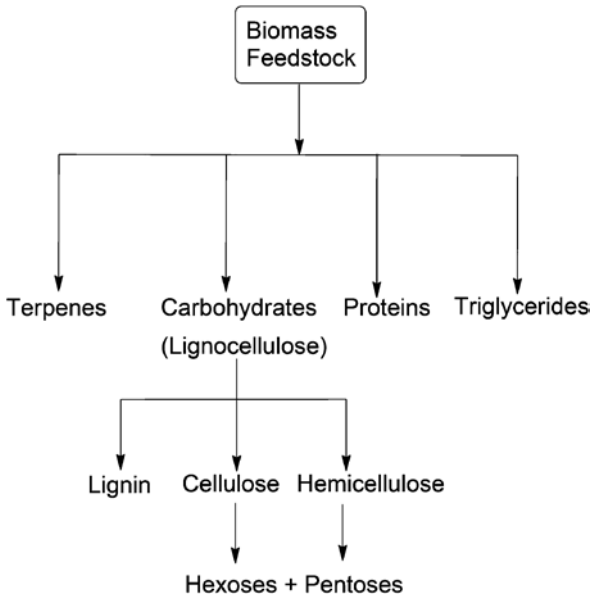


Figure 1.10- Primary components of biomass.[39]

Cellulose:

Cellulose is the main polysaccharide in biomass and glucose is the fundamental unit of cellulose. The open form of glucose is in equilibrium with the corresponding hemiacetal pyranosidic or furanosidic cyclic forms (anhydroglucose). These species link together end to end with strong β-1,4-glycosidic bonds to form the polysaccharide chain $(C_6H_{10}O_5)_n$. Owing to the specific opposite arrangements of the side hydroxymethyl groups in cellulose the real monomer is the dimer cellobiose (Figure 1.12). The degree of polymerization of cellulose varies between 5000 and 10,000. The abundant hydrogen bonds, both inside and between chains, organize secondary, tertiary and quaternary structures affording a quite peculiar fibrillary network compatible to form high crystalline region of the resulting materials. For example, the cellulose content accounts of almost 100 % for cotton.

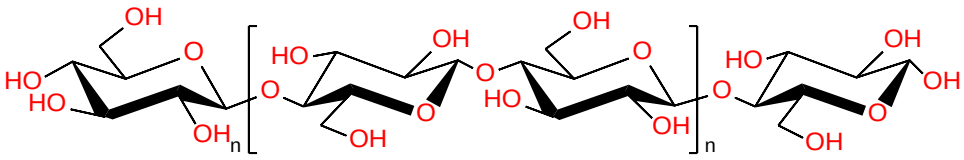


Figure 1.11 - Primary structure of cellulose (cellobiose monomer and terminal reducing and non reducing glucose).

The strong beta bonds in cellulose make its degradation difficult and only strong acids and selected enzymes (cellulases) can break down the polymer to the monomer.[37] Chemical methods by acids are known to induce extensive degradation products, so they can be used only in the limited cases in which

feedstock of impure glucose are compatibles. More attractive and extensively studied are the biotechnological processes which uses microorganism of purified hydrolysis enzymes, even if until now only limited industrial applications are known. There are three main types of cellulose enzymes useful for this scope, frequently used in mixtures with selective β-glucosidase devoted to break down the cellobiose dimer (Figure 1.13).

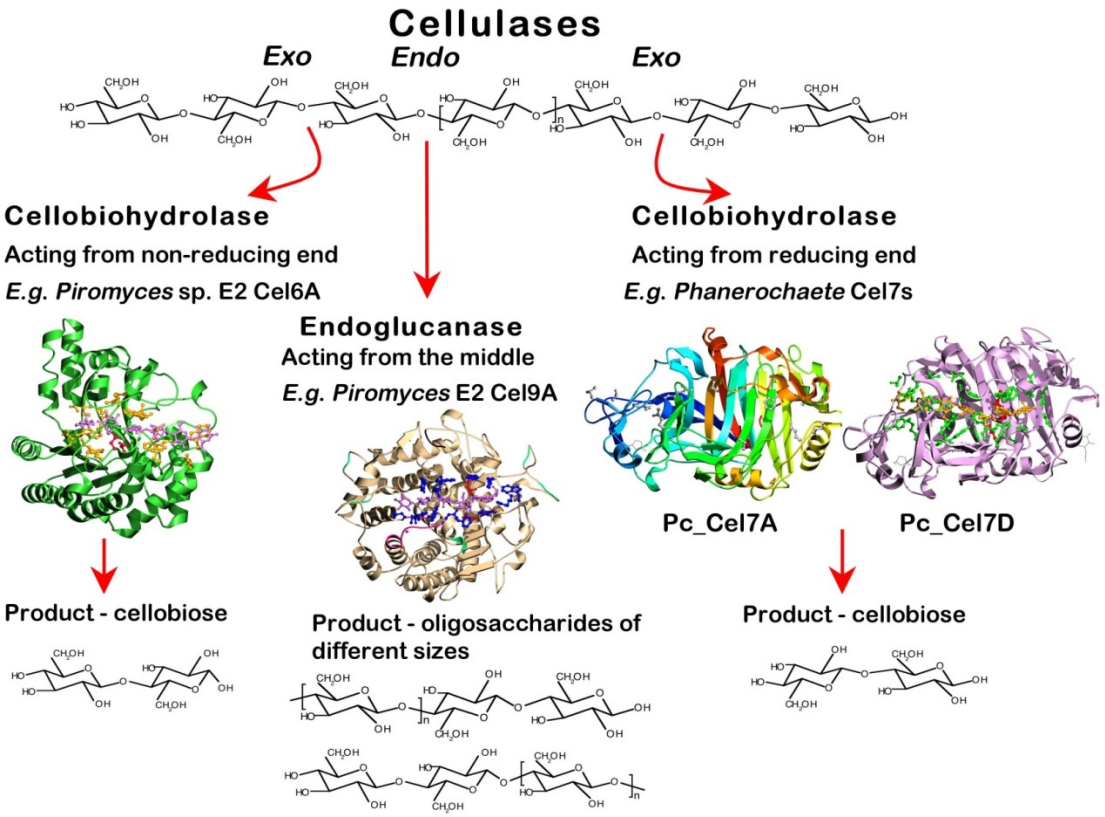


Figure 1.12 - Main enzymes involved in the cellulose breakdown and used in bioprocesses to recover glucose.

In future, it is expected that the bioprocesses resulting from this hydrolysis will provide the main source of carbon feedstock per organic chemicals and materials in a context generally called biorefinery.

Hemicellulose:

The second component for importance in plant fiber is hemicellulose. Compared to cellulose, hemicellulose is a hetero-copolymer composed of several different saccharide molecules, i.e. glucose and several other hexoses (galactose, mannose, etc.) and pentoses (xylose, arabinose, etc.). The proportion of these monomers varies plant to plant with a variable degree of polymerization from 50 to 300. Generally, hemicellulose is more abundant in soft wood. The polymers has branched chains of D-xylose, D-mannose, D-glucose, D-galactose and other sugars (Figure 1.14). Moreover, the polymer is complicated by the fact that the main chain of hemicellulose may consist of one or more diverse glycosyls units (xylan, xyloglucan, glucomannan, mannan, galactomannan, callose, and so on), and that the connections between glycosyl can differ in anomeric carbon and hydoxyl groups involved.

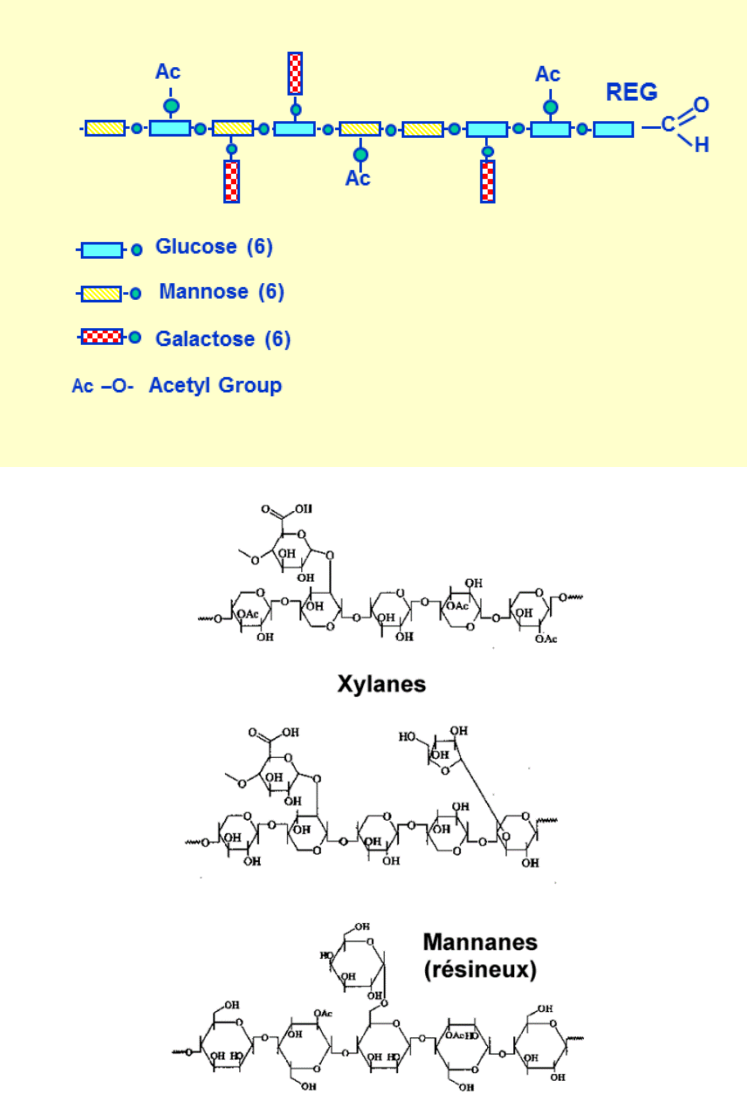


Figure 1.13 - Representative structure of hemicellulose and primary structure of three classes of hemicelluloses.

Hemicelluloses are insoluble in water but easily solubilized in alkali or hot, dilute mineral acids giving monosaccharides.

Particularly relevant to this thesis are Galactan and Arabinogalactan. A galactan hemicellulose, also termed pectic galactan, is particularly abundant in compression wood and pectin. This galactan has a backbone of β-(1,4)-linked D-galactose units (an epimer at C-4 of glucose), partly substituted at the hydroxyl group of C-6 with galacturonic acid units (Figure 1.15).[37] Arabinogalactan and galactan in the cell walls may be independent polymeric molecules or as the side chains on the polysaccharide molecules of pectin, which we discuss in more detail in chapter 2 as source of galactaric acid.

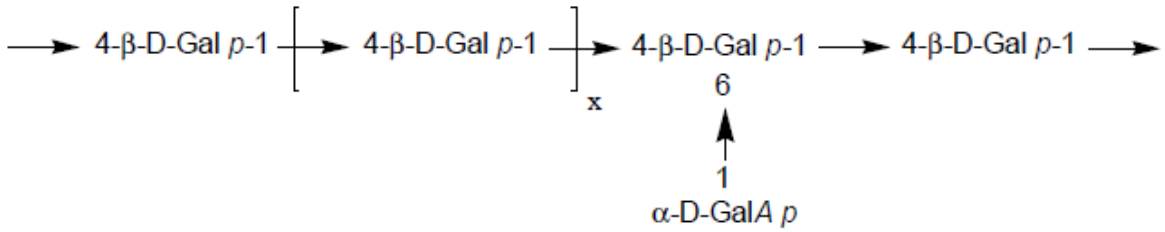
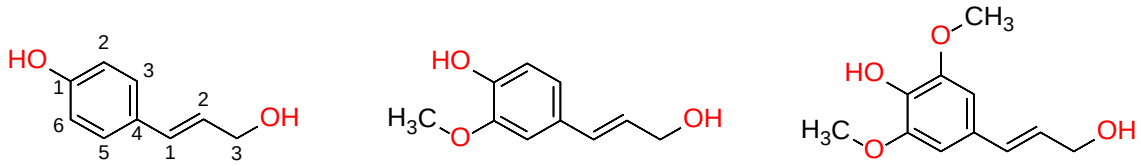


Figure 1.14 - "Pectic" galactan hemicellulose.[37]

Lignin:

Lignin is a complex and high molecular weight phenolic polymer consisting of three main phenylpropane units. It is composed of p-hydroxy-cinnamyl alcohols, such as p-coumaryl, coniferyl, and sinapyl alcohols (Figure 1.16), polymerized by oxidative free-radical de-hydrogenation between the cells and cell walls. Different combination of these monomers occurs in different plant groups. Its phenolic nature makes the material a natural antioxidant, but its complex three dimensional structure makes challenging its isolation and use. Deposited during lignification of the plant tissue, it gets intimately associated within the cell walls with cellulose and hemicellulose, and imparts the plant an excellent strength and rigidity, along with water repellency. [37]



p-Coumaryl alcohol (H lignin) **Coniferyl alcohol** (G Lignin) **Sinapyl alcohol** (S Lignin)

Figure 1.15 - The three major monomers of Lignin.

Lignin polymer has a molecular weight which is strongly dependent on plant source and on biomass treatment.[40] This is due to chemical reactivity towards acid and bases of the propyl side chain of the polymer but also to the existing ester, ether and glycosydic bonds with hydroxyl groups of polysaccharides hemicellulose and cellulose. All this makes challenging the recovery of both saccharides and lignin itself. Only treatments with strong bases at high pH or with sulfur derivatives (e.g. bisulfite) make possible to dissolve lignin, even if strongly modified. Solubilisation of lignin by sulfur derivatives represents the more common method to access lignin and its derivatives on industrial scale via the so-called pulping process (Kraft lignin). In Figure 1.17 a representative structure of lignin with the main different structural elements until now described are summarized.

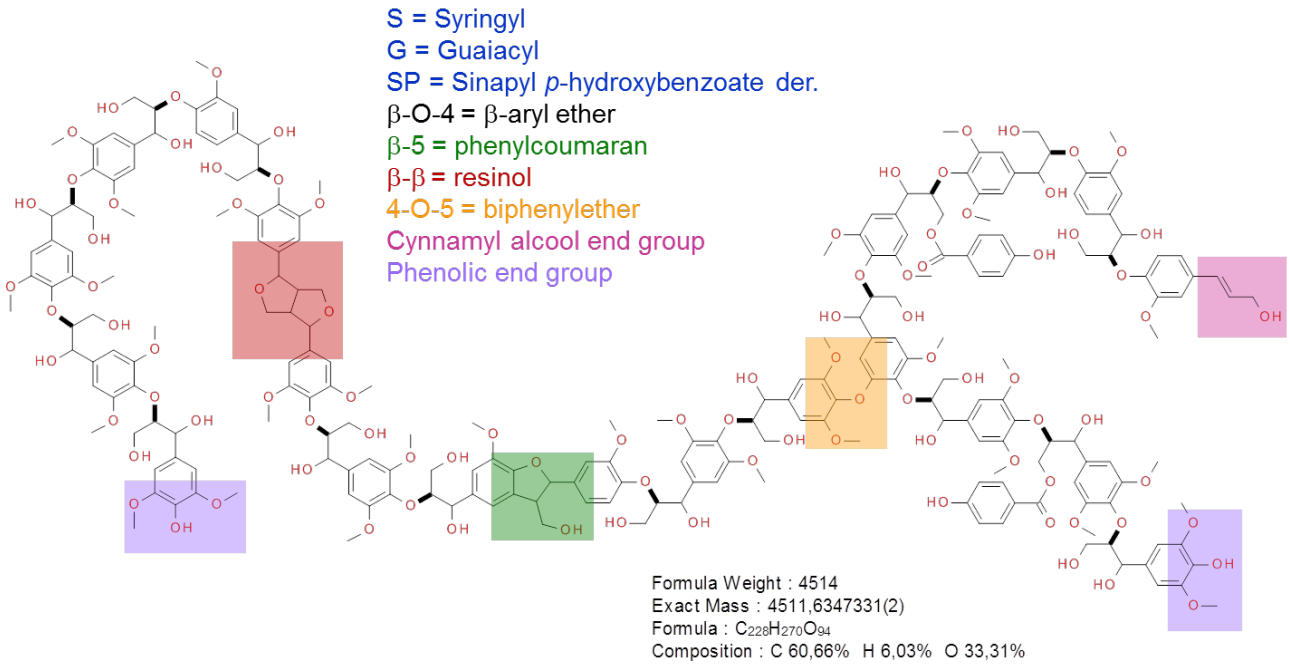


Figure 1.16 - Representative chemical motifs present in the lignin polymer.

1.3 - Bio-refinery

As we have just previously introduced, one of the severe challenges human being facing is the critical problem of fossil resource depletion and climate changing due to the rising demand of world's mobility and chemical need as the population grow. Moreover, considering the annual worldwide biomass production that is estimated to exceed 100 trillion kilograms (1000 times more than the global total consumption of 100 billion kilograms for organic chemicals, polymers, and fibers), there is great potential for bio-based chemicals to share markets with their fossil based counterparts. In addition, the European chemical manufacturing and user industries also feel the pressure of intensive legislation on chemical testing, the representative one which, REACH (namely the registration, evaluation, authorisation and restriction of chemicals), have started to affect the entire chemical supply chain. REACH regulation aims to improve the protection of human health and the environment through a better and earlier identification of the intrinsic properties of chemical and substances, so enhancing innovation and competitiveness of the EU chemicals industry. According to the requirement of REACH, it is needed to develop greener substitutes of non-renewable or toxic chemicals and materials by taking use of renewable resources from biological origin. This had led to a deep investigation of bio-refinery concept and strategy to replace the old and existing fossil based feedstock for an integrated production of food, fuels, chemicals and materials of the future.

Oil or petroleum refinery is a complex interplay of industrial plants and processes developed in 70 years to transform the complex mixtures hydrocarbons and derivatives present in the crude oil and to

refine it into more useful products, such as petroleum naphtha, gasoline, diesel fuel, and commodity chemicals. As an analogue of petro refinery, bio-refinery aims to convert and separate the analogously complex biomass instead of crude oil as feedstock into a variety of goods that can be used as fuels or platform chemicals for the chemical industry. A simplified comparison of these two processes is shown in Figure 1.18. The main difference in the chemistry involved is that carbon in oil is involved in a reduced form whereas in biomass it is present in medium or high oxidation state. This means that chemistry developed for oil is hardly functioning in bio-refinery. Moreover, the large energy accumulated as entropy in complex biomass don't need to be dispersed and transformation processes requires therefore selective and low temperature chemistry, therefore the use of biological organisms and enzymes appears to be the more suitable solutions. Furthermore, all bio-refineries should be assessed for the entire value chain to meet the requirement of environmental, economic, and social sustainability during the whole life cycle. It means that sustainable, environment friendly technologies are obligatory for the integrated biorefinery process. As an emerging field of bioeconomy, the definition of bio-refinery is normally in parts controversial, two of which are introduced below.

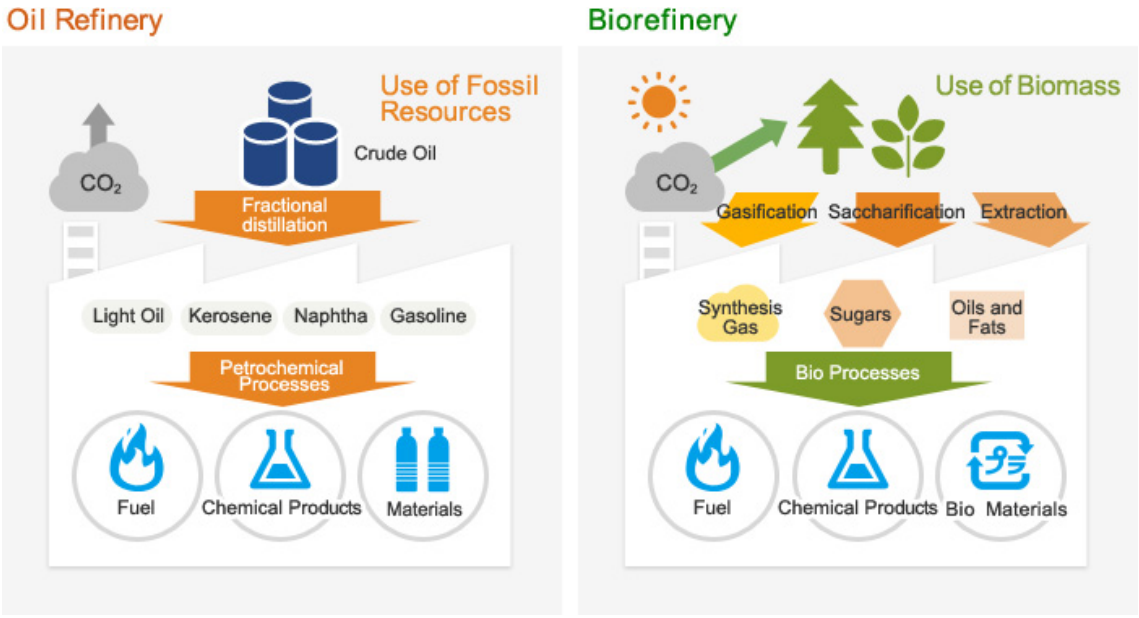


Figure 1.17- Comparison between oil refinery and biorefinery.

The United States Department of Energy (DOE) has introduced this definition: "*a biorefinery is an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products, based on the petrochemical refinery*". International Energy Agency (IEA) - Bioenergy Task 42, gave a more restrictive definition, which claims: "*Biorefinery is the sustainable processing of biomass into a spectrum of marketable products and energy*". According to both definitions, biorefinery is an integral processing of biomass into a spectrum of products. In fact, biorefinery has long tradition, mainly in connection to food and feed conversion, such as the edible oil, sugar, starch as well as paper industry, which has been considered as incomplete biorefineries.[41]

We are interested to focus on modern biorefinery, the so-called second-generation biorefinery (Figure 1.19). The task is the use of a series of biomass, including food or agriculture waste and energy crops, as starting materials in order to take full advantage of nonedible biomass such as agro-processing residues or other wastes to avoid the land use competition with food crops. Agricultural waste includes sugar cane bagasse, sugar beet pulp, orange peels, wheat and rice straw. Biorefineries can be a rational and sustainable solution for the reuse of all these wastes, frequently originating pollution problems. Furthermore, the combination of different transformation technologies, particularly biotechnological and chemical catalytic conversion of substances, will play an essential role in order to avoid human and environment hazardous influence. In the next generation biorefinery, the biomass feedstock will be first fractionated further into valuable components by traditional methods like extraction, fermentation and controlled pyrolysis and the platform chemical produced could be further converted into higher value products (e.g. via esterification, oxidation, reduction or polymerization). Though one major limitation of the current biomass refinery is that it is not as economically competitive as the petroleum refinery, further technological developments devoted to the direct generation of value-added chemicals need to be introduced to provide the necessary financial incentive to promote the flourishing of biorefining industry. This strategy is similar to the separation in oil refinery to convert part of heavier hydrocarbons into lighter one, which are feedback for producing petroleum platform commodity molecules such as p-xylene, toluene, benzene, butadiene, ethylene etc. Many bulk chemicals are derived from these platform molecules. However, as mentioned before, biomass cracking is essentially more complicated and difficult than thermal and chemical cracking of crude oil and biotechnological solution need to be investigated, developed and introduced in the market. The obtained bio-based platform molecules have some more advantages for valuable bulk chemicals and other useful materials than their petroleum competitors mainly due to their high functionality. The conservation of functional groups can greatly simplify the transformation for complex value-added products, which is in accordance with the principles of green chemistry (Figure 1.19). For example, glucose from starch and sugar (and more recently from cellulose) has been just widely transformed by fermentation or chemical transformation into basic chemicals providing access to the more recognized C-2 – C6 platform molecules. However, economics remains challenging, in part because integrated approaches were difficult to develop and competition with oil-derived products is again significant.

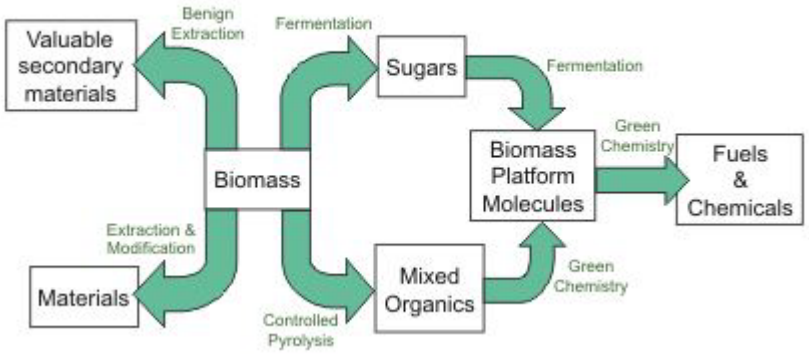


Figure 1.18 - Green chemistry and the biorefinery.[42]

In conclusion, the modern biorefinery need to investigate deeply first the chemistry, particularly the organic chemistry and biochemistry, involved in the synthesis of platform molecules, and then the alternative strategies to convert them into value-added sustainable products and materials by a combination of biotechnological and chemical methods. Green engineering will then adopt the best technology developed to design industrial processes with appropriate environmental, safety and economic outcomes. Some of the most promising platform molecules will be examined in next section.[42, 43]

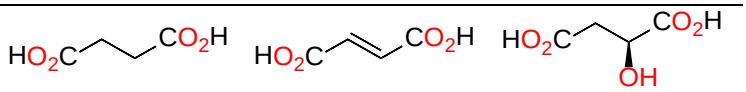
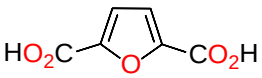
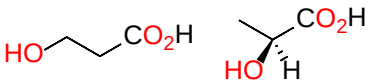
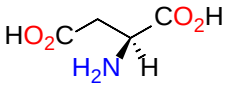
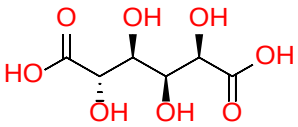
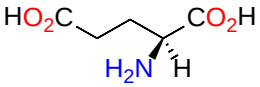
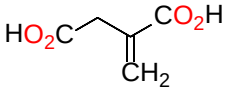
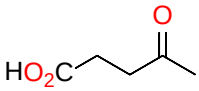
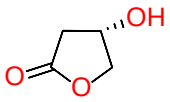
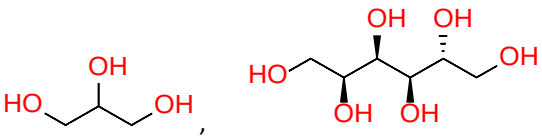
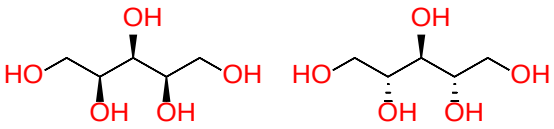
1.4 - Platform chemicals (value-added products) derived from biomass

Platform chemicals are defined as the molecules with high potential to be converted into various valuable chemicals and materials. The origin from biomass determines their high potentiality. The biomass route through platform chemicals to produce value added chemicals and advanced materials has been examined in last section. Considering that carbohydrates in form of polysaccharides are the main substrates in biomass, the wide range of sugar derivatives such as starch, sucrose, cellulose, hemicelluloses, pectin, etc., are accepted as the potential feedstock for modern biorefineries similar to the role of hydrocarbons play in oil refineries. In this case, various more complex oxygen-containing platform chemicals can be obtained by conversion of bio-based carbohydrates. As introduced in the section of biomass composition, different C5 and C6 sugar building blocks and structural polysaccharides containing repeating units of D-glucose, D-galactose, D-mannose, D-xylose, D-arabinose, etc., constitute the major components of biomass. Therefore, monosaccharides are suitable to be the raw material for building blocks synthesis due to their low cost and large availability in biomass. What's more, abundance of oxygen in molecules introduces high levels of functionality, which avoid the further drastic oxidation process in the conversion into final products. In order to escape the risk of targets overabundance for biobased chemical production, a core group of platforms

including already existed commercial products in petrochemical industry and promising new molecules from biomass, should be rationally selected based on comprehensive criteria. Therefore, a limited number of platform chemicals for further commodities and bulk chemicals production will to large extent decide the success of modern bio-refineries.

One of the most widely accepted portfolio of platform molecules was identified by the US Department of Energy (DOE) in 2004. After analyzing over 300 potential platform chemicals from biomass, the group of Pacific Northwest National Laboratory (PNNL) and National Renewable Energy Laboratory (NREL) presented a report with 30 most promising candidates from a chemical market perspective and identifying twelve top platform chemicals potentially derived from biomass carbohydrates, as shown in Table 1.3. The final list was confirmed by analyzing chemical and market data, properties, performance of the potential building block chemicals as well as examining their potential markets and the feasibility of the synthesis routes. The synthesis in the assessment system mainly contains the pathway from sugar to the object molecule and the transformation of platform molecules to possible valuable chemicals. All these chemicals with multi-functional groups can be subsequently converted to diverse high value chemicals and materials. Starting from this list as a preliminary direction, significant progress and commercial success have been accomplished to further evaluate their potentiality for producing multiple products by both chemical and biological processes.[32, 44] At moment, some biorefinery plants at industrial scale have already been successful installed. For example, there are 98 biodiesel plants around the world with capacity of 2.4 billion gallons per year. The bioethanol production capacity has increased to about 8.5 billion litres per year in 2012, with an actual annual production of about 4.8 billion litres or 57% of the total capacity. In 2013 the first industrial-scale plant of second generation bioethanol from lignocellulosic biomass was installed in North Italy with production capacity of 75 million litres/year. This plant is based on the Biolife Project with Proesa™ technology by Chemtex Company. In this process the wheat straw and *arundo donax* plant are sent first to the double-steps steam explosion pre-treatment following with the enzymatic liquefaction at high dry matter level, and finally the treated liquid was simultaneously saccharised and fermented with diverse microorganisms.[45] Meanwhile, the platform-based biorefinery also experienced a significant development. In Pomacle France, for instance, the world's first large scale bio-based succinic acid plant with 3,000 ton/year capacity from glucose, lignocellulosic sugars and glycerine has been built by ARD in 2009.[46] The top fourteen representative platform chemicals are summarized in Table 1.3 and will be briefly introduced in below.

Table 1.2 - Top 14 platform molecules according to DOE. [44]

Platform molecules	Structures
1,4-succinic, fumaric and malic acids	
2,5-furandicarboxylic acid	
3-hydroxypropionic acid, L-lactic acid	
L-aspartic acid	
D-glucaric acid (Aldaric acids)	
L-glutamic acid	
itaconic acid	
levulinic acid	
3-hydroxybutyrolactone	
Glycerol, D-sorbitol	
D-xylitol/L-arabinitol	

1.4.1 - Three-carbon platform chemicals

3-Hydroxypropionic acid

3-Hydroxypropionic acid is an important biomass based platform molecules for many value added products. It is a 3-carbon, non-chiral organic molecule and a structural isomer of lactic acid. A group of high volume valuable derivatives such as 1,3-propanediol, acrylic acid, malonic acid, methyl acrylate, acrylamide have been obtained from 3-hydroxypropionic acid. 1,3-Propanediol is one of the

largest scale application of 3-hydroxypropionic acid by catalytic hydrogenation. Furthermore, the industrial precursor of acrylic acid, acrolein, has been synthesized by heating 3-hydroxypropionic acid water solution. Some researchers have developed also direct biotechnological conversion of 3-hydroxypropionic acid to acrylic acid derivatives. In March 2012, OPX Biotechnologies said it was able to successfully demonstrated its fermentation process to produce acrylic acid from sugar-based 3-HP at a 3,000 liter-scale (equivalent to 60,000 lbs/year) in a pilot facility. Until now, the industrial application is still unavailable due to the low yields of different products, the complex workup procedures, and its toxicity. An interesting route proposed for the preparation of 3-hydroxypropionic acid starts from glycerol, but, also for this approach, no industrial application has appeared. Nowadays some improvements has been made in the glucose fermentation by *Lactobacillus reuteri*, which takes advantage from the removal of products continuously to avoid product inhibition.[32]

1,2,3-Propantriol (Glycerol)

For last two decades, glycerol has gained increasing interest for its strong potential to become a future bio-based platform chemical. It is produced in high quantity with low price from the biodiesel industry as a co-product, which assures its availability for further transformation. A large portfolio of reaction pathways for the catalytic conversion of glycerol is available and more than 1000 different products incorporating glycerol are known in the market. As a promising building block for valuable chemicals, various reaction pathways such as reduction, dehydration and fermentation have been investigated and successfully applied to produce ethylenglycol, propanol, propandiols, glyceric acid, glycerates, acrolein, branched polymers, etc. One of the industrial application of glycerol as platform molecule is the conversion of glycerol to epichlorohydrin, which is widely used for plastics, epoxy glues and resins, and elastomers production. One 100,000 ton/year epichlorohydrin plant was recently built in Thailand by means of this route. [31]

2-Hydroxypropionic acid (L-Lactic acid)

Another molecule of great interests as platform chemical is 2-hydroxypropanoic acid – more known with the trivial name of lactic acid (LA). Swedish chemist Scheele has first discovered lactic acid in acid milk in 1780.[47] Until now, it has been widely applied in different industrials, such as an acidulant, preservative and gelling agent for food industry. After 2000, polylactic acid (PLA, the polycondensation product of lactic acid) was poised to play a big role as a viable, biodegradable replacement of traditional polyesters and polyolefins from both researchers and industries. PLA plastic is made from fermented plant starch (usually corn) or sugar cane and the production process involves the polycondensation of two basic monomers, lactic acid and its lactide dimer. As one of the major raw materials used in the production of the bioplastics (10%), the global production of PLA polymer has reached 200,000 tons in 2017. One of the biggest player of PLA product is NatureWorks LLC which

built a plant with 140,000 ton/year capacity in Nebraska, USA.[48] One promising feedstock is non-edible cellulose by innovative catalytic hydrolysis method. Furthermore lactic acid has been added into the updated US DOE's selection of top 15 (platform) chemicals.[32] Although lactic acid already has long been treated as commodity chemical, its high reactivity still makes it excellent as an intermediate for other useful chemicals through selective transformation. With the right catalyst and reaction condition, lactic acid has been approved to be converted into other intermediates such as propylene glycol, 2,3-pentanedione, acrylic acid, acetaldehyde, pyruvic acid, and lactide (the monomer in PLA synthesis). Besides, the alkyl esters of lactic acid (ethyl lactate) have been demonstrated as a promising green solvent thanks to theirs biodegradability and good solvation properties.[49] Taking into account of the increasing production of PLA polymer and the possible application as platform molecule, the demand of lactic acid was estimated to reach 600,000 ton/year in 2020.[50]

1.4.2 - Four-carbon platform chemicals

1,4-Succinic acid

Succinic acid (butanedioic acid) is a dicarboxylic acid that occurs naturally in plant and animal tissues. Biotechnological production of 1,4-succinic acid by sugar fermentation is now a well consolidated process which make this compound an important starting material for high-valued chemicals and materials (mainly polymers). Esterification of 1,4-succinic acid can lead to an huge variety of succinate esters. Lower chain esters are used as flavoring base materials, plasticizers, solvent carriers and coupling agents. Longer chain compounds are used as components in metalworking fluids, surfactants, lubricants, detergents, oiling agents, emulsifiers, wetting agents, textile treatments and emollients. Some of these esters are intermediates for the production of some important oil refinery products, such as tetrahydrofuran, γ-butyrolactone, 1,4-butanediol and pyrrolidinone derivatives. Succinic acid is also a valuable monomer of some polyamides and polyesters. Some researches of succinic acid based biodegradable functional polyester for coating uses have been carried out with high yield.[51] Succinic acid is traditionally produced from maleic anhydride derived from petroleum. The biorefinery of 1,4-succinic acid is based on the biological conversion of carbohydrates by engineered microorganisms such as *Anaerobiospirillum succiniciproducens* and *Mannheimia succiniciproducens* with yields higher than 1.35 mol/mol of sugar, as well as recombinant *Escherichia. coli* with 1.3 mol succinate/mol glucose. The industrial production has just been realized in capacity of 2000 tons per year production by Roquette/DSM and Bioamber.[32] In Italy the Reverdia's bio-based succinic acid plant is active from 5 years. Massachusetts-based Myriant Technologies is constructing the world's biggest bio-based succinic acid facility in Lake Providence, Louisiana, in the United States. The estimated 2016 worldwide use of succinic acid was around 35,000 to 50,000 tons per year and this is on the increase by around 10 percent a year.

1.4.3 - Five-carbon platform chemicals

Levulinic acid

Levulinic acid is one of the more recognized biobased platform molecule for a large variety of key compounds due to versatility of the functional groups present in its structure, ketone and carboxylic group. Levulinic acid and its derivatives are used as precursors for levulinate esters applied as solvents, fuel additives and plasticizers. Levulinic acid is also used in polyester resins to increase mechanical performance for interior and exterior coatings. Gamma-valerolactone and 1,4-pentanediol derived from levulinic acid can be interesting monomers for polyester and polyurethanes. Levulinic acid can be easily produced by acidic hydrolysis of C6 sugars from brewery waste, biomass and manure among others. The difficulty for industrial production lied in the presence of coproducts such as formic acid and acetic acid, which cause problem in the working-up processes. The optimization of isolation and purification was researched by introducing Amberlite LA-2 to extract levulinic acid from solution reaching 53% of yield. Until now the commercial production of levulinic acid has been realised by companies auch as Biofine, DSM, GFBiochemicals and Segetis. In Caserta, Italy, one lignocellulose biomass based levulinic acid plant of 10,000 t/year capacity was built by GFBiochemicals in 2015.[32, 51]

D-Xylitol

The traditional application of xylitol is as sugar substitute, thanks to its high sweetening capacity and non-diabetic properties. The world demand of xylitol is about 60 kt equivalent to 340 million dollars annually. Nowadays the industrial production of xylitol is made by catalytic hydrogenation of xylose on Raney nickel catalyst with a market price of 5 dollar/kg. For applying xylose as a biobased platform chemicals, hemicellulose from biomass can substitute pure xylose as the raw material for xylitol production. In order to obtain xylose from biomass, biochemical processes with *Candida* yeasts have obtained relatively high yields of xylitol. By introducing *E. coli* expressed by xylose reductase, the formation of arabinose as byproduct during process was avoided.[32] In 2013, Xylitol Canada announced they had completed pilot demonstration of its cellulosic xylose process.

1.4.4 - Six-carbon platform chemicals

2,5-furandicarboxylic acid

2,5-Furandicarboxylic acid together with furfural and 5-hydroxymethylfurfural are treated as main representatives of furan derivatives which has huge potential for renewable building blocks. 2,5-furandicarboxylic acid has received significant attention due to its wide application in many fields, particularly as a substitute of petrochemical-derived terephthalic acid in the synthesis of polyester. The

2,5-furandicarboxylic acid is synthesized by catalytic oxidation from 5-hydroxymethylfurfural (obtained by acid catalyzed dehydration of glucose) with 99% selectivity. Furthermore galactaric acid can react with butanol to form furandicarboxylic acid dibutyl eater in yields higher than 50%.[32, 44] Dutch company, Wageningen UR Food & Biobased Research, has produced 5-hydroxymethylfurfural from agricultural biomass in large scale, which can be further transformed into furandicarboxylic acid by fermentation. Another Dutch company, Avantium, has already introduced the biobased plastic materials derived from their sugar-based platform furan derivatives via chemical routes. DuPont has announced the production of FDCA for use in PTF.[52] In March 2016, Avantium and BASF created the joint venture Synvina to erect a 50,000 t/a plant for the production of FDCA based on fructose at BASF's Verbund-site in Antwerp, Belgium.[53]

D-Glucaric acid

D-Glucaric acid, also called saccharic acid, belongs to a large family of chemicals known as oxidized sugars named aldaric acid. It has huge potential to synthesize adipic acid as important monomers for polymers like renewable nylon-6,6 and branched polyester due to its high functionality. As a potential platform molecule, glucaric acid can also be used for bio-detergents, anticorrosion additives, cement additive, coating materials, etc. Glucaric acid is formally produced by oxidation of glucose, using nitric acid as a catalyst, which has drawback of NO_x release and moderate yield (50%). Some improved oxidation processes using alternative catalysts have been introduced by companies Rivertop, Rennovia, and Johnson Matthey Process Technologies. Rennovia and Johnson Matthey Process Technologies are running a mini-plant for the catalytic oxidation of glucose to biobased glucaric acid. An alternative greener process makes the oxidation of glucose with oxygen as terminal oxidant in presence of enzymes or microorganism. The more attractive, even not yet economical compared with the chemical source, involve the use of recombinant *Escherichia coli* and was first introduced by company Kalion. The industrial application of this last technology is still in the early stage.[44]

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Chapter 2

2.1 - Aldaric acids as platform molecules in bio-based economy

Since from last chapter, we have broadly discussed the increasing interests of producing value-added chemicals rather than only biofuel, in connection to evolution of biorefinery and decrease of crude oil price, but also the need for substitution of petroleum based chemicals with bio-based chemicals, which has made this area a new promising research sector for both academic and industrial players. Considering the history of biomass usage for value-added chemicals, organic acids derived from carbohydrates has been long used in many different applications such as foods and beverages industries in the purpose of preservation, flavoring agents and so on. The utilization of acetic acid and lactic acid in vinegar and other food products can be dated back to 5000 years ago. Beyond that, malonic acid, succinic acid, citric acid, adipic acid are also largely used in the food and beverages markets. The possible replacement of petroleum organic acids by biomass exploitation and processing improvement has attracted attention of researchers and organic acid producers, which expanded the application of renewable organic acids as platform molecules for large variety of products such as polymer, surfactants, solvents, pharmaceuticals and other useful materials. The current industrial scale production of renewable organic acid is summarized in Table 2.1. By 2021 the estimated market for renewable derived organic acids is expected to reach 9.29 billion dollars.[1]

Table 2.1- Current industrial development of renewable organic acids.

Company	Products	Potential markets
Myriant	succinic acid, muconic acid, acrylic acid, fumaric acid	Resins, plasticisers and polyester polyols
BioAmber Inc.	succinic acid, adipic acid	Resins, plasticisers and polyester polyols
Cargill	citric acid	Preservations, flavouring, stabilizer, detergents, pharmaceuticals
Natureworks LLC	lactic acid	Polylactic acid
Corbion	lactic acid	Polylactic acid
Dow Chemicals	acetic acid	Vinyl acetate
Dupont/ADM	2,5-furandicarboxylic acid	PTF replacement for PET
Avantium Technology	2,5-furandicarboxylic acid	PTF replacement for PET
Renovia	adipic acid	Nylon and polyurethanes
Rivertop Renewables	glucaric acid	Detergents, corrosion inhibitors, additives

The efforts of major manufacturers for developing renewable organic acid are mainly stay in the stage of smaller investments in order to balance the pressure of environmental regulation and their traditional petroleum business as a common strategy for biorefinery. The main difficulty of biomass derived organic acid is its highly mature and competitive market, which is very sensitive to organic acids price.

Therefore, there is still a long way to go to make renewable organic acid commercialized at high volume.

However, at the same time with the continuous financial support and regulatory efforts from European and US government, as well as the significant advances in technology, commercialization routes of some sugar based carboxylic acid have been developed for platform chemical applications. Furthermore, the recent recovery of crude oil price also encourages the industries to develop acids from alternative carbon sources of non-edible sugars biomass. For example, lactic acid has been used in large scale for industrial production of polylactic acid (PLA), which has been treated as the next generation bio-plastic packaging material.[2] The global PLA market was above USD 1,650 million in 2015, with above USD 698 million, by revenue in 2017, and it is anticipated to reach USD 2,091 million by 2023.[3]

Dicarboxylic acids are also widely present in cell metabolism, which make them relevant platform chemicals and target for important bio-based chemicals transformations. Between them, the aldaric acids are of particular interest. Aldaric acids (in the old literature referred as saccharic acids) are polyhydroxy dicarboxylic acids that are formally produced by oxidation of aldoses at both termini. In Figure 2.1 are reported the isomeric C-6 aldaric acids (sometime indicated as hexaric acids) of the D series (**1a**-**1h**). In the figure are also reported two representative identities arising from the end-to-end symmetry of these compounds, which allows internal compensation, so that various *meso* (optically inactive) forms exist, and different aldoses may afford identical aldaric acids (i.e. the equivalence of D-galactaric acid (**1a**) with L-galactaric acid and of D-glucaric acid (**1b**) with L-gularic acid). In general for C-6 aldaric acids exist $N = 2^{(4/2)-1} \cdot (2^{(4/2)}+1) = 10$ isomers.

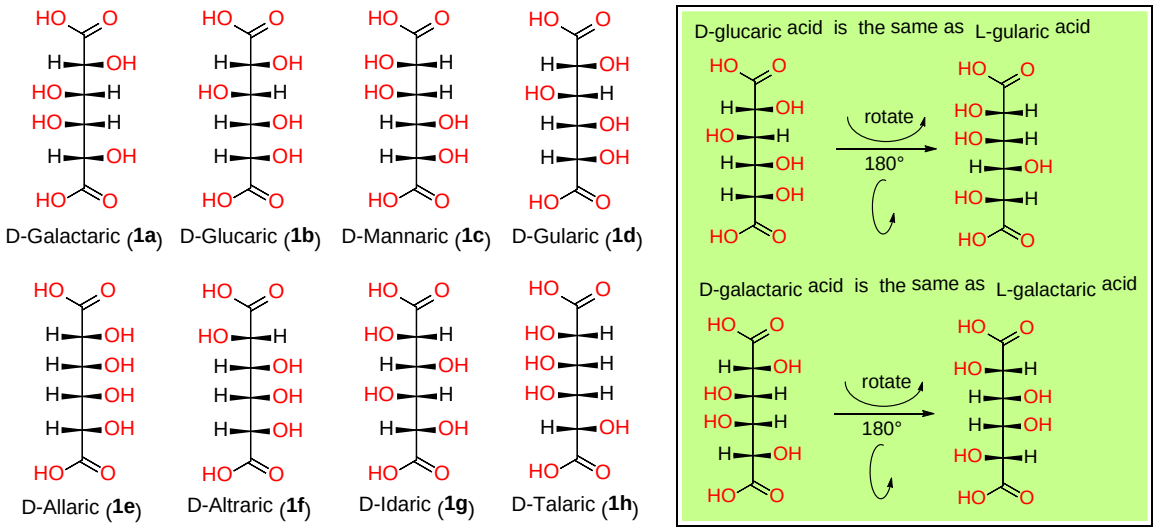


*Figure 2.1 - Chemical structure of C-6 D-aldaric acids (**1a – 1h**) and related stereo-chemical equivalence.*

Aldaric acids are primarily obtained from sugars, aldonic acids, and oligo- or poly-saccharides by reaction with strong oxidizing agents or via enzymatic synthesis. They are relatively strong dicarboxylic acids in the primary and secondary acidity constants, as reported in Table 2.2 for the corresponding pK.

Table 2.2 - Acidity of representative bio-derived dicarboxylic acids.

Acidity constants	**Tartaric acid**	**Glucaric acid (1a)**	**Galactaric acid (1b)**	**Adipic acid**
pK_{a1}	3.036	3.15	3.08	4.41
pK_{a2}	4.366	4.03	3.63	5.41

Aldaric acids show quite variable solubility in water, where they form several complexes with practically any metal cation. Some of these compounds have been specified among the twelve most promising sugar-based building blocks for the chemical industry by the US Department of Energy.[4] They are promising raw materials for biodegradable detergents, metal complexation agents, and monomers. However, until now they are produced on limited scale and at significant price, as indicated by the data in Table 2.3 related to the main C-6 aldaric acids (glucaric, galactaric, and mannaric acids) and the corresponding C-5 acids (xylaric and arabinaric acids), compared with the corresponding monocarboxylic aldonic acids.

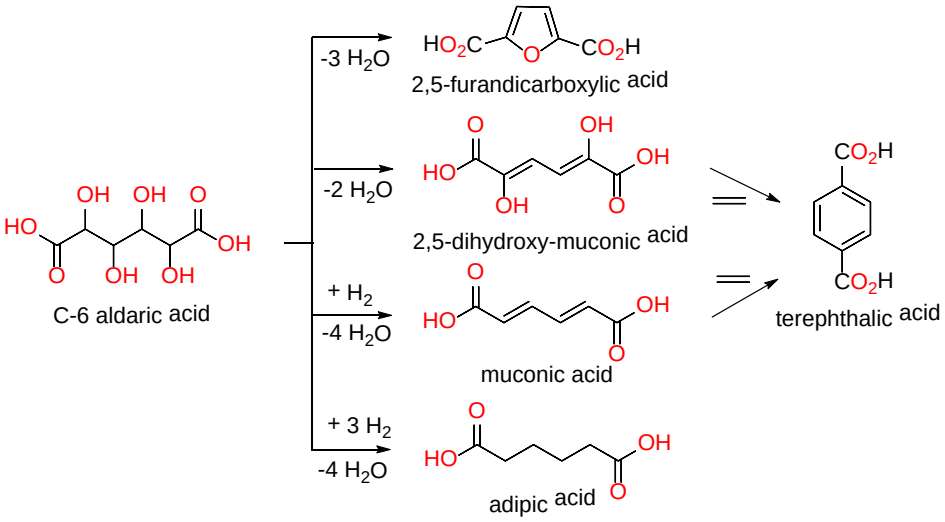
Table 2.3 - Production and price of the main aldonic and aldaric acids.

Aldonic Acid	Production (ton/a)	Aldaric Acid	Production (ton/a)
Gluconic	250,000 (1.20 $/kg)	**Glucaric**	100,000 (6-40 $/kg)*
Galactonic	20,000	**Galactaric**	38,000 (4-10 $/kg)*
Mannonic	4,000	**Mannaric**	n.a
Xylonic	5,000	**Xylaric**	n.a.
Arabinonic	3,000	**Arabinaric**	n.a.

*dependent from purity and applications

The relatively complex structure and the specific stereochemistry make these compounds susceptible of several transformations involving both the hydroxy and carboxylic groups. The main targeted transformations, until now more intensively investigated for their industrial potentialities, refer mainly to achiral compounds related to dehydration and reduction processes. In Scheme 2.1 the stoichiometry

of these routes are summarised which make access to three important monomers: adipic acid, 2,5-furandicarboxylic acid, and terephthalic acid..



Scheme 2.1 - Achiral target monomeric acids potentially available from C-6 aldaric acids.

Moreover, hydroxy and carboxylic groups can be more or less easily functionalized, providing access to other chiral dicarboxylic acid derivatives (esters, ethers, amides, salts, etc.) of controlled polarity. In turn, these compounds are difunctional monomers susceptible to be transformed into polymers, (i.e. polyamides, polyesters, polyanhydrides) either directly or through blending with suitable co-monomers.[5] Furthermore, evidences have been accumulated that ester, amides and salts derivatives can be applied as lubricants, plasticizers, and specialty products in food and bioremediation.[6]

Aims of This Research

All the facts, along with the limited knowledge of the chemistry and biochemistry of these compounds, have oriented the choice of aldaric acids as research subject for this thesis. The study was centred mainly on galactaric acid as the more representative aldaric acid, with minor attention also to glucaric acid and mannaric acid. The main aims of the research can be summarised as an increase of the knowledge in the:

- dehydration of aldaric acids, identifying lactones and unsaturated intermediates involved;
- reduction of aldaric acid and their unsaturated intermediates;
- acylation of hydroxy groups of aldaric acids and role of esters in the elimination of acyl groups;
- role of aldarate salts (both inorganic and organic) in the above mentioned processes;
- use of possible efficient transformations into value-added products and materials, (e.g.):
  - five membered aromatic derivatives of furan and pyrrole
  - six membered derivatives of pyrones

- amide salts, diamides and polyamides.

In the subsequent part of this chapter, we will report representative information from literature on the main aldaric acids (glucaric acid, mannaric acid, and galactaric acid) as an introduction on their chemistry/ biochemistry and process development to frame the new investigations carried out in this thesis.

2.2 - D-Glucaric acid

D-Glucaric acid [(2R,3S,4S,5S)-tetrahydroxyhexanedioic acid] is one of the most important aldaric acid, directly connected to the more abundant aldose sugar, glucose. The compound is touted for its dietary value, particularly as a cancer preventative agent[7] and in cholesterol reduction[8]. Also known as saccharic acid, it is found in small amounts as end-product of the D-glucuronic acid pathway in mammals and in a variety of vegetables and fruits.[8, 9] D-Glucaric acid was reported to occur as magnesium salt in the sap of *Ficus elastica*, and 3-deoxy-manno-heptaric acid has been found to be widely distributed in the *Cereus* and *Trichocereus* genera of the *Cactaceae* family. However, the abundance of D-glucaric acid in living organisms is so low that no direct extraction process has been attempted.

The more easy way to access glucaric acid is by oxidation of the naturally occurring glucuronic acid, both by chemical and enzymatic processes. Oxidation of glucose at both terminals is more challenging even if of higher industrial interest. Different terminal oxidants and chemical catalysts were investigated, and a large variety of intermediate acids were isolated (Figure 2.1).[10]

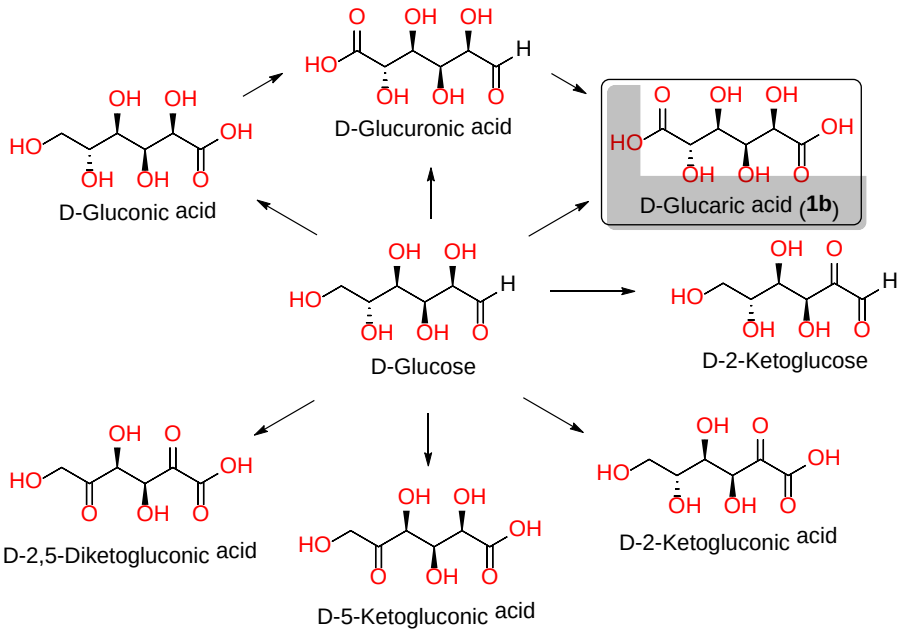


Figure 2.2 - Intermediates involved in the oxidation of D-glucose to D-glucaric acid.

In these direct chemical conversions of glucose, glucaric acid is generally isolated and purified as monopotassium salt (Figure 2.3).

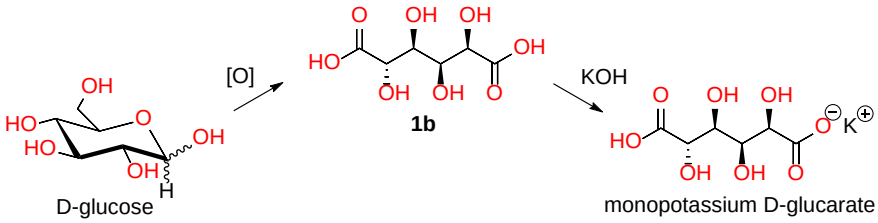


*Figure 2.3 - Oxidative conversion of D-glucose to D-glucaric acid (**1b**), isolated as D-glucarate salt.*

The biosynthesis of glucaric acid in microorganisms and mammalians involve a complex interplay of metabolic pathways (more than ten), as summarized in Figure 2.4 from the studies on *Escherichia coli.*[11]

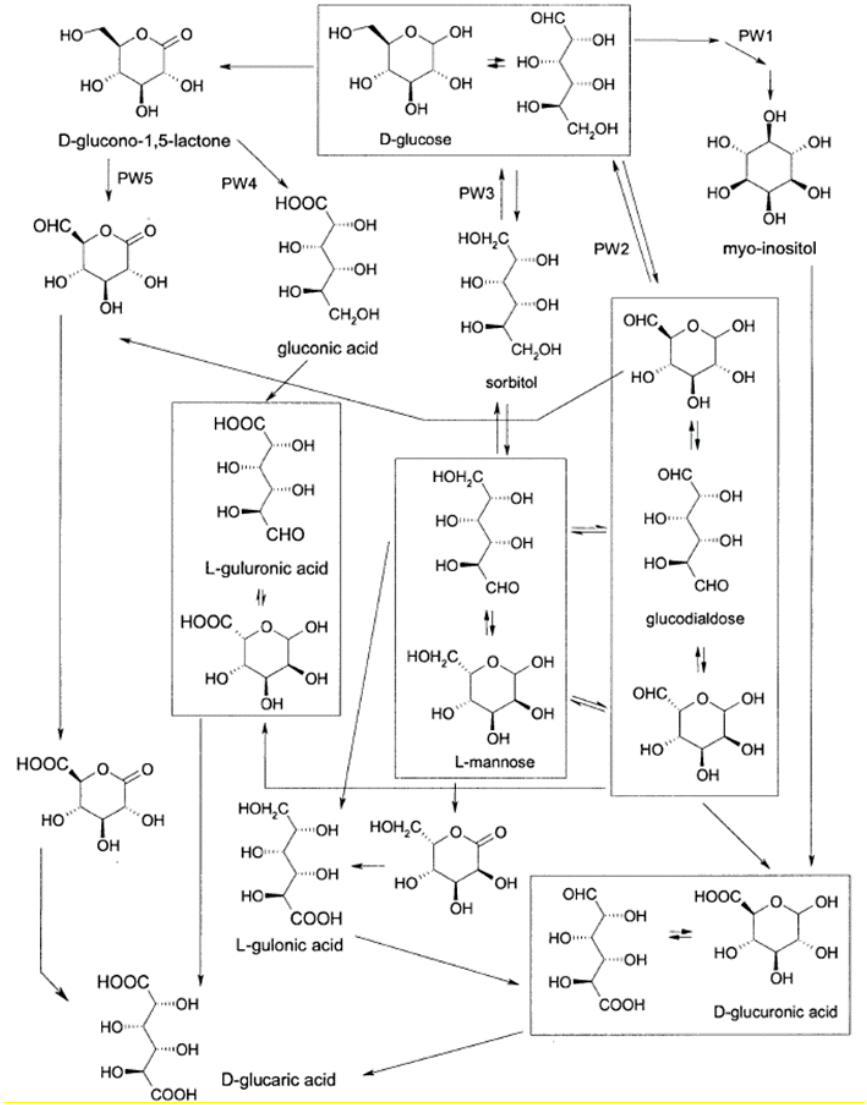


*Figure 2.4 – Biosynthetic pathways for producing D-glucaric acid (**1b**) in Escherichia coli.*[11]

In chemical synthesis, issues arise from the modest yield and the complex isolation procedure, whereas, in biological systems, the modest yield and lengthy downstream processing require engineered microorganisms for practical application.

2.2.1 - Properties of Glucaric Acid (1b)

Because the molecules of aldaric acids have carboxyl groups at both ends, there is potential for numbering from both sides, which results in the different absolute structure (e.g., D-glucaric acid and L-glucaric acid). Selection is applied in order to have the configuration of C-2 as the one of natural glucose, so that D-glucaric acid is directly connected to D-glucose. Glucaric acid adopts a bent structure in the crystalline state, a conformation that is devoid of destabilizing eclipsed 1,3-hydroxyl interactions in an extended conformation (Figure 2.5).[12]

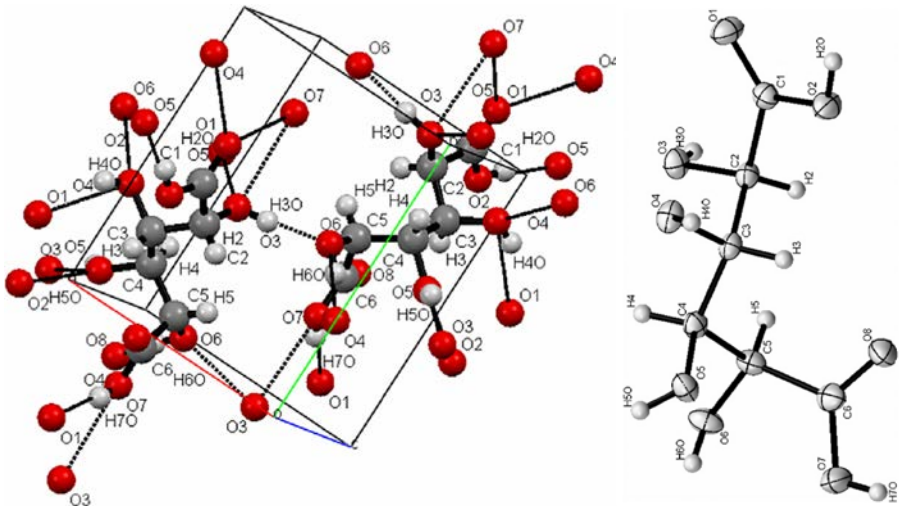


*Figure 2.5 - The unit cell of crystalline D-glucaric acid (**1b**) showing the hydrogen bonding system associated to the crystal structure.*[12]

A crystalline form of D-glucaric acid was generated by Rehorst by treating the silver salt with hydrochloric acid,[13] whereas Hirasaka et al. converted the monopotassium salt to **1b** using a cation exchange resin,[14] a procedure further developed recently to obtain good crystals for analysis by X-ray single crystal diffraction.[12]

In Table 2.4 are reported the main physico-chemical properties of D-glucaric acid. A peculiarity of this acid is its extremely high solubility in water, which, along with the extensive intramolecular lactonization, makes the purification of compound so difficult that commonly mono or di-glucarate salts are isolated.

Table 2.4 – Main physico-chemical properties of D-glucaric acid.

Property name	Property value
Molecular weight (g/mol)	210.14
CAS Number	87-73-0
Melting point (°C)	119.3 (lit. 118, 126)
Density at 25 °C (Mg/m^3)	1.654
pK_{a1}; pK_{a2}	3.15; 4.03[15, 16]
Water solubility (g/L)	510
Optical rotatory power (°) in water, 23 °C	58.2

The conformations of D-glucaric acid in solution were investigated by NMR spectroscopy.[17] The coupling constants suggested a mixing of the $_3G^+$ and $_2G^-$ sickle forms, together with the planar (P),

zigzag conformation. Similar studies were carried out on the five-membered glucaro mono- and di-lactones, evidencing the existence of a conformational equilibrium between two conformers with the OH-5 group occupying preferentially the position over the lactone ring. The ^{1}H-NMR spectra of D-glucaric acid, D-glucaro-1,4-lactone and D-glucaro-1,4,6,3-dilactone in D_2O are reported for comparison in Figure 2.6.

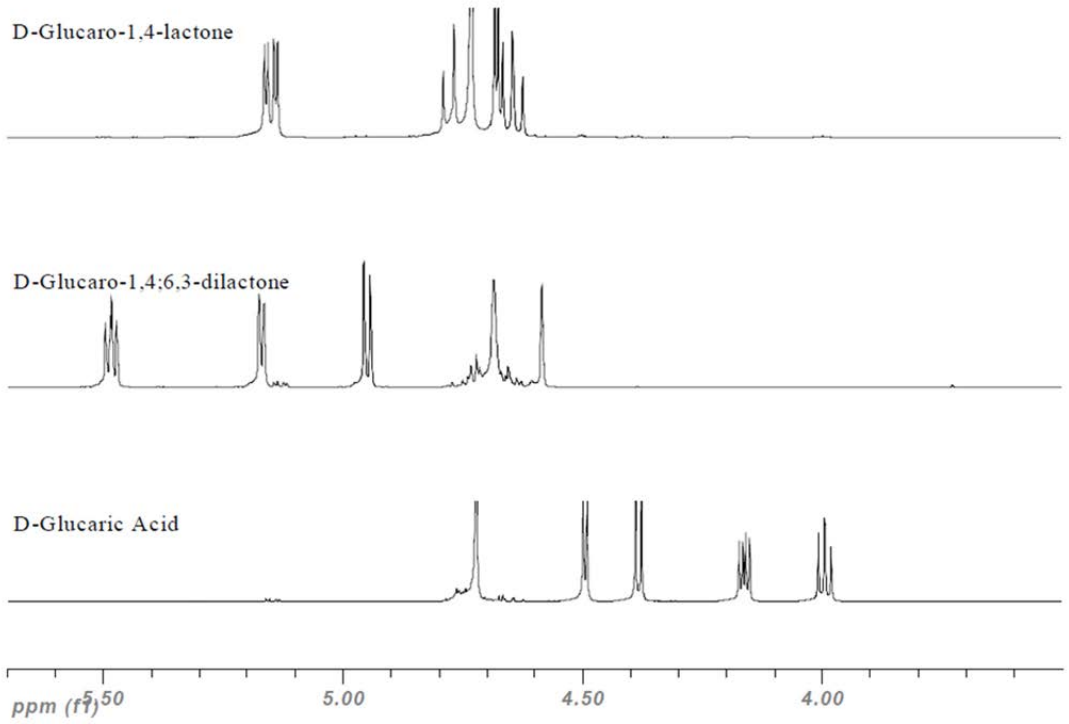
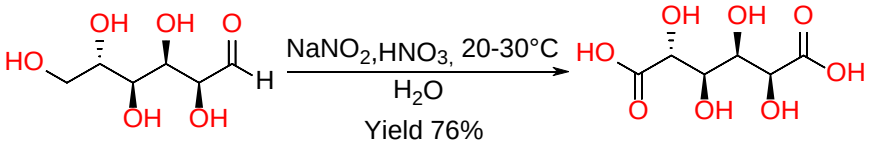


Figure 2.6 – ^{1}H-NMR spectra (400 MHz, D_2O) of D-glucaric acid and its main two lactones.

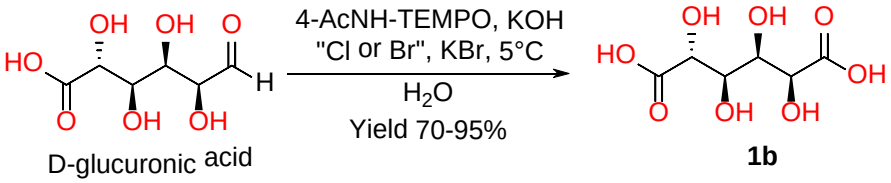
2.2.2 - Synthesis of Glucaric Acid

D-Glucaric acid was first isolated as its monopotassium salt by Shost and Tollens in 1888, via the nitric acid oxidation of D-glucose.[18] The potassium salt was converted to a calcium salt, which upon treatment with acid afforded impure D-glucaric acid, as reported by German chemist H. Kiliani in 1925 and subsequently by Smith in 1944.[19] The yields of original nitric acid method were normally lower than 45%. Then, some improved routes were developed with higher yields, mainly in the extensive work of Kiely group.[20, 21] The best yield reported by one alternative nitric oxidation route (Scheme 2.2) was 76.7% as disodium D-glucarate salt,[22] but this high yield was never confirmed. Recently in 2015,[23] the Montana based company Rivertop Renewables has adopted a similar procedure for the industrial production of the acid in a process with production capacity at 4500 ton/year and yield of 55%. Both molasses and starch can be used as source of glucose.



Scheme 2.2 - Nitric acid oxidation of glucose according to the best method of Kiely and Hash.[24]

Nitric acid oxidation leaves the secondary hydroxyl groups mainly unchanged, providing moderate yields of analogous aldaric acid as the main product. Afterward, some researchers develop other methods involving alternative terminal oxidants and catalysts instead of nitric acid, in the attempt to overcome the issues of toxicity and safety connected to nitric acid and the byproduct nitrogen oxides (NO_x) generated in the process. So, the halogen aided TEMPO oxidation of glucuronic acid, firstly introduced by Merbouh et al.,[25] affords a 70-95% yields of glucaric acid and related salts (Scheme 2.3).



Scheme 2.3 - TEMPO oxidation of D-glucuronic acid according to method by Merbouh et al.[25]

Oxidation processes rather than the traditional syntheses are based on electrochemical treatment,[26-29] molecular oxygen (as pure compound or in air) as terminal oxidant under ultrasound irradiation[30] or catalysis such as Pt, Rh[31] or Au/C[32], and hydrogen peroxide in the presence of iron salts catalysts.[33] None of those synthetic approaches has reached the industrial plant scale.

As indicated before, the biochemical approach to the conversion of D-glucose into D-glucaric acid is more challenging. However, this has not inhibited researchers to study this complex conversion. If the use of purified enzymes is seen actually as an impracticable route, the possibility of using microorganisms appears promising. The best results in this direction were obtained by Prather and coworkers, which constructed a recombinant D-glucaric acid producing *E. coli* strain by heterologous expression of the myo-inositol-1-phosphate synthase (Ino1) from *S. cerevisiae* and myoinositol oxygenase (MIOX) from mice with a titer of 0.3 g/l.[34] The activity of MIOX was identified as rate limiting in the pathway, resulting in the accumulation of both myo-inositol and D-glucuronic acid. Co-expressing the urinate dehydrogenase from *Pseudomonas syringae* that facilitates the conversion of D-glucuronic acid into D-glucaric acid improved the production titer to more than 1 g/l. In a follow-up

study, synthetic scaffolds were introduced into the recombinant system to help improve the effective concentration of myo-inositol.[11] Specifically, polypeptide scaffolds built from protein–protein interaction domains were used to co-localize three heterologous pathway enzymes, involved in D-glucaric acid synthesis, in a complex. The synthetic scaffolds increased the specific activity of MIOX and resulted in a recombinant strain with a D-glucaric acid production titer improved to 5 g/l.

Until now, Renovia (Santa Clara, CA) and Rivertop Renewables (Missoula, MT) are the more active companies to promote the commercialization of D-glucaric acid production, mainly for further conversion into bio-adipic acid.

Significant researches were also devoted to the synthesis of D-glucaric acid by oxidation of glucuronic acid, owing the commercial availability of this uronic acid by hydrolysis of some abundant natural polysaccharides, i.e. pectins. Both chemical and biotechnological routes were investigated and the process appears easier to be applied on industrial scale, even somewhat more expensive.[35] The use of specific enzymes (mainly engineered uronate dehydrogenases or oxidases) has made this conversion attractive, mainly for its selectivity and compatibility also with mixture of sugar derivatives.[36]

2.2.3 - Chemical Reactivity of D-Glucaric acid

The acidic nature of D-glucaric acid make its salification an easy process by both strong and weak bases, affording a large variety of the corresponding mono- and di-glucarate salts. As indicated before, the large-scale purification of the compound involves commonly the corresponding monopotassium salt. One of the current direct application of D-glucaric acid is its conversion in the sodium salt for formulations of dishwasher detergents thanks to its chelating property to tie up calcium and magnesium ions presented in hard-water. For this reason and the relevance of the acid in the mammalian metabolism, the complexing ability of D-glucaric acid with inorganic cation was extensively investigated.[15, 37, 38]

Glucaric acid in different solvents, including water, equilibrates easily to the corresponding intramolecular dehydration products (lactones).**Error! Bookmark not defined.** The unsymmetrical nature of glucaric acid can so form two different five-membered monolactones (D-glucaro-1,4-lactone and D-glucaro-3,6-lactone), two six-membered monolactones (D-glucaro-1,5-lactones) and one five-membered dilactone (D-glucaro-1,4:6,3-dilactone) in a distribution that depends on acid concentration and reaction conditions (Figure 2.7). The 5-membered lactones appear to be much less stable than the 6-membered lactones. D-glucaro-1,4:6.3-dilactone was synthesized by repeated lyophilization of glucaric acid from dioxane, as reported by Hashimoto, et al..[39] Gehret et al. in 2009 has reported a more viable large scale synthesis of this compound.[40] Both chemical and biological catalysis are used to controls the formation rate of lactones. For the biological routes, the enzyme D-glucuronolactone dehydrogenase has been found and identified as the key catalyst for the D-glucuronic acid oxidation

into D-glucaro-l,4;6,3-dilactone.[35] The dilactone can be selectively hydrolyzed in water solution to D-glucaro-l,4-lactone, providing an interesting access to this relevant derivative.[35]

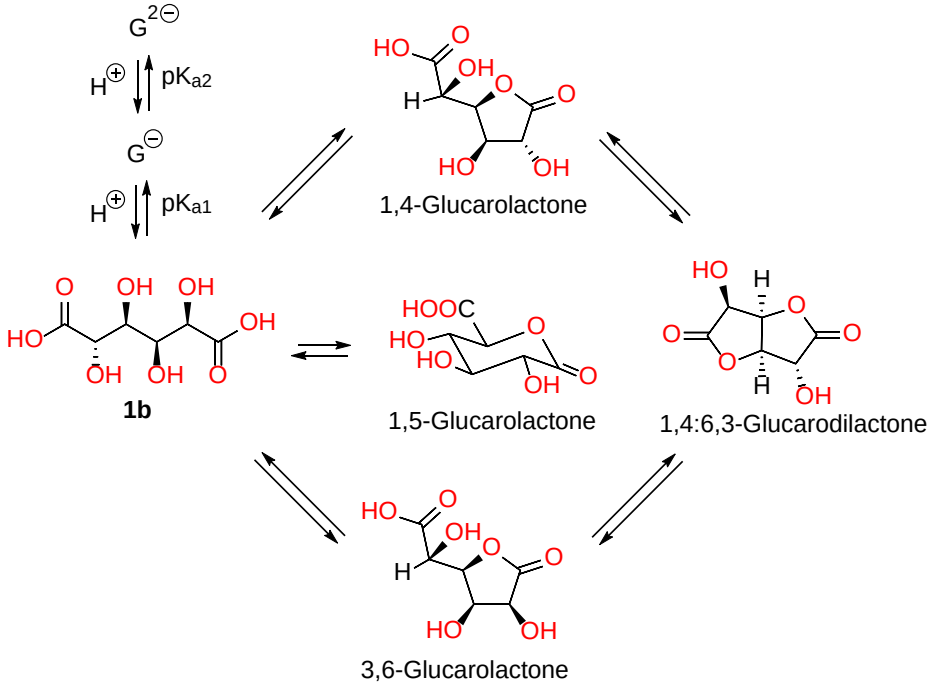


Figure 2.7 – Intramolecular dehydration equilibria of glucaric acid to the corresponding glucarolactones.

In fact, as a β-glucuronidase inhibitor, the anti-cancer function of D-glucaro-l,4-lactone and its precursor has been demonstated.[35]

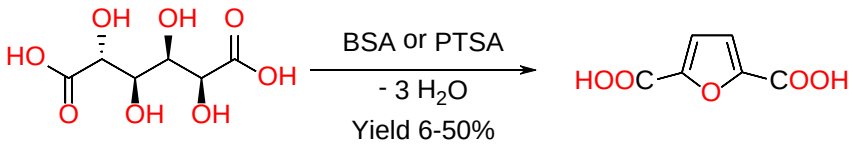
Thermodynamic proprieties of the lactonisation process of glucaric acid were also determined and compared with those of other saccharic acids.[41]

The esterification reaction of the carboxylic functions of glucaric acid by alcohols has been the subject of extensive attention. Conversion of the Na salts into the respective methyl esters was achieved smoothly and efficiently (> 85% yield) by stirring a suspension in dry methanol in the presence of MeOH-washed Amberlite IR-120 (H^+form) and molecular sieve at ambient temperature for 2-5 hours. Intra and inter molecular esterification of D-glucaric acid is easily carried out in acidic methanol solution yielding a mixture of dimethyl D-glucarate, methyl D-glucaro-1,4-1actone, methyl D-glucaro-6,3-lactone, and D-glucaro-l,4:6,3-dilactone.[42] Similar equilibrium mixtures of alkyl D-glucaro-1,4-lactone and D-glucaro-6,3-lactones, dialkyl D-glucarate, and small amount of D-glucaro-1,4:6,3-dilactone was also described with other alcohols.[43] These ester/lactone mixtures are useful building blocks for further polycondensation reactions with a variety of diamines to provide useful polyhydroxypolyamides (PHPAs) of varying structures, molecular weights, and properties.[42]

Amides of glucaric acid can be smoothly obtained from the acid and several primary amines. One or two carboxylic function can be converted in this way, with preference for mono-amidation at moderate temperature and di-amidation at high temperatures. Both the acid and its alkali salt can be used with primary amines under mild conditions with the possible intermediacy of 5-membered lactones.[44] Several primary α,ω-diamines react analogously to give monomeric glucaramides, which were subsequently converted by condensation polymerizations to the corresponding stereoregular poly(D-glucaramides).[45] The interesting properties of these polymers were exploited until commercial level, despite the conformationally complex structures related to the repeating asymmetric D-glucaryl unit of the four chiral carbons.

Some attention was also placed on the functionalization of the hydroxyl groups both as a method to protect these functionalities and to provide access to other functional groups. Acylation was easily obtained with acyl anhydrides and acyl chlorides. After protection of the alcoholic groups, activation of the acid groups to the corresponding acyl chloride was obtained by reaction with thionyl chloride, $POCl_3$ or oxalyl chloride. The resulting D-glucaroyl dichloride derivatives were used for the preparation of several esters, amides, azides or polycondensed with polyols, diamines and dihydrazides to prepare hyperbranched glucarate esters, urethanes, and poly(D-glucaramides. As for other saccharides, the vicinal hydroxyl groups can be protected by chetalization with carbonyl compounds and diisopropylidenglucaric acid and benzylideneglucaric acid were developed in good yield with acetone and benzaldehyde, respectively.

In addition, the dehydration of D-glucaric acid received attention as a method to obtain open chain unsaturated and carbonyl derivatives, along with aromatic derivatives of the furan series. The main target of these researches was the 2,5-furandicarboxylic acid, an useful monomer for polymers analog to terephthalic acid. Acid catalysis is essential for this conversion, and benzene sulfonic acid (BSA) or p-toluene sulfonic acid (PTSA) were found effective with yields ranging from 6 to 50 %, depending on the amount of catalysts used (Scheme 2.4).[46, 47]

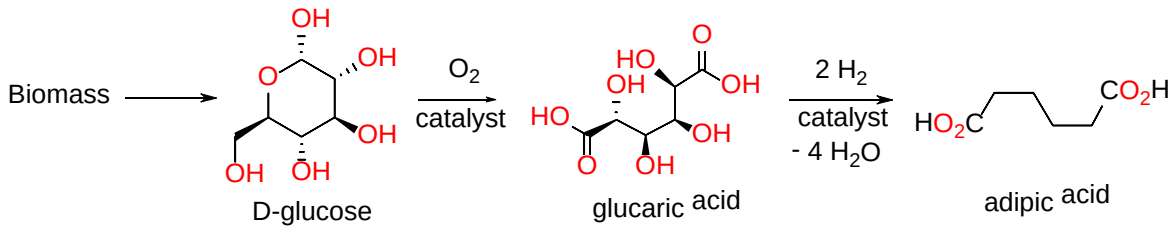


Scheme 2.4 - Synthesis of 2,5-furandicarboxylic acid from D-glucaric acid.[47]

The dehydration process appears a complex sequence with relevant side reactions, which explain the limited yield and the relevant by-products observed. To improve the reaction, several heterogeneous and homogeneous catalysts were investigated. Under appropriate conditions, 2,5-dihydroxytetrahydro-

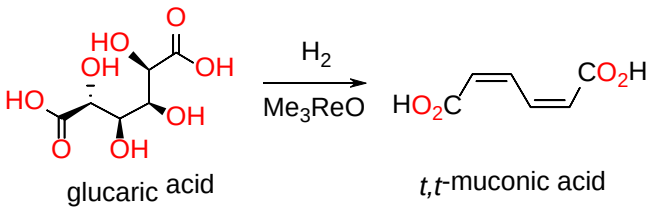
2-furancarboxylic acid (glutraldehyde hydrate) and 4,5-dihydroxy-2-oxo-adipic acid were detected as intermediates.[48]

Oxidation or reduction of glucaric acid and its salts were also investigated with the aim to obtain unsaturated and saturated molecules of interest. Specific attention was deserved to selective catalytic hydrogenation of D-glucaric acid to adipic acid by molecular hydrogen.[49] The method was developed in a pilot plant by Rennovia starting from lignocellulosic biomass (Scheme 2.5). This biobased adipic acid is an important building block for producing various polyurethanes, non-phthalate plasticizers and biodegradable polyesters, as well as renewable nylon-6,6 polymer.[49]



Scheme 2.5 - Rennovia's Two Step Process for Production of Bio-based Adipic Acid from Glucose.

Under less drastic conditions and catalysis of methyltrioxyrhenium ($MeReO_3$) the reduction stops at the stage of muconic acid, mainly as *trans-trans* isomer (Scheme 2.6).[50]



Scheme 2.6 - Me_3ReO catalysed hydro-deoxygenation of glucaric acid to muconic acid.

Muconic acid is a biobased compound of potential interest to access, between others, adipic acid and terephthalic acid. This selective hydro-deoxygenation process was applied with success also for other carbohydrates.

2.2.4 - Known Uses of Glucaric Acid and its Potential as Platform Molecule

Given its straightforward manufacture and the low cost of D-glucose as a direct precursor, the full potential of D-glucaric acid has not yet been fully exploited. Most of the applications of D-glucaric acid involve consumption of this compound on relatively small scale, and therefore do not make use

of the prospective economy of scale associated with its precursor D-glucose.[21] However, the range of miscellaneous uses of D-glucaric acid and its derivatives reported in literature is very widespread. All forms of D-glucaric acid are human metabolites of D-glucuronic acid and this has led to their use in medical and cosmetic preparations. Particular relevance has found in cancer treatments and preventatives,[51-53] but also in a variety of formulations to treat and prevent illnesses ranging from hair loss to heart disease.[54, 55] D-glucaric acid is also known as precursor of 2,5-dihydroxytetrahydro-2-furancarboxylic acid, a compound claimed as a powerful anticancer agent.[48]

Uses of D-glucaric acid in industrial processes are known,[56] especially as an additive or builder in detergents.[57-59] Other uses ranging from an energy-containing treatment for plants exposed to pesticides,[60] to a low-sugar and low flour base for food products[61] and a composition for cleaning egg shells.[62] The use of the disodium salt as a substitution of phosphates compounds in detergents is now common. The ban on the use of phosphates in detergents due to their toxic nature and stringent regulations restricting the use of harmful chemicals has increased glucaric acid demand in this application.

The global glucaric acid market size was systematically growing in the last years (Figure 2.8), with an estimated value of USD 550.4 million in 2016 thanks to increasing demand from detergents, soaps, food ingredients, corrosion inhibitors, and de-icing applications.[63] Growing liquid detergent industry, which was the largest consumer of this product, is expected to play a key role in propelling market demand.

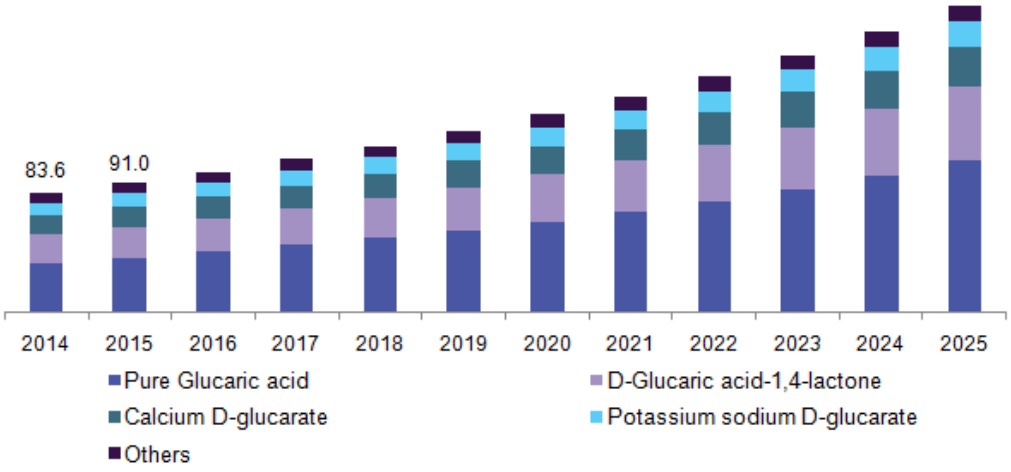


Figure 2.8 - Global market size of glucaric acid and its main derivatives.[63]

Some of the major products of glucaric acid include pure glucaric acid, calcium D-glucarate, potassium sodium D-glucarate, and D-glucaro-1,4-lactone, with a market share indicated in Figure 2.9. This market is dominated by pure glucaric acid accounting for 41.3% of the revenue in 2016 due to low price and easy availability as compared to other products.

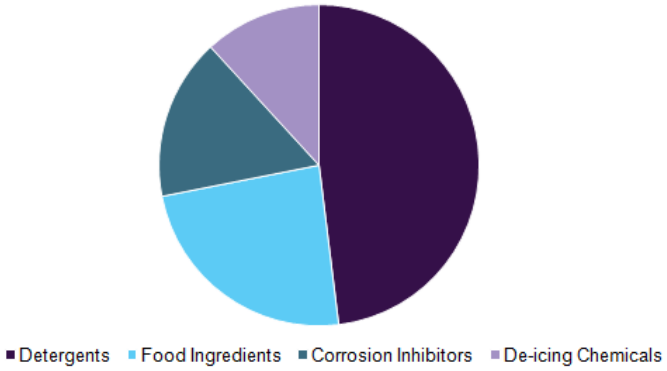


Figure 2.9 - Global glucaric acid market revenue by application, 2016 (%).[63]

Calcium D-glucarate is projected to grow at a Compound Annual Growth Rate (CAGR) of 8.4% by value, from 2018 to 2025 because of wide applications in the food industry optimizing the levels of vitamins and minerals in the body, thus, safeguarding vital organs such as lungs and liver. D-glucaro-1,4-lactone, one of the widely used derivatives, is projected to grow at the second-highest CAGR of 10.4% by value, from 2018 to 2025. This product, which is used in the treatment of cancer has witnessed growing demand from the emerging medical sector, especially from countries with a high prevalence of this disease.

Finally, glucaric acid has a key potential as platform molecule in the preparation of three relevant biobased diacid monomers, i.e. furan-2,5-dicarboxylic acid, adipic acid and terephthlic acid. The polyester, polyurethane and polyamide polymers obtained from these monomers are expected to grow fast in the next years, along with the development of the above mentioned poly(D-glutaramides) and poly(alkyl D-glutarates). Moreover, the results obtained in this thesis suggest that other potentialities exist in the direction of pyrone derivatives and other aromatic five membered compounds, i.e. pyrrole-2,5-dicarboxylic acid and thiophene-2,5-dicarboxylic acid and related product of decarboxylation. All these potentialities are summarized in Figure 2.10 to show the quite broad functionalization that this acid provides. Obviously, achiral aliphatic and aromatic compounds can be in principle accessed from all aldaric acids, whereas the chiral nature of glucaric acid will provide specific chiral compounds (i.e. polyamides and polyesters) different from other achiral aldaric acid.

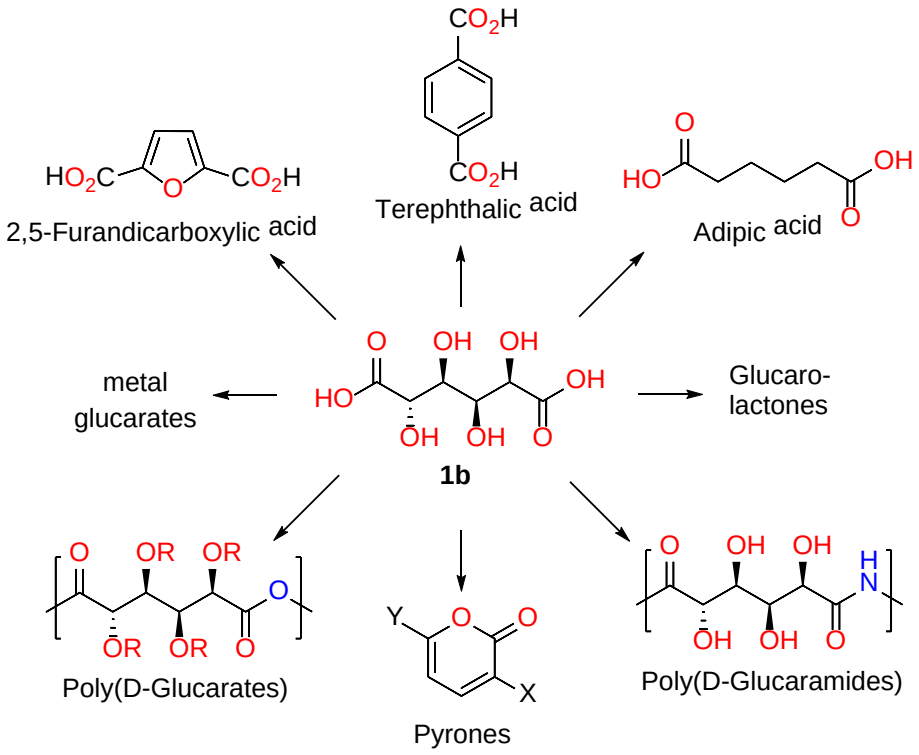


*Figure 2.10 – Potential use of D-glucaric acid (**1b**) as platform molecule for relevant products and materials.*

2.3 - D-Mannaric acid

D-Mannaric acid **1c** [IUPAC name (2S,3S,4S,5S)-2,3,4,5-tetrahydroxyhexanedioic acid] is the aldaric acid derived from D-mannose. Since D-mannose is widely distributed in carbohydrates biomass as the third abundant aldohexose after the monosaccharides D-glucose and D-galactose, D-mannaric acid as its oxidation product has significant potential to be applied as useful renewable resources and platform chemicals.[64] The acyclic D-mannaric acid is in equilibrium with its dilactone (**A**) by heating under reduced pressure. The dilactone **A** has already been isolated as a crystalline powder, while the acyclic D-mannaric acid **1c** was never retrieved in solid form. The structures of these two compounds are shown in Figure 2.11.

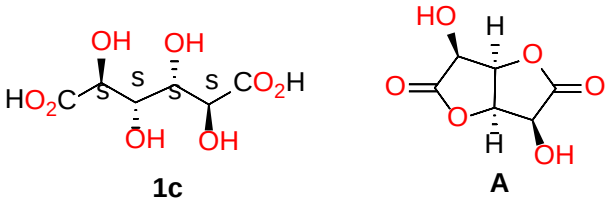


*Figure 2.11 - Absolute stereochemistry of D-mannaric acid (**1c**) and D-mannaric-1,4:3,6-dilactone (**A**).*

2.3.1 - Properties of Mannaric Acid

From the structure of D-mannaric acid, it is clear to see that it has 4 S chiral centers and a C-2 axis of symmetry, while there is no plane of symmetry as a chiral molecule.[64] D-mannaric acid exists in an extended conformation being free from the steric interaction between the 1,3-parallel hydroxyl groups, present in glucaric acid.[64] Table 2.5 summarizes some relevant properties of D-mannaric acid.

Table 2.5- Properties of Mannaric acid.

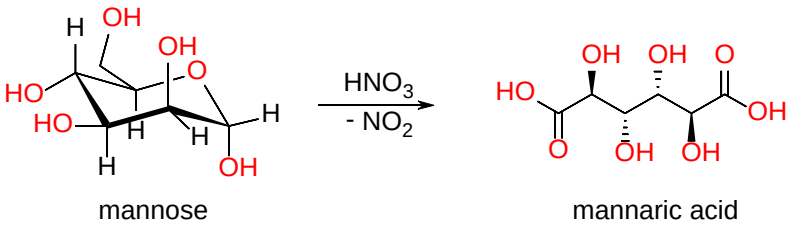
Property name	Property value
Molecular weight (g/mol)	210.14
CAS Number	6543-97-1
Melting point (°C)	Not known
pK_{a1} , pK_{a2}	2.99 , 3.7
Water solubility (g/L at 14°C)	>100
Optical rotatory power (°) in water, 25 °C	+50

Mannaric acid when heated under reduced pressure converts easily in D-mannaric-1,4:6,3-dilactone, a stable crystalline compound only experiencing a slow hydrolysis process of the lactone ring. However, the compound is particularly sensitive to water elimination to give an unsaturated lactone.[65]

2.3.2 - Synthesis of Mannaric Acid

D-Mannaric acid was first synthesized from D-mannose by benchtop oxidation with nitric acid, but impure non crystalline acid form was first isolated (in 24% yield) by hydrolysis of the corresponding D-mannaro-1,4:6,3-dilactone by Haworth et al. in 1944.[66] The oxidation of mannose with nitric acid

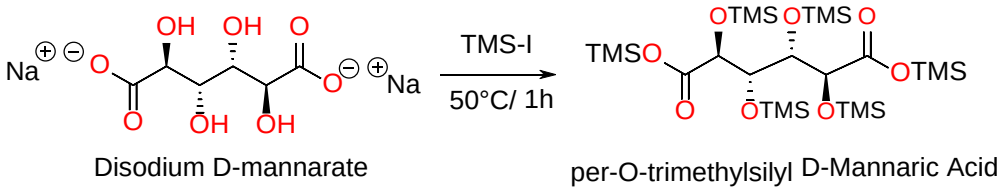
was improved by Kiely et al. using $NaNO_2$ as catalyst to reach yield of dilactone up to 62% thanks to efficient crystallization followed by extraction of nitric acid with diisopropyl ether (Scheme 2.7).[67]



Scheme 2.7- Synthesis of D-mannaric acid according to Kiely et al. [67]

By introducing bromine as the terminal oxidant, D-mannose oxidation gave a yield of 70 % in D-mannaric acid, which was isolated as its crude sodium salt impure of 15 % tartrate and 10 % unidentified product.[25] DL-mannaro-1,4:6,3-dilactone was also synthesized in 46-50% yield by thermal dehydrative lactonization of DL-mannaric acid in a water/acetone medium. The product was isolated by removal of water by azeotropic distillation.[68] Oxidations with oxygen using transition metal catalysts supported on TiO_2 or Al_2O_3 was less selective affording mainly mannonic acid.[21]

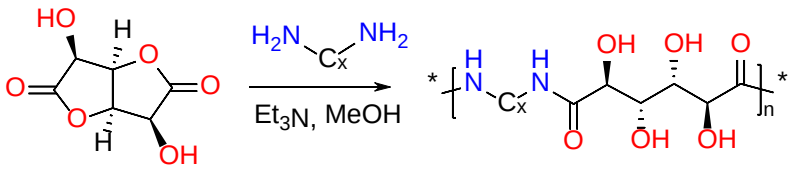
Separation and identification of mannaric acid is commonly carried out by gas chromatography (GC-MS), after conversion into its trimethylsilyl derivative (Scheme 2.8), or by HPLC and ion chromatography.[64] D-Mannaric acid and D-mannonic acid are clearly differentiated as TMS derivatives by their mass fragmentation patterns in EI spectra.[69]



Scheme 2.8 - Synthesis of per-O-trimethylsilyl derivative of D-mannaric acid used in the analysis.[65]

2.3.3 - Chemical Reactivity and Potential as Platform Molecule of Mannaric acid

The D-mannaro-1,4:6,3-dilactone is a potential building block for chiral material, protease inhibitors and polymers.[68] The disodium D-mannarate was prepared by sodium hydroxide hydrolysis of the dilactone in low yield 34% due to the β-elimination side reaction to produce enol and ketone by-products.[64]



Scheme 2.9 - Synthesis of poly(mannaramides) from D-mannaro-1,4:6,3-dilactone.[70]

The acyclic diamide of D-mannaric acid was synthesized by first esterification of D-mannaro-1,4:6,3-dilactone with hydrogen chloride in methanol to give methyl ester lactone and dimethyl mannarate, followed by aminolysis with amine in methanol to form mannarodiamide in 53% yield. The diamide was further hydrolysed to dialkylammonium mannarate salt by NaOH and polymerised into stereoregular poly(alkylene D-mannaramides) with trimethylamine in 75.7% yield and Degree of polymerization (DP) 4.96.[64] Varela *et al.* also reported the synthesis of stereoregular poly(mannaramides) by reaction of the dilactone with even-numbered alkylenediamines (Scheme 2.9).[70]

Protection of C-2, C-5 hydroxyls of L-mannaric acid was obtained by O-benzylation. The benzylated molecule was further coupled with amino acid and amines in the presence of pyridine or tetrabutylammonium fluoride, to form the C2-symmetric bisamide product in 67-96% yield by C-terminal duplication.[71] An alternative route by nucleophilic ring opening was reported with D-mannaro-1,4:3,6-dilactone with various amines and amino acids: bisamides were obtained in 22-76% yield (Figure 2.12).[71] These C2-symmetric products were proved to be potent HIV protease inhibitors.[71] The rate of addition of amines to D-mannaro-1,4:3,6-dilactone is significantly slower than to D-glucaro-1,4:3,6-dilactone.

Mannaric acid was also converted to 2,5-furandicarboxylic acid (FDCA) by dehydration and cyclization under catalysis of acids, such as sulfuric acid, nitric acid, etc. or organic sulfonic acid. A medium free from alcohol was used for the reaction to avoid the formation of furandicarboxylic acid esters.[72]

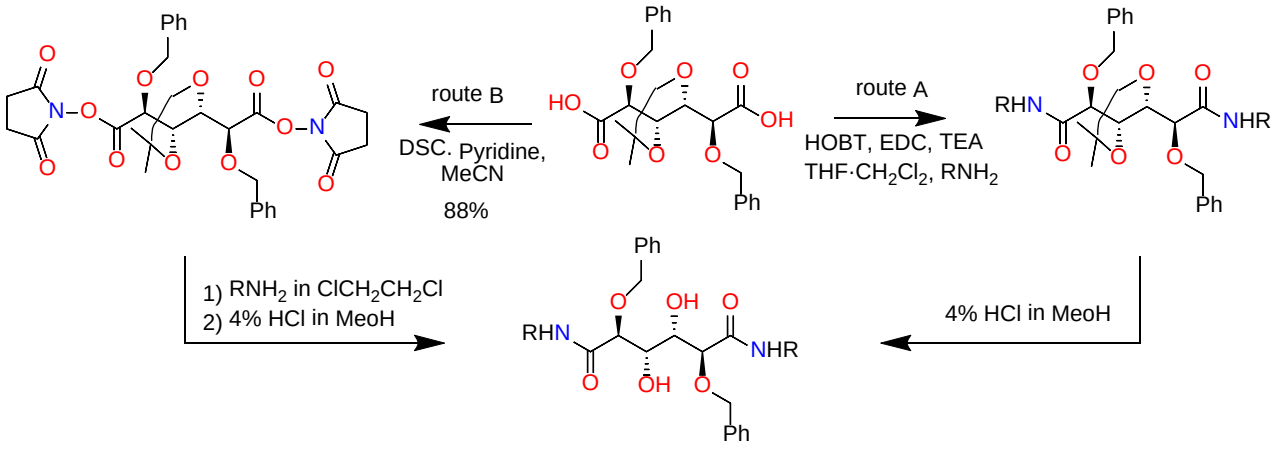


Figure 2.12 – Approaches to the synthesis of fully or partially protected D-mannaric diamides.[71]

2.3.4 - Applications

Limited uses of mannaric acid has been found until now, mainly for the difficulty in its isolation and purification. As derivative (dilactone, disalts, diesters, and diamides) it has found some applications as metal complexing agent, surfactant and as monomer for polyesters and polyamides. This last class of compounds demonstrates remarkable biological activity and was patented as potent HIV protease inhibitors.[71] Amphiphilic derivatives of mannaric acid has been applied as surfactant agent in cosmetic and food industry.

2.4 - Galactaric acid

Galactaric acid (**1a**, (2R,3S,4R,5S)-2,3,4,5-tetrahydroxyhexanedioic acid, also known as mucic acid) is an aldaric acid, which can be easily prepared from C-6 sugar galactose, or from galacturonic acid. It presents a symmetrical structure (Figure 2.13) and therefore is achiral.

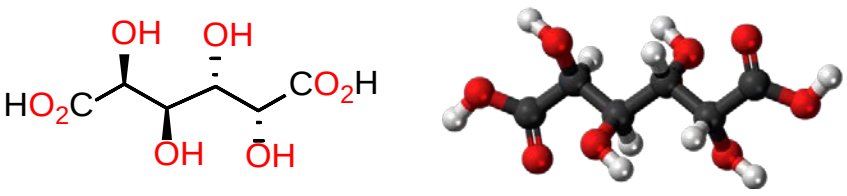


Figure 2.13- Molecular structure of galactaric acid and its 3-D model.

Galactaric acid can be prepared by nitric acid oxidation of milk, sugar, dulcite, quercite and most varieties of gum. These last materials belongs to a broad class of acid polysaccharides (pectins) containing monomeric unit of uronic acid derivatives. Between them in specific plants the monomeric galacturonic acid is accumulated, and, therefore, these were first used as source of galactaric acid, after hydrolysis and oxidation of galacturonic acid. Nowadays, galactaric acid plays the role of a specialty chemical in the food, cosmetic, pharmaceutical and construction industries. In this section, the synthetic methods/processes and the chemical reactivity of the compound will be revised with the aim to bring out the potentiality of this natural sugar derivative as platform chemical. Advancements in chemical and biotechnology are promising to leads to cost-effective production of galactaric acid extending market and application of this acid and its derivatives. Conversion of galactaric acid from biomass appears a promising route to access chemicals and materials for a future sustainable development.

Even if galactaric acid has been numbered in the list of the strategic platform chemicals for the future green chemistry,[73] it is still considered as a fine chemical, with a limited market and few commercial applications. Moreover, even if the compound is known since the 18th century, the available data on its chemical, biological reactivity and physico-chemical properties are limited and incomplete on several aspects.

Galactaric acid was first found and isolated from Arabic gum by German pharmacist Scheele in 1780 and was later characterized by Malaguti, Berzelius and Limpricht in the first half of the19th century.[74] Main sources of galactaric acid were identified in natural saccharides containing galactose, typically in the forms of oligo- or polysaccharides (e.g. lactose and pectins). After the first isolation, galactaric acid experienced the further exploitation in the same century resulting in the first production technique from pectin hydrolysis until the introduction of the first industrial production process patented in 1929.[75, 76] Some minor application of galactaric acid was explored until the beginning of “petro-chemical era”. After World War II, galactaric acid was left almost forgotten: few and fragmentary contributions were given to the knowledge of this compound, both from the point of view of characterization and industrial production. For this reason, galactaric acid has remained a quite expensive specialty chemical for more than a century. Only recently, with the growth of interest for alternative raw materials from biomasses, the compound has started to draw the attention of the scientific community.

2.4.1 - Chemical and Physical Property of Galactaric Acid

Galactaric acid is a crystalline solid with high melting point (220–230 °C). The high stability in crystalline state is closely related to the high crystal density of the solid (1.79 g/cm^3), which explains

also the low solubility of this aldaric acid in cold water (3.3 g/l). The behavior is remarkably different from that of glucaric acid, which is well soluble in water (510 g/l) at the same temperature. Galactaric acid is insoluble in ethyl alcohol and other conventional solvents, but partially soluble in DMSO and other chaotropic solvents. As a *meso* compound with chiral centers, it is optically inactive thanks to its symmetric structure. Some of the general properties of galactaric acid are shown in Table 2.6.

Table 2.6 –Properties of Galactaric Acid.

Property name	Property value
Molecular weight (g/mol)	210.14
CAS Number	526-99-8
Melting point (°C)	220-230 (dec.)
Density at 25 °C	1.79[77]
pK_{a1}, pK_{a2}	3.08, 3.63[78]
Water solubility at 14°C (g/L)	3.3
Optical rotatory power (°) in water, 25 °C	0.0

As indicated above for the conformation of D-mannaric acid, D-galactaric acid also lacks 1,3-parallel hydroxyl group eclipsing steric interactions which contributes to its extended conformation in the crystal structure producing an extended hydrogen bond network.[79] Detail of the crystal structure of galactaric acid are reported in Figure 2.14.[77]

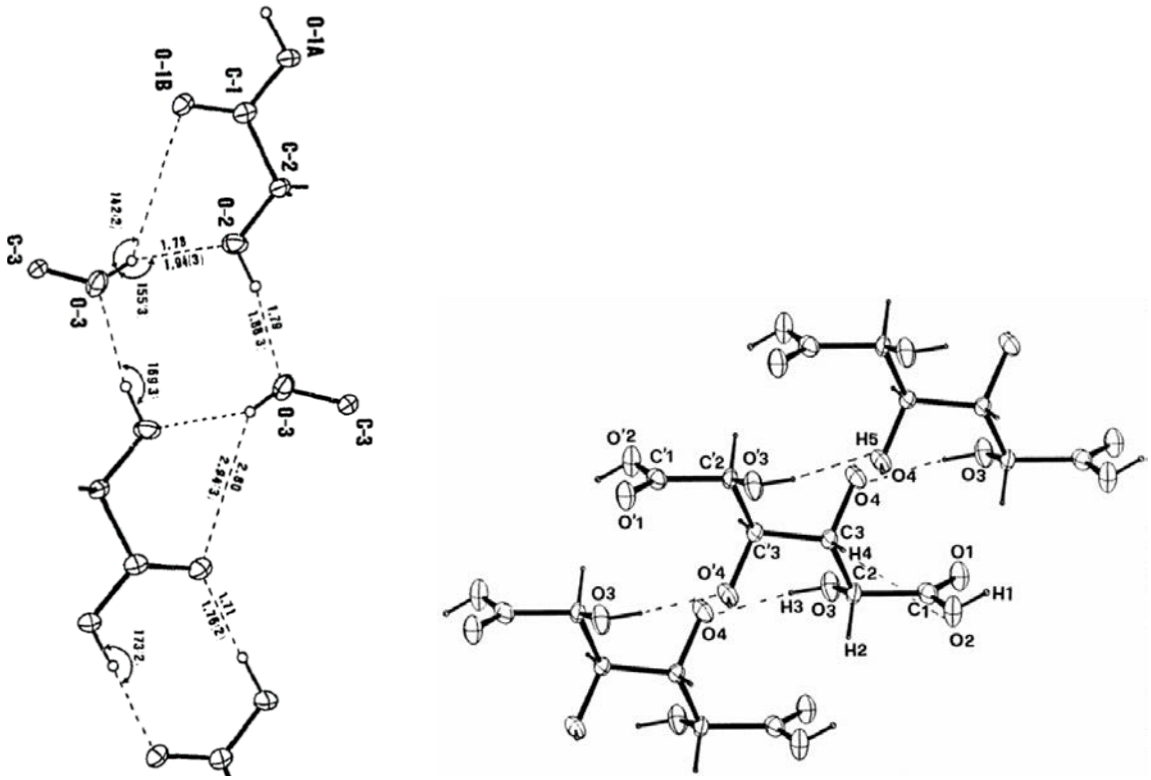


Figure 2.14 - ORTEP view (50% ellipsoids) of galactaric acid crystal structure showing inter- and intramolecular hydrogen bonds.[77]

2.4.2 - Synthesis of Galactaric Acid

Galactaric acid was first discovered and separated from milk whey date back to 1826. The milk was remain aside for few hours, a thick layer of cream was the first collected from its surface. Then the remaining bluish white liquid was heated to about 100 °C to separate whey, the principal constituents of milk. Afterward the whey was transformed into lactose by evaporation. When lactose was treated with nitric acid, galactaric acid was formed. To recover the acid, one part of lactose was digested in excess nitric acid with moderate heating. Then the flask was placed in cool place, some white powder subsided and galactaric acid was collected by filtation.[80] Since then, several processes to produce galactaric acid appeared based on both chemical routes and biotechnological conversion of substrates containing the four CHOH aldose groups of appropriate R,S configuration (mainly pectin, pure galactose or galacturonic acid). Although galactaric acid has a huge potential to be applied as a platform molecule, the main demand for galactaric acid is still limited to pharmaceutical and cosmetic industry, thus the current production of galactaric acid is mainly based on the traditional routes characterized by relatively high price with limited process engineering.

In the following paragraphs the main processes investigated will be summarized, with some details for the ones applied on industrial scale. They are allocated in two categories: a) catalytic chemical process and b) biotechnological processes.

a) Catalytic chemical processes

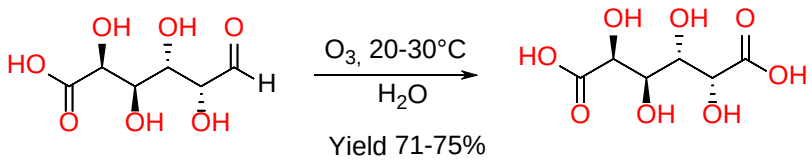
Along with the discovery of galactosan (11-23% weight) in dried western larch woods,[75] the first patent for galactaric acid production based on nitric acid oxidation appeared in 1929, disclosing a big scale process to obtain a fairly pure (90%) galactaric acid powder from western larch aqueous extract rich in lactose. The nitric acid was added in weight ratio 3:1 in a jacketed vessel at temperature 90-100 °C and the process was optimized by introducing a cascade of absorption towers in which the nitrous gases released in fresh lactose solution during oxidation was recovered.[76] For a long time, these processes based on nitric acid were accepted as the standard preparation method for commercial galactaric acid. Although the use of nitric acid offers the advantages of playing as solvent and unexpected selective oxidising agent at the same time, this oxidation still had some drawback. The main issues identified were: i) the process is highly exothermic and runaway reactions are difficult to control; ii) it produce large amount of nitrous gases, which are toxic and dangerous for the environment; iii) the unreacted nitric acid is difficult to isolate and recycle; iv) complex mixture of different organic acids are formed, making complex the isolation procedure at good purity level.[81]

In order to improve the sustainability of nitric acid process, optimizations was attempted mainly by the Kiely group in a series of patents applied to the main aldaric acid. In 2008 an improved process was introduced, which was characterized by moderate conditions, better temperature control (20-45°C, for 8 hours), and use of molecular oxygen as terminal oxidant and nitric acid as catalysts.[24] Also the downstream processing to recover nitric acid and galactaric acid was modified from the early nitric acid process. Different techniques, like neutralization, distillation and addition of 2-propanol, diffusion dialysis, etc., were proposed as a low energy separation technique to recover unreacted nitric acid, before proceeding with the product crystallization.[82] Some patents applied similar downstream processing to glucaric acid and galactaric acid, even starting from different raw materials, i.e. galactose or arabinogalactan.[83] As a consequence, even though galactaric acid has not yet produced in large scale by this improved nitric acid oxidation, this technology is ready to provide the acid from galactose at a price of 650 $/ton. Referring to the process patented by Kiely,[67] the conversion of the initial sugar is always 100%, while the maximum yield of the target aldaric acids is around 40% (44% for glucaric acid), which is restricted by the complex downstream separations. In addition, the nitric acid process, though technologically improved, is still cumbersome for safety and environmental aspects resulting in high fixed costs. The only example of large scale production of aldaric acid (glucaric acid) was developed by Rivertop Renewables with production capacity of 4500 ton/year at a price close to 30 $/kg.[84] Different oxidants were investigated as substitute of nitric acid and oxygen for the oxidation of galactose. Merbouh has patented a process in which a basic aqueous solution of galactose was oxidized

using chlorine gas in the presence of a nitroxide catalyst (4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxyl) at 0-5°C, resulting in a 75-78% overall yield.[85]

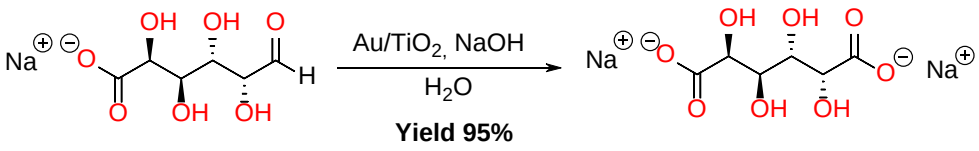
Apart from aldoses, other starting materials to prepare aldaric acids are the alduronic acids, monomers commonly present in hemicellulose polysaccharides of fruit peel. So, galacturonic acid can be isolated from these polysaccharides and oxidized to galactaric acid by a combination of a variety of terminal oxidants and catalyst. Nitric acid was the first used in this oxidation, followed by an electrolytic process of aqueous solution of galacturonic acid in the presence of a redox mediator.[86] Électricité de France (EDF-France) has applied this electrochemical oxidation in one electrolytic cell as modified Grinard reactor in the presence of Br^-/Br_2 couple and 0.5 M HBr).[87] French company Givaudan is currently using this electrolytic oxidation to produce galactaric acid from D-galacturonic acid for cosmetic application as an antioxidant (Trade name: Muciliance® Fruit).

Ozone can also be use as oxidant, to obtain a highly pure crystalline galactaric acid without further purification treatment.[88] Ozone is provided at 20-30°C in the absence of any catalyst at a pressure of about 2 bar, and it is produced in situ from liquid oxygen. The starting solution consists of 12% galacturonic acid in water (in case obtained separately through partial oxidation of galactose[89] or better from pectic acids). The main limit of this method, comparing with other oxidation process for commercial galactaric acid, is that is less cost-efficient. Scheme 2.10 summarizes the approach.



Scheme 2.10 - Oxidation of galacturonic acid by ozone according to method by Bonhoure et al.[88]

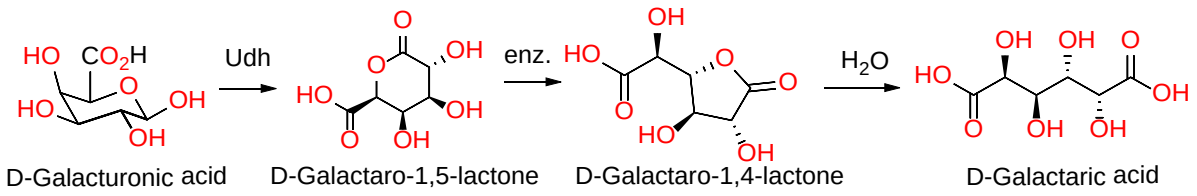
One of the latest development in uronic process is the use of oxygen and a gold catalysts on various supports in basic medium to produce the galacatarate salt[90,91] Typical conditions were pH 8–10 and 40–60 °C under heterogeneous catalysis of Au/Al_2O_3. Improvements were later reported with Au/C catalyst in acidic condition resulting in higher selectivity at C1 and good recyclability of the catalyst. With this catalyst, a maximum galactaric acid yield of 95% with 100% selectivity was reached. The Au/C catalyst was recyclable without losing activity and selectivity after five successive batch runs.[92] The employing of gold catalysts have been also applied in the oxidation of galactose.[93] Scheme 2.11 summarizes this approach.



Scheme 2.11 - Catalytic oxidation of sodium galacturonate according to the method of van der Klis.[91]

b) Biotechnological processes

Although nitric acid oxidation has been used as the main process for commercial galactaric acid, all the catalytic methods introduced above need to use oxidizing agents mainly with poor environmental performances. Nitric acid and halogen substances are difficult to handle. Ozone and gold catalyst are expensive chemicals concerning the exploration of galactaric acid as platform molecule. Thanks to the improvement of biological engineering, attempts to overcome these drawbacks were made adopting biotechnological processes. Because the biosynthesis of galactaric acid from galactose is a multistep challenging process,[94] the new bio-based approaches include commonly some modified microorganisms. More simplified biotechnological approaches were adopted starting from D-galacturonic acid and pectin. The need to use genetically modified organism (GMO) derives from the fact that anabolic pathways for galactaric acid formation are expressed normally in superior vegetal organisms instead of microorganism strains. Even so, some promising catabolic steps are just present in microorganisms. For example, D-galacturonic acid ($pK_a = 3.51$)[95] can be first oxidized by an uronate dehydrogenase enzyme (EC 1.1.1.203) to D-galactaro-1,5-lactone, which fast rearranges to the corresponding D-galactaro-1,4-lactone, followed by a slow hydrolysis to galactaric acid, as summarized in Scheme 2.12.[96,97]



Scheme 2.12 - Fungal catabolic D-galacturonic acid pathway and a heterologous uronate dehydrogenase (UDH) for galactaric acid production.

In particular, Mojzita et al. produced for the first time fungal strains able to oxidize D-galacturonate to L-galactonic acid[98] and *meso*-galactarate salt.[99] The selection of fungal strains is because eukaryotic microorganisms living in decaying plant have several pathways to catabolize D-galacturonate and its derivatives in the biomass, in which have the ability to produce *meso*-galactarate salts. For galactaric acid production, the genes of *Aspergillus Niger* and *Hypocrea jecorina* were engineered by deleting

those encoding D-galacturonic acid reductase and expressing those encoding a D-galacturonic acid dehydrogenase, in order to avoid fungi to use galacturonic acid as source of carbon. The study showed that both the strains were efficient in the conversion to galactarate, and, in particular, *H. jecorina* reached the theoretical yield of 80-90% (1.08 g of galactarate/g galacturonate, pH 5.5 in pure D-galacturonate solution). On the contrary, *A. niger* did not result in efficient galactaric acid production. In all cases, the low solubility of galactaric acid in water contributes to an easy recovery of the product. In alternative, another eukaryotic transport protein for D-galacturonic acid, originally from *Nerospora crassa*, was expressed in *Saccharomyces cerevisiae* as fermentation host. Benz et al. has developed this process by using the genes in the yeast devoted to the conversion of D-galacturonic acid into D-galactaric acid.[100] Thanks to the peculiar resistance to inhibitors and easy industrialization of this microorganism, the feasibility of using *S. cerevisiae* was finally proved.

For the bio-approaches above, galactaric acid was mainly produced from D-galacturonic acid as a pure chemical, but a more economical process is expected from the use of the hydrolysis mixtures of pectins containing D-galacturonic acid. As a polymer of non-woody plant primary cell wall, commercial pectin (e.g. in food and cosmetics products) is currently produced from fruit peel, as well as sugar beet pulp and other plant waste.[101] In general, these pectin-rich waste biomass streams are available and currently used as animal feed or remains as unused waste. So pectin-rich biomasses can be a good source for galactaric acid production, which can be a new valorisation of pectin derivatives more than colloid for common application. Therefore, in order to apply commercial galactaric acid as platform chemical on large scale at relatively low price, biorefinery processes must be further improved and optimized. Efforts were mainly addressed to the largely underexploited resource of pectin rich biomass from fruit and vegetable wastes with again significant but not able to provide galactaric acid at acceptable prices. The main target in this direction is the complete use of all components present in these hydrolysates.

Among all the waste from fruit and vegetable transformation industry, the citrus peel waste (CPW) from juice production has high organic contents (95% of total solids) including fats, free sugars (glucose, fructose, and sucrose), organic acids, carbohydrate polymers (pectin, cellulose and hemicellulose), enzymes (pectinesterase, phosphatase, and peroxidase), flavonoids, essential oils (mainly limonene), and pigments, among which 25% is typically pectin.[102] As approximately the one-third of world citrus fruits production is consumed in industrial transformations, the volume of citrus waste generated is of the order of 10 Mton/year.[103] This quantity is higher than any other pectin sources from food by-products. While the demand of food grade pectin for pharmaceutical, cosmetics and food industries was only 69 Kton for the year 2017, more than 90% of pectin production potential is currently not exploited.[104] Since galactaric acid production has been technically proved from CPW by fermentation, the future need of galactaric acid as platform molecule can possibly make use of this huge amount of biomass, which will also contribute to the development of the bioeconomy. Besides this, it can in turn reduce the negative impacts of CPW on environment and challenges for producers.

The traditional waste management approaches for citrus waste are normally composting, anaerobic digestion, incineration and gasification. CPW currently is pelletized to low-value animal feed,[105] biogas under harsh conditions or with low yields due to the inhibition effect of acids on fermenting bacteria,[106-108] and in most cases they are burnt with energy losses due to the high water content (80-90%).[109] For these reasons, citrus peel is nowadays a cost more than a resource for citrus processing industries. In order to protect the environment in addition to bioeconomy development, some attempts of green valorisation were recently proposed by using miscellaneous biochemical technologies. Limonene, as a citrus-derived hydrocarbon solvent, has been studied to explore the possible replacement of toluene as cleaning agents, but the substitution at global level is still unrealistic based on the current results. CPW extraction of flavonoids and terpenes is often practiced, but these treatments actually do not solve the disposal issues of the spent biomass. CPW is also used as fermentation substrate for enzyme and active pharmaceutical ingredient (API) production.[110] Finally, spent biomass can undergo the saccharification processes to increase the fermentability for second-generation biofuels production (via steam explosion, acid or enzymatic hydrolysis).[103, 105, 111, 112] However, the production of biofuels, even if with enhanced fermentation conditions, has been already estimated as little profitable,[113] while API and enzyme production can absorb only a small amount of the available biomass.

As the scale-up production process introduced above, it is important to underline that there is no need of such a complex process as for food-grade pectin, to recover galacturonic acid and then galactaric acid, concerning the current price of D-galacturonic acid (more than € 3000 per kg) and pectin (€10–100 per kg). Except for the advantage of achieving a high purity standardized product, many of the unit operations to preserve pectin chain length and extraction of pectin are not necessary, as the monomer is the target. Hence, the abovementioned saccharification techniques become a valid option to release galacturonic acid directly from the CPW, and other peel wastes, as plant biomass. Enzymatic treatments to break pectin chains are well known and widely applied in food and paper industry. Fruit juice and wine clarification, cellulose degumming, and vegetable extract stabilization are some of the many unit operations that require the decomposition of the complex structures of pectin (CP-Kelco, Dupont).[114-117] By investigating individual efficiency of different pectinase in catalysing galacturonic acid formation from citrus peel pectin, pectinase 62L as mixture of polygalacturonase, pectin lyase and pectin esterase, has been proved as best. Moreover, thanks to the high cost of commercial enzymes, an integral approach including the solid state fermentation of fungi on citrus peels and the hydrolysis of fermented substrate, has been explored in order to get galacturonic acid for further conversion. Thus, the idea to apply enzymes such as a combination of pectinase with lyase and esterase to the citrus waste biomass is a prospective solution to the galactaric acid production. And some preliminary studies, mainly in the direction of the recovery of fermentable substrates, has been carried out on other substrates like rice straw, sugar beet pulp, citrus peels, etc.[103, 110, 118-121]

Although the CPW derived galacturonic acid together with arabinose and glucose are all considered as "fermentable sugars", any integration with the galactaric acid production was never developed. Recently, a particularly interesting approach was established by the Cosun company from Netherland, one of the few producers of commercial galacturonic acid and galactaric acid.[118] The process consists of enzymatic hydrolysis of sugar-beet pulp (a by-product of sugar industry) in which 79% of total galacturonic acid and 82% of arabinose in sugar beet pulp are recovered. Enzymatic hydrolysis of citrus peel has been extensively studied in the seminal work of Grohmann et al,[122-124] however no explicit yields in galacturonic acid was reported, since their purpose was the direct fermentation of the crude hydrolysate to bio-fuel. Li et al [125] and Khamseh and Miccio[126] addressed more recently the production of galacturonic acid from citrus waste and offered a clear insight of the new developments in the field. In particular, Khamseh and Miccio provided detailed depolymerisation kinetics for commercial enzymes. A 63% yield of hydrolysed galacturonic acid is reported, in line with the saccharification yields mentioned in literature (e.g. 70% from CPW-orange,[122] 63.8% from CPW grapefruit,[127] and 78.7% from CPW by *A. japonicus* crude enzyme[125]).

Furthermore, concerning the direct conversion of CPW to galactaric acid, one approach was adding commercial pectinase mixture into citrus waste first, without the pectin isolation for typical pectin industry.[104,128] In order to convert pectin-rich biomass to galactaric acid, one approach is applying a consolidated process by using the mould *Aspergillus Niger* without the isolation of pectin. The advantage of using moulds is that these organisms often have the ability of producing enzymes to hydrolyse the biomass. This makes conceivable a continuous process in which pectin-rich biomass is hydrolysed and converted to galactaric acid in one step. However, when strains of *Aspergillus Niger* were used for galactaric acid production, an unknown pathway catabolized the formed acid. In order to solve the problem for efficient galactaric acid production, Kuivanen et al.[129] disrupted the galactaric acid catabolism by CRISPR/Cas9 mediated gene deletion technology together with in vitro synthesized sgRNA. A 4.3 g/l yield of galactaric acid was so reached, again insufficient for a production of galactaric acid if compared with the results of *Trichoderma reesei*. This last mesophilic and filamentous fungus was found to produce galactaric acid in the presence of commercial pectinase mixture on sugar beet pulp or citrus waste, after pre-processing to hydrolyse pectin. One of the constraints for microbial and fungi host is the low solubility of galactaric acid and its salts in water. The first report on production of galactaric acid by engineered strains of *Trichoderma reesei* got a relatively low 5.9 g/l yield at 25°C.[99] Later, Barth et al. found that galactaric acid was most soluble at pH 4.7 in the presence of ammonium or sodium ions at higher temperatures. By controlling the temperature, pH and medium composition, a productivity of 20 g/l in galactaric acid was obtained by *Trichoderma reesei* D-161646 at pH 4 and 35 °C. [130] Furthermore, the first scale-up process was carried out by using food grade pectin instead of plant biomass to preliminarily explore the possibility of large amount galactaric acid production.[131] With *Trichoderma reesei* QM6a galacturonate dehydrogenase, *Trichoderma reesei* produced 14 g/l of galactaric acid (yield 0.77 g/(g D-galacturonate used)) at 250

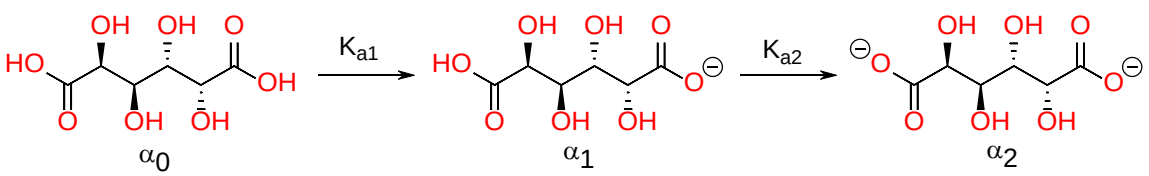
litre scale with recovery of approximately 2.8 kg of solid galactaric acid. So far, *Trichoderma reesei* appears to be the more promising agent for industrial bioconversion of both pectin and D-galacturonic acid to galactaric acid, and strain improvement of *T. reesei* is a continuous effort in order to harness fully the cellulolytic and hemicellulolytic potentials in this strain. Further process optimization of bioreactor and downstream processing of biomass is necessary for a cost-effective industrial scale process. Concerning the loss of pre-crystallized galactaric acid on biomass during the separation process, a Soxhlet extraction of fungal biomass was carried out with the culture supernatant from previous biotechnological process in order to get a cell-free suspension.[132] By this method, up to 24% of total galactaric acid produced was recovered, suggesting how to improve the efficiency in large-scale production. One promising production process of galactaric acid would be one single fermentation continuous process converting pectin-rich biomass to final sugars mixture, with integrated downstream separation process to ensure at same time ecological compatibility and sustainability. At moment, the Finnish company VTT has patented a process to convert by biochemical processing citrus wastes to galactaric acid. The first step consists of the oxidation of galacturonic acid, a constituent of pectin, to galactaric acid with a fungal biocatalyst. The conversion efficiency is high and this step has been scaled up to pilot scale (300 l), delivering kilogramme amounts of galactaric acid for the further downstream conversion of this monomer to polymers or other key chemicals.

2.4.3 - Chemical and Biological Reactivity of Galactaric Acid

This section is devoted to summarize the chemical and biological reactivity of galactaric acid to introduce the specific experimental work carried out in this thesis and offering a vision on possible uses of this compound as platform molecule.

2.4.3.1 - Acid-base behavior of galactaric acid

Galactaric acid is a diprotic acid with an equilibrium dissociation constants of $8.32 \cdot 10^{-4}$ (K_1) and $2.34 \cdot 10^{-4}$ (K_2), respectively. Based on these values, the graph of Figure 2.15 was drawn for the three forms in which this compound can exist in water depending on the pH of the medium ($\alpha 0$ indissociated. $\alpha 1$ monoanion, and $\alpha 2$ dianion). Remarkable is the limited amount of the monoanion, with a maximum concentration at pH = 3.36, a peculiarity that makes difficult to isolate the mono salts of this acid, owing to the nearly equimolar co-presence of the acid and dianion forms.



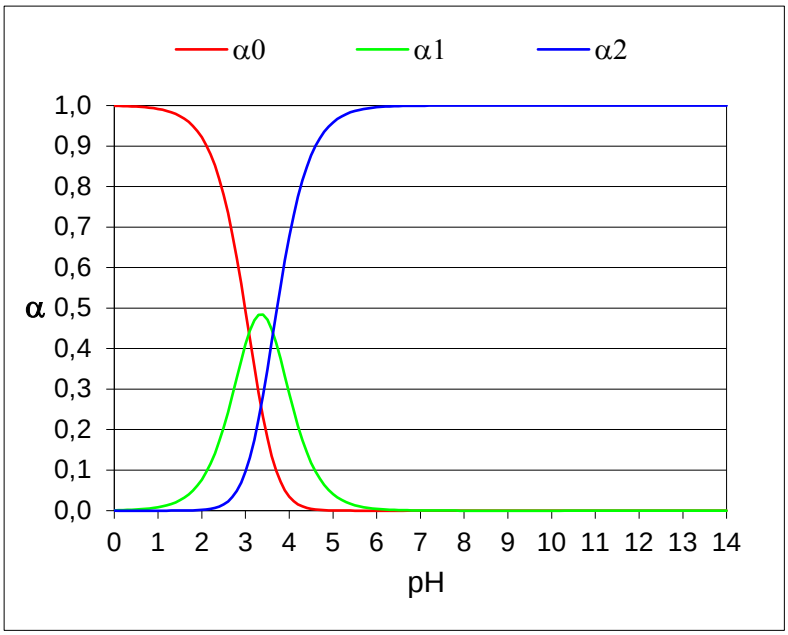
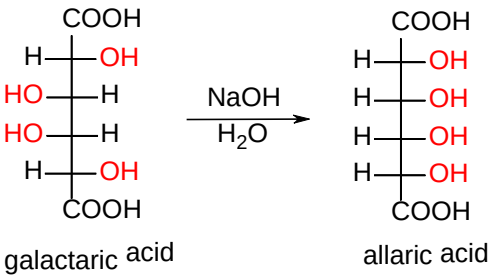


Figure 2.15 - Distribution on pH of acid (a0), monoanion (a1) and dianion (a2) of galactaric acid in water.

In highly basic medium, galactaric acid can be equilibrated to allaric acid by inversion at C-3 and C-4 positions in yield of 33% (Scheme 2.14).[133] The reaction was never studied in details but we consider that is the result of water elimination from carbons at position 3 and 4 to the corresponding alpha-enol derivatives and unselective re-hydration.



Scheme 2.13 – Equilibration of galactaric acid to allaric acid in sodium hydroxide water solution.

A large variety of galactarate salts were synthetized from galactaric acid or its lactone. So, barium galactarate was isolated from galactaro-1,4-lactone by hydrolysis with $Ba(OH)_2$ in quantitative yield,[133, 134] whereas ammonium galactarate was prepared in 40% yield by reacting galactaric acid with

aqueous ammonia in neat conditions by heating on a steam bath.[135] The ammonium salt was unstable on heating and converts in low yield to pyrrole and other substances by dry distillation.

The ability of galactaric acid to form complexes with a large variety of metal ions have received particular attention.[136, 137] It is well known that simple sugars and their derivatives with oxygen, nitrogen, sulphur or phosphorous anchoring donor groups are easily involved in metal complexation. Composition and stability of these coordination compounds are mainly affected by their polyfunctionality, leading to different possible coordination sites with metal ions. For aldaric acid, the electron-withdrawing effect of the carboxyl group increases significantly the acidity of the α-hydroxyl group. Hence the α-hydroxycarboxylate moiety of aldaric acid can be an attractive ligand also in acidic and neutral solutions comparing with the less stable complexes formed by common sugar alcohols, especially with metals of weak Lewis acidity. Meanwhile, in alkaline solution, the good regioselectivity and stability of alcoholic hydroxyl remains valid for both complexes of sugar-alcohol and aldaric acid. So, as an aldaric acid with flexible chain, galactaric acid is an important biopolyhydroxydicarboxylic acid ligand of variable coordination mode. For this reason, its metal complexing property was explored in different fields, i.e. as separation agent of rare earths, as a surfactant, chelator or sequestrant.[138] Besides, rare earths and galactaric acid can also form metal-organic frameworks (MOFs) with porous structure, which have potential to be applied in sensory material, selective molecular storage, shape catalysis and luminescence.[93] Until now a series of lanthanides (Ln^{III})-based galactaric acid MOFs have been synthesized including terbium(III), europium(III) and terbium(III)−europium(III) mixed galactarates frameworks.[139, 140] N-Substituted diammonium salts were reported by reaction of galactaric acid with organic amines, and the reaction was found particularly useful for the preparation of the alkylenediammonium salts, useful intermediates for condensation to polyglutaramides.[44]

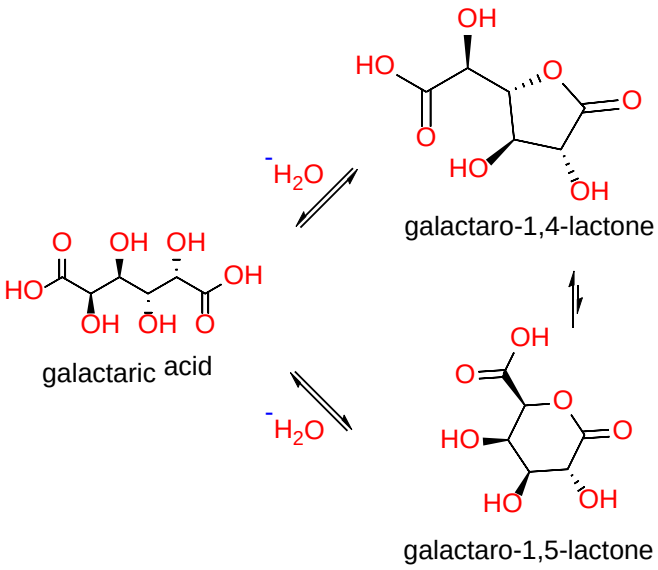
2.4.3.2 - Reactivity at the carboxyl group

2.4.3.2.1. Intramolecular dehydration to lactones

The intramolecular dehydration of galactaric acid to form the corresponding lactones was firstly reported by Taylor et al. in 1915,[141] and the equilibrium between galactaric acid and its lactone was investigated by chemical methods (mainly titrations) during the research of galactaric acid solubility in water. From the experiment, a 66.7% yield of one galactarolactone was obtained at 100 °C in 30 minutes, but purification from the mixture was unsuccesful.[141] Afterward, the kinetics of the equilibrium was further studied by Levene et al.,[142] which hypothesized the formation of two different types of galactaric acid lactone (5-membered ring γ-lactone and 6-membered ring δ-lactone). The lactones of galactaric acid were also synthesized directly from galacturonic acid by bromine oxidation, proving the optical activity of galactaric acid lactone. Based on the changes in optical rotation during

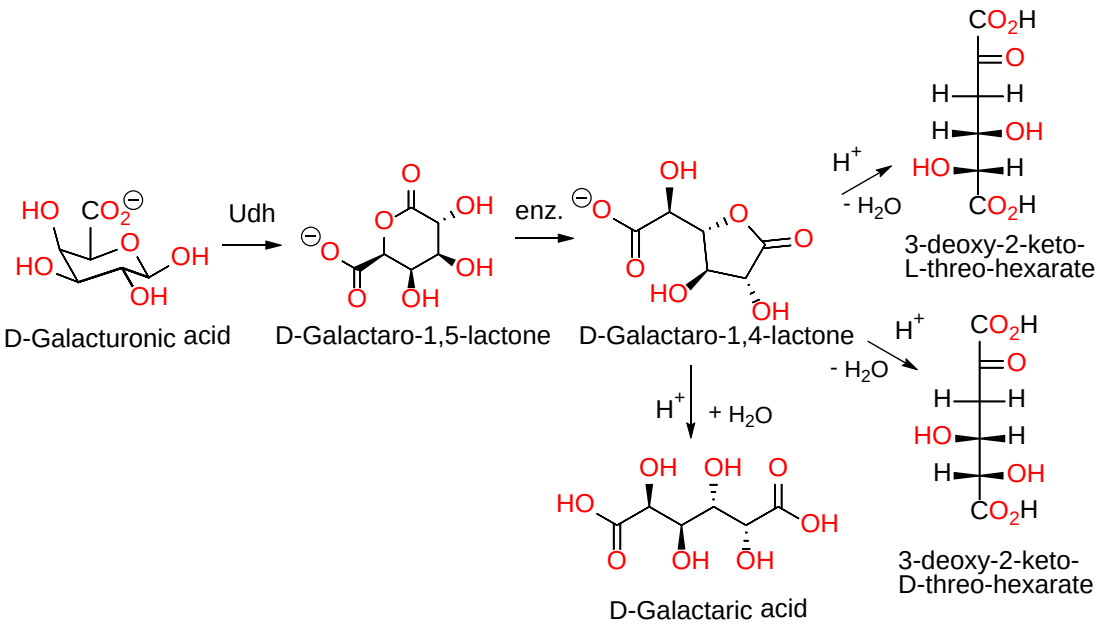
the reaction, a kinetic rough ratio 2:1 between δ-lactone and γ-lactone was deduced by titration, while the more stable product was the levorotatory γ-lactone.[134] The equilibrium between galactaric acid and its lactone derivatives is shown in Scheme 2.14. It must be remembered that the galactaro-1,4-lactone is now known to be the highly prevalent specie in well equilibrated systems, as expected from the general preference for γ-lactones over the δ-lactones. Therefore, the reported change in rotation power is due to side reaction and not to equilibration of different lactones. On the other hand, lactone rings due to their resonance structures, contains two sp^2 atoms (carbon C-1 and oxygen ring atom), therefore, their pertinent four ring atoms are placed in a plane and five-membered rings suit much better such planar arrangements than the six-membered rings do.

Attempt to prepare galactarolactones and other aldaric acids by gas sparging water removal are described in patent literature,[143] but industrial application were never reported.



Scheme 2.14- Intramolecular dehydration of galactaric acid to lactone derivatives.

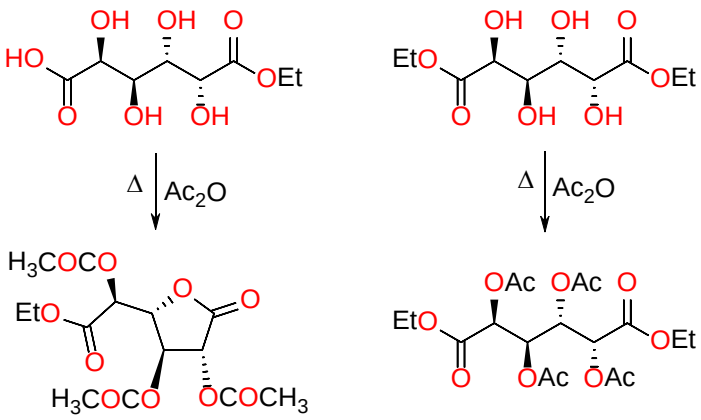
Substituted galactaro-1,4-lactones (i.e. the 3-O-ferulate derivative) were found in plants and the synthesis of their methyl and ethyl esters was reported.[69, 144] The galactaro-1,4-lactone is also involved in the catabolic pathway of D-galacturonate, a relevant component of hemicellulose polysaccharides. In this pathway, the D-galacturonate is first oxidized into D-galactaro-1,5-lactone by NAD^+-dependent galacturonate dehydrogenase (EC 1.1.1.203), and the δ-lactone is experiencing a isomerization reaction to γ-lactone by galactaro δ-lactone isomerase. The resulting D-galactaro-1,4-lactone is dehydrated to 3-deoxy-2-keto-hexarate isomers by a galactarate dehydratase or to galactaric acid by hydrolysis (Scheme 2.15).[145, 146] The 3-deoxy-2-keto-hexarate is a potential building block for heterocycles.



Scheme 2.15 - D-Galacturonate oxidative catabolic pathway in A. Tumefaciens.[146]

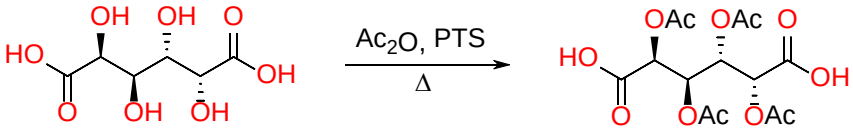
2.4.3.2.2. Ester derivatives

Esterification of galactaric acid was investigated extensively as a method to activate and protect this acid. The general conclusion was that quite specific condition must be carefully taken in order to reach high yield and selectivity owing to the presence of several competitive processes. So, Malaguti and Morgan prepared the ethyl hydrogen galactarate and diethyl galactarate by esterification of galactaric acid with alcohol containing sulfuric acid or 1% of hydrochloric acid at 100 °C giving yield of 42% and 58%, respectively.[147, 148] Dimethyl galactarate (m.p. 199-202°C) was prepared in 80% yield by treatment of a methanol solution of the acid with an excess of methyl iodide and sodium carbonate at 25°C for 30 min.[149] More recently it was synthetized on larger scale in similar yield using sulfuric acid as catalyst.[150, 151] Galactaric acid dibutyl ester was also synthesized by esterification of galactaric acid in refluxing 1-butanol and HCl mixture.[50] Monoethyl galactarate can be obtained by partial hydrolysis of the diethyl ester with potassium hydroxide in yield of 84%.[152] Moreover, acetylation of both diethyl and monoethyl galactarate were made by reaction with acetic anhydride and sodium acetate at 100 °C to form diethyl tetra-*O*-acetyl-galactarate and tri-*O*-acetyl-galactaro-1,4-lactone but in yields lower than 10% (Scheme 2.16).[148]



Scheme 2.16 - Synthesis of diethyl tetra-O-acetyl-galactarate and ethyl tri-O-acetyl-galactaro-1,4-lactone.

Galactaric acid itself can be tetra-*O*-acetylated in acetic anhydride and acetic acid with catalytic amount of *p*-toluenesulfonic acid (PTS) at 85 °C with a reported yield of 29% (Scheme 2.17).[153]



Scheme 2.17 - Synthesis of tetra-O-acetyl-galactaric acid.[153]

Thanks to the biodegradability of galactaric acid, the ester derivatives of galactaric acid have drew industry attention in recent times. Petit et al.[154] patented a process to produce biodegradable detergents (Figure 2.16), constituted by a central molecule of galactaric acid with functional substituents R_1, R_2 or R_3 hydrogen, alkenyl, alkyl or charged groups.

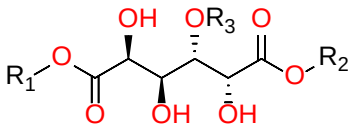


Figure 2.16 – General formula of alkyl galactarates proposed as surfactant agents.[154]

Galactaric acid derivatives esterified with acyl chain and long hydrophilic chains (e.g. polyethyleneglycol) are also of interest for pharmaceutical industry. These compounds have in fact amphiphilic and nanoscale self-assembled properties capable to organise in water micelles with hydrophobic core and hydrophilic shell at low critical concentration useful for delivering drugs in

biomedical application. As an example, the alkylation of the hydroxyl groups of galactaric acid affords multi-branched bio-compatible compounds suitable for the synthesis of the so called "scorpion like macromolecules" (Figure 2.17) and "star-like macromolecules".[155] These compounds have been recently proposed as parenteral drugs carriers and liposomal delivery systems for targeted therapy to counteract macrophage-engendered atherosclerosis mechanisms and to inhibit the inflammation of macrophages.[156, 157]

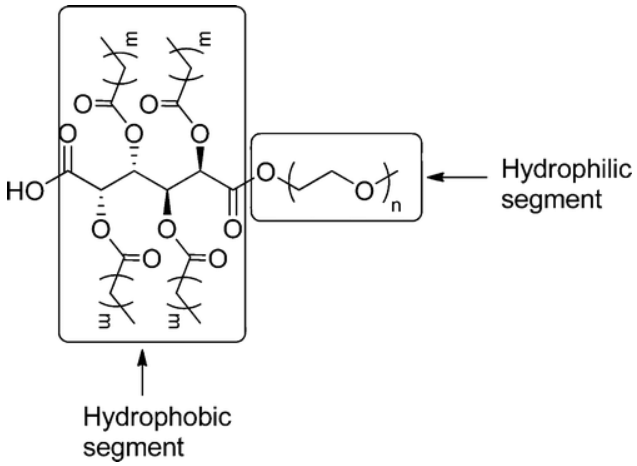
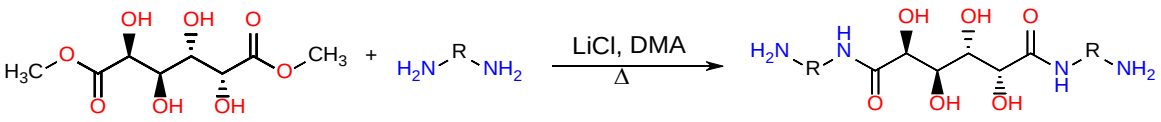


Figure 2.17 - Structure of amphiphilic polymers based on galactaric acid.[156]

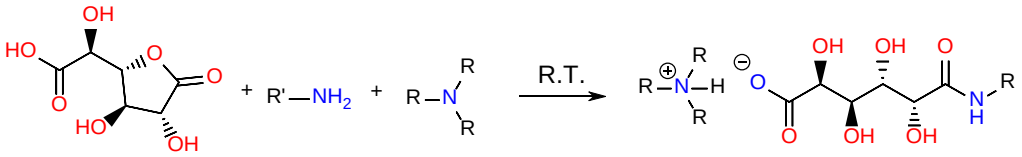
2.4.3.2.3. Amidation

Galactaramide was reported to be formed from ethyl galactaro-1,4-lactone or diethyl galactarate by treatment with concentrated ammonia.[148] N-Substituted mono galactaramides were obtained by reaction of the crude galactaro-1,4-lactone with amines for 12-24 hr at r.t. and filtration of the resulting solid.[158] More recently, a patent discloses the synthesis of diverse difunctional galactaramides from dimethyl galactarate under various reaction conditions (Scheme 2.18).[159] These compounds are useful monomers for crosslinked functional polymers.



Scheme 2.18 - Synthesis of galactarodiamides from the dimethyl galactarate and representative diamines.[159]

Amide salt of galactaric acid were also described by direct condensation of crude galactaro-1,4-lactone with a mixture of tertiary amine and primary amine (Scheme 2.19).[151]

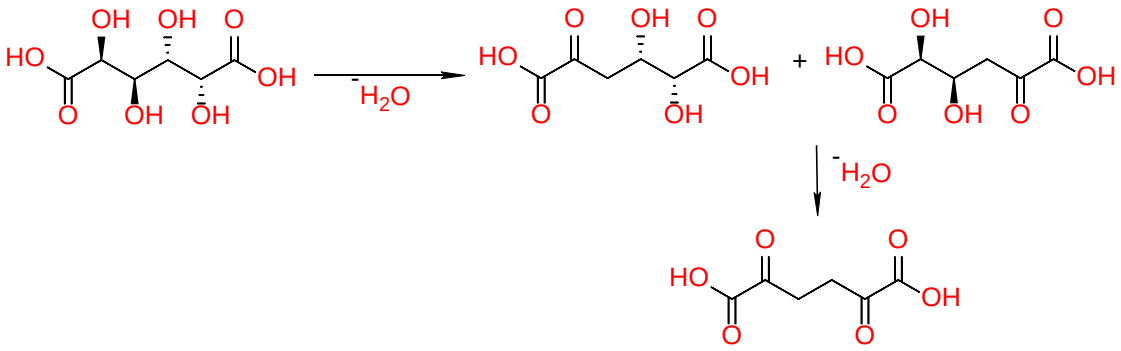


Scheme 2.19 - Synthesis of ammonium galactaramide salts from galactaro-1,4-lactone and mixture of tertiary amine and primary amines.[151]

2.4.3.3 - Reactions involving the hydroxyl groups

2.4.3.3.1. Dehydration

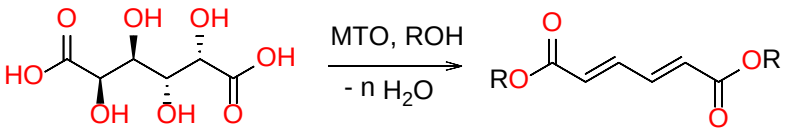
The loss of water from aldaric acid involving the OH substituents but not the acid group is a complex process with relevant potential for accessing unsaturated derivatives precursors of valuable products and materials. Water loss can be obtained thermally or under catalysis by protonic acids as well as by Lewis acids, but the process proved to be not easy to control owing to the intra- and inter-molecular reactivity of the two functional groups COOH and OH. A large variety of compounds was identified in these thermal and catalytic dehydration reactions, with distribution strongly dependent from the presence of co-reagents, as reported in subsequent paragraph. In living organisms, the enzymes sugar acid dehydratase control the process that splits off a water molecule from a sugar acid to generate a 2-keto-3-deoxy derivative. More common in fungi and other eukaryotic cells, these enzymes are relevant part of the catabolism of monocarboxylic sugars acid. Limited examples are also known for dicarboxylic acids. For example, selective mono- and di-dehydration of galactaric acid was identified in the catabolism of D-galacturonate and D-galactarate by a variety of bacteria (i.e. using *Agrobacterium tumefaciens* cell-free extract) to give isomeric 2-keto-3-deoxy derivatives (from the loss of a first water molecule) and 2,5-diketoadipate (from elimination of two water molecules) (Scheme 2.20).[160] The 2,5-diketoadipate is a useful intermediate for further synthesis of pyrone derivatives and adipic acid.[161] The exploration of this type of chemistry will represent a significant part of this thesis.



Scheme 2.20 - Enzymatic dehydration route of galactaric acid to 2,5-diketoadipate.

2.4.3.3.2. Deoxydehydration and hydrogenation

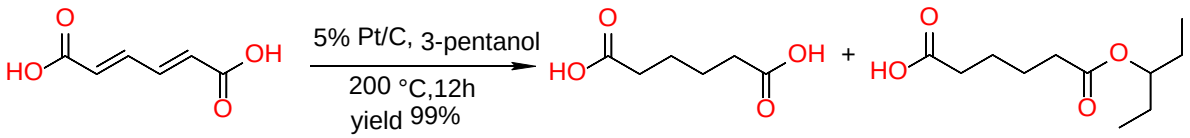
Deoxygenation of oxygen-rich saccharides to the corresponding hydrocarbons are of interest to provide alternatives to current petroleum-based commercial chemicals. The search of efficient deoxygenation methods resulted in growing interest in the catalytic deoxydehydration reaction to remove two adjacent hydroxyl group from vicinal diol to afford alkenes. Application of these methods to aldaric acid are scantly, but in 2012 appeared a report in which methyltrioxorhenium (MTO) was found to promote a deoxydehydration process of galactaric acid to muconic acid esters using a sacrificial alcohol.[50] The process is dependent from Brønsted acids[50, 162] and was broadly applied to other sugars,[163] and polyols. Mechanistic studies and the possible use of other metal catalyst were recently revised to better define the potentiality of the method.[164] Scheme 2.21 summarizes the transformation.



Scheme 2.21 - Deoxydehydration and esterification reaction of galactaric acid to alkyl galactarate.

Muconic acid esters can be easily converted to adipic acid,[165] a relevant dicarboxylic acid of 2.5 billion kilograms of annual production as a precursor for the production of nylon. Recently some different routes starting from galactaric acid to produce adipic acid derivatives, has been published. First, Shiramizu and Toste reported the conversion of galactaric acid into adipic acid ester, by using a deoxydehydration and hydrogenation reaction, with yields of 62%.[162] Then, the adipic acid was obtained from the dehydrated muconic acid by transfer hydrogenation with Pt/C catalyst in high yield of 99% (Scheme 2.22). These two steps can be realized in both independent or condensed process.[166]

This synthesis proved that galactaric acid has the potential to be a bio-alternative resource to prepare renewable adipic acid, which nowadays originates entirely from petro-chemical resources.



Scheme 2.22 - Transfer-hydrogenation of trans,trans-muconic acid to adipic acid and its ester.

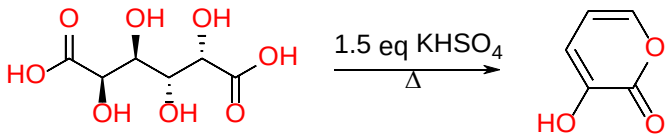
2.4.3.3.3. Hydroxyl protection and derivatization

Protection of secondary hydroxyl groups in aldaric acids is necessary to avoid branching or cross-linking reactions when used in polymerization processes or for selective functionalization. O-methylation was the protecting method mostly used because it does not affect the sugar configuration and provides an ether group that is stable during the functionalization or polycondensation reaction as well as during product handling.[167] However, methyl groups are not easily to remove selectively, so other protection approaches were used with benzyl and similar groups to be removed by hydrogenolysis. Also acetalization was used to protect vicinal OH groups of galactaric acid derivatives with carbonyl compounds with good success.[168] More recently, biacetalized galactaric acid derivatives (i.e. 2,3:4,5-di-O-methylene-galactarate and 2,3:4,5-di-O-isopropylidene-galactarate) were found appropriate to induce crystallinity in the derived polyesters and polyamides.[169] The Netherland based Cosun Biobased Products has very recently developed a >100 kg scale process to produce 2,3:4,5-di-O-isopropylidene-galactarate.[170] Similarly, protection with benzyl chloride and acetone was found useful in the preparation of 2,5-dibenzyl-3,4-O-isopropylidene-galactarate as precursor for protected polygalactaramides.[71] Moreover, tert-butyldimethylsilylation of OH groups was proved useful in the analysis of galactaric acid and its derivatives.[171]

2.4.3.3.4. Heterocycles derived from galactaric acid

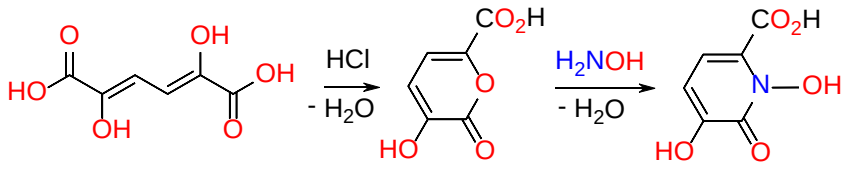
Some sporadic reports suggest that galactaric acid can be an interesting source of heterocycles. The high functionalities of this acid and the relatively easy dehydration can provide an easy access to conjugated unsaturated compounds, which can incorporate heteroatoms to provide heterocycle molecules. Representative of this reactivity is the synthesis of α-Pyrone, a six membered heterocycle diene useful for the synthesis of aromatics via Diels–Alder reaction and as signaling molecules in bacterial communication. The first report on the conversion of galactaric acid to 3-hydroxy-2-pyrone was released more than 100 year ago and was obtained by heating the acid with potassium bisulfate at

350 °C and distillation at 100-170°C for 3.5 hours (Scheme 2.23). A modest overall yield of 20-40% was obtained by ether extraction and crystallization.[172, 173] Similar low yield of 4-acetoxy-6-ethoxycarbonyl-α-pyrone was reported by heating at 100 °C for 90 min the synthetically prepared ethyl galactaro-1,4-lactone not derived from natural precursor.[174]



Scheme 2.23 - Synthesis of 3-hydroxy-2-pyrone by thermal decomposition of galactaric acid.[172]

An indirect evidence of the possible involvement of dehydrated forms of the galactaric acid in this thermal process can be found in a 2009 seminal paper of Wiedemann and co-workers.[174] In the attempt to clarify contradictory results from previous literature, these authors developed a low yield chemical synthesis of 2,5-dihydroxymuconic acid and demonstrated its conversion to 2-hydroxypyrone-5-carboxylic acid and corresponding pyridone derivatives (Scheme 2.24). In this thesis we will prove that 2-hydroxypyrone-5-carboxylic acid can be easily obtained from galactaric acid derivatives and is the precursor of 2-hydroxypyrone by decarboxylation.



Scheme 2.24 - Synthesis of pyrone and pyridone derivatives from 2,5-dihydroxymuconic acid.

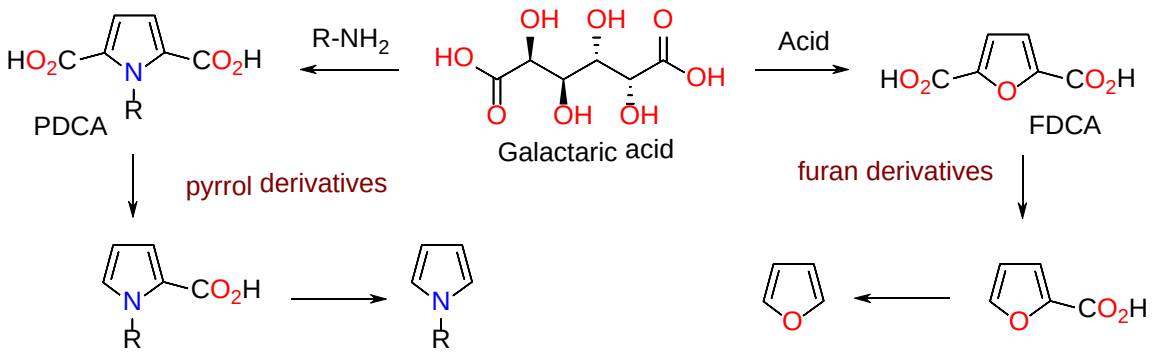
Aldaric acids have long history as intermediates to produce 2,5-furandicarboxylic acid (FDCA), a promising building block among the sugars-derivatives. This compound in combination with a glycol co-monomer can provide by condensation polymerization poly(alkylenefuranoates) (PAF), bio-based alternatives for polyethylene terephthalate (PET).[175, 176] In 1876 Fitting and Heinzelman synthesized FDCA from galactaric acid using concentrated hydrobromic acid.[177] Later on, several other mineral acids were tested as dehydration catalysts (e.g. HCl, H_2SO_4, HNO_3)[178, 179] on a variety of substrates.[180-185] Representative conditions and yields are summarized in Table 2.7. All the reactions required severe conditions (highly concentrated acids in large molar excess, temperature higher than 120 °C, long time (more than 20 hours)) and yields were commonly below 50%, owing to several competitive reactions.[186] Only one paper mentions good yields (95%) from diethyl α,α'-dihydroxygalactarate.[187]

Table 2.7 – Representative conditions used in the FDCA synthesis from galactaric acid.

Catalyst	T (°C)	Time (h)	Yield (%)
HCl 36%	150	8	55[180]
HBr 50-60%	150	16-36	40-55[184]
HBr 48%	120	8-26	45-50[183]
Methanesulfonic acid (MSA)	120	24	64

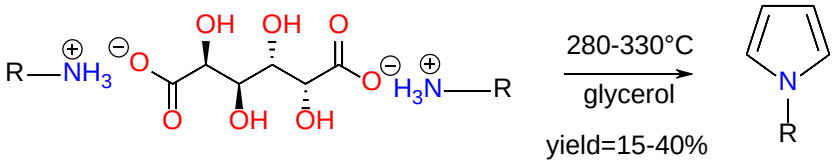
Some of the FDCA derivatives such as dibutyl 2,5-furandicarboxylate was prepared in nearly quantitative yields by reacting galactaric acid and 1-butanol using sulfuric acid, p-toluenesulfonic acid or heteropolyacids as catalysts.[186,188-190] The galactaric acid based synthesis of FDCA has a big potential but appears to be much less explored than other approach to this acid from less oxidized sugar derivatives.[191] The limited data available on this area have suggested part of the research reported in this thesis. In March 2016, Avantium and BASF created the joint venture Synvina to erect a 50,000 t/a plant for the production of FDCA (based on fructose) at BASF's Verbund-site in Antwerp, Belgium.

Other five membered heteroaromatics, i.e. pyrroles and thiophenes, are known to be accessed in low yield via galactaric acid, through a dehydrated intermediate in analogy to furan-2,5-dicarboxylic acid. Scheme 2.25 summarizes the sequence of steps in the furan and pyrrole series.



Scheme 2.25 - Synthesis of pyrrole and furan derivatives from galactaric acid via pyrroldicarboxylic acid (PDCA) and furandicarboxylic acid (FDCA)

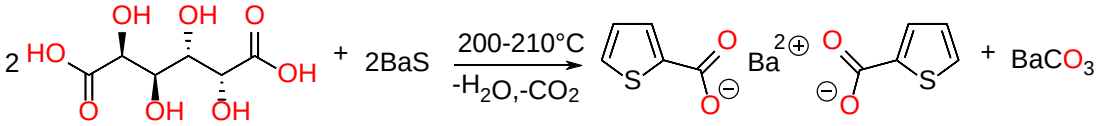
Moderate yield (15-40%) were reported of pyrrole (R = H) and N-alkyl pyrroles (R = alkyl) by heating the corresponding diammonium galactarate in presence of glycerol (Scheme 2.26).[192] Despite the limited yield, the reaction was proved useful to prepare ^{13}C-labeled pyrrole.[192]



Scheme 2.26 - Syntheses of N-substituted pyrroles from substituted diammonium galactarates.

Similarly heating of galactaric acid and dibutylamine afford moderate yield of N-n-butyl-pyrrole-2-n-butylcarboxamide.[193]

Thiophene and 2-thiophenecarboxylic acid were formed by heating galactaric acid together with barium sulphide. The method was used in analysis to trace the sulfur element.[194] The proposed stoichiometry is shown in Scheme 2.27.



Scheme 2.27- Synthesis of 2-thiophene carboxylic acid from galactaric acid.

Despite the interest for pyrrole and thiophene derivatives is remarkable in several fields, i.e. pharmaceuticals, agrochemicals, dyes, polymers and other organic compounds (including tolmetin, pyrrolidine, and polypyrrole derivatives),[195, 196] this approach to the synthesis of these heterocycles was never used on industrial scale for the intrinsic low atom economy (AE < 0.5) and the complexity of downstream processing.

2.4.3.3.5. Polymerization

Yet, the biggest potential of galactaric acid is still in the field of polymer technology. Since aldaric acids containing two terminal carboxylic acid functionalities, they can be widely applied in condensation polymerization to prepare polyamides and polyesters. With appropriate substituents, these polymers can in fact have full biodegradability and biocompatibility, along with huge abundance and low cost of sugar precursors. Therefore, several polymers derived from galactaric acid (i.e. polyamides, polyesters and polyanhydrides etc,) have been synthesized and have attracted increasing attention from both academia and industry as promising alternative to petroleum-based plastics to solve environment concerns. The first syntheses dated back to 1958 to the work Butler and al..[197] Lavilla et al. studied the synthesis of linear polycyclic polyesters with MW 35000-45000 by reacting alkanediols

(6 to 12 carbons) with 2,3:4,5-di-O-methylenegalactarate.[198] It was observed that the obtained polyesters showed a higher T_g and rigidity, with a lower ductility if compared to other poly(adipates), in consequence of the introduced stiff bicyclic groups of 2,3:4,5-di-O-methylenegalactarate. However, the polymer showed enhanced biodegradability following the method of stress-strain essay, lipase incubation and SEM analysis.[199] The same author studied copolymerization in melt between 2,3:4,5-di-O-methylenegalactarate and dimethyl terephthalate in presence of either 1,6-hexanediol, 1,12-dodecanediol, 1,4 butanediol or 2,3:4,5-di-O-methylene-galactitol to produce copolyester. The effects of galactaric acid content in the final co-polyester, considering physical/mechanical properties and biodegradability were evaluated, concluding that the substitution induces better solubility in halogenated solvents and better thermal stability.[199-201]

The interest for poly(galactaramide) materials was even higher than poly(galactarate) in relation to the solubility behaviour, biological activity and biodegradability found for the general family of polyhydroxypolyamides. The first compound of this series was synthesized through melt and solution polycondensation of diethyl glacatarate and alkelenediamines in 1976 by Ogata and coworkers,[202] The product was found to be a linear polymer decomposing at 200°C. Moreover, under solid-phase conditions, higher molecular weight polyamide was formed. Later, the same group studied also the co-polycondensation reactions between galactaric acid diethylester, active diester and hexamethylenediamine under different solvent condition to obtain copolymer composition.[23] More recently, a comparison between several polyhydroxypolyamides (D-glucaric, meso-xylaric, meso-galactaric, and D-mannaric) was published, to have a critical assessment of different polymer properties (e.g. melting point, water solubility, etc.) useful for choosing the best practical applications. Among the polyamides tested, galactaramides owned higher melting points and lower water solubility due to its extended zigzag conformation of monomer unit.[203] In order to obtain high molecular weight, a procedure to produce poly-galactaramides was developed in 2004 by using stoichiometric molar ratio of starting material as prepolymer.[204] The glass transition temperature (T_g) of galactaric acid based polyamide was further increased by using aldehyde or acetone-protected galactaric acid derivatives as building blocks, whose bicyclic structure improves stiffness of the polymer backbone.[169] Concerning the high hydrophilicity of aldaric acid based polymer, galactaric acid derivatives have also been used to produce novel carbohydrate-segmented silicone polyamides. The hydrophilicity modified polydimethylsiloxanes showed strong microphase separation, which can be useful in applications as cosmetic additives or as textile finishing agents.[205, 206]

Apart from the normal polymers introduced above, galactaric acid based copolyanhydrides has also been synthesized for clinical application. The galactaric acid derived monomer was prepared by O-acetylation of the secondary hydroxyl groups and subsequent condensation to diacetyl mixed anhydride by acetic anhydride. This monomer was then copolymerized with acetylated galactaric acid monomer and adipic acid at 150°C.[153] Furthermore, polyurethanes with galactaric acid derived monomers

containing protected and free hydroxyl groups have been prepared by polycondensation with α,ω-diamines (2-10, 12 carbon).[207]

2.4.4 - Known Uses of Galactaric Acid and its Potential as Platform Molecule

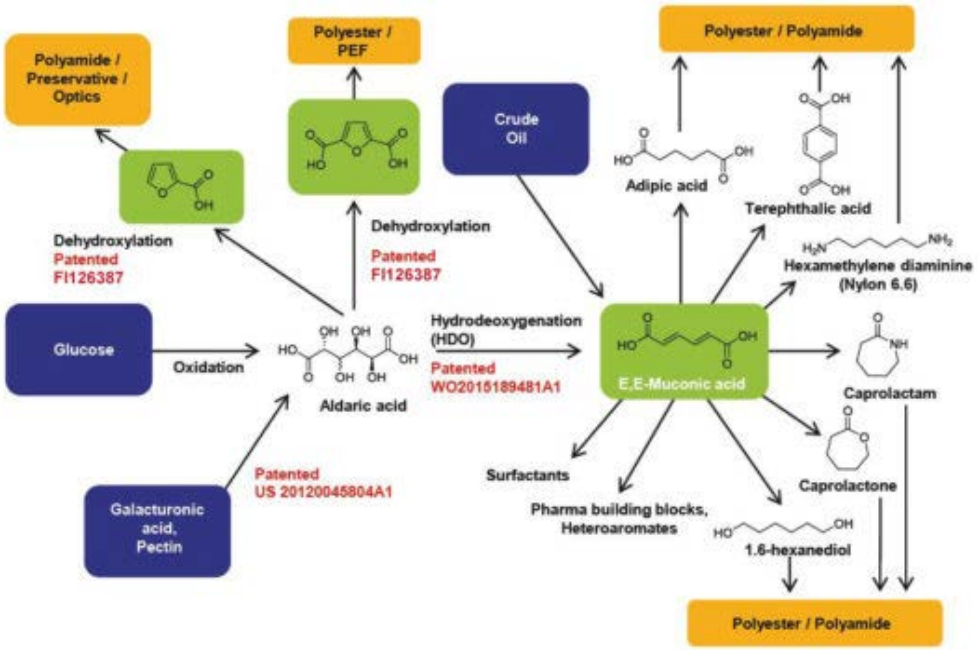
The uses of galactaric acid are similar to the ones of glucaric acid and mainly restricted to specialty products.[208] However, a number of interesting chemical properties that, together with its peculiar biocompatibility, makes this acid suitable for very different practical applications, such as additive for metal complexation, for skin care and medical products, as well as precursor for the synthesis of polymers and materials. Its market is at present mainly limited to the pharmaceutical, cosmetic and additive industries. Examples of use as drug component are the isometheptene mucate, a known as powerful central analgesic, and L(-)carnitine and alkanoyl L(-)carnitine mucate.[209] Galactaric acid has found uses in food technology to replace tartaric acid in self-rising flour or fizzies and to replace potassium bitartrate in baking powder. Typical practice can also be found in the manufacture of granular effervescing salts. The relevant complexing ability of galactaric acid toward metal ions has found extensive applications, and some specific metallic complexes of galactaric acid have their own market, e.g. in the production of stable electrodes for Ni-H secondary batteries[210] or in enhancing the properties of hydraulic cements.[211] As a reducing agent, galactaric acid is used in formulation of a hard-surface detergent to remove metallic stains,[212] and as corrosion scavenger for cooling water systems.[213] Metal chelation is also used in formulations for environmental and medical applications. Galactaric acid has been used for decontamination of heavy metal-polluted soils[214] and to treat the industrial ashes containing high amounts of hazardous metals (such as Cd, Pb, Cr, Zn and Cu) with the aims to reduce disposal cost of by-product incineration.[215] The metal galactarate complexes as reagents can also remove heavy metals (such as Al, Cu, Pb) from the body for intoxication treatment.[216] One of the first application in pharmaceutical usage is the spectrophotometric determination of bismuth(III) by the formation of galactaric acid complex.[217] Another advantage of galactaric acid as metal ion binders is the stability even in physiological pH range. For this reason, vanadium(IV,V) galactarate complexes were prepared for various physiological application.[218]

For the same metal-complexation capacity but for an opposite purpose, galactaric acid can also be used to enhance biocompatibility and skin permeation of specific metallic compounds, which can treat topical conditions such as acne, xerosis, age spots, and so on.[219]

Cosmetic companies use galactaric acid in the formulation of skin care products with anti-aging properties. This stays because galactaric acid sequestrates free metallic ions present as skin impurities, so inhibiting dangerous free-radicals chain processes involved in aging and other undesirable skin diseases.[220] For instance, the French company Soliance commercializes a galactaric acid based product, obtained from citrus pulp, disclosed as useful additive in the formulation of personal care products. It

acts as an antioxidant to protect skin from oxidative stress and prevents cosmetic products from bacteria proliferation by potently sequestering calcium ions.[221-223]

Unfortunately, the high price of galactaric acid has hampered until now its utilization for high volume consumer products, and restricts its use only to specialty chemicals. An improvement in the production techniques of this acid is strongly acclaimed, as it would unlock its full potential as a platform chemical in the market, and not only for the previously cited applications. Indeed, galactaric acid can be the precursor of many high value derivatives, such as adipic acid, furandicarboxylic acid, terephthalic acid, as indicated in section above, and novel synthetic procedures have been recently disclosed in this direction.



Scheme 2.28 – Potential pathways for the valorization of galactaric acid.

The need for bio-plastics is growing. Brand owners are looking for sustainable solutions for packaging, fibres, paints, inks and plastics. This creates a need for high-performance bio-plastics such as polyamides (PA) and polyesters (PET). The total global production of PET polymers was over 50 MT and that of PA over 10 MT in 2015. Furan dicarboxylic acid (FDCA) based polyethylene furanoate (PEF) polymers offer a bio-based alternative to petroleum-based PET polymers. Polyamides are used in applications calling for high durability and strength. At moment, the main constraints of these applications are the relatively high price of galactaric acid, even if some companies continue to develop processes to convert biochemically citrus processing wastes to galactaric acid. In addition, the step to convert aldaric acid into furancarboxylic acid (FCA) and FDCA need further improvement for a realistic industrial application. On the contrary, techno-economic analysis and life cycle analysis show

that pricing can be competitive and that carbon footprint can be lower than petroleum-based alternatives for both monomers. A summary of the main potential uses of galactaric acid, and in general C-6 aldaric acids, is shown in Scheme 2.28.

Finally, turning back to the established uses of galactaric acid, the global market size of this compound has systematically growth in the last years six years (Figure 2.18), with an estimated value of USD 110 million in 2016 on account of increasing demand from food ingredients, concrete additives, detergents, and pharma applications. Growing construction industry, cosmetic and polymer industry are expected to play a key role in propelling market demand. Important industry producing GA on modest scale are located in China (Shanghai Hanhong Chemical Co.), India, USA, Germany, Nederland, and France. The increase in polymers incorporating galactaric acid was mainly related to composites and co-polymers, whereas interest for furandicarboxylate polymers derived from galactaric acid is considered yet undeveloped.

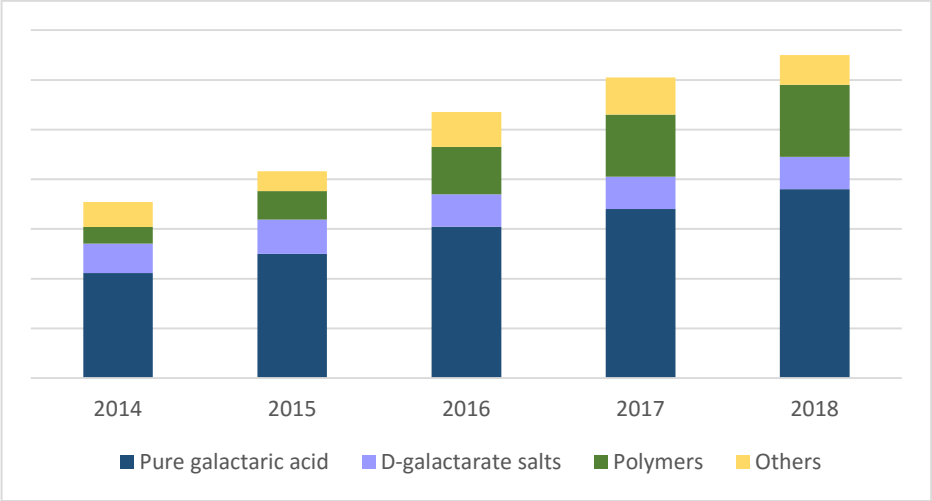


Figure 2.18 - Global market size of galactaric acid and its main derivatives.[208]

2.5 - Reference to Chapter 2

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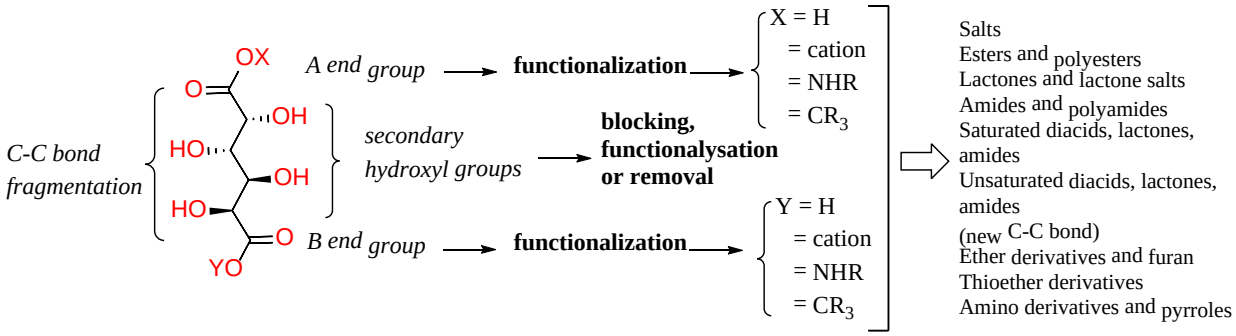
Chapter 3

3.1 - Introduction

The literature analysis on aldaric acid reported in chapter 2 was the starting root of the study undertaken in this thesis. A large part of the extensive data available on this class of sugar acids appears not deeply analysed resulting mainly from sporadic unoptimized investigations with limited focus on the chemistry involved and even less on potential applications and valorisation. The only exception can be found in the work of Kiely's group, which has provided relevant contributions on two aspects: a) the chemical synthesis of these compounds by oxidation of monosaccharides, and b) the preparation and characterisation of linear polymers originated from aldaric acid, in particular polyhydroxyesters and polyhydroxyamides. We decided to re-analyse some of the reported chemistry in order to identify deeply the basis of the chemical reactivity of this class of compounds. To simplify the analysis, we focused on one definite type of aldaric acid, the galacatric acid, owing to its peculiarity to be optically inactive and therefore able to reduce the number of diasteroisomers and epimers formed in the reactions, allowing, on the contrary, an easy control of the induced chirality in final unsymmetric products via rotatory power measurements.

The chemistry of aldaric acid is intrinsically complex owing to the presence of several functional groups. If we limit the analysis to C-6 aldaric acids, we can identify two main types of reactivity: one related to the terminal carboxyl groups and the other related to the internal hydroxyl groups (Scheme 3.1).

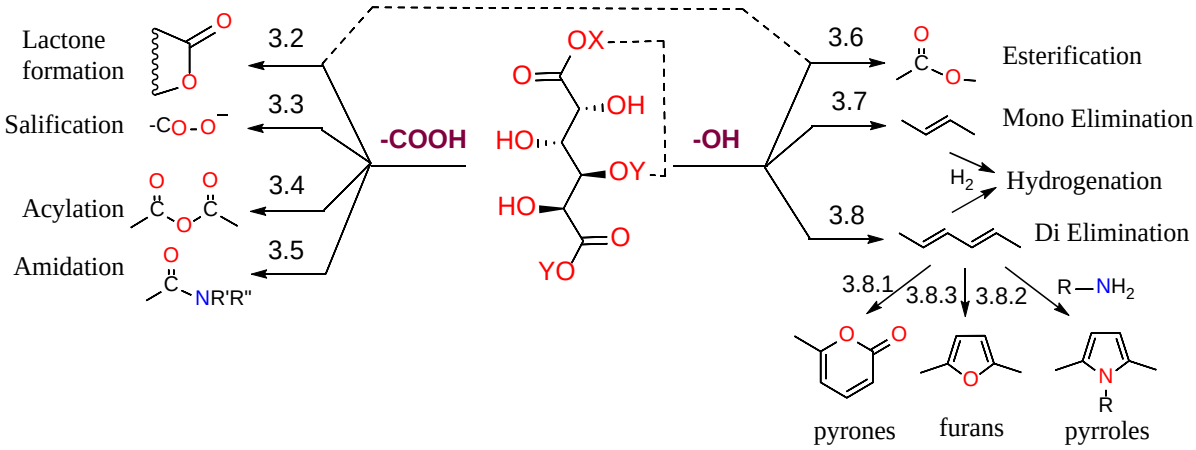


Scheme 3.1 – Overview of the reactivity of aldaric acids

Modification of the carbon backbone can also occur via fragmentation of C-C bond and can be expected mainly by carbon dioxide loss from carboxylic groups or by retro aldol reactions of the polyhydroxy fragment. For reason of time, this part of the reactivity was not investigated specifically, even if carbon dioxide elimination was seldom observed during the research activity, mainly in connection with the

intermediate formation of alpha-carbonyl derivatives or from partially aromatized diunsaturated derivatives.

The results of the research activity will be reported as classes of reaction following the general framework indicated in Scheme 3.2.



Scheme 3.2 – Types of reactions of aldaric acid investigated in the thesis.

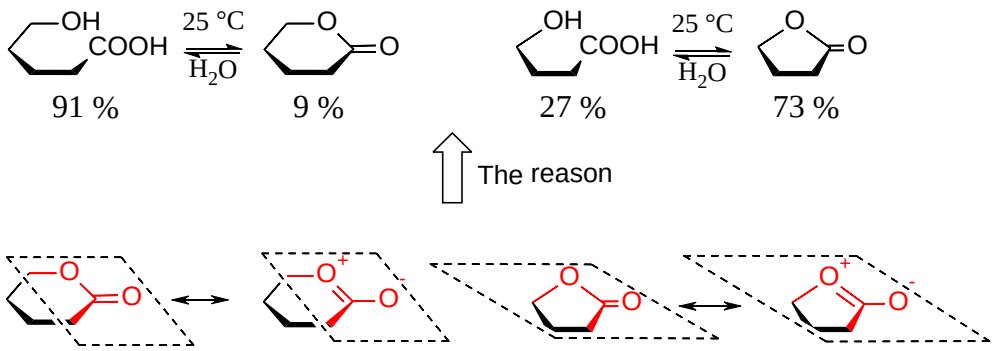
The numbering in Scheme 3.2 refers to the paragraphs of chapter 3, where the type of reaction is discussed and related results are reported.

3.2 - Lactonization of Galactaric Acid

Carbohydrate lactone derivatives have been recognized as relevant building blocks derived from biomass, whose reactivity was explored mainly in the biosynthesis of sugars and for the synthesis of important bioactive compounds and natural products. Sugar acids form easily lactones by intramolecular esterification of the corresponding hydroxyl and carboxyl groups, which can take place more easily when the formed ring is five- or six-membered. These classes of compounds offer interesting properties of both protection and activation of the two functional groups. Moreover, allying the inherent chirality of the sugar to the reactivity of the lactone functionality, they constitute useful chemical intermediates towards a variety of purposes and valuable synthons for diverse types of transformations.[1-6] Not like hemiacetal derivatives of aldehydic sugars, which prefer the six member intramolecular cyclization, carbohydrate lactones preferentially exist in a five-membered cyclic form. This is related to the nearly planar arrangement of the five membered lactone compared with the six-

membered one, a stereochemical condition that stabilises the interaction of the two sp^2 atoms (carbon C-1 and oxygen ring atom) via a π-system, as suggested in Scheme 3.3.

Investigations concerning aldaric acid lactones are generally less extensive than lactones of aldose and aldonic acids, but useful applications are just known, in part because they are biologically active and sometimes used as drugs (i.e. glucaro-1,4-lactone). Among epimers and diastereomers of C-6 aldaric acid, galacataric acid and its salts have an unusual low solubility in water (3.3 g/l the acid), while for instance D-mannaric and D-glucaric acid are very soluble in water (> 500 g/l). This, in combination with its high melting point, creates some difficulties in the applications of galactaric acid as a platform molecule, despite its apparently more simple chemistry, and is the main reason of the harsh reaction conditions (and moderate to low selectivity) frequently reported in literature of this acid.



Scheme 3.3 – Preferential planar arrangements of five-membered lactone than the six-membered.

Based on the study of Jeffrey et al., the low solubility of galactaric acid in water is mainly due to its crystal structure characterized by strong intermolecular hydrogen-bonding, as reported in Figure 2.14 of Chapter 2.[7] This suggests that elimination of some polar groups, as for the intramolecular esterification, would increase the solubility in various media. As already reported in chapter 2, aqueous D-glucaric acid, D-mannaric acid, and D-galactaric can form mixtures of mono-lactones and, by further dehydration, the first two, but not the third, afford also the corresponding di-lactone. At present, these last compounds are commercially available in pure form (and are stable enough in water solution),[8] whereas the corresponding mono-lactones are commercialised as the equilibrium mixture. This last route cannot access galactarolactone, unless as minor components, owing to the extreme insolubility of the galactaric acid in water. In fact, based on the literature data (summarized in chapter 2), it is clear that equilibrium mixture in water contains only limited amount of this compounds, which however was found to increase at higher concentrations under reflux condition. However, the purification procedure proved to be quite expensive and never reach a purity higher than 70%.[9] Also the alternative methods of lactone preparation starting from cyclic galacturonic acid involving the oxidation by bromine or

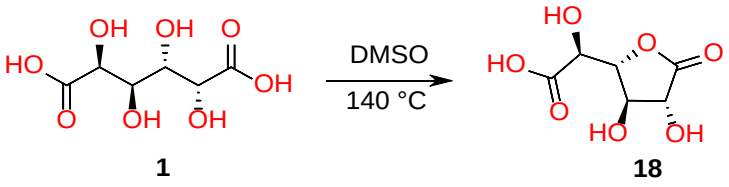
oxygen (including biological processes) work in water buffered medium and is useful only in small scale.[10]

On the other hand, comparing galactarolactone with its diastereomers and epimers, the symmetrical nature of the starting acid results in only one six membered and one five membered lactone derivative, with the latter largely prevalent in water and hopefully also in other solvents. This will avoid the expensive purification methods of chromatography or re-crystallization.

At the same time, it is known from the single-crystal x-ray crystal structure analysis that *meso*-galactaric acid adopts an extended (rather than bent or sickle) conformation in the crystalline state, free of destabilizing 1,3-parallel hydroxyl group eclipsing steric interactions, and that this persist also in solution.[7] Different from glucaric acid derivatives, the open form of any galactaric acid derivative must adopt a similar extended conformation, a peculiarity quite useful in polymers to prevent stiff behaviour.

All these considerations induced us to investigate better the synthesis of lactones of galactaric acid in aprotic media with the aim to develop a robust and affordable method to access this compound also at the industrial level and define its potential use as platform chemical.

Synthesis of Galactaro-1,4-lactone (18) in DMSO



1 **18**

Scheme 3.4 - Lactonization reaction of galactaric acid in DMSO.

Table 3.1 - Preparation of D-galactaro-1,4-lactone by thermal dehydration in DMSO.

Substrate (g)	DMSO (ml)	Conc. (%)	T (°C)	Time (h)	1 (Conv.%)	18 (Yield %)
0.04	0.5	8	140	4	100	98
0.16	0.5	32	140	1	100	99
0.80	0.5	160	100	3	88	87
6	20	30	140	0.33	100	100
7.5	25	30	140	1	95	95
1	1	100	140	0.75	99	97
6	4	150	140	7	87	85

50	100	50	140	0.33	100	99

Several experiments were carried out in different polar solvents (i.e. dimethyl sulfoxide, N,N-dimethylformmamide, N,N-dimethylacetamide, sulfolane, etc.) at 90-140°C. In all cases the galactarolactones were formed above 80°C and galactaro-1,4-lactone (Scheme 3.4) was always the main or exclusive product. Dimethyl sulfoxide was proved to be particularly effective in the reaction, reaching 100% conversion and nearly 100% yield of galactaro-1,4-lactone in 30 minutes at 140°C (Table 3.1).

Following a design of experiment approach, the reaction time was identified as the more critical factor, inducing at higher time the formation of dark by-products. The procedure was validated into a robust and well reproducible method (see Chapter 4).

The main reason of this preparative result is certainly related to the higher solubility of galactaric acid in DMSO, evaluated by us to be as high as 189 g/L at room temperature, far above the value of 3.3 g/L in water.[11] This increased solubility is mainly related to the effective competitive interaction of this polar solvent with monomeric galactaric acid, reducing the importance of intermolecular hydrogen bonding in its aggregated form. Moreover, this solvent appears to be completely miscible with the product galactaro-1,4-lactone, allowing to reach an optimised ratio between galactaric acid and DMSO as 50 g/100 mL for a full conversion in 20 minutes (Table 3.1).

The reaction mixture proved to be stable for month as evidenced by ^{1}H and ^{13}C-NMR analysis of the viscous solution, where the typical pattern of the four hydrogens of C-H bonds remains well resolved and does not decay in the presence of minor amount of water (Figure 3.1).

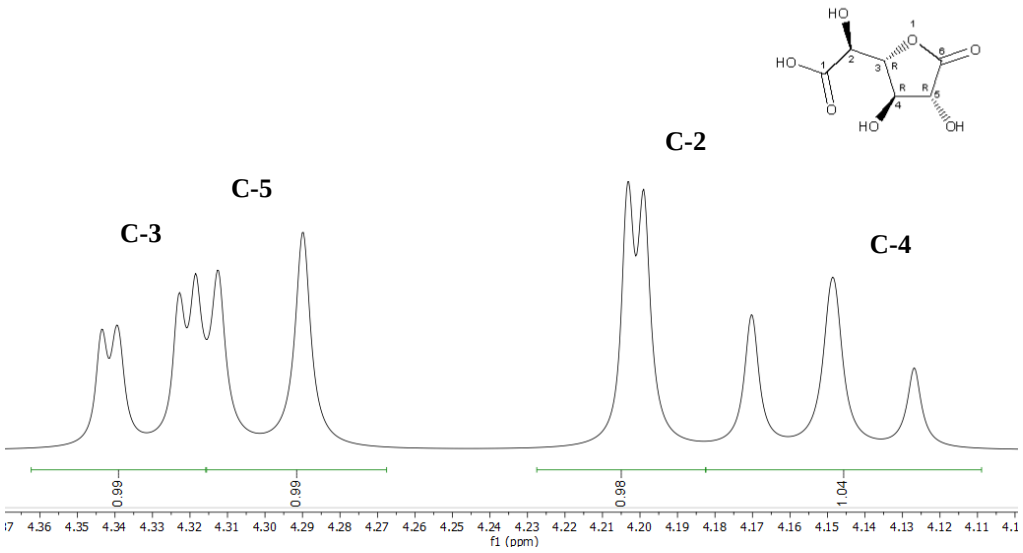


Figure 3.1 - Assigned ^{1}H-NMR Spectrum (DMSO-d6, 400 MHz, ppm) of galactaro-1,4-lactone.

The absence of other picks at high signal to noise ratio proved that isomeric 1,5-lactone must be lower than 1%. Due to the asymmetric structure of the galactaro-1,4-lactone, the ^{1}H-NMR spectra shows four signal for four non-equivalent protons at the C-H bond in the range 4.15-4.4 ppm. The lowest field signal at 4.33 ppm ($J_{2,3}$ = 1.7 Hz and $J_{3,4}$ = 8.3 Hz) is assigned to H-3 proton of C-H bond bearing the oxygen of the lactone ring on the basis of the general rule of proton assignment of carbohydrate δ-lactones.[12] The left three signals are assigned to H-2 (4.19 ppm), H-4 (4.15 ppm), H-5 (4.29 ppm), respectively, based on coupling constants. Similarly, ^{13}C-NMR spectra (Figure 3.2) shows two signals in the downfield region (172 -175 ppm) attributed to the non-equivalent carbonyl carbon (consistent with that of the ^{13}C acyclic diacid spectrum in D_2O),[13] meanwhile the four signals of the four saturated carbon are located in the range 67 – 83 ppm, which confirm the presence of one asymmetric structural form of product. The assigned ^{1}H and ^{13}C-NMR are consistent with literature report, taking into account the different D_2O solvent used.

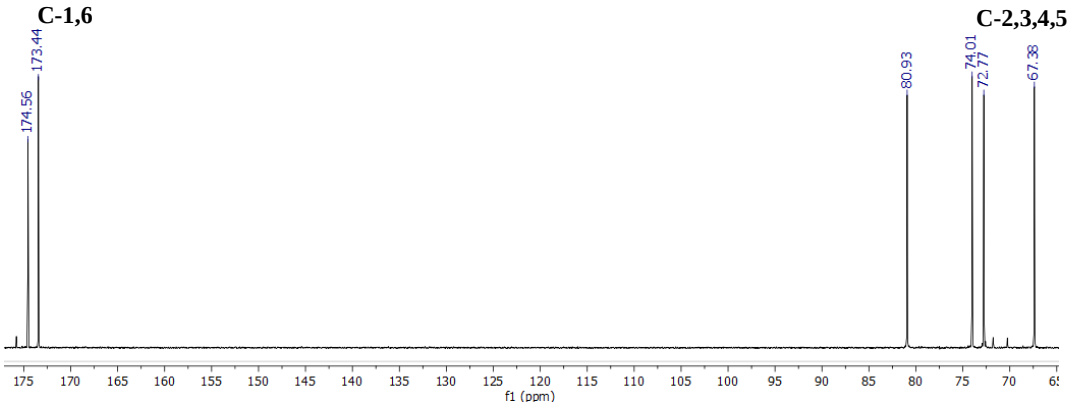


Figure 3.2 - Assigned ^{13}C-NMR Spectrum (DMSO-d6, 400 MHz, ppm) of galactaro-1,4-lactone.

To understand better the stability of the lactone toward water and the role of DMSO, we investigate the equilibration reaction between **1** and **18** in aqueous solution by ^{1}H-NMR spectroscopy. Lactone **18** in the crude DMSO solution was diluted (1:1-5) with deuterium oxide at 25°C and ^{1}H-NMR spectra were taken at defined time. The initial relative concentration of **18** was determined with respect to the signal of the internal standard 1,3,5-trioxane, added at the start and proved to be stable under the condition of the analysis. The plot against time of the integrated signal of galactaro-1,4-lactone protons is reported in Figure 3.3 and confirms that galactaro-1,4-lactone hydrolyses relatively slowly in this homogeneous solution, reaching a stable final ratio of 2% after eight hours. After 1.5 hours, only 29% of lactone was hydrolysed and, after 6.5 hours, 6% of lactone was still present in the D_2O solution.

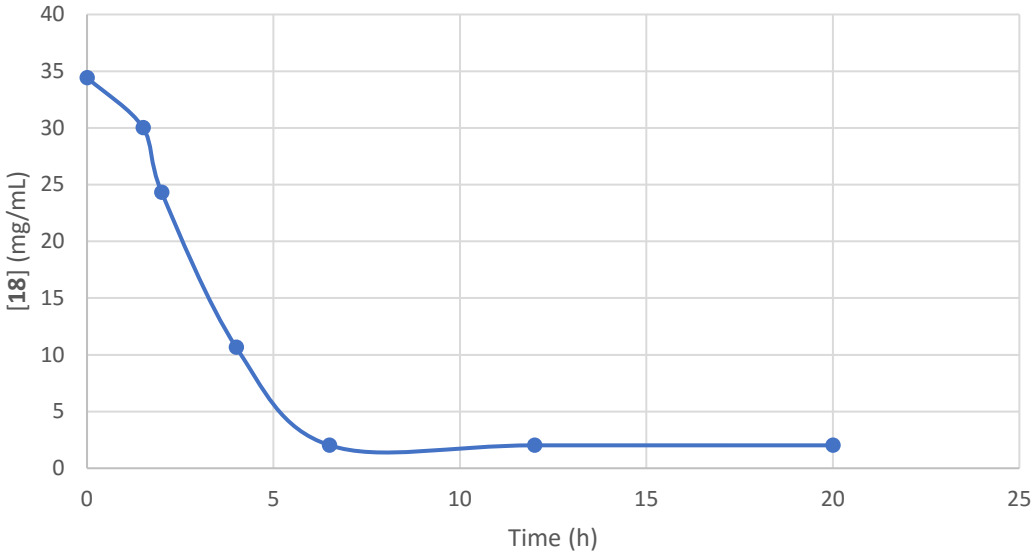


Figure 3.3 – Kinetic of the hydrolysis of galactaro-1,4-lactone to galactaric acid in DMSO/D_2O solution at 25°C.

In addition, mass spectra (negative ions and positive ions) of the obtained DMSO solution of **18** was consistent with the formation of the delta lactone (Figure 3.4). The molecular anion at m/z = 191 [M-H] is significant and the only relevant other signal corresponds to the dimeric anion at m/z = 383 [2M-H]. The importance of this last ion confirms the remarkable tendency of aldaric acid derivatives to aggregate forming dimers also in gas phase, when stabilising solvents are removed.

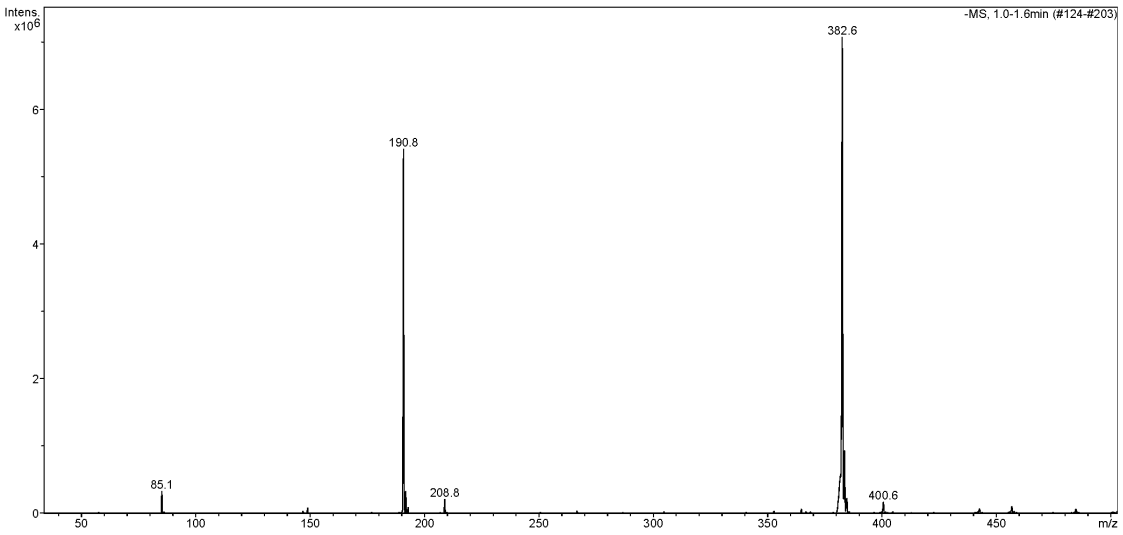


*Figure 3.4 – Negative ESI-MS spectra of galactaro-1,4-lactone (**18**) in methanol.*

3.3 - Salification Reaction of Galactaric acid (1) and Galactaro-1,4-lactone (18)

Formation of the salts of an organic acid is an ordinary process, which in general does not require specific analysis. However, in the case of aldaric acid the situation appears to be more complex, demanding specific attention and development of specific procedures. This can be appreciated easily from the fact that hemi-salts of galactaric acid are nearly unknown and no report exists in literature on isolated salt of any galactarolactone, even if in solution they were easily detected. The easy availability of the DMSO galactaro-1,4-lactone solution was assumed by us as a good opportunity to acquire more insight on this class of compounds, comparing its behaviour with the one of the galactaric acid itself. In fact, it was expected that under hydrolytic conditions the lactone could provide all possible salts of galactaric acid.

From previous studies in POLIMI laboratory, it was clear that alkali and alkali-earth disalt of galactaric acid can be obtained from water solution of the acid only by refluxing with more than stoichiometric amount of metal hydroxide at 100 °C. The salts were isolated by slow evaporation at 600 mmHg pressure of the resulting solution at 65 °C until crystallization occurs. Dissolution of the starting acid **1** was in any case slow and, with the stoichiometric amount of sodium hydroxide, it takes four hours. With less powerful bases (i.e. ammonia and sodium acetate), the salification process proved to be even more difficult. Despite several attempts, the isolation of mono-salt of galactaric acid was impossible both by using one equivalent of the base or by contacting the di-salt with starting galactaric acid. On the contrary, the salification of lactone **18** in DMSO solution was observed easy when it was heated with stoichiometric amount of sodium acetate or DABCO at 140 °C. The process appears to be not completely selective, as evident from the ^{1}H-NMR of Figure 3.5. Signals in the spectrum marked in green colour refer to the salt of the lactone, whereas the blue signals refers to one aromatic products, to be discussed in the next section.

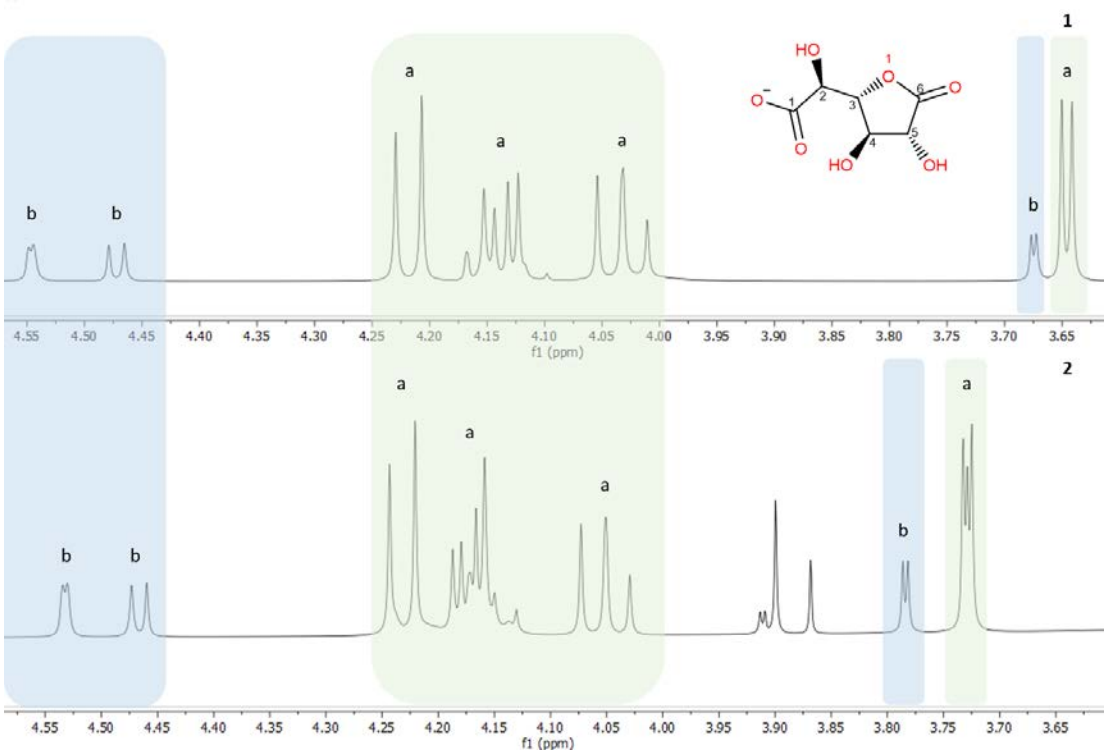
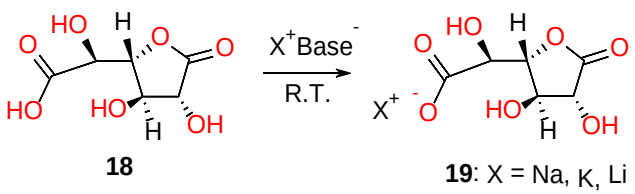


*Figure 3.5 - Assigned ^{1}H-NMR spectra (DMSO, 400 MHz) of **1**) of sodium salt of galactaro-1,4-lactone (**19a**) (sodium acetate for 8h); **2**) DABCO salt of galactaro-1,4-lactone (**20a**) (DABCO for 8h).*

The emerging aspect was the relative stability of the lactone salts at so high temperature. In order to avoid the formation of by-products, the reaction was exploited further at room temperature. By using diluted DMSO solution of **18** at 20-30 °C, the salification process occurs easily with different alkaline bases. By using concentrated DMSO solution of **18**, the reaction conditions depend mainly on the different solubility of the bases and their basicity as reported in Scheme 3.5. With sodium hydroxide and sodium bicarbonate, the reaction mixture need to be heated to higher than 90 °C in order to obtain a homogeneous solution, affording only moderate yield of lactone salt. Meanwhile, with more soluble sodium methoxide, potassium t-butoxide and n-butyllithium, nearly quantitative yield (> 90%) of the salt can be obtained. As any salification process, the reaction was exothermic and temperature need to be controlled when carried out on large scale.



*Scheme 3.5 - Preparation of galactaro-1,4-lactone inorganic salts (**19**).*

Table 3.2 - Salification reaction of D-galactaro-1,4-lactone by different alkaline bases.

Entry	[Subst.] (mmol)	Base	Solvent	[Base] /[sub]	T (°C)	t (h)	18 (Conv. %)	19 (Yield %)
JL152	2.6	CH_3ONa	DMSO	1.3	28	1	100	95
GICR08	1.56	$NaHCO_3$	DMSO	1	90	0.67	100	86
GICR04	9.8	NaOH	H_2O	1	100	14	100	60
JL154	2.6	^tBuOK	DMSO	1	20	2	100	96
GICR07	1.56	n-C_4H_9Li	DMSO	1.17	20	4	100	90

^{1}H and ^{13}C-NMR Characterization of Galactaro-1,4-lactone Salts.

By comparing the chemical shifts of lactone and its alkali metal salts (in Table 3.3), it is clearly to identify the signal moving from downfield (4.2 ppm) to high field (3.6 ppm) as the hydrogen H-2 of β position of carboxylic group. Furthermore, the four proton signals of lactone derivatives in D_2O were assigned in lower field than the one in DMSO-d_6, due to the fact that protons of multi-hydroxyl group are exchangeable with D_2O. This is also one of the common properties of carbohydrates derivatives.

Table 3.3- Chemical shifts of galactaro-1,4-lactone and related salts under different conditions.

Name	Compound	Solvent	H-2 or H-5 (ppm, *Hz*)	H-3 or H-4 (ppm, m, Hz)	H-3 or H-4 (ppm, m, *Hz*)	H-2 or H-5 (ppm,m, Hz)
18	Lactone	DMSO	4.30 d *J=9.2*	4.33 dd *J=1.7, J=8.3*	4.15 dd *J=8.7*	4.20 d *J=1.7*
19a	Na salt	DMSO	4.21 d *J= 8.9*	4.09 dd *J=4.0, J= 8.3*	4.01 dd *J= 8.8*	3.61 d *J= 4.0*
19b	K salt	DMSO	4.22 d *J= 9.2*	4.12 dd *J=3.7, J= 8.3*	4.03 dd *J=8.8*	3.69 d *J= 3.8*
19c	Li salt	D_2O	4.40 d *J= 8.6*	4.59 dd *J=2.1,J= 8.4*	4.63 dd *J=9.2*	4.23 d *J= 2.0*
19a	Na salt	D_2O	4.63 d *J= 9.2*	4.59 dd *J= 2.0, J=8.4*	4.40 dd *J= 8.4,J=9.2*	4.24 d *J=2.0*
Ref. [14]	Lactone	D_2O	4.65 (H2) *J=2.1*	4.61(H4) *J=9.2*	4.41(H3) *J=2.1*	4.24 (H5) *J=9.2*

All isolated alkaline salts of Galactaro-1,4-lactone display in FT-IR spectra the characteristic absorption band around 1780 cm^{-1} typical of 5-membered lactone carbonyl group. Figure 3.6 reports the FT-IR spectra of the sodium salt **19a**. The other main IR bands were assigned based on related alkaline galactarate salts. The intense band at 1617 cm^{-1} is attributed to carboxylate anion, whereas the

complex pattern of bands between 2000 and 2600 cm^{-1} is related to the inter-and intramolecular hydrogen bonds of the hydroxyl groups and residual water. The pattern was more complex than the one observed in the FT-IR spectra of the disodium salt of galactaric acid, whose carboxylate group absorbs at 1607 cm^{-1}, slightly lower than what observed in the lactone salt **19a**. Comparison of the two sets of bands seems to indicate an intermolecular H-bonding network for the structure of these lactone salts.

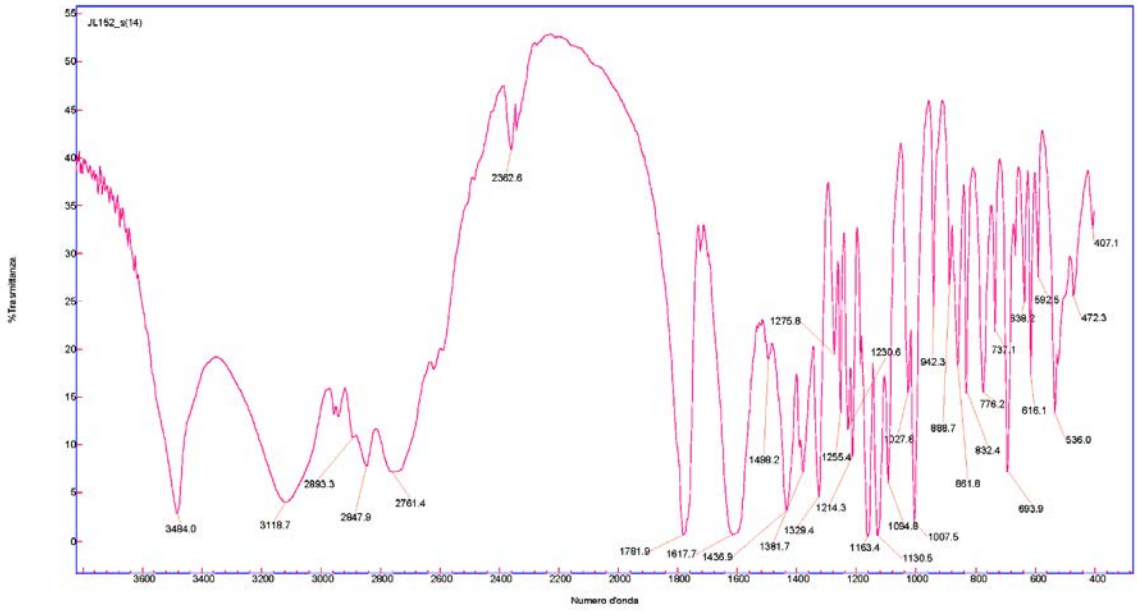


Figure 3.6 - FT-IR spectra of sodium (S)-2-((2R,3R,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetate (sodium galactaro-1,4-lactone salt).

The salts have also ESI mass spectra (negative ions and positive ions) fully compatible with the lactone salt structure. For example, with the sodium salt **19a** (Figure 3.7) the MS positive spectra shows the molecular ion at m/z = 215 [M+H], along with two complexes of sodium cation with two or three molecules of the lactone at m/z = 451 [2M+Na], 665 [3M+Na]. The formation of these complexes is a characteristic also of salts of galactaric itself and evidence the strong coordination ability of this class of compound, being useful in several technological applications as reported in Chapter 2.

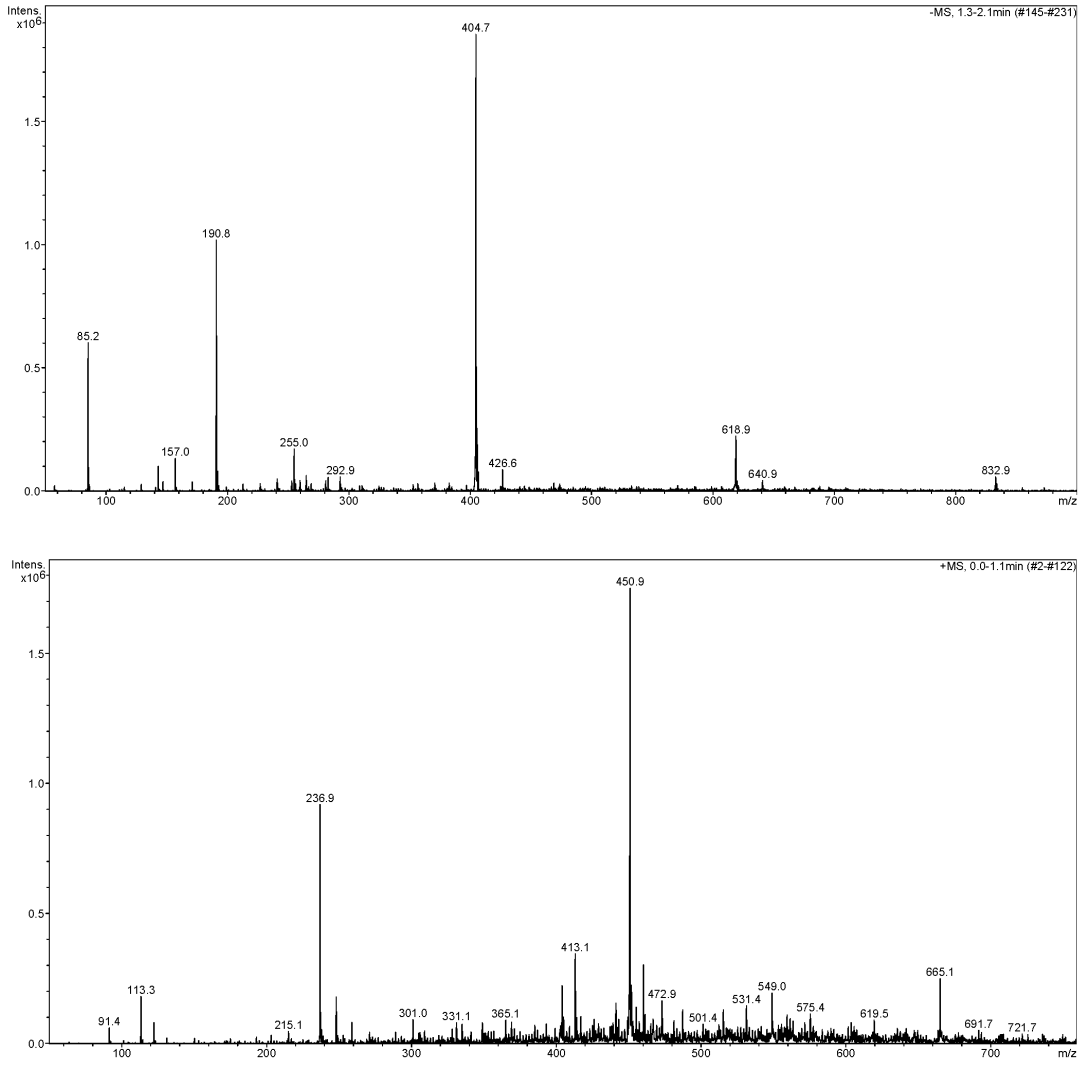
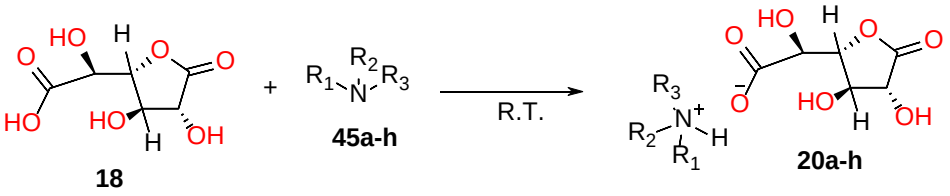


*Figure 3.7 – Negative and positive ESI-MS spectra of **19a** in methanol.*

Synthesis of ammonium salts of galactaro-1,4-lactone (18)

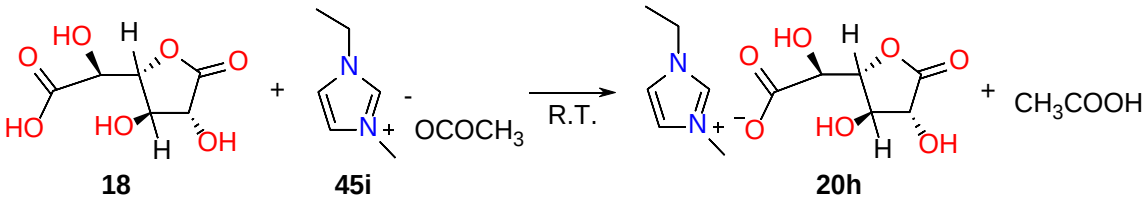
Based on the results of lactone **18** salification with alkaline bases, analogous reaction were experimented with some nitrogen centered bases, starting with ammonia itself and extending to primary, secondary, tertiary amines (Scheme 3.6). In these experiments the molar ratio between amines and lactone was controlled to around 1:1. Table 3.4 summarizes the good results of salification reactions with hindered primary, secondary, and tertiary amines, along with the results of exchange reaction with quaternary ammonium acetate. Frequently, the galactaro-1,4-lactone salt precipitates from the DMSO solution and was recovered in high yield by addition of acetone or acetonitrile as co-solvents. By distillation of the filtrated liquid it was possible to recover large part of the used solvents, in particular the DMSO, which can be recycled for other batch processes. This way solved the difficulty to isolate the galactaro-1,4-lactone **18** from the DMSO solution when salts are of interest.



a: R_1=R_2=H; R_3= $CH(CH_2OH)_2$
b: R_1=R_2=H; R_3= $CH(Ph)CH_3$
c: R_1=H; R_2=R_3= $CH(CH_3)_2$
d: R_1=H; R_2= R_3=CH_3; R_3=CH_2Ph
e: R_1,R_2,R_3 = DABCO
f: R_1,R_2,R_3 = Pyridine
g: R_1=R_2=R_3=H
h: R_1=R_2=H; R_3= CH_2CH_2OH

Scheme 3.6 – Synthesis of galactaro-1,4-lactone ammonium salts by hindered primary or secondary and tertiary amines.

Furthermore, preparation of quaternary ammonium salt of the lactone was verified starting from the DMSO solution of **18** with quaternary ammonium acetates i.e. 1–ethyl–3–methylimidazolium acetate **45i** (Scheme 3.7).



*Scheme 3.7 - Synthesis of galactaro-1,4-lactone quaternary ammonium salt **20h** via exchange with 1–ethyl–3–methylimidazolium acetate **45i**.*

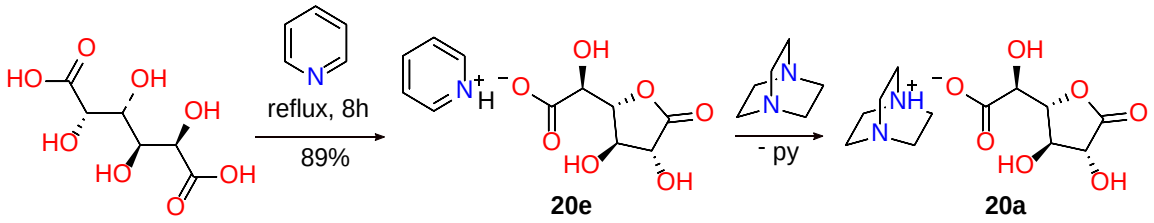
*Table 3.4 - Conditions and yields in the synthesis of ammonium galactaro-1,4-lactone salts (**20**) with some nitrogen bases **45a-i** (25°C, DMSO).*

Entry	[Subst.](mmol)	Amine	[Amine]/[sub]	T (h)	Conv. (%)	20 (Yield %)
JL157	2.1	**45a**	1.3	2	100	**20i** (85)
JL106	2.1	**45b**	1.0	4	100	**20b** (80)
JL150	2.1	**45c**	1.1	4	100	**20c** (100)
JL104	2.1	**45d**	1.3	4	100	**20d** (87)
JL139	2.1	DABCO (**45e**)	1.3	14	100	**20a** (95)
JL140	2.1	Pyridine (**45f**)*	1.3	10	100	**20e** (89)*
JL137	2.1	NH_3 (**45g**)	1.3	8	100	**20g** (100)
JL178	2.6	**45i**	1.1	10	100	**20h** (79)

* By reaction refluxing galactaric acid in pyridine solvent for 12 h.

The pyridinium lactone salt **20e** was peculiar. This compound apparently cannot be isolated by crystallization from the DMSO solution and the ^{1}H-NMR spectra does not evidence the typical shift in C-H proton close to the carboxylate group. In order to verify the real existence of the salt, a different preparative procedure was developed by refluxing galactaric acid in pyridine for 8 hours. The system became homogeneous after 6 hours and, by cooling and adding acetonitrile, a 89% yield of a solid was isolated. ^{1}H-NMR and mass spectra proved that the solid is compatible with the structure of the pyridinium galactaro-1,4-lactone salt. Interestingly, however, the ^{1}H-NMR spectra taken in DMSO-D6 of the solid does not evidence the shift typical of the lactone salts. Moreover, when dissolved in DMSO the solid cannot be recovered by crystallization or addition of several co-solvents, as verified in direct experiments in DMSO solution. A further evidence of the peculiarity of this salt was its low melting point (121 °C), much lower than the other ammonium galactarolactone salts, and its stability for long time at the temperature of reflux of pyridine.

Morever, the pyridinium salt **20e** offered the possibility to prepare other galactaro-1,4-lactone salts by exchange the base (i.e. DABCO in Scheme 3.8), harnessing the low basicity of pyridine. The exchange reaction was found general with inorganic and organic nitrogen bases, unless with primary amines.



*Scheme 3.8 – Exchange of the base between the pyridinium lactone salt **20e** and DABCO resulting in precipitation of the DABCO lactone salt **20a**.*

The X-ray crystal structure of DABCO galactaro lactone salt **20a** was then determined to better understand its insolubility in DMSO. The structure is reported in Figure 3.8.

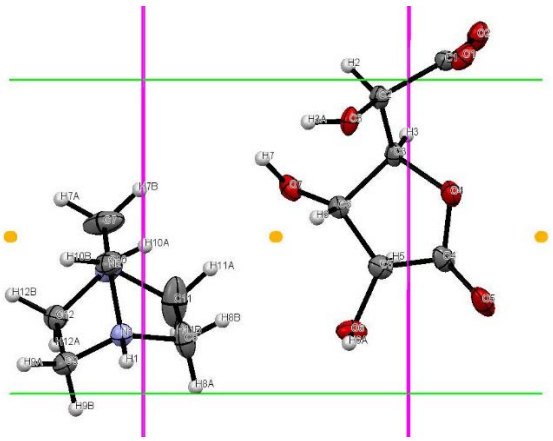


*Figure 3.8 – An ORTEP representation of crystal structure of **20a** (with symmetry elements).*

The individual bond lengths and angles fell within expected values, so these are not tabulated herein but are available from the Supplementary material. In each case the atoms of the lactone and carboxylate group is very close to planarity, generally to within ±0.01 Å. The following discussion is therefore restricted to conformational aspects and to an analysis of hydrogen bonding networks. Figure 3.9 shows the hydrogen bonding schematic for **20a**.

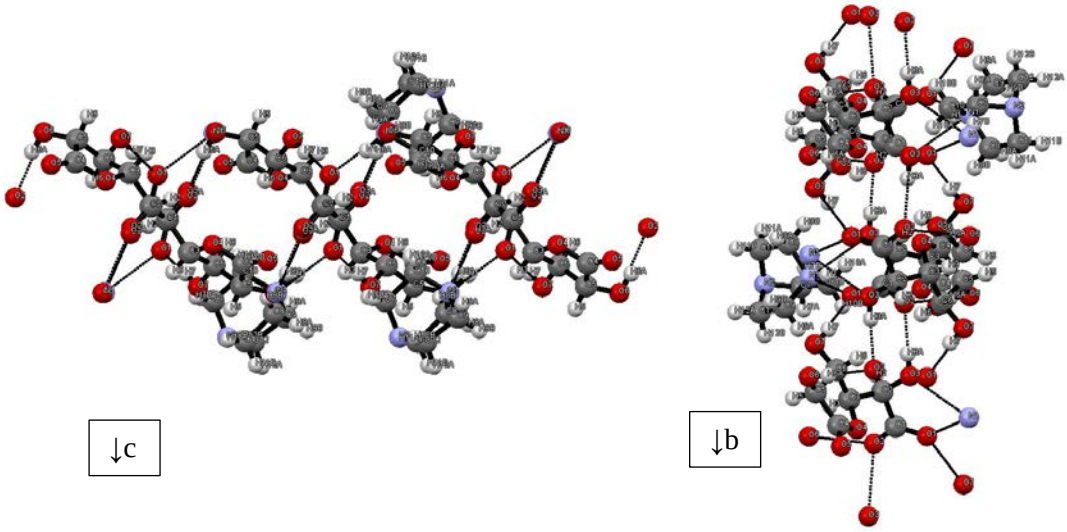
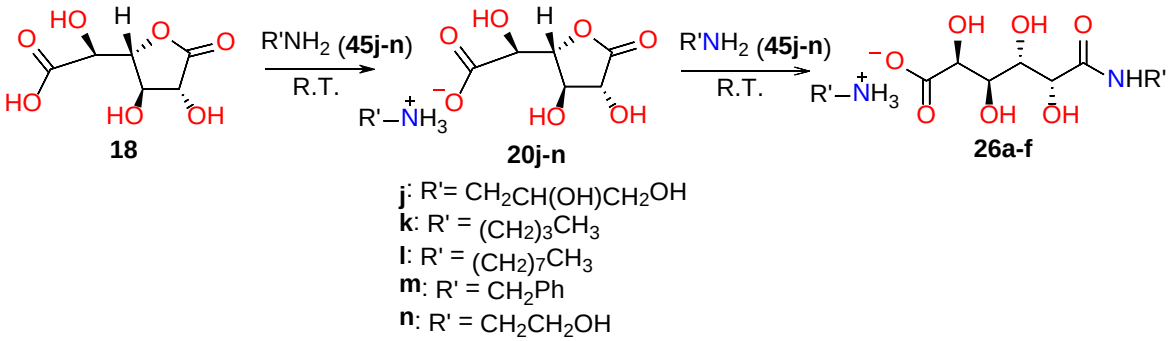


*Figure 3.9 – Hydrogen bonding schematic of **20a** (along b and c axis).*

The hydroxyl groups on C(2) and C(3) are intermolecularly hydrogen bonded with the carboxylate anions and hydrogen on ammonium nitrogen of DABCO is bonded to one oxygen of carboxylate anion and to the vicinal hydroxyl hydrogen. The arrangement recall what observed in alkaline salt of galactaric acid, where chelation of carboxylate and ortho hydroxyl group is a common feature. The hydrogen bond network organises the crystal in a layered compact structure with DABCO molecules on both side of the polar layer of sugars. The carbonyl of lactone ring is not involved in hydrogen bonding and is oriented within the sugar layer. The structure is an excellent example of the generalization that in crystals hydrogen bonding will control packing such that all proton donors will form hydrogen bonds, most hydrogen acceptors will form hydrogen bonds, and the best donors will seek the best acceptors[15]. The C=O distance of the lactone ring (1.206 Å) is typical of five membered lactones (1.198(7) Å is the average structural data derived from the Cambridge Structural Database; values in parentheses are standard deviations in the last digits), as the C-O distance inside the lactone (1.348 Å) (1.350(9) Å from the same source) suggesting a relevant contribution of electron delocalization owing to the nearly planar arrangement of this part of the lactone ring (torsion angle (O)C-O-C-C 1.61 degree). The erythro conformation of the vicinal hydroxyl group and the torsion angle between the cycle and side chain (-71.70 degree) confirm that the stereochemistry of chiral centres of galactaric acid is preserved.

The formation of lactone salts **20** with primary amines was more challenging, due to competition between salification and nucleophilic addition of the amine to lactone carbonyl group, resulting in the formation of amidogalactarate salts **26a-f** (Scheme 3.9). The suggested hypothesis that salification precedes the amidation in the scheme was inferred by the fact that the amidation process strongly declines decreasing the temperature.



*Scheme 3.9 – Synthesis of galactaro-1,4-lactone salts of primary amines (**20j-n**) and competitive ring opening by a second molecule of primary amine (**45j-n**) to amidogalactarate salt **26a-f**.*

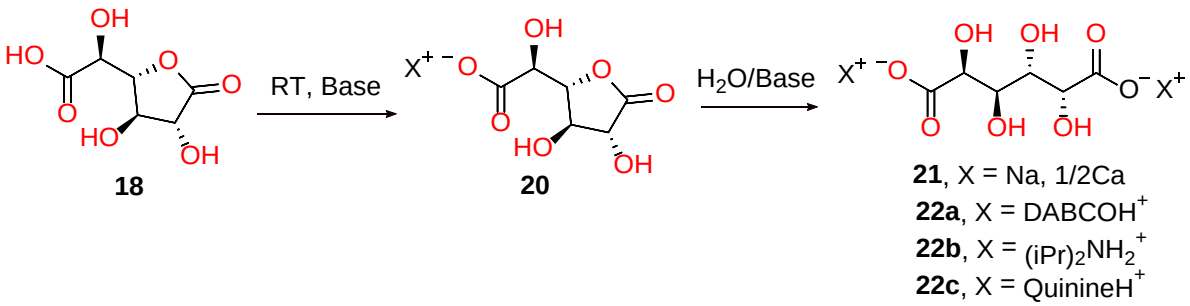
The results of salification with representative primary amines are reported in Table 3.5. The trend in rate was dominated by the kinetic of the salification and amidation reactions and by the relative solubility of amidogalactarate salt **26a-f** and lactone salt **20j-n** in DMSO. When the first compound has a solubility lower than the corresponding lactone salt (i.e. in the case of benzylamine, octylamine, and butylamine), the lactone salt was isolated only in moderate or low yield. On the contrary, when the lactone salt was relatively insoluble (i.e. in the case of isoserinol and ethanolamine) high yields of **20j-n** were observed. The preparative aspects related to the amidogalactarate salts **26a-f** will be discussed in the section devoted to the amidation reactions.

*Table 3.5 - Conditions and yields in the synthesis of galactaro-1,4-lactone salts (**20**) with primary amines (**45j-n**) (DMSO, 25 °C).*

Entry	[Subst.] (mmol)	Amine	[Amine]/[sub]	t (h)	18 (Conv %)	20 (Yield %)	26 (%)
JL151	2.1	**45j**	1.3	4	96.3	**20j** (93)	**26e** (-)
JL147	2.1	**45k**	1.3	4	78	**20k** (56)	**26f** (35)
GICR33	1.56	**45l**	1.0	0.25	60.2	**20l** (20)	**26c** (70)
JL146	2.1	**45m**	1.3	2	58.4	**20m** (17)	**26d** (78)
JL186	9	**45n**	1.1	4	93.7	**20n** (83)	**26b** (10)

Synthesis of galactarate salts

The reactivity of galactaro-1,4-lactone salts towards primary amines clearly indicates that these compounds are sensitive to ring opening by nucleophiles. If more than two equivalent of a base is present in a hydrolytic media, there is high potentiality to obtain the galactarate salts. This was verified with experiments in which the base, temperature, and amount of co-solvent water were changed. Galactarate salts identical to the one obtained from galactaric acid (i.e. **21**, sodium and calcium galactarate) were so obtained in the presence of more than stoichiometric amount of water. Also organic salts **22-c** proved to be synthetized by this method (Scheme 3.10).



*Scheme 3.10 – Synthesis of inorganic galactarate salts **21** and ammonium galactarate salts **22** via galactaro-1,4-lactone (**18**) and its salts (**20**).*

In Table 3.6 we report some representative reaction conditions, which evidence the good yields obtained by the method.

*Table 3.6 – Synthesis of galactarate salts from galactaro-1,4-lactone **18** and its salts **20** (DMSO/H_2O 9:1)*

Substrate	Base	[Base]/[sub]	T (°C)	t (min)	Conv. (%)	Salt (Yield %)
18	NaOH	2	25	30	100	**21a** (90)
19a	NaOH	1	25	20	100	**21a** (82)
18	$Ca(OH)_2$	2.1	40	30	100	**21b** (90)
20e	DABCO (**45e**)	1	25	30	-	-
20e	DABCO* (**45e**)	1.1	100	180	88	**22a** (90)
20c	$HN(iPr)_2$ (**45c**)	1	40	60	100	**22b** (83)
18	Quinine (**45o**)	2	25	120	100	**22c** (91)
1*	**45i**	2	25	120	100	**23** (90)

The procedure demonstrates further the potentiality of our approach via galactaro-1,4-lactone, overcoming the issues related to the low solubility of galactaric acid in water and many other solvents

and the need to use high temperature to end the salification. The main limit is the low solubility of the lactone salts (i.e. DABCO). In these cases, heating at 100 °C and longer reaction time was necessary.

A different situation was found with tertiary base quinine. In this case, attempts to prepare the lactone salt from galactaro-1,4-lactone (**18**) and this base failed and we isolate only the quininium galactarate **22c**. The course of the reaction was also peculiar, because the solid quinine dissolved in the DMSO solution of **18** in 30 minutes and a white crystalline solid appeared after 2 hours. The analytical data (^{1}H-NMR and mass spectra) for this solid confirmed its structure as quininium galactarate **22c**. No quininium galactaro-1,4-lactone salt was obtained also by elimination of the solvent. The more reasonable explanation is that, in this case, the lactone salt is fully soluble in the medium and its opening occurs with further salt formation by the base, providing the more insoluble galactarate salt **22**.

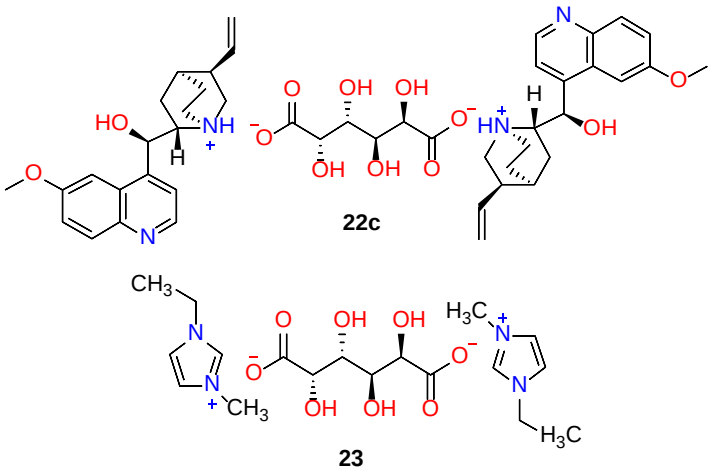


*Figure 3.10 – Chemical structures of quininium galactarate **22c** and 1–ethyl–3–methylimidazolium galactarate **23**.*

Finally, quaternary ammonium galactarate was found to be prepared efficiently from galactaric acid itself in low melting point quaternary ammonium acetates (ionic liquids). For example di(1–ethyl–3–methylimidazolium) galactarate salt **23** was prepared in nearly quantitative yield by addition at room temperature of galactaric acid **1** to the ionic liquid 1–ethyl–3–methylimidazolium acetate (**45i**) used as solvent (molar ratio 6:1). After 1 hour from the mixing, a solid starts to precipitate and precipitation is nearly complete after 2 hours, affording 88% yield after isolation. The structure of the salt was determined by ^{1}H-NMR, ^{13}C-NMR, and IR spectra and by mass spectrometry.

Characterization of Galactarate Salts

Compound **21** was obtained as a light yellow powder. The molecular formula of $C_6H_8Na_2O_8$ was identified by the mass spectrum analysis at m/z 209 [M^{2-}+H], 231 [M^{-}], 277 [M+Na], 509 [2M+H], 531 [2M+Na]. ^{1}H and ^{13}C-NMR spectrum were carried out to characterize the compound (Figure 3.11). From the ^{13}C-NMR spectrum, two pair of symmetric carbon of CH (δ 71.8, 71.5 ppm) and equivalent carbon of carbonyl group (δ 179.8 ppm) were identified. The ^{1}H-NMR spectrum showed two signals at δ 4.21 ppm (s, 2H), 3.89 ppm (s, 2H), which are close to the assignment of CH group proton on galactaric acid molecule. The structure was further confirmed by the IR data with carboxylate carbonyl absorbed at 1611 cm^{-1}.

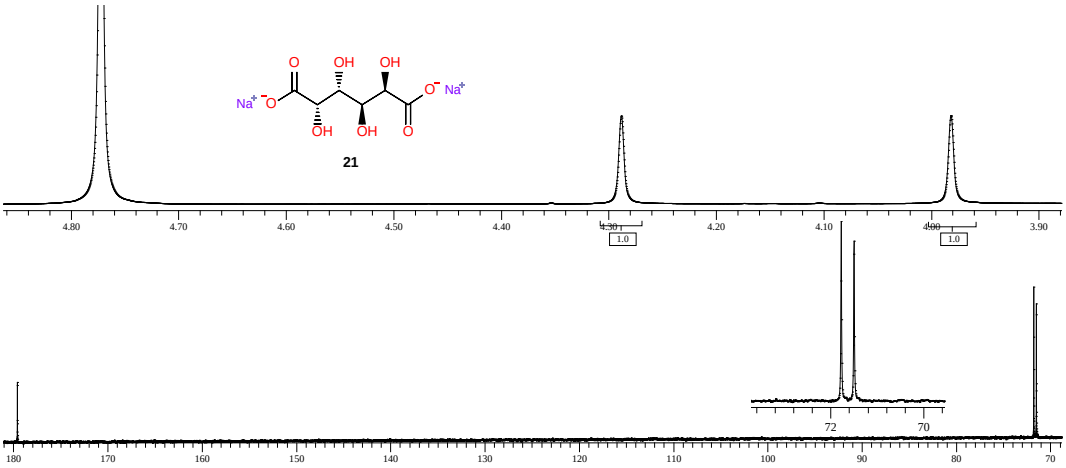


*Figure 3.11 - ^{1}H and ^{13}C -NMR spectrum of sodium galactarate (**21**) in D_2O.*

Compound **22c** was obtained as white solid. The molecular formula of $C_{46}H_{58}N_4O_{12}$ is identified by MS analysis at m/z 191 [M^{2-} + H], 209 [M^{2-} + H_2O + H]. From the ^{13}C-NMR spectrum, one CH_3 (δ 19.9 ppm), two unsaturated CH (δ 110.0, 130.9 ppm), two quaternary C-atoms (δ 139.5, 146.7 ppm) and three carbonyl group (δ 167.5, 159.7 and 155.9 ppm) are identified. The ^{1}H-NMR spectrum shows two signals at δ 4.09 ppm (s, 2H), 3.72 ppm (s, 2H), attributed to the symmetric CH group proton on galactarate, which are similar but slightly displaced to the signal on galactaric acid (Figure 3.12).

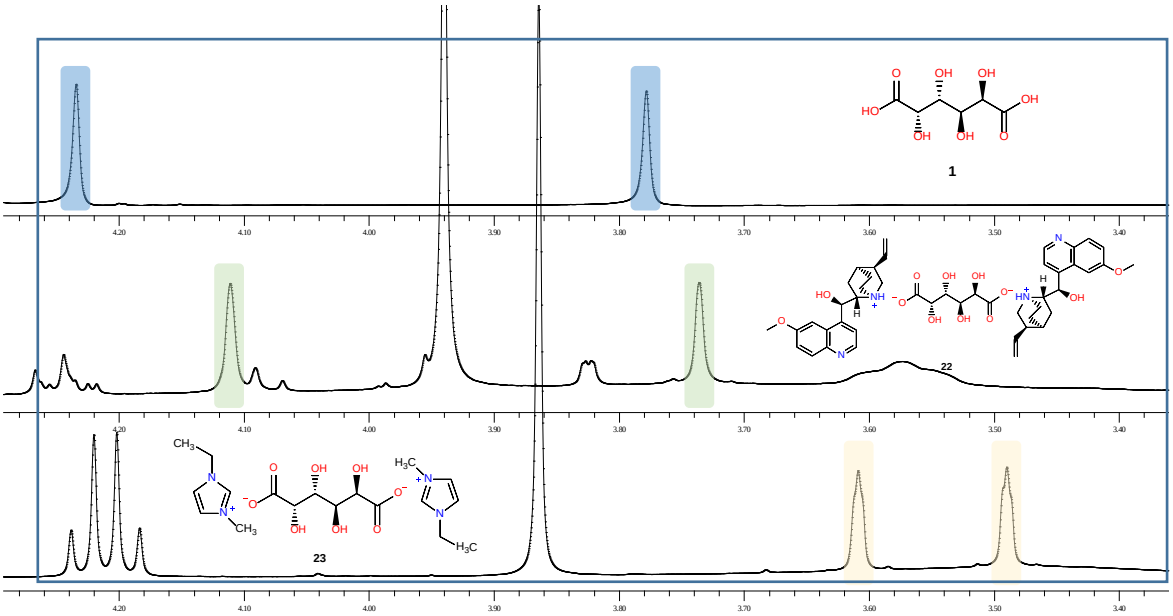


*Figure 3.12 - Comparison of [1]H-NMR spectra of galactaric acid **1** and its disalts **22c** and **23**.*

Compound **23** was obtained as a white solid. The molecular formula of $C_{18}H_{30}N_4O_8$ is identified by the mass spectrum analysis at m/z 191 [M^{2-}—H_2O+H], 209 [M^{2-} + H], 111 [imidazolium], 431 [M+H], 453 [M+Na] (Figure 3.13). The ^{1}H-NMR spectrum (Figure 3.12) shows two signals at δ 3.62 ppm (s, 2H), 3.41 ppm (s, 2H), typical of the linear galactarate system, aand two dublets at δ 7.79 ppm (s, 2H) and δ 7.70 ppm (s, 4H), of imidazolium ring protons. Compared with the spectra in Figure 3.12, it is clear that the chemical shift of galactarate is sensitive to the structure of the cation with deviation at higher field in the imidazolim salt. Also in the IR the absorption of the carboxylate is shifted at 1596 cm^{-1}.

The positive ESI mass spectra shows essentially only the imidazolium cation at m/e 113, whereas the negative ESI mass spectra show more ions with prevalence of the monogalactarate anion at m/e 209 [MH^-] and the corresponding fragment of water elimination at m/e 191 [MH^- - H_2O].

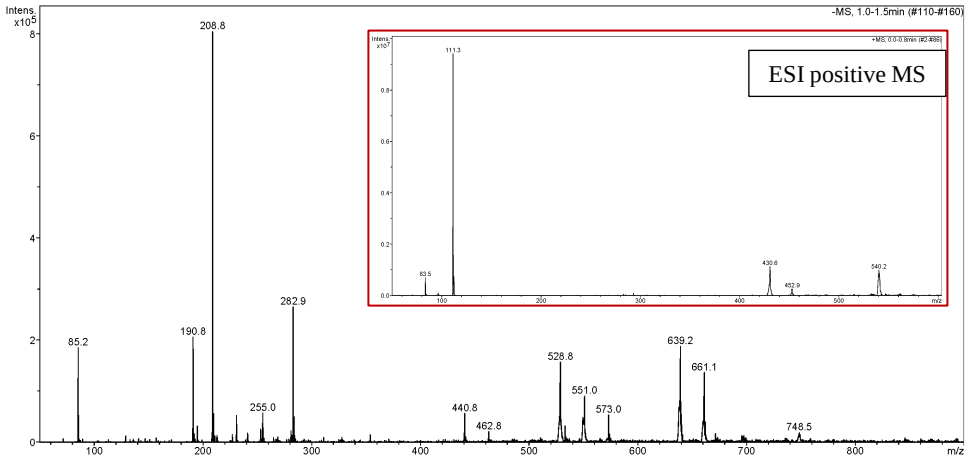


*Figure 3.13 - ESI-MS negative and positive (in the insert) spectra of **23** in methanol.*

3.4 - Acylation of Carboxylic Group of Galactaric Acid Derivatives

The esterification reaction of galactaric acid is described in literature as preparative methods without mechanistic considerations.[16] However, some peculiarities of the process deserve specific attention. Firstly, even in excess of acetic anhydride the reaction is sluggish and under reflux conditions stops at the stage of tri-acetylated 1,4-galactarolactone. In order to get the tetra-acetylated derivative, the presence of a strong acid in significant amount (10% on starting galactaric acid) is essential. Second, during the process in the absence of acid at 40-70 °C, the unsubstituted galactaro-1,4-lactone was detected along with one of its possible mono-acetylated derivatives. We decided to investigate further the reaction even with the scope to optimize the process for large-scale preparation of these intermediates, useful also for other part of the research. The experiments were carried out with excess of acetic anhydride up to molar ratio 5:1.

To recover more insight on the esterification, we attempt also to prepare formate esters of galactaric acid using formic−acetic anhydride mixture, a well-known reagent for the formylation of alcohols,[17] because the formic anhydride is not stable. Formate esters of aldaric acids were never reported in the literature and these experiments could provide further details on the mechanism of the esterification. However, formylation processes by this reagent have a drawback in the known reactivity between anhydrides and DMSO (the solvent used in the preparation of galactaro-1,4-lactone) resulting in a variety of reactions, including oxidation of the hydroxyl groups, generation of formaldehyde derivatives, and dialkylsulfide species by Pummerer rearrangement.[18-21] Therefore, also this transformation was challenging.

3.4.1 - Acetylation of galactaric acid with acetic anhydride

The acetylation of galactaric acid was verified by using excess of acetic anhydride and under different reaction conditions. The analytical technique selected to detect and quantify the progress of the reaction was the proton or carbon nuclear magnetic resonance (^{1}H-NMR or ^{13}C-NMR). Preliminarily it was verified that recognition of the main reaction products was possible by comparing the ^{1}H-NMR of the starting galactaric acid (**1a**) and the previously prepared galactaro-1,4-lactone (**18**), tetra-acetylgalactaric acid (**2**), and tri-acetyl-1,4-galactarolactone (**3**) (Figure 3.14). Next, a reaction was followed in the NMR tube with external reference standard to follow the decay of signals of starting reagent and the formation of final products and any possible acetate intermediates with the aim to define the role of competitive and consecutive reactions

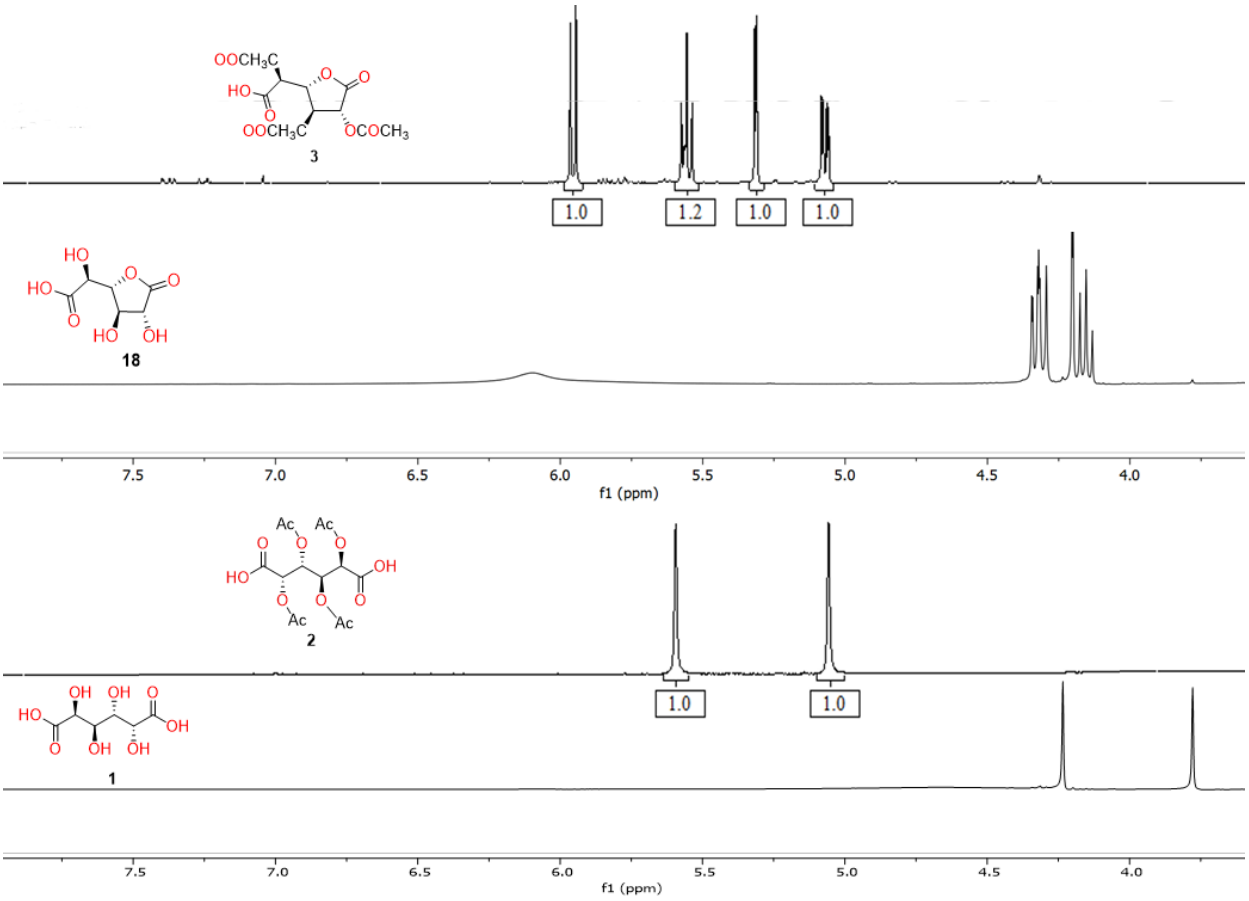


*Figure 3.14 - Comparison between the ^{1}H-NMR spectra (DMSO-d_6) of tri-acetylated **3**, tetra-acetylated **2**, galactaro-1,4-lactone (**18**), and starting galactaric acid (**1a**).*

We soon clarified that the slugging nature of the process is strictly related to the insolubility of the starting galactaric acid, while the acetylation of the lactone **18** is much faster than the acyclic acid itself. So, acetylation occurs mainly on this intermediate and under reflux condition the lactone reacts as soon it is formed. Three kinetic experiments were carried out to follow the decay of the lactone **18** (0.02 M) in acetic anhydride/DMSO 5:1 at three different temperatures: 80, 110 and 130 °C. Figure 3.15 summarises the results of these experiments, which indicate an exponential decay of the lactone. The deduced pseudo-first order kinetic is probably due to the excess of the solvent acetic anhydride.

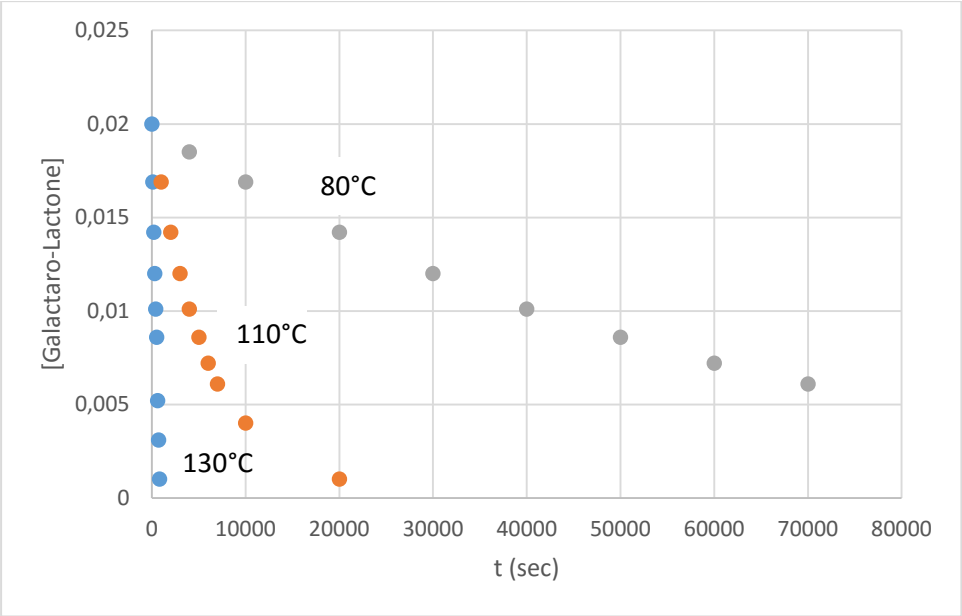
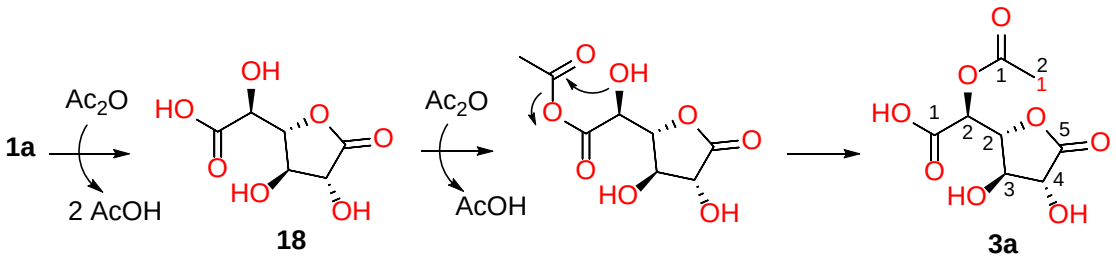


Figure 3.15 – Temperature dependence of the kinetic of disappearance of galactaro-1,4-lactone (0.2 M) in acetic anhydride/DMSO 5:1.

From the data, an activation energy of 22 kJ·mol^{-1} was estimated. In these experiments, it was noticed that, for reaction at lower temperatures, one mono-acetate was prevalent and only at high temperature and longer time the polysubstitution occurs, clearly suggesting a stepwise selective acetylation process. Analysis of the ^{1}H-NMR spectra supports the structure of the first product as the (S)-2-acetoxy-2-((2R,3R,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl)acetic acid (**3a**). The only reasonable explanation for this selective acetylation can arise from an anchimeric assistance by the carboxyl group via the formation of a mixed galactaric-acetic anhydride (Scheme 3.11).



*Scheme 3.11 – Proposed mechanism for the selective intramolecular acetylation of galactaro-1,4-lactone to the acetate **3a** via an intermediate galactaric-acetic mixed anhydride.*

The hypothesis seems curious but not unexpected owing to the proved existence of several mixed anhydrides and supported further by the recently reported preparation of co-polyanhydrides of the peracetylgalactaric acid, involving the same acetic anhydride as activating species.[22]

Mixed anhydrides have found relevant interest in polymers owing to their fast hydrolysis, which make them suitable for biodegradation in living systems and therefore useful materials for clinical applications.[23, 24]

Analysis of the spectra at lower temperature evidences new minor signals at 2.24 ppm in the ^{1}H-NMR spectrum and at 22.1 ppm in the ^{13}C-NMR spectrum compatible with the formation with a mixed carboxylic/acetic anhydride. Similar groups were identified when tetraacetyl galactaric acid is dissolved in acetyl anhydride (Figure 3.16) and in oligomeric co-polyanhydrides with terminal acetyl groups and are typically used to evaluate the degree of polymerization in these materials.[25] These signals were present along all the reaction and disappeared only after addition of water to quench the reaction.

As can be seen in the spectra of Figure 3.16 the signals of the mixed anhydride at 22 ppm is more clearly seen in the ^{13}C-NMR than in ^{1}H-NMR, but the longer acquisition time, necessary for these spectra to see intermediates, prevent their use to follow kinetics of the reaction.

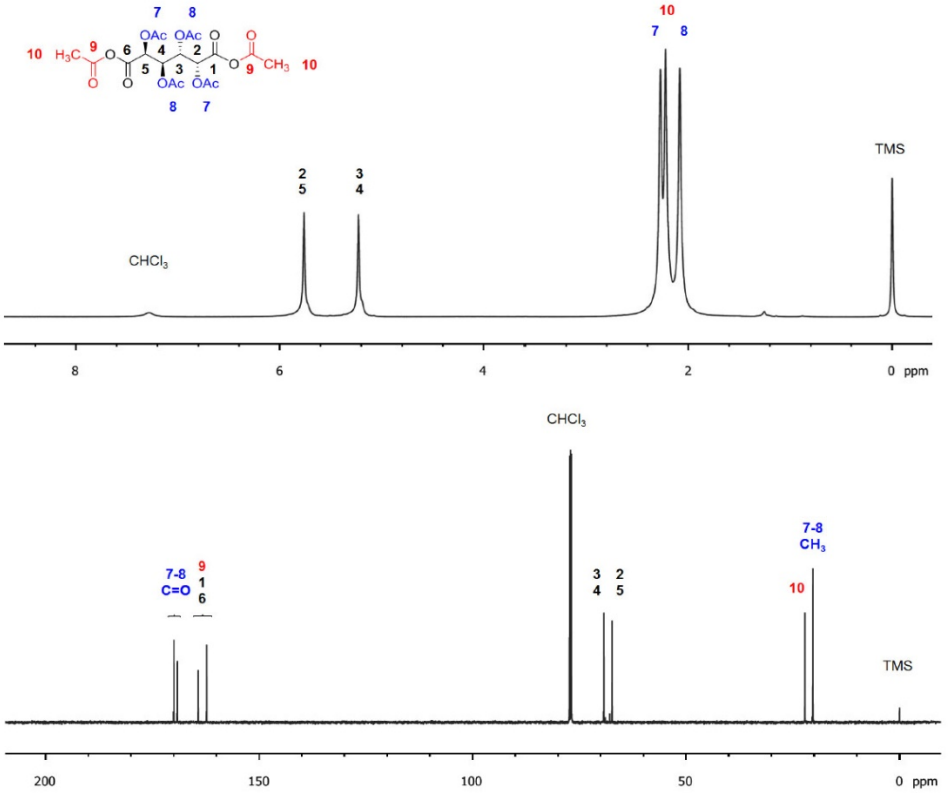


Figure 3.16 - ^{1}H-NMR and ^{13}C-NMR spectra of tetraacetylgalactaric acid in acetic anhydride.

Moreover, the hypothesis of the lactone formation at the first stage reaction is substantiated by the ^{1}H-NMR analysis. Lactone **18** was detected in the reaction mixture at 80-100 °C, where it can reach

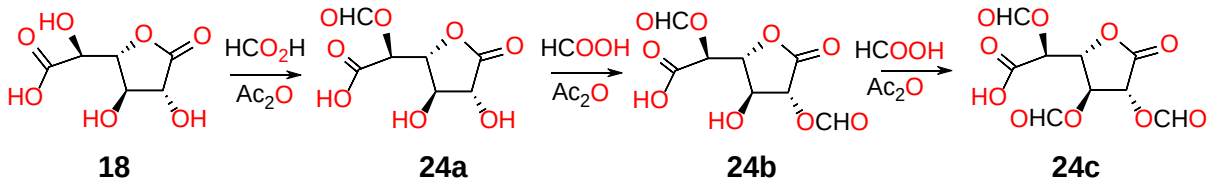
appreciable concentration owing to the better solubility in acetic anhydride than starting galactaric acid **1**.

The next acetylation stages were ascertained less definitively owing to superposition of signals in ^{1}H-NMR, but some insights were obtained, which point out to a preferential formation of the diacetylated product close to the two carbonyl of the galactaro lactone. If this hypothesis is correct, it probably means that the lactone can open to exchange the acyl group, generating a new alfa hydroxyacid, which can be further acetylated by a similar intramolecular process via a new mixed galactaric/acetic anhydride.

It must be remembered that in typical preparative experiments, the peracetylation of galactaric acid is carried out at reflux of acetic anhydride with or without acid catalyst, but these conditions promote also the degradation of formed acetates and induce acetic acid elimination processes. From the above reported ^{1}H-NMR spectra (Figure 3.14), for instance in the central spectrum (the spectra of tetracetylated galactaric acid **2** at the bottom and the spectra of tri-acetylated lactone **3** at the top), we can distinguish two zones: a first zone with chemical shift between 4.8 and 6 ppm and a second one between 6 and 8 ppm. In the first interval it was possible to identify the galactaric acid tetracetate (**2**), the galactaro-1,4-lactone triacetoxylate (**3**), and the intermediates of partial acetylation, whereas the signals in the range 6-8 ppm were attributed to products of acetic acid mono- and di-elimination, for which a specific analysis will be carried out in detail in next subchapter.

3.4.2 - Formylation of Galactaro-1,4-lactone and its salts

Afterward, we studied the formylation reaction of galactaro-1,4-lactone (**18)** in DMSO solution with excess of mixed acetic-formic anhydride at 20-30 °C. Parallel reactions were carried also on the lactone salts **19** and **20**. Also this process appears to occur stepwise, involving firstly the –OH near to the carboxylic group and then to the –OH near to the carbonyl of the lactone group (Scheme 3.12). It must be noticed that in all cases, no interference of hydroxyl groups oxidation by the mixed anhydride was observed and the competitive Pummerer reaction did not inhibit the formylation.



*Scheme 3.12 - Stepwise formylation of **18** with acetic-formic anhydride.*

In this case, the reaction was easier to follow by ^{1}H-NMR owing to the specific effect of formyl group, which allow to distinguish all the intermediate mono-, di- and tri-formylated products. In fact, by using the DMSO solution of **18**, diluted by 1:10 with acetic formic anhydride, we found the trend observed in Figure 3.17 for the ^{1}H-NMR spectra of samples taken at different time intervals. As typical of the mixed acetic formic anhydride, the largely prevalent products were the formylation product and only trace of acetylation products were detected. Identification of the main products as the mono-formylated lactone (**24a**), the di-formylated lactone (**24b**) and the tri-formylated lactone (**24c**) as final products was obtained comparing the signals of formyl groups around 8-8.4 ppm with the signals of the sugar moiety. No other mono-formylation product nor other di-formylation products were observed in yield higher than 3%, substantiating further the selective introduction of the acyl group. Details of the attribution of the ^{1}H-NMR spectra are summarized in Table 3.7.

Table 3.7 – ^{1}H-NMR chemical shifts of mono-, di- and tri-formylated galactaro-1,4-lactone (DMSO-D6).

Name	**Compounds**	**H-2 o H-5 (ppm, m, Hz)**	**H-3 o H-4 (ppm, m, Hz)**	**H-3 o H-4 (ppm, m, Hz)**	**H-2 o H-5 (ppm,m, Hz)**
24a	Galactaro-1,4-lactone mono-formate	5.29 dd *J=2.19,1.04*	4.55 ddd *J=8.4, 2.1, 0.89*	4.02 t *J=8.8*	4.36 d *J=9.0*
24b	Galactaro-1,4-lactone di-formate	5.89 d *J= 8.9, 0.9*	4.84 ddd *J=8.4, 2.2, 0.9*	4.39 t *J= 8.7*	5.36 dd *J= 2.1, 1.0*
24c	Galactaro-1,4-lactone tri-formate	6.12 d *J= 7.8*	5.10 dd *J=2.3, 7.8*	5.62 t *J=7.6*	5.43 d *J= 2.7*

It must be noticed that in the NMR spectrum can be easily seen two siglet signals between 5.0 and 5.3 ppm (marked in blue colour in Figure 3.17) of variable intensity during the reaction. These singlets are also present, and in larger amount, when the reaction is carried out in the absence of substrate **18** simply mixing the acetic-formic anhydride with DMSO. The signals are due to the products of the Pummerer reaction assigned on the basis of previous work as the methyl thiol protons of methylthiomethyl acetate.[26]

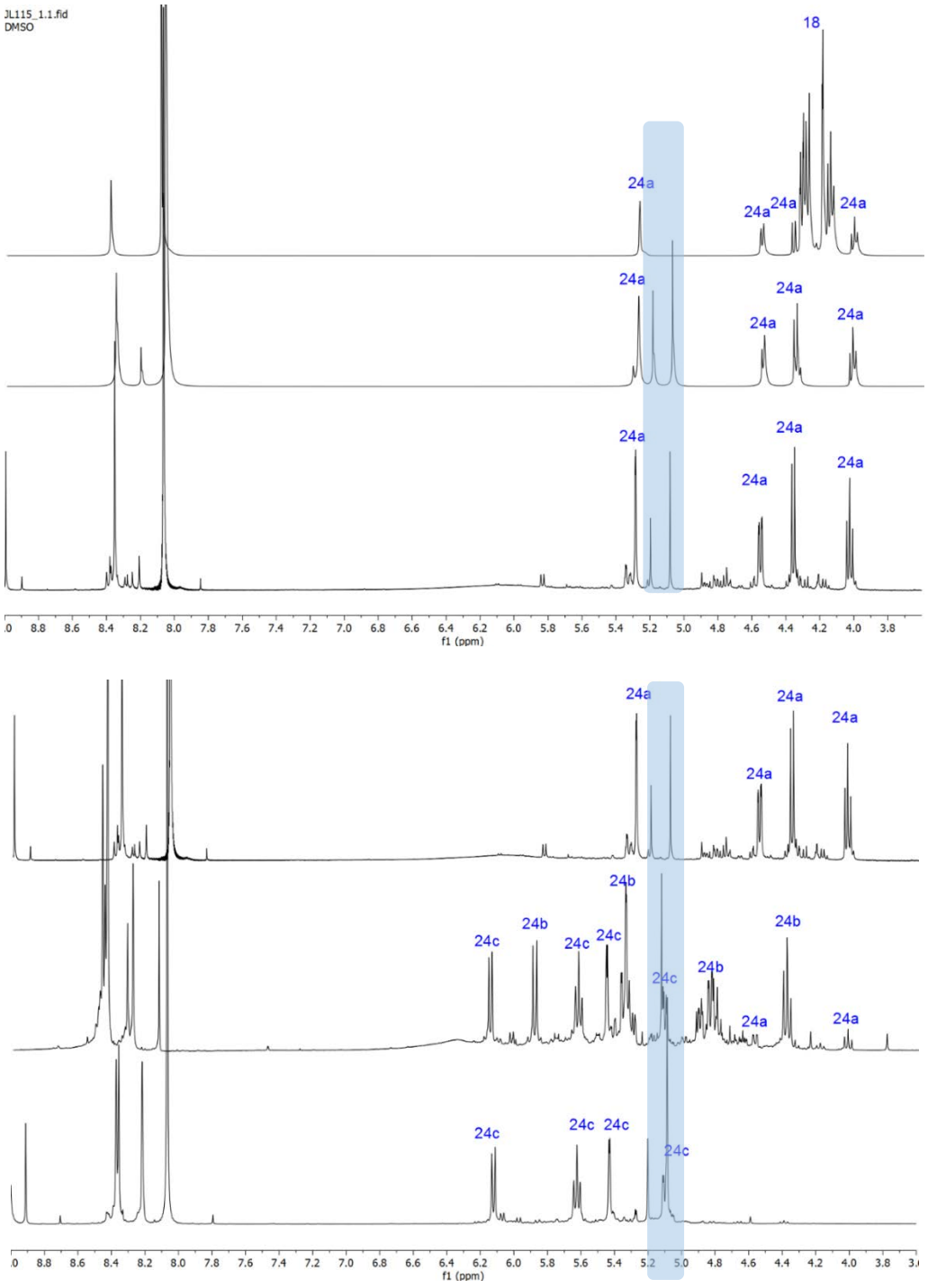


*Figure 3.17 - ^{1}H-NMR spectra of the formylation reaction of **18** taken at different reaction time.*

Also the mass spectrum (negative ions, Figure 3.18) of the reaction mixture is consistent with the formation of the three formylated compounds **24a**, **24b** and **24c**. The molecular ion of m / z = 219 [M_{24a}-H], 247 [M_{24b}-H] and 275 [M_{24c}-H] can be recognized in the spectrum. In fact the ion of m / z = 495 [$2M_{24b}$-H], 523 [M_{24b}+M_{24c}-H] and 551 [$2M_{24c}$-H] can be distinguished in the spectrum which

are compatible with an adduction between two molecules of **24b** and **24c**. Their fragments 219 [M_{24b} -CHO-H]$^-$, 203 [M-COOH-H]$^-$ are also compatible with the polyformylated molecules.

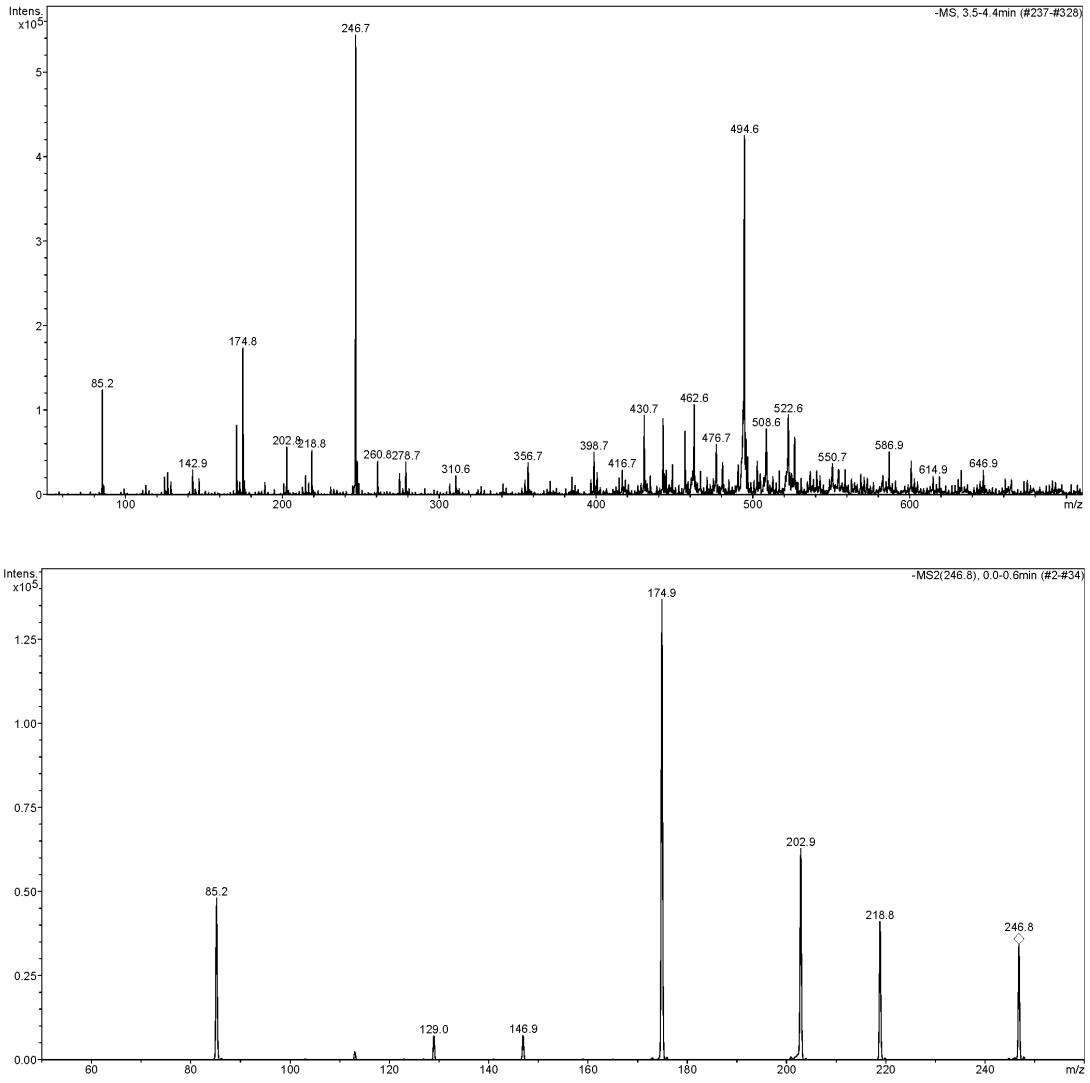
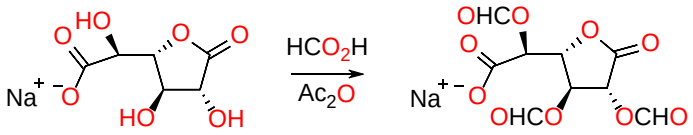


*Figure 3.18 - MS((-)-ESI) (1:100 MeOH) of products from formylation reaction of **18**.*

3.4.3 - Isolation of Sodium galactaro-1,4-lactone triformate

Considering the fact that galactaro-1,4-lactone triformate **24c** is difficult to be isolated due to its high solubility in DMSO and its sensitivity to water, attempts were made to synthesize the salt of performylated lactone by reacting the isolated sodium galactaro-1,4-lactone salt **19a** with acetic formic anhydride at room temperature.



19a **25**

*Scheme 3.13 - Formylation of **19a** with acetic formic anhydride.*

The reaction proved to be effective and the sodium triformylgalactaro-1,4-lactone salt **25** was easily isolated and purified as a white solid in 72% yield by adding diethyl ether as co-solvent with following filtration and drying. The trend in formylation sequence with **19a** was similar to one observed with the corresponding acid derivative **18**, with initial preference for the formyl group close to the carboxylate group. Mixed anhydrides are expected to be more easily formed from carboxylate anion than from the carboxyl group itself.

Compound **25** was characterized by ^{1}H-NMR on the crude reaction mixture (Figure 3.19). The chemical shifts of protons in the sugar region of **25** are located between 4.90 and 6.10 ppm, specifically at 6.09 ppm (d, J=7.7 Hz), 5.56 ppm (t, J=7.7 Hz), 5.11 ppm (dd, J=7.7, 2.8 Hz), 4.90 ppm (d, J=2.8 Hz). Three formyl protons are assigned at 8.2-8.4, along with remaining formic acid and the remaining acetic formic anhydride. From the assignments, it is apparent that protons in the lactone ring of **25** are shifted to lower field than the ones in **19a**, which absorbs in the range 3.60~4.20 ppm, and the ones in its acid form **24c**. The electron-withdrawing inductive effect of formyl substituent and the effect of carboxylate anion can explain the observed trend, with larger influence for proton in position 2 of the lactone ring.

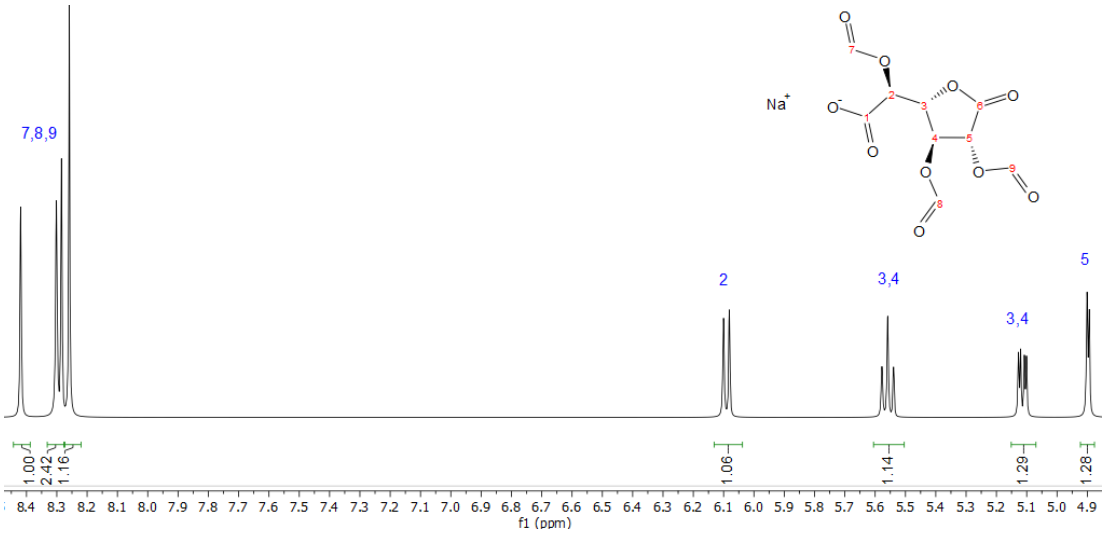


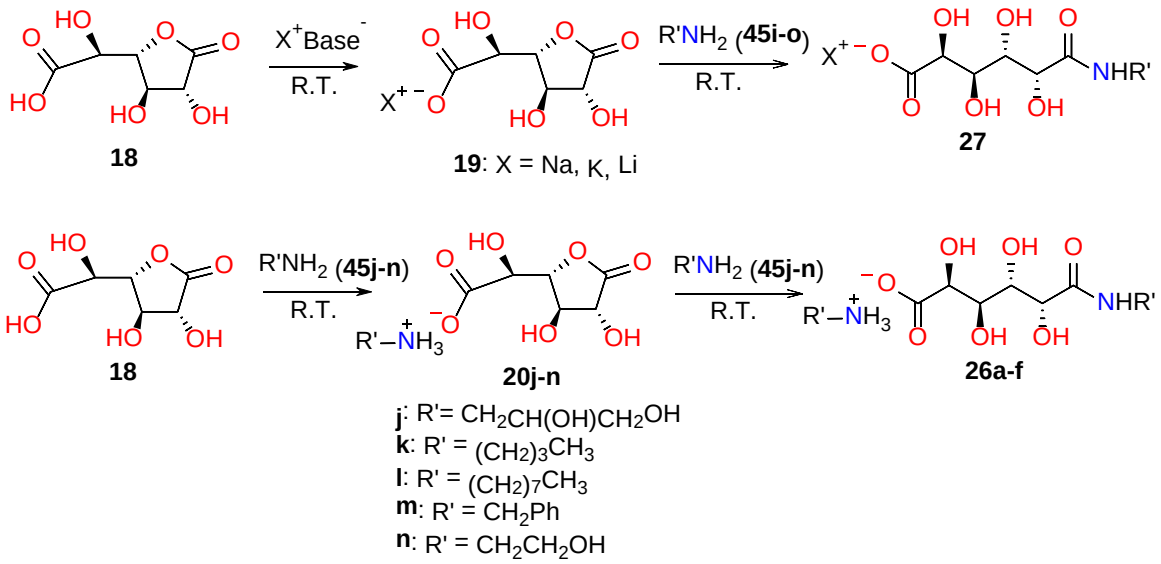
*Figure 3.19 - Assigned ^{1}H-NMR spectrum of sodium triformate salt **25** in DMSO-d_6.*

3.5 - Amidation Reactions of Galactaro-1,4-lactone

As indicated in the previous chapter and discussed in cited literatures, the formation of amide bonds in the aldaric series seems to be particularly easy. Despite the interest developed in literature for this reaction as preparative method to obtain the recognized useful materials polyhydroxypolyamides, we were interested in clarifying the reasons of the fast kinetic of the process and its apparent limitation to primary amines. Frequently literature suggested that lactones are intermediates of the reaction and this has been proved with glucaric acid, but no specific experiment was carried out to prove the intermediary of galactarolactones in the reactions with galactaric acid. Therefore, we decided to use our preparative method of galacatro-1,4-lactone (**18**) to provide more insight on the amidation reaction with this reagent and to compare the results with the more common methods of amidation with acyclic galactaric acid and its diester derivatives or lactone esters.[27]

3.5.1 - Synthesis of amide galactarate salts from galactaro-1,4-lactone

The aminolysis of **18** was easily observed by a simple modification of the preparative method adopted for the synthesis of lactone salts **19** and **20**. As just indicated in previous section 3.4, the reaction occurs easily with primary amines and galactaramide salts **27**, which are easily obtained both from lactone **18** as well as its inorganic salts **19** and organic salts **20** at slightly higher temperature or longer time (Scheme 3.14).



*Scheme 3.14 - Synthesis of amidogalactarate salts from galactaro-1,4-lactone **18**.*

The ammonium galactaramide salts **26** were in fact easily prepared by using 2 equivalent (or more) of primary amine **45j-n** from **18** in a direct procedure or adding 1 equivalent (or more) of the same amine **45j-n** on the in-situ preformed ammonium salt **20j-n**. The reaction was fast enough to be concluded at room temperature in less than 3 hours in the direct approach. With this procedure ammonium salt of galactaramide **26** were formed having the same substituent at both nitrogen atoms (to simplify the notation this compound are termed symmetrical in the sense that the amino residue is in identical species, to be differentiated from the asymmetrical one when the structure of cation differs from the amide substituent). Typical reaction conditions of the procedure developed are reported in Table 3.8. Isolation of the products was carried out by direct precipitation from the DMSO solution or by addition of the appropriate co-solvent with moderate to good yield.

*Table 3.8 - Synthesis of galactaramide salt **26** by reaction of **18**, with primary amines (DMSO, 25-30°C).*

Run	[Subst.] (mmol)	Base	[Base]/[sub]	t (min)	18 (Conv. %)	26 (Yield %)
1	2.1	Serinol	2.2	120	100	**26a** (87)
2	2.1	Isoserinol	2.1	120	100	**26e** (95)
3	2.1	Butylamine	2	20	100	**26f** (77)
4	2.1	Benzylamine	2.2	20	100	**26d** (77)
5	2.1	Octylamine	2	120	100	**26c** (94)
6	9	Ethanolamine	2	120	100	**26b** (93)

By using the inorganic salt **19a-c**, the amidation process produces unsymmetrical galactaramide salts **27**. The procedure works also for the preparation of fully organic unsymmetric galactaramide salts

starting from ammonium galactaro-1,4-lactone salts **20** arising from primary (hindered), secondary, and tertiary amines or from quaternary ammonium salts. The versatility of the method is evident by the mild conditions used and the high (even not optimized) yields of a large variety of mixed salts by using strictly stoichiometric amount of primary amines. Representative examples of this procedure are summarized in Table 3.9. This isolation procedure turned out to be the preferred method for isolation of unsymmetric amide galactarate salts with isolated yields higher than 70% for **27**.

*Table 3.9 - Synthesis of N-substituted galactaramide salts **27** by reaction of salt **19** or **20** with primary amines (DMSO, 25°C).*

X / Amine	[Base]/[sub]	t (min.)	19 or 20 (Conv. %)	27 (Yield %)
EMI / Butylamine	1	120	100	**27a** (87)
Na / Octylamine	2	120	100	**27b** (95)
Na / Dodecylamine	1	20	100	**27c** (77)
Et_3N/Butylamine	1	100	98	**27d** (90)
DABCO/Butilamine	1	240	80	**27e** (70)

All the isolated galactaramide salts were characterized by ^{1}H- and ^{13}C-NMR spectroscopy. Taking the butylamine derivative **26f** as an example, it is evident from Figure 3.20 that the structure is asymmetric. The four protons of acyclic galactaric acid (H-2 to H-5) have chemical shifts in the range from 3.55 to 4.10 ppm, and specifically at 3.55 (dd, J=3.30, 9.54 Hz), 3.69 (dd, J=0.73, 9.30 Hz), 3.73 (d, J=3.55 Hz), 4.10 (d, J=0.73 Hz). The two methylene protons at the α and β position on both amide and salt side are well differentiated, with the ones in position 1' and 1" resonating at 3.08 and 2.75 ppm. Protons in position 2' and 2" are assigned at 1.53 and 1.35 ppm, respective. Meanwhile the rest methylene proton 3', 3" and methyl proton 4', 4" are superimposed and located at 1.28 and 0.84 ppm. The amide proton resonates at 7.50 ppm as a triplet, a value typical of all galactaramide derivative. The asymmetric nature of the molecule can be appreciated ^{13}C-NMR spectrum in D_2O, where all 14 carbons shows distinct signals. The terminal methyl carbons C-4' and C-4" fall at 12.8 and 13.0 ppm, the methylene carbons C-3' and C-3" at 19.3 and 18.8 ppm, the methylene carbons C-2' and C-2" at 30.6 and 28.7 ppm, the methylene carbon bearing nitrogen C-1' and C-1" at 39.3 and 38.8 ppm), the carbonyl carbon C-1 and C-6 at 179.3 and 175.2 ppm, the carbon from C-2 to C-5 in the range of 70.7 to 71.4 ppm. The assignment of C1-6 is consistent with the ^{13}C-NMR spectrum of acyclic diacid **1** in D_2O.[13]

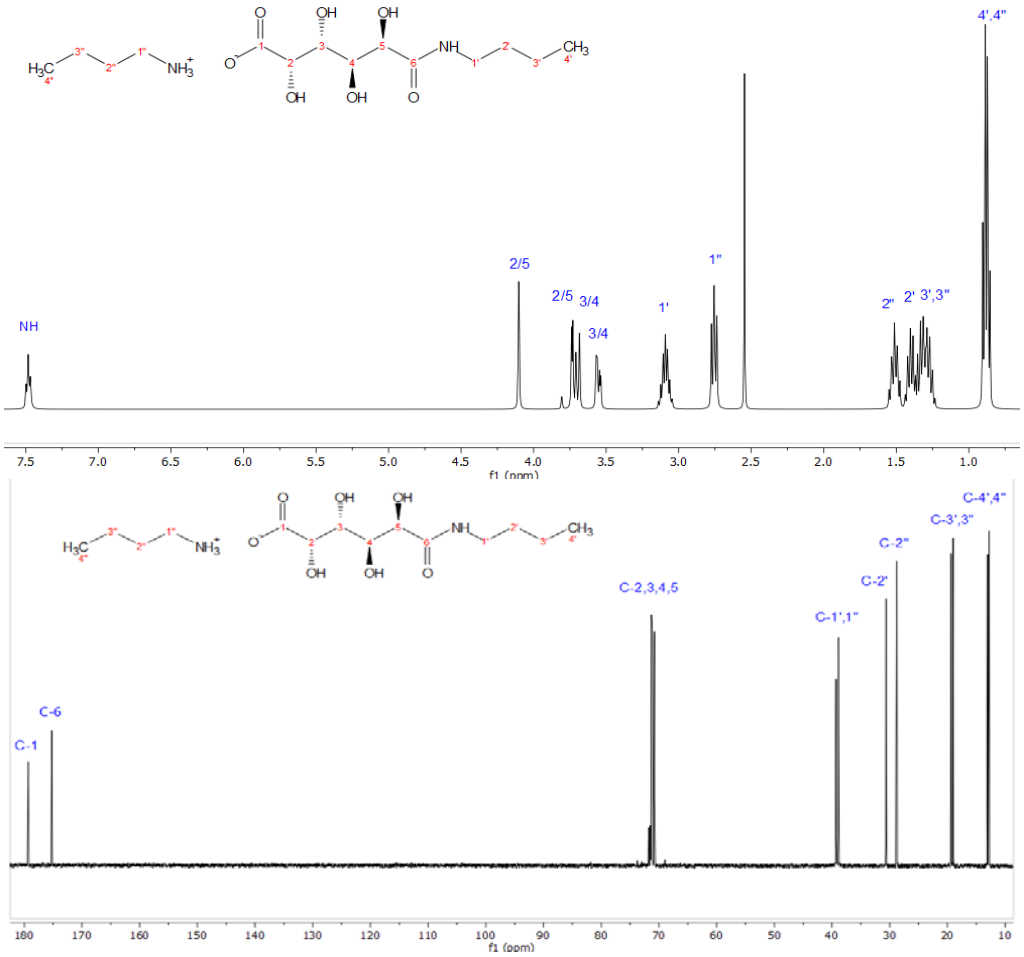


*Figure 3.20 - Assigned ^{1}H and ^{13}C -NMR spectrum of **26f** in DMSO-d_6 and D_2O.*

As concern the mass spectra (positive ions) of the isolated white solid **26f**, they are consistent with the proposed structure, even if somewhat complex (Figure 3.21). The molecular ion at m/z = 339 [M+H] is present in the negative spectra but at low intensity. The base peak at m/e 266 corresponds to the protonated form of the amidogalactaric acid, and the ion at m/e 248 corresponds to a loss of water from m/e 266. The ion at m/e 321 has the structure of protonated N-butyl galactaramide, whereas the ions at m/e 553 and 575 belong to dimeric species. The fragmentation spectra of peak 339 confirms futher the structure affording the peak base at m/e 266 by loss of the butylamine.

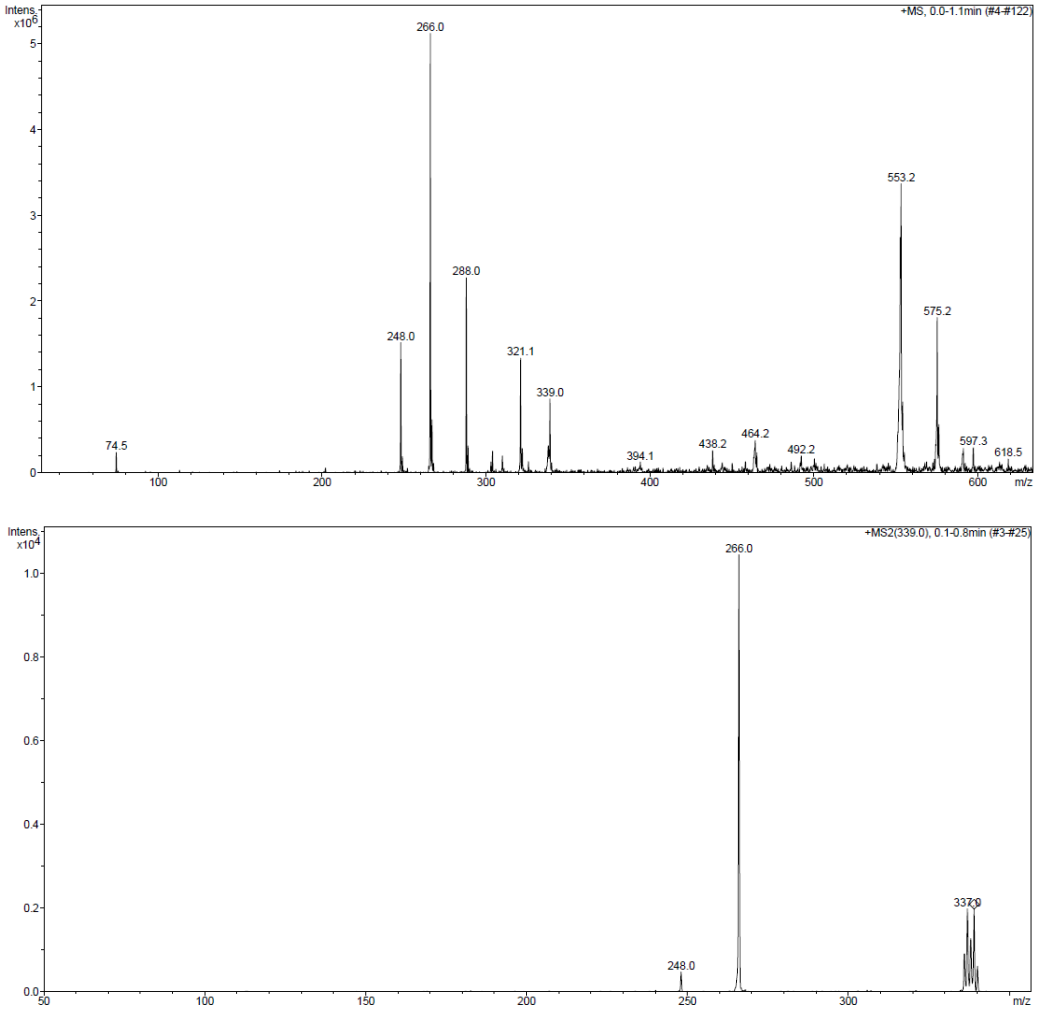


*Figure 3.21 – Positive ESI-MS spectra (direct and MS-MS of 339 ion) of **26f** in methanol.*

Moreover, consistent data were obtained also from the infrared spectra of compound **26f** (Figure 3.22). The broad band covering the range from 2800 to 3400 cm^{-1} is identified as the O-H stretching of the sugar moiety superimposed to H-N stretching of the amide group (at 3387 cm^{-1}) and the NH_3^+ stretching of the ammonium ion. At the same time two stake-shaped band at 1701 and 1642 cm^{-1} were observed and assigned at the C=O stretching of carboxylate and carboxamide groups, respectively.

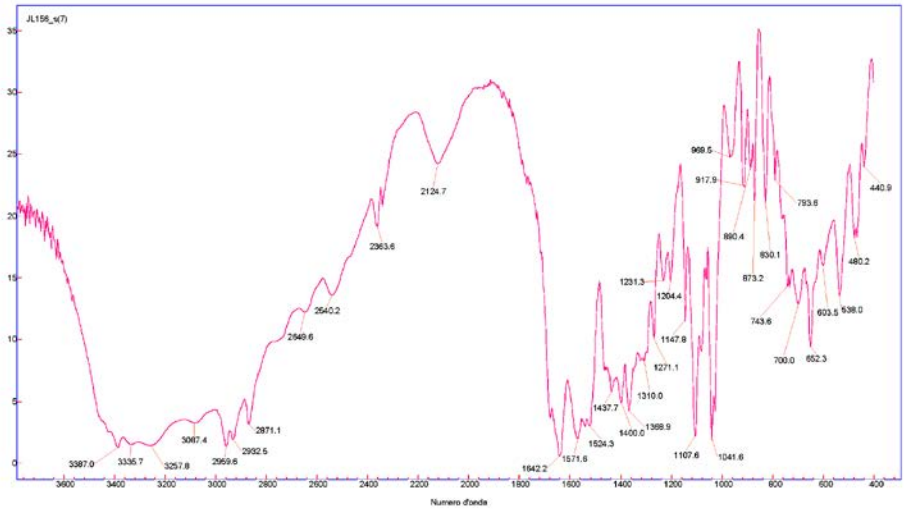
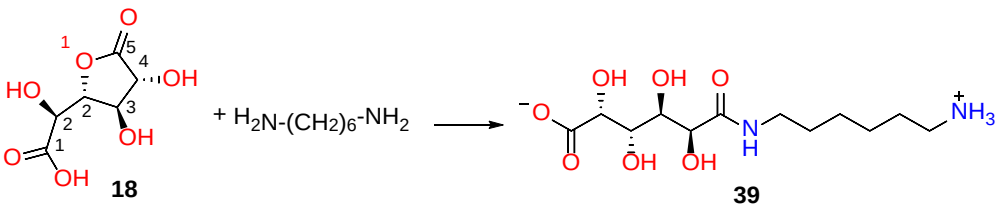


*Figure 3.22 - FT-IR spectrum of compound **26f**.*

3.5.2 - Synthesis of zwitterionic salt 39 ((2R,3S,4R,5S)-6-((6-ammoniohexyl)amino)-2,3,4,5-tetrahydroxy-6-oxohexanoate).

A special mention deserves the behaviour of primary alkylenediamines (i.e. hexamethylendiamine **37p**). Reaction of this compound with DMSO solution of lactone **18** provides in excellent yield the corresponding internal amide salt **39** ((2R,3S,4R,5S)-6-((6-ammoniohexyl)amino)-2,3,4,5-tetrahydroxy-6-oxohexanoate) in strictly 1:1 reaction (Scheme 3.15).



*Scheme 3.15 – Symthesis of zwitterionic salt **39** by condensation of **18** with hexamethylenediamine in DMSO.*

The reaction occurs at room temperature at molar ratio 1:1 and the internal salt **39** can be isolated in nearly quantitative yield. The reaction was carried out at high concentration of substrates in DMSO and after 2 hours the initial heterogeneous system became homogeneous as a jelly-like viscous liquid. A sample, analysed by ^{1}H-NMR, confirms the end of the reaction and the presence of the switterionic compound (**39**). The procedure is similar to the one used by Kiely on the synthesis of N'-alkylammonium D-aldaramic acid terminal carboxylate zwitterionic salt from D-glucaric acid.[28] The method has the advantage to provide a strictly 1:1 ratio of basic and acid functional groups in a solid,

which can be purified at high level, so that it can represent a remarkable precursor for polycondensation processes to polygalactaramides of high molecular weight.

Analytical data for compound **39** fully agrees with the proposed zwitterionic structure. So, the ^{1}H-NMR spectra reported in Figure 3.23 in DMSO-D6 and D_2O, show amide protons at 8.0-8.1 ppm in the DMSO spectra but not in water where they exchange. The four protons of sugar portion of the molecule appear also quite different in the two solvents, with more complex patterns and shift at higher field in DMSO. On the contrary, better resolution can be seen in the D_2O spectra for the protons of the amine portion in molecule, with clearly different methylene protons close to the amide group (at 3.09 ppm in DMSO-D6 and 3.32 n D_2O) from the ones close to the ammonium group (at 2.71 ppm in DMSO-D6 and 2.88 n D_2O).

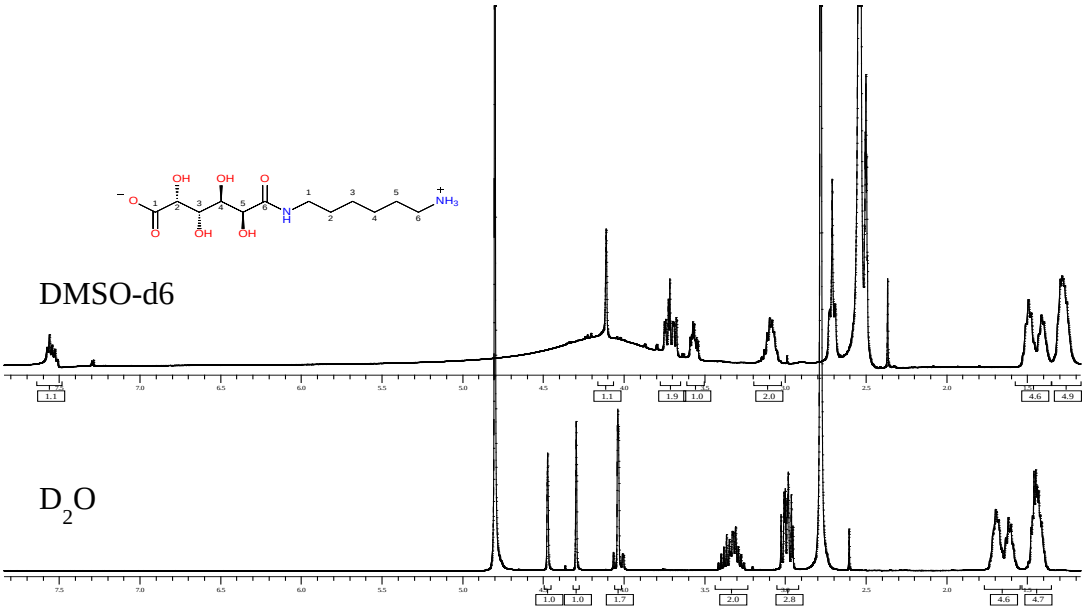


*Figure 3.23 - Assigned ^{1}H-NMR spectrum of **39** in DMSO-d_6 and D_2O.*

In addition, the mass spectrum (negative ions in the insert, shown in Figure 3.24) of the solid is consistent with the formation of the switterionic compound **39**. Between the relatively high number of ions, the molecular ion at m/e 307 [M-H] is recognized but also the dimeric ion at m/z 615 [2M-H] and the fragment at m/z 249 [M – $C_4H_9NH_2$]. The positive mass spectrum is simpler, with the base peak of molecular ion at m/e 309 [M+H].

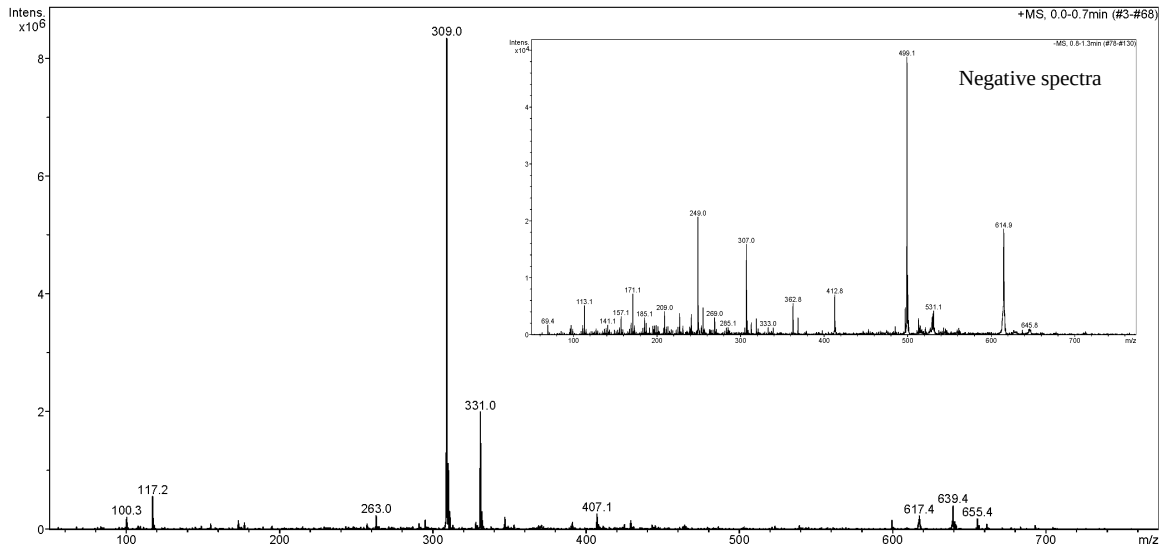
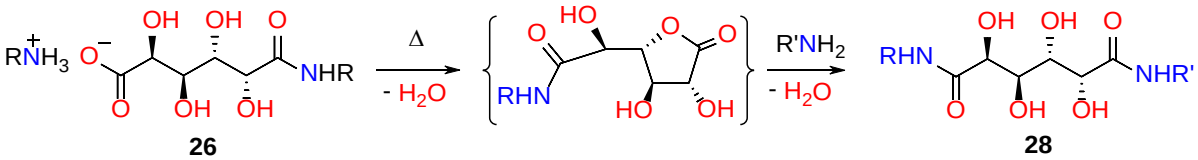


*Figure 3.24 – ESI-MS negative and positive spectra of compound **39** in methanol.*

3.5.3 - Synthesis of N-substituted galactaramides.

The availability of a large variety of amide salts evidenced in previous section provides the potentiality to access symmetrical galactaramides **28** in highly efficient way via the formation of the corresponding amidelactones (Scheme 3.16). In principle, the sequence can also be used to synthesize unsymmetrical galactaramides.



Scheme 3.16 - Thermal aminolysis of ammonium galactaramides **26** to unsymmetrical or symmetrical N-substituted galactaramides **28**.

The amidation process occurs by heating the salt at moderate temperatures (80-120°C) in condition fully compatible with the hydroxyl group of the sugar backbone and possible substituents on the amide residue. By tracking the aminolysis reaction through ^{1}H-NMR spectra, the mechanistic hypothesis of Scheme 3.16 was verified by identifying the amide substituted galactaro-1,4-lactone as intermediate. From the NMR spectrum (Figure 3.25) of the reaction mixture, compared with the one of the starting phenylmethanaminium mono amide galactarate **26d** and of the product galactarodiamide **28d**, an

intermediates with proton signal in the range 4.15~4.45 ppm can be identified. The proton pattern observed is very similar to the assignment of amide glucaro-1,4-lactone reported in the Kiely's work.[27] The better condition for galactaramides synthesis was to heat the salt **26** in DMSO at 100 °C in a thermostat. The waxy-like initial mixture first became homogeneous and then some solid precipitates. The reaction end after about 4 hours, as for ^{1}H-NMR analysis. A homogeneous sample obtained by dissolving all system in excess of DMSO, analysed by the same technique, confirms that the N-substituted galactaramides **28** were formed in high yield (70-90%). The products were precipitated largely from DMSO solution as white solids and can be purified in high yield by recrystallization using acetone or acetonitrile as co-solvent. Different kinds of mono amide galactarate salts were used successfully for this indirect galactaramide syntheses. Representative examples of the procedure, with the specific reaction conditions investigated, are reported in Table 3.10.

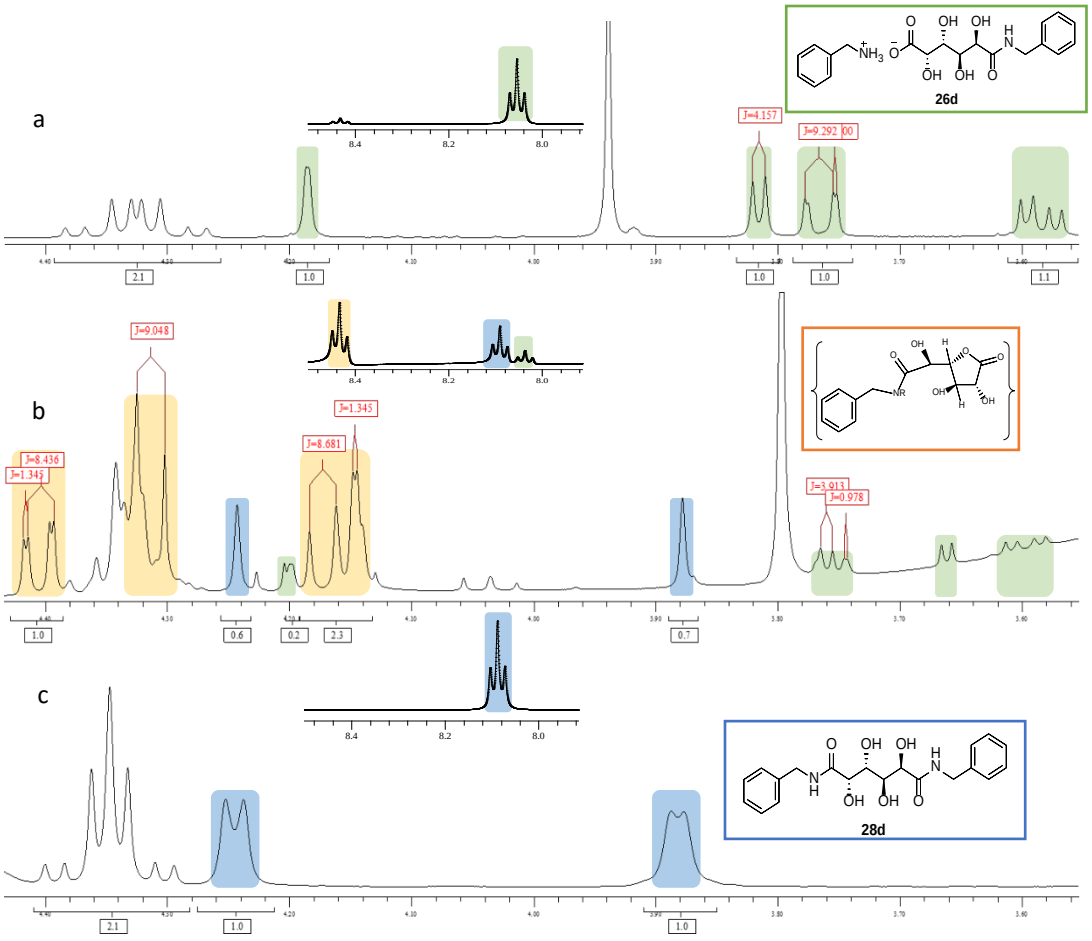


Figure 3.25 - ^{1}H-NMR spectra of: ***a)*** *starting material* ***26d****;* ***b)*** *thermal aminolysis reaction mixture;* ***c)*** *product* ***28d****.*

We have also verified that the aminolysis reaction can be otained from the acyclic galactaric acid and primary amines in neat condition, but at higher temperatures. The best condition found were at 160 °C for 4-6 hours, in 70-90% yields depending on the molar ratio of amine to acid (2-5) used. Despite the

good analytical results, isolation of pure galataramides from these reaction mixtures was more challenging, affording yellow colored products contaminated by variable amount of by-products. Only recrystallization from an appropriate solvent (acetonitrile, methanol) affords finally a product with purity higher than 98%.

*Table 3.10 - Indirect synthesis of galactaramides **28** by thermal decomposition of **26**.*

Entry	[v.](mmol)	Amine	T(°C)	t (h)	28 (Yield %)
GICR28b	1.56	n-$C_8H_{17}NH_2$	130	2	80.7
JL193	8.8	$C_6H_5CH_2NH_2$	130	2	92
JL194	1.8	$C_6H_5CH_2NH_2$	100	4	90
JL185	9.5	$HOCH_2CH_2NH_2$	140	4	88
JL195	1.8	$H_2N(CH_2)_6NH_2$	100	2	86.5

The symmetrical structure of galactaramides **28** are easily identified by ^{1}H and ^{13}C-NMR analysis on isolated samples. Figure 3.27 and Figure 3.26 show the the ^{1}H-NMR and ^{13}C-NMR spectra of the galactaramide of serinol (N^1,N^6–bis(1,3–Dihydroxy–2–propanyl) galactaramide) **28a**. In the ^{13}C-NMR spectrum there are equivalent carbons C-3 and C-6 at 71.25 ppm, C-4 and C-5 at 71.10 pp), C-2 and C-7 at 173.70 ppm, C-2', C-2", C-4', C-4" at 60.15 ppm, and C-3' and C-3" at 52.55 ppm. From the ^{1}H-NMR of **28a** in both D_2O and DMSO-d_6 (Figure 3.27), the proton signals assigned in the range 4.3 to 5.4 ppm in DMSO are the signals of –OH groups on galactaric acid chain and the ones of serinol, which disappear in D_2O solvent due to the exchangement of OH with D_2O. The rest signal assignment is consistent with a symmetrical galactarodiamide. The equivalent protons are H-3 and H-6 are at 4.14 ppm and the H-4and H-5 at 3.79 ppm, with $J_{3,4}$=$J_{5,6}$= 4.9 Hz, and $J_{4,5}$=4.0 Hz.

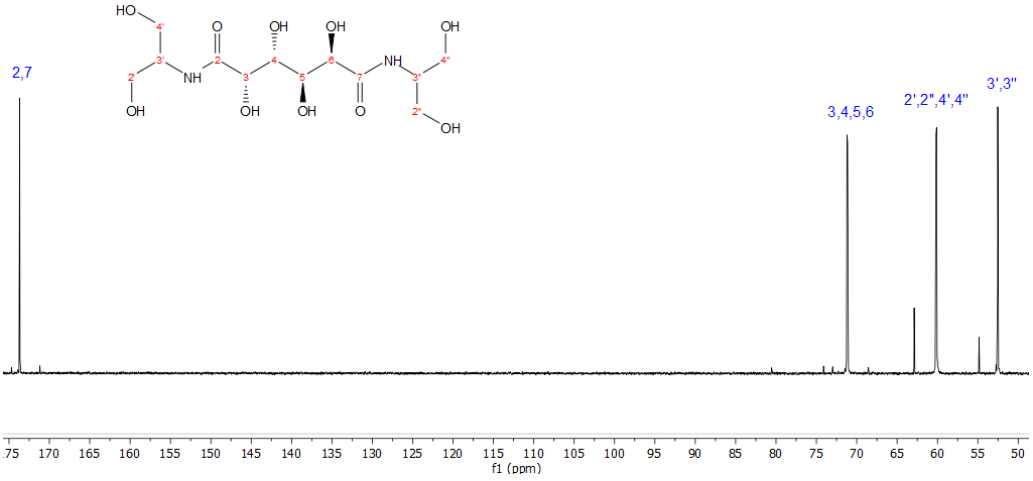


*Figure 3.26- Assigned ^{13}C-NMR spectrum of **28a** in D_2O.*

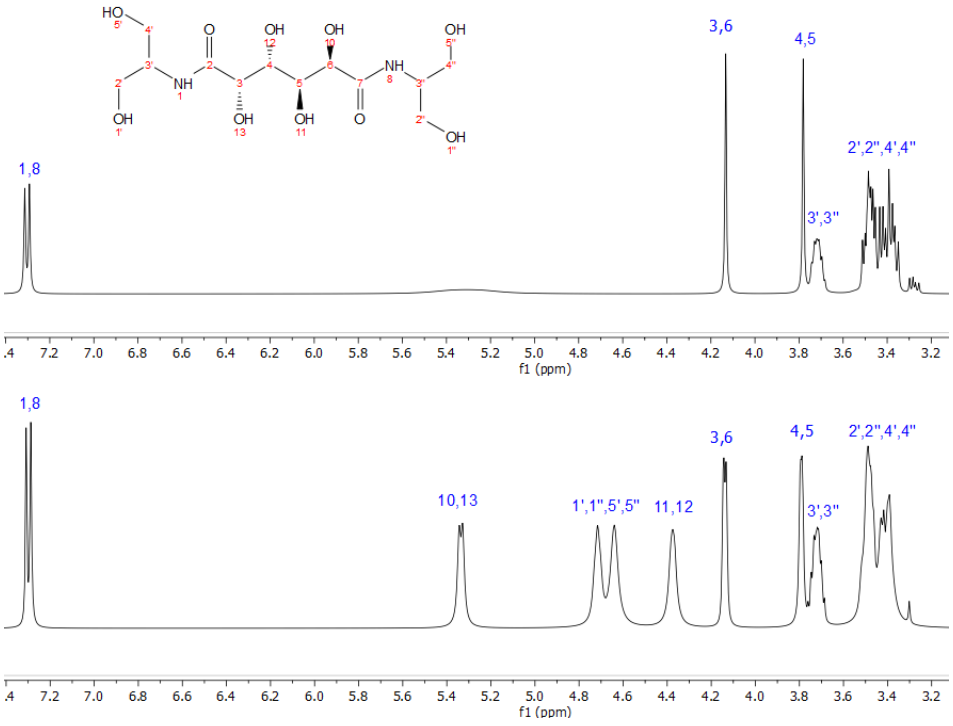


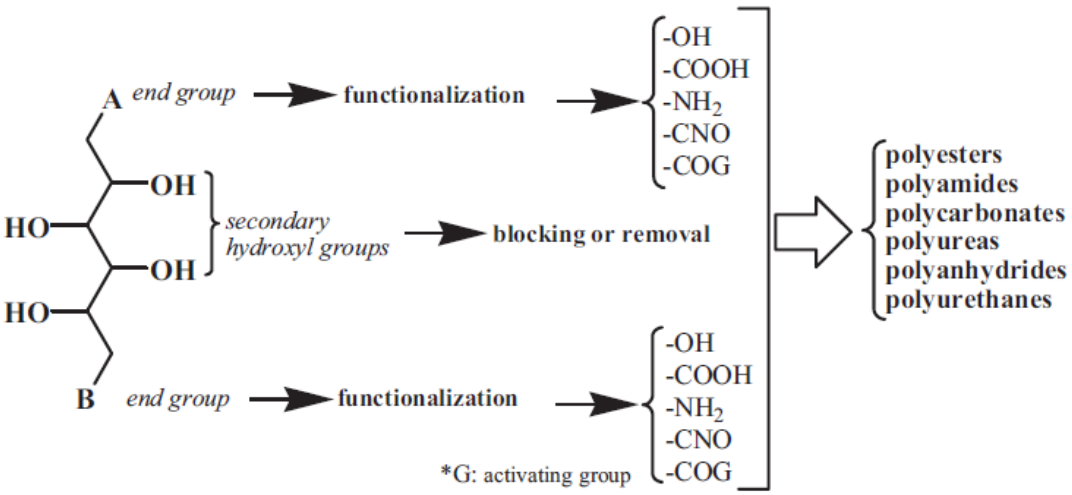
*Figure 3.27- Assigned ^{1}H-NMR spectrum of **28a** in D_2O and DMSO-d_6.*

Also the mass spectrum (negative ions) of the reaction mixture is consistent with the formation of **28a**. The molecular ion of m / z = 355 [M-H] can be recognized in the spectrum. Their fragments 282 [M – serinol+H_2O] -, 265 [M-serinol] are also compatible with an acyclic galactaramide.

3.5.4 - Synthesis of polyalkylengalactaramides

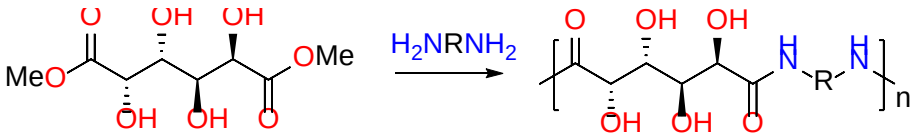
The easy availability of amide salts **26**/**27**, diamides **28** and switterionic alkylenamido salts **39** of galactaric acid has finally stimulated our attention on the preparation of polygalactaramides and specifically the polyalkylengalactaramides. This study is a part of the more general attention to polymers derived from aldaric following the general vision depicted in Scheme 3.17.

Aldaric acids are treated as a potential building block for synthesizing valuable monomers and polymer crosslinking agents thanks to their high functionality. Amide derivatives such as diaminoaldaramides, dihydroxyaldaramides, bis(alkoxycarbonylalkyl)aldaramides, and bis(carboxyalkyl)aldaramides were synthesized as potential monomers and crosslinking agents for further polymeric application.[30]



Scheme 3.17 - Linear polycondensates from carbohydrates.[29]

The very first synthesis of hydroxylated nylons from aldaric acid were carried out by Ogata [31, 32] and coworkers in 1970s. The diesters of galactaric acid were first prepared and then polycondensed with primary diamines to form the polymer (Scheme 3.18). Then, more examples of polyhydroxypolyamides syntheses from esterified galactaric, xylaric and D-glucaric acid were reported at laboratory scale.[33, 34] For mannaric acid, Carpenter and Kiely[35] started from the D-Mannaro-1,4:6,3-dilactone, after esterification with methanolic hydrogen chloride to a mixture of methyl ester lactone and acyclic dimethyl mannarate, and aminolysis by methylamine in methanol with an overall yield of 52.9% in diamide. Using diester of aldaric acid as activated monomer precursor, the yield was lower and the mean molecular weight of the polymer was less than 6,000, owing to various side reactions.

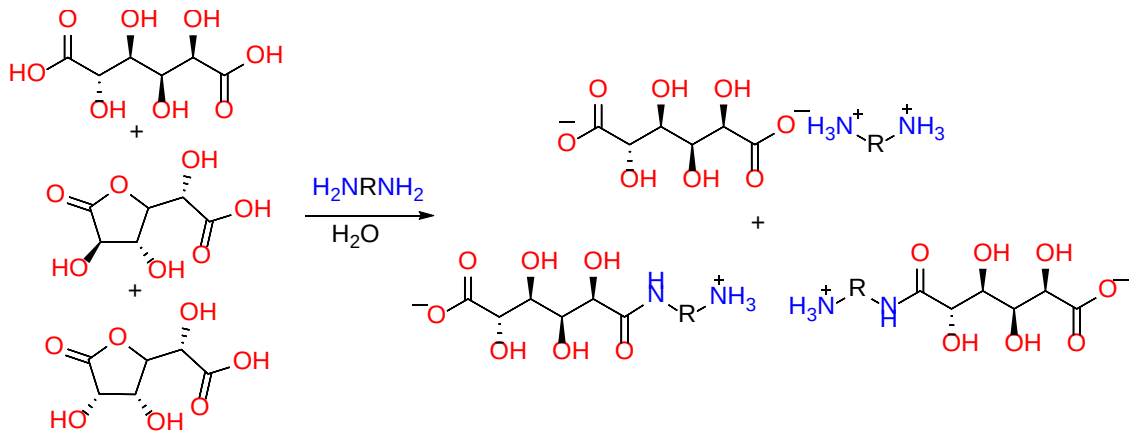


Scheme 3.18 - Polycondensation between dimethyl galactarate ester and alkylenediamine.[34]

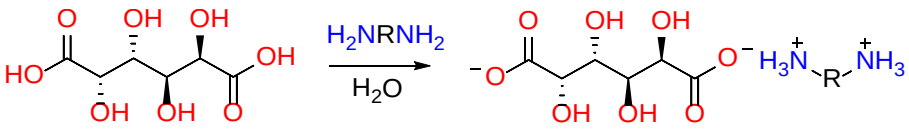
It has already been reported that the 1,4-lactonization was just observed during the aminolysis of aldaric acid diester, suggesting that all esters of aldaric acids (i.e. acyclic dialkyl aldarates, aldarate ester/lactones or aldarate dilactones) can similarly undergo diaminolysis and further polymerization.[36]

Furthermore Kiely [28, 30] and co-workers have developed the direct use of D-glucaric acid (and seldom galactaric acid) without pre-esterification in order to prepare polyhydroxypolyamides. D-glucaric acid, together with the equilibrium lactone and dilactone forms, were reacting with alkylene based diamine

to synthesize a mixture of diamine salt and a N'-alkylammonium (or alkylderived)-D-aldaramic acid terminal carboxylate zwitterionic salt (Scheme 3.19). By a careful selection of conditions, also the galactarate salts of the diamine were converted into polyhydroxypolyamide (Scheme 3.20). With galactaric acid, only the first approach was verified and amides were obtained in moderate yield. By comparing the two processes, the differences could be interpreted, on the basis the results reported in previous section, as due to the presence of the lactone form of D-glucaric acid in the first conditions, which can led to the formation of ammonium mono amide mono glucarate zwitterionic salt.



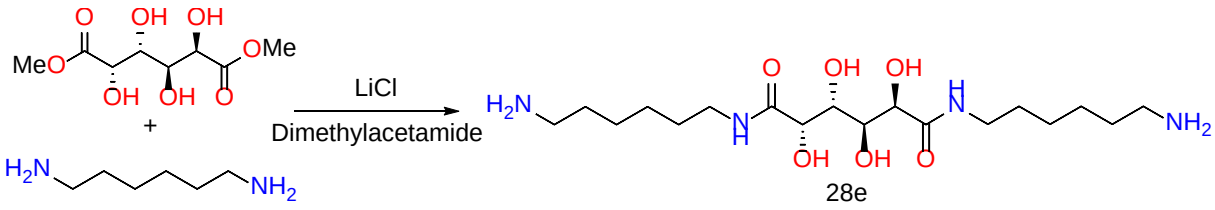
Scheme 3.19 - The approach of Kiely to the synthesis of glucaramides via the conversion of the equilibrium mixture of D-glucaric acid and its lactone forms in water to alkylene diammonium D-glucarates or mixtures of 1 and 6-N'- ammoniumalkyl (or alkyl derived)-D-glucaramates.[28]



Scheme 3.20 - The conversion of galactaric acid in water to alkylene diammonium galactarate.[28]

Furthermore, diamides of aldaric acid were also prepared to be used as monomers or polymer crosslinking agents. Some early trials of diamidation followed the protection route of diamine by esterification in order to avoid the formation of intramolecular oligopeptides.[37] The hydroxyl groups of corresponding dicarboxylic acid such as tartaric acid needed also to be protected. The carboxyl groups were activated conventionally as acyl chloride and was reacted with protected amino acid esters. Then, the diamides was obtained after deprotected.[38]

By the dialkyl ester approach, difunctional galactaramides and glucaramides were synthesized from the corresponding methyl esters and different diamines at temperature higher than 55 °C. For example, N^1,N^6-Bis(6-aminohexyl)galactaramide was synthesized with 96% yield (Scheme 3.21). [30]



Scheme 3.21 - Kiely's route for N^1,N^6-Bis(6-aminohexyl)galactaramide synthesis. [30]

We verified the possibility to obtain the polyalkylenegalactaramides by using the zwitterionic salt **39** as reagent and found that polymers can be obtained at moderate temperature (100-140°C) in solvent DMSO and DMA. The high melting point of the internal salt **39** has prevented untill now the melt polymerization without extensive darking. Some representative results of this polymerization process are reported in Table 3.11.

*Table 3.11 – Synthesis of poly(alkylene)galactaramides by thermal decomposition of salts **39a-c**.*

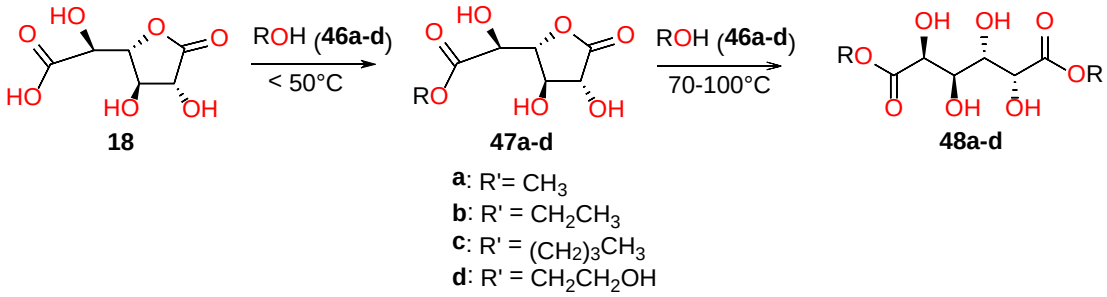
Entry	# of methylene groups in amine	[Subst.] M	T (°C)	t (h)	Polymer (Yield %)	M_n	DP
1	6	0.1	130	2	81	1660	5.7
2	6	1.0	130	2	86	3200	11.4
3	6	1.5	130	2	91	7020	25.0
4	6	1.5	100	6	82	2200	7.8
5	6	1.5	160	1	80	6700	23.9
6	2	1.5	130	2	91	5600	25.1
7	4	1.5	130	2	85	6110	24.2

The number average molecular weight (M_n) and the degree of polymerization (DP) was determined by end-group analysis calculating the ratio of the integrals for methylene protons adjacent to terminal amine groups with those for methylene protons adjacent to internal amide nitrogens and applying the formula DP = (Ratio + 1)/2. The DP values observed are higher than the ones generally observed starting from the diester and the disalt. This is a further example of the potentiality of our approach

which is adressed to isolate relevant intermediates to better undestand and control the polymerisation process. Moreover, it must be noticed that the polymerisation can be obtained at the relatively low temperatures. Under these condition in fact the darkening of the reaction generally seen in the preparation of polyhydroxyaldaramides from esters or acids can be strongly prevented and the polymer appears as very light yellow solid (or viscous liquid when the DP was low).

3.6 - Esterification

The esterification of OH groups of galactaric acid is discussed on section 3.4 and is really identified mainly as an intramolecular process occurring via the mixed carboxylic acetic anhydride. The esterification of the COOH groups was experimented in this thesis only to synthetize esters of galactaro-1,4-lactone or some galactarate esters to be used as references or as reagents to reproduce previous reported synthesis based on these compounds. The more appropriate conditions found to synthetize the galactarolactone alkyl esters were the addition of the concentrated DMSO solution of galactaro-1,4-lactone **18** to an excess of the alcohol (**46a-d**) and then addition of 1-5% of a strong acid (H_2SO_4 or heterogeneous Amberlist 15 resin) at 0-5°C and then warming at 40-50 °C for 3-6 hours. The obtained yield were never quantitative and allowed to isolate 65-75 % of the lactone esters **47a-d**. On more drastic conditions (80-100 °C) the main product obtained were the diesters **48a-d** along with some lactone **47a-d**. Good yield of dimethyl and diethyl esters were obtained when distillation of the alcohol was applied during the reaction. Quantitative yield of dimethyl galactarate were obtained when diazomethane was used as reagent for the analytical determination of galactaric acid.



*Scheme 3.22 – Esterification reaction of **18** with alcohols.*

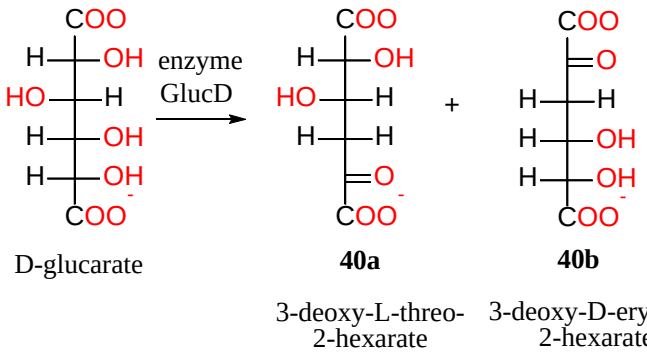
*Table 3.12 - Synthesis of alkyl galactarato1,4-lactone ester **47a-d** or alkyl galacatarates **48a-d** by esterification with alcohols **46a-d**.*

Entry	Alcohol	[46]/[Subst.]	Catalyst (%)	T (°C)	t (h)	47 (Yield %)	48 (Yield %)
1	**46a**	15	H_2SO_4 (5)	40	6	50	2
2	**46b**	10	H_2SO_4 (5)	50	4	75	7
3	**46b**	10	H_2SO_4 (2)	80	3	11	81
4	**46b**	15	H_2SO_4 (2)	80*	4	8	88
5	**46b**	10	Amb. 15	80	4	14	77
6	**46c**	10	H_2SO_4 (3)	45	10	80	6
7	**46c**	10	H_2SO_4 (3)	118	3	3	91
8	**46d**	1.5	H_2SO_4 (5)	50	4	69	9

3.7 - Mono(elimination) of Water in Galactaric Acid Derivatives

As discussed in Chapter 2, the elimination reaction of hydroxyl group represents an important part of the reactivity of aldaric acids and their derivatives but was never investigated in detail. Water elimination from carbohydrates is a well-known process in biological systems, offering routes for their anabolism or catabolism. In aldaric acid the reaction is also of industrial interest because it represents an essential step for the conversion to acyclic mono-eliminated derivatives (i.e. in the case of glucaric acid, 5-dehydro-4-deoxy-glucarate (DDG, 3-deossi-L-treo hexarate, **40a**) and 5-keto-4-deoxyglucarate (5-KDG, 3-deossi-D-treo hexarate, **40b**)) into 2,5-furandicarboxylic acid and its derivatives. For instance, potassium DDG in acetic acid/water medium and HBr as catalyst at 80°C has been reported to give 2,5-furandicarboxylic acid in 92% yield on lab scale.[39-41]

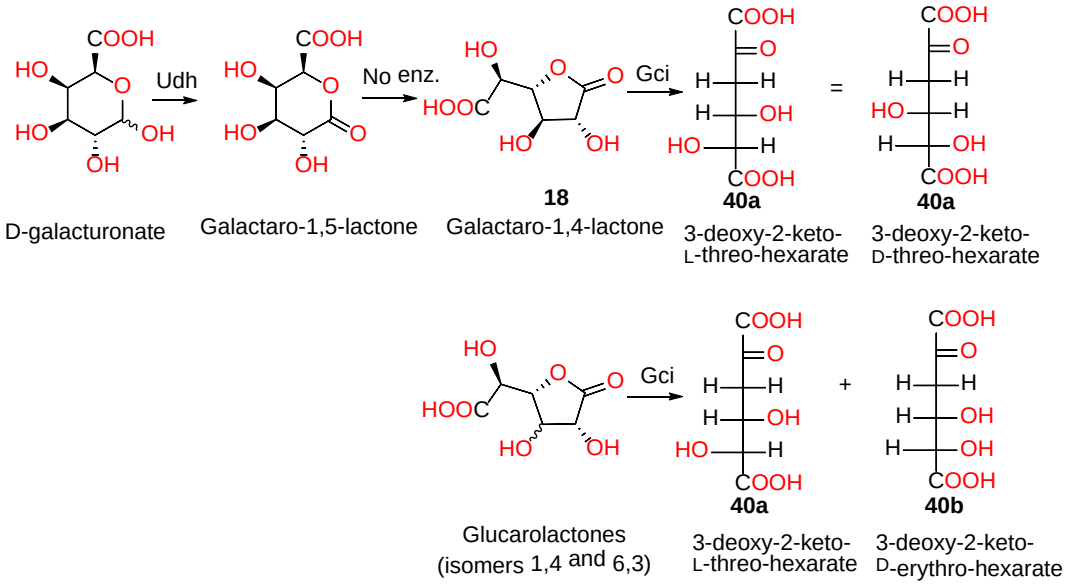
The first synthesis of these acyclic monoeliminated derivatives was reported in 1968 by the enzyme Glucarate dehydratase (GlucD) from *P. putida.* This enzyme catalyses the dehydration of D-glucarate to a 92:8 mixture of keto derivatives **40a** and **40b** (Scheme 3.23).



Scheme 3.23 – Enzyme catalysed water elimination from D-glucarate ion in 5-keto-4-deoxyglucarate (3-deoxy-L-threo-2-hexarate, 5-KDG) ***40a*** *and 2-keto-3-deoxyglucarate (3-deoxy-D-erythro-2-hexarate, 2-KDG)* ***40b****.*

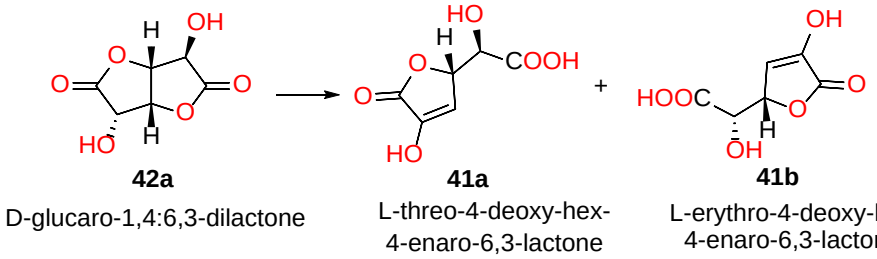
Our investigation on this point started from galactaric acid protected at the hydroxyl groups because literature analysis indicates that no selective removal of water from the unprotected acid occurs at low temperature and that a quite complex pattern of reactions starts at high temperatures. We decided to test for this scope the easily prepared tetracetylgalactaric acid (**2**) and the acetyl and formyl derivative of galactaro-1,4-lactone (**3** and **25**, respectively).

Concerning the biotechnological route, instead of glucaric acid, a novel enzyme galactaro-lactone cycloisomerase (At Gci) from *A. tumefaciens* strain was identified in 2012 for the direct conversion of D-galactaro-1,4-lactone into 3-deoxy-L-threo-2-ketohexarate. This purified enzyme is active both on both D-glucarolactones and galactaro-1,4-lactone (**18**) but does not work on the corresponding linear hexaric acid form (Scheme 3.24).[42] During this process, the starting material D-galacturonic acid was first oxidised into galactaro-1,5-lactone and finally converted to the more stable galactaro-1,4-lactone (**18**) by catalysis of an uronate dehydrogenase (Udh) from *A. tumefaciens* C58 strain. Next step was the opening of the lactone to the acyclic 3-deoxy-2-keto-galactarate under catalysis of the At Gci enzyme. The reaction is initiated by the base induced proton-abstraction from C-H bond α to the carboxylic acid coordinated to exogenous Mg^{2+} ions. Products formed from D-galactarolactone **5** or D-glucarolactone are shown in Scheme 3.24. Thanks to the meso form of galactaric acid, the configuration of 3-deoxy-2-keto-L-threo-hexarate **40a** is the same of 3-deoxy-2-keto-D-threo-hexarate **40a**, which has the advantage to simplify its purification for further syntheses.



*Scheme 3.24 - Oxidative pathway of galacturonate to D-galactaro-1,4-lactone **18** and its further conversion to compound **40a** by Gci enzyme, compared with the isomeric glucarolactones.*

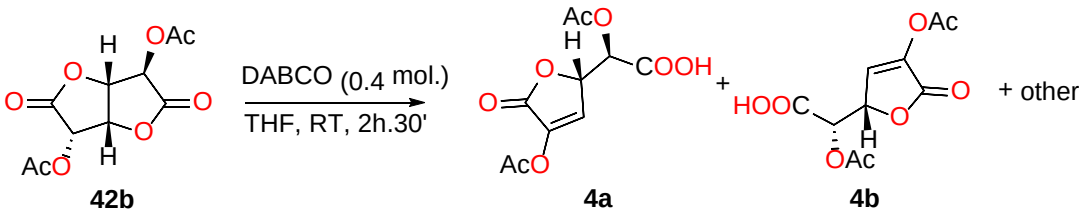
Isolation of carbonyl compounds 40a or 40b was not reported, but Gehret in 2009 isolated the cyclic derivatives of **40a** and **40b** (i.e. L-threo-4-deoxy-hex-4-enaro-6,3-lactone (**41a**) and L-erythro-4-deoxy-hex-4-enaro-6,3-lactone (**41b**), respectively) starting from D-glucaro-1,4:6,3-dilactone **42a**. (Scheme 3.25).[43] For this reason these lactones should be interesting intermediates for the synthesis 2,5-furandicarboxylic acid, when hydrolysed in water to keto derivatives **40**.



*Scheme 3.25 - Synthesis of **41a** and **41b** from D-glucaro-1,4:6,3-dilactone **42a**.*[41]

In our previous work, we prepared via chemical routes mainly two derivatives of lactones **41a** and **41b**, namely L-threo-4-deoxy-hex-4-enaro-6,3-lacton-2yl tetracetate **4a** and L-erythro-4-deoxy-hex-4-enaro-6,3-lacton-2yl tetracetate **4b** starting from D-glucaro-1,4:6,3-dilactone diacetate **42b** (Scheme 3.26). The process was carried out in THF under catalysis of DABCO (0.4 equiv) at room temperature for 2.5 hours. The analysis by ^{1}H-NMR showed that diacetylated lactones **4a** and **4b** were present in

the resulting crude reaction mixture in 10% and 11% yield, respectively, with a 20% of another monoeliminated product.



*Scheme 3.26 - Synthesis of **4a** and **4b** by rearrangement of D-glucaro-1,4:6,3-dilactone diacetate **42b**.*

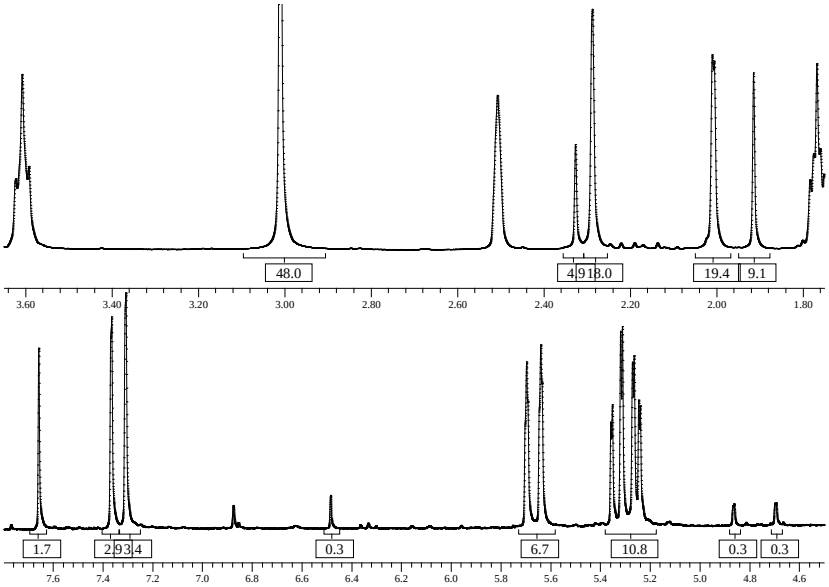
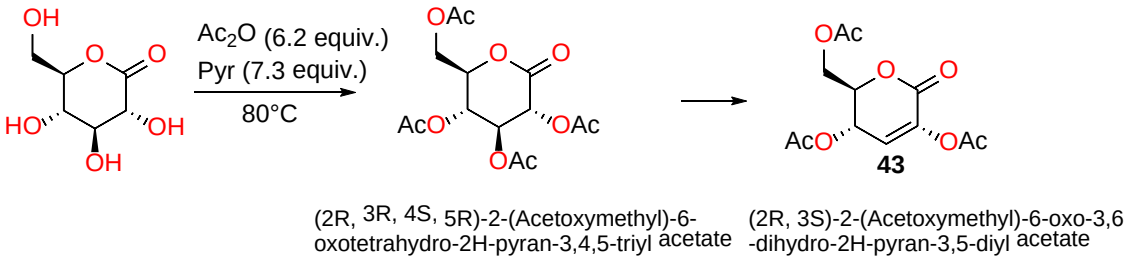


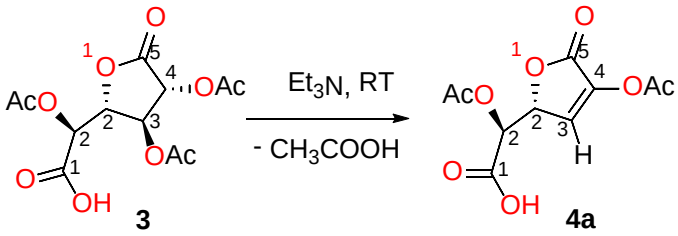
Figure 3.28 - ^{1}H-NMR (DMSO-d6) of the reaction mixture of D-glucaro-1,4:6,3-dilactone diacetate and DABCO in THF (25 °C, 2.5 h).

Then we started to explore the synthesis of **4a** from galactaro-1,4-lactone triacetate **3**. Baumann and co-workers have reported that heating a crude solution of tetraacetylgluconolactone at 80 °C for 10 min in the presence of a catalytic amount of Et_3N (0.25 equiv) resulted in selective formation of the monodeacetylated intermediate **43** (96% conversion, 93% isolated yield by column chromatography) (Scheme 3.27).[44]



*Scheme 3.27 - Synthesis of 5,6-dihydropyrone **43** from tetraacetyl delta gluconolactone according to Baumann.*[44]

In our case, compound **3** in acetic acid was heated with different amount of DABCO (0.8~1 equiv.), or triethylamine, (0.6~1.2 equiv) for different reaction time. Following the reaction by ^{1}H-NMR spectroscopy, diacetylated lactone **4a** was detected in yield from 10 to 38%. The specific reaction conditions used are shown in Table 3.13.

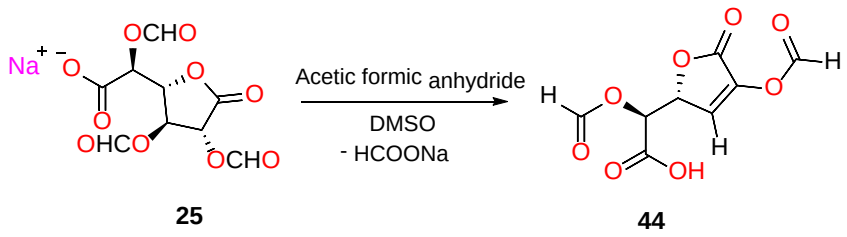


*Scheme 3.28 - Synthesis of compound **4a** from triacetyl galactaro-1,4-lactone* 3.

*Table 3.13 - Base induced elimination of acetic acid from unsaturated lactone **4a** to compound **3**.*

Base	[3] (mmol)	[Base]/[3] ratio	AcOH (ml)	T (°C)	t (h)	3 (Conv. %)	4a (Yield %)
Et_3N	0.69	0.62	4l	80	11	39	28
Et_3N	3.10	0.62	20	80	24	60	33
Et_3N	0.69	1.2	0.17	50	48	90	38
DABCO	1.00	1	5	63	4	50	20
DABCO	0.64	0.8	7	RT	17	57	10

Meanwhile, the sodium galactaro-1,4-lactone triformate **25** in DMSO in the presence of acetic-formic anhydride was converted into L-threo-4-deoxy-hex-4-enaro-6,3-lacton-2yl tetraformate **44** spontaneously at room temperature (Scheme 3.29). ^{1}H-NMR analysis indicated that the conversion of **25** was 100% and the yield of **44** was nearly quantitative.



25 **44**

*Scheme 3.29 - Selective mono-elimination of triformylated lactone **25** into **44**.*

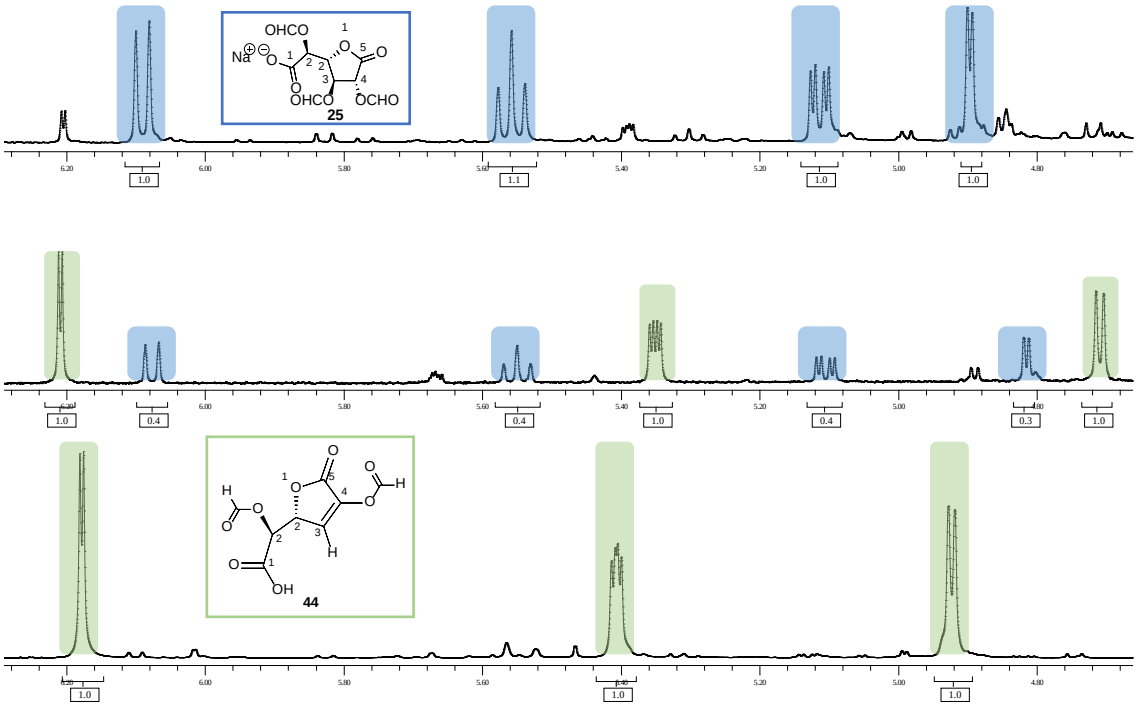
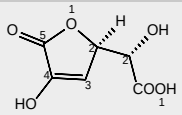
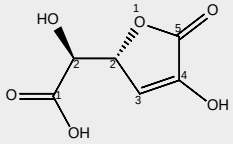
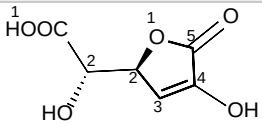
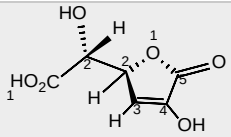
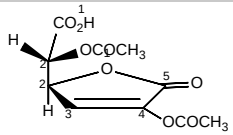
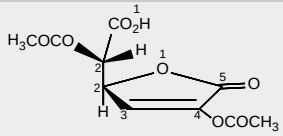
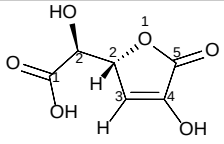
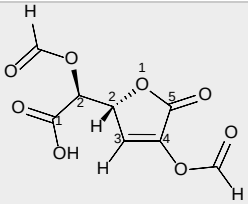


*Figure 3.29 - ^{1}H-NMR spectra (DMSO-D6) of the selective formic acid elimination from triformate product **25** to compound **44** at different reaction time (0, 1h, 4 h).*

For comparison reasons, the ^{1}H-NMR and ^{13}C-NMR chemical shifts of the unsaturated lactones obtained in this study are collected in Table *3.14*. It can be seen that the doublet of vinyl proton occurs in the range 6.0-6.3 ppm in hydroxyl derivatives and at 7.3-7.4 ppm in acetoxy compounds **10** and **11**. The difference is in line with the electron-withdrawing effect of the acetoxy group as typically seen also on saturated derivatives of galactaric acid and, more in general, in sugar derivatives. In accord

with this observation, also the proton on the carbon resonating at lower field shifts from 4.3-4.5 ppm to about 5.3 ppm, and the proton on carbon bearing the lactone oxygen shifts from 5.2-5.3 ppm to 5.7-5.9 ppm.

Table 3.14 - Comparison of ^{1}H and ^{13}C-NMR of unsaturated galactarolactone derivatives.

Products	Solvent	δ H_{vin} (H_d)	δ H_{cen} (H_c)	δ H_{ext} (H_b)	δ ^{13}C	Stereo
 (*S*)-2-hydroxy-2-((*R*)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	acetone [41]	6.25 (d, *J*=2.1 Hz)	5.29 (t, *J*= 2.3 Hz)	4.44 (d, *J*=2.6 Hz)	172.7, 169.4, 144.9, 117.6, 79.7, 70.6	L-eythro
 (*S*)-2-hydroxy-2-((*R*)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid						
 (*S*)-2-hydroxy-2-((*S*)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	Acetone [41]	6.11 (d, *J*=2.1 Hz)	5.21 (dd, *J*= 2.1, 3.7 Hz)	4.52 (d, *J*=3.7 Hz)	-	L-threo
 (*R*)-2-hydroxy-2-((*S*)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	D_2O [45]	6.09 (d, *J*=2.0 Hz)	5.21 (dd)	4.32 (d, *J*=2.5 Hz)	173.5, 171, 142, 117, 79.5, 69	L-erythro
 (*R*)-2-acetoxy-2-((*S*)-4-acetoxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	DMSO	7.36 (d, *J*=1.8 Hz)	5.69 (dd, *J*=1.8, 2.9 Hz)	5.18 (d, *J*=2.9 Hz)	-	(From glucaric acid) L-erythro
 (*S*)-2-acetoxy-2-((*S*)-4-acetoxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	DMSO	7.30 (d, *J*=1.8 Hz)	5.63 (dd, *J*=1.8, 3.1 Hz)	5.31 (d, *J*=3.1 Hz)	-	(From glucaric acid) L-threo
 (*S*)-2-hydroxy-2-((*R*)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	DMSO	6.18 (d, *J*=2.1 Hz)	5.41 (dd, *J*=2.2, 3.5 Hz)	4.92 (d, *J*=3.5 Hz)	-	L-erythro
 (*S*)-2-(formyloxy)-2-((*R*)-4-(formyloxy)-5-oxo-2,5-dihydrofuran-2-yl)acetic acid	DMSO	7.37 (d, *J*=1.8 Hz)	5.72 (t, J=2.3 Hz)	5.21 (d, *J*=3.1 Hz)		L-erythro

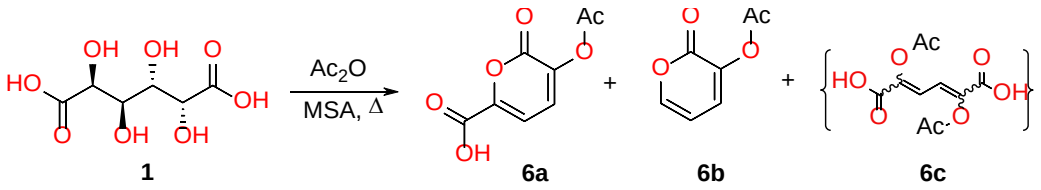
3.8 - Bis(elimination) of Water in Galactaric Acid Derivatives

The next session of the thesis concerned the study of dehydration reactions of the galactaric acid and its derivatives to the corresponding di-unsaturated derivatives, useful precursors for the synthesis of important monomers, such as adipic acid, or for the direct use in the synthesis of new polymers. The choice of the galactaric acid as a model is connected to the commercial availability of this product directly in acyclic acid form, unlike the glucaric acid isomer, which is instead mainly marketed as calcium salt. Free glucaric acid can only be obtained by a medium-duty procedure (actually used also to isolate the corresponding lactone) in mixture with its lactone forms. The loss of two molecules of water from aldaric acid, removing the stereochemistry of the sugar moiety, con provide access to stabilized products by aromatization or pseudo-aromatization, bringing selectivity to the overall process. Moreover, application of this approach on different aldaric acids can produce the same final products improving the economics of these products when inexpensive mixture of aldaric acids are available from biomass residues.

The study on bis(elimination) process was addressed in three different direction to provide three different classes of products which will be discussed separately in the following paragraphs: a) pyrones (section 3.8.1), b) furans (section 3.8.2), and c) pyrroles (section 3.8.3).

3.8.1 - Synthesis of pyrone derivatives from galactaric acid and its lactone

Based on literature data, direct dehydration of galactaric acid is not a viable reaction. A more favorable way involves the conversion of the OH groups into a better leaving group with respect to the hydroxyls, and the subsequent dehydroelimination reaction under acidic or basic catalysis. The approach we followed, not present in the literature, go through the conversion of the hydroxyl groups into an ester groups, followed by catalyzed acid dehydroacylation. At the beginning of the research on di-elimination reactivity of galactaric acid, we exploit the acid catalysis of methanesulfonic acid (MSA) in acetic anhydride. Galactaric acid (**1**) under these conditions afford a mixture of 3-acetoxypyron-2-oxo-6-carboxylic acid (**6a**), its decarboxylation product 2-acetoxypyrone (**6b**), and a small amount of 2,5-diacetoxymuconic acid (**6c**) (Scheme 3.30). This combination was initially evaluated for a scale-up preparative production of compound **6a**.



Scheme 3.30 – Dielimination products in the reaction of galactaric acid with acetic anhydride in the presence of methanesulfonic acid (120°C, 5h).

As mentioned previously, triacetylation reaction of galactaric acid produces the dielimination products, even not selectively. The presence of these 2-pyrone derivatives is clearly established by ^{1}H-NMR on samples of the reaction mixture (Figure 3.30) from the signals at chemical shift between 6 and 8 ppm.

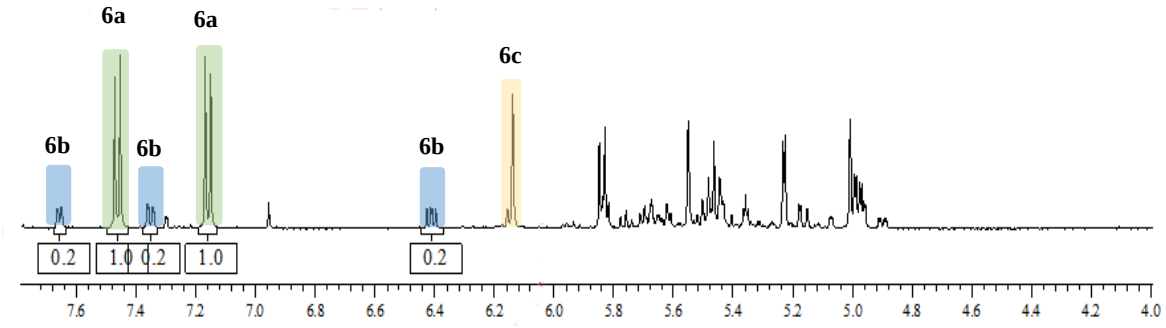


*Figure 3.30 – Di-unsaturated products identified in the ^{1}H-NMR spectra of the reaction mixture of **1** with acetic anhydride and methanesulfonic acid.*

In this region of the ^{1}H-NMR spectrum, it was possible to identify two products: the 3-acetoxy-2-oxo-2H-pyran-6-carboxylic acid (**6a**), with two doublets at 7.16 and 7.46 ppm, and its decarboxylated product 2-oxo-2H-pyran-3-yl acetate (**6b**), with signals assigned at 6.45, 7.36 and 7.66 ppm. The singlet at 6.2 ppm was assigned to one of the stereoisomers of the 2,5-diacetoxyhexa-2,4-dienedioic acid (**6c**) according to literature[46] and our isolation by column chromatography.

Following these reactions by ^{1}H-NMR spectroscopy, it was clear that polyacetylated products was firstly formed as intermediates (Figure 3.30), which were further converted into the corresponding unsaturated 2-pyrone derivatives. From screening experiments under different conditions, it was inferred that product **6a** is thermally sensitive toward decarboxylation and is converted into **6b**, lowering the yield of **6a** and the atom economy of the overall process. In order to optimise the reaction conversion and selectivity, reaction temperature and reagents ratio were chosen as the most significant parameters. The dehydroacetylation reaction was found to start only at temperature higher than 120 °C, meanwhile pyrone **6a** starts to decarboxylate at temperature higher than 100 °C. Furthermore, the effect of the molar ratios between the reagents: substrate, acetic anhydride and methanesulfonic acid, was exploited, providing the results summarized in Table 3.15. Compound **6a** was purified by

crystallization from water or from a mixture of acetic acid and formic acid ad looks as a white microcrystalline powder. The best yields of **6a** were obtained with a ratio MSA/galactaric acid of 0.8:1 at 128 °C for 9 hours with simultaneous higher yield of decarboxylated derivative **6b** and reduced amount of the 2,5-diacetoxymuconic acid **6c**. The low amount of this compound at higher reaction times suggested that equilibration of **6c** to **6a** could occur via *trans-cis* isomerization.

In addition, the reaction was verified also on pure tetraacetylgalactaric acid (**2**) and triacetylgalactaro-1,4-lactone (**3**) as starting material in acetic anhydride, since these compounds were certainly formed in the direct reaction with galactaric acid. Our conviction was that the dehydroacetoxylation could be more selective in the absence of the mixture of the hydroxyacetate intermediates.

Table 3.15 – Synthesis of 3-acetoxy-2-oxo-2H-pyran-6-carboxylic acid under different conditions.

Substrate	$[(Ac)_2O]$/ [sub]	[MSA]/[sub]	T (°C)	t_{tot} (h)	Yields (%)		
					6a	6c	6b
1	8	3	140	4	54	15	12
1	8	3	120	9	57	15	15
1	8	3	120	4	49	18	12
1	8	0.8	128	9	73	5	14
1	8	0.6	128	9	60	3	12
1	8	0.4	128	9	33	2	8
1	16	0.8	132	6.7	56	3	8
2	4	2	120	4.8	46	13	10
3	4	2	120	4.8	69	17	14

From the results shown in Table 3.15, the yield of **6a** from **3** is higher than the reaction from **2** and can improve further by increasing time. From the ^{1}H-NMR of the reaction mixture (Figure 3.31) it is clearly seen that compound **2** was first lactonized into **3** and then further dehydroacetylated into the final product **6a**.

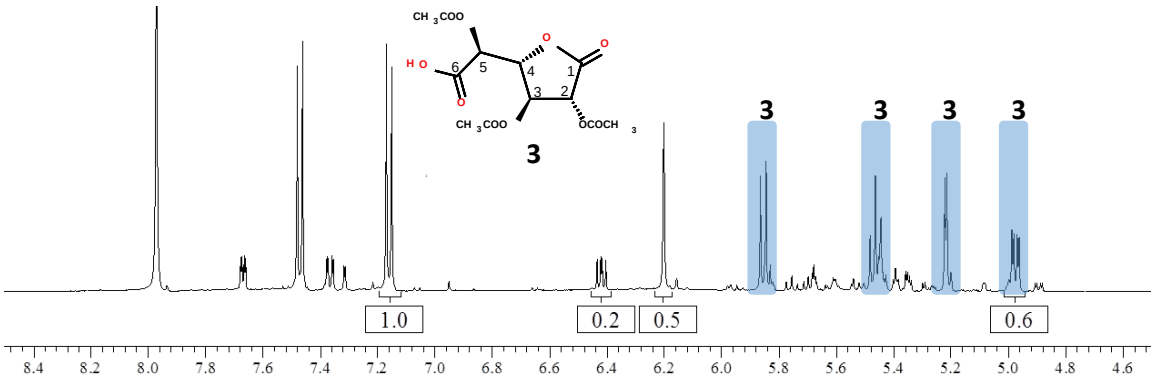
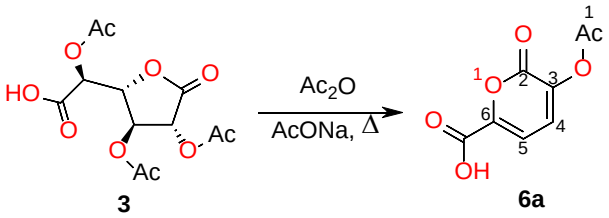


*Figure 3.31 - ^{1}H-NMR spectrum of reaction mixture of **2** with acetic anhydride and MSA.*

Finally yet importantly, the decarboxylation reaction was significantly favoured by the high acidity and high temperature, but selectivity towards **6a** remains moderate.

In order to increase further the selectivity of the process and recover more information on the mechanism of formation of **6a**, we investigated the dehydroacetylation reactivity of acetyl galactaric acid derivatives in a buffered medium adding a base. A previous literature report[47] suggested that an *α*-pyrone, probably 4-acetoxy-6-ethoxycarbonyl-α-pyrone, can be obtained by reaction between monoethyl galactarate (or its monolactone) in acetic anhydride with sodium acetate at 100 °C, but the yield reported were always lower than 30% and the product was not well characterized.



*Scheme 3.31 – Synthesis of pyrone **6a** by bis(dehydroacetylation) of triacetyl derivative **3** in acetyc anhydride in the presence of sodium acetate.*[45]

We verify these conditions on isolated galactaro-1,4-lactone triacetate **3** by using acetic anhydride as solvent and stochiometric amount of sodium acetate, verifying that product **6a** was more easily formed and in yield comparable with the best one obtained in acidic medium. We also verify the use of bases different from sodium acetate, such as DABCO, 4-dimethylaminopyridine (DMAP), lithium acetate, and sodium hydroxide. The specific reaction conditions tested are shown in Table 3.16. By optimising the reaction condition according to the description in Chapter 4, the product **6a** was synthesized by reacting **3** with 10 equivalent of acetic anhydride and 0.1 equivalent of sodium acetate (respect to reagent **3**) at 100 °C for 8 hours in 74% yield. The lower temperature and the basic catalyst prevented the formation of decarboxylated by-product **6b**.

*Table 3.16 - Synthesis of **6a** and its salts (**7a** and **7b**) by reacting **1** or **2** with different bases.*

Substrate	[(Ac)$_2$O]/[sub]	Base	[Base]/[sub]	T (°C)	t_{tot} (h)	Product (Yield %)
1	6.67	DABCO	0.15	100	15	**6a** (75)
1	6.67	DMAP	0.15	100	15	**6a** (52)
1	6.67	AcOLi	0.15	120	9	**6a** (65)
1	6.67	AcONa	0.15	100	9	**6a** (74)
1	6.67	NaOH	0,15	100	9	**6a** (61)
1	8.9	DABCO	1	80	4	**7b** (52)

1	6.7	NaOH	1	95	4	**7a** (71)
1	6.7	NaOH	1	70	4.5	**7a** (39)
1	10	AcONa	1.5	70	4.5	**7a** (32)
2	15	AcONa	1.1	135	4	**7a** (98)

Very exciting was the result obtained with tetracetylgalactaric acid (**2**), where nearly quantitative yield of **6a** was observed. Because these conditions prevent the presence of traces of water, it was assumed that this reagent could induce side reactions or that mixed galactaric acetic anhydride could be involved in the process.

Compound **6a** was isolated as a white solid having the ^{1}H-NMR spectrum of Figure 3.32, with two doublets in the range 7.0 to 7.5 ppm assigned to vicinal aromatic protons on the six membered pyrone ring. The signals are typical of aromatic protons, as expected from the resonance structures of the 2-hydroxypyrone ring.

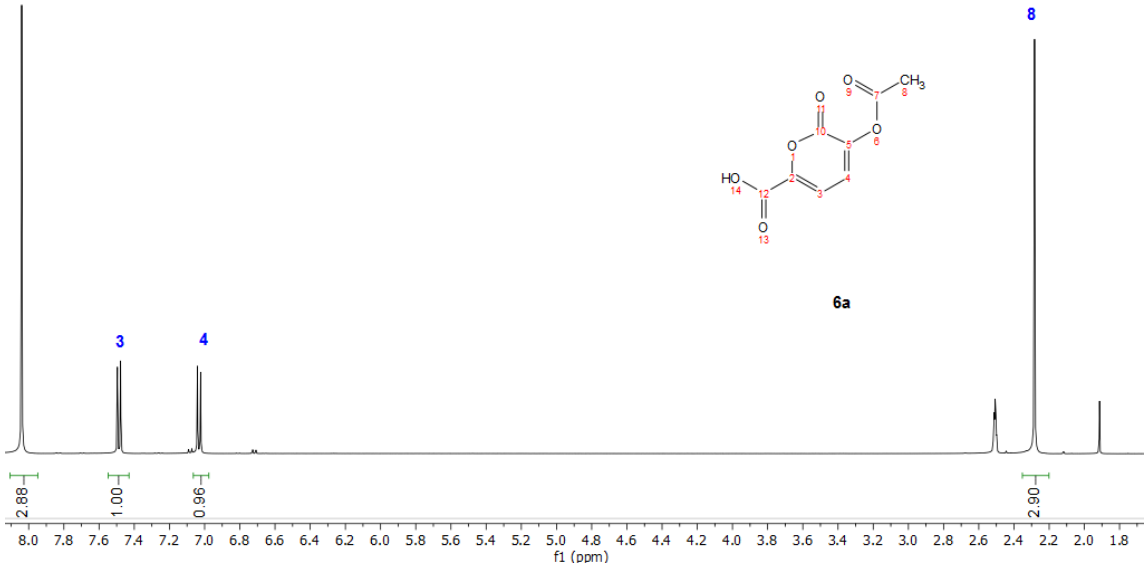
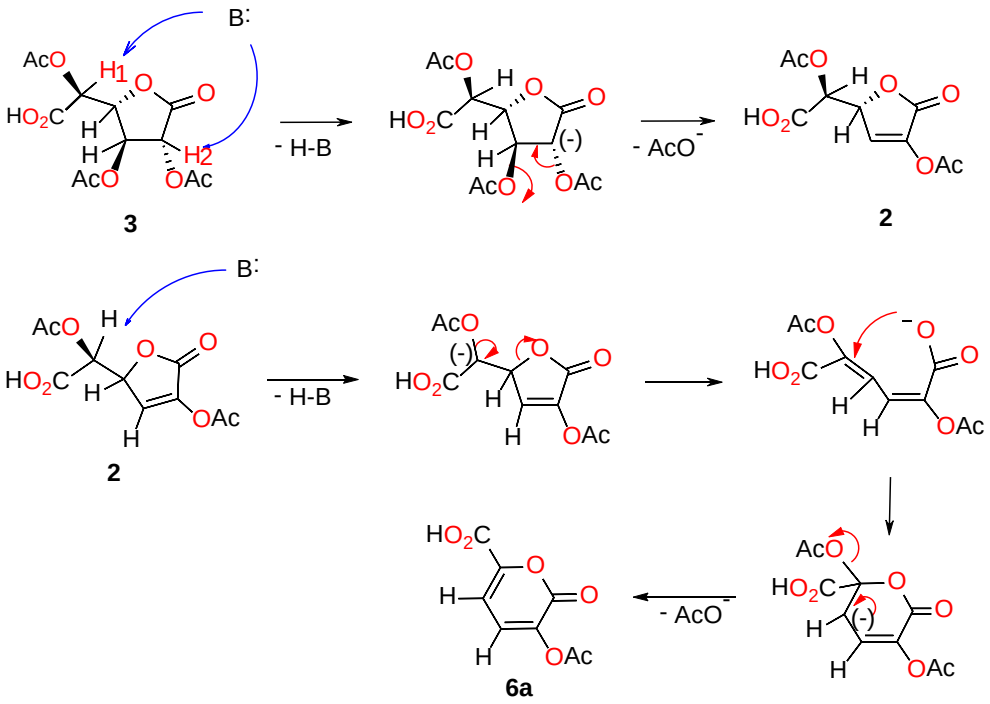


*Figure 3.32 - ^{1}H-NMR spectrum (DMSO-d6) of **6a** synthesized from reaction of galactaric acid (**1**) with acetyc anhydride and catalytic amount of sodium acetate.*

Moreover, under similar conditions but using a stoichiometric amount of sodium acetate, it was possible to isolate the sodium salt of **6a**, (sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate (**7a**)). The specific reaction conditions are reported in Table 3.16. Other base were investigated and the best yields of salt **7a** were obtained by reacting galactaric acid with sodium hydroxide in 1:1 molar ratio. The preferred conditions were obtained by adding sodium hydroxide in 10 batches over a period of 100 minutes to a stirred suspension of galactaric acid in acetic anhydride. The internal reactor temperature was adjusted to 90-95 °C for 4 hours, controlling the temperature with a cooling bath, because the reaction of NaOH with the medium is quite exothermic. Care must be applied to avoid build-up of

solid NaOH in the reactor, owing to the relative insolubility of this compound in the medium, and the possibility to develop runaway reactions. Then, after filtration, concentration of mother liquor, and further purification, white crystals of product **7a** were isolated in 70.8% yield. In all cases, the isolated salt was a bis-acetic acid solvate. A similar reaction, in which DABCO was used as a base instead of sodium acetate, affords the corresponding DABCO salt **7b**. As before indicated, a nearly quantitative yield of compound **7a** was obtained by selecting under similar conditions as substrate the acid **2**.

Form the data collected we delineate a possible mechanism for the conversion of **1** to **6a** or **7a**, which is summarized in Scheme 3.32. The sequence of reactions proposed is: a) the substrates are fully acetylated both as lactone or as open chain form, b) the base operates not only in fast acid-base proton transfer with all the acid intermediates but also as proton abstractor from C-H position to the carbonyl groups; c) the resulting anion eliminates acetate anion from the beta position affording the mono-unsaturated compound **2**, or, better, opens the lactone ring affording via beta elimination an open chain carboxylate anion; d) this last has now the chance to add at position 2 of the open system (probably via the keto form) giving through a new beta acetate elimination the pyrone nucleus. We prefer this hypothesis because lactones have C-H bonds more acid than simple esters.



*Scheme 3.32 – Proposed mechanism for the base catalyzed elimination of AcOH from **3** to pyrone **6a**.*

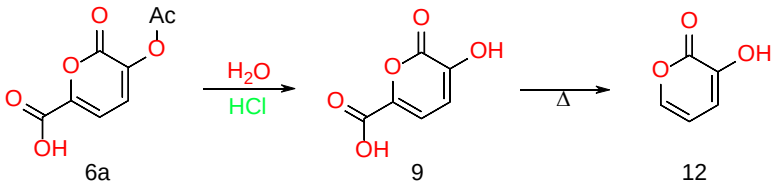
Overall these results represents a new approach to the synthesis of 2-hydroxypyrone derivatives from aldaric acids and opens new potentiality to this class of compounds, presently available from other methodologies at high cost. The possibility to isolate the substituted 2-acetoxypyrone acid **6a**, or better

its salt **7a** and **7b**, directly from the reaction medium reduce the impact of using large excess of acetic acid and of recovering large amount of acetic acid. The best conditions found, starting directly from galactaric acid, were exploited on larger scale (1 kg) and found to be well reproducible with an yield of 75-80% in **7a**.

Hydrolysis and decarboxylation of 3-acetoxy-2-oxo-2H-pyran-6-carboxylic acid

The availability of **6a** and **7a** has stimulated our interest to explore simple transformations of these compounds to access derivatives of potential interest. The investigation were oriented in two different directions, the first was the hydrolysis of acetoxy groups on lactone nucleous, and the second the opening of the pyrone ring to access open derivatives of muconic acid. The complex equilibration present in these last compounds con be in principle directed toward new and old condensation or reduction reactions. The diene nature of muconic acid derivatives was considered of limited interest until recent time when accessibility of the precursor from glucose was ascertained in good yield by biotech approaches. These studies have incresed the interest of the entire class including the alpha-pyrone derivatives. Our synthetic method to access **6a** was a stimulus to investigate this quite versatile chemistry, until now only partially explored.

The hydrolysis of derivatives **6a** and its salt **7a** was investigated in water under both acid and basic conditions (Scheme 3.33). In Table 3.17 are summarized the reaction conditions adopted and the yield obtained of the 2-hydroxypyrone derivative **9**.



*Scheme 3.33 – Synthesis of 2-hydroxypyrone **12** by hydrolysis of **6a** and decarboxylation of **9**.*

*Table 3.17 – Hydrolysis condition of **6a** and **7a** to compound **9**.*

Substrate	Acid or Base	Equiv.	Solvent	T (°C)	t (h)	9 (yield %)
6a	HCl (37%)	10	Water	85	5	65
6a	NaOH (0.1N)	10		25	5	30
6a	HCl (10%)	3	Water	25	>24	70
7a	HCl (37%)	10	Water	25	6	90

The acetate group was found to be relatively sensitive to hydrolysis with excess of acid or bases at room temperature, giving, after acidic work-up, the 5-hydroxy-6-oxo-pyran-carboxylic acid (**9**) in yield from good to nearly quantitative. Under these conditions, no evidence for the opening of pyrone ring was obtained. Moreover, heating the system at high temperature (140-170°C) reduces the yield of **9** and induces the formation of decarboxylation product 3-hydroxy-2H-pyran-2-one (**12**). After minor optimisation efforts, isolation of **9** and its thermal decarboxylation in a sublimation apparatus at 170 °C proved that the sequence can provide access of **12** in nearly quantitative yield.

2-Pyrone is widely used for the synthsis of more complex chemical structures by cycloaddition reacton to form bicyclic lactones, in which the most important reaction is the formation of useful substituted benzene by Diels-Alder reactions with alkynes.[48] As a *cis* diene, 3-hydroxy-2-pyrone (**12**) is highly reactive in Diels-Alder reaction, particularly when the hydroxyl group on position 2 is converted to its phenate like anion. In a separate work, we have just demonstrated that these pyrone products afford cycloadducts with different electron-deficient dienophiles and rearomatization to terephthalic acid derivatives in nearly quantitative yields.[49]

Esterification of 5-hydroxy-6-oxo-pyran-carboxylic acid (9)

Because 2-pyrone-6-carboxylic acid can be a key intermediate for 2-pyrone chemistry, some of its derivatives (e.g. the acyl chloride, amides, and simple ester derivatives were synthetized for evaluation as potential inhibitors of tumour growth.[50]) For instance, product **9** was used as starting material to synthesize the methyl and ethyl ester derivatives (**10** and **11**, respectively), mainly with the aim to overcome the insolubility of this acid in several organic solvents. The conditions used in these reactions and the yield obtained are reported in Table 3.18.

*Table 3.18 – Reaction condition used in the esterification of **9**.*

Substrate (M)	Acid (%)	Solvent	T (°C)	t_{tot} (h)	9a/9b (Yield %)
9 (0.15)	H_2SO_4 (3%)	MeOH	65	5	**9a** (75)
9 (0.11)	H_2SO_4 (3%)	EtOH	78	24	**9b** (89)

Characterization of Pyrone derivatives from Galactaric acid

All the pyrone derivatives isolated in this thesis were characterized in term of structure and purity by using the analytical tools available in the Department, mainly ^{1}H-NMR, ^{13}C-NMR and Mass spectra, but some X-ray diffraction studies were carried out to better understand the solubility behaviour and

the tendency to form solvates in crystal state. In Figure 3.34 are reported the ^{13}C-NMR spectrum of acid **6a** and its sodium salt **7a**.

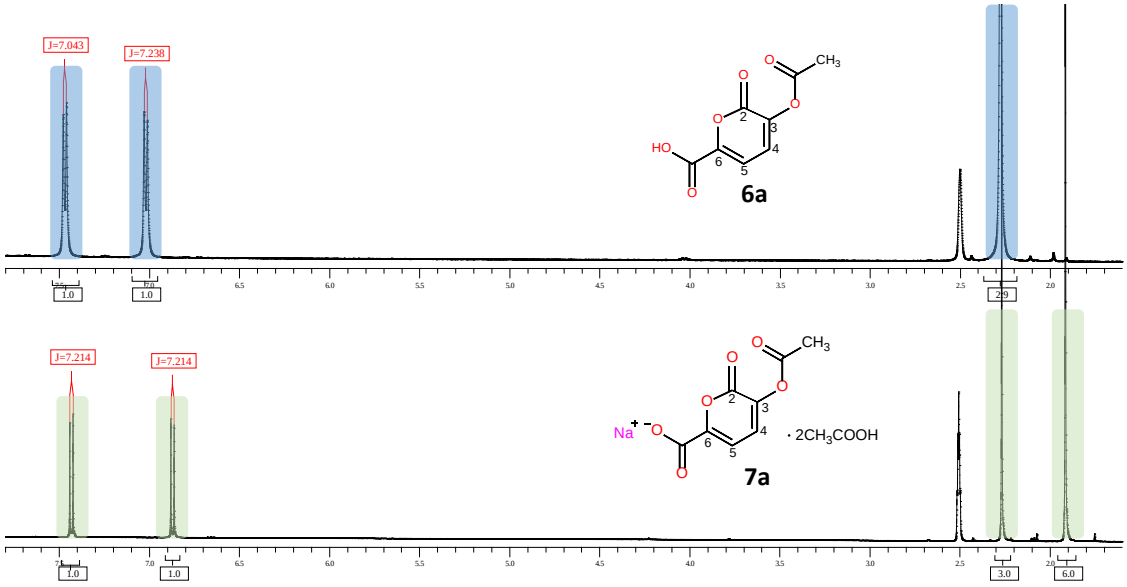


*Figure 3.33 - ^{1}H-NMR spectrum of pyrone derivatives **6a** and **7a** in DMSO-d6.*

From the spectra, the following signals were assigned: terminal CH_3 (δ 19.9 ppm), two unsaturated methyne CH (δ 110.0, 134.9 ppm), two quaternary C-atoms (δ 139.5, 146.7 ppm) and three carbonyl group (δ 167.5, 159.7, and 155.9 ppm). The ^{1}H-NMR spectrum (Figure 3.33) showed two signals at δ 7.02 (d, J = 7.1 Hz) and 7.47 (d, J = 7.1 Hz), attributed to the two vicinal protons in a typical 3,6-disubstituted α-pyrone system.The molecular formula of $C_8H_6O_6$ was identified by the mass spectrum analysis at m/z 199 $[M + H]^+$.

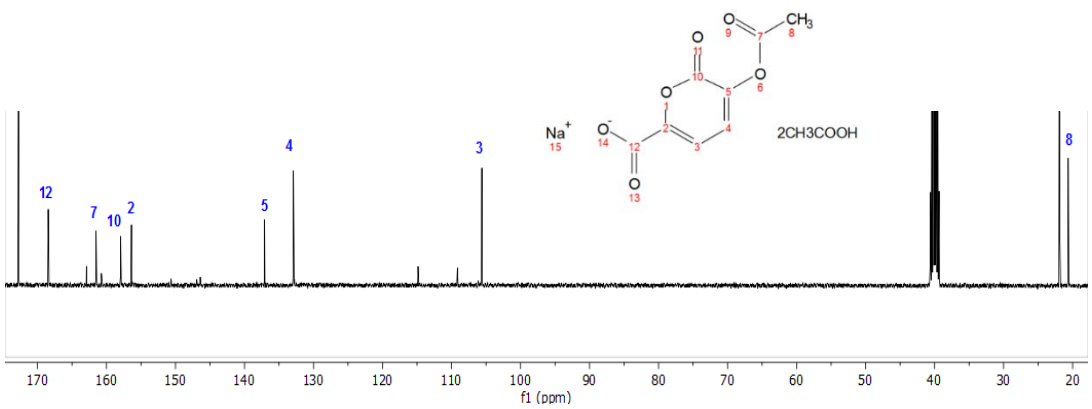


*Figure 3.34 - ^{13}C-NMR spectrum of **7a** in DMSO-d6.*

FT-IR data further supports the pyrone structure with two typical carbonyl groups at 1781 and 1619 cm^{-1} and acetate bending at 1377 cm^{-1}.

Also the colourless crystalline compound **7a** was characterised by NMR, FT-IR, MS and single crystal X-ray diffraction. Figure 3.34 shows its ^{13}C-NMR spectra. The molecule is asymmetric and the following carbon signals were assigned: CH_3 (δ 20.3 ppm), two methyne CH (δ 105.7 and 132.9 ppm) at higher field compared to **6a** owing to the inductive effect of carboxylate anion, two quaternary C-atoms (δ 136.8 and 156.4 ppm) and three carbonyl group (δ 168.6, 161.8, and 158.0 ppm). The proton signal ^{1}H-NMR spectrum of Figure 3.33 are consistent with a 3,6-disubstituted α-pyrone structure, with minor shift at higher field of one of two methene protons due to the effect of carboxylate anion. The FT-IR spectrum of **7a** shows two carbonyl bands at 1772 and 1619 and the acetate bending at 1372 cm^{-1}. The crystal structure of compound 7a was definitely confirmed by X-ray crystal diffraction analysis (see next paragraph).

Because a different crystalline material was recovered by crystallization **7a** in water, the appropriately growth crystals were also investigated by X-ray single crystal diffraction to define the structure. The data will be discussed in next specific paragraph and confirm that the structure of the pyrone salt **7** is the same but the two acetic acid molecules of solvation are replaced by five water molecules.

Compound **9** was obtained as a white crystal by sublimations and its analytical data agree with the one reported in literature. In particular, the ^{13}C-NMR spectrum shows two unsaturated CH at δ 113.8 and 115.0 ppm, two quaternary C-atoms ay δ 141.2 and 147.7 ppm, and two carbonyl group at (δ 161.1 and 158.9 ppm, respectively. The ^{1}H-NMR spectrum showed two signals at δ 6.70 (d, J = 7.3 Hz) and 7.12 (d, J = 7.3 Hz), attributed to the two vicinal protons in a typical 3,6-disubstituted α-pyrone system. The IR data further support the structure with the enol OH group at 3308 cm^{-1} and the pyrone carbonyl at 1659 cm^{-1}. The structure of sodium salt of compound **8** was definitely confirmed by X-ray crystal diffraction analysis (see next).

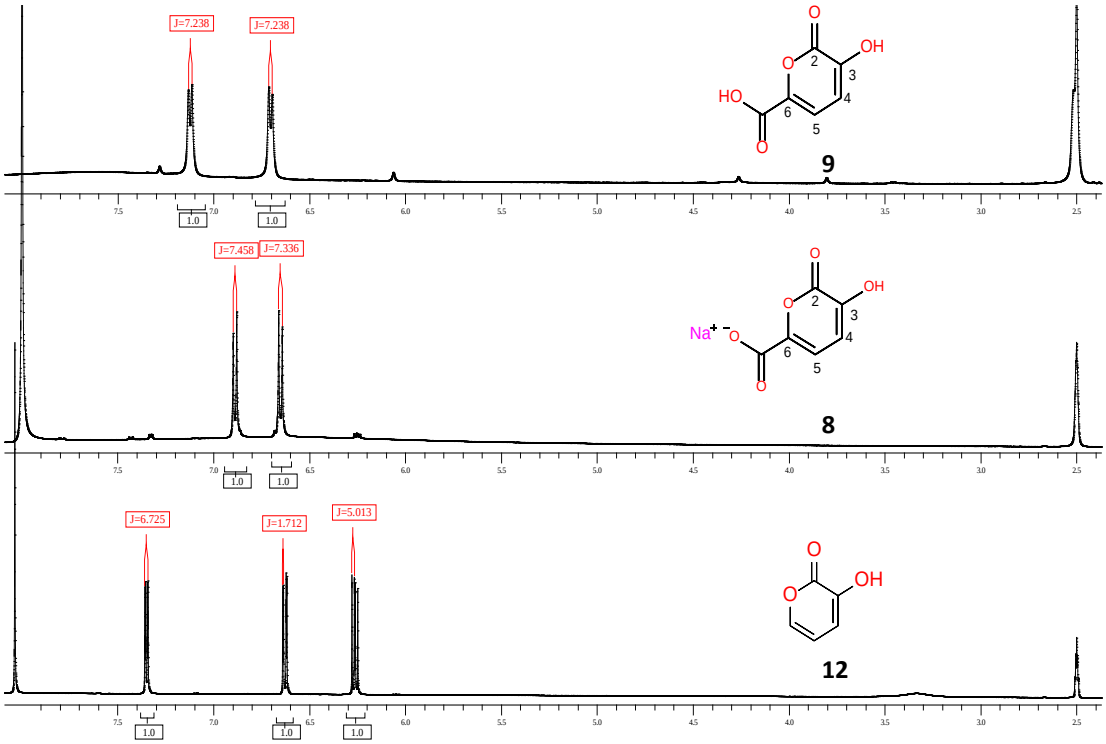
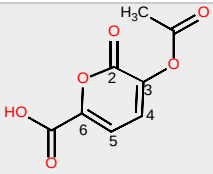
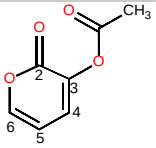
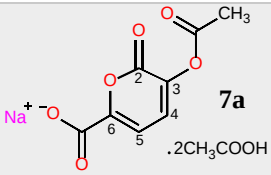
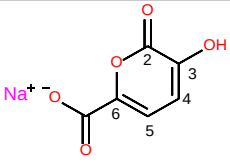
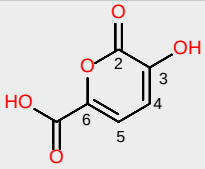
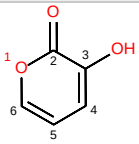
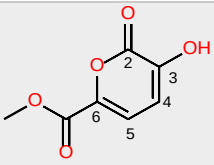
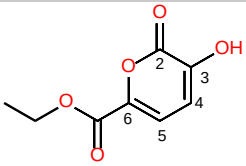


*Figure 3.35 - ^{1}H-NMR spectra of compounds **8**, **9**, and **12** in DMSO-d6.*

Similarly compound **12** has molecular formula of $C_5H_4O_3$ as identified by the mass spectrum analysis at m/z 111 $[M - H]^-$. Three unsaturated CH (δ 106.7, 115.7 and 142.6 ppm), one quaternary C-atoms (δ 143.0 ppm) and one carbonyl group (δ 159.8 ppm) are present in the ^{13}C-NMR spectra to confirm the structure of a 3-substituted α-pyrone system, along with the proton signal assigned in the spectrum of Figure 3.35. For comparative scopes, the different signals in the ^{1}H- and ^{13}C-NMR spectra of pyrone derivatives are summarized

Table 3.19 - Comparison of Pyrone derivetives ^{1}H and ^{13}C-NMR.

Product	Solvent	δ H_4 (ppm)	δ H_5 (ppm)	δ H_6 (ppm)	δ ^{13}C
 **6a**	DMSO-d6	7.51 (d, *J*=7.1 Hz)	7.19 (d, *J*= 7.1 Hz)		167.52, 159.67, 155.86, 146.65, 139.47, 130.97, 110.02, 19.99
 **6b**	DMSO-d6	7.36	6.45	7.66	
 **7a**	DMSO-d6	7.45 (d, *J*=7.1 Hz)	6.91 (d, *J*= 7.1 Hz)		172.91, 168.57, 161.78, 157.97, 156.35, 136.78, 132.95,105.66, 21.8, 20.7
 **8**	DMSO-d6	7.12 (d, *J*=7.3 Hz)	6.71 (d, *J*=7.3 Hz)		173.5, 171, 142, 117, 79.5, 69
 **9**	DMSO-d6	7.12 (d, *J*= 7.3 Hz)	6.70 (d, *J*=7.3 Hz)		161.1, 158.9, 147.7, 141.2, 115.0, 113.8
 **12**	$CDCl_3$	7.14 (dd, *J*= 1.76, 5.28 Hz)	6.19 (dd, *J*= 5.28, 7.04 Hz)	6.65 (dd, *J*= 1.76, 7.04 Hz)	(DMSO-d6) 106.7, 115.7, 142.6, 143.0, 159.8
 **10**	DMSO-d6	7.15 (d, *J*=7.5 Hz)	6.68 (d, *J*=7.5 Hz)		159.45, 157.74, 147.34, 139.21, 114.04, 113.69, 52.46
 **11**	DMSO-d6	7.15 (d, *J*=7.5 Hz)	6.69 (d, *J*=7.5 Hz)		($CDCl_3$) 159.60, 159.12, 145.77, 140.89, 113.12, 113.00, 62.13, 14.10

X-ray Crystallographic Analysis of Pyrone derivatives

As indicated in previous paragraph, the isolated solid from the reaction mixture was initially challenging because if the pyrone structure was clear from the NMR and MS analyses, the form of the crystals and their solubility was strongly dependent from the condition of purification. The situation was more evident with pyrone derivatives **6a**, **7a** and **8**. In order to reach more insight on the problem we decide to recover the structure of these compounds by X-ray single crystal diffraction.

X-ray crystallography is the most common experimental method to obtain a detailed picture of a molecule in the solid state. This technique also provides structural information such as molecule absolute configuration, bond lengths and angles, and symmetry of the molecule. The main limitations of the technique to require a crystal of good quality and size and finding the appropriate conditions to produce good crystals of a specific molecule often requires many careful trials.

A crystal consists of atoms arranged in a pattern repeated in three dimensions. The unit cell is the simplest and smallest volume element that is completely representative of the whole crystal (Figure 3.36).

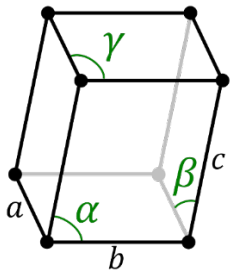


Figure 3.36 - Representation of a unit cell showing length and angle parameters.

The length of the unit cell is described by the vectors a, b, and c wherea the angles of the unit cell are described by the parameters α, β. and γ. The location of an atom within the unit cell is given in terms of the length and angle coordinates.

X-ray Crystallographic Analysis of Pyrone Salts 7a and 7b.

Good crystals, in term of quality and size, of the sodium salt of 3-acetoxy-2-oxo-2H-pyran-6-carboxylic acid (**7a –** Lidia 118) were obtained directly from the crude reaction mixture and proved to be stable for month at room temperature is closed vial. Figure 3.37 shows the labeling of atoms in the x-ray crystal structure. The unit cell associated with the crystal structure, with dashed lines representing hydrogen bonding and heavy red lines representing the ionic bonding, is shown in Figure 3.38.

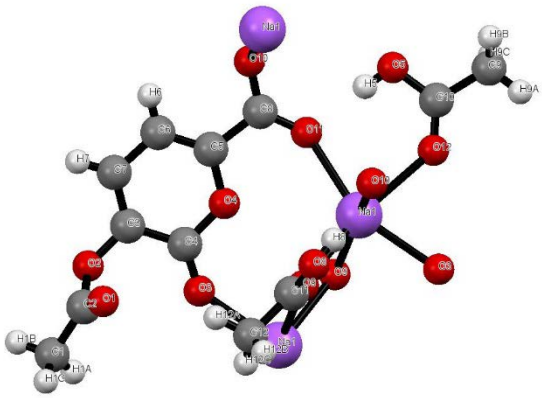


*Figure 3.37 - Ortep drawing showing the labeling of atoms in the crystal structure of pyrone salt **7a***

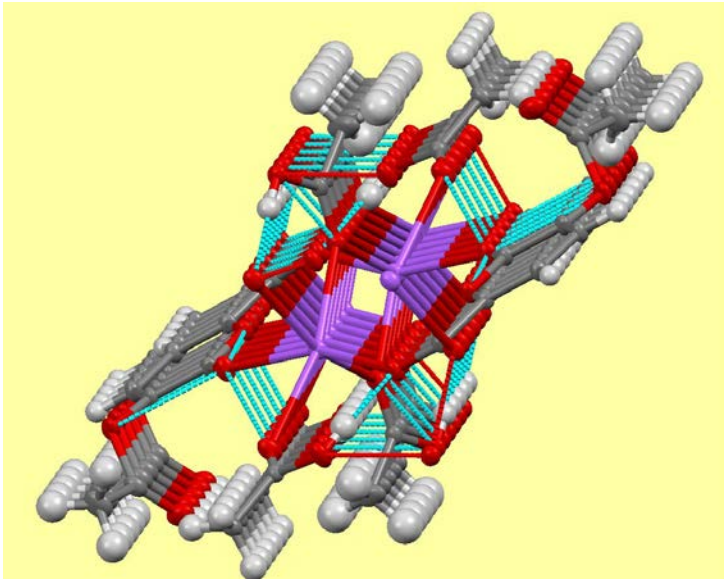


*Figure 3.38 - Mercury drawing of the unit cell of pyrone sodium salt **7a** as viewed along the a axis (slightly tilted for a better vison of the stacking) showing the hydrogen (in light blue) and ionic bonding (in violet) associated with the crystal structure.*

The structure confirms that the compound is a sodium salt and that two molecules of acetic acid are associated via hydrogen bonding to the sodium cation and oxygen atom of the pyrone. The remarkable tight association of the hydrogen and ionic interaction visualised in Figure 3.38 explain the stability of the crystals and the remarkable tendency to crystallize from the reaction mixture. The acetic acid at the perifery of the polar network organises the crystal in a sort of layered system, with the larger vibrational amplitude of methyl group of acetic acid associated to the apolar layer surrounding the polar ionic network. The sodium cations are alined along the a axis of the crystal, each organized in a distorted octahedral coordination with six oxygen atoms, two in axial position belong to two opposite acetic acid

molecules and four equatorial oxygen belong two to carboxylate groups and and two to carbonyl groups of the pyrone molecule. The proton of acetic acid form an hydrogen bond with the the pyrone carbonyl. The structure of the aromatic portion of the molecule is similar the the known compound of pyrones with less than 10% variability in bond lenghth and bond angles. The strong interaction with sodium ion of the carbonyl of the pyrone nucleous confirm the high negative charge density in this compound and suggest relevant contibution of aromaticity and the relevance of the limit structure with a partial positive charge on pyrone oxygen. The competition for sodium cation between the carboxylic group of pyrone and the carboxylic group of acetic acid clearly indicate that the acidity constant of the first is higher (probably 2 order of magnitude) than the one of acetic acid.

Crystallization of compound **7b** from water allows to isolate another crystalline material which was clearly laking of acetic acid but containing some water (between 2 and 3 mol from the elemental analysis). In order to define its structure and the stoichiometry, we submit also this compound to X-ray diffraction analysis. Figure 3.39 shows the labeling of atoms in the x-ray crystal structure. The unit cell associated with the crystal structure, with dashed light blue lines representing hydrogen bonding and heavy red lines representing the ionic bonding, is shown in Figure 3.40.

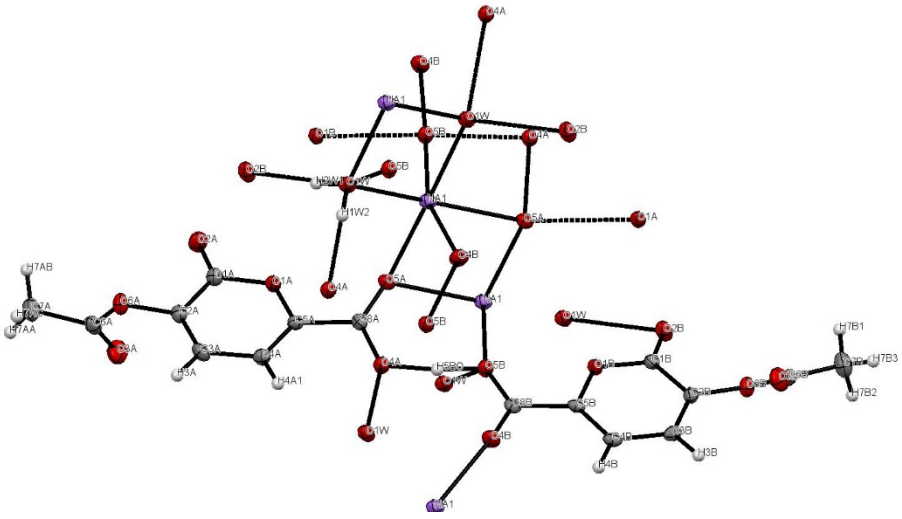


*Figure 3.39 - An ORTEP representation of crystal structure of pyrone **9**.*

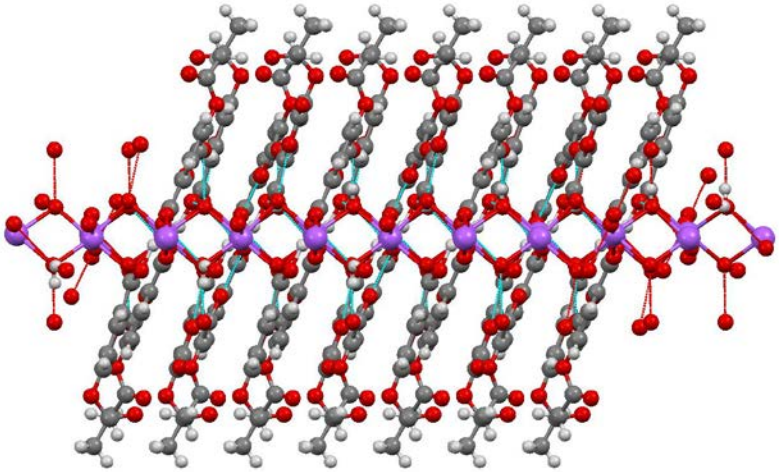


*Figure 3.40 - Mercury drawing of the unit cell of pyrone sodium salt **7b** as viewed along the b axis showing the hydrogen bonding (in light blue) and ionic bonding (in red) associated with the crystal structure.*

The structure confirm that the compound is a 1:1 mixture of pyrone acid and the corresponding sodium salt with one molecule of water of crystallization. The network present in the system can be seen from the Figure 3.40 where the vision along the b axis evidence the layered structure with the acetate group on border and the polar arrangenment in the middle where are located the sodium cations. The coordination around sodium is again near perfectly octahedral even if the system is not fully symmetrical owing the the stoichiometry and the presence of the water molecule. Both distances and angles on pyrone system confirm that also this molecule is planar and with a relevant aromatic contribution.

X-ray Crystallographic Analysis of Pyrones 8.

Good crystals, in term of quality and size, of the sodium salt of 3-hydroxy-2-oxo-2H-pyran-6-carboxylic acid (**8** , Lidia 119) were obtained by slow crystallization from water. The x-ray single structure was determined to ascertain the stoichiometry and the presence of water. Figure 3.41 shows the labeling of atoms in the x-ray crystal structure. The unit cell associated with the crystal structure, with dashed light blue lines representing hydrogen bonding and heavy red lines representing the ionic bonding, is shown in Figure 3.39.

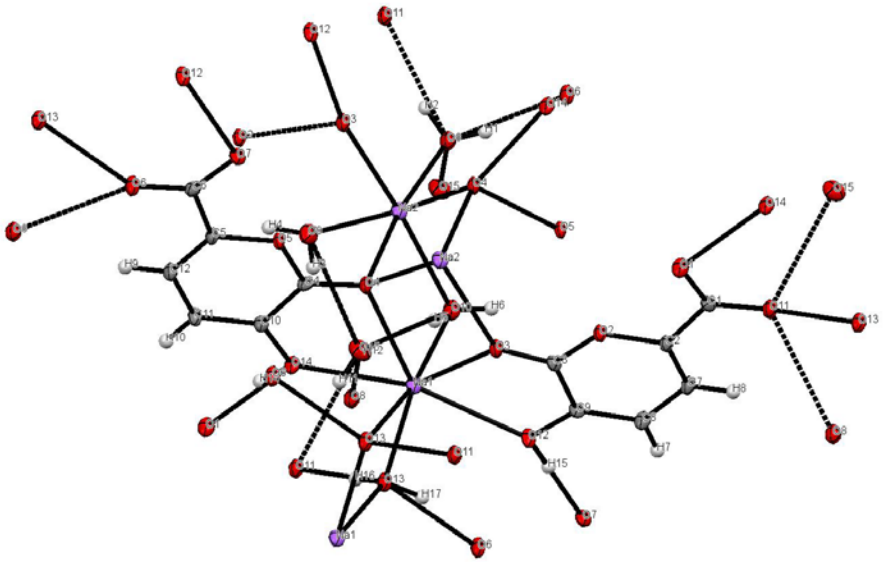


Figure 3.41 - An ORTEP representation of crystal structure of pyrone salt 7

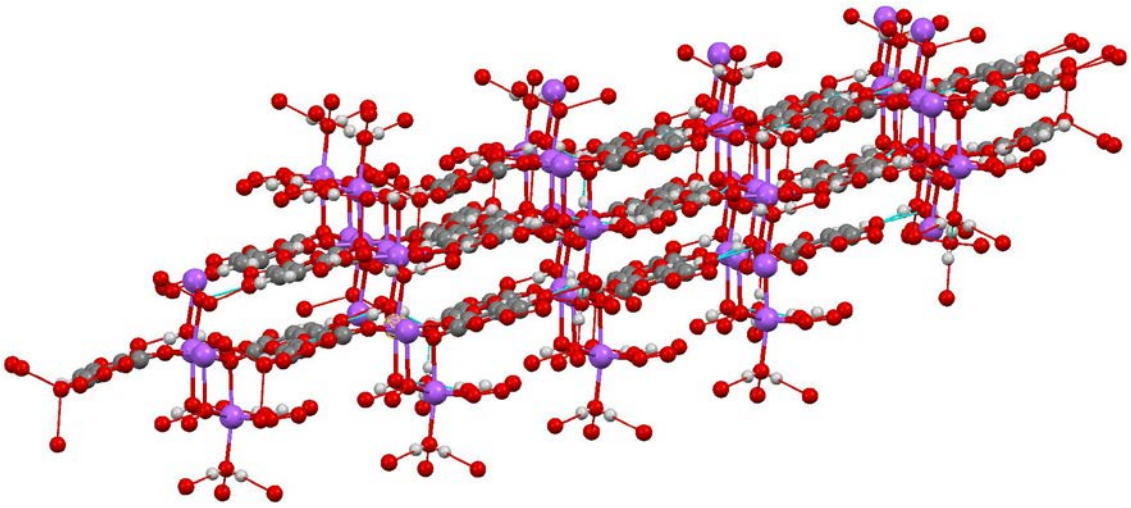


*Figure 3.42 - Mercury drawing of the unit cell of pyrone sodium salt **6a** as viewed along the a axis (slightly tilted for a better vison of the stacking) showing the hydrogen (in light blue) and ionic bonding (in red) associated with the crystal structure.*

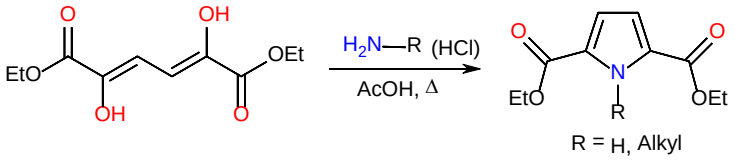
The salt is again organised in a strong network but now 2,5 water molecules are inside the crystal (five molecules for two molecules of pyrone) and the acetate substituent on pyrone is not present, replaced by an hydroxy groups. Several finding are anomalous for this structure. Firstly, the high amount of water create an extended hydrogen bonding framework, and next the sodium cation does not appear connected with the carboxylate anion even this seem to be present because the its distances C-O are similar and quite different from the one of carboxylic group of an acid. On the other part, the carboxylate group is linked by hydrogen bonding to the hydroxyl group in position 2 of the pyrone and

to a water molecule in an arrangement recalling the classical dimer of carboxylic acids. The strong hydrogen bonding present in the system is oriented along the lined flat pyrone molecules, whereas the ionic bonds are organized perpendicular to the pyrone network. Despite the anormaly that sodium ions are not linked to oxygen of carboxylate group, their coordination remain similar to the one observed in the acetate derivative. It presents octahedral arrangement with the axial oxygens belonging to two consecutive layer of pyrone molecules. The four equatorial oxygen ligand belong two at two water molecules and due to the hydroxy-carbonyl moiety of the pyrone via a five membered ring. This unusual arrangement can be also deduced from the distances between carbonyl of pyrone and C-H bond of the hydroxy substituent. The pyrone ring appear to be flat with torsion angles close to zero, clearly indicating the aromatic nature of the molecule.

3.8.2 - Pyrrole Derivatives from Galactaric Acid

The natural occurrence and aromatic properties of pyrrole derivatives have triggered vital interest of both pharmaceutical and chemical industries. The aromatic pyrrole ring can be found in many important biological compounds, such as chlorophyll and tryptophan.[51, 52] Furthermore, pyrrole and its derivatives are widely used as precursor in synthesis of pharmaceuticals, agrochemicals, dyes, polymers and other organic compounds, including tolmetin, pyrrolidine, and polypyrrole.[53] Among all these pyrrole derivatives, pyrrolecarboxylic acid are important building block for the synthesis of biologically active substances such as porphyrins.[54] Different strategies of cyclization reaction have been applied in order to synthesize these compounds, such as Fischer, Saegusa–Ito, Hantzsch and Paal-Knorr syntheses, Friedel–Crafts acylation, carbonation of arylmetal species, and so on. A more direct route is still waiting to be developed to avoid these multi-step reaction sequences and/or prefunctionalization of raw material.[55] Until now the main published data on the synthesis of pyrrole carboxylic acid are derived from review and texts in the pyrrole series and mention very sporadically carbohydrate acid as raw materials.[56] However, it is generally recognised that C-6 sugars in the presence of ammonia or amines, are precursor of C-substituted and N-substituted pyrroles in low yields. So, for instance, Koo et al. was able to condense D-glucose with N-benzylamine to provide under mild nonaqueous acidic conditions the pyrrole-2-carbaldehydes with maximum 40% yield.[57] An old method mention the possibility to prepare pyrrole itself by pyrolysis of ammonium galactarate, but again in low yield. The method was judged synthetically useful with a 35% yield for the preparation of ^{13}C labelled pyrrole in position 2.[58]

Of specific interest for our work was the method developed by Kuhn and Dury in which 1-Alkyl-1H-pyrrole-2,5-dicarboxylates were synthesized from ethyl 2,5-dihydroxymuconate (Scheme 3.34). This muconic derivative was prepared by reductive dimerization of bromopyruvate and not from a bio-precursor.[59]



Scheme 3.34 – Synthesis of diethyl substituted 1H-pyrrole-2,5-dicarboxylates from 2,5-dihydroxymuconic acid by Kuhn and Dury.[59]

In fact, the studies discussed in this thesis, on the reactivity of amine toward galactaro-1,4-lactone and on elimination to unsaturated derivatives, combine well with this finding, strengthening our believe that there exist wide possibilities to extend and improve the conversion of galactaric acid into pyrrole derivatives. This part of the research activity ended with an operationally simple, sustainable autocatalytic Paal-Knorr pyrrole condensation of appropriate galactaric acid derivative with primary amines, under neat conditions, which allows the synthesis of N-substituted pyrrole-2,5-dicarboxylic acid and pyrrole-2-carboxylic acid derivatives in good yield.[60]

For clarity reasons, in the following we will discuss in separate sections the results obtained using different galactaric acid derivative as precursor for the condensation with amines to give pyrroles.

Pyrones as Reagents for the Synthesis of Pyrroles

During our mechanistic studies on the synthesis of pyrones from galactaric acid, we identify by ^{1}H-NMR in the raw reaction mixture small yield of the acyclic di-dehydration product 2,5-diketoadipic acid. The compound exist in solution mainly as the tautomeric derivative 2,5-diacetoxymuconic acid in all the possible forms, *cis-cis*, *cis-trans* and *trans-trans*. The latter prevails at higher temperatures owing to its higher thermodynamic stability. The structure of compound was isolated and further confirmed by ^{1}H-NMR and mass spectrometry. The ^{1}H-NMR spectrum of this compound is shown in Figure 3.43, in which the enol CH proton is assigned at 6.98 ppm to be compared with the reported enol CH of 2,5-dihydroxymuconic acid assigned at 6.42 ppm.[61] Also the mass spectrum (positive and negative ions) of the reaction mixture is consistent with the formation of the diacetylated compounds **6c**. The ESI-MS spectra is shown in Figure 3.44. The molecular ion of m/z = 257 [M-H] and 281 [M+Na] can be recognized in the spectrum. Moreover, the ions at m/z = 537 [2M+Na-2H], 539 [M+Na] and 551[2M_{24c}-H] are compatible with the formation of a dimer.

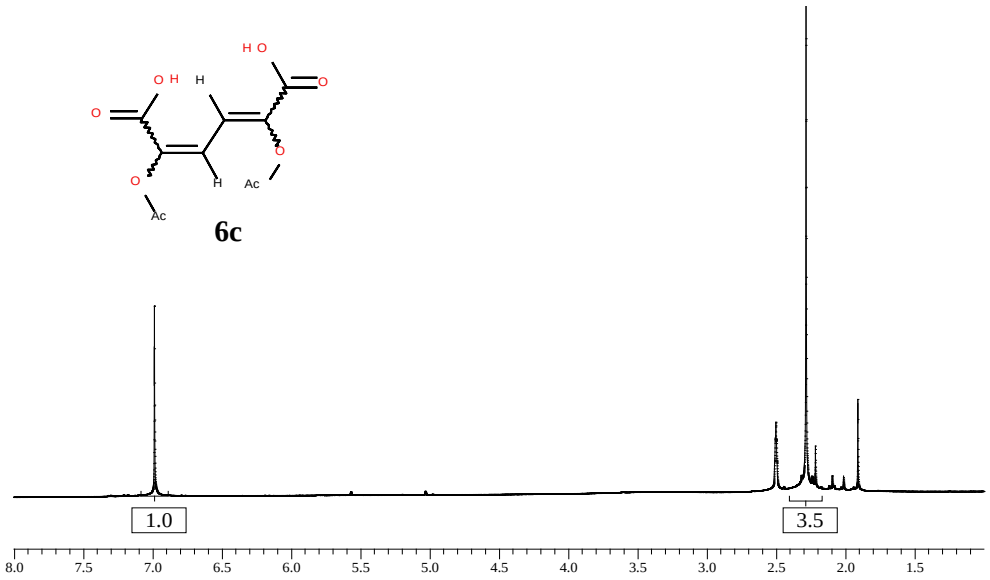
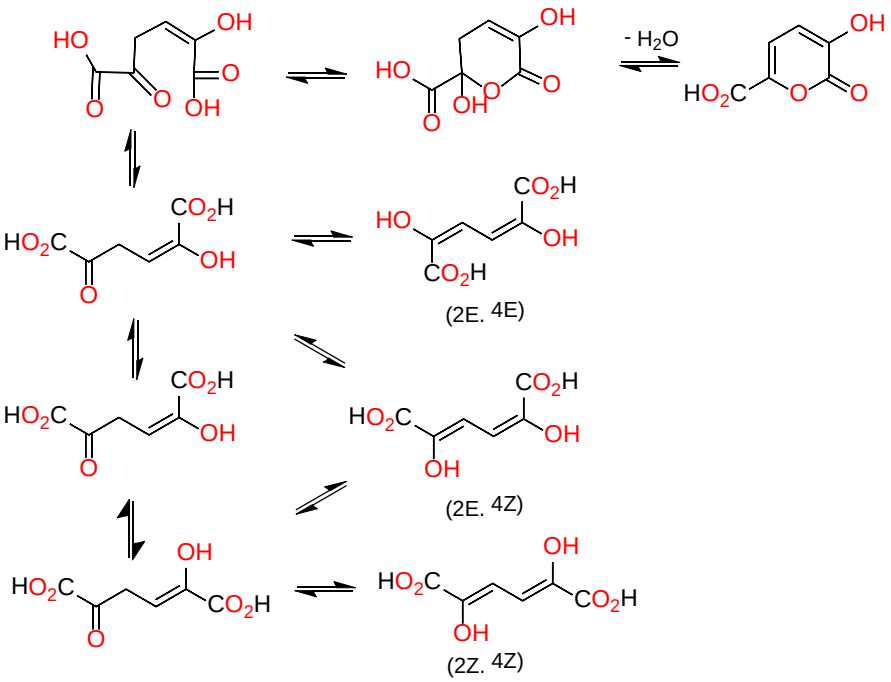


*Figure 3.43 – ^{1}H-NMR spectrum (400 Hz, DMSO-d6) of 2,5-diacetoxymuconic acid (**6c**).*

The keto-enol equilibration assures the possibility of ring closure to stabilized pyrone derivatives (**8**, **9**, **12**) via an intramolecular nucleophilic addition of the carboxylate anion to carbonyl carbon at position 2 (or 5) (Scheme 3.35). The isolation of the open form in *trans* configuration (2Z, 4Z) of **6c** indicates that the equilibration was not fully realized under our experimental condition but assures that carbonyl isomeric forms exist along with hydrated forms.



Scheme 3.35 - Equilibration of 2,5-diketoadipic acid via enol forms to 2-hydroxypyrone-5-carboxylic acid.

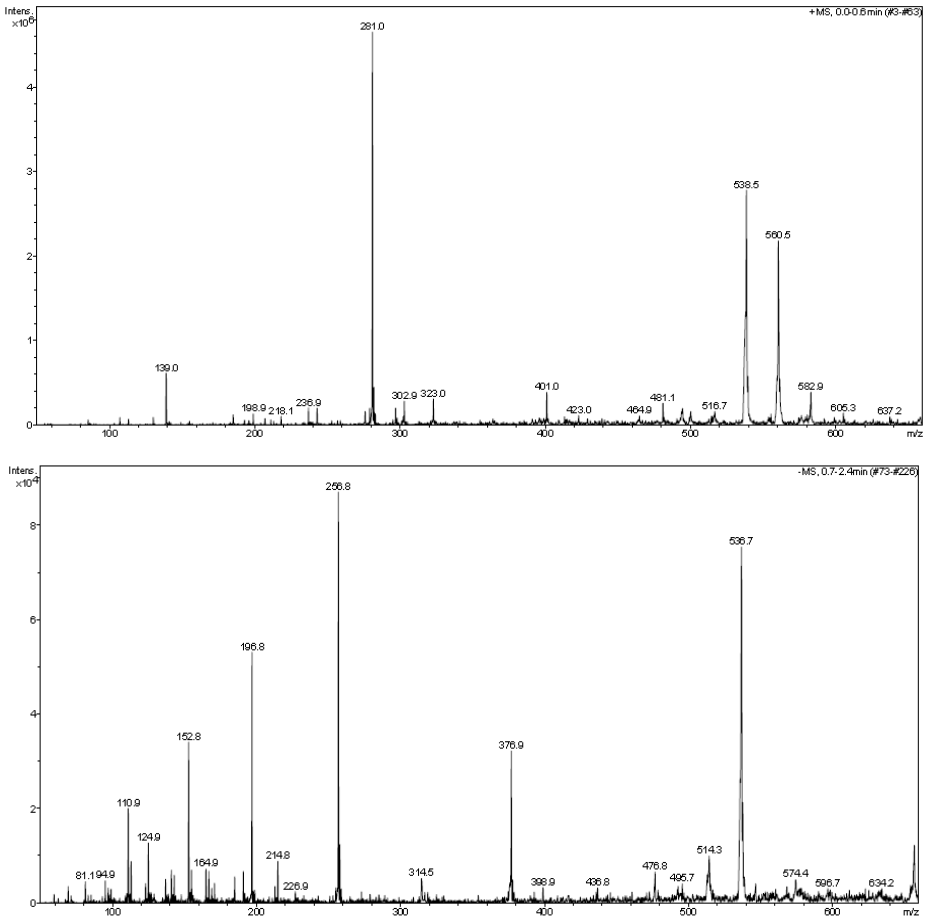
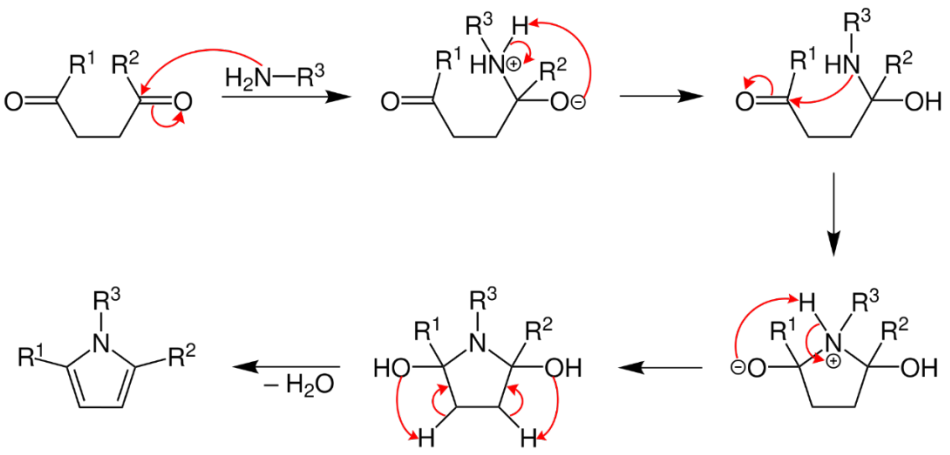


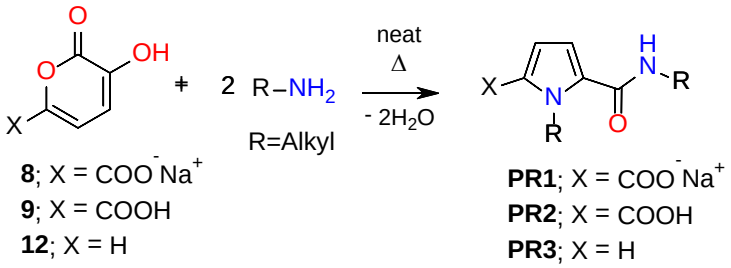
Figure 3.44 - Positive and negative ESI-MASS spectrum (1:100 MeOH) of 2,5-diacetoxymuconic acid.

We conclude that, under equilibration conditions and in the presence of amines, 2-hydroxypyrone derivatives could be useful precursor of pyrroles through a cyclisation similar to the classical Paal-Knorr mechanism involving the open form of 2,5-dihydroxymuconic acid intermediates.[60, 62] The mechanism of the Paal-Knorr reaction has been clarified (by V. Amarnath et al. in 1991)[63] as a sequence of processes involving the addition of the amine to the protonated carbonyl to form an hemi-aminal, followed by a further intramolecular addition of the nitrogen base to the second carbonyl with final dehydration and aromatization to the corresponding substituted pyrrole. The sequence is summarized in Scheme 3.36.



Scheme 3.36 - Mechanism of the Paal-Knorr pyrrole synthesis proposed by V. Amarnath et al.[63]

So we decided to explore the reactivity of the pyrone derivatives obtained in previous chapter (i.e. sodium 3-hydroxy-2-oxo-2H-pyran-6-carboxylate (**8**), 5-hydroxy-6-oxo-pyran-carboxylic acid (**9**) and 3-hydroxy-2H-pyran-2-one (**12**)) towards primary amines with the aim to identify a Paal-Knorr like reaction useful for the synthesis of substituted pyrroles. The reaction was firstly verified under neat conditions with variable amount of the amine and by changing the amine (Scheme 3.37).



*Scheme 3.37 - Synthesis of 2,5-substitued pyrroles from pyrones **8**, **9** and **12** under neat conditions.*

The effect of substituents on the amine, reaction temperature and time were screened to optimize the process. Depending on substrates and conditions, 2,5-disubstituted, 2-substituted and unsubstituted N-alkyl and N-aryl pyrroles were obtained in 0-95% yields. The best yields on 2,5-disubstituted pyrroles **PR1** with the stoichiometry from 5 to 10 were obtained under neat and mild conditions at 50-70 °C (Table 3.20). A further increase in temperature (> 80°C) with substrates **8** and **9**, respectively, induced the decarboxylation of pyrrole **PR1** and **PR2** to 2-pirrole carboxamide **PR3**. Meanwhile, the decarboxylated pyrrole **PR3** was formed directly by reacting 3-hydroxy-2H-pyran-2-one (**12**) with amines at 60 °C in 70-80% yield.

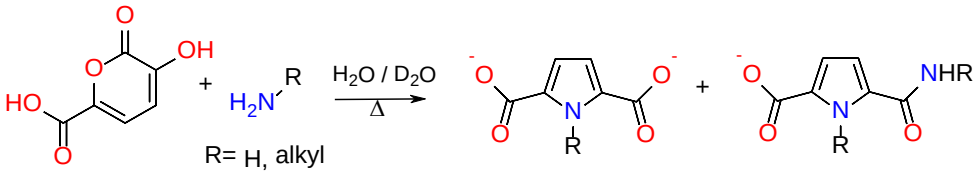
In the case of reaction with alkanolamines, such as with isoserinol, serinol and alaninol, the corresponding pyrrole-lactones were also formed as a result of intramolecular esterification. Simple

primary amines on disubstituted carbons, like cyclohexylamine and isopropylamine, do not react to form pyrroles. In contrast, β-hydroxylethylamines (e.g. serinol and alaninol) show significant reactivity even if the carbon bearing the primary amine is disubstituted.

*Table 3.20 - Synthesis of Pyrroles by reaction of Pyrones **8**, **9**, and **12** with amines in neat conditions.*

Substrates	Amine (equiv.)	*T* (°C)	t (h)	Conversion (%)	PR1 (Yield %)	PR2 (Yield %)	PR3 (Yield %)
8	Ethanolamine (10)	50	63	98	**94**	-	**0.6**
8	Ethanolamine (5)	50	24	45	**35**	-	**6**
8	Octylamine (10)	50	43	79	**76**	-	**3**
9	Ethanolamine (6.0)	50	24	100	-	**95**	**0.6**
9	Isoserinol (5.9)	50	26	98	-	**95**	**0**
9	Serinol (5.9)	60	20	100	-	**35**	**10**
9	Serinol (5.9)	70	21	97	-	**93**	**6**
9	Benzylamine (5.4)	60	20	11	-	**11**	**0**
9	Benzylamine (5.4)	75	20	92	-	**71**	**21**
9	Octylamine (4.9)	75	24	100	-	**20**	**16**
9	Octylamine (10)	65	23	100	-	**87**	**7**
9	Alaninol (5.9)	70	36	96	-	**40**	**19**
9	Cyclohexylamine (5.0)	95	27	0	-	**0**	**0**
9	Isopropylamine (10)	65	24	0	-	**0**	**0**
12	Ethanolamine (6)	50	24	100	-	**0**	**75**
12	Octylamine (10)	65	24	100	-	**0**	**80**

A further investigation was carried out by using water as solvent (for analytical scopes D_2O) to check if the hydrolysis of pyrone derivatives **8** – **10** could overcome the limits evidenced in the experiments under neat conditions. The main product obtained with the base in excess was the ammonium pyrrole-2,5-dicarboxylate, **PR4 (**from which the acid was recovered by treatment with ion-exchange resin) and the pyrrole monoamide **PR2** in lower amount (Scheme 3.38). Once more, under reflux condition partial decarboxylation was observed, recovering 2-pyrrolecarboxylic salts in moderate yield.



*Scheme 3.38 - Synthesis of pyrrole-2,5-dicarboxylate salts from pyrone **9** in H_2O (D_2O).*

Reagent conversion and product yield were quantified by carrying out parallel reactions under the same conditions in deuterated water adding at the end known amount of terephthalic acid as an internal standard. Different from reactions in neat condition, the reactions in water need temperatures of about 100 °C and a significantly long time in order to reach a complete conversion of pyrone derivative. Once more, under these conditions partial decarboxylation to 2-pyrrolcarboxylate salts was observed (Table 3.21).

*Table 3.21 - Synthesis of pyrroles by reaction of pyrone **9** with primary amines in H_2O/D_2O.*

Substrate	Amines (equiv.)	Solvent	*T* (°C)	t (h)	9 (Conv. %)	PR2 (Yield %)	PR4 (Yield %)
9	NH_3 (10)	H_2O	25	89	80	**30**	**10**
9	NH_3 (10)	H_2O	70	10	100	**7**	**35**
9	NH_3 (4.4)	H_2O	70	22	84.8	**16**	**19**
9	NH_3 (4.5)	H_2O+D_2O	70	14	79	**13**	**16**
9	NH_3 (6.3)	H_2O+D_2O	80	6.2	77.4	**6.5**	**7**
9	Serinol (6)	H_2O	70	24	59	**44**	**1.5**
9	Serinol (6)	H_2O	100	10	100	**80**	**-**
9	$HO(CH_2)_2NH_2$ (4)	H_2O	70	14	83.3	**-**	**25.8**
9	$HO(CH_2)_2NH_2$ (4.6)	D_2O	70	21	90	**21.8**	**40.7**
9	Lysine (5)	H_2O	60	27	68	**21**	**21.5**
9	Serine (10)	H_2O	85	11.5	100	**2**	**2**

All pyrrole derivatives obtained were characterized by ^{1}H-NMR, ^{13}C-NMR, and FT-IR spectroscopy and further confirmed by ESI-MS spectrometry. For example, compound **30** [1-(octyl)-5-(octylcarbamide)-1H-pyrrole-2-carboxylic acid] was obtained as yellow crystals by flash chromatography. Positive ESI-MS indicate a molecular formula of $C_{22}H_{38}N_2O_3$ as deduced from the protonated and sodiated ions at m/z 379 [M+H] and 401 [M+Na]. ^{1}H and ^{13}C spectra of isolated compound **30** are shown in Figure 3.45. The ^{13}C-NMR spectrum shows several carbon absorptions due to its asymmetric structure: two pyrrole enol CH (δ 131.54 and 125.95 ppm), two pyrrole quaternary C-atoms (δ 115.59, 110.72 ppm) and two carbonyl group (δ 161.92 and 160.90 ppm). The ^{1}H-NMR spectrum showed two pyrrole proton signals at δ 6.75 ppm (d, 1H, J= 4.16 Hz); 6.59 ppm (d, 1H, J=4.16 Hz), attributed to the two vicinal protons in a typical 2,5-substituted pyrrole system. The proton signal on the amide NH of carboxamide group was assigned at 8.23 ppm as a triplet (J=5.75 Hz).

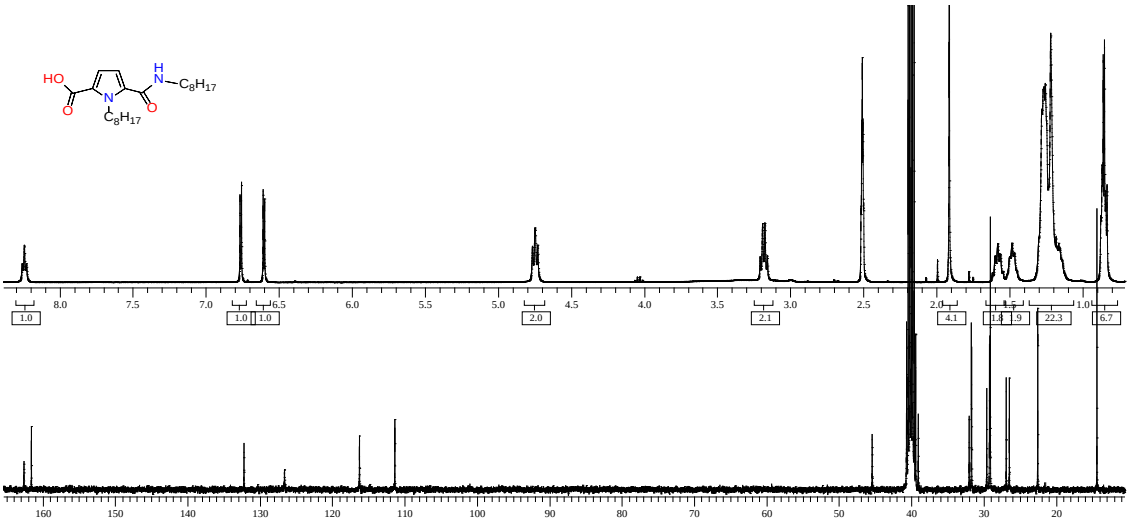


*Figure 3.45 - ^{1}H and ^{13}C-NMR spectra (400 Hz, DMSO-d6) of pyrrole derivative **30**.*

The decarboxylated form N,1-dioctyl-1H-pyrrole-2-carboxamide (**31**) was obtained as a yellow crystalline powder. The molecular formula of $C_{22}H_{38}N_2O_3$ was identified by ESI mass spectrometry at m/z 336 [M+H]. The ^{1}H-NMR spectrum (Figure 3.46) showed three pyrrole proton signals at δ 6.89 (dd, 1H pyrrole, J=1.96, 2.45 Hz); 6.68 (dd, J=1.71, J=4.16 Hz); 5.97 (dd, 2H, J=2.57 Hz, 3.79 Hz), attributed to the three vicinal protons in a typical 2-substituted pyrrole system. The proton signal on the carboxamide NH group was assigned at 7.88 ppm as a triplet (J=7.09 Hz).

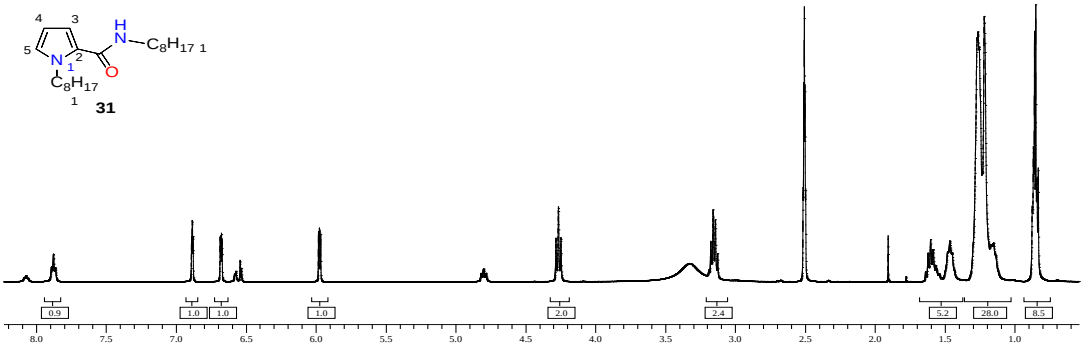


*Figure 3.46 - ^{1}H-NMR spectrum (400 Hz, DMSO-d6) of compound **31**.*

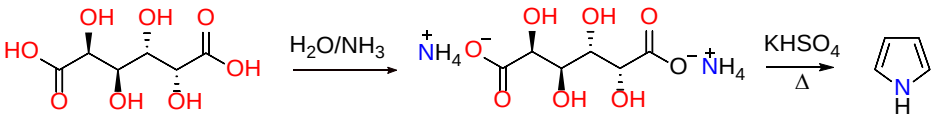
In Table 3.22 are collected the data of ^{1}H-NMR spectra related to the aromatic hydrogens of different pyrrole derivatives, obtained in the reactions of pyrone substrates.

Table 3.22 – Comparison of the chemical shift of CH pyrrole hydrogens and CH-NRCO hydrogens in substituted 2-pyrroleamides (DMSO-d6, 25 °C)

R-NH_2	**Substituents on Pyrrole ring**	**δ Pyrrole hydrogen (m,nH, J Hz)**	**δ Hydrogen Cα (m,nH, J Hz)**
$HOCH_2CH_2$-	2-COOH, 5-CONHR	6.62 (d, 1H, J= 3.9 Hz) 6.31 (d, 1H, J=3.9 Hz)	4.80 (t, 2H, J=5.1 Hz)
$PhCH_2$-	2-COO^-, 5 CONHR	6.73 (d,1H, J=3.9 Hz) 6.59 (d, 1H, J=3.9 Hz)	4.34 (d, J=6.1 Hz)
$HOCH_2CH(OH)$-CH_2-	2-COOH, 5 CONHR	6.63 (d, 1H, J=4.0 Hz) 6.35 (d, 1H, J=4.0 Hz)	4.86 (2t, 1H J=13.2, J=3.4 Hz); 4.63 (m, 1H)
$(HOCH_2)_2CH$-	2-COOH, 5-lactone	6.78 (d, 1H, J=4.0 Hz) 6.48 (d, 1H, J=4.0 Hz)	5.37 (m, 1H, J=7.1, J=1.9 Hz)
n-C_8H_{17}-	2-COOH, 5-CONHR	6.75 (d, 1H, J=4.2 Hz) 6.59 (d, 1H, J=4.2 Hz)	4.74 (t, 2H; J=7.2 Hz)
n-C_8H_{17}-	2-CONHR, 5-H	6.89 (dd, 1H J= 2.0, 2.5 Hz) 6.68 (dd, 1H, J= 1.7, 4.2 Hz) 5.97 (dd, 2H, J=2.6. 3.8 Hz)	4.27 (t 2H, J= 7.1 Hz)
$CH_3CH(OH)CH_2$-	2-COOH, 5-lactone	6.93 (d, 1H, J=4.2 Hz) 6.85 (d, 1H, J=4.2 Hz)	5.26 (m, 1H J=6.8, J=2.6 Hz, J=2.9 Hz)
$CH_3CH(OH)CH_2$-	2-CONHR, 5-H	7.05 (dd, 1H, J=2.5, 1.8 Hz) 6.69 (dd, 1H, J=3.7, 1.2 Hz) 6.01 (dd, 1H, J=3.7, 2.8 Hz)	5.31 (m, 1H, J= 6.7 Hz)
H	2,5-H	6.74 (d, 2H, J=1.7 Hz)	

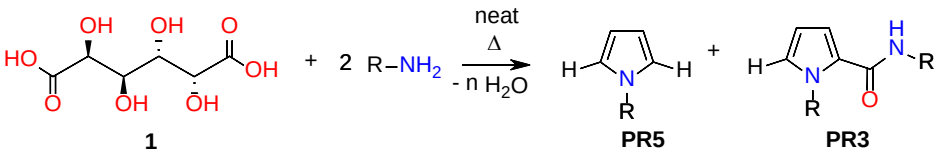
Galactaric acid as starting material

Since previous work[64] on ammonium salts of galactaric acid proved that unsubstituted pyrroles can be synthesized in low yield by thermal decomposition at temperatures between 180 and 240 °C (Scheme 3.39), we explored further this reaction to improve knowledge on the mechanism of this poorly investigated reaction.



Scheme 3.39 – Synthesis of pyrrole by thermal decomposition of ammonium galactarate.[64]

We decided to decrease the reaction temperature to 160°C, working under neat conditions with a molar ratio 1:1 of galactaric acid to amine. Unsubstituted N-alkylpyrrole **PR5** was initially formed, then compound **PR3** progressively increased up to 60-70% yield (Scheme 3.40 and Figure 3.47).



*Scheme 3.40 - Synthesis of N-substituted pyrroles **PR5** and pyrrole-2-carboxamides **PR3** by thermal reaction of galactaric acid (**1**) and primary amines (1:1 mol ratio, 160°C).*

*Table 3.23-Synthesis of Pyrroles **PR3** by reaction of galactaric acid (**1**) with Amines.*

Entry	amine (equiv)	*T* (°C)	t (h)	1 (Conv. %)	PR3 (Yield %)
1	n-C_8H_{17}-NH_2 (1.0)	160	20	100	**66**
2	isoserinol (1.0)	160	20	100	**58**
3	NH_3 (1.0)	160	20	100	**52**

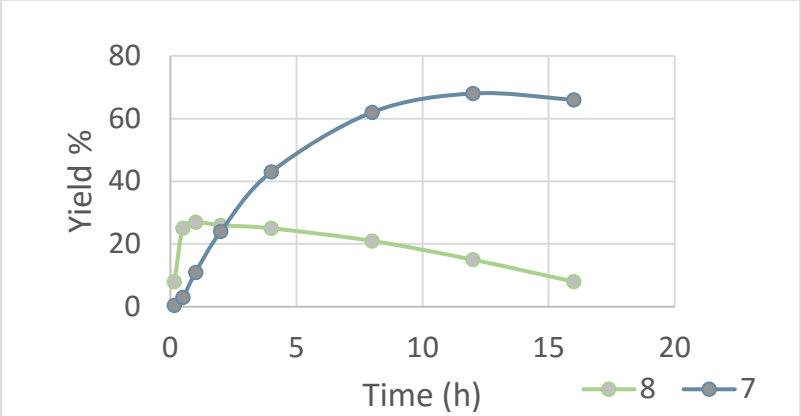
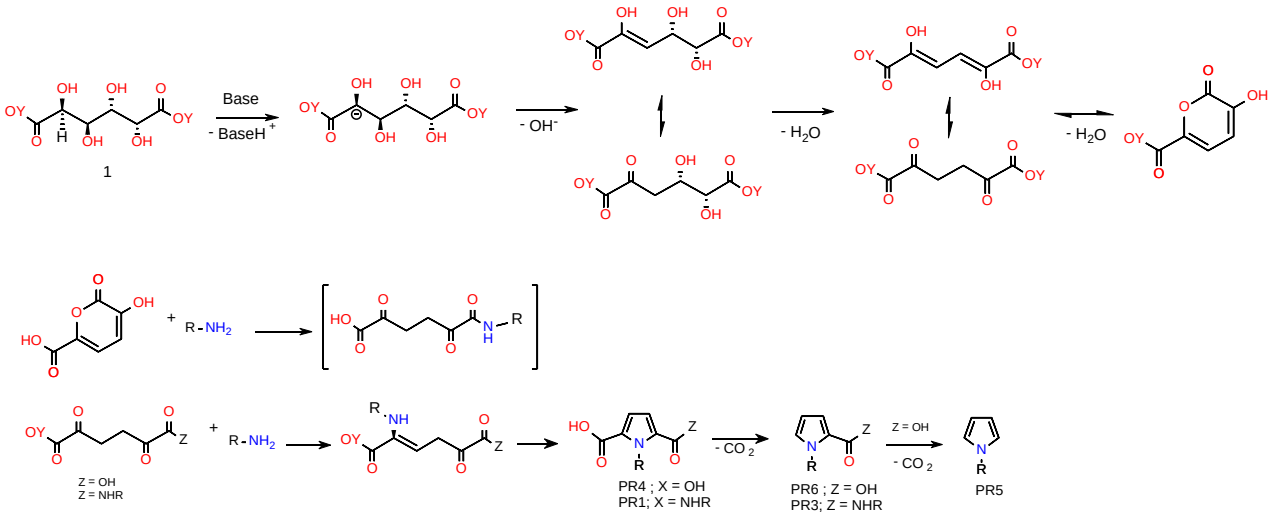


*Figure 3.47 – Trend in the distribution of pyrrole product **PR3** and **PR5** in the thermal reaction of **1** with isoserinol.*

Base induced dehydration of galactaric acid provides 2,5-dihydroxymuconic acid (and the corresponding 2,5-diketo-adipic acid), which is able to give a Paal-Knorr addition of alkylamine to form N-substituted pyrroles. Subsequent dehydration of 2,5-dihydroxymuconic acid to pyrone derivatives **8**, **9** opens the alternative route to the acyclic monoamide carboxylic acid by nucleophilic addition of the amines. Thermal decarboxylation of pyrroles **PR1** and **PR4** provides access to corresponding 2-pyrrole carboxylic acid **PR6** and pyrrole carboxamide **PR3**, the **PR6**, which can be further decarboxylated to unsubstituted pyrroles **PR5** (Scheme 3.41).



Scheme 3.41 - Proposed mechanism for the formation of pyrroles from aldaric acid derivatives.

Reactivity of galactaric acid with serinol under neat condition

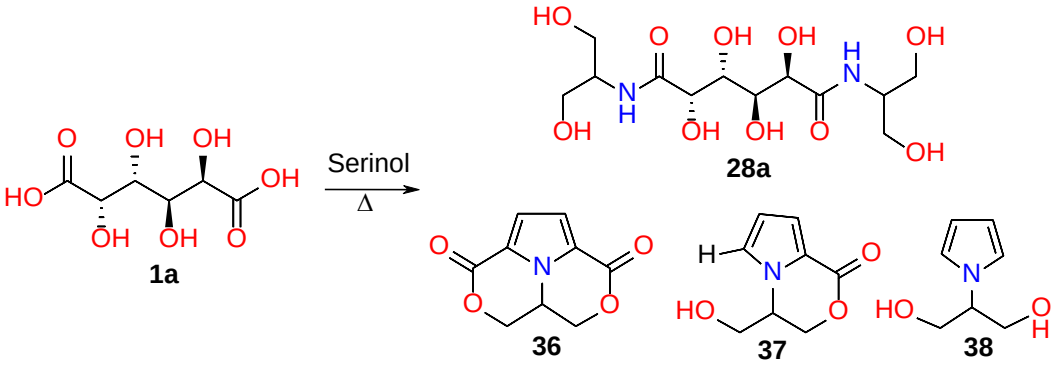


Figure 3.48 – Distribution of products formed in the thermal reaction of galactaric acid with serinol at high temperature under neat condition.

In the reaction of galactaric acid (**1**) with serinol, **1a** was first well mixed together with stoichiometric amount of solid serinol by a ball milling apparatus for 2 hours. Then, the mixture was heated at 160 °C for 8 hours. The reaction afforded the distribution of products reported in Figure 3.48. The first isolated product was the N^1,N^6-bis(1,3-dihydroxy-2-propanyl) D-galactaramide (**28a**), which was previously synthesized by aminolysis of galactaro-1,4-lactone (**18**) with serinol and well characterized in previous subsection. Then, separation by flash chromatography afforded three different pyrrole compounds, identified as 5a,6-dihydro-5H-4,7-dioxa-2a1-azaacenaphthylene-3,8-dione (**36**), 4-(hydroxymethyl)-3,4-dihydro-1H-pyrrol[2,1-c][1,4]oxazin-1-one (**37**) and 2-(1H-Pyrrol-1-yl)propan-1,3-diol (**38**), respectively. Compounds **36** and **37** are formed via intramolecular lactonisation of alcoholic groups of the serinol moiety with carboxylic acid or carboxylic amides substituents. Similar delta-lactones were formed with all 2-alkanolamine.

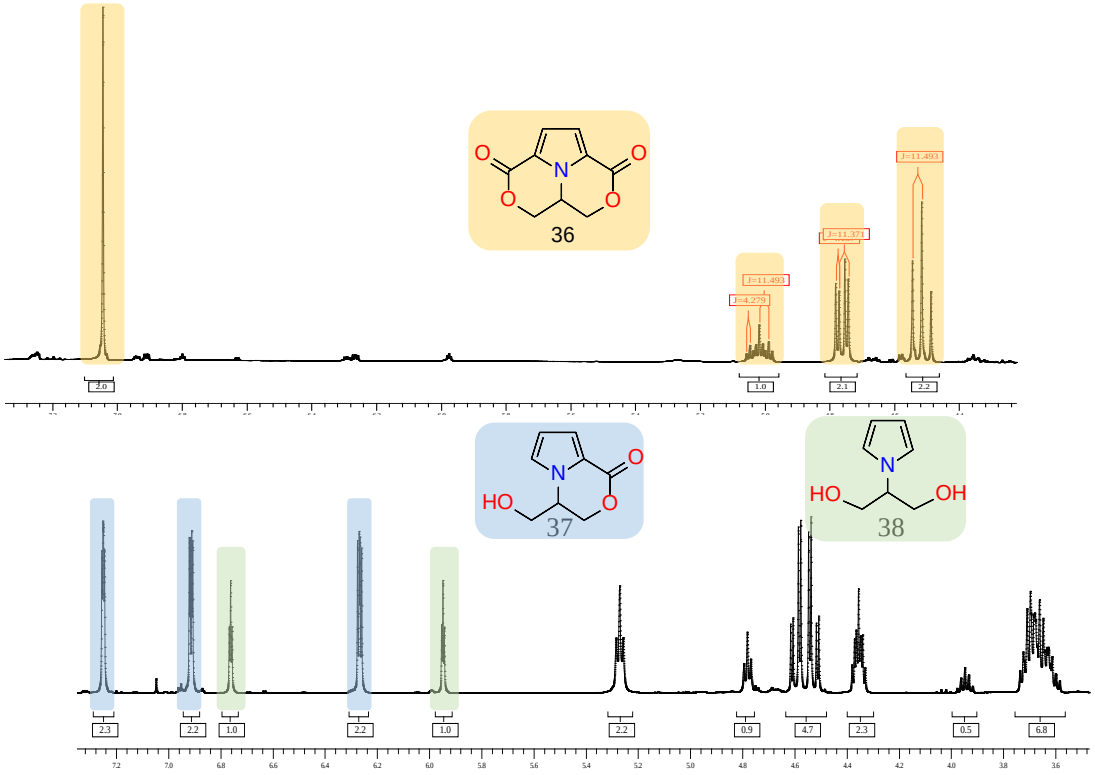


*Figure 3.49 - ^{1}H-NMR spectra (DMSO-d6) of compounds **36**, **37**, and **38** isolated from reaction between **1a** and serinol (140°C , neat conditions).*

Compound **36** was obtained as white crystals of molecular formula $C_9H_7NO_4$ according to the ESI(+) mass analysis at m/z 194 $[M + H]^+$. The ^{13}C-NMR spectrum of Figure 3.50 is diagnostic of a symmetrical structure: two equivalent CH_2 (δ 68.1 ppm), one CH (δ 46.7 ppm), two equivalent pyrrole

CH (δ 115.9 ppm), two equivalent pyrrole quaternary carbons (δ 120.5 ppm) and two equivalent carbonyl carbons (δ 157.5 ppm) attributed to the lactone ring. The ^{1}H-NMR spectrum (Figure 3.49) confirms the structure, with two signals at δ 7.05 (s, 2H pyrrole), attributed to the two vicinal protons of a 2,5-substituted pyrrole system. FT-IR spectroscopy shows absorption maxima at 1536 (C=O) and 1113, 1059, 1023 cm^{-1}.

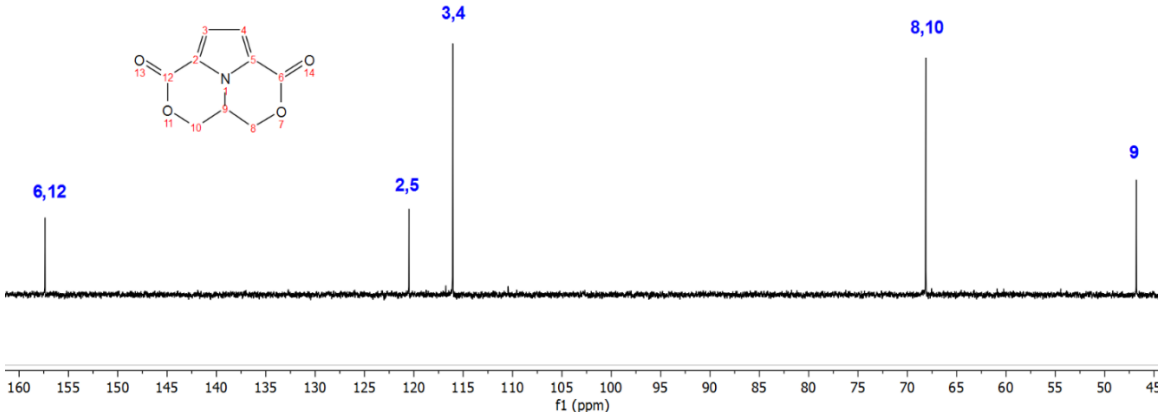


*Figure 3.50 - ^{13}C-NMR spectrum of **36** in DMSO-d6.*

Compound **37** was obtained as a light yellow crystalline powder with a molecular weight of $C_8H_9NO_3$ as deduced from the ESI mass spectra at m/z 168 $[M + H]^+$. ^{1}H (Figure 3.49) and ^{13}C-NMR spectra were carried out to characterize isolated compound. The ^{13}C-NMR spectrum (Figure 3.51) shows several absorptions compatible with an asymmetric structure with two CH_2 (δ 67.5, 54.2 ppm), one CH (δ 60.9 ppm), three pyrrole aromatic CH (δ 126.7, 116.9, 110.5 ppm), one pyrrole quaternary C-atoms (δ 119.8 ppm), and one carbonyl group (δ 158.5 ppm), attributed to the intramolecular ester. The ^{1}H-NMR spectrum is fully compatible with a 2-substituted pyrrole as deduced from the three pyrrole proton signals at δ 7.25 (dd, J=1.6, 2.3 Hz), 6.92 (dd, J=1.6, 3.9 Hz), 6.27 (dd, J=2.6, 3.9 Hz), attributed to the three vicinal protons.

Compound **38** was also a light yellow crystalline powder with molecular weight $C_7H_{11}NO_2$ as deduced from ESI mass spectrometry at m/z 140 $[M - H]^-$, 281 $[2M - H]^-$. ^{1}H (Figure 3.49) and ^{13}C-NMR (Figure 3.51) spectra agree with the proposed structure. The ^{13}C-NMR spectrum shows a simple assignment of carbon signal due to its symmetric structure, two equivalent CH_2 (δ 62.1 ppm), one CH (δ 63.5 ppm), two equivalent pyrrole unsaturated CH (δ 107.4 ppm), and two equivalent pyrrole quaternary C-atoms (δ 120.2 ppm). The ^{1}H-NMR spectrum showed two pair of symmetric pyrrole proton signals at δ 6.76 (t, J=2.1 Hz), 5.95 (t, J=2.1 Hz).

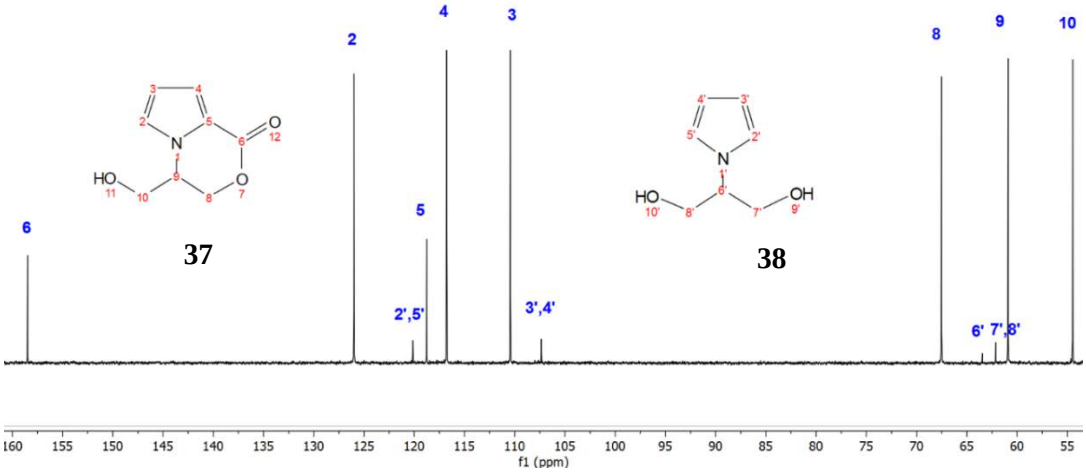
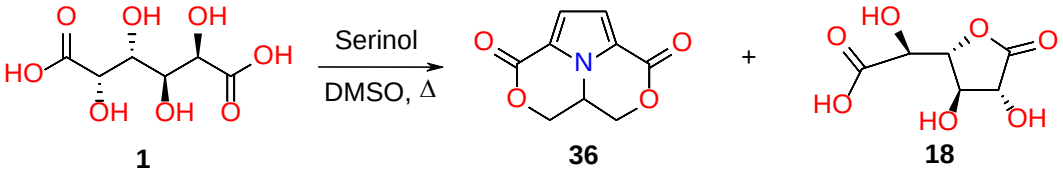


*Figure 3.51 – Assigned ^{13}C-NMR spectra of compounds **37** and **38** in DMSO-d6.*

Reactivity of galactaric acid with serinol in DMSO

The selectivity of reaction between galactaric acid and serinol was further investigated by introducing DMSO as solvent and adjusting the temperature profile. A better yield of product **36** was synthesized in condition described in Chapter 4, following the stoichiometry of Scheme 3.42.



*Scheme 3.42 - Synthesis of pyrrole dilactone **36** from galactaric acid and serinol in DMSO.*

By analysing the reaction mixture with ^{1}H-NMR at different reaction stages. A 52% yield of the dilactone pyrrole derivative (**36**) was detected along with some galactaro-1,4-lactone (**18**). The analytical data for the isolated pyrrole dilactone **36** and galactaro-1,4-lactone **18** are identical to the one reported in the previous section.

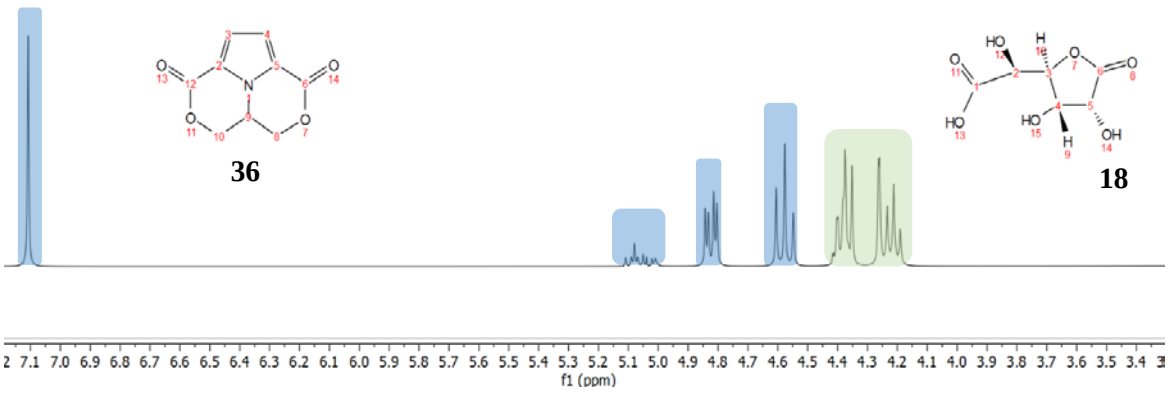
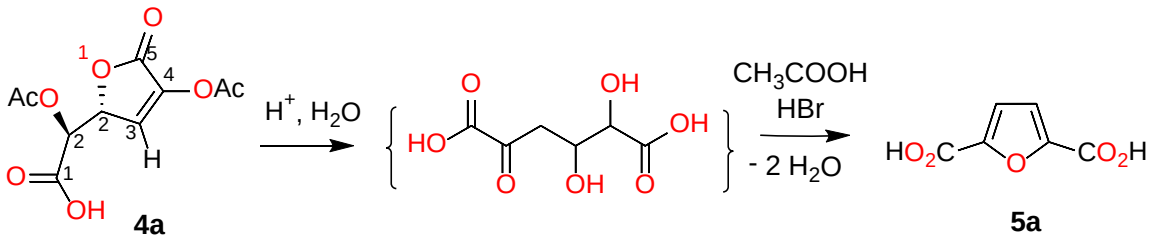


Figure 3.52 - ^{1}H-NMR spectrum of the reaction mixture between galactaric acid and serinol in DMSO-d6.

3.8.3 - 2,5-Furandicarboxylic Acid from Galactaric acid

Another possible useful dieliminated product from galactaric acid is the 2,5-furandicarboxylic acid (**5**). We have explored the synthesis of **5** from L-threo-4-deoxy-hex-4-enaro-6,3-lacton-2yl tetracetate (**4a**) which has been synthesized and discussed in the previous section. Lactone **4a** was preliminarily studied in literature and from one of these works, the sequence of rections of Scheme 3.43 were applied to convert **4a** to **5**.



*Scheme 3.43 – Synthesis of 2,5-furandicrboxylic acid (**5a**) from L-threo-4-deoxy-hex-4-enaro-6,3-lactone-2-yl tetracetate (**4a**).*

In our work, as reported in experimental part of Chapter 4, a solution of **4a** (0.607 ml, 0.07 M, 0.06 mmol) in acetic acid was prepared with 10% by volume of water. HBr was added to the solution in water at 48% and the reaction was heated at 60 ° C under stirring. A cold sample was diluted with DMSO-d6 and analysed by ^{1}H-NMR, which is shown in Figure 3.52. It can be seen that the signal at 7.29 ppm, assigned to equivalent hydrogens of 2,5-furandicarboxylic acid **5a** (highlighted in yellow) increases with time and at the same time the monohydrated and hydrolyzed substrate **4a** (highlighted with two shades of blue) are consumed.

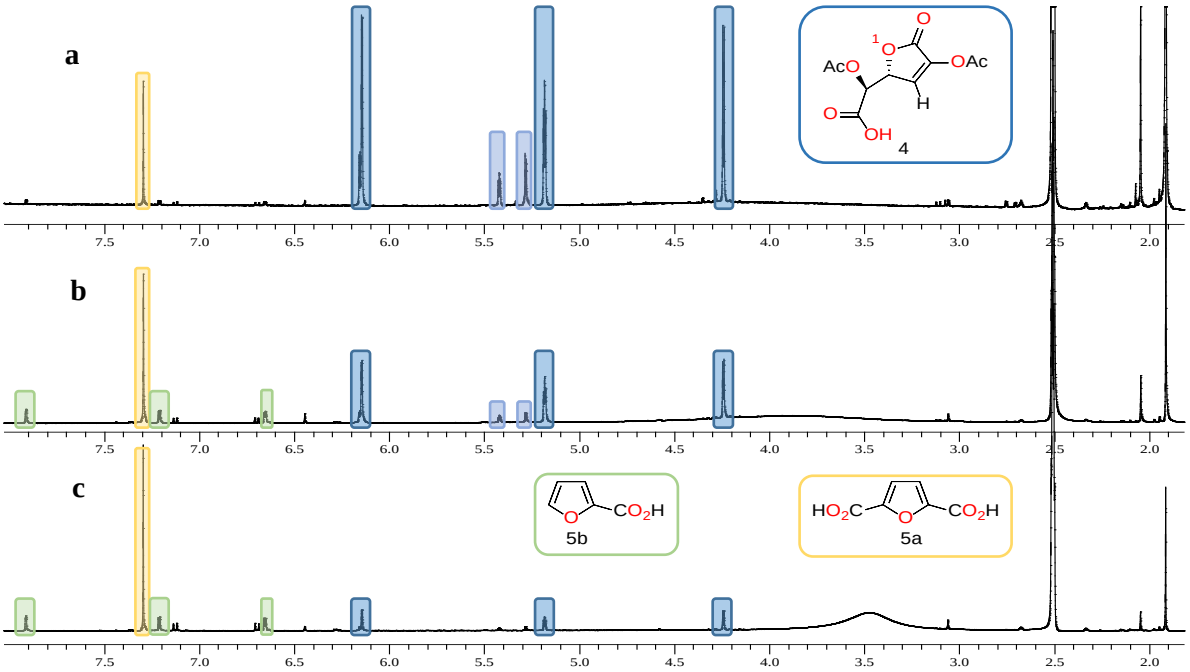


Figure 3.53 - ^{1}H-NMR (400 Hz, DMSO-d6) of reaction mixture in the synthesis of 2,5-furandicarboxylic acid at 60°C, a) 2 h, b) 24 h, c) 48 h.

Under these conditions, the ^{1}H-NMR analysis allowed to characterize two products other than the desired 2,5-furandicarboxylic acid, the 3-acetoxy-2-oxo-2H-pyran-6-carboxylic acid (**6a**) just discussed in previous section and a product containing three hydrogens with a structure compatible with 2-furandicarboxylic acid (**5b**) (Figure 3.53). It is at present difficult to explain the observed decarboxylation to **5b** at the moderate temperature of the experiment (60 °C). A possible explanation could be found in the involvement of an open alpha ketoacid which are known unstable compounds and can easily decarboxylate in this acidic medium at moderate temperature (pyruvic is a typcal example of this behaviour). Following the reaction by ^{1}H-NMR against time, the distribution of products (Table 3.24) evidences that all three compounds increase at the same level, so that they appear to arise from competative reactions more than consecutive reactions. This can exclude that decarboxylation of 2,5-furandicarboxylic acid can occurs under the experimented condition tested and the loss of yield arises mainly from some intermediates, again to be characterised. In any case, the trend observed provide preliminary evidences that compound **4** is a possible substrate for the conversion of aldaric acid to 2,5-furandicarboxylic acid (**5a**).

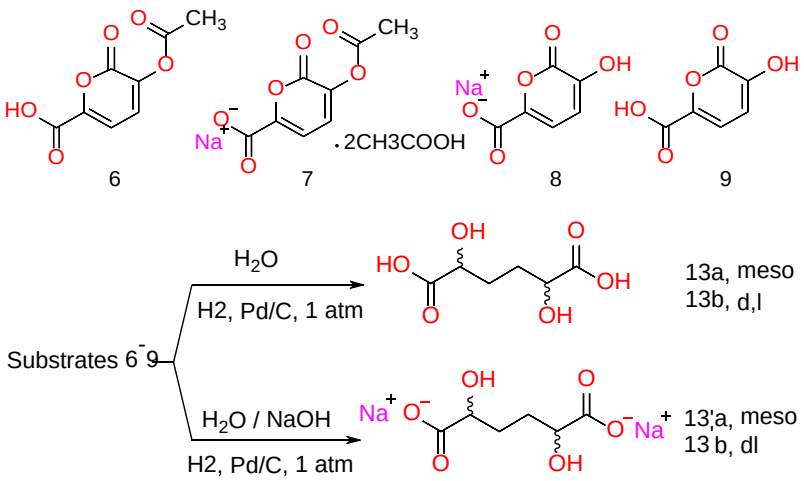
*Table 3.24 - Conversion trend and yields of 2,5-furandicarboxylic acid (**5a**) and 2-furancarboxylic acid (**5b**) in the acid treatment of **4** (50% HBr, 60°C).*

Reaction time (h)	4 (Conv. %)	5a (Yield %)	5b (Yield %)	Other (%)
2	14	11	2	1
24	46	29	14	3
48	75	46	23	6

Attempt to improve the yield of 2,5-furandicarboxylic acid by direct acid treatment of galactaric acid in different solvents and conditions, evidenced that the maximum yield was 55% and black side-products are formed. Because these data does not improve the known conversion of glucaric acid to 2,5-furandicarboxilic acid, details of these experiments will not be reported in this thesis.

3.9 - Reduction Reactivity of Pyrone Derivatives from Galactaric acid

Finally, a last reaction of pyrone derivatives, developed in the study, was investigated, the catalytic hydrogenation. The aim initially was to improve existing methods to convert aldaric acid into adipic acid, but we soon realized that also the pyrone substrates **6**-**9** require very drastic condition to be hydrogenated to adipic acid. For example, under catalysis of 5% Rh/C in diethyl succinate at 180 °C only 5% of adipic acid was obtained after 18 hours, whereas in water at 200 atm and 180°C no conversion was observed. However, these experiments proved that pyrone derivatives were cleanly converted to a mixture of open dicarboxylic acid, dominated by the 2,5-dihydroxyadipic acid (**13**). Therefore, we address the attention to this derivative, trying to define the more mild condition for its preparation. The reactions were carried out in water by using neutral derivative **6** and **9** and the salts **7**, **8** whereas as catalyst were used mainly Pd/C and Nickel Raney (Scheme 3.44).



*Scheme 3.44 – Synthesis of 2,5-dihydroxyadipic acid (**13**) and its sodium salt (**13a**) by hydrogenation of pyrone derivatives **6**, **7**, **8**, **9**.*

It was soon identified that the hydrogenation of pyrone compounds **6**, **7**, **8**, **9** occurs relatively easily also at atmospheric pressure and at temperature lower than 50°C. We decided to carry out the main part of the study at 35 °C in water in the presence Pd/C as catalyst and hydrogen at 1 bar of pressure. Attempts to optimise the reaction were carried out by checking different substrate concentrations, reaction time and catalyst to substrate ratios, as reported in Table 3.25.

*Table 3.25 - Synthesis of 2,5-dihydroxyadipic acid (13) by hydrogenation of pyrones **6-9** (H_2, Pt/C, 35°C, 1 atm).*

Reaction	Substrates (mg)	[Sub] M	[Pd]/[subst] ratio	t (h)	Sub. Conv. %	13a (Yield %)	Mono-alkene (Yield%)
JL023	**7** (205.5)	0.29	0.197	20	100	11	0
JL024	**6** (203.1)	0.51	0.179	20	100	29	0
JL025	**7** (101.2)	0.29	0.225	3	100	23	0
JL026	**6** (99.1)	0.50	0.195	3	70	11.2	0.25
JL029	**8** (209)	0.58	0.167	5	70	15.1	0
JL032	**9** (202.5)	0.065	0.143	6	100	58.8	0
JL042	**9 (**9700)	0.12	0.197	10	100	59.6	0

Figure 3.54 shows the ^{1}H-NMR of the reactions JL025, JL026, JL029 and JL032 starting from substrates **7**, **6**, **8**, **9**, respectively. Substrates **6** and **8** show moderate conversion (70% after 6 hours) in the reaction JL026 and JL029, respectively; all other substrates were converted quantitatively in less

than 10 hours. The result with **8** can be ascribed to its low solubility in water. Mono-reduced product was detected at low conversion, reaching a maximum yield of 15.1% based on the 2,5-dihydroxyadipic acid in reaction JL026. Substrate **6** is soluble in water, meanwhile the hydrogenation reached only a 70% conversion. Reaction JL025, in which the substrate **6** is crystallized with two mole of acetic acid, shows a conversion of 100%, with preferential formation of 2,5-dihydroxyadipic acid. The acid medium seems to favour the reduction as confirmed by the results with **9**.

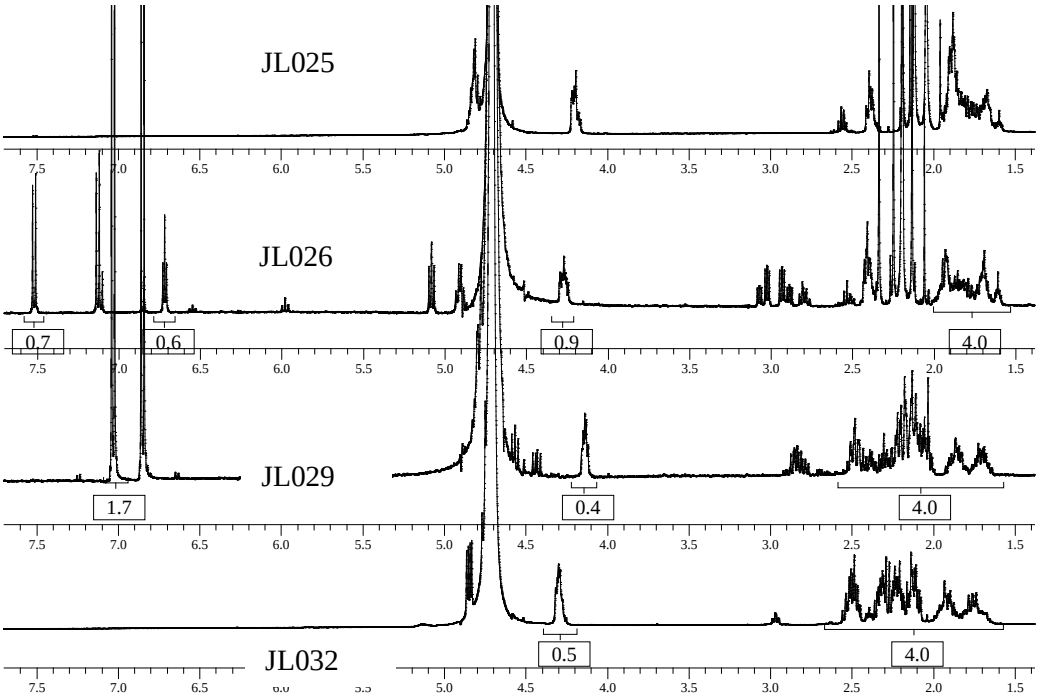


Figure 3.54 - ^{1}H-NMR spectra (400 MHz, DMSO-d6) of reaction mixture JL025, 26, 29, 32.

Reaction JL032 evidences after 6 hours a 100% conversion. Substrate **9** is very little soluble in water and its concentration was fixed at 0.065 M to start the reaction under homogeneous conditions.

The mixture coming from reaction JL032 became soluble in DMSO after filtration of the catalyst and removal of water because no salt was present. In Figure 3.55 are collected the ^{1}H-NMR spectra taken in D_2O, in DMSO-d6 and in NaOD to verify whether lactone forms were presented in the reaction mixture.

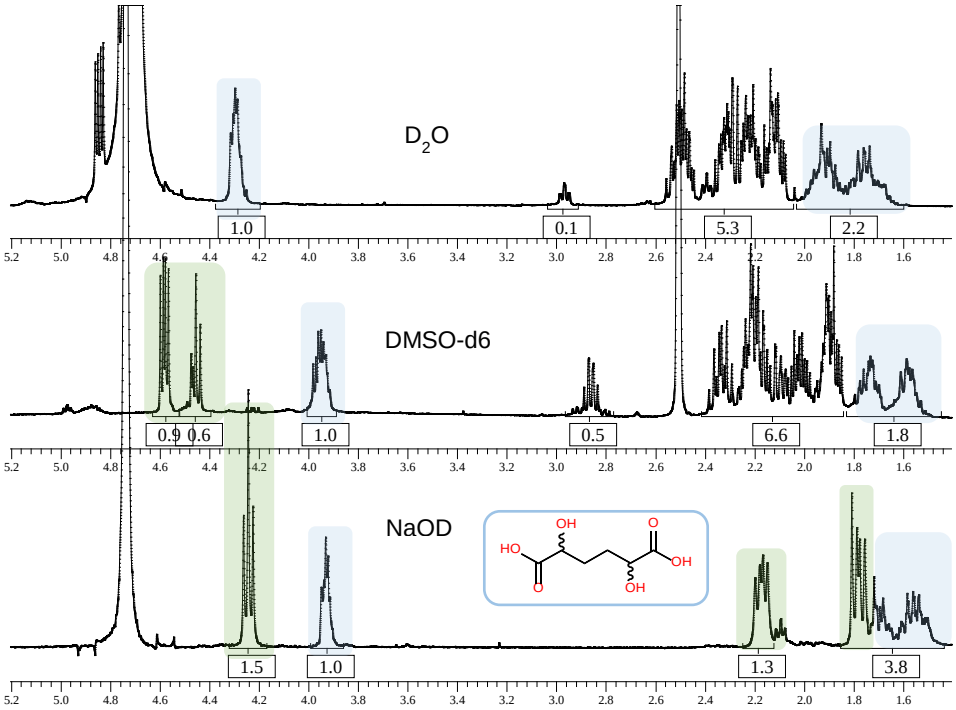


Figure 3.55 - ^{1}H-NMR spectra of JL032 reaction in D_2O, DMSO-d6 and NaOD solution (1M).

As can be seen from Figure 3.55, the ^{1}H-NMR spectrum in DMSO revealed the presence of a third signal in the range 3.8 - 4.7 ppm compatible with CH hydrogens of CHOHCOOH. It was hypothesized that together with the two diastereoisomers of 2,5-dihydroxydipic acid (**13**) (*meso* and *d,l* - coloured in green and light blue) there were also lactone derivatives. Registration of the NMR after addition of NaOD to the sample determines the disappearance of only one signals suggesting that only one lactone was present and confirming that the other set of signals were insensitive to the base, as expected for the isomers of 2,5-dihydroxyadipic acid.

*Table 3.26 – Hydrogenation of substrate **9** (reaction JL035) at different stages.*

Reaction	Reagent (g)	Reagent (mmol)	Pd/C 10% (mg)	H_2 (mL)	H_2 (mmol)	Water (mL)	t (h)
JL035_1	0.9911	6.35	101.7			90	6.5
JL035_2	0.9822	6.29	98.5	250	10.4	90	15
JL035_3	1.0482	6.72	66.1	260	10.8	60	21
JL035_4	0.5251	3.34	42.1	295	12.27	30	27
JL035_5	0.4486	2.88	44.2	350	14.56	30	33.5

The trend observed in Figure 3.56 indicates that at high reaction times the spectra become simpler, suggesting that 2,5-dihydroxyadipic acid diastereoisomers equilibrate to the more stable *meso* form (**13a**). This was confirmed by experiments on larger scale (100 g) at 50°C under 5 bar of hydrogen pressure on substrate **9** were a 85% yield of *meso*-2,5-dihydroxyadipic acid (**13a**) was obtained after recrystallization, with less than 2% of residual d,l diastereoisomer. The Pd catalyst seems to favour the equilibration through a mechanism not yet clear, but certainly not involving a dehydration reaction because no adipic acid or 2-hydroxyadipic acid was observed under the conditions investigated.

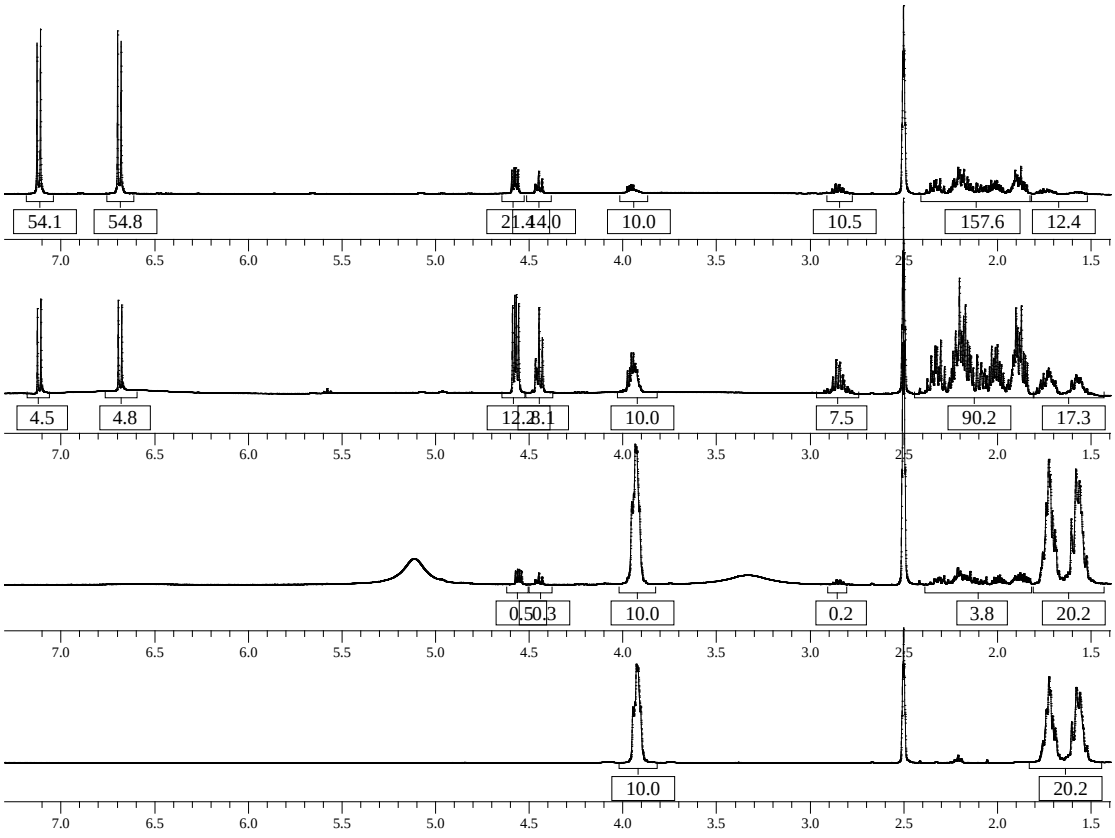


Figure 3.56 - ^{1}H-NMR of samples of reaction JL035 taken at different times (DMSO, 2°C).

It must be emphasised that despite the preparative interest for the production of 2,5-dihydroxyadipic acid, our finding that only the *meso* form of the acid can be easily accessed in good yield was discouraging from another point of view. We consider in fact that the *d,l*-2,5-dihydroxyadipic acid has relevant potentiality because it can give the bridged dilactone 2,5-dioxabicyclo[2.2.2]octane-3,6-dione (**50**) by internal lactonization, whereas the *meso* derivative can stop at the stage of monolactone (2S,5R)-5-hydroxy-6-oxotetrahydro-2H-pyran-2-carboxylic acid (**51**) owing to the *trans* stereochemistry of the OH and COOH groups..

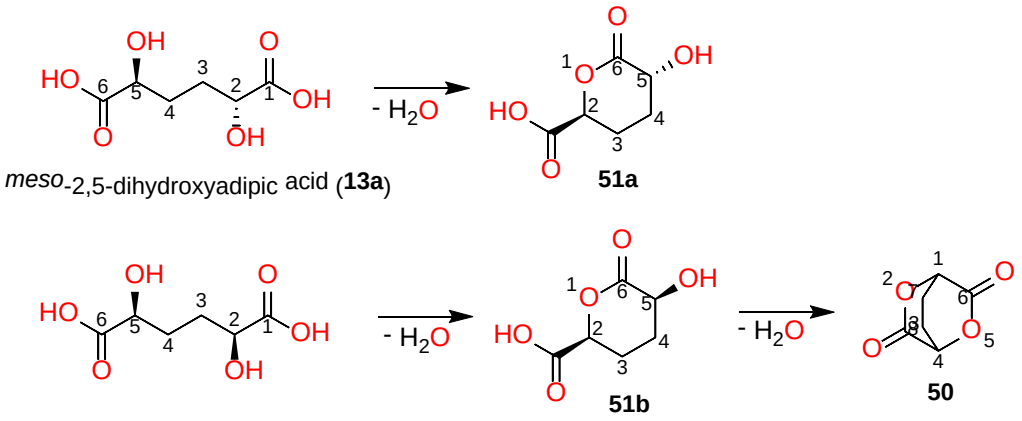


Figure 3.57 – Diastereoisomers of 2,5-dihydroxyadipic acid and their conversion into lactones.

In fact, the optically active compounds can serve as potential chiral synthons in asymmetric organic synthesis, as do other optically active δ-lactones,[65] and in the preparation of chiral polymers analogue of polylactic acid (PLA). Compound **50** was prepared as Racemate more than 100 years ago[66] and their enantiomers were more recently isolated.

Characterization of dihydroxyadipic acid

Compound **13a** (meso isomer) was obtained as white crystalline solid. Colourless crystals were grown from water. The molecular formula of $C_6H_{10}O_6$ was identified by mass spectra at m/z 177 [M - H], 159 [M - H_2O - H], 219 [M + H_2O + Na^+]. The ^{13}C-NMR spectrum shows two equivalent CH_2 at δ 29.02 ppm, two symmetric CH-OH at δ 69.97 ppm and two symmetric carbonyl group at δ 177.53 ppm. The ^{1}H-NMR spectrum showed two signals at 4.3 ppm (m, 2H, C*H*COOH) and at 1.95-1.7 ppm (2m, 4H). The melting point recorded was 170 °C which is consistent with the reported melting point of *meso*-2,5-dihydroxyadipic acid by Rosenlew.[66] Also the infrared spectrum (Figure 3.58) agrees with the one reported for *meso*-2,5-dihydroxyadipic acid.

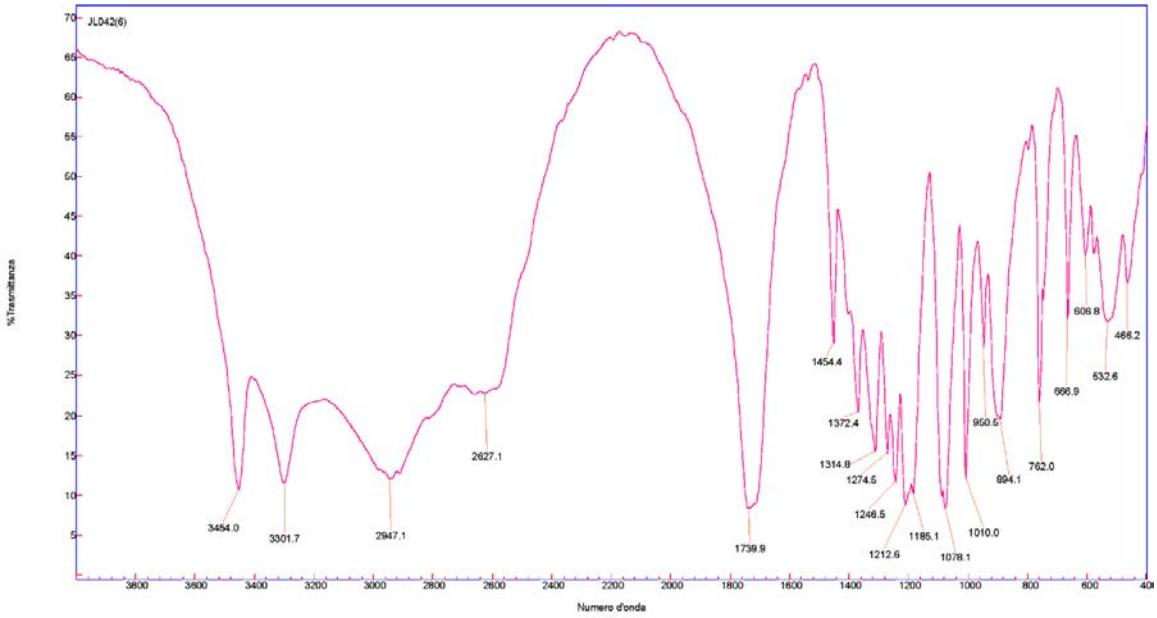


*Figure 3.58 – FTIR (KBr pellet) spectrum of 2,5-dihydroxyadipic acid (**13**).*

3.10 - References to Chapter 3

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Chapter 4

Experimental part

4.1 - Materials

Reagents and solvents, commercially available, were mostly selected at the highest degree of purity and used without further purification.

Solvents: Acetic acid (Sigma-Aldrich, 99.8%), Ethyl acetate (Sigma-Aldrich, 99.8%), Tetrahydrofuran (THF) (Sigma-Aldrich, 99.9%), Methanol (Sigma-Aldrich, >99.8%), Ethanol (Sigma-Aldrich, ≥99.8%), Dimethyl sulfoxide (Carlo Erba, 99.5%), Dimethylformamide (Sigma-Aldrich, 99.8%), Acetone (Sigma-Aldrich, 99.5%), Acetonitrile (Sigma-Aldrich, >99.9%), Diethylether (Sigma-Aldrich, >99.8%), 1,1,1,3,3,3-Hexafluoro-2-propanol (Sigma-Aldrich, ≥99%), Hexane (Sigma-Aldrich, >99%), Hexadeuterodimethyl sulfoxide (Sigma-Aldrich, ≥99.00%, 99.9 atom % D), Deuterium oxide (Sigma-Aldrich, 99.9 atom % D).

Reagents: Galactaric Acid (Sigma-Aldrich, 97%), Acetic Anhydride (Carlo Erba, 98%), 1,4-diazabicyclo[2.2.2]octane (DABCO) (Sigma-Aldrich, 98%), 4-(Dimethylamino)pyridine (DMAP) (Merck, 99%), Sodium acetate anhydrous (Sigma-Aldrich), Sodium hydroxide (ACS reagent, ≥97.0%, pellets), Potassium glucarate (Sigma-Aldrich, ≥98%), Sulfuric Acid (Sigma-Aldrich, 98%), Hydrochloric Acid (Sigma-Aldrich, 37%), Pd/C (Engelhard, 10%), Trimethyl orthoformate (Merck/Aldrich, 99%), *p*-Toluenesulfonyl chloride (Sigma-Aldrich, 99%), Triethylamine (Sigma-Aldrich, ≥99.5%), Trifluoroacetic acid (Sigma-Aldrich, 99%), Octylamine (Sigma-Aldrich, 99%), Hexamethylenediamine (Sigma-Aldrich, 98%), 2-Amino-1,3-propanediol (Bracco), Formic acid (Sigma-Aldrich, 95-97%), Benzylamine (Sigma-Aldrich, 99%), *N*-Benzylmethylamine (Merck Schuchardt OHG, 97%), (S)-(−)-α-Methylbenzylamine (Dipharma, 98%), Pyridine (Sigma-Aldrich, 99.8%), 1-Ethyl-3-methylimidazolium acetate (Io-li-tec, >95%), Quinine (Sigma-Aldrich, ≥98.0%), Benzylamine (Fluka, ≥99%), Ethanolamine (Sigma-Aldrich, ≥98%), Butylamine (Sigma-Aldrich, 99%), Dodecylamine (Sigma-Aldrich, ≥99%), Diisopropylamine (Sigma-Aldrich, ≥99%), Aniline (Sigma-Aldrich, ≥99.5%), Isoserinol (Bracco), Sodium methoxide (Fluka, ≥97%, powder), Sodium bicarbonate (Sigma-Aldrich, ACS reagent, ≥99.7%), Potassium butoxide (Fluka, ≥98%), n-Butyllithium (Sigma-Aldrich, 2.0 M solution in cyclohexane), Methylenediphenyl-diisocyante (Sigma-Aldrich, 98%), Hexamethylene diisocyanate (Sigma-Aldrich, 99%).

Analytical reagent: Pancaldi solution (molybdatophosphorus acid and Ce(IV)sulphate in 4% sulphuric acid), KBr (FT-IR grade, ⩾99% trace metals basis (EMD Millipore)), Ninhydrin (97%, Sigma-Aldrich) 0.2% solution in ethanol for TLC.

4.2 - Equipments

Melting point: BIBBY Melting Point SMP1 (capillary) device by Stuart Scientific. The melting points obtained have not been corrected.

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.6 MHz): NMR-spectra were recorded at 298 K on a Bruker AV 400 MHz (100 MHz for ^{13}C) instrument equipped with a 5 mm multinuclear probe with reverse detection at 305 K. Chemical shifts were reported in ppm with the solvent residual peak as internal standard (DMSO-d_6: δ H= 2.50 ppm, $CDCl_3$: δ H= 7.26 ppm) or adding terephthalic acid, 1,3,5-trioxane or tetramethylsilane (TMS) as internal standard for quantitative analysis.

Mass spectrometry: (MS): ESI-MS spectra were recorded by a Bruker Esquire 3000 plus ion-trap mass spectrometer instrument equipped with an ESI Ion Trap LC/MSn System. Tandem mass spectrometry (MS/MS) was also carried out by using the same instrument to analyse the fragmentation of parent ions. The results were presented as **MS** ((-)-ESI) and **MS** ((+)+ESI) for negative and positive ion mode, respectively.

Infrared spectroscopy (FT-IR): KBr pellets of solid samples were used to record IR absorption spectra by a FT-IR Varian 640 instrument.

Single Crystal X-ray Diffraction (X-ray): the X-ray diffraction data were collected using Bruker-AXS SMART-APEXII CCD diffractometer, Mo-K α radiation (λ = 1.54179 Å), θ/2θ geometry was used. Absorption correction was performed by multi-scan method implemented in SADABS.[1]

Gas Chromatograph/Mass Spectrometer (GC-MS): an Agilent 6890A instrument was used, which is equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) operating at constant pressure with a specific temperature program (e.g. initial temp. 40 °C hold for 1 min, ramp rate 10 °C/min to 300 °C, final temp. 300 °C hold for 10 min, gradient time 26 min, hold-up time 1.43 min) and with a Agilent 5973N quadrupole mass detector.

Flash chromatography: for the purification of analytical samples, column chromatography was used: as direct stationary phase, Merck 60, 270-400 mesh silica gel was used, at a pressure of 0.4 atm of compressed air, following the general procedure,[2] using a mixture of ethyl acetate and acetic acid (95: 5) as eluent.

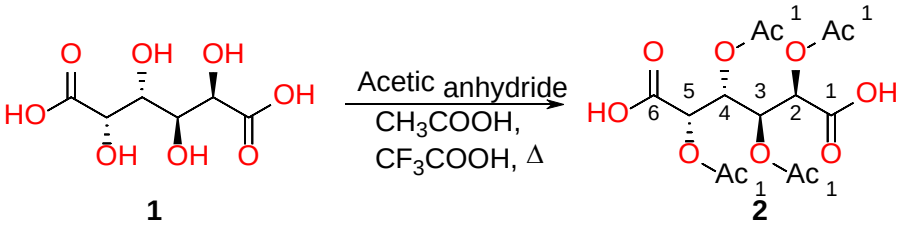
Thin layer chromatography (TLC): Analytical thin-layer chromatography was performed on precoated silica gel (0.25 mm thickness, 60 Fluka 99571) glass plates or on reverse phase silica gel C-18 sheet (Aldrich). The eluents used were: ethyl acetate, acetic acid, acetonitrile, methanol, hexane, and acetone in selected ratio. Compounds were visualized with UV lamp or by treatment with a solution of phosphomolybdic acid in ethanol (Pancaldi solution) followed by heating (organic substances are oxidised giving blue spots on a white to light blue background).

Vacuum pump: Edwards mechanical pump capable of producing a vacuum up to 0.1 mmHg.

Analytical Balance: Gibertini E 50 S/2 with five decimal places.

4.3 - Experimental Procedure and Compounds Characterization

1. Preparation of (2R,3S,4R,5S)-2,3,4,5-tetraacetoxyhexanedioic acid (galactaric acid tetracetate)



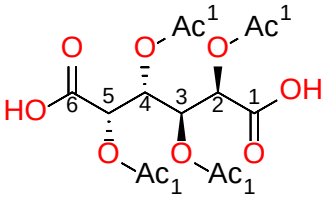
1 **2**

Procedure:

Galactaric acid (**1**, 2.0 g), acetic anhydride (9 ml), acetic acid (1 ml), and trifluoroacetic acid (1 ml) were charged in the sequence in a flask equipped with a condenser and magnetic stirrer. The flask was heated to 140 ° C by means of a heating plate and an oil bath, and the temperature was kept in constant for 8 hours, at the end of reaction the reaction mixture was cooled down to room temperature. From the ^{1}H-NMR analysis with terephthalic acid as an internal standard, the yield of **2** was 95%. The solid precipitated from the reaction was filtered, dispersed in ethyl acetate (4 ml) and re-filtered two times to give 3.0 g of a white solid (80.6 % yield). The compound can be recrystallized from ethyl acetate and is soluble in THF.

The reaction was repeated (a) at room temperature, then at 70 °C without acetic acid and (b) increasing the amount of trifluoroacetic acid to 5 ml. In the first case a yield of 5% was obtained after 33 hours of reaction, whereas in the second experiment the yield after 3 hours was 95%.

Analytical data for (2R,3S,4R,5S)-2,3,4,5-tetraacetoxyhexanedioic acid (2):

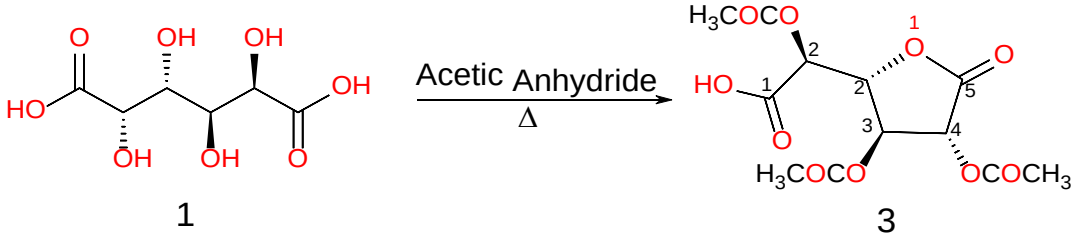


2

CAS: 5469-75-0; **MW**: 378.29 g/mol ($C_{14}H_{18}O_{12}$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 5.64 (s, 2H), 5.17 (s, 2H), 2.076 (s, 6H), 1.96 (s, 6H); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 169.4 (CO), 168.6 (CO), 167.7 (CO), 69.21 (CH_3), 57.7 (CH_3)**; FT-IR** (KBr, ν_{max}, cm^{-1}): 3406, 3058 (OH), 2916 (CH),

1757 ($C=O_{Acid}$), 1737 ($C=O_{ester}$), 1420, 1376, 1328, 1230, 1107, 1058, 948, 898, 846, 769, 675, 634, 593, 561; -**MS** ((-)-ESI) m/z [attribution]: 377 [M - H], 317 [M - H – CH_3COOH], 273 [M - H – CH_3COOH – CO_2], 213, 171, 141.

2. Preparation of (S)-2-acetoxy-2-((2R,3S,4R)-3,4-diacetoxy-5-oxotetrahydrofuran-2-yl)acetic acid [Galataro-1,4-lactone-triacetate]

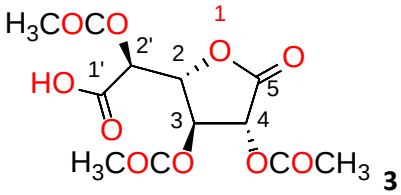


1 3

Procedure:

Galactaric acid (**1**, 50 g**)** and acetic anhydride (224.91 ml, molar ratio 9:1) were loaded in a three-necked bottom flask equipped with a magnetic stirring bar as well as a thermometer and a coil reflux condenser. The flask was heated at 140 °C with the use of an oil bath (150 °C) on a stirring/heating plate, and kept in constant temperature for 5 hours. At the end, a green/brown oil was obtained by evaporation of the acetic acid and residual acetic anhydride on the rotary evaporator at 60 °C. The oil was stirred with 250 ml of diethyl ether for 24 hour. The so formed white precipitate (galataro-1,4-lactone-triacetate) was recovered by filtration (47.2 g, yield 94.5% based on galactaric acid).

Analytical data for (S)-2-acetoxy-2-((2R,3S,4R)-3,4-diacetoxy-5-oxotetrahydrofuran-2-yl)acetic acid [galactaro-1,4-lactone-triacetate] **(3):**



3

MW: 318.23 g/mol ($C_{12}H_{14}O_{10}$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 5.89 (d, 1H, H-2', $J_{4,5}$ = 7.7 Hz), 5.49 (dd, 1H, H-2, $J_{2,2'}$ = 7.7 Hz, $J_{2,3}$ = 7.3 Hz), 5.26 (d, 1H, H-4, $J_{3,4}$ = 2.8 Hz), 5.01 (dd, 1H, H-3, $J_{3,4}$ = 2.8 Hz $J_{2,3}$ = 7.3 Hz), 2.14 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.07 (s, 3H, CH_3); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 170.5 (C-5), 169.8 (C-1), 168.9, 167.9, 77.3 (C-4), 72.5 (C-2), 72.0 (C-3), 70.0 (C-5), 20.8 (–**C**H_3); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3487 (OH), 2947 (CH), 1852 ($C=O_{Lactone}$), 1817 ($C=O_{Acid}$), 1758 ($C=O_{Ester}$), 1645, 1432, 1375, 1323, 1226, 1171, 1118, 1047, 959, 926, 894, 782, 741, 708, 605.

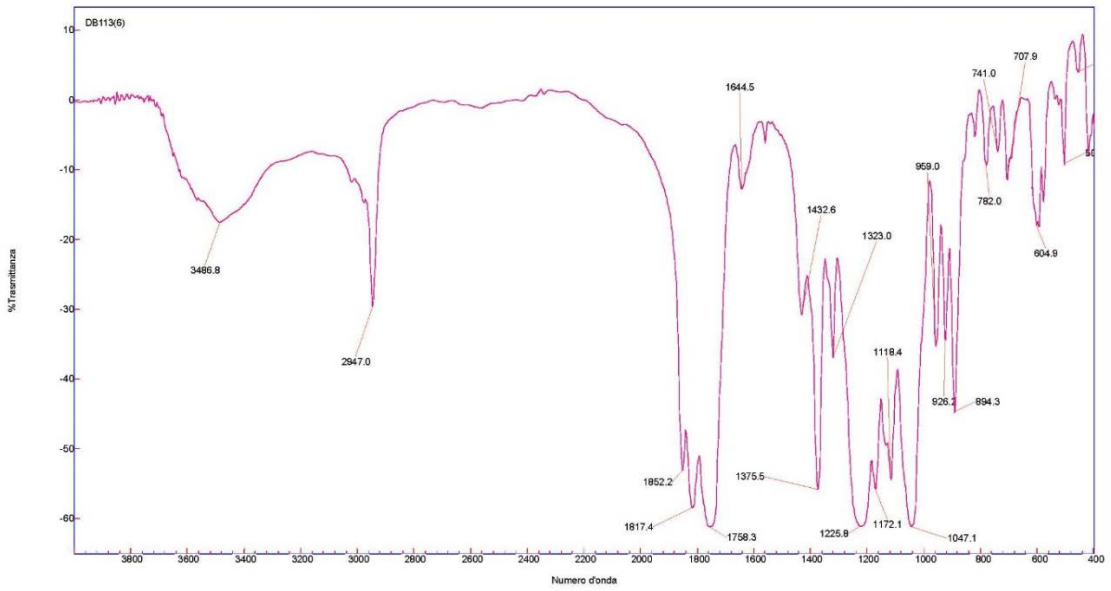
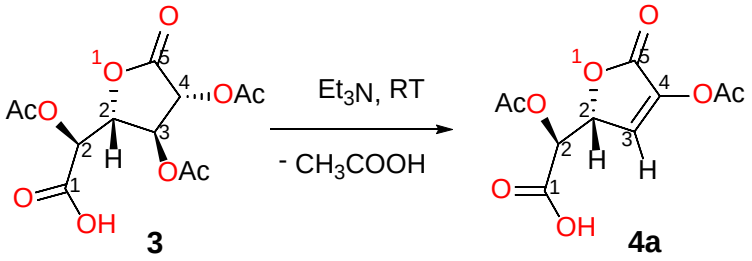


*Figure 4.1 - FT-Infrared spectrum of galactaro-1,4-lactone-triacetate (**3**)*

-**MS**(-ESI) (CH_3CN) m/z: 634 $[2M-H]^-$, 317 $[M-H]^-$.

3. Preparation from 3 of (S)-2-acetoxy-2-((R)-4-acetoxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid (4a)



Procedure:

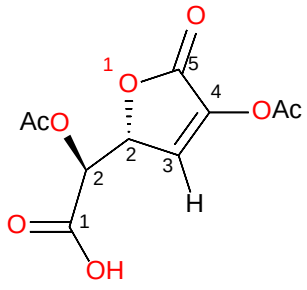
Galactaro-1,4-lactone triacetate [((S)-2-acetoxy-2-((2R,3S,4R)-3,4-diacetoxy-5-oxotetrahydrofuran-2-yl)acetic acid] (**3,** 220 mg, 0.694 mmol) and triethylamine (0.116 mL, 0.215 mmol) were loaded into a single necked flask equipped with a magnetic stirrer. Acetic acid (0.167 ml, 2.92 mol) was then added to mixture in molar ratio of 1: 4.2 as solvent. The reaction was carried out at room temperature for 4 days. After 4 days, a first sample was taken and a ^{1}H-NMR test was performed. The result showed a conversion of 30% with a yield 26% of the monoeliminated lactone (**4a**), while a 5% of the converted reagent goes to give a second mono-unsaturated isomer of **4a**. The reaction mixture showed a pale orange appearance. We decided to let the reaction run until the end of the week. After one week, a further ^{1}H-NMR test was performed. The results, however, didn't show a significant change: the conversion results in fact only increased to 39%, while the yield in **4a** is 34% + 2% (for the second mono-unsaturated isomer). The reaction mixture was dried at rotary evaporator and mechanical pump. The product **4a** [(S)-2-acetoxy-2-((R)-4-acetoxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid] of purity

higher than 70% was recovered from the raw product by flash chromatography separation (eluent AcOEt/CH_3COOH (100:1.25 ml). Other experiments afford the yield reported in Table 4.1.

*Table 4.1 - Synthesis of unsaturated lactone (**4a**) by elimination of acetic acid from **3**.*

Base	[3] (mmol)	[Base]/[3] ratio	AcOH (ml)	T (°C)	t (h)	Conv. (%)	4a (Yield %)
Triethylamine	0.69	0.62	4	80	11	39	28
Triethylamine	3.10	0.62	20	80	24	60	33
Triethylamine	0.69	1.2	0.17	50	48	90	38
DABCO	1.00	1	5	63	4	50	20
DABCO	0.64	0.8	7	RT	17	57	10

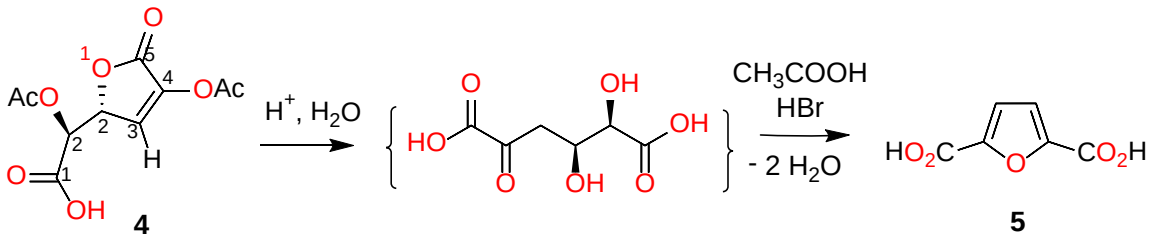
Analytical data for [(S)-2-acetoxy-2-((R)-4-acetoxy-5-oxo-2,5-dihydrofuran-2-yl)acetic acid] (4):



4a

MW: 258.04 g/mol ($C_{10}H_{10}O_8$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.36 (d, 1H, J=1.71 Hz); 5.69 (dd, 1H, J= 1.71 Hz, J=3.06 Hz); 5.18 (d, 1H, J=3.06 Hz); 2.28 (s, 3H); 1.99 (s, 3H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 169.2, 167.3, 167.0, 166.2, 137.3, 133.8, 78.5, 70.8, 20.35, 20.28; **+MS**(+ESI) m/z: 281 $[M+Na]^+$, 303 $[M - H +2 Na]^+$; **-MS**(-ESI) m/z: 257 $[M-H]^-$, 515 $[2M-H]^-$, 537 $[2M-2H+Na]^-$.

4. Preparation of 2,5-furandicarboxylic acid (5) from 4

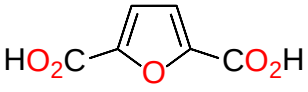


4 **5**

Procedure:

A solution of **4** (0.607 ml, 0.07 M, 0.06 mmol) in acetic acid (containing 10% by volume of water) is mixed with of conc. HBr (5.1 molar solution, 0.034 ml, 0.31 mmol). The system is heated at 60 °C for 2 hours. A cold sample was diluted with DMSO-d6 and analysed by ^{1}H-NMR. Then, the sampling was repeated after 6 hours detecting the typical peak at 7.29 ppm of aromatic hydrogens of 2,5-furandicarboxylic acid (**5**). Quantitative analysis by NMR with terephthalic acid as internal standard indicates that **5** was formed in yield of 11%.

Analytical data for 2,5-Furandicarboxylic acid (5):

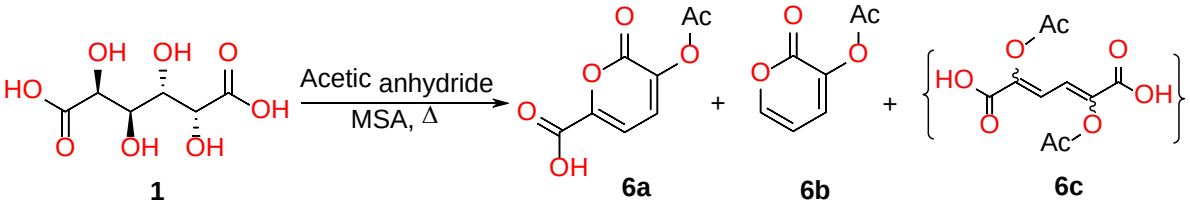


5

CAS: 3238-40-2; **MW:** 156.09 g/mol ($C_6H_4O_5$); **Melting Point**: 342 °C (with decomposition); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.29 (s, 2H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 158.9 (C-1, C-6), 147.1 (C-2, C-5), 118.4 (C-3, C-4); **FTIR** (KBr, ν_{max}, cm^{-1}): 3147 (C–H), 3125 (C–H), 2878 (br, OH), 1667 (C=O), 1571, 1523, 1417, 1268, 1224, 1187, 1163 and 1041; **GC-MS (EI):** found 156.0513. $C_6H_4O_5$ requires 156.0059.

5. Preparation of 3-Acetoxy-2-oxo-2*H*-Pyran-6-Carboxylic Acid (6a) from 3

a. Methanesulfonic acid as catalyst

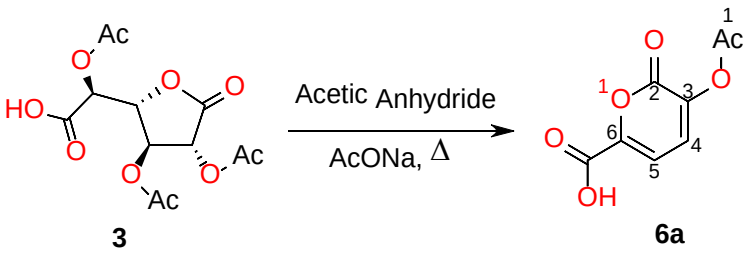


1 **6a** **6b** **6c**

Procedure:

In a flask equipped with a condenser and magnetic stirrer, compound **3** (250 mg), acetic anhydride (320 mg) and methanesulphonic acid (151 mg) were loaded. The flask was brought to 120 °C by means of a heating plate and oil bath. The system was kept in temperature for 4.5 hours, after which it was allowed to cool to room temperature. The yield of product **6a**, taken from the ^{1}H-NMR analysis with internal standard, was 69%.

b. Sodium acetate as catalyst



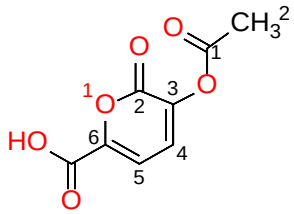
3 **6a**

Procedure:

Galactaro-1,4-lactone-triacetate (**3**) (5 g, 15,7 mmol) and acetic anhydride (14.83 ml, 157 mmol) were mixed in a flask equipped with condenser and magnetic stirrer. Sodium acetate (0.213 g, 1,57 mmol) was added to the flask and, then, the system was heated at 100 °C by electrical oil bath, and then kept the mixture at this constant temperature for 8 hours. At the end, after evaporating acetic acid and residual acetic anhydride on the rotary evaporator at 60 °C, a brown homogeneous solution was obtained, which was dissolved in THF (20 ml). The stirrer mixture was cooled to room temperature, concentrated at 45°C to 10 mL by evaporating acetic acid and residual acetic anhydride on the rotary evaporator. Then the mixture was left under stirring for 1 hour. A white solid was precipitated which was recovered by filtration and washing with acetic acid and, then, with diethyl ether. The mother liquors were concentrated obtaining a yellow oil. Then, by repeating the work-up procedure, 4 batches of solid precipitates were collected. The total yield in **6** is 73.8 %.

In the dehydration and acetylation reaction reported above, acetic anhydride was used as an acylation agent and sodium acetate worked as basic catalyst. The procedure improves the one previously developed by us with methanesulfonic acid as catalyst.

Analytical data for 3-Acetoxy-2-oxo-2H-Pyran-6-Carboxylic Acid (6a)



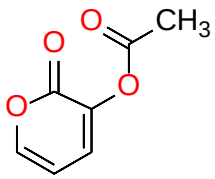
6a

MW: 198.13 g/mol ($C_8H_6O_6$); **Rf:** 0.3 (Eluent: ethyl acetate/acetic acid 95:5) by UV detection; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.47 (d, 1H, H-5, J = 7.1 Hz), 7.02 (d, 1H, H-4, J = 7.1 Hz), 2.76 (s, 3H, CH_3); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 167.52 (HO-**C=O**), 159.67 (C2), 155.86 (CH_3-**C=O**), 146.65 (C-6), 139.47 (C-3), 130.97 (C-5), 110.02 (C-4), 19.99 (CH_3); **FT-IR** (KBr, ν_{max}, cm^{-1})**:** 3561, 3446, 3141, 3102, 3064, 2893, 2831, 2763, 2598, 2654, 2598, 2511, 2485, 2189, 1978, 1950, 1781 ($C=O_{Lactone}$), 1735 ($C=O_{Ester}$), 1708 ($C=O_{Acid}$), 1644, 1619, 1438, 1377, 1268, 1232, 1194, 1141,

1121, 1070, 1007, 980, 902, 877, 796, 774, 759, 671, 597, 529, 458; -**MS** ((-)-ESI) m/z [attribution]: 197 [M - H], 153 [M - H - CO_2], 111 [153 - CH_2CO], 81; +**MS** ((+)+ESI) - m/z [attribution]: 199 [M + H], 181 [M + H – H_2O], 157 [M + H - CH_2CO], 139 [181 - CH_2CO].

From the reaction mixture, compounds **6b** and **6c** were also isolated by column chromatography.

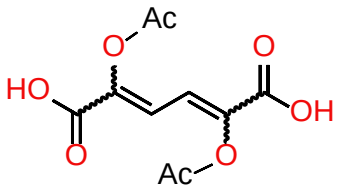
Analytical data for 2-oxo-2H-pyran-3-yl acetate (6b)



6b

MW: 154.12 g/mol ($C_7H_6O_4$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.69 (dd, 1H, J = 1.66 Hz J = 5.14 Hz), 7.38 (dd, 1H, J = 1.65 Hz J = 7.15 Hz), 6.43 (dd, 1H, J = 5.14 Hz, J = 7.15 Hz), 2.24 (s, 3H, CH_3).

Analytical data for 2,5-diacetoxymuconic acid [(2Z,4Z)-2,5-diacetoxyhexa-2,4-dienedioic acid] (6c)

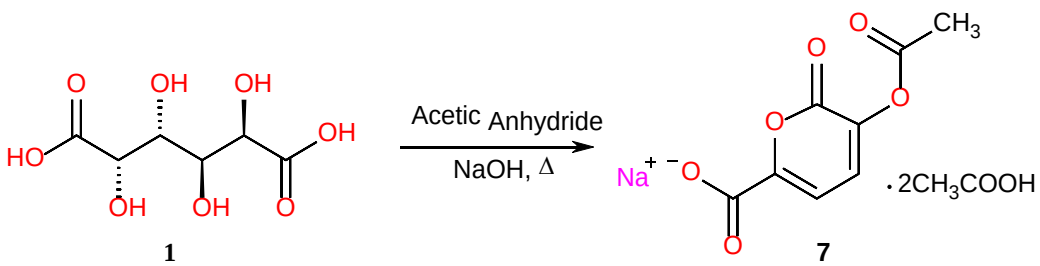


6c

MW: 258.18 g/mol ($C_{10}H_{10}O_8$); **Melting point**: 228 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 6.98 (s, 2H, =CH), 2.29 (s, 6H, CH_3); -MS ((-)-ESI) m/z: 257 [M - H], 537 [2M + Na - 2H]; +MS ((+)+ESI) - m/z [attribution]: 281 [M + Na], 539 [2M + Na].

6. Preparation of Sodium 3-Acetoxy-2-oxo-2H-Pyran-6-Carboxylate (7) starting

a) from galactaric acid (1)



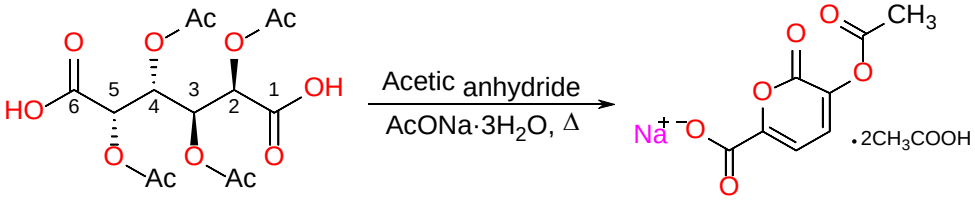
1 **7**

Procedure (JL019):

In a three-necked round flask, equipped with a thermometer and a coil reflux condenser, galactaric acid (5.00 g, 23.80 mmol) and acetic anhydride (30 mL, 317 mmol) were mixed together and the system was stirred and heated at 90 °C by an oil bath. Then, sodium hydroxide (0.997 g, 24.93 mmol) was

added over a period of 1.7 hour to the stirring suspension. The reaction was kept at 90-95 °C (during the addition of NaOH the temperature was increased and it was necessary to cool the mixture by lifting the bottom flask from the oil bath) for 4 hour. Then, the mixture was cooled to room temperature when it became a viscous brown liquid. After one night a precipitate was formed and it was filtered, washed two time with a mixture of acetic acid/acetic anhydride (95/15, 10 mL) and dried under vacuum:, obtaining 4.6 g of sodium 3-acetoxy-2-oxo- pyran-6-carboxylate *bis*-acetic acid solvate ($NaC_8H_5O_6 \cdot 2\ CH_3COOH$) with 98.24% purity (overall yield 59.7%). The filtrate was concentrated at 45°C to 10 mL by evaporation and, then, cooled to room temperature. The resulting solid was filtered, washed two times with a mixture of acetic acid/acetic anhydride (95/15, 1 mL) and dried under vacuum affording sodium 3-acetoxy-piran-2-one-6-carboxylate · 2 CH_3COOH (1.12 g) in 11.1 % yield. Overall yield 70.8% based on galactaric acid.

b) from galactaric acid tetraacetate (2)



2 **7**

Procedure:

Galactaric acid tetraacetate **2** (88% purity, 2.0 g, 4.65 mmol) and acetic anhydride (15 ml) were charged in a flask equipped with a condenser and magnetic stirrer. The flask was heated up to the reflux temperature of acetic anhydride, by using a heating plate with oil bath. When the reaction mixture reached 110 °C (in 10 minutes), the substrate dissolved completely to form a colourless solution. Sodium acetate ($AcONa \cdot 3H_2O$, 719 mg, 5.28 mmol) was added to the hot solution. Sodium acetate remains insoluble and after a few minutes the amount of out-of-phase solid significantly increased by precipitation of the sodium tetraacetyl galactarate. The heterogeneous reaction mixture was heated at 135 °C and in four hours becomes a homogeneous yellow solution. The reaction mixture was then cooled to room temperature giving a liquid. This was concentrated by reducing the volume by half and from this cold solution a white solid precipitated (602 mg, yield: 46%, purity: 82%), whose ^{1}H-NMR spectrum is shown in Figure 4.2. The mother liquors were concentrated to obtain a brown solid (yield: 52%, purity: 77%).

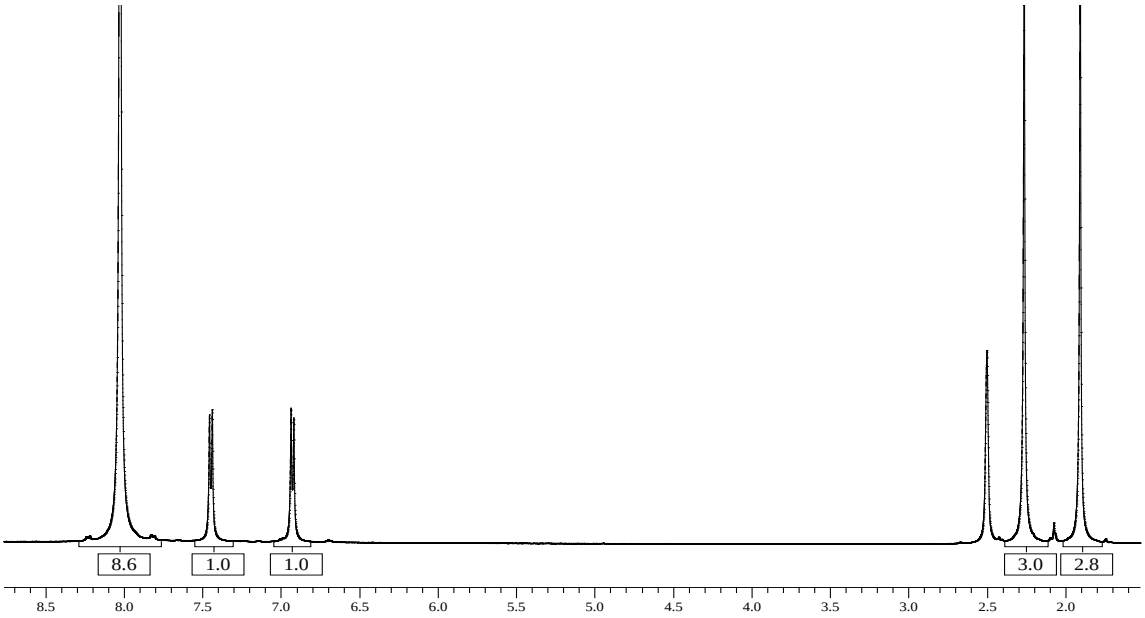
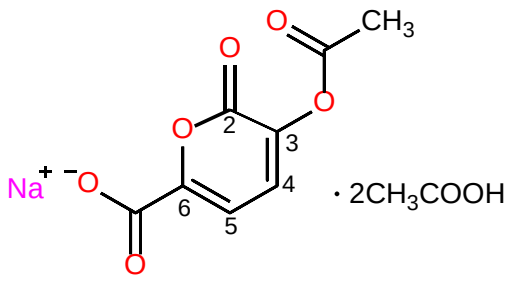


*Figure 4.2 - ^{1}H-NMR (DMSO (237_pre)): precipitated solid (**7**) from reaction solution (11.45 mg) with addition of terephthalic acid (12.48 mg) as internal standard (δ 8.0 ppm).*

Analytical data for Sodium 3-Acetoxy-2-oxo-2H-Pyran-6-Carboxylate (7) Acetic Acid solvate.



7

MW: 219.78 g/mol (unsolvated); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.45 (d, 1H, H-5, J = 7.1 Hz), 6.91 (d, 1H, H-4, J = 7.1 Hz), 2.27 (s, 3H, CH_3), 1.91 (s, 6H, CH_3); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 172.91 ($^{-}$O-**C=O**), 168.57 (HO-**C=O**), 161.78 (C-2), 157.97 (CH_3-**C=O**), 156.35 (C-6), 136.78 (C-3), 132.95 (C-5),105.66 (C-4); **FT-IR (KBr**, ν_{max}, cm^{-1}**):** 3416, 3104, 3076, 3022, 2941, 2900, 2840, 2774, 2667, 2607, 2539, 2365, 2165, 1772 ($C=O_{Lactone}$), 1727 ($C=O_{Ester}$), 1651 ($C=O_{Acid}$), 1619, 1412, 1372, 1332, 1289, 1200, 1135, 1100, 1062, 1007, 972, 150, 887, 855, 811, 784, 747, 675, 654, 599, 577, 565, 529, 468, 429, 408; **MS**((-)-ESI): m/z 197 [M - H], 153 [M - H - CO_2], 111 [153 - CH_2CO], 81.

The compound was characterized by X-ray single crystal analysis (Table 4.2).

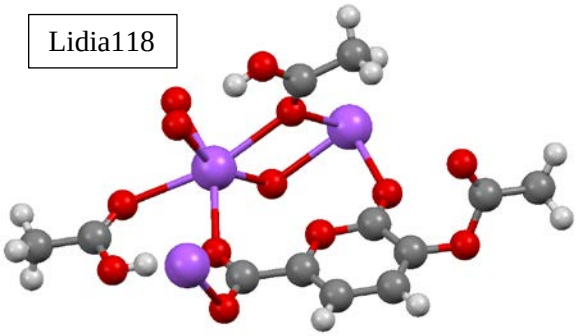


*Table 4.2 - Single crystal X-ray diffraction structure and data of sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate acetic acid solvate (**7a**).*

Crystal data: **LIDIA118**	
Chemical formula	$C_{12}H_{13}NaO_{10}$
M_r	340.21
Crystal system, space group	Triclinic, *P*1
Temperature (K)	100
a, *b*, *c* (Å)	8.2167 (8), 9.3536 (10), 10.3921 (11)
α, β, γ (°)	95.567 (3), 96.894 (4), 105.333 (3)
V (Å^3)	757.75 (14)
Z	2
Radiation type	Mo *K*α
μ (mm^{-1})	0.16
Crystal size (mm)	0.42 × 0.12 × 0.06
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	Multi-scan
T_{min}, T_{max}	0.659, 0.746
No. of measured, independent	14392, 4441, 3262
R_{int}	0.039
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.707
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.044, 0.123, 1.03
No. of reflections	4441
No. of parameters	213
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.41, −0.32

Dissolving **7a** in water and crystallization afforded the sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate hydrate **7b**. The compound was characterized by X-ray single crystal analysis (Table 4.3).

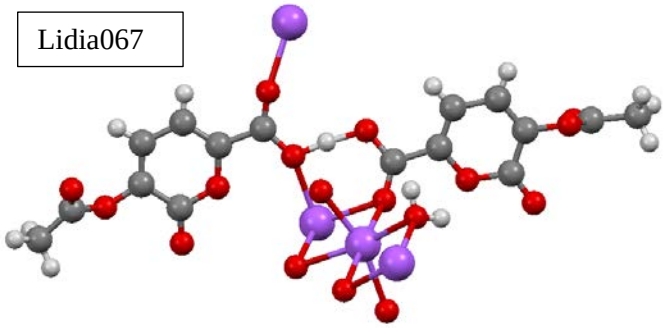
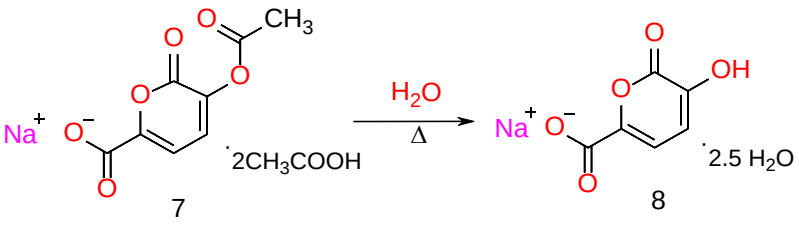


*Table 4.3 - Single crystal X-ray diffraction structure and data of sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate hydrate **7b**.*

Crystal data: **LIDIA067**	
Chemical formula	$C_{16}H_{13}NaO_{13}$
M_r	436.25
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	150
a, b, c (Å)	19.929 (7), 12.975 (4), 6.935 (3)
β (°)	96.510 (12)
V (Å^3)	1781.7 (11)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.16
Crystal size (mm)	0.35 × 0.28 × 0.03
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	Multi-scan
T_{min}, T_{max}	0.626, 0.746
No. of measured, independent	21305, 4387, 3154
R_{int}	0.043
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.675
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.039, 0.089, 1.02
No. of reflections	4387
No. of parameters	285
H-atom treatment	H atoms treated by a mixture of independent and constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.32, −0.24

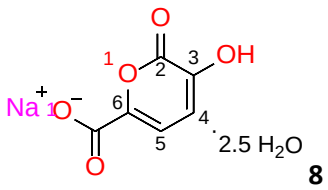
7. Preparation of Sodium 3-Hydroxy-2-oxo-2H-pyran-6-carboxylate (8)



Procedure:

Hydrolysis of sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate (**4)** to 3-hydroxy-2-oxo-2H-pyran-6-carboxylate (**8**) was carried out by mixing compound **7** (2 g) with water (10 ml) in a round bottom flask equipped with a magnetic stirring bar, as well as a thermometer and coil reflux condenser. The system was heated to 100 °C by electric heating plate for 5 hours. The reaction mixture was concentrated at 60 °C to 5 mL by rotatory evaporator and, then, cooled to room temperature. A light brown solid was formed from the concentrated solution. 130 mg of crystals was recovered by filtration and dried in vacuum for 4 hours. Structure **8** was consistent with the data of ^{1}H-NMR and single-crystal X-ray diffraction.

Analytical data for Sodium 3-Hydroxy-2-oxo-2H-pyran-6-carboxylate hemihydrate (8).



MW: 177.99 g/mol (anhydrous); **^{1}H-NMR** (400 MHz, D_2O, ppm): δ 7.02 (d, 1H, H-5, J = 7.5 Hz), 6.84 (d, 1H, H-4, J = 7.5 Hz); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.12 (d, 1H, H-5, J = 7.34 Hz), 6.71 (d, 1H, H-4, J = 7.34 Hz); **-ESI-MS(-):** m/z 155 [M-2.5H_2O-Na], 333 [2M^{-}-5H_2O-Na].

A selected crystal of the compound was submitted to single crystal X-ray diffraction, recovering the structure and the data collected in the next page (Table 4.4). The analysis has confirmed that the isolated solid is the pentahydrate derivative of **8**.

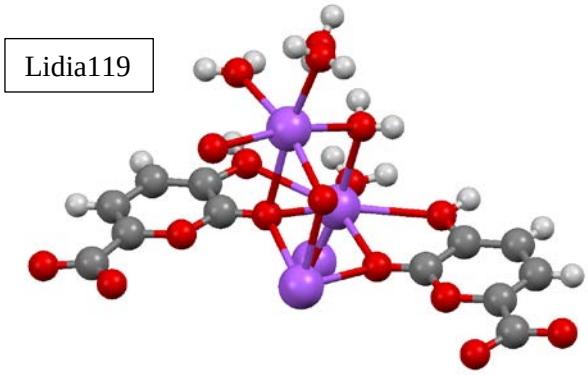
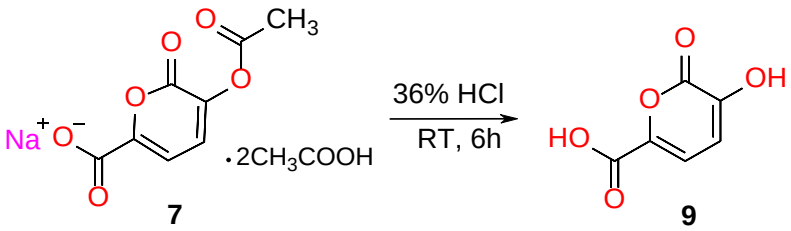


*Table 4.4 - Single crystal X-ray diffraction structure and data of sodium 5-hydroxy-6-oxo-pyran-carboxylate pentahydrate **(8)**.*

Crystal data: **LIDIA 119**	
Chemical formula	$C_{12}H_{16}Na_2O_{15}$
M_r	446.23
Crystal system, space group	Triclinic, *P*1
Temperature (K)	103
a, *b*, *c* (Å)	9.3955 (14), 10.3523 (14), 11.0073 (14)
α, β, γ (°)	113.687 (6), 102.299 (6), 107.499 (6)
V (Å^3)	864.1 (2)
Z	2
Radiation type	Mo *K*α
μ (mm^{-1})	0.20
Crystal size (mm)	0.24 × 0.10 × 0.08
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	SADABS
T_{min}, T_{max}	0.604, 0.746
No. of measured, independent	10157, 5082, 3960
R_{int}	0.042
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.708
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.042, 0.124, 1.01
No. of reflections	5082
No. of parameters	302
No. of restraints	12
H-atom treatment	H atoms treated by a mixture of independent and constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.54, −0.30

8. Preparation of 3-Hydroxy-2-oxo-2H-pyran-6-carboxylic acid (9)

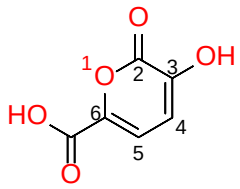


7 9

Procedure:

The raw salt from previous preparation (35.07 g, containing 32.65 g [96 mmol] of acetic acid solvate of sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate) was added in a 50 mL round flask at room temperature to a concentrated solution of hydrochloric acid (36%, 33 mL) and the heterogeneous system was left to stir for 2 days. Then, the precipitated solid was filtered recovering 25.52 g of a solid. ^{1}H-NMR analysis allows to identify the solid as 3-hydroxy-2-oxo-2H-pyran-6-carboxylic acid with 67% of purity. Then water was used to solubilise the residual NaCl as by-product inside the solid. After filtration, 15.25 g of solid was recovered, which is mainly 3-hydroxy-2-oxo-2H-pyran-6-carboxylic acid of 94% purity. The overall yield calculated from the NMR data was 101%, which could be explained by the water in the product crystal structure.

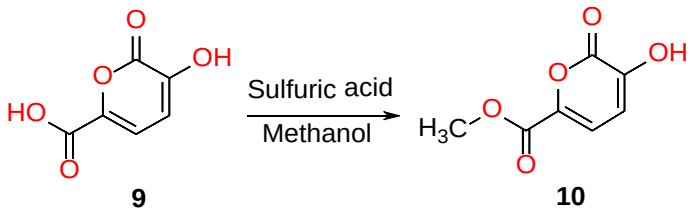
Analytical data for 5-hydroxy-6-oxo-pyran-carboxylic acid pentahydrate (9) [Consistent to the one reported in D. Wiedemann, A Grohmann Z. Naturforsch. 2009, 64b, 1276 – 1288].[3]



9

CAS: 93905-59-0; **MW:** 156.093 g/mol ($C_6H_4O_5$); **Melting Point**: 215 °C (with decomposition); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 13.41 (br s, 1H, COOH); 11.02 (br s, 1H, OH); 7.12 (d, 1H, H-5, ^{3}J = 7.3 Hz); 6.70 (d, 1H, H-4, ^{2}J = 7.3 Hz); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 161.13 (HO-C=O), 158.86 (C-2), 147.74 (C-6), 141.18 (C-3), 115.02 (C-5), 113.84 (C-4); **IR** (KBr, ν_{max}, cm^{-1}**):** 3308 (OH_{Enol}), 3116, 3080 (CH), 2921 (OH_{Acid}), 1715 ($C=O_{Acid}$), 1659 ($C=O_{Lactone}$), 1554, 1437, 1267, 1227, 1161, 1074, 871, 756, 705, 600, 466; **-MS**((-)-ESI): m/z 155 [M - H], 127 [M - CHO], 111 [M - H - CO_2], 83 [M - CO_2 - CHO].

9. Synthesis of Methyl 3-Hydroxy-2-oxo-2H-Pyran-6-Carboxylate (10)

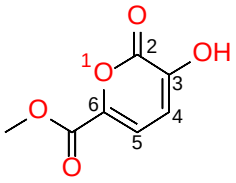


9 **10**

Procedure:

3-Hydroxy-2-oxo-2H-pyran-6-carboxylic acid (**9**, 2.16 g, 10.9 mmol)) was mixed together with methanol (20 mL) in a round bottom flask. Then, sulfuric acid (65 mg) was added to the suspension and the resulting mixture was refluxed by electric heater for 5 hours under stirring. Then the reaction mixture was cooled down and water (90 mL) was added. The resulting mixture was extracted with dichloromethane (3×30 mL). The combined organic layers were dried on sodium sulphate, filtered and then the solvent was removed by evaporation under reduced pressure to obtain product **10** as a white solid with 90 % purity (yield 75 %).

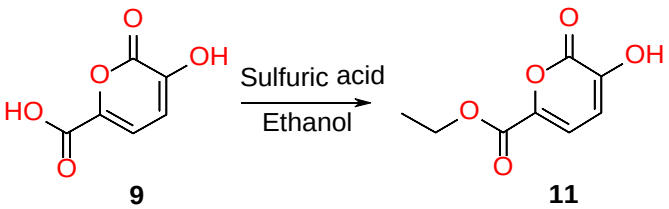
Analytical data for methyl 3-hydroxy-2-oxo-2H-pyran-6-carboxylate (10)



10

MW: 170.02 g/mol ($C_7H_6O_5$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.15 (1H, d, J = 7.5 Hz), 6.68 (1H, d, J = 7.5 Hz), 3.79 (s, 3H); **^{13}C-NMR** (100.6 MHz, DMSO-d6, ppm): δ 159.45, 157.74, 147.34, 139.21, 114.04, 113.69, 52.46; **+MS**((+)+ESI): m/z 171 [M-H], 193 [M+Na].

10. Synthesis of Ethyl 3-hydroxy-2-oxo-2H-pyran-6-carboxylate (11)



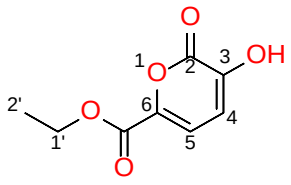
9 **11**

Procedure:

3-Hydroxy-2-oxo-2H-pyran-6-carboxylic acid (**9**, 1.50 g, 9.63 mmol)) was added to ethanol (15 mL) in a round bottom flask and the system was allowed to stir with a magnetic stirrer for 10 minutes. Then, sulfuric acid (136 mg) was added to the suspension and the resulting mixture was refluxed by electric heater for 24 h under stirring. Then, the reaction mixture was cooled down at R.T. and water (90 mL) was added. The resulting mixture was extracted with dichloromethane (3×40 mL). The combined

organic extracts were dried on Na_2SO_4, filtered and, then, the solvent was removed by evaporation at reduced pressure, recovering product **11** as a white solid (1.57 g, 8.54 mmol, 89% yield).

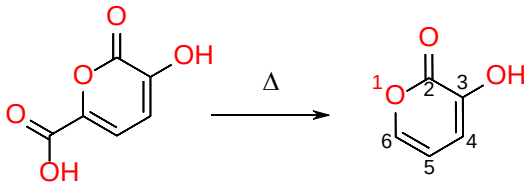
Analytical data for ethyl 3-hydroxy-2-oxo-2H-pyran-6-carboxylate (11)



11

CAS: 1254758-51-4; **MW:** 184.04 g/mol ($C_8H_8O_5$); **Melting point:** 105-108 °C; **Crystalline form**: very soft colorless needles; **^{1}H-NMR** (400 MHz, $CDCl_3$, ppm): δ 7.18 (1H, d, J=7.5 Hz), 6.73 ppm (1H, d, J=7.5 Hz), 4.35 (2H, q, J=7.0 Hz), 1.36 (3H, t, J=7.0 Hz); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 11.15 (1H, bs), 7.15 (1H, d, J=7.5 Hz), 6.69 (1H, d, J=7.5 Hz), 4.27 (2H, q, J=7.1 Hz), 1.28 (3H, t, J=7.1 Hz); **^{13}C-NMR** (100.6 MHz, $CDCl_3$, ppm): δ 159.60, 159.12, 145.77, 140.89, 113.12, 113.00, 62.13, 14.10; **IR** (KBr, ν_{max}, cm^{-1}**):** 3325, 1739, 1729, 1687; +**MS** ((+)+ESI): m/z: 207 $[M+Na]^+$; **Elemental analysis**: calculated. C 52.18, H 4.38; recorded: C 51.99, H 4.31.

11. Decarboxylation of 5-Hydroxy-6-oxo-pyran-carboxylic acid pentahydrate (9) to 3-Hydroxy-2H-pyran-2-one (12)

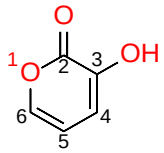


9 **12**

Procedure:

The acid **9** (1.06 g) was introduced in the bottom of a sublimation apparatus. The lower part of the apparatus was immersed in an oil bath heated at 170 ° C by means of an electric heating plate. The sublimation product, which started to form when the bath temperature reached 140 °C, was deposited on the surface of the cold finger in which cold water was circulated. At the end of the reaction, 3-hydroxy-2H-pyran-2-one (**12**) was recovered from the cold finger surface (750.78 mg, 98% yield).

Analytical data for 3-Hydroxy-2*H*-pyran-2-one (12)

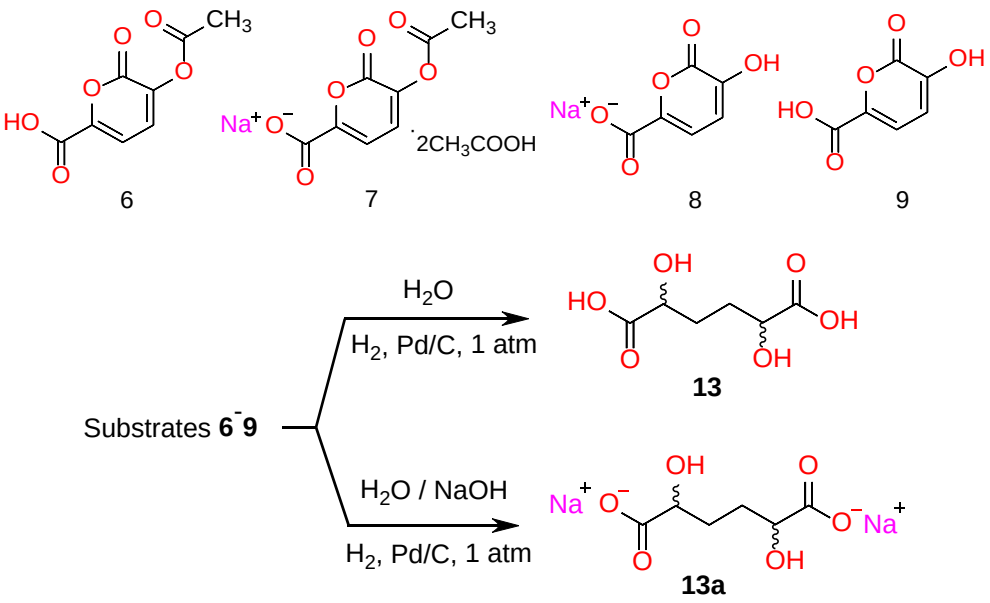


12

CAS: 496-64-0; **MW:** 112.084 g/mol ($C_5H_4O_3$); **Melting point**: 90-91°C (lit. 92 °C) (sublimes); **^{1}H-NMR** (400 MHz, $CDCl_3$, ppm): δ 7.14 (dd, 1H, J=1.76, 5.28 Hz), 6.65 (dd, 1H, J=1.76, 7.04 Hz), 6.19

(dd, 1H, J=5.28, 7.04 Hz), 6.18 (bs, 1H(OH)). Identical to the one reported in ref. W.V. Turner, W.H. Pirkle, J. Org. Chem., 39, 1935 (1974)[4] but different from the one reported in DMSO solvent (Inorganica Chimica Acta 360, 264–272 (2007))[5]; **^{13}C-NMR** (100 MHz, DMSO-d6, 25 °C, ppm): δ 106.7 (Ar–C), 115.7 (Ar–C), 142.6 (Ar–C), 143.0 (C–OH), 159.8 (C=O); **-MS** ((-)-ESI): m/z: 111.01 [M-H].

12. Preparation of 2,5-dihydroxyadipic acid (13) starting from 2-pyrone compounds 6-9.



Procedure:

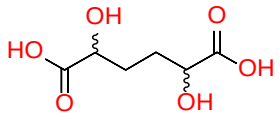
In a two-necked flask equipped with magnetic stirring bar, thermometer and a gas inlet connector, water was added under nitrogen and then the different 2-pyrone substrates (**6**, **7**, **8**, **9**) followed by the Pd/C catalyst (in the amount shown in Table 4.3) in 2 mL of water. Since some of the substrates are not very soluble in water, the concentration of substrate used was around 0.065 M in order to reach solubility just from the start of reaction. Hydrogen gas was supplied from the connector inlet through a hydrogen delivery ramp and a mechanical pump. The atmosphere was changed by removing nitrogen and adding hydrogen for three times, and then the flask was left in a hydrogen atmosphere for different time from 3 hours to 20 hours at 35 °C. The specific reaction conditions are shown in Table 4.5.

*Table 4.5 - Synthesis of 2,5-dihydroxyadipic acid (**13**) by hydrogenation of pyrones **6-9** (H_2, Pt/C, 35°C, 1 atm).*

Reaction	Substrates (mg)	[Sub] M	[Pd]/[Sub.] ratio	t (h)	Sub. Conv. %	13 Yield %	Mono-alkene Yield%
JL023	**7** (205.5)	0.29	0.197	20	100	11	0
JL024	**6** (203.1)	0.51	0.179	20	100	29	0
JL025	**7** (101.2)	0.29	0.225	3	100	23	0
JL026	**6** (99.1)	0.50	0.195	3	70	11.2	0.25
JL029	**8** (209)	0.58	0.167	5	70	15.1	0
JL032	**9** (202.5)	0.065	0.143	6	100	58.8	0
JL042	**9 (**9700)	0.12	0.197	10	100	59.6	0

Similar conditions (but adding 5% H_2SO_4) applied on purified **6** at larger scale (100 g) afford 2,5-dihydroxyadipic acid (**13**) in isolated yield of 95% (85.4 g).

Analytical data for 2,5-dihydroxyadipic acid (13) [similar to the one reported in Dewaele, A., et al., ACS Sustainable Chemistry & Engineering, 2016. **4**(11): p. 5943-5952].[6]



13

CAS: 13544-77-9**;** **MW:** 178.14 g/mol ($C_6H_{10}O_6$); **Melting Point**: 170 °C comparing with the recorded melting point 173 °C in the work of Rosenlew [7]; **^{1}H-NMR** (400 MHz, D_2O, ppm): δ 4.3 (m, 2H, C*H*COOH), 1.95-1.7 (2m, 4H); **^{13}C-NMR** (100 MHz, D_2O, ppm): δ 177.53 (HO-**C=O**), 69.793 (C-2), 29.02 (C-3); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3454, 3301, 3080 (CH), 2947 (OH_{Acid}), 2661，2627, 1740 ($C{=}O_{Acid}$), 1540, 1454, 1372, 1315, 1274, 1247, 1213, 1078, 1010, 951, 894, 762, 667, 607, 533, 466; -**MS**((-)-ESI): m/z 177 [M - H], 159 [M - H_2O - H]; +**MS** ((+)+ESI): m/z: 219 [M + H_2O + Na^+]. The compound isolated was in all cases a mixture of two diastereoisomers with a preference for the *meso* form.

The published analytical data of derivatives of dihydroxyadipic acid as follows,
(±)-2,5-Dihydroxyadipic acid: m.p. 146 °C (from diethyl ether), **FT-IR** (KBr, ν_{max}, cm^{-1}): 3400, 3000-2500 (broad), 1700, 890, 830, 800, and 760 cm^{-1}, as prepared following the method of Le Sueur.[7]

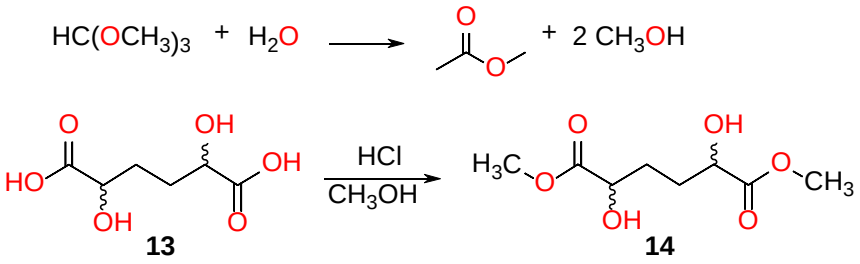
meso-2,5-Dihydroxyadipic acid: m.p. 173-174 °C (from ethanol), **FT-IR** (KBr, ν_{max}, cm^{-1}): 3450, 3000-2500 (broad), 1710, 890, 760, and 745 (Found: C, 40.3; H, 5.8. Calc. for $C_6H_{10}O_6$: C, 40.5; H, 5.7%) as prepared following the method of Le Sueur.[7]

Diethyl *meso*-2,5-diacetoxyadipate, m.p. 79.5-80 °C (from ethyl acetate) (Found: C, 52.85; H, 6.9. $C_{10}H_{18}O_6$, requires C, 52.8; H, 7.0%). (ref. Brettle, R & W. Latham, D. (1968). Aliphatic hydroxy-

acids. Part III. Syntheses with alkyl 2-acetoxy-3-carboxypropionates. Journal of The Chemical Society C: Organic. 10.1039/j39680000906)[8].

Diethyl (±)-2,5-dihydroxyadipate (prepared by use of sulphuric acid (3%) in ethanol) had m.p. 58-59 °C [from chloroform-pentane] (Found: C, 51.4; H, 8.0. $C_{10}H_{18}O_6$ requires C, 51.3; H, 7.8%).

13. Esterification of 2,5-Dihydroxyadipic acid to dimethyl ester (14) or diethyl ester (15)



Reagents	MW	Amount	mmol	Equivalent
13	178	0.46 g	2.6	1
Hydrochloric acid	36.46	0.022 ml	0.26	0.1
Trimethyl orthoformate	106.12	0.67 ml	6.11	2.35
methanol	32.04	10 ml	247.4	95.17

Procedure:

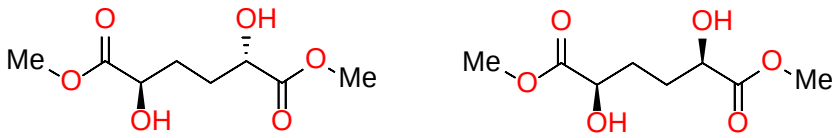
The reaction was carried out in a flask equipped with a magnetic stirrer bar adding 10 ml of methanol (together with a catalytic amount of 37% hydrochloric acid), substrate **13** and, then, trimethyl orthoformate, and water in the amount reported in the above table. The reaction mixture was reacted overnight at room temperature. The reaction was carried out at a concentration of around 0.065 M in order to have at start a homogeneous system. At the end, a white solid (insoluble in chloroform) was obtained by evaporation of trimethyl orthoformate and methanol on rotary evaporator. The product was crystalized from the reaction mixture after 48 hours. Better crystals can be obtained by recrystallization from diethyl ether.

Ethyl ester was similarly obtained (reaction **JL039**) by using ethanol and triethyl orthoformate instead of methanol and trimethyl orthoformate as solvents. Reaction conditions are shown in Table 4.6.

*Table 4.6 - Esterification of **13** by trialkyl orthoformates and hydrochloric acid to methyl ester (**14**) and ethyl ester (**15**)*

Reaction	Reagent (g)	$HC(OR)_3$ (ml)	HCl (ml)	H_2O (ml)	Solvent (ml)	Ester (Yield %)
JL036	0.461	0.67	0.02	0.09	Methanol (10)	**14** (86.6)
JL039	0.298	0.43	0.014	0.06	Ethanol (5)	**15** (29.1)

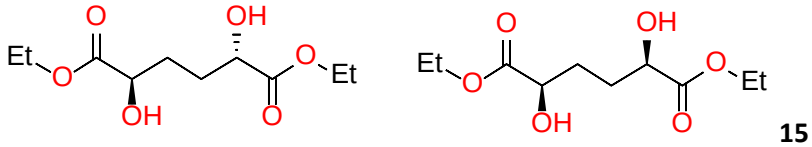
Analytical data for (2R,5S)-dimethyl 2,5-dihydroxyadipate (14)



14

MW: 206.08 g/mol ($C_8H_{14}O_6$); **Melting Point**: 75 °C; **^{1}H-NMR** (DMSO-d6, 400 MHz, ppm): δ 3.70 (s, 6H, CH_3), 4.12 (m, 2H, C**H**-OH), 1.76 (m, 2H, CH_2-), 1.58 (m, 2H, CH_2-); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 52.5, 71.4, 72.3, 175.9; **FT-IR** (KBr, ν_{max}, cm^{-1}**):** 3307 (O-H), 2971 (C-H), 1726 (C=O_{ester}), 1382 (C-O_{ester}).

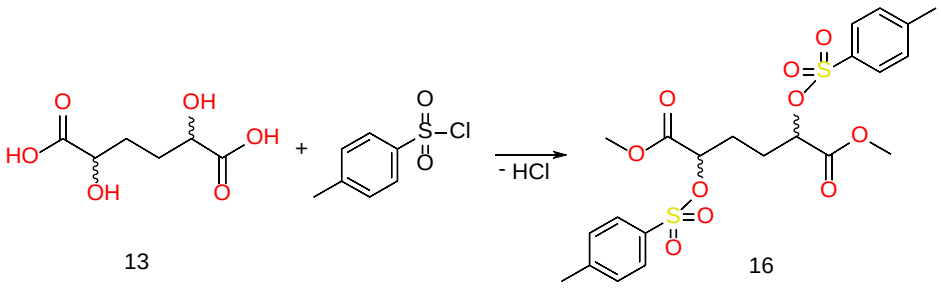
Analytical data for (2R,5S)-Diethyl 2,5-Dihydroxyadipate (15)



15

CAS: 855909-49-8; **MW**: 234.25 g/mol ($C_{10}H_{18}O_6$); **M.p.** (from diethyl ether): 105 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 4.31 (m, 2H, C**H**-OH), 3.86 (m, 4H, CH_2), 1.74 (m, 2H, CH_2-), 1.55 (m, 2H, CH_2-), 1.25 (t, 6H, CH_3); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 175.5, 72.5, 59.5, 32.5, 13.8.

14. Reaction between dihydroxyadipic acid and *p*-toluenesulfonyl chloride (PTSC)



13 16

Procedure (JL046):

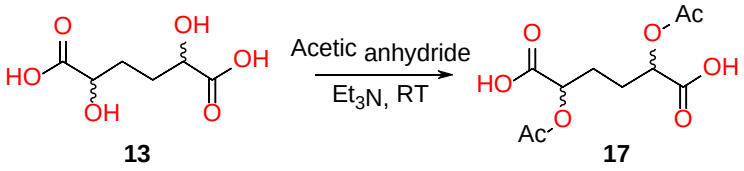
2,5-Dihydroxyadipic acid (0.25 g, 1.4 mmol, MW 178) was mixed together with p-toluenesulfonyl chloride (0.59 g, 3.08 mol, MW 190.65) and trimethylamine (0.43 ml, 3.08 mol, MW 101.19) in molar

ratio 1 : 2.2 : 2.2 in acetonitrile as solvent. The reaction was carried out at room temperature for 24 hours. The ^{1}H-NMR analysis shows that product **16** was formed in 31% yield.

Analytical data for 16

^{1}H-NMR (400 MHz, DMSO-d6, ppm): δ 7.66 (d, J = 8.1Hz, 4H, -CH=), 7.35 (d, J = 8.1Hz, 4H, -CH=), 2.38 (s, 6H, H_3C-Ar), 3.70 (s, 6H, CH_3), 4.29 (m, 2H, C**H**-OH), 1.95 (m, 2H, CH_2), 1.76 (m, 2H, CH_2).

15. Base catalysed esterification of 13 to 2,5-diacetoxyadipic acid (17).



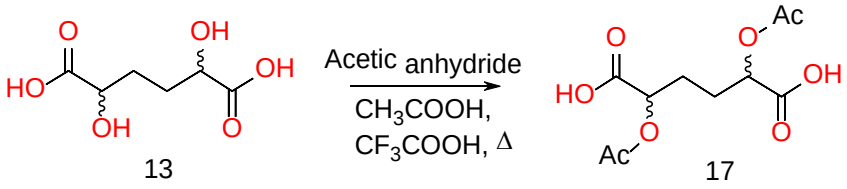
13 **17**

Reagents	MW	Amount	mmol	Equivalent
13	178	0.25 g	1.4	1
Acetic anhydride	102.09	0.27 ml	3.08	2.2
Triethylamine	101.19	0.43 ml	3.08	2.2

Procedure (JL047):

2,5-Dihydroxyadipic acid (0.25 g, 1.4 mmol) was mixed together with acetic anhydride and trimethylamine in molar ratio 1 : 2.2 : 2.2 in acetonitrile as solvent. The reaction was carried out at room temperature for 24 hours. ^{1}H-NMR analysis shows that product **17** was formed in 35% yield.

16. Acid catalyzed esterification of 13 to 2,5-diacetoxyadipic acid (17)



13 17

Reagents	MW	Amount	mmol	Equivalents
13	178	0.25 g	1.4	1
Acetic anhydride	102.09	1.32 ml	14	10
Acetic acid	60.05	0.143 ml	2.49	1.78
Trifluoroacetic acid	114.02	0.141 ml	1.9	1.36

Procedure (JL053):

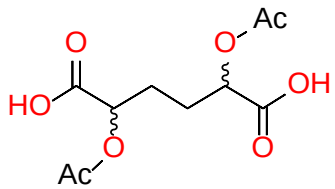
Compound **13** (0.25 g), acetic anhydride (1.32 ml), acetic acid (0.143 ml), and trifluoroacetic acid (0.141 ml) were charged in sequence in a flask equipped with a condenser and a magnetic stirring bar. The flask was brought to 140 °C by means of a heating plate and oil bath, and temperature was kept for 8 hours. At the end the mixture was allowed to cool to room temperature and analysed by ^{1}H-NMR. A 95% yield of compound **17** was obtained. By increasing the amount of trifluoroacetic acid, similar yield can be fulfilled at lower temperature (70 °C).

Alternative preparation of 2,5-diacetoxyadipic acid (17) by hydrogenation of 2-pyrone 7

Procedure (XCAR222):

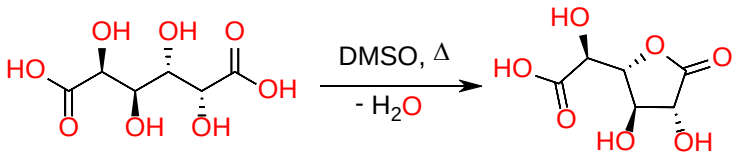
Compound **7** (30 g) was suspended in acetic anhydride (100 ml) and acetic acid (20 ml) in a 500 ml flask equipped with mechanical stirrer, thermometer, reflux condenser and inlet for hydrogen. The system was stirred and heated at 55°C, then flushed with nitrogen for 5 minutes until the system became homogeneous, and the catalyst 10% Pd/C was added. After 10 minutes of stirring at 55°C, hydrogen was supplied for 1 minute and then the system pressurized at 1.1 atm from a continuous reserve. The reaction was run for 4 hours, monitoring the H_2 absorption. When about 4.3 equivalent of H_2 was absorbed the reaction stoped, the system was depressurized and cooled at 20°C. Evaporation of the solvent after addition of 1 equivalent of metansulfonic acid at 25°C allows to obtain a viscous liquid from which a solid formed after standing for a night at 5°C. The solid was dispersed in water (40 ml) at 5°C and stirred for 15 minutes, then was filtered and washed with acetone (20 ml). After drying at 50°C for 3 hour the 2,5-diacetoxyadipic acid (**17**) was obtained in 97% purity and 86% yield (30.1 g).

Analytical data for 2,5-diacetoxyadipic acid (17)



MW: 262.21 g/mol ($C_{10}H_{14}O_8$ Exact Mass: 262.07); **Melting Point**: 75 °C; **^{1}H-NMR** (DMSO-d6, 400 MHz, ppm): δ 4.82 (m, 2H, CH-O), 2.07 (s, 2H, CH_3), 1.86 (m, 4H, CH_2); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 174.3 (-COOH), 173.6 (-C-O-**C**=O), 72.6 (-**C**-O-C=O), 26.2 (-CH_2), 20.0 (-CH_3); **FTIR** (KBr, ν_{max}, cm^{-1}): 3398, 3045 (OH), 2916 (CH), 1757 ($C{=}O_{Acid}$), 1737 ($C{=}O_{ester}$), 1420, 1376, 1328, 1250, 1105, 1058, 950, 898, 858, 769, 676, 634, 593, 561; **-MS((**-)-ESI, m/z): 261 (M-H).

17. Preparation of D-galactaro-1,4-lactone (18) by heating galactaric acid in DMSO – (S)-2-((2R,3S,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetic acid



1 **18**

Procedure:

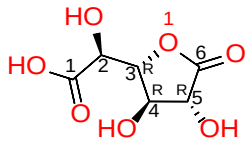
Galactaric acid (50 g, 0.238 mol) and 100 ml of dimethyl sulfoxide (DMSO) were loaded in a three-necked flask equipped with thermometer, condenser, and mechanical stirrer. The reaction mixture was first stirred at room temperature for 30 minutes. Then, the flask was heated to 140 °C. During the reaction, the temperature was controlled by the thermometer inserted into the flask. The water, formed from the reaction, was removed from the system with a nitrogen stream at 70 °C and condensed in a cold condenser. After 15 minutes the reaction mixture became a homogeneous solution. The reaction was stopped after 25 minutes and cooled down. Analysis by ^{1}H-NMR shows that the product was formed in 98% yield. The amount of water collected from the system was 2.6 g. Recovery of the solid lactone from the DMSO solution was found difficult and only by the use of 1,1,1,3,3,3-hexafluoro-2-propanol was possible to isolate a white solid (m.p. 220 °C). Alternative isolation procedure was carried out by using trifluoroacetic acid and diethyl ether as solvent to precipitate lactone as a white solid under protection of nitrogen in order to avoid the formation of starting galactaric acid.

The dehydration reactivity of mucic acid was also explored by using different reaction condition (Table 4.7), where are also reported the preliminary kinetic data obtained in DMSO-d6 in NMR tube).

*Table 4.7 - Preparation of D-galactaro-1,4-lactone (**18**) by thermal dehydration of **1** in DMSO.*

Reaction	1 (g)	DMSO (ml)	T °C	Time (h)	Conv. %	18 Yield %
JL077	0.04	0.5	140	4	100	98
JL081	0.16	0.5	140	1	100	99
JL089	0.80	0.5	100	3	88	87
JL143	6	20	140	0.33	100	100
JL099	7.5	25	140	1	95	95
JL119	1	1	140	0.75	99	97
JL129	6	4	140	7	87	85

Analytical data for the D-galactaro-1,4-lactone - (2S)-[(2R,3R,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl](hydroxy)acetic acid (18)



18

CAS: 909890-05-7**; MW:** 192.12 g/mol ($C_6H_8O_7$); **Melting Point**: 220 °C decomposed; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 4.33 (dd, J=1.7, 8.3 Hz, 1H, H-3), 4.295 (d, J = 9.2 Hz, 1H, H-5), 4.195 (d, J=1.7 Hz, 1H, H-2), 4.15 (dd, J=8.7 Hz, 1H, H-4), the unsymmetrical pattern of the 4 hydrogens of **18** can be easily identified by ^{1}H-NMR (see Figure 4.3); **^{13}C-NMR** (100 MHz, DMSO-d6) - δ 174.75 (C-6), 173.46 (C-1), 80.82 (C-3), 74.02 (C-5), 72.87 (C-4), 67.23 (C-2); **-MS((**-)-ESI, m/z): 191 [M - H], 209 [M + H_2O - H], 383 [2M$^-$ + H], 401 [2M$^-$ + H_2O + H]; **+MS(**(+)+ESI, m/z): 215 [M +Na], 233 [M +H_2O +Na], 407 [2M +Na], 429 [2M$^-$ +2Na +H], 447 [2M$^-$ +H_2O +2Na +H].

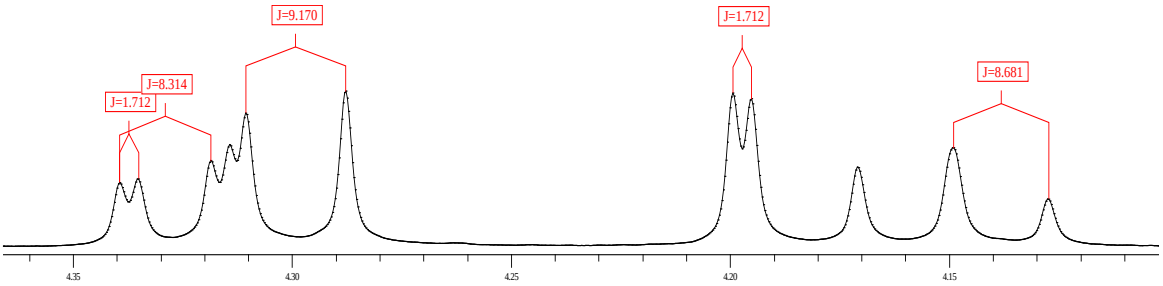
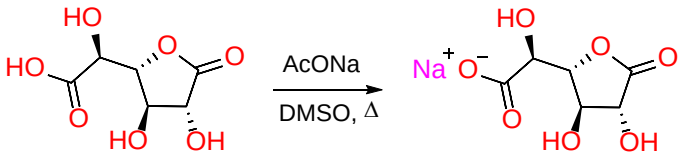


*Figure 4.3. ^{1}H-NMR spectrum (DMSO, 400 MHz, ppm) of galactaro-1,4-lactone **(JL077_4h)***

17. Preparation of sodium salt of D-galactaro-1,4-lactone (19a) from D-galactaro-1,4-lactone.

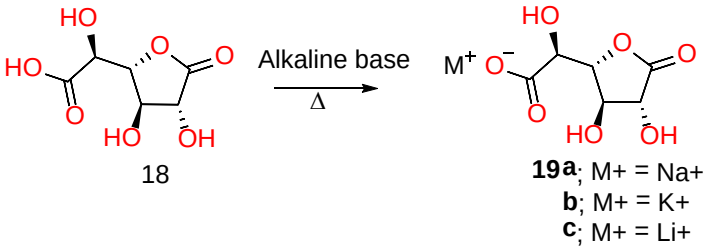


18 **19a**

A solution of galactaro lactone (0.384 mmol) in DMSO (2 ml) was introduced in a round bottom flash at room temperature, and then sodium acetate (31.5 mg, 0.384 mmol) was added under stirring. The system was heated under stirring at 140 °C for 8 hours. At the end, the system was cooled at R.T. and the solid obtained was analysed by ^{1}H-NMR to confirm that the products is the sodium salt of D-galactaro-1,4-lactone (see Figure 4.3). An acid-base titration with 0.1 N HCl affords a 98% purity of the sample. Remarkable was the stability of the lactone salt under the drastic conditions used. Some unsaturated products were detected in a similar reaction after 24 hours (JL084_2).

The analytical data of this sample were coincident with the ones reported in the subsequent experiments under more mild conditions. The ^{1}H-NMR was found sensitive to concentration and excess of the base.

18. General procedure for the synthesis of alkaline salts of galactaro-1,4-lactone (19a-c).



18

19a; M+ = Na+
b; M+ = K+
c; M+ = Li+

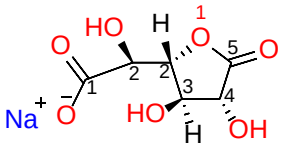
Procedure:

Alkali metal bases (sodium methoxide, sodium bicarbonate, sodium hydroxide, potassium t-butoxide, n-butyllithium) were mixed together with DMSO solution of galactaro-1,4-lactone in a round bottom flask equipped with magnetic stirrer in the ratio as reported in Table 4.8. The molar ratio between bases and galactaro-1,4-lactone was extended from 1 to 2. The salification reaction was carried out at room temperature and the reaction was finished in different time depending on the types of base and their solubility in DMSO. The alkali metal salts of galactaro-1,4-lactone **19a-c** were mostly or partially precipitated from DMSO solution as white solids. The salts were purified by recrystallization using acetone or acetonitrile as co-solvent (DMSO : acetone/acetonitrile in ratio 1:5) and then by filtration and drying.

*Table 4.8 - Synthesis of alkaline salts of D-galactaro-1,4-lactone **19a-c** from **18** and alkaline bases in DMSO.*

Entry	18 (mmol)	Base	Solvent	Ratio [Base]/[sub]	T °C	t (h)	18 Conv. (%)	19a-c Yield (%)
JL152	2.6	$NaOCH_3$	DMSO	1.3	RT	1	100	95.2
GICR08	1.56	$NaHCO_3$	DMSO	1	90	0.67	100	86
GICR04	9.8	NaOH	H_2O	1	100	14	100	60
JL154	2.6	$NaOBu^t$	DMSO	2.0	RT	2	100	96
GICR07	1.56	n-BuLi	DMSO	1.17	RT	4	100	90

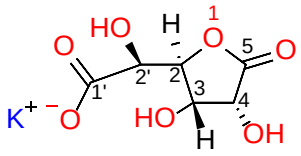
Analytical data for sodium salt of D-galactaro-1,4-lactone – Sodium (S)-2-((2R,3R,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetate (19a)



19a

MW: 214.10 g/mol ($C_6H_7NaO_7$); **Melting Point**: 223 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 4.21 (d, J=8.93 Hz, 1H), 4.09 (dd, J=4.04, 8.31 Hz, 1H), 4.01 (dd, J=8.80, 1H), 3.61 (d, J=4.04, 1H); **^{1}H-NMR** (400 MHz, D_2O, ppm): δ 4.59 (dd, J=2.00, 8.40 Hz, 1H), 4.63 (d, J=9.20 Hz, 1H), 4.24 (d, J=2.00, 1H), 4.40 (dd, J=9.20, 8.40 Hz, 1H); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3484, 3315, 3120, 2960, 2945, 2894, 2847, 2761, 2604, 2362, 1783, 1603, 1497, 1436,1393, 1329, 1275, 1255, 1230, 1214, 1162, 1129, 1094, 1049, 1007, 942, 889, 862, 832, 776, 737, 694, 638, 616, 593, 536, 494, 465, 408; **-MS(**(-)-ESI, m/z): 191 [M^-], 405 [$2M^-$ + Na]; **+MS**((+)+ESI, m/z): 215 [M + H], 237 [M + Na], 451 [2M + Na].

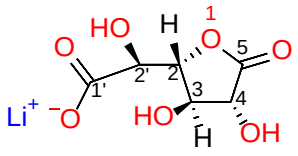
Analytical data for potassium salt of galactaro-1,4-lactone (19b)



19b

MW: 228.00 g/mol ($C_6H_7KO_7$); **Melting Point**: 185 °C (decomposes); **^{1}H-NMR** (DMSO-d6, 400 MHz, ppm) - δ 4.12 (dd, J=3.67, 8.31 Hz, 1H), 4.22 (d, J=9.170 Hz, 1H), 3.69 (d, J=3.79 Hz, 1H), 4.03 (dd, J=8.80 Hz, 1H); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3286, 3179, 2957, 2927, 2881, 2739, 2645, 2530, 2364, 2321, 1742, 1595, 1432, 1367, 1315, 1281, 1245, 1198, 1109, 1049, 971, 871, 844, 807, 767, 712, 669, 636, 568, 523, 475; **-MS(**(-)-ESI, m/z): 191 [M^-], 209 [M^- + H_2O].

Analytical data for lithium salt of galactaro-1,4-lactone (19c)



19c

MW: 198.06 g/mol ($C_6H_7LiO_7$); **Melting Point**: 220 °C (decompose); **^{1}H-NMR** (400 MHz, D_2O, ppm): δ 4.63 (dd, J=9.170 Hz, 1H), 4.59 (dd, J=2.08, 8.44 Hz, 1H), 4.40 (dd, J=8.56 Hz, 1H), 4.23 (d, J=1.96 Hz, 1H); **-MS(**(-)-ESI, m/z): 191 [M-].

19. Preparation of DABCO salt of D-galactaro-1,4-lactone

A solution of galactaro1,4-lactone (0.384 mmol) in DMSO (2 ml) was introduced in a round bottom flash at room temperature, and then DABCO (42.5 mg, 0.384 mmol) was added under stirring. The mixture was heated under stirring at 140 °C for 8 hours. At the end, the system was cooled at R.T. and analysed by ^{1}H-NMR in DMSO-d6. The main product was identified as the DABCO salt of galactaro-1,4-lactone (**20a**). (see ^{1}H-NMR of Figure 4.3). Some aromatic products were formed when the reaction time was increased to 8 hour (JL083_3).

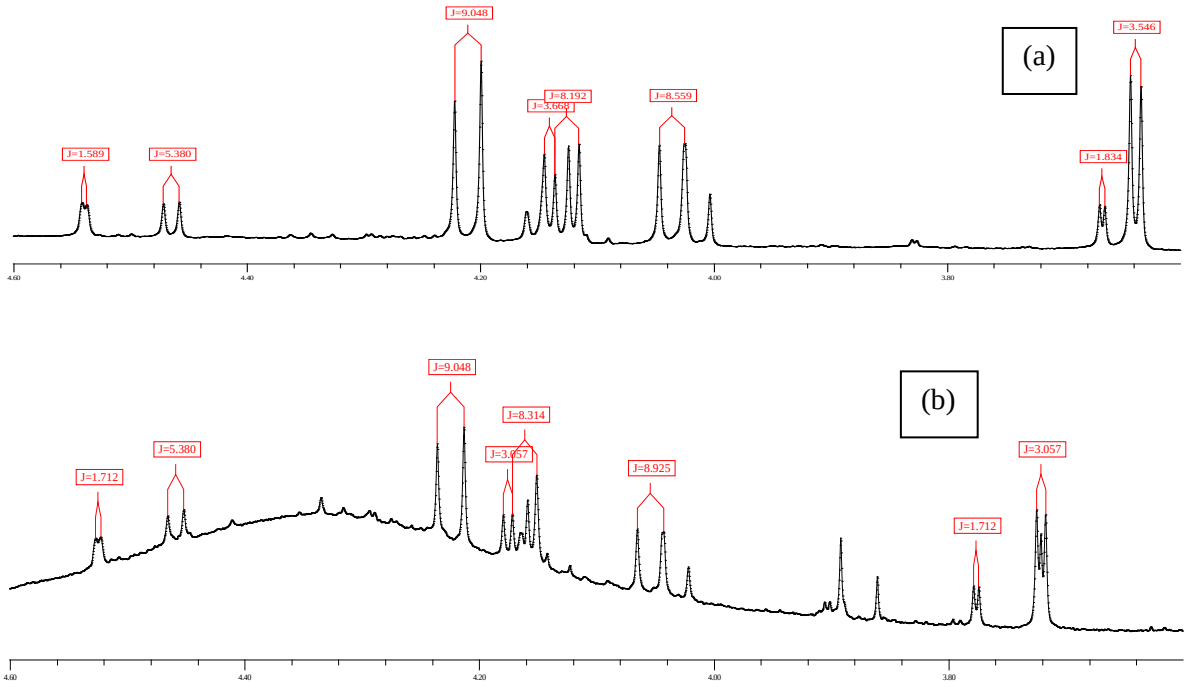


*Figure 4.4 - ^{1}H-NMR spectra (DMSO, 400 MHz) of (a) sodium salt of galactaro-1,4-lactone from sodium acetate for 8h; (**JL083_2**). (b) DABCO salt of galactaro-1,4-lactone from DABCO for 8h (**JL084_2**).*

Unsaturated products were observed in minor amount under these conditions and a compound with the ^{1}H-NMR spectrum reported in Figure 4.5 was isolated by flash column chromatography. The product was identified as a pyrone derivative based on the analysis of ^{1}H-NMR, ^{13}C-NMR, and mass analyses.

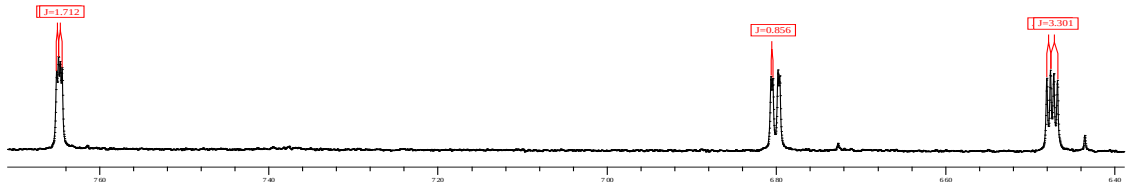
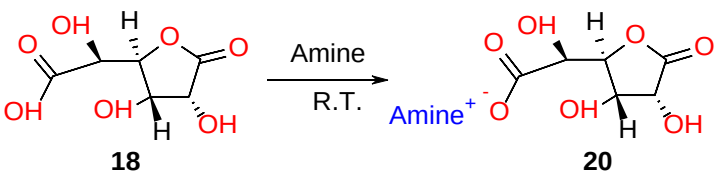


*Figure 4.5 - ^{1}H-NMR (DMSO, 400 MHz) spectrum of the unsaturated product observed in the reaction of galactaro-1,4-lactone with DABCO for 8 hours (**JL084_2**).*

20. General procedure for the synthesis of ammonium salts of galactaro-1,4-lactone (20a-l)



18 **20**

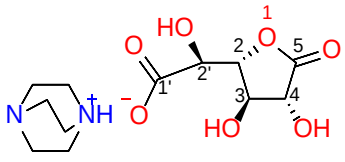
Procedure:

Amines were mixed together at 20-25°C with a DMSO solution of galactaro-1,4-lactone in a round bottom flask equipped with a magnetic stirrer. The molar ratios between amine and galactaro-1,4-lactone were ranging from 1 to 2. The salification reaction was carried out at room temperature and the reaction was finished in different time depending on the types of amine and their solubility in DMSO. The amine salts of galactaro-1,4-lactone were, mainly or partially, precipitated from DMSO solution as white to pale yellow solids. The salts were purified by recrystallization using acetone or acetonitrile as co-solvent (DMSO:Acetone/Acetonitrile 1:5) and then filtered and dried under vacuum for 12 hours. For primary amines, in order to obtain the amine salt of the lactone, it is necessary to control strictly the molar ratio between lactone and amines to 1:1 and control the temperature. The primary amines were added dropwise to the DMSO solution of lactone during the whole reaction. Different amines were applied in this series of reactions; the specific reaction conditions are reported in Table 4.9.

Table 4.9 - Conditions and yields for the synthesis in DMSO of ammonium salts of galactaro-1,4-lactone.

Entry	18 (mmol)	Amine	Ratio [Amine]/[sub]	T (°C)	t (h)	Conv. (%)	20a-g (Yield %)
JL157	2.1	Serinol	1.3	RT	2	100	**20g** (85)
JL151	2.1	Isoserinol	1.3	RT	4	100	**20i** (93)
JL147	2.1	Butylamine	1.3	RT	4	78.0	**20j** (56)
GICR33	1.56	Octylamine	1.0	RT	0.25	60.2	**20h** (20)
JL146	2.1	Benzylamine	1.3	RT	2	58.4	**20k** (17)
JL106	2.1	L-Phenylethylamine	1.0	RT	4	100	**20d** (80)
JL104	2.1	*N*-Benzyl-N-methylamine	1.3	RT	4	100	**20b** (87)
JL150	2.1	Diisopropylamine	1.1	RT	4	100	**20c** (100)
JL139	2.1	DABCO	1.3	RT	14	100	**20a** (95)
JL140	2.1	Pyridine	1.3	RT	10	100	**20e** (89)
JL178	2.6	1-Et-3-Me-imidazolium acetate	1.1	RT	10	100	**20f** (79)
JL186	9	Ethanolamine	1.1	RT	4	93.7	**20l** (83)

Analytical data for the DABCO salt of galactaro-1,4-lactone (20a) - 1,4-diazabicyclo[2.2.2]octan-1-ium (S)-2-((2R,3R,4R)-3,4-dihydroxy-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetate



20a

MW: 304.30 g/mol ($C_{12}H_{20}N_2O_7$); **Melting Point**: 206 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 4.23 (d, J=9.048 Hz, 1H), 4.16 (dd, J=3.06, 8.31 Hz, 1H), 4.05 (dd, J=8.80 Hz, 1H), 3.76 (d, J=3.18 Hz, 1H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 175.2 (C-1), 174.7 (C-6), 81.5 (C-4), 74.1 (C-2), 73.8 (C-3), 68.7 (C-5), 44.8 (-N-**C**H_2-**C**H_2-NH^+-); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3172, 3014, 2946, 2871, 2706, 2637, 2372, 2185, 2104, 2043, 1785, 1605, 1474, 1466, 1412, 1380, 1335, 1311, 1296, 1254, 1238, 1157, 1114, 1052, 1012, 952, 876, 839, 764, 691, 597, 526; **-MS(**(-)-ESI, m/z): 191 [M^-], 383 [$2M^-$ + H], 405 [$2M^-$ + Na]; **+MS**((+)+ESI, m/z): 113 [DABCO + H].

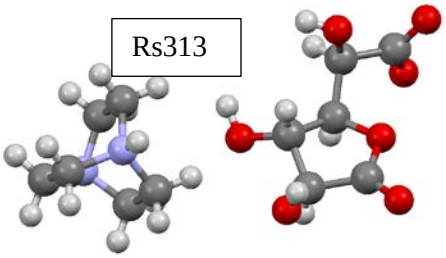
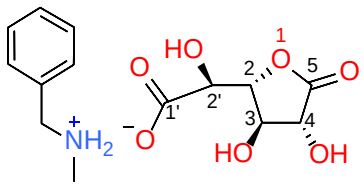


*Table 4.10 - Single crystal X-ray diffraction data for **20a.***

Crystal data: **rs313**	
Chemical formula	$C_6H_7O_7 \cdot C_6H_{13}N_2$
M_r	304.30
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	103
a, b, c (Å)	10.6216 (8), 10.5370 (8), 12.9296 (8)
β (°)	105.529 (2)
V (Å^3)	1394.25 (17)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.12
Crystal size (mm)	0.22 × 0.2 × 0.12
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	Multi-scan
T_{min}, T_{max}	0.702, 0.746
No. of measured, independent	10248, 3897, 2471
R_{int}	0.044
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.695
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.047, 0.113, 1.01
No. of reflections	3897
No. of parameters	196
H-atom treatment	H atoms treated by a mixture of independent and constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.27, −0.26

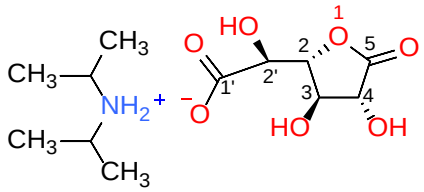
Analytical data for N–benzyl-N-methylammonium salt of galactaro-1,4–lactone (20b)



20b

MW: 313.07 g/mol ($C_{14}H_{19}NO_7$); **Melting Point**: 150 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.43 (m, 5H, C_6H_5–), 4.24 (d, J=9.2 Hz, 1H), 4.19 (dd, J=2.9, 8.3 Hz, 1H), 4.06 (dd, J=8.6 Hz, 1H), 4.04 (s, 2H, –CH_2–), 3.75 (d, J=3.1 Hz, 1H), 2.50 (s, 3H, –CH_3); **FTIR** (KBr, ν_{max}, cm^{-1}): 3653, 3368, 3279, 3080, 3031, 2957, 2833, 2792, 2700, 2414, 2390, 1967, 1905, 1784, 1603, 1497, 1427, 1398, 1308, 1213, 1197, 1107, 1046, 969, 930, 902, 879, 827, 780, 746, 699, 669, 617, 527, 472, 416; **-MS(**(-)-ESI, m/z): 312 [M – H], **+MS**((+)+ESI, m/z): 336 [M + Na], 122 [N–$PhCH_2NHCH_3$ + Na].

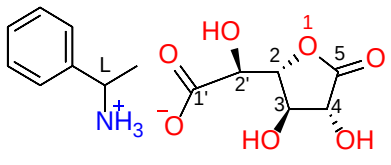
Analytical data for diisopropylammonium salt of galactaro-1,4–lactone (20c)



20c

MW: 293.15 g/mol ($C_{12}H_{23}NO_7$); **Melting Point**: 162 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm) - δ 4.21 (dd, J=2.45, 8.3Hz, 1H), 4.23 (d, J=9.1 Hz, 1H), 3.71 (d, J=2.5, 1H), 4.06 (dd, J=8.7, 1H), 3.31 (m, 2H, –$CH(NH_2)$–), 1.22 (d, J=6.5 Hz, 12H, –CH_3); **FTIR** (KBr, ν_{max}, cm^{-1}): 3413, 3331, 3289, 3161, 2968, 2902, 2836, 2756, 2722, 2533, 2474, 2414, 2282, 2189, 2132, 2081, 1965, 1599, 1542, 1474, 1434, 1404, 1374, 1313, 1283, 1247, 1199, 1183, 1151, 1112, 1048, 972, 953, 872, 840, 767, 559,636, 525, 502, 464; **-MS(**(-)-ESI, m/z): 191[M^-], 209[M^- +H_2O]; **+MS**((+)+ESI, m/z): 102 [$(CH_3)_2CH)]_2NH$ +H], 312 [M + H_2O +H].

Analytical data for L-phenylethylamine salt of galactaro-1,4–lactone (20d)

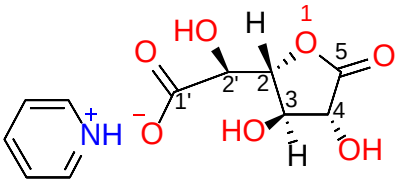


20d

MW: 313.31 g/mol ($C_{14}H_{19}NO_7$); **Melting Point**: 196 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3400, 3245, 3033, 2926, 2737, 2697, 2629, 2512, 2369, 2155, 1879, 1637, 1578, 1517, 1456, 1417, 1353, 1310, 1236,

1196, 1142, 1097, 1050, 965, 915, 874, 829, 771, 699, 669,648, 588, 531, 464, 484; **-MS(**(-)-ESI, m/z): 312 [M – H], 209 [M^- + H_2O]; **+MS**((+)+ESI, m/z): 336 [M + Na].

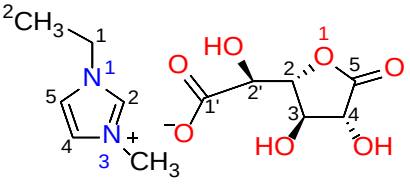
Analytical data for the pyridinium salt of galactaro-1,4–lactone (20e)



20e

MW: 271.23 g/mol ($C_{11}H_{13}NO_7$); **Melting Point**: 121 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.56 (dd, J=1.6, 5.624 Hz, 2H, -C**H**=NH^+), 7.78 (tt, J=1.8 Hz, 7.580 Hz, 1H, -CH=), 7.37 (m, 2H, -CH=), 4.35 (dd, J=1.6, J=8.3 Hz, 1H), 4.31 (d, J=9.0 Hz, 1H), 4.21 (d, J=1.6 Hz, 1H), 4.16 (dd, J=8.7, 1H); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3422, 3362, 3221, 3104, 2886, 2705, 2582, 2523, 2167, 2042, 1785, 1759, 1638, 1595, 1544, 1488, 1385, 1329, 1285, 1225, 1204, 1173, 1134, 1106, 1017, 990, 957, 918, 879, 830, 761, 735, 685, 642, 599, 517, 478; **-MS(**(-)-ESI, m/z): 191 [M^-], 209 [M^-+H_2O], 383 [$2M^-$+H], 405 [$2M^-$+Na], 423 [$2M^-$+Na+H_2O]; **+MS**((+)+ESI, m/z): 215 [M^-+H+Na], 237 [M^-+2Na], 407 [$2M^-$+2H+Na], 429 [$2M^-$+H+2Na], 451 [$2M^-$+H+2Na], 643 [$3M^-$+H+3Na], 665 [$3M^-$+4Na].

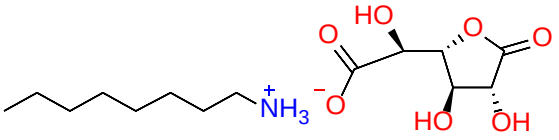
Analytical data for the 1–ethyl–3–methylimidazolium salt of galactaro-1,4–lactone (20f)



20f

MW: 302.28 g/mol ($C_{12}H_{18}N_2O_7$); **Melting Point**: 169 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 9.19 (s, 1H, N–CH=N^+), 7.78 (s, 1H, N^+–CH=CH), 7.69 (s, 1H, N–CH=CH), 4.20 (quartet, J=7.2 Hz, 2H, CH_3–CH_2–N), 4.21 (d, J=8.6 Hz, 1H), 4.05 (dd, J=4.4, J=8.1 Hz, 1H), 3.99 (dd, J=8.7 Hz, 1H), 3.85 (s, 3H, N^+–CH_3), 3.56 (d, J=4.3, 1H), 1.41 (t, 3H, J=7.4 Hz, –CH_3); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3284, 3150, 3055, 2880, 2856, 2732, 2575, 2398, 2358, 1784, 1606, 1468, 1399, 1352, 1323, 1285, 1237, 1169, 1147, 1107, 1024, 958, 922, 878, 794, 767, 678, 648, 623, 601; **-MS(**(-)-ESI, m/z): 191 [M^- – H], 383 [$2M^-$ + H]; **+MS**((+)+ESI, m/z): 111 [1–Et–3–Me-imidazolium$^+$], 325 [M + Na].

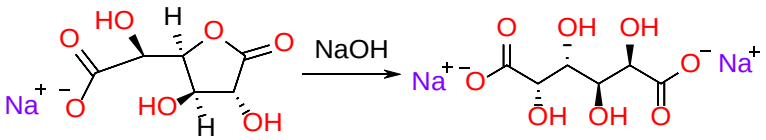
Analytical data for n-octylammonium salt of D-galactaro-1,4–lactone (20h)



20h

MW: 321.37 g/mol ($C_{14}H_{27}NO_7$); **Melting Point**: 160 °C;

21. Preparation of disodium D-galactarate from D-galactaro-1,4-lactone (21a)

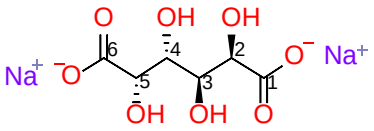


19a **21a**

Procedure:

In a round bottom flask, equipped with a stirrer, the sodium salt of galactarate-1,4-lactone (**19a**, 334 mg, 1.56 mmol) was first solubilised in 0.1 N NaOH (15.6 ml) solution in a round bottom flask equipped with a stirrer. The molar ratio between bases and galactaro-1,4-lactone were 1:1. The hydrolysis reaction was carried out at room temperature and was monitored by ^{1}H-NMR. The reaction was finished in 20 minutes and the system became homogeneous, showing a light yellow colour. The disodium galactarate salt was isolated and dried by removing residual water from the reaction solution using rotary evaporator. The yield of disodium salt was 82.05% (by ^{1}H-NMR analysis with internal standard). The sample exhibits analytical data similar to the ones reported in the work of Taga et al. [9]

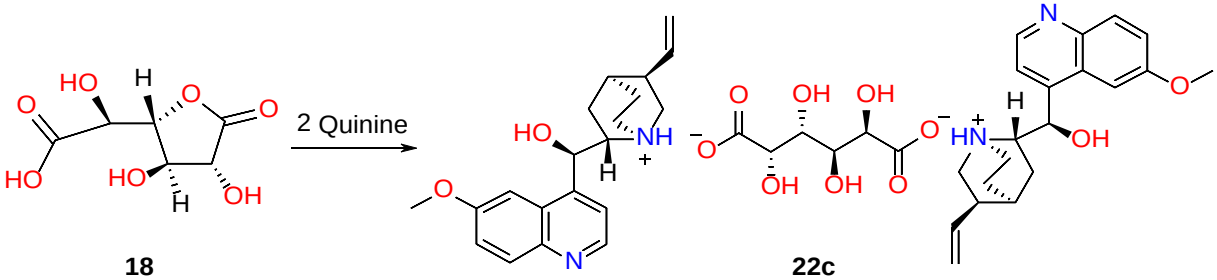
Analytical data for sodium D-galactarate - [Di-Sodium (2R,3S,4R,5S)-2,3,4,5-tetrahydroxy-hexanedioate (21a)



21a

MW: 254.10 g/mol ($C_6H_8Na_2O_8$); **Melting Point**: >270 °C; **^{1}H-NMR** (400 MHz, D_2O, ppm): δ 4.21 (s, 2H), 3.89 (s, 2H); **^{13}C-NMR** (100 MHz, D_2O, ppm): δ 179.8 (-O-**C=O**), 71.8 (-CO-**C**H-OH), 71.5 (-**C**H-OH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3505, 3124(broad), 2943, 2868, 2741, 2536, 2372, 2279, 2086, 1611, 1443, 1372, 1318, 1286, 1215, 1099, 1033, 984, 875, 827, 763, 670, 639, 536, 473; **-MS**((-)-ESI, m/z %): 209 [M^{2-}+H], 231 [M^-], **+MS**((+)+ESI, m/z %): 277 [M+Na], 509 [2M+H], 531 [2M+Na].

22. Preparation of diquininium galactarate salt (22)

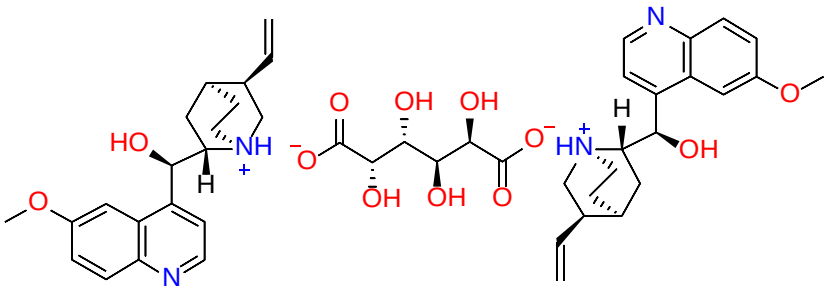


18 **22c**

Procedure (JL142):

Quinine (2.03 g, 6.25 mmol) was mixed together with a DMSO solution of galactaro-1,4-lactone (0.5 g, 3.125 mmol) in the round bottom flask equipped with magnetic stirrer. The molar ratio between amines and galactaric acid-1,4-lactone was close to 2:1. The salt forming reaction was carried out at room temperature and the reaction was finished in 2 hours when the reaction mixture became a waxy solid. The diquininium galactarate salt was nearly quantitatively precipitated from DMSO solution as a white solid. In order to remove the DMSO solvent, the filtered product was recrystallized by adding acetonitrile as co-solvent in (DMSO:Acetone/Acetonitrile = 1:5) and then filtered. The DMSO was recovered by removing acetonitrile inside the mother liquor by rotary evaporator.

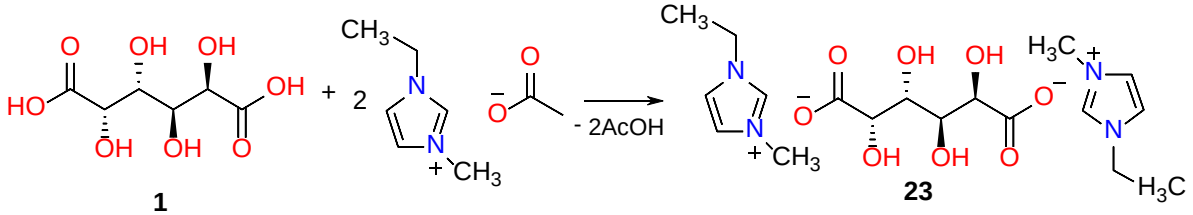
Analytical data for diquininium D-galactarate (22c)



22c

MW: 858.99 g/mol ($C_{46}H_{58}Na_4O_{12}$); **Melting Point**: 268 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.71 (d, J=4.4 Hz, 2H, -N=CH-), 7.95 (d, J=9.2 Hz, 2H, CH-C**H**=C), 7.58 (d, J=4.5 Hz, 2H, -C=C**H**-CH=N), 7.50 (d, J=2.6 Hz, 2H, O-C-C**H**=C-), 7.40 (dd, J = 2.7, 9.2 Hz, 2H, O-C=C**H**-CH=), 5.85 (m, 2H, CH_2=C**H**-), 5.05 (d, J = 17.1 Hz, 2H, C**H$_2$**-CH-), 4.95 (d, J = 10.4 Hz, 2H, -C**H$_2$**-CH-), 3.93 (s, 6H, CH_3), 4.09 (s, 2H), 3.72 (s, 2H); **-MS**((-)-ESI, m/z %): 191 [M^{2-} + H], 209 [M^{2-} + H_2O + H]; **+MS**((+)+ESI, m/z %): 325 [Quinine + H].

23. Preparation of di(1–ethyl–3–methylimidazolium) D-galactarate salt (23) from galactaric acid

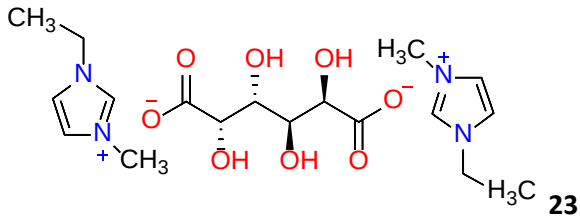


1 **23**

Procedure (JL145):

1–Ethyl–3–methylimidazolium acetate (2.5 ml, 14.7 mmol, MW 170.2) was mixed together with galactaric acid powder (0.50 g, 2.38 mmol) in the round bottom flask equipped with magnetic stirrer. The molar ratio between ionic liquid and galactaric acid was 6:1. The reaction was carried out at room temperature. After 20 minutes the reaction mixture became homogeneous, then start to precipitate a solid and, after 1 hour, the system became a wax-like white solid. The di(1–ethyl–3–methylimidazolium) galactarate salt was purified by recrystallization using acetonitrile as co-solvent (DMSO:acetonitrile ≈ 1:5).

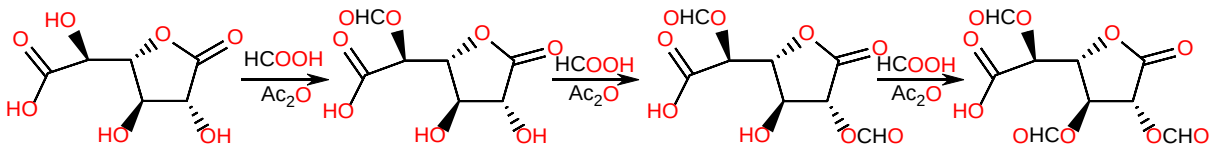
Analytical data for di(1–ethyl–3–methylimidazolium) D-galactarate salt (23)



23

MW: 430.46 g/mol ($C_{18}H_{30}N_4O_8$); **Melting Point**: 65 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 9.39 (s, 1H, N-CH=N+), 7.79 (s, 1H, N^+-C**H**=CH), 7.70 (s, 2H, N-CH=C**H**), 4.20 (quartet, J=7.214 Hz, 4H, CH_3-C**H**$_2$-N), 3.87 (s, 6H, N^+-CH_3), 3.62 (s, 2H), 3.41 (s, 2H), 1.40 (t, J=7.336 Hz, 6H, –CH_3); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3442, 3163, 2958, 2861, 2737, 2529, 2101, 1596, 1432, 1367, 1315, 1283, 1246, 1198, 1170, 1109, 1050, 971, 873, 844, 767, 669, 636, 525, 475; **-MS**((-)-ESI, m/z %): 191 [M^{2-} – H_2O+H], 209 [M^{2-} + H]; **+MS**((+)+ESI, m/z %): 111 [1-Ethyl-3-methylimidazolium], 431 [M+H], 453 [M+Na].

24. Formylation of galactaro-1,4-lactone up to tri-formylated derivative (24)



18 **24a** **24b** **24c**

Reagent	MW	Quantity	mmol	Equivalent
19	192	1.5 g	7	1
Acetic anhydride	102.09	2.7 ml	28	4
Formic acid	46.03	1.08 ml	28	4

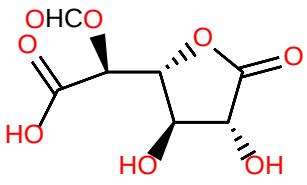
Procedure:

The formylation reaction was investigated by reacting galactaro-1,4-lactone (**18**) in DMSO solution (as above indicated) with a sample of preformed acetic/formic anhydride. In order to prepare this mixed anhydride, formic acid was added to acetic anhydride drop by drop (molar ratio 1:1) in a round bottom flask under magnetic stirring at 15-20°C for 30 min. Then, the DMSO lactone solution was added to the resulting solution and the reaction was allowed to run under stirring at 30 °C for the desired time. Different molar ratios between substrates were adopted for different time from 3 hours to 20 hours at 30 °C. The specific reaction conditions are shown in the Table 4.11. Recovery of the tri-formylated lactone from the DMSO solution was found difficult and only by the use of 1,1,1,3,3,3-Hexafluoro-2-propanol was possible to isolate the compound as a white solid.

Table 4.11 - Synthesis of poly-formylated lactone of galactaric acid.

Entry	18 (mmol)	Ratio [mix-anhydride]/[sub]	T (°C)	t (h)	Conv. (%)	24a-c Yield (%)
JL107	1.1	2	100	2	100	**24a** (21)
JL112	1.1	4	RT	4	100	**24a** (49), **24b** (12)
JL113	2.1	1.3	RT	4	24.5	**24a** (22)
JL115	7.1	4	30	4	100	**24a** (67.2), **24b** (21), **24c** (9)
JL124	2.1	10	RT	48	100	**24c** (92), **24b** (5)

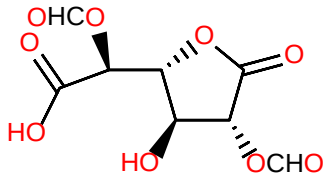
Analytical data for D-galactaro-1,4-lactone monoformate (24a)



24a

MW: 220.13 g/mol ($C_7H_8O_8$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.37 (s, 1H), 5.29 (dd, J=2.2, 1.0 Hz, 1H), 4.55 (ddd, J=8.4, 2.1, 0.9 Hz, 1H), 4.36 (d, J=9.0 Hz, 1H), 4.02 (t, J=8.8 Hz, 1H); **-MS**((-)-ESI, m/z %): 219 [M - H].

Analytical data for D-galactaro-1,4-lactone diformate (24b)

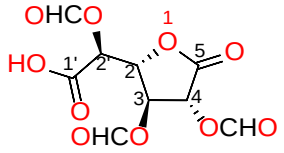


24b

MW: 248.14 g/mol ($C_8H_8O_9$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.37 (s, 1H), 8.21 (s, 1H), 6.89 (d, J=8.9, 0.9 Hz, 1H), 5.36 (dd, J=2.1, 1.0 Hz, 1H), 4.84 (ddd, J=2.2, 0.9, 8.4 Hz, 1H), 4.39 (t, J=8.7 Hz, 1H); **-MS**((-)-ESI, m/z %): 495 [2M – H], 247 [M - H].

Analytical data for D-galactaro-1,4-lactone triformate (24c)

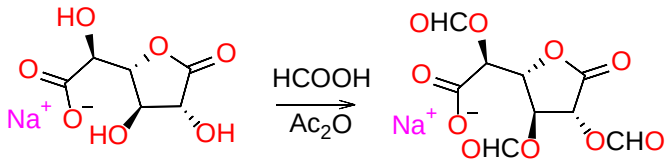
[(S)-2-((2R,3S,4R)-3,4-bis(formyloxy)-5-oxotetrahydrofuran-2-yl)-2-(formyloxy)acetic acid]



24c

MW: 276.01 g/mol ($C_9H_8O_{10}$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.38 (s, 1H), 8.37 (s, 1H), 8.21 (s, 1H), 6.11 (d, J=7.8 Hz, 1H), 5.62 (t, J=7.6 Hz, 1H), 5.42 (d, J=2.2 Hz, 1H), 5.09 (dd, J=2.3, 7.6 Hz, 1H); **-MS**((-)-ESI, m/z %): 551 [2M – H], 275 [M - H]; **Elemental Analysis**: C, 39.02; H, 2.98; O, 58.00, (calculated for $C_9H_8O_{10}$ C, 39.14; H, 2.92; O, 57.94).

25. Preparation of sodium salt of triformylated D-galactaro-1,4-lactone from the sodium salt of galactaro-1,4-lactone.



19a **25**

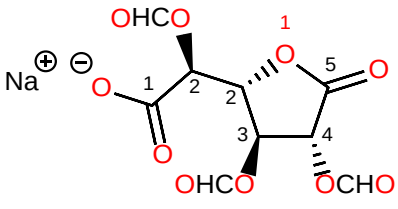
Run	Reagent	MW	Quantity	mmol	Equivalent
1	**19a**	214	0.49 g	2.3	1
2	Acetic anhydride	102.09	1.08 ml	23.1	10
3	Formic acid	46.03	1.72 ml	23.1	10

Procedure:

The formyl substituted derivatives can also be produced by isolated salt of galactaric-1,4-lactone (**19**) and acetic formic anhydride. In order to prepare the acetic formic anhydride, Formic acid was adding to acetic anhydride drop by drop with molar ratio 1:1. The acetic formic anhydride was mixed together for 45 minutes before adding solid sodium salt of galactaro-1,4-lactone. The reaction was carried out in a round bottom flask with presence of magnetic stirrer. Reaction temperature was set at room temperature for 24 hours. At beginning the solid of lactone salt was solubilised in mixed anhydride immediately with heat and bubbles formed. At the end of the reaction, the system appears as a light orange solution. The solvent is concentrated at low pressure without heating. Treatment of the solution with Et_2O leads to the precipitation of the product as a white solid (72% yield).

Analytical data for the sodium salt of D-galactaro-1,4-lactone triformate (25)

[Sodium (S)-2-((2R,3S,4R)-3,4-bis(formyloxy)-5-oxotetrahydrofuran-2-yl)-2-(formyloxy)acetate]

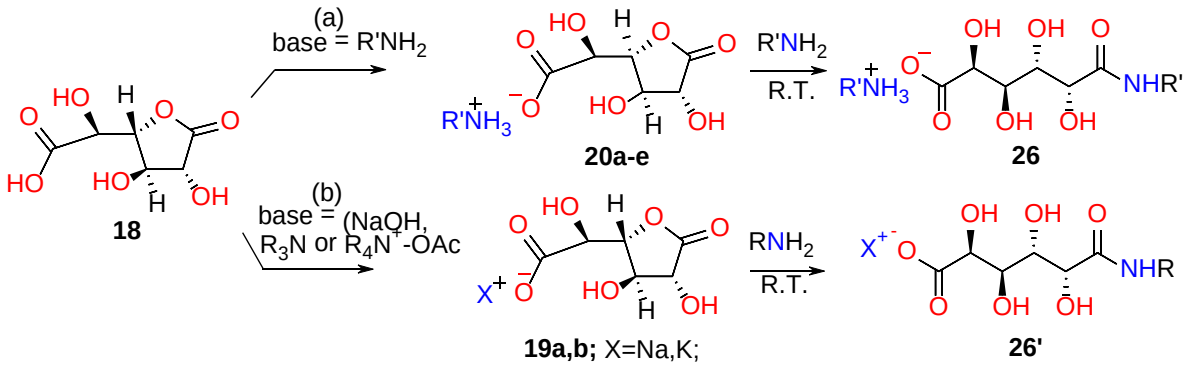


25

MW: 298.13 g/mol ($C_9H_7NaO_{10}$); **Melting Point:** 121 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.29 (s, 1H), 8.41 (s, 1H), 8.28 (s, 1H), 6.09 (d, J=7.7 Hz, 1H), 5.55 (t, J =7.7 Hz, 1H), 5.09 (dd, J=2.7 Hz, J=7.7 Hz, 1H), 4.87 (d, J=2.7 Hz, 1H); **^{13}C-NMR** (100 MHz, DMSO-d6): δ 179.2 (NH-**C=O**), 175.6 (**O**=**C**-O$^-$), 71.4, 70.8, 60.0, 57.8, 41.1; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3447, 2993, 2940, 2831, 2565, 2357, 2170, 1809, 1730, 1701, 1643, 1412, 1334, 1209, 1143, 1077, 1014, 958, 903, 821, 786, 766, 723, 698, 606, 570, 501, 474; **+MS**((+)+ESI, m/z): 321 [M+Na].

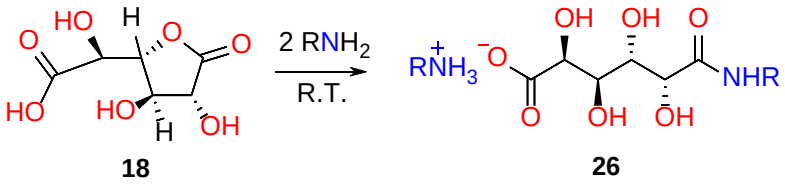
26. Synthesis of salts of mono galactaramide [(2S,3R,4S,5R)-6-amino-2,3,4,5-tetrahydroxy-6-oxohexanoate] (26)

Salts of mono galactaramide were synthesized in almost quantitative yields by using the following two procedures. (a) The DMSO solution of lactone **18** was treated with stoichiometric amount (two moles) of primary amine. (b) The DMSO solution of lactone **18** was treated alkali metal bases or amines (only tertiary and quaternary in 1:1 molar ratio to stop the process to the mono ammonium salts) at 25°C for 1-4 hours, and then, a stoichiometric amount of a primary amine was added at 20-50 °C.



The isolation procedure of the mono galactaramide salt from the DMSO solution was dependent on the reagents used. Some salts were directly precipitated as white solid from the DMSO solution (i.e. with cations sodium, potassium, DABCO, etc.). For others, white solids were isolated by addition of a co-solvent.

a. Synthesis of ammonium mono galactaramide (26) using primary amines.



The high reactivity of primary amines in the addition to the lactone ring of **18** made possible the one-step preparation of mono galactaramide ammonium salts by addition of two moles of the amine to the DMSO solution of **18**. The reaction was fast enough to be concluded at room temperature in 3 hours. Typical examples of the procedure developed are reported in following pages.

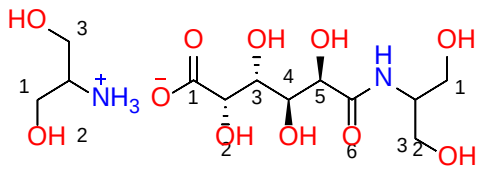
Procedure:

Primary amines were mixed together with galactaric acid-1,4-lactone DMSO solution in the round bottom flask equipped with magnetic stirrer at room temperature. The molar ratio between amines and galactaric acid-1,4-lactone were 2:1. The amidation and salification reaction occurs with similar rate and the reaction was finished in time dependent on the types of amine and the solubility of the salt products in DMSO. The mono galactaramide salts were largely precipitated from DMSO solution as white solids. The final reaction mixture looked as a wax-like solid. Mono galactaramide salts were purified by recrystallization using acetone or acetonitrile as cosolvent (DMSO : Acetone/Acetonitrile ≈ 1:5) and, then, by filtration and drying. The specific reaction conditions used with the different primary amines tested are summarized in Table 4.12.

*Table 4.12 - Synthesis of substituted ammonium salts of mono galactaramide by reaction of **18** with primary amines in DMSO.*

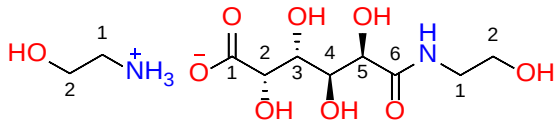
Entry	18 (mmol)	Base	[Base]/ [sub]	T °C	t (min)	18 (Conv. %)	26a-f (Yield %)
JL157	2.1	Serinol	2.2	RT	120	100	**26a** (87)
JL187	9	Ethanolamine	2	RT	120	100	**26b** (93)
JL105	2.1	Octylamine	2	RT	120	100	**26c** (94)
JL155	2.1	Benzylamine	2.2	RT	20	100	**26d** (77)
JL148	2.1	Isoserinol	2.1	RT	120	100	**26e** (95)
JL156	2.1	Butylamine	2	RT	20	>95	**26f** (77)

Analytical data for serinol salt of (1,3-dihydroxypropan-2-yl)galactaramide (26a) 1,3-dihydroxypropan-2-aminium (2S,3R,4S,5R)-6-((1,3-dihydroxypropan-2-yl)amino)-2,3,4,5-tetrahydroxy-6-oxohexanoate



MW: 374.34 g/mol ($C_{12}H_{26}N_2O_{11}$); **Melting Point**: 228 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.33 (d, J=8.4 Hz, 1H, -NH-CO-), 4.14 (d, J=0.4 Hz, 1H), 3.83 (d, J=1.8 Hz, 1H), 3.72 (dd, 1H), 3.65 (dd, J=1.8, 9.66 Hz, 1H), 3.52 (dd, J =4.9, 11.25 Hz, 2H, –C**H**-NH-), 3.42 (m, 4H, –CH_2); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3288, 2950, 2859, 2664, 2361, 2063, 1653, 1611, 1557, 1435, 1412, 1364, 1324, 1260, 1216, 1118, 1066, 1035, 968, 900, 878, 853, 813, 677, 654, 597, 540, 517, 276, 418; **-MS**((-)-ESI, m/z): 355 [M – H_2O – H], 282 [M^-].

Analytical data data for ethanolamine salt of 2-hydroxyethylgalactaramide (26b) – [2-hydroxyethan-1-aminium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-((2-hydroxyethyl)amino)-6-oxohexanoate]

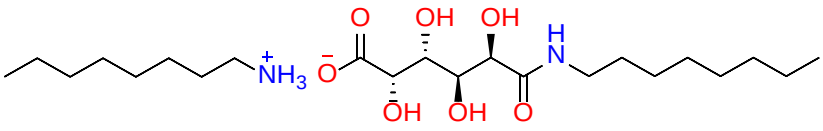


26b

MW: 374.34 g/mol ($C_{10}H_{22}N_2O_9$); **Melting Point**: 150 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.53 (t, J=5.6 Hz, 1H, -NH-CO-), 4.11 (d, J=1.0 Hz, 1H), 3.75 (d, J=3.6 Hz, 1H), 3.57 (dd, 1H), 3.71 (dd, J=0.86, 9.4 Hz, 1H), 3.56 (m, 2H, –CH_2-), 3.42 (t, J=6.0 Hz, 2H, –CH_2-), 3.16 (m, 2H, –CH_2–), 2.81 (t, J=5.1 Hz, 2H, –CH_2-); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 179.2 (NH-**C=O**), 1175.6 (**O**=**C**-O^-), 71.4, 70.8, 60.0, 57.8, 41.1; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3347, 3180, 2954, 2879, 2732,

2597, 2377, 2036, 1655, 1601, 1542, 1485, 1408, 1354, 1314, 1199, 1122, 1069, 1040, 963, 929, 895, 878, 837, 737, 657, 596, 562, 517, 479; **-MS**((-)-ESI, m/z): 252 [M – $HOCH_2CH_2NH_2$ – H], 505 [2M – 2 $HOCH_2CH_2NH_2$ –H]; **+MS** ((+)+ESI, m/z): 315 [M + H], 297 [M – H_2O + Na].

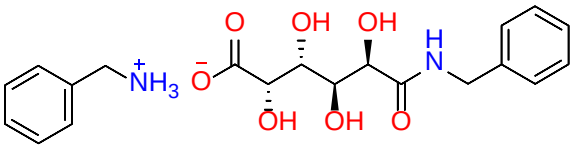
Analytical data for the octylamine salt of N-octylgalactaramide (26c)
Octane-1-aminium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-((2-hydroxyethyl)amino)-6-oxohexanoate



26c

MW: 450.33 g/mol ($C_{22}H_{46}N_2O_7$); **Melting Point**: 201 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.46 (t, J=5.9, 1H, –CO–NH–),4.07 (d, J=1.1 Hz, 1H), 3.68 (dd, J=1.2, 9.8 Hz, 1H), 3.66 (d, J=5.1 Hz, 1H), 3.468 (dd, J=5.1, 9.2 Hz, 1H), 3.07 (m, 2H, –C**H**$_2$–NH_3^+), 2.74 (1t, J=7.3 Hz, 2H, –NH–C**H**$_2$), 1.50 (m, 2H, -NH–CH_2–C**H**$_2$–), 1.41 (m, 2H, –C**H**$_2$–CH_2– NH_3^+), 1.26 (t-broad, 6H, –CH_3), 1.26 (m, 20H, –CH_2–); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3881, 2923, 2850, 2558, 2358, 1681, 1642, 1567, 1520, 1469, 1368, 1239, 1149, 1107, 1040, 971, 926, 888, 826, 754, 723, 657, 627, 544, 482, 419; **-MS**((-)-ESI, m/z): 320 [M – n-$C_8H_{17}NH_2$ – H], 641 [2M – 2 n-$C_8H_{17}NH_2$ – H]; **+MS** ((+)+ESI, m/z): 451 [M + H], 322 [M – n-$C_8H_{17}NH_2$ + H], 344 [M – n-$C_8H_{17}NH_2$ + Na].

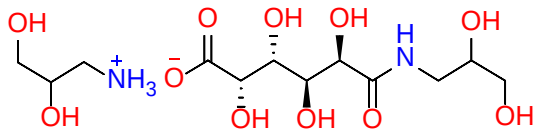
Analytical data for the Benzylaminium salt of N-benzylgalactaramide (26d)
Phenylmethanaminium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-((2-hydroxyethyl)amino)-6-oxohexanoate



26d

MW: 406.44 g/mol ($C_{20}H_{06}N_2O_7$); **Melting Point**: 224 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.04 (t, 1H, NH–CO), 7.35 (m, 10H, C_6H_5), 4.33 (m, 2H, C_6H_5–CH_2–N), 4.19 (d, J=0.7 Hz, 1H), 3.82 (d, J=4.2 Hz, 1H), 3.94 (s, 2H, C_5H_6–CH_2–N), 3.76 (dd, J=1.1, J=9.3 Hz, 1H), 3.60 (dd, J=4.0, 9.3 Hz, 1H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 176.9, 174.2, 140.1, 136.4, 129.2, 128.8, 128.4, 127.7, 127.1, 72.7, 71.7, 71.6, 71.2, 43.2, 42.3; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3984, 3338, 3078, 3041, 2922, 2844, 2780, 2748, 2636, 2249, 2134, 1952, 1878, 1810, 1659, 1615, 1573, 1534, 1442, 1390, 1308, 1250, 1202, 1146, 1107, 1038, 959, 933, 885, 816, 784, 732, 695, 664, 602, 562, 521, 464, 417; **-MS**((-)-ESI, m/z): 298 [M – $PhCH_2NH_2$ – H], 597 [2M – 2 $PhCH_2NH_2$ – H]; **+MS** ((+)+ESI, m/z): 407 [M + H], 389 [M – H_2O + H].

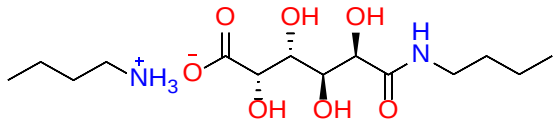
Analytical data for the galactaric acid mono salt mono amide of isoserinol (26e) 2,3-dihydroxypropan-1-aminium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-((2-hydroxyethyl)amino)-6-oxohexanoate



26e

MW: 374.34 g/mol ($C_{12}H_{26}N_2O_{11}$); **Melting Point**: 211 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.58 (m, 1H, NH-CO), 4.25 (dd, J=2.3, 8.3 Hz, 1H), 4.23 (d, J=9.1 Hz, 1H), 4.07 (t, J=8.7 Hz, 1H), 3.76 (d, J=2.0 Hz, 1H); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3430, 3288, 2959, 2859, 2664, 2565, 2361, 2150, 2063, 1653, 1611, 1557, 1435, 1412, 1364, 1324, 1260, 1216, 1118, 1066, 1035, 968, 912, 900, 878, 853, 813, 677, 654, 597, 540, 517, 476, 418; **-MS**((-)-ESI, m/z): 355 [M – H_2O – H], 282 [M^-].

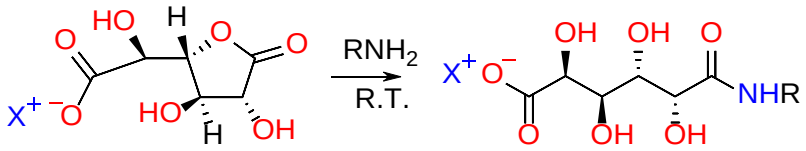
Analytical data for butylamine salt of N-butylgalactaramide (26f) [Butane-1-aminium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-((2-hydroxyethyl)amino)-6-oxohexanoate]



26f

MW: 338.40 g/mol ($C_{14}H_{30}N_2O_7$); **Melting Point**: 199 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.50 (t, J=7.87 Hz, 1H, NH–CO), 4.10 (d, J=0.7 Hz, 1H), 3.73 (d, J=3.6 Hz, 1H), 3.69 (dd, J=0.7, 9.3 Hz, 1H), 3.55 (dd, J=3.3, 9.5 Hz, 1H), 3.08 (m, 2H, –CH_2–NH–), 2.75 (t, J=7.5 Hz, 2H, –NH_3^+–CH_2–), 1.53 (m, 2H, –CH_2–), 1.35 (m, 2H, –CH_2–) 1.28 (m, 4H, –CH_2–CH_3), 0.84 (q, J=7.2 Hz, 6H, -CH_3); **^{13}C-NMR** (100 MHz, D_2O, ppm): δ 179.3 ($^-$O-C=O), 175.2 (HN-C=O), 71.4 (CH-OH), 71.3 (CH-OH), 71.2 (CH-OH), 70.8 (CH-OH), 39.3 (CH_2-NH), 38.9 (CH_2-NH_3^+), 30.6 (CH_2-), 28.7 (CH_2-), 19.4 (CH_2-), 19.0 (CH_2-), 13.0 (CH_3-), 12.7 (CH_3-); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3387, 3336, 3258, 3087, 2960, 2933, 2871, 2758, 2650, 2540, 2364, 2342, 2125, 1642, 1572, 1324, 1438, 1400, 1369, 1310, 1271, 1231, 1204, 1148, 1108, 1042, 970, 918, 890, 873, 830, 794, 744, 700, 652, 604, 538, 480, 441; **-MS**((-)-ESI, m/z): 264 [M^-], 529 [$2M^-$ + H]; **+MS** ((+)+ESI, m/z): 339 [M + H], 321 [M + H – H_2O], 266 [M^- + 2H].

b. Synthesis of salts of mono galactaramide by reaction of galactaro-1,4-lactone salts with primary amines.



19, 20 **27**

Procedure:

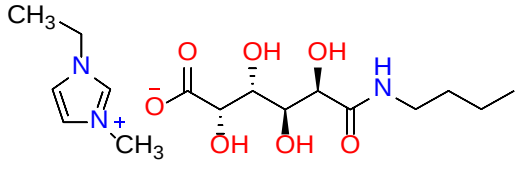
The previously isolated solid salt of galactaric acid-1,4-lactone (**19**, **20**) was first added to DMSO in a round bottom flask equipped with magnetic stirrer at 20-25°C. The primary amine was then added into the lactone salt solution in equimolar ratio to galactaric acid-1,4-lactone salt. The amidation reaction was carried out at room temperature and the reaction time was selected depending on the types of amine and the solubility of products in DMSO, following the process by ^{1}H-NMR analysis. The reaction was stopped when the conversion of starting salt was higher than 95%. The mono amide galactarate salts were largely precipitated from DMSO solution as white solids. The final mixture of reaction became a wax-like solid. The products were purified by recrystallization using acetone or acetonitrile as co-solvent (DMSO:acetone/acetonitrile = 1:5) and then further filtration and drying. The specific substrates and reaction conditions used are reported collectively in Table 4.13.

*Table 4.13 - Synthesis of salt of galactaramides (**27**) by reaction of salts **19** with primary amines in DMSO (procedure b).*

Entry	[Subst.] (mmol)	X / Amines	solvent	Ratio [Base]/[sub]	T °C	t (min)	Subt. Conv.(%)	27a-c (Yield %)
JL182	**20f** (1)	1–Et–3–Meimidazolium / Butylamine	DMSO	1	RT	120	**20f** (>95)	**27a** (87)
JL160	**19a** (1)	Na / n-$C_{12}H_{25}NH_2$	DMSO	2	RT	120	**19a**(100)	**27b** (95)
JL163	**20a** (1)	Na / n-$C_{12}H_{25}NH_2$	DMSO	1	RT	20	**19a**(100)	**27c** (77)

Analytical data for the compound 27a

1-ethyl-3-methyl-1H-imidazol-3-ium (2S,3R,4S,5R)-6-(butylamino)-2,3,4,5-tetrahydroxy-6-oxohexanoate

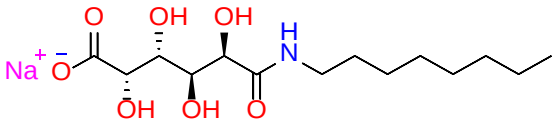


27a

MW: 375.42 g/mol ($C_{16}H_{29}N_3O_7$); ^{1}H-**NMR** (400 MHz, DMSO-d6, ppm): δ 9.22 (s, 1H, N–CH=N^{+}), 7.79 (s, 1H, N^{+}–CH=CH), 7.71 (s, 1H, N–CH=CH), 3.68 (dd, J=0.6, 9.3 Hz, 1H), 4.11 (d, J=0.6 Hz,

1H), 3.64 (d, J=4.5 Hz, 1H), 7.48 (t, J=5.9 Hz, 1H, -NH-CO-), 4.20 (dd, J=7.3, 14.6 Hz, 2H, CH_3–CH_2–N), 3.51 (dd, J=4.52, 9.29 Hz, 1H), 3.85 (s, 3H, N^+–CH_3), 3.08 (m, 2H, -HN-CH_2-), 1.41 (t, 3H, J=7.2 Hz, –CH_3), 1.37 (m, 2H, -CH_2-), 1.27 (m, 2H, -CH_2-), 0.86 (t, J=7.2 Hz, 3H, -CH_2-CH_3-); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3387, 3334, 3264, 2959, 2932, 2871, 2549, 2129, 1641, 1544, 1439, 1369, 1309, 1271, 1240, 1212, 1148, 109, 1041, 973, 914, 874, 830, 743, 704, 653, 540, 472; **-MS**((-)-ESI, m/z): 264 [M^-], 529 [$2M^-$+H], 551 [$2M^-$+Na], **+MS** ((+)+ESI, m/z): 288 [M^-+Na], 553 [$2M^-$+2H+Na], 575 [$2M^-$+2Na+2H], 597 [$2M^-$+3Na].

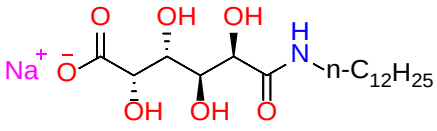
Analytical data for the sodium salt *N*–octyl galactaramide (27b)
[Sodium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-(octylamino)-6-oxohexanoate]



27b

MW: 343.35 g/mol ($C_{16}H_{26}NNaO_7$); **Melting Point**: 228 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.45 (t, J=6.0 Hz, 1H, -NH-CO-), 4.85 (d, J=7.2 Hz, 1H, -OH), 4.08 (d, J=7.0 Hz, 1H, –C**H**–OH), 3.65 (dd, J=8.9, 1.0 Hz, 1H, –C**H**–OH), 3.55 (d, J=5.9 Hz, 1H, –C**H**–OH), 3.40 (dd, J=6.0, 8.9 Hz, 1H, –C**H**–OH), 3.15 (sep, J=6.1 Hz, 2H, –CH_2–), 1.40 (t, J=5.6 Hz, 2H, –CH_2–), 1.28 (m, 10H, –CH_2–), 0.86 (t, J=6.5 Hz, 3H, –CH_3); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3412, 3325, 3255, 2956, 2922, 2850, 2738, 2645, 2619, 2526, 2367, 2345, 2251, 2170, 1677, 1625, 1542, 1475, 1444, 1421, 1379, 1311, 1240, 1214, 1113, 1089, 1041, 965, 918, 881, 858, 825, 754, 720, 658, 558, 526, 474, 432; **--MS**((-)-ESI, m/z): 320 [M - Na], 663 [2M–Na]; **+MS** ((+)+ESI, m/z): 366 [M + Na], 130 [n-$C_8H_{17}NH_2$ + H], 451 [2M + Na].

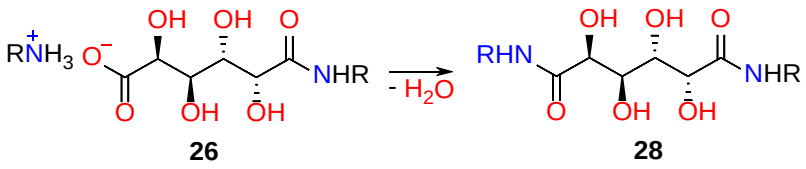
Analytical data for sodium salt *N*–dodecyl galactaramide (27c)
[Sodium (2S,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-(dodecylamino)-6-oxohexanoate]



27c

MW: 399.46 g/mol ($C_{18}H_{34}NNaO_7$); **Melting Point**: 221 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.45 (t, J=5.9 Hz, 1H, -NH-CO-), 4.06 (d, J=1.0 Hz, 1H, –C**H**–OH), 3.65 (dd, J=8.8, 1.0 Hz, 1H, –C**H**–OH), 3.55 (d, J=6.0 Hz, 1H, –C**H**–OH), 3.40 (dd, 1H, –C**H**–OH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3510, 3411, 3314, 3255, 2960, 2920, 2849, 2740, 2623, 2366, 2345, 1677, 1626, 1545, 1471, 1443, 1421, 1379, 1312, 1262, 1240, 1214, 1114, 1041, 963, 875, 842, 820, 751, 719, 659, 568, 526, 474; **+MS** ((+)+ESI, m/z): 400 [M + H], 422 [M + Na], 186 [n-$C_{12}H_{25}NH_2$ + H].

27. Synthesis of N-alkyl derivatives of galactaro diamide (2R,3S,4R,5S)-2,3,4,5-tetra-hydroxyhexanediamide (R = H) (28)



26 **28**

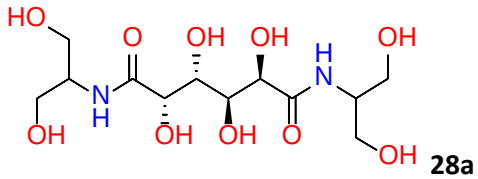
Procedure:

Primary amines were mixed together with a DMSO solution of galactaro-1,4-lactone in a round bottom flask equipped with magnetic stirrer. The molar ratio between amines and galactaro-1,4-lactone were 2:1. After completion of the salification and amidation reaction, the mixture was heated at 100 °C with an electric heating plate. The waxy-like mixture first became homogeneous and then some solid precipitates. The reaction was completed in 4 hours. The N-substituted galactaramides were formed in high yield according to the ^{1}H-NMR and specific isolation methods were developed. The products were largely precipitated from DMSO solution as white solids. The final products were purified by recrystallization using acetone or acetonitrile as co-solvent (DMSO:acetone/acetonitrile = 1:2~5) and then further filtration and drying. Different mono galactaramide salts were successfully used for this indirect galactarodiamides synthesis. The specific reaction condition investigated are reported in Table 4.14.

*Table 4.14 - Synthesis of galactarodiamides **28** by thermal decomposition of the amide salts **26** in DMSO.*

Entry	[26] (mmol)	Amine	T (°C)	t (h)	26 (conv. %)	28 (yield %)
JL065	5.0	Serinol	140	4	**26a** (90)	**28a** (80)
JL185	9.5	Ethanolamine	140	4	**26b** (100)	**28b** (88)
JL158	2.1	Octylamine	120	12	**26c** (100)	**28c** (63)
GICR28b	1.56	Octylamine	130	2	**26c** (100)	**28c** (81)
JL193	8.8	Benzylamine	130	2	**26d** (100)	**28d** (92)
JL194	1.8	Benzylamine	100	4	**26d** (100)	**28d** (90)
JL195	1.8	Hexamethylene diamine	100	2	**26h** (100)	**28h** (87)

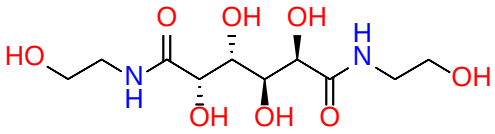
Analytical data for *N*^{1},*N*^{6}–bis(1,3–dihydroxypropan–2–yl)galactaramide [(2R,3S,4R,5S)-N1,N6-bis(1,3-dihydroxypropan-2-yl)-2,3,4,5-tetrahydroxyhexanediamide] (28a)



28a

MW: 356.33 g/mol ($C_{12}H_{24}N_2O_{10}$); **Melting Point**: 246 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.31 (d, J=8.5 Hz, 2H, -HN-CO-), 4.75 (t, J=5.3 Hz, 2H), 4.69 (t, J=5.5 Hz, 2H), 4.39 (dd, J=2.2, 5.8 Hz, 2H), 4.35 (d, J=6.9 Hz, 2H), 4.14 (d, J=6.7 Hz, 2H), 3.78 (dd, J=1.6, 5.9 Hz, 2H), 3.72 (m, 2H), 3.49 (m, 2H), 3.40 (m, 4H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 173.8 (O=**C**-NH-), 71.2 (-**C**H-OH), 60.1 (-CH-**C**H_2-OH), 52.5 (-NH-**C**H-); **FT-IR** (KBr, ν_{max}, cm^{-1}): 2956, 2929, 2892, 2820, 2726, 2666, 2618, 2537, 2508, 2452, 2362, 2317, 2169, 2150, 2111, 2064, 1954, 1656, 1539, 1444, 1411, 1370, 1342, 1313, 1284, 1256, 1215, 1198, 1133, 1106, 1077, 1045, 1005, 979, 911, 881, 853, 829, 684, 631, 605, 543, 491, 459, 409; **-MS** ((-)-ESI, m/z): 355 [M – H]; **+MS** ((+)+ESI, m/z): 393 [M + 2H_2O + H].

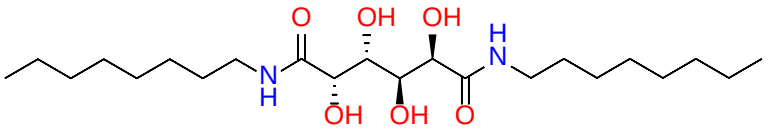
Analytical data for *N*^{1},*N*^{6}–bis(2–Hydroxyethyl)galactaramide [(2R,3S,4R,5S)-2,3,4,5-tetra-hydroxy-N1,N6-bis(2-hydroxyethyl)hexanediamide] (28b)



28b

CAS: 80714-42-7; **MW**: 296.28 g/mol ($C_{10}H_{20}N_2O_8$); **Melting Point**: 203 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.55 (d, J=5.6 Hz, 2H, -HN-CO-), 5.20 (s, 2H, -OH), 4.68 (s, 2H, -OH), 4.36 (s, 2H, -OH), 4.06 (s, 2H, -OH), 4.14 (s, 2H, -C**H**-OH), 3.79 (s, 2H, -C**H**-OH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3407 (-NH-), 3258 (-NH-), 2983, 2951 (-OH), 2881 (-OH), 2698, 2534, 2360, 2105, 1633 (C=O), 1547 (C=O), 1447, 1395, 1314, 1264, 1218, 1117, 1060, 998, 944, 912, 886, 849, 797, 704, 648, 588, 540, 475; **-MS** ((-)-ESI, m/z): 295 [M – H]; **+MS** ((+)+ESI, m/z): 297 [M + H], 319 [M + Na], 279 [M – H_2O + Na].

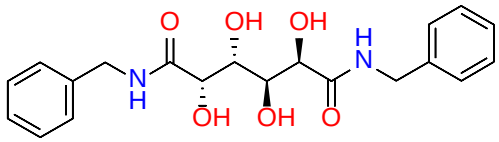
Analytical data for *N*^{1},*N*^{6}–Dioctyl galactarodiamide [(2R,3S,4R,5S)-2,3,4,5-tetrahydroxy-N1,N6-dioctylhexanediamide] (28c)



28c

MW: 432.60 g/mol ($C_{22}H_{44}N_2O_6$); **Melting Point**: 199 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): **δ** 7.51 (t, J=5.9 Hz, 2H, -HN-CO-), 4.10 (s, 2H, -C**H**-OH-), 3.77 (s, 2H, -C**H**-OH-), 3.08 (septet, J=7.0 Hz, 4H, -NH-C**H**$_2$-), 1.25 (m, 24H, -CH_2-), 0.86 (t, J=5.62 Hz, 6H); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3396 (-NH-), 3197 (-NH-), 2925, 2850, 1650 (-**CO**-NH-), 1623 (-**CO**-NH-), 1551, 1468, 1376, 1350, 1293, 1254, 1189, 1158, 1102, 1078, 1046, 926, 883, 796, 723, 690, 651, 626; **-MS** ((-)-ESI, m/z): 431 [M – H], 320 [M– n-$C_8H_{16}NH_2$ +H_2O–H], **+MS** ((+)+ESI, m/z): 433 [M + H], 455 [M + Na].

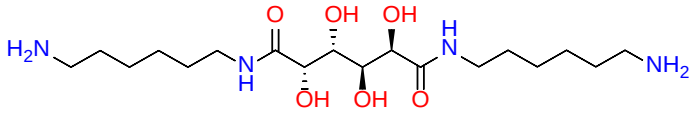
Analytical data for N^1,N^6–Dibenzyl galactarodiamide [(2R,3S,4R,5S)-N1,N6-dibenzyl-2,3,4,5-tetrahydroxyhexanediamide] (28d)



28d

MW: 388.42 g/mol ($C_{20}H_{24}N_2O_6$); **Melting Point**: 228 °C (decompose); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.09 (t, J=6.2 Hz, 2H, -HN-CO-), 7.30 (d, J=4.4 Hz, 8H), 7.23 (sextet, J=4.0 Hz, 2H), 5.34 (d, J=6.7 Hz, 4H, O**H**-CH-), 4.46 (s, 2H, O**H**-CH-), 4.34 (ddd, J=6.4, 14.8, 20.9 Hz, 4H, -C**H**$_2$-NH-), 4.24 (d, J=5.9 Hz, 2H, -C**H**-OH), 3.88 (d, J=4.0 Hz, 2H, -C**H**-OH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3395, 3299, 3219, 3061, 3032, 2952, 2808, 2688, 2641, 2484, 2356, 2177, 1642, 1546, 1496, 1454, 1435, 1412, 1360, 1326, 1279, 1200, 1118, 1086, 1046, 1031, 931, 905, 861, 801, 737, 694, 660, 609, 573, 505, 458, 420; **-MS** ((-)-ESI, m/z): 387 [M – H]; **+MS** ((+)+ESI, m/z): 411 [M + Na], 433 [M – H + 2Na].

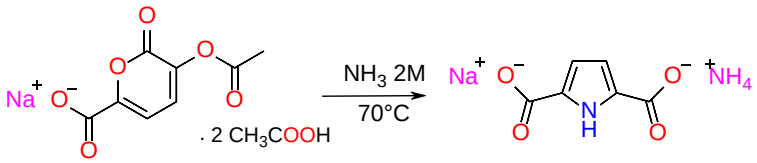
Analytical data for N^1,N^6–Bis(6-aminohexyl)galactaramide [(2R,3S,4R,5S)-N1,N6-bis(6-aminohexyl)-2,3,4,5-tetrahydroxyhexanediamide] (28e)



28e

MW: 406.52 g/mol ($C_{18}H_{38}N_4O_6$); **Melting Point**: >270 °C, which is not compatible with the reported data T_m=178 °C;[10] **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.55 (t, J= 5.3 Hz, 2H, -HN-CO-), 4.12 (s, 2H), 3.78 (s, 2H), 3.09 (sep, J = 6.6 Hz, 4H, -C**H**$_2$-NH-), 1.53 (m, 8H, -CH$_2$-), 1.26 (m, 12H, -CH$_2$-); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3343, 3338, 2929, 2855, 2107, 1627, 1542, 1437, 1375, 1330, 1212, 1169, 1107, 1083, 1045, 954, 876, 824, 728, 649, 540, 474; **+MS** ((+)+ESI, m/z): 407 [M+H].

28. Synthesis of salt of 1H-pyrrole-2,5-dicarboxylate (29) and 1H-pyrrole-2,5-dicarboxylic acid (29b)



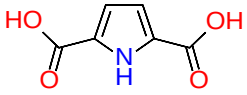
7 **29**

Procedure:

A three necked round bottom flash was charged with solid sodium 3-acetoxy-2-oxo-2H-pyran-6-carboxylate (crystallized with two moles of acetic acid) (**7**, 340 mg, 1 mmol) and then with 2 M solution of NH_3 (5 mL) under stirring. The reaction was heated at at 70 °C and kept under stirring for 10 hours.

The disappearance of starting lactone was followed until completion by ^{1}H-NMR analysis of a sample. At the end, the solvent was removed and the analysis by ^{1}H-NMR in DMSO-d6 with internal standard showed that 1H-pyrrole-2,5-dicarboxylate **29** was the main product (35% yield), along with a pyrrole monoamide salt (7%) and the 2-pyrrolecarboxylate (1.5 %). The reaction raw product was acidified to pH 3. After removal of the water by rotatory evaporator, the obtained brown solid was mixed together with ethanol. The organic phase was filtered and dried in rotatory evaporator, obtaining 1H-pyrrole-2,5-carboxylic acid (**29a**, 28 mg, 18% yield). From the water by colum chromatography were isolated 5-carbamoyl-1H-pyrrole-2-carboxylic acid (**29b**) and 1H-pyrrole-2-carboxylic acid (**29c**).

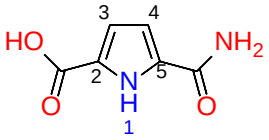
Analytical data for 1H-pyrrole-2,5-dicarboxylic acid (29a)



29a

CAS: 937-27-9; **MW**: 155.11 g/mol ($C_6H_5NO_4$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 12.7 (s, br 2H, COOH); 12.2 (s, 1H, NH); 6.74 (d, 2H pyrrole, J=1.7 Hz)[11]; **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): 161.2; 127.2; 114.9; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3496, 3432, 3357, 1670, 1552, 1442, 1423, 1261, 1228, 1125, 1111, 1066, 1038, 984, 962, 873, 823, 759, 617, 574, 531, 484, 460; **-MS** ((-)-ESI, m/z): 154 [M-H^{+}]$^{-}$ (peak base), 110, 66.

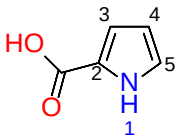
Analytical data for 5-carbamoyl-1H-pyrrole-2-carboxylic acid (29b)



29b

MW: 154.13 g/mol; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): **δ** 12.7 (s, br 2H, COOH); 12.2 (s, 1H, NH); 6.74 (d, 2H, J=1.7)[11]; **^{13}C-NMR** (100 MHz, DMSO-d6): 162.2; 127.2; 114.9; **FTIR** (KBr, ν_{max}, cm^{-1}): 3496, 3432, 3357, 1670, 1552, 1442, 1423, 1261, 1228, 1125, 1111, 1066, 1038, 984, 962, 873, 823, 759, 617, 574, 531, 484, 460; **-MS** ((-)-ESI, m/z): 154 [M-H^{+}]$^{-}$ (peak base), 110, 66.

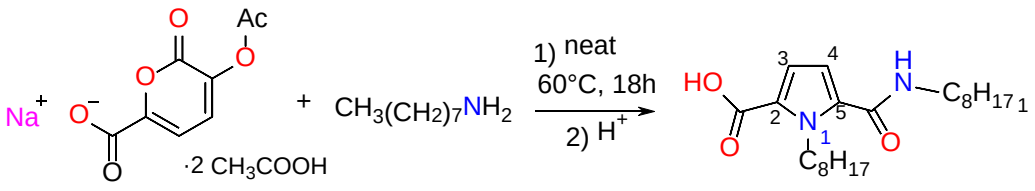
Analytical data for 1H-pyrrole-2-carboxylic acid (29c)



29c

MW: 111.10 g/mol; **M.p.**: 204-208 °C (decomp.); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): **δ** 12.5 (s, br 1H, COOH); 11.7 (s, 1H, NH); 6.96 (dd, 1H, J=2.7, 2.3 Hz), 6.82 (d, 1H, J=2.7 Hz), 6.17 (d, 1H, J=2.3 Hz); **^{13}C-NMR** (100 MHz, DMSO-d6): 162.2, 126.2, 125.1, 114.6, 109.2; **FTIR** (KBr, ν_{max}, cm^{-1}): 3152, 3130, 1630, 1460, 1403, 1200, 1111, 1095, 964, 893, 475; **-MS** ((-)-ESI, m/z): 110 [M-H^{+}]$^{-}$ (peak base), 66.

29. Synthesis of 1-octyl-5-(octylcarbamoyl)-1H-pyrrole-2-carboxylic acid by reaction of compound 7 with octylamine.

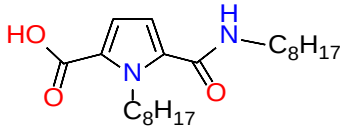


8 **30**

Procedure:

In a flask equipped with magnetic stirrer were charged under magnetic stirring the sodium 5-acetoxy-6-oxo-pyran-2-carboxylate (as acetic acid solvate) (**7**, 0.5 g, 1.46 mmol) and octylamine (2.41 ml, 14.6 mmol). The mixture was heated at 60 °C and maintained under stirring for 20 hours. Analysis by ^{1}H-NMR of a sample indicates a conversion of 79% and the formation of the pyrrole derivative **30** as the main product in yield of 76%. The pure product of 1-octyl-5-(octylcarbamoyl)-1H-pyrrole-2-carboxylic acid (**30**) was isolated by column chromatography in yield 36%.

Analytical data for 1-octyl-5-(octylcarbamoyl)-1H-pyrrole-2-carboxylic acid (30)

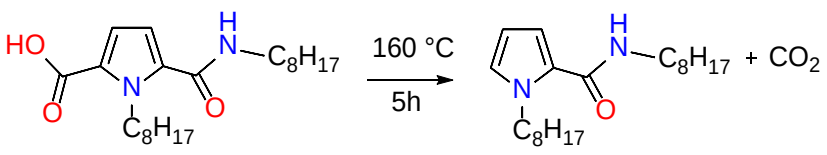


30

MW: 378.29 g/mol ($C_{22}H_{38}N_2O_3$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.23 (t, J=5.8 Hz, 1H of –HN-CO-); 6.75 (d, 1H pyrrole, J=4.2 Hz); 6.59 (d, 1H pyrrole, J=4.2 Hz); 4.74 (t, 2H, J=7.2 Hz); 3.17 (dd, 2H, J=6.6, J=12.8 Hz); 1.57 (q, 2H); 1.48 (dd, 2H); 1.22 (m, 20H); 0.85 (2t, 6H; J=7.0, 7.1 Hz); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 161.92, 160.90, 131.54 (=C-H), 125.95 (=C-H), 115.59 (=C-N), 110.72 (=C-N), 44.81, 38.44, 31.42, 31.11, 31.07, 28.98, 28.61, 28.55 (x 2), 28.49, 26.30, 25.86, 21.95, 21.93, 13.76; **+MS** ((+)+ESI, m/z): 379 [M+H], 401 [M+Na].

30. Preparation of N,1-dioctyl-1H-pyrrole-2-carboxamide (31):

a. By decarboxylation of 1-octyl-5-(octylcarbamoyl)-1H-pyrrole-2-carboxylic acid

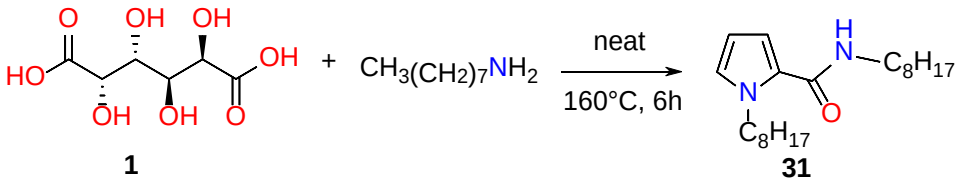


30 **31**

Procedure:

250 mg of compound **30** was heated under neat conditions at 160 °C in a round bottom flask. The N,1-dioctyl-1H-pyrrole-2-carboxamide (**31**) was formed after 5 hours in nearly quantitative yield (>90%).

b. By reaction between galactaric acid and octylamine under neat condition

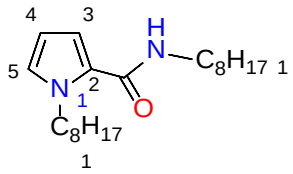


1 **31**

Procedure (JL061):

Galactaric acid (1 g, 4.76 mmol) and octylamine (1.57 ml, 9.52 mmol) were mixed and heated in round-bottomed flask first at 160 °C for 20 hours. At the end, a brown viscous liquid was obtained by cooling at room temperature. The ^{1}H-NMR analysis of a sample shows that **31** was the main product in 67% yield from ^{1}H-NMR analysis with terephthalic acid as the internal standard. A flash chromatography was carried out to purify product **31** and to separate by-products. Water and other solvents in the mixture were removed on rotary evaporator. The sample was first solubilised in the minimum amount of solvent and mixed together with silica gel. After removal of the solvent by evaporation, the remained brown powder was carefully introduced to the top of the prepared column. Elution was carried out using a mixture of ethyl acetate:acetic acid in ratio 1:1, obtaining mixtures of pyrrole derivatives and pure compound **31** in the last fractions.

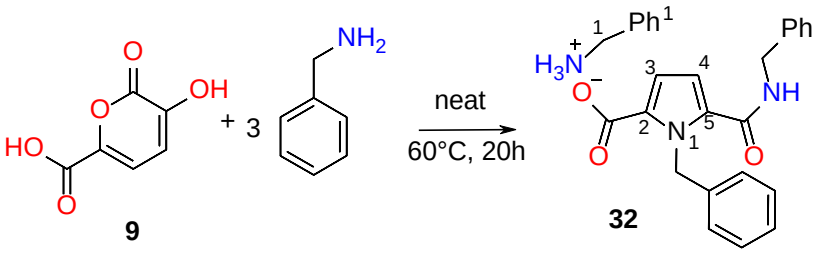
Analytical data for N,1-dioctyl-1H-pyrrole-2-carboxamide (31)



31

MW: 334.55 g/mol ($C_{21}H_{38}N_2O$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.88 (t, J=7.1 Hz, 1H of -HN-CO-); 6.89 (dd, 1H pyrrole, J=2.0, 2.5 Hz); 6.68 (dd, J=1.7, J=4.2 Hz); 5.97 (dd, 2H, J=2.6, 3.8 Hz); 4.27 (t, 2H, J= 7.1 Hz); 3.17 (dd, 2H, J=6.6, 12.8 Hz); 1.57 (q, 2H); 1.48 (dd, 2H); 1.22 (m, 20H); 0.85 (2t, 6H; J=7.0, 7.1 Hz); **+MS** ((+)+ESI, m/z): 336 [M+H].

31. Synthesis of phenylmethanaminium 1-benzyl-5-(benzylcarbamoyl)-1H-pyrrol-2-carboxylate (32)



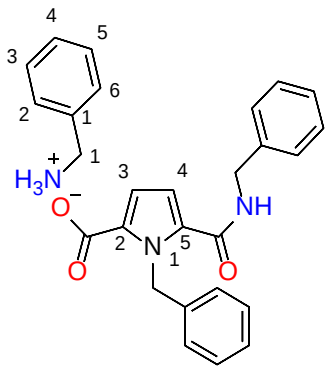
9 **32**

Procedure:

3-Acetoxy-2-oxo-2H-pyran-6-carboxylate acetic acid solvate (135 mg, 0.87 mmol) and distilled benzylamine (540 mg, 5 mmol) were loaded into a 3 mL tube. The heterogeneous system was heated under stirring at 60 °C and kept closed for 20 hours and, then, at 70 °C for other 20 hours. ^{1}H-NMR analysis of a sample showed that starting pyrone was disappeared and that compound **32a** and the related product of decarboxylation (**32b**) were formed in a relative ratio of 75:25. Minor amount of a sublimated white solid (85 mg) was also formed and proved to be the benzylamine self-condensation product. The cooled crude reaction mixture (brown coloured solid) was dissolved in ethanol (10 ml) and a white solid of **32a** was precipitated by addition of ethyl acetate. Recrystallization and drying allowed getting pure **32a** in 58% yield.

In a parallel reaction (RG24), the crude final mixture was fractionated by column chromatography and the fraction 18 was identified by ^{1}H-NMR as 1-benzyl-5-(benzylcarbamoyl)-1H-pyrrole-2-carboxylic acid (**32**) (the areas of aromatic signals account for 10 hydrogens, while in the benzylammonium salt **32a** they are 15).

Analytical data for phenylmethanaminium 1-benzyl-5-(benzylcarbamoyl)-1H-pyrrol-2-carboxylate (32a)

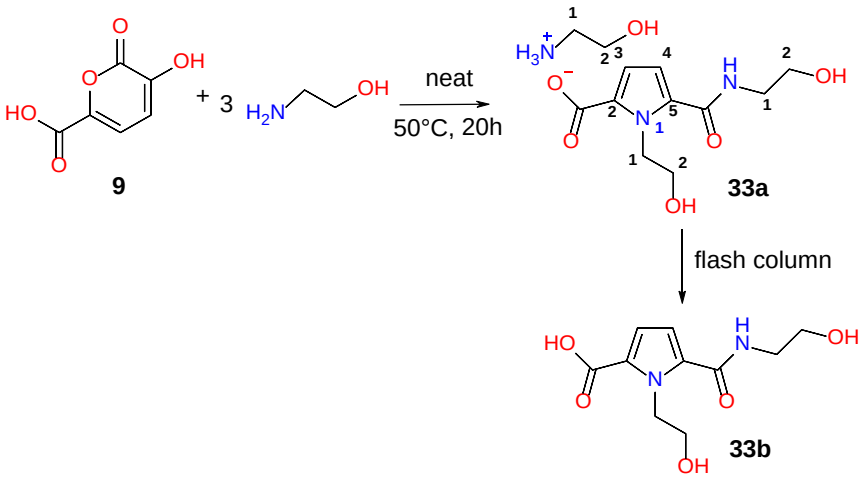


32

MW: 441.53 g/mol ($C_{27}H_{27}N_3O_3$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 8.61 (t, J=6.1 Hz, 1H, NH-CO), 7.29 (m, 13H, Ph), 6.96 (d, J=6.7 Hz, 2H, Ph), 6.73 (d, J=3.9 Hz, 1H, pyrrole); 6.59 (d, J=3.9 Hz, 1H, pyrrole), 6.32 (s, 2H, -CH_2-, $PhCH_2NH_3^+$), 4.34 (d, J=6.1 Hz, 2H, -CH_2-, $PhCH_2NH_2$), 3.93 (s, 2H , -CH_2- of benzylpyrrole); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 164.32, 161.50, 140.85 (=C-H), 139.77 (=C-H), 128.24, 128.22, 127.99, 127.79, 127.53, 126.35, 126.09, 112.94 (=C-N), 111.50

(=C-N), 47.04, 42.81, 41.65; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3260, 3061, 3032, 2980, 2853, 2742, 2627, 2177, 1627, 1583, 1544, 1495, 1454, 1432, 1401, 1368, 1315, 1302, 1258, 1240, 1203, 1177, 1159, 1076, 1064, 1029, 1014, 962, 929, 881, 813, 775, 731, 710, 695, 620, 588, 548, 506, 473, 420; **-MS** ((-)-ESI, m/z): 334 [M- $PhCH_2NH_3^+$].

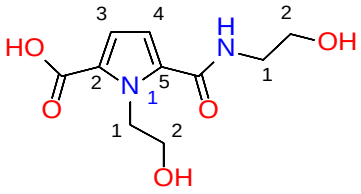
32. Synthesis of 2-hydroxyethan-1-aminium 1-(2-hydroxyethyl)-5-((2-hydroxyethyl)-carbamoyl)-1H-pyrrol-2-carboxylate



Procedure:

3-Hydroxy-2-oxo-2H-pyran-6-carboxylic acid (135 mg, 0.87 mmol) and ethanolamine (305 mg, 5.0 mmol) were mixed in a mortar and decanted into a 3 mL glass tube. The system, which is heterogeneous, was heated at 50 °C, then closed and kept under stirring at this temperature for 20 hours. The reaction was followed by ^{1}H-NMR (in DMSO-D6 as solvent) and stopped when the lactone was disappeared. The salt **33a** was formed as the main product in yield higher than 90%. Flash chromatographic separation affords the 1-(2-hydroxyethyl)-5-((2-hydroxyethyl)carbamoyl)-1H-pyrrole-2-carboxylic acid **33b** in 66% yield, instead of the salt **33a**.

Analytical data for 1-(2-Hydroxyethyl)-5-((2-hydroxyethyl)carbamoyl)-1H-pyrrol-2-carboxylic acid (33b)

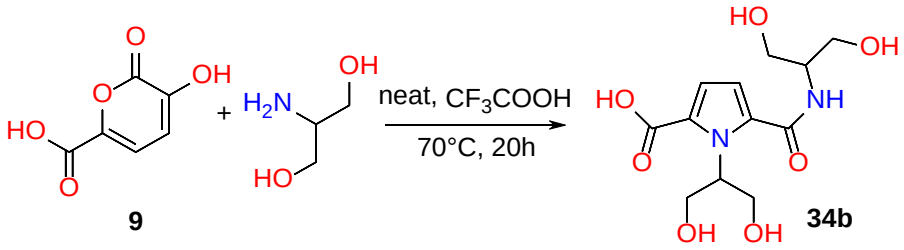


33b

MW: 242.23 g/mol ($C_{10}H_{14}N_2O_5$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.91 (t, J=5.6 Hz, 1H, NH-CO), 6.62 (d, J=3.9 Hz, 1H pyrrole), 6.31 (d, J=3.9 Hz, 1H pyrrole), 4.80 (t, 2H, J=5.1 Hz), 3.66

(t, 2H, J=5.1 Hz), 3.46 (t, 2H, J=6.2 Hz), 3.23 (dd, 2H, J=6.0, 6.2 Hz); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 165.48, 161.88, 137.40 (=C-H), 126.18 (=C-H), 111.03 (=C-N), 110.83 (=C-N), 62.66 (-CH_2-OH), 59.88 (-CH_2-OH), 47.00, 41.45; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3365, 3253, 2977, 2940, 2870, 1645, 1566, 1556, 1518, 1455, 1434, 1408, 1381, 1311, 1263, 1166, 1102, 1066, 963, 942, 905, 860, 821, 774, 742, 689, 632, 581, 485, 448; **-MS**((-)-ESI, m/z): 241 [M-H^+]$^-$, 223, 197 (peak base), 179, 110.

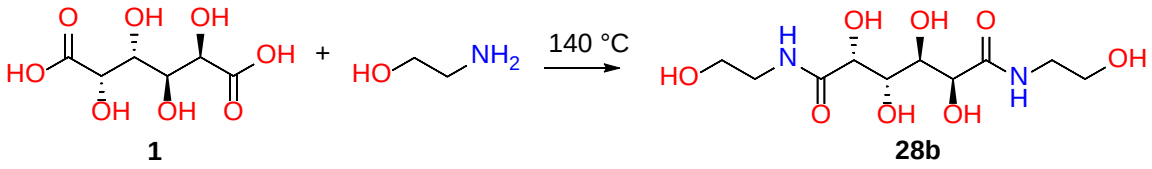
33. Synthesis of 1-(1,3-dihydroxypropan-2-yl)-5-((1,3-dihydroxypropan-2-yl)carbamoyl)-1H-pyrrole-2-carboxylic acid (34) from pyrone 9.



Procedure:

3-Hydroxy-2-oxo-2H-pyran-6-carboxylic acid (135 mg, 0.87 mmol) and serinol (455 mg, 4.87 mmol) were loaded into a 3 ml tube. The heterogeneous system was heated at 70 °C and, then, closed and maintained under stirring at this temperature for 21 hours. ^{1}H-NMR analysis of a sample of the reaction mixture indicate that the pyrone conversion was 97% and pyrrole salt derivative **34a** was formed in 85% yield, along with a small quantity of pyrrole lactone (**35**), pyrrole amide (**36**) and pyrrole amide carboxylic acid (**34b**). To the mixture was then added trifluoroacetic acid (0.382 ml, 5.1 mmol) and the reaction was stirred further for 23 hours at 40 °C and for 20 hours at 60 °C. ^{1}H-NMR analysis confirmed that conversion of pyrone **9** was quantitative and that pyrrole lactone **35**, pyrrole amide (**36**), and pyrrole amide carboxylic acid (**34b**) were formed in 12, 23, and 10% yield, respectively.

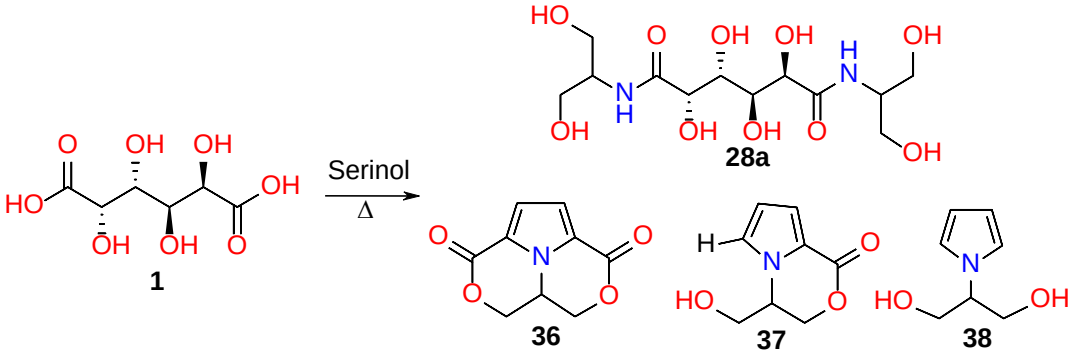
33. Synthesis of (2R,3S,4R,5S)-2,3,4,5-tetrahydroxy-*N1*,*N6*-bis(2-hydroxyethyl)-hexanediamide (28b) from galactaric acid and ethanolamine (neat conditions)



Procedure:

Galactaric acid (2 g, 9.52 mmol) and excess of ethanolamine (5.74 ml, 95.2 mmol) were mixed and heated under magnetic stirring in a round-bottomed flask at 140 °C for 24 hours. The process affords a yellow viscous mixture. The analysis by ^{1}H-NMR of a sample indicate that the main product was the galactaramide derivative (**28b**). A white solid was precipitated by adding the reaction mixture into 20 ml of acetonitrile. The solid obtained by filtration was further purified by using methanol. After drying for 12 h, a white powder was obtained. Analytical data for the [(2R,3S,4R,5S)-2,3,4,5-tetrahydroxy-N1,N6-bis(2-hydroxyethyl)hexanediamide] was identical to those found for compound **28b**.

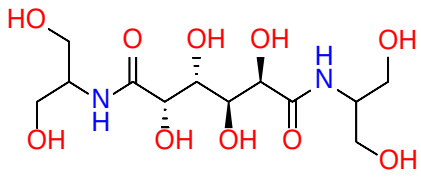
34. Reactivity of galactaric acid with serinol uder neat conditions



Procedure (JL064)

Galactaric acid (10 g, 47.6 mmol, MW 210) and serinol (4.4 g, 47.6 mmol, MW 91.1) were first mixed in a ball milling apparatus for 2 hours. Then, the mixture was heated in round-bottomed flask first at 130 °C for 24 hours and, then, the temperature was increased to 160 °C for further 8 hours. The final dark brown mixture was first poured in water and the solid formed was filtered, washed with water (2 × 5 ml), and dried by vacuum pump to obtain 9 grams of a white solid (identical to compound **28a**). The mixture of water and other solvents was concentrated at rotary evaporator and the residue was first solubilised in the minimum amount of solvent and then mixed together with silica gel. After removal of solvent by evaporation, the remained brown powder was placed on the top of a prepared SiO_2 column and fractionated by flash chromatography to separate different by-products. As eluents were used firstly ethyl acetate/hexane 9:1, then ethyl acetate/acetic acid 1:1, and lastly pure acetic acid) and fractions of 30 ml were collected. The more pure fractions were collected affording four samples (JL064_C1, JL064_C1C2, JL064_C2, JL064_C3), which were identified as follow.

Analytical data for (2R,3S,4R,5S)-*N1,N6*-bis(1,3-dihydroxypropan-2-yl)-2,3,4,5-tetrahydroxy-hexanediamide (28a)



28a

MW: 356.33 g/mol (Exact Mass for $C_{12}H_{24}N_2O_{10}$: 356,14); **Melting Point**: 246°C (decomposes); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.31 (1d, J=8.4 Hz, 2H, -HN-CO-), 4.35 (1 d, J=6.8 Hz, 2H), 4.75 (1t, J = 5.3 Hz, 2H), 4.69 (1t, J = 5.5 Hz, 2H), 4.39 (1dd, J = 2.2, 5.7 Hz, 2H), 4.14 (1d, J = 6.7 Hz, 2H), 3.78 (1dd, J = 1.6, 5.9 Hz, 2H), 3.72 (1m, 2H) 3.49 (1m, 2H), 3.40 (1m, 4H) (identical to **28a**, see Figure 4.6); Identical were also the ^{13}C-NMR, FTIR, –MS((–)–ESI), and –MS((–)–ESI) spectra.

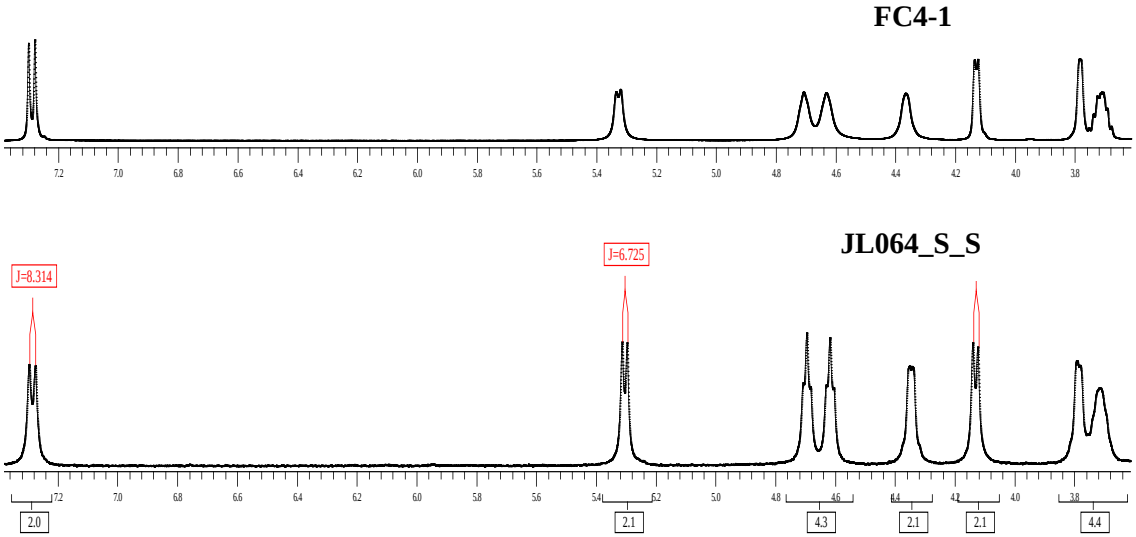
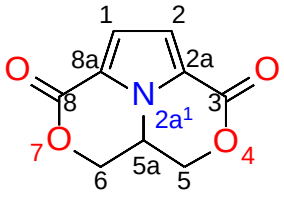


*Figure 4.6 - ^{1}H-NMR (DMSO, 400 MHz) spectra of the water insoluble solid from reaction JL064 (**JL064_S_S**) compared with sample **28a** from **FC4-1**.*

Analytical data for 5a,6-dihydro-5H-4,7-dioxa-2a1-azaacenaphthylene-3,8-dione (36)



36

MW: 193.16 g/mol ($C_9H_7NO_4$); **^{1}H-NMR** (DMSO-d6, 400 MHz, ppm): δ 7.05 (s, 2H pyrrole), 5.0 (tt, J=4.28, 11.49 Hz, 1H), 4.75 (dd, J=4.16, 11.37 Hz, 2H), 4.15 (t, J=11.49 Hz, 2H), shown in Figure 4.6; **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 157.7 (C-1), 120.6 (C-2), 115.8 (C-3), 67.6 (C-4), 47.2 (C-5); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3388, 3115, 2939, 2880, 2778, 2360, 2271, 2105, 2018, 1679, 1536, 1477, 1459, 1408, 1379, 1322, 1265, 1243, 1200, 1113, 1059, 1023, 985, 964, 891, 842, 773, 750, 704, 635, 567, 526, 480; **+MS** ((+)+ESI, m/z): 194.

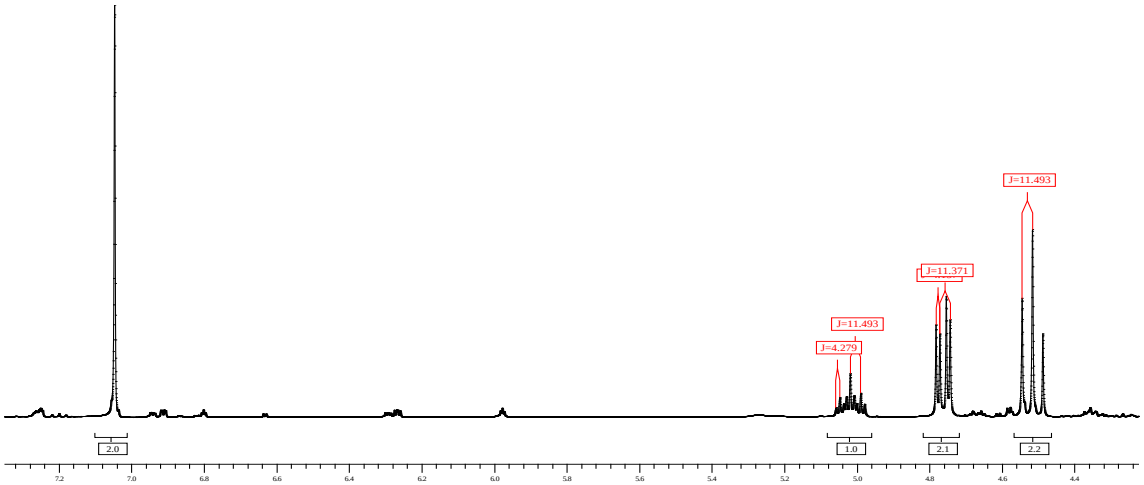
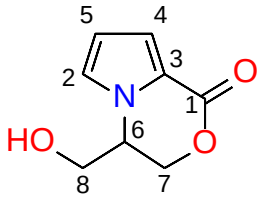


*Figure 4.7 - ^{1}H-NMR (DMSO, 400 MHz) spectrum of the first chromatographic fraction of reaction JL064 (**JL064_C1**).*

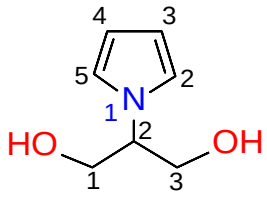
Analytical data for 4-(hydroxymethyl)-3,4-dihydro-1H-pyrrol[2,1-c][1,4]oxazin-1-one (37)



37

MW: 167.16 g/mol ($C_8H_9NO_3$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.25 (dd, J=1.59, 2.32 Hz, 1H pyrrole), 6.92 (dd, J=1.59, 3.91 Hz, 1H pyrrole), 6.27 (dd, J=2.57, 3.91 Hz, 1H pyrrole), 5.26 (t, J=5.38 Hz, 1H, -OH), 4.55 (ddd, J=3.67, 11.86, 15.77 Hz, 2H), 4.35 (m, 1H), 3.67 (m, 2H); **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 158.5 (C-1), 126.7 (C-2), 119.8 (C-3), 116.9 (C-4), 110.5 (C-5), 67.5 (C-7), 60.9 (C6), 54.2 (C-8); **+MS** ((+)+ESI, m/z): 168 (M + H), 190 (M + Na), 357 (2M + Na).

Analytical data for 2-(1H-Pyrrol-1-yl)propane-1,3-diol (38)



38

CAS: 1566958-13-1; **MW**: 141.17 g/mol ($C_7H_{11}NO_2$); **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 6.76 (t, J=2.079 Hz, 2H), 5.95 (t, J=2.079 Hz, 2H), 4.78 (t, J=5.380 Hz, 2H, -OH), 3.95 (tt, J=6.113 Hz, J=5.869 Hz, 1H), 3.62 (m, 4H), see the ^{1}H-NMR spectrum in Fig. 4.8; **^{13}C-NMR** (100 MHz, DMSO-d6, ppm): δ 120.2 (C-2, C-5), 107.4 (C-3, C-4), 63.5 (C-2), 62.1 (C-1, C-3); **-MS**((-)-ESI, m/z): 140 [M-H], 281 [2M-H].

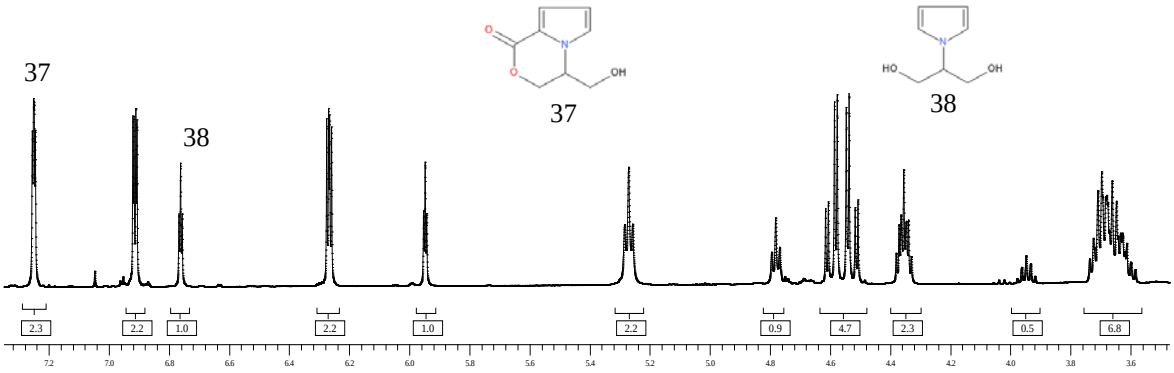


*Figure 4.8 - ^{1}H-NMR spectrum of the mixture of compounds **36** and **37**.*

35. Improved synthesis of 5a,6-dihydro-5H-4,7-dioxa-2a^{1}-azaacenaphthylene-3,8-dione (36) by reaction of D-galactaric acid with serinol in DMSO

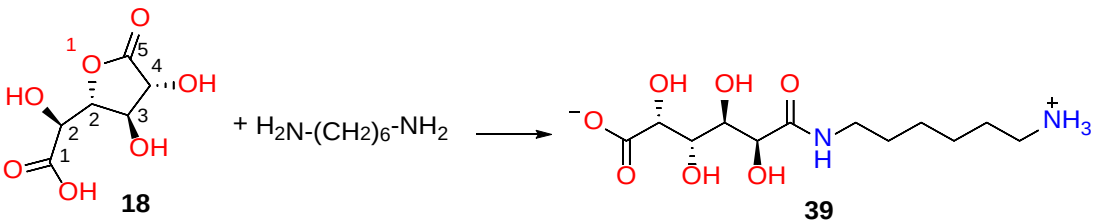
1 **36**

Reagent	MW	Amount	mmol	Equivalent
1	210	40 mg	0.19	1
Serinol	91.11	17.5 mg	0.19	1
DMSO	78.13	0.5 ml	7.06	37

Procedure (JL070):

Serinol (17.5 mg, 0.19 mmol) was first mixed together with 0.5 ml of DMSO in a round bottom flask equipped with magnetic stirrer and electrical heater. The mixture was then heated at 86 °C until it becomes homogeneous before adding galactaric acid (40 mg, 0.19 mmol). Afterward, the flask was heated at 140 °C for 20 hours. The reaction mixture was cooled at R.T. and analysed by ^{1}H-NMR. A 52% yield of the dilactone pyrrole derivative (**36**) was detected along with some galactaro-1,4-lactone. The analytical data for the isolated dilactone **36** was identical to the one reported in the previous paragraph.

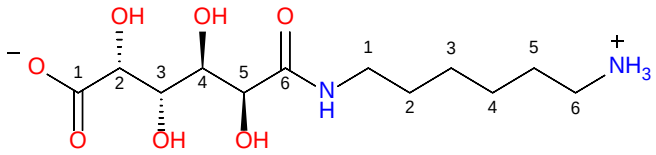
36. Reactivity of galactaro-1,4-lactone with hexamethylenediamine at 25 °C



Procedure:

Hexamethylenediamine (0.26 ml, 1.83 mmol) was mixed together with the DMSO solution of galactaro-1,4-lactone (1 ml, containing 350 mg of lactone) in a round bottom flask equipped with magnetic stirrer. The molar ratio between the amine and galactaro-1,4-lactone was strictly controlled to 1:1. The amidation and salification reaction was carried out at room temperature. After 2 hours the reaction mixture became a homogeneous jelly-like viscous liquid. A sample, analysed by ^{1}H-NMR, confirms the presence of the switterionic compound (**39**). The reaction mixture was left again at RT for 12 hours. Then, some solid was precipitated from the mixture, which became a hard wax-like solid. The mono amide galactarate salts was purified by recrystallization using acetone or the co-solvent DMSO: Acetone/Acetonitrile in ratio 1:3, and then by further filtration and drying. The isolated solid was found to be more soluble in water than in DMSO.

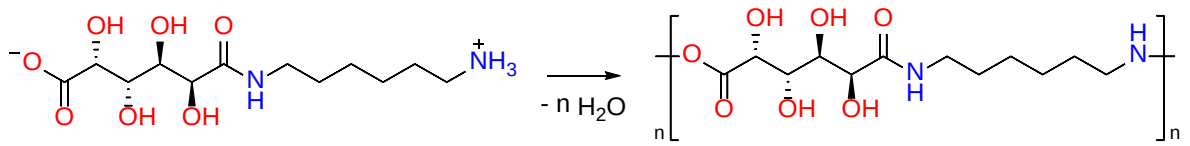
Analytical data for [(2R,3S,4R,5S)-6-((6-ammoniohexyl)amino)-2,3,4,5-tetrahydroxy-6-oxohexanoate] (39)



39

MW: 308.33 g/mol ($C_{12}H_{24}N_2O_7$); **Melting Point**: > 270 °C; **^{1}H-NMR** (400 MHz, DMSO-d6, ppm): δ 7.52 (t, 1H, NH-CO), 4.10 (s, 1H, C**H**-OH), 3.72 (dd, 1H, –C**H**-OH), 3.65 (s, 1H, –C**H**-OH), 3.55 (m, 1H, -C**H**–OH), 3.05 (m, 2H, C**H$_2$**-) 2.67 (m, 2H, C**H$_2$**-), 1.51 (m, 2H, C**H$_2$**-), 1.49 (m, 2H, C**H$_2$**-), 1.48 (m, 4H, C**H$_2$**-).**^{13}C-NMR** (100 MHz, D_2O, ppm): δ 179.2 (C=O), 175.3 (C=O), 71.3 (CH-OH), 70.9 (CH-OH), 28.2, 28.1, 27.7, 27.1, 25.6, 25.2; **FT-IR** (KBr, ν_{max}, cm^{-1}**):** 3391, 3314, 2932, 2856, 2107, 1646, 1583, 1541, 1466, 1401, 1371, 1313, 1209, 1108, 1039, 955, 876, 827, 729, 655, 541, 477; **-MS**((-)-ESI, m/z %): 307 [M-H], 615 [2M-H]; **+MS**((+)+ESI, m/z %): 309 [M+H], 331 [M+Na], 617 [2M+H], 639 [2M+Na].

37. Convenient procedures for hydroxylated nylons (polyhydroxypolygalactaramides) preparation from D-galactaro-1,4-lactone



The switterionic compound **39** was heated at 120-140 °C under neat conditions or in DMSO to ensure the polycondensation process to give the corresponding polyamide (a polyhydroxypolyamide (PHPA)). Higher temperatures were not investigated owing the possible involvement of esterification processes and, therefore, cross linking. The resulting diamine copolymer was crystalline and water insoluble.

For the characterization of the polyhydroxypolyamide was followed the method suggested by Kiely (Kiely, E. D.; Vishwanathan, A.; Jarman, B. P.; Manley-Harris M., Journal of Carbohydrate Chemistry, 28:6, 348-368)[12] which uses ^{1}H-NMR analysis. The number average molecular weight (M_n) and the degree of polymerization (DP) was determined by end-group analysis by calculating the ratio of the integrals for methylene protons adjacent to terminal amine groups with those for methylene protons adjacent to internal amide nitrogens and applying the formula DP = (Ratio + 1)/2. The mean value obtained for M_n and DP for the sample tested were in the range 1700-3000 and 7-10, respectively.

General procedure for the aminolysis kinetic of 19 and/or 20 by ^{1}H- and ^{13}C-NMR monitoring.

The aminolysis of **19** or **20** was typically carried out and monitored in a ^{1}H- or ^{13}C-NMR tube. Typically, a spectrum of the starting lactone (0.44 M) in 1 mL of DMSO-d6 was obtained at 31°C. The sample tube was then removed from the instrument and the appropriate 2 molar amount of the amine was pipetted into the tube which was then vigorously shaken by hand. The NMR tube was then inserted into the instrument and spectra acquired at specific time intervals. The scan rate for both ^{1}H or ^{13}C-NMR spectra was 16 scans/min, with ca. 1 min processing at designated time points. Aminolysis of a solution of **19** and **20** was carried out as above, but with each at a concentration of 0.22 M.

4.4 - References to Chapter 4

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Chapter 5

Conclusions

Overall this work represents a thorough investigation into some of the aspects of the chemistry of D-galactaric acid and D-galactaro-1,4-lactone in solution of DMSO and other solvents. The main goal was to develop new and old pathways to apply aldaric acid as new platform chemicals potentially derived from biorefinery biomass.

Firstly, we have demonstrated that galactaro-1,4-lactone (**18**) is efficiently prepared by a simple method in quantitative yields, opening the possibility to become a potential platform molecule. Inorganic and organic salts of galactaro-1,4-lactone are easily prepared and isolated under mild conditions from this lactone overcoming the issue of low solubility of galactaric acid in most solvents. Salts of galactaramides and galactarodiamides can be synthesized in high yields by treatment of galactaro-1,4-lactone with primary amines. High potential comes from ammonium galactarate zwitterionic salts as potential precursor for polycondensation processes to polygalactaramides of high molecular weight, thanks to its strict stoichiometry 1:1 between acid and amine. These results rationalize some literature data on reactions of other aldaric acids with bases, and were applicable to the production of poly(D-galactaramides). The extension of these conclusions need again to be extended to homo- and co-polyamides PHPA of other aldaric acids.

Galactaro-1,4-lactone can be selectively mono-, di- and tri-formylated in consecutive reactions and a mechanism involving mixed galactatic acetic or formic anhydride is proposed to explain the observed selectivity. Esterification or esterification/internal lactonization of the hydroxyl groups of galactaric acid proved to be essential for elimination processes, occurring under catalysis of both acids and bases. These reactions provide access to quite versatile mono-unsaturated and di-unsaturated derivatives of galacrtaric acid. The more relevant result is a new efficient method for the synthesis of 2-hydroxypyrone derivatives, whereas 2,5-dihydroxymuconic acid derivatives are proved to be relevant intermediates in these dehydration. Care must be exercised with pyrone carboxylic derivatives at high temperature (> 120°C) owing to decarboxylation of side chain carboxylic acid, especially in basic media. On the contrary, the 2-hydroxy pyrone nucleus is relative stable and it can preserved in several further derivatisations.

Under mild conditions the per-acylated galactaric acid derivates undergo selective monoelimination by a base to mono-unsaturated galactaro lactone. These derivatives are key intermediates for further elimination under acid catalysis to di-unsaturated derivatives 2,5-furandicarboxylic acid, an important renewable monomer per bio-derived plastics, potential substitute of terephthalic acid, via 2-ketohexaric acids. These last compounds and the 2,5-dihydroxymuconic acid derivatives proved to be key intermediated in the conversion of galactaric acid and its 1,4-lactone in the presence of primary amines to pyrrolecarboxylic acid derivatives. As for pyrrole derivatives, N-alkylpyrrolecarboxylic acids and their salts decarboxylate at high temperature, providing access to unsubstituted pyrroles. The study has clarified the mechanistic details involved in the formation of pyrroles from galactaric acid and amines

and the synthetic potentiality of these reactions to access new monomers for polymers (i.e. serinolpyrrole and substituted 2,5-pyrroldicarboxylic acids).

Moreover, the study has proved the potentiality of the reduction of pyrone derivative **7** to access 2,5-dihydroxyadipic acid and its derivatives (including its intra molecular dilactone). This difunctional nature of these derivatives makes them useful monomers for homo- and co-polymers. Preference in the reduction for the *meso*-form of the 2,5-dihydroxyadipic acid was unpredicted.

Further work that is possible includes a more general investigation of the chemistry of D-galactaric acid and D-galactaro-1,4-lactone as starting species with sulfur and phosphorous species. A specific attention need to be placed on the analogies between chemical and biochemical behavior of the intermediates found to better characterize the catalytic steps and how can be improved. This aspect is relevant in the context to recover galactaric acid and its derivatives form selected biomass.

In the future, special attention will be devoted to the polymerization of the different monomers derived from galactaric acid in the area of polyamides, polyesters, polyamides and polyurethanes as an extension of the preliminary investigations carried out in this thesis. Furthermore, kinetic data and separation technologies will be identified in order to transfer the more promising lab reactions to a potential industrial process with a preliminary cost evaluation and green metrics. Special attention will be devoted to adjust the process condition in order to satisfy the requirement of industrial scale production in term of equipment, post-reaction operations and safety.