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The first synthesis of glycosylflavanones catalyzed by praseodymium triflate: a straightforward approach to potential antidiabetic agents

RUI MIGUEL GALHANO DOS SANTOS LOPES

DOUTORAMENTO EM QUÍMICA

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TESE ORIENTADA

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To my grandmother,

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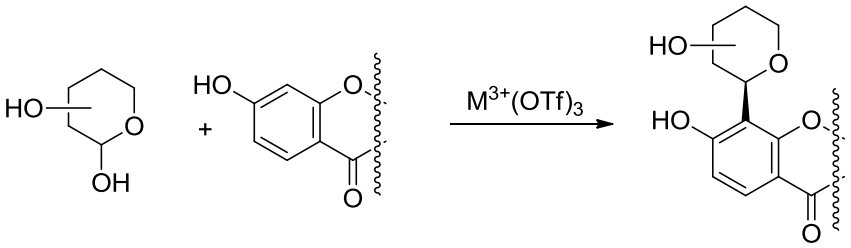
Abstract

The work developed in this thesis was focused on the development of simple and direct strategies aiming at the synthesis of glycosylflavanones by Fries-type rearrangement promoted by Lewis acids.

The total synthesis of this type of compounds involves usually multi-step approaches that are time consuming as well as very expensive. Hence we envisaged to outline a methodology for obtaining those molecules in a fast, cheap and easy way (scheme i).



Scheme i - General reaction for the coupling of sugars to flavonoids *via* Fries-type rearrangement.

Glycosylation of (±)-naringenin, as flavanone template, with D-glucose catalyzed by scandium triflate in acetonitrile:water was conducted leading to the desired glycosylflavanone in low yield.

Reaction conditions such as solvent mixture, reaction time, energy source and also the catalyst were studied aiming at the increase of the reaction yield. Lanthanide triflates, which were commercially available, were evaluated as catalysts and praseodymium triflato proved to be the most suitable one for this coupling reaction. Other glycosyl donors such as D-mannose, D-galactose and L-rhamnose led to the corresponding glycosylnaringenin in reasonable good yield. The structure of the compounds obtained was confirmed by ^{1}H and ^{13}C NMR experiments including two-dimensional experiments COSY, HMQC and HMBC. Disaccharides were also investigated as glycosyl donors. Lactose and maltose were successfully coupled to naringenin being subjected to NMR experiments for their structure characterization. Ultrasounds were used to enhance the outcome of the coupling reaction leading to higher yields and a significant decrease in reaction time. This work resulted in a simple, direct and clean method for coupling sugars to naringenin.

Preliminary toxicity evaluation of the synthesized compounds showed a moderate level of cytotoxic effects, significantly different from the positive control (hydrogen peroxide). The preliminary genotoxicity results showed a low genetic injury potential for the compounds tested, within the blank range. These results encourage pursuing further studies of these compounds as biological tools or lead compounds for drug discovery.

Resumo

O trabalho levado a cabo durante este doutoramento teve como objetivo o desenvolvimento de estratégias de síntese simples e eficazes para a preparação de glicosilflavonóides, recorrendo a reações de rearranjo de tipo Fries catalisadas por triflatos de lantanídeos em meio aquoso. A investigação direcionada para estes compostos surgiu após o estudo aprofundado do perfil fitoquímico de uma planta endémica da Ilha da Madeira, *Genista tenera*, utilizada pela população para o combate à diabetes. Esta investigação, liderada pela Professora Doutora Amélia Pilar Rauter, conduziu inicialmente à preparação de extratos e à avaliação da sua atividade antidiabética. Alguns destes extratos revelaram ser bastante promissores para o combate a *Diabetes mellitus*, apresentando uma atividade hipoglicémica e anti-hiperglicémica significativa. Os estudos dos componentes dos extratos da planta conduziram à identificação de um flavonóide glicosilado, nomeadamente a 8-(β-D-glucopiranosil)genisteína como composto maioritário do extrato com maior atividade antidiabética.

Os flavonóides estão amplamente disseminados na Natureza, maioritariamente no *Reino Plantae*, possuindo diversas atividades

biológicas. Estes compostos polifenólicos são identificados em extratos de plantas, em muitos dos casos com açúcares ligados por ligações covalentes à sua estrutura constituindo os glicósidos de flavonóide ou os flavonóides glicosilados. Nos primeiros, o açúcar está ligado ao flavonóide hidroxilado através de uma ligação acetálica, enquanto que no segundo grupo o açúcar está ligado, através da sua posição anomérica, diretamente ao esqueleto carbonado do flavonóide por meio de uma ligação C-C, geralmente, com o carbono C-6 ou C-8 da aglícona. Estes derivados apresentam propriedades biológicas interessantes, desempenhando a ligação C-C um papel de grande importância uma vez que não é hidrolisada enzimaticamente *in vivo* tornando-se mais estável em condições fisiológicas, ao contrário da ligação glicosidíca O-C que, fazendo parte de um acetal, é facilmente hidrolisada em meio ácido e por enzimas.

Estas moléculas têm sido exaustivamente estudadas nas últimas décadas demonstrando que as suas propriedades biológicas têm um enorme potencial como coadjuvantes na alimentação e saúde humana. As suas propriedades antioxidantes têm sido exploradas através da introdução deste tipo de compostos em alimentos funcionais. Sendo o recurso a estes produtos naturais limitado, pois estão disponíveis em pequenas quantidades através do seu isolamento a partir de plantas, estes compostos despertam nos

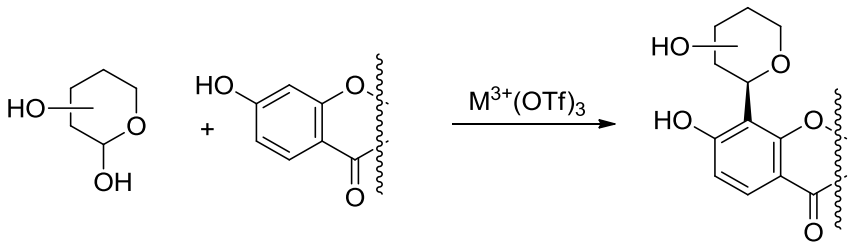
químicos orgânicos um grande interesse do ponto de vista sintético.

Inicialmente a formação da ligação C-C entre o açúcar e a estrutura polifenólica ativada eletronicamente foi conseguida através de uma reação de Friedel-Crafs. Hoje em dia estão à disposição uma grande variedade de métodos para a C-glicosilação, nomeadamente o ataque nucleófilo de reagentes de Grignard a haletos de glicosilo, de aniões anoméricos *via* compostos litiados, reações mediadas por metais de transição ou iodeto de samário, reações radicalares intermoleculares, entre outros. Uma das últimas abordagens foi desenvolvida pelos grupos de investigação de Suzuki e Kometani. Esta estratégia consiste numa metodologia para a C-glicosilação de fenóis catalisada por ácidos de Lewis através de um rearranjo de *O*-glicósido para *C*-glicósilderivados denominada rearranjo de tipo Fries. Este método tem vindo a ser amplamente estudado e aplicado, apresentando grande sucesso para a obtenção de glicosilflavonóides, bem como de outros compostos complexos glicosilados de origem natural. Este procedimento envolve numa primeira fase a formação de um *O*-glicósido que por sua vez sofre um rearranjo que conduz a formação do derivado *C*-glicosilado pretendido. Há ainda um passo final que consiste no deslocamento

do equilíbrio reacional que leva à formação do anómero mais estável, pelo que esta reação é regio- e estereosselectiva.

Como foi referido anteriormente, a síntese total de flavonóides glicosilados envolve, geralmente, estratégias sintéticas com muitos passos reacionais consumindo assim muito tempo e subsequentemente sendo muito dispendiosos. *Assim, porquê não apostar em estratégias simples e diretas para este tipo de compostos?*

O primeiro objetivo consistiu em otimizar a metodologia que envolve a reação de tipo rearranjo de Fries promovido por triflatos de lantanídeo como ferramenta para o acoplamento direto de açúcares a flavonóides, resultando assim numa metodologia simples e rápida para alcançar os compostos pretendidos (esquema i).



Esquema i – Reação geral para o acoplamento de açúcares a flavonóides via rearranjo do tipo Fries.

Como modelo de flavonóides foi usada a (±)-naringenina, estando este polifenol disponível nos fornecedores de produtos químicos a

um preço acessível. Este método revelou-se potencialmente bom quando se recorreu à D-glucose como dador de glicosilo, necessitando de ser otimizado uma vez que apresentou rendimentos baixos. Esta metodologia foi então otimizada alterando as condições reacionais nomeadamente a mistura de solventes, o tempo de reação, a fonte de energia e também o catalisador. Para estender esta metodologia a outras entidades moleculares foram testados outros modelos de açúcares e fenóis, pretendendo-se estabelecer um protocolo genérico para o acoplamento de açúcares desprotegidos a flavonóides, podendo, posteriormente, ser avaliada a sua bioactividade.

A elucidação estrutural do composto, obtido pela reação da D-glucose com (±)-naringenina catalisada por triflato de escândio em meio aquoso, foi levada a cabo por experiências de RMN de protão e carbono e bidimensionais COSY, HMQC e HMBC.

Uma gama de triflatos de lantanídeo, disponíveis comercialmente, que atuam como ácidos de Lewis, foram avaliados como o intuito de aferir qual deles seria o mais indicado para este tipo de reações, revelando-se o triflato de praseodímio, utilizado pela primeira vez neste tipo de reações, como o mais eficiente, uma vez que a reação por ele catalisada conduziu a um rendimento mais elevado que os demais testados.

Após a otimização do sistema, o acoplamento de outros açúcares prosseguiu com a D-frutose e a L-sorbose. Contudo com estes modelos a reação revelou-se ineficaz pois nenhum produto foi obtido, o que se deve ao facto de ambos os açúcares terem na sua posição anomérica um grupo hidroximetilo, dificultando assim a aproximação do flavonóide ao ião oxónio intermediário.

D-Manose, L-ramnose e D-galactose foram também utilizados levando à obtenção das respectivas flavanonas glicosiladas, que foram isoladas com um rendimento aceitável. Os dissacáridos, lactose e maltose foram ainda testados, e os compostos pretendidos obtidos com sucesso. A estrutura dos compostos obtidos foi caracterizada por ressonância magnética nuclear.

Foram ainda testados outros flavonóides tais como flavonas, isoflavonas e flavonóis. Genisteína, quercetina, 5,7-di-hidroxiflavona e 6-hidroxiflavona foram estudadas como aceitadores de glicosilo, contudo estas moléculas demonstraram ser inativas nas condições reacionais testadas, pois além da sua fraca solubilidade estão desativadas eletronicamente para a substituição electrófila no anel A do flavonóide.

A investigação com o objetivo de acelerar as reações orgânicas recorre frequentemente à sonoquímica, ou seja ao uso de ultra-sons. Através de um fenómeno denominado cavitação, a

velocidade da reação poderá ser aumentada levando assim a uma diminuição do tempo de reação bem como ao aumento do rendimento da mesma face ao método comum de agitação e aquecimento. Assim a aplicação de ultrasons à metodologia utilizada na síntese dos derivados glicosilados conduziu a um decréscimo substancial do tempo de reação bem com a um aumento significativo do rendimento da reação.

Uma avaliação das propriedades biológicas dos compostos obtidos, uma vez que possuem estruturas similares às de princípios ativos, é ambicionada como forma de valorizar o trabalho elaborado. Foram conduzidos estudos preliminares da toxicidade em linhas celulares, recorrendo à avaliação da citotoxicidade e genotoxicidade. Os compostos revelaram ser pouco cito- e genotóxicos quando comparados com os controlos positivos.

O trabalho desenvolvido nesta dissertação representa uma primeira fase de uma linha de investigação levada a cabo no Grupo da Química de Glúcidos para o desenvolvimento de compostos antidiabéticos derivados de polifenóis. A abordagem desenvolvida apresenta enorme potencial para ser utilizada na obtenção direta de glicosilflavanonas, as quais por si só podem ter bioactividades promissoras ou servir de intermediários químicos relevantes para grande diversidade de estruturas de flavonóides glicosilados, de

difícil acesso através das metodologias correntemente descritas na literatura.

Keywords

Glycosylflavanones

Synthesis

Fries-type rearrangement

Lanthanide triflatos

Carbohydrates

Polyphenols

Palavras-chave

Glicosilflavanonas

Síntese

Rearranjo de tipo Fries

Triflatos de Lantanídeo

Carbo-hidratos

Flavonóides

Abbreviations

^{13}C-NMR	Carbon Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
2D-NMR	Two Dimensional Nuclear Magnetic Resonance
ADA	American Diabetes Association
AE	adverse event
brd	broad doublet
brt	broad triplet
cat	catalyst
CC	column chromatography
COSY	Correlation Spectroscopy
CVD	cardiovascular disease
d	doublet
dd	Double doublet
ddd	doublet of double doublet
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DM	*Diabetes mellitus*
DMSO	dimethylsulfoxide
DPP-IV	dipeptidyl peptidase-IV

DTBP	2,6-di-*tert*-butylpyridine
eq	equivalent
EtOH	ethanol
FCT	Fundação para a Ciência e a Tecnologia
GLP-1	glucagon-like peptide-1
h	hours
HbA1c	glycated haemoglobin
HMBC	Heteronuclear Multiple-Bond Correlation
HMQC	Heteronuclear Multiple-Quantum Correlation
HPLC	High Performance Liquid Chromatography
IC_{50}	Inhibitory Concentration (50%)
IDF	International Diabetes Federation
Int.	Integration
J	Coupling constant
kHz	kiloHertz
m	multiplet
MeCN	acetonitrile
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Mult.	Multiplicity
n.h.	no hydrolysis

NMR	Nuclear Magnetic Resonance
nr	no reaction
OHAs	oral hypoglycemic agents
Ph.D.	Doctor of Philosophy
ppm	parts per million
pyr	pyridine
Rf	retention factor
RSD	Relative Standard Deviation
s	singlet
SU	sulphonylurea
t	triplet
T1DM	type 1 *Diabetes mellitus*
T2DM	type 2 *Diabetes mellitus*
td	triplet of doublets
temp	temperature
THF	tetrahydrofuran
TLC	thin layer chromatography
TTN	Thallium trinitrate
TZDs	thiazolidinediones
UKPDS	United Kingdom Prospective Diabetes Study

UV	ultraviolet
UV-Vis	ultraviolet-visible
W	watts
δ	chemical shift

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Introduction

This Ph.D. thesis was based on the outlining and optimization of simple methodologies employing Fries-type rearrangement catalyzed by rare-earth triflates in aqueous medium as a tool for the synthesis of glycosylflavonoids. These compounds are known for their multi-bioactivities including antidiabetic properties and studies have been conducted exploring their potential application for the treatment of diabetes. The hypoglycemic effect of flavonoids using different experimental models and treatments have been proven. Some of those compounds have shown to exert beneficial effects against this disease, throughout their capacity to avoid glucose absorption or towards the improvement of glucose tolerance. As a result, bio-flavonoids are currently referred as promising and significantly attractive natural substances to enrich the current therapeutic agents against *Diabetes mellitus* [1]. Compounds of this type are found as major components of a plant endemic to Madeira Island, *Genista tenera*, which is used by the local population for the treatment of diabetes. Research on the plant active principles and development of new methodologies to access these molecular entities and analogues has been conducted under the supervision of Prof. Amélia P. Rauter and funded by a

FCT project (PTDC/QUI/67165/2006). Preliminary biological evaluation of the plant extracts and their commercially available flavonoid aglycones and glycosides showed some attractive biological activities, in particular the hypo- and antihyperglycemic effects. To access components identified in the plant and their analogues that can not be purchased, some novel, simple and efficient approaches are needed, enabling to proceed with their biological evaluation and to establish new and promising antidiabetic compounds.

The present introduction is organized in subchapters containing, two of them, reviews published within the scope of this subject. Further relevant information/data not included in these surveys concerning *Diabetes mellitus* prevalence in the world and the pharmacotherapy associated with the disease will also be presented as well as an overall view of the previous work related to *Genista tenera* chemical composition and bioactivity.

The general survey on *C*-Glycosylflavonoids, published in Natural Product Communications, illustrates the relevance of these compounds for human health benefits. The other review, recently published in Current Organic Chemistry, focus on Fries-type reactions, which are exploited in this Ph.D.. Both reviews were developed and accomplished by the author of this thesis, being the role of other co-authors, besides the supervisors of this thesis, explained at the beginning of each subchapter.

The results and discussion chapter includes the studies conducted regarding the efficacy of a variety of lanthanide triflates for the direct coupling of D-glucose, D-galactose, D-manose, and L-rhamnose to naringenin, as well as that of dissacharides, namely maltose and lactose. Optimization of the reaction conditions will be also discussed ending up with a new environmentally friendly method to easily access glycosylflavanones. Preliminary toxicity was screened to facilitate the selection of the most promising non-toxic agents for further biological tests.

In Conclusion and Perspectives, the last chapter of this thesis, emphasis is given on the efficacy of the synthetic methodology developed and on future perspectives from either chemical or biological point of view.

1.1 *Diabetes mellitus*

The American Diabetes Association Expert Committee considers *Diabetes mellitus* (DM) a group of metabolic diseases described by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycemia can lead to long-term damage, dysfunction and failure in kidney, nerves, heart, blood vessels and especially the eyes [2-4]. Patients with DM are divided

in two groups, those who suffer from type 1 DM (T1DM) or from type 2 DM (T2DM), being the former the less common one.

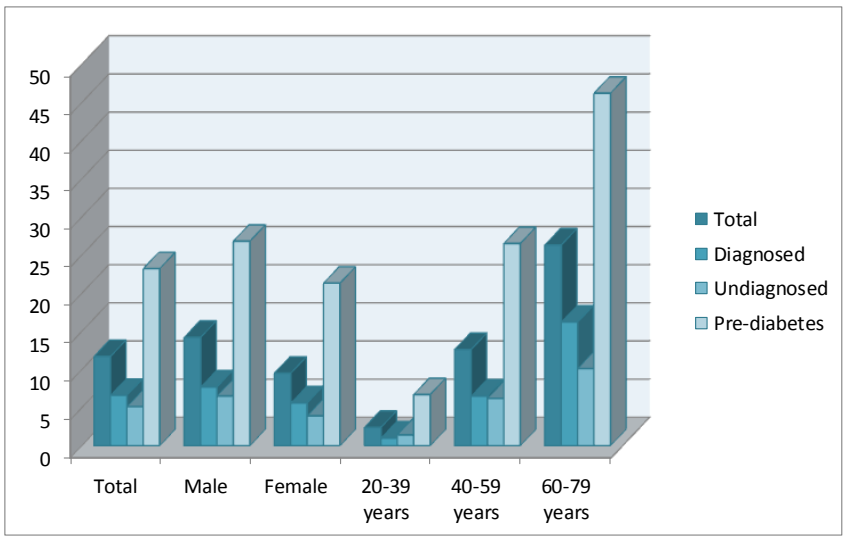
The main cause for T1DM is the absolute insulin deficiency overcoming from autoimmune destruction of pancreatic β-cells. On the other hand T2DM is a heterogeneous metabolic disorder, characterized by a relative insulin deficiency resulting from a reduced sensitivity of tissues to insulin and impairment of insulin secretion from pancreatic β-cells[4-5].

1.1.1 Prevalence across the world

The approximate population incidence of T1DM is 25/100000 being the highest incidence observed at the age of 13 to 15. However, some significant differences were found in China and Japan with 0.4 and 1.6/100000, respectively, while in Finland the figures arise to 40/100000 [4]. Unfortunately for those who suffer from this disease the substitution of the missing insulin is presently the only therapeutic option available. Concerning the T2DM, this group represents more than 80% of the DM cases, and the number is increasing every day due to nutritional disorders related to obesity [4].

Portuguese prevalence, in 1995, was estimated in about 5.1% of the population. However, in 2010 a study revealed new numbers.

This survey was divided in total prevalence, diagnosed vs. undiagnosed, male vs. female and into age classes. The results are depicted in graph 1. The total prevalence of the illness is now 11.7% showing an increase for more than the double in just 15 years. At younger age (20-39 years) 2.4% of participants in this study had diabetes, making type 2 diabetes a real concern nowadays. This investigation also indicated that 34.9% of the population aging from 20 to 79 years were diabetic or "pre-diabetic", which represents one-third of the adult population [6].



Graph 1 - Distribution of the prevalence of *Diabetes mellitus* within the Portuguese population

1.1.2. Glycemic control

Being one of the primary causes of increased cardiovascular morbidity and mortality in Western countries, DM constitutes a large burden to health care systems in terms of both direct and indirect costs. A good glycemic control plays a crucial role in the prevention of chronic microvascular and probably also of macrovascular complications. The United Kingdom Prospective Diabetes Study (UKPDS) stated that 1% decrease in glycated haemoglobin (HbA1c) was associated with a risk reduction of 37% for microvascular disease and 14% for myocardial infarction [7]. Therefore, efficient glucose control is essential to the prevention of life-threatening complications of the disease. Although most patients due to life-style modifications fail to sustain long-term and adequate control of glucose levels and even combined oral medications (biguanides, sulfonylureas, meglitinides, glitazones, incretin mimetics, or DPP- 4 inhibitors) can become ineffective. In fact, multidrug therapies are being re-evaluated, with recent studies questioning their long-term therapeutic benefit over side effects or disease complications [8].

Many organizations and associations around the globe such American Diabetes Association (ADA), the European Association for the Study of Diabetes and the International Diabetes Federation (IDF) recommend strict control of glycemia.

Nevertheless the long-term achieving and sustaining such glycemic control is highly challenging and leads to side effects, particularly hypoglycaemia [7].

1.1.3. Pharmacotherapy in Diabetes mellitus

T2DM patients are likely to be overweight or obese and are unable to achieve or sustain a near normoglycemia without oral medication. Physicians are offered with a wide range of oral antidiabetic drugs for the treatment of T2DM. The main classes are heterogeneous in their modes of action, safety profiles and tolerability [9]. Concerning the pharmacotherapy for T2DM, sulphonylureas (SUs) and metformin are the current most used oral hypoglycemic agents (OHAs). Metformin and glibenclimide (sulphonylurea) are the only OHAs included on the World Health Organization list of essential medicines. Over the last years, several new OHAs have become available, some of them resulting from modifications of former drugs while others belong to new classes of antidiabetic agents [7].

Currently, the approved medicines for T2DM comprise SUs, metformin, thiazolidinediones (TZDs), non-SU secretagogues, α-glucosidase inhibitors, glucagon-like peptide-1 (GLP-1) agonists, amylin analogues and dipeptidyl peptidase-IV (DPP-IV) inhibitors.

With the increasing prevalence of T2DM, there will be in the future an increased exposure to OHAs leading to an increase in the frequency of associated adverse events (AEs). Side effects, such as renal/hepatic impairment or cardiovascular disease (CVD) may intensify the adverse events of diabetic drugs, in particular, in those patients with long-standing T2DM. For traditional drugs such as metformin and SUs most AEs are predictable and well known, while recent agents can be associated with unforeseen side effects. Long-term clinical exposure is missing for many new agents being the risk-benefit data limited for agents such as GLP-1 analogues and DPP-IV inhibitors. The development and availability of new antidiabetic agents are welcome, nevertheless long-term outcome studies are required to deduce the safety of such compounds, until such data become available it is difficult to evaluate risk-benefit profile of new agents in comparison to traditional drugs. Although their efficacy decreases over time metformin and SUs remain a relatively safe and effective treatment option for T2DM.

In the future, due to the population ageing and the global increased incidence of obesity, the world healthcare systems will not be able to deal with the increased incidence of chronic diseases such as diabetes, cancer and vascular disorders [10]. A novel strategy to be considered is the intake of plant biophenols in order to prevent or reduce such chronic diseases. Flavonoids are

widely present in the human diet [11] and modern medicine values their therapeutic potential to treat many common diseases [12-13]. A wide variety of mechanisms can explain the antidiabetic properties of flavonoids [12, 14], however their antioxidant capacity plays an important role in the prevention of hyperglycemia and related complications [15]. Flavonoids possess antioxidant activity and pancreatic cells are sensitive to oxidative stress [16]. Hence these compounds may have the ability to prevent the progressive impairment of pancreatic β-cells function [17-18], regenerating the damaged pancreatic cells or stimulating the secretion of insulin by β–cells of the pancreas [19], thus reducing the occurrence of diabetes.

Despite the advances in the quality of life improvement of those who suffer from this disease, new drugs are needed for those who existing drugs stopped working or have no effect.

1.2 *Genista tenera*

Genista tenera is plant endemic to Madeira Island, Portugal. This plant was used in folk medicine as an adjuvant to diabetes treatment. The phytochemical profile of the plant was studied by Dr. Alice Martins (post-doctoral member of the Carbohydrate Chemistry Group) under the supervision of Prof. Amélia P. Rauter.

Over a former ethanolic extract, subsequent extractions were performed and bioactivity profile of each extract was then studied [20-22]. Concerning the bioactivity of the ethyl acetate extract, treatment of diabetic induced streptozotocin rats with this extract revealed a significant hypoglycemic and antihyperglycemic effect. Moreover, it was noticed that this extract did not lower the normal levels of glucose in healthy rats, and an increase in body weight was registered [23-24]. After identification of the extract constituents, the bioactivities of the flavonoid standards, commercially available, were also evaluated. They revealed a protective effect of those compounds over the liver, kidney and pancreas lesions caused by the administration of streptozotocin, and prevention of the vascular diseases was additionally observed [22-23, 25] as well as a significant hypo- and antihyperglycemic effect but not as good as the extract itself. The main constituent of this extract, 8-β-D-glucopyranosylgenistein, is not commercially available and access to this compound and study of its antidiabetic activity is mandatory. A strategy was envisaged by us to prepare it in March 2006 in this Ph.D. program. However Sato et al, in August 2006, described the first synthesis of this compound using a similar approach to that we had proposed. Hence, new synthetic approaches are needed to obtain this type of compounds and analogues for biological evaluation and structure/bioactivity relationship studies. We have focused our attention on flavanones, some of which, when glycosylated, are known for their

antidiabetic activity. In addition they can be valuable precursors for the synthesis of 8-β-D-glucopyranosylgenistein and analogues. The outlined synthetic methodology for flavanones *C*-glycosylation adopted for this work will be further presented and discussed.

1.3. *C-Glycosylflavonoids: Identification, Bioactivity and Synthesis*

The next subchapter was published as a review on Natural Products Communication as:

"*C*-glycosylflavonoids: Identification, bioactivity and synthesis" Rauter, A.P.; Lopes, R. G.; Martins, A. *Nat. Prod. Commun*., **2007**, 2, 1175-1196.

This paper gives an overview on the identification, bioactivity and synthesis of the *C*-glycosylflavonoids. Dr Alice Martins as co-author has played an important role in helping on the gathering and discussing the information regarding biological activities. At the end of this subchapter an update on relevant new molecules and bioactivities will be given, concerning research papers published recently, since publication of this review in 2007.

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C-Glycosylflavonoids: Identification, Bioactivity and Synthesis

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This paper is dedicated to Professor Tom J. Mabry for his 75th birthday.

C-Glycosylflavonoids are a group of compounds widespread in nature which have gained much interest due to their biological properties. This review focuses on the research of the last ten years concerning detection and structural characterization of *C*-glycosylflavonoids, their bioactivities and synthesis.

Keywords: *C*-Glycosylflavonoids, identification, bioactivity, synthesis.

Flavonoids occur widely in nature and usually possess a chromene-type skeleton with a phenyl group in either position C-2 or C-3 (Figure 1). They are also present as *O*-glycosides and, less frequently, as *C*-glycosylflavonoids, in which the bond is established between the anomeric carbon of the sugar and a carbon of the flavonoid, usually C-6 or C-8. *C*-glycosylflavonoids are not rare, more than 300 being known by 1999 [1]. Flavonoids have been extensively studied during the last decades [2-6] and their bioactivities make them promising compounds for human nutrition and health. The limited natural sources and the unique structural features of *C*-glycosylflavonoids have motivated organic chemists to develop synthetic approaches leading to such bioactive molecules. Thus, we present herein the latest findings on *C*-glycosylflavonoids, including their synthesis. This review summarizes the research on bioactive compounds of this type, over the last ten years.

C-glycosylflavonoids can be classified as mono-*C*-glycosylflavonoids, di-*C*-glycosylflavonoids, *C*-glycosyl-*O*-glycosylflavonoids and acyl-*C*-glycosylflavonoids [1], examples of which and their natural occurrence are presented in Table 1.

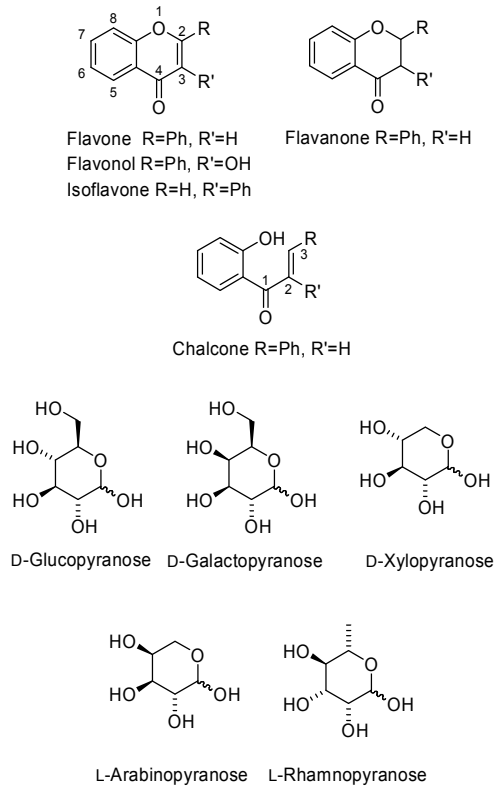


Figure 1: Flavonoid-type skeletons and the most common sugars in *C*-glycosylflavonoids.

The IUPAC names of flavonoids are derived from "flavan" or "isoflavan" in the present review, but common names are used for *C*-glycosylflavonoids to avoid long and unwieldy names. The use of "*C*-glycosylflavonoid" instead of "flavonoid *C*-glycoside" is based on the IUPAC recommendations to name these compounds using the appropriate 'glycosyl-' prefixes and avoiding '*C*-glycoside' terminology [7]. IUPAC and common names, as well as the substitution pattern of flavonoids, are given in Table 2. In order to facilitate understanding of *C*-glycosylflavonoid nomenclature, the structures of these compounds are shown in Table 3, with the numbering system used and two typical examples (structures of compounds **36** and **37**) depicted in Figure 2.

The most common flavonoids to occur as *C*-glycosylcompounds are flavones, although isoflavones, flavonols, flavanones and chalcones are also known (Figure 1, Tables 1-3). The range of sugars involved includes D-glucose, by far the most common one, although D-galactose, L-rhamnose, D-xylose and L-arabinose are also common (Figure 1). In addition, some unusual sugar residues are encountered, namely the deoxy sugar moieties in compounds **1**, **20**, **30** and **33**. Recently a complex *C*-glycosylflavone possessing an anthocyanin moiety in its structure was reported (compound **70**, Figure 2), which was isolated from the leaves of *Oxalis triangularis* [8]. *C*-Glycosylflavones also act as natural copigments of anthocyanins, for example the blue color of the marguerite daisy (*Fellicia amelloides*) petals has been found to arise from copigmentation between an anthocyanin and compound **65** [9].

An important feature of the distribution of flavonoids in plants is the strong tendency for taxonomically related plants to produce similar types of flavonoids [3]. The presence of *C*-glycosylflavonoids in some genera and plant species can be of chemotaxonomic significance. For example, 2''-*O*-glucopyranosyl-vitexin (**59**) was reported as a viable chemotaxonomic marker of the genus *Cryptocoryne* (Araceae) [10] and of *Podocarpus* spp. [11].

C-glycosylflavonoids were also identified in chemotaxonomic studies of the following genera: *Ocimum* [12], *Cotoneaster* [13], *Asplenium* [14] *Huberia* [15], *Asarum* [16], *Genista* [17], *Abrus* [18] and *Fortunella*, with the accumulation of 3',5'-di-*C*-β-D-glucopyranosylphloretin (**19**), being a generic trait of this genus [19]. However, in this review, emphasis will be given to other relevant aspects such as the updated analytical techniques for flavonoid identification, as well as bioactivities and synthesis.

Separation and identification

Separation and analysis of *C*-glycosylflavonoids from crude extracts continues to be performed by classical techniques such as mono- and bi-dimensional paper chromatography (1D- and 2D-PC) [12,14,15], analytical and preparative thin layer chromatography (TLC and PTLC), low and medium pressure column chromatography (CC) [21], as well as by paper electrophoresis (PE) [55], capillary zone electrophoresis (CZE) [24,29] and high-speed counter-current chromatography [30]. Recently, analytical separation and detection methods for flavonoids have been reviewed [58]. The identification of isolated compounds can be achieved by the combination of spectroscopic methods such as mass spectrometry (MS), nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis), infrared (IR) spectroscopy and by determination of physical data such as melting points and optical rotations as shown, for example, in references 18,30,33,50,51,54,59.

Table 1: Natural occurrence of *C*-glycosylflavonoids (covering data reported in the last ten years).

***C*-Glycosylflavonoid**	**Source**			**Ref.**
	Plant species	**Plant part**	**Plant family**	
Mono-6-*C*-glycosylflavonoids				
6-*C*-2'',6''-Dideoxy-β-L-*xylo*-hexopyranosylchrysoeriol (**1**)	*Zea mays*	Style	Poaceae	[20]
Isoorientin (6-*C*-β-D-glucopyranosylluteolin) (**2**)	*Sasa borealis*	Leaves	Poaceae	[21]
	Gentiana arisanensis	Whole plant	Gentianaceae	[22]
	Gentiana piasezkii	Whole plant	Gentianaceae	[23,24]
	Cymbopogon citratus	Aerial parts	Poaceae	[25]
	Gentiana olivieri	Aerial parts	Gentianaceae	[26]
	Viola yedoensis	Whole plant	Violaceae	[27]
	Alliaria petiolata	Seeds	Brassicaceae	[28]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
	Patrinia villosa	Whole plant	Valerianaceae	[30]

Table 1 (*continued*): Natural occurrence of *C*-glycosylflavonoids.

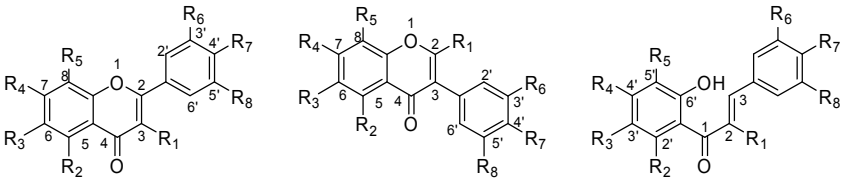
***C*-Glycosylflavonoid**	**Source**			**Ref.**
	Plant species	**Plant part**	**Plant family**	
Isoscoparin (6-*C*-β-D-glucopyranosylchrysoeriol) (**3**)	*Cymbopogon citratus*	Aerial parts	Poaceae	[25]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
Isovitexin (6-*C*-β-D-glucopyranosylapigenin) (**4**)	*Gentiana arisanensis*	Whole plant	Gentianaceae	[22]
	Patrinia villosa	Whole plant	Valerianaceae	[30]
	Ziziphus jujuba var.*spinosa*	Seeds	Rhamnaceae	[31]
	Xanthosoma violaceum	Leaves	Araceae	[32]
	Cladogynos orientalis	Aerial parts	Euphorbiaceae	[33]
Moghanin A (6-*C*-β-D-Glucopyranosylsyringetin) (**5**)	*Moghania macrophylla*	Roots	Leguminosae	[34]
Swertisin (Swertish) (7-*O*-Methyl-6-*C*-β-D-glucopyranosylapigenin) (**6**)	*Alliaria petiolata*	Seeds	Brassicaceae	[28]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
	Ziziphus jujuba var.*spinosa*	Seeds	Rhamnaceae	[31]
Swertiajaponin (7-*O*-Methyl-6-*C*-β-D-glucopyranosylluteolin) (**7**)	*Cymbopogon citratus*	Aerial parts	Poaceae	[25]
	Alliaria petiolata	Seeds	Brassicaceae	[28]
Mono-8-*C*-glycosylflavonoids				
8-*C*-β-D-Glucopyranosylorobol (**8**)	*Dalbergia monetaria*	Bark	Fabaceae	[35]
8-*C*-Glucopyranosyldiosmetin (**9**)	*Saccharum officinarum*	Leaves	Poaceae	[36]
8-*C*-Glucopyranosyl-4',5'-dimethylluteolin (**10**)	*Saccharum officinarum*	Leaves	Poaceae	[36]
8-*C*-β-D-Glucopyranosylgenistein (**11**)	*Genista tenera*	Aerial parts	Leguminosae	[37]
Orientin (8-*C*-β-D-Glucopyranosylluteolin) (**12**)	*Cymbopogon citratus*	Aerial parts	Poaceae	[25]
	Saccharum officinarum	Leaves	Poaceae	[36]
Puerarin (8-*C*-β-D-Glucopyranosyldaidzein) (**13**)	*Pueraria lobata*	Roots	Fabaceae	[39,40]
Vitexin (8-*C*-β-D-Glucopyranosylapigenin) (**14**)	*Passiflora incarnata*	Leaves	Passifloraceae	[29]
	Xanthosoma violaceum	Leaves	Araceae	[32]
	Saccharum officinarum	Leaves	Poaceae	[36]
	Luehea divaricata	Aerial parts	Tiliaceae	[38]
	Combretum quadrangulare	Leaves	Combretaceae	[41]
6,8-Di-*C*-glycosylflavonoids				
6-*C*-α-L-Arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin (**15**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
6-*C*-α-L-Arabinopyranosyl-8-*C*-β-D-xylopyranosylapigenin (**16**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
6,8-Di-*C*-α-L-arabinopyranosylapigenin (**17**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
6,8-Di-*C*-β-D-glucopyranosyldiosmetin (**18**)	*Citrus* spp.	Fruits	Rutaceae	[42]
3',5'-Di-*C*-β-D-glucopyranosylphloretin (**19**)	*Fortunella*	Fruits	Rutaceae	[19]
Diandraflavone (7-*O*-methyl-6-*C*-2'',6''-dideoxy-β-D-*lyxo*-hexopyranosyl-4'-*C*-β-D-glucopyranosylapigenin) (**20**)	*Drymaria diandra*	Whole plant	Caryophyllaceae	[43]
Carlinoside (6-*C*-β-D-Glucopyranosyl-8-*C*-α-L-arabinopyranosylluteolin) (**21**)	*Hordeum vulgare*	Leaves	Poaceae	[53]
6-*C*-β-D-Glucopyranosyl-8-*C*-β-D-apiofuranosylapigenin (**22**)	*Xanthosoma violaceum*	Leaves	Araceae	[32]
Isocarlinoside (6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylluteolin) (**23**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
Isoschaftoside (6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin) (**24**)	*Abrus mollis*	Aerial parts	Leguminosae	[18]
	Viola yedoensis	Whole plant	Violaceae	[27]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
	Saccharum officinarum	Leaves	Poaceae	[36]
	Camellia sinesis	Leaves	Theaceae	[44]
Lucenin-2 (6,8-di-*C*-β-D-glucopyranosylluteolin) (**25**)	*Passiflora incarnata*	Leaves	Passifloraceae	[29]
Neoschaftoside (6-*C*-β-D-glucopyranosyl-8-*C*-β-L-arabinopyranosylapigenin) (**26**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
Schaftoside (6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranosylapigenin) (**27**)	*Abrus mollis*	Aerial parts	Leguminosae	[18]
	Viola yedoensis	Whole plant	Violaceae	[27]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
	Saccharum officinarum	Leaves	Poaceae	[36]
	Piper methysticum	Leaves	Piperaceae	[45]
Vicenin-2 (6,8-di-*C*-β-D-glucopyranosylapigenin) (**28**)	*Ocimum americanum var. pilosum*	Leaves	Lamiaceae	[12]
	Asplenium normale	Fronds	Aspleniaceae	[14]
	Abrus mollis	Aerial parts	Leguminosae	[18]
	Viola yedoensis	Whole plant	Violaceae	[27]
	Passiflora incarnata	Leaves	Passifloraceae	[29]
	Xanthosoma violaceum	Leaves	Araceae	[32]
	Citrus spp.	Fruits	Rutaceae	[42]
	Phoenix dactylifera	Fruits	Arecaceae	[46]
	Salvia officinalis	Whole plant	Labiatae	[47]
	Glinus lotoides	Fruits	Molluginaceae	[48]
Violanthin (6-*C*-β-D-glucopyranosyl-8-C-α-L-rhamnopyranosylapigenin) (**29**)	*Viola arvensis*	Aerial parts	Violaceae	[49]
Violarvensin (6-*C*-β-D-glucopyranosyl-8-*C*-β-D-6-deoxygulopyranosylapigenin) (**30**)	*Viola arvensis*	Aerial parts	Violaceae	[49]

Table 1 (*continued*): Natural occurrence of *C*-glycosylflavonoids.

***C*-Glycosylflavonoid**	**Source**			**Ref.**
	Plant species	**Plant part**	**Plant family**	
6-*C*-β-D-Xylopyranosyl-8-*C*-β-D-glucopyranosylapigenin (**31**)	*Sasa borealis*	Leaves	Poaceae	[21]
6-*C*-β-D-Xylopyranosyl-8-*C*-α-L-arabinopyranosylapigenin (**32**)	*Viola yedoensis*	Whole plant	Violaceae	[27]
***C*-Glycosyl-*O*-glycosylflavonoids**				
6-*C*-2'',6''-Dideoxy-β-L-*xylo*-hexopyranosyl-7-*O*-β-D-glucopyranosylchrysoeriol (**33**)	*Zea mays*	Style	Poaceae	[20]
Lutonarin (6-*C*-β-D-glucopyranosyl-7-*O*- β-D-glucopyranosylluteolin) (**34**)	*Gentiana piasezkii*	Whole plant	Gentianaceae	[23,24]
Saponarin (7-*O*-β-D-glucopyranosylisovitexin) (**35**)	*Gentiana piasezkii*	Whole plant	Gentianaceae	[23]
Acyl-*C*-glycosylflavonoids				
4´-*O*-Acetyl-8-*C*-2''-*O*-(β-D-glucopyranosyl)-β-D-xylopyranosylchrysoeriol (**36**)	*Scleranthus uncinatus*	Whole plant	Caryophyllaceae	[52]
6'''-Coumaroylsaponarin {7-*O*-β-D-[6'''-*O*-(*E*)-*p*-coumaroyl]glucopyranosylisovitexin} (**37**)	*Hordeum vulgare*	Leaves	Poaceae	[53]
2''-*O*-(3''',4'''-Dimethoxy)benzoylorientin (**38**)	*Trollius ledebouri*	Flowers	Ranunculaceae	[54]
2''-*O*-(3''',4'''-Dimethoxy)benzoylvitexin (**39**)	*Trollius ledebouri*	Flowers	Ranunculaceae	[54]
6'''-Feruloylisospinosin (**40**)	*Ziziphus jujuba* var.*spinosa*	Seeds	Rhamnaceae	[31]
7-*O*-Feruloylorientin (**41**)	*Gentiana piasezkii*	Whole plant	Gentianaceae	[23,24]
6'''-Feruloylspinosin (**42**)	*Ziziphus jujuba* var.*spinosa*	Seeds	Rhamnaceae	[31]
7-*O*-β-D-[6'''-*O*-(*E*)-Feruloyl]glucopyranosylisoorientin (**43**)	*Hordeum vulgare*	Leaves	Poaceae	[53]
7-*O*-β-D-[6'''-*O*-(*E*)-Feruloyl]glucopyranosylisovitexin (**44**)	*Hordeum vulgare*	Leaves	Poaceae	[53]
6-*C*-2''-*O*-Galloyl-β-D-glucopyranosylapigenin (**45**)	*Cladogynos orientalis*	Aerial parts	Euphorbiaceae	[33]
6''-*O*-Glucopyranosylisoorientin peracetate (**46**)	*Gentiana arisanensis*	Whole plant	Gentianaceae	[22]
6''-*O*-Malonoyl-2''-*O*-β-D-xylopyranosylvitexin (**47**)	*Beta vulgaris* ssp. *cycla*	Leaves	Chenopodiaceae	[55]
2''-*O*-(2'''-Methylbutanoyl)isoswertisin (**48**)	*Trollius ledebouri*, *Trollius chinensis*	Flowers	Ranunculaceae	[54,56]
3''-*O*-(2'''-Methylbutanoyl)isoswertisin (**49**)	*Trollius ledebouri*	Flowers	Ranunculaceae	[54]
2''-*O*-(2'''-Methylbutanoyl)orientin (**50**)	*Trollius ledebouri*	Flowers	Ranunculaceae	[54]
2''-*O*-(2'''-Methylbutanoyl)vitexin (**51**)	*Trollius ledebouri*	Flowers	Ranunculaceae	[54]
Others				
Dulcinoside [6-*C*-6''-*O*-(α-D-rhamnopyranosyl)-β-D-glucopyranosylapigenin] (**52**)	*Garcinia dulcis*	Fruits	Guttiferae	[57]
2''-*O*-β-D-Glucopyranosylisoscoparin (**53**)	*Alliaria petiolata*	Seeds	Brassicaceae	[28]
2''-*O*-Glucopyranosylisoorientin (**54**)	*Passiflora incarnata*	Leaves	Passifloraceae	[29]
6''-*O*-Glucopyranosylisoorientin (**55**)	*Gentiana arisanensis*	Whole plant	Gentianaceae	[22]
2''-*O*-β-D-Glucopyranosylisovitexin (**56**)	*Passiflora incarnata*	Leaves	Passifloraceae	[29]
	Ziziphus jujuba var.*spinosa*	Seeds	Rhamnaceae	[31]
6''-*O*-Glucopyranosylisovitexin (**57**)	*Gentiana arisanensis*	Whole plant	Gentianaceae	[22]
	Xanthosoma violaceum	Leaves	Araceae	[32]
8-*C*-2''-*O*-(β-D-glucopyranosyl)-β-D-xylopyranosylchrysoeriol (**58**)	*Scleranthus uncinatus*	Whole plant	Caryophyllaceae	[52]
2''-*O*-Glucopyranosylvitexin (**59**)	*Cryptocoryne* spp.	Leaves	Araceae	[10]
	Glinus lotoides	Fruits	Molluginaceae	[48]
Isospinosin [7-*O*-methyl-8-*C*-2''-*O*-(β-D-glucopyranosyl)-D-glucopyranosylapigenin] (**60**)	*Ziziphus jujuba* var.*spinosa*	Seeds	Rhamnaceae	[31]
Spinosin (2''-*O*-glucopyranosylswertisin) (**61**)	*Felicia amelloides*	Flower petals	Asteraceae	[9]
	Ziziphus jujuba var.*spinosa*	Seeds	Rhamnaceae	[31]
2''-*O*-α-L-Rhamnopyranosylisorientin (**62**)	*Sasa borealis*	Leaves	Poaceae	[21]
	Cymbopogon citratus	Aerial parts	Poaceae	[25]
2''-*O*-Rhamnopyranosylvitexin (**63**)	*Piper methysticum*	Leaves	Piperaceae	[45]
4'-*O*-α-L-Rhamnopyranosylisovitexin (**64**)	*Xanthosoma violaceum*	Leaves	Araceae	[32]
4'-*O*-Glucopyranosyl-2''-*O*-rhamnopyranosylswertisin (**65**)	*Felicia amelloides*	Petals	Asteraceae	[9]
6-C-6''-Deoxy-2''-*O*-(α-L-rhamnopyranosyl)-β-L-galactopyranosylchrysoeriol (**66**)	*Zea mays*	Style	Poaceae	[50]
8-*C*-2''-*O*-(β-D-Glucopyranosyl)-β-D-xylofuranosylchrysoeriol (**67**)	*Scleranthus uncinatus*	Whole plant	Caryophyllaceae	[51]
2''-*O*-Xylopyranosylvitexin (**68**)	*Beta vulgaris* ssp. *cycla*	Leaves	Chenopodiaceae	[55]
8-*C*-2''-*O*-(D-Xylopyranosyl)-β-D-glucopyranosylchrysoeriol (**69**)	*Scleranthus uncinatus*	Whole plant	Caryophyllaceae	[51]

The use of HPLC in combination with spectroscopic techniques such as UV, MS and NMR [60] has become routine in most phytochemical laboratories. The high sensitivity, reproducibility and easy quantification of metabolites in crude extracts, made these hyphenated techniques a method of choice over other chromatographic procedures. HPLC-UV analysis has been extensively used after the introduction of the diode array detector (DAD), since UV-Vis spectra can be readily obtained in the same sample injection, without using stopped-flow techniques. An example is the quantitative

Table 2: Structures of *C*-glycosylated flavonoids (Table 1), their IUPAC and common names.



A B C

IUPAC and common name	Skeleton -type	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
5,7,4'-Trihydroxyflavone [Apigenin (**A_1**)]	A	H	OH	H	OH	H	H	OH	H
5,7,4'-Trihydroxy-3'-methoxyflavone [Chrysoeriol (**A_2**)]	A	H	OH	H	OH	H	OMe	OH	H
5,7,3'-Trihydroxy-4'-methoxyflavone [Diosmetin (**A_3**)]	A	H	OH	H	OH	H	OH	OMe	H
5,7,3',4'-Tetrahydroxyflavone [Luteolin (**A_4**)]	A	H	OH	H	OH	H	OH	OH	H
3,5,7,4'-Tetrahydroxy-3',5'-methoxyflavone [Syringetin (**A_5**)]	A	OH	OH	H	OH	H	OMe	OH	OMe
7,4'-Dihydroxyisoflavone [Daidzein (**B_1**)]	B	H	H	H	OH	H	H	OH	H
5,7,4'-Trihydroxyisoflavone [Genistein (**B_2**)]	B	H	OH	H	OH	H	H	OH	H
5,7,3',4'-Tetrahydroxyisoflavone [Orobol (**B_3**)]	B	H	OH	H	OH	H	OH	OH	H
(*E*)-3-(4-Hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)prop-2-en-1-one.[Phloretin (**C**)]	C	H	OH	H	OH	H	H	OH	H

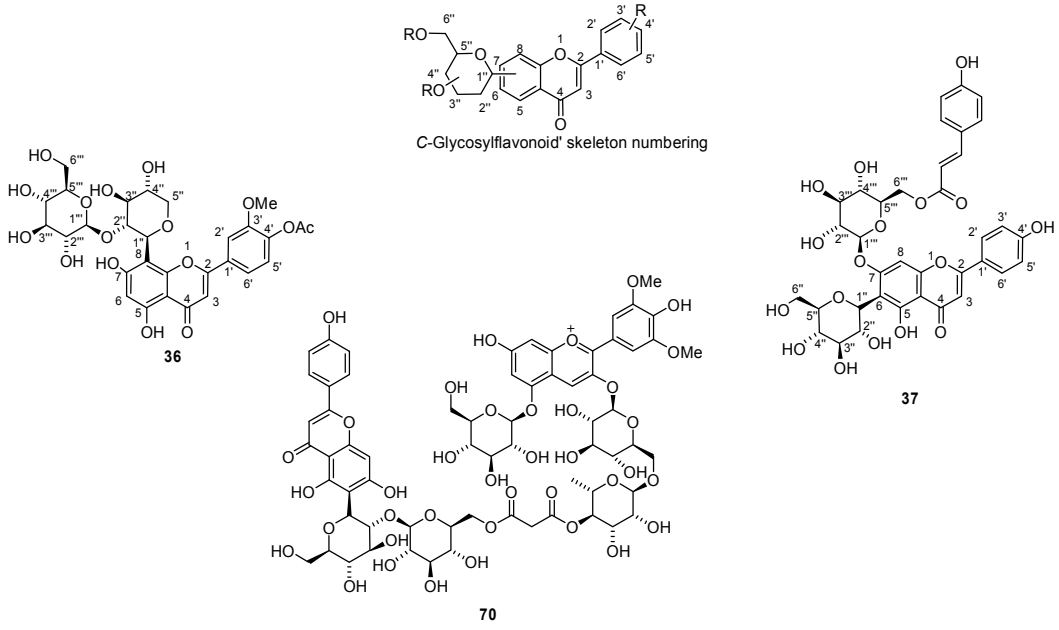


Figure 2: Skeleton numbering and structure of the *C*-glycosylflavonoids **36**, **37** and **70**.

determination of vicenin-2 (**28**) and 2''-*O*-glucopyranosylvitexin (**59**) in *Glinus lutoides* seeds extracts, used in traditional medicine and in tablet formulation for the treatment of tape worm infection [48]. Furthermore, a post-column addition of shift reagents [36] provides more precise structural information about flavonoids (oxidation patterns, position of free phenolic and methoxyl groups, position of the sugar moieties). This on-line UV technique derives from the classical application of reagents to induce characteristic shifts of the absortion maxima in the UV spectra of flavonoids [2,3].

HPLC-MS analysis allows the identification of compounds according to their molecular weight. Its ability to detect ions with a specific mass-to-charge (*m/z*) ratio makes MS invaluable for the analysis of complex mixtures, since, generally, electrospray ionization mass spectrometry (ESI-MS) provides a mass spectrum with little or no fragmentation. Currently, LC-MS using ESI, FAB

Table 3: Structure of *C*-glycosylflavonoids **1-69**.

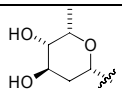
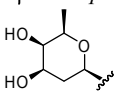
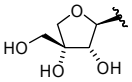
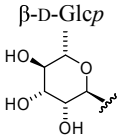
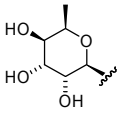
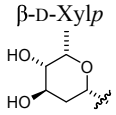
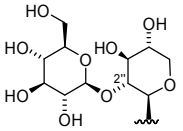
Compound	Flavonoid-type	R_3	R_4	R_5	R_7
1	A_3		OH	H	OMe
2	A_4	β-D-Glc*p*	OH	H	OH
3	A_2	β-D-Glc*p*	OH	H	OH
4	A_1	β-D-Glc*p*	OH	H	OH
5	A_5	β-D-Glc*p*	OH	H	OH
6	A_1	β-D-Glc*p*	OMe	H	OH
7	A_4	β-D-Glc*p*	OMe	H	OH
8	B_3	H	OH	β-D-Glc*p*	OH
9	A_3	H	OH	β-D-Glc*p*	OMe
10	A_4	H	OH	β-D-Glc*p*	OMe
11	B_2	H	OH	β-D-Glc*p*	OH
12	A_4	H	OH	β-D-Glc*p*	OH
13	B_1	H	OH	β-D-Glc*p*	OH
14	A_1	H	OH	β-D-Glc*p*	OH
15	A_1	α-L-Ara*p*	OH	β-D-Glc*p*	OH
16	A_1	α-L-Ara*p*	OH	β-D-Xyl*p*	OH
17	A_1	α-L-Ara*p*	OH	α-L-Ara*p*	OH
18	A_3	β-D-Glc*p*	OH	β-D-Glc*p*	OMe
19	C	β-D-Glc*p*	OH	β-D-Glc*p*	OH
20	A_1		OMe	H	β-D-Glc*p*
21	A_4	β-D-Glc*p*	OH	α-L-Ara*p*	OH
22	A_1	β-D-Glc*p*	OH		OH
23	A_4	α-L-Ara*p*	OH	β-D-Glc*p*	OH
24	A_1	α-L-Ara*p*	OH	β-D-Glc*p*	OH
25	A_4	β-D-Glc*p*	OH	β-D-Glc*p*	OH
26	A_1	β-D-Glc*p*	OH	β-L-Ara*p*	OH
27	A_1	β-D-Glc*p*	OH	α-L-Ara*p*	OH
28	A_1	β-D-Glc*p*	OH	β-D-Glc*p*	OH
29	A_1	β-D-Glc*p*	OH		OH
30	A_1	β-D-Glc*p*	OH		OH
31	A_1	β-D-Xyl*p*	OH	β-D-Glc*p*	OH
32	A_1	β-D-Xyl*p*	OH	α-L-Ara*p*	OH
33	A_3		β-D-Glc*p*-O	H	OMe
34	A_4	β-D-Glc*p*	β-D-Glc*p*-O	H	OH
35	A_1	β-D-Glc*p*	β-D-Glc*p*-O	H	OH
36	A_2	H	OH		OAc

Table 3 (*continued*): Structure of *C*-glycosylflavonoids **1-69**.

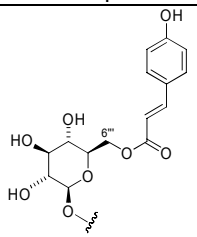
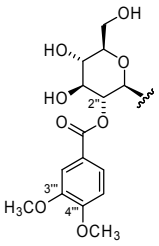
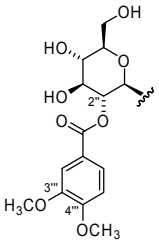
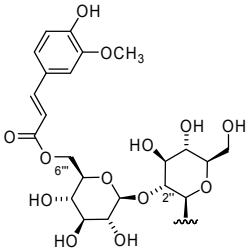
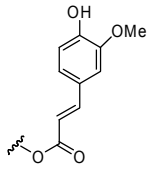
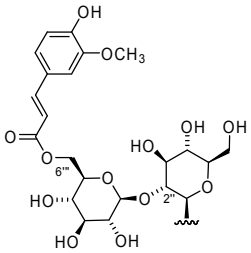
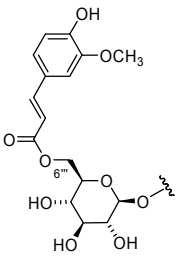
Compound	Flavonoid-type	R_3	R_4	R_5	R_7
37	A_1	β-D-Glc*p*		H	OH
38	A_4	H	OH		OH
39	A_1	H	OH		OH
40	A_1	H	OMe		OH
41	A_4	H		β-D-Glc*p*	OH
42	A_1		OMe	H	OH
43	A_4	β-D-Glc*p*		H	OH

Table 3 (*continued*): Structure of *C*-glycosylflavonoids **1-69**.

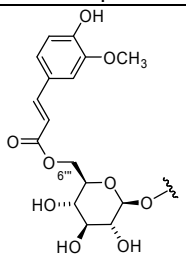
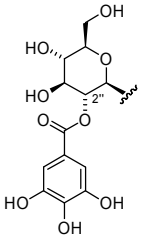
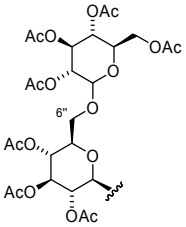
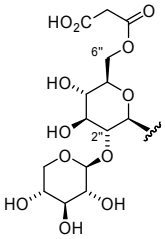
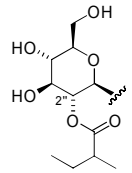
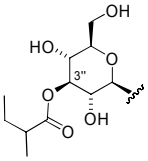
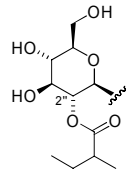
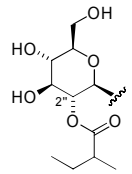
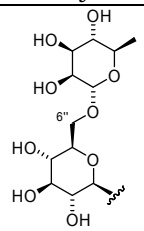
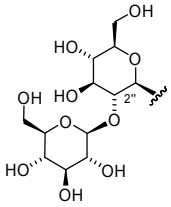
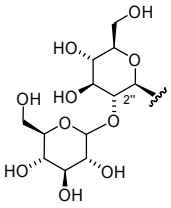
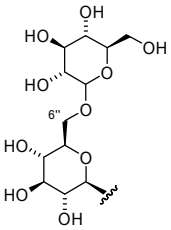
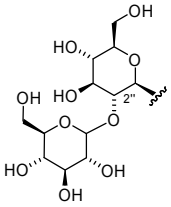
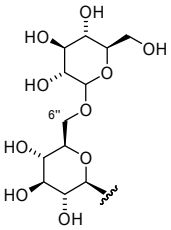
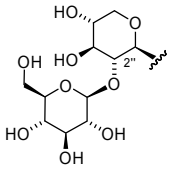
Compound	Flavonoid-type	R_3	R_4	R_5	R_7
44	A_1	β-D-Glc*p*		H	OH
45	A_1		OH	H	OH
46	A_4		OH	H	OH
47	A_1	H	OH		OH
48	A_1	H	OMe		OH
49	A_1	H	OMe		OH
50	A_4	H	OH		OH
51	A_1	H	OH		OH

Table 3 (*continued*): Structure of *C*-glycosylflavonoids **1**-**69**.

Compound	Flavonoid-type	R_3	R_4	R_5	R_7
52	A_1		OH	H	OH
53	A_2		OH	H	OH
54	A_4		OH	H	OH
55	A_4		OH	H	OH
56	A_1		OH	H	OH
57	A_1		OH	H	OH
58	A_2	H	OH		OH

and TSP interfaces, with a combination of tandem mass spectrometry (MS/MS) and UV detection, is a powerful tool to investigate rapidly the chemical composition of complex flavonoid extracts. The application of these hyphenated techniques to the analysis and identification of *C*-glycosylflavonoids is well documented in the literature. For example HPLC-DAD-ESI-MS-MS was employed to evaluate the di-*C*-glycosylflavone content in the juice of cultivars of various *Citrus* species [42]. These compounds are characteristic minor components differentiating orange juice, which contains mainly

Table 3 (*continued*): Structure of *C*-glycosylflavonoids **1**-**69**.

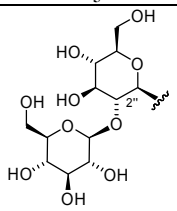
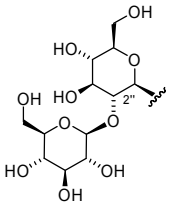
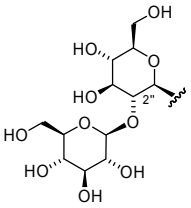
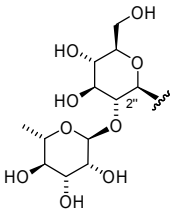
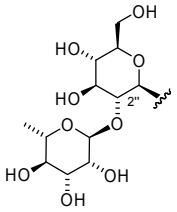
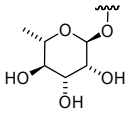
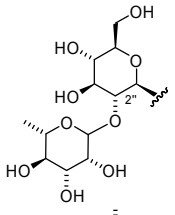
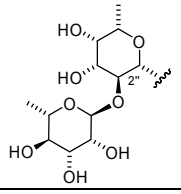
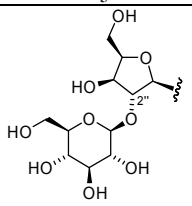
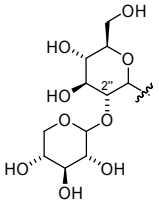
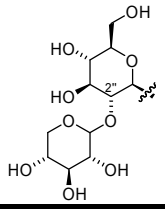
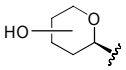
Compound	Flavonoid-type	R_3	R_4	R_5	R_7
59	A_1	H	OH		OH
60	A_1	H	OMe		OH
61	A_1		OMe	H	OH
62	A_4		OH	H	OH
63	A_1	H	OH		OH
64	A_1	β-D-Glc*p*	OH	H	
65	A_1		OMe	H	β-D-Glc*p*-O
66	A_2		OH	H	OH

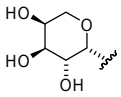
Table 3 (*continued*): Structure of *C*-glycosylflavonoids **1**-**69**.

Compound	Aglycone	R_3	R_4	R_5	R_7
67	A_2	H	OH		OH
68	A_1	H	OH		OH
69	A_2	H	OH		OH

[a] The following abbreviations are used:
β-D-Glc*p* (β-D-glucopyranosyl group)
β-D-Xyl*p* (β-D-xylopyranosyl group)
α-L-Ara*p* (α-L-arabinopyranosyl group)



β-L-Ara*p* (β-L-arabinopyranosyl group)



6,8-di-*C*-β-D-glucopyranosylapigenin (**28**), from lemon and citron juices, in which the most important *C*-glycosylflavone is 6,8-di-*C*-β-D-glucopyranosyl-diosmetin (**18**), while bergamot juice contains both the di-*C*-glycosylflavones in similar concentrations and clementine juice has only minor amounts of both of them. Other examples of the application of this technique include the identification of the 8-*C*-glycosylflavones **9**, **10**, **12** and **14** and the 6,8-di-*C*-glycosylflavones **24** and **27** (Table 1) present in sugar cane (*Saccharum officinarum*) complex extracts [36], and of a *C*-glycosylapigenin in the fruit of date palm (*Phoenix dactylifera*) [46]. Profiling and quantification of isoflavonoids in Kudzu (*Pueraria* sp.) dietary supplements, which include puerarin (**13**) as one of the major components responsible for their therapeutical properties, was also successfully achieved by this method [61]. Recently, *C*-glycosyl-isoflavones were detected and tentatively identified by HPLC-MS/MS analysis of methanol extracts of *Pueraria lobata* leaf tissues, *callus*, suspension and root cultures aiming at developing an experimental system in which puerarin could be synthesized *in vitro* [40]. The quantification of puerarin in human plasma was also conducted using HPLC in combination with solid phase extraction (SPE) and LC-ESI-MS experiments [62].

Capillary electrophoresis-mass spectrometry (CE-MS) is another analytical method reported in the literature for the separation and identification of the *C*-/*O*-glycosylflavonoid composition, namely that of a *Genista tenera* extract [63]. MALDI-TOF-MS was also used for the study of the *C*-glycosylflavonoids **37**, **43** and **44** isolated by HPLC from the leaves of barley (*Hordeum vulgare*) [53].

MS/MS of *C*-glycosylflavonoids is also a powerful method to differentiate 1→2 and 1→6 glycose linking in disaccharides [58], and has been used to distinguish 6-*C*- and 8-*C*-glycosylflavones by comparing spectra of particular fragments [64-66]. The study of isoorientin (**2**), orientin (**12**) and vitexin (**14**) has also been carried out using ESI-MS/MS at high mass accuracy [67]. In conclusion, mass spectrometry has proven to be a very powerful technique in the analysis of *C*-glycosylflavonoids, owing to its high sensitivity and the possibility of coupling with different chromatographic techniques and especially with LC, because it allows both

qualitative and quantitative determinations. Structural information can be obtained on the flavonoid moiety, the types of carbohydrates or other substituents present, the stereochemical structure of terminal monosaccharide units, the sequence of the sugar units, interglycosidic linkages and attachment points of the substituents to the flavonoid [68]. However the structure of the 8-*C*-β-D-glucopyranosylgenistein (**11**), a major constituent of the ethyl acetate extract of *Genista tenera*, could not be unambiguously assigned only by HPLC-DAD-ESI-MS-MS. The final structure was elucidated by means of two-dimensional nuclear magnetic resonance (2D-NMR) and *J*-resolved spectra of the mixture [37]. This is one of the first successful examples of the application of NMR to the structure characterization of a *C*-glycosylflavonoid present in a mixture. However NMR has been largely employed for the structure elucidation of flavonoids, in particular of *C*-glycosylflavonoids after their successful isolation from the crude mixture. Information about the nature and position of glycosyl groups and type of linkage in complex glycosides has been provided by this spectroscopic tool. The 2D-NMR techniques such as COSY, HMQC, HSQC, HMBC, NOESY, and less frequently TOCSY have allowed the unambiguous identification of a variety of *C*-glycosylflavonoids [8-10,19,20,22,27,31-33,38,43,44,49,51,53,54,69,70].

Biological Activity

A wide range of biological properties were attributed to *C*-glycosylflavonoids, the pharmacological profile of which is quite different from that of the corresponding *O*-glycosides and aglycones, since the C-C bond linking the sugar to the flavonoid is not cleaved under normal physiological conditions [69]. Hence, they have better bioavailability than the *O*-glycosides, which are hydrolysed to aglycone and sugar under acid conditions.

As can be observed in Table 4, the antioxidant effect of *C*-glycosylflavonoids is well documented. Their activity as free radical scavengers in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is frequently reported. For the 6-*C*-glycosylflavones **2**, **6**, **7**, and **53**, isolated from garlic mustard (*Alliaria petiolata*), the IC_{50} values were of the same order of magnitude as that of trolox, a well-known antioxidant. In addition, they were tested in the brine shrimp lethality assay, an effective and rapid assay to screen compounds for potential cytotoxicity. The high LD_{50} values obtained, when compared to that of the control used, indicated a very low general toxicity for these compounds [28]. In addition to the DPPH radical scavenging activity exhibited by 6,4'-di-*C*-glycosylflavone **20**, this compound also inhibited significantly the induced superoxide anion generation from human neutrophils, which are extensively involved in inflammatory processes [43]. Scavenging of the superoxide anion generated by xanthine oxidase and inhibition of lipoperoxidation were also evaluated for compounds **2**, **3**, **7**, **12** and **62** isolated from lemongrass (*Cymbopogon citratus*) [25], which infusion has widespread use in folk medicine to treat inflammation, diabetes and nervous disorders. Isoorientin (**2**) and orientin (**12**) presented both similar activities either towards the DPPH assay or the inhibition of lipid peroxidation. The sugar position at C-6 or C-8 did not influence any of the activities, which were higher than those of the diglycosyl derivative **62**. The 6-*C*-glycosylflavonoids isoorientin (**2**) and isoscoparin (**3**) presented the best superoxide anion scavenger activity. The presence of a diglycosyl moiety at C-6, methoxylation at C-7 or a glycosyl group at C-8 reduced the superoxide anion scavenger effect. No cytotoxic effect of the compounds was assessed on cell lines derived from human lung at doses up to 1 mM, supporting the beneficial properties of lemongrass tea and soft drinks [25]. More recently, isoorientin (**2**) and 2''-*O*-α-L-rhamnopyranosylisoorientin (**62**) also showed strong cytoprotective effects against *t*-BOOH-induced oxidative damage in HepG2 cells, at very low concentrations [21]. The free radical scavenging properties of *Xanthosomona violaceum* extracts also seem to be correlated with the structure of the *C*-glycosylflavones, which are the apigenin derivatives **4**, **14**, **22**, **28** and **64** [32].

The 8-*C*-glucosylisoflavones **8** and **11** have important bioactivities, which are different from those of the corresponding isoflavones that show biological activities analogous to the female hormone estrogen [71]. The glucosylgenistein **11** inhibits HOCl-induced damage to human erythrocytes [92] and would be expected to be useful as an antioxidant and a radioprotective agent, since it prevents the destruction of cytochrome P-450 in a dose-dependent manner and exerts a protective effect against gamma irradiation in rats [72]. On the other hand, the glucosylorobol **8** was investigated regarding its potential immunomodulating effect. Both the strong mitogenic activity and the induction of colony-stimulating factor (CSF) suggest that it might act on the hematopoietic system [35]. Among the *C*-glycosylisoflavones, puerarin (**13**) is one of the

Table 4: Bioactivity of *C*-Glycosylflavonoids.

Activity	Compound	Ref.
Antioxidant	Isoorientin (**2**)	[21,23, 25,28]
	Isoscoparin (**3**)	[25]
	Swertisin (**6**)	[28]
	Swertiajaponin (**7**)	[25,28]
	8-*C*-β-D-Glucosylgenistein (**11**)	[72,92]
	Orientin (**12**)	[25]
	Puerarin (**13**)	[73,74]
	Diandraflavone (**20**)	[43]
	6-*C*-β-D-Xylopyranosyl-8-*C*-β-**D**-glucopyranosylapigenin (**31**)	[21]
	7-*O*-Feruloylorientin (**41**)	[23]
	Dulcinoside (**52**)	[57]
	2´´-β-D-Glucopyranosylisoscoparin (**53**)	[28]
	2´´-*O*-α-L-Rhamnosylisoorientin (**62**)	[21,25]
Hypoglycaemic	Isoorientin (**2**)	[26,77]
Antihyperglycaemic	Puerarin (**13**)	[78]
Glycation inhibition	6-*C*-2'',6''-Dideoxy-β-L-*xylo*-hexopyranosylchrysoeriol (**1**)	[20]
	Puerarin (**13**)	[39]
	6-*C*-2'',6''-Dideoxy-β-L-*xylo*-hexopyranosyl-7-*O*-β-D-glucopyranosylchrysoeriol (**33**)	[20]
Hypocholesterolemic	Puerarin (**13**)	[79]
Hepatoprotective	Puerarin (**13**)	[81,82]
	Vitexin (**14**)	[41]
	Isoschaftoside (**24**)	[44]
Anti-inflammatory	Sugar-fused flavones **104** and **105**	[84,85]
Antiviral	2´´-*O*-(2´´´-methylbutanoyl)isoswertisin (**48**)	[56]
Antibacterial	2''-β-D-Glucopyranosylisoscoparin (**53**)	[28]
Sedative	Swertisin (**6**)	[31]
	Spinosin (**66**)	[31]
Anxiolytic	Isovitexin (**4**)	[90]
	Puerarin (**13**)	[87]

most studied compounds for its effects on hyperglycaemic, cardiovascular and neurological diseases. Its antioxidant activity has been evaluated using the Briggs-Rauscher reaction [73] and the DPPH assay [73,74]. The radical scavenger efficiency of its OH-4' group was found to be more effective by a factor of 2 than that of its OH-7 group [75]. In addition, puerarin was reported to protect neurons from oxidative stress, a property that may be determinant for its potential use against Alzheimer's disease [76].

The antioxidant properties of flavonoids are related to their potential to prevent disorders associated with oxidative stress caused by free radicals and other reactive oxygen species. Hyperglycaemia results in the generation of free radicals, which may lead to disruption of cellular functions, oxidative damage to cell membranes and enhanced susceptibility to lipid peroxidation. The significant hypoglycaemic activity of isoorientin (**2**) [77] may be related to its potent inhibitory effect on lipid peroxidation. The presence of the chemically stable sugar moiety may facilitate the transport and accummulation of the molecule in pancreas β-cells and restore or prevent further oxidative dammage due to its potent antioxidant activity [26]. This 8-*C*-glycosylflavone also prevented hyperlipidemia, an important risk factor in diabetics for many diseases, particularly cardiovascular disorders [26]. The antihyperglycaemic effect of puerarin (**13**) in streptozotocin-induced diabetic rats has also been reported [78].

Some diabetic complications arise when reducing sugars, such as glucose, react nonenzymatically with the amino groups of proteins, nucleic acids, and lipids forming Schiff's bases and Amadori products in a process known as glycation which leads to the formation of AGE (advanced glycation end products) [20]. A potent glycation inhibitory activity was exhibited by puerarin (**13**) [39] and by the 6-*C*-deoxyglycosylflavones isolated from the style of *Zea mays* L., which is known as "corn silk" and used in folk medicine for diuretic treatment [20,50].

Hypercholesterolemia is a dominant risk factor for the development and progression of atherosclerosis and cardiovascular diseases. Puerarin (**13**) has been reported to have atheroscleroprotective potential and hypocholesterolemic function [79]. In addition, its effect on vascular relaxation was also investigated [80].

Hepatoprotective effects of *C*-glycosylflavonoids were also reported. Isoschaftoside (**24**), isolated from green tea, suppressed the D-galactosamine-induced increase of plasma alanine aminotransferase and aspartate aminotransferase activities in rats, indicating that these compounds had a liver injury-suppressing effect [44]. Vitexin (**14**), isolated from the methanol extract of *Combretum quadrangulare* leaves [41], and puerarin (**13**) [81] also showed hepatoprotective properties. The latter compound could reverse alcohol induced liver fibrosis in experimental rats via recovery of hepatic injury [82].

Puerarin may also act as a chemopreventive and/or chemotherapeutic agent in colon cancer cells by reducing cell viability and inducing apoptosis [83]. The sugar-fused *C*-glycosylflavones **104** and **105** (Scheme 3) exhibited anti-inflammatory activity, based on the suppression of contact hypersensitivity in mice induced by treatment with 2,4-dinitro-fluorobenzene, indicating that **104** is c.a. 1000 times

stronger than dexamethasone, a conventionally used anti-inflammatory drug [84,85].

Puerarin (**13**) was identified as the major component of kudzu extract responsible for the suppression of alcohol drinking [62,86]; it reduces the anxiogenic effects of alcohol withdrawal as a weak benzodiazepine site antagonist [87]. This *C*-glycosyl-isoflavone can cross the blood-brain barrier as evidenced by its presence in the brain tissues of puerarin treated rats [88] which supports the concept that it works on the central nervous system [62]. In addition, its hypothermic and antipyretic effects acting through brain serotonergic mechanisms have been described [89]. Anxiolytic and sedative-like properties were described for the aqueous fraction of a methanol extract from the leaves of *Passiflora actinia*, which contained isovitexin (**4**) [90]. A significant sedative activity was also reported for swertisin (**6**) and spinosin (**61**) [31]. Although antimicrobial activities are not extensively reported for this group of compounds, the antibacterial activity against *Citrobacter ferundii* was exhibited by the 6-*C*-glycosylflavone **53** [28] and antiviral properties were attributed to the 8-*C*-glycosylflavone **48** which showed a moderate activity against influenza virus A [56].

Apart from the above described *C*-glycosylflavonoid bioactivities of potential interest to human nutrition and health, a role for these compounds in plant defense has been described in oats (*Avena sativa*). Plant invasion with the nematodes *Heterodera avenae* and *Pratylencus neglectus* induced the production of a *C*-deoxyhexosyl-*O*-hexosyl-*O*-methylapigenin which provided protection against these major cereal nematodes [91].

Synthesis

Synthesis of the active principles extracted from natural sources is quite often the next step after their isolation and identification. Exploring the synthetic access to natural products-based drugs is important because either the natural resources are limited or new lead structures with better properties can be obtained by decorating the unique natural scaffolds with suitable functional groups.

Flavonoids are promising raw materials for the synthesis of compounds derived from a chromene-type skeleton, with various substitution patterns. The bioactivities of the natural *C*-glycosylflavonoids have led to the development of methodologies for the

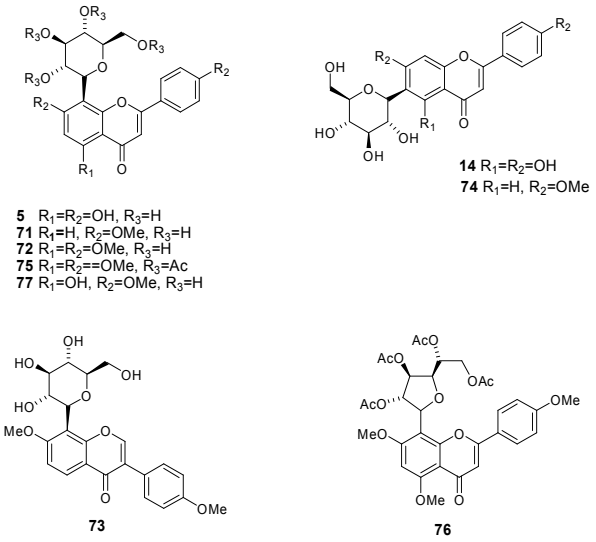
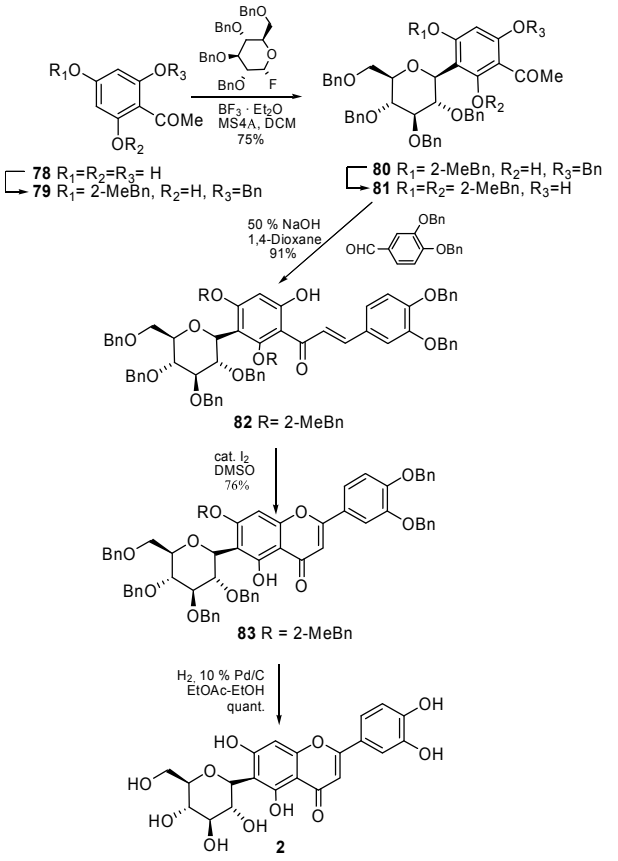


Figure 3: Structure of synthesized *C*-glycosylflavonoids prior to 1994.

C-glycosylation of flavonoids. Eade and co-workers reported the total synthesis of the 8-*C*-glucosylflavones **71** [93] and **72** (Figure 3), where the *C*-glycosylation step was performed via a zinc oxide-promoted coupling using peracetyl glucosyl bromide as glycosyl donor [94]. For the synthesis of 7,4'-di-*O*-methylpuerarin (**73**), thallium nitrate in methanol followed by treatment with acid or base were used to transform a chalcone precursor into the desired isoflavone [95]. Harborne and Mabry also made an important contribution to the development of the synthesis of this type of compound by studying the reactions of 5,7-hydroxyflavones with glycosyl bromides, which afforded *C*-glycosylflavones in one step [96]. Tschesche and Widera described the first synthesis of the 6-*C*-glycosylflavone **74** [97], while Frick and Schmidt reported the preparation of **75** and **76** carrying out *C*-glycosylation by arylation of a protected glucose, in its acyclic carbonyl form, with a lithiated aromatic ring [98]. Schmidt and co-workers also described the synthesis of isovitexin (**4**), vitexin (**14**) and isoembigenin (**77**) (8-*C*-β-D-glucopyranosyl-5-hydroxy-7,4'-dimethoxy-flavone) using trichloro-acetimidates as glycosyl donors and Fries-type rearrangement to convert *O*-glycosides into *C*-glycosyl derivatives, prior to the construction of the flavone system by application of a Baker-Venkataraman-type rearrangement [99]. New pathways to *C*-glycosylflavonoid involve the synthesis of 6-*C*-glycosylflavones, including sugar-fused and 4'-*O*-glycosyl derivatives, 8-*C*-glycosylated flavones and isoflavones, and 6,8-di-*C*-glycosylflavonoids with chalcone-, flavanone- and flavone-type structures.

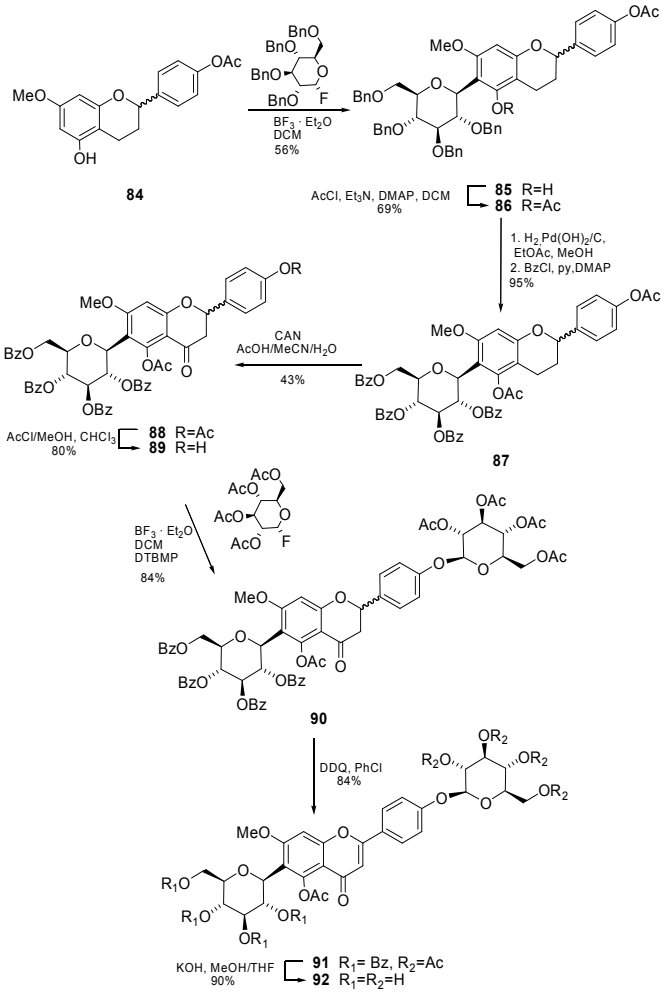
6-*C*-glycosylflavones: The total synthesis of isoorientin (**2**) was investigated by Kumazawa and co-workers [100] (Scheme 1) who succeeded in obtaining this compound in 15% overall yield, starting from the commercially available phloroacetophenone **78**, which was first conveniently protected leading to the derivative **79**. One of the crucial steps consists of the coupling of this moiety to the sugar. The method commonly used is the Fries-type rearrangement which involves an *O*- to *C*-glycosyl migration. The glycosyl donor 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl fluoride reacted with **79** in the presence of $BF_3{\cdot}Et_2O$ to give an intermediate *O*-glycoside which rearranged to afford the *C*-glycosyl derivative **80** in good yield. Protection of the free OH and deprotection of the benzyl group afforded compound **81**, which was subjected to aldol condensation with 3,4-bis(benzyloxy)benzaldehyde leading to the key intermediate chalcone **82**. Its cyclization was accomplished using a method developed by Cavaleiro *et al*. [101], in which the chalcone was oxidized in the presence of a catalytic amount of iodine in DMSO under reflux, followed by cleavage of the 2-methylbenzyl group, *ortho*-positioned to the cinnamoyl moiety, affording **83**.



Scheme 1: Synthesis of isoorientin (**2**) [100].

Hydrogenolysis with 10% palladium-on-charcoal under hydrogen atmosphere gave isoorentin (**2**).

6-*C*-Glycosyl-4'-*O*-glycosylflavones: The first total synthesis of flavocommelin **92** was accomplished by Oyama and Kondo [102] in 8% overall yield, using the 5-hydroxy-7,4'-protected flavan **84** as starting material (Scheme 2). Its direct 6-*C*-glycosylation was accomplished by reaction with perbenzyl glucosyl fluoride in the presence of $BF_3{\cdot}Et_2O$. Acetylation of the free OH group, debenzylation by hydrogenolysis and benzoylation afforded the protected flavan **87**, which was oxidized to the flavanone **88** by cerium ammonium nitrate (CAN). Regioselective deacetylation of OH-4' with AcCl/MeOH gave **89**, glycosylation of which with peracetyl glucosyl fluoride in dichloromethane (DCM) in the presence of $BF_3{\cdot}Et_2O$ and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), led to the diglucosylflavanone **90**. Dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in PhCl afforded the flavone

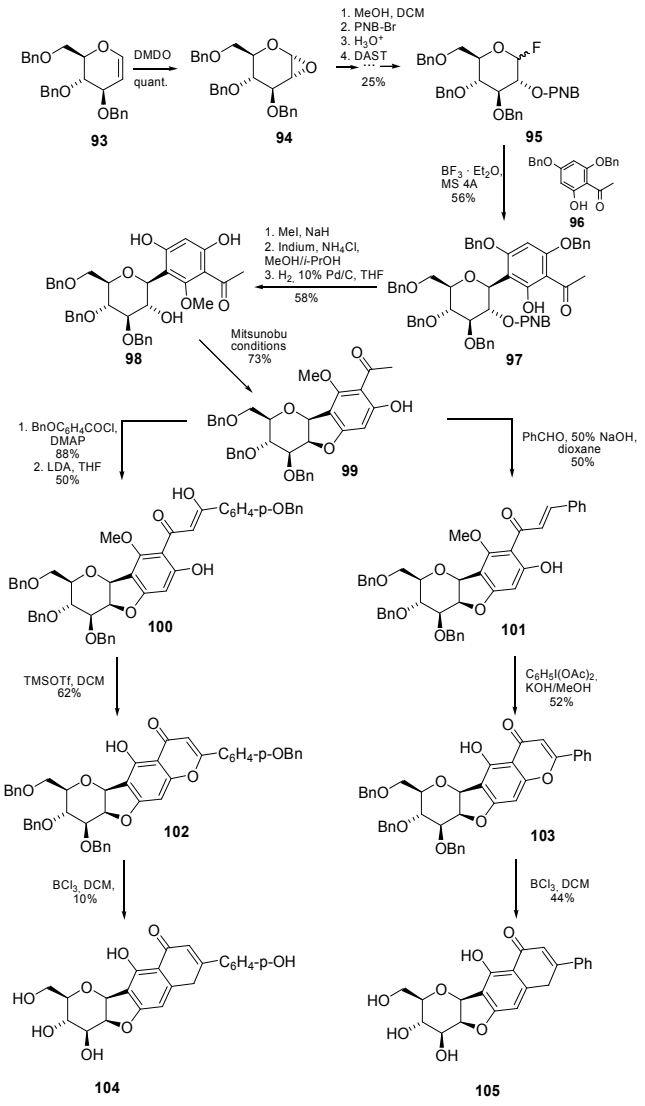


Scheme 2: Synthesis of the 6-*C*-4'-*O*-diglucosylflavone **92** [102].

91, which was deprotected with KOH/MeOH to give the target molecule **92** in 90% yield.

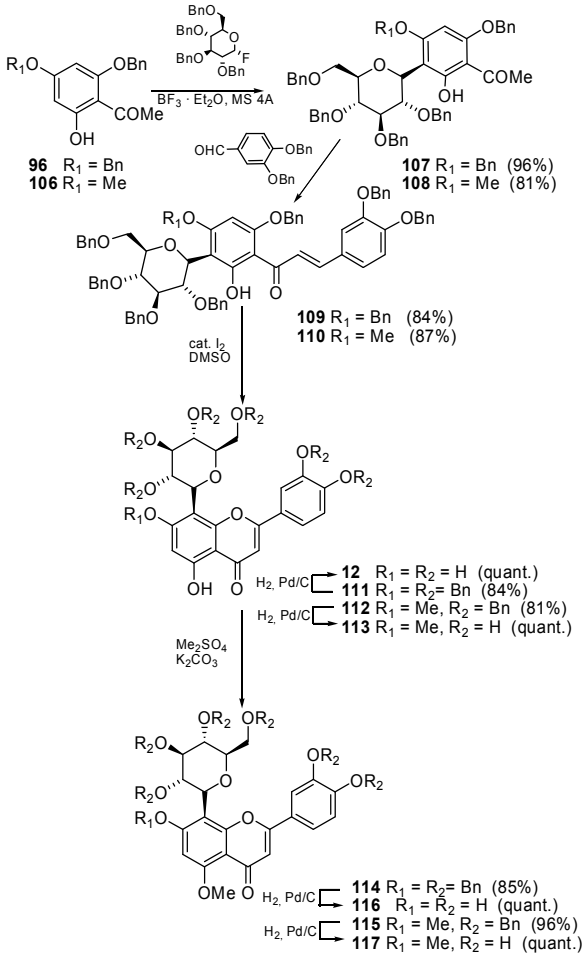
Sugar-fused 6-*C*-glycosylflavones: A synthetic pathway for the first total synthesis of two flavones with a strong anti-inflammatory effect was published by Nakatsuka *et al.* [85] and Furuta *et al*. [84]. The glycosyl donor was obtained by stereoselective epoxidation of the 3,4,6-tri-*O*-benzyl-D-glucal **93** with dimethyldioxirane (DMDO) followed by treatment with methanol, protection of OH-2 with the *p*-nitrobenzyl group, acid hydrolysis of the intermediate methyl glycoside and reaction with (diethylamino)sulfur trifluoride (DAST) to give the glucosyl fluoride **95** [85] (Scheme 3).

Glycosylation of 1-[2,4-bis(benzyloxy)-6-hydroxyphenyl]ethanone (**96**) activated with $BF_3{\cdot}Et_2O$ in DCM, *via* Fries-type rearrangement, gave the *C*-glycosyl derivative **97**. Methylation of the free OH group, followed by cleavage of the *p*-nitrobenzyl group and deprotection of both the phenolic benzyl ether groups by Pd/C-H_2 in THF gave the triol **98**. Cyclization under Mitsunobu conditions with 1,1'-azobis(*N*,*N*-dimethylformamide)/tributylphosphorane in benzene led to the tricyclic compound **99**, isolated in good yield. This compound is a key intermediate for the synthesis of both sugar-fused derivatives **104** and **105**. The chalcone precursor **100** was obtained by acylation of OH-2' with *p*-benzyloxybenzoyl chloride and dimethylaminopyridine (DMAP), followed by treatment with lithium diisopropylamide (LDA) to give a carbanion, which promoted the intramolecular addition-elimination reaction with the ester function. Its cyclization to the flavone **102** was accomplished with trimethylsilyl triflate (TMSOTf). For the synthesis of the flavone **103**, condensation of **99** with benzaldehyde was followed by flavone formation with hypervalent iodine reagent. Debenzylation to give the target molecules **104** and **105** was achieved by treatment with BCl_3.



Scheme 3: Synthesis of the sugar-fused flavones **104** and **105** [85].

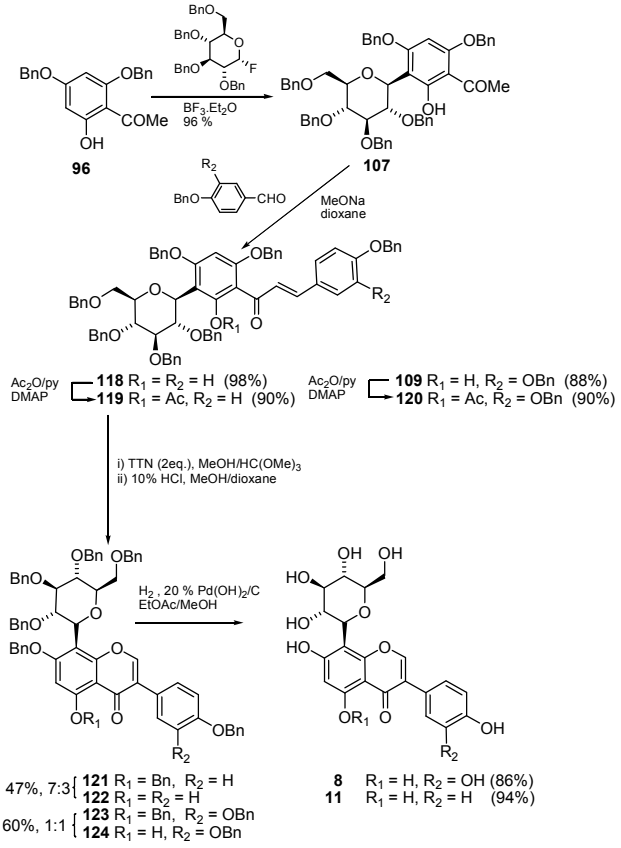
8-*C*-glycosylflavones: Based on the Fries-type rearrangement, Kumazawa *et al.* [103] developed an approach for the synthesis of orientin (**12**), isoswertiajaponin (**113**), parkinsonin A (**116**) and parkinsonin B (**117**). A benzyl protected glucopyranosyl fluoride reacted with **96** or **106** (Scheme 4) in the presence of $BF_3{\cdot}Et_2O$ as an activator to give the *C*-glycosyl intermediates **107** and **108**, respectively, in high yield. Further aldol condensation with 3,4-bis(benzyloxy)aldehyde led to the formation of the chalcones **109** and **110**, which were transformed into the 8-*C*-glycosylflavones **111** and **112** by oxidative cyclization in the presence of a catalytic amount of iodine in DMSO, which also promoted cleavage of the benzyl group in the *ortho* position to the cinnamoyl group. Orientin (**12**) was then obtained by hydrogenolysis of **111** in the presence of 10% Pd/C in quantitative yield. Methylation of **111** with dimethylsulfate afforded the benzyl-protected parkinsonin A **114** in very good yield. Hydrogenolysis under the conditions used for orientin led to the synthesis of parkinsonin A (**116**) in 85% yield. On the other hand, compound **112,** which possesses the methoxy group on position 7, was debenzylated leading to isoswertiajaponin (**113**) in high yield. In addition, methylation of **112** with dimethyl sulphate converted it to compound **115** in 96% yield, which gave parkinsonin B (**117**) in quantitative yield when subjected to hydrogenolysis.



Scheme 4: Synthesis of the 8-*C*-glycosylflavones **12**, **113**, **116** and **117** [103].

Wang *et al.* [104] presented a similar approach for the synthesis of parkinsonin B starting with the coupling of 1-[2,4-bis(methoxy)-6-hydroxyphenyl]ethanone to perbenzyl glucosyl trichloroacetimidate, followed by aldol condensation prior to the cyclization with iodine/DMSO, affording the benzyl protected Parkinsonin B **115**, hydrogenolysis of which gave the target molecule **117** in 32% overall yield.

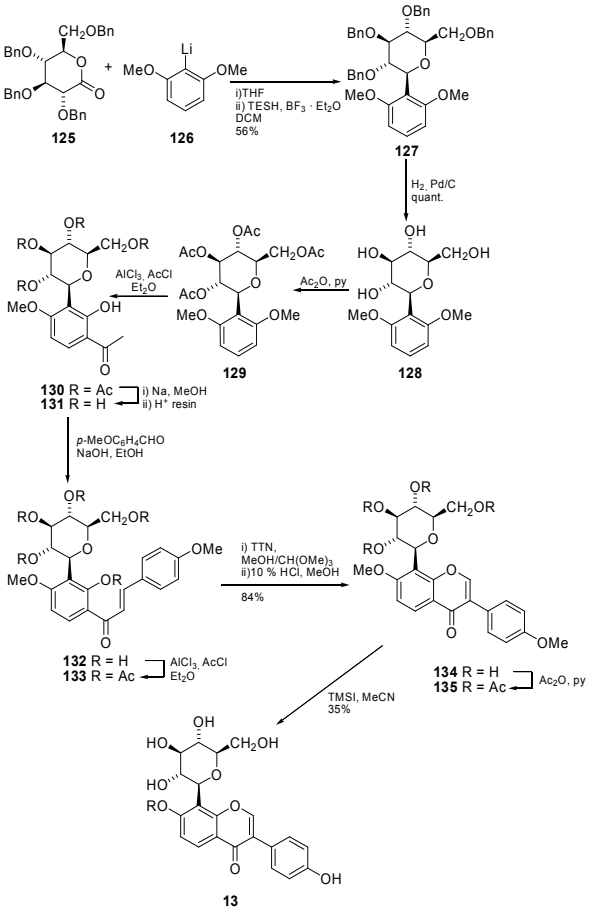
8-*C*-glycosylisoflavones: 8-*C*-Glucosylorobol (**8**) and 8-*C*-glucosylgenistein (**11**) are bioactive naturally occurring glucosylisoflavones (Table 1), the total syntheses of which were described by Sato *et al*. [105]. The synthetic strategy used for both isoflavones is very similar to those previously described for 8-*C*-glycosylflavones with the exception of the chalcone oxidative rearrangement, which was promoted by thallium (III) nitrate (TTN)/trimethyl orthoformate leading to the formation of intermediate dimethyl acetals as shown



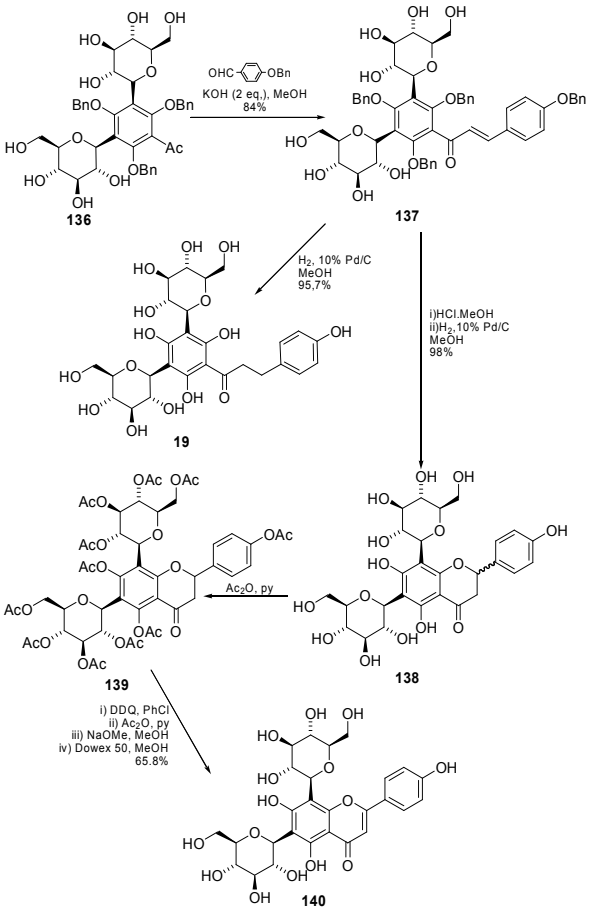
Scheme 5: Synthesis of 8-*C*-glucosylorobol (**8**) and 8-*C*-glucosylgenistein (**11**) [105].

for similar syntheses [106, 107]. Their subsequent acid-catalyzed cyclization gave the isoflavones **121** and **123** and their 5-*O*-debenzylated products **122** and **124**, due to the acidic conditions used, obtained in 47% yield (**121**/**122**: 7/3) and 60% yield (**123**/**124**: 1/1), respectively (Scheme 5). The subsequent debenzylation of these compounds was conducted by hydrogenolysis with $Pd(OH)_2/C$ under hydrogen atmosphere leading to the target molecules **8** and **11** in 86%.and 94% yield, respectively.

Puerarin (**13**) is another natural occurring 8-*C*-glucosylisoflavone with a variety of promising bioactivities (Tables 1 and 4). Its synthesis was reported by Lee and co-workers in 10% overall yield [69]. The starting material was the benzyl protected glycopyranolactone **125**, which reacted with the lithiated aromatic compound **126** (Scheme 6). Further dehydroxylation with triethylsilane (TESH) in the presence of $BF_3{\cdot}Et_2O$ gave the β-anomer **127** in 56% yield. The benzyl groups were then cleaved by hydrogenolysis with Pd/C in methanol to afford the *C*-glucosyl derivative **128**, acetylation of which was followed by Friedel-Crafts acylation, to give compound **130**, with a free OH group resulting from



Scheme 6: Synthesis of puerarin (**13**) [69].



Scheme 7: Synthesis of 6,8-di-*C*-glycosylflavonoids [108].

the cleavage of the methoxy group *ortho*-positioned to the acyl group. After removal of the acetyl protecting groups, the chalcone **132** was obtained by condensation with *p*-methoxybenzaldehyde. Acetylation of the free OH in **132** was carried out prior to the oxidative rearrangement with TTN in methanol/trimethyl orthoformate, followed by acid catalyzed cyclization to give methylpuerarin **134** in 84% yield. Demethylation of this compound was tried using several reaction conditions and succeeded only with Me_3SiI in acetonitrile under reflux for 5 days, converting **134** to puerarin (**13**) in 35% yield.

6,8-di-*C*-glycosylflavonoids: The total synthesis of compounds **19**, **138** and **140**, the 6,8-di-*C*-β-D-glucopyranosyl derivatives of phloretin (dihydrochalcone), naringenin (flavanone) and apigenin (flavone), respectively, was reported by Sato *et al*. [108]. The methodology adopted starts with direct *C*-glycosylation of phloroacetophenone with unprotected D-glucose using water as solvent, an environmentally friendly medium, and scandium (III) trifluoromethanesulfonate as catalyst, affording the bis-*C*-glucosylderivative **136** [109], aldol condensation of which with *p*-benzyloxybenzaldehyde in the presence of potassium hydroxide led to the chalcone **137** in 84% yield (Scheme 7). Hydrogenolysis in the presence of Pd/C in methanol led to deprotection of the benzyl groups and reduction of the cinnamoyl double bond giving the dihydrochalcone **19** in 95.7% yield. When **137** was treated with HCl-MeOH under reflux for 30 min, cyclization to a flavanone occurred, hydrogenolysis of which afforded **138** in quantitative yield. Its conversion to the 6,8-di-*C*-glucosylflavone was achieved by treating the acetyl protected derivative **139** with DDQ in chlorobenzene to afford the flavone acetate, deacetylation of which gave the target flavone **140** in 35% overall yield, while the di-*C*-glucosylphloretin **19** and the di-*C*-glucosylnaringenin **138** were synthesized in 52.3% and 53.5% overall yield, respectively.

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1.3.1 Glycosylflavonoids: Identification and Bioactivity– An Update

In this subchapter a short update on the isolation and bioactivity of glycosylflavonoids reported from 2007 until present is given.

As previously stated, isolation and identification of glycosylflavonoids is embodied of great interest due to their pharmacological properties. When the review "*C*-Glycosylflavonoids: Identification, Bioactivity and Synthesis" was published, comprising the years from 1997 to 2007, an average of 4.4 papers was published each year. Over the last five years this number increased to 6.8 papers per year, showing the increasing interest on those compounds. Table 1 summarizes the known compounds isolated from new plant sources as well as 18 new molecules isolated from plants since 2007.

Table 1– Natural occurrence of glycosylflavonoids (covering the last five years)

Entry	C-Glycosylflavonoid*	Source		Ref
		Plant	Plant part	
Known compounds				
1	2''-(4'''-Hydroxybenzoyl)isorientin	*Centaurea gigantea*	aerial	[26]
2	2''-*O*-galloylvitexin	*Clidemia sericea*	leaves	[27]
3	2''-*O*-rhamosylvitexin	*Celtis africana*	aerial	[28]
4	2''-*O*-α-D-rhamnopyranosylisorientin	*Cymbopogon citratus*	Leaves	[29]
5	2''-*O*-α-rhamnosyl-4'-*O*-methylvitexin	*Piper ossanum*	leaves	[30]
6	2''-*O*-β -L-galactopyranosylorientin	*Trollius ledebourii*	flower	[31]
7	2''-*O*-β-L-galactopyranosylvitexin	*Trollius ledebourii*	flower	[31]
8	2''-O-α-L-rhamnopyranosylisovitexin	*Averrhoa carambola*	leaves	[32]
9	6,8-di-*C*-β-D-glucopyranosylchrysin	*Passiflora edulis*	pericarp	[33]
10	6-*C*-(2''-*O*-α-L-rhamnopyranosyl)-β-L-fucopyranylapigenin	*Averrhoa carambola*	leaves	[32]
11	6-*C*-α-D-glucopyranosylisoorientin	*Primula spectabilis*	leaves	[34]
12	(2*R*,3*R*)-6-C-β-D-glucopyranosyltaxifolin	*Garcinia buchananii*	bark	[35]
13	(2*R*,3*R*)-6-C-β-D-glucopyranosylaromadendrin	*Garcinia buchananii*	bark	[35]
14	6-*C*-β-L-fucopyranosylapigenin	*Averrhoa carambola*	leaves	[32]
15	8-*C*-β-glucopyranosylkaempferol	*Primula spectabilis*	leaves	[34]
16	Desmodin [apigenin 6-*C*-β-D-xylopyranosyl-2''-*O*-β-D-glucopyranoside]	*Lespedeza cuneata*	Aerial part	[36]
17	Homoadonivernite [luteolin 6-*C*-β-D-xylopyranosyl-2''-*O*-β-D-glucopyranoside]	*Lespedeza cuneata*	Aerial part	[36]
18	Isoorientin	*Passiflora edulis*	pericarp	[33]
		Clematis rehderiana	Aerial parts	[37]
		Araucaria excelsa	needles	[38]
		Centaurea gigantea	aerial	[26]
		Ficus microcarpa	leaves	[39]
		Oxalis corniculata	leaves	[40]
		Cymbopogon citratus	Leaves	[29]
19	Isosaponarin	*Ficus microcarpa*	leaves	[39]
20	Isoschaftoside	*Dendrobium huoshanense*	Leaves and stems	[41]
		Desmodium uncinatum	roots	[42]
21	Isospinosin	*Ziziphus jujuba mill*	seeds	[43]
22	Isoswertiajaponin	*Trollius ledebourii*	flower	[31]
		Celtis africana	aerial	[28]
23	Isoswertisin	*Trollius ledebourii*	flower	[31]
		Celtis africana	aerial	[28]
24	Isovitexin	*Clidemia sericea*	leaves	[27]
		Araucaria excelsa	needles	[38]
		Wilbrandia ebracteata	roots	[44]
		Ficus microcarpa	leaves	[39]
		Lespedeza cuneata	Aerial	[45]
		Petrosavia sakuraii	aerial	[46]
		Oxalis corniculata	leaves	[40]
		Trigonella foenum-graecum	seeds	[47]
25	Orientin	*Trollius ledebourii*	flower	[31]
		Araucaria excelsa	needles	[38]
		Centaurea gigantea	aerial	[26]

Entry	C-Glycosylflavonoid*	Source		Ref
		Plant	Plant part	
		Potentilla astracanica	aerial	[48]
		Celtis africana	aerial	[28]
		Astragalus corniculatus	aerial	[49]
26	Spinosin	*Passiflora edulis*	pericarp	[33]
		Ziziphus jujuba mill	seeds	[43]
27	Swertiajaponin	*Cymbopogon citratus*	Leaves	[29]
		Knautia montana	flower	[50]
28	Swertisin	*Wilbrandia ebracteata*	roots	[44]
		Ziziphus jujuba mill	seeds	[43]
		Enicostemma hyssopifolium	Aerial parts	[51]
		Gentiana algida	flower	[52]
		Oxalis corniculata	leaves	[40]
		Astragalus corniculatus	aerial	[49]
29	Vicenin-2	*Peperomia blanda*	Aerial part	[53]
		Passiflora edulis	pericarp	[33]
		Petrosavia sakuraii	aerial	[46]
		Trigonella foenum-graecum	seeds	[47]
30	Vitexin	*Clidemia sericea*	leaves	[27]
		Araucaria excelsa	needles	[38]
		Petrosavia sakuraii	aerial	[46]
		Celtis africana	aerial	[28]
		Trigonella foenum-graecum	seeds	[47]
New compounds				
31	2'',6''-*O*-digalloylvitexin	*Clidemia sericea*	leaves	[27]
32	2''-*O*-α-L-Rhamnopyranosyl-6''''-*O*-dihydoferuloylsaponarin	*Dianthus japonicus*	aerial	[54]
33	6'''-(4''''-*O*-β-D-glucopyranosyl)vanilloylspinosin	*Ziziphus jujuba mill*	seeds	[43]
34	6''-*O*-acetylisoorientin	*Comastoma pedunlulatum*	aerial	[55]
35	6''-*O*-(*E*)-*p*-coumaroylisovitexin	*Clematis rehderiana*	Aerial parts	[37]
36	6-*C*-(α-D-arabinopyranosyl)-8-*C*-[(2''-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl]apigenin	*Dendrobium huoshanense*	Leaves and stems	[41]
37	6-*C*-(α-D-arabinopyranosyl)-8-*C*-[(2''-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranosyl]apigenin	*Dendrobium huoshanense*	Leaves and stems	[41]
38	6-*C*-(β-D-xylopyranosyl)-8-*C*-[(2''-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl]apigenin	*Dendrobium huoshanense*	Leaves and stems	[41]
39	6-*C*-[(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-8-*C*-(α-D-arabinopyranosyl)apigenin	*Dendrobium huoshanense*	Leaves and stems	[41]
40	6-*C*-α-D-arabinofuranosylapigenin	*Primula spectabilis*	leaves	[34]
41	6-*C*-β-D-(2'''-sinapoyl)arabinopyranosyl-8-*C*-β-D-galactopyrasoyl-apigenin	*Triticum aestivum*	bran	[56]
42	6-*C*-β-D-galactopyrasoyl-8-*C*-β-(2'''-sinapoyl)arabinopyranosylapigenin	*Triticum aestivum*	bran	[56]
43	6-*C*-β-D-glucosyl-7-*O*-methylchrysin	*Sphaeranthus indicus*	Aerial part	[57]
44	7-*O*-methyl-2''-*O*- α-L-rhamnopyranosylorientin	*Celtis africana*	aerial	[28]

Entry	C-Glycosylflavonoid*	Source		Ref
		Plant	Plant part	
45	7-*O*-methyl-6''-*O*-α-L-rhamnopyranosylvitexin	*Celtis africana*	aerial	[28]
46	7-*O*-α-D-arabinofuranosylorientin	*Primula spectabilis*	leaves	[34]
47	7-*O*-β-D-xylopyranosylvitexin	*Peperomia blanda*	Aerial part	[53]
48	8-*C*-(2''-*O*-β-D-apiofuranosyl)-β-D-glucopyranosylapigenin	*Ficus microcarpa*	leaves	[39]

*Compound names given are those reported in the original paper.

The bioactivity studies, conducted over the last years have shown their potential as new leads for human health. The antioxidant capability still is the main property of these molecules but other bioactivities were also reported (table 2, entry 1). Biological properties such as hypoglycemic, antihyperglycemic and anti-inflammatory (table 2, entry 2-5) have been once again reported for this type of compounds, although new activities have been assigned to glycosylflavonoids such has antimalarial and urease inhibitors (table 2, entry 6 and 7).

Table 2 – Bioactivity of C-glycosylflavonoids (covering the last five years)

Entry	Activity	Compound	Ref.
1	Antioxidant	2''-*O*-rhamosylvitexin	[28]
		6''-*O*-(*E*)-*p*-coumaroylisovitexin	[37]
		(2*R*,3*R*)-6-*C*-β-D-glucopyranosyltaxifolin	[35]
		(2*R*,3*R*)-6-*C*-β-D-glucopyranosylaromadendrin	[35]
		7-*O*-methyl-2''-*O*- α-D-Rhamnopyranosylorientin	[28]
		7-*O*-methyl-6''-*O*-α-D-Rhamnopyranosylvitexin	[28]
		8-*C*-(2''-*O*-β-D-apiofuranosyl)-β-D-glucopyranosylapigenin	[39]
		Isoorientin	[29, 37, 39]
		Isosaponarin	[39]
		Isoswertiajaponin	[28]
		Isoswertisin	[28]
		Isovitexin	[39, 47]
		Orientin	[28]
		Vicenin-2	[47]
		Vitexin	[28, 47]
2	Hypoglycemic	Swertisin	[51]
3	Antihyperglycemic	Isovitexin	[44]
		Swertisin	[44]
4	Anti-inflammatory	Isovitexin	[47]
		Vicenin-2	[47]
		Vitexin	[47]
5	Anti-inflammatory	Spinosin	[58]
6	Antimalarial	2'',6''-*O*-digalloylvitexin	[27]
		2''-*O*-galloylvitexin	[27]
7	Urease inhibitors	Isoswertiajaponin	[28]
		Isoswertisin	[28]
		Orientin	[28]
		Vitexin	[28]

1.4 Fries-type Reactions for the *C*-Glycosylation of Phenols

The next subchapter was published as a review on Current Organic Chemistry as:

"Fries-type Reactions for the *C*-Glycosylation of Phenols" Rui G. dos Santos. , Ana R. Jesus, João M. Caio, Amélia P. Rauter, *Curr. Org. Chem*., **2011**, 15, 128-148.

This review gives an extended overview on the work developed so far, regarding the use of the Fries-Type Rearrangement for the coupling of sugars to phenols. João Caio gave his contribution on data regarding boron trifluoroetherate catalysis, while Ana Rita Jesus worked on ionic liquids containing a protic acid and montmorillonite K-10 as catalysts.

Fries-type Reactions for the *C*-Glycosylation of Phenols

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Abstract: *C*-Glycosylphenols and -polyphenols occur widely in nature and present a variety of biological properties, namely antitumor, antibacterial and antidiabetic activities. Synthetic access to such structures relies mainly on efficient methodologies for phenols *C*-glycosylation. In the past few years major advances have been described addressing the use of Fries rearrangement to obtain a diversity of *C*-glycosyl compounds. Herein we survey the glycosyl donors and the activators used for this reaction, covering both early work and recent developments in the area. Reaction mechanism and reaction outcome control, aiming at regio- and stereoselectivity are also discussed.

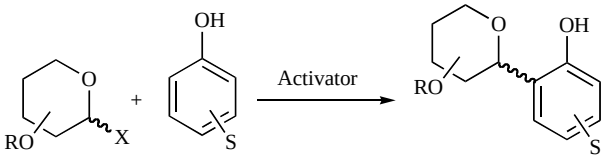
Keywords: Phenols *C*-glycosylation, Fries-type rearrangement, activator.

1. INTRODUCTION

The Fries-type reaction is a very straightforward and useful tool for the synthesis of *C*-glycosylphenols starting from a glycosyl donor, a phenol and an activator (Chart **1**). This one-pot reaction proceeds through an initial glycoside rapidly formed at low temperature, which undergoes, by warming up, an *in situ* *O*→*C*-rearrangement to give regioselectively an *ortho*-hydroxy *C*-glycosyl aromatic derivative in good yield [1].

Compounds possessing a sugar attached to a polyphenol moiety by a C-C bond at the anomeric position are quite common in nature as plant or bacteria secondary metabolites [2,3].

This structure-type is also found in insects, e.g. the red dye carminic acid **1** (Fig. **1**), isolated from the cochineal insects (*Dactylopius* sp.) [3].



X- Leaving group
S- Substituent

Chart 1. Phenol *C*-glycosylation via Fries-type rearrangement.

The plant *C*-glycosylpolyphenols are typically derivatives of six chromophore types: flavones (e.g. isoorientin **2**) [4,5], isoflavones (e.g. puerarin **3**) [5,6], chromones anthrones (e.g. aloin **5**), xanthones (e.g. mangiferin **6**), and gallic acids (e.g. bergenin **7**) [3] (Fig. **1**).

The bioactivities exhibited by *C*-glycosylpolyphenols have already been reviewed [2,5]. This group of compounds includes the antibiotics pluramycins (e.g. Pluramycin A **8)** [7] and the angucyclines (e.g. aquayamycin **9**) [8], some of which exhibiting notable antibacterial and antitumor activities [2]. Angucyclines were also reported to act as inhibitors of oxidative enzymes and to be potent inhibitors of blood platelet aggregation [2,8].

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The unique *C*-glycosylphenol moiety is embodied in a variety of biologically important natural products and the C-C bond appears to be of great importance since it is not enzymatically degraded *in vivo* and is stable under physiological conditions, while the glycosidic O-C bond, part of an acetal, is easily cleaved in acidic medium and by enzymes.

The synthesis of this type of compounds has become a challenge for organic chemists, who have developed various approaches aiming at a direct access to such bioactive principles [2, 9-13]. Firstly, formation of the C-C bond between carbohydrate templates and electron-rich aromatic moieties was accomplished by Friedel-Crafts reaction [9, 14-16]. Nowadays a variety of methods for *C*-glycosylation is known, including nucleophilic attack of aromatic Grignard reagents to glycosyl halides [10,11], the use of anomeric anions via lithiated compounds [6], reactions mediated by transition metals or samarium iodide [17], intermolecular free radical reactions and *C*-glycosylation through intramolecular aglycon delivery [12]. The latter approach covers the strategy first developed by the Suzuki [18] and Kometani [19] groups, which consists of a straightforward methodology for the *C*-glycosylation of phenols by a Lewis acid catalysed rearrangement of a glycoside to a *C*-glycosyl derivative, known as Fries-type reaction. It has been exploited by various authors up to the present days and successfully applied to the synthesis of *C*-glycosylflavonoids [20-23] and of other complex natural products [24,25].

2. REACTION MECHANISM

The Fries-type reaction involves a glycosyl donor, an acceptor and an activator and takes advantage of a migration of the glycosyl moiety from the oxygen atom to the carbon in *ortho* position, which proceeds in highly regio- and stereoselective manner. The reaction mechanism involves two crucial steps. The first one consists of the activation of the glycosyl donor (type **10**) with a Lewis acid and subsequent coupling with a phenol derivative **11** to afford the glycoside **12** (Scheme **1**). The following step is based on the rearrangement of **12** leading to the *C*-glycosyl derivative **13** via ion pair **A**, which undergoes an irreversible Friedel-Crafts coupling, regioselectively at the *ortho* position to the phenolic hydroxyl group. The first stage is conducted at low temperature (commonly ranging from -78 ºC to -20 ºC), which allows the prompt formation of the glycoside, and is followed by a slow increase of the temperature permitting the *in situ* rearrangement to its *C*-congener [26].

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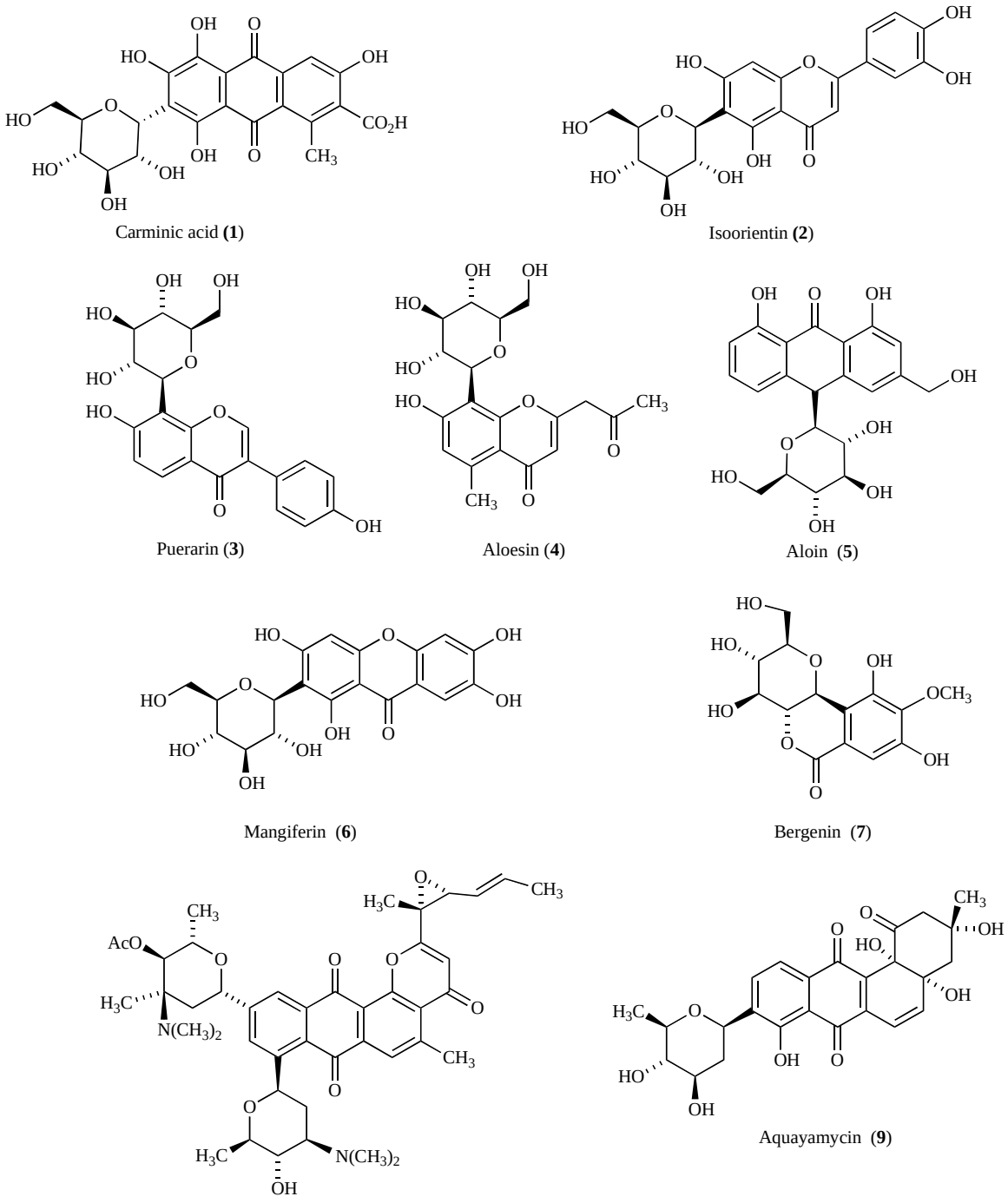
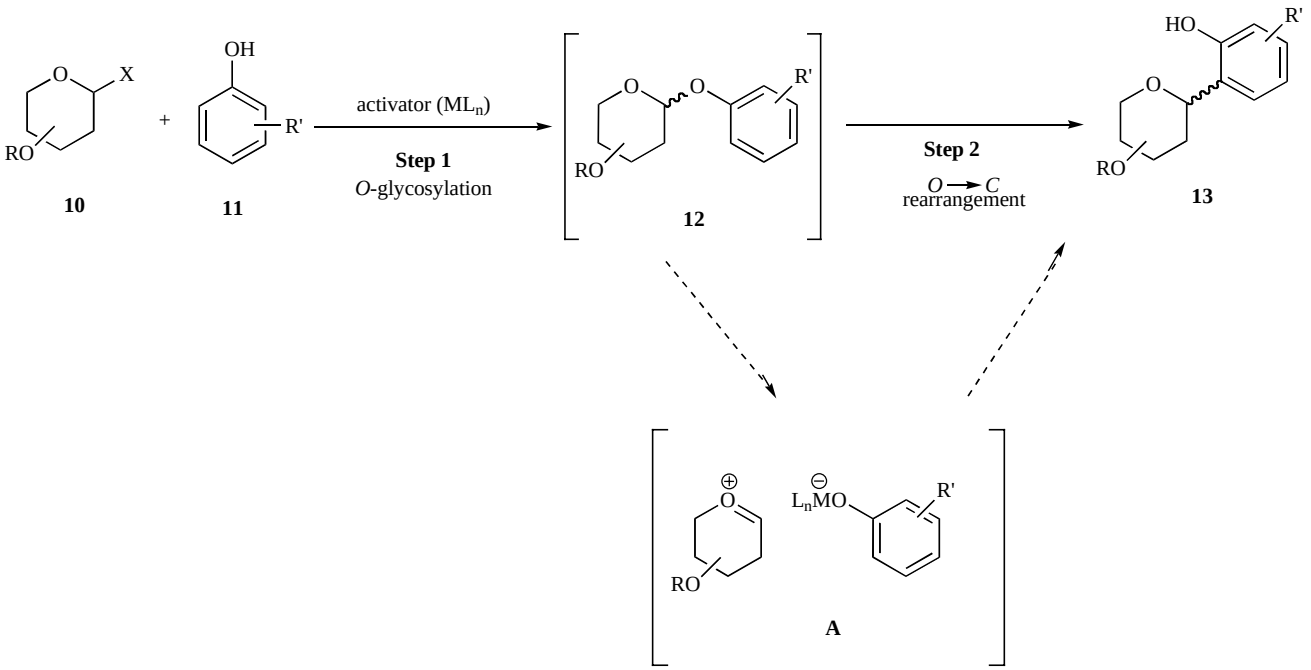


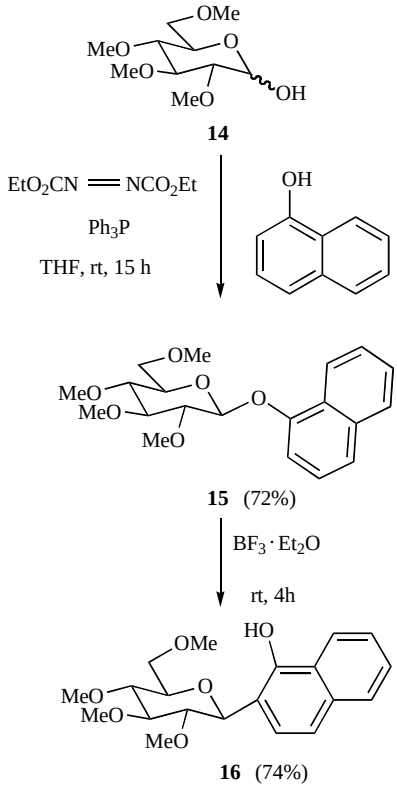
Fig. (1). Examples of natural *C*-glycosylpolyphenols.

The latter step was confirmed by an experiment described on Kometani's first papers concerning these reactions [19]. The glycoside **15**, accessed under the Mitsunobu conditions, was treated with boron trifluoride etherate at room temperature leading to the corresponding *C*-glycosylnaphthol **16** (Scheme **2**).

The stereochemical outcome of the reaction is known, and in most cases one anomer is produced predominantly. The β-configuration is thermodynamically favored for D-glucose [27-29], D-galactose [30] and D-*arabino*-hexopyranose series [1,15,28,29,31]. However, Suzuki & co-workers [32] have shown that when the reaction is quenched at low temperature, a mixture of both anomers is found. The explanation for this relies on the Lewis acid mediated ring opening-closure of the compound formed under kinetic control to give the thermodynamically controlled product. Hence, this additional step can be outlined as an anomeric conversion of the kinetically formed α/β-mixture to the more stable thermodynamical form with an equatorial anomeric substituent, proceeding via the *ortho*-quinone methide, since at this point the anomeric effect is no longer present [15,26,31] (Scheme **3**). Kumazawa *et al*. [33] showed that the α-configuration was preferred in *C*-glycosylation of L-mannose derivatives, while for D-mannose scaf-



Scheme 1. Mechanism proposed for phenols *C*-glycosylation by Fries-type rearrangement [26].



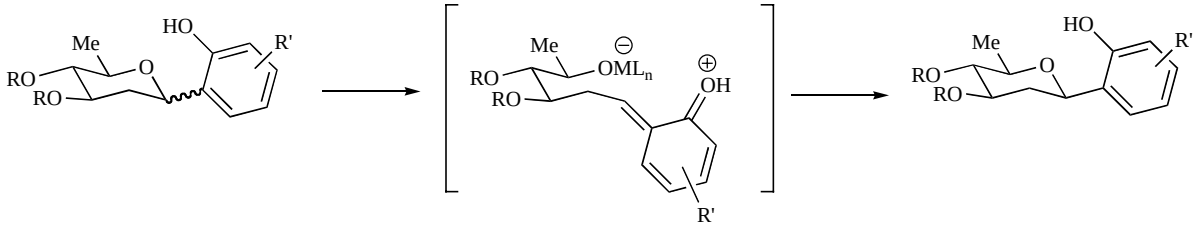
Scheme 2. *O*→*C*-Glycosyl rearrangement promoted by boron trifluoride etherate [19].

folds, the anomeric configuration of the major product formed depended on the reactants and reaction conditions used.

The exclusive formation of the α-*C*-mannosylphenols was described by Palmacci & Seeberger [17] when perbenzyl D-mannosyl phosphate reacted with electron-rich phenol acceptors, in the presence of trimethylsilyl triflate (1.2 equiv.) at 0 ºC for 30 min. However, reaction of perbenzyl D-mannosyl fluoride mediated by boron trifluoride etherate (2.1 equiv.) gave mainly the β-*C*-mannosyl anomer in 2.5 h reaction time and temperature increase from -78 ºC to 0 ºC [33]. These authors suggested that an intermediate α-*C*-mannosyl compound is formed resulting from the attack of the nucleophile to the α-face of the oxonium ion, obtained by cleavage of the glycoside promoted by the Lewis acid, since the β-face is blocked by the benzyloxy group. It adopts the conformation in which the aglycon is oriented equatorially in order to avoid 1,3-diaxial interactions resulting from the aglycon in axial orientation. At higher temperature, anomerization occurs in order to avoid 1,3-diaxial interactions between C-3 and C-5, leading to the β-anomer with the aglycon equatorially oriented, in which no such diaxial interactions are possible. The occurred anomerization may provide an experimental evidence for the strong 1,3-diaxial interaction between substituents at C-3 and C-5 of the α-*C*-mannosyl moiety. Also the anomeric configuration resulting from *C*-glycosylation of *p*-methoxyphenol with D-ribofuranosyl fluoride strongly depends on the Lewis acid and the number of equivalents used [18]. At the same temperature, both the activators boron trifluoride etherate and tin chloride promoted α-selectivity, which increased when the equivalents used decrease from 3 to 0.5, while for hafnium cyclopentadienyl chloride/silver perchloride a β-selectivity was observed. However, when the reaction was promoted by boron trifluoride etherate, a temperature increase resulted also in β-selectivity. Hence, glycosyl donor, activator and temperature have to be carefully selected for the synthesis of the target *C*-glycosylphenol.

3. *O*-/*C*-GLYCOSYL RATIO CONTROL

The *O*-/*C*-glycosyl ratio depends on the glycosyl donor and nucleophile reactivity, and can be controlled by selecting the temperature at which the reaction is quenched. However, glycosyl donor and acceptor protecting groups as well as the activator and sometimes the solvent also play an important role. Raising the temperature promotes, in general, the formation of the *C*-glycosyl com-



Scheme 3. Conversion of the kinetically formed α/β-mixture into the β-D-*C*-glycosylphenol (thermodynamically controlled product).

pound. For the same equivalents of boron trifluoride etherate, the increase of the final reaction temperature from -20 ºC to 15 ºC led to the exclusive formation of the *C*-glycosyl compound, while at -20 ºC the ratio *O*-/*C*-glycosyl derivative was about 1:1, as reported by Matsumoto and co-workers [18] for the Fries-type rearrangement of *p*-methoxyphenol with D-ribofuranosyl fluoride. If acceptors are less electron rich phenolic compounds, namely *p*-methylphenol or 7-hydroxycoumarin, only glycosides are obtained by reaction with benzyl protected trifluoro- or trichloroacetimidates promoted by TMSOTf [34,35].

Regarding the activator, the number of equivalents used and its strength are the issues to be considered. In the presence of boron trifluoride etherate, glycosides could be isolated even when the reaction temperature was raised to room temperature. Hence, this activator does not seem to be so effective for *C*-glycosylation as tin chloride or biscyclopentadienyl hafnium dichloride/silver perchlorate, which led exclusively to the desired C-glycosyl derivative using the same number of Lewis acid equivalents [15,18,32].

The choice of the glycosyl donor protecting groups may also be decisive for *C*-glycosylation. It was reported that, depending on the promoter and the reaction conditions used, sugar donors which possess acyl protecting groups at non-anomeric positions may lead to *C*-glycosyl derivatives in lower yield than that obtained with benzyl or methyl ether protected donors [27,36]. Schmidt and co-workers [37] have shown that reaction of phenols with acetyl protected sugar trichloroacetimidates in the presence of TMSOTf afforded only the glycosides, while benzyl protected sugar trichloroacetimidates gave mainly *C*-glycosyl compounds.

Another parameter to be controlled is the reaction time. Seeberger and co-workers [17] have shown that with glycosyl phosphates as donors and TMSOTf as promoter, short reaction times afforded mostly the glycoside derivatives. After 15 min, the glycoside was the only product isolated, whereas 30 min to 3 h were needed to afford *C*-glycosylphenols as the main products.

For a successful synthesis of *C*-glycosylphenols/polyphenols, the matching of the glycosyl donor and the aromatic acceptor reactivity, as well as the choice of the activator become crucial, as illustrated by the examples presented in this review. In the next sections a survey on the activators will be given and discussed in terms of efficiency, reaction stereoselectivity and application for the synthesis of a variety of natural and synthetic products.

4. ACTIVATORS

The activators play an important role in promoting both the *O*-glycosylation and the *O*→*C* rearrangement. The most common promoters are Lewis acids but recently a protic acid in ionic liquids [38] was also reported. Also the heterogeneous catalyst Montmorillonite K-10 [39] demonstrated to be efficient in this reaction.

One of the first Lewis acids employed was boron trifluoride etherate ($BF_3{\cdot}Et_2O$) [19], which is presently still in use [23]. Tin chloride, trimethylsilyl trifluoromethanesulfonate (TMSOTf), the system biscyclopentadienyl hafnium dichloride/silver perchlorate (Cp_2HfCl_2 – $AgClO_4$), and more recently scandium (III) trifluoromethanesulfonate have also succeeded to promote the Fries-type rearrangement. Their efficacy will be addressed and illustrative examples, reported in the literature, will be given.

a. Boron Trifluoride Etherate

Activation of glycosyl fluorides with $BF_3{\cdot}Et_2O$ for Fries-type rearrangement is very well documented [5,19,23,26,27,40]. The reaction is conducted mainly in dichloromethane or dichloroethane as solvent in the presence of molecular sieves (4 Å or 5 Å) or drierite. The initial temperature is often -78 ^oC, and then raised to the temperature for which the *C*-glycosyl derivative is the major or single product. *C*-Glycosylation of phloroglucinol derivatives has been widely studied and the results presented in Table **1** show the influence of the temperature control and the acceptor nucleophilicity on the reaction outcome. *C*-glycosyl compounds are the major products formed when the reaction starts at -78 ^oC (Table **1**, entries 3, 4, 7) while reaction with less reactive nucleophiles, starting at -20 ^oC or at higher temperature, gives only glycosides (Table **1**, entries 1, 6) or a mixture of glycosides and *C*-glycosyl compounds (Table **1**, entries 2 and 8). This effect is noticed even in the presence of acyl protected donors and reactive acceptors are again required for a successful *C*-glycosylation reaction. Hence, nucleophile substitution and protection influence the reaction products obtained (Table **1**, entries 2-8). For example phloroglucinol nucleophiles with an acetyl group in *ortho* position to the free hydroxyl group afforded exclusively *C*-glycosyl derivatives in good yield (Table **1**, entries 3, 4), while phloroglucinol with the acetyl group in *para* position to the free OH (Table **1**, entry 5) led mainly to glycoside synthesis [27].

Reaction with the flavan (Table **1**, entry 8) gave as major product the 6-*C*-glycosylflavan, the precursor used for total synthesis of Flavocommelin, a component of the blue supramolecular pigment from *Commelina communis* [22].

Preparation of *C*-glycosylphenols with permethylated ribosyl fluoride was also promoted by this mild activator to afford the target *C*-glycosyl compounds in high yield and stereoselectivity (Table **1**, entries 16-18). When the reaction of *p*-methoxyphenol was stopped at +15 ºC, the target *C*-glycosyl phenol was obtained in 85% yield (Table **1**, entry 16), while the reaction stopped at -20 ºC gave a mixture of the glycoside precursor in 41% yield and the *C*-glycosylphenol in 50 % yield [18], confirming the expected temperature control for the reaction outcome. However, the electron richer resorcinol monomethyl ether gave a complex mixture of products under the same conditions, from which diarylated products were also isolated in low yield [18]. Nevertheless, the electron poorer corresponding ester protected compounds (Table **1**, entries 17, 18) could be converted into their *C*-glycosyl derivatives in high yield in a regio- and stereoselective manner. The low yield for phe-

Table 1. Fries-type Rearrangement Promoted by $BF_3{\cdot}Et_2O$ in Dichloromethane

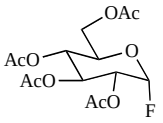
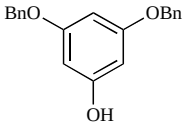
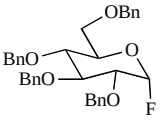
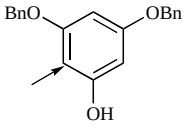
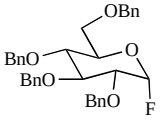
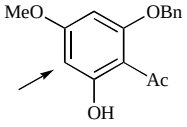
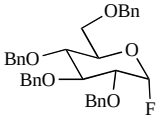
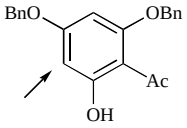
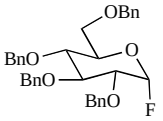
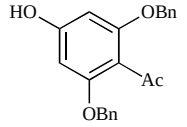
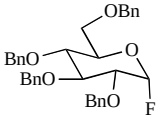
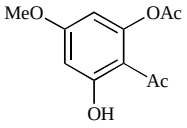
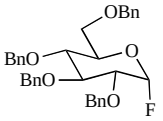
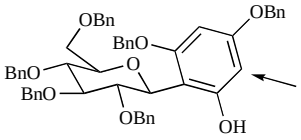
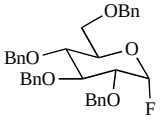
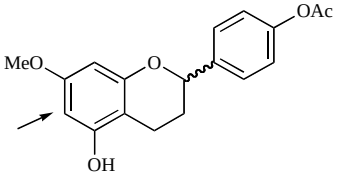
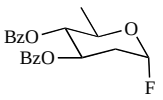
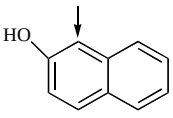
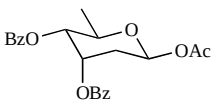
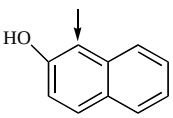
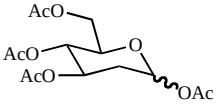
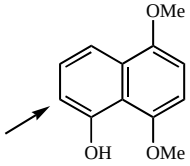
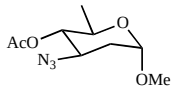
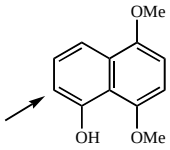
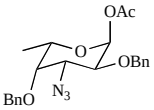
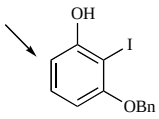
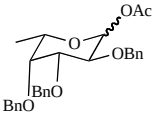
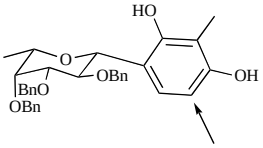
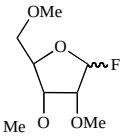
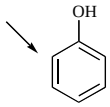
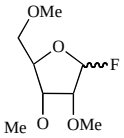
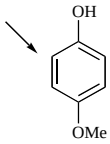
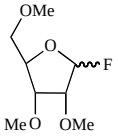
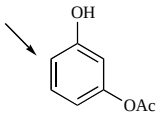
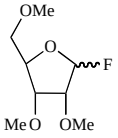
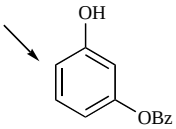
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (ºC)	*C*-/*O*-Glycosyl Derivatives Yield (%)
1 [27]			- 20	95 (*O*,α)
2 [40]			-20 → rt	24 (*C*,β) 12 (*O*,α) 6 (*O*,β)
3 [27]			-78 → rt	78 (*C*,β)
4 [23]			-78 →0	96 (*C*,β)
5 [27]			-78 → -5	58 (*O*,α)
6 [27]			-20 → rt	52 (*O*,α)
7 [40]			-78 →0	88 (*C*,β)
8 [22]			-10 → rt	56 (6-*C*,β) 7 (8-*C*,β) 13 (*O*,α) 6 (*O*,β)
9 [26]			-78 →0	70 (*C*, α/β = 3.4/1) 28 (*O*, α/β = 4.6/1)
10 [32]			-78 → rt	75 (*C*, α/β = 1/4)
11 [41][a]			rt	94(*C*,β)

Table 1. contd…

Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-/*O*-Glycosyl Derivatives Yield (%)
12 [42][a]			0	60 (*C*,β)
13 [43]			-78 → +40	39 (*C*,β) 9 (*C*,α)
14 [44]			-78 → +10	83 (*C*,β)
15 [18][b]			-78 →-5	45 (*C*,α/β = 5/1) 28 (*O*,α/β)
16 [18][b]			-78 → +15	85 (*C*,α/β = 1/9)
17 [1][b]			-78 → -10	81(*C*,α/β = 1/17)
18 [1][b]			-78 → -20	77 (*C*,α/β = 1/19)

[a]Acetonitrile was the solvent used; [b]α/β ratio of the glycosyl fluoride = 1/1

nol *C*-glycosylation (Table **1**, entry 15) may be rationalized in terms of the low reactivity of the nucleophile and/or the low temperature at which the reaction was quenched, preventing conversion of the glycoside initially formed into the *C*-glycosyl derivative and further α/β anomerization. Major formation of the kinetically controlled *C*-olivosyl naphthol α-anomer (Table **1**, entry 9) occurred when the reaction was accomplished using 2,6-dideoxy benzoyl protected fluoride as starting material and quenched at 0 °C [26].

Anomeric acetates proved to be efficient donors for *C*-glycosylation promoted by $BF_3{\cdot}Et_2O$, e.g. the acyl protected form of D-digitoxose (Table **1**, entry 10), which is a 2,6-dideoxy sugar present in *Digitalis* glycosides [32].

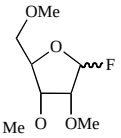
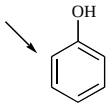
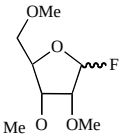
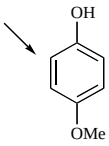
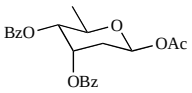
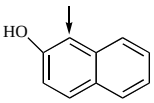
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranose (Table 1, entry 11) was used for β-*C*-glycosylation of 5,8-dimethoxy-naphtalen-1-ol in acetonitrile, the key step in synthetic approaches to the angucycline antibiotics [41]. The fucosyl and 3-azido fucosyl acetates (Table **1**, entries 13, 14) were also successfully *C*-linked to resorcinol derivatives in the presence of this activator [43, 44]. 3-Azido methyl glycosides of D and L series could be *C*-linked to naphthols in high yield using $BF_3{\cdot}Et_2O$ (2.0 equiv.) in acetonitrile at 0 °C, giving the β-*C*-glycosyl compounds, as illustrated in entry 12 [42]. The use of acetonitrile and highly reactive naphthols allowed *C*-glycosylation to be performed at room temperature in 15 min (Table **1**, entry 11) or at 0 °C in 2 h (Table **1**, entry 12) resulting in a clean reaction and formation of a single β-*C*-glycosyl compound.

In conclusion, boron trifluoride etherate proved to efficiently promote reaction even with non-activated sugars (e.g. methyl glycosides) leading to β-*C*-glycosylation. The structure of nucleophile and donor, the temperature and the solvent must be controlled to achieve the highest regio- and stereoselectivity.

b. Tin Chloride

Another Lewis acid tested for this type of reaction was tin tetrachloride ($SnCl_4$), a promoter which led to some significant α-selectivity for phenols *C*-glycosylation with ether protected ribofuranosyl fluoride, as shown in Table **2** (Entries 1-5).

Table 2. Fries-type Rearrangement Promoted by $SnCl_4$ or $BF_3{\cdot}Et_2O$ in Dichloromethane

Entry	Glycosyl Donor	Glycosyl Acceptor	Activator	Temp (°C)	*C*-/*O*-Glycosyl Derivatives Yield (%)
1 [18][a]			$BF_3{\cdot}Et_2O$	-78 → -5	45 (*C*,α/β=5/1) *28* (*O*)
2 [18][a]			$SnCl_4$	-78 → -10	51 (*C*,α/β=4/1) 9 (*O*)
3 [18][a]			$BF_3{\cdot}Et_2O$	-78 → -20	50 (*C*,α/β=1/1) 41 (*O*, α/β=1/7)
4 [18][a]				-78 → 15	85 (*C*, α/β=1/9)
5 [18][a]			$SnCl_4$	-78 → -15	73 (*C*,α/β=5/1) 6 (*O*)
6 [32]			$SnCl_4$	-78 →-25	99 (*C*, α/β = 1/14)

[a] α/β ratio of the glycosyl fluoride = 1/1.

When comparing $SnCl_4$ with $BF_3{\cdot}Et_2O$, the first promoter seems to be slightly more efficient than the second one for *C*-glycosylation (Table **2**, entries 2 and 5), when quenching the reaction at low temperature. The *O*→*C* rearrangement depends on the nature of the promoter as judged by the temperature required for completion of the reaction (Table **2**, entries 1 and 2, 4 and 5) [18]. Matsumoto *et al.* [32] have shown that this promoter is more efficient than $BF_3{\cdot}Et_2O$ for *C*-glycosylation of 2-naphthol with 1-*O*-acetyl-D-olivose donors leading to a higher yield and β-selectivity. These results can be explained by the thermodynamic equilibration to the more stable β-anomer from the initially formed anomeric mixture, in which the α-anomer was the major compound, detected when the reaction mixture was quenched at -35 °C (α/β = 2.8/1). (Table **1**, entry 10 and Table **2**, entry 6) [32].

c. Biscyclopentadienyl Hafnium Dichloride/Silver Perchlorate

Suzuki and co-workers [15] introduced the system biscyclopentadienyl hafnium dichloride/silver perchlorate (Cp_2HfCl_2-$AgClO_4$) as promoter for the *C*-glycosylation of phenols with glycosyl fluorides in dichloromethane. This highly regio- and stereoselective reaction afforded β-*C*-glycosylnaphthols and β-*C*-glycosylanthracenol (Table **3**, entries 1-3) in good yield starting from the D-olivosyl donor. Among the compounds synthesized, emphasis should be given to the β-*C*-olivosylanthracene, which corresponds to the *C*-glycosyl moiety of the antitumor antibiotic vineomycin B_2 (Table **3**, entry 3).

Both the *O*→*C* rearrangement and the anomerization step are facilitated by Cp_2HfCl_2-$AgClO_4$ more efficiently than by $BF_3{\cdot}Et_2O$, which promoted the reaction in lower yield and stereoselectivity (Table **1**, entries 9 and 15, Table **3** entries 1 and 4) [15].

O-Glycosylation with furanosyl fluorides is quite rapid in the presence of both the activators. However the *O*→*C* rearrangement seems to be highly dependent on the nature of the promoter, the metallocene-based promoter being the most efficient one for phenols *C*-glycosylation, leading to a higher β-stereoselectivity (Table **3**, entry 1 and Table **1**, entry 9) or to comparable results but at a lower final temperature (Table **3**, entry 5 and Table **1**, entry 16) [18].

d. Trimethylsilyl Trifluoromethanesulfonate

Trimethylsilyl trifluoromethanesulfonate (TMSOTf) is used in catalytic amount for the Fries-type rearrangement, which proceeds with high regio- and stereoselectivity, resulting in β-*C*-glycosyl derivatives in good yield (Table **4**). It is frequently the most used catalyst for this type of reaction. *O*-Benzyl protected trichloroacetimidates were used as glycosyl donors in reaction with phenol and naphthol derivatives (Table **4**, entries 1-10) [21, 34, 37] allowing the preparation of precursors of *C*-glycosylflavones [20, 37], *C*-glycosylflavanones [37], and bis(*C*-glycosyl)flavonoids (Table **4**, entry 10) [21]. More recently, perbenzylated gluco-, galacto- and mannopyranosyl trifluoroacetimidates, which are more stable than trichloroacetimidates, were reported as glycosyl donors.

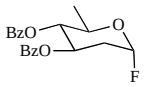
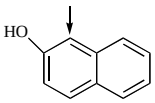
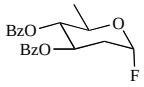
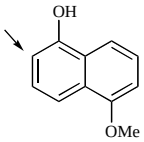
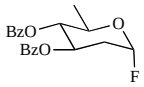
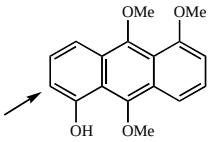
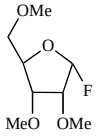
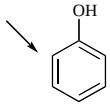
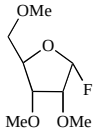
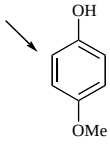
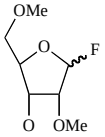
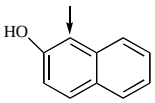
Depending on the nature of the nucleophile, *C*-glycosylation with mannosyl-/galactosyl donors takes place in higher yield than with glucosyl donors (Table **4**, entries 11-21) [35].

The regioselectivity of the *C*-glycosylation with trifluoroacetimidates was fully determined by the phenolic acceptor and the stereoselectivity was controlled by the glycosyl donor to give 1,2-*trans*-glycosylation products, namely the β-*C*-gluco-, β-*C*-galacto- and α-*C*- mannopyranosyl derivatives [35].

The use of glycosyl phosphate donors also succeeded in constructing the *C*-aryl linkage in the presence of electron rich phenolic acceptors and TMSOTf [17].

The mannosyl phosphates led to higher yields than the glucosyl phosphates and the rearrangement was stereospecific leading to the exclusive formation of the α-*C*- aryl and β-*C*-aryl linkage, respectively (Table **4**, entries 22-26). Schmidt and co-workers [37] demonstrated that ether protected glucosyl donors are much more efficient for *C*-glycosylation of naphthols than esters when the reaction is promoted by TMSOTf. However the *C*-glycosylation of naph-

Table 3. Fries-type Rearrangement Promoted by Cp_2HfCl_2-$AgClO_4$

Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivatives Yield (%)
1 [26]			-78 →0	98 (α/β = 0/1)
2 [15]			-78 →0	78 (α/β = 0/1)
3 [15]			-78 →0	86 (α/β = 0/1)
4 [18][a]			-78 → -20	71 (α/β = 1/14)
5 [18][a]			-78 → -20	79 (α/β = 1/9)
6 [18][a]			-78 →0	92 (α/β = 1/9)

[a] α/β Ratio of the glycosyl fluoride = 1/1.

thols could be achieved with acyl protected 2-deoxyglycosyl donors in low to moderate yields (Table **4**, entries 27, 28) [45].

TMSOTf is also an efficient activator for *C*-glycosylation with unprotected free sugars and unprotected methyl glycosides in high yields (Table **4**, entries 33-49).

The combined use of TMSOTf-$AgClO_4$ allowed the activation of acyl protected glycosyl donors giving *C*-glycosyl compounds in good yield (Table **4**, entries 50-53, 58-60) [36, 46]. This system is also very efficient in promoting β-*C*-glycosylation with unprotected 2-deoxy sugars (Table **4**, entries 61 and 62) or unprotected methyl 2-deoxy glycosides (Table **4**, entries 54-57) [36].

A particular attention has been given over the last years to the use of these conditions, which avoid protection/deprotection steps and involve eco-friendly and shorter pathways to the target molecules, which include some key subunits for the synthesis of *C*-glycosylangucycline antibiotics [24, 36, 45-47].

e. Scandium(III) Trifluoromethanesulfonate

This transition metal-based Lewis acid is a highly efficient catalyst of the Fries-type rearrangement, in contrast to the previously mentioned Lewis acid promoters, which act stoichiometrically. Glycosyl acetates, including 2-deoxy- and 3-azido-3-deoxy derivatives were used as donors for the *C*-glycosylation of phenols (Table **5**). The β-stereoselectivity of the reaction is very high for all donors studied, with the exception of rhamnosyl acetate (Table **5**, entry 6). With this donor the α/β ratio depended on the reaction time, ranging from 1/1.9 after 1 h to 1/20 after 22 h. The efficiency of this catalyst depended also on the drying agent and the solvent used, and the best results were achieved with the addition of Drierite in the presence of dichloromethane as solvent [43].

When phenols with *ortho*-hydrogen bond acceptors were the nucleophiles used (Table **5**, entries 12-14), the intermediate glycosides could not be obtained by early quenching experiments at low temperature, indicating that these hydrogen bonded substrates undergo very easily *C*-glycosylation under these reaction conditions [43].

The antitumor pluramycins are bis-*C*-glycosylated aromatic compounds used as probes in biochemical research, due to their highly sequence-selective intercalation into DNA resulting in specific alkylation [7].

Their bioactivities and multiple applications have encouraged the development of synthetic methods targeting the bis-*C*-glycosylation of phenols, an attractive challenge for organic chemists.

Table 4. Fries-type Rearrangement in Dichloromethane Catalysed by TMSOTf (Entries 1 - 49) or by TMSOTf-$AgClO_4$ (Entries 50 – 62)

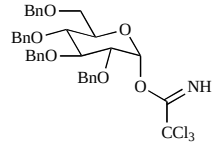
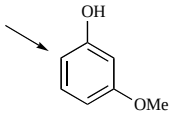
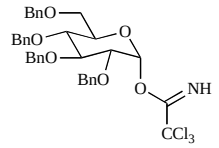
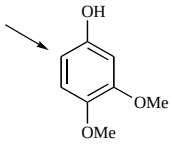
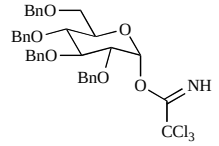
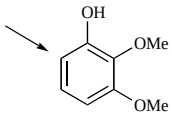
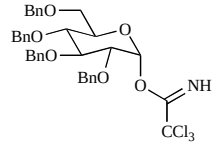
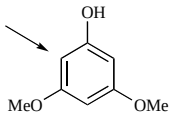
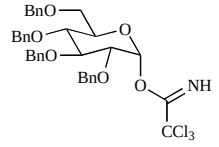
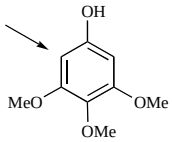
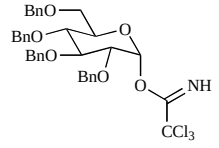
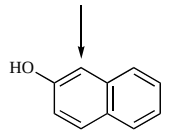
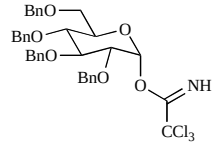
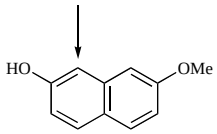
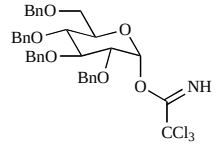
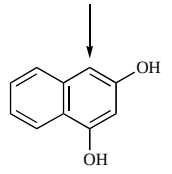
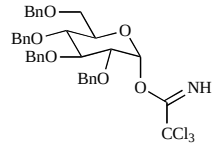
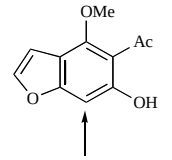
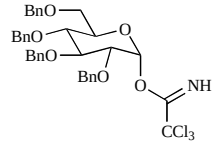
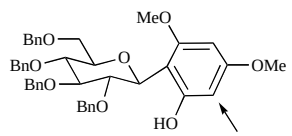
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivative(s) Yield (%)
1 [34]			-30 →rt	69 β only
2 [34]			-30 →rt	59-63 β only
3 [34]			-30 →rt	53-55 β only
4 [34]			-30 →rt	65-71 β only
5 [48]			-20 → rt	67 β only
6 [34]			-30 →rt	65 β only
7 [34]			-30 →rt	34 β only
8 [34]			-30 →rt	59 β only
9 [37]			-30 →rt	77 β only
10 [21]			-65 →rt	93 β only

Table 4. contd…

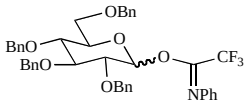
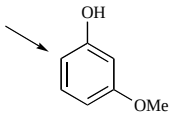
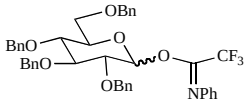
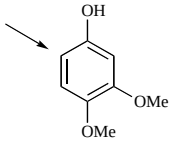
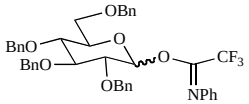
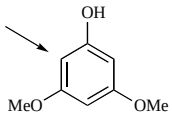
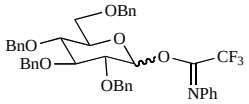
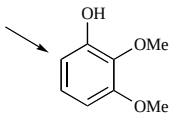
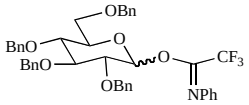
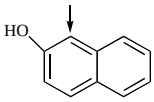
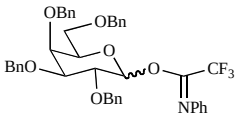
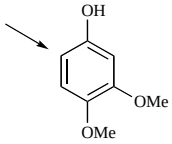
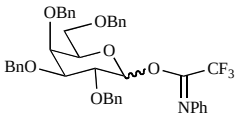
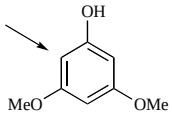
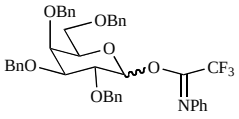
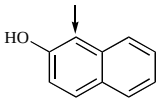
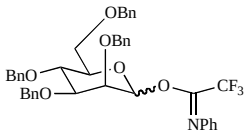
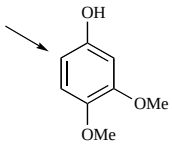
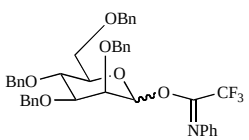
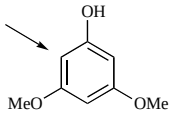
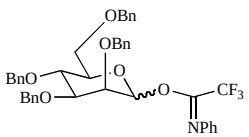
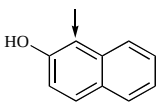
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivative(s) Yield (%)
11 [35]			0 → rt	27 β
12 [35]			0 → rt	21 β
13 [35]			0 → rt	37 β
14 [35]			0 → rt	71 β
15 [35]			0 → rt	69 β
16 [35]			0 → rt	45 β
17 [35]			0 → rt	67 β
18 [35]			0 → rt	76 β
19 [35]			0 → rt	48 α only
20 [35]			0 → rt	76 α only
21 [35]			0 → rt	96 α only

Table 4. contd…

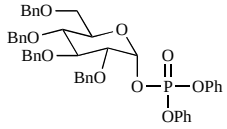
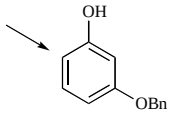
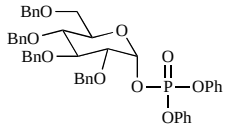
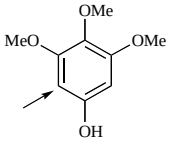
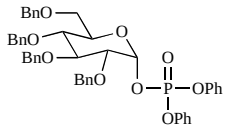
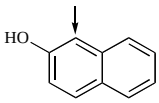
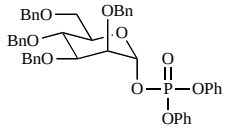
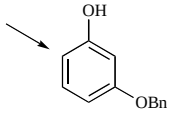
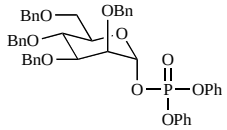
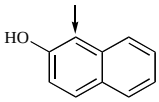
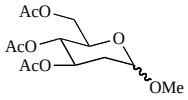
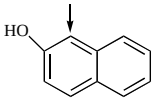
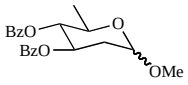
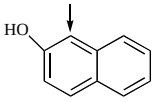
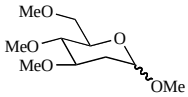
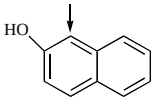
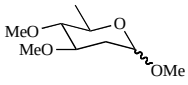
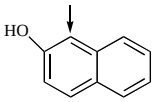
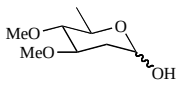
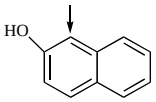
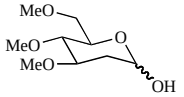
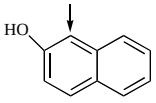
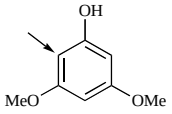
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (ºC)	*C*-Glycosyl Derivative(s) Yield (%)
22 [17]			0 → rt	62[a] β only
23 [17, 48]			0 → rt	57[b] β only
24 [17]			0 → rt	60[c] β only
25 [17]			0	82 α only
26 [17]			0	79 α only
27 [36,45]			25	19 β only
28 [36,45]			25	57 β only
29 [45]			25	99 β only
30 [45]			25	89 β only
31 [36]			25	89 α/β =1/>99
32 [36]			25	99 α/β =1/>99
33 [45]			25	98 β only

Table 4. contd…

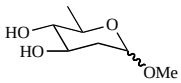
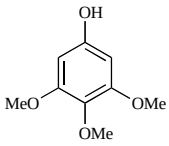
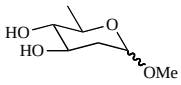
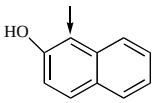
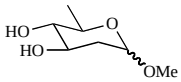
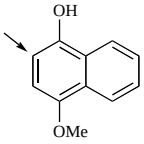
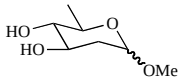
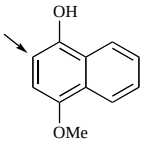
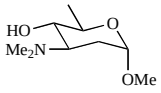
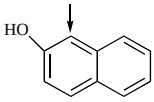
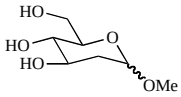
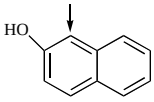
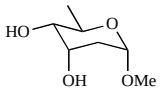
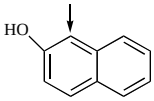
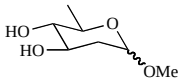
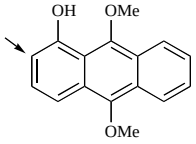
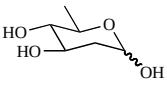
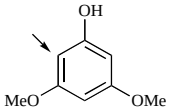
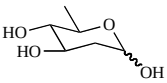
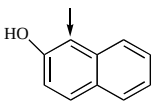
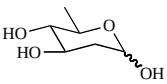
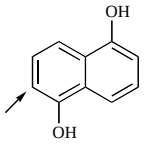
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivative(s) Yield (%)
34 [36]			25	91 α/β =1/>99
35 [45]			25	98 β only
36 [45]			25	79 β only
37 [49] [d]			25	64 β only
38 [45]			40	93 β only
39 [45] [d]			40	89 β only
40 [45]			25	91 β only
41 [36]			25	64 α/β = 1/>99
42 [45] [d]			25	71 β only
43 [45]			25	97 β only
44 [47] [d]			25	65 β only

Table 4. contd…

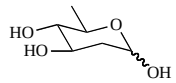
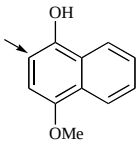
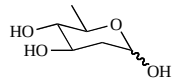
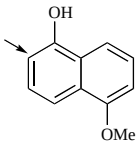
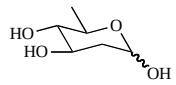
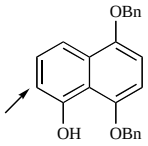
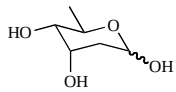
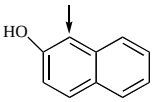
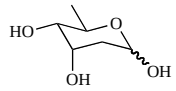
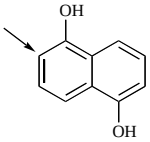
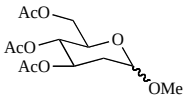
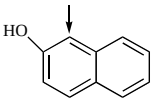
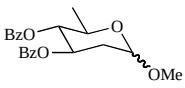
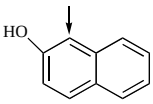
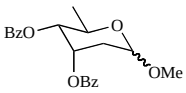
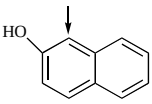
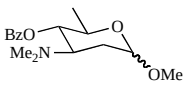
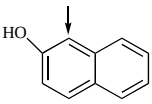
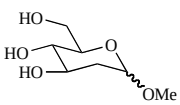
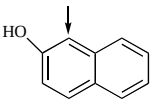
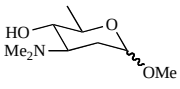
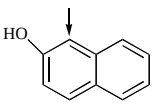
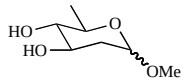
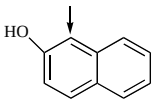
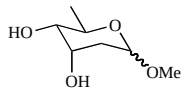
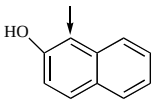
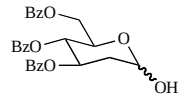
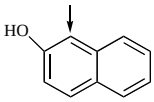
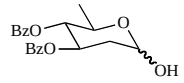
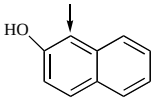
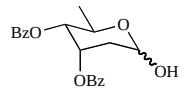
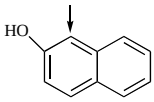
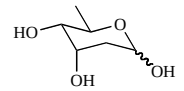
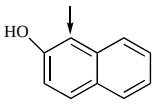
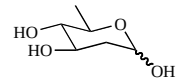
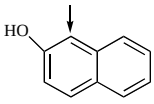
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivative(s) Yield (%)
45 [45][d]			25	63 β only
46 [45][d]			25	59 β only
47 [47][d]			25	27 β only
48 [45]			25	72 β only
49 [49][d]			25	61 β only
50 [36]			25	99 α/β = 1/>99
51 [36]			25	99 α/β = 1/>99
52 [36]			25	98 α/β = 1/87
53 [36]			40	99 α/β = 1/>99
54 [36][d]			25	86 α/β = 1/>99
55 [36]			40	72 α/β = 1/>99

Table 4. contd…

Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (ºC)	*C*-Glycosyl Derivative(s) Yield (%)
56 [36] [d]			25	91 α/β = 1/>99
57 [36] [d]			25	92 α/β = 1/32
58 [36]			25	85 α/β = 1/>99
59 [36]			25	99 α/β = 1/70
60 [36]			25	90 α/β = 1/15
61 [36][d]			25	84 α/β 1/97
62 [36][d]			25	92 α/β 1/>99

[a] *O*-glycoside in traces; [b] 13 % (*O*-glycoside,α/β=1/0) [c] 9 % (*O*-glycoside, α/β=1/0; [d] The solvent was acetonitrile

Matsumoto *et al.* [50] developed a highly stereoselective one-pot double *C*-glycosylation method to obtain bis(*C*-glycosyl)resorcinol derivatives under catalysis of $Sc(OTf)_3$ in the presence of Drierite (Table **5**, entries 2, 4, 5, 7-9, 15). Rhamnose-derived acetates, deoxy sugar and azido sugar acetates gave the corresponding bis(β-*C*-glycosyl) derivatives in good to high yield.

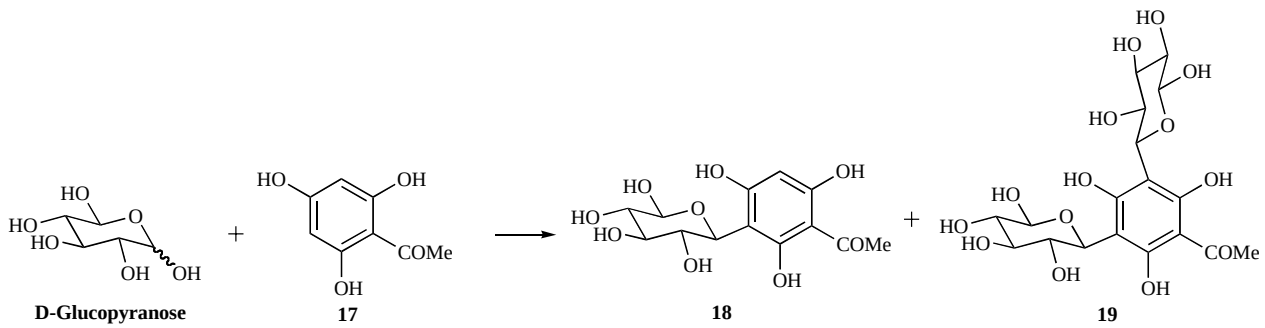
The intermediate mono-glycoside, mono-*C*-glycosyl and mono-*O*-mono-*C*-glycosyl derivatives could be detected although they were all converted throughout the temperature increase into the bis(β-*C*-glycosyl) derivative as the sole product. The bis(glycoside) was never detected, probably because the rearrangement of mono-glycoside to the mono-*C*-glycosyl isomer is faster than bis(glycoside) formation [50].

The reaction does not seem to be highly affected by conjugation of the aromatic ring with electron accepting groups such as the carbonyl group of ketones and esters, as shown in entries 8 and 9.

Direct *C*-glycosylation of unprotected polyphenols with unprotected sugars in aqueous media was achieved by Sato *et al.* [51] using $Sc(OTf)_3$ as reaction catalyst.

The most efficient solvent system was EtOH/water (2:1) and the reaction was run under reflux for 6.5 h– 9 h in the presence of the catalyst (0.2 -0.24 equiv.) to give mono-*C*-glycosyl- and bis(*C*-glycosyl) derivatives in ca. 40% yield each (Scheme **4**).

Despite the moderate yield, this methodology proceeds with high regio- and stereoselectivity, is simple and environmentally



D-Glucopyranose **17** **18** **19**

Scheme 4. *C*-glycosylation of phloroacetophenone (**17**) with D-glucose catalysed by $Sc(OTf)_3$ in water/ethanol (2/1). Conditions: $Sc(OTf)_3$ (0.2 equiv.), reflux, 9 h, **18** (43%), **19** (38%) or $Sc(OTf)_3$ (0.4 equiv.), reflux, 6.5 h, **18** (39%), **19** (40%).

Table 5. Fries-type Rearrangement Promoted by $Sc(OTf)_3$

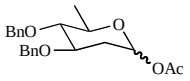
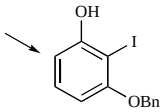
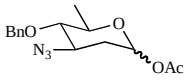
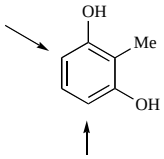
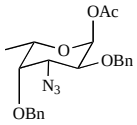
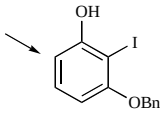
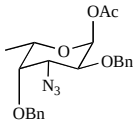
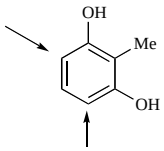
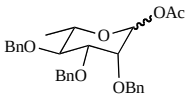
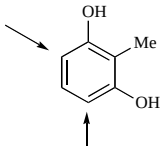
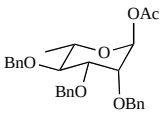
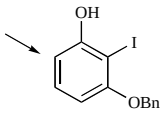
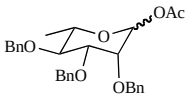
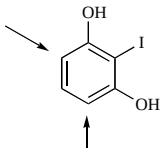
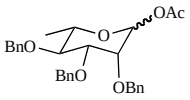
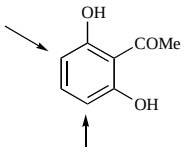
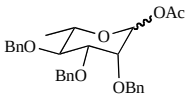
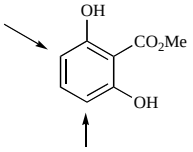
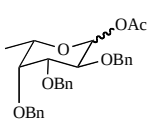
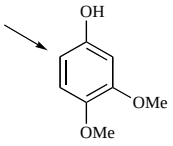
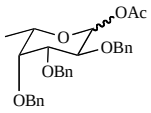
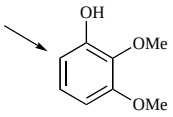
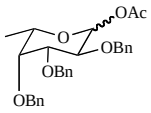
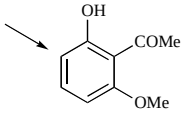
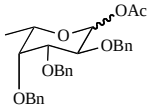
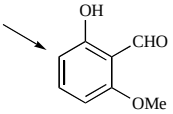
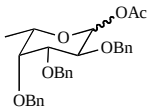
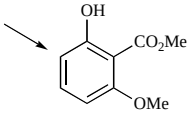
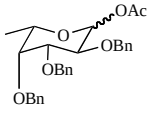
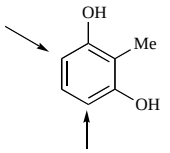
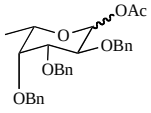
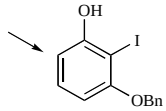
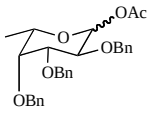
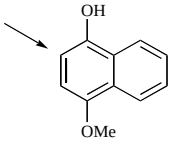
Entry	Glycosyl Donor	Glycosyl Acceptor	Temp. (°C)	*C*-Glycosyl Derivatives Yield (%) (α:β Ratio)
1 [43]			-30 → -10	89 (1:99)
2 [50]			20	62 β only
3 [43]			-30 → 12	78 (3:97)
4 [50]			25	86 β only
5[50]			25	79 β only
6 [43]			-30 → 25	82 (1:20)
7 [50]			25	78 β only
8 [50]			20	71 β only
9 [50]			15	79 β only
10 [43]			-30 → 25	95 (1:99)

Table 5. contd…

Entry	Glycosyl Donor	Glycosyl Acceptor	Temp (°C)	*C*-Glycosyl Derivatives Yield (%) (α:β Ratio)
11[43]			-30 → 25	80 (1:99)
12 [43]			-30 → 25	85 (1:99)
13 [43]			-30 → 25	84 (1:99)
14 [43]			-30 → 25	82 (1:99)
15 [50]			0	99 β only
16 [43]			-30 → 25	81 (1:99)
17 [43]			-30 → 25	91 (1:99)

friendly, having considerable potential for the synthesis of bis(*C*-glycosyl) phenolic compounds.

f. Protic Acid in Ionic Liquids

C-Glycosylation of different phenols (Table **6**, entries 1-5) and different sugars (Table **6**, entries 6-10) with ionic liquids containing a protic acid was reported by Toshima and co-workers in 2007 [38].

These liquids have unique properties, are non-volatile and immiscible with some organic solvents and/or water and proved to be reusable for a wide variety of organic transformations. The two most promising ionic liquid/protic acid systems used were 1-hexyl-3-methylimidazolium tetrafluoroborate (C_6mim-[BF_4]/HBF_4) and 1-hexyl-3-methylimidazolium tetrafluoromethanesulfonimide (C_6mim-[NTf_2]/$HNTf_2$). The latter proved to be the more efficient, leading to the target compounds in high yield (entries 1-5).

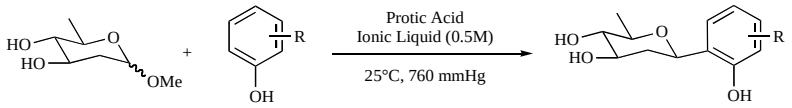
Reaction of glucosyl fluoride (Table **6**, entries 6 and 7) with 3,4,6-trimethoxyphenol in C_6mim[BF_4] containing HBF_4 (1 mol %/IL) at 60 ºC proceeded smoothly to afford the β-*C*-glycosyl derivative. However, at 760 mmHg considerable amount of the hydrolysis product (1-OH sugar) was formed. Hence, the reaction was accomplished under reduced pressure, taking advantage of the systems' non-volatility, ensuring the anhydrous reaction conditions (Table **6**, entry 7). The reactions presented in entries 8-10 were also carried out at reduced pressure and under anhydrous conditions. Coupling of mannosyl fluoride yielded α-*C*-glycosyl derivative in 82 % yield (Table **6**, entry 8). In addition, reaction of 2-deoxyglycosyl fluoride and 2,6-dideoxyglycosyl acetate afforded the corresponding β-*C*-glycosyl derivative in high yield and stereoselectivity (Table **6**, entries 9 and 10). These results reveal the potential of the system ionic liquid/protic acid to promote phenols *C*-glycosylation and encourage further research in the field.

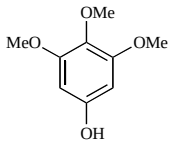
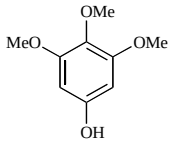
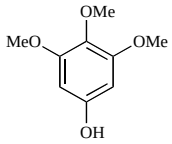
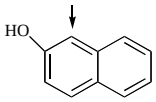
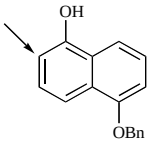
g. Heterogeneous Catalysts: Montmorillonite K-10

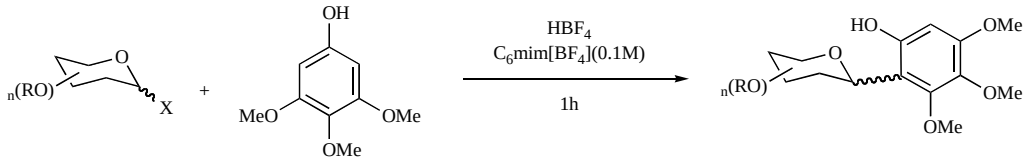
Several methods have been developed for stereo- and regioselective phenols *C*-glycosylation. However, the use of Montmorillonite K-10, an environmentally benign catalyst, became an alternative for this type of reactions. This material is readily available, easy to use, non-corrosive and one of its most important characteristics is that it is a reusable acidic clay [39].

Toshima and co-workers exploited Montmorillonite K-10 for *C*- glycosylation with unprotected sugars as glycosyl donors and phenol and naphthol derivatives as glycosyl acceptors (Table **7**).

Table 6. Fries-type Rearrangement in Ionic Liquids Containing a Protic Acid [38]



Entry[a]	Acceptor	Ionic Liquid (IL)	Protic Acid (mol % to IL)	Yield (%)
1		C_6mim-[BF_4]	HBF_4 (4)	66
2		C_6mim-[NTf_2]	$HNTf_2$ (4)	74
3		C_6mim-[NTf_2]	$HNTf_2$ (1)	85
4		C_6mim-[NTf_2]	$HNTf_2$ (4)	73
5		C_6mim-[NTf_2]	$HNTf_2$ (4)	71



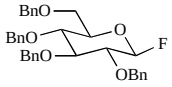
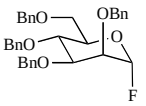
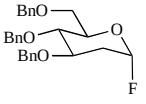
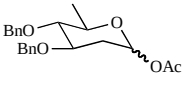
Entry	Donor	HBF4/mol % to IL	Pressure (mmHg)	Yield (%)
6		1.0	760	40 (*C*, β)
7		1.0	2	60 (*C*, β)
8		0.5	2	82 (*C*, α)
9		0.5	2	93 (*C*, β)
10		1.0	2	98 (*C*, β)

Table 7. Aryl *C*-Glycosylation with Montmorillonite K-10 [39]

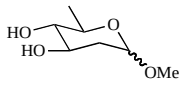
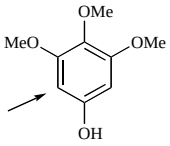
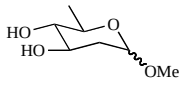
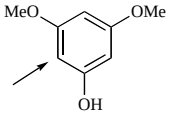
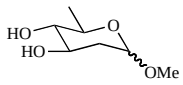
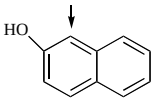
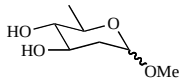
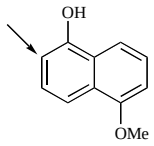
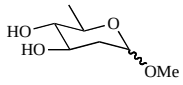
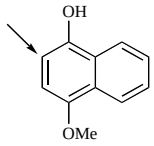
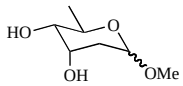
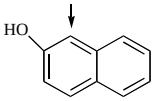
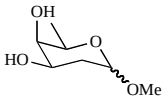
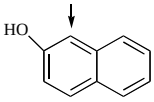
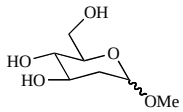
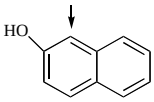
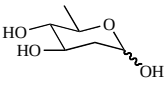
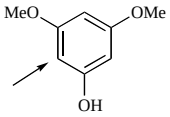
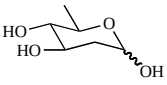
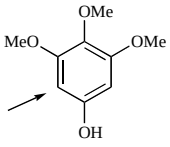
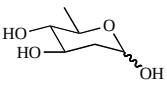
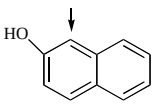
Entry	Glycosyl Donor	Glycosyl Acceptor (2.0 equiv.)	Weight % of Catalyst	Temp (ºC)	*C*-Glycosyl Derivatives Yield (%) (α:β Ratio)
1			400	50	85 (1:99)
2			300	50	80 (1:99)
3			500	50	98 (1:99)
4			500	50	66 (1:99)
5			500	50	71 (1:99)
6			300	50	91 (1:99)
7			300	50	92 (1:99)
8			500	50	68 (1:99)
9			500	50	73 (1:99)
10			400	50	82 (1:99)
11			500	50	85 (1:99)

Table 7. contd….

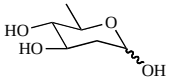
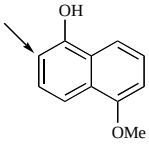
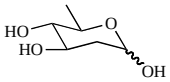
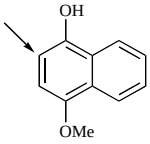
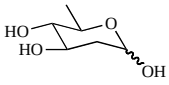
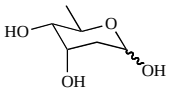
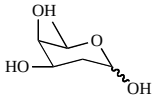
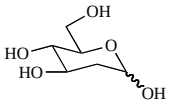
Entry	Glycosyl Donor	Glycosyl Acceptor (2.0 equiv.)	Weight % of Catalyst	Temp (°C)	*C*-Glycosyl Derivatives Yield (%) (α:β Ratio)
12			500	50	65 (1:99)
13			500	50	70 (1:99)

Table 8. 2,4-Dimethoxyphenol *C*-Glycosylation with Montmorillonite K-10 in Different Solvents [39]

Entry	Glycosyl Donor	Solvent	Weight % of Catalyst	Temp (°C)	*C*-Glycosyl Derivatives Yield (%) (α:β Ratio)
1		Dry $CHCl_3$	500	50	73 (1:99)
		$CHCl_3$	500	50	72 (1:99)
		H_2O	500	70	70 (1:99)
2		Dry $CHCl_3$	300	50	78 (1:99)
		$CHCl_3$	300	50	77 (1:99)
		H_2O	300	70	72 (1:99)
3		Dry $CHCl_3$	300	50	79 (1:99)
		$CHCl_3$	300	50	79 (1:99)
		H_2O	400	70	75 (1:99)
4		H_2O	500	80	61 (1:99)

In a first attempt to assay the efficacy of this material for *C*-glycosylation, reaction of methyl olivoside and olivose, both unprotected, with phenols, naphthol and derivatives was explored.

These reactions proceeded smoothly in dry $CHCl_3$ for 24 h to afford the respective *C*-glycosylnaphthol and its derivatives with high stereoselectivity and yield. However the amount of catalyst used for naphthol and derivatives was higher than that needed for phenols.

Deoxy glycosyl donors with different stereochemistry were also used for naphthol *C*-glycosylation (Table **7**, entries 3, 6-8) and were effectively coupled to afford the respective *C*-glycosyl derivative in high yield and stereoselectivity. In the presence of the hydroxymethyl group, the yield is lower than that obtained with 6-deoxy donors. The results also suggest that configuration of the C-3 and C-4 centers is irrelevant for reaction yield and stereoselectivity.

Also fully unprotected deoxy sugars were used for *C*-glycosylation of 2,4-dimethoxyphenol (Table **8**), in both $CHCl_3$ or H_2O, in the presence of Montmorillonite K-10. The reaction was successful in both solvents and yield and stereoselectivity were very similar when H_2O, $CHCl_3$ and dry $CHCl_3$ were used. Hence, anhydrous conditions are not necessary for the *C*-glycosylation of unprotected 1-OH sugars using this catalyst.

CONCLUSIONS

Bioactive aglycones, particularly polyphenols, appear in nature quite often *C*-glycosylated and the synthesis of such molecules, often with a very complex structure, is quite demanding and challenging for organic chemists. Among the methods developed so far, the Fries-type rearrangement proved to be very useful to give a

variety of *C*-glycosyl phenolic compounds in high regio- and stereoselective manner.

Control of the reaction outcome can be made by appropriate choice of the glycosyl donor and activator, the solvent, the temperature and the reaction time.

The most frequent activators are Lewis acids, and among them TMSOTf and $Sc(OTf)_3$ used in catalytic amount are efficient to transform a variety of phenolic compounds into their *C*-glycosylated form.

Regioselectivity of the reaction depends upon the structure of the acceptor, while the stereoselectivity relies mainly on the catalysts and the protected or unprotected glycosyl donor. The method using water or alcohol as solvent and avoiding sugar protection/deprotection steps is quite appealing regarding environmental issues. Ionic liquids in the presence of a protic acid and Montmorillonite K-10 also proved to be appropriate for the environmentally friendly *C*-glycosylation of phenolic compounds.

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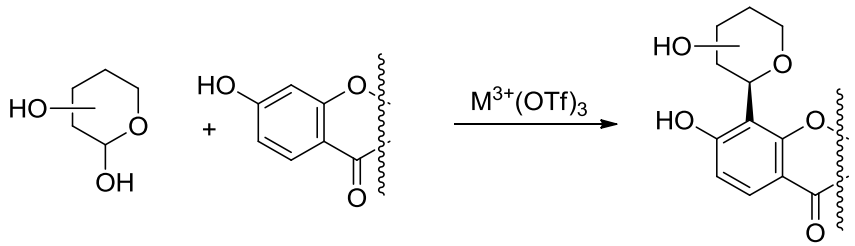
Results and discussion

As presented, the total synthesis of glycosylflavonoids involves, usually, multistep approaches which are time consuming as well as very expensive. *So, why multipath way synthesis?* Our first aim was to optimize a methodology involving the Fries-type rearrangement promoted by lanthanide triflates as a tool for direct coupling of free sugars to a flavonoid resulting in a simple and fast methodology for achieving the desired products (scheme 1).



Scheme 1 - General reaction for the coupling of sugars to flavonoids *via* Fries-type rearrangement

The flavanone (±)-naringenin (F1), was used as template since it is readily available from chemical suppliers at low cost. The glycosylflavanone obtained from the (±)-naringenin can then be further transformed into various glycosylated flavonoid types,

namely chalcone, flavone and isoflavone. In order to extend the methodology to other molecular entities, several glycosyl donors were tested aiming to establish a general procedure for coupling sugars to flavonoids for later evaluation of its potential biological activity. A whole series of lanthanide triflates, commercially available, which act as Lewis acids in aqueous mediums, were screened in order to find out the most suitable one for this type of rearrangement. Solvent system water:acetonitrile ratio and reaction time were controlled and the use of ultrasounds was also investigated.

The results obtained will be presented and discussed in this chapter.

2.1 Synthesis of 8-(β-D-Glucopyranosyl)naringenin (P1)

An approach carried out by Sato and coworkers [1]showed that stirring glucose (S1) and (±)-naringenin (P1) in acetonitrile:water in the presence of scandium triflate under reflux for 2 days led to a mixture of 6,8-diglucosylflavonoid and 6- or 8-glucosylflavonoid. To test and prove the effectiveness of this procedure, a mixture of (±)-naringenin (P1) and glucose (S1) in acetonitrile/water (2:1) was stirred overnight in the presence of scandium triflate. After this period of time, the formation of a single spot was detected by

TLC. The reaction was quenched with water and filtered throughout column packed with MCI gel CHP20P to separate the phenolic content from the unreacted sugar and catalyst. Naringenin and the reaction products were recovered from the gel column with an aqueous solution of acetone and afterwards with acetone. The combined acetone fractions were then concentrated under vacuum and the product isolated by column chromatography in 12% yield. Compound structure elucidation was accomplished by NMR experiments.

2.1.1. Structure elucidation by NMR

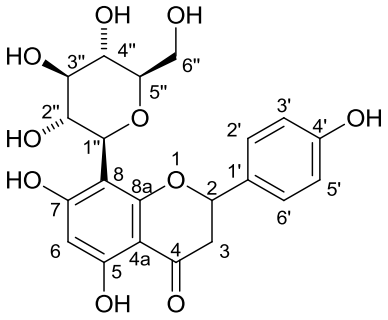


Figure 1 - Structure of 8-(β-D-glucopyranosyl)naringenin (P1)

Compound P1 structure (figure 1) was established by examination of its ^{1}H-NMR and ^{13}C-NMR data and 2D-NMR spectra (COSY, HMQC, and HMBC). The atoms were numbered as indicated in figure 1. In particular ^{1}H-NMR spectrum (figure 2) was analyzed

in order to evaluate the signal purity and to obtain exact coupling constants. Moreover, this data allowed us to confirm the formation of the mono-glycosyl derivative.

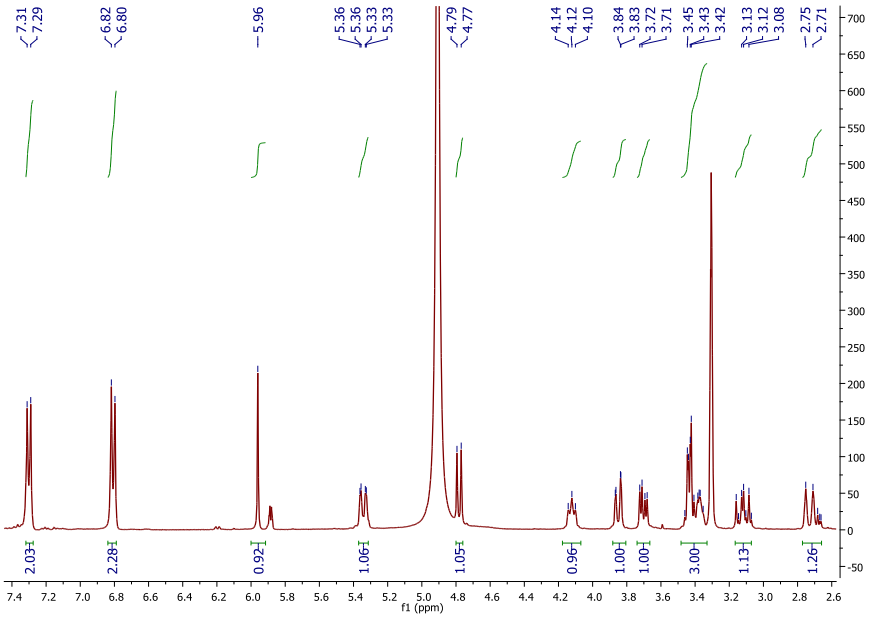


Figure 2 - ^{1}H-NMR spectrum of compound P1 in methanol-d^{4}.

Comparison of naringenin spectrum (F1) with that of the reaction product P1 shows undoubtedly the formation of the mono-substituted product. The signal at δ 5.89 (figure 3a) of naringenin spectrum integrates two protons, namely H-6 and H-8, while for

compound P1, a singlet at δ 5.97 integrates just one proton (figure 3b) in agreement with mono-glycosylation.

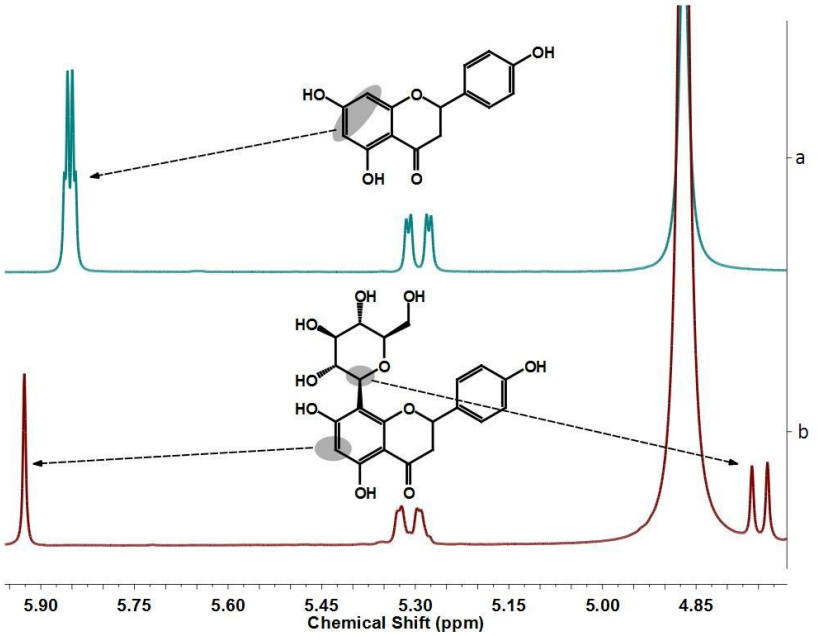


Figure 3 - 1H-NMR spectrum comparison between: a) Naringenin and b) Compound P1.

The HMQC spectrum presents a correlation for H-1'' at δ 4.78, with C-1'' at δ 75.2, a resonance characteristic for glycosyl derivatives while for *O*-glycosides the anomeric proton signal appears between δ 90 to 100 (figure 4).

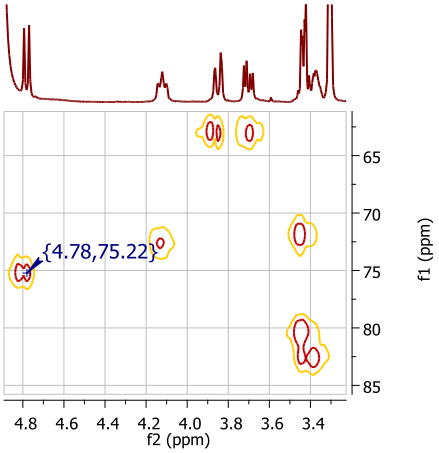


Figure 4 - HMQC correlation of H-1'' with C-1''of compound P1

Furthermore the doublet at δ 4.78, assigned to H-1'', also showed a correlation in the bi-dimensional NMR experiment COSY with a broad triplet at δ 4.12 corresponding to H-2'' presenting $J_{1'',2''}$ = 9.85 Hz (figure 5), which confirms stereoselectivity to the β-anomer, the single product obtained. This data allowed us to propose that the product obtained was a mono-β-D-glucopyranosylnaringenin.

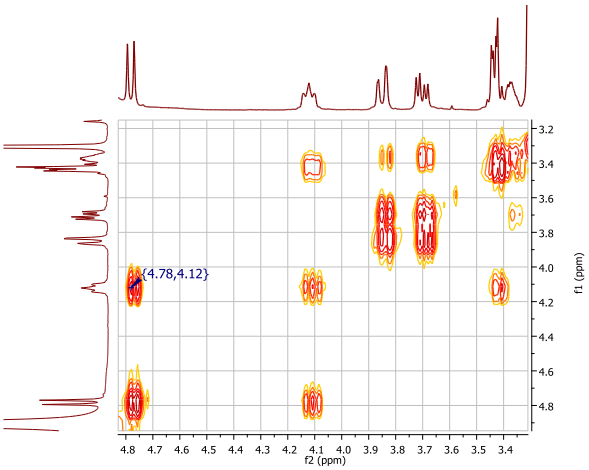


Figure 5 - COSY correlation of H-1'' with H-2''of compound P1

The HMBC correlations observed (figure 6) were decisive for the assignment of the structure providing the information needed to assign where the substitution took place on the flavonoid A ring. Correlations of H-1'' with C-7, C-8 and C-8a were detected corroborating that the *C*-glucosyl moiety is indeed linked to position 8. Assignment of C-8a was confirmed by its HMBC correlation with H-2. Moreover, the broad doublet at δ 2.73 and the singlet at δ 5.97 assigned to H-3e and H-6, respectively, present a HMBC correlation with C-4a, clearly indicating that the position 6 was not glycosylated. Table 1 summarizes NMR full characterization of 8-(β-D-glucosyl)naringenin (P1).

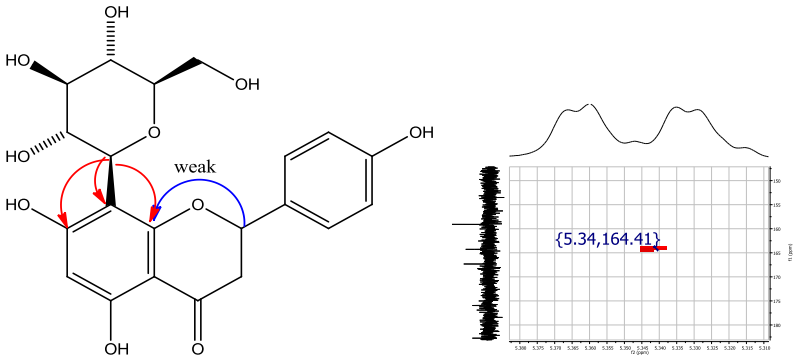


Figure 6 - Compound P1 - HMBC correlations of H-1'' with the flavonoid carbon atoms, and of H-2 with C-8a, and HMBC spectrum showing correlation of H-2 with C-8a

Table 1 - ^{1}H-NMR and ^{13}C-NMR data for compound P1 (in methanol-d^4)

	^{1}H-NMR				**^{13}C-NMR**
Proton	**δ in ppm**	**Int.**	**Mult.**	**J (Hz)**	**δ in ppm**
2	5.34	1	dd	$J_{2,3e}$= 2.40 $J_{2,3a}$ = 12.38	80.2
3a	3.13	1	dd	$J_{3a,3e}$= 17.18	44.9
3e	2.73	1	brd		44.9
4	-	-	-	-	198.2
5	-	-	-	-	164.3
6	5.97	1	s	-	96.4
7	-	-	-	-	167.3
8	-	-	-	-	106.3
4a	-	-	-	-	103.9
8a	-	-	-	-	164.4
1'	-	-	-	-	130.9
2',6'	7.30	2	d	$J_{2',3'}$= 8.34	129.1
3',5'	6.81	2	d	$J_{5',6'}$= 8.34	116.4
4'	-	-	-	-	159.1
1''	4.78	1	d	$J_{1'',2''}$= 9.85	75.2
2''	4.12	1	brt	$J_{2'',3''}$=9.65	71.0
3''					80.5
4''	3.46-3.35	3	m		72.6
5''					82.6
6a''	3.85	1	Part A, ABX	$J_{6a,6b}$=12.38 $J_{6a,5}$=1.64	62.9
6b''	3.70	1	Part B, ABX	$J_{6b,5}$ =5.31	62.9

Int – integration; Mult - multiplicity

In our hands, this methodology revealed to be a remarkable and selective way to achieve stereo- and regioselective *C*-glycosylation, since only one product, the 8-(β-D-glucosyl)naringenin, was formed in contrast with the complex mixture obtained by Sato and coworkers [1]. Further studies concerning the optimization of this methodology were conducted in order to enhance reaction yield. Catalyst, reaction time and solvent mixture ratio were changed in order to improve reaction outcome. Other sugars and flavonoids were also investigated as starting materials and the results are described in the next sub-chapters.

2.2 Catalyst, Reaction Time and Solvent Mixture

After testing the coupling reaction procedure and validating it for product P1, evaluation of the performance of other commercially available rare earth metal triflates was conducted, in order to identify other acid Lewis that could lead to higher yields. The validated experimental procedure was repeated with each of the new catalysts, which was added to a solution of (±)-naringenin/glucose and stirred under reflux for 12 hours. After removal of the unreacted glucose and catalyst, the phenolic content was recovered and fractionated by HPLC using previously

synthesized 8-(β-D-glucopyranosyl)naringenin (P1) as standard. For HPLC analysis a stock solution (1.000 mg L^{-1}) of individual standard and of each reaction residue were prepared in methanol. The mobile phase consisted on a mixture of acetonitrile and 0.1% *o*-phosphoric acid aqueous solution in 2:8 ratio and analysis was conducted with a flow of 1.0 mL min^{-1} at 26 ^{0}C, injection volume 20 μL with a draw speed of 200 μL min^{-1}. For identification purposes, standard addition method was used by spiking the samples with the pure standards, as well as by comparing the retention parameters and UV-visible spectral reference data, obtained for the synthesized standard. The retention time obtained for the compound P1 was 3:55 minutes being the maximum absorvance observed at 226 nm. For quantification purposes, the external standard methodology was performed, using six calibration standard solutions with concentrations ranging from 10.0 to 1000.0 mg L^{-1}.

The yields obtained (by HPLC analysis) for the studied catalysts are summarized in table 2. When the reaction was promoted by praseodimium triflate an improvement in the outcome was clear given that reaction yield was significantly higher (41.2 %) then all other yields.

Table 2 – Yields coupling reaction of naringenin with D-glucose in acetonitrilo:water (2:1) (by HPLC analysis) *via* Fries-type rearrangement of promoted by rare-earth triflates

Entry	Sugar (eq.)	Cat. eq.	Cat. $M^{3+}(OTf)_3$	Temp.	Time	Yield[a](%)	RSD[b](%)
1			Sc			18.9	1.1
2			Y			18.0	1.1
3			La			6.2	0.7
4			Ce			2.3	0.7
5			**Pr**			**41.2**	**2.2**
6			Nd			9.5	0.8
7			Sm			9.0	0.8
8	3	0.2	Eu	Reflux	22	16.9	1.1
9			Gd			21.4	1.2
10			Tb			4.3	0.7
11			Dy			13.5	0.9
12			Ho			18.8	1.1
13			Er			14.8	1.0
14			Tm			17.5	1.1
15			Yb			2.2	0.7
16			Lu			15.4	1.0

[a] Obtained by HPLC analysis
[b] Relative Standard Deviation (RSD)

For optimization of reaction yield under $Pr(OTf)_3$ catalysis, the solvent ratio acetonitrile:water, the amount of glycosyl acceptor and reaction time were evaluated. Concerning the reaction time, after increasing the time to 24 and 48 hours, the isolated yield was 32 % and 34 %, respectively (Table 3). Hence, reaction time ranging from 24 to 48 hours does not significantly changes the yield.

Table 3 – Results of the optimization of reaction time for the coupling of glucose to naringenin

Entry	Solvent	$Pr(OTf)_3$ Eq.	Time (h)	Yield (%)
1	MeCN:H_2O 2:1	0.2	24	32
2	MeCN:H_2O 2:1	0.2	48	34

As for the solvent ratio, Table 4 summarizes the reactions conducted with several solvent ratios. The solvent ethanol was not used because this type of reactions run with scandium triflate in ethanol give usually complex mixtures and diglycosylated products. It was found that the best result was observed for the mixture acetonitrile:water (2:1). Above this ratio the glucose was not completely dissolved turning the reactional medium into a suspension rather than a solution, which resulted in a decrease in the amount of product obtained. When the amount of water increased, naringenin started to be insoluble becoming the reaction mixture very cloudy thus leading to lower yields.

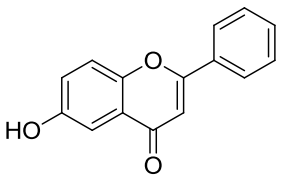
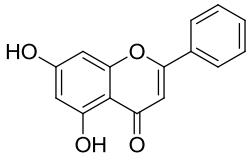
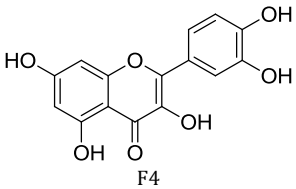
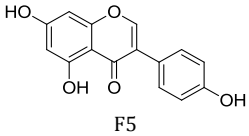
Table 4 – Solvent studies towards the optimization of solvent mixture of the coupling reaction of naringenin with D-glucose.

Entry	MeCN: H_2O	$Pr(OTf)_3$ eq.	Time (h)	Yield (%)
1	1:0	0.2	24	9
2	3:1	0.2	24	28
3	**2:1**	**0.2**	**24**	**32**
4	1:1	0.2	24	29
5	1:2	0.2	24	23
6	1:3	0.2	24	16
7	0:1	0.2	24	0

2.3. Flavones and isoflavones

As glycosyl acceptor other flavonoids were tested for coupling with D-glucose. 6-Hydroxyflavone and 5,7-dihydroxyflavone were tried under several reaction conditions using acetonitrile, ethanol and acetonitrile:water mixtures, but no reaction products were obtained even after longer reaction times (entries 1-7, table 5). However the non reactivity of those flavones may be due to the electron-withdrawing effect of the conjugated system of the flavone disfavoring the electrophilic aromatic substitution reaction or to solubility problems. Quercetin, more soluble than 5,7-dihydroxyflavone or 6-hydroxyflavone, was then used but, as expected, no reaction occurred leading to the conclusion that electronic effects are determinant for the reactivity (entries 8-11, table 5). Also 8-(β-D-glucosyl)genistein, which is believed to possess antidiabetic activity, was also tried, but unfortunately the reaction of glucose with genistein did not succeed. Ethanol, acetonitrile and a mixture of acetonitrile and water were used as solvent, longer reaction times were tested but there was no formation of any product whatsoever. As before the main reason for its unreactivity is the electron withdrawal effect of the chromen-4-one skeleton (entries 12-17, table 5).

Table 5 – Reaction of D-glucose with 6-hydroxyflavone (F2); 5,7-dihydroxyflavone (F3); quercetin (F4) and genistein (F5) promoted by $Pr(OTf)_3$.

Entry	Flavonoid	Solvent	Catalyst eq.	Time (h)	Yield (%)
1	 F2	MeCN:H_2O 2:1	0.2	24	nr
2		MeCN:H_2O 2:1	0.2	72	nr
3		MeCN	0.2	24	nr
4		MeCN	0.2	72	nr
5	 F3	MeCN:H_2O 2:1	0.2	24	nr
6		MeCN	0.2	24	nr
7		EtOH	0.2	24	nr
8	 F4	MeCN:H_2O 2:1	0.2	24	nr
9		MeCN:H_2O 2:1	0.2	72	nr
10		MeCN:H_2O 2:1	0.5	72	nr
11		MeCN	0.2	72	nr
12	 F5	MeCN:H_2O 2:1	0.2	24	nr
13		MeCN	0.2	24	nr
14		EtOH	0.2	24	nr
15		MeCN:H_2O 2:1	0.2	72	nr
16		MeCN	0.2	72	nr
17		EtOH	0.2	72	nr

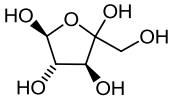
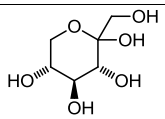
2.4. Glycosyl donors

We endeavor to extend this methodology to other sugars beside glucose in order to establish a general procedure that will lead to several analogues for later bioactivity evaluation. Regarding the glycosyl donors a wide variety of sugars were used.

2.4.1. D-Frutose and L-Sorbose

Ketosugars, namely D-fructose (S2) (entries 1-5, table 6) and L-sorbose (S3) (entries 6-10, table 6), were evaluated as glycosyl donors but no reaction product was obtained, even after longer reaction time (up to 72 hours) and increasing the amount of catalyst. The non reactivity of those sugars may be tentatively explained by the presence of the hydroxymethyl group at the anomeric position hampering formation of the key intermediate *O*-glycoside that would suffer further rearrangement to its *C*-congener.

Table 6 – Coupling reaction of D-fructose (S2) and L-sorbose (S3) to naringenin promoted by $Pr(OTf)_3$

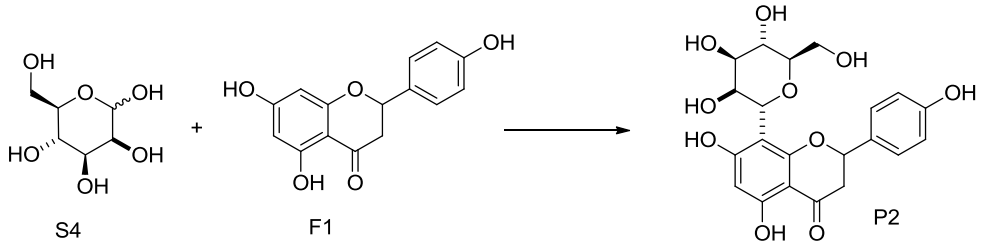
Entry	Sugar	Catalyst eq.	Time (h)	Yield (%)
1	 S2	0.2	24	nr
2		0.2	48	nr
3		0.2	72	nr
4		0.3	72	nr
5		0.4	72	nr
6	 S3	0.2	24	nr
7		0.2	48	nr
8		0.2	72	nr
9		0.3	72	nr
10		0.4	72	nr

nr – no reaction

2.4.2. D-Manose, D-Galactose, L-Rhamnose

The aldosugars D-manose (S4), L-rhamnose (S5), D-galactose (S6), and D-glucosamine (S7) were considered as scaffolds. The coupling reaction for S4, S5 and S6 afforded the desired products while for glucosamine the reaction did not occur.

2.4.2.1 Synthesis of 8-(α-D-Mannopyranosyl)naringenin (P2)



Scheme 2 - Synthesis of 8-α-(D-mannopyranosyl)naringenin via Fries type rearrangement. Reagents and conditions : $Pr(TfO)_3$, MeCN: Water (2:1), reflux, 24h.

Coupling of D-mannose with (±)-naringenin in the presence of the catalyst, for 24 hours, gave a single product detected by TLC. After quenching the reaction and purification of the reaction crude by CC, compound P2 (figure 7) was isolated in 38% yield and subjected to NMR for structure elucidation.

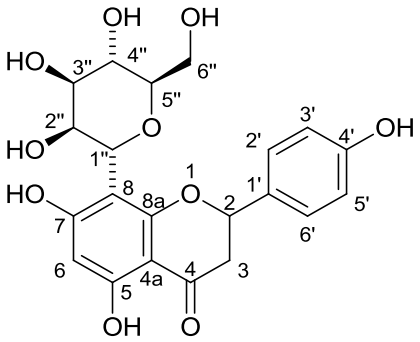


Figure 7 – Structure for compound P2.

NMR characterization of P2 followed the same guidelines used for the structure elucidation of 8-(β-D-glucopyranosyl)naringenin (P1). ^{1}H-NMR revealed a signal at δ 5.05 due to H-1'' with coupling constant $J_{1'',2''}$= 9.20 Hz, characteristic for α-anomer configuration of mannose with a $^{1}C_{4}$ chair conformation (figure 8). The resonance of H-1'' presented a HMQC correlation with C-1'' at δ 77.0, typical for *C*-glycosylflavonoids. COSY and HMQC experiments allowed the assignment of the signals of each sugar

proton and carbon (Table 7). HMBC correlations were also very useful for confirming substitution at position 8 on A ring of the flavonoid demonstrating the significant correlations as explained previously for compound P1.

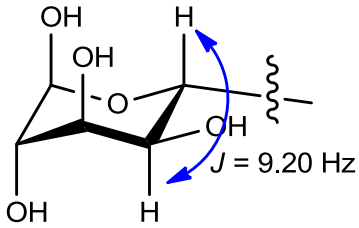


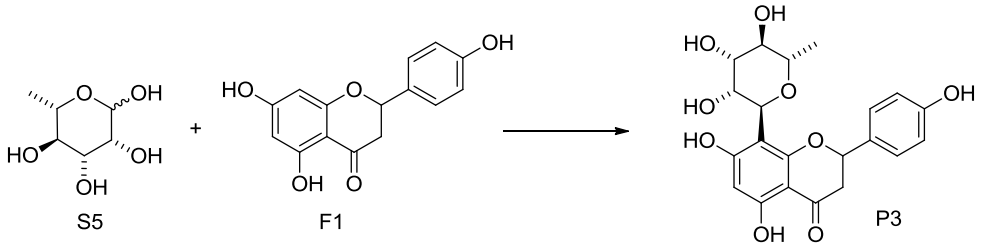
Figure 8 – Conformer of mannose moiety in P2.

Table 7 - ^{1}H-NMR and ^{13}C-NMR data for compound P2 (in methanol-d^4)

			^{1}H-NMR		^{13}C-NMR
Proton	**δ in ppm**	**Int.**	**Mult.**	***J* (Hz)**	**δ in ppm**
2	5.30	1	dd	$J_{2,3e}$= 2.95 $J_{2,3a}$ = 12.68	80.6
3a	3.07	1	dd	$J_{3a,3e}$= 17.29	44.1
3e	2.69	1	dd		44.1
4	-	-	-	-	198.1
5	-	-	-	-	162.4
6	5.87	1	s	-	97.4
7	-	-	-	-	166.3
8	-	-	-	-	103.9
4a	-	-	-	-	101.2
8a	-	-	-	-	160.3
1’	-	-	-	-	130.2
2’,6’	7.25	2	d	$J_{2',3'}$= 8.47	129.1
3’,5’	6.76	2	d	$J_{5',6'}$= 8.47	116.3
4’	-	-	-	-	157.9
1’’	5.05	1	d	$J_{1'',2''}$= 9.20	77.0
2’’	3.93	1	brt	$J_{2'',3''}$=2.97	73.7
3’’	3.58	1	ddd	$J_{3'',4''}$=9.47	76.1
4’’	3.61	1	t	$J_{4'',5''}$=9.47	68.3
5’’	3.32	1	ddd		83.4
6a’’	3.85	1	Part A, ABX system	$J_{6a,6b}$=12.00 $J_{6a,5}$=1.89	62.7
6b’’	3.72	1	Part B, system ABX	$J_{6b,5}$ =5.41	62.7

Int – integration; Mult - multiplicity

2.4.2.2. Synthesis of 8-(α-L-rhamnopyranosyl)naringenin (P3)



Scheme 3- Synthesis of 8-(α-L-rhamnopyranosyl)naringenin via Fries type rearrangement. Reagents and conditions: $Pr(TfO)_3$, MeCN: Water (2:1), reflux, 24h

To access the L-rhamnose coupling product (figure 9) with (±)-naringenin, the procedure adopted for the D-mannosyl derivative (P2) was applied. After stirring the reaction mixture for 24 hours, it was possible to identify by TLC a spot sensible to ferric chloride which was then purified by CC. Compound P3 was isolated with 33% yield and subjected to NMR for confirmation of its structure.

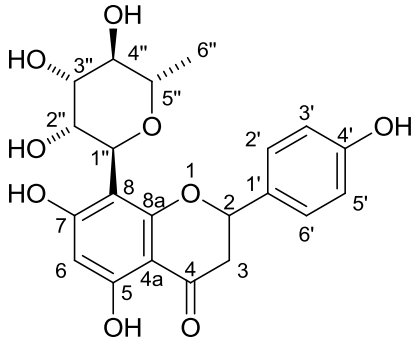


Figure 9 - Structure for compound P3.

For the full NMR characterization of the 8-α-L-rhamnosylnaringenin (P3) ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC and HMBC experiments were again used.

In the ^{1}H-NMR spectrum a resonance at δ 5.04 was identified for H-1'' having a coupling constant $J_{1'',2''}$= 10.36 Hz, (figure 10) consistent with the α configurated L-rhamnosyl residue with a $^{4}C_{1}$ conformation (figure 11).

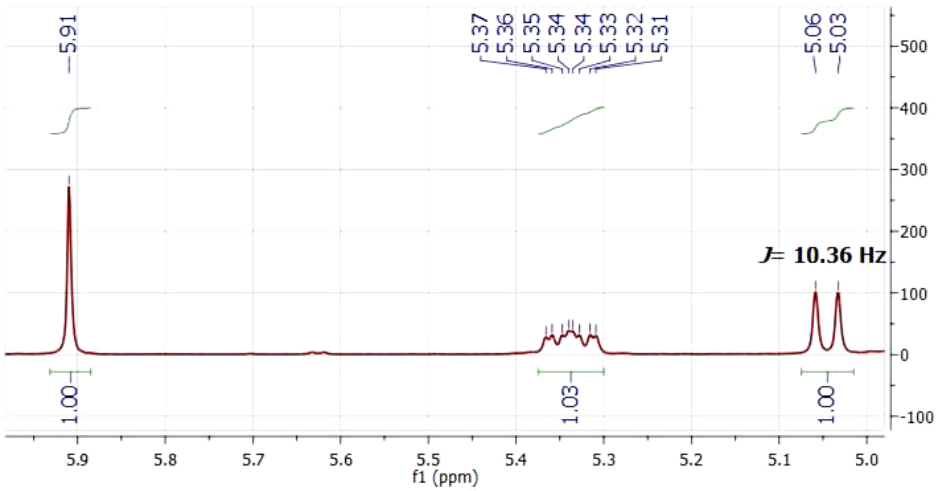


Figure 10 – ^{1}H-NMR spectrum (from 6 ppm to 5 ppm) of compound P3

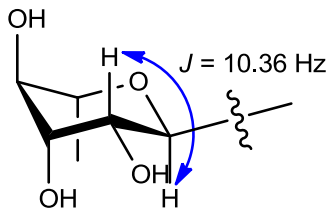


Figure 11 - Conformer of mannose moiety in P3.

The singlet corresponding to H-6 was identified at δ 5.91 indicating the absence of the AB system (H6 and H8) found in the naringenin spectrum and demonstrating that a substitution in the aromatic ring has occurred (figure 12). Moreover, the anomeric proton, H-1'', presented a HMQC correlation with C-1'' at δ 76.8, the common chemical shift for *C*-glycosylflavonoids while for *O*-glycosides this value is usually located at δ 90-100.

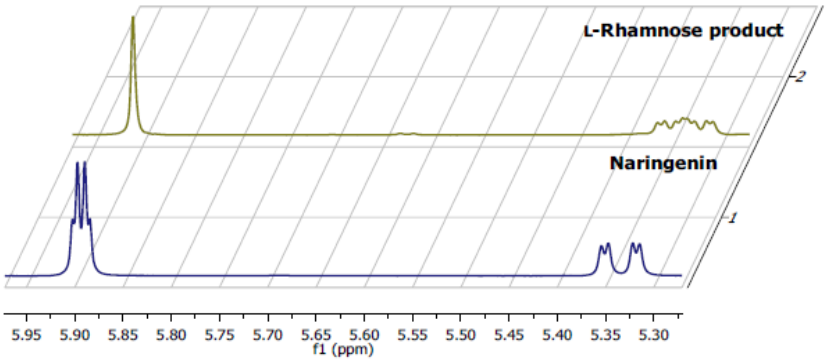


Figure 12 – Comparison of 1H-NMR spectrum (H-6, H-8 and H-2) between naringenin and compound P3

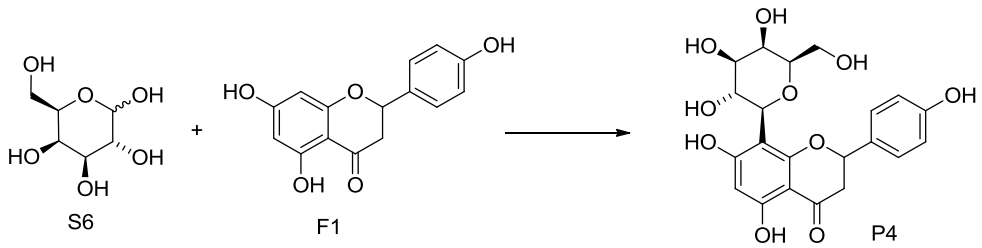
Once again the HMBC correlations, similar to those for the glucosyl and mannosyl derivatives, were essential for the confirmation of the substitution in the flavonoid A Ring. The full NMR data for the compound P3 is presented in table 8.

Table 8 - ^{1}H-NMR and ^{13}C-NMR data for compound P3 (in methanol-d^4)

		^{1}H-NMR			^{13}C-NMR
Proton	**δ in ppm**	**Int.**	**Mult.**	***J* (Hz)**	**δ in ppm**
2	5.34	1	dd	$J_{2,3e}$= 2.91 $J_{2,3a}$ = 12.20	80.6
3a	3.12	1	dd	$J_{3a,3e}$= 17.17	43.6
3e	2.73	1	dd		43.6
4	-	-	-	-	198.4
5	-	-	-	-	163.9
6	5.91	1	s	-	97.6
7	-	-	-	-	160.8
8	-	-	-	-	105.6
4a	-	-	-	-	102.9
8a	-	-	-	-	161.9
1’	-	-	-	-	131.4
2’,6’	7.30	2	d	$J_{2',3'}$= 8.35	130.0
3’,5’	6.81	2	d	$J_{5',6'}$= 8.35	116.2
4’	-	-	-	-	159.3
1’’	5.04	1	d	$J_{1'',2''}$= 10.36	76.8
2’’	3.97	1	brt	$J_{2'',3''}$=3.55	73.3
3’’	3.58	1	brd	$J_{3'',4''}$=9.14	75.6
4’’	3.46	1	t	$J_{4'',5''}$=9.37	73.5
5’’	3.39	1	m		78.5
6’’(CH_3)	1.36	3	d	$J_{6'',5''}$=5.90	18.1

Int – integration; Mult - multiplicity

2.4.2.3. Synthesis of 8-(β-D-Galactopyranosyl)naringenin (P4)



Scheme 4 - Synthesis of 8-(β-D-galactopyranosyl)naringenin via Fries type rearrangement. Reagents and conditions: $Pr(TfO)_3$, MeCN: Water (2:1), reflux, 24h

Regarding the reaction with D-galactose, the protocol previously used was followed, a single product was detected, and product P4 was isolated in 35% yield. NMR spectrum was run in deuterated acetone because the product was not reasonably soluble in deuterated methanol. The chemical shift found in ^{1}H-NMR spectrum for H-1'' (δ 4.81) and the coupling constant $J_{1'',2''}$ = 9.73 Hz (Table 9), indicate that the compound obtained was a β-anomer. Furthermore, this coupling constant combined with the observed HMQC correlation of H-1'' with C-1'' at δ 74.7, and the HMBC correlations clearly suggest the structure of an 8-(β-D-galactosyl) derivative.

Table 9 - ^{1}H-NMR and ^{13}C-NMR data for compound P4 (in Acetone – d^{6})

	^{1}H-NMR				**^{13}C-NMR**
Proton	**δ in ppm**	**Int.**	**Mult.**	***J* (Hz)**	**δ in ppm**
2	5.45	1	dd	$J_{2,3e}$= 3.06 $J_{2,3a}$ = 12.50	79.1
3a	3.18	1	dd	$J_{3a,3e}$= 15.78	42.8
3e	2.75	1	dd		42.8
4	-	-	-	-	191.0
5	-	-	-	-	162.2
6	5.95	1	s	-	95.7
7	-	-	-	-	164.4
8	-	-	-	-	105.2
4a	-	-	-	-	102.3
8a	-	-	-	-	160.9
1'	-	-	-	-	129.4
2',6'	7.37	2	d	$J_{2',3'}$= 8.68	128.2
3',5'	6.88	2	d	$J_{5',6'}$= 8.68	115.1
4'	-	-	-	-	158.1
1''	4.81	1	d	$J_{1'',2''}$= 9.73	74.7
2''	4.06	1	d	$J_{2'',3''}$=2.35	68.9
3''	3.65	1	dd	$J_{3'',4''}$=9.53	75.1
4''	4.03	1	td	$J_{4'',5''}$=2.99	70.2
5''					78.9
6a''	3.77-3.68	3	m		61.3
6b''					61.3

Int – integration; Mult - multiplicity

^{1}H and ^{13}C NMR data for the synthesized monoglycosylated compounds is shown in tables 11 and 12, for comparison purposes.

Table 10 - ^{1}H-NMR data for compound P1, P2, P3 (in Methanol – d^4) and P4 (in Acetone – d^6)

	^{1}H-NMR			
	δ (ppm)			
Proton	**P1**	**P2**	**P3**	**P4**
2	5.34	5.30	5.34	5.45
3a	3.13	3.07	3.12	3.18
3e	2.73	2.69	2.73	2.75
4	-	-	-	-
5	-	-	-	-
6	5.97	5.87	5.91	5.95
7	-	-	-	-
8	-	-	-	-
4a	-	-	-	-
8a	-	-	-	-
1’	-	-	-	-
2’,6’	7.30	7.25	7.30	7.37
3’,5’	6.81	6.76	6.81	6.88
4’	-	-	-	-
1’’	4.78	5.05	5.04	4.81
2’’	4.12	3.93	3.93	4.06
3’’	3.37	3.58	3.58	3.65
4’’	3.37	3.61	3.46	4.03
5’’	3.37	3.32	3.39	
6a’’	3.85	3.85	1.36	3.77-3.68
6b’’	3.70	3.72	(CH3)	

Table 11 - ^{13}C-NMR data for compound P1, P2, P3 (in Methanol – d^4) and P4 (in Acetone – d^6)

	^{13}C-NMR			
	δ (ppm)			
Carbon	**P1**	**P2**	**P3**	**P4**
2	80.2	80.6	80.6	79.1
3a	44.9	44.1	43.6	42.8
3e	44.9	44.1	43.6	42.8
4	198.2	198.1	198.4	191.0
5	164.3	162.4	163.9	162.2
6	96.4	97.4	97.6	95.7
7	167.3	166.3	160.8	164.4
8	106.3	103.9	105.6	105.2
4a	103.9	101.2	102.9	102.3
8a	164.4	160.3	161.9	160.9
1’	130.9	130.2	131.4	129.4
2’,6’	129.1	129.1	130.0	128.2
3’,5’	116.4	116.3	116.2	115.1
4’	159.1	157.9	159.3	158.1
1’’	75.2	77.0	76.8	74.7
2’’	71.0	73.7	73.3	68.9
3’’	80.5	76.1	75.6	75.1
4’’	72.6	68.3	73.5	70.2
5’’	82.6	83.4	78.5	78.9
6a’’	62.9	62.7	18.1	61.3
6b’’	62.9	62.7		61.3

A final remark has to be made concerning the multiplicity obtained for H-2, H-3e and H-3a. H-3a and H-3e are expected to show a ABX pattern as observed in the ^{1}H-NMR spectrum of (±) naringenin. However, since naringenin has a stereogenic center at C-2, a mixture of two diastereoisomers was obtained leading to a more complex signal for those protons. Instead of one ABX system we can, in fact, observe two systems overlapped. The same observation can be made regarding the signal assigned to H-2,

where instead of a double doublet, two such signals appear, one of each isomer, leading to a more complex signal (figure 13).

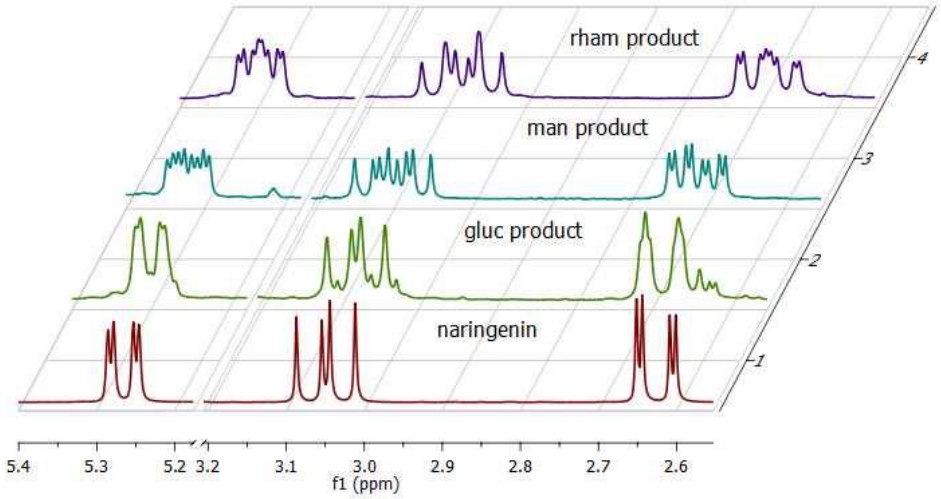


Figure 13 – Comparison between ^{1}H-NMR spectrum of naringenin and compound P1, P2 and P3 (the D-galactosyl derivative was not included since the NMR experiments were performed in acetone-d^{6})

2.5. Sonochemistry

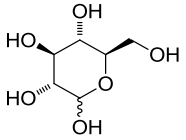
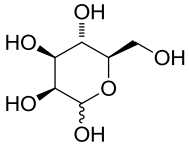
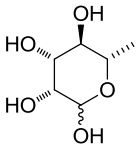
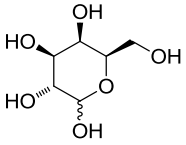
Since the early 30's that sonication is known to provide the needed energy to enhance reactivity and to assist organic synthesis. Taking advantage of a phenomenon called cavitation, and having in mind that acoustic energy cannot be absorbed by molecules, ultrasounds are transformed into a chemically usable

form of energy that could accelerate the reaction rate as well as improve the reaction outcome [2-3].

Therefore, in the search to improve the yield and to shorten the reaction time, ultrasounds were tested as an alternative energy source in this approach. A common commercial ultrasound heating bath was used at 80 ºC and after 8 hours the 8-(β-D-glucopyranosyl)naringenin P1 was isolated in 56% of yield (table 13, entry 1). This methodology was applied to the remaining sugar scaffolds S4-S6 and the glycosylation yield was considerably improved (table 13).

In summary in this work we have established a procedure that can be useful for the expedite synthesis of *C*-glycosylnarigenin through a simple and inexpensive approach.

Table 12 - Fries-type rearrangement promoted by ultrasounds.

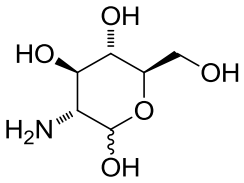
Run	Sugar	Catalyst $[M^{3+}(OTf)_3]$	Product	Yield (%)
1	 S1	Pr	P1	56
2	 S4		P2	46
3	 S5		P3	51
4	 S6		P4	49

2.6 Glucosamine as glycosyl donor

Another sugar donor tested was glucosamine (S7). However the result obtained was not the expected since no product was detected even when subjected to higher amounts of catalyst and longer reaction times (table 10). The explanation relies on the fact that it is believed that the active catalytic species is the triflic acid. Bizier *et al* [4] reported that triflic acid, as well as metal(III)

triflates promote the acetylation of galactose. However, in the presence of 2,6-di-*tert*-butylpyridine (DTBP) the reaction did not proceed as previously. Glucosamine was described as having the same role as DTBP, inhibiting the effect of the catalyst. Hence, N-acetylglucosamine coupling reaction was tested and by TLC a product was formed confirming that aldosamines are not suitable donors for the Fries type rearrangement under these conditions due to their basicity.

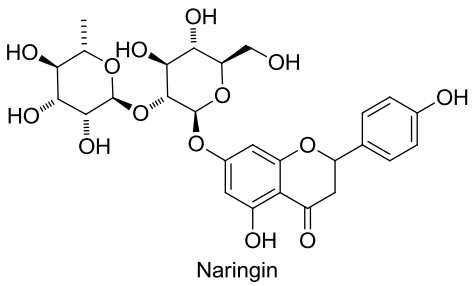
Table 13 – Reaction of glucosamine with glucose in aqueous solution of acetonitrile

Entry	Sugar	$Pr(OTf)_3$ eq.	Time (h)	Yield (%)
1		0.2	24	N.R.
2		0.4	24	N.R.
3		0.2	48	N.R.
4	S7	0.4	48	N.R.

2.7. Disaccharides

Some citrus bioflavonoids have gained some importance over the years due to their versatile bioactivities and high consumption by humans[5], namely the polyphenol dissacharides naringin and hesperidin [6-7], (figure 14). It has been suggested that supplementation with hesperidin or naringin decreases hyperglycemia in type 2 diabetic *db/db* mice (animal model for diabetes) by increasing glucose utilization, which apparently is

mediated via elevated glycolysis and hepatic glycogen concentration resulting from their effect on glucokinase[6].



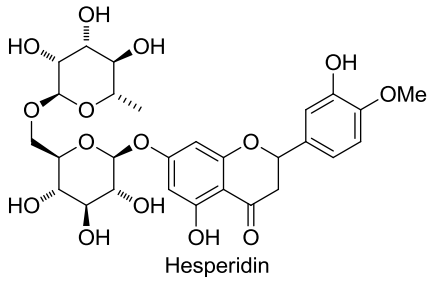


Figure 14 – Structures of naringin and hesperidin.

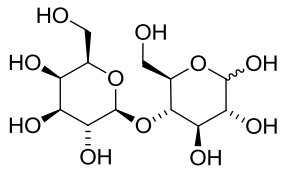
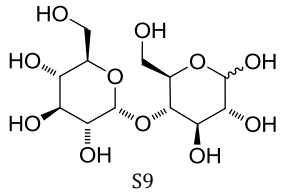
In particular, naringin regulates gluconeogenesis. Furthermore, the levels of plasma insulin in the hesperidin- and naringin-supplemented groups were significantly higher than those in the control group[6]. A study conducted by Jung *et al.* [8]demonstrated that naringin and hesperidin played an important role in improving the lipid and glucose metabolism in *db/db* mice, exhibited antidiabetic properties and were able to reduce certain diabetic complications related to hyperlipidemia. Naringin was already indicated as potential anticancer agent

[7]and is reported to be able to protect mouse liver and intestine against the radiation-induced damage by elevating the antioxidant status and reducing the lipid peroxidation [5]. In addition, along with other flavonoids, was described as a neuroprotective agent [9]. This encouraged us to test the previously established conditions for the coupling of disaccharides to naringenin.

2.7.1. Synthesis of 8-(β-Lactosyl)naringenin (P5) and 8-(β-Maltosyl)naringenin (P6)

Lactose (S8) and maltose (S9) were used as glycosyl donors. However we have to ensure that the disaccharide glycosidic bond is stable under the reaction conditions, since as stated, the reaction mechanism involves the formation of glycosidic bond between a sugar and a phenol and its subsequent hydrolysis. A few tests were run consisting on stirring a solution of lactose or maltose in acetonitrile: water in the presence of the catalyst. The stability of the disaccharides under these conditions was detected by TLC (table 14). The disaccharide glycosidic bond hydrolysis occurred only after 96 hours and by increasing the amount of catalyst (entries 4 and 8, table 14). Since the reaction generally takes no more than 24 hours, we proceeded with the coupling of disaccharides to naringenin.

Table 14 – Stability test of the glycosidic bond of maltose and lactose evaluated by TLC

Entry	Dissacharide	Cat. Eq.	Time (h)	Result[a)]
1	 S8	0.2	48	n.h.
2		0.4	48	n.h.
3		0.2	96	n.h.
4		0.4	96	h.
5	 S9	0.2	48	n.h.
6		0.4	48	n.h.
7		0.2	96	n.h.
8		0.4	96	h.

n.h. – no hydrolysis, h. – hydrolysis, a) – Observed by TLC

The coupling reaction of lactose and maltose to naringenin followed the procedure adopted for monossacharides but the reaction time was extended to 48 hours. After this period the reactions were quenched with water. The 8-(β-lactosyl)naringenin (P5) and the 8-(β-maltosyl)naringenin (P6) were isolated by column chromatography with a yield of 28% and 34%, respectively (entries 1 and 3, table 15). The purified samples were then studied by NMR for their structure elucidation. HMQC and HMBC correlations were very useful in the assignment of the protons of the A ring. In the lactose derivative two anomeric

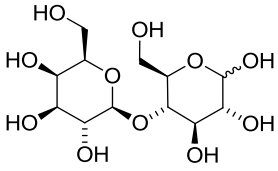
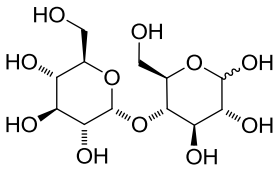
protons were found, as expected, confirming that the dissacharide was not cleaved. The doublet at δ 4.80 presented a coupling constant of 10.05 Hz, a β-anomer, was atributed to glucose attached directly to naringenin (H-1''), given that it showed HMQC correlation with the signal at δ 73.5, characteristic of the *C*-glycosyl derivatives. In contrast the signal of the other anomeric proton of the galactosyl moiety, (H-1''') was a doublet at δ 4.43, correlating with a carbon at δ 103.5, clearly indicating an *O*-glycoside structure. Moreover, the signal of the D-galactosyl residue anomeric proton (H-1''') presents also the coupling constant of a β-anomer, confirming that the lactosyl moiety has suffered no modification in its structure. As for the 8-(glucosyl)naringein, the analysis of HMBC correlations of H-6 and H-1'' allowed to establish the structure of an 8-glycosylated flavonoid.

Likewise, the maltosyl product showed the anomeric proton chemical shift at δ 4.81 which correlates, in HMQC experiment, with a carbon at δ 73.5, while for the glucosyl residue the anomeric proton and carbon resonances appear at δ 5.23 and δ 101.6, respectively. The coupling constants for both anomeric protons were 10.01 Hz and 3.76 Hz corresponding to a β- and an α-anomer, respectively, according to the expected structure. The full assigment of NMR spectral data for 8-(β-lactosyl)naringenin

and 8-(β-maltosyl)naringenin is shown in tables 16 and 17, respectively.

Ultrasounds, as for the monosaccharides, were also applied to the dissacharides coupling reaction (entries 2 and 4, table 15). Instead of a commercial ultrasound heating bath, an ultrasonic homogenizer with 200W of power operating at a frequency of 24 kHz, equipped with a titanium ultrasonic horn, was used without heating, although the reaction mixture reached 71 ^{o}C. Under these conditions, reaction yield was increased and reaction time reduced to 12 hours. After column chromatography purification, compounds P5 and P6 were isolated in 43% and 51% yield, respectively. This methodology revealed, once again, to be a good tool to accelerate the reaction and improve its outcome.

Table 15 – Coupling reaction of lactose and maltose to naringenin *via* Fries-type rearrangement with different energy sources catalyzed by $Pr(OTf)_3$

Run	Sugar	Product	Energy Source	Yield (%)
1	 S8	P5	Heat	28
2			US	43
3	 S9	P6	Heat	34
4			US	51

US – ultrasounds

Table 16 - 1H-NMR and 13C-NMR data for compound P5 (in methanol– d^4)

	^{1}H-NMR				^{13}C-NMR
Proton	**δ in ppm**	**Int.**	**Mult.**	***J* (Hz)**	**δ in ppm**
2	5.36	1	dd	$J_{2,3e}$= 1.52 $J_{2,3a}$ = 12.43	78.8
3a	3.13	1	dd	$J_{3a,3e}$= 16.96	42.3
3e	2.74	1	dd		
4	-	-	-	-	196.6
5	-	-	-	-	162.5
6	5.97	1	s	-	95.1
7	-	-	-	-	166.0
8	-	-	-	-	104.4
4a	-	-	-	-	101.7
8a	-	-	-	-	162.9
1’	-	-	-	-	129.2
2’,6’	7.31	2	d	$J_{2',3'}$= 8.13	127.7
3’,5’	6.82	2	d	$J_{5',6'}$= 8.13	114.8
4’	-	-	-	-	157.4
1’’	4.80	1	d	$J_{1'',2''}$= 10.05	73.5
2’’	4.25	1	brt	$J_{2'',3''}$= 9.30	70.5
1’’’	4.43	1	d	$J_{1''',2'''}$= 7.44	103.5
3’’					75.8
4’’					71.1
5’’					79.7
6a’’					
6b’’					60.0
6a’’’	3.45-3.96	11	m	---	61.1
6b’’’					
2’’’					73.0
3’’’					78.7
4’’’					68.7
5’’’					77.0

Int – integration; Mult - multiplicity

Table 17 - ^{1}H-NMR and ^{13}C-NMR data for compound P6 (in methanol– d^4)

		^{1}H-NMR			^{13}C-NMR
Proton	**δ in ppm**	**Int.**	**Mult.**	***J* (Hz)**	**δ in ppm**
2	5.38	1	dd	$J_{2,3e}$= 2.98 $J_{2,3a}$ = 12.66	78.6
3a	3.16	1	dd	$J_{3a,3e}$= 17.12	42.1
3e	2.76	1	dd		
4	-	-	-	-	181.5
5	-	-	-	-	162.4
6	5.99	1	s	-	95.0
7	-	-	-	-	166.4
8	-	-	-	-	104.6
4a	-	-	-	-	101.1
8a	-	-	-	-	163.3
1’	-	-	-	-	127.5
2’,6’	7.33	2	d	$J_{2',3'}$= 8,73	127.3
3’,5’	6.84	2	d	$J_{5',6'}$= 8.73	114.9
4’	-	-	-	-	157.9
1’’	4.81	1	d	$J_{1'',2''}$= 10.01	73.7
2’’	4.23	1	brt	$J_{2'',3''}$=9.23	70.1
4’’					78.7
6a’’	3.94-3.80	3	m		61.0
6b’’					61.0
3’’					80.3
3’’’					73.3
4’’’	3.63-3.78	5	m		72.9
6a’’’					61.3
6b’’’					61.3
1’’’	5.23	1	d	$J_{1''',2'''}$= 3.76	101.6
2’’’	3.44-3.53	2	m		73.3
5’’					79.8
5’’’	3.37-3.27		m		70.1
CH_3OD					

int – integration; mult - multiplicity

2.8. Preliminary toxicity screening – Cytotoxicity and genotoxicity evaluation

The toxicity screening for human cells was performed, using mammalian in vitro cell culture, namely rodent hepatocytes (H-II-4-E cells). Direct cytotoxicity was evaluated by the MTT cell survival assay. Genotoxicity was assessed by the in vitro cytokinesis-blocked micronuclei assay.

2.8.1. MTT cell survival assay

Concerning the cytotoxicity of the glycosylflavanones obtained, the exponentially growing eukaryotic cells (HII4E, rat hepatocytes) were exposed to test compounds (20 mg/mL in DMSO, final concentrations ranging from 2 mg/mL to 2x10-5 mg/mL), to negative (DMSO) and positive (H_2O_2) controls for 24 h. A cell blank control was also conducted. Cells were then exposed to MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] at a final concentration of 1mg/mL and incubated at 37 °C. The mitochondrial activity in cells transforms the MTT to a purplish water-insoluble pigment called formazan. The amount of formazan formed, proportional to cell viability in each well, is determined using a microplate reader and the results obtained are

presented in table 18. The acute toxicity assay showed a moderate level of cytotoxic effects in all the compounds studied, significantly different from the positive control (hydrogen peroxide). The less toxic compounds are P4 and P3 with IC_{50} ranging from 0.341 to 0.724 mg/mL, similar to that of commercial drug chloramphenicol (0.255 mg/mL in our cell model).

Table 18 – Cytotoxicity for the compounds synthesized in comparison with H_2O_2, chloramphenicol, DMSO and naringenin

Compounds	**IC_{50} (mg/mL)**	**Standard Deviation**
DMSO	3.2220	0.066
8-Galactosylnaringenin (P4)	0.7235	0.015
8-Rhamnosylnaringenin (P3)	0.3411	0.007
Chloramphenicol	0.2550	0.005
Naringenin (F1)	0.1502	0.003
8-Mannosylnaringenin (P2)	0.1123	0.002
8-Glucosylnaringenin (P1)	0.0532	0.001
8-Lactosylnaringenin (P5)	0.0398	0.001
8-Maltosylnaringenin (P6)	0.0218	0.000
H_2O_2	0.0080	0.001

2.8.2. In vitro cytokinesis-blocked micronuclei assay

The slide cell cultures were inoculated in petri dishes with the test compounds and controls. After 44 h of incubation, a solution of cytochalasin-B in DMSO was added. Subsequently, the medium was remove by aspiration, and washed. After drying and staining, codes and score binucleated cells from slides were screened for micronuclei in a microscope with a lens of 500x magnification, according to criteria set in Kirsch-Volders *et al* [10]. The preliminary genotoxicity results show a low genetic injury potential for the compounds tested, within the blank range. More experiments are currently underway to confirm these data.

Table 19 - Genotoxicity for the compounds synthesized in comparison with H_2O_2 and DMSO.

Compounds	**Micronuclei per 1000 cells**
8-Glucosylnarigenin (P1)	2
DMSO	2
8-Mannosylnarigenin (P2)	6
8-Rhamanosylnarigenin (P3)	6
8-Maltosylnarigenin (P6)	7
H_2O_2	30
8-Galactosylnarigenin (P4)	Culture failed

2.9 References

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Conclusion and future perspectives

Fries-type rearrangement was exploited as a tool for the direct coupling of sugars to naringenin as a very simple, economical and environmentally friend synthetic pathway.

The obtained *C*-glycosylnaringenin molecular entities were prepared by a single reaction step approach comprising formation of an *O*-glucoside, promoted by a Lewis acid, suffering a further rearrangement to give the desired C-glycosyl congener. Lewis acid lanthanide triflates were evaluated as catalysts of this reaction for the first time. The most effective one, that is also amongst the less expensive Lewis acids, was $Pr(OTf)_3$. It succeeded to catalyze direct coupling of free sugars, namely D-glucose, D-manose, D-galactose, L-rhamnose, lactose and maltose to naringenin in a stereo- and regio-selective manner to give the corresponding 8-glycosylnaringenin in good yield. The solvent used was a mixture of acetonitrile and water. However this approach showed a strong dependency on the amount of water present in the medium and the proportion of both solvents was optimized to be 2:1 for the coupling of monosaccharides and 1:1 for that of disaccharides to allow dissolution of the starting materials. Ultrasounds as energy

source were also tried for the first time in Fries-type reactions catalyzed by praseodymium triflate and reaction yields were frankly increased while reaction times were largely shortened.

As expected, isolation of the synthesized compounds was very difficult due to their hydrophilicity. Hence a lot of time was spent for optimization of the isolation procedures. The target molecules could have been acetylated for an easier separation, but this alternative would have introduced protection/deprotection steps, which could be avoided in the methodology developed in this thesis.

Full NMR characterization of synthesized compounds was accomplished using both mono- and bidimensional techniques confirming that only *C*-glycosylation at position 8 of naringenin occurred. Reaction stereoselectivity towards the β-anomer for all compounds with the expected exception of the D-mannosyl and L-rhamnosyl derivatives, which major product was the corresponding α-anomer, was corroborated by these techniques.

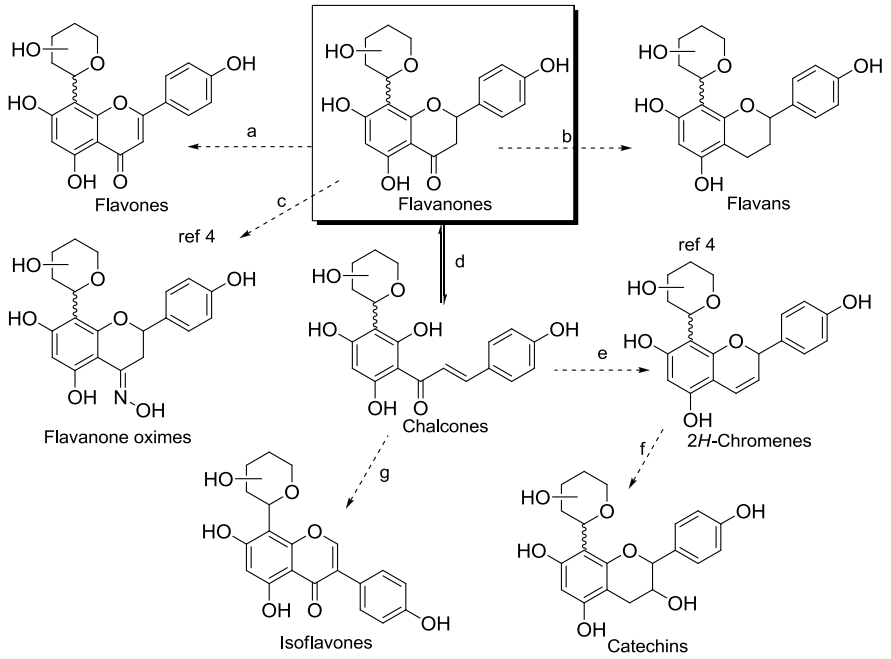
The results presented herein led to a direct and straightforward methodology for the coupling of free sugars to naringenin, a clean reaction, easy to run and giving a single major product isolated in yields ranging from 43% to 56%. Sonochemistry proved essential to optimize yield and reaction time of this regio- and

stereoselective reaction yielding the 8-glycosylnaringenin with α- or β-configuration, depending on the sugar starting material.

Cytotoxicity screening of the glycosylflavanones was accomplished. The acute toxicity results clearly indicated a moderate level of cytotoxicity of all the compounds studied, significantly different from that of the positive control (hydrogen peroxide). The preliminary genotoxicity results show a low genetic injury potential for the compounds tested, within the blank range.

This synthetic approach was widened to flavones, isoflavones and flavonols, but their skeleton embodying a chromen-4-one structure seems to hamper saccharide coupling possibly due to electron withdrawing effects. Nevertheless the easily accessed 8-glycosylnaringenin can be further chemically derivatized into other flavonoid derivatives by well-known procedures. Oxidation with DDQ and subsequent saponification converts glycosylflavanones into the corresponding flavones (scheme 1, a)[1]. Flavans are easily accessed by reduction of flavanones with sodium borohydride (scheme 1, b)[1]. Witczak *et al* [2]described the reaction of flavanones with hydroxylamine to afford flavanone oximes (scheme 1, c). In basic medium flavanones are interconverted into chalcones (scheme 1, d)[3], which can then be

cyclized into the respective 2*H*-chromenes (scheme 1, e)[4] and further hydroboration leads to a catechin type structure (flavan-3-ols) (scheme 1, f)[4]. To achieved the desired isoflavones the chalcone can undergo an oxidative rearrangement, by treatment with TTN in dimethoxymethane and methanol gave the dimethyl acetals, which in the presence of a HCl–methanol solution in 1,4-dioxane afford the desired isoflavones (scheme 1, g)[5].



Scheme 1. Chemical conversion of Flavanones into: a) Flavones[1]i. DDQ, PhCl; ii. NaOMe, MeOH. (b) Flavans [1] [1]$NaBH_4$, THF-H_2O. c) Flavanone oximes [2] $NH_2OH.HCl$, EtOH/pyr; d) Chalcones [3], Base. e) 2*H*-chromenes[4], $NaBH_4$, THF/EtOH. f) Cathechins [4] BH_3.THF. g)Isoflavones [5] i. TTN, $(MeO)_2CH_2$; ii. 10% HCl, MeOH.

Glycosylnaringenins are the *C*-congeners of naringenin glycosides. From the biological point of view, the latter exhibit a variety of potent bioactivities. For example prunin, a naringenin glucoside (figure 1), exhibited a remarkable and significant hypoglycemic effect in diabetic rats [6]. Naringenin 7-*O*-neohesperidoside (trivial name: naringin), possesses anxiolytic, sedative [7], antihyperglycemic and antioxidant effects [8]. Recent studies provided evidence that naringin ameliorates insulin resistance, dyslipidaemia, β-cell dysfunction, hepatic steatosis and kidney damage in type 2 diabetic rats by partly regulating oxidative stress, inflammation and adipocytokines production [9]. Another naringenin glycoside, namely its 7-*O*-rutenoside (trivial name: narirutin), is described as a possible new tool for the treatment of bronchial asthma [10]. Hesperidin, a 7-*O*-rutinoside of hesperitin, a tetrasubstituted flavanone presented anti-inflammatory, analgesic [11], hypolipidemic [12], antihypertensive and diuretic activity [13]. Therefore a detailed biological evaluation of the synthesized molecules is quite promising for their valorization as potentially new pharmacological leads.

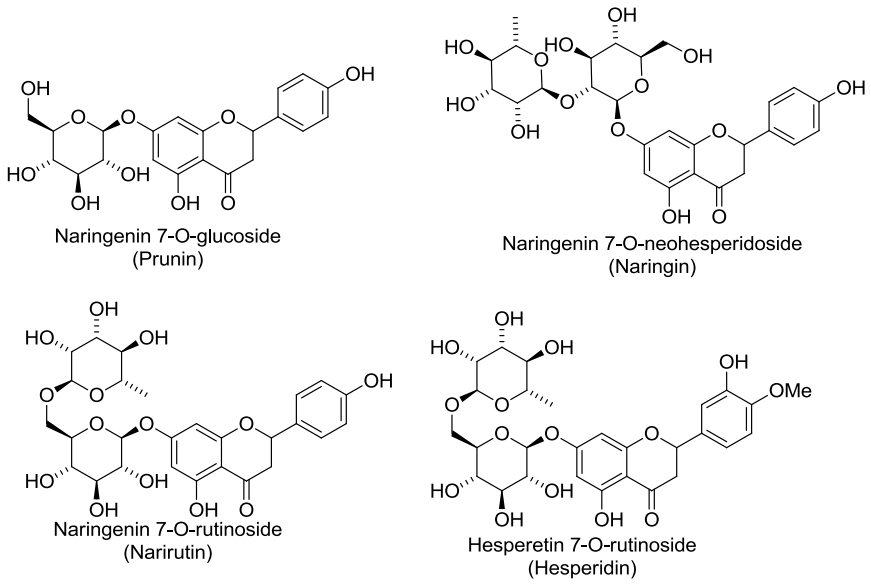


Figure 1-Structure of some bioactive flavanone glycosides

In addition, the methodology developed in this thesis allows an easy and environmentally friendly access to glycosylflavanones encouraging future studies to extend this approach to other sugars as well to other flavanone polyphenols for further biological properties evaluation.

3.1 References

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Experimental

4.1. General Methods

4.1.1 Solvents and Reagents

Solvents and reagents were purchased from Fluka, Sigma, Aldrich or Alfa-Aeser.

4.1.2. Ultrasound Experiments

The ultrasound experiences for coupling monosaccharides to flavonoids were carried out in the commercial ultrasound cleaning bath USC200T from VWR Collection with a frequency of 45 kHz (power: 80 W) set for 80 ºC. Concerning the coupling reaction with disaccharides those were performed with the ultrasonic processor UP200S from Hielscher with a variable working frequency of 24 kHz set to 100% (power: 200 W).

4.1.3 Evaporation and concentration

Büchi rotary evaporator R-215 coupled to a vacuum controller Büchi V-850 and to a vacuum pump V-700 was used to concentrate, under vacuum, the reaction mixtures as well as the final products. A heating bath Büchi B-491 was used to heat the solutions. The system was chilled with a cooling bath RTE100 from NESLAB set to 0 °C.

4.1.4. Thin-Layer Chromatography

Thin-layer chromatography (TLC) was performed on Silica Gel 60-F_{254} plates (Merck). The spots were detected with UV light chamber set to 254 nm, or by using a developing Sulfuric solution (H_2SO_4 conc. /EtOH/H_2O in a ratio of 5:45:45 followed by heating at 180 °C) or a solution of 1% Ferric (III) Chloride in Methanol/water (1:1).

4.1.5. Gel Chromatography

Gel column chromatography was performed on a 2 cm diameter column packed with 20 cm height of MCI GEL® CHP20P (Mitsubishi Chemical Corp) purchased from Sigma-Aldrich.

4.1.6. Column Chromatography

Flash column chromatography was performed on Silica Gel 230-400 mesh (Merck). The diameter, height and eluents were adapted for each reaction mixture.

4.1.7. HPLC

CHEMICALS AND STANDARDS

HPLC grade acetonitrile (ACN, 99.9%) and methanol (MeOH, 99.9%) were purchased from Panreac (Spain). *o*-Phosphoric acid (85.0 %) was purchased from Aldrich (Germany). Ultra-pure water was obtained from Milli-Q water purification systems (Millipore, USA).

PREPARATION OF STANDARDS AND SAMPLES

The stock solutions (1,000 mg L^{-1}) of individual standard and samples were prepared in HPLC grade MeOH. To prevent photodegradation, the stock solutions and working standards

after experimental procedure were wrapped in aluminium foil for storage at -4 ºC.

INSTRUMENTATION SETTINGS

HPLC-DAD analyses were carried out on an Agilent 1100 Series LC system (Agilent Technologies, Waldbronn, Germany), constituted by the following modules: vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostatted column compartment (G1316A) and the diode array detector (G1315B). The data acquisition and instrumental control were performed by the software LC3D ChemStation (version Rev.A.10.02[1757]; Agilent Technologies). Analyses were performed on a Tracer excel 120 ODS-A column, 150 mm × 4.0 mm, 5 μm particle size (Teknokroma, Spain).

The mobile phase consists on a mixture of ACN and 0.1% *o*-phosphoric acid aqueous solution with ratio of 20/80% (25 ºC) and a flow of 1.0 mL min^{-1}. The analyses were performed at 26 ºC and the injection volume was 20 μL with a draw speed of 200 μL min^{-1}. The detector was set at 226 nm.

For identification purposes, standard addition method was used by spiking the samples with the pure standards, as well as by comparing the retention parameters and UV-visible spectral reference data.

For quantification purposes, the external standard methodology was performed, using six calibration standards solutions having concentrations ranging from 10.0 to 1000.0 mg L^{-1}.

4.1.8. NMR Spectroscopy

NMR spectra were recorded on Brüker spectrometers: ^{1}H-NMR spectra were recorded at 400 MHz with Avance 400 (fitted with a QNP probe) for solution in acetone-d^6 and methanol-d^4 at room temperature. Assignments were confirmed by COSY experiments. ^{13}C-NMR spectra were recorded at 100.57 MHz with a Avance 400 (fitted with a QNP probe). Assignments were confirmed by HMQC and HMBC. Chemical shifts (δ) are given in ppm relative the residual solvent signal. Coupling constants (*J*) are reported in Hertz (Hz).
Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), [multiplicity, integration, coupling constant (Hz)]. Multiplicities are indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), brs (broad singlet), etc.

4.1.9 High-Resolution Mass Spectrometry

HRMS spectra were acquired with an Apex Ultra FTICR Mass Spectrometer equipped with an Apollo II Dual ESI/MALDI ion source (Bruker Daltonics), and a 7T actively shielded magnet (Magnex Scientific).

4.2 Synthesis

4.2.1 Evaluation of the catalyst efficiency

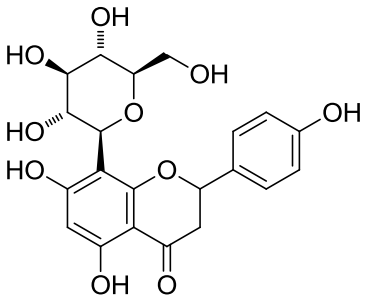
To a 2 mL acetonitrile/water (2:1) solution (±)-naringenin (150mg, 0.551 mmol), D-glucose (168.76 mg, 0.94 mmol, 1.7 eq) and lanthanide triflate (0.110 mmol, 0.2eq) were added. After stirring for 22 hours under reflux the reaction was quenched with 150 mL of water, the unreacted glucose and the catalyst were remove throughout a 2 centimeter diameter filtration column packed with MCI gel CHP20P, being the phenolic content recovered with 200 mL of 50% acetone aqueous solution and 100 mL of acetone. The phenolic fractions were combined and evaporated under reduced pressure. The residues were then subjected to HPLC analysis.

4.2.2.Monossacharide derivatives

4.2.2.1 General Procedure

To a 6 mL acetonitrile:water (2:1) solution of (±)-naringenin (500mg, 1.84 mmol), monosaccharide (1.7 eq) and praseodymium triflate (216 mg, 0.2 eq) were added. After stirring for 24 hours under reflux the reaction was concentrated under vacuum for further CC purification.

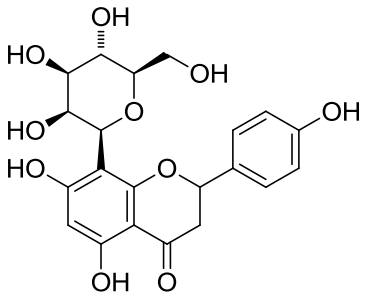
4.2.2.2. Synthesis of 8-β-D-Glucopyranosylnaringenin (P1)



8-β-D-Glucopyranosylnaringenin (P1). Starting with D-Glucose (563.5 mg, 3.14 mmol) using general procedure 1.2.2.1 the obtained residue was chromatographed (EtOAc/Acetone/AcOH/Water 45:15:2:1), to afford the glucose

derivative as yellowish oil (255 mg, 0.59 mol, 32 %). R_f = 0.45 (EtOAc/Acetone/AcOH/Water 30:15:2:1), **^{1}H-NMR** (400 MHz, Methanol-d^6): δ(ppm) 7.30 (d, 2H, $J_{2',3'}$= $J_{5',6'}$= 8.34 Hz, H-2', H-6'), 6.81 (d, 2H, H-3', H-5'), 5.97 (s, 1H, H-6), 5.34 (dd, 1H, $J_{2,3e}$= 2.40 Hz, $J_{2,3a}$ = 12.38 Hz, H-2), 4.78 (d, 1H, $J_{1'',2''}$= 9.85 Hz, H-1''), 4.12 (brt, 1H, $J_{2'',3''}$=9.65 Hz, H-2''), 3.85 (part A, ABX system, 1H, $J_{6a'',6b''}$=12.38 Hz, $J_{6a'',5''}$=1.64 Hz, H-6a''), 3.70 (part B, ABX system, 1H, $J_{6b'',5''}$=5.31 Hz, H-6b''), 3.37 (m, 3H, H-3'', H-4'', H-5''), 3.13 (dd, 1H, $J_{3a,3e}$= 17.18 Hz, H-3a), 2.73 (brd, 1H, H-3e) **^{13}C-NMR** (100.57 MHz, Methanol-d^6): δ(ppm) 198.2 (C-4), 167.3 (C-7), 164.4 (C-8a), 164.3(C-5), 159.1 (C-4'), 130.9 (C-1'), 129.1 (C-2',C-6'), 116.4 (C-3', C-5'), 106.3 (C-8), 103.9 (C-4a), 96.4 (C-6), 82.6 (C-5''), 80.5 (C-3''), 80.2 (C-2), 75.2 (C-1''), 72.6 (C-4''), 71.0 (C-2''), 62.9 (C-6''), 44.9 (C-3). **HRMS,** m/z: $[M+Na]^+$ calcd 457.11052; found 457.11013 (error 0.38 ppm)

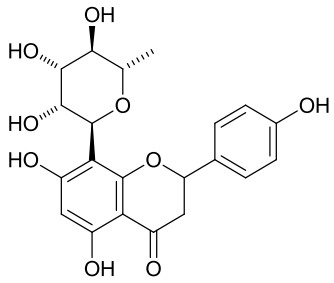
4.2.2.3. 8-α-D-Mannopyranosylnaringenin (P2)



8-α-D-Mannopyranosylnaringenin (P2). Starting with D-Mannose (564 mg, 3.14 mmol) using general procedure 1.2.2.1 the obtained residue was chromatographed (EtOAc/Acetone/AcOH/Water 45:15:2:1), to afford the desired product as a yellow oil (303 mg, 0.70 mol, 38 %). R_f = 0.56 (EtOAc/Acetone/AcOH/Water 30:15:2:1), **^{1}H-NMR** (400MHz, Methanol-d^6): δ(ppm) 7.25 (d, 2H, $J_{2',3'}$= $J_{5',6'}$=8.47 Hz,H-2', H-6'), 6.76 (d, 2H, H-3', H-5'), 5.87 (s, 1H, H-6), 5.30 (dd, 1H, $J_{2,3e}$= 2.95, $J_{2,3a}$ = 12.68 Hz, H-2), 5.05 (d, 1H, $J_{1'',2''}$= 9.20 Hz, H-1''), 3.93 (brt, 1H, $J_{2'',3''}$=2.97 Hz, H-2''), 3.85 (part A, ABX system, 1H, $J_{6a'',6b''}$=12.00 Hz, $J_{6a,5}$=1.89 Hz, H-6a''), 3.72 (part B, ABX system, 1H, $J_{6b'',5''}$=5.41 Hz, H-6b''), 3.61 (t, 1H, H-4'', $J_{4'',5''}$=9.47 H), 3.58 (ddd, 1H, H-3'', $J_{3'',4''}$=9.47 H), 3.32 (ddd, 1H, H-5''), 3.07 (dd, 1H, $J_{3a,3e}$= 17.29 Hz, H-3a), 2.69 (dd, 1H, H-3e) **^{13}C-NMR** (100.57MHz, Methanol-d^6): δ(ppm) 198.1 (C-4), 166.3 (C-7), 162.4 (C-5), 160.3 (C-8a), 157.9 (C-4'), 130.2 (C-1'), 129.1 (C-2',C-6'), 116.3 (C-3', C-

5'), 103.39 (C-8), 101.2 (C-4a), 97.4 (C-6), 83.4 (C-5''), 80.6 (C-2), 77.0 (C-1''), 76.1 (C-3''), 73.7 (C-2''), 68.3 (C-4''), 62.7 (C-6''), 44.1(C-3). **HRMS**, m/z: $[M+Na]^+$ calcd 457.11052; found 457.11118 (error -0.66 ppm)

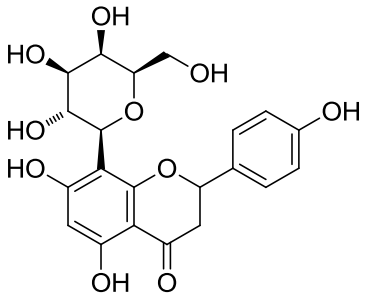
4.2.2.3. 8-α-L- Rhamnopyranosylnaringenin (P3)



8-α-L-Rhamnopyranosylnaringenin (P3). Starting with L-Rhamnose (514mg, 3.14 mmol) using general procedure 1.2.2.1 the obtained residue was chromatographed (EtOAc/Acetone/AcOH/Water 45:15:2:1), to afford the product as light brown solid (264 mg, 0.64 mol, 33 %). R_f = 0.52 (EtOAc/Acetone/AcOH/Water 30:15:2:1), **^{1}H-NMR** (400MHz, Methanol-d^6): δ(ppm) 7.30 (d, 2H, $J_{2',3'} = J_{5',6'}$ =8.35 Hz, H-2', H-6'), 6.81 (d, 2H, H-3', H-5'), 5.91 (s, 1H, H-6), 5.34 (dd, 1H, $J_{2,3e}$= 2.91 Hz, $J_{2,3a}$ = 12.20 Hz, H-2), 5.04 (d, 1H, $J_{1'',2''}$= 10.36 Hz, H-1''), 3.97 (brt, 1H, $J_{2'',3''}$=3.55 Hz, H-2''), 3.58 (brt, 1H, $J_{3'',4''}$=9.14 Hz, H-3''),

3.46 (t, 1H, $J_{4'',5''}$=9.37 H, H-4''), 3.39 (m, 1H, H-5''), 3.12 (dd, 1H, $J_{3a,3e}$= 17.17 Hz, H-3a), 2.73 (dd, 1H, H-3e) 1.36 (d, 3H, $J_{6'',5''}$=5.90 Hz, CH_3), **^{13}C-NMR** (100.57MHz, Methanol-d^6): δ(ppm) 198.4 (C-4), 163.9 (C-7), 161.9 (C-8a), 160.8 (C-5), 159.3 (C-4'), 131.4 (C-1'), 130.0 (C-2',C-6'), 116.2 (C-3', C-5'), 105.6 (C-8), 102.9 (C-4a), 97.6 (C-6), 80.6 (C-2), 78.5 (C-5''), 76.8 (C-1''), 75.6 (C-3''), 73.5 (C-4''), 73.3 (C-2''), 43.6 (C-3) , 18.1 (C-6''). **HRMS**, m/z: $[M+Na]^+$ calcd 441.11560; found 441.11513 (error 0.47 ppm)

4.2.2.4 8-β-D-Galactopyranosylnaringenin (P4)



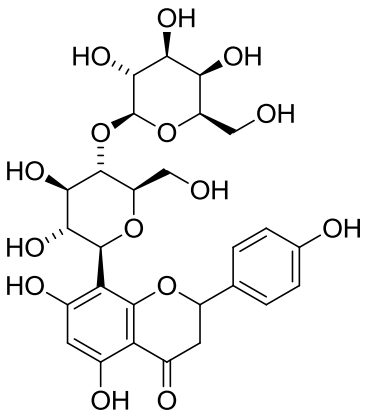
8-β-D-Galactopyranosylnaringenin (P4). Starting with D-Galactose (566 mg, 3.14 mmol) using general procedure 1.2.2.1 the obtained residue was chromatographed (EtOAc/Acetone/AcOH/Water 45:15:2:1), to afford the targeted product as dark yellow oil (279 mg, 0.64 mol, 35 %). R_f = 0.49 (EtOAc/Acetone/AcOH/Water 30:15:2:1), **^{1}H-NMR** (400MHz, Methanol-d^6): δ(ppm) 7.37 (d, 2H, $J_{2',3'}$= $J_{5',6}$' =8.68 Hz, H-2', H-6'),

6.88 (d, 2H, H-3', H-5'), 5.95 (s, 1H, H-6), 5.45 (dd, 1H, $J_{2,3e}$= 3.06 Hz, $J_{2,3a}$ = 12.50 Hz, H-2), 4.81 (d, 1H, $J_{1'',2''}$= 9.73 Hz, H-1''), 4.06 (d, 1H, $J_{2'',3''}$=2.35 Hz, H-2''), 4.03 (td, 1H, H-4'', $J_{4'',5''}$=2.99 H), 3.77-3.68 (m, 3H, H-5'', H-6a'', H-6b''), 3.65 (dd, 1H, H-3'', $J_{3'',4''}$=9.53 H), 3.18 (dd, 1H, $J_{3a,3e}$= 15.78 Hz, H-3a), 2.75 (dd, 1H, H-3e) **^{13}C-NMR** (100.57MHz, Methanol-d^6): δ (ppm) 191.0 (C-4), 164.4 (C-7), 162.2 (C-5), 160.9 (C-8a), 158.1 (C-4'), 129.4 (C-1'), 128.2 (C-2',C-6'), 115.1 (C-3', C-5'), 105.2 (C-8), 102.3 (C-4a), 95.7 (C-6), 79.1 (C-2), 78.9 (C-5''), 75.1 (C-3''), 74.7 (C-1''), 70.2 (C-4''), 68.9 (C-2''), 61.3 (C-6''), 42.8 (C-3). **HRMS**, m/z: $[M+Na]^+$ calcd 457.11052; found 457.11043 (error 0.08 ppm)

4.2.3. Dissacharides

To a 6 mL acetonitrile:water (2:1) solution naringenin (500mg, 1.84 mg), monossaccharide (1.7 eq) and praseodymium triflate (216 mg, 0,2eq) were added. After stirring for 48 hours under reflux the reaction was concentrated under vacuum for further CC purification.

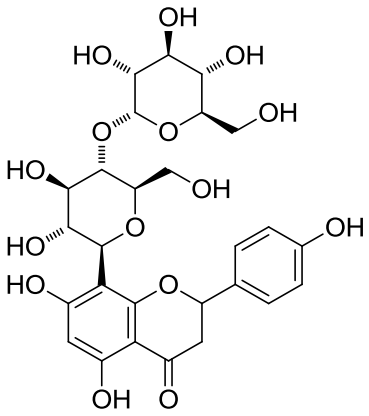
4.2.3.1. Synthesis of 8-β-Lactosylnaringenin (P5)



8-β-Lactosylnaringenin (P5). Starting with lactose (1071 mg, 3.13 mmol) using the general procedure for disaccharides the obtained residue was chromatographed (EtOAc/ MeOH/Water 10:4:1) to afford desired product as pale yellow oil (307 mg, 0.52 mmol, 28 %). R_f = 0.31(EtOAc/ MeOH/Water 7:2:1), **^{1}H-NMR** (400MHz, Methanol-d^6): δ(ppm) 7.31 (d, 2H, $J_{2',3'}$=$J_{5',6'}$= 8.13 Hz, H-2', H-6'), 6.82 (d, 2H, H-3', H-5'), 5.97 (s, 1H, H-6), 5.36 (dd, 1H, H-2, $J_{2,3a}$= 12.43 Hz, $J_{2,3e}$= 1.52 Hz), 4.80 (d, 1H, $J_{1'',2''}$= 10.05 Hz, H-1''), 4.25 (bt, 1H, $J_{2'',3''}$= 9.30 Hz, H-2''), 4.43 (d, 1H, H-1''', $J_{1''',2'''}$=7.44 Hz), 3.45-3.96 (m, 11H, 3'', 4'', 5'', 6a'', 6b'', 2''', 3''', 4''', 5''', 6a''', 6b'''), 3.13 (dd, 1H, $J_{3a,3e}$= 16.96 Hz, H-3a), 2.74 (dd, 1H, H-3e) **^{13}C-NMR** (100.57MHz, methanol-d^6): δ(ppm) 196.6 (C-4), 166.0 (C-7), 162.5 (C-5), 162.9 (C-8a), 157.4 (C-4'), 129.2 (C-1'), 127.7 (C-2',C-6'), 114.8 (C-3', C-5'), 104.4 (C-8), 103.5 (H-1'''), 101.7 (C-4a), 95.1

(C-6), 79.7 (C-5''),78.8 (C-2), 78.7 (C-3'''), 75.8 (C-3''), 77.0 (C-5'''), 73.5 (C-1''), 73.0 (C-2'''), 71.1 (C-4''), 70.5(C-2''), 68.7 (C-4'''), 61.1 (C-6'''), 60.0 (C-6''), 42.3 (C-3). **HRMS**, m/z: $[M+Na]^+$ calcd , 619.16334; found 619.16185 (error 1.49 ppm).

4.2.3.2. Synthesis of 8-βMaltosylnaringenin (P6)



8-(β-Maltosyl)naringenin (P6). Starting with maltose (1065 mg, 3.11 mmol) using the general procedure for disaccharides the obtained residue was chromatographed (EtOAc/ MeOH/Water 10:4:1) to afford desired product as pale yellow oil (373 mg, 0.63 mol, 34 %). R_f = 0.37 (EtOAc/ MeOH/Water 7:2:1), **^{1}H-NMR** (400MHz, Methanol-d^6): δ (ppm) 7.33 (d, 2H, $J_{5',6'}$= $J_{2',3'}$= 8,73 Hz, H-2', H-6'), 6.84 (d, 2H, H-3', H-5'), 5.99 (s, 1H, H-6), 5.38 (dd, 1H, H-2, $J_{2,3a}$= 12.66 Hz, $J_{2,3e}$= 2.98 Hz), 5.23 (d, 1H, H-1''', $J_{1''',2'''}$=3.76 Hz), 4.81 (d, 1H, $J_{1'',2''}$= 10.01 Hz, H-1''), 4.23 (bt, 1H, $J_{2'',3''}$= 9.23 Hz, H-

2''), 3.94-3.80 (m, 3H, 4'', 6a'', 6b''), 3.63-3.78 (m, 5H, 3'', 3''', 4''', 6a''', 6b'''), 3.44-3.53 (m, 2H, 2''', 5''), 3.37-3.27 (m, 5''', MeOD), 3.16 (dd, 1H, $J_{3a,3e}$= 17.12 Hz, H-3a), 2.76 (dd, 1H, H-3e) **^{13}C-NMR** (100.57 MHz, Methanol-d^6): δ(ppm) 181.5 (C-4), 166.4 (C-7), 163.3 (C-8a), 162.4 (C-5), 157.9 (C-4'), 127.5 (C-1'), 127.3 (C-2',C-6'), 114.9 (C-3', C-5'), 104.6 (C-8), 101.6 (H-1'''), 101.1 (C-4a), 95.0 (C-6), 80.3 (C-3''), 79.8 (C-5''), 78.7 (C-4''), 73.7 (C-1''), 73.3 (C-3'''), 73.3 (C-2'''), 72.9 (C-4'''), 70.1 (C-2), 70.1 (C-5'''), 70.1 (C-2''), 61.3 (C-6'''), 61.0 (C-6''), 42.1 (C-3). **HRMS,** m/z: $[M+Na]^+$ calcd 619.16334; found 619.16528 (error -1.94 ppm).

4.2.4. Ultrasounds

Concerning the monossacharides, to a 4 mL acetonitrile:water (2:1) solution naringenin (500mg, 1.84 mg) saccharide (1.7 eq) and praseodymium triflate (216 mg, 0,2eq) were added. After 8 hours in an ultrasonic bath at 80 ^{0}C the reaction was concentrated under vacuum for further CC purification. Regarding the reaction with dissacharides, the reaction mixture was subjected to ultrasounds for 12 hours. Compound P1, P2, P3, P4, P5 and P6 were isolated with a yield of 56%, 46%, 51%, 49%, 43% and 51%, respectively.

4.2.5.Toxicological Evaluation

The toxicity screening for human cells was performed, using mammalian in vitro cell culture, namely rodent hepatocytes (H-II-4-E cells). Direct cytotoxicity was evaluated by the MTT cell survival assay. Genotoxicity was assessed by the in vitro cytokinesis-blocked micronuclei assay.

4.2.5.1. MTT ASSAY

MTT assay was performed according to Invittox Method nº 17. Exponentially growing eukaryotic cells (HII4E, rat hepatocytes) were seeded into 96-well microplates at $5x10^4$ cell number/well and allowed to attach for 24h. Cells were exposed to test compounds (20mg/mL in DMSO, final concentrations ranging from 2mg/mL to $2x10^{-5}$mg/mL), negative control (DMSO) and positive controls (H_2O_2), or cell blank for 24h.

Cells were then exposed to MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] at a final concentration of 1mg/mL and incubated at 37°C for a short time (3h); mitochondrial activity in cells transforms the MTT to a purple – colored water-insoluble pigment called formazan. The medium was then removed and 100µL DMSO was added to each well to disrupt cell membrane and solubilize formazan. The amount of formazan formed,

proportional to cell viability in each well, was determined using a microplate reader (modified spectrophotometer), set to 540nm reading wavelength and 630nm reference wavelength.

The number of cells present in all wells is expressed as a percentage of cell blank (cells in full growth without added compounds or controls). Results are expressed as IC50, the 50% inhibitory concentration, which is interpolated form the logarithmic dose-response curves.

4.2.5.2. IN VITRO CYTOKINESIS-BLOCKED MICRONUCLEI ASSAY

CELL CULTURES

The cells were sub-culture in MEM complete medium (Sigma Aldrich, St. Louis, USA) supplemented with 10% fetal calf serum, penicillin (100 IU/ml), streptomycin (100µg/ml), 1% L-glutamine (all from (Sigma, St Louis, MO, USA), in petri dishes containing a 20mm by 20mm sterile glass slide. Test compounds and controls were added and incubated. After 44h of incubation, cytochalasin-B (Sigma, St. Louis, MO, USA) was added to a final concentration of 12.5 µM. (6µg/ml, stock solution 8.34 mM prepared in DMSO).

SLIDES PREPARATION

After a total of 72 hours of culture the medium was removed by aspiration, and the slides in the petri dish gently washed twice with cold PBS and 5 ml of cold methanol/acetic acid (3:1). The slides were dried before staining with 4% Giemsa in 0.01 M. phosphate buffer pH 6.8 for 10minutes cover slips on to microscopy slides, with Entellan® (Merck, Darmstadt, Germany), were attached.

SCORING OF SLIDES

The slides were coded and scored at 500x magnification, according to criteria set in Kirsch-Volders *et al.* [1].

4.3. References

1. Kirsch-Volders, M., et al., *Report from the In Vitro Micronucleus Assay Working Group.* Environmental and Molecular Mutagenesis, 2000. **35**(3): p. 167-172.