Send Reprints Orders on reprints@benthamscience.org

Mini-Reviews in Medicinal Chemistry, 2012, *12*, 1465-1476

Diels-Alder Cycloaddition in the Synthesis of 1-Azafagomine, Analogs, and Derivatives as Glycosidase Inhibitors

Daniela A.L. Salgueiro, Cristina E.A. Sousa, A. Gil Fortes and M. José Alves*

Departamento de Quimica, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Abstract: This comprehensive review deals with the synthesis of 1-azafagomine, analogs, and derivatives having the Diels-Alder cycloaddition as the key step. Most of the compounds referred are racemic or have been resolved by lipase transesterification. There are two asymmetric cycloadditions leading to 1-azafagomine or to an analog. In one case both enantiomers of 1-azafagomine were prepared together with a pair of derivatives. The study comprises glycosidase inhibition studies of the target compounds to a set of glycosidic enzymes, and evidenced molecular features that enhance or diminish their activity as glycosidase inhibitors.

Keywords: Azafagomine, biological activity, Diels-Alder cycloaddition, glycosidase inhibitors, azasugars.

1. INTRODUCTION

Glycosidases are crucial enzymes in all living organisms because they control many biological processes. Potent and selective inhibition of these enzymes is a very important topic that deals with finding the right chemical entities, mainly entrusted to synthetic chemists. Azasugars are major targets as glycosidase inhibitors due to their ability to mimic the transition state of saccharides in the enzymes. Two azasugars are currently being used in clinics: Miglitol against diabetes type II from Gliset®, and Miglustat against Gaucher disease from Zavesca®. Many other azasugars have been shown to be active against diabetes [1], cancer [2], hepatitis [3], Gaucher's disease [3], AIDS [4,5] and influenza [6]. In some cases the target azasugars reached advanced clinical trials, as did D-swainsonine that passed the clinical trial phase II [7], but for one or two undesirable secondary effects they have failed to be introduced as pharmaceuticals. Fig. **1** shows the five groups of natural azasugars known. This brief review deals only with synthetic piperidine and indolizidine analogs, containing an N-N unit incorporated in the molecules, in which the crucial process in its synthesis is a Diels-Alder cycloaddition.

Azasugars are able to disguise sugars because the cleavage of the anomeric group develops a positive charge that can be stabilized either by oxygen or nitrogen atoms, attached to the anomeric carbon. Fig. **2** shows the developing positive charges on the transition state of glucosides in glucosidases active sites. The cleavage of the anomeric oxygen group develops a positive charge at the anomeric carbon (**A**), and the resonance effect creates a second transition state (**B**). Which of these transition states seems more important depends on the enzyme, although it is likely that most enzymes have a component of each [8-10].

*Address correspondence to this author at the Departamento de Quimica, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal; Tel: 253 604376; Fax: 263 604382; E-mail: mja@quimica.uminho.pt

It had been explained that the stereoelectronic effect that assists the α-cleavage is due to the *anti*-periplanar disposition of the axial electron pair at the glucopyranose oxygen atom to give type **B** transition state, with the positive developing charge at the oxygen. On the other hand, the β-cleavage has to occur with no stereoelectronic assistance, giving transition state type **A** with the positive charge developing at the anomeric carbon atom [11] (Fig. **2**). As a consequence glucose analogs that develop a positive charge at the oxygen/nitrogen atoms will be α-inhibitors, and those that develop a positive charge on the anomeric carbon will be β-inhibitors.

2. SYNTHESIS OF RACEMIC 1-AZAFAGOMINE, AND ANALOGS

(±)-1-Azafagomine (**1**) potently inhibits yeast α-glucosidase and almond β-glucosidase with very low K_i values as will be presented ahead. This means that there is a close connection in the ability of 1-azafagomine to accept a proton at the active site of the enzyme and the transition states **A** and **B**. In fact the mixture of the hydrazonium ions clearly resembles transition states **A** and **B** (Fig. **3**). On this basis is to be expected that they will fit well in the active site of α- and β-glucosidases, explaining the potential of such compounds over these enzymes.

2.1. Synthesis of 1-Azafagomine, and Monocyclic Analogs

Racemic 1-azafagomine (±)-**1** was obtained through a synthetic sequence based on a Diels-Alder cycloaddition between achiral materials: 2,4-pentadien-1-ol (**2**) and 4-phenyl-1,2,4-triazole-3,5-dione (PTAD, **3**). The racemic cycloadduct **4** was epoxidized with trifluoromethyl (methyl)dioxirane generated *in situ* to furnish a 3:1 ratio of isomers, from which the major epoxide, **5**, was isolated in 62 % yield. The hydrolysis of epoxide **5** under perchloric acid afforded triol **6** in 73 % with a high degree of stereoselectivity. Treatment of **6** with hydrazine at 100 ºC gave finally compound (±)-**1** in 84 % yield [11] (Scheme **1**).

1875-5607/12 $58.00+.00

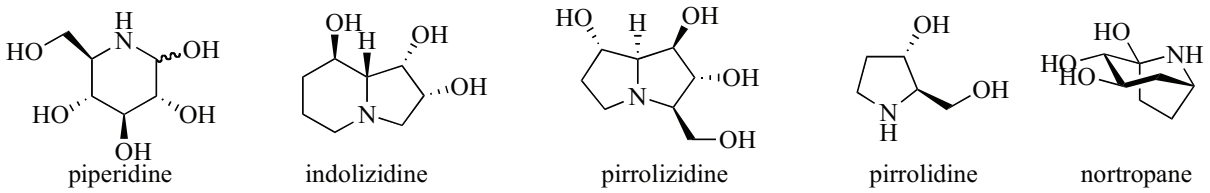


Fig. (1). Natural azasugar groups.

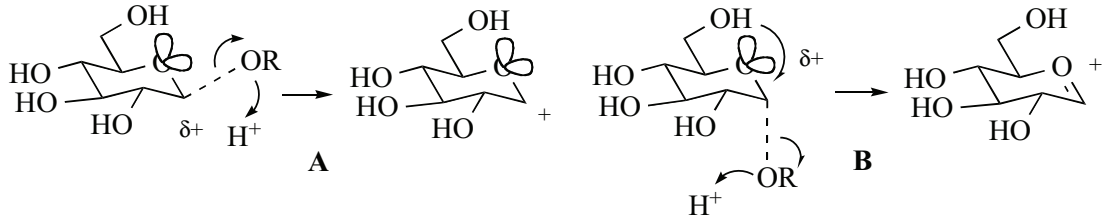


Fig. (2). Two possible transition states of a D-glucose unit in a saccharide.

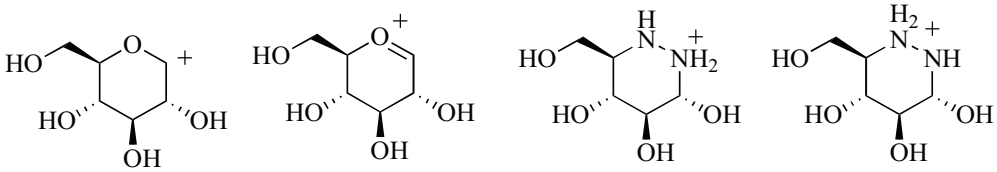
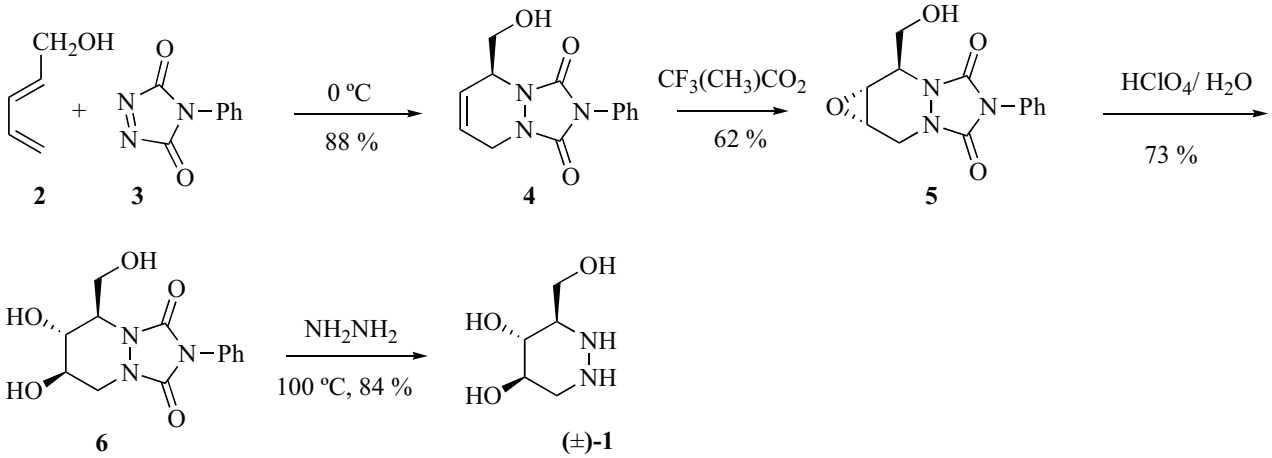


Fig. (3). Structural resemblance between D-glucose transition states **A** and **B** with the two hydrazonium ions of protonated 1-azafagomine.



Scheme (1). Synthesis of racemic 1-azafagomine **1**.

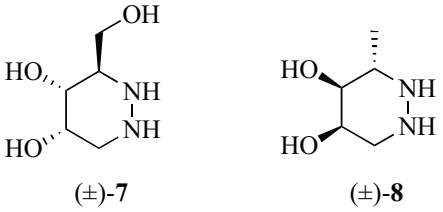
A high biological activity was found in racemic 1-azafagomine, inhibiting both α-and β-glucosidases. The almond β-glucosidase inhibition is demonstrated by the K_i = 0.65 μM at pH=6.8, but curiously, the results are not much different with pH ranging from 5.0 to 7.5: at pH = 5.0, K_i = 0.76 μM, and pH = 7.5, K_i = 1.09 μM. To explain the independence of the inhibition potential of 1-azafagomine (**1**) to the medium pH, compound (±)-**1** was protonated with aqueous acidic solution and titrated with NaOH; the pK_a was revealed to be 3.9. Hydrazine is very weak base, largely unprotonated even at pH=5.0. This means that the unprotonated 1-azafagomine is the enzyme substrate even at pH=5. 1-Azafagomine is also a potent inhibitor of yeast α-glucosidase K_i= 3.9 μM at pH= 6.8 [11]. Isomaltase from backer's yeast and phosphorylase A are too highly inhibited by 1-azafagomine with K_i = 1.06 μM and IC_{50} = 13.5 μM respectively. Other glycosidases were tested including α- / β-galactosidases and α-mannosidases, but the results were very poor. Interestingly compound **1** combines the strong α-inhibition of deoxynojirimycine (DNJ) with the strong β-inhibition of isofagomine showing that 1-azafagomine is able to mimic both the transition state **A** and **B**, and can be considered a hybrid of those azasugars. But, on the contrary, deoxynojirimycine and isofagomine are highly dependent of

Table 1. Inhibition Constants (K_i) in μM of Various Enzymes on Target Compounds Measured at 25 ºC

Enzyme	 (±)-1	 DNJ	 isofagomine	 (±)-7	 (±)-8	 (±)-13	 (±)-15	 (±)-11a, R=H 11b, R= Me
α-glucosidase (yeast, pH 6.8)	3.9	2.5	86	> 1000	> 1000	> 1000	340	3600 (**11a**) 92 (**11b**)
β-glucosidase (almonds, pH 6.8)	0.65	47	0.11	137	41	78.8	46	540 (**11a**) 3 (**11b**)
β-glucosidase (almonds, pH 5.0)	0.76	330	---	---	---	---		---
β-glucosidase (almonds, pH 7.5)	1.09	---	---	---	---	---		---
Isomaltase (baker´s yeast, pH 6.8)	1.06	11	7.2	3080	> 3000	---	95	690 (**11a**) 4 (**11b**)
α-galactosidase (*E. coli.* pH 6.8)	934	> 1000	---	---	---	---		---
β-galactosidase (*E. coli.* pH 6.8)	702	> 1000	---	149	> 1000	---		---
α-mannosidase (jack bean pH 5.0)	3306	270 (pH 4.5)	770 (pH 4.5)	323	185	---		---
Phosphorylase A	13.5 (IC_{50})	55000	---	---	---	---		---

the pH experiment, which agrees with their much higher basicity (See Table **1**).

Pyridazines **7** and **8** do not have the configuration of any natural sugar, but they were moderately competitive inhibitors of β-glucosidase and α-mannosidase; compound **7** is in some extent also a moderate inhibitor of β-galactosidase. Compound **7** was obtained in 79 % yield by osmilation of compound **4**; compound **8** was obtained in 55 % overall yield, following the steps described in Scheme **1**, but using piperylene as the diene (Fig. **4**).



(±)-**7** (±)-**8**

Fig. (4). Structures of compounds (±)-**7** and (±)-**8**.

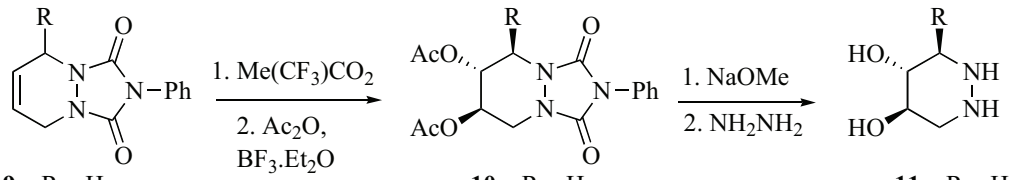
Using piperylene or 1,3-butadiene as dienes and PTAD as dienophile, were obtained cycloadducts **9a** and **9b** [12]. The synthetic process includes first the synthesis of the oxirane with trifluoromethyl(methyl)dioxirane which was then treated with acetic anhydride in acetic acid in the presence of trifluoroboro etherate to yield compounds **10a** (80.8 %) and **10b** as 6 (*trans*) : 1 (*cis*) mixture of isomers (80.9 %). The diacetates were deprotected with sodium methoxide in methanol, followed by hydrazinolysis to afford compound **11a** (56 %) and **11b** (35 %) over the two final steps [12] (Scheme **2**).

The biological activity of compound **11b** as a pure enantiomer (3*R*,4*R*,5*R*) [13] is one order of magnitude less active than (±)-1-azafagomine relatively to β-glucosidase (K_i = 3.0 μM) and to α-glucosidase (K_i = 92.0 μM), and 4 times less active than isomaltase (K_i = 4.0 μM). Compound **11a** shows a much lower activity than (±)-1-azafagomine (See Table **1**).

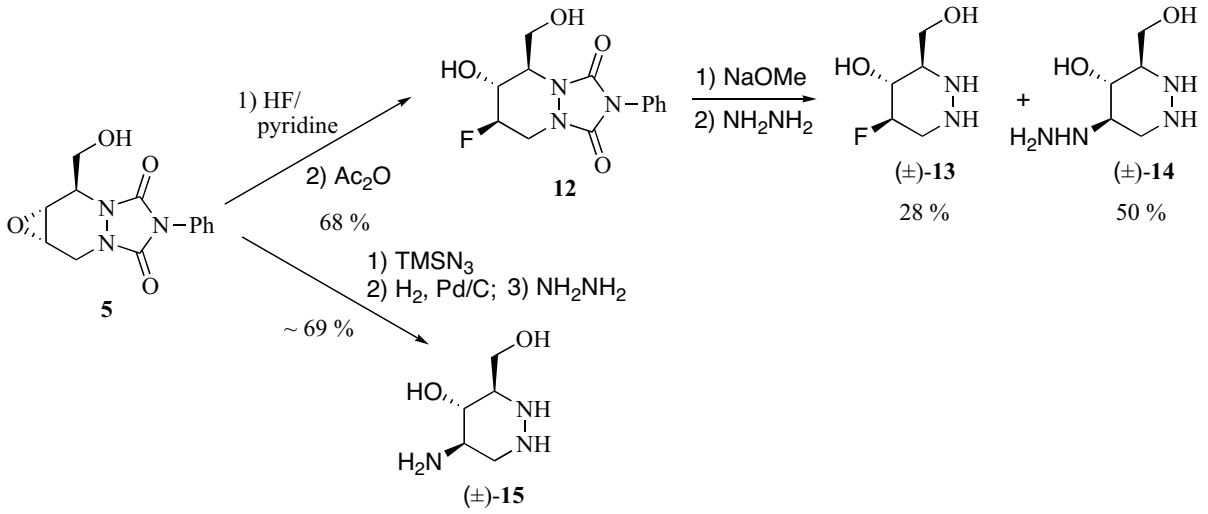
To investigate the role of the hydroxyl groups in the binding to the enzyme and find if other groups could successfully substitute the hydroxyl groups, fluoride, amino, and hydrazine were introduced at position 3. Fluorine is a bioisostere of the hydroxy group with similar polarity and shape [14]. The amino group was introduced because it strongly enhanced the activity of neuraminic acid derivatives on neuraminidase and the same trend could happen with 1-azafagomine derivatives relatively to glycosidases [15].

The hydroxy group at C-3 in compound **1** was displaced with fluorine by using the synthetic intermediate epoxide **5**. Reaction of compound **5** with HF and pyridine furnished a fluorinated compound, which was peracetylated to promote isolation giving compound **12** in 68 % yield. Deacetylation of **12** with sodium methoxide followed by hydrazinolysis yield compound **13** (28 %) together with compound **14** (50%) as by-product. It had been demonstrated that the fluorine work as a leaving group under hydrazinolysis conditions, giving back the epoxide then opening again with hydrazine (Scheme **3**).

The amino group at the position 3 was introduced by opening the epoxide with trimethylsilylazide followed by reduction of the azido group and hydrazinolysis of the urazol moiety. Compound **15** was obtained in 69 % yield after the 3 steps [16, 17] (Scheme **3**).



Scheme (2). Synthesis of racemic compounds **11a** and **11b**.



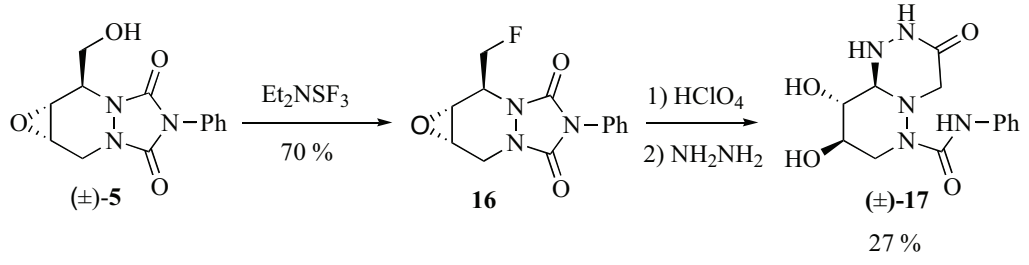
Scheme (3). Synthesis of 3-fluoro-, 3-hydrazinyl-, and 3-amino-1-azafagomines.

Displacement of the primary hydroxyl group in compound **5** with fluorine was carried out with diethylaminosulfur trifluoride (DAST). This reagent is known to be a powerful reagent for direct displacement of 6-hydroxyl group for fluorine in unprotected glucosides. The fluorine compound, **16**, was obtained with 70 % yield. The epoxide hydrolysis occurs under aqueous perchloric acid and is followed by hydrazynolysis with defluorination to give compound **17** as the only product formed in 27 % yield from **16** [17] (Scheme **4**).

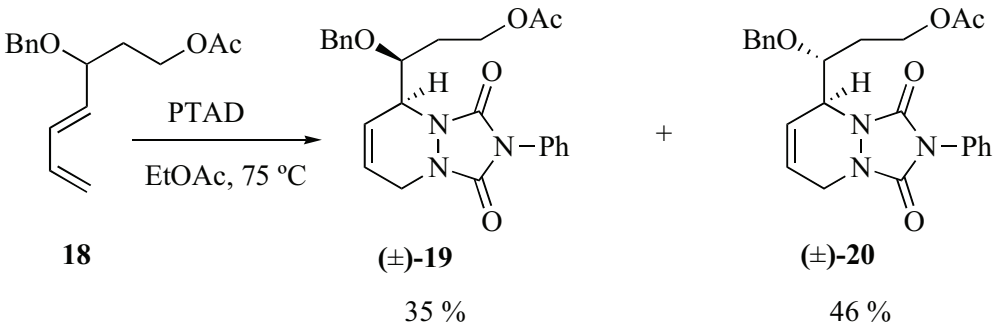
Compound **13** as is shown in the Table **1** is poor inhibitor of both the α- and β-glucosidases which suggests that the hydroxy group binds in the enzyme as a proton donor rather than a proton acceptor, which of course the fluoride atom has no ability to do. The substitution of the hydroxyl group for the amino group (compound **15**) has shown a diminution of activity either to α- and β-glucosidases. The authors also concluded that the exocyclic amino group do not act as a hydrogen acceptor and its hydrogen donor ability is not affected by protonation. As happen with 3-amino-isofagomine [16] the amino group is acting as a poor hydrogen-bond donor compared to the hydroxyl group at the same position. Comparing **15** to the fluoro analog **13** to which no hydrogen bond donor effect can be described it seems that the inhibitory potential of compound **15** is between the excellent hydrogen bond donor ability of compound **1** and the poor effect displayed by compound **13**.

2.2. Synthesis of Fused Bicyclic Azafagomine Analogs (Castanospermine Analogs)

Two epimers of 5-aza-6-deoxycastanospermine analogs were obtained through Diels-Alder cycloaddition in the first step, from 5-benzyloxy-7-acetoxyhepta-1,3-diene **18** and PTAD [18] (Scheme **5**).



Scheme (4). Attempt to substitute the hydroxymethyl group for fluoromethyl of 1-azafagomine.



18 **(±)-19** **(±)-20**

35 % 46 %

Scheme (5). Cycloaddition of the diene **18** to PTAD.

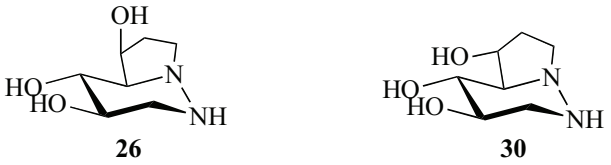
Each of the epimeric adducts (±)-**19** and (±)-**20** underwent functional group transformations to give the azacastanospernine analogs. Scheme **6**, briefly summarizes the synthetic sequence of one of the epimers, compound (±)-**26**.

Compound (±)-**19** was subjected to epoxidation followed by ring opening in the presence of trifluoroboro etherate, and acetic anhydride / acetic acid. The *anti* relationship of the acetyl groups was obtained, as expected. The acetyl groups were removed in compound **21** with sodium metoxide/ methanol. Oxidation with TEMPO and sodium hypochlorite gave δ–lactone **22**. Reduction with diisobutylaluminium hydride, followed by acetylation with acetic anhydride in the presence of triethyamine and 4-dimethylaminopyridine afforded compound **23**. Treatment of **23** with benzylic alcohol in the presence of trifluoroboro etherate gave the acetal **24**. This compound was deacetylated and then treated with hydrazine to give **25**. Hydrogenolysis under Pd/C in methanol cleaved the benzyl groups and turn the aldehyde function free to suffer a reductive amination forming 5-aza-6-deoxycastanospermine **26** (Scheme **6**).

Compound (±)-**21** was subjected to deacetylation with sodium metoxide, followed by hydrazinolysis to yield the triol **27**. The unprotected monocyclic **28** was obtained by hydrogenation under Pd in the presence of HCl (Scheme **7**). The same sequence was applied to the product obtained from compound (±)-**20**: epoxidation followed by epoxide opening in the presence of trifluoroboro etherate, under acetic anhydride / acetic acid to yield the respective diastereomer **29** (See Table **2**).

Epimer **26** showed a poor α-glucosidase inhibition to the yeast source (K_i >600 μM) and a much better inhibition to α-glucosidase from rice (K_i= 15 μM). The values for β-glucosidase obtained from almonds are also good (K_i= 10 μM). Even so the best result of castanospermine is for α-glucosidase from rice (K_i= 0.015 μM), three orders of magnitude more active than **26** for the same enzyme. The epimer of **26**, **30** having the hydroxyl group of the five-membered ring at the equatorial position showed poorer values to the same enzymes: between K_i= 570 – 690 μM, except for α-glucosidase from rice to which the value is even poorer K_i >1000 μM. This strongly suggests a clear preference of glucosidases for binding substrates with the hydroxy group at the axial position [18] (Fig. **5**).

The best results obtained for compounds **29** and **30** goes for α-glucosidases either from rice and yeast, even so the K_i are between 150 and 380 μM.



26 **30**

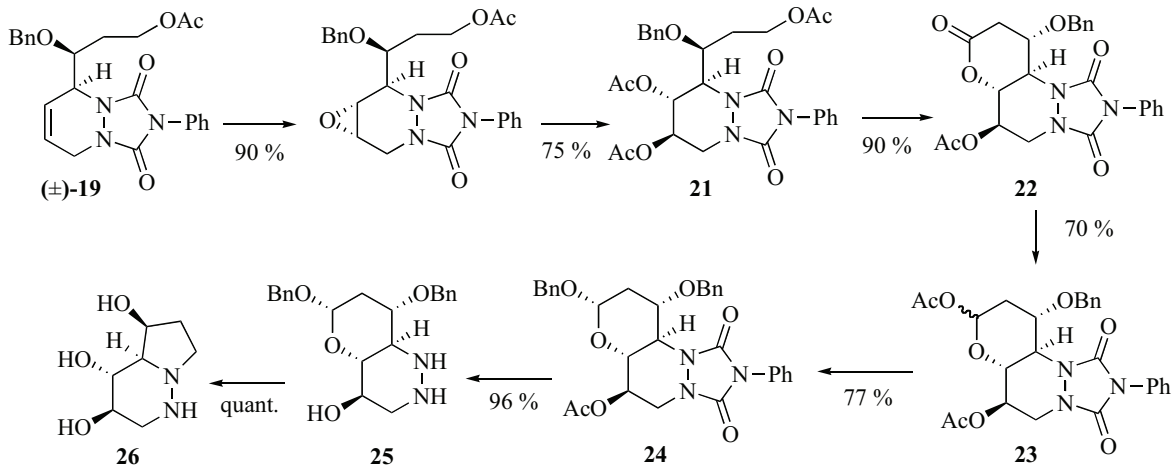
Fig. (5). Haworth representations of 5-aza-6-deoxy castanospermine analogs.

3. CHIRAL 1-AZAFAGOMINES, DERIVATIVES, AND ANALOGS

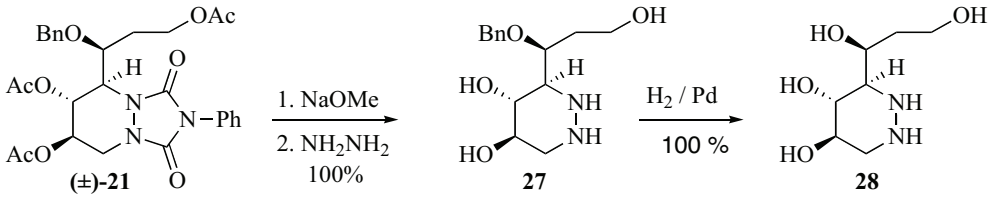
3.1. Chemoenzymatic Resolution of 1-Azafagomine, and Analogs

Racemic 1-azafagomine described in (Scheme **1**) was submitted to biocatalytic kinetic resolution using lipases [19]. An array of lipases was screened with poor results. Literature [20,21] refers a considerable number of reports using lipases for hydroxymethyl piperidines resolution; in all cases a less bulky group was attached at the nitrogen atom. And so, a parallel cycloaddition using 4-methyl-1,2,4-triazole-3,5-dione (MTAD), instead of PTAD, prepared *in situ* from methyl urazol and *tert*-butyl hypochlorite, was used as dienophile. The procedure gave cycloadduct **31** in 87 % yield (Scheme **8**).

Lipase-catalysed transesterification of compound **31** used vinyl acetate as the acetyl donor. Two enzymes out of over 20 were selected: *Candida antartica lipase* showed a good conversion but low selectivity; the best selectivity was achieved from lipase R (obtained from *Penicillium roqueforti*) after 17 h (12 % conversion, 96 % *ee*). The scale-up of the reaction with lipase R turned the process to be much slower, being stopped after *ca* 40 % conversion. This provides compound (*S*)-**32** in 38 % yield and 86 % *ee* and (*R*)-**31** in 60 % yield and 59 % *ee* [18] (Scheme **9**). The enantiopurity of the enantiomer (*S*)-**32** could not be improved by crystallization, but after saponification of (*S*)-**32**, (*S*)-**31** was obtained in enantiopure form. Unreacted compound **31**, from the crude enzyme esterification, was

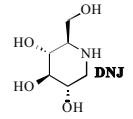
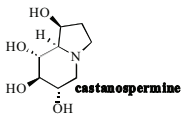
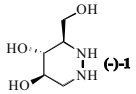
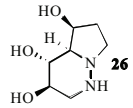
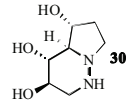
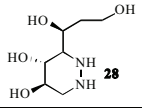
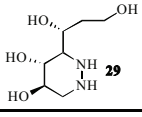


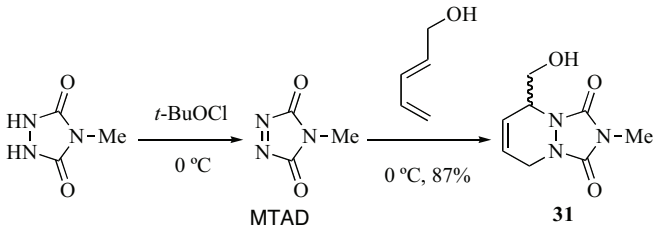
Scheme (6). Formation of 5-aza-6-deoxycastanospermine analog **26**.



Scheme (7). Synthesis of monocyclic compound **28**.

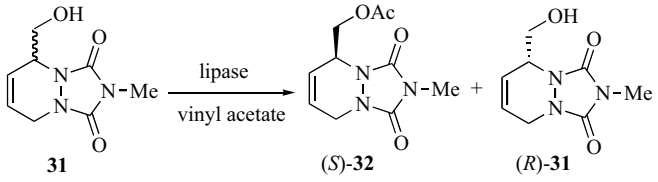
Table 2. Inhibition Constants (K_i) in μM of Target Compounds with Various Enzymes, Measured at 25 °C and pH 6.8, Unless noted Otherwise

Compound	α-Glucosidase (Yeast)	α-Glucosidase (Rice)	Isomaltase (Yeast)	β-Glucosidase (Almonds)
 DNJ	25	0.01 (pH not given)	11	47
 castanospermine	> 1500	0.015 (pH not given)	---	1.5 (pH 5.0)
 (-)-1	2.0	6	0.27	0.33
 26	> 600	15	79	10
 30	570	> 1000	550	690
 28	275	250	> 1000	660
 29	380	150	> 1000	820



Scheme (8). Synthesis of cycloadduct **31**.

enantiomerically enriched in the (*R*)-alcohol. The sample was treated with *Candida antartica* lipase, which having a lower selectivity than lipase R, had a greater reaction rate. After 50 % conversion, 48 % of the starting material was (*R*)-**31**, isolated with 99 % *ee*. The resolved alcohols were submitted to chemical group transformation. First, the epoxide was formed as a mixture of isomers: the compound with the *anti* disposition of the epoxide/hydroxymethyl group was the major product formed in 68 % (the minor isomer was isolated in 13 % yield). After, the epoxide was hydrolyzed to give the triol, and at last it was submitted to hydrazinolysis to give (-)-**1** from (*S*)-**31** and (+)-**1** from (*R*)-**31** [19].



Scheme (9). Lipase-catalysed transesterification of racemic cycloadduct **31**.

This strategy has an overall yield of 18 % after 5 steps, 9 % yield to each enantiomer. The optical purity of the compounds **31** was carried out by means of esterification of the alcohols (-)-**31** and (+)-**31** with camphanoyl chloride in the presence of dimethylaminopyridine and triethylamine. The two diastereomeric forms differ substantially from one another in their ^{1}H NMR spectra.

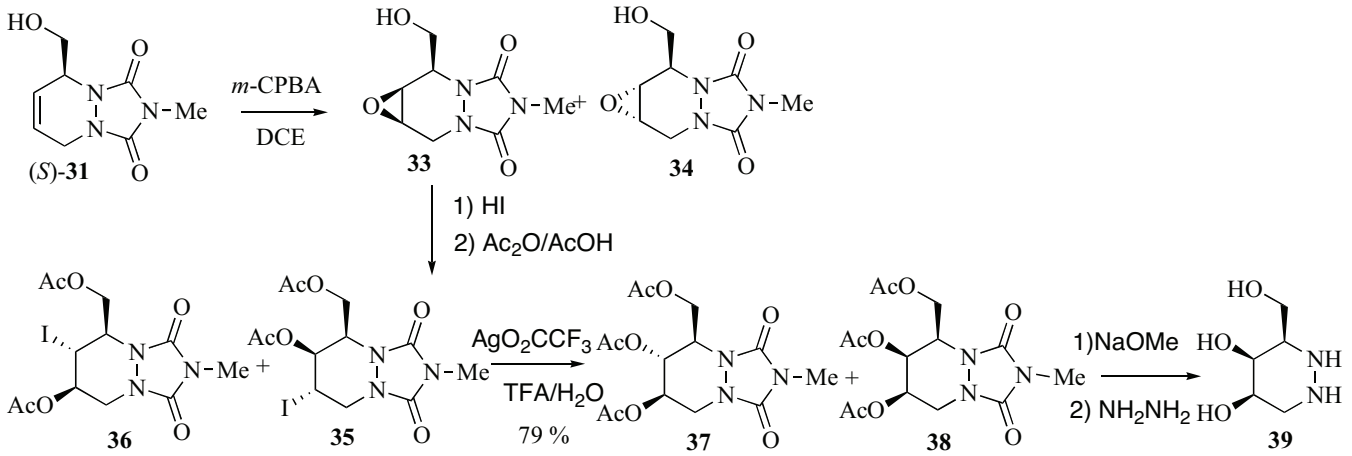
1-Azagalactofagomine was synthetized from achiral 2,4-pentadien-1-ol (**2**) and MTAD by Diels-Alder cycloaddition followed by enzymatic resolution with lipase R as the key steps of the strategy to give compounds (*S*)-**31** and (*R*)-**31** [22]. Compound (*S*)-**31** underwent epoxidation with *m*-chloroperoxybenzoic acid at 80 ºC for 18 h affording a mixture of epoxides **33** and **34** in a 2:1 ratio, from which the major *syn* epoxide **33** was isolated in 42 % yield, and then opened with 57 % aqueous HI in acetic acid followed by *in situ* peracetylation with acetic anhydride to afford a mixture of acetylated iodides **35** and **36** in a 1 (**35**) : 3 (**36**) ratio and 75 % yield. The preference for the formation of **36** could be explained by geometrical constraints in the bicyclic system that are in favour of 3*R*,4*R*-diaxial opening. The mixture of acetates **35** and **36** was treated with silver acetate in 6 % aqueous acetic acid and further acetylated with acetic anhydride and triethylamine affording a mixture of acetates 7 (**37**) : 3 (**38**) ratio. The formation of **37** as the major isomer was a drawback of this reaction. This was suppressed by using silver trifluoroacetate in 6 % aqueous trifluoroacetic acid. The stereoselectivity was improved to 96 (**38**): 4 (**37**) in 79 % yield. Compound **38** contaminated with 4 % of **37** was reacted with sodium methoxide in methanol followed by treatment with hydrazine hydrate at 100 ºC to give 1-azagalactofagomine **39** in 56 % yield for the last two steps [22] (Scheme **10**).

Azagalactofagomine **39** was tested against a series of glycosidases. Not surprisingly compound **39** is a poor α-glucosidase inhibitor, but in contrast is a strong β-glucosidase and β-galactosidase inhibitor of several sources, together with α-galactosidase from green coffee beans. It is noteworthy that compound **39** is a slightly weaker inhibitor of β-galactosidase and β-glucosidase than is isogalactofagomine, but more potent than *galacto*deoxynojirimycine. In contrast, compound **39** is a stronger α-galactosidase inhibitor than isogalactofagomine, but weaker than *galacto*deoxynojirimycine (Table **3**).

3.2. Synthesis of Homochiral 1-Azafagomine, Derivatives, and Analogs

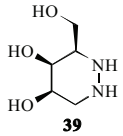
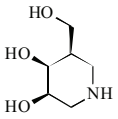
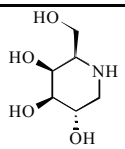
Most of the reported synthesis of azasugars has carbohydrates, amino acids and tartaric acid as starting materials [23-25]. *E.g.* (-)-1-Azafagomine and (+)-1-azafagomine have also been prepared from L- and D-xylose respectively [26]. More recently the Diels-Alder cycloaddition as the key synthetic step, has been appealing to several authors. Combining Stoodleys's cycloaddition of (*E*)-1-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-1,3-butadiene **40** to PTAD with Bols's olefin functionalization of the six-membered-ring and cleavage of the urazol moiety was obtained homochiral (-)-1-azafagomine. Cycloadduct **41** was prepared first in 70 % yield and in a high degree of diastereoselectivity. Treatement of **41** with triethylsilane in trifluoroacetic acid according to Stoodley's protocol generated (-)-**42** [27,28]. Epoxidation of compound (-)-**42** with oxone/trifluoroacetone in the presence of sodium carbonate formed a 3:1 ratio of oxiranes, from which the major isomer **43** was crystallised. The epoxide moiety has an *anti* relationship to the ester group and was obtained in 65 % yield. The epoxide **43** was opened in refluxing aqueous sulfuric acid with total *regio*- and *stereo*-selectivity to afford the *trans*-diol **44**. On the other hand, osmilation of compound **42** produced the *cis*-diol **45** with total *stereo*-selectivity. Selective reduction of *trans*-diol **44** and *cis*-diol **45** with sodium borohydride gave compounds **46** and **47**, respectively. Reflux of these compounds with hydrazine hydrate gave the target compounds (-)-1-azafagomine (-)-**1** in 14 % overall yield and (+)-5-*epi*-1-azafagomine (+)-**48** in 26 % overall yield, from alkene (-)-**42** (Scheme **11**).

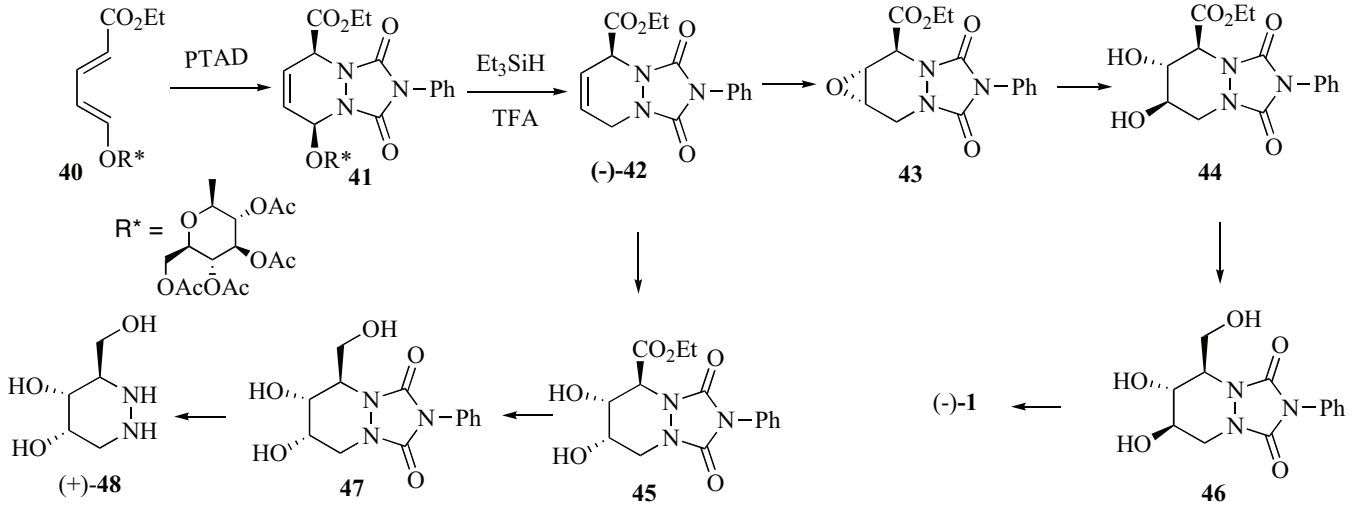
It is known that 2-*N*-alkyl-1-azafagomines are poorer glycosidase inhibitors than 1-azafagomine [29], but 1-*N*-alkylated compounds display a higher inhibitory potential together with a largely enhanced α/β selectivity [30]. A series of 1-*N*-alkylated 1-azafagomines has been prepared to study the structure activity relationship as glycosidase inhibitors. Scheme **12** represents the reaction sequence to obtain these compounds. An intermediate in the synthesis of 1-azafagomine from L-xylose, compound **49** was used as



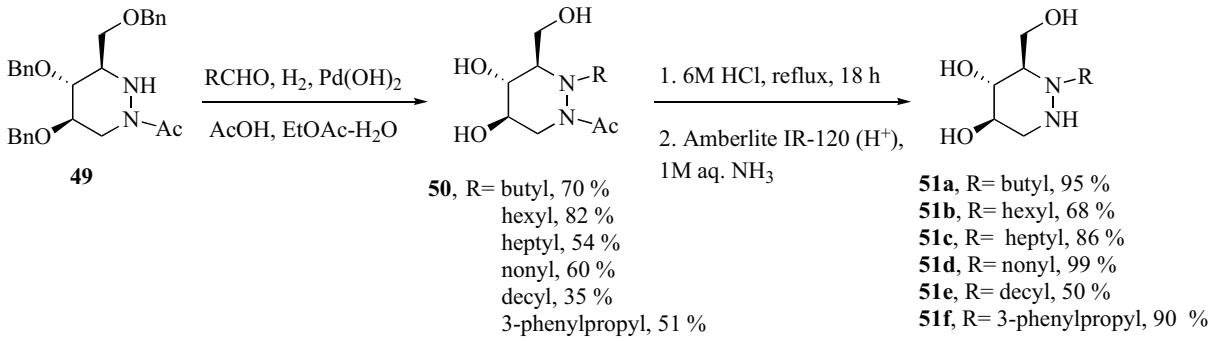
Scheme (10). Synthesis of 1-Azagalactofagomine.

Table 3. Inhibition Constants (K_i) in μM of Target Compounds to Various Enzymes, Measured at 25 ºC and pH 6.8

Enzyme	 **39**	 **isogalactofagomine**	 **galactodeoxynojirimycine**
α-glucosidase (baker´s yeast)	570	> 2000 (IC_{50})	---
β-glucosidase (almonds)	0.13	0.097	540
β-galactosidase (*Aspergillus oryzae*)	0.04	0.004	---
β-galactosidase (*E. coli.*)	0.30	0.2 (racemate measure)	12.5
β-galactosidase (*Saccharomyces fragilis*)	7.8	0.33	81
α-galactosidase (green coffee beans)	0.28	50	0.0016



Scheme (11). Diastereoselective synthesis of 1-azafagomine (-)-**1** and 5-*epi*-1-azafagomine (+)-**48**.



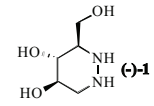
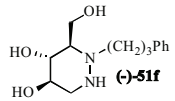
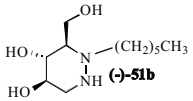
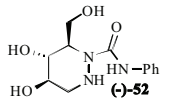
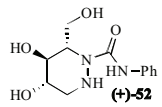
Scheme (12). Synthesis of 2-*N*-alkylated 1-azafagomines.

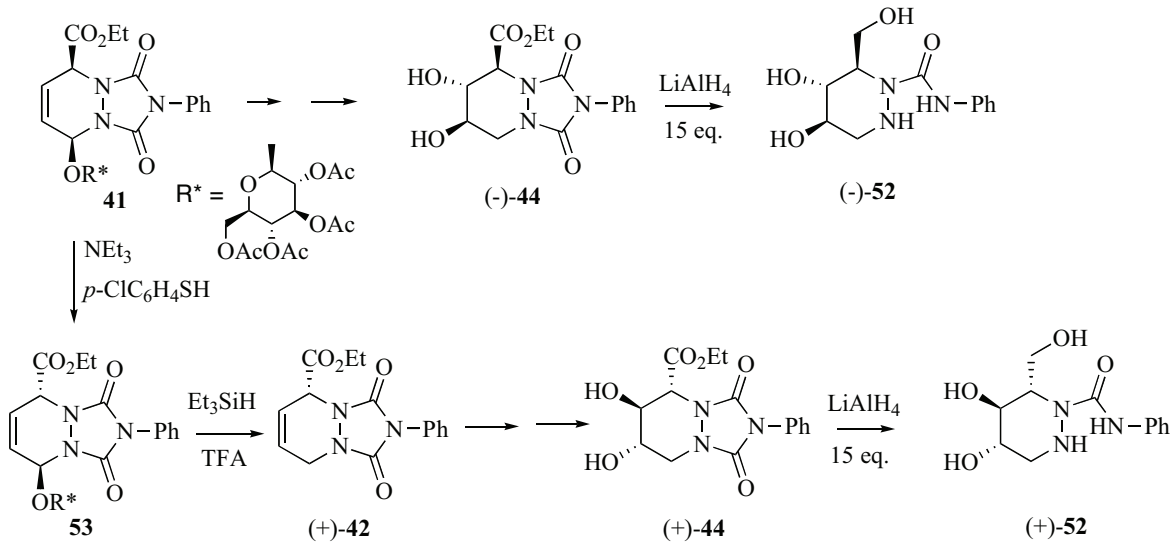
starting material. Alkylation occurs readily at *N*-2 because this atom is more basic, using compound **49** as the starting material the problem is avoided [12]. Alternatively, 1-azafagomine could be directly acetylated with acetic anhydride in methanol giving a 4:1 ratio of 1-*N*- *versus* 2-*N*-acetyl *regio*-isomers [17]. After separation the major compound 1-*N*-acetyl could be used as the starting material for the 2-*N*-alkylation. The introduction of the alkyl group at *N*-2 was tried by direct alkylation with *e.g.* alkyl halides but the reactions failed due to the low basicity of this nitrogen atom. Alkylation occurs by reductive amination with an aldehyde in the presence of Pd (II). Under these conditions the benzyl groups were simultaneously cleaved to afford compounds **50**. The acetyl group was removed by ion-exchange acidic resine and compounds **51** were obtained in good yields (Scheme **12**).

Compounds **51** were investigated for inhibition of α-glucosidase from yeast source and β-glucosidase from almonds. All compounds showed to be competitive inhibitors and the K_i were determined. The inhibition of α-glucosidase was 10-30 times weaker than 1-azafagomine, showing that a free nitrogen atom at *N*-2 is important for the enzyme-substrate interaction. On the other hand, all compounds **51** revealed a much better activity against β-glucosidase than 1-azafagomine. The *N*-hexyl (**51b**) and the *N*-(3-phenypropyl) (**51f**) are the preeminent compounds with five- to tenfold increased binding to β-glucosidase enzymes relatively to 1-azafagomine (See Table **4**).

A new homochiral derivative of 1-azafagomine, 2-*N*-phenyl carboxamide hexahydropyridazine (-)-**52** was obtained from the intermediate (-)-**42** [31] (Scheme **11**). Treatment of compound (-)-**42** with freshly opened $LiAlH_4$ (15 eq.) selectively reduced one of the carbonyl groups of the phenyltriazolidinone moiety to afford compound (-)-**52** (Scheme **13**). The synthesis of its enantiomer, compound (+)-**52**, was obtained from **41** by epimerization of H-5

Table 4. Inhibition Constants (K_i) in μM of Target Compounds with Various Enzymes, Measured at 25 °C and pH 6.8, Unless Noted Otherwise

Compound	α-Glucosidase (Backer´s Yeast)	β-Glucosidase (Almonds)	α/β Selectivity
 (-)-1	6.90 (pH 6.8)	0.32 (pH 6.8)	22
 (-)-51f	158 (pH 6.8)	0.032 (pH 6.8)	4938
 (-)-51b	278 (pH 6.8)	0.55 (pH 6.8)	5054
 (-)-52	3.36 (pH 7.0) --- (pH 5.0)	14.7 (pH 7.0) 67.4 (pH 5.0)	0.23
 (+)-52	10.6 (pH 7.0) --- (pH 5.0) enzyme inactive	25.2 (pH 7.0) 90.0 (pH 5.0)	0.42



Scheme (13). Synthesis of (-)- and (+)-2-*N*-carboxamide hexahydropyridazines.

inducted with NEt_3 and *p*-chlorothiophenol to give compound **53** (Scheme **13**). The glucosyl moiety was removed afterwards with triethylsilane in trifluoroacetic acid to yield (+)-**42** in 84 %. The reactive sequence followed the same route of the levorotatory isomer (-)-**42** being obtained (+)-2-*N*-phenyl carboxamide hexahydropyridazine (+)-**52** from enantiomer (+)-**42** (Scheme **13**). Compounds (-)-2-*N*-phenyl carboxamide hexahydropyridazine (-)-**52** and (+)-2-*N*-phenyl carboxamide hexahydropyridazine (+)-**52** were obtained in 29 % and 10 % overall yield, starting from compounds **41** and **53** respectively.

Both enantiomers (-)-**52** and (+)-**52** were tested against yeast α-glucosidase and almonds β-glucosidase. Curiously the α-glucosidase inhibition of compound (-)-**52** display a K_i = 3.36 μM and (+)-**52** a relatively similar result K_i = 10.6 μM, in strict contradiction with the results of α-glucosidase inhibition in the (-)-2-*N*-alkyl hexahydropyridazine derivatives. Another curious achievement is the question raised against the established knowledge that there is a big difference between the left- and dextrorotatory enantiomers in respect to the inhibition activity. In this case it just do not apply. It was found in the past that the dextrorotatory 1-azafagomine was practically inactive towards α- and β-glucosidases [26]. The relationship of inhibition activities between the two enantiomers (-)-**52** and (+)-**52** is *ca* 1:3, in favour of levorotatory isomer relatively to α-glucosidase from backer's yeast source, and *ca* 1:2 relatively to β-glucosidase from almonds. The interaction of both enantiomers (-)-**52** and (+)-**52** with α-glucosidase was studied by molecular modelling. The aromatic ring seems to efficiently pack into a hydrophobic pocket in the enzyme's active site, which could be responsible for the improved binding affinity of these compounds in relation to underivatized (-)-1-azafagomine and (+)-1-azafagomine [31].

Reaction of optically active compound **34** with HI in acetic acid gave a single iodinated product **54**, with the attack occurring at the more accessible carbon atom. The product **54** was obtained with 73 % yield. This iodide was subjected to radical reduction with tribuylstannane-azobisisobutyronitrile (Bu_3SnH-AIBN) to give the 4-deoxy derivative **55** in 65 % yield. Deacetylation with sodium methoxide in methanol followed by hydrazinolysis gave compound **56** in 57 % yield. The diastereomer of compound **34**, epoxide **33** under HI in acetic acid followed by treatment with acetic anhydride yield two *regio*-isomers: compounds **35** and **36**. These were subjected to the reductive conditions to which compound **54** was submitted, giving a mixture of compounds **57** and **58**. The mixture was separated to give **58** in 46 % yield and **57** in 21 % yield. After deacetylation and hydrazinolysis the deoxy- compounds **59** and **60** were obtained in 65 % and 63 % yield, respectively [13] (Scheme **14**).

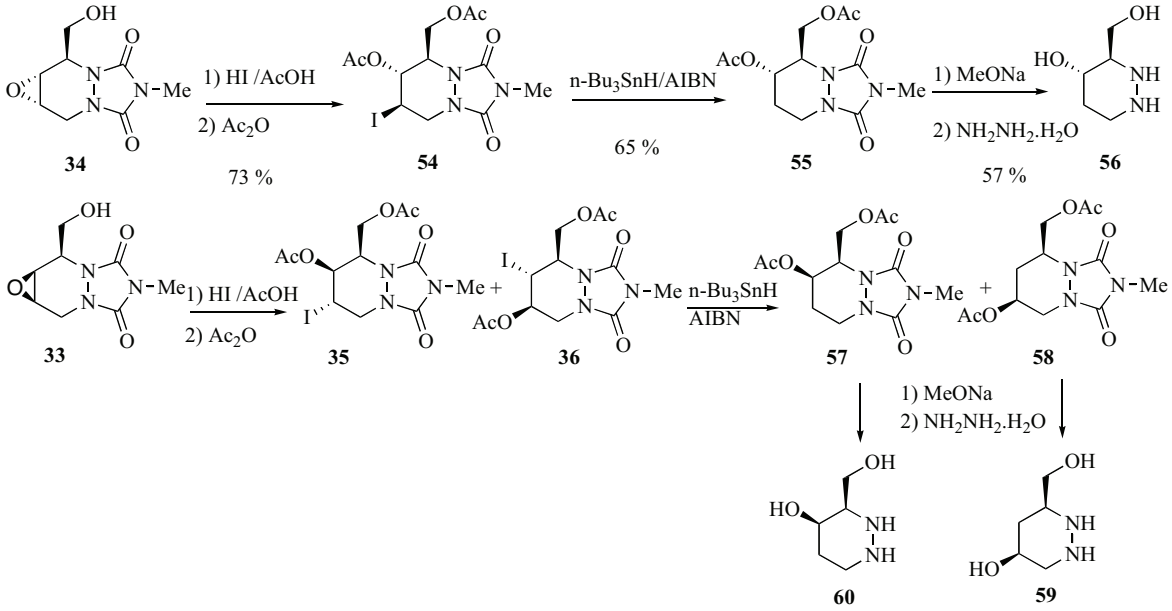
1-Azagulofagomine analog **61** was obtained in a 5 steps strategy with 25 % overall yield [32]. The key step is an asymmetric hetero Diels-Alder cycloaddition of chiral 1-*p*-tolylsulfinyl-1,3-pentadiene **62** to MTAD (Scheme **15**). The reaction occurs at -10 ºC generating cycloadduct **63**, which underwent a [2,3] sigmatropic rearrangement of the sulfinyl group leading to compound **64**. Compound **64** was treated with trimethylphosphite as a thiophilic agent to form the alcohol **65** in 98 % yield and 91 % *ee* [33]. Compound **65** was silylated with ^{t}-butyldimethylsilyl triflate in presence of triethylamine to give the derivative **66**. Oxidation of the double bond with osmium tetroxide and *N*-methylmorpholine oxide gave a mixture of *cis* diols in a ratio 9:1 and 80 % *de*, from which the major isomer **67** was isolated in 46 %. Surprisingly the same compound **67**, but in better yield and higher *de* (64 % yield, 98 % *de*) was obtained by epoxidation of the double bond with 3-methyl-3-trifluoromethyldioxirane followed by hydrolysis under basic conditions.

Although hydrazine has been used to cleave *N*-methyl urazole units in several compounds of type **67** [19,34], it did not work in compound **67**. To overcome this problem, the two vicinal hydroxyl groups of diol **67** were protected as an acetal to form **68**, which was then treated with NH_2NH_2 to

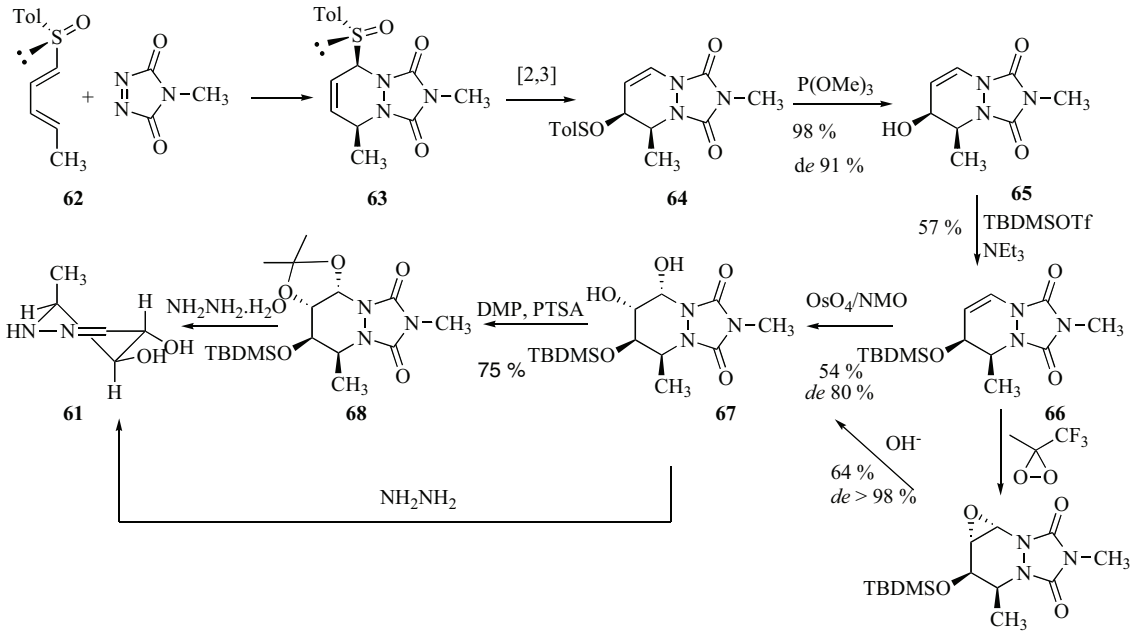
give a 1-azagulofagomine analog **61** in quantitative yield (Scheme **15**).

An explanation for the unexpected result obtained in the epoxide opening was suggested to be an assisted opening by a lone pair of electrons of the oxygen in the OTBS group, that would occur before the nucleophilic attack promoted by the hydroxide anion, according to (Fig. **6**).

No glycosidase inhibition study was found for compound **64**.



Scheme (14). Synthesis of deoxyazasugars **56**, **59** and **60**.



Scheme (15). Formation of 1-azagulofagomine analog **61**.

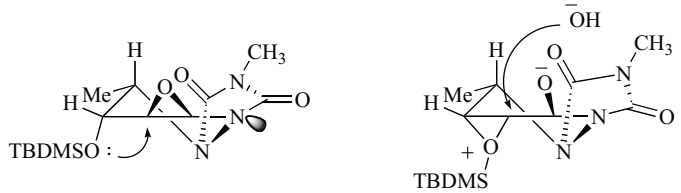


Fig. (6). Assistance of the OTBS group in the epoxide opening leading to *cis* diol **70**.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Scott, L.J.; Spencer, C.M. Miglitol: A Review of its Therapeutic Potential in Type 2 Diabetes Mellitus. *Drugs*, **2000**, *59*, 521-549.

[2] Bernacki, R.J.; Niedbala, M.J.; Koerytnyk, W. Glycosidases in cancer and invasion. *Cancer and Metastasis Reviews*, **1985**, *4*, 81-101.

[3] Alper, J. Searching for Medicine's Sweet Spot. *Science*, **2001**, *291*, 2338-2343.

[4] Jacob, G.S.; Scudder, P.; Butteres, T.D.; Jones, I.; Tiemeier, D.C. In: *Natural Products as Antivaral Agents;* Chu, C.K. and Cutler, H.G. Ed.; Plenum Press, New York, **1992**; pp 137-151.

[5] Karpas, A.; Fleet, G.W.J.; Dwek, R.A.; Fellows, L.E.; Tymes, A.S.; Petursson, S.; Namgoong, S.K.; Ramsden, N.G.; Jacob, G.S.; Rademacher, T.W. Aminosugar derivatives as potential anti-human immunodeficiency virus agents. *Proc. Natl. Acad. Sci. U.S.A.*, **1988**, *85*, 9229-9233.

[6] Laver, W.G.; Bischofberger, N; Webster, R.G. Disarming flu viruses. *Sci.Am.,* **1999**, *280*, 78-87.

[7] Tian, Y-S.; Joo, J-E.; Kong, B-S.; Pham, V-T.; Lee, K-Y.; Ham, W-H. Asymmetric synthesis of (-)-swainsonine. *J. Org. Chem.*, **2009**, *74*, 3962-3965.

[8] Heightman, T.D., Vasella, A.T., Recent insights into inhibition, structure, and mechanism of configuration-retaining glycosidases. *Angew. Chem. Int. Ed.*, **1999**, *38*, 750-770.

[9] Zechel, D.L., Withers, S.G. Glycosidase Mechanisms: Anatomy of a Finely Tuned Catalyst. *Acc. Chem. Res.*, **2000**, *33*, 11-18.

[10] Bols, M. 1-Azasugars, apparent transition state analogs of equatorial glycoside formation/cleavage. *Acc. Chem. Res.*, **1998**, *31*, 1-8.

[11] Bols, M.; Hazell, R.G.; Thomsen, Ib B. 1-Azafagomine: a hydroxyhexahydropyridazine that potently inhibits enzymatic glycoside cleavage. *Chem. Eur. J.*, **1997**, *3*, 940-947.

[12] Jensen, H.H.; Lyngbye, L.; Jensrn, A.; Bols, M. Stereoelectronic substituent effects in polyhydroxylated piperidines and hexahydropyridazines. *Chem. Eur. J.*, **2002**, *8*, 1218-1226.

[13] Jensen, H.H.; Jensen, A.; Hazell, R.G.; Bols, M. Synthesis and investigation of L-fuco- and D-glucurono-azafagomine. *J. Chem. Soc., Perkin Trans. 1*, **2002**, 1190-1198.

[14] Tsuchiya, T. Chemistry and developments of fluorinated carbohydrates. *Adv. Carbohydr. Chem. Biochem.*, **1990**, *48*, 91-277.

[15] Von Itzstein, M.; Wu, W.-Y.; Kok, G.B.; Pegg, M.S.; Dyason, J.C.; Jin, B.; Phan, T.V.; Smythe, M.L.; Oliver, S.W.; Colman, P.M.; Varghese, J.N.; Ryan, D.M.; Woods, J.M.; Bethell, R.C.; Hotham, V.J.; Cameron, J.M.; Penn, R.C. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature*, **1993**, *363*, 418-423.

[16] Lohse, A.; Jensen, H.H.; Bach, P.; Bols, M. Synthesis of 3-substituted isofagomine analogs using an unusual *syn* hydrogenation reaction. *J. Chem. Soc., Perkin Trans. 1*, **2000**, 659-665.

[17] Thomsen, Ib; Ernholt, B.V.; Bols, M. Synthetic studies of fluorinated analogs of 1-azafagomine: remarkable nucleophilic substitution of fluorine by hydrazine. *Tetrahedron,* **1997**, *53*, 9357-9364.

[18] Søndergaard, K.; Liang, X.; Bols, M. Synthesis of 5-azacastanospermine, a conformationally restricted azafagomine analog. *Chem. Eur. J.*, **2001**, *7*, 2324-2331.

[19] Liang, X.; Bols, M. Chemoenzymatic synthesis of enantiopure 1-azafagomine. *J. Org. Chem.*, **1999**, *64*, 8485-8488.

[20] Ling, L.; Ozaki, S. Enzyme aided synthesis of D-*myo*-inositol 1,4,5-trisphosphate. *Tetrahedron Lett.*, **1993**, *34*, 2501-2504.

[21] Altenbach, H.-J.; Blanda, G. A novel building block for the synthesis of isofagomine analogs. *Tetrahedron: Asymmetry*, **1998**, *9*, 1519-1524.

[22] Jensen, H.H.; Bols, M. Synthesis of 1-azagalactofagomine, a potent galactosidase inhibitor. *J. Chem Soc., Perkin Trans 1*, **2001**, 905-909.

[23] Alves. M.J.; Azoia, N.G. "Stereoselective Methods towards the Synthesis of Azasugars", In: *Stereochemistry Research Trends*, M.A. Horvat and J.H. Golob Ed.; Nova Science Publishers, **2008**; pp 1-50.

[24] Afarinkia, K.; Bahar, A. Recent advances in the chemistry of azapyranose sugars. *Tetrahedron: Asymmetry*, **2005**, *16*, 1239-1287.

[25] Asano, N.; Nash, R. J.; Molyneux, R.J.; Fleet, G.W.J. Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application. *Tetrahedron: Asymmetry*, **2000**, *11*, 1645-1680.

[26] Ernholt, B.V.; Thomsen, Ib B.; Lohse, A.; Plesner, I.W.; Jensen, K.B.; Hazell, R.G.; Liang, X.; Jakobsen, A.; Bols, M. Enantiospecific synthesis of 1-azafagomine. *Chem. Eur. J.*, **2000**, *6*, 278-287.

[27] Aspinall, I.H.; Cowley, P.M.; Mitchell, G.; Stoodley, R.J. Asymmetric synthesis of (3*S*)-2,3,4,5-tetrahydropyridazine-3-carboxylic acid. *J. Chem. Soc., Chem. Commun.,* **1993**, 1179-1180.

[28] Cowley, P.M.; Stoodley, R. J. *Regio*- and *stereo*-selective intermolecular interceptions of a conjugated *N*-acylhydrazonium ion. *Tetrahedron Lett.,* **1994**, *35*, 7853−7856.

[29] Lohse, A.; Jensen, K.B.; Bols, M. The first combinatorial library of azasugar glycosidase inhibitors. *Tetrahedron Lett.,* **1999**, *40*, 3033-3036.

[30] Lopez, O.L.; Bols, M. Anomer-selective glycosidase inhibition by 2-*N*-alkylated 1-azafagomines. *ChemBioChem*., **2007**, *8*, 657-661.

[31] Alves, M.J.; Costa, F.T.; Duarte, V.C.M.; Fortes, A.G.; Martins J.A.; Micaelo, N.M. Advances in the Synthesis of homochiral (−)-1-azafagomine and (+)-5-epi-1-azafagomine. 1-*N*-phenyl carboxamide derivatives of both enantiomers of 1-azafagomine: leads for the synthesis of active ***α***-glycosidase inhibitors. *J. Org. Chem.* **2011**, *76*, 9584-9592.

[32] Arroyo, Y.; Rodriguez, J.F.; Santos, M.; Sanz Tejedor, M.A.; Vaca, I.; Garcia Ruano, J.L. Asymmetric synthesis of (3*S*,4*R*,5*R*)-4,5-dihydroxy-3-methyl-2,3,4,5-tetrahydropyridazine: a formal synthesis of 1-azagulofagomine analogs. *Tetrahedron: Asymmetry*, **2004**, *15*, 1059-1063.

[33] Carreño, M. C.; Cid, M. B.; García Ruano, J. L.; Santos, M. Short enantioselective approach to substituted triazolopyridazines from [(*S*)*R*]-1-(1*E*,3*E*)-1-*p*-tolylsulfinyl-1,3-pentadiene. *Tetrahedron Lett.*, **1998**, *39*, 1405-1408.

[34] Hansen, S. U.; Bols, M. Synthesis of labelled 1-azafagomine. *J. Chem. Soc., Perkin Trans. 1*, **1999**, 3323-3325.

Received: November 23, 2011 Revised: December 20, 2011 Accepted: December 23, 2011