Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 543–553

TETRAHEDRON

Asymmetric synthesis of potent glycosidase and very potent α-mannosidase inhibitors: 4-amino-4-deoxy-L-erythrose and 4-amino-4,5-dideoxy-L-ribose

Jean-Bernard Behr,[†] Carine Chevrier, Albert Defoin,* Céline Tarnus* and Jacques Streith

Laboratoire de Chimie Organique et Bioorganique, Ecole Nationale Supérieure de Chimie de Mulhouse, Université de Haute-Alsace.3, rue Alfred Werner, F-68093 Mulhouse Cédex, France

Received 17 October 2002; accepted 21 November 2002

Abstract—Pyrrolidine amino-sugars, cyclic iminoalditols as well as linear aminoalditols in 4-amino-L-erythrose and 4-amino-5-deoxy-L-ribose series were synthesised by asymmetric hetero-Diels–Alder reaction followed by chemical transformations. 4-Amino-4-deoxy-L-erythrose and 4-amino-4,5-dideoxy-L-ribose were potent β-D-glucosidase, α-D-mannosidase, α- and β-D-galactosidase inhibitors. We have shown that the ribose derivative was a very potent inhibitor of α-D-mannosidase. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Glycosidases are involved in the metabolism of a wide range of biologically essential oligo- and polysaccharides,[1] making these enzymes interesting targets for the development of potent and selective inhibitors.[2–4]

Among well known glycosidase inhibitors, amino-sugars belong to the most potent compounds. It is now well established that the replacement of the intracyclic oxygen atom by a nitrogen atom in sugar moieties leads to a protonated species at physiological pH which mimics the oxocarbenium intermediate formed during enzymatic catalysis.[2,5,6]

5-Membered cyclic amino-sugars like 4-amino-4-deoxy-pentoses, which mimics the furanose form of carbohydrates, prove to be important glycosidase inhibitors. They have been described for the first time in the L-lyxose and L-xylose series by Paulsen.[7,8] The natural occurring D-arabinose derivative *nectrisine* (**1**) is a well known inhibitor of α-and β-glucosidase as well as of α-mannosidase.[9–12] Its D-ribose[13] and L-xylose[11] stereoisomers are both active against α-glucosidase. More recently, 5-deoxy-analogs bearing a methyl group as for the D-arabinose derivative **2**[11] have been synthesised and show an interesting spectrum of inhibitory properties toward various glycosidases.[14–16]

In this study, we first report of the rapid and efficient stereospecific synthesis of 4-amino-4-deoxy-L-erythrose (L-**14a**), as well as its methylated derivative 4-amino-4,5-dideoxy-L-ribose (L-**14b**) and their sulfite adducts L-**13a**,**b** bearing a sulfonic acid group in 1-position. Synthesis of some corresponding cyclic or linear amino-alditols are also described. All these chiral compounds were synthesised from *N*-dienyl-L-pyroglutamates **3a–c** according to a methodology we had previously set up in the racemic series[17] starting from *N*-dienylpyrrolidones **3d**,**e**. The key step of the synthetic route, an asymmetric hetero-Diels–Alder cycloaddition, had been essentially described in a previous article[18] and proceeded with complete regioselectivity and a good asymmetric induction. The first results to the synthesis of optically pure amino-sugars have been described as short communication in enantiomeric D-series[16] and the present work complete these data.

In addition, we discuss the structure–activities relationship of these compounds towards various glycosidases. Furthermore, we propose a possible mode of binding for these amino-sugars. Finally the stability of the described amino-sugars in various buffered solutions were also examined.

2. Results and discussion

2.1. Diels–Alder reaction and osmylation in diols

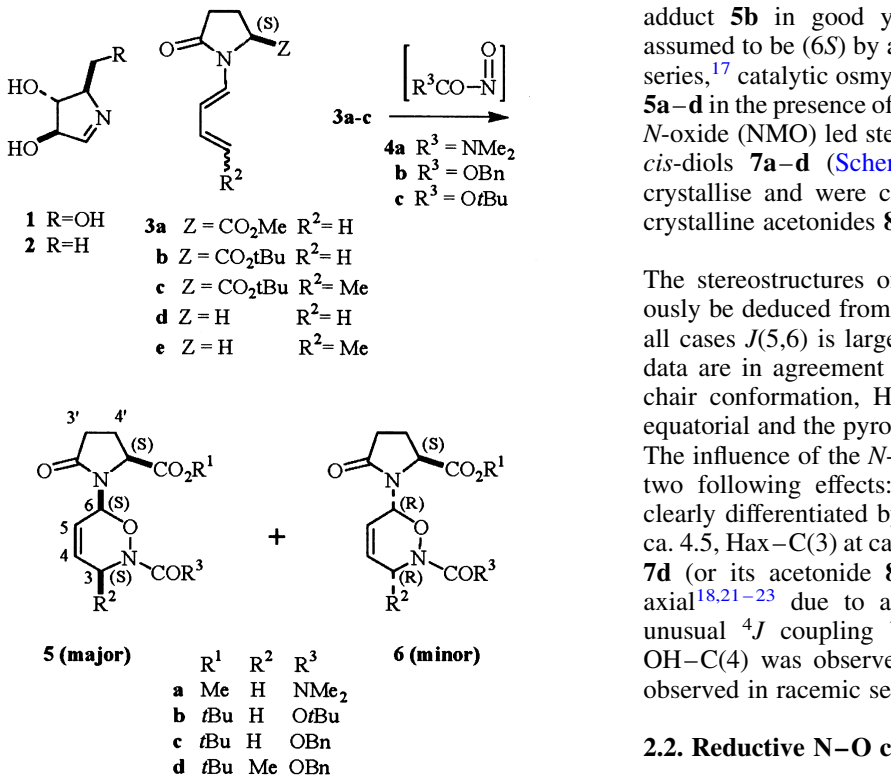
We had previously shown that chiral adducts **5a**,**c**,**d** could be obtained by asymmetric hetero-Diels–Alder reaction of *N*-dienyl pyroglutamates **3a–c** and nitroso compounds **4a**,**b** with good yield and good diastereoisomeric excess

Keywords: amino-sugars; aminoalditols; 3,4-dihydroxypyrrolines; α-mannosidase inhibitor; glycosidase inhibitors.

* Corresponding authors. Tel.: +33-3-89-33-6864; fax: +33-3-89-33-6815; e-mail: a.defoin@uha.fr

† Present address: Laboratoire de Chimie Bioorganique, UMR 6519, UFR de Sciences, F-51687 Reims Cédex 02, France.

PII: S0040-4020(02)01512-0



Scheme 1.

(70–90%), together with the corresponding minor isomers of type **6**[18] (Scheme 1). The (6*S*) configuration for **5a**,**c** and (3*S*,6*S*) for **5d** had also been ascertained.

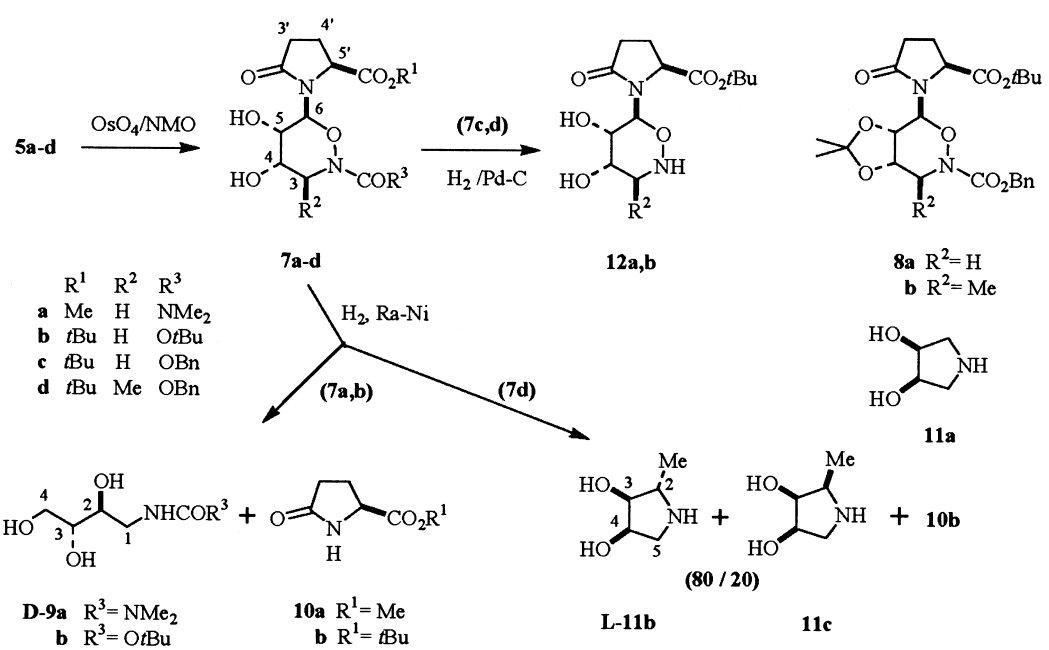
t-Butylated acylnitroso dienophile **4c** had already been used in Diels–Alder reaction[19,20] and was prepared in situ by oxidation of the corresponding hydroxamic acid. A simple preparation of this latter compound is described herein. Reaction of diene **3b** with **4c** gave adducts **5b** and **6b** in a 86:14 ratio. Chromatographic separation led easily to pure adduct **5b** in good yield (75%). Its configuration was assumed to be (6*S*) by analogy with **5a**.[18] As in the racemic series,[17] catalytic osmylation (OsO_4) of chiral cycloadducts **5a–d** in the presence of the co-oxidant *N*-methylmorpholine *N*-oxide (NMO) led stereospecifically to the corresponding *cis*-diols **7a–d** (Scheme 2). Diols **7c**,**d** did not easily crystallise and were characterised as their corresponding crystalline acetonides **8a**,**b**.

The stereostructures of **7a–d** and **8a**,**b** could unambiguously be deduced from their ^{1}H NMR spectra (Table 1). In all cases J(5,6) is large (9–10 Hz) and J(4,5) small; these data are in agreement with the oxazinane ring being in a chair conformation, H–C(5) and H–C(6) axial, H–C(4) equatorial and the pyroglutamate moiety strictly equatorial. The influence of the *N*-acyl residue was responsible for the two following effects: in diols **7a–c**, both H–C(3) are clearly differentiated by magnetic anisotropy Heq–C(3) at ca. 4.5, Hax–C(3) at ca. 3.4 ppm[17,22] and in methylated diol **7d** (or its acetonide **8b**) the Me–C(3) group is strictly axial[18,21–23] due to a strong steric effect. A clear and unusual ^4J coupling between both axial Hb–C(3) and OH–C(4) was observed for **7b**,**c**. Same effects had been observed in racemic series.[17]

2.2. Reductive N–O cleavage

Catalytic hydrogenolysis of oxazinane–diol **7a–d** provided acyclic or cyclic aminoerythritols according to the nature of the substitution at N(2) (COR^3) as already observed in previous studies[17,24,25] (Scheme 2). In all cases either methyl **10a** or *t*-butyl **10b** L-pyroglutamates were liberated during hydrogenolysis and were recovered in excellent yield.

Diols **7a**,**b**. Reductive cleavage of the *O*,*N*-disubstituted *N*-acylated N–O bond is difficult when not strained and is only described with SmI_2[26] or Na-amalgam.[27] In the present case, it was easily achieved over active Raney-nickel and



Scheme 2.

Table 1. ^{1}H NMR data of oxazinanes **7a–d**, **8a**,**b** and **12a**,**b** ($CDCl_3$, 250 MHz, 300 K, δ in ppm, J in Hz)

	Ha–C(3)	Hb–C(3)[a]	H–C(4)	H–C(5)	H–C(6)	2H–C(3′)	2H–C(4′)	H–C(5′)	R^1	R^3
7a[b]	4.01	3.28	4.14	3.49	5.58	2.68, 2.40	2.40, 2.19	4.43	3.82	2.95
7b[b]	4.35	3.34	4.14	3.35	5.84	2.47	2.47, 2.08	4.34	1.52	1.53
7c[b]	4.39	3.39	4.15	3.38	5.86	2.47	2.47, 2.08	4.34	1.51	5.27, 5.20[c]
7d[b]	4.56	1.28	4.00	3.60	5.82	2.57–2.40	2.38, 2.08	4.35	1.51	5.24, 5.20[c]
8a[d]	4.40	3.65	4.31	4.31	5.47	2.63, 2.32	2.32, 2.06	4.18	1.47	5.27, 5.18[c]
8a[d,e]	4.35	3.08	3.60	4.35	5.84	2.34, 1.86	1.45	4.08	1.35	5.13, 5.11[c]
8b[d]	4.67	1.37	4.10	4.47	5.40	2.66, 2.35	2.35, 2.07	4.18	1.49	5.26, 5.18[c]
12a[f]	3.30	3.09	4.16	3.97	5.29	2.72, 2.45	2.45, 2.18	4.41	1.50	
12b[f]	3.20	1.26	3.99	4.11	5.27	2.70, 2.45	2.45, 2.18	4.44	1.50	

	J(3a,3b)[a]	J(3a,4)	J(3b,4)	J(4,5)	J(5,6)	J(3b, OH-4)	J(4, OH-4)	J(5, OH-5)
7a[b]	14.6	2.5	2.0	3.7	9.8			
7b[b]	14.7	2.6	1.7	3.1	9.7	2.2	1.0	3.0
7c[b]	14.6	2.5	1.6	3.1	9.5	2.3	0.5	3.1
7d[b]	7.2	2.2		3.2	9.8			3.2
8a[d,e]	15.1	3.9	2.0	5.0	8.6			
8b[d]	7.2	1.1		5.0	8.9			
12a[f]	14.8	1.7	3.0	3.3	9.8			
12b[f]	7.0	3.1		3.2	9.1			

[a] Or Me–C(3) and J(Me,3a) for **7d**, **8b**, **12b**.
[b] OH–C(4) at δ=3.45 for **7a**, 2.60–2.75 for **7b–d**, OH–C(5) at δ=4.10–4.20.
[c] $5H_{arom}$ at δ=7.45–7.25; J_{benzyl}=12.2 Hz.
[d] Acetonide signals for **8a** at δ=1.37, 1.31 in $CDCl_3$, 1.35, 1.37 in C_6D_6; for **8b** at δ=1.40, 1.31.
[e] In C_6D_6.
[f] In D_2O.

led to acyclic 1-acylamino-1-deoxy-D-erythritols D-**9a**,**b**. Racemic (±)-**9a** had been obtained by us in a similar way.[24]

Benzylated diols **7c**,**d**. Hydrogenolysis of these compounds under mild conditions (rt, Pd/C) gave the *N*-deprotected oxazinanes **12a**,**b**. However, at 50°C further reductive cleavage of the N–O bond occurred. For instance, double hydrogenolysis of diol **7c** should lead to a *meso* compound, i.e. to *cis*-3,4-dihydroxypyrrolidine (**11a**) as in similar cases[24,25] and was not studied any further. Hydrogenolysis of **7d** was more efficiently performed over Raney-nickel and afforded cyclic L-aminoribitol L-**11b** together with pyroglutamate **10b**; both compounds were then easily separated by aqueous treatment and characterised. Small amounts (20%) of lyxose isomer **11c** were also formed (probably the D-enantiomer, by methyl isomerisation during the hydrogenolysis process). D-Enantiomer of L-**11b**[28,29] and L-enantiomer of **11c**[16,28] were already known compounds.

2.3. L-Aminoerythrose and L-aminoribose

Attempts to prepare amino-sugars L-**14a**,**b** by partial hydrogenolysis of deprotected diols **12a**,**b** or of acetonides **8a**,**b**, by analogy with our previous work in non-optically active series,[17] gave only mixtures of products. For this reason, we developed an original method based on both acidic and reducing properties of aqueous sulfurous oxide (Scheme 3). Treatment of the unprotected diols **12a** and **12b** in water at 60°C under SO_2 atmosphere, in the presence of *p*-toluenesulfonic acid, led to the following reaction sequence: (a) acid induced cleavage of the pyroglutamate moiety, (b) reductive N–O bond cleavage, (c) formation of the crystalline sulfite adduct (sulfonate inner salts) of 4-amino-4-deoxy-L-erythrose L-**13a** and of 4-amino-4,5-dideoxy-L-ribose L-**13b**, respectively. A more rapid approach was the reaction with the *N*-Boc compound **7b** which gave directly L-**13a**, avoiding the hydrogenolysis step. Racemic (±)-**13a**,**b** had already been prepared[17] and were shown to appear exclusively as β-anomers.

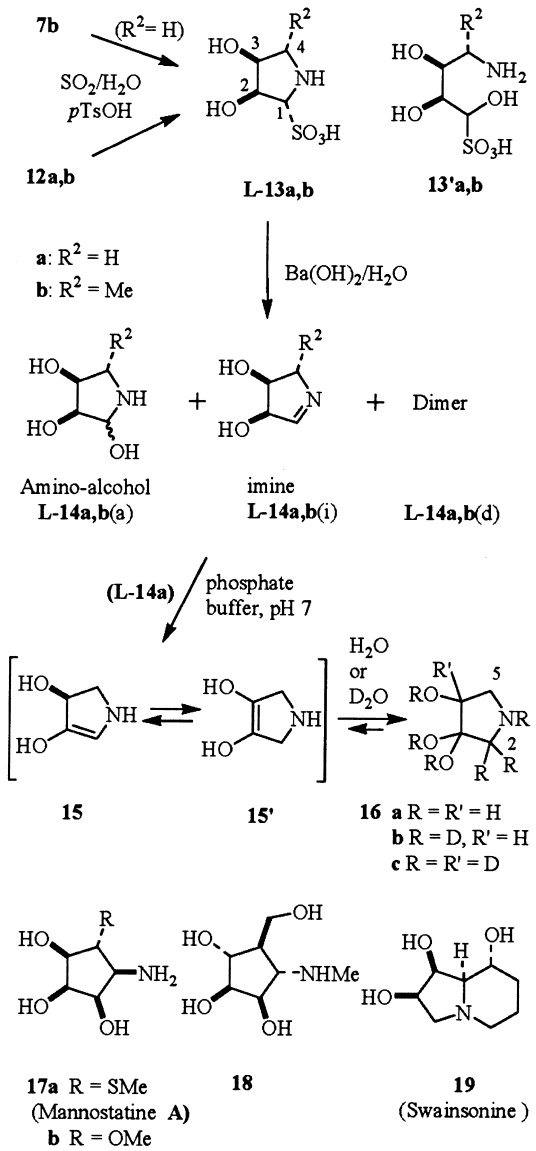
The corresponding amino-sugars could easily be obtained in aqueous solution by saponification of these sulfonates with baryte and precipitation of barium sulfite, leading thereby to amino-sugars L-**14a**,**b** as equilibrium mixtures of the corresponding imine (i), amino-alcohol (a) and dimer (d) (Scheme 3). Structures of these different species were the same as in the racemic series[17] as determined by ^{1}H NMR.

2.4. Stability of amino-sugars

We have evaluated the stability of amino-sugars L-**13a**,**b**, L-**14a**,**b** in the experimental conditions required for the various enzyme essays. Results given by NMR studies were completed by those observed in enzyme inhibition essays.

Sulfite adducts L-**13a**,**b**. Sulfite adducts L-**13a**,**b** were stable in water at pH ca. 4 (natural pH) or in acetate buffer (pH ca. 4.0) as well as in phosphate or Hepes buffer (pH ca. 7.0). In Hepes buffer for both series, a minor species was observed beside L-**13a**,**b** and it was assumed to be the linear sulfite adducts **13′a**,**b** (ratio L-**13a**/**13′a** 65:35, L-**13b**/**13′b** 85:15) for two reasons: (a) the H–C(1) NMR signal was deshielded of 0.4 ppm at 4.8 ppm, (b) acidification of the solution at ca. pH 4 with AcOH gave again L-**13a**,**b** as only species with the same ^{1}H NMR data as in acetate buffer.

Amino-sugars L-**14a**,**b**. We have observed various behaviours of the imine (i), amino-alcohol (a), dimer (d) mixture according to the pH value and the ionic species of the buffers. Without buffer at pH ca. 8, these compounds appeared in ratio 60:18:22 for L-**14a** and 66:0:33 for L-**14b** at ca. 10^{-2} M as stable mixture at rt. At pH ca. 4.0 in acetate buffer, dimer was the major species and a reversible modification was only observed. In contrast, at pH ca. 7.0



Scheme 3.

in phosphate buffer, phosphate ions seemed to favorise an irreversible isomerisation which was studied below. A half life around 5 and 20 h for L-**14a** and L-**14b**, respectively have been estimated at 25°C by both enzyme inhibitions and ^{1}H NMR studies. At the same pH value, in Hepes buffer which are a typical non-nucleophilic buffer, L-**14a**,**b** were stable up to one day at 25°C, as determined by enzyme inhibitions essays (Section 4) and by ^{1}H NMR studies, corresponding to the utilised conditions for determining the various K_i values reported herein

Transformation of L-**14a**,**b** *in phosphate buffer*. It was studied in details in the case of L-**14a**: the ^{1}H NMR spectra of its H_2O solution after 6 h at rt showed new major signals between 4.13 and 3.34 ppm which were attributed to the compound **16a**, i.e. an isolated CH_2(2) and a CH–CH_2 moiety. The ^{13}C NMR spectra of the H_2O solution showed a quaternary signal at 100 ppm, a carbinol signal near 75 ppm and two NCH_2 signals near 50 ppm. These data were in agreement with those of the cetol hydrate **16a**. Indeed it resulted from an isomerisation by imine–enamine tautomerie of the imine/amino-alcohol function of L-**14a** leading to the intermediary enol **15**, following by hydration in the observed cetol **16a**. In D_2O solution, the deuteriated compound **16b** was the only species observed, the CH_2(2) group did not appeared by deuteriation, indicating a rapid equilibrium between **15** and **16b**. This isomerisation is also related to the Amadori transposition of amino-sugars in acidic medium[30] and an example in piperidine serie was recently described.[31]

In addition, the slow deuteriation of the CH(4) signal ($t_{1/2}$ ca. 7 h at rt) in D_2O leading to **16c**, showed a slower equilibrium with the isomer **15'** where the double bond had migrated further along the molecule. Consequently, the circulation of this double bond should gradually lead to a deuteriation of the whole molecule. In the case of a such isomerisation sequence, the much slower transformation of the methylated compound L-**14b** would lead in D_2O to a mixture of partially deuteriated stereoisomers; we have effectively observed a complex mixture of compounds which was not studied.

Table 2. Glycosidase inhibition values for amino-sugars L-**13a**, L-**14a**, D-**13a**,[16] D-**14a**,[16] L-**13b**, L-**14b** and for amines **11a**,[32] L-**11b** [IC_{50} or K_i (in μM)]

Enzymes	4-Amino-erythroses					4-Amino-riboses		
	L-**13a**	L-**14a**	D-**13a**	D-**14a**	**11a**	L-**13b**	L-**14b**	L-**11b**
α-Glucosidase (yeast)[a]	ni	nd	ni	150	ni	ni	nd	ni
α-Glucosidase (yeast)[b]	ni	ni	nd	nd	nd	[c]	[c]	nd
β-Glucosidase (almond)	**0.5**	**0.3**	ni	ni	600	**0.2**	**0.4**	340
α-Mannosidase (Jack bean)	**1**	**2**	**1**	**20**	400	**0.05**	**0.05**	670
β-mannosidase Snail	ni	nd	ni	nd	ni	ni	nd	ni
α-Galactosidase (green coffee bean)[a]	**0.2**	**0.1**	ni	nd	ni	**0.1**	**0.1**	**16**
α-Galactosidase (green coffee bean)[b]	**0.2**	**0.2**	nd	nd	nd	**0.15**	**0.15**	nd
β-Galactosidase (*Escherichia coli*)[a]	**0.2**	**0.3**	ni	1 mM	[c]	**30**	**3**	1 mM
β-Galactosidase (*Escherichia coli*)[b]	**0.06**	**0.2**	nd	nd	nd	**40**	**1.5**	nd
α-Fucosidase (bovine kidney)	1 mM	nd	**4**	**3**	ni[d]	[c]	[c]	nd

nd—not determinated; ni—no inhibition or inhibition <30% at 1 mM.
[a] Phosphate buffer.
[b] Hepes buffer.
[c] ca. 40% Inhibition at 1 mM.
[d] α-Fucosidase from Bovine epididymis.

2.5. Inhibition of glycosidases

Amino-sugars L-**14a**,**b** and their sulfite adducts L-**13a**,**b** were evaluated as glycosidases inhibitors, as well as diol L-**11b** which was obtained almost pure by crystallisation of its hydrobromide. All these compounds were found to be reversible competitive inhibitors of the various glycosidases evaluated. The kinetic constants (K_i values) determined for the most potent inhibitors are reported in Table 2. These studies were performed within times scales assuming a complete stability of our compounds, particularly in phosphate buffer.

The enantiomers D-**13a**, D-**14a**[16] and D-**11b**[28,29] have been recently synthesised and their glycosidase inhibition values as well as those of *meso*-diol **11a**[32] have been published. They have been reported here for structure–activity analysis and comparison with our active compounds.

4-*Amino-erythrose derivatives*. Amino-sugar L-**14a** as well as its sulfite adduct L-**13a** showed interesting inhibitory activity on the glycosidases presented in this study. A similar activity profile for both compounds was observed. In both cases μM to sub-μM range K_i values were determined for β-glucosidase, α-mannosidase, α- and β-galactosidase, but no significant activity was observed for the other studied enzymes. We have also reported for comparison the inhibitions constants of the enantiomers D-**14a** and D-**13a**. As already mentioned[16] these compounds inhibited only α-mannosidase and α-fucosidase, which emphasised a more narrow selectivity for those enzymes when compared to the L-series.

4-*Amino-ribose derivatives*. Addition of a methyl group on the 4-position of the above mentioned inhibitors did not change significantly the selectivity profile for the various enzymes, nevertheless it is interesting to underline the observed differences in their inhibitory potency. Indeed ribose derivatives L-**14b** and L-**13b** were ca. 10–100 times less active on β-galactosidase whereas they were ca. 20–40 times more active on α-mannosidase, leading to very potent (K_i=50 nM) inhibitors of this particular enzyme. These 5-membered ring amino-sugar are therefore as potent as for example the well known mannostatine A (**17a**),[33–37] its derivative **17b**,[35] the methylamino-pentacyclitol **18**[38] or swainsonine (**19**).[39–41]

Comparison with iminoalditols **11a**, L-**11b**. We have reported in Table 2 the inhibitory activity of cyclic amines **11a** (from Ref. 32) and L-**11b** in order to compare with activities of amino-sugars L-**14a**,**b** and D-**14a** (from Ref. 16). It appeared clearly that cyclic amines **11a** and L-**11b** were very weak inhibitors of β-glucosidase and α-mannosidase with IC_{50} values in the millimolar range. L-**11b** was a weak inhibitor of α-galactosidase and no significant affinity was observed for the other evaluated glycosidases. These results were in clear contrast with the potency of amino-sugars L-**14a**,**b** and D-**14a**. Similar observations were reported in *nectrisine* series where pyrroline **2a** itself is a potent glycosidase and mannosidase inhibitor[10,11] whereas the corresponding pyrrolidine showed less affinity for α-glucosidase and poor affinities for the other hydrolases. Similar results were reported in D-ribose,[13] 5-deoxy-L-lyxose[15,16] and 5-deoxy-L-arabinose[14] series. The negative effect of the loss of one hydroxyl group had been already observed for mannostatine A.[33]

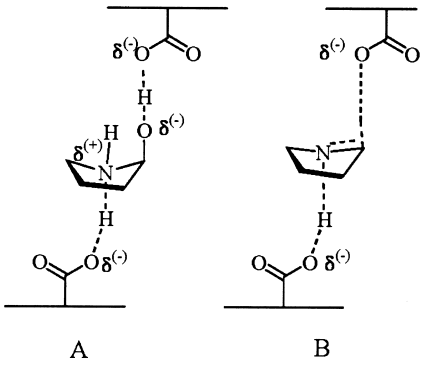


Figure 1. Possible modes of binding of amino-sugars in glucosidase active site: (A) with amino-alcohol species, (B) with imine species.

Indeed 5-membered ring amino-sugars as cyclic imines were quite reactive structures and they easily dimerise[7,8] at 10^{-2} M. It was also reported, that L-arabinose analogue inhibited irreversibly a rhamnosidase.[14] Nevertheless, in our case, the observed inhibitions were clearly reversible (see also Ref. 16). One has to keep in mind that these compounds appeared in aqueous solution as an equilibrium of amino-alcohol, imine and dimeric form[7,8,13,16,17] (Scheme 3). Thus it is difficult to predict the precise structure which will promote an optimal binding in a given glycosidase active site. However, one can assume that, at the concentration used in our enzyme assays (nanomolar to micromolar), no dimer occurred. Thus the anomeric center could provide additional binding interactions either through the 1-hydroxyl group of the amino-alcohol species or through a direct reversible bond with the C=N function of the corresponding imine, as depicted in Figure 1A and B, respectively.

We have observed that the sulfite adducts L-**13a**,**b** exhibited a similar behaviour than L-**14a**,**b** towards all the glycosidases evaluated in this study. The same results were find in the enantiomeric series[16] for D-**13a** and D-**14a**. We could hypothesise that the corresponding amino-sugars were generated within the active site of enzymes with release of SO_2, meaning that the same chemical structure was finally evaluated.

3. Conclusion

The synthesis of 5-membered ring amino-sugars in the L-erythrose and L-ribose series was achieved with an overall yield for their sulfite adducts of 41–63% for L-**13a** and 21% for L-**13b** from *N*-dienyl-L-pyroglutamates **3b**,**c**. These amino-sugars have demonstrated interesting potent inhibitory activities towards various glycosidases. They could be used as lead structures and be further modified to improved their selectivity and/or for the design of novel inhibitors for other classes of enzymes where sugar recognition is an essential feature.

4. Experimental

General remarks. Flash chromatography (FC): silica gel (Merck 60, 230–400 mesh). TLC: Al-roll silica gel (Merck 60, F_{254}). Mp Kofler hot bench, corrected. IR spectra (cm^{-1}): Perkin–Elmer 157 G. ^1H and ^{13}C NMR spectra: Bruker AC-F250, tetramethylsilane (TMS) or sodium trimethylsilylpropionate-D_4 (TSP-D_4) in D_2O (^1H NMR) and $CDCl_3$ or (in D_2O) CH_3OH or dioxane (^{13}C NMR; $\delta(CDCl_3)=77.0$ ppm, $\delta(CD_3OD)=49.0$, in D_2O $\delta(CH_3OH)=49.5$, δ(dioxane)=67.6 with respect to TMS) as internal references; δ in ppm and J in Hz; spectra in H_2O with D_2O as external lock. $[\alpha]_D$ values were determined with a Schmidt Haensch Polartronic Universal polarimeter. High resolution (HR) MS were measured on a MAT-311 spectrometer at the University of Rennes. Microanalyses were carried out by the Service Central de Microanalyses du CNRS, Vernaison or by the Service de microanalyse de l'ICSN, Gifs/Yvette.

Reagents and solvents. Raney-Ni (slurry in H_2O), 5% Pd/C catalyst, 2,2-dimethoxypropane (DMP), *N*-methylmorpholine-*N*-oxide (NMO) in 5 M aqueous solution, OsO_4, *t*-butyl hydroperoxide, *p*-toluenesulfonic acid, di-*t*-butyl dicarbonate from Fluka, *N*-methylmorpholine-*N*-oxide (NMO) from Aldrich, sulfur dioxide gas from Esseco, Hepes (4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonique acide) from Avocado. Amberlyst-15 (H^+) was a gift from Rohm and Haas. Usual solvents were freshly distilled, CH_2Cl_2 was kept over Na_2CO_3.

Wet Raney-Ni was washed by stirring in MeOH under H_2 atmosphere and decantation before use.

4.1. Diels–Alder reaction

4.1.1. *t*-Butyloxycarbohydroxamic acid (*t*-butyl *N*-hydroxycarbamate). Procedure according to lit.[24] A suspension of $NH_2OH{\cdot}HCl$ (9.6 g, 0.14 mol, 1.5 equiv.) and K_2CO_3 (7.2 g, 0.07 mol, 1.5 equiv.) in Et_2O (60 ml) and H_2O (2 ml) was stirred for ca. 1 h at rt with evolution of CO_2. A solution of *t*-butyl dicarbonate (20.0 g, 92 mmol) in Et_2O (40 ml) was then added dropwise at 0°C and the suspension was stirred at rt from 4 to 16 h. The organic phase was decanted, the solids were washed with Et_2O and the organic solutions evaporated to dryness. Crystallisation in cyclohexane or cyclohexane/toluene provided hydroxamic acid (10.3–12.7 g, 85–100%), in several crops. Mp 60–62°C. (lit.:[42] mp 58–59°C). ^1H NMR ($CDCl_3$, 300 K): 1.48 (s, *t*Bu); 6.55, 7.01 (2 br s, NHOH).

4.1.2. *t*-Butyl (6*S*)-6[(5′*S*)-5′-*t*-butylcarbonyl-2′-oxo-pyrrolidin-1′-yl]-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (5b) and *t*-butyl (6*R*)-6[(5′*S*)-5′-*t*-butylcarbonyl-2′-oxo-pyrrolidin-1′-yl]-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (6b). Procedure according to lit.[17] To a stirred solution of **3b** (1.6 g, 6.75 mmol) in MeOH (20 ml) at 0°C was added $(BnMe_3N)IO_4$[43] (0.92 g, 2.7 mmol, 0.4 equiv.) and portionwise over 0.5 h *t*-butyl *N*-hydroxycarbamate (1.08 g, 8.1 mmol, 1.2 equiv.). After 1 h at 0°C, the brown solution was diluted with AcOEt (50 ml) and washed with aqueous 1 M Na_2CO_3 (containing enough $NaHSO_3$ to remove I_2), and brine (5×5 ml), the aqueous phases were extracted with AcOEt and the combined organic solutions dried ($MgSO_4$) and evaporated. The resulting orange oil (3.0 g, **5b/6b** ratio 86:14) was resolved by FC (Et_2O, on 150 g silica gel) to give successively **5b** (1.80 g, 72%), a mixture of **5b**, **6b** (0.10 g, 4%) and **6b** (0.30 g, 12%).

Compound **5b**. Colorless crystals. Mp 87–89°C (iPr_2O). $[\alpha]_D^{21}=-109$ (c=1, $CHCl_3$). IR (KBr): 2970, 2930, 1730, 1700, 1658, 1460, 1410, 1390, 1365, 1322, 1230, 1220, 1150, 1100, 1022, 990, 940, 852, 830, 775, 758, 748. ^1H NMR ($CDCl_3$, 300 K): 1.46, 1.51 (2s, 2 *t*Bu); 1.99 (m, Hb–C(4′)); 2.30 (m, Ha–C(4′)); 2.20–2.60 (m, 2H–C(3′)); 4.06 (m, Hb–C(3)); 4.09 (m, Ha–C(3)); 4.31 (d, J=8.7 Hz, H–C(5′)); 5.74 (dq, H–C(5)); 6.07 (ddt, H–C(4)); 6.20 (quint, H–C(6)). J(3a,3b)=17.6 Hz, J(3a,4)=3.5 Hz, J(3b,4)=3.1 Hz, J(3a,5)=2.1 Hz, J(3b,5)=2.2 Hz, J(3a,6)=2.0 Hz, J(3b,6)=2.5 Hz, J(4,5)=10.2 Hz, J(4,6)=1.8 Hz, J(5,6)=2.8 Hz, ^{13}C NMR ($CDCl_3$, 300 K): 24.2 (C(4′)); 27.8, 28.2 (2 C*Me*$_3$); 29.3 (C(3′)); 44.3 (C(3)); 58.3 (C(5′)); 77.4 (C(6)); 82.0, 82.0 (2 *C*Me$_3$); 123.3 (C(4)); 127.8 (C(5)); 154.7 (CO–N(2)); 172.0 (CO–C(5′)); 175.7 (CO(2′)). Anal. calcd for $C_{18}H_{28}N_2O_6$ (368.43): C 58.68, H 7.66, N 7.60; found: C 58.8, H 7.7, N 7.5.

Compound **6b**. Colorless needles. Mp 129–130°C (iPr_2O). $[\alpha]_D^{21}=+4$ (c=1, $CHCl_3$). IR (KBr): 2975, 2915, 1727, 1705, 1655, 1430, 1407, 1390, 1367, 1353, 1290, 1250, 1235, 1220, 1180, 1154, 1092, 1015, 945, 910, 870, 817, 792, 760, 690. ^1H NMR ($CDCl_3$, 300 K): 1.46, 1.50 (2s, 2 *t*Bu); 2.08 (m, Hb–C(4′)); 2.20–2.40 (m, Ha–C(4′), Hb–C(3′)); 2.60 (m, Ha–C(3′)); 3.97 (dm, Hb–C(3)); 4.13 (dm, Ha–C(3)); 4.29 (dd, J=2.4, 4.6 Hz, H–C(5′)); 5.86 (m, H–C(5)); 5.90 (m, H–C(6)); 6.10 (m, H–C(4)). J(3a,3b)=17.6 Hz, J(3a,4)=3.8 Hz, J(3b,4)=2.8 Hz, J(3a,5)=1.9 Hz, J(3b,5)=2.3 Hz, J(3a,6)=2.5 Hz, J(3b,6)=2.3 Hz, J(4,5)=10.2 Hz, J(4,6)=1.8 Hz, J(5,6)=2.2 Hz. ^{13}C NMR ($CDCl_3$, 300 K): 23.4 (C(4′)); 27.8, 28.2 (2 C*Me*$_3$); 30.2 (C(3′)); 44.4 (C(3)); 58.6 (C(5′)); 78.9 (C(6)); 81.8, 82.0 (2 *C*Me$_3$); 123.6 (C(4)); 126.9 (C(5)); 154.9 (CO–N(2)); 171.2 (CO–C(5′)); 175.7 (CO(2′)). Anal. calcd for $C_{18}H_{28}N_2O_6$ (368.43): C 58.68, H 7.66, N 7.60; found: C 58.4, H 7.8, N 7.7.

4.2. *cis*-Glycol formation by osmylation

General procedure.[24,44] To a solution of adduct **5** (10 mmol) in acetone (10 ml) and H_2O (6 ml) was added NMO (2.7 g of solid or 4 ml of aqueous solution, 20 mmol, 2 equiv.) and the OsO_4 solution (3–10 ml) (prepared from OsO_4 (1 g) in *t*BuOH (200 ml) and 1 ml of 70% *t*-BuOOH[45]). The solution was stirred at 40°C until the adduct disappeared (1–2 days.). Some Na_2SO_3 was added, the solution diluted with AcOEt (100 ml) and washed with brine (4×5 ml). The aqueous phases were extracted again with AcOEt/acetone and the combined organic phases dried over $MgSO_4$ and evaporated. A second work-up has also been used: the solution was evaporated, dissolved in AcOEt (10 ml), filtered over a bed of silica gel and evaporated.

4.2.1. (4*S*)-*r*-4,*c*-5-Dihydroxy-*t*-6-[(5′*S*)-5′-(methoxycarbonyl-2′-oxo-pyrrolidine-1′-yl]-*N*,*N*-dimethyl-1,2-oxazinane-2-carboxamide (7a). General procedure with compound **5a** (0.417 g, 1.64 mmol), NMO (0.37 g,

2.7 mmol, 1.7 equiv.), the OsO_4 solution (2 ml) in acetone (1.5 ml) and H_2O (1 ml) for one day. Standard work-up gave **7a** (0.22 g, 40%).

Compound **7a**. Colorless crystals. Mp 171°C (AcOEt/Et_2O). $[\alpha]_D^{20}=+31$ (c=0.85, $CHCl_3$). IR (KBr): 3480, 3350, 2950, 1730, 1700, 1635, 1390, 1380, 1270, 1230, 1200, 1100, 995, 980. ^1H NMR ($CDCl_3$): Table 1. Anal. calcd for $C_{13}H_{21}N_3O_7$ (331.32): C 47.13, H 6.39, N 12.68; found: C 47.4, H 6.4, N 12.7.

4.2.2. *t*-Butyl (4*S*)-*t*-6-[(5′*S*)-5′-(*t*-butyloxycarbonyl)-2′-oxo-pyrrolidine-1′-yl]-*r*-4,*c*-5-dihydroxy-1,2-oxazinane-2-carboxylate (7b). General procedure with compound **5b** (1.8 g, 4.9 mmol) in acetone (10 ml) and water (2 ml), 5 M aqueous NMO solution (1.8 ml, 9 mmol, 2 equiv.) and the OsO_4 solution (2.4 ml and again 2.4 ml after 8 h) for one day. Standard work-up gave a mixture of **7b** and **5b** (2.1 g) which was resolved by FC (AcOEt/cyclohexane, 8:2) and gave successively **5b** 0.30 g, 16%) and **7b** (1.50 g, 75%). **7b** crystallised difficulty, but easily in AcOEt as solvate.

Compound **7b**. Colorless crystals. Mp 118–122°C (Et_2O/iPr_2O) or 80–85°C (solvated with 1 mol AcOEt). $[\alpha]_D^{20}=-22$ (c=1, $CHCl_3$). IR (KBr): 3470, 3330, 2965, 1700, 1450, 1390, 1365, 1245, 1273, 1240, 1225, 1158, 1118, 985, 898, 860, 820, 760; solvated with AcOEt: 3500, 3370, 2980, 1735, 1722, 1690, 1395, 1368, 1335, 1285, 1243, 1220, 1150, 1130, 1095, 1035, 970, 938, 815, 800. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$, 300 K): 24.9 (C(4′)); 27.8, 28.0 (2 C*Me*$_3$); 29.2 (C(3′)); 50.4 (C(3)); 56.2 (C(5′)); 66.8, 67.9 (C(4), C(5)); 78.9 (C(6)); 82.4, 84.0 (2 *C*Me$_3$); 156.2 (CO–N(2′)); 174.1 (CO–C(5′)); 176.4 (CO(2′)). Anal. calcd for $C_{18}H_{30}N_2O_8$ (402.45): C 53.72, H 7.51, N 6.96; found C 53.9, H 7.7, N 6.9; calcd for $C_{18}H_{30}N_2O_8+C_4H_8O_2$ (solvate, 490.56): C 53.87, H 7.81, N 5.71; found: C 53.7, H 7.8, N 5.8.

4.2.3. Benzyl (4*S*)-*t*-6-[(5′*S*)-5′-(*t*-butyloxycarbonyl)-2′-oxo-pyrrolidine-1′-yl]-*r*-4,*c*-5-dihydroxy-1,2-oxazinane-2-carboxylate (7c). General procedure with compound **5c** (2.4 g, 6 mmol), NMO (1.61 g, 12 mmol, 2 equiv.) and the OsO_4 solution (4.8 ml) in acetone (9.6 ml) and H_2O (2.4 ml) for one day. Standard work-up gave **7c** (2.42 g, 93%) as a colorless resin which crystallised with difficulty.

Compound **7c**. Colorless crystals. Mp 126–128°C (AcOEt). $[\alpha]_D^{18}=-33$ (c=1, $CHCl_3$). IR (KBr): 3445, 2980, 1735, 1715, 1460, 1392, 1370, 1332, 1282, 1258, 1205, 1150, 1137, 1093, 1050, 963, 820, 752, 700. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$, 300 K): 24.8 (C(4′)); 27.7 (C*Me*$_3$); 29.2 (C(3′)); 50.6 (C(3)); 56.4 (C(5′)); 66.4, 67.7, 67.9, (C(4), C(5), *CH*$_2$Ph); 79.7 (C(6)); 83.7 (*C*Me$_3$); 135.8, 128.2, 128.1, 127.9 (Ph); 156.5 (CO–N(2)); 173.7 (CO–C(5′)); 176.6 (CO(2′)). Anal. calcd for $C_{21}H_{28}N_2O_8$ (426.37): C 57.79, H 6.47, N 6.57; found: C 57.8, H 6.5, N 6.4.

4.2.4. Acetonide 8a. A solution of **7c** (0.24 g, 0.55 mmol) in acetone (3.2 ml) and 2,2-dimethoxypropane (0.7 ml, 5.8 mmol) was stirred with Amberlyst-15 (H^+) (in beads, 30 mg) for 4 h at 35°C, then filtered and evaporated. The resulting crystals were washed with iPr_2O to give **8a** (0.26 g, quantitative).

Compound **8a**. Colorless crystals. Mp 176°C (AcOEt/iPr_2O). $[\alpha]_D^{20}=+6$ (c=0.4, $CHCl_3$). IR (KBr): 2980, 1728, 1705, 1440, 1408, 1390, 1378, 1362, 1270, 1242, 1232, 1212, 1160, 1140, 1100, 972, 720. ^1H NMR ($CDCl_3$) Table 1. ^{13}C NMR ($CDCl_3$, 300 K): 24.5 (C(4′)); 26.2 (Me); 27.6 (C*Me*$_3$); 27.7 (Me); 29.6 (C(3′)); 46.3 (C(3)); 58.6 (C(5′)); 68.0 (*CH*$_2$Ph); 71.3, 70.9 (C(4), C(5)); 82.1 (C(6)); 82.5 (*C*Me$_3$); 110.3 (*C*Me$_2$); 135.8, 128.6, 128.4, 128.2 (Ph); 156.5 (CO–N(2)); 171.3 (CO–C(5′)); 176.0 (CO(2′)). Anal. calcd for $C_{24}H_{32}N_2O_8$ (476.51): C 60.49, H 6.77, N 5.88; found: C 60.5, H 6.9, N 5.9.

4.2.5. Benzyl (3*S*)-*c*-6-[(5′*S*)-5′-(*t*-butyloxycarbonyl)-2′-oxo-pyrrolidine-1′-yl]-*t*-4,*t*-5-dihydroxy-*r*-3-methyl-1,2-oxazinane-2-carboxylate (7d). General procedure with compound **5d** (3.61 g, 8.7 mmol), NMO (2.34 g, 17.4 mmol, 2 equiv.) and the OsO_4 solution (7.1 ml) in acetone (14 ml) and H_2O (3.5 ml) for 16 h at 40°C. Standard work-up and filtration over a bed of silica gel (AcOEt) gave **7d** (3.87 g, 99%) which was characterised as its acetonide **8b**.

Compound **7d**. Colorless resin. $[\alpha]_D^{20}=-32$ (c=1, $CHCl_3$). IR (KBr): 3460, 2975, 1718, 1708, 1401, 1370, 1300, 1325, 1141. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$, 300 K): 14.4 (Me–C(3)); 24.8 (C(4′)); 27.8 (C*Me*$_3$)); 29.3 (C(3′)); 55.4 (C(3)); 56.6 (C(5′)); 65.0 C(5)); 68.0 (*CH*$_2$Ph); 70.7 C(4); 80.4 (C(6)); 83.8 (*C*Me$_3$); 136.0, 128.4, 128.2, 128.0 (Ph); 156.3 (CO–N(2)); 173.9 (CO–C(5′)); 176.5 (C(2′)).

4.2.6. Acetonide 8b. A solution of **7d** (0.44 g, 0.99 mmol) in acetone (6 ml) and 2,2-dimethoxypropane (1.4 ml) was stirred with Amberlyst-15 (H^+) (in beads, 57 mg) for 4 h at 40°C, then filtered and evaporated. The resulted crystals were washed with iPr_2O to give **8b** (0.43 g, 89%).

Compound **8b**. Colorless crystals. Mp 133°C (AcOEt/iPr_2O). $[\alpha]_D^{20}=+20$ (c=0.55, $CHCl_3$). IR (KBr): 2981, 1709, 1415, 1303, 1278, 1222, 1170, 1142, 1119, 1091, 1075, 1028, 996, 754. ^1H NMR ($CDCl_3$): Table 1. Anal. calcd for $C_{25}H_{34}N_2O_8$ (490.54): C 61.21, H 6.99, N 5.71; found: C 61.2, H 7.0, N 5.7.

4.3. Hydrogenolysis to aminoalditols

4.3.1. 1-[(*N*,*N*-Dimethylcarbamoyl)-amino]-1-deoxy-D-erythritol (D-9a). Compound **7a** (0.24 g, 0.72 mmol) was hydrogenated at rt in MeOH (5 ml) over Raney-Ni (wet 1.2 g) for 5–6 h. The solution was filtered over celite and evaporated. The resulting oily crystals were washed with hot AcOEt to give D-**9a** (0.12 g, 90%). The AcOEt solution contained the methyl L-pyroglutamate **10a** (0.105 g, quant.) which was identified by its NMR data.

Compound D-**9a**. Colorless crystals. Mp 130°C (MeOH/Et_2O). $[\alpha]_D^{20}=+8.9$ (c=1, MeOH). IR (KBr): 3350, 3300, 3200, 2930, 1625, 1450, 1380, 1350, 1255, 1245, 1060, 1040. ^1H NMR (D_2O, 300 K): 2.87 (s, NMe_2), 3.23 (dd, Ha–C(4)), 3.40 (dd, Hb–C(4)), 3.60 (m, Ha–C(1), H–C(2),

H–C(3)), 3.74 (m, Hb–C(1)); J(3,4a)=6.5 Hz, J(3,4b)=3.1 Hz, J(4a,4b)=14.4 Hz. Anal. calcd for $C_7H_{16}N_2O_4$ (192.21): C 43.74, H 8.39, N 14.58; found: C 43.5, H 8.4, N 14.3.

4.3.2. Triacetate of D-9a. Compound D-**9a** (29 mg, 0.15 mmol) was acetylated in pyridine (0.2 ml) with Ac_2O (90 μl, 0.95 mmol) for 8 h at rt. Addition of MeOH (1 ml) followed by evaporation gave its triacetate (53 mg, quant.) as a colorless resin. Same IR and ^{1}H NMR data as for the racemic one.[24]

4.3.3. 1-[(*t*-Butyloxycarbonyl)-amino]-1-deoxy-D-erythritol (D-9b). Compound **7b** (solvated, 0.10 g, 0.25 mmol) was hydrogenated at 40°C in MeOH (4 ml) over Raney-Ni (wet 1.0 g) for 20 h. The catalyst was discarded by centrifugation and the solution evaporated. The resulting oily crystals were extracted with hot cyclohexane (3×5 ml) to give insoluble crystals of D-**9b** (49 mg, quant.) and a solution of L-pyroglutamate **10b** (40 mg after evaporation, quant., mp 104–106°C; lit.:[18] mp 108–109°C, identified by its NMR data).

Compound D-**9b**. Colorless needles. Mp 99–100°C (iPr$_2$O). $[\alpha]_D^{20}=-7$ (c=0.5, $CHCl_3$). IR (KBr): 3280, 2975, 2930, 1670, 1550, 1365, 1272, 1250, 1172, 1078, 1048, 938, 890. ^{1}H NMR (D_2O, 300 K): 1.45 (s, CMe_3); 3.14 (dd, Ha–C(1)); 3.40 (dd, Hb–C(1)); 3.63 (m, H–C(2), 2H–C(4)); 3.75 (dt, H–C(3)); J(1a,1b)=14.2 Hz, J(1a,2)=7.1 Hz, J(1b,2)=3.2 Hz, J(2,3)=8.6 Hz, J(3,4)=6.2 Hz. Anal. calcd for $C_9H_{19}NO_5$ (221.26): C 48.85, H 8.66, N 6.33; found: C 48.9, H 8.9, N 6.2.

4.3.4. (2*S*)-*r*-2-Methyl-pyrrolidine-*t*-3,*t*-4-diol (1,4-imino-1,4,5-tridesoxy-L-ribitol, L-11b). A solution of **7d** (0.99 g, 2.2 mmol) in MeOH (35 ml) was hydrogenated over Raney-Ni (wet, 3.9 g) for 3.5 h at rt. The catalyst was removed by centrifugation and the organic solution evaporated. The resulting crystalline oil was dissolved in H_2O (2–3 ml) and the solution extracted with $CHCl_3$. Evaporation of the organic phase gave **10b** (0.367 g, 90%, mp 107°C; lit.:[18] mp 108–109°C, identified by its NMR data). Lyophilisation of the aqueous phase gave L-**11b** (0.22 g, 85%) as slightly brownish hygroscopic crystals (containing ca. 20% of minor isomer **11c**), or (when the solution was neutralised with conc. HBr) as its hydrobromide (oily crystals). Recrystallisation of the crude hydrobromide in nPrOH gave pure L-**11b**·HBr (containing 6% of **11c**·**HBr**).

Compound L-**11b**. ^{1}H NMR (CD_3OD, 300 K): 4.10 (dt, H–C(4)) 3.53 (dd, H–C(3)); 3.29 (dd, Hb–C(5)); 3.09 (quint., H–C(2)); 2.87 (dd, Ha–C(5)); 1.25 (d, Me–C(2)); J(2,Me)=6.6 Hz, J(2,3)=7.8 Hz, J(3,4)=5.0 Hz, J(4,5a)=3.2 Hz, J(4,5b)=5.4 Hz, J(5a,5b)=12.4 Hz. ^{13}C NMR (CD_3OD, 300 K): 79.1 (C(3)); 71.8 (C(4)); 58.3 (C(2)); 51.9 (C(5)); 17.2 (Me–C(2)). MS (m/z (%)): 117 (M^+, 9), 99 (12), 71 (6), 57 (100). HRMS: mass calcd for $C_5H_{11}NO_2$: 117.07897; found: 117.0789.

Compound L-**11b**·*HBr*. Cream crystals. Mp 130°C (nPrOH) (lit.[28] mp 134–135°C; lit.[29] mp 126–128°C for the D-enantiomer). $[\alpha]_D^{20}=-43$ (c=0.28, MeOH) (lit.:[28,29] $[\alpha]_D^{20}=+45$, c=0.41, MeOH for the D-enantiomer). IR (KBr): 3320, 3240, 2930, 2770, 1595, 1450, 1415, 1375, 1355, 1260, 1135, 1120, 1090, 1055, 970 (same data[28] or similar data[29] as for the D-enantiomer).^{1}H NMR (D_2O, 300 K): 1.44 (d, Me–C(2)); 3.30 (dd, Ha–C(5)); 3.57 (dd, Hb–C(5)); 3.61 (m, H–C(2)); 3.99 (dd, H–C(3)); 4.40 (dt, H–C(4)). J(2,Me)=7.0 Hz, J(2,3)=8.4 Hz, J(3,4)=4.2 Hz, J(4,5a)=2.2 Hz, J(4,5b)=4.9 Hz, J(5a,5b)=13.1 Hz (similar data as for the D-enantiomer[28,29]).

Compound **11c**·*HBr*. Only characterised by ^{1}H NMR (D_2O, 300 K): same data as for the D-enantiomer.[16,28]

4.4. Sulfite adducts

4.4.1. (4*S*)-*t*-6-[(5′*S*)-5′-(*t*-Butyloxycarbonyl)-2′-oxo-pyrrolidine-1′-yl]-*r*-4,*c*-5-dihydroxy-1,2-oxazinane (12a). A solution of **7c** (2.37 g, 5.44 mmol) in MeOH (40 ml) was hydrogenated over 5% Pd/C (0.33 g) for 1.7 h at rt. The catalyst was removed by centrifugation and the solution evaporated to give, after washing with Et_2O, **12a** (1.57 g, 96%)

Compound **12a**. Colorless crystals. Mp 197–198°C (AcOEt/MeOH, 5:1). $[\alpha]_D^{20}=-58$ (c=1, MeOH). IR (KBr): 3498, 3261, 2985, 2881, 1716, 1408, 1371, 1244, 1160, 1095, 1044, 1003. ^{1}H NMR (D_2O): Table 1. ^{13}C NMR (D_2O, 300 K): 24.8 (C(4′)); 27.6 (CMe_3), 30.8 (C(3′)); 53.0 (C(3)); 60.4 (C(5′)); 66.9, 67.4 (C(4), C(5)); 82.5 (C(6)); 85.1 (*C*Me$_3$); 173.9 (CO–C(5′)); 181.4 (CO(2′)). Anal. calcd for $C_{13}H_{22}N_2O_6$ (302.32): C 51.64, H 7.34, N 9.27; found: C 51.7, H 7.5, N 9.3.

4.4.2. (3*S*)-*c*-6-[(5′*S*)-5′-(*t*-Butyloxycarbonyl)-2′-oxo-pyrrolidine-1′-yl]-*t*-4,*t*-5-dihydroxy-*r*-3-methyl-1,2-oxazinane (12b). Same procedure with **7d** (2.38 g, 5.3 mmol) in MeOH (40 ml) over 5% Pd/C (0.33 g) for 2 h at rt, to give, after washing with iPrOH/iPr$_2$O **12b** (1.20 g, 72%).

Compound **12b**. Colorless crystals. Mp 197°C (dec.) (AcOEt/iPrOH). $[\alpha]_D^{20}=-69$ (c=1, MeOH). IR (KBr): 3480, 3220, 3160, 2979, 1740, 1718, 1412, 1370, 1240, 1220, 1160, 1150, 1100, 969. ^{1}H NMR (D_2O): Table 1. ^{13}C NMR (D_2O, 300 K): 14.3 (Me–C(3)); 24.6 (C(4′)); 27.6 (CMe_3); 30.7 (C(3′)); 57.7 (C(3)); 60.6 (C(5′)); 64.6 (C(4)); 70.7 (C(5)); 83.3 (C(6)); 85.1 (*C*Me$_3$)); 173.8 (CO–C(5′)); 181.4 (CO(2′)). Anal. calcd for $C_{14}H_{24}N_2O_6$ (316.35): C 53.15, H 7.65, N 8.86; found: C 53.1, H 8.0, N 8.8.

4.4.3. 4-Amino-1,4-dideoxy-L-erythrose-1-sulfonic acid (L-13a). *From* **12a**. A solution of **12a** (0.75 g, 2.18 mmol) and pTsOH (0.53 g, 2.8 mmol, 1.3 equiv.) in H_2O (27 ml) was stirred under SO_2 atmosphere at 60°C for 6 days.[23] The yellowish solution was then evaporated and the residue crystallised in MeOH, to give L-**13a** (0.40 g, 87%), as brown crystals, purified by filtration over carbon black in H_2O.

From **7b**. Same procedure with **7b** (0.36 g, 0.89 mmol) and pTsOH (0.1 g, 0.5 mmol, 0.56 equiv.) in H_2O (5 ml) for 6 days to give L-**13a** (0.12 g, 76%) as brown crystals.

Compound L-**13a**. Colorless crystals. Mp dec. above 150°C (H_2O/MeOH). $[\alpha]_D^{20}=+47$ (c=2, H_2O). IR (KBr): 3520, 3420, 2970, 2780, 1597, 1440, 1425, 1295, 1240, 1210,

Table 3. ^{1}H NMR data of L-**13a**,**b**, L-**14a**,**b** (major species: imine (i), amino-alcohol (a) and dimer (d)) in 0.01 M solution (D_2O, 250 MHz, 300 K, δ in ppm, J in Hz) without buffer or in acetate, phosphate or Hepes buffer

	Buffer[a]	H–C(1)	H–C(2)	H–C(3)	Ha–C(4)	Hb–C(4)[b]	J(1,2)	J(2,3)	J(3,4a)	J(3,4b)	J(4a,4b)[b]
L-**13a**	No	4.41	4.60	4.47	3.55	3.47	7.2	4.1	3.6	2.2	12.6
	P, H	4.09	4.44	4.27	3.16	3.02	5.3	4.7	4.3	3.5	11.9
	A	4.39	4.59	4.46	3.52	3.43	7.0	4.1	3.5	2.2	12.5
L-**13′a**	H	4.81	4.46	4.58	3.66	3.42	1.7	4.6	6.8	6.6	10.7
L-**13b**	No	4.45	4.62	4.09	3.75	1.46	4.9	4.8	6.5		6.8
	P, H	4.08	4.35	3.73	3.22	1.22	2.8	5.3	8.1		6.4
	A	4.43	4.60	4.07	3.72	1.44	4.8	5.0	6.5		6.9
L-13′b	H	4.87	4.41	4.25	3.63	1.39	1.0	4.6	7.0		6.2
L-**14a**(i)[c]	No	7.64	4.73	4.31	3.85	3.85	0.6	5.4	3.3	3.3	[d]
L-**14a**(a)	No	3.15	3.96	4.30	3.45	2.47	5.8	6.9	6.9	4.6	10.3
L-**14a**(d)	No	4.96	4.16	4.31	3.20	2.91	1.6	4.8	4.2	5.5	11.3
		4.80	4.53	4.10	3.07	2.61	4.8	5.0	6.7	9.2	10.6
L-**14a**(i)	H	7.65	4.72	4.33	3.85	3.85	ca. 0	5.2	2.7	2.7	[d]
L-**14a**(d)	A	5.08	3.58	3.99	3.30	3.09	4.2	6.0	3.3	9.2	13.2
		5.28	4.17	4.45	3.65	3.28	ca. 5	4.2	5.5	3.5	12.8
L-**14b**(i)[e]	No	7.61	ca. 4.8	3.92	4.06	1.18	0.6	5.4	1.8		7.1
L-**14b**(d)	No	4.66	4.60	3.48	2.89	1.16	4.9	4.9	9.4		6.2
		5.01	4.17	3.68	2.89	1.22	4.1	5.2	8.2		6.2
L-**14b**(i)	H	7.61	ca. 4.8	3.93	4.03	1.18	ca. 0	5.5	1.8		7.3
L-**14b**(d)	H	ca. 4.8	4.62	3.55	[d]	1.22	5.0	5.0	9.3		ca. 6.0
		5.05	4.16	3.69	[d]	1.22	4.0	5.0	8.0		ca. 6.0
L-**14b**(d)	A	5.23	ca. 4.80	3.92	3.46	1.23	5.1	4.6	9.8		6.3
		5.26	4.09	3.80	3.19	1.38	3.5	3.9	7.9		6.3

[a] no—without buffer; A—acetate buffer pH ca. 4.5; H—Hepes buffer pH ca. 7.0; P—phosphate buffer pH ca. 7.0.
[b] Me(5) or J(4a,Me) for **13b**, **14b**.
[c] J(1,4a)=J(1,4b)=2.4 Hz.
[d] Not determinated.
[e] J(1,4)=1.7 Hz, J(2,4)=1.1 Hz.

1176, 1045, 828. Same ^{1}H NMR (Table 3) and ^{13}C NMR data (D_2O) as for the racemic one.[17] Anal. calcd for $C_4H_9NO_5S$ (183.13): C 26.23, H 4.95, N 7.65, O 43.66, S 17.51; found: C 26.5, H 5.0, N 7.4, O 43.5, S 17.1.

4.4.4. 4-Amino-1,4,5-trideoxy-L-ribose-1-sulfonic acid (L-13b). A solution of **12b** (0.825 g, 2.6 mmol) and *p*TsOH (0.59 g, 3.1 mmol, 1.2 equiv.) in H_2O (29 ml) was stirred under SO_2 atmosphere at 60°C for seven days. The same work-up as above gave L-**13b** (0.235 g, 45%).

Compound L-**13b**. Colorless crystals. Mp 174°C (dec.) (H_2O/MeOH). $[\alpha]_D^{20}$=+2 (c=1, H_2O). IR (KBr): 3490, 3020, 1607, 1260, 1196, 1172, 1140, 1115, 1043. ^{1}H NMR (D_2O, 300 K): Table 3 (similar data as for the racemic one[17]). Anal. calcd for $C_5H_{11}NO_5S$ (197.21): C 30.45, H 5.62, N 7.10, O 40.57, S 16.26; found: C 30.5, H 5.6, N 7.2, O 39.8, S 16.5.

4.5. Stability of amino-sugars L-13a,b, L-14a,b

The stability of sulfite adducts **13a**,**b** and amino-sugars **14a**,**b** was assessed at pH ca. 4.5 in acetate buffer and at pH ca. 7 in phosphate buffer and in the non-nucleophilic Hepes buffer.

4.5.1. Sulfite adducts L-13a,b. To a ca. 10^{-2} M solution of L-**13a** or L-**13b** (1.0 mg) in D_2O (0.5 ml) were added AcOK (5.0 mg) and AcOH (4.5 μl) (acetate buffer, pH ca. 4.5) or NaH_2PO_4 (5.0 mg) and $Na_2HPO_4{\cdot}3H_2O$ (8.0 mg) (phosphate buffer, pH ca. 7) or Hepes (5.0 mg) and $NaHCO_3$ (5.0 mg) (Hepes buffer, pH ca. 7). ^{1}H NMR data at natural pH (without buffer, pH ca. 4) or in buffered solutions are reported in Table 3. In acetate and phosphate buffer only one species was observed in ^{1}H NMR (D_2O, 300 K); in Hepes buffer, a minor species **13′a**,**b** was observed in addition to L-**13a**,**b** (ratio **13a/13′a**: 65:35, **13b/13′b**: 85:15). No transformations of the solutions were detected with the time.

In order to compare the spectra in various conditions with a standard spectrum, the spectrum of each solution of L-**13a**,**b** after incubation in phosphate or Hepes buffer was always performed as control at pH 4–5 by acidification with AcOH. In all cases the same ^{1}H NMR spectra as in acetate buffer was observed.

4.5.2. Amino-sugars L-14a,b. Their solutions were prepared by addition of $Ba(OH)_2{\cdot}8H_2O$ (2.0 mg) to a 10^{-2} M solution of L-**13a** or L-**13b** (1.0 mg) in D_2O (0.5 ml), stirring for 2 h, elimination of solids by centrifugation and then addition of the buffer components as above.

4.5.3. ^{1}H NMR studies. ^{1}H NMR spectral data of the main species in D_2O or in studied buffered solutions were consigned in Table 3. In D_2O solution at 300 K (natural pH ca. 8), these compounds were a mixture of three main species imine (i), amino-alcohol (a) and a dimer (d) in proportions of 60:18:22 for L-**14a**, 33:0:66 for L-**14b**. In Hepes buffer L-**14a** were a complex mixture whose main analysable species were imine, L-**14b** were a 1:2 mixture of imine and dimer. No notable evolution of these solutions was observed after one day at rt.

In acetate buffer, dimer was the major species in both cases, but other species appeared after one day at 0°C. In both Hepes or acetate buffer, ulterior basicification to pH 8–9 with $Ba(OH)_2$ led to a similar ^{1}H NMR-spectra as without

buffer, with as main compounds imine (i) for L-**14a**, or imine (i) and dimer (d) for L-**14b**.

In phosphate buffer (pH 7), a irreversible evolution was observed with a $t_{1/2}$ of ca. 3 h for L-**14a** and of 10–20 h for L-**14b** at rt. In the case of L-**14a** the ketone hydrate **16** was only formed, whose NMR data are presented below. In the case of L-**14b** this evolution was more complex and was not studied.

4.5.4. 4-Hydroxy-pyrrolidine-3-one, hydrate (16). *Compound* **16a**. ^{1}H NMR (H_2O, 400 MHz, 300 K): 3.34 (dd, Hb–C(5), 3.36 (d, Hb–C(2), 3.38 (d, Ha–C(2), 3.68 (dd, Ha–C(5), 4.13 (dd, H–C(4); J(2a,2b)=12.0 Hz, J(4,5a)=4.8 Hz, J(4,5b)=1.8 Hz, J(5a,5b)=13.0 Hz. ^{13}C NMR (H_2O, 100 MHz, 300 K): 51.7, 51.6 (C(2), C(5)); 73.7 (C(4)); 100.9 (C(3)).

Compound **16b**. ^{1}H NMR (D_2O, 400 MHz, 300 K): 3.35 (dd, Hb–C(5), 3.68 (dd, Ha–C(5), 4.13 (dd, H–C(4); J(4,5a)=4.8 Hz, J(4,5b)=1.7 Hz, J(5a,5b)=12.9 Hz. **16c**: ^{1}H NMR (D_2O, 400 MHz, 300 K): 3.34 (d, Hb–C(5), 3.68 (d, Ha–C(5); J(5a,5b)=12.9 Hz.

4.5.5. Inhibition studies. We have used a simple enzyme assay with α-galactosidase to determine the concentration of inhibitor at a given time in various buffered solution at 25°C. The half-life of L-**14a**,**b** was then estimated. At concentration near their IC_{50} values L-**14a**,**b** was incubated in phosphate or Hepes buffer at pH 7.0 and 25°C, control aliquot were stored at −20°C. Addition of enzyme and substrate (1 mM) allowed to determine the variation of inhibition and then to estimate a concentration of the inhibitor (see Section 4.6). A decrease in the inhibitory potency was observed in phosphate buffer with a $t_{1/2}$ of ca. 5 h for L-**14a** and ca. 20 h for L-**14b** at 25°C. No change was detected in Hepes buffer for both amino-sugars after 17 h at this temperature.

4.6. Enzyme inhibitions

Solutions of amino-sugars L-**14a**,**b** are prepared by stirring a solution in H_2O of L-**13a**,**b** in adequate concentration with $Ba(OH)_2{\cdot}8H_2O$ (1.1 equiv.) for 2 h at rt and by removing insoluble $BaSO_3$ by centrifugation. The concentration of L-**14a**,**b** was assumed to be the same as the initial concentration of L-**13a**,**b**.

Glycosidase activities were determined at 25°C at the optimal pH of each enzyme[46] with the corresponding *p*-nitrophenyl glycopyranoside as substrate against α-D-glucosidase (EC 3.2.1.20) from baker's yeast (nine units per mg of protein, K_m=0.25 mM, pH=7.0, phosphate or Hepes buffer), β-D-glucosidase (EC 3.2.1.21) from almonds (20–40 units per mg of protein, K_m=1.3 mM, pH=5.0, acetate buffer), α-D-mannosidase (EC 3.2.1.24) from Jack beans (ca. 20 units per mg of proteins, K_m=2 mM, pH=4.5, acetate buffer), β-D-mannosidase (EC 3.2.1.25) from snail acetone powder (ca. 7 units per mg of proteins, K_m=1.3 mM, pH=4.0, acetate buffer). α-L-Fucosidase (EC 3.2.1.51) from bovine kidney (5–15 units per mg, K_m=0.15, pH=5.5, acetate buffer), α-D-galactosidase (EC 3.2.1.22) from green coffee beans (10 units per mg, K_m=0.25, pH=6.5, phosphate or Hepes buffer), β-D-galactosidase (EC 3.2.1.23) from *Escherichia coli* (1000 units per ml of proteins, K_m=0.65 mM, pH=7.0, phosphate or Hepes buffer). Glycosidases and corresponding *p*-nitrophenyl glycopyranosides were obtained from Sigma chemical Co. The release of *p*-nitrophenol was measured continuously at 405 nm (or 390 nm for pH=4.0) to determine initial velocities.[47] All kinetics were started by enzyme addition in a 1 ml assay medium using substrate concentrations around the K_m value of each enzyme. K_i values were determined for the most potent inhibitors using the Dixon graphical method,[48] IC_{50} values were determined for weak inhibitions (at a substrate concentration equal to K_m value) and correspond to the inhibitor concentration required for 50% inhibition of the enzyme in our experimental conditions.

Acknowledgements

The support of the Centre National de la Recherche Scientifique (UMR-7015), and of the Ministère de l'Enseignement Supérieur et de la Recherche for PhD grants to J.-B. Behr and C. Chevrier is gratefully acknowledged. We thank also Dr Joaquim Pires for his cooperation, Dr Muriel Joubert, Dr Isabelle Dosbaâ and the diploma students Sebastien Albrecht, Laetitia Briand, Audrey Ihler, Amandine Martin, Didier Rombach, Sylvie Teixeira, Thomas Tricotet for some determinations of inhibition values.

References

1. Elbein, A. D. *Ann. Rev. Biochem.* **1987**, *56*, 497–534.
2. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319–384.
3. Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199–210.
4. Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645–1680.
5. Sinnott, M. E. *Chem. Rev.* **1990**, *90*, 1171–1202.
6. Zechel, D. L.; Withers, St. G. *Acc. Chem. Res.* **2000**, *33*, 11–18.
7. Paulsen, H.; Brüning, J.; Heyns, K. *Chem. Ber.* **1970**, *103*, 1621–1630.
8. Paulsen, H.; Propp, K.; Brüning, J. *Chem. Ber.* **1969**, *102*, 469–487.
9. Shibata, T.; Nakayama, O.; Tsurumi, Y.; Okuhara, M.; Terano, H.; Kohsaka, M. *J. Antibiot.* **1988**, *41*, 296–301.
10. Kayakiri, H.; Nakamura, K.; Takase, Sh.; Setoi, H.; Uchida, I.; Terano, H.; Hashimoto, M.; Tada, T.; Koda, Sh. *Chem. Pharm. Bull.* **1991**, *39*, 2807–2812.
11. Kim, Y. J.; Takatsuki, A.; Kogoshi, N.; Kitahara, T. *Tetrahedron* **1999**, *55*, 8353–8364.
12. Tsujii, E.; Muroi, M.; Shiragami, N.; Takatsuki, A. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 549–566.
13. Witte, J. F.; McClard, R. W. *Tetrahedron Lett.* **1991**, *32*, 3927–3930.
14. Provencher, L.; Steensma, D. H.; Wong, Ch.-H. *Bioorg. Med. Chem.* **1994**, *2*, 1179–1188.
15. Wong, Ch.-H.; Provencher, L.; Porco, Jr. J. A.; Jung, S.-H.;

Wang, Y.-F.; Chen, L.; Wang, R.; Steensma, D.; H, *J. Org. Chem.* **1995**, *60*, 1492–1501.
16. Joubert, M.; Defoin, A.; Tarnus, C.; Streith, J. *Synlett* **2000**, 1366–1368.
17. Behr, J.-B.; Defoin, A.; Mahmood, N.; Streith, J. *Helv. Chim. Acta* **1995**, *78*, 1166–1177.
18. Behr, J.-B.; Defoin, A.; Pires, J.; Streith, J.; Macko, L.; Zehnder, M. *Tetrahedron* **1996**, *52*, 3283–3302.
19. Bach, P.; Bols, M. *Tetrahedron Lett.* **1999**, *40*, 3461–3464.
20. Zhang, D.; Gosh, A.; Süling, C.; Miller, M. J. *Tetrahedron Lett.* **1996**, *37*, 3799–3802.
21. Defoin, A.; Fritz, H.; Geffroy, G.; Streith, J. *Helv. Chim. Acta* **1988**, *71*, 1642–1658.
22. Paulsen, H.; Todt, K. *Chem. Ber.* **1967**, *100*, 3385–3396.
23. (a) Defoin, A.; Sarazin, H.; Streith, J. *Tetrahedron* **1997**, *53*, 13769–13782. (b) Defoin, A.; Sarazin, H.; Streith, J. *Tetrahedron* **1997**, *53*, 13783–13796.
24. Defoin, A.; Pires, J.; Streith, J. *Helv. Chim. Acta* **1991**, *74*, 1653–1670.
25. Defoin, A.; Pires, J.; Streith, J. *Synlett* **1991**, 417–419.
26. Keck, G. E.; Wager, Tr. T.; McHardy, St. F. *Tetrahedron* **1999**, *55*, 11755–11772.
27. Keck, G. E.; Fleming, St.; Nickell, D.; Weider, P. *Synth. Commun.* **1979**, *9*, 281–286.
28. Bierer, L. Dissertation. PhD Thesis, Stuttgart, Germany, 1999.
29. Palmer, A. M.; Jäger, V. *Eur. J. Org. Chem.* **2001**, 2547–2558. This enantiomer was found inactive against glycosidases.
30. Paulsen, H. *Liebigs Ann. Chem.* **1965**, *683*, 187–198.
31. Kondo, K.-I.; Adachi, H.; Shirata, E.; Kojima, F.; Nishimura, Y. *Bioorg. Med. Chem.* **2001**, *9*, 1091–1095.
32. Popowycz, Fl.; Gerber-Lemaire, S.; Demange, R.; Rodriguez-Garcia, E.; Carmona Asenjo, A. T.; Robina, I.; Vogel, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2489–2493.
33. Ogawa, S.; Morikawa, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1499–1504.
34. Nishimura, Y.; Umezawa, Y.; Adachi, H.; Kondo, Sh.; Takeuchi, T. *J. Org. Chem.* **1996**, *61*, 480–488.
35. Ogawa, S.; Morikawa, T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1047–1050.
36. Tropea, J. E.; Kaushal, G. P.; Pastuszak, I.; Mitchell, M.; Aoyagi, T.; Molineux, R. J.; Elbein, A. D. *Biochem.* **1990**, *29*, 10062–10069.
37. Cho, S. J.; Ling, R.; Kim, A.; Mariano, P. S. *J. Org. Chem.* **2000**, *65*, 1574–1577.
38. Farr, R. A.; Peet, N. P.; Kang, M. S. *Tetrahedron Lett.* **1990**, *31*, 7109–7112.
39. Schneider, M. J.; Ungemach, Fr. S.; Broquist, H. P.; Harris, Th. M. *Tetrahedron* **1983**, *39*, 29–32.
40. Tulsiani, D. R. P.; Broquist, H. P.; Touster, O. *Arch. Biochem. Biophys.* **1985**, *236*, 427–434.
41. Fleet, G. W. J.; Smith, P. W.; Evans, St. V.; Fellows, L. E. *J. Chem. Soc, Chem. Commun.* **1984**, 1240–1241.
42. Carpino, L. A.; Giza, Ch. A.; Carpino, B. A. *J. Org. Chem.* **1959**, *81*, 955–957.
43. Dang, H.-Sh.; Davies, A. G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 721–734.
44. VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973–1976.
45. Akashi, K.; Palermo, R. E.; Sharpless, K. B. *J. Org. Chem.* **1978**, *43*, 2063–2066.
46. Lundblad, G.; Bjare, U.; Lybing, S.; Måhlén, A. *Int. J. Biochem.* **1983**, *15*, 835.
47. La, Y.-T. *J. Biol. Chem.* **1967**, *242*, 5474–5480.
48. Segel, I. H. *Enzyme Kinetics*; Wiley: New York, 1975; pp 109–144.