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Amino(hydroxymethyl)cyclopentanetetriols, an Emerging Class of Potent Glycosidase Inhibitors—Part II: Synthesis, Evaluation, and Optimization of β-D-Galactopyranoside Analogues

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KEYWORDS:

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From efforts to rationalize the strong activity of mannostatins, the related structures of amino(hydroxymethyl)cyclopentanetriols were derived and shown to be highly active glycosidase inhibitors, first in the α-D-*manno* and then in other series (see Part I[1] and ref. [2]). Concerning the β-D-*galacto* series,[2] to be detailed here, the parent compound **13** and the *N*-methyl derivative **14** (prepared and assayed first) showed remarkable inhibitory effects as well as distinct changes in activity and selectivity when substituting NH_2 for $NHCH_3$.[2a] Thus, some 20 related structures were chosen and prepared for optimization.[2, 3] This led to several compounds with K_i values near or below the nanomolar range, which is reported herein.

The starting material for the cycloadditions was the protected L-*arabino*-5-hexenose **1**, obtained in three steps from D-galactose.[2–4] The hexenose **1** was converted into the corresponding isoxazolidines (**2** – **7**) on treatment with *N*-substituted hydroxylamines via intermediate nitrones,[2–4] following a route proposed earlier by Vasella et al.[5] (Scheme 1). Here, the diastereomeric ratio (d.r.) of the *syn*-/*anti*-cyclopentanoisoxazolidines (**2**:**5**, **3**:**6**, **4**:**7**) varied strongly, depending on the solvent used for the cycloaddition.[2, 3] In polar solvents the *syn*-tricycle was preferred, independent of R. For the cycloaddition with PhNHOH in methanol, d.r. values up to 87:13 (*syn*/*anti*) were observed (Scheme 1).[2b] In contrast, when the reaction was performed with BnNHOH in a nonpolar solvent like chloroform, a d.r. value of $<$ 5:95 in favor of the *anti* isomer resulted.[2b]

The free amino(hydroxymethyl)cyclopentanetriols **13** – **17** were obtained in the form of their hydrobromide salts from

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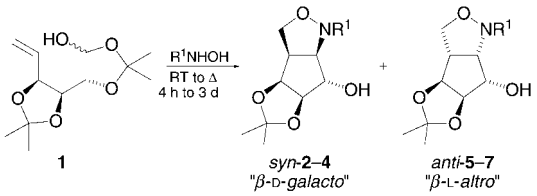
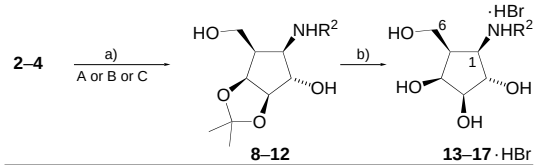


Scheme 1. *Synthesis of cyclopentanoisoxazolidines* ***2*** *–* ***7****. R^1 = Bn: EtOH/H_2O (4:1):* ***2*** *:* ***5*** *= 79:21, 66 %* ***2****,* ***5*** *n.i.; $CHCl_3$:* ***2*** *:* ***5*** *= < 5:95,* ***2*** *n.i., 85 %* ***5****. R^1 = Me: EtOH/H_2O (4:1):* ***3*** *:* ***6*** *= 79:21, 54 %* ***3****,* ***6*** *n.i.; $CHCl_3$:* ***3*** *:* ***6*** *= < 5:95,* ***3*** *n.i., 72 %* ***6****. R^1 = Ph: MeOH:* ***4*** *:* ***7*** *= 87:13, 77 %* ***4****,* ***7*** *(11 % from different run); $CHCl_3$:* ***4*** *:* ***7*** *= 48:52. n.i. = not isolated.*

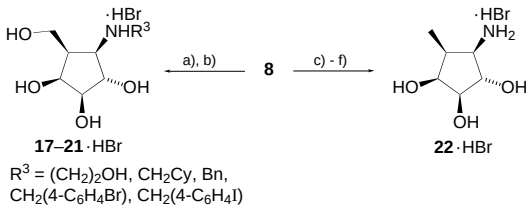
the respective isoxazolidines **8** – **12** by catalytic hydrogenation with $Pd(OH)_2$ on charcoal or with zinc in acetic acid and subsequent removal of the acetonide protecting group by treatment with HBr in methanol (Scheme 2). Unexpectedly, the *N*-phenyl substituent in **4** was saturated to *N*-cyclohexyl in the product **11** by catalytic reduction; the *N*-benzyl compound **17** was, therefore, secured from zinc reduction.



educt	R^1	method in a)	R^2	compound	yield [%]	compound	yield [%]
2	Bn	B	H	**8**	91	**13**	94
3	Me	B	Me	**9**	–[a]	**14**	90
4	Ph	C	Ph	**10**	63	**15**	96
4	Ph	A	Cy	**11**	92	**16**	48
2	Bn	C	Bn	**12**	75	**17**	91

Scheme 2. *Synthesis of amino(hydroxymethyl)cyclopentanetriols* ***13*** *–* ***17****. a) Method A: $Pd(OH)_2$/C, H_2 (4 bar), MeOH, RT; method B: as in A, but with HBr (48 %); method C: Zn, HOAc, RT, 4 – 5 h; b) HBr (48 %), MeOH, RT. [a] Not isolated; hydrogenation in acidic medium affords* ***14****. Cy = cyclohexyl.*

Some further *N*-substituted derivatives, **18** – **21** (and again **17**), were accessible by reductive amination of the respective aldehydes with the amine **8** (Scheme 3). In order to clarify the role of the 6-OH group for inhibition activity, it was of interest to also have the corresponding 6-deoxy derivatives such as **22**



Scheme 3. *Synthesis of* N*-substituted derivatives* ***17*** *–* ***21*** *and of the 6-deoxy compound* ***22****. a) 1. R^3CHO, Al_2O_3,[7] CH_2Cl_2, RT; 2. $NaBH_4$, MeOH, reflux, 47 – 85 %; b) HBr (48 %), MeOH, RT, 91 – 98 %; c) Boc_2O, $NaHCO_3$, dioxane, RT, 3 h, 90 %; d) ArOC(S)Cl, HOSu, Py, benzene, RT, 4 h; e) Bu_3SnH, AIBN, benzene, reflux, 3 h, 75 %; f) HBr (48 %), MeOH, RT, 1 d, 99 %. AIBN = 2,2′-azobisisobutyronitrile, Boc =* tert*-butoxycarbonyl, Su = succinimidyl.*

available for tests. To this purpose, the amine **8** was protected as the *N*-Boc carbamate, followed by a two-step Barton reduction[6] and deprotection as described above, thus affording **22**. This protocol likewise led to the "β-L-*fuco*" compound *ent*-**22** when starting from L-galactose.[2b]

Comprehensive tests with up to 28 commercially available glycosidases were carried out with all derivatives synthesized and also with some epimers and enantiomers of **13**.[2, 3] The parent compound **13**, gratifyingly, showed strong inhibition of all five β-D-galactosidases tested, but also of one (of three tested) of the α series, and further of the two β-D-glucosidases used (Table 1, entry 1). For the latter, even higher activities were found with **13** than those observed with the 2-epimer, the β-D-*gluco* parent compound (see Part I[1a]), which is in line with previous reports and classifications.[8] The basic amino group again is essential for good inhibition, since *N*-acetylation of **13** and derivatives resulted in K_i values that were higher by two orders of magnitude or more throughout.[2c] The role of the 6-hydroxy group was also evaluated, by testing the 6-deoxy compound **22**, with rather different results for the respective enzymes: no activity or distinctly decreased activity with the α-D- and two of the four β-D-galactosidases (*A. niger* and *A. oryzae*), similar or increased inhibition of the other two β-D-galactosidases (*E. coli*, bovine liver) and of the two β-D-glucosidases tested (Table 1, entry 2). Thus, hydroxymethyl and methyl groups are equivalent in the latter cases, suggesting that there is no or little hydrogen

Table 1. *Selected glycosidase inhibition activities of* ***13***[13] *and its derivatives.*[a]

			α-D-Galactosidase		β-D-Galactosidase				β-D-Glucosidase	
Entry	Inhibitor	R^2 or R^3	(c.b.)	(E.c.)	(b.l.)	(A.n.)	(A.o.)	(j.b.)	(alm.)	(C.s.)
1	**13**	H	12	4.5	3.3	0.85	0.6	0.145	2.2	0.17
2	**22**	H (6-deoxy)	n.i.	8	4	ca. 1000	13	n.d.	0.8	0.08
3	**14**	Me	n.i.	0.22	0.09	0.4	0.033	0.068	0.45	0.35
4	**18**	$(CH_2)_2OH$	n.i.	0.087	0.48	0.11	0.085	0.16	5	14
5	**15**	Ph	n.i.	185	n.d.	80.7	102	n.d.	113	23
6	**16**	Cy	n.i.	2.4	3	n.i.	20	0.035	0.83	7
7	**17**	Bn	n.i.	0.002	0.006	2.4	0.038	0.14	1.39	0.165
8	**19**	CH_2Cy	22	0.02	0.004	0.05	0.03	n.d.	0.104	0.12
9	**20**	$CH_2(4\text{-}C_6H_4Br)$	n.i.	0.0006	0.0007	0.035	0.004	n.d.	0.017	0.12
10	**21**	$CH_2(4\text{-}C_6H_4I)$	n.i.	0.008	0.003	0.148	0.057	n.d.	0.053	0.187

[a] K_i values are given in μM. n.i. = no inhibition for $IC_{50} > 1$ mM; n.d. = not determined. In all cases competitive inhibition was observed. [b] Enzyme sources: c.b. = coffee beans, E.c. = *Escherichia coli*, b.l. = bovine liver, A.n. = *Aspergillus niger*, A.o. = *Aspergillus oryzae*, j.b. = jack beans, alm. = almonds, C.s. = *Caldocellum saccharolyticum*.

bonding to this position in the active site of the respective enzymes. This is in agreement with different classifications of the *E. coli* and *A. niger* enzymes (families 2 and 35, respectively).[8]

Introducing a methyl group at the amino nitrogen atom, that is, going from **13** to **14**, caused substantial alterations of inhibitory activity with all of these enzymes, which can be discussed only briefly here. With the α-D-galactosidase, the K_i value of **14** rose to about 1 mM, but all other enzymes in the β-D-*galacto* and -*gluco* series except one responded readily to this, by decreasing K_i values by factors of 2 to 35 (Table 1, entry 3). Consequently, variation of *N*-substituents was undertaken. Increased hydrophilicity, as seen with the *N*-hydroxymethyl group (successful in the α-D-*gluco* series, see miglitol),[9] led to slight changes only (Table 1, entry 4). Turning to more lipophilic attachments to the amino nitrogen atom, a *tert*-butyl group was introduced, but this modification eliminated most activities (ca. 200 μM with the *E. coli* and the *A. niger* β-D-galactosidases).[2d] Furthermore, an *N*,*N*-dimethylamino moiety proved less effective throughout (K_i values of 0.3 to 7.8 μM for the β-glycosides shown).[2c] Thus, derivatives with only one lipophilic group at the nitrogen atom deemed more promising, but both *N*-phenyl (**15**) and *N*-cyclohexyl substituents (**16**) did not improve inhibition, except for the case of the jack bean galactosidase (Table 1, entries 5, 6). This changed much for the better when the cyclohexyl and phenyl rings were linked to the amino group by means of a methylene spacer (compounds **17** and **19**, respectively). Inhibitions were improved dramatically, although not in a uniform way (Table 1, entries 7, 8). Looking at the first two β-D-*galacto* cases from truly distinct sources—*E. coli* and bovine liver—it is remarkable that both the *N*-benzyl and the *N*-cyclohexylmethyl derivatives **17** and **19**, respectively, caused similarly potent inhibition, now in the low nanomolar range!

At this stage, applications of these extremely powerful inhibitors were envisaged: Many open questions concerning three-dimensional structures of most glycosidases, which are not available or classified yet,[8] might be solved in specific cases by cocrystallization with such strongly binding unnatural inhibitors. In the field of β-D-galactosidases, the *E. coli* enzyme had been crystallized, but the structure was not well resolved (3.5 Å) due to its size (1023 amino acids) and crystal form (four tetramers in the unit cell, ca. 2×10^6 Da).[10] In order to simplify such analyses, halogen-substituted derivatives of the *N*-benzyl compound **17** seemed particularly promising, since introduction of a halogen atom onto the aromatic ring would just necessitate the use of appropriate aldehydes for condensation with the amine **8**. Thus, the *p*-bromo and *p*-iodo derivatives **20** and **21**, respectively, were prepared (Scheme 3), with the bromo compound **20** now showing even better inhibition values than the benzyl analogue **17**: K_i values for the *E. coli* and bovine liver enzymes went down to 0.6 and 0.7 nM, respectively, setting a new low in this area as far as we are aware of! In the β-D-*gluco* series, again the best activities were recorded with the bromo compound **20**: 17 and 120 nM for the almond and the *Caldocellum* enzymes, respectively (entry 9 in Table 1).

A brief comparison with some known structures of the most potent D-galactosidase inhibitors is appropriate: The 1,5-iminoglycitols (piperidines) with D-*galacto* configuration inhibit strongly in the α-anomer series, but much less with β-galactosidases [D-galacto-nojirimycin: α (coffee beans): $K_i = 0.0007$; β (*E. coli*): $K_i = 0.045$ M].[11] D-Galactopyranosylamines with *N*-benzyl or *N*-heptyl substituents may be as powerful as the new benzylaminocyclopentane structures (**17**, **20**, **21**), as judged from the rather limited data available; however, assay conditions were different (Mg^{2+} added) and *N*-alkyl derivatives in aqueous solution are not stable (anomerization and hydrolysis).[12]

Implications concerning the mechanism of such enzymatic hydrolyses and the binding of the amino(hydroxymethyl)cyclopentanetriols should await the determination of specific three-dimensional structures of enzyme – inhibitor complexes;[10] our working hypothesis presently is that *exo*-protonated glycosides are well mimicked by these new structures (and also pyranosylamines) present as ammonium ions, that is, that *intermediates*, not transition states or pyranosyl cations, may be the key structures. The above results with the cyclopentane structures of "β-D-*galacto*" configuration to yield "1,5-deoxypyranosyl amines" provide a promising general concept to generate new, powerful, unnatural, and stable glycosidase inhibitors. Applications are manifold, and fine-tuning with regard to the specific enzyme addressed is feasible by varying the *N*-substituents.

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