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Competitive inhibition of glycoside hydrolases by 1,3-diamino-1,3-dideoxy-tetritols and their cyclic carbonic and thiocarbonic amides

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ABSTRACT

1,3-Diamino-1,3-dideoxy-D-threitol (**1**) and the corresponding 1,3-diamino-1,3-dideoxy-D-erythritol (**2**) were synthesised starting from D-glucose and L-arabinose, respectively. These acyclic diamines inhibited competitively both β-D-glucosidase from sweet almond emulsin and β-D-galactosidase from *E. coli* with K_i-values ranging from 3 to 10 mM. When the suitably blocked diamines were reacted with activated carbonic and thiocarbonic acid derivatives, cyclic urea 5(*R*)-hydroxy-4(*R*)-hydroxymethyl-tetrahydropyrimidin-2-one (**13**), 5(*S*)-hydroxy-4(*R*)-hydroxymethyl-tetrahydropyrimidin-2-one (**15**) and thiourea 5(*S*)-hydroxy-4(*R*)-hydroxymethyl-tetrahydropyrimidin-2-thione (**18**) derivatives were obtained, which conformationally resemble the envelope structure of the D-glucopyranosyl or the D-galactopyranosyl cation. The cyclic carbonamides showed extremely weak competitive inhibition but only with their corresponding enzymes. Compounds **15** and **18** exist, as indicated by ^{1}H NMR spectroscopy, in an unexpected *E*-conformation with axial substituents. Upon per-*O*-acetylation the expected conformation with equatorial substituents is adopted.

INTRODUCTION

Innumerable substrates and competitive inhibitors for glycoside hydrolases have been synthesised and tested, all having in common a heterocyclic structure. It was accepted that a fairly rigid skeleton for the glyconic part of a sugar derivative, preferentially flattened in the area of the anomeric carbon like the δ-lactones[1], lactams[2], or the corresponding lactone oximes[3] would be necessary for the enzyme to recognize a good competitive inhibitor, because it resembles the glycosyl cation **A** (Fig. 1) of enzymic glycoside cleavage. Ideally the combination of a flattened conformation and a positive charge as in the cyclic guanidinium structure **B** would create an optimal inhibitor, generally referred to as a "transition state analogue". Very close to this optimal structure is the cyclic amidinium structure **C** (ref 4).

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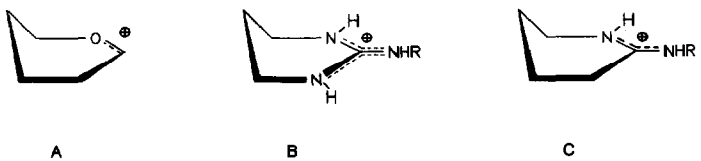
Recently it was shown that a positive charge and a flattened, flexible ring conformation as in several hydroxy pyrrolidines of varying configuration, are the major structural elements required for good competitive inhibition of different glycoside hydrolases[5]. The stereochemistry of the different compounds has hardly any influence. This fact was attributed to the flexibility of conformations, other than the rigid chair conformations of glycosides, hydroxypiperidines, or glycosylamines[6]. In the present study, we have investigated the minimal structural requirements for competitive inhibitors of two glycoside hydrolases, β-D-glucosidase from sweet almond emulsin, and β-D-galactosidase from *E. coli*.

RESULTS AND DISCUSSION

The active site of a glycoside hydrolase is generally equipped with a proton-donating, acidic group. The presence of such a group is one reason why some basic monosaccharide derivatives are extremely potent competitive inhibitors[5]. The formation, in addition to ordinary hydrogen bonding, of a strong salt bridge apparently causes significant enhancement in binding. Under the assumption that the acidic group at the active site of a glycoside hydrolase would have a coordinating effect on amino groups attached to structurally fitting acyclic polyols, we prepared 1,3-diamino-1,3-dideoxy-D-threitol (**1**) and 1,3-diamino-1,3-dideoxy-D-erythritol (**2**) as potential competitive inhibitors, starting from 1,3-*O*-benzylidene-L-erythritol and 1,3-*O*-benzylidene-L-threitol[7] respectively. The former was converted, after 2,4-di-*O*-benzylation, removal of the benzylidene group, and 2,4-di-*O*-tosylation, into the 1,3-diazido-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol (**9**), and the latter after 2,4-di-*O*-tosylation[7] into 1,3-diazido-2,4-*O*-benzylidene-1,3-dideoxy-L-erythritol (**6**). Removal of the blocking groups and reduction of the azido groups gave the diamines **1** and **2**. The two diamines, or suitable derivatives thereof, were also designed to prepare cyclic urea (**13** and **15**), thiourea (**18**) and, finally, strongly basic guanidine derivatives (**B**) (Fig. 1), which mimic the flattened structures of configurationally related glycosyl cations (**A**). These compounds, especially structure **B** which could not yet be realised, were expected to be potent competitive inhibitors of the configurationally related glycoside hydrolases.

Compounds **13** and **15** were prepared by cyclising the diamines of **9** and **6** with the reagent di-(succinimidyl)-carbonate and removal of the blocking groups. The



A B C

Fig. 1. Flattened *E*-conformation and positive charge make amidinium (**C**) and guanidinium ion (**B**) resemble a glycosyl cation (**A**)

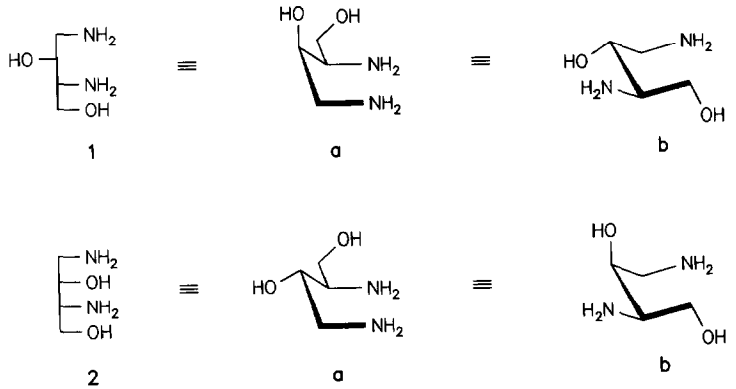


Fig. 2. Two different types of conformations of the 1,3-diamino-1,3-dideoxytetritols **1** and **2**. Types **b** seem to be preferentially bound by the corresponding glycoside hydrolases

thiourea derivative **18** was obtained by reacting the diamine of **6** with 1,1′-thiocarbonyl-diimidazole.

We expected the neutral, cyclic compounds **13**, **15** and **18**, resembling the general structure **A**, to competitively and specifically inhibit their corresponding β-D-glycoside hydrolase, e.g., compound **13**, β-D-galactosidase, and compounds **15** and **18**, β-D-glucosidase. The poor affinities of compounds **15** and **13** compared to those of their corresponding lactames[5] demonstrate the importance of the hydroxyl groups at C-2 and C-3 in pyranosides and analogous derivatives for effective binding. The diamines **1** and **2** may have inhibitory properties due to their basic properties. This was in fact demonstrated by Huber and Gaunt[8] who showed 1-amino-1-deoxy- and 2-amino-2-deoxy-D-glycerol to competitively inhibit *E. coli* β-D-galactosidase. Folded in conformations **a** (Fig. 2), with the amino groups eclipsed on the zig-zag carbon chain, compound **1** bears a resemblance to the nonreducing side of D-galactose and compound **2** to that of D-glucose. These conformations could be complementary to the binding areas of the corresponding enzymes. Stabilisation may be derived mainly from a salt bridge with the enzyme, formed after protonation of one of the properly oriented amino groups, and by hydrogen bonding through the hydroxyl groups (Fig. 3). Kinetic parameters for the

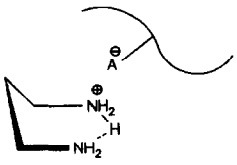


Fig. 3. An acyclic 1,3-diamine protonated by a glycoside hydrolase may adopt a conformation, which resembles a glycosyl cation

3 $R^1 = R^3 = OBzl$ $R^2 = R^4 =$ 

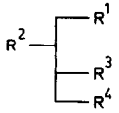
4 $R^1 = R^3 = OBzl$ $R^2 = R^4 = OH$

5 $R^1 = R^3 = OBzl$ $R^2 = R^4 = OTos$

6 $R^1 = R^3 = N_3$ $R^2 = R^4 =$ 

7 $R^1 = R^3 = N_3$ $R^2 = R^4 = OH$

8 $R^1 = R^3 = NHAc$ $R^2 = R^4 = OAc$



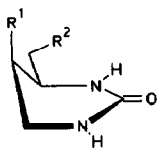
9 $R^1 = R^3 = N^3$ $R^2 = R^4 = OBzl$

10 $R^1 = R^3 = N^3$ $R^2 = R^4 = OH$

11 $R^1 = R^3 = NHAc$ $R^2 = R^4 = OAc$

cyclic and acyclic compounds were measured as described under Experimental and inhibition constants determined by a Dixon plot[9], giving the values listed in Table V. The mean deviation in three independent measurements for each compound was not more than 15%.

Values for the cyclic compounds, although quite insignificant, are at least consistent with our expectations. Compound **13** being a D-galactopyranosyl analogue binds better to β-D-galactosidase than to β-D-glucosidase and compounds **15** and **18**, being D-glucopyranosyl analogues, competitively inhibit β-D-glucosidase action, but only noncompetitively the action of β-D-galactosidase. The relative low stereospecificity of almond β-D-glucosidase may be an explanation for the fact that **13** is a competitive inhibitor for β-D-glucosidase at all. It seems that the D-glucopyranosyl analogues **15** and **18**, in contrast to their epimer **13**, are thermodynamically more stable in their alternate conformation **15a** and **18a** (Fig. 4), perhaps due to stabilising noncovalent interaction of an axial hydroxyl group with the amido group. The conformations of compound **15a** and **18a** shown in Fig. 4, are indicated by the coupling constants $^3J_{4,5}$ (3.5 Hz) and the long-range coupling constants $^4J_{4,6}$



12 $R^1 = R^2 = OBzl$

13 $R^1 = R^2 = OH$

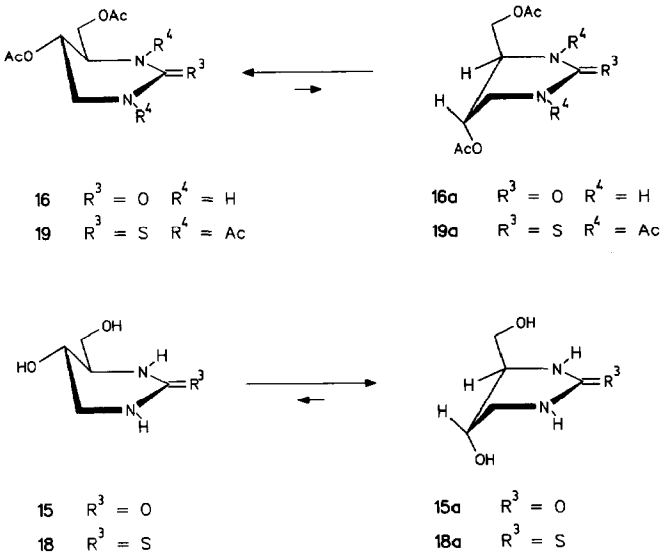
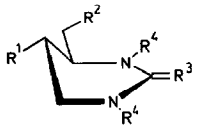


Fig. 4. Acetylated cyclic urea (**16**) and thiourea (**19**) derivatives configurationally resembling a D-glucosyl cation prefer, as expected, equatorial substituents in solution. On deacetylation compounds **15** and **18** adopt the unexpected conformations **15a** and **18a** in aqueous solution.

(2.0 Hz) (see Table III B). On per-*O*-acetylation the expected conformation with equatorial substituents in compounds **16** and **19** are preferred. The wrong position of the hydroxyl substituents in the alternative conformation of **15a** and **18a** would cause steric hindrance on binding. The inhibition constants for the acyclic diamines **1** and **2** correspond with those found for aminoglycerols[8], but are much higher than those of cyclic analogues. Clearly free rotation in compounds **1** and **2** is adverse to binding[10]. The small but reproducible and therefore significant differences in inhibition constants of the diastereomers **1** and **2** for the two enzymes suggests that binding occurs not in the depicted conformation **1a** and **2a**, but rather in conformations **1b** and **2b** (Fig. 2).



14	$R^1 = R^2 =$ 	$R^3 = O$	$R^4 = H$
15	$R^1 = R^2 = OH$	$R^3 = O$	$R^4 = H$
16	$R^1 = R^2 = OAc$	$R^3 = O$	$R^4 = H$
17	$R^1 = R^2 =$ 	$R^3 = S$	$R^4 = H$
18	$R^1 = R^2 = OH$	$R^3 = S$	$R^4 = H$
19	$R^1 = R^2 = OAc$	$R^3 = S$	$R^4 = Ac$

Since 1,3-diaminopropane (**20**) has no inhibitory effect at all on neither enzyme, this is an indication that some minimal structural relation has to exist between an artificial, synthetic ligand and a natural one of any given enzyme.

EXPERIMENTAL

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck) and column chromatography was performed on Silica Gel 32–63, 60 A (ICN). Melting points were measured with a Büchi apparatus and are uncorrected. Optical rotations were obtained with a Schmidt & Haensch Polartronic I polarimeter and IR spectra with a Perkin–Elmer 1320 spectrophotometer. ^{1}H NMR spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz and a Bruker AM 400 spectrometer at 400 MHz for solutions in $CDCl_3$ (internal Me_4Si), CD_3OD (internal Me_4Si), pyridine-d_5 (internal Me_4Si) or D_2O (internal DSS). Kinetic data were obtained with an Eppendorf photometer at 405 nm connected with a transformation unit and an SE 120 recorder (BBC).

Enzymes.—β-D-Galactosidase [EC 3.2.1.23, 600 U/mg protein] from *E. coli* and β-D-glucosidase [EC 3.3.1.21, 20 U/mg protein] from sweet almonds were purchased from Boehringer Mannheim.

Determination of the inhibition constants (K_i).—*o*-Nitrophenyl-β-D-galacto- and -gluco-pyranoside were used as substrate (0.1–10 mM, K_M 116–137 μM and 0.7–25 mM, K_M 7.2–8.1 mM) in 200 mM sodium-potassium-phosphate buffer (pH 6.8, 1 mM $MgCl_2$) at 30°C. Inhibitors were used in the following concentrations (mM): **1** 1.8, 2.5, 4.0, and 1.5, 3.0, 4.0; **2** 1.0, 3.0, 5.0, 7.0, 10.0 and 3.0, 5.0; **13** 19.0, 10.0, 6.0 and 10.0, 20.0, 28.0; **15** 5.0, 10.0, 50.0 and 10.0, 30.0, 50.0, 80.0; **17** 5.0, 10.0 and 2.5, 5.0, 7.5, 10.0; **18** 20.0, 50.0, 100.0 and 10.0, 20.0, 40.0; **19** 5.0 for both enzymes. The amount of enzyme added in each assay was adjusted so that at the highest substrate concentration $\Delta\epsilon$/min was ~ 0.8. K_i-Values were determined by a Dixon plot[9].

1,3-Di-O-benzyl-2,4-O-benzylidene-D-erythritol (**3**).—To a solution of 1,3-*O*-benzylidene-L-erythritol[7] (11.5 g, 54.7 mmol) in dry DMF (280 mL), NaH (6.0 g, 150 mmol) was added and the mixture was stirred for 2 h at room temperature. A solution of benzylchloride (18.8 mL) in dry DMF (200 mL) was added dropwise. Stirring was continued for 3 h; the excess hydride was destroyed by adding MeOH (30 mL), and the mixture was diluted with water (500 mL) and extracted with ether (4 × 200 mL). The combined extracts were washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1 : 10 EtOAc–cyclohexane) of the residue gave **3** as a syrup (**14.7 g, 69%**); $[\alpha]_D^{23}$ −23°(*c* 0.43, $CHCl_3$); R_f 0.27 (1 : 10 EtOAc–cyclohexane). For ^{1}H NMR data, see Tables IA and IB. Anal. Calcd for $C_{25}H_{26}O_4$: C, 76.9; H, 6.71. Found: C, 76.76; H, 6.69.

1,3-Di-O-benzyl-D-erythritol (**4**).—A solution of **3** (14 g, 35.86 mmol) in 60% AcOH (300 mL) was kept at 50°C for 4 h. The solution was concentrated in vacuo and coevaporated with water (3 × 100 mL). Column chromatography (1 : 1

TABLE IA

^{1}H NMR data for compounds **3–7**

Proton	Compound				
	3	**4**	**5**	**6**	**7**
H-1	3.62 dd	3.57 dd	3.63 dd	3.48 dd	3.50 dd
H-1′	3.72 dd	3.64 dd	3.67 dd	3.63 dd	3.58 dd
H-2	4.32 dd	3.92 ddd	4.64 ddd	3.77 ddd	3.86 ddd
H-3	3.87 m	3.51 ddd	4.18 m	4.43 ddd	3.55 ddd
H-4	3.70–3.85 m	3.80 dd	3.97 dd	3.70 d	3.90 dd
H-4′	3.70–3.85 m	3.75 dd	3.95 m	3.70 d	3.95 dd
CH_2-Ar	4.50–4.66 m	4.46–4.66 m	4.32–4.51 m		
CH-Ar	5.48 s			5.51 s	
H-Ar	7.24–7.40 m	7.26–7.36 m	7.12–7.33 m	7.33–7.52 m	
	7.44–7.52 m		7.68–7.74 m		
O-H		2.86 s			2.50–2.80 s
		2.54 s			
Me-Ar			2.38 s		
			2.40 s		

TABLE IB

^{1}H NMR data, coupling constants for compounds **3–7**

Proton	Compound				
$J_{H,H}$	**3**	**4**	**5**	**6**	**7**
1,2	9.75	6	4.5	5	6
1′,2	9.75	3.75	4.5	2.4	3.5
2,3	4.2	7.2	4.5	10.5	7.5
3,4		4.2	6	8	4.5
3,4′		4.2		8	5
1,1′	9.75	9.75	11.25	13.5	12.5
4,4′		11.25	13.5		12

EtOAc–cyclohexane) of the residue gave **4** as a colourless oil (6.5 g, 67%); R_f 0.14 (1 : 1 EtOAc–cyclohexane); $[\alpha]_D^{23}$ −25° (*c* 1.17, $CHCl_3$). For ^{1}H NMR data see Tables IA and IB. Anal. Calcd for $C_{18}H_{22}O_4$: C, 71.51; H, 7.34. Found: C, 71.63; H, 7.28.

*1,3-Di-*O*-benzyl-2,4-di-*O*-*p*-tolylsulfonyl-*D*-erythritol* (**5**).—To a solution of **4** (6.0 g, 19.85 mmol) in dry pyridine (130 mL) was added *p*-toluenesulfonyl chloride (9.8 g, 51.6 mmol), and the mixture was kept for 20 h at room temperature. The solution was poured into ice–water (200 mL) and after 1 h extracted with CH_2Cl_2 (4 × 100 mL). The combined extracts were neutralised with satd aq $NaHCO_3$, (150 mL), washed with water (100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1 : 3 EtOAc–cyclohexane) of the residue gave **5** (11.0 g, 90%) as a light yellow oil; $[\alpha]_D^{23}$ +33° (*c* 1.8, $CHCl_3$); R_f 0.4 (1 : 2 EtOAc–cyclohexane). For ^{1}H NMR data see Tables IA and IB. Anal. Calcd for $C_{32}H_{34}O_{10}S_2$: C, 59.80; H, 5.33; S, 24.88. Found: C, 59.91; H, 5.30; S, 24.84.

TABLE IIA

^{1}H NMR data for compounds **8–11**

Proton	Compound			
	8	**9**	**10**	**11**
H-1	2.87 ddd	3.33 dd	3.44 dd	2.96 ddd
H-1′	4.05 ddd	3.45 dd	3.49 dd	3.79 ddd
H-2	4.77 ddd	3.71 ddd	3.56 ddd	5.08 ddd
H-3	4.39 dddd	3.66 m	3.86–4.00 m	4.47 dddd
H-4	4.08 dd	3.62 dd	3.86–4.00 m	4.06 dd
H-4′	4.36 dd	3.65 dd	3.86–4.00 m	4.17 dd
CH_2-Ar		4.50–4.63 m		
H-Ar		7.28–7.35 m		
O-H			2.15 s	
			2.59 s	
N-H	6.42 d			5.90 dd
	6.69 dd			6.38 d
OAc	2.00 s			2.07 s
	2.07 s			2.08 s
NAc	2.08 s			2.00 s
	2.10 s			2.05 s

TABLE IIB

^{1}H NMR data, coupling constants for compounds **8–11**

Proton $J_{H,H}$	Compound			
	8	**9**	**10**	**11**
1,2	4	6	5.7	7.5
1′,2	3.5	4.5	6.3	6
2,3	9	5.25	6	3
3,4	3	4.5		6
3,4′	4.5	1.5		7.5
1,1′	15	12.75	12.75	14.25
4,4′	11	10.5		11.25
3,NH	8			9
1,NH	4			4.5
1′,NH	8			7.5

*1,3-Diazido-2,4-di-O-benzyl-1,3-dideoxy-*D*-threitol* (**9**).—To a solution of **5** (11.0 g, 18 mmol) in dry DMF (200 mL) NaN_3 was added (1.3 g, 20.0 mmol) and the mixture was stirred for 14 h at 50°C. The solvent was evaporated and the residue filtered through a column of silica gel with EtOAc and evaporated in vacuo. Column chromatography (1:5 EtOAc–cyclohexane) of the residue gave syrupy **9** (5.7 g, 80.8% mmol); $[\alpha]_D^{23}$ −29° (*c* 1.48, $CHCl_3$); R_f 0.61 (1:1 EtOAc–cyclohexane); ν_{max}^{film} 2110 cm^{-1} (N_3). For ^{1}H NMR data see Tables IIA and IIB. Anal. Calcd for $C_{18}H_{20}O_2N_6$: C, 61.35; H, 5.72; N, 23.85. Found: C, 61.42; H, 5.71; N, 23.74.

1,3-Diazido-1,3-dideoxy-D-threitol (**10**).—To a solution of **9** (270 mg, 0.766 mmol) in 18:1 CH_2Cl_2–H_2O (19 mL) was added 2,3-dichloro-4,5-dicyano-*p*-benzochinone (1.04 g, 4.58 mmol) and the mixture was stirred for 5 days at room temperature. Saturated aq $Na_2S_2O_3$ (5 mL) was added, and the solvents were evaporated in vacuo. The residue was filtered through a column of silica gel with MeOH and evaporated. Column chromatography (1:1 EtOAc–cyclohexane) afforded **10** as a colourless oil (42 mg, 33%); R_f 0.23 (1:1 EtOAc–cyclohexane); $[\alpha]_D^{23}$ −26° (*c* 1.0, $CHCl_3$). For ^{1}H NMR data see Tables IIA and IIB. Anal. Calcd for $C_4H_8O_2N_6$: C, 27.91; H, 4.88; N, 48.83. Found: C, 27.73; H, 4.51; N, 48.24.

1,3-Diamino-1,3-dideoxy-D-threitol (**1**).—A solution of **10** (33 mg, 0.197 mmol) in MeOH (4 mL) was hydrogenated (1.013×10^{-5} Pa) over Pt (4 mg PtO_2) with stirring, then filtered, and concentrated in vacuo. The product **1** (23 mg, 97%) was obtained as a colourless oil; R_f 0.23 [20:25:12 $CHCl_3$–MeOH–NH_3 (23% in H_2O)]. For an analytical sample compound **1** (10 mg, 0.103 mmol) was acetylated in the usual way with Ac_2O (1 mL) in dry pyridine (2 mL) for 3 h and then concentrated. Column chromatography (20:2:1 EtOAc–MeOH–H_2O) gave amorphous 1,3-diacetamido-2,4-di-*O*-acetyl-1,3-dideoxy-D-*threitol* (**11**) (21 mg, 70%); R_f 0.47 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ −2.5° (*c* 0.18, $CHCl_3$). For ^{1}H NMR data see Tables IIA and IIB. Anal. Calcd for $C_{12}H_{20}O_6N_2$: C, 50.00; H, 6.99; N, 9.72. Found: C, 49.86; H, 7.04; N, 9.65.

5(R)-Benzyloxy-4(R)-benzyloxymethyl-tetrahydropyrimidin-2-one (**12**).—Compound **9** (3.172 g, 9.0 mmol) was dissolved in EtOH (40 mL) and hydrogenated (1.013×10^{-5} Pa) over 10% Pd–C (80 mg) with stirring for 24 h, then filtered, and concentrated in vacuo. To the residue, 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-*threitol*, dissolved in dry MeCN (85 mL), was added dropwise for 9 h a solution of di-(*N*-succinimidyl)-carbonate (DSC) (2.31 g, 9 mmol) in dry MeCN (85 mL). The solution was stirred for 15 h at room temperature, the solvent evaporated in vacuo. Column chromatography (20:1 EtOAc–MeOH) of the residue gave **12** as a syrup (1.5 g, 51%); R_f 0.21 (10:1 EtOAc–MeOH); $[\alpha]_D^{23}$ + 2° (*c* 1.03, $CHCl_3$); ν_{max}^{film} 3250 (NH), 3100 (NH), and 1670 cm^{-1} (C=O). For ^{1}H NMR data see Tables IIIA and IIIB. Anal. Calcd for $C_{19}H_{22}O_3N_2$: C, 69.92; H, 6.79; N, 8.58. Found: C, 69.53; H, 6.98; N, 8.69.

5(R)-Hydroxy-4(R)-hydroxymethyl-tetrahydropyrimidin-2-one (**13**).—Compound **12** (270 mg, 0.827 mmol) in AcOH (8 mL) was hydrogenated (1.013×10^{-5} Pa) over 10% Pd–C (50 mg) with stirring for 2 days, then filtered and concentrated in vacuo. Column chromatography (10:2:1 EtOAc–MeOH-H_2O) gave **13** (103 mg, 85%); mp 155–157°C (from MeOH); R_f 0.19 (7:2:1 EtOAc–MeOH-H_2O); $[\alpha]_D^{23}$ + 43° (*c* 0.93, H_2O); ν_{max}^{KBr} 3350 cm^{-1} (OH), 3250 cm^{-1} (NH), 1685 cm^{-1} (C=O). For ^{1}H NMR data, see Tables IIIA and IIIB. Anal. Calcd for $C_5H_{10}O_3N_2$: C, 41.09; H, 6.90; N, 19.17. Found: C, 40.60; H, 6.71; N, 18.82.

1,3-Diazido-2,4-O-benzylidene-1,3-dideoxy-D-erythritol (**6**).—1,3-*O*-Benzylidene-2,4-di-*O*-*p*-tolylsulfonyl-L-threitol[7] (6.40 g, 12.3 mmol) was treated with NaN_3 (3.2 g, 49 mmol) in dry DMF (100 mL), as described for **9**, for 3 days at 80°C. Column

TABLE IIIA

^{1}H NMR data for compounds **12–16**

Proton	Compound				
	12	**13**	**14**	**15**	**16**
α	3.61 dd	3.77 dd	3.89 dd	3.59 dd	4.04 dd
α′	3.64 dd	3.58–3.68 m	4.53 dd	3.59 dd	4.15 dd
4	3.73 ddd	3.58–3.68 m	3.70 ddd	3.44 dddd	3.75 dddd
5	3.78 dd	4.18 ddd	4.08 ddd	4.08 ddd	5.00 ddd
6	3.27 dd	3.29 dd	3.50 dd	3.25 ddd	3.38 ddd
6′	3.44 dd	3.46 ddd	3.68 dd	3.44 dd	3.58 dd
CH_2-Ar	4.43–4.65 m				
CH-Ar			5.86 s		
H-Ar	7.25–7.36 m		7.36–7.50 m		
			7.70–7.80 m		
N-H	5.28 s				4.94 s
	5.53 s				5.06 s
OAc					2.11 s
					2.13 s

TABLE IIIB

^{1}H NMR data, coupling constants for compounds **12–16**

Proton	Compound				
$J_{H,H}$	**12**	**13**	**14**	**15**	**16**
α,α′	9	9.75	10.5	11	11
α,4	9	9	10.5	5.7	6
α′,4	4.5		4.5	6.5	6
4,5	3.75	3	9	3.5	8
5,6	2.7	3	10.5	4.0	4
5,6′	3.7	3	5.7	3.4	2
6,6′	12.5	13	10.5	13	13
4,6				1.5	1.5
4,6′		0.8			

chromatography (1 : 10 EtOAc–cyclohexane) and recrystallisation from light petroleum gave **6** as colourless needles (1.40 g, 43%); mp 34–35°C (from light petroleum); R_f 0.61 (1 : 2 EtOAc–cyclohexane); $[\alpha]_D^{23}$ −108° (*c* 2.35, $CHCl_3$); ν_{max}^{KBr} 2120 cm^{-1} (N_3). For ^{1}H NMR data see Tables IA and IB. Anal. Calcd for $C_{11}H_{12}O_2N_6$: C, 50.76; H, 4.65; N, 32.29. Found: C, 50.49; H, 4.54; N, 32.09.

*1,3-Diazido-1,3-dideoxy-*D*-erythritol* (**7**).—To a solution of **6** (1.0 g, 4.1 mmol) in MeOH (40 mL) was added 37% HCl (0.5 mL) and the mixture was kept for 1 h at room temperature. Sodium hydrogencarbonate (0.5 g) was added and the mixture stirred for 1 h, then filtered and concentrated to dryness. Column chromatography (1 : 1 EtOAc–cyclohexane) of the residue gave **7** as a syrup (0.52 g, 71%); R_f 0.24 (1 : 1 EtOAc–cyclohexane); $[\alpha]_D^{23}$ −41° (*c* 9.16, acetone); ν_{max}^{film} 2120 cm^{-1} (N_3). For ^{1}H NMR data see Tables IA and IB. Anal. Calcd for $C_4H_8O_2N_6$: C, 27.91; H, 4.68; N, 48.82. Found: C, 27.91; H, 4.69; N, 48.42.

1,3-Diamino-1,3-dideoxy-D-erythritol (**2**).—A solution of **7** (154 mg, 0.895 mmol) in MeOH (5 mL) was treated as described for **1**. The product **2** (107 mg, 100%) was obtained as a colorless amorphous solid; R_f 0.27 [20:25:12 $CHCl_3$–MeOH–NH_3 (23% in H_2O)]. For an analytical sample, compound **2** (42 mg, 0.35 mmol) was acetylated in the usual way with Ac_2O (2 mL) in dry pyridine (4 mL) for 3 h and then concentrated. Column chromatography (20:2:1 EtOAc–MeOH–H_2O) and recrystallisation from $CHCl_3$–ether gave colorless crystals of 1,3-diacetamido-1,4-di-*O*-acetyl-1,3-dideoxy-D-erythritol (**8**); R_f 0.49 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ −32° (*c* 1.6, acetone). For ^1H NMR data see Tables IIA and IIB. Anal. Calcd for $C_{12}H_{20}O_6N_2$: C, 50.00; H, 6.99; N, 9.72. Found: C, 49.91; H, 7.02; N, 9.49.

Benzylidene-5(S)-oxy-4(R)-oxymethyl-tetrahydropyrimidin-2-one (**14**).—Compound **6** (142 mg, 0.547 mmol) was dissolved in EtOAc (10 mL) and hydrogenated (1.013×10^{-5} Pa) over PtO_2 (10 mg) with stirring for 3 h, then filtered and concentrated in vacuo. To the resulting, 1,3-diamino-2,4-*O*-benzylidene-1,3-dideoxy-D-*erythritol*, dissolved in dry MeCN (25 mL), was added dropwise for 3 h a solution of di-(*N*-succinimidyl)-carbonate (DSC) (141 mg, 0.551 mmol) in dry MeCN (17 mL). The solution was stirred for 15 h at room temperature. Filtration of the product and washing with MeCN (5 mL) gave **13** as a colorless solid (85 mg, 65%); mp >240°C, R_f 0.55 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +110° (*c* 0.37, Me_2SO); ν_{max}^{KBr} 3240 (NH), 3090 (NH), 1690 (C=O), and 1640 cm^{-1} (C=O). For ^1H NMR data see Tables IIIA and IIIB. Anal. Calcd for $C_{12}H_{14}O_3N_2$: C, 61.52; H, 6.02; N, 11.96. Found: C, 60.82; H, 5.81; N, 11.59.

5(S)-Hydroxy-4(R)-hydroxymethyl-tetrahydropyrimidin-2-one (**15**).—Compound **14** (750 mg, 3.20 mmol) was dissolved in 20% AcOH (50 mL) at 70°C. The solution was concentrated in vacuo and coevaporated with water (4 × 50 mL). Recrystallisation from MeOH gave **15** as colorless crystals (356 mg, 76%); mp 138–141°C (from MeOH); R_f 0.25 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +103° (*c* 0.96, H_2O); ν_{max}^{KBr} 3300 (OH), 3250 (NH), 3140 (NH), and 1655 cm^{-1} (C=O). For ^1H NMR data see Tables IIIA and IIIB. Anal. Calcd for $C_5H_{10}O_3N_2$: C, 41.09; H, 6.90; N, 19.17. Found: C, 40.88; H, 6.79; N, 18.78.

5(S)-Acetoxy-4(R)-acetoxymethyl-tetrahydropyrimidin-2-one (**16**).—Compound **15** (57 mg, 0.39 mmol) was acetylated in the usual way with Ac_2O (2 mL) in dry pyridine (4 mL) for 5 h and then concentrated. Recrystallisation from EtOH gave **16** as colorless crystals (56 mg, 62%); mp 177–180°C (from EtOH); R_f 0.55 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +72° (*c* 1.18, MeOH). For ^1H NMR data see Tables IIIA and IIIB. Anal. Calcd for $C_9H_{14}O_5N_2$: C, 46.95; H, 6.13; N, 12.17. Found: C, 47.04; H, 5.90; N, 11.98.

Benzylidene-5(S)-oxy-4(R)-oxymethyl-tetrahydropyrimidin-2-thione (**17**).—1,3-Diamino-2,4-*O*-benzylidene-1,3-dideoxy-D-erythritol (743 mg, 3.57 mmol), prepared as described for **14**, was dissolved in dry MeCN (150 mL), and a solution of 1,1'-thiocarbonyldiimidazole (664 mg, 3.73 mmol) in dry MeCN (50 mL) was added dropwise for 3 h. The solution was stirred for 15 h at room temperature,

TABLE IVA

^{1}H NMR data for compounds **17–19**

Proton	Compound		
	17	**18**	**19**
α	3.29 dd	3.61 dd	4.23 dd
α′	4.64 dd	3.62 dd	4.31 dd
4	3.68 ddd	3.45 dddd	4.87 ddd
5	4.04 ddd	4.16 ddd	5.08 ddd
6	3.48 dd	3.28 ddd	3.01 dd
6′	3.75 dd	3.45 dd	4.97 dd
CH-Ar	5.86 s		
H-Ar	7.36–7.78 m		
N-H	5.1 s		
	5.1 s		
OAc			2.04 s
			2.10 s
NAc			2.63 s
			2.72 s

TABLE IVB

^{1}H NMR data, coupling constants for compounds **17–19**

Proton $J_{H,H}$	Compound		
	17	**18**	**19**
α,α′	10.5	11	11.7
α,4	10.5	5.5	6
α′,4	4.5	6	5
4,5	9	3.5	2.5
5,6	10.5	3.5	9.3
5,6′	5	3.5	7
6,6′	10.5	14	12
4,6		2	

concentrated in vacuo to a volume of 50 mL, and water (50 mL) was added. Filtration of the product and recrystallisation from EtOH gave **17** as colourless needles (429 mg, 48%); mp 242–244°C (dec) (from EtOH); R_f 0.57 (17:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +62°(*c* 0.97, pyridine); ν_{max}^{KBr} 3210 (NH), 1570 (C=S/C–N), and 1530 cm^{-1} (C=S/C–N). For ^{1}H NMR data see Tables IVA and IVB. Anal. Calcd for $C_{12}H_{14}O_2N_2S$: C, 57.58; H, 5.64; N, 11.19; S, 12.81. Found: C, 57.35; H, 5.58; N, 11.06; S, 12.55.

5(S)-Hydroxy-4(R)-hydroxymethyl-tetrahydropyrimidin-2-thione (**18**).—Compound **17** (880 mg, 3.52 mmol) was dissolved in 20% AcOH (100 mL) at 70°C. The solution was concentrated in vacuo and coevaporated with water (4 × 100 mL). Recrystallisation from water gave **18** as colourless crystals (463 mg, 81%); mp 215–217°C (dec) (from H_2O); R_f 0.29 (17:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +79° (*c* 1.58, Me_2SO); ν_{max}^{KBr} 3320 (OH), 3260 (NH), 3210 (NH), and 1550 cm^{-1}

TABLE V

Inhibition constants K_i (mM) for compounds **1**, **2**, **13**, **15**, **18** and **20**

Enzyme	Compound					
	1	**2**	**13**	**15**	**18**	**20**
β-D-Galactosidase	10	3.2	15	non competitive	non competitive	
β-D-Glucosidase	5.0	4.0	90	130	90	

(C=S/C–N). For ^{1}H NMR data see Tables IVA and IVB. Anal. Calcd for $C_5H_{10}O_2N_2S$: C, 37.02; H, 6.21; N, 17.27; S, 19.77. Found: C, 37.06; H, 6.09; N, 17.17; S, 19.95.

5(S)-Acetoxy-4(R)-acetoxymethyl-1,3-diacetyl-tetrahydropyrimidin-2-thione (**19**). —Compound **19** (49 mg, 0.30 mmol) was acetylated in the usual way with Ac_2O (2 mL) in dry pyridine (4 mL) for 5 h at 70°C and then concentrated. Column chromatography (1:1 EtOAc–cyclohexane) gave a yellow syrup **19** (29 mg, 30%); R_f 0.51 (1:1 EtOAc–cyclohexane). For ^{1}H NMR data see Tables IVA and IVB.

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