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Carbohydrate Research 278 (1995) 167–180

CARBOHYDRATE RESEARCH

Analogues of disaccharides and glycosides containing a cyclic guanidinium structure show varying inhibitory effects on glycoside hydrolases

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Received 20 February 1995; accepted 18 April 1995

Abstract

By condensation of 1,3-diamino-2,4-(*R*)-*O*-benzylidene-1,3-dideoxy-D-erythritol (**3**) and 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol (**4**) with methyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-isothiocyanato-β-D-glucopyranoside (**9**) the (1 → 4)-linked disaccharide analogues 4-deoxy-4-[(4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**15**) and 4-deoxy-4-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**18**) were synthesized. By the same reaction sequence, using **3** and methyl isothiocyanate, the glycoside analogue (4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-2-methylamino-1,4,5,6-tetrahydropyrimidine hydrochloride (**20**) was obtained. All compounds possess in their 'glyconic' moiety the flat guanidinium group, mimicking a glucopyranosyl cation. Together with the previously synthesized (1 → 6)-linked disaccharide analogues 6-deoxy-6-[(4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**1**) and 6-deoxy-6-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**2**), a possible inhibitory effect on the action of α-D-glucosidase, β-D-glucosidase, α-D-galactosidase, and β-D-galactosidase was investigated. All compounds, except **20** with α-D-glucosidase where no inhibition could be detected, showed either competitive or mixed competitive inhibition with all enzymes. The effects of the disaccharide analogues were generally weaker as compared to the effect of the previously synthesized configurationally related nitrophenyl glycoside analogues (4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-2-(*p*-nitrophenyl)amino-1,4,5,6-tetrahydropyrimidine hydrochloride (**21**) and (4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-2-(*p*-nitrophenyl)amino-1,4,5,6-te-

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SSDI 0008-6215(95)00170-0

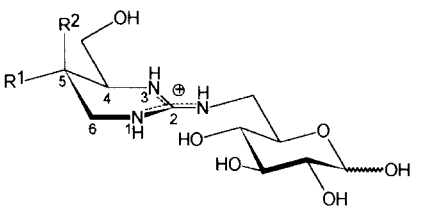
trahydropyrimidine hydrochloride (**22**). On the basis of experimental results, different binding modes of competitive inhibitors to the active site of corresponding enzymes are discussed.

Keywords: Transition-state analogues; Cyclic guanidines; Glycoside hydrolases; Competitive inhibition

1. Introduction

It is generally assumed that the transition state of the heterolysis of a glycoside, or an oligosaccharide, has a half-chair or envelope conformation regarding the glyconic unit, with a developing positive charge around its anomeric centre [1]. With a pair of partly functionalised cyclic phenyl guanidinium ions, varying inhibitory effects with a series of glycosidases could be measured [2]. Similar cyclic guanidinium ions were prepared as glycosidase inhibitors [3,4]. Expecting that changing structural elements not only in the glyconic, but also in the aglyconic unit of glycoside as well as disaccharide analogues, would give some insight into factors influencing affinity of so-called transition-state analogues, some more cyclic guanidinium derivatives resembling glycosides and also disaccharides were synthesized and tested for their inhibitory potential towards a number of glycoside hydrolases.

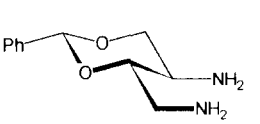
Syntheses of 6-deoxy-6-[(4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**1**) and 6-deoxy-6-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**2**) were previously described [5] and this approach was essentially adopted for the preparation of 4-deoxy-4-[(4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**15**) and 4-deoxy-4-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]-amino-α,β-D-glucopyranose hydrochloride (**18**) as cationic analogues of (1 → 4)-linked oligosaccharides and (4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-2-methylamino-1,4,5,6-tetrahydropyrimidine hydrochloride (**20**) as a cationic glycoside analogue.



	R^1	R^2
1	OH	H
2	H	OH

2. Results

All six-membered cyclic guanidines were prepared by reacting a 1,3-diamine with an isothiocyanate and subsequent ring closure using yellow lead oxide [6]. Syntheses of the diamines, 1,3-diamino-2,4-(*R*)-*O*-benzylidene-1,3-dideoxy-D-erythritol (**3**) and 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol (**4**), were previously described [7].



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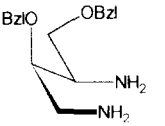

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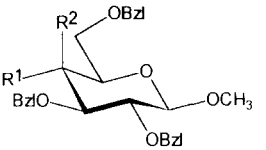
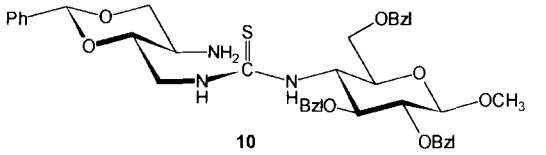
The preparation of the disaccharide analogues **15** and **18** was initiated by the functionalisation at C-4 of methyl glucoside with an isothiocyanate group. Methyl 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranoside (**7**) [8], prepared by an improved method from methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**5**) [9], gave on reduction with $NaBH_4$ in ethanol [10] methyl 4-amino-2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranoside hydrochloride (**8**), which was converted into methyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-isothiocyanato-β-D-glucopyranoside (**9**) by reacting with carbon disulfide in the presence of dicyclohexylcarbodiimide in pyridine [11].



	R^1	R^2
5	H	OH
6	H	OMs
7	N_3	H
8	NH_2	H
9	NCS	H

Compound **9** was coupled to diamine **3** and **4**, respectively, to give *N*-[3-amino-2,4-(*R*)-*O*-benzylidene-1,3-dideoxy-D-erythritol-1-yl]-*N'*-(methyl 2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranosid-4-yl)thiourea (**10**) and *N*-[3-amino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol-1-yl]-*N'*-(methyl 2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranosid-4-yl)-thiourea (**11**), which were cyclized with yellow lead oxide to the derivatised cyclic guanidines methyl 2,3,6-tri-*O*-benzyl-4-[(4*R*,5*S*)-benzylidene-5-oxy-4-oxymethyl-1,4,5,6-tetrahydropyrimidin-2-yl]amino-4-deoxy-β-D-glucopyranoside hydrochloride (**13**) and methyl 2,3,6-tri-*O*-benzyl-4-[(4*R*,5*R*)-5-benzyloxy-4-(benzyloxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-4-deoxy-β-D-glucopyranoside (**16**). The blocking groups in **13** and **16** were removed by hydrogenolysis using Pd/C to give methyl 4-deoxy-4-[(4*R*,5*S*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-β-D-glucopyranoside hydrochloride (**14**) and methyl 4-deoxy-4-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-β-D-glucopyranoside hydrochloride (**17**). Hydrolysis of the glucosides **14** and **17** with 2 M hydrochloric acid gave **15** and **18** as anomeric mixtures.



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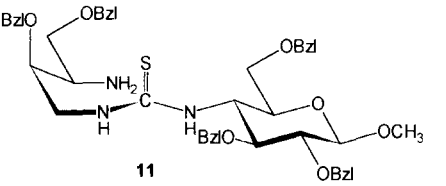

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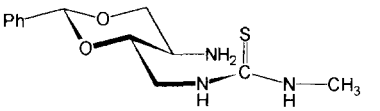

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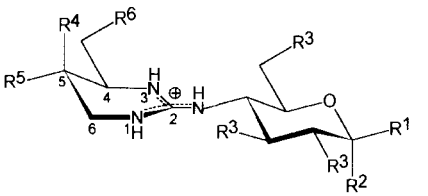
Reaction of diamine **3** with methyl isothiocyanate, following the procedure described for the preparation of **13** and **16** via *N*-(3-amino-2,4-(*R*)-*O*-benzylidene-1,3-dideoxy-D-erythritol-1-yl)-*N'*-methylthiourea (**12**), yielded (4*R*,5*S*)-benzylidene-5-oxy-4-oxymethyl-2-methylamino-1,4,5,6-tetrahydropyrimidine (**19**). Compound **19** was deprotected with 2 M HCl to give the glycoside analogue **20**.



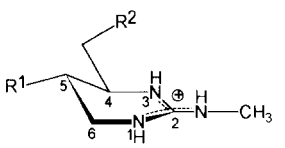
	R^1	R^2	R^3	R^4	R^5	R^6
13	OCH_3	H	OBzl	H		
14	OCH_3	H	OH	H	OH	OH
15 (α)	H	OH	OH	H	OH	OH
(β)	OH	H	OH	H	OH	OH
16	OCH_3	H	OBzl	OBzl	H	OBzl
17	OCH_3	H	OH	OH	H	OH
18 (α)	H	OH	OH	OH	H	OH
(β)	OH	H	OH	OH	H	OH

Table 1
Inhibition constants K_i (mM) for compounds **1, 2, 14, 15, 18**, and **20-24**

Compound	Enzyme			
	α-D-Glucosidase	β-D-Glucosidase	α-D-Galactosidase	β-D-Galactosidase
1	m.c.	0.45 ± 0.05	m.c.	m.c.
2	m.c.	m.c.	5 ± 0.5	4 ± 0.5
14	–	35 ± 5	–	–
15	m.c.	10 ± 2	m.c.	7.5 ± 1
18	m.c.	10 ± 2	m.c.	5.5 ± 1
20	n.i.	1.8 ± 0.2	m.c.	m.c.
21 [2]	2 ± 1	0.03 ± 0.01	–	n.i.
22 [2]	6 ± 1	m.c.	–	0.3 ± 0.1
23 [2]	m.c.	2 ± 1	–	m.c.
24 [2]	m.c.	m.c.	–	0.08 ± 0.01

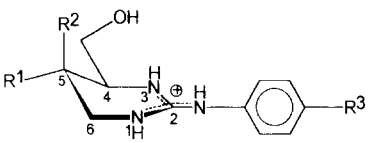
m.c., mixed competitive inhibition; n.i., no inhibition.
–, not determined.

Inhibition constants of **1**, **2**, **15**, **18** and **20** were determined for a number of glycosidase-catalysed reactions and compared with those of previously described cyclic guanidinium derivatives **21-24** [2] (Table 1).



	R^1	R^2
19	O	O, Ph
20	OH	OH

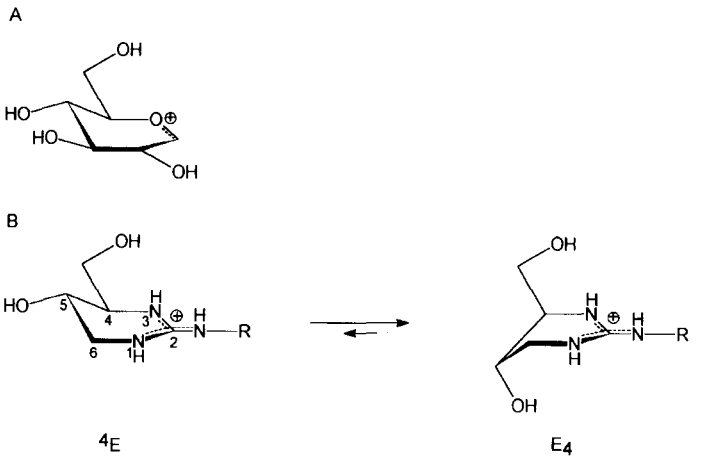
Judged by the effect of several standard cationic inhibitors like the substituted piperidines, including amidines and pyrrolidines [12–15], guanidinium derivatives proved to be rather ineffective, except for the aromatic derivatives **21** and **23**, which significantly inhibit emulsin β-D-glucosidase. This inhibitory effect is probably mainly due to a known interaction of the aglycon binding-site.



	R^1	R^2	R^3
21 [2]	OH	H	NO_2
22 [2]	H	OH	NO_2
23 [2]	OH	H	NH_3^+
24 [2]	H	OH	NH_3^+

3. Discussion

There are several reasons why such seemingly ideal transition-state analogues as **1** and **2**, **15** and **18**, and **20** may not be as effective as expected. Although the



	R
1	glucose (6'-linked)
15	glucose (4'-linked)
20	CH_3
21 [2]	*p*-nitrophenyl
23 [2]	*p*-aminophenyl

Fig. 1. ^4E Glucopyranosyl ion A and conformational equilibrium of '*gluco*' cyclic guanidinium systems B.

configuration of asymmetric carbons in the six-membered guanidinium ions are fixed, either '*gluco*' or '*galacto*', the former systems **1**, **15**, **20**, **21**, and **23** do not adopt the 'normal' conformation (Fig. 1) of a ^4E glucopyranosyl oxocarbenium ion. The low ^1H NMR coupling constants $J_{4,5}$ of 3.2 Hz in **15** and **20** and in the analogous compounds **1**, **21**, and **23** [2] contradict a diaxial conformation of the two hydrogen atoms at C-4 and C-5. Apparently the alternative conformation resembling the E_4 system is stabilised, even in aqueous medium, by polar attractive forces between the oxygen at C-5 and the positive charge, not counteracted by repulsive 1,3-diaxial interactions between hydrogens and functional groups normally governing the equilibrium between two conformers of a pyranose derivative. Since compounds where the C-5 hydroxyl group is alkylated do also show this strange conformational behaviour [16], the attraction must be between the oxygen lone-pair and the positive charge of the guanidinium ion. Assuming that the 'natural' transition-state is bound as ^4E conformer, binding a E_4 analogue has to be compensated by additional binding energy on complex formation. The '*galacto*' analogues **2, 18, 22**, and **24** have a 'normal' conformation because the polar interaction does not require conformational change (Fig. 1). A *gauche* arrangement of the hydrogen atoms at C-4 and C-5 with a coupling constant $J_{4,5}$ of 2.1 Hz proves the ^4E conformation in compound **18**. A major reason for the relatively low affinities of all cyclic guanidines is probably the missing hydroxyl groups in positions 1 and 6 corresponding to C-2 and C-3 in an ordinary glycoside, which seem to be very important

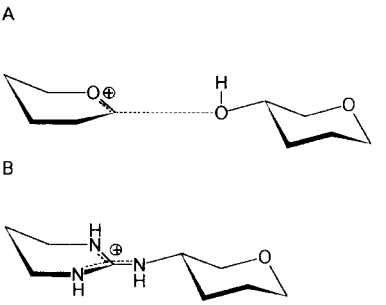


Fig. 2. Hypothetical transition-state of disaccharide heterolysis A in comparison with a disaccharide analogue as transition-state mimic B. In the latter, the cyclic units are pulled together by the guanidinium system, in the former they should drift apart.

for binding interaction in the transition-state. Very surprising is the fact that the disaccharide analogues **1, 2, 15**, and **18**, with an additional monosaccharide unit, bind less than their glycosidic counterparts **20–24**. The poor 'fitting' may be attributed to a significant change in the relative positions of the functional groups, normally responsible for binding in the transition-state. We can assume that the guanidinium system, with its shorter bond lengths [17] compared to the transition-state of oligosaccharide cleavage, 'shrinks' the molecule in the '> C-N-C-axis' (Fig. 2) pulling 'glyconic' and 'aglyconic' part together instead of separating them as it must be in the true transition-state '> C–O-C'.

A last great difference between the basic systems in the amidines, piperidines, and glycosylamines on one hand, and the guanidinium system on the other, is the fairly localised positive charge on the former, corresponding to the oxocarbenium ion, but the widely delocalised charge in the latter, which in comparison to a potentially hydrogen bond-accepting hydroxyl group at C-2 of a pyranoside [14] may be unfavourable for optimal binding interaction with a complementary group or groups in the active site.

One last set of results still remains to be explained: The low but almost equal inhibiting effect of the (1 → 4)-linked (and only those) analogues **15** and **18** on β-D-glucosidase (**15**, $K_i = 10 \pm 2$ mM; **18**, $K_i = 10 \pm 2$ mM) as well as β-D-galactosidase (**15**, $K_i = 7.5 \pm 1$ mM; **18**, $K_i = 5.5 \pm 1$ mM) (Table 1). It may be speculated that the diastereomers **15** and **18** are bound to the glycon binding sites of both enzymes through their reducing 'aglyconic' ends, which are unlike their 'glyconic' ends identical. The free lactol group could then mimic the Gal-configuration as α- and Glc-configuration as β-anomer.

4. Experimental

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) and column chromatography was performed on Silica Gel 32-63, 60 Å (ICN). Ion exchange resin (Dowex 50W-X8) was purchased from Fluka. Melting points were measured with a Büchi apparatus and are uncorrected. Optical rotations were obtained

with a Schmidt and Haensch Polartronic I polarimeter at 23°C 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). H-H COSY was used to characterize anomeric mixtures. ^{13}C NMR spectra (APT) were recorded with a Bruker AM 400 spectrometer at 100 MHz for solutions in $CDCl_3$ (internal Me_4Si). Elemental analyses were obtained with a Perkin–Elmer 240 analyzer. Mass spectra were recorded on Finnigan MAT 312. Kinetic data were obtained at 405 nm with an Eppendorf photometer connected with a transformation unit (Samson Arugolanu) and a SE 120 recorder (BBC).

Enzymes.—α-D-Galactosidase [EC 3.2.1.23, suspension of 5 mg/mL in 3.2 M NH_4OAc pH 6, 10 U/mg] from green coffee beans was purchased from Boehringer Mannheim. α-D-Glucosidase [EC 3.2.1.20, 3.9 U/mg] from yeast, β-D-glucosidase [EC 3.2.1.21, 6.9 U/mg] from sweet almonds, and β-D-galactosidase [EC 3.2.1.23, 640 U/mg] from *E. coli* were purchased from Sigma.

Determination of the inhibition constants (K_i).—*p*-Nitrophenyl α-D-gluco- (0.08–1.25 mM, K_M 0.19–0.25 mM), *p*-nitrophenyl β-D-gluco- (1.11–8.00 mM, K_M 3.4–4.0 mM), and *o*-nitrophenyl β-D-galactopyranoside (0.10–1.00 mM, K_M 0.10–0.14 mM) were used as substrate in 100 mM sodium potassium phosphate buffer (pH 6.8, 1 mM $MgCl_2$) at 30°C and *p*-nitrophenyl α-D-galactopyranoside (0.20–1.00 mM, K_M 0.24–0.47 mM) was used in 100 mM potassium phosphate buffer (pH 6.5) at 25°C. Inhibitors were used in the following concentrations (mM): α-D-glucosidase: **1** 3.0, 7.0, 11.0; **2** 9.0, 10.0, 15.0; **15** 3.0, 5.0, 8.0; **18** 2.5, 5.0, 7.5, 10.0; **20** 2.0, 5.0, 10.0; β-D-glucosidase: **1** 0.15, 0.24, 0.53, 1.9; **2** 0.67, 1.55, 3.00; **14** 0.05, 5.0, 10.0; **15** 3.0, 5.0, 10.0, 12.5; **18** 5.0, 7.5, 10.0, 12.5; **20** 1.0, 2.0, 3.0, 4.0; α-D-galactosidase: **1** 1.5, 3.0, 4.2; **2** 1.0, 3.0, 5.0, 6.5; **15** 1.5, 3.0, 5.0, 7.0; **18** 2.0, 5.0, 8.0, 11.0; **20** 5.0, 10.0, 15.0, 20.0; β-D-galactosidase: **1** 4.0, 7.5; **2** 2.0, 4.0, 6.0, 7.5; **15** 3.0, 5.0, 7.0, 10.0; **18** 2.5, 5.0, 7.5, 10.0; **20** 5.0, 10.0, 15.0, 20.0. Enzymes used had the following activities (U/mL): α-D-glucosidase 0.042; β-D-glucosidase 0.015; α-D-galactosidase 0.042; β-D-galactosidase 0.100. K_i-Values were determined by a Dixon plot [18].

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylsulfonyl-β-D-galactopyranoside* (**6**).—Mesyl chloride (12.5 mL, 18.6 g, 162 mmol) was added to a solution of methyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**5**) [8] (37.6 g, 81 mmol) in dry pyridine (200 mL) at 0°C. The mixture was stirred for 3 days at room temperature, hydrolysed with ice–water (200 mL) and extracted with EtOAc (3 × 200 mL). The combined extracts were washed with water (100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:3 EtOAc–cyclohexane) of the residue gave **6** as a light yellow solid (44.0 g, 100%); mp 63°C; R_f 0.45 (1:3 EtOAc–cyclohexane); $[\alpha]_D$ + 41° (*c* 1.3, $CHCl_3$). For ^{1}H NMR data (400 MHz, $CDCl_3$), see Table 2. ^{13}C NMR data (100 MHz, $CDCl_3$): δ 104.43 (C-1), 79.12 (C-2), 78.51 (C-3), 76.42 (C-4), 75.15 (CH_2-Ar), 73.98 (CH_2-Ar), 73.36 (CH_2-Ar), 71.88 (C-5), 68.11(C-6), 57.24 (CH_3-O), 39.15 (CH_3-S). Anal. Calcd for $C_{29}H_{34}O_8S$: C, 64.18; H, 6.33; S, 5.91. Found: C, 64.40; H, 6.65; S, 5.75.

*Methyl 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranoside* (**7**).—To a solution of **6** (44.0 g, 81 mmol) in dry DMF (300 mL) was added NaN_3 (10.5 g, 162 mmol). The mixture was heated for 1 day at 120°C, diluted with water (200 mL), and extracted with

Table 2
^{1}H NMR data for compounds **6–9**

Proton	Compound			
	6	**7**	**8**	**9**
H-1	4.30 d	4.21 d	4.32 d	4.30 d
H-2	3.73 dd	3.44 dd	3.44 dd	3.39 dd
H-3	3.69 dd	3.50 t	3.33 t	3.58 t
H-4	5.28 d	3.61 t	2.93 t	3.89 t
H-5	3.55 d	3.26 ddd	3.34 ddd	3.49 ddd
H-6a	3.56 dd	3.68 dd	3.68 dd	3.69 dd
H-6b	3.70 dd	3.76 dd	3.74 dd	3.78 dd
OCH_3	3.55 s	3.55 s	3.57 s	3.56 s
SCH_3	2.97 s			
N*H*			2.35 s	
CH_2-Ar	4.49–4.89 m	4.55–4.91 m	4.53–4.97 m	4.57–5.03 m
H-Ar	7.24–7.40 m	7.24–7.36 m	7.23–7.39 m	7.15–7.35 m
$J_{H,H}$	Compound			
	6	**7**	**8**	**9**
1,2	7.5	7.5	7.5	7.8
2,3	9.0	9.0	9.0	9.0
3,4	1.7	9.2	9.0	9.2
4,5	1.0	10.2	9.0	10.0
5,6a	1.0	4.5	4.5	4.5
5,6b	1.0	2.3	3.3	2.0
6a,6b	10.2	10.7	12.0	10.8

Et_2O (3 × 150 mL). The combined extracts were washed with water (150 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:8 EtOAc–cyclohexane) of the residue gave **7** as a white solid (36.3 g, 92%); mp 58°C; R_f 0.57 (1:3 EtOAc–cyclohexane); $[\alpha]_D$ +84° (*c* 0.8, $CHCl_3$); ν^{KBr} 2100 cm^{-1} (N = N). For ^{1}H NMR data (400 MHz, $CDCl_3$), see Table 2. Anal. Calcd for $C_{28}H_{31}N_3O_5$: C, 68.68; H, 6.39; N, 8.58. Found: C, 69.05; H, 6.67; N, 8.42.

Methyl 4-amino-2,3,6-tri-O-*benzyl-4-deoxy-β*-D-*glucopyranoside* (**8**).—To a solution of **7** (33.0 g, 67 mmol) in EtOH (250 mL) and water (25 mL) was added in small portions $NaBH_4$ (2.6 g, 69 mmol). The mixture was stirred for 3 days at 70°C, diluted with water (200 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were washed with water (100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (5:1 EtOAc–MeOH) of the residue gave **8** as a viscous oil (15.6 g, 50%); R_f 0.09 (1:3 EtOAc–cyclohexane); $[\alpha]_D$ −96° (*c* 1.0, $CHCl_3$). For ^{1}H NMR data (400 MHz, $CDCl_3$), see Table 2. Anal. Calcd for $C_{28}H_{34}ClNO_5$; C, 67.25; H, 6.87; N, 2.80. Found: C, 67.30; H, 6.77; N, 2.91.

Methyl 2,3,6-tri-O-*benzyl-4-deoxy-4-isothiocyanato-β*-D-*glucopyranoside* (**9**).—To a solution of DCC (8.2 g, 40 mmol) and CS_2 (14.0 mL, 232 mmol) in dry pyridine (200 mL) was added at −10°C a solution of **8** (11.0 g, 24 mmol) in dry pyridine (50 mL). The mixture was stirred for 30 min at 0°C, diluted with water (250 mL), stirred for 30

min at room temperature, and extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were washed with water (100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:10 EtOAc–cyclohexane) gave **9** as a white solid (12.1 g, 100%); mp 66°C; R_f 0.57 (1:3 EtOAC–cyclohexane); $[\alpha]_D$ −5° (*c* 1.1, $CHCl_3$); ν^{KBr} 2020 cm^{-1} (NCS). For ^1H NMR data (400 MHz, $CDCl_3$), see Table 2. Anal. Calcd for $C_{29}H_{31}NO_5S$: C, 68.88; H, 6.19; N, 2.77; S, 6.34. Found: C, 69.06; H, 6.23; N, 3.21; S, 6.12.

N-[3-Amino-2,4-(R)-O-benzylidene-1,3-dideoxy-D-erythritol-1-yl]-N′-(methyl 2,3,6-tri-O-benzyl-4-deoxy-β-D-glucopyranosid-4-yl)thiourea (**10**).—To a solution of 1,3-diamino-2,4-(*R*)-O-benzylidene-1,3-dideoxy-D-erythritol (**3**) [5] (1.7 g, 8.2 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise at 0°C a solution of **9** (4.2 g, 8.3 mmol) in dry CH_2Cl_2 (40 mL) within 45 min. The mixture was concentrated and purified by column chromatography (EtOAc) to afford **10** as a colourless foam (3.4 g, 59%), which was used without further characterisation to prepare **13**; R_f 0.68 (17:2:1 EtOAc–MeOH–H_2O).

Methyl 2,3,6-tri-O-benzyl-4-[(4R,5S)-benzylidene-5-oxy-4-oxymethyl-1,4,5,6-tetrahydropyrimidin-2-yl]amino-4-deoxy-β-D-glucopyranoside hydrochloride (**13**).—The thiourea **10** (3.0 g, 4.2 mmol) was dissolved in dry EtOH (150 mL) and yellow PbO (4.2 g, 17.6 mmol) was added. The mixture was heated under reflux for 2 days at 70°C, filtered and concentrated. Column chromatography [20:4:4:1 Et_2O–petroleum ether (bp 60–80°C)–MeOH–aq 25% NH_3] of the residue yielded the blocked guanidine **13** as a white solid (2.7 g, 95%); mp 58°C; R_f 0.70 (5:4:1 EtOAc–MeOH–aq 25% NH_3); $[\alpha]_D$ + 98° (*c* 1.0, pyridine); ν^{KBr} 3330 (NH) and 1640 cm^{-1} (CN). For ^1H NMR data (400 MHz, pyridine-d_5, 110°C), see Table 3. Anal. Calcd for $C_{40}H_{45}N_3O_7$: C, 70.66; H, 6.69; N, 6.18. Found: C, 70.47; H, 6.58; N, 6.12.

Methyl 4-deoxy-4-[(4R,5S)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-β-D-glucopyranoside hydrochloride (**14**).—The blocked guanidine **13** (2.5 g, 3.7 mmol) was dissolved in a mixture of MeOH (100 mL) and aq HCl (1 M, 20 mL) and hydrogenated over Pd/C for 1 day. The mixture was filtered, concentrated and purified by column chromatography (4:2:1 EtOAc–MeOH–AcOH). The product was dissolved in aq HCl (1 M, 25 mL), evaporated under reduced pressure, and coevaporated with water (3 × 20 mL) to afford **14** as a white solid (1.1 g, 83%); mp 53°C; R_f 0.40 (2:2:1 EtOAC–MeOH–aq 25% NH_3, saturated with NH_4OAc); $[\alpha]_D$ +18° (*c* 0.8, H_2O). For ^1H NMR data (400 MHz, D_2O), see Table 3.

4-Deoxy-[(4R,5S)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**15**).—The guanidine **14** (841 mg, 2.35 mmol) was dissolved in aq HCl (2 M, 5 mL) and heated for 4 days at 90°C. The product was purified by passing through ion exchange resin (H^+-form) and eluated with 2 M HCl. The solvent was evaporated under reduced pressure. Coevaporation with water (3 × 20 mL) gave **15** as a white solid (590 mg, 73%); mp 85°C; R_f 0.20 (2:2:1 EtOAc–MeOH–aq 25% NH_3, saturated with NH_4OAc); $[\alpha]_D$ +100° (*c* 0.3, H_2O). For ^1H NMR data (400 MHz, D_2O, H-H COSY), see Table 3. FABMS: *m/z* 308 $[M + H]^+$.

N-[3-Amino-2,4-di-O-benzyl-1,3-dideoxy-D-threitol-1-yl)-N′-(methyl 2,3,6-tri-O-benzyl-4-deoxy-β-D-glucopyranosid-4-yl)thiourea (**11**).—To a solution of 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol (**4**) [6] (1.8 g, 6.1 mmol) in dry CH_2Cl_2 (100

Table 3
^{1}H NMR data for compounds **13–16**

Proton	Compound				
	13	**14**	**15**(α)	(β)	**16**
H-4	4.01 m	3.62 m	3.57 m		3.74 m
H-5	3.75 ddd	4.21 dd	4.20 dd		3.87 ddd
H-6_a	3.24 t	3.36 ddd	3.36 ddd		3.10 dd
H-6_b	3.46 dd	3.52 dd	3.50 dd		3.43 dd
H-α_a	3.67 t	3.64 dd	3.62 dd		3.78 dd
H-α_b	4.42 m	3.71 dd	3.69 dd		3.95 dd
H-1′	4.49 d	4.41 d	5.27 d	4.66 d	4.41 d
H-2′	3.56 dd	3.34 dd	3.60 dd	3.30 dd	3.42 dd
H-3′	4.03 dd	3.63 t	3.80 t	3.61 t	4.02 t
H-4′	3.90 t	3.55 t	3.51 t	3.52 t	3.80 t
H-5′	3.43 ddd	3.57 m	3.95 ddd	3.87 m	3.70 ddd
H-$6'_a$	3.95 dd	3.72 dd	3.71 dd	3.70 dd	3.97 dd
H-$6'_b$	3.96 dd	3.88 dd	3.78 dd	3.83 dd	3.98 dd
OCH_3	3.51 s	3.59 s			3.46 s
CH_2-Ar	4.67–5.03 m				4.42–5.02 m
C*H*-Ar	5.68 s				
H-Ar	7.15–7.35 m				7.08–7.41 m
$J_{H,H}$	Compound				
	13	**14**	**15**(α)	(β)	**16**
4,5	6.0	3.4	3.2		11.3
4,α_a	10.8	5.8	9.0		2.4
4,α_b		4.0	5.7		4.2
4,6_a		1.5	1.5		
5,6_a	10.2	3.9	4.4		4.5
5,6_b	9.2	3.4	3.2		3.4
α_a,α_b	10.8	12.0	13.5		15.0
6_a,6_b	10.5	13.5	13.5		13.2
1′,2′	7.5	7.8	3.8	7.9	7.5
2′,3′	8.6	9.0	9.6	9.2	9.0
3′,4′	9.0	9.3	10.2	9.8	9.6
4′,5′	9.0	9.3	10.4	9.8	9.6
5′,$6'_a$	10.5	4.2	4.0	4.4	4.4
5′,$6'_b$	4.8	2.0	2.3	1.8	3.8
$6'_a$,$6'_b$	10.5	12.5	12.3	12.8	12.0

mL) was added dropwise at 0°C a solution of **9** (3.1 g, 6.1 mmol) in dry CH_2Cl_2 (50 mL) within 45 min. The mixture was concentrated and purified by column chromatography (EtOAc) to afford **11** as a colourless foam (3.5 g, 64%), which was used without further characterisation to prepare **16**; R_f 0.70 (5:4:1 EtOAc–MeOH–25% NH_3).

*Methyl 2,3,6-tri-*O*-benzyl-4-[(4*R*,5*R*)-5-benzyloxy-4-benzyloxymethyl-1,4,5,6-tetrahydropyrimidin-2-yl]amino-4-deoxy-β-*D*-glucopyranoside* (**16**).—The thiourea **11** (3.5 g, 4.3 mmol) was dissolved in dry EtOH (120 mL) and yellow PbO (3.1 g, 13.0 mmol) was added. The mixture was heated under reflux for 3 days at 70°C, filtered and concen-

trated. Column chromatography (20:4:4:1 Et_2O–petroleum ether (60–80°C)–MeOH–25% NH_3) of the residue yielded the blocked guanidine **16** as a yellow oil (1.4 g, 42%); R_f 0.54 (5:4:1 EtOAc–MeOH–25% NH_3); $[\alpha]_D$ +70° (*c* 0.9, pyridine); ν^{film} 3200 (NH) and 1600 cm^{-1} (CN). For ^1H NMR data (400 MHz, pyridine-d_5, 110°C), see Table 3. Anal. Calcd for $C_{47}H_{53}N_3O_7$: C, 73.12; H, 6.93; N, 5.44. Found: C, 72.86; H, 7.10; N, 5.19.

*Methyl 4-deoxy-4-[(4*R*,5*R*)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-β-*D*-glucopyranoside hydrochloride* (**17**).—The blocked guanidine **16**

Table 4
^1H NMR data for compounds **17–20**

Proton	Compound				
	17	**18**(α)	(β)	**19**	**20**
H-4	3.66 m	3.69 m		3.29 ddd	3.52 ddt
H-5	4.29 dd	4.31 dd		3.78 ddd	4.19 dd
H-6_a	3.41 dd	3.42 dd		3.20 t	3.34 ddd
H-6_b	3.49 dd	3.51 dd		3.49 dd	3.48 dd
H-α_a	3.67 t	3.70 dd		3.70 t	3.61 dd
H-α_b	3.85 dd	3.83 dd		4.38 dd	3.66 dd
NHCH_3				2.69 s	2.81 s
H-1′	4.38 d	5.28 d	4.67 d		
H-2′	3.32 t	3.60 dd	3.30 dd		
H-3′	3.60 t	3.81 t	3.62 t		
H-4′	3.51 t	3.52 t	3.54 t		
H-5′	3.57 m	3.95 ddd	3.88 m		
H-$6'_a$	3.72 dd	3.73 dd	3.72 dd		
H-$6'_b$	3.88 dd	3.78 dd	3.85 dd		
OCH_3	3.56 s				
CH_2-Ar				4.42–5.02 m	
C*H*-Ar				5.68 s	
H-Ar				7.31–7.51 m	
$J_{H,H}$	Compound				
	17	**18**(α)	(β)	**19**	**20**
4,5	2.3	2.1		9.0	3.2
4,α_a	8.3	8.3		10.5	6.2
4,α_b	3.5	2.0		4.8	5.7
4,6_a					1.8
5,6_a	3.0	3.0		10.2	4.1
5,6_b	2.6	2.1		5.9	3.2
α_a,α_b	9.6	13.4		10.5	12.1
6_a,6_b	13.3	13.5		10.5	13.5
1′,2′	7.8	3.8	7.8		
2′,3′	9.0	9.6	9.0		
3′,4′	9.3	9.6	9.5		
4′,5′	9.3	10.4	9.8		
5′,$6'_a$	3.8	3.5	4.4		
5′,$6'_b$	2.1	2.3	1.8		
$6'_a$,$6'_b$	12.6	12.3	12.8		

(1.4 g, 1.8 mmol) was dissolved in a mixture of MeOH (40 mL) and aq HCl (1 M, 5 mL) and hydrogenated over Pd/C for 6 days. The mixture was filtered and evaporated under reduced pressure. Coevaporation with water (3 × 20 mL) afforded **17** as a yellow solid (518 mg, 80%); mp 77°C; R_f 0.43 (2:2:1 EtOAc–MeOH–25% NH_3, saturated with NH_4OAc); $[\alpha]_D$ +46° (*c* 1.1, H_2O). For ^1H NMR data (400 MHz, D_2O), see Table 4.

4-Deoxy-4-[(4R,5R)-5-hydroxy-4-(hydroxymethyl)-1,4,5,6-tetrahydropyrimidin-2-yl]amino-α,β-D-glucopyranose hydrochloride (**18**).—The guanidine **17** (506 mg, 1.41 mmol) was dissolved in aq HCl (1 M, 5 mL) and heated for 4 day at 90°C. The product was purified by passing through ion exchange resin (H^+-form) and eluted with 2 M HCl. The solvent was evaporated under reduced pressure. Coevaporation with water (3 × 20 mL) gave **18** as a white solid (409 mg, 84%); mp 120°C; R_f 0.22 (2:2:1 EtOAc–MeOH–25% NH_3, saturated with NH_4OAc); $[\alpha]_D$ + 60° (*c* 0.5, H_2O). For ^1H NMR data (400 MHz, D_2O, H-H COSY), see Table 4. FABMS: *m/z* 308 $[M + H]^+$.

N-*(3-Amino-2,4-(R)-O-benzylidene-1,3-dideoxy-D-erythritol-1-yl)-N'-methylthiourea* (**12**).—To a solution of 1,3-diamino-2,4-(*R*)-*O*-benzylidene-1,3-dideoxy-D-erythritol (**3**) [6] (1.6 g, 7.7 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise at 0°C a solution of methyl isothiocyanate (0.6 g, 8.2 mmol) in dry CH_2Cl_2 (25 mL) within 2 h. The mixture was concentrated and purified by column chromatography (4:4:1 EtOAc–MeOH–cyclohexane) to afford **12** as a colourless foam (1.7 g, 79%), which was used without further characterisation to prepare **19**; R_f 0.38 (17:2:1 EtOAc–MeOH–H_2O).

(4R,5S)-Benzylidene-5-oxy-4-oxymethyl-2-methylamino-1,4,5,6-tetrahydropyrimidine (**19**).—The thiourea **12** (1.7 g, 6.0 mmol) was dissolved in dry EtOH (100 mL) and yellow PbO (5.1 g, 21.3 mmol) was added. The mixture was heated under reflux for 5 days at 70°C, filtered and concentrated. Column chromatography (3:3:1 CH_2Cl_2–MeOH–25% NH_3) of the residue yielded the blocked guanidine **19** as a white solid (1.3 g, 86%); mp 160°C; R_f 0.38 (5:4:1 EtOAc–MeOH–25% NH_3); $[\alpha]_D$ + 48° (*c* 0.9, MeOH). For ^1H NMR data (250 MHz, CD_3OD), see Table 4.

(4R,5S)-5-Hydroxy-4-(hydroxymethyl)-2-methylamino-1,4,5,6-tetrahydropyrimidine hydrochloride (**20**).—The blocked guanidine **19** (1.2 g, 4.9 mmol) was dissolved in a mixture of MeOH (25 mL) and aq HCl (1 M, 5 mL) and heated for 1 h at 50°C. The mixture was evaporated under reduced pressure. Coevaporation with water (5 × 5 mL) afforded **20** as a colourless oil (1.0 g, 100%); R_f 0.25 (5:4:1 EtOAc–MeOH–25% NH_3); $[\alpha]_D$ + 58° (*c* 0.8, H_2O). For ^1H NMR data (400 MHz, D_2O), see Table 4. FABMS: *m/z* 160 $[M + H]^+$.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie for financial support.

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