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ANALOGUES OF URIDINE 5'-DIPHOSPHATE GLUCOSE. INHIBITION OF GLYCOLIPID BIOSYNTHESIS.

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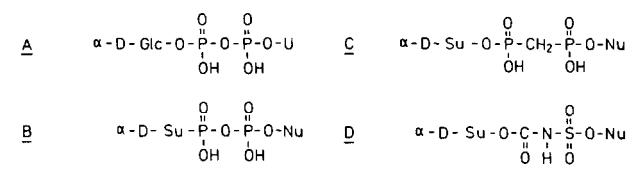
Summary: Phosphorylation of 2',3'-*O*-benzoyl-uridine with four different phosphorylating reagents using the hydroxybenzotriazole method gave the fully-protected intermediates which could be converted into the corresponding analogues of UDP-Glc, after addition of 3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-2-deoxy-D-erythro-L-gulo-octitol and removal of the protecting groups.

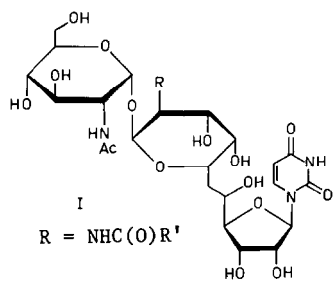
Glycosyltransferases are enzymes that catalyze the transfer of a glycosyl residue from a donor to an acceptor substrate. The donor molecules are nucleoside diphosphate sugars (e.g. compound **A**, Su = α-D-Glc, Nu = U) which donate, in a stereospecific manner, glycosyl residues in the biosynthesis of polysacharides, glycolipids, teichoic acids and glycoproteins[1]. The nucleoside antibiotic tunicamycin (i.e. **I**, R' = $CH{=}CH(CH_2)_nCH(CH_3)_2$) is known to inhibit[2a] UDP-*N*-acetylglucosamine:dolichyl-phosphate-*N*-acetylglucosamine-1-phosphate transferase and thus prevents the formation of dolichyl-pyrophosphoryl-*N*-acetylglucosamine[2b]. This lipid intermediate participates in the first step of the pathway leading to



glycoprotein synthesis. The interference of this antibiotic with protein glycosylation gives also rise, apart from other biological activities[3], to antiviral activity against enveloped viruses[4]. Unfortunately, the high toxicity of this antibiotic severely limits its clinical use. A possible way to overcome the latter disadvantage would be the development of analogues of nucleoside diphosphate sugars having a potent inhibitory effect on protein and other glycosylation processes, and(or) showing a high antiviral activity. The above described approach was recently explored by Vaghefi *et al.*[5,6] who synthesized the analogues **B** (Su = α-D-Glc, Nu = A) and **C** (Su = α-D-Glc/α-D-Man, Nu = A and Su = α-D-Gal, Nu = U) in which the potential sites for enzymatic cleavage (i.e., the ester linkage between sugar and phosphate and the anhydride linkage of the pyrophosphate) were replaced by more stable *C-P* bonds. On the other hand, Camarasa *et al.*[7] prepared the neutral analogue **D** (Su = α-D-Glc, Nu = U) in which the five-atom diphosphate bridge was replaced by an esosteric $-OC(O)NHSO_2O-$ bridge.

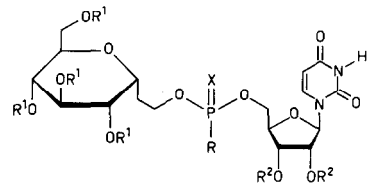
We now report the design, synthesis and biological activity of the analogues **IIa-d** in which the α-D-glucose and uridine are linked by a charged (R = OH) or neutral (R = alkyl/aryl) five-atom $-CH_2CH_2OP(X)RO-$ bridge.

The design of analogues II is based on the following concepts. Introduction of a two-carbon linkage between C-1 of the sugar and the 5'-phosphate of uridine will result in a molecule which should be resistant toward glycosyltransferase activity[5,6]. Futhermore, the presence of one ionic phosphate (see analogue IIa) or nonionic modified phosphate bonds (see analogues IIb-d) mimics[7,8] more or less the neutral five-atom bridge arrangement in tunicamycin that is still capable of binding to the site which has to accommodate a tetraanionic



I

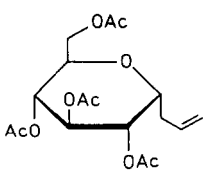
R = NHC(O)R'



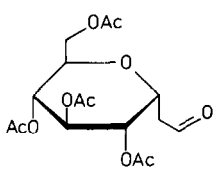
II a X=O, R=OH, $R^1=R^2=H$
b X=O, R=CH_3, $R^1=R^2=H$
c X=S, R=CH_3, $R^1=R^2=H$
d X=O, R=C_6H_5, $R^1=R^2=H$

transition state[9]. Molecular modelling (MacroModel[10]) studies indicated that the spatial arrangement of the five atoms spanning the distance between C-3 of the octitol and the C-5' of uridine in analogues II was in good agreement with the corresponding spatial arrangements in UDP-Glc (**A**) and tunicamycin (I).

The synthesis of analogues **IIa-d** could easily be accomplished by removal of the base-labile protecting groups from **7a-d**, which were obtained by phosphorylation of 2',3'-di-*O*-benzoyl-uridine (4) with the bifunctional reagents **5a-d** followed by the addition of 3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-2-deoxy-D-erythro-L-gulo-octitol (3). Key intermediate **3** was prepared in two steps by converting the readily accessible allyl *C*-glucoside **1**[11] into **2** followed by reduction. Thus compound **1** (17 mmol) in dioxane/water was oxidized[12] with osmium tetroxide (0.17 mmol) and sodium periodate (34 mmol). Work-up, after 1 h at 20°C, and purification by silica gel chromatography gave aldehyde **2**[13] (m.p. 62-64°C) in a yield of 90%. Reduction of **2** (4.7 mmol) in tetrahydrofuran/*iso*-propanol with sodium borohydride (4.7 mmol) for 30 min at 0°C furnished octitol **3**[12] (m.p. 85-88°C) in a yield of 49%. The low yield of **3** could not be increased by using the reducing reagent lithium tri-*tert*-butoxy-aluminohydride[14] which has been advocated[15] to be compatible with the presence of acetyl groups.



1



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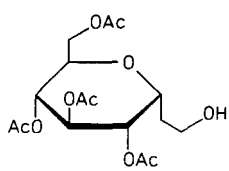

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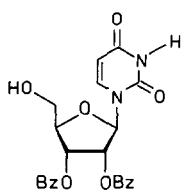
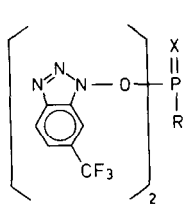
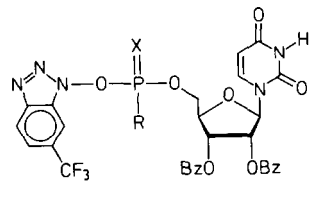


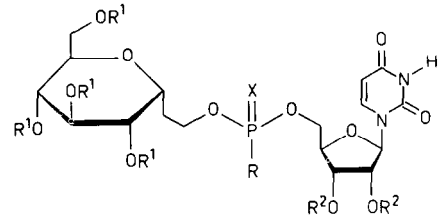
4

The introduction of the four different phosphate bonds could be realized by using the earlier by us developed oxybenzotriazolide activated reagents **5a**[16], **5b**[17], **5c**[18] and **5d**[19]. Thus phosphorylation of **4** (0.4 mmol) in pyridine (0.7 mL) with **5a-d** [(0.48 mmol in dioxane (2.3 mL)] resulted in the formation of the corresponding intermediates **6a-d**. A solution of **3**

(0.3 mmol) containing *N*-methylimidazole (5 eq.) in pyridine was now added to the individual intermediates to afford the corresponding diastereoisomers of: phosphatetriester **7a** (84%, δ_P -6.14 and -6.53), methylphosphonate **7b** (78%, δ_P 32.88 and 32.82), methylphosphorothioate **7c** (75%, δ_P 98.87 and 90.40) and phenylphosphonate **7d** (73%, δ_P 20.66). Deblocking of **7a** (0.1 mmol) to give **IIa**[13] (0.07 mmol, δ_P 0.85) was effected by removal of the 2-chlorophenyl group with oximate-ion[20], and subsequently basic hydrolysis (water/ammonia) of the acetyl and benzoyl groups. On the other hand, removal of the same protecting groups from compounds **7b-d**







5 a X=O, R=OC_6H_4Cl
b X=O, R=CH_3
c X=S, R=CH_3
d X=O, R=C_6H_5

6 a X=O, R=OC_6H_4Cl
b X=O, R=CH_3
c X=S, R=CH_3
d X=O, R=C_6H_5

7 a X=O, R=OC_6H_4Cl, R^1=Ac, R^2=Bz
b X=O, R=CH_3, R^1=Ac, R^2=Bz
c X=S, R=CH_3, R^1=Ac, R^2=Bz
d X=O, R=C_6H_5, R^1=Ac, R^2=Bz

can be performed most effectively[17] with dry methanolic ammonia. In this way analogues **IIb-d**[13] could be isolated as colourless solids in a yield of 60-80%, after silica gel chromatography.

Preliminary biological studies[21] using permeabilized cells indicated that analogues **IIa-d** had a more or less distinct inhibiting effect on glycolipid biosynthesis. This result is, to our knowledge[22], the first example of a synthetic analogue of a nucleoside diphosphate sugar that exerts an inhibitory effect on the biosynthesis of glycolipids. At present, we are studying in detail whether the observed effect may be attributed to one or both diastereoisomers of analogues **IIb-d** and, further, whether the replacement of R = Me by R = $(CH_2)_nMe$ in analogues **IIb,c** has an increasing or decreasing influence on the inhibition of the biosynthesis of glycolipids.

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13. Compound 2: ^{1}H NMR ($CDCl_3$): δ 9.74 (t, 1 H, H-1, $J_{1,2}$= $J_{1,2'}$= 1.9 Hz), 5.27 (t, 1 H, H-5, $J_{5,6}$= 8.6 Hz), 5.13 (dd, 1 H, H-4, $J_{4,5}$= 8.9 Hz), 4.98 (t, 1 H, H-6, $J_{6,7}$= 8.6 Hz), 4.86 (dt, 1 H, H-3, $J_{3,4}$= $J_{3,2}$= 5.8 Hz, $J_{3,2'}$= 8.0 Hz), 4.28 (dd, 1 H, H-8, $J_{8,8'}$= 12.3 Hz), 4.08 (dd, 1 H, H-8'), 3.90 (ddd, 1 H, H-7, $J_{7,8}$= 5.7 Hz, $J_{7,8'}$= 2.9 Hz), 2.92-2.76 (ABMX, 16 lines, 2 H, H-2 and H-2') and 2.08-2.04 (4 x s, 12 H, 4 x CH_3COO). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): δ 198.0 (C-1), 170.4, 169.6, 169.3 and 169.2 (4 x $CH_3\underline{C}OO$), 69.9, 69.5, 68.9, 67.8 and 67.0 (C-3, C-4, C-5, C-6 and C-7), 61.5 (C-8), 41.3 (C-2) and 20.4 ($\underline{C}H_3COO$). Anal. calcd for $C_{17}H_{24}O_9$: C, 51.34; H, 5.92. Found: C, 50.86; H, 5.90.
Compound 3: ^{1}H NMR ($CDCl_3$): δ 5.29 (t, 1 H, H-5, $J_{5,6}$= 8.4 Hz), 5.05 (dd, 1 H, H-4, $J_{4,5}$= 8.6 Hz), 4.94 (t, 1 H, H-6, $J_{6,7}$= 8.6 Hz), 4.40 (ddd, 1 H, H-3, $J_{3,2}$= 3.1 Hz, $J_{3,2'}$= 11.6 Hz, $J_{3,4}$= 6.1 Hz), 4.29 (dd, 1 H, H-8, $J_{8,8'}$= 12.1 Hz), 4.10 (dd, 1 H, H-8'), 3.99 (ddd, 1 H, H-7, $J_{7,8}$= 6.5 Hz, $J_{7,8'}$= 2.6 Hz), 3.78 (b t, 2 H, 2 x H-1, $J_{1,2}$= $J_{1,2'}$= 5.8 Hz), 2.27 (b s, 1 H, *OH*), 2.14-2.00 (m, 1 H, H-2), 2.10, 2.07, 2.06 and 2.05 (4 x s, 12 H, 4 x CH_3COO) and 1.78-1.68 (m, 1 H, H-2'). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): δ 170.5, 170.4, 169.3 and 169.2 (4 x $CH_3\underline{C}OO$), 69.6, 69.53, 69.47, 69.0 and 68.2 (C-3, C-4, C-5, C-6 and C-7), 61.8 and 58.3 (C-1 and C-8), 28.0 (C-2) and 20.2 ($\underline{C}H_3COO$). Anal. calcd for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.43. Found: C, 52.05; H, 6.81.
Compound IIa (atoms attached to the 3,7-anhydro-2-deoxy-D-erythro-L-gulo-octitol residue are marked with an asterisk). ^{1}H NMR (D_2O, pD= 9.0, 305K): δ 7.88 (d, 1 H, H-6), 5.96 (d, 1 H, H-1', $J_{1',2'}$= 4.2 Hz), 5.90 (d, 1 H, H-5, $J_{5,6}$= 8.0 Hz), 4.31 (m, 2 H, H-2' and H-3'), 4.23 (m, 1 H, H-4'), 4.15 (dd, 1 H, H-3*, $J_{3,4}$= 6.0 Hz), 4.14 (m, 1 H, H-5'), 4.06 (ddd, 1 H, H-5", J= 12 Hz, J= 3 Hz and J= 5 Hz), 4.02-3.89 (m, 2 H, 2 x H-1*), 3.80 (dd, 1 H, H-8*, $J_{8,8'}$= 12.8 Hz), 3.72 (dd, 1 H, H-4*, $J_{4,5}$= 9.7 Hz), 3.68 (dd, 1 H, H-8*), 3.61 (t, 1 H, H-5*, $J_{5,6}$= 9.3 Hz), 3.52 (ddd, 1 H, H-7*, $J_{7,8}$= 2.4 Hz, $J_{7,8'}$= 5.4 Hz), 3.35 (t, 1 H, H-6*, $J_{6,7}$= 9.6 Hz) and 2.05-1.96 (m, 2 H, 2 x H-2*). $^{13}C\{^{1}H\}$ NMR (D_2O): δ 168.4 (C-4), 153.8 (C-2), 142.1 (C-6), 103.3 (C-5), 89.5 (C-1'), 83.7 (d, C-4', $^{3}J_{P,C-4'}$= 8.8 Hz), 74.6, 74.0, 73.5, 73.0, 71.6, 70.9 and 70.3 (C-2', C-3', C-3*, C-4*, C-5*, C-6* and C-7*), 65.1 (d, C-1*, $^{2}J_{P,C-1*}$= 4.4 Hz), 63.1 (d, C-5', $^{2}J_{P,C-5'}$= 3.9 Hz), 61.7 (C-8*) and 25.8 (d, C-2*, $^{3}J_{P,C-2*}$= 7.3 Hz). ^{31}P NMR (D_2O): δ 0.85.
Relevant NMR data of compound IIb (diastereoisomeric mixture): ^{1}H NMR (D_2O): δ 7.75 (d, 0.5 H, H-6, $J_{6,5}$= 8.1 Hz), 7.73 (d, 0.5 H, H-6, $J_{6,5}$= 8.1 Hz), 5.91-5.82 (m, 2 H, H-5 and H-1'), 4.44-4.11 (m, 8 H), 3.83-3.56 (m, 6 H), 2.14-1.86 (m, 2 H, H-2*), 1.68 (d, 3 H, CH_3PO, $^{3}J_{P,H}$= 17.5 Hz); $^{13}C\{^{1}H\}$ NMR (D_2O): δ 167.0 (C-4), 152.4 (C-2), 142.5 (C-6), 103.2 (C-5), 90.9 (C-1'), 82.7 (C-4'), 61.9 (C-8*), 25.7 (C-2*), 9.8 (d, CH_3PO $^{2}J_{P,C}$= 139 Hz); ^{31}P NMR (D_2O): δ 34.69 and 34.60.
Relevant NMR data of compound IIc (diastereoisomeric mixture): ^{1}H NMR (D_2O): δ 7.82 (d, 0.5 H, H-6, $J_{6,5}$= 8.1 Hz), 7.79 (d, 0.5 H, H-6, $J_{6,5}$= 8.1 Hz), 5.92-5.89 (m, 2 H, H-5 and H-1'), 4.48-4.06 (m, 5 H), 3.86-3.30 (m, 9 H), 2.10-1.78 (m, 5 H, H-2* and CH_3PS, $^{3}J_{P,H}$= 17.4 Hz); $^{13}C\{^{1}H\}$ NMR (D_2O): δ 167.2 (C-4), 152.3 (C-2), 142.6 (C-6), 103.1 (C-5), 90.8 (C-1'), 25.5 (C-2*), 20.5 and 20.4 (2 x d, CH_3PO $^{2}J_{P,C}$= 110 Hz); ^{31}P NMR (D_2O): δ 100.57 and 100.36.
Relevant NMR data of compound IId (diastereoisomeric mixture): ^{1}H NMR (D_2O): δ 7.85-7.56 (m, 5 H, $H_{arom.}$ phenyl), 7.53 and 7.53 (2 x d, 1 H, H-6), 5.80 and 5.79 (2 x d, 1 H, H-1', $J_{1',2'}$= 3.3 Hz), 5.61 (d, 0.5 H, H-5, $J_{5,6}$= 8.1 Hz), 5.53 (d, 0.5 H, H-5, $J_{5,6}$= 8.1 Hz), 4.50-4.08 (m, 8 H), 3.75-3.31 (m, 6 H), 2.10-1.98 (m, 2 H, H-2*); $^{13}C\{^{1}H\}$ NMR (D_2O): δ 127.1 and 123.4 ($C_{arom.}$ *P*-phenyl), 90.9 (C-1'), 61.6 (C-8*), 25.2 (C-2*); ^{31}P NMR (D_2O): δ 22.85 and 22.73.
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19. Compound **5d** was prepared[16,17] from commercially available phenylphosphonic dichloride.
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21. The experiments were executed by Drs. J. J. Neefjes and H. L. Ploegh (Netherlands Cancer Institute, Amsterdam, The Netherlands).
22. Analogues D (Su = α-D-Glc, Nu = U) still carrying protected groups at het sugar and uridine moieties (see ref. 7a) showed antiviral activity and inhibition of protein glycosylation.

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