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Carbohydrate Research 282 (1996) 293–298

CARBOHYDRATE RESEARCH

Note

4-(Arylamino)phenyl α-D-glucopyranosides as potential anti-HIV agents

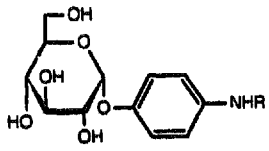
Josie C. Briggs, Alan H. Haines *

School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom

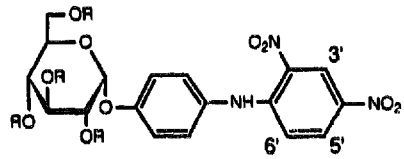
Received 21 August 1995; accepted 30 October 1995

Keywords: Aryl glucopyranosides; Glucosides; Anti-HIV agents

Our recent interest in aryl-substituted phenyl α-D-glucopyranosides as potential anti-HIV agents [1–3] led us to examine 4-(arylamino)phenyl α-D-glucopyranosides (**1**) in this context, since we regarded these glycosides as a logical modification of 4-(arylsulfonylamino)phenyl α-D-glucopyranosides (**2**), one of which had been shown [3] to possess weak anti-HIV activity against HIV-1 IIIB in infected cell cultures. Glycosides of the type **2** were found [3] to be remarkably effective competitive inhibitors of yeast α-D-glucosidase, an observation of relevance in regard to anti-HIV activity since nojirimycin, castanospermine, and some of their derivatives which show such antiviral activity also act competitively against certain glycosidases [4]. We now report the synthesis of a series of glycosides **3**–**6** and some representative examples of lipophilic *O*-acyl derivatives, compounds **7**–**10**, and results of anti-HIV tests with these compounds.



1 R = Ar
2 R = SO_2Ar
3 R = 2,4-$(NO_2)_2C_6H_3$-
4 R = 2,6-$(NO_2)_2C_6H_3$-
5 R = 3,4-$(NO_2)_2C_6H_3$-
6 R = 2,4,6-$(NO_2)_3C_6H_2$-

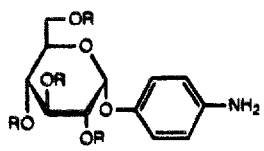


7 R = MeCO-
8 R = EtCO-
9 R = PrCO-
10 R = $Me(CH_2)_{14}CO$-

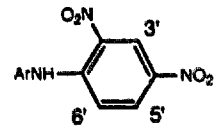
* Corresponding author.

SSDI 0008-6215(95)00379-7

Glycosides **3–6** were prepared by reaction of 4-aminophenyl α-D-glucopyranoside (**11**), readily prepared by reduction of commercially available 4-nitrophenyl α-D-glucopyranoside with hydrogen over palladium–charcoal, with 2,4-dinitrofluorobenzene, 2,6-dinitrochlorobenzene, 3,4-dinitrochlorobenzene, and 2,4,6-trinitrochlorobenzene, respectively. Compound **7** was prepared by reaction of 4-aminophenyl tetra-*O*-acetyl-α-D-glucopyranoside (**12**) with 2,4-dinitrofluorobenzene. Conventional acylation of **3** with propanoyl chloride, butanoyl chloride, and hexadecanoyl chloride afforded esters **8–10** although extensive column chromatography was required to purify these compounds which were obtained, as a result, in only low yields.



11 R = H
12 R = MeCO-



13 Ar = 4-HOC_6H_4-
14 Ar = 4-$MeOC_6H_4$-
15 Ar = 4-$Me(CH_2)_7OC_6H_4$-
16 Ar = 4-$EtCO_2C_6H_4$-
17 Ar = 4-$Me(CH_2)_8CO_2C_6H_4$-

Surprisingly, in view of the inhibition shown by glycosides **2** towards yeast α-D-glucosidase [3], compounds **3–6** did not inhibit this enzyme although results of anti-HIV tests (see Table 1) showed a small but significant activity. In order to investigate the role of lipophilicity in influencing antiviral activity in this type of compound we examined a series of tetra-*O*-acyl derivatives of **3** having increasing chain lengths, compounds **7–10**, and found a considerable increase in therapeutic index (TI) values for the acetyl and propanoyl derivatives, compounds **7** and **8**, respectively, compared to the parent compound.

Table 1
Anti-HIV activity [a] of 4-(arylamino)phenyl α-D-glucopyranosides (**3–6**), tetra-*O*-acyl derivatives (**7–10**), and *N*-aryl-2,4-dinitroaniline derivatives (**13–17**)

Compound	EC_{50}	TC_{50}	TI	Compound	EC_{50}	TC_{50}	TI
3	2–4	50	12.5–25	**10**	50	100	2
4	8–10	80	8–10	**13**	8	100	12.5
5	8–16	100–200	6.25–25	**14**	8–10	400	40–50
6	1	5–10	5–10	**15**	40	40	1
7	4	400	100	**16**	1.6	80	50
8	4	500	125	**17**	16	200	12.5
9	250	500	2				

[a] Tests carried out in HIV-1 IIIB-infected cell (C8166) cultures. The parameters EC_{50} and TC_{50} represent, respectively, the concentration of the compound that reduces the antigenic glycoprotein gp120 by 50% in infected cells and the concentration of compound which reduces normal cell growth by 50%. The parameter TI (therapeutic index) = TC_{50}/EC_{50}. Typically, 3'-azidothymidine (AZT) gives a TI value of > 50,000 in such tests.

In view of the probability that the mode of action of these compounds against HIV did not involve glycosidase inhibition [1], we investigated the biological activity of the aglycon moiety in **3** through a series of simple *N*-aryl derivatives of 2,4-dinitroaniline, compounds **13–17**. The highest TI values were observed with the 4-methoxyphenyl derivative **14** and the 4-propanoyloxyphenyl derivative **16**, both of which were more effective than the 4-hydroxyphenyl compound **13**. However, in both types of derivative, an increase in lipophilicity led to a decrease in TI values (compare data for **14** and **16** with those for the 4-octyloxyphenyl and 4-octanoyloxyphenyl derivatives, compounds **15** and **17**, respectively. Wide-ranging further modifications in the basic structure of the aglycon [6] led to no further improvement of anti-HIV activity.

1. Experimental

^{1}H NMR spectra were recorded for solutions in $CDCl_3$ or CD_3OD (internal Me_4Si) either at 60 MHz with a JEOL PMX60si spectrometer or at 270 MHz with a JEOL EX270 spectrometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. TLC and column chromatography were performed on silica gel (Machery–Nagel, SIL G-25UV$_{254}$) and Silica Gel 60 (Merck, 70–230 mesh), respectively. 4-Nitrophenyl α-D-glucopyranoside was purchased from Lancaster Synthesis and 4-nitrophenyl tetra-*O*-acetyl-α-D-glucopyranoside was prepared [7] by acetylation of this compound with acetic anhydride in pyridine. Enzyme assays were monitored with a Pye–Unicam PU 8800 UV/Visible spectrophotometer fitted with a cell temperature controller. α-D-Glucosidase (type VI from brewer's yeast) was purchased from Sigma Chemical Co. Ltd.

4-Aminophenyl α-D-glucopyranoside (**11**).—A solution of 4-nitrophenyl α-D-glucopyranoside (3 g, 9.96 mmol) in 95% EtOH (100 mL) was reduced under 1 atmosphere pressure of hydrogen in the presence of 10% Pd–C (0.08 g). The residue obtained on concentration of the filtered reaction mixture was crystallised from EtOH to give **11** (2.27 g, 84%); mp 183–185 °C; $[\alpha]_D$ +195° (*c* 1.5, H_2O); lit. [8] mp 185–186 °C, $[\alpha]_D$ +194.1° (*c* 1.4, MeOH).

4-Aminophenyl tetra-O-acetyl-α-D-glucopyranoside (**12**).—A suspension of 4-nitrophenyl tetra-*O*-acetyl-α-D-glucopyranoside (4 g, 8.52 mmol) and 10% Pd–C (0.08 g) in EtOH (130 mL) was stirred rapidly under a slight over-pressure of hydrogen. During the reaction, H_2 was absorbed and the starting material dissolved. The suspension was filtered through a plug of silica gel and the filtrate concentrated to give a syrup which solidified on storage under vacuum to give **12** (3.68 g, 98%); mp 49–50 °C; $[\alpha]_D$ +141° (*c* 0.3, EtOH); ^{1}H NMR data (60 MHz, $CDCl_3$): δ 2.00–2.20 (complex, 12 H, 4 × MeCO), 2.99 (br s, 2 H, NH_2), 4.02–4.50 (complex, 3 H, H-5,6a,6b), 4.85–4.94 (complex, 2 H, H-2,4), 5.52–5.88 (complex, 2 H, H-1,3), 6.49–7.03 (m, 4 H, AA'BB' system of 4ROC$_6H_4$NH$_2$). Anal. Calcd for $C_{20}H_{25}NO_{10}$: C, 54.7; H, 5.7; N, 3.2. Found: C, 54.7; H, 5.7; N, 3.1.

[1] The mode of action of these compounds is unknown although it appears that they are not reverse transcriptase inhibitors [5].

4-(2,4-Dinitrophenylamino)phenyl α-D-glucopyranoside (**3**).—To a solution of glucoside **11** (0.3 g, 1.11 mmol) in aq 50% EtOH (18 mL) was added a solution of 2,4-dinitrofluorobenzene (0.3 g, 1.6 mmol) in EtOH (9 mL) followed by $NaHCO_3$ (0.99 g, 11.8 mmol). The mixture was rapidly stirred for 1 h then filtered, the filtrate was concentrated, and the residue was purified by column chromatography (19:1 EtOAc–MeOH) to give **3** (0.26 g, 53%); mp 225–226 °C; $[\alpha]_{546}$ +51.9° (*c* 1, Me_2SO); ^1H NMR data (270 MHz, CD_3OD): δ 3.45 (t, 1 H, $J_{3,4} = J_{4,5} = 8.9$ Hz, H-4), 3.61 (dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.9 Hz, H-2), 3.64–3.76 (complex, 3 H, H-5,6a,6b), 3.88 (dd, 1 H, H-3), 5.34 (d, 1 H, H-1), 7.08 (d, 1 H, J_o 9.56 Hz, H-6')[2], 7.31 (s, 4 H, 4-ROC$_6H_4$NH-), 8.18 (dd, 1 H, J_m 2.64 Hz, H-5'), 9.03 (d, 1 H, H-3'). Anal. Calcd for $C_{18}H_{19}N_3O_{10}$: C, 49.4; H, 4.4; N, 9.6. Found: C, 49.4; H, 4.3; N, 9.4.

4-(2,6-Dinitrophenylamino)phenyl α-D-glucopyranoside (**4**), *4-(3,4-dinitrophenylamino)phenyl α-D-glucopyranoside* (**5**), *and 4-(2,4,6-trinitrophenylamino)phenyl α-D-glucopyranoside* (**6**).—These compounds were prepared similarly to glycoside **3**, using 2,6-dinitrochlorobenzene, 3,4-dinitrochlorobenzene, and 2,4,6-trinitrochlorobenzene, respectively, in place of 2,4-dinitrofluorobenzene.

4 (63%); mp 156–159 °C; $[\alpha]_{546}$ +60.4° (*c* 1, Me_2SO). Anal. Calcd for $C_{18}H_{19}N_3O_{10}$: C, 49.4; H, 4.4; N, 9.6. Found: C, 49.7; H, 4.5; N, 9.2.

5 (63%); mp 111–113 °C; $[\alpha]_D$ +158° (*c* 0.1, EtOH). Anal. Calcd for $C_{18}H_{19}N_3O_{10}$: C, 49.4; H, 4.4; N, 9.6. Found: C, 49.4; H, 4.4; N, 9.4.

6 (13%); mp 220–222 °C; $[\alpha]_{546}$ +36.4° (*c* 0.1, Me_2SO). Anal. Calcd for $C_{18}H_{18}N_4O_{12}$: C, 44.8; H, 3.8; N, 11.6. Found: C, 44.9; H, 3.6; N, 11.3.

4-(2,4-Dinitrophenylamino)phenyl tetra-O-acetyl-α-D-glucopyranoside (**7**).—A solution of 2,4-dinitrofluorobenzene (0.12 g, 0.64 mmol) in EtOH (4 mL) was added to a solution of **12** (0.2 g, 0.455 mmol) in aq 50% EtOH (7 mL) and to the rapidly stirred solution $NaHCO_3$ (0.41 g, 4.92 mmol) was added. After 1 h the solvent was decanted from the orange gum which had separated, the latter was triturated with water and dissolved in CH_2Cl_2, and the organic solution was then dried and concentrated. Column chromatography (6:4 light petroleum–EtOAc) gave a gum which solidified on storage, to give **7** (0.27 g, 96%); mp 87–89 °C; $[\alpha]_{546}$ +151° (*c* 2.4, CH_2Cl_2); ^1H NMR data (270 MHz, $CDCl_3$): δ 2.06 (s, 3 H, MeCO), 2.07 (s, 6 H, 2 × MeCO), 2.10 (s, 3 H, MeCO), 4.07–4.17 (complex, 2 H, H-5,6a), 4.27 (dd, 1 H, $J_{5,6b}$ 4.29, $J_{6a,6b}$ 12.54 Hz, H-6b), 5.06 (dd, 1 H, $J_{1,2}$ 3.63, $J_{2,3}$ 10.23 Hz, H-2), 5.19 (t, 1 H, $J_{3,4} = J_{4,5} = 9.90$ Hz, H-4), 5.72 (br t, 1 H, H-3), 5.79 (d, 1 H, H-1), 7.06 (d, 1 H, J_o 9.57 Hz, H-6'), 7.20–7.28 (m, 4 H, AA'BB' system of 4-ROC$_6H_4$NH-), 8.17 (dd, J_m 2.64 Hz, H-5'), 9.18 (d, 1 H, H-3'), 9.88 (br s, 1 H, NH). Anal. Calcd for $C_{26}H_{27}N_3O_{14}$: C, 51.6; H, 4.5; N, 6.9. Found: C, 52.0; H, 4.4; N, 6.9.

4-(2,4-Dinitrophenylamino)phenyl tetra-O-propanoyl-α-D-glucopyranoside (**8**), *4-(2,4-dinitrophenylamino)phenyl tetra-O-butanoyl-α-D-glucopyranoside* (**9**), *and 4-(2,4-dinitrophenylamino)phenyl tetra-O-hexadecanoyl-α-D-glucopyranoside* (**10**).—Acylation of **3** (0.20 g, 0.457 mmol) with propanoyl chloride, butanoyl chloride, or hexadecanoyl chloride (23 mmol) in pyridine (10 mL) in the usual manner gave, after column

[2] Primed numbers here and elsewhere refer to the 2,4-dinitrophenyl group.

chromatography (9:1 light petroleum–EtOAc), compounds **8**, **9**, and **10**, respectively, each having a ^{1}H NMR spectrum similar to that of **7** in the region δ 4.00–10.00.

8 (7%); syrup; mass spectrum (EI): m/z 661.2150 (M).

9 (25%); syrup; $[\alpha]_{546}$ +59.3 (c 0.3, CH_2Cl_2). Anal. Calcd for $C_{34}H_{43}N_3O_{14}$: C, 56.9; H, 6.0; N, 5.9. Found: C, 57.3; H, 5.8; N, 5.8.

10 (11%); syrup; mass spectrum (FAB): m/z 1413.0246 (M + Na).

N-(4-Hydroxyphenyl)-2,4-dinitroaniline (**13**).—A solution of 2,4-dinitrofluorobenzene (2.56 g, 13.7 mmol) in EtOH (50 mL) was added to a solution of 4-aminophenol (1 g, 9.17 mmol) in aq 50% EtOH (100 mL) followed by $NaHCO_3$ (8 g, 95 mmol). The mixture was stirred rapidly for 1 h, then filtered, and the filtrate concentrated to afford a solid which on column chromatography (7:3 light petroleum–EtOAc) gave, as a crystalline solid, **13** (0.46 g, 18%); mp 193–195 °C; ^{1}H NMR data (60 MHz, CD_3OD): δ 6.80–7.40 (complex, 5 H, AA'BB' system of HOC_6H_4NH- and H-6'), 8.16 (dd, 1 H, J_m 3, J_o 9.6 Hz, H-5'), 9.02 (d, 1 H, H-3'). Anal. Calcd for $C_{12}H_9N_3O_5$: C, 52.4; H, 3.3; N, 15.3. Found: C, 52.2; H, 3.2; N, 15.0.

N-(4-Methoxyphenyl)-2,4-dinitroaniline (**14**).—A solution of 2,4-dinitrofluorobenzene (10 g, 54 mmol) in EtOH (100 mL) was added to a solution of 4-methoxyaniline (3.98 g, 32.3 mmol) in aq 50% EtOH (200 mL) followed by $NaHCO_3$ (30 g, 435 mmol). The mixture was stirred rapidly for 1 h during which time an orange solid precipitated which was collected, washed with water, dried under vacuum, and crystallised from EtOAc–hexane to give **14** (7.4 g, 79%); mp 143–144 °C; lit. [9] mp 141 °C; ^{1}H NMR data (60 MHz, $CDCl_3$): δ 3.87 (s, 3 H, MeO), 6.86–7.42 (complex, 5 H, AA'BB' system of $MeOC_6H_4NH$- and H-6'), 8.14 (dd, 1 H, J_m 2.8, J_o 9.6 Hz, H-5'), 9.15 (d, 1 H, H-3'), 9.88 (br s, 1 H, NH). Anal. Calcd for $C_{13}H_{11}N_3O_5$: C, 54.0; H, 3.8; N, 14.5. Found: C, 54.4; H, 3.7; N, 14.4.

2,4-Dinitro-N-(4-octyloxyphenyl)aniline (**15**).—A stirred solution of **13** (0.1 g, 0.363 mmol) in 1,2-dimethoxyethane (4 mL) was treated with sodium hydride (0.02 g, 0.83 mmol); after 15 min, octyl iodide was added and the mixture was stirred for a further 48 h. EtOH (1 mL) was added and the mixture was then filtered through kieselguhr and the filtrate concentrated. Isolation of the product by column chromatography (19:1 light petroleum–EtOAc) gave **15** (0.05 g, 34%); mp 64 °C; ^{1}H NMR data (60 MHz, $CDCl_3$): δ 0.70–1.60 (complex, 15 H, $Me(CH_2)_6$-), 4.04 (t, 2 H, J 6 Hz, $-CH_2O-$), 6.92–7.44 (complex, 5 H, AA'BB' system of ROC_6H_4NH- and H-6'), 8.22 (dd, 1 H, J_m 2.4, J_o 9.6 Hz, H-5'), 9.04 (d, 1 H, H-3'), 9.96 (br s, 1 H, NH). Anal. Calcd for $C_{20}H_{25}N_3O_5$: C, 62.0; H, 6.5; N, 10.9. Found: C, 62.3; H, 6.4; N, 10.7.

2,4-Dinitro-N-(4-propanoyloxyphenyl)aniline (**16**) *and 2,4-dinitro-N-(4-octanoyloxyphenyl)aniline* (**17**).—Acylation of **13** with propanoyl or octanoyl chloride in pyridine in the usual manner and isolation of the product by column chromatography gave esters **16** and **17**, respectively.

16 (25%); mp 132–134 °C. Anal. Calcd for $C_{15}H_{13}N_3O_6$: C, 54.4; H, 3.95; N, 12.7. Found: C, 54.1; H, 3.8; N, 12.4.

17 (72%); mp 94–96 °C. Anal. Calcd for $C_{20}H_{23}N_3O_6$: C, 59.8; H, 5.8; N, 10.5. Found: C, 59.8; H, 5.7; N, 10.4.

Enzyme assays.—Assays with yeast α-D-glucosidase were performed at 30 °C and pH 6.5 [buffer: 10 mM disodium piperazine-*N,N'*-bis(ethane-2-sulfonate) (PIPES di-

sodium salt), 20 mM NaOAc, 0.1 mM EDTA; pH adjusted with a Rapide Intruments AGB-M1 meter with 5 mM HCl] using 4-nitrophenyl α-D-glucopyranoside as a substrate (K_m 0.20 mM). Assays were performed using substrate concentrations of 0, 0.01, 0.02, and 0.05 mM by monitoring the initial rate of release of 4-nitrophenol from the substrate at 400 nm. Compounds **3–6** did not inhibit this enzyme.

Acknowledgements

This work was supported under the MRC AIDS Directed Programme. We thank Dr. N. Mahmood of the MRC Collaborative Centre for conducting the anti-HIV experiments, and the SERC Mass Spectrometry Service Centre, Swansea for determination of the high-resolution mass spectra.

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