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Note

Synthesis of 7-[3-bromo-3,4-dideoxy-6-*O*-(2-hydroxyethyl)-β-D-*glycero*-hex-3-enopyranosyl-2-ulose]theophylline

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In recent years, attempts have been made to increase the selectivity of chemotherapeutic agents by binding them to targetting macromolecules, such as DNA, concanavalin A, antibodies against tumor-specific surface antigens, carcinoembryonic antigen, and fibrin[1–7]. Another approach[8] used the ability of alpha-fetoprotein (AFP) to bind polyunsaturated fatty acids (namely, eicosatetraenoic and docosahexaenoic acids) strongly and selectively even in the presence of high concentrations of serum albumin. In order to improve the therapeutic action of unsaturated ketonucleosides, the latter approach has been chosen since various cancer cells have specific AFP receptors that are absent on normal cells[9,10]. Uptake of the AFP–fatty acid complex delivers fatty acid to the cell, and the delipidated AFP released by the cell is able to transport and concentrate other fatty acid molecules inside the cell. Moreover, the unsaturated bromoketonucleoside **1**, besides having a significant antitumor activity, had a high toxicity to the host[11], due, in part, to its reaction with glutathione[12], leading to a high decrease in the intracellular P_H. This undesirable side-effect can be turned to advantage by targetting derivatives of **1** towards cancer cells. As a part of our programme on carrier-bound unsaturated ketonucleosides attached by spacers of various lengths, we now report the synthesis of 7-[3-bromo-3,4-dideoxy-6-*O*-(2-hydroxyethyl)-β-D-*glycero*-hex-3-enopyranosyl-2-ulose]theophylline (**12**), which could be linked through its spacer-arm to polyunsaturated fatty acids.

Treatment of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with sodium hydride and 2-(2-bromoethoxy)tetrahydropyran in *N,N*-dimethylformamide gave 1,2:3,4-di-*O*-isopropylidene-6-*O*-[2-(tetrahydropyran-2-yloxy)ethyl]-α-D-galactopyranose (**2**) in good yield. Hydrolysis of **2** with 0.1M hydrochloric acid in methanol at 90° afforded 6-*O*-(2-hydroxyethyl)-α,β-D-galactose (**3**), acetylation of which yielded syrupy 6-*O*-(2-acetoxyethyl)-1,2,3,4-tetra-*O*-acetyl-D-galactose (**4**). Reaction of **4** with trimethylsilyltheophylline, using stannic chloride as catalyst[13], afforded 7-[6-*O*-(2-acetoxyethyl)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl]theophylline (**5**). The ^{1}H-n.m.r. data ($J_{1',2'} = J_{2',3'} = 9$ Hz) showed that **5** was β.

Deacetylation of **5** with methanolic ammonia gave 7-[6-*O*-(2-hydroxyethyl)-

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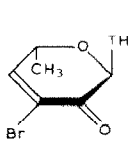

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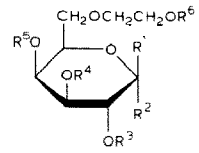
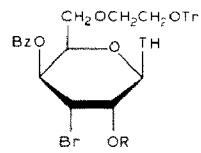
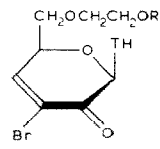


2 $R^1 = H, R^2, R^3 = R^4, R^5 = Me_2C, R^6 = THP$
3 $R^1, R^2 = H, OH, R^3 = R^4 = R^5 = R^6 = H$
4 $R^1 = OAc, R^2 = H, R^3 = R^4 = R^5 = R^6 = Ac$
5 $R^1 = TH, R^2 = H, R^3 = R^4 = R^5 = R^6 = Ac$
6 $R^1 = TH, R^2 = R^3 = R^4 = R^5 = R^6 = H$
7 $R^1 = TH, R^2 = R^3 = R^6 = H, R^4, R^5 = PhCH$
8 $R^1 = TH, R^2 = R^3 = H, R^4, R^5 = PhCH, R^6 = Tr$

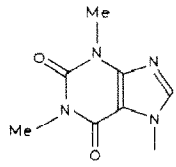


9 R = H
10 R = Ac



11 R = Tr
12 R = H

TH =



THP =

β-D-galactopyranosyl]theophylline (**6**). Benzylidenation of **6** with α,α-dimethoxytoluene by a modified[14] procedure of Evans[15] gave the 3′,4′-*O*-benzylidene derivative **7** as a crystalline mixture of diastereoisomers. The ^{1}H-n.m.r. spectrum [$(CD_3)_2CO$] of **7** showed two benzylidene methine signals at δ 6.28 and 5.98 (ratio of intensities, ~10:1) indicating[16] that the *endo*-H isomer preponderated. Purification to give a single diastereoisomer was not necessary for the next step.

Treatment of **7** with chlorotriphenylmethane afforded 7-[3,4-*O*-benzylidene-6-*O*-(2-triphenylmethoxyethyl)-β-D-galactopyranosyl]theophylline (**8**). Regiospecific cleavage of the benzylidene acetal ring of **8** with *N*-bromosuccinimide in refluxing carbon tetrachloride[17] yielded the expected 3′-bromonucleoside **9**. The ^{1}H-n.m.r. spectrum ($CDCl_3$) of **9** contained a signal for H-1′ at δ 6.35 ($J_{1,2}$ 8.5 Hz) and a signal at δ 5.72 (J 1 and 2 Hz) indicative of a BzOCH group (*trans*-diaxial opening of the acetal ring). The ^{1}H-n.m.r. spectrum [$(CD_3)_2CO$] of the acetate **10** of **9** was consistent with the structure assigned, since the signal for H-2′ now appeared at δ 6.13 and that (δ 5.67) for H-4′ occurred at lower field than that (δ 5.22) of H-3′; consequently, the *O*-benzoyl group was situated at C-4′ and the bromine atom at C-3′. The small value (~1 Hz) of $J_{3,4}$ indicated a *trans*-diaxial relationship of BzO-4′ and Br-3′.

Further evidence for the structure of **9** was provided by the next step in the synthesis. Oxidation of **9** with pyridinium dichromate–molecular sieve[18] or methyl sulfoxide–acetic anhydride[19] was accompanied by elimination of the benzoate group, indicating it to be β to the keto group and yielding 60% of 7-[3-bromo-3,4-dideoxy-6-*O*-(2-triphenylmethoxyethyl)-β-D-*glycero*-hex-3-enopyranosyl-2-ulose]-theophylline (**11**). The ^{1}H-n.m.r. spectrum ($CDCl_3$) of **11** contained, *inter alia*, signals for an olefinic proton at δ 7.65 ($J_{4,5}$ 1.7 Hz), for H-1′ at δ 6.68 ($J_{1,5}$ 1.5 Hz), and for H-5′ (a poorly resolved multiplet) at δ 4.93. The doublet for H-1′ collapsed

to a singlet when the H-5′ resonance was irradiated, indicating a six-bond coupling between the two protons, rarely encountered in the carbohydrate field[20].

Removal of the trityl group from **11** with aqueous 70% acetic acid afforded the desired 7-[3-bromo-3,4-dideoxy-6-*O*-(2-hydroxyethyl)-β-D-*glycero*-hex-3-enopyranosyl-2-ulose]theophylline (**12**). An account on the coupling of **12** with polyunsaturated fatty acids and the cytotoxic activity of these compounds will be published elsewhere.

EXPERIMENTAL

General methods. — U.v. spectra were recorded with a Varian UV-VIS M 635 spectrophotometer and ^{1}H-n.m.r. spectra (internal Me_4Si) with a Varian T-60 instrument. Optical rotations were determined with a Roussel-Jouan Quick polarimeter. Melting points are uncorrected. T.l.c. was performed on silica gel 60 F_{254} (Merck), and silica gel 60 (230–400 mesh, Merck) was used for flash chromatography[21], with ethyl acetate (*A*), and 2:8 (*B*), 5:5 (*C*), and 7:3 (*D*) ethyl acetate–hexane.

*1,2:3,4-Di-*O*-isopropylidene-6-*O*-[2-(tetrahydropyran-2-yloxy)ethyl]-α-*D*-galactopyranose* (**2**). — A mixture of sodium hydride (3.6 g, 150 mmol) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13 g, 50 mmol) in dry *N,N*-dimethylformamide (100 mL) was stirred for 2 h under nitrogen. 2-(2-Bromoethoxy)tetrahydropyran (41.8 g, 200 mmol) was then added slowly and stirring was continued for 1 h at room temperature. The mixture was poured into ice–water and extracted with dichloromethane, and the extract was washed with water, dried (Na_2SO_4), and concentrated. The oily residue was purified by flash chromatography (solvent *A*, followed by solvent *B*) to give pure **2** as a syrup (14.74 g, 76%), $[\alpha]_D^{22}$ −32° (*c* 0.15, methanol).

Anal. Calc. for $C_{19}H_{32}O_8$: C, 58.76; H, 8.25. Found: C, 58.41; H, 8.10.

*6-*O*-(2-Hydroxyethyl)-*D*-galactose* (**3**). — To a solution of **2** (13.58 g, 35 mmol) in methanol (15 mL) was added 0.1M hydrochloric acid (150 mL). The mixture was stirred for 1 h at 90°, then cooled, neutralised with Amberlite IR-45 (HO^-) resin, filtered, concentrated to ~50 mL, and treated with activated charcoal. Concentration of the filtered solution yielded a clear syrup which was triturated with ether. The residue was dried *in vacuo* to give **3** as a syrup (6.35 g, 81%), $[\alpha]_D^{22}$ +15° (*c* 0.1, water).

Anal. Calc. for $C_8H_{16}O_7$: C, 42.86; H, 7.14. Found: C, 43.06; H, 7.25.

*6-*O*-(2-Acetoxyethyl)-1,2,3,4-tetra-*O*-acetyl-*D*-galactose* (**4**). — Treatment of **3** (5.11 g, 22.82 mmol) with acetic anhydride (50 mL) in pyridine (100 mL) and column chromatography (solvent *C*) of the product yielded **4** as a syrup (7.46 g, 75%), which was essentially the β-pyranose form containing traces of the furanose form and had $[\alpha]_D^{22}$ +24° (*c* 0.1, methanol).

Anal. Calc. for $C_{18}H_{26}O_{12}$: C, 49.77; H, 6.0. Found: C, 50.12; H, 6.06.

*7-[6-*O*-(2-Acetoxyethyl)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranosyl]theophylline*

(**5**). — A mixture of **4** (8.025 g, 18.49 mmol), trimethylsilyltheophylline [from 3.66 g (20.34 mmol) of theophylline], and acetonitrile (110 mL) was treated with $SnCl_4$ (0.72 mL) for 6 h at 80°. The mixture was then diluted with dichloromethane, neutralised with saturated aqueous $NaHCO_3$, filtered, washed with water, dried, and concentrated. The residue was purified by flash chromatography (solvent *D* followed by solvent *A*) to yield **5** (6.62 g, 65%), m.p. 159.5–160° (from ethanol), $[\alpha]_D^{22}$ +4° (*c* 0.15, chloroform), $\lambda_{max}^{CHCl_3}$ 279 nm (ε 9563). ^{1}H-N.m.r. data [$(CD_3)_2CO$]: δ 8.18 (s, 1 H, H-8), 6.33 (d, 1 H, *J* 9 Hz, H-1′), 5.82 (t, 1 H, *J* 9 Hz, H-2′), 5.58 (dd, 1 H, *J* 1.5 Hz, H-4′), 5.45 (dd, 1 H, *J* 3.5 Hz, H-3′), 4.52 (m, 1 H, *J* 6 Hz, H-5′), 4.15 (m, 2 H, CH_2CH_2OAc), 3.82–3.55 (m, 4 H, H-6′,6′ and CH_2CH_2OAc), 3.52 and 3.33 (2 s, 6 H, NMe-1,3), 2.23–1.9 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{23}H_{30}N_4O_{12}$: C, 49.82; H, 5.42; N, 10.11. Found: C, 50.06; H, 5.49; N, 9.78.

7-[6-O-(2-Hydroxyethyl)-β-D-galactopyranosyl]theophylline (**6**). — Compound **5** (3.48 g, 6.28 mmol) was treated overnight at room temperature with methanol saturated with ammonia at 0°. The solution was then concentrated, and the residue was crystallised from methanol and recrystallised from aqueous methanol, to yield **6** (2.07 g, 85%), m.p. 151–152°, $[\alpha]_D^{22}$ +16° (*c* 0.15, water), $\lambda_{max}^{H_2O}$ 275 nm (ε 9262).

Anal. Calc. for $C_{15}H_{22}N_4O_8 \cdot CH_3OH$: C, 45.93; H, 6.22; N, 13.40. Found: C, 45.75; H, 6.01; N, 13.52.

7-[3,4-O-Benzylidene-6-O-(2-hydroxyethyl)-β-D-galactopyranosyl]theophylline (**7**). — To a stirred solution of **6** (1.85 g, 4.42 mmol) in dry *N,N*-dimethylformamide were added α,α-dimethoxytoluene (0.864 mL, 5.76 mmol) and anhydrous toluene-*p*-sulfonic acid (28 mg). The mixture was kept for 4 h at 68–70° under diminished pressure and then poured with stirring into ether (130 mL), and the mixture was stored overnight at 4°, The crystalline material was collected, stirred again with ether, and then crystallised from water–ethanol (8:2) to give a 10:1 mixture of diastereomers (1.322 g, 63%). Recrystallisation from water–acetone gave the pure *endo*-H isomer of **7**, m.p. 186–188°, $[\alpha]_D^{22}$ +57° (*c* 0.15, chloroform), $\lambda_{max}^{CHCl_3}$ 279 nm (ε 8897). ^{1}H-N.m.r. data [$(CH_3)_2CO$]: δ 8.25 (s, 1 H, H-8), 7.58–7.3 (m, 5 H, Ph), 6.28 (s, 1 H, C*H*Ph), 5.93 (d, 1 H, H-1′).

Anal. Calc. for $C_{22}H_{26}N_4O_8 \cdot 0.5\ H_2O$: C, 54.66; H, 5.59; N, 11.59. Found: C, 54.65; H, 5.67; N, 11.36.

7-[3,4-O-Benzylidene-6-O-(2-triphenylmethoxyethyl)-β-D-galactopyranosyl]-theophylline (**8**). — To a stirred solution of dry **7** (948 mg, 1.96 mmol) in dichloromethane (5 mL) were added chlorotriphenylmethane (669 mg, 2.4 mmol) and dimethylaminopyridine (12 mg, 0.1 mmol). The solution was kept overnight at room temperature, then diluted with dichloromethane, washed with water, dried, and concentrated. Flash chromatography (solvent *A*) of the residue gave **8** (1.09 g, 77%), which, after recrystallisation from benzene–di-isopropyl ether, had m.p. 117–119°, $[\alpha]_D^{22}$ +44° (*c* 0.15, chloroform), $\lambda_{max}^{CHCl_3}$ 279 nm (ε 8068).

Anal. Calc. for $C_{41}H_{40}N_4O_8$: C, 68.72; H, 5.59; N, 7.82. Found: C, 68.39; H, 5.85; N, 7.38.

*7-[4-O-Benzoyl-3-bromo-3-deoxy-6-O-(2-triphenylmethoxyethyl)-β-*D-gulopyranosyl]*theophylline* (**9**). — To a solution of **8** (859 mg, 1.2 mmol) in carbon tetrachloride were added barium carbonate (1.8 g) and *N*-bromosuccinimide, and the suspension was boiled under reflux with stirring for 2 h. The solids were removed, and the filtrate was diluted with dichloromethane, washed with cold aqueous $NaHCO_3$ and water, dried, and concentrated. The residue was crystallised from dichloromethane–light petroleum to give **9** (830 mg, 87%), m.p. 80–84° (dec.), $[\alpha]_D^{22}$ +28° (*c* 0.1, chloroform), $\lambda_{max}^{CHCl_3}$ 278 nm (ε 7773). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.4–7.1 (m, 21 H, H-8 and 4 Ph), 6.35 (d, 1 H, *J* 8 Hz, H-1′), 5.73 (dd, 1 H, *J* 1 and 2 Hz, H-4′).

Anal. Calc. for $C_{41}H_{39}BrN_4O_8$: C, 61.89; H, 4.91; Br, 10.05; N, 7.04. Found: C, 62.01; H, 4.78; Br, 9.91; N, 7.18.

The 2′-acetate **10** had m.p. 187–188° (from di-isopropyl ether), $[\alpha]_D^{23}$ +27° (*c* 0.1, chloroform). ^{1}H-N.m.r. data [$(CD_3)_2CO$]: δ 8.5–7.1 (m, 21 H, H-8 and 4 Ph), 6.52 (d, 1 H, *J* 9 Hz, H-1′), 6.13 (dd, 1 H, *J* 4 Hz, H-2′), 5.67 (dd, 1 H, *J* 2 Hz, H-4′), 5.22 (dd, 1 H, *J* 1 Hz, H-3′), 5.0 (m, 1 H, *J* 6.5 Hz, H-5′), 3.82–3.0 (m, 6 H, H-6′,6′ and OCH_2CH_2O), 3.53 and 3.37 (2 s, 6 H, NMe-1,3), 1.87 (s, 3 H, OAc).

*7-[3-Bromo-3,4-dideoxy-6-O-(2-triphenylmethoxyethyl)-β-*D-glycero-*hex-3-enopyranosyl-2-ulose]theophylline* (**11**). — To a solution of **9** (398 mg, 0.5 mmol) in dry dichloromethane (3 mL) were added molecular sieve Type 3A (560 mg) and pyridinium dichromate (290 mg). The suspension was stirred for 2.5 h and then filtered through silica gel G (Merck), the silica gel was washed with 1:1 ethyl acetate–ether, and the combined filtrate and washings were concentrated. Flash chromatography (1:1 ethyl acetate–di-isopropyl ether) of the residue gave **11** (200 mg, 60%) as a foam, $[\alpha]_D^{22}$ −15° (*c* 0.15, chloroform), $\lambda_{max}^{CHCl_3}$ 271 nm (ε 8480). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.72 (s, 1 H, H-8), 7.65 (d, 1 H, *J* 1.7 Hz, H-4′), 7.6–7.0 (m, 15 H, 3 Ph), 6.68 (d, 1 H, *J* 1.5 Hz, H-1′), 4.93 (m, 1 H, *J* 6 Hz, H-5′), 4.0–3.0 (m, 6 H, H-6′,6′ and OCH_2CH_2OTr), 3.63 and 3.38 (2 s, 6 H, NMe-1,3).

Anal. Calc. for $C_{34}H_{31}BrN_4O_6$: C, 60.81; H, 4.62; Br, 11.91; N, 8.35. Found: C, 60.88; H, 4.87; Br, 11.03; N, 8.16.

*7-[3-Bromo-3,4-dideoxy-6-O-(2-hydroxyethyl)-β-*D-glycero-*hex-3-enopyranosyl-2-ulose]theophylline* (**12**). — A mixture of **11** (150 mg, 0.22 mmol) and aqueous 70% acetic acid (1.5 mL) was stirred for 1 h at 55° and then for 1 h at 0°. Triphenylmethanol was removed, the filtrate was concentrated, and column chromatography (8:2 ethyl acetate–ethanol) of the residue yielded **12** (75 mg, 78%), m.p. 110–112° (from ethanol–ether), $[\alpha]_D^{22}$ −64° (*c* 0.15, chloroform), $\lambda_{max}^{CHCl_3}$ 270 nm (ε 9556). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.77 (s, 1 H, H-8), 7.58 (d, 1 H, *J* 1.7 Hz, H-4′), 6.62 (d, 1 H, *J* 1.3 Hz, H-1′), 4.95 (m, 1 H, H-5′), 4.2–3.2 (m, 6 H, H-6′,6′ and OCH_2CH_2OH), 3.63 and 3.38 (2 s, 6 H, NMe-1,3).

Anal. Calc. for $C_{15}H_{17}BrN_4O_6 \cdot EtOH$: C, 42.96; H, 4.84; Br, 16.83; N, 11.79. Found: C, 43.20; H, 4.88; Br, 16.71; N, 12.11.

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