2,3,6-Trideoxy-5-*O*-(4-nitrobenzoyl)-3-trifluoroacetamido-L-*ribo*-hexofuranosyl Bromide – A Suitable Furanoid Ristosamine Glycosylation Reagent

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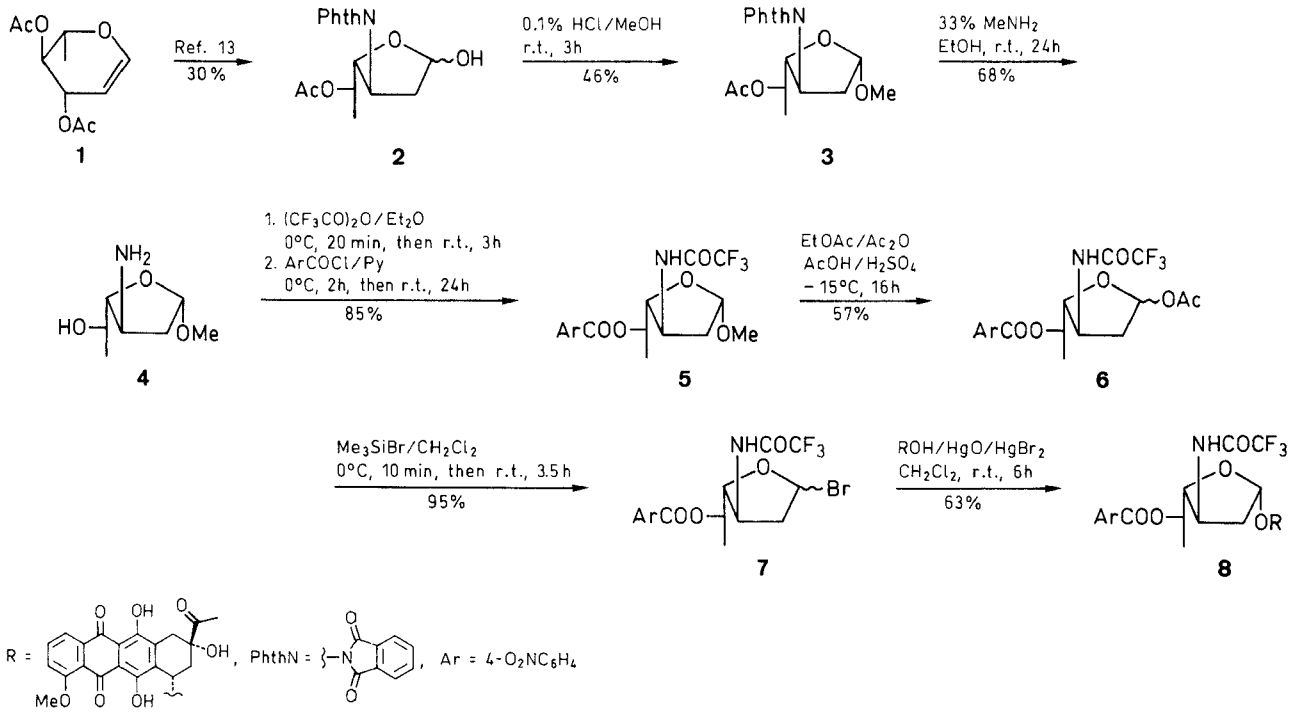
5-*O*-Acetyl-2,3,6-trideoxy-3-phthalimido-β-L-*ribo*-hexofuranose **(2)** was converted to its corresponding methyl glycoside **3**. After deprotection with 33% methylamine in ethanol and subsequent protection of the amino group with trifluoroacetic anhydride and the hydroxy group with 4-nitrobenzoyl chloride, the resulting compound **5** was transformed into the appropriate glycosyl bromide **7** via the 1-*O*-acetyl derivative **6**. Compound **7** was coupled with daunomycinone in the presence of yellow mercury(II) oxide and mercury(II) bromide.

The 3-amino-2,3,6-trideoxyhexoses are distributed in nature as the glysosidic moiety of several important antibiotics which exhibit anticancer activity.[1-3] In addition, anthracycline antibiotics such as doxorubicin, daunorubicin and their analogues showed strong inhibitory effect on HIV-reverse transcriptase.[4,5] Their administration is, however, accompanied by various undesirable side-effects, especially a cumulative, dose-related cardiopathogenicity,[6] which seriously impedes their broader utilization in chemotherapy. Daunosamine, the sugar component of these antibiotics, has been replaced by other aminodeoxy sugars,[7,8] as well as by neutral sugars,[9,10] all in their pyranoid form. The first glycosylation with a furanoid analogue at 7-*O* in carminomycinone was performed by Medgyes et al.[11] with a 3-amino-3,5-dideoxy-L-*lyxo*-hexofuranosyl derivative. Shortly after, the synthesis and the cytostatic activity of daunorubicin analogues with furanoid structures was also published.[12] The prerequisite of the described routes is the multistep synthesis of the appropriate furanosides with easily removable protecting groups. In our laboratory, we have previously synthesized an L-ristosamine derivative possesing the furanose configuration.[13] In this investigation we synthesize its glycosyl bromide with the same protecting



Scheme

groups that previously have been reported useful[12] for other configurations of 3-amino-2,3,6-trideoxyhexoses when used in glycosylation reactions.

The L-*ribo* isomer of 5-*O*-acetyl-2,3,6-trideoxy-3-phthalimidohexofuranose (**2**) was chosen as the starting material because it could be easily obtained in two steps from di-*O*-acetyl-L-rhamnal (**1**).[13] Glycosidation of **2** in 0.1% hydrogen chloride afforded an anomeric mixture from which the β-anomer **3** crystallized in 46% yield by addition of diethyl ether. Compound **3** was deprotected with 33% methylamine in absolute ethanol to give the corresponding methyl 3-aminohexofuranoside **4** which was treated with trifluoroacetic anhydride and then with 4-nitrobenzoyl chloride in dry pyridine to give methyl 5-*O*-(4-nitrobenzoyl)-2,3,6-trideoxy-3-trifluoroacetamido-β-L-*ribo*-hexofuranoside (**5**) in 85% yield. Treatment with acetic anhydride in the presence of acetic acid and sulfuric acid[14] resulted in formation of an anomeric mixture (α/β = 1:2) of the 1-*O*-acetylated hexofuranose **6** in 57% yield which on bromination with bromotrimethylsilane in dichloromethane afforded the glycosyl bromide under the same conditions as described by Kumar et al.[15] An example of using the latter for glycosylation reactions was performed with daunomycinone in dichloromethane in the presence of mercury(II) oxide and mercury(II) bromide. From the anomeric mixture formed, the β-anomer **8** was isolated in 63% yield by column chromatography.

The structural assignment of the glycoside **8** was confirmed by ^{1}H-^{1}H 2D COSY and ^{1}H NOE experiments. Due to the *ribo*-configuration, irradiation of 3'-H resulted in 7% NOE in 2'β-H and 4% NOE in 5'-H. The latter was in accordance with 6% NOE in 3'-H when 5'-H was irradiated. The β-configuration was assigned by the 11% NOE in 2'-α-H when 1'-H was irradiated. In conformity with this assignment we found J(1',2'β) close to zero in the ^{1}H NMR spectrum. In a similar way ^{1}H-^{1}H 2D COSY and ^{1}H NOE experiments on compound **5** confirmed β-configuration of the compounds **3**–**5**.

NMR spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 250 MHz for ^{1}H NMR and 62.9 MHz for ^{13}C NMR with TMS as an internal standard. FAB mass spectra were obtained on a Kratos MS 50 TS spectrometer. Analytical silica gel TLC plates $60F_{254}$ and silica gel (230–400 mesh) were purchased from Merck. 3,4-Di-*O*-acetyl-6-deoxy-L-glucal (di-*O*-acetyl-L-rhamnal) was purchased from Pfanstiehl Laboratories INC, Waukegan. Satisfactory microanalyses obtained for **3**, **5**, **6**: C ± 0.19, H ± 0.30, N ± 0.34.

Methyl 5-*O*-Acetyl-3-phthalimido-2,3,6-trideoxy-β-L-*ribo*-hexofuranoside (3):

Compound **2** (3.6 g, 11.3 mmol) was dissolved in dry MeOH (50 mL) containing 0.1% HCl (w/w) and stirred at r.t. for 3 h. Ag_2CO_3 (1 g) was added and the resulting suspension was filtered after 40 min through a pad of Celite. After evaporation in vacuo the resulting oil was treated with dry Et_2O (200 mL) and the precipitate filtered off to give pure **3** (0.75 g). An additional precipitate of **3** was obtained from the mother liquor on standing at 5°C for one week to give a total yield of 1.75 g (46%); mp 111–113°C.

^{1}H NMR ($CDCl_3$): δ = 7.88–7.73 (m, 4H, PhthN), 5.20 (d, 1H, J = 5.1 Hz, 1-H), 5.01–4.87 (m, 2H, 3-H, 5-H), 4.34 (t, 1H, J = 7.0 Hz, 4-H), 3.38 (s, 3H, MeO), 2.85 (ddd, 1H, J = 5.1, 8.5, 12.6 Hz, 2α-H), 2.17 (dd, 1H, J = 8.5, 12.6 Hz, 2β-H), 1.96 (s, 3H, Ac), 1.30 (d, 3H, J = 6.3 Hz, 6-H).

^{13}C NMR ($CDCl_3$): δ = 170.12 (Ac), 167.58 (C-2'), 134.07 (C-5'), 131.60 (C-3'), 123.19 (C-4'), 105.10 (C-1), 81.93 (C-4), 72.28 (C-5), 54.82 (MeO), 50.85 (C-3), 35.67 (C-2), 20.98 (Ac), 16.72 (C-6).

Methyl 3-Amino-2,3,6-trideoxy-β-L-*ribo*-hexofuranoside (4):

Compound **3** (1.30 g, 3.9 mmol) was dissolved in 33% solution of $MeNH_2$ in abs. EtOH (65 mL) and left at r.t. overnight. The solvent was evaporated in vacuo and the residual sirup purified by silica gel column chromatography (2 × 20 cm) with MeOH/CH_2Cl_2 (1:9); yield: 0.43 g (68%) which was used for the next step.

Methyl 5-*O*-(4-Nitrobenzoyl)-2,3,6-trideoxy-3-trifluoroacetamido-β-L-*ribo*-hexofuranoside (5):

A solution of **4** (0.46 g, 2.7 mmol) in dry Et_2O (20 mL) was cooled in an ice bath and treated with cold $(CF_3CO)_2O$ (5.6 mL). The clear

solution was kept in the ice bath for 20 min and at r. t. for 3 h. After evaporation of the solvent, the resulting sirup was coevaporated twice with toluene (2 × 20 mL). The residual oil was dissolved in ice-cooled MeOH (15 mL). The solution was treated with a few drops of pyridine and stirred at r.t. for 12 h. The solvent was evaporated in vacuo followed by coevaporation twice with pyridine (2 × 10 mL). To the solution was added dry pyridine (8.5 mL) and the solution was cooled to 0 °C. 4-Nitrobenzoyl chloride (0.81 g) was added and the mixture was stirred at 0 °C for 2 h and then at r. t. for 24 h. The mixture was poured into ice-water and extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were washed with sat. aq $NaHCO_3$ (10 mL) and H_2O (10 mL), dried (Na_2SO_4) and the solvent evaporated in vacuo. Silica gel column chromatography with petroleum ether/EtOAc (7 : 3) afforded pure **5**; yield: 0.58 (85 %); mp 109–110 °C.

^{1}H NMR ($CDCl_3$): δ = 8.31–8.19 (m, 4 H, H_{arom}), 6.52 (d, 1 H, J = 7.0 Hz, NH), 5.29 (quint, 1 H, J = 6.4 Hz, 5-H), 5.10 (dd, 1 H, J = 1.2, 5.2 Hz, 1-H), 4.77 (quint, 1 H, J = 7.8 Hz, 3-H), 4.04 (t, 1 H, J = 6.0 Hz, 4-H), 3.30 (s, 3 H, Ac), 2.45 (ddd, 1 H, J = 1.2, 7.8, 13.4 Hz, 2β-H), 2.07 (ddd, 1 H, J = 5.2, 7.8, 13.4 Hz, 2α-H), 1.43 (d, 3 H, J = 6.4 Hz, 6-H).

^{13}C NMR ($CDCl_3$): δ = 163.92 (ArCO), 156.41 ($CF_3\underline{C}O$), 150.54 (C-1′), 135.43 (C-4′), 130.68 (C-3′), 123.42 (C-2′), 117.80 (CF_3), 104.85 (C-1), 85.48 (C-4), 72.47 (C-5), 55.26 (MeO), 51.19 (C-3), 39.19 (C-2), 16.41 (C-6).

1-*O*-Acetyl-5-*O*-(4-nitrobenzoyl)-2,3,6-trideoxy-3-trifluoroacetamido-L-*ribo*-hexofuranose (6):

Compound **5** (0.50 g, 1.2 mmol) was dried by coevaporation with benzene and dissolved in EtOAc (10 mL). The solution was cooled to −25 °C and mixed with a cold (−25 °C) solution of EtOAc (19.5 mL), Ac_2O (11.0 mL), AcOH (8.3 mL) and conc. H_2SO_4 (0.05 mL). The solution was kept at −15 °C for 16 h, poured into ice-water and extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with H_2O (15 mL) and dried (Na_2SO_4). After evaporation of the solvent, the product was coevaporated with dry toluene; yield: 0.30 g (57 %).

Pure β-(80 mg) and α-(40 mg) anomers of **6** were isolated from 0.12 g of the anomeric mixture by silica gel column chromatography with CH_2Cl_2/MeOH (97 : 3).

Compound **6** (β-anomer): mp 113–114 °C.

^{1}H NMR ($CDCl_3$): δ = 8.32–8.20 (m, 4 H, H_{arom}), 6.87 (d, 1 H, J = 8.0 Hz, NH), 6.40 (d, 1 H, J = 4.3 Hz, 1-H), 5.31 (quint, 1 H, J = 6.4 Hz, 5-H), 4.82 (quint, 1 H, J = 8.0 Hz, 3-H), 4.13 (t, 1 H, J = 6.0 Hz, 4-H), 2.55 (ddd, 1 H, J = 1.0, 8.0, 13.8 Hz, 2β-H), 2.27 (ddd, 1 H, J = 4.3, 8.0, 13.8 Hz, 2α-H), 1.95 (s, 3 H, Ac), 1.45 (d, 3 H, J = 6.4 Hz, 6-H).

^{13}C NMR ($CDCl_3$): δ = 169.72 (Ac), 163.85 (ArCO), 150.54 (C-1′), 135.19 (C-4′), 130.71 (C-3′), 123.49 (C-2′), 97.29 (C-1), 86.14 (C-4), 72.07 (C-5), 50.58 (C-3), 38.30 (C-2), 20.86 (Ac), 16.04 (C-6).

Compound **6** (α-anomer): mp 146–148 °C.

^{1}H NMR ($CDCl_3$): δ = 8.31–8.19 (m, 4 H, H_{arom}), 7.08 (d, 1 H, J = 8.3 Hz, NH), 6.47 (d, 1 H, J = 4.7 Hz, 1-H), 5.21 (quint, 1 H, J = 6.4 Hz, 5-H), 4.77 (t, 1 H, J = 8.2 Hz, 3-H), 4.26 (dd, 1 H, J = 1.8, 5.4 Hz, 4-H), 2.52 (ddd, 1 H, J = 4.7, 8.2, 13.7 Hz, 2β-H), 2.13 (d, 1 H, J = 13.7 Hz, 2α-H), 2.11 (s, 3 H, Ac), 1.47 (d, 3 H, J = 6.4 Hz, 6-H).

^{13}C NMR ($CDCl_3$): δ = 168.81 (Ac), 163.73 (ArCO), 156.41 ($CF_3\underline{C}O$), 150.59 (C-1′), 135.12 (C-4′), 130.62 (C-3′), 123.46 (C-2′), 98.17 (C-1), 88.68 (C-4), 71.29 (C-5), 49.58 (C-3), 37.53 (C-2), 20.90 (Ac), 16.21 (C-6).

5-*O*-(4-Nitrobenzoyl)-2,3,6-trideoxy-3-trifluoroacetamido-L-*ribo*-hexofuranosyl Bromide (7):

Compound **6** (0.20 g, 0.46 mmol) was dissolved in 3 mL of dry alcohol-free CH_2Cl_2 and the solution was cooled to 0 °C. Me_3SiBr (0.2 mL) was added and the mixture was stirred for 10 min and then at r.t. for 3.5 h. When silica gel TLC with $CHCl_3$/MeOH (19 : 1) showed complete conversion of **6** into a more polar product, the solvent was evaporated in vacuo at r.t. The product, an odorless solid was immediately used in the following step; yield: 0.20 g (95 %).

7-*O*-[5-*O*-(4-Nitrobenzoyl)-2,3,6-trideoxy-3-trifluoroacetamido-L-*ribo*-hexofuranosyl]daunomycinone 8:

Daunomycinone (0.10 g, 0.25 mmol) was dissolved in dry alcohol-free CH_2Cl_2 (15 mL) and finely ground preignited type 3Å molecular sieves (0.4 g) was added. Dry yellow HgO (0.21 g) and $HgBr_2$ (0.084 g) was added. To this mixture was added **7** (100 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) with stirring. After 30 min another batch of molecular sieves (0.21 g), yellow HgO (0.13 g) and $HgBr_2$ (0.032 g) and finally **7** (100 mg, 0.22 mmol) were added. After 6 h, the mixture was diluted with CH_2Cl_2 (50 mL) and filtered. The solid was rinsed with CH_2Cl_2 (20 mL) which was combined with the filtrate and evaporated. The residual red solid was purified by silica gel column chromatography with MeOH/benzene/$CHCl_3$ (1 : 5 : 10) to give **8**; yield: 0.12 g (63 %); mp 178–180 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 14.07 (s, 1 H, OH), 13.20 (s, 1 H, OH), 8.05, 7.80 and 7.42 (d, t, and d, 3 H, J = 7–8 Hz, 1,2,3-H), 8.04–8.0 (m, 4 H, H_{arom}), 6.49 (d, 1 H, J = 7.4 Hz, NH), 5.75 (d, 1 H, J = 5.8 Hz, 1′-H), 5.50 (d, 1 H, J = 3.0 Hz, 7-H), 5.30 (quint, 1 H, J = 6.4 Hz, 5′-H), 4.68 (quint, 1 H, J = 8.0, 3′-H), 4.11 (s, 3 H, OMe), 4.00 (t, 1 H, J = 6.4 Hz, 4′-H), 3.16 and 3.02 (2 × d, 2 H, J = 19.0 Hz, 10-H), 2.51 (dd, 1 H, J = 8.0, 13.9 Hz, 2′β-H), 2.41 (m, 1 H, partially hidden by Ac, 8e-H), 2.38 (s, 3 H, Ac), 2.25 (ddd, 1 H, J = 5.8, 8.0, 13.9 Hz, 2′-α-H), 2.00 (dd, 1 H, J = 3.0, 15.0 Hz, 8a-H), 1.33 (d, 1 H, J = 6.4 Hz, 6′-H).

FAB (MeOH + 1 % AcOH + 3-$O_2NC_6H_4CH_2OH$): m/z (%) = 773 ($M + H^+$, 1).

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