Efficient preparation of 2-nitroimidazole nucleosides as precursors for hypoxia PET tracers

Petra Krizková1 · Anna Wieczorek1 · Friedrich Hammerschmidt1

Received: 21 October 2016 / Accepted: 6 November 2016 / Published online: 7 December 2016 © The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract 2-Deoxy-D-ribose was converted to α/β-mixtures of methyl 3-O-acetyl- and methyl 3-O-benzoyl-2-deoxy-5-(p-toluenesulfonyl)-D-ribofuranosides. These were reacted with boron trichloride to generate ribofuranosyl chlorides, which afforded precursors for tracers to image tumor hypoxia on substitution with salts of 2-nitroimidazole. The anomeric ratio of the nucleosides was delicately influenced by the reaction conditions.

Keywords Hypoxia · 2-Deoxy-D-ribose nucleosides · 2-Nitroimidazole · Alkylation · Halogenides

Introduction

Tumor hypoxia has a negative prognosis predictive value for solid tumors, because it is associated with tumor aggressiveness, metastasis, and aberrant angiogenesis [1–3]. It reflects increased resistance to anticancer treatment by radio- and chemotherapy. Therefore, it is in the interest of cancer patients to identify and target hypoxic areas in solid tumors [4, 5]. Non-invasive in vivo quantification of hypoxic areas of solid tumors with radiolabeled tracers attracted much attention and was studied extensively in recent years [6, 7]. Fluorine-18 containing tracers derived from 2-nitroimidazole (azomycin) are the most important ones used for positron emission tomography (PET) to image hypoxia for diagnostic purposes. Under hypoxic conditions in cells, the 2-nitroimidazole moiety of the tracer is reduced stepwise by electron transfers via reactive intermediates [8, 9]. These attack low-molecular weight compounds, preferably glutathione, and to a lesser extent high molecular weight compounds, and the nitro group ends up as amino group. The modified compounds with the bound 18F, which is detected by PET, stay in the cells and are accumulated. Figure 1 is a compilation of those tracers, nucleosides derived from carbohydrates, such as various D-pentoses and D-hexoses, except compounds 1 and 2. The first azomycin-based tracer and, at the same time, the gold standard up to now for imaging tumor hypoxia are [18F]fluoromisonidazole (FMISO, 1) [7, 10]. A homologue thereof is [18F]fluoroerythronitroimidazole (2) [11]. From the [18F]fluoro nucleosides 3-8 derived from α-arabinose, tracer 3 [12, 13], from β-arabinose, tracer 4 [14], from β-xylene, tracer 5 [14], and from β-glucose, tracer 6 [15], only 3 gained prominence. Recently, we synthesized 2-nitroimidazole precursors derived from α- and β-2-deoxy-D-ribose and α- and β-D-allofuranose. The β-anomers were radiolabeled and deprotected to give tracers 7 [16] and 8 [17] so far and evaluated for imaging tumor hypoxia.

Results and discussion

The synthesis of the precursors for tracers α- and β-8 is given in Scheme 1 [16]. In brief, it started from 2-deoxy-D-ribose, which was converted via methyl glycosides 10 to...
fully protected methyl glycosides 11. Their mixture was treated with 8 M HCl/Et₂O/CH₂Cl₂ at 0 °C to form a mixture of glycosyl chlorides which was reacted with the tetrabutylammonium salt of 2-nitroimidazole. The two nucleosides, α- and β-12, were separated by flash column chromatography and individually desilylated and finally tosylated to give the two desired precursors α- and β-14. This sequence was selected, because we thought that introduction of the tosyl group right from the beginning would not be tolerated by 8 M HCl in Et₂O/CH₂Cl₂. However, if that worked, the synthesis of both precursors could be shortened. Furthermore, we wanted to replace the tedious preparation of 8 M HCl in Et₂O by a commercially available and more reactive reagent, such as BCl₃, for the conversion of the methyl glycosides into the glycosyl chlorides.

The improved synthesis is given in Scheme 2. Although the mixture of methyl glycosides α- and β-10 [18] was tosylated [19] at −25 °C for 3 days in 59% yield (α/β = 1.2/1), some ditosylate 16 was formed as well (11%, α/β = 1.4/1). Analytical samples of the anomers for characterization could not be obtained by column flash chromatography. However, they could be obtained in homogeneous form by deacetylation of (+)- and (−)-17 and allowed to assign the anomeric configuration as will be shown later. Acetylation of the mixture of tosylates α- and β-15 with Ac₂O in dry pyridine delivered a mixture of acetates α- and β-17 in 92% (α/β = 1.2/1) yield. This mixture could be separated by flash

![Scheme 1](image-url)
column chromatography and Zemplen saponification of acetates (+)- and (−)-17 delivered homogenous samples of α- and β-15, respectively. The latter one is a literature known compound whose β-configuration has been determined by 2D NMR methods [20]. It allowed to assign α-configuration to (+)-15 and α and β to (+)- and (−)-17, respectively. As glycosides α- and β-17 were less reactive with HCl/Et2O in CH2Cl2, BCl3 in CH2Cl2 (1 M) was found to be an alternative to generate the glycosyl chlorides at 0 °C (general procedure A). Rapid aqueous work up at 0 °C allowed to isolate the labile chlorides, which were immediately reacted in two ways with 2-nitroimidazole. In the first case (general procedure B), the tetrabutylammonium salt of 2-nitroimidazole [21] was mixed with a solution of the 2-deoxy-D-ribofuranosyl chloride at −30 °C in CH3CN. Work up after 2 h and purification delivered 12% of nucleoside α-14 and 36% of β-14 starting from methyl glycosides. Satisfyingly, the two complementary procedures gave either preferably α- or β-anomer 14 [16].

We aimed to increase the yields of the nucleosides by replacing the acetyl protecting group by the more stable benzoyl group (Scheme 3). Therefore, the mixture of tosylates α- and β-15 was benzoylated and gave again a mixture of globally protected 2-deoxy-D-ribofuranosyl chloride at −30 °C in CH2Cl2. The reaction mixture was allowed to warm slowly to 0 °C within 2 h and was then extractively worked up. Flash chromatography furnished known anomers α- and β-14 over two steps in 41 and 11% yield, respectively. When the reaction was started at −50 °C, the α/β-14 ratio was 5/1 (by NMR) and only the α-anomer was isolated in 53% yield. In the second case (general procedure C), the mixture of glycosyl chlorides was added to a mixture of 2-nitroimidazole/K2CO3/excess tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as phase transfer catalyst [22] in CH3CN at 0 °C. Work up after 2 h and purification delivered 12% of nucleoside α-14 and 36% of β-14 starting from methyl glycosides. Satisfyingly, the two complementary procedures gave either preferably α- or β-anomer 14 [16].
warm to ambient temperature. The mixture of the nucleosides α- and β-19 was isolated in 81% yield (α/β = 2/1) by flash chromatography. The individual anomers were obtained by a second flash chromatography. When general procedure C was used for the preparation of the nucleosides from the chlorides, the yield of α-19 was 14% and that of β-19 was 69%. As envisioned, the yields with the benzoyl protecting group were higher than with the acetyl version. The anomeric configuration of α- and β-19 (1H NMR; α-1-H: d, J = 6.6 Hz; β-1-H: dd, J = 7.5 and 5.6 Hz) was assigned in analogy to nucleosides α- and β-14 (1H NMR; α-1-H: dd, J = 6.0 Hz; β-1-H: dd, J = 6.1 Hz) and the literature known analogue [19] of β-14 with two 4-toluoyl protecting groups (1H NMR; β-1-H: t, J = 5.6 Hz) instead of the acetyl and benzoyl group. The 1-H hydrogen atoms of the α-anomers resonate as doublets or as doublets of doublets with one coupling constant being very small. However, the 1-H' hydrogen atoms of the β-anomers resonate as doublets of doubles or as triplet with two similar coupling constants.

Conclusions

The synthesis of known 2-nitroimidazole nucleosides derived from 2-deoxy-D-ribose used as precursors for tracers was shortened if tosylation is performed at the beginning instead of at the end of the reaction sequence. The yield was further improved using BCl3 for generation of 2-deoxy-D-ribofuranosyl chlorides and benzoyl instead of acetyl group as protecting group for OH at C-3.

Experimental

1H, 13C (J-modulated; not J-modulated spectra were recorded of 2-nitroimidazole derivatives) NMR spectra were recorded in CDCl3 on a Bruker AV III 400 (1H: 400.27 MHz, 13C: 100.65 MHz), AV 400 (1H: 400.13 MHz, 13C: 100.61 MHz), and AV III 600 (1H: 600.13 MHz, 13C: 150.90 MHz) spectrometer at 25 °C, respectively. Chemical shifts δ (ppm) were referenced to residual CHCl3 (δH = 7.24 ppm) and CDCl3 (δC = 77.00 ppm). IR spectra were recorded on a Bruker VERTEX 70 IR spectrometer as ATR spectra or of films on a silicon disc [23] on a Perkin Elmer 1600 FT-IR spectrometer. Optical rotations were measured at 20 °C on a Perkin Elmer 351 polarimeter in a 10 cm cell. Melting points were determined on a Reichert Thermovar instrument. Elemental analyses (C, H, N, S) were conducted using the Euro EA 3000 Elemental Analyser (for oxygen in combination with a high temperature pyrolysis furnace (1480 °C) and reduction with carbon) from Eurovector. Their results were found to be in good agreement (±0.3%) with the calculated values.

Flash (column) chromatography was performed with Merck silica gel 60 (230–400 mesh). TLC was carried out on 0.25 mm-thick Merck plates, silica gel 60 F254. Spots were visualized by UV and/or dipping the plate into a solution of 23.0 g (NH4)6Mo7O24·4H2O and 1.0 g Ce(SO4)2·4H2O in 500 cm3 10% aqueous H2SO4, followed by heating with a heat gun. Pyridine was dried by refluxing over powdered CaH2, then distilled and stored over molecular sieves (4 Å). Dichloromethane was dried by storage over molecular sieves (3 Å). All other chemicals and solvents were of the highest purity available and used as received.

Mixture of methyl 2-deoxy-5-O-(p-toluenesulfonyl)-α- and methyl 2-deoxy-5-O-(p-toluenesulfonyl)-β-D-ribofuranoside (α- and β-15, C13H18O6S) and mixture of methyl 3,5-bis(p-toluenesulfonyl)-α- and methyl 3,5-bis(p-toluenesulfonyl)-β-D-ribofuranoside (α- and β-16, C20H24O8S2) Dry pyridine (2.20 cm3, 27.24 mmol) was added to a mixture of 1.345 g methyl glycosides α- and β-10 (9.08 mmol) [18] in 17 cm3 dry CH2Cl2 under Ar. The stirred reaction mixture was cooled to 0 °C and 1.868 g p-toluenesulfonyl chloride (9.08 mmol) was added. The flask was stored at –25 °C for 3 days and afterwards 1 cm3 water was added. After stirring for 15 min, the reaction mixture was concentrated under reduced pressure and 10 cm3 EtOAc was added. The organic phase was washed
with 10 cm³ 2 M HCl, 10 cm³ water, and 10 cm³ NaHCO₃, then dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc = 1/1; Rₜ = 0.34 for monotosylates, Rₜ = 0.75 for ditosylates) giving 1.631 g mixture of monotosylates α- and β-15 (59%; α/β = 1.22/1) and 0.458 g mixture of ditosylates α- and β-16 (11%), both as colorless oils. The data of the individual isomers are given later. Mixture of ditosylates α- and β-16: 

\[ \text{[α]D}^{20}_p = +24.3 \text{ cm}^2 \text{ g}^{-1} (c = 1.55, \text{acetone}) \]; IR (ATR, NMR sample in CDCl₃): \( \nu = 1359, 1190, 1174, 1096, 976 \text{ cm}^{-1} \).

\( ^1\text{H} \) NMR (400.27 MHz, CDCl₃): \( \delta/\beta = 1.4/1.0; \) contained 5% by weight of toluene; \( \alpha-16 \): \( \delta = 7.76–7.77 (m, 4H, HAr), 7.36–7.30 (m, 4H, HAr), 4.92 (bd, J = 5.2 Hz, 1H, 1-H), 4.77 (dd, J = 8.3, 3.4, 2.0 Hz, 1H, 3-H), 4.29 (q, J = 3.4 Hz, 1H, 4-H), 4.09 (d, J = 3.4 Hz, 2H, 5-H), 3.27 (s, 3H, OCH₃), 2.43 (s, 6H, CH₃), 2.13 (dd, J = 14.8, 8.3, 5.2 Hz, 1H, 2-H), 1.96 (dd, J = 14.8, 2.0, 0.8 Hz, 1H, 2-H) ppm; \( \beta-16 \): \( \delta = 7.76–7.78 (m, 4H, HAr), 7.36–7.30 (m, 4H, HAr), 5.04 (dd, J = 5.4, 2.0 Hz, 1H, 1-H), 4.19–2.13 (m, 2H, 4-H and 5-H), 4.04 (dd, J = 9.7, 6.6 Hz, 1H, 5-H), 3.21 (s, 3H, OCH₃), 2.42 (s, 3H, CH₃), 2.31 (dd, J = 14.0, 7.3, 2.0 Hz, 1H, 2-H), 2.09 (d, J = 14.0, 5.3 Hz, 1H, 2-H), 2.01 (s, 3H, CH₂CO) ppm; \( ^{13}\text{C} \) NMR (150.90 MHz, CDCl₃): \( \delta = 171.0 (\text{C}=\text{O}), 144.9 (\text{C}₉), 132.8 (\text{C}₈), 129.8 (2 \text{CH}), 127.9 (2 \text{CH}), 105.2 (2 \text{C}), 86.0 (3 \text{C}), 80.9 (3 \text{C}), 74.0 (2 \text{C}), 69.5 (2 \text{C}), 55.2 (\text{OCH}_3), 38.8 (2 \text{C}), 21.6 (\text{CH}_3), 21.0 (\text{CH}_2) \) ppm; and IR (ATR): \( \nu = 2925, 1737, 1360, 1235, 1175, 1047, 973, 955 \text{ cm}^{-1} \).

\(^{13}\text{C} \) NMR (150.90 MHz, CDCl₃): \( \delta = 171.0 (\text{C}=\text{O}), 144.9 (\text{C}₉), 132.8 (\text{C}₈), 129.8 (2 \text{CH}), 127.9 (2 \text{CH}), 105.2 (2 \text{C}), 86.0 (3 \text{C}), 74.0 (2 \text{C}), 69.5 (2 \text{C}), 55.2 (\text{OCH}_3), 38.8 (2 \text{C}), 21.6 (\text{CH}_3), 21.0 (\text{CH}_2) \) ppm; and IR (ATR): \( \nu = 2925, 1736, 1364, 1240, 1177, 1070, 1020, 978 \text{ cm}^{-1} \).

**Methyl 2-deoxy-5-O-(p-toluenesulfonyl)-α-D-ribofuranoside (α-15, C₁₅H₁₃O₄S)**

A solution of 0.291 g acetate (+)-17 (0.84 mmol, [α]D²⁰ = +93.6 (c = 1.05, acetone), 4.25 cm³ dry MeOH, and 0.43 cm³ NaOMe/MeOH (0.425 mmol, 1 M) was stirred for 30 min at 0 °C (TLC). Dry ice was added to neutralize base. The reaction mixture was concentrated under reduced pressure. Water (10 cm³) and 5 cm³ CH₂Cl₂ were added. The organic phase was separated and the aqueous one extracted with CH₂Cl₂ (2 × 5 cm³). The combined organic layers were dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc = 1/1; Rₜ = 0.47) to yield 0.199 g alcohol α-15 (77%) as colorless oil. [α]D²⁰ = +95.09° g cm⁻² (c = 1.12, acetone); \(^{1}\text{H} \) NMR (400.13 MHz, CDCl₃): \( \delta = 7.78–7.73 (m, 2H, H^{\text{H}}), 7.35–7.30 (m, 2H, H^{\text{H}}), 5.04 (bd, J = 4.4 Hz, 1H, 1-H), 4.18 (zd, J = 4.1, 1.8 Hz, 1H, 4-H), 4.10 (bd, J = 5.9 Hz, 1H, 3-H), 4.04 (AB part of ABX system, Jₐₜ = 10.7 Hz, J = 4.3, 3.8 Hz, 2H, 5-H), 3.32 (s, 3H, CH₃O), 2.75 (bs, 1H, OH), 2.43 (s, 3H, CH₃), 2.05 (dd, J = 13.9, 5.9, 4.4 Hz, 1H, OH).
2-H), 1.96 (dd, J = 13.9, 0.8 Hz, 1H, 2-H) ppm. $^{13}$C NMR (100.61 MHz, CDCl$_3$): δ = 145.0 (Cq, CO$_3$O), 132.7 (Cq$^{s}$O), 129.9 (2 HC$_{22}$O), 127.9 (2 HC$_{176}$O), 105.7 (C-1), 84.6 (C-4), 72.8 (C-3), 69.4 (C-5), 55.0 (OCH$_3$), 41.0 (C-2), 21.6 (CH$_3$O) ppm; and IR (Si): ν = 3445, 2923, 1354, 1173, 1081, 961, 908 cm$^{-1}$.

**Methyl 2-deoxy-5-O-(p-toluenesulfonyl)-β-D-ribofuranoside (β-15, C$_{176}$H$_{22}$O$_7$S)**

A mixture of 0.066 g acetate (–)–17 (0.19 mmol, less polar acetate, [z]$^{20}$D = –40.7 cm$^2$ g$^{-1}$ (c = 1.01, acetone), 2 cm$^3$ dry MeOH, and 0.064 cm$^3$ MeONa/MeOH (0.064 mmol, 0.33 equiv, 1 M) was stirred at –30 °C for 2 h (TLC: hexanes/EtOAc = 1/1; virtually no starting material was used immediately for the next step after withdrawing a sample for $^1$H NMR spectroscopy; ratio of chlorides: α/β = 3.6/1.0. (200 C, HC$^s$O), 127.9 (2 HC$^s$O), 105.7 (C-1), 84.6 (C-4), 72.65 (C-3), 70.14 (C-5), 50.02 (CH$_3$O), 41.32 (C-2), 21.64 (CH$_3$ tol) ppm.

**Preparation of anomeric 3-O-acetyl-2-deoxy-5-O-(p-toluenesulfonyl)-D-ribofuranosyl chlorides (general procedure A) and their conversion to 1-(3'-O-acetyl-2'-deoxy-5'-O-(p-toluenesulfonyl)-α- and 1-(3'-O-acetyl-2'-deoxy-5'-O-(p-toluenesulfonyl)-β-D-ribofuranosyl)-2-nitroimidazole (α- and β-14)**

General procedure A: To a solution of 0.507 g methyl glycosides, α- and β-17 (1.47 mmol) in 4.5 cm$^3$ dry Et$_2$O at 0 °C under Ar 3.68 cm$^3$ BCl$_3$ (3.68 mmol, 2.5 equiv, 1 M in CH$_2$Cl$_2$) was added. The reaction mixture was stirred for 2 h (TLC: hexanes/EtOAc = 1/1; virtually no starting material was present; new strong spot with $R_f$ = 0.34) at 0 °C. CH$_2$Cl$_2$ (12 cm$^3$, 0 °C) was added and the mixture was washed with 4 cm$^3$ cold brine (–18 °C), which was then extracted with 5 cm$^3$ cold CH$_2$Cl$_2$ (0 °C). The combined organic phases were washed with 5 cm$^3$ cold aqueous solution of NaHCO$_3$ (0 °C), dried (Na$_2$SO$_4$) at 0 °C, and concentrated first to 5–10 cm$^3$ on a rotavapor without warming with the water bath and then the remaining solvent was removed on the vacuum pump (1 mbar) within a few min without warming. The clear somewhat coloured solution was used immediately for the next step after withdrawing a sample for $^1$H NMR spectroscopy; ratio of chlorides: α/β = 3.6/1.0.

$^1$H NMR of anomeric 2-deoxy-D-ribofuranosyl chlorides (400.27 MHz, CDCl$_3$): δ = 6.21 ppm (d, J = 5.3 Hz, 1-H of α-chloride), 1-H of β-chloride overlapping with 1-H of α-chloride, integration was referenced to resonance at 4.46 ppm (q, J = 2.9 Hz, 4-H).

**Reaction of anomeric 2-deoxy-D-ribofuranosyl chlorides with tetrabutylammonium salt of 2-nitroimidazole (general procedure B)**

A solution of the above 2-deoxy-D-ribofuranosyl chlorides derived from α- and β-17 in 3.5 cm$^3$ dry CH$_2$Cl$_2$ (0 °C) was added to a solution of the 0.450 g tetrabutylammonium salt of 2-nitroimidazole (1.32 mmol, 0.9 equiv. relative to methyl glycosides) [21] in dry 4 cm$^3$ CH$_2$Cl$_2$ at –30 °C under Ar. Stirring was continued for 2 h, while the cooling bath was allowed to reach 0 °C. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in 15 cm$^3$ EtOAc and washed with water (2 × 5 cm$^3$). The organic phase was dried (MgSO$_4$) and concentrated under reduced pressure. The residue (α/β = 2/1 by $^1$H NMR) was flash chromatographed (hexanes/EtOAc = 1/1, x: $R_f$ = 0.29; β: $R_f = 0.49$) to yield 0.060 g β-14 (11%) and 0.231 g α-14 (41%), both spectroscopically ($^1$H, $^{13}$C NMR) identical to the ones described in Ref. [14].

Similarly, 0.536 g mixture of anomeric methyl glycosides (1.56 mmol) were converted via chlorides to nucleosides (reaction was started at –50 °C); ratio of α/β = 5/1 by $^1$H NMR in crude product. Flash chromatography furnished 0.318 g α-14 (53%).

**Reaction of 3-O-acetyl-glycosyl chlorides with 2-nitromimidazole/K$_2$CO$_3$/tris[2-(2-methoxyethoxy)ethyl]amine (TDA-I) (general procedure C)**

A mixture of 0.118 g 2-nitromimidazole (1.04 mmol, 0.8 equiv.), 0.225 g K$_2$CO$_3$ (1.63 mmol), 10 mm$^3$ TDA-I [23], and 20 cm$^3$ dry CH$_2$CN was stirred for 10 min at RT under Ar and then cooled to 0 °C. The chlorides prepared from 0.449 g methyl glycosides α- and β-17 (1.30 mmol) by the above given general procedure A were dissolved in dry CH$_2$CN at 0 °C and added. Stirring was continued for 2 h at 0 °C and then the reaction mixture was filtered through Celite (washing with CH$_2$Cl$_2$). The filtrate was concentrated under reduced pressure and 20 cm$^3$ EtOAc was added to the residue. The mixture was washed with water (2 × 10 cm$^3$), dried (MgSO$_4$), and concentrated under reduced pressure. The residue (α/β = 1/3, by $^1$H NMR) was purified by flash chromatography (hexanes/EtOAc = 1/1) to yield 54 mg α-14 (12%) and 160 mg β-14 (36%).

**Mixture of methyl 3-O-benzoyl-2-deoxy-5-O-(p-toluene-sulfonyl)-α- and methyl 3-O-benzoyl-2-deoxy-5-O-(p-toluene-sulfonyl)-β-D-ribofuranoside (α- and β-18, C$_{20}$H$_{22}$O$_7$S)**

To 0.800 g, mixture of anomeric monotosylates 15 (2.65 mmol) and 0.64 cm$^3$ dry pyridine (7.95 mmol) in dry CH$_2$Cl$_2$ (6.3 cm$^3$) under Ar was added 0.64 cm$^3$ benzyl chloride. The reaction mixture was stirred at RT.
for 18 h. After addition of 0.5 cm³ water, stirring was continued for 15 min. The mixture was concentrated under reduced pressure and 15 cm³ EtOAc and 5 cm³ water were added. The organic phase was separated and washed with 5 cm³ 2 M HCl, 5 cm³ water, and 5 cm³ saturated aqueous solution of NaHCO₃, dried (MgSO₄), and concentrated under reduced pressure.

The residue was purified by flash chromatography (hexanes/EtOAc = 2/1, Rf = 0.76) to yield 0.972 g mixture of anomeric benzoates 18 (90%): α/β = 1.2/1.0 by ¹H NMR as a colorless oil possibly containing some benzoic acid; [α]D²⁰ = +41.0 g cm⁻² (c = 1.35, acetone). The individual anomers of 18 for characterization were prepared by esterification of homogeneous anomers α- and β-15 with PhC(O)Cl/pyridine.

*Methyl 3-O-benzoyl-2-deoxy-5-O-(p-toluenesulfonyl)-α-and methyl 3-O-benzoyl-2-deoxy-5-O-(p-toluenesulfonyl)-β-D-ribofuranoside (α- and β-18, C₁₉₂H₁₈₂O₁₉S)*

Benzylic chloride (0.143 g, 1.02 mmol, 0.118 cm³) and 0.120 g dry pyridine (1.52 mmol, 0.122 cm³) were added to 0.153 g alcohol α-15 (0.51 mmol) dissolved in 1.5 cm³ dry CH₂Cl₂ and the solution was stirred for 20 h at RT. Water (0.5 cm³) was added and the reaction mixture was stirred for 15 min. The mixture was concentrated under reduced pressure, 10 cm³ water was added, and it was extracted with ethyl acetate (3 × 5 cm³), dried with Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexanes/ethyl acetate = 1/1, Rf = 0.55) to yield 0.183 g benzoate α-18 (88%) as a colorless oil. Similarly, 0.096 g alcohol β-15 (0.32 mmol) was converted to 0.104 g benzoate β-18 (81%).

**α-18:** [α]D²⁰ = +98.23 (c = 1.015, acetone); ¹H NMR (400.13 MHz, CDCl₃): δ = 8.01–7.96 (m, 2H, H²ᵖᵇ), 7.81–7.76 (m, 2H, H⁴ᵖᵇ), 7.58–7.52 (m, 1H, H⁶ᵖᵇ), 7.45–7.38 (m, 2H, H⁷ᵖᵇ), 7.34–7.28 (m, 2H, H⁸ᵖᵇ), 5.22–5.16 (m, 1H, 3-H), 5.06 (d, J = 5.2 Hz, 1H, 1-H), 4.35–4.27 (m, 3H, 4-H, 5-H), 3.34 (s, 3H, CH₃O), 2.41 (s, 3H, CH₃), 1.35 (acetone). The individual anomers of 18 were separated by flash chromatography (CH₂Cl₂/EtOAc = 20/1: α: Rf = 0.42; β: Rf = 0.36) using a long column to yield homogeneous anomers and mixture of anomers.

**β-18:** [α]D²⁰ = −16.75 (c = 0.83, acetone); ¹H NMR (400.13 MHz, CDCl₃): δ = 7.98–7.93 (m, 2H, H²ᵖᵇ), 7.83–7.77 (m, 2H, H⁴ᵖᵇ), 7.59–7.53 (m, 1H, H⁶ᵖᵇ), 7.46–7.38 (m, 2H, H⁷ᵖᵇ), 7.33–7.27 (m, 2H, H⁸ᵖᵇ), 5.32 (dd, J = 7.5, 5.4, 5.3 Hz, 1H, 3-H), 5.13 (dd, J = 5.4, 2.0, Hz, 1H, 1-H), 4.32 (dd, J = 7.1, 5.1, 3.3 Hz, 1H, 4-H), 4.26 (dd, J = 10.1, 5.1 Hz, 1H, 5-H), 4.14 (dd, J = 10.1, 7.1 Hz, 1H, 5-H), 3.26 (s, 3H, OCH₃), 2.43 (ddd, J = 14.2, 7.3, 2.0 Hz, 1H, 2-H), 2.39 (s, 3H, CH₃⁴ᵖᵇ), 2.25 (td, J = 14.2, 5.4 Hz, 1H, 2-H) ppm; ¹³C NMR (100.61 MHz, CDCl₃): δ = 165.95 (CO), 144.90 (CSO₃), 133.28 (HC⁴ᵖᵇ), 132.85 (CH₂⁴ᵖᵇ), 129.86 (2C, HC⁵ᵖᵇ), 129.73 (2C, HC⁶ᵖᵇ), 129.58 (CCO), 128.39 (2C, HC⁷ᵖᵇ), 127.99 (2C, HC⁸ᵖᵇ), 105.26 (C-1), 80.945 (C-4), 74.94 (C-3), 69.56 (C-5), 55.14 (CH₃O), 38.90 (C-2), 21.62 (CH₃⁴ᵖᵇ) ppm; and IR (Si): ν = 2924, 1721, 1674, 1274, 1178, 1110 cm⁻¹.

**Preparation of mixture of anomeric 3-O-Benzoyl-2-deoxy-5-O-(p-toluenesulfonyl)-D-ribofuranosyl chlorides and their conversion to 1-(3-O-benzyloxy-2-deoxy-5'-O-(p-toluenesulfonyl)-α- and 3'-O-benzoyl-2-deoxy-5'-O-(p-toluenesulfonyl)-β-D-ribofuranosyl)-2-nitroimidazole (α- and β-19, C₁₅₈H₁₇₅N₄O₈S)**

A mixture of 0.609 g methyl glycosides α- and β-19 (1.50 mmol) was converted to 3-O-benzoyl-glycosyl chlorides (TLC: hexanes/EtOAc = 1/1, Rf = 0.58) by the procedure used for methyl glycosides α- and β-17 (general procedure A). The crude product was used immediately for the next step. ¹H NMR spectrum of crude 3-benzoyl-2-deoxy-D-ribofuranosyl chlorides (400.27 MHz, CDCl₃): δ = 6.31 (d, J = 5.0 Hz, 1-H of α-chloride), 6.28 (dd, J = 5.5, 1.6 Hz, 1-H of β-chloride), integration referenced to resonance at 4.60 ppm (q, J = 2.6 Hz, 4-H); α/β = 1/0.13; fairly pure.

The above mixture of chlorides was converted to a mixture of α- and β-19 using the procedure (general procedure B) given for the corresponding 3-O-acetyl-glycosyl chlorides. Tetrabutylammonium salt of 2-nitroimidazole (0.481 g, 1.36 mmol) was used; the reaction was started at −50 °C; and the reaction mixture was allowed to warm to RT in 18 h. The crude product (α/β = 2/1, by ¹H NMR) was flash chromatographed (hexanes/EtOAc = 2/1, α-19: Rf = 0.25; β-19: Rf = 0.21) to yield 0.536 g mixture (81%, α/β = 2/1) of α- and β-19. The anomers were separated by flash chromatography (CH₂Cl₂/EtOAc = 20/1: α: Rf = 0.42; β: Rf = 0.36) using a long column to yield homogeneous anomers and mixture of anomers.
1H, 4′-H), 4.37 (AB part of ABX system, $J_{AB} = 11.4$ Hz, $J_{AX} = J_{BX} = 3.0$ Hz, 2H, 5′-H), 3.05 (td, $J = 15.5, 6.6$ Hz, 1H, 2′-H), 2.48 (d, $J = 15.5$ Hz, 1H, 2′-H), 2.45 (s, 3H, CH₃) ppm; $^{13}$C NMR (100.61 MHz, CDCl₃): δ = 165.7 (CO), 145.6 (Cq tos), 143.7 (Cq im), 133.9 (HC), 132.43 (Cq), 130.1 (2HC tos), 129.5 (2HC ar), 128.6 (2HC ar), 128.4 (Cq ar), 128.2 (HC im), 127.9 (2HC ar), 122.2 (HC im), 91.4 (C-1′), 86.4 (C-4′), 74.6 (C-3′), 69.0 (C-5′), 41.1 (C 2′), 21.7 (CH₃im) ppm; and IR (ATR, NMR sample): $\nu = 2971, 1709, 1535, 1476, 1352, 1279, 1226, 1174, 1096, 1075$ cm⁻¹.

β-19 [α]D₂⁰ = −16.89 g cm⁻² (c = 1.06, acetone); m.p.: 90 °C (decomp., CH₂Cl₂CH₂Cl/Pr₂O, solution not heated above 50 °C); $^1$H NMR (400.13 MHz, CDCl₃): δ = 8.03–7.96 (m, 2H, H⁻); 7.82–7.75 (m, 2H, H⁺); 7.63–7.57 (m, 2H, H⁻); 7.60 (d, $J = 1.0$ Hz, 1H, H⁺); 7.50–7.43 (m, 2H, H⁻); 7.38–7.32 (m, 2H, H⁺); 7.17 (d, $J = 1.0$ Hz, 1H, H⁺); 6.78 (dd, $J = 7.6, 5.6$ Hz, 1H, 1′-H); 5.42 (td, $J = 6.6, 2.3$ Hz, 1H, 3′-H); 4.48–4.37 (m, 3H, 5′-H and 4′-H); 2.45 (s, 3H, CH₃), 2.41 (dd, $J = 14.3, 7.6, 6.6$ Hz, 1H, 2′-H); 2.43 (s, 3H, CH₃), 2.41 (dd, $J = 14.3, 5.6, 2.3$ Hz, 1H, 2′-H), 1.06, acetone); m.p.: 88°C.

Acknowledgements Access open funding provided by University of Vienna. The authors thank S. Felsinger for recording NMR spectra and J. Theiner for combustion analyses.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References
1. Fyles AW, Milosevic M, Wong R, Kavanagh MC, Pintilie M, Sun A, Chapman W, Levin W, Manchui L, Keane TJ, Hill RP (1998) Radiother Oncol 48:149
2. Hanahan D, Weinberg RA (2011) Cell 144:646
3. Eales KL, Hollinshead KE, Tennant DA (2016) Oncogenesis 5:e190
4. Vaupel P, Mayer A (2007) Cancer Metastasis Rev 26:225
5. Dhani N, Fyles A, Hedley D, Milosevic M (2015) Semin Nucl Med 45:110
6. Kelada OJ, Carlson DJ (2014) Radiat Res 181:335
7. Kumar P, Bacchu V, Wiebe LJ (2015) Semin Nucl Med 45:122
8. Nunn A, Linder K, Strauss HW (1995) Eur J Nucl Med 22:265
9. Masaki Y, Shimizu Y, Yoshioka T, Numata Y, Yamaguchi Y, Tamaki N, Kuge Y (2015) Nature Sci Rep 5:16802
10. Rasey JS, Koh WJ, Evans ML, Peterson LM, Lewellen TK, Graham MM, Krohn KA (1996) Int J Radiat Oncol Biol Phys 36:417
11. Lehtio K, Oikonen V, Gronroos T, Eskola O, Kalliokoski K, Bergman J, Solin O, Grennan R, Nuutila P, Minn H (2001) J Nucl Med 42:1643
12. Pietr M, Machulla H-J, Picchio M, Reischl G, Ziegler S, Kumar P, Wester H-J, Beck R, McEwan AJB (2005) J Nucl Med 46:106
13. Halms OB, Bruine de Bruin L, Langendijk JA, van der Laan BF, Pruim J, Steenbakkers RJB (2014) Clin Nucl Med 39:44
14. Kumar P, Emami S, Kresoek Z, Yang J, McEwan AJB, Wiebe LI (2009) Med Chem 5:118
15. Patt M, Sorger D, Scheunemann M, Stücklin G (2002) Appl Radiat Isot 57:705
16. Schreiber A, Maier F, Ehrlichmann W, Laparter D, Kneillling M, Pichler JB, Hammerschmidt F, Reischl G (2016) Mol Med Biol 43:759
17. Weihe T, Kreis K, Krizková P, Scheifers A, Denk C, Stanek J, Mairinger S, Filip T, Sauberer M, Edelhofer P, Traxl A, Muchitsch VE, Mereiter K, Hammerschmidt F, Cass CE, Damaraju VL, Langer O, Kuntner C (2016) Bioorg Med Chem 24:5326
18. Bath CC (1968) In: Zorbach WW, Tipson RS (eds) Synthetic procedures in nucleic acid chemistry. Wiley, New York, p 521
19. Wang D, Nugent WA (2007) J Org Chem 72:7307
20. Schmidt L, Pedersen EB, Nielsen C (1994) Acta Chem Scand A, Chapman W, Levin W, Manchui L, Keane TJ, Hill RP (1998) Radiother Oncol 48:149
21. Searcey M, Pye PL, Lee JB (1989) Synth Commun 19:1309
22. Rao P, Benner SA (2001) J Org Chem 66:5012
23. Mikenda W (1992) Vib Spectrosc 3:327