Aminonucleotides and Their Derivatives; XIII[1]. Synthesis of Benzimidazole Azidonucleotides

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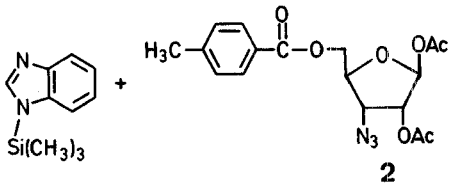
Nucleosides having benzimidazole as the heterocyclic base are known to possess mutagenic properties[2,3]. In continuation of our studies on the syntheses of derivatives of 3'-amino-3'-deoxynucleosides we prepared benzimidazole aminonucleosides of the ribo- and 2'-deoxyribo series.

1-(3-Azido-3-deoxy-β-D-ribofuranosyl)-benzimidazole **(1)** was obtained via glycosidation of silylated benzimidazole with 3-azido-3-deoxy-1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-β-D-ribofuranose **(2)**[4] in 60% yield following the method of Ref.[5].



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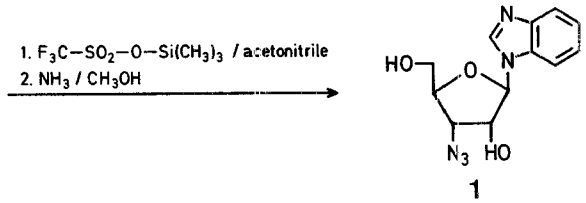

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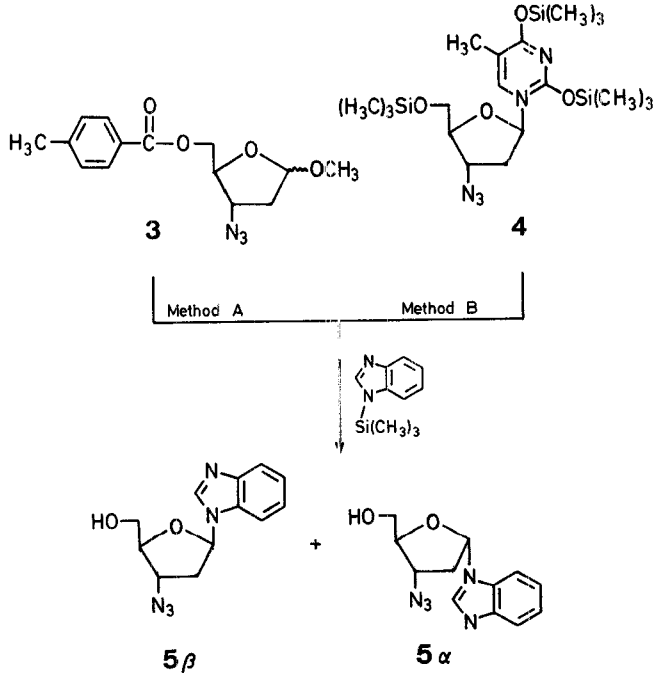
Two alternative routs were used to prepare 1-(3-azido-2,3-dideoxy-β-D-ribofuranosyl)-benzimidazole **(5)**: glycosidation of silylated benzimidazole with 1-*O*-methyl-3-azido-2,3-dideoxy-5-*O*-(4-methylbenzoyl)-D-ribofuranose **(3)**[1] according to the method of Ref.[6] (Method A), and transglycosidation (Method B). For the latter reaction, we chose the readily available 3'-azido-3'-deoxythymidine **(4)**[8] as the initial azidonucleoside. The yield of the β-anomer **(5β)** was 18% and that of the α-anomer **(5α)** 20% in both cases.



5β **5α**

It should be noted that 1-silylated benzimidazole tends to form a stable complex with some Lewis acids used as the glycosidation and/or transglycosidation catalysts. Thus, the tin(IV) chloride-1-(trimethylsilyl)-benzimidazole complex seems to be insoluble in the reaction solvent acetonitrile. We therefore performed the synthesis of azidonucleotide **1** from 1-trimethylsilylbenzimidazole and the protected azidofuranose **2** in the presence of 4–5 molecular equivalents of trimethylsilyl triflate; attempts to use only catalytic amounts of trimethylsilyl triflate led only to very low yields of product **1**.

T.L.C. was performed on Kieselgel F 60_{254} plates (Merck) in chloroform/methanol (9/1). Column chromatography was run on L 40/100 silica gel (Cavalier, CSSR). Preparative H.P.L.C. was performed on a Zorbax ODS (21.2 mm × 25 cm) column. A duPont 8800 chromatograph with a 2 ml sample loop was used to separate the anomers **5β** and **5α**. Elution was performed with a linear gradient of methanol in 0.1 molar ammonium acetate (64% methanol in 0.1 molar NH_4OAc → 80% methanol in 0.1 molar NH_4OAc, 30 min) at a flow rate of 5 ml/min. The eluates were U.V.-monitored at $\lambda = 290$ nm.

The $[\alpha]_D^{20}$ values were measured in methanol using a Perkin-Elmer 241 polarimeter. I.R. spectra were recorded with a Perkin-Elmer 250 spectrophotometer, U.V. spectra with a Beckman 25 spectrophotometer. ^{1}H-N.M.R. spectra were recorded on a Bruker Spectrospin instrument at 360 MHz.

1-(3'-Azido-3'-deoxy-β-D-ribofuranosyl)-benzimidazole (1):
Benzimidazole (345 mg, 3 mmol) is heated at reflux temperature in a mixture of hexamethyldisilazane (10 ml) and chlorotrimethylsilane (1 ml) until the solid material has completely dissolved. The mixture is then evaporated. The residue is coevaporated with toluene (2 × 10 ml), and dissolved in acetonitrile (15 ml) containing sugar derivative **2** (565 mg, 1.5 mmol). Trimethylsilyl trifluoromethanesulfonate (1 ml, 6 mmol) is added and the mixture is heated at reflux temperature for 6 h, then cooled, and poured into saturated sodium hydrogen carbonate solution (30 ml). The protected nucleoside is extracted with chloroform (3 × 25 ml), the extracts are washed with water (2 × 10 ml), dried with sodium sulfate, and concentrated to a small volume. The residue is applied to a silica gel column (2 × 10 cm). Elution is performed with chloroform/methanol (98/2). The fractions containing protected **1** are combined and evaporated to dryness. A saturated (at 4°C) solution of ammonia in methanol (30 ml) is added to the residue. The solution is kept at room temperature overnight, then evaporated to dryness. The deprotected nucleotide is purified on a silica gel column (2 × 10 cm) using chloroform/methanol (97/3) as eluent. The ribonucleotide **1** is obtained as an oil; yield: 130 mg (60%); R_f: 0.43; $[\alpha]_D^{20}$: + 51° (*c* 0.34, methanol).

$C_{12}H_{13}N_5O_3$	calc.	C 52.36	H 4.76	N 25.45
(275.3)	found	52.17	4.59	25.62

I.R. (KBr): $\nu = 2100\ cm^{-1}$ (N_3).

U.V. (CH_3OH): $\lambda_{max} = 246$ ($\varepsilon = 6000$); 253 (5600); 268 (2900); 277 (3350); 284 nm (3300).

^{1}H-N.M.R. (CD_3OD/TMS_{int}): $\delta = 8.46$ (s, 1H, 2-H); 7.73–7.60, 7.36–7.20 (2m, $4H_{arom}$); 5.94 (d, 1H, $J_{1',2'} = 5.5$ Hz, 1'-H); 4.74 (t, 1H, $J_{2',3'} = 5.7$ Hz, 2'-H); 4.20 (dd, 1H, $J_{3',4'} = 4.6$ Hz, 3'-H); 4.14–4.07 (m, 1H, $J_{4',5a'} = J_{4',5b'} = 3.0$ Hz, 4'-H); 3.88 (dd, 1H, $J_{5a',5b'} = 12.2$ Hz, 5a'-H); 3.77 ppm (dd, 1H, 5b'-H).

1-(3-Azido-2,3-dideoxy-D-ribofuranosyl)-benzimidazole (5α and 5β):
Method A, Glycosidation: Benzimidazole (283 mg, 2.4 mmol) and the sugar derivative **3** (344 mg, 1.2 mmol) are suspended in acetonitrile (10 ml), *N,O*-bis[trimethylsilyl]-acetamide (trimethylsilyl *N*-trimethylacetamidate; 0.4 ml) is then added and the mixture is heated at reflux temperature until the solid has dissolved completely.

The solution is cooled, trimethylsilyl trifluoromethanesulfonate (1 ml, 6 mmol) is added, the mixture is heated at reflux temperature for 2 h, poured into the saturated sodium hydrogen carbonate solution (30 ml), and extracted with chloroform (3 × 25 ml). The extracts are dried with sodium sulfate and evaporated to dryness. A saturated (at 4 °C) solution of ammonia in methanol (10 ml) is added to the residue. This solution is kept at room temperature overnight and evaporated to dryness. The residue is dissolved in 50% aqueous methanol and passed through a Dowex 1 × 8 ($OH^{\ominus}$) column (5 × 6 ml). The eluate is concentrated to a volume of 2 ml and the anomers are separated by preparative H.P.L.C., using two 1 ml injections. The retention times are 19.4 min for α-anomer **5**α and 21.3 min for β-anomer **5**β. The fractions containing the nucleosides are evaporated to dryness and water (10 ml) and chloroform (10 ml) are added to the residue. The chloroform layer is separated, dried with sodium sulfate, and evaporated to dryness to give colorless crystals: yield of anomer **5**α: 62 mg (20%); yield of anomer **5**β: 58 mg (18%).

Method B, Transglycosidation: 3′-Azido-3′-deoxythymidine (**4**; 400 mg, 1.5 mmol) and benzimidazole (354 mg, 3 mmol) are suspended in acetonitrile (15 ml); *N,O*-bis[trimethylsilyl]-acetamide (0.5 ml) is added and the mixture is heated at reflux temperature until the solid has dissolved. The solution is cooled, trimethylsilyl trifluoromethanesulfonate (1 ml, 6 mmol) is added, and the mixture is heated at reflux temperature for 3 h. The solution is again cooled, aqueous ammonia (5 ml) is added dropwise, and after 15 min the mixture is evaporated to dryness. Isolation of the anomers **5**α and **5**β is performed as in Method A; yield of anomer **5**α: 90 mg (20%); yield of anomer **5**β: 80 mg (18%).

α-Anomer **5**α: m.p. 77–79 °C; R_f: 0.68; $[\alpha]_D^{20}$: +107° (*c* 0.28, methanol).

$C_{12}H_{13}N_5O_2$	calc.	C 55.59	H 5.05	N 27.02
(259.3)	found	55.43	5.17	26.80

I.R. (KBr): $\nu = 2100\ cm^{-1}$ (N_3).

U.V. (methanol): λ_{max} = 248 (ε = 5900); 253 sh (5600); 268 (2900); 277 (3300); 284 nm (3200).

^{1}H-N.M.R. (CD_3OD/TMS_{int}): δ = 8.32 (s, 1H, 2-H); 7.73–7.71, 7.38–7.27 (2m, $4H_{arom}$); 6.40 (dd, 1H, $J_{1',2a'}$ = 7 Hz, $J_{1',2b'}$ = 3.8 Hz, 1′-H); 4.49–4.43 (m, 1H, $J_{3',4'}$ = 3.9 Hz, 3′-H); 4.27–4.21 (m, 1H, $J_{4',5a'}$ = 4.3 Hz, $J_{4',5b}$ = 4.0 Hz, 4′-H); 3.73 (dd, 1H, 5a′-H); 3.69 (dd, 1H, $J_{5a',5b'}$ = 14.4 Hz, 5b′-H); 3.05–2.92 (m, 1H, $J_{2a',3'}$ = 8.0 Hz, 2a′-H); 2.63–2.54 (m, 1H, $J_{2b',3'}$ = 4.37 Hz, $J_{2a',2b'}$ = 15 Hz, 2b′-H).

β-Anomer **5**β: m.p. 106–107 °C; R_f: 0.73; $[\alpha]_D^{20}$: +1.84 (*c* 0.27; methanol).

$C_{12}H_{13}N_5O_2$	calc.	C 55.59	H 5.50	N 27.02
(259.3)	found	55.47	4.92	27.25

I.R. (KBr): $\nu = 2100\ cm^{-1}$ (N_3).

U.V. (methanol): λ_{max} = 249 (ε = 6100); 255 sh (5600); 269 (2900); 278 (3400); 284 nm (3300).

^{1}H-N.M.R. (CD_3OD/TMS_{int}): δ = 8.45 (s, 1H, 2-H); 7.72–7.61, 7.37–7.27 (2m, $4H_{arom}$); 6.33 (t, 1H, $J_{1',2a'} = J_{1',2b'}$ = 6.4 Hz, 1′-H); 4.52–4.44 (m, 1H, $J_{3',4'}$ = 5.0 Hz, 3′-H); 4.11–2.96 (m, 1H, $J_{4',5a'} = J_{4',5b'}$ = 3.8 Hz, 4′-H); 3.83–3.71 (m, 2H, $J_{5a',5b'}$ = 12.3 Hz, 5a′-H, 5b′-H); 2.85–2.74 (m, 1H, $J_{2a',3'}$ = 7.3 Hz, 2a′-H); 2.62–2.51 ppm (m, 1H, $J_{2b',3'}$ = 5.0 Hz, $J_{2a',2b'}$ = 13.6 Hz, 2b′-H).

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