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SHORT COMMUNICATIONS

Synthesis of Nucleosides Containing a Photolabile 2-(2-Nitrophenyl)propoxycarbonyl Group

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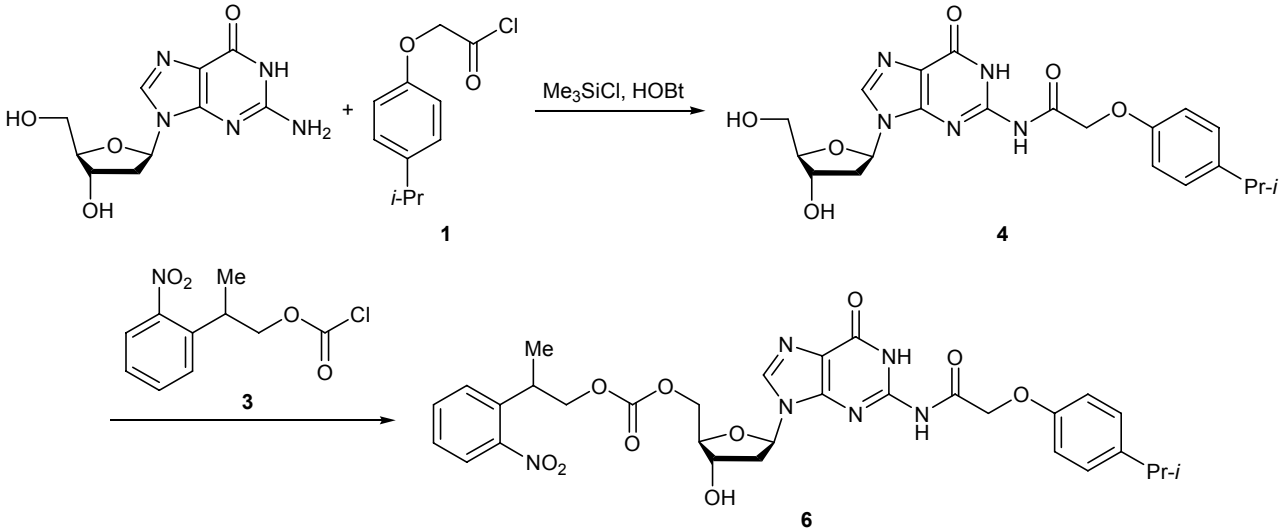
At present, DNA biochips are widely used in medicine as diagnostic systems [1, 2]. Oligonucleotide biochips are often obtained with the aid of photolabile protecting groups [3–7]. The latter should be stable under the conditions of oligonucleotide synthesis but should be readily removed by photolysis without involving the protected moiety. A widely used photolabile protecting group is the *o*-nitrobenzyl group [3, 8–10]. The goal of the present work was to extend the series of nucleosides protected by the photolabile 2-(2-nitrophenyl)propoxycarbonyl group and containing readily removable protecting groups in the aromatic heteroring, which can subsequently be used in the design of oligonucleotide biochips.

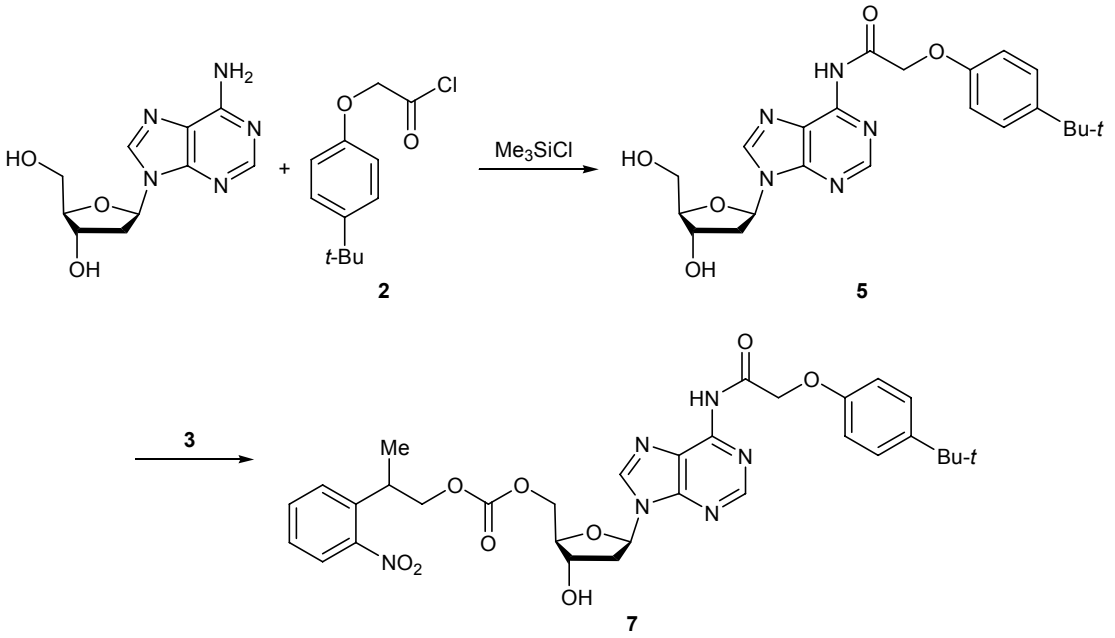
We have synthesized previously unknown nucleosides **4**–**7**. Compounds **4** and **5** were obtained by acylation of 2′-deoxyguanosine and 2′-deoxyadenosine with phenoxyacetyl chlorides **1** and **2**, respectively. Nucleosides **6** and **7** protected by photolabile 2-(2-nitrophenyl)propoxycarbonyl groups were synthesized by treatment of compounds **4** and **5**, respectively, with 2-(2-nitrophenyl)propyl chloroformate (**3**) which was prepared as described in [10]. Compounds **6** and **7** were isolated as mixtures of diastereoisomers.

Scheme 1.



HOBt is 1-hydroxybenzotriazole.

Scheme 2.



***N*^{2}-(4-Isopropylphenoxyacetyl)-2′-deoxyguanosine (4).** 2′-Deoxyguanosine, 1.14 g (4 mmol), was dried by double vacuum evaporation of its mixture with 20 mL of anhydrous pyridine and was dispersed in 20 mL of anhydrous pyridine, 2.52 g (3 mL, 20 mmol) of chloro(trimethyl)silane was added, and the mixture was stirred for 25 min at room temperature. 1-Hydroxybenzotriazole (HOBt), 0.86 g (6.4 mmol), was dried by triple distillation of its mixture with 3 mL of anhydrous acetonitrile and dispersed in a mixture of 3 mL of anhydrous acetonitrile and 3 mL of anhydrous pyridine, and 1.28 g (6 mmol) of (4-isopropylphenoxy)acetyl chloride was added to the mixture. Both reactant mixtures were cooled on an ice bath, and the suspension of 2′-deoxyguanosine was added to the second mixture. The resulting mixture was stirred for 8 h at room temperature (TLC), cooled with ice-cold water, and treated with 2 mL of water and 2.5 mL of concentrated aqueous ammonia (TLC). The precipitate was filtered off, the filtrate was evaporated, and the residue was combined with the precipitate and treated with water. The white voluminous solid was filtered off, washed with water and diethyl ether, and dried in air until constant weight. Yield 1.75 g (99%), mp 234–235°C (decomp.). IR spectrum (KBr), ν, cm^{-1}: 3501, 3426, 3221, 3117, 3049, 2951, 1686, 1614, 1555, 1514, 1483, 1418, 1366, 1329, 1242, 1221, 1150, 1105, 1088, 1059, 976, 928, 833, 787, 772, 644. UV spectrum (EtOH): λ_{max} 261 nm (logε 4.05). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.16 d (6H, CH_3, J = 6.8 Hz), 2.29 m (1H, CH), 2.58 m (1H, CH), 2.82 m (1H, CH), 3.55 m (2H, CH_2), 3.84 m (1H, CH), 4.38 m (1H, CH), 4.83 m (2H, CH_2), 6.22 m (1H, CH), 6.88 d and 7.16 d (2H each, H_{arom}, J = 8.5 Hz), 11.79 s (1H, NH), 11.83 s (1H, NH). Found, %: C 56.99; H 5.32; N 15.83. $C_{21}H_{25}N_5O_6$. Calculated, %: C 56.87; H 5.68; N 15.79.

***N*^{6}-(4-*tert*-Butylphenoxyacetyl)-2′-deoxyadenosine (5).** 2′-Deoxyadenosine, 3.96 g (15 mmol), was dried by double vacuum evaporation of its mixture with 50 mL of anhydrous pyridine and was dissolved on heating in 110 mL of anhydrous pyridine. The solution was cooled in a stream of argon on an ice bath, 7.7 g (9 mL, 71 mmol) of chloro(trimethyl)silane was added dropwise, and the mixture was stirred for 30 min at room temperature. The mixture was then cooled again on an ice bath, 7 g (31 mmol) of (4-*tert*-butylphenoxy)acetyl chloride was added dropwise, the mixture was stirred for 2 h at room temperature and kept for 15 h at room temperature, 200 mL of a saturated solution of sodium hydrogen carbonate was added, and the mixture was stirred until carbon dioxide no longer evolved. The resulting solution was partially evaporated under reduced pressure, and the residue was extracted with methylene chloride. The extract was dried over $MgSO_4$ and evaporated, and residual pyridine was removed by evaporation with toluene. The viscous residue, 11.37 g, was purified by silica gel column chromatography (gradient elution with chloroform–methanol, 0 to 5% of the latter). Yield 4.13 g

(62%), mp 88–89°C. IR spectrum (KBr), ν, cm^{-1}: 3371, 2961, 2868, 1716, 1614, 1585, 1512, 1462, 1225. UV spectrum (EtOH), λ_{max}, nm (log ε): 213 (4.46), 272 (4.28). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.28 s (9H, *t*-Bu), 2.37 d.d (1H, CH, *J* = 12, 6 Hz), 2.97 m (1H, CH), 3.80 d (1H, CH, *J* = 12 Hz), 3.94 d (1H, CH, *J* = 12 Hz), 4.19 m (1H, CH), 4.78 d (1H, CH, *J* = 4.5 Hz), 4.82 s (2H, CH_2), 6.39 d.d (1H, CH, *J* = 8, 6 Hz), 6.94 d and 7.32 d (2H each, H_{arom}, *J* = 8 Hz), 8.22 s (1H, CH), 8.71 s (1H, CH), 9.59 s (1H, NH). Found, %: C 59.47; H 5.93; N 15.67. $C_{22}H_{27}N_5O_5$. Calculated, %: C 59.86; H 6.12; N 15.87.

***N*^{2}-(4-Isopropylphenoxyacetyl)-5′-*O*-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyguanosine (6).** Nucleoside **4**, 1.77 g (4 mmol), was dried by triple distillation of its mixture with 15 mL of anhydrous pyridine and was dissolved on heating in 15 mL of anhydrous pyridine. The solution was cooled to –40°C, a solution of 1.31 g (5.4 mmol) of compound **3** in 10 mL of anhydrous methylene chloride was added dropwise, and the mixture was stirred for 5 h at a temperature not exceeding –40°C. The mixture was then treated with water (40 mL) and extracted twice with methylene chloride. The combined extracts were dried over $MgSO_4$ and evaporated, and the residue was dried by triple evaporation of its mixture with 40 mL of toluene and subjected to silica gel column chromatography (successive elution with methylene chloride and methylene chloride–methanol, 100:1, 100:2, 100:4). Yield 1.46 g (56%), mp 71–72°C. IR spectrum (KBr), ν, cm^{-1}: 3485, 3395, 3200, 2959, 1753, 1680, 1610, 1528, 1514, 1356, 1252. UV spectrum (EtOH): λ_{max} 256 nm (log ε 4.30). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.17 d (6H, CH_3, *J* = 6.9 Hz), 1.27 d (3H, CH_3, *J* = 7.0 Hz), 2.17 s (1H, OH), 2.52 m (1H, CH), 2.59–2.71 m (1H, CH), 2.83 m (1H, CH), 3.66 m (1H, CH), 4.11–4.36 m (5H, CH_2, CH), 4.69 s (2H, CH_2), 4.74 m (1H, CH), 6.27 m (1H, CH), 6.85 d and 7.12 d (2H each, H_{arom}, *J* = 8.5 Hz), 7.29 m (1H, H_{arom}), 7.40 m (1H, H_{arom}), 7.51 m (1H, H_{arom}), 7.67 m (1H, CH), 7.89 s and 7.94 s (1H, 8-H). Found, %: C 57.23; H 4.99; N 12.97. $C_{31}H_{34}N_6O_{10}$. Calculated, %: C 57.23; H 5.23; N 12.92.

***N*^{6}-(4-*tert*-Butylphenoxyacetyl)-5′-*O*-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyadenosine (7).** Nucleoside **5**, 3.3 g (7.5 mmol), was dried by double evaporation of its mixture with 15 mL of anhydrous pyridine and was dissolved in 20 mL of anhydrous pyridine. The solution was cooled to –50°C, a solution of 2.61 g (10.7 mmol) of compound **3** in 25 mL of anhydrous methylene chloride was added dropwise, and the mixture was stirred for 5 h at –20 to –50°C and kept for 10 h in a refrigerator. The mixture was then treated with 50 mL of water, stirred for 30 min, and extracted with methylene chloride, and the extract was dried over $MgSO_4$ and evaporated. The residue was subjected to silica gel column chromatography (gradient elution with methylene chloride–methanol, 0 to 10% of the latter). Yield 2.1 g (44%), mp 76–77°C. IR spectrum (KBr), ν, cm^{-1}: 3370, 2960, 1749, 1612, 1525, 1263, 1244. UV spectrum (EtOH): λ_{max} 273 nm (log ε 4.35). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 s (9H, *t*-Bu), 1.35 d and 1.33 d (3H, CH_3, *J* = 3 Hz), 2.56 m (1H, CH), 2.83 m (1H, CH), 3.76 m (1H, CH), 4.15–4.40 m (5H, CH_2, CH), 4.69 m (1H, CH), 4.81 s (2H, CH_2), 6.51 t (1H, CH, *J* = 6 Hz), 6.96 d (2H, H_{arom}, *J* = 10 Hz), 7.33 m (3H, H_{arom}), 7.45 d (1H, H_{arom}, *J* = 8 Hz), 7.55 t (1H, H_{arom}, *J* = 8 Hz), 7.73 d (1H, H_{arom}, *J* = 8 Hz), 8.16 s and 8.21 s (1H, 8-H), 8.74 s and 8.75 s (1H, 2-H), 9.47 s and 9.49 s (1H, NH). Found, %: C 59.29; H 5.56; N 12.59. $C_{32}H_{36}N_6O_9$. Calculated, %: C 59.26; H 5.56; N 12.96.

The analytical and spectral data were obtained at the Joint Chemical Service Center, Siberian Branch, Russian Academy of Sciences. The IR spectra were recorded on a Bruker Vector 22 spectrometer. The UV spectra were measured on a Hewlett Packard 4853 spectrophotometer. The ^1H NMR spectra were obtained on a Bruker AV-400 instrument using the residual proton signal of the solvent as reference ($CHCl_3$, δ 7.26 ppm; DMSO-d_5, δ 2.50 ppm). 2′-Deoxyguanosine, 2′-deoxyadenosine, 1-hydroxybenzotriazole, (4-*tert*-butylphenoxy)acetyl chloride, (4-isopropylphenoxy)acetyl chloride, and chloro(trimethyl)silane were commercial products. Pyridine, acetonitrile, and methylene chloride were dried according to standard procedures.

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