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A NEW SYNTHESIS OF 3-DEAZATHYMIDINE AND OF A RELATED PHOSPHORAMIDITE SYNTHON

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Key Words: Barton-McCombie deoxygenation; 3-deazathymine; 3-deazathymidine; triisopropylphenylsulfonate; oligonucleotide synthesis.

Abstract: *3-Deazathymine (**6**), a DNA base analog, was synthesized in a new way. The β-selective glycosylation to the deoxynucleoside derivative and the use of the uncommon triisopropylphenylsulfonyl (TIPS) protecting group (→**7**) lead to a high yield of 3-deazathymidine (**8**). The TIPS-nucleoside derivative **9** is readily converted into the phosphoramidite **10**, a suitable derivative for automatic DNA synthesis.*

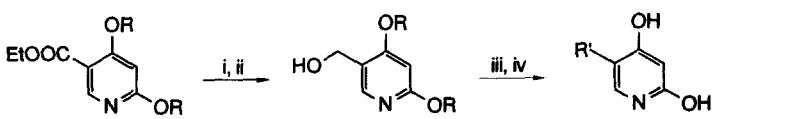
INTRODUCTION

For our studies of the specificity of replication by DNA polymerase I [1] a phosphoramidite derivative of 3-deazathymidine was required. 3-Deazapyrimidine (pyridine) nucleoside analogs have been prepared since the late sixties [2,3], but a shorter and more selective procedure was needed for our purpose.

RESULTS

Our synthesis of 3-deazathymine (**6**) started from ethyl 2,4-dihydroxypyridine-5-carboxylate [4] (**1**) (Scheme 1).

Scheme 1



1 R = H
2 R = TBDMS

3 R = TBDMS
4 R = H

5 R' = p-Tol-OCSOCH$_2$
6 R' = CH$_3$

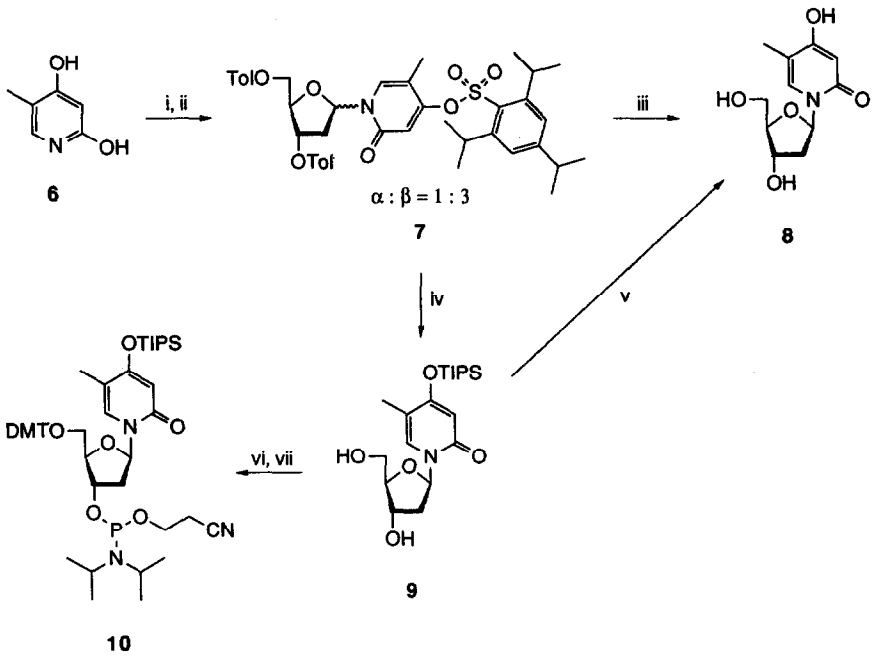
Reagents:

(i) TBDMSCl, imidazole; (ii) $LiAlH_4$, then 80% AcOH; (iii) *O-p*-tolyl chlorothioformate; (iv) Bu_3SnH, AIBN.

Diol **1** was disilylated with TBDMSCl and reduced with $LiAlH_4$ to alcohol **3**, which is sensitive to hydrolysis. This alcohol was desilylated quantitatively to triol **4** by reaction with 80% acetic acid (60% from **1**). The poorly soluble compound **4** was converted into the corresponding thiocarboxylate **5** as a 1% v/w solution in pyridine. The intermediate **5** was reduced *in situ* with Bu_3SnH to 3-deazathymine (**6**). The overall yield of this *Barton-McCombie* deoxygenation, taken recovered **5** into account, was 48%.

The glycosylation of **6** was carried out with anomerically pure (^1H NMR) *O*^{3},*O*^{5}-*di*-*p*-tolyl-2-deoxy-α-D-ribofuranosyl chloride [5] (Scheme 2).

Scheme 2



Reagents:

(i) $(TMS)_2NH$, then *O*^{3},*O*^{5}-*di*-*p*-tolyl-2-deoxy-α-D-ribofuranosyl chloride, $ZnCl_2$, then MeOH; (ii) NaH, then triisopropylphenylsulfonyl chloride (TIPSCl); (iii) NaOMe/MeOH (RT, 6 h); (iv) NaOMe/MeOH (0°, 100 min.); (v) conc. NH_3 (H_2O/MeOH 9:1); (vi) 4,4'-dimethoxytrityl chloride; (vii) chloro β-cyanoethyl *N*,*N*-diisopropylphosphoramidite, $(i\text{-}Pr)_2NEt$.

For this purpose, base **6** was bis-trimethylsilylated with hexamethyldisilazane. Then a solution of the chloro sugar was added to the silyl ether. After methanolysis and extraction, the diastereoisomeric ratio of the reaction mixture was investigated by ^1H NMR [6]. The ratio could be increased from 1:1 to 3:1 in favour of the desired β-anomeric product by presence of 0.1 equiv. $ZnCl_2$. The crude glycosylation mixture was treated first with NaH in THF and then with TIPSCl [7]. The β-anomer of **7** was easily separated by chromatography on silica gel. The overall yield of the silylation, glycosylation and TIPS-protection amounted to 45%. For characterization, triester **7** was completely deprotected to form 3-deazathymidine (**8**) with NaOMe (RT, 6h). After chromatography on silica gel, the nucleoside analog was isolated in a

yield of 70% and crystallized from MeOH/diisopropyl ether. TIPS-protected 3-deazathymidine (**9**) was required for the following sequence of reactions. This compound was obtained in a yield of 82% by selective methanolysis of **7** under mild conditions (0°,100 min.). The clean aminolysis of **9** to form **8** within 6 hours with conc. NH_3 at 55° verifies the compatibility of the TIPS protecting group with the oligonucleotide synthesis by most conventional approaches. Compound **9** was 4,4'-dimethoxytritylated (89%) and phosphitylated to synthon **10** (72%) using standard reaction conditions [8].

The uncommon use of TIPS for protection provides several advantages. Other nucleoside derivatives, such as the *O*^{4}-*tert.*-butyldimethylsilyl or the *O*^{4}-acetyl derivative, either turned out to be chemically unstable in solution or on silica gel. The solubility and chromatographic separation of the anomers after glycosylation has been considerably improved by TIPS protection. Finally, the TIPS group can be used as permanent 3-deazathymidine protection during oligonucleotide synthesis.

EXPERIMENTAL SECTION

General methods: For the recording of 60 MHz ^{1}H NMR spectra a Varian EM 360 spectrometer was used. 400 MHz ^{1}H, 101 MHz ^{13}C and 162 MHz ^{31}P NMR spectra were recorded on a Varian VXR-400 spectrometer, 300 MHz ^{1}H NMR spectra on a Varian Gemini 300 spectrometer. Optical rotations were measured with a Perkin Elmer 141 polarimeter. Melting points were obtained on a hot-plate apparatus and are corrected. Mass spectra were recorded on a mass spectrometer VG 70-250 by Dr. H. Nadig. Elemental analyses were carried out by Dr. L. Strauch in the microanalytical laboratory.

Ethyl 2,4-di-(*tert.*-butyldimethylsilyloxy)-pyridine-5-carboxylate (2)

Ester **1** (32.0 g, 175 mmol), imidazole (47.6 g, 699 mmol) and TBDMSCl (52.7 g, 350 mmol) were stirred in DMF (930 ml) at room temperature overnight. The reaction mixture was partitioned between diethyl ether and water. Evaporation of the dried (Na_2SO_4) organic layer afforded the water-sensitive product **2** in a yield of 89% (64.2 g, 156 mmol). **2**: Oil; CI-MS (NH_3): m/z 412 (100, [M+H]+), 371, 355, 354; ^{1}H NMR (60 MHz, $CDCl_3$): 8.55 (s, 1H), 6.05 (s, 1H), 4.3 (q, J = 7, 2H), 1.3 (t, J = 7, 3H), 1.0 (s, 9H), 0.95 (s, 9H), 0.3 (s, 6H), 0.25 (s, 6H) ppm.

2,4-Di-(*tert.*-butyldimethylsilyloxy)-5-hydroxymethyl-pyridine (3)

A solution of the silyl ether **2** (24.0 g, 58.4 mmol) in Et_2O (75 ml) was added dropwise to a suspension of $LiAlH_4$ (4.43g, 117 mmol) in Et_2O (400 ml). The reaction mixture was kept between -40 and -50° during 3 h. After destruction of the excess $LiAlH_4$ with ethyl acetate (60 ml), H_2O (7 ml), 15% NaOH (7 ml) and H_2O (25 ml) were subsequently added. The reaction mixture was partitioned between ether and water. The organic layer was separated, washed with sat. NH_4Cl-solution (3·50 ml) and dried (Na_2SO_4). After removal of the solvent, the silylated compound **3** was obtained as a colourless oil in a yield of 78% (16.9 g, 45.7 mmol).

2,4-Dihydroxy-5-hydroxymethyl-pyridine (4)

An emulsion of the crude silyl ether **3** (16.9 g, 45.7 mmol) in 80% acetic acid was stirred at room temperature for 2 h. Then the solvent was removed under reduced pressure. Repeated coevaporation with benzene yielded 86% (5.52 g, 39.1 mmol) of the pure alcohol **4**

as beige crystals; m. p. 230° (DSC); FAB-MS: m/z 142 ([M+H]+), 124, 112; ^{1}H NMR (400 MHz, DMSO-d_6): 10.8 (br s, 1H), 7.09 (s, 1H), 5.54 (s, 1H), 4.57 (br s, 1H), 4.21 (d, J = 1 Hz, 2H) ppm.

3-Deazathymine (6)

O-*p*-tolyl chlorothiolformate (3.37 ml, 21.8 mmol) in abs. THF (2 ml) was added dropwise to a solution of the alcohol **4** (2.80 g, 19.8 mmol) in abs. pyridine (280 ml) of 0°. After stirring overnight at 5°, the solvent was removed under reduced pressure. The resulting thiocarboxylate **5** was isolated from a sample of the obtained residue for analytical purposes by column chromatography (CH_2Cl_2/MeOH 9:1). **5**: Colourless crystals; m. p. 300° (DSC); CI-MS (NH_3): m/z 126 ([M+H]+), 112, 84; ^{1}H NMR (400 MHz, DMSO-d_6): 10.75 (br s, 2H), 7.02 (s, 1H), 5.56 (s, 1H), 1.83 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 166.7, 163.7, 133.0, 107.0, 98.1, 12.1 ppm; elemental analysis calcd. for $C_6H_7NO_2$: C 57.59, H 5.64, N 11.19%; found: C 57.35, H 5.31, N 11.34%.

Last traces of pyridine were removed by repeated coevaporation with diglyme (10 ml) and toluene (50 ml) under exclusion of moisture. Bu_3SnH (15.7 ml, 59.2 mmol) and AIBN (650 mg, 3.96 mmol) were added to a degassed suspension of the residue in diglyme (255 ml). The reaction mixture was stirred at 66° for 72 h. Additional AIBN (650 mg, 3.96 mmol) was added every 24 h. After distilling off the solvent under high vacuum at 66°, 3-deazathymine (**6**) was isolated by flash chromatography (EtOAc/MeOH/H_2O 4:0.6:0.1) in 24% from **4** (602 mg, 4.81 mmol). In addition, 50% of the thiocarboxylate **5** could be recovered. **6**: Colourless crystals; m. p. 300° (DSC); CI-MS (NH_3): m/z 126 ([M+H]+), 112, 84; ^{1}H NMR (400 MHz, DMSO-d_6): 10.75 (br s, 2H), 7.02 (s, 1H), 5.56 (s, 1H), 1.83 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 166.7, 163.7, 133.0, 107.0, 98.1, 12.1 ppm; elemental analysis calcd. for $C_6H_7NO_2$: C 57.59, H 5.64, N 11.19%; found: C 57.35, H 5.31, N 11.34%.

4-O-Triisopropylphenylsulfonyl-3',5'-*di*-O-*p*-tolyl-3-deazathymidine (β-7)

3-Deazathymine (**6**) (50.0 mg, 400 µmol) was refluxed in hexamethyldisilazane (1.8 ml) for 2 h. After evaporation of the excess reagent the disilylated 3-deazathymine was dissolved in abs. $CHCl_3$ (1.1 ml). $ZnCl_2$ (5.5 mg, 40 µmol) and *O*^{3},*O*^{5}-*di*-*p*-tolyl-2-deoxy-α-D-ribofuranosyl chloride (156 mg, 401 µmol) in abs. $CHCl_3$ (1.1 ml) were added. After stirring at room temperature for 2 h, MeOH (0.5 ml) was added to the reaction mixture and it was stirred overnight. Then the solvent was removed and the residue was partitioned between $CHCl_3$ and water. The organic phase was dried (Na_2SO_4) and evaporated. A suspension of the obtained residue in abs. THF (11 ml) was added to NaH (45.8 mg, 1.91 mmol) in abs. THF (5 ml). After 30 min. TIPSCl (121 mg, 400 µmol) was added. The reaction mixture was stirred at room temperature for 1 h and poured to 60 ml $CHCl_3$ / 20 ml sat. NH_4Cl-solution. The organic layer was separated, dried (Na_2SO_4) and evaporated. The resulting TIPS-ester β-**7** was isolated from the residue by flash chromatography (petroleum ether/ether 7:3 and 6:4) in an overall yield (from **6**) of 45% (134 mg, 180 µmol). β-**7**: Amorphous solid; $[\alpha]_D^{24}$ = +17° (c = 1.05, $CHCl_3$); FAB-MS: m/z 744 ([M+H]+), 392, 353, 307, 267, 192, 154; ^{1}H NMR (300 MHz, $CDCl_3$): 7.95 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.5 Hz, 2H), 7.52 (s, 1H), 7.23-7.27 (m, 6H), 6.55 (dd, J = 8.5, 5.5 Hz, 1H), 5.92 (s, 1H), 5.62 (d, J = 6.5 Hz, 1H), 4.81 (dd, J = 12, 3 Hz, 1H), 4.68 (dd, J = 12, 3.5 Hz, 1H), 4.59 (m, 1H), 4.07 (sept, J = 6.5 Hz, 2H), 2.87-3.02 (m, 2H), 2.41 (s, 6H), 2.14-2.27 (m, 1H), 1.85 (s, 3H), 1.29 (d, J = 7 Hz, 6H), 1.25 (d, J = 6.5 Hz, 12H) ppm.

3-Deazathymidine (8)

A solution of the sulfonate β-**7** (96.9 mg, 130 μmol) in 0.1 N NaOMe/MeOH (2.6 ml) was stirred at room temperature for 6 h. Then Dowex® 50WX8 (H^+) (270 mg) was added. 10 min. later the reaction mixture was transferred into a column and eluated with MeOH (60 ml). After removal of the solvent, the residue was purified by column chromatography (EtOAc/MeOH/H_2O 4:0.75:0.1). 3-Deazathymidine (**8**) was obtained in a yield of 70% (21.9 mg, 90.8 μmol). **8**: Colourless crystals; m. p. 200.5-201.5° (MeOH/diisopropyl ether); $[\alpha]_D^{25}$ = +49° (c = 1.00, MeOH); FAB-MS: m/z 242 ([M+H]$^+$), 126, 117; ^{1}H NMR (400 MHz, DMSO-d_6): 10.80 (br s, 1H), 7.55 (s, 1H), 6.35 (dd, J = 7, 7 Hz, 1H), 5.58 (s, 1H), 5.19 (br s, 1H), 5.01 (br s, 1H), 4.22 (m, 1H), 3.76 (m, 1H), 3.53-3.63 (m, 2H), 2.06-2.13 (m, 1H), 1.89-1.99 (m, 1H), 1.87 (s, 3H) ppm; ^{13}C NMR (101 MHz, CD_3OD): 169.0, 165.7, 133.2, 112.3, 98.6, 88.8, 86.3, 72.0, 62.8, 42.5, 12.8 ppm; elemental analysis calcd. for $C_{11}H_{15}NO_5$: C 54.77, H 6.27, N 5.81%; found: C 54.68, H 6.01, N, 5.52%.

3-Deaza-4-O-triisopropylphenylsulfonyl-thymidine (9)

A suspension of the sulfonate β–**7** (2.26 g, 3.04 mmol) in 0.1 N NaOMe/MeOH (61 ml) was stirred at 0° for 100 min. Then Dowex® 50WX8 (H^+) (6.3 g) was added. After 20 min. the reaction mixture was eluated with MeOH (2.6 l). Column chromatography (CH_2Cl_2/MeOH 94:6) of the evaporated eluate afforded the product **9** in a yield of 82%. **9**: Amorphous solid; $[\alpha]_D^{24}$ = +59° (c = 1.07, MeOH); FAB-MS: m/z 508 ([M+H]$^+$), 392, 267,203, 175, 117; ^{1}H NMR (300 MHz, $CDCl_3$): 7.74 (s, 1H), 7.23 (s, 2H), 6.29 (dd, J = 6.5, 6.5 Hz, 1H), 6.05 (s, 1H), 4.48 (m, 1H), 4.42 (br s, 1H), 3.98-4.13 (m, 3H), 3.73-3.93 (m, 3H), 2.94 (sept, J = 7 Hz, 1H), 2.39-2.51 (m, 1H), 2.13-2.25 (m, 1H), 2.05 (s, 3H), 1.28 (d, J = 7 Hz, 6H), 1.24 (d, J = 7 Hz, 12 H) ppm.

β-Cyanoethyl-3-deaza-5'-O-dimethoxytrityl-thymidine-3'-O-*N,N*-diisopropylamino-phosphoramidite (10)

A solution of the sulfonate **9** (400 mg, 788 μmol) and dimethoxytrityl chloride (294 mg, 868 μmol) was stirred at room temperature for 3 h. Then MeOH (2 ml) was added. 10 min. later the solvent was removed and the residue was suspended in $CHCl_3$ (15 ml). After washing with H_2O and 2% $NaHCO_3$-solution, the organic phase was dried (Na_2SO_4) and evaporated. Flash chromatography (CH_2Cl_2/MeOH 98:2) furnished the resulting dimethoxytrityl ether in a yield of 89% (566 mg, 699 μmol). This compound was dried by evaporation of abs. pyridine, abs. toluene and abs. THF (each 10 ml). A solution of this dimethoxytrityl ether, (*i*-Pr)$_2$NEt (430 μmol, 2.51 mmol) and chloro β-cyanoethyl *N,N*-diisopropylamino phosphoramidite (280 μmol, 1.26 mmol) was stirred at room temperature for 4 h. Then the reaction mixture was filtered and evaporated. The residue was suspended in toluene/Et_3N 9:1 (30 ml), washed twice with cold 10% Na_2CO_3-solution and dried (Na_2SO_4). The solvent was removed and the residue was purified by flash chromatography (petroleum ether/EtOAc/Et_3N 5:3:2). The phosphoramidite synthon **10** was obtained in a yield of 72% (from the dimethoxytritylated intermediate) (451 mg, 446 mmol). **10** (2 diastereoisomers): Amorphous solid; FAB-MS: m/z 1048 ([M+K]$^+$), 792, 706, 690, 488, 392, 319, 303, 201; ^{1}H NMR (300 MHz, $CDCl_3$ + traces py-d_5): 7.84/7.78 (s, 1H), 7.41 (m, 2H), 7.19-7.36 (m, 9H), 6.83 (dd, J = 9, 3.5 Hz, 4H), 6.40-6.50 (m, 1H), 5.90/5.91 (s, 1H), 4.53-4.66 (m, 1H), 4.19 (m, 1H), 4.08 (sept, J = 6.5 Hz, 2H), 3.794/3.787 (s, 6H), 3.46-3.64 (m, 4H), 3.24-3.43 (m, 2H), 2.94 (sept, J = 7 Hz, 1H), 2.51-2.63 (m, 2H), 2.40 (t, J = 6.5 Hz, 1H), 2.13-2.26 (m, 1H), 1.71/1.69 (s, 3H), 1.28 (d, J = 7 Hz, 6H), 1.25 (d, J = 6.5 Hz, 12H), 1.16/1.15 (d, J = 7 Hz, 6H), 1.05 (m, 6H) ppm; ^{31}P NMR (162 MHz,

referenced to external $(PhO)_3PO$, which is -18 ppm rel. to 85% H_3PO_4, py-d_5): 13.69 (s, 10%, side product), 7.62/7.54 (s, 90%, product) ppm.

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   b) ^1H NMR (300 MHz, $CDCl_3$): α-H(1') of β-chlorodeoxyribose deriv.: 6.48 ppm (d, J = 5 Hz); β-H(1') of α-chlorodeoxyribose deriv.: 7.25 ppm (dd, J = 8, 8 Hz).
6. ^1H NMR (300 MHz, $CDCl_3$): α-H(1') of β-nucleoside: 6.54 ppm (dd, J = 7, 6 Hz); β-H(1') of α-nucleoside: 6.34 ppm (dd, J = 5.5, < 0.5 Hz).
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