Syntheses of Fluorescent Pteridinyl Base-Containing Phosphoramidite Analogs for Use in Ribosyl Oligonucleotide Syntheses

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Background

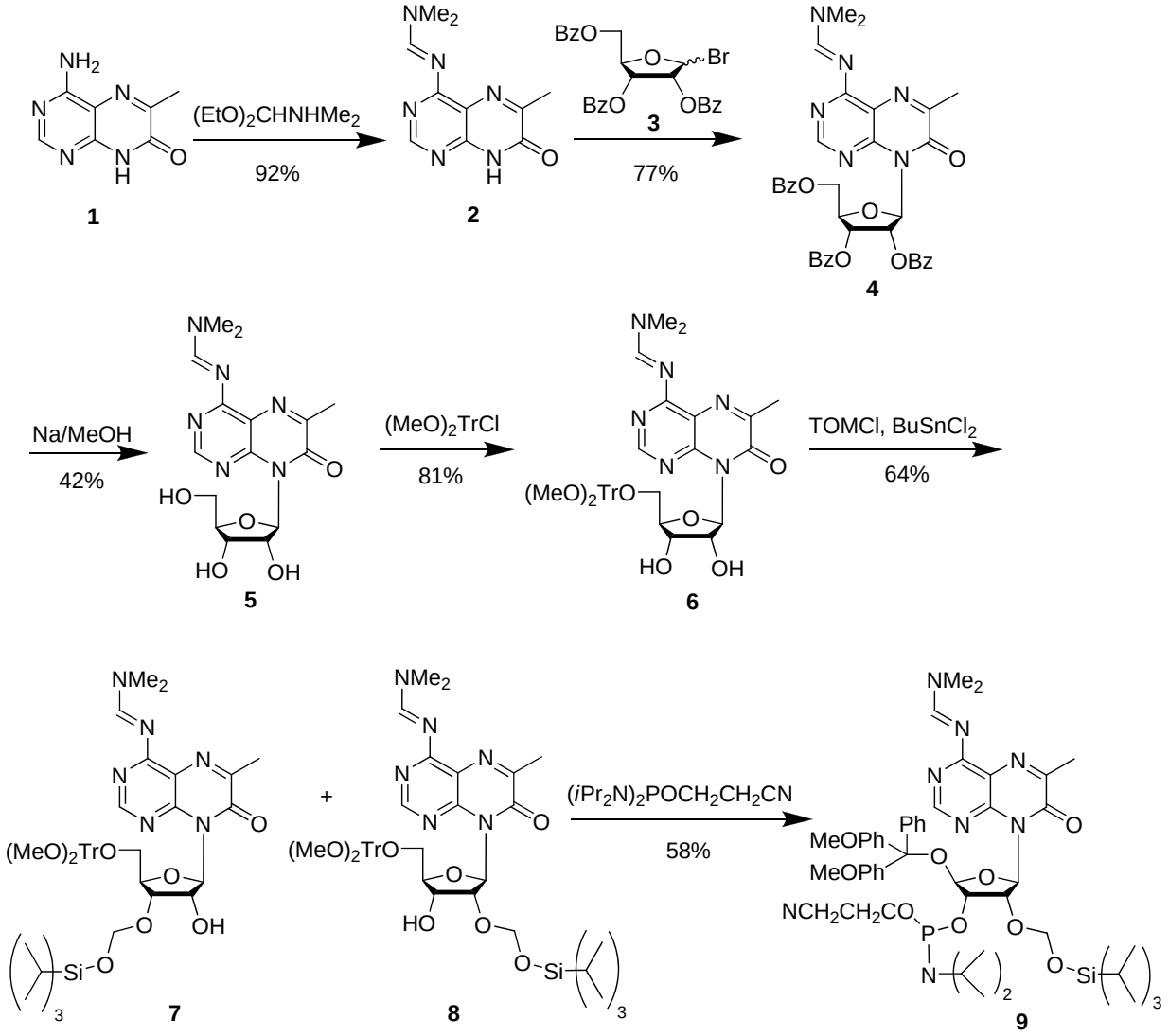
Pteridines are fused ring compounds that are similar in overall size and shape to purines, but can also be strongly fluorescent. This enables their possible use as nucleoside analogs that can be inserted intimately and site-specifically into the backbones of oligonucleotides, enabling unique studies of oligonucleotide interactions to be performed.[1] In related work as described,[2] and partially reproduced by IPDC, pteridine nucleoside analogs of deoxyribonucleic acid were made for incorporation into synthetic DNA strands, via solid phase phosphoramidite chemistry.[3] In this work, the synthetic methodology is extended to the preparation of a ribosyl-pteridine analog intended for use in pteridinyl-RNA oligonucleotide syntheses.

Chemistry

Based on the methods reported in the literature for syntheses of pteridine typed nucleosides,[2; 3] the amino function of the pteridine (**1**), previously synthesized by us for the DNA analog work, was converted into a dimethylaminomethyl Schiff base, in order to improve its solubility in organic solvents. Pteridine (**2**) was synthesized by reacting (**1**) with dimethylformamide diethylacetal, in 92% yield. Conjugation of the modified pteridine (**2**) with the protected ribosyl bromide (**3**) was performed in 1 equivalent of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) to produce the desired β-anomer of nucleoside (**4**) in 77% yield, along with trace amount of the α-anomer. This experimental result was obtained due to the pteridine amide anion's S_N2 attack on the acyloxonium ion which is stabilized *via* a neighboring group. Ribosyl-6MAP (**5**) was obtained after sequential deprotection using sodium methoxide. After the 5'-hydroxy group was protected as a trityl ether, single and non-selective protection of the 2' and 3' hydroxyls with the recently described protecting moiety TOM (triisopropylsilyloxymethyl-) was performed,

resulting in a product mix of (**7**) and (**8**) in a combined yield of 64%, without regioselectivity.[4] Selection of TOM as the 2' protecting group was based on the report that it allowed for higher yields of RNA oligonucleotides. Finally, phosphoramidation of (**8**) resulted in the final pteridinyl-RNA mono phosphoramidite agent (**9**) in 58% yield, suitable for use in automated RNA syntheses (Scheme 1).

Scheme 1



Experimental

General. Silica gel 60 F254 TLC plates were from Merck. Flash chromatography was done on an Analogix Intelliflash Workstation with a SuperFlash Sepra Si 50 SF column. Mass spectral analyses were done on an Agilent 1200 mass spectrometer. ^{1}H-NMR were obtained on a Varian

400 MHz. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS). Starting materials are commercially available from Aldrich and were used without further purification.

(E)-*N,N*-dimethyl-N'-(6-methyl-7-oxo-7,8-dihydropteridin-4-yl)formimidamide (2). A mixture of compound (**1**) (4.20 g, 23.8 mmol) and 1,1-diethoxy-*N,N*-dimethylmethanamine (24 mL) was stirred at 60°C for 2 hours. After cooling to room temperature, the mixture was evaporated under high vacuum and washed with *i*-PrOH (isopropanol) to afford (**2**) as an orange solid (5.06 g, yield 92%). ^{1}H NMR (d^{6}-DMSO, 400 MHz) δ 8.70 (s, 1H), 8.38 (s, 1H), 3.20 (s, 3H), 3.14 (s, 3H), 2.37 (s, 3H). MS (*m/z*): 233.9 $(M+H)^{+}$.

1-(2',3',5'-Tri-*O*-benzoyl-*β*-D-ribofuranosyl)-(E)-*N,N*-dimethyl-N'-(6-methyl-7-oxo-7,8-dihydropteridin-4-yl)formimidamide (4). Compound (**2**) (1.38 g, 5.95 mmol) was suspended in anhydrous acetonitrile (50 mL) under an argon atmosphere. To this suspension was added dropwise DBU (0.9 mL, 6.0 mmol) and the mixture was stirred for 20 minutes at room temperature after which it became a clear orange solution. To this solution was added a solution of 1-Bromo-2,3,5-tri-*O*-benzoyl-*β*-D-ribose[5] (compound (**3**), 5.95 mmol) in anhydrous acetonitrile (20 mL) and the resulting solution was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was purified by flash chromatography using silica gel and a gradient of CH_2Cl_2:MeOH (40:1 to 20:1) to give the product 3.11 g (77%) as a light yellow solid. R_f = 0.73 (dichloromethane:methanol = 10:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.74 (s, 1H), 8.51 (s, 1H), 8.07-7.31 (m, 16H), 6.47 (m, 1H), 6.38 (m, 1H), 4.84 (dd, 1H, J = 3.5 Hz, J = 11.8 Hz), 4.78 (m, 1H), 4.65 (dd, 1H, J = 5.5 Hz, J = 11.7 Hz), 3.31 (s, 3H), 3.23 (s, 3H), 2.61 (s, 3H); MS (*m/z*): 677.4 $(M+H)^{+}$.

4-Amino-6-methyl-8-(*β*-D-ribofuranosyl)-8*H*-pteridin-7-one (5). Compound (**4**) (676 mg, 1 mmol) was added to a solution of sodium (28 mg, 1.22 mmol) in anhydrous methanol (20 mL). This suspension was stirred at room temperature overnight. After neutralization with acetic acid to pH = 7, the solvent was removed *in vacuo* and the residue was purified by flash chromatography using silica gel and a gradient of CH_2Cl_2:MeOH (20:1 to 10:1) to give the product 131 mg (42%) as a white solid. R_f = 0.24 (dichloromethane:methanol = 10:1). ^{1}H NMR

(d^6-DMSO, 400 MHz) δ 8.20 (s, 1H), 7.70 (br, 1H), 7.50 (br, 1H), 6.68 (d, 1H, J = 3.1 Hz), 5.07 (d, 1H, J = 5.3 Hz), 4.93 (d, 1H, J = 6.7 Hz), 4.68-4.64 (m, 2H), 4.26 (q, 1H, J = 6.3 Hz), 3.77 (m, 1H), 3.64 (m, 1H), 3.47 (m, 1H), 2.38 (s, 3H). MS (*m/z*): 309.9 $(M+H)^+$.

4-Amino-6-methyl-8-[5-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-8*H*-pteridin-7-one (6). Compound (**5**) (126 mg, 0.41 mmol) was dissolved in anhydrous pyridine (6 mL) under an argon atmosphere. To this solution was added 4,4'-dimethoxytrityl chloride (166 mg, 0.49 mmol) and the resulting solution was stirred for 12 hours at room temperature. After the solvent was removed *in vacuo*, the residue was dissolved in dichloromethane (100 mL), washed with saturated sodium bicarbonate (2 x 10 mL), and dried over anhydrous sodium sulfate. After the solvent was taken to dryness, the residue was purified by flash chromatography on silica gel using a gradient of CH_2Cl_2:MeOH (50:1 to 30:1) to give 209 mg (81%) of product (**6**) as a white solid. R_f = 0.52 (dichloromethane:methanol = 10:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (s, 1H), 7.45-7.18 (m, 8H), 6.92 (d, 1H, J = 1.8 Hz), 6.79 (d, 2H, J = 9.0 Hz), 6.78 (d, 2H, J = 9.2 Hz), 4.83-4.78 (m, 2H), 4.03 (m, 1H), 3.78 (s, 6H), 3.50 (dd, 1H, J = 5.7 Hz, J = 8.8 Hz), 3.42 (dd, 1H, J = 5.3 Hz, J = 9.8 Hz), 2.46 (s, 3H).

4-Amino-6-methyl-8-{5'-*O*-(4,4'-dimethoxytrityl)-(2'or3')-*O*-[(triisopropylsilyl)oxymethyl]-*β*-D-ribofuranosyl}-8*H*-pteridin-7-one (7, 8). To a solution of (**6**) (206 mg, 0.337 mmol) and DIPEA (diisopropylethylamine) (0.25 mL, 1.47 mmol) in anhydrous 1,2-dichlorethane (4 mL) was added Bu_2SnCl_2 (dibutyltin dichloride, 139 mg, 0.46 mmol) under an argon atmosphere. The resulting solution was stirred at room temperature for 1 hour and then heated to 80°C. To the above solution was added TOM-Cl (triisopropylsilyloxymethyl chloride) (0.12 mL, 0.50 mmol) and the mixture was stirred for 90 minutes. After cooling to room temperature, the reaction mixture was diluted with 50 mL dichloromethane, washed with saturated aqueous sodium bicarbonate (20 mL), and dried over anhydrous magnesium sulfate. After the solvent was removed *in vacuo*, the residue was purified *via* silica gel flash chromatography using a gradient of 0.5 % TEA (triethylamine)in hexanes:ethyl acetate (6:1 to 3:1) to give 83 mg of (**7**) (31%) and 88 mg (**8**) (33%), both as white solids. (**7):** R_f = 0.77 (hexane:acetate = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (s, 1H), 7.45-7.16 (m, 8H), 6.98 (d, 1H, J = 2.0 Hz), 6.76 (d, 2H, J = 9.0 Hz), 6.74 (d, 2H, J = 9.0 Hz), 5.11 (d, 1H, J = 4.5 Hz), 4.89 (d, 1H, J = 4.5 Hz), 4.84 (m, 1H), 4.66

(m, 1H), 4.23 (m, 1H), 3.67 (s, 6H), 3.42-3.39 (m, 2H), 3.29 (d, 1H, J = 2.0 Hz), 2.46 (s, 3H), 1.16-0.98 (m, 21 H). (**8):** R_f = 0.87 (hexanes:ethyl acetate = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (s, 1H), 7.47-7.14 (m, 8H), 6.96 (br, 1H), 6.77 (d, 2H, J = 8.8 Hz), 6.75 (d, 2H, J = 9.0 Hz), 5.04 (d, 1H, J = 4.7 Hz), 5.01 (m, 1H), 4.96 (d, 1H, J = 4.9 Hz), 4.69 (m, 1H), 4.07 (m, 1H), 3.79 (s, 6H), 3.45 (dd, 1H, J = 4.0 Hz, J = 10.1 Hz), 3.39 (dd, 1H, J = 6.3 Hz, J = 10.2 Hz), 2.97 (d, 1H, J = 7.5 Hz), 2.47 (s, 3H), 1.11-0.89 (m, 21 H). MS (*m/z*): 798.5 $(M+H)^+$.

4-Amino-6-methyl-8-{3'-*N*-Diisopropylamino(2-cyanoethyl)-phosphoramido-5'-*O*-(4,4'-dimethoxytrityl)-(2'or3')-*O*-[(triisopropylsilyl)oxymethyl]-*β*-D-ribofuranosyl}-8*H*-pteridin-7-one (9). To a solution of compound (**8**) (70 mg, 0.088 mmol) in anhydrous dichloromethane (2 mL) was added 2-cyanoethyl tetraisopropylphosphorodiamidite (0.046 mL, 0.144 mmol) and 3% 1-*H*-tetrazole (0.12 mL, 0.048 mmol) under an argon atmosphere. The solution was stirred at room temperature for 12 hours, then diluted with dichloromethane (50 mL), washed with saturated aqueous sodium bicarbonate (2 x 10 mL) and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent, the residue was purified *via* silica gel flash chromatography using a gradient of hexanes:ethyl acetate (4:1 to 2:1) to give 40 mg (58%) of product (**9**) as a white solid. R_f = 0.81 (hexane:ethyl acetate = 1:2). ^{1}H NMR (d^6-DMSO, 400 MHz) δ ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (s, 1H), 7.76 (br, 1H), 7.57 (br, 1H), 7.38-7.16 (m, 9H), 6.84-6.79 (m, 5H), 4.98-4.93 (m, 2H), 4.89 (d, 1H, J = 4.9 Hz), 4.85 (d, 1H, J = 4.8 Hz), 4.11 (m, 1H), 3.66 (s, 6H), 3.60-3.40 (m, 6H), 2.75 (t, 2H, J = 5.8 Hz), 2.36 (s, 3H), 1.24-0.79 (m, 33 H).

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