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

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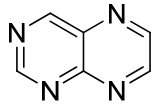
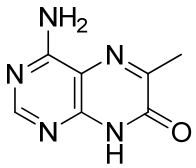
Background

The ordering of only four organic bases imparts chemical complexity to DNA, and the electronic status of each individual base in any given sequence is dependant to some extent on the status of neighboring bases. Subtle electronic variations may be detected with fluorescently labeled analogs thereby providing an understanding of sequence-dependant electronic interactions between bases.[1] Most commercially available fluorescent probes are so structurally dissimilar to natural bases that they interfere with the macromolecular structure of DNA. Also, most DNA probes are attached to oligonucleotides via a multi-atom linker and are not an integral part of the base pairing in double stranded nucleic acids, making them of no use in determining base-base electronic effects in any given sequence. Thus, commercially available fluorescent DNA probes are of limited use in studying the biophysical properties and enzyme chemistry of deoxyribose oligonucleotides.[1] Pteridine **1** is structurally similar to the purine bases guanosine and adenosine, and the pteridone analogs, 6MAP **2** and 3MI **3** are desirable for studies where the fluorescent label must be an integral part of a DNA chain and yet have minimal interference in the macromolecular structure of the oligonucleotide. These analogs are stable through automated nucleic acid syntheses involving deprotection and annealing at elevated temperatures[2; 3] and their fluorescent properties are sensitive enough to detect subtle changes in their electronic environment due to neighboring bases in a sequence.[4] They also have spectral properties distinct from tryptophan fluorescence, facilitating their detection in assays in protein-rich environments.

Figure 1. The structures of pteridine (**1**), 6MAP (**2**), and 3MI (**3**).



1



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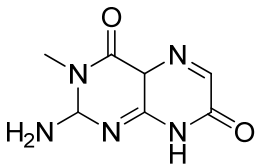

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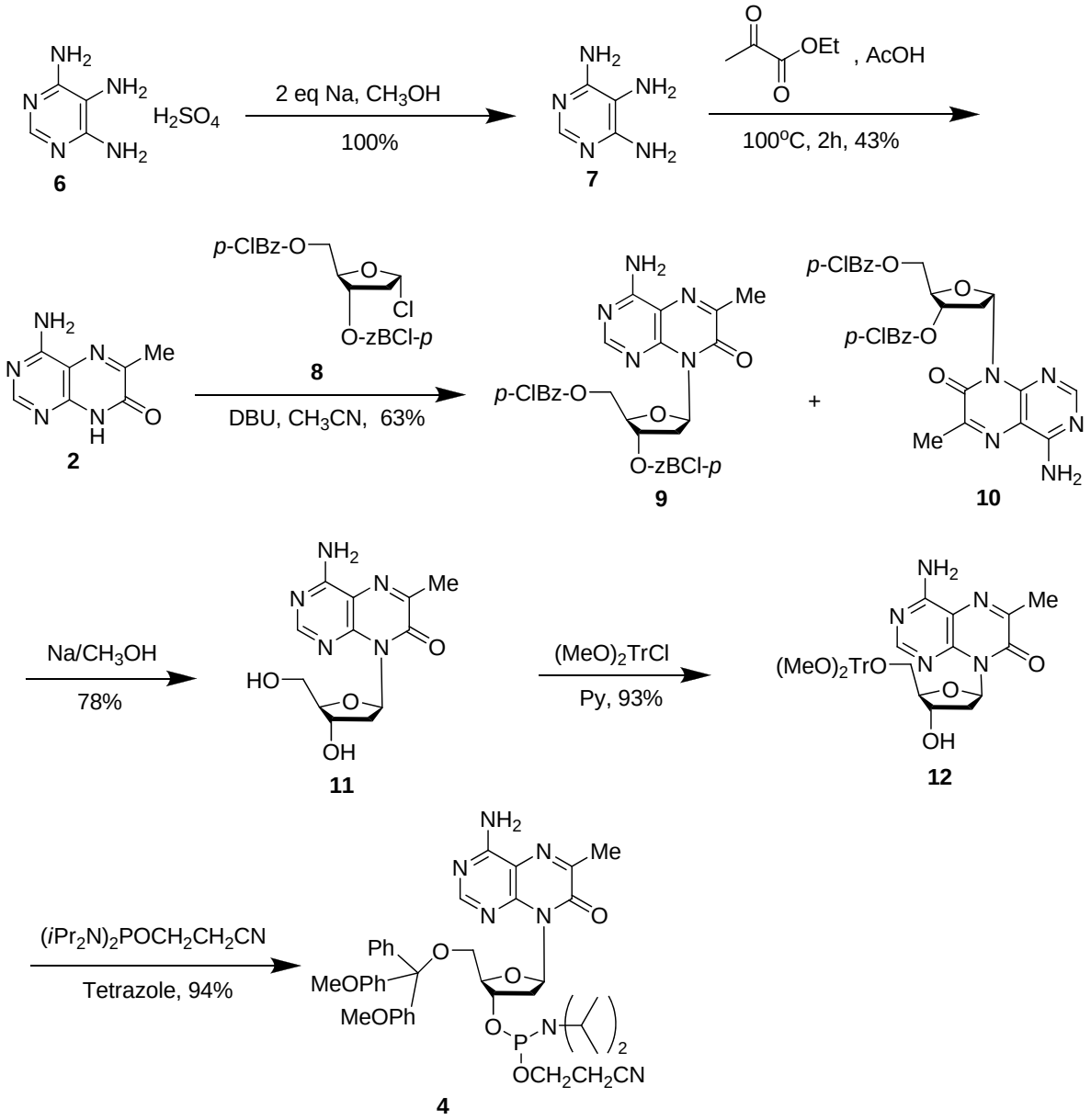
Chemistry

As they attach to nucleotides through a bond at the C1 of deoxyribose, these fluorescent imaging probe monomers are useful as phosphoramidites for incorporation into automatically synthesized deoxyribonucleic acids. Syntheses of phosphoramidite monomers of 6MAP **4** and 3MI **5** were previously described by Hawkins, Pfleiderer, *et al.,*[3; 5] and were repeated here with some modifications and yield improvements (**Schemes 1** and **2**). In the initial synthesis of 6MAP, commercially available 4,5,6-triaminopyrimidine sulfate salt **6** was used in an acid catalyzed annulation with ethyl pyruvate but resulted in only a trace of impure pteridone **2** due to the sulfate salt's poor solubility. We improved the yield of the pteridone **2** from ~ 4% to 43% by first converting the 4,5,6-triaminopyrimidine sulfate salt **6** to its free amine using sodium methoxide in methanol (**Scheme 1**). Pteridone **2** was converted to its free base and in the presence of one equivalent of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in anhydrous acetronitrile,[6] is reacted with α-chlorodeoxyribose derivative **8** to yield the β-epimer of the N8 substituted pteridone deoxyribose derivative **9** in a 3:1 ratio with the β-epimer **10** in 63% combined yield. Separation of **9** and **10** was achieved by precipitation in methanol after the subsequent deprotection step using dilute sodium methoxide, yielding deprotected nucleoside **11**. Following the previously described methods,[5] subsequent dimethoxytritylation of the 5'-hydroxy group of **11** (93% yield) and the phosphoramidation of the 3'-hydroxy group of **12** (94% yield) leads to the final pteridone phosphoramidite **4**. Of note, due to phosphoramidite sensitivity to acid and moisture, its column chromatography purification is carried out with 0.5% triethylamine (TEA) added to the eluting solvents. The 6MAP phosphoramidite **4** is stored dry at -78°C.

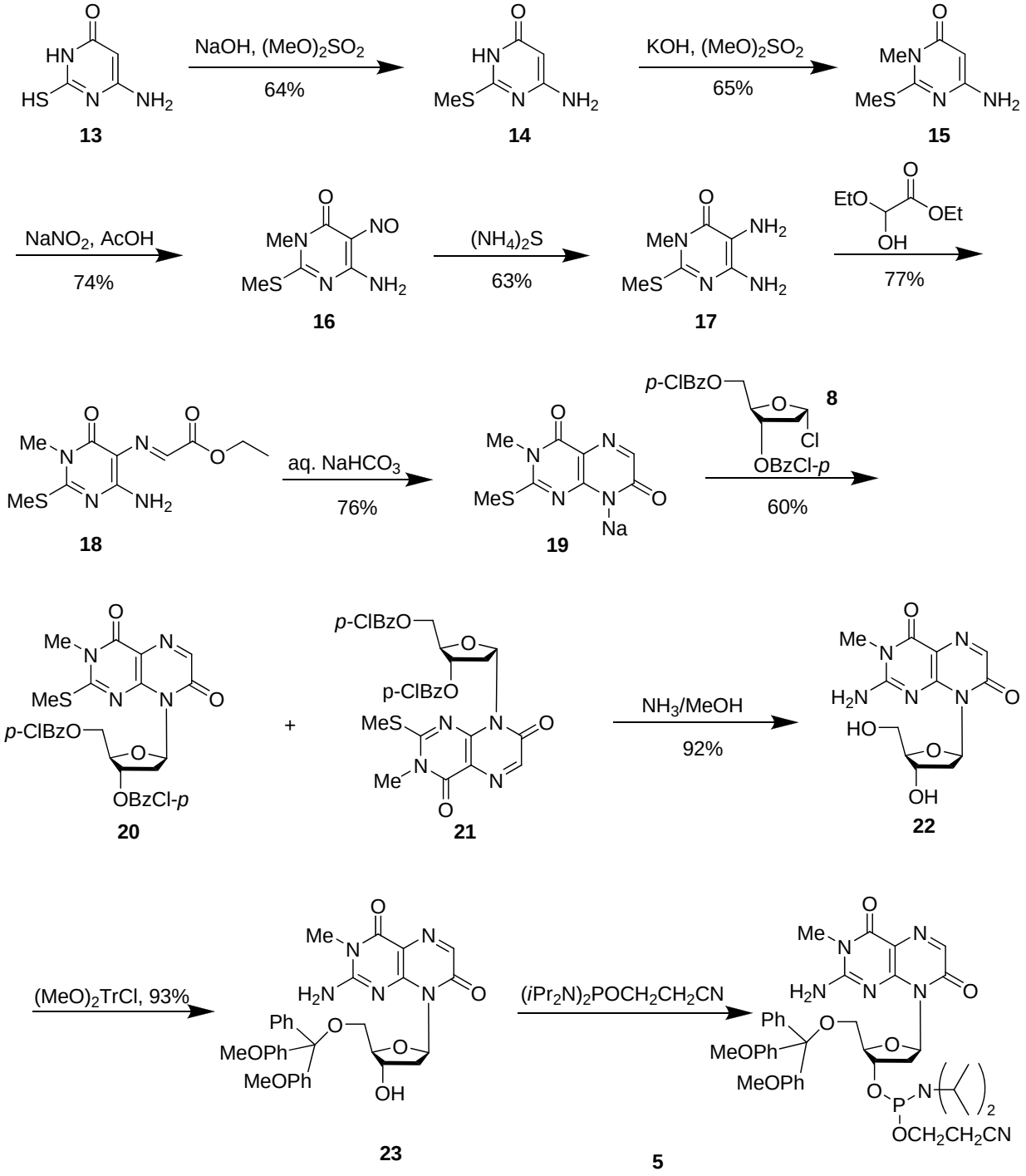
Synthesis of 3MI phosphoramidite **5** is somewhat similar (**Scheme 2**). It differs in that the synthesis of pteridone intermediate **19** involves additional steps and there is a transformation of the 2-thiomethyl group into a 2-amino group after coupling the pteridone to deoxyribose. 6-

Amino-2-mercapto-3*H*-pyrimidin-4-one **13** is methylated in a stepwise manner at the 2-mercapto and 3-amide groups using dimethyl sulfate to yield intermediates **14** and **15** (Note: Dimethyl sulfate is highly toxic and protection from skin exposure and inhalation is required to avoid injury or death.) Amination of C-5 was accomplished *via* nitrosation with sodium nitrite to yield **16** (74% yield) followed by reduction with diammonium sulfide to give **17** (63% yield), which was then condensed with ethyl gyloxylate, yielding **18**. Intermediate **18** was subsequently treated with sodium bicarbonate to produce the annulated pteridone 8-*N*-sodium salt **19** (59% yield, two steps) which was used to substitute α-chlorodeoxyribose **8** to form a mixture of the *β*- and *α*-epimers **20** and **21** in a 3:1 ratio, respectively (60% combined yield). The *β*-epimer **20** was isolated by flash chromatography and purified by recrystallization from toluene and ethyl acetate. Treatment of **20** with ammonia in methanol transforms the 2-thiomethyl group to an amino group, and cleaves the *p*-chlorobenzoyl protecting groups to reveal nucleoside **22**. Subsequent dimethyltritylation of **22** yields penultimate product **23** and phosphoramidation yields the final 3MI phosphoramidite **5**.

Scheme 1. Synthesis of 6MAP (**2**) and its phosphoramidite (**4**)



Scheme 2. Synthesis of 3MI phosphoramidite (**5**)



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 API 2000 and ^{1}H-NMR obtained on a Bruker 360MHz. All chemicals are commercially available from Aldrich.

Pyrimidine-4,5,6-triamine (7). Pyrimidine-4,5,6-triamine sulfate **6** (4.46g, 20 mmol) was added to a solution of sodium (920 mg, 40 mmol) in methanol (400 mL). This suspension was heated under reflux for 20 minutes. After cooling to room temperature, the suspension was filtered and the filtrate evaporated and dried in vacuum to give 2.50 g (100%) of free base.

4-Amino-6-methyl-8*H*-pteridin-7-one (2). Compound **7** (2.50 g, 20 mmol) and ethyl pyruvate (2.75 mL, 25 mmol) were dissolved in acetic acid (25 mL) at 50°C and then heated at 100°C for 2 hours, producing a heavy brown precipitate. After cooling to room temperature, the precipitate was collected, washed successively with cold water (5 mL), cold saturated sodium bicarbonate solution (3 mL), cold water (3 mL), cold ethanol (3 mL), cold ether (2x4 mL) and dried *in vacuo* to give 1.51 g of **2** (43%) as a brown solid. R_f 0.73 in dichloromethane[DCM]:methanol (5:1). ^{1}H NMR (d^{6}-dimthylsulfoxide (DMSO), 360 MHz) δ 2.31 (s, 3H, CH_3-C(6)), 7.20 (br, 2H, NH_2), 8.08 (s, 1H, H-C(2)); MS (*m/z*): 175.9 $(M-H)^-$.

4-amino-6-methyl-8-(2-deoxy-3,5-di-O-*p*-chlorobenzoyl-D-ribofuranosyl)-7(8*H*)-pteridin-7-one (9, 10). Compound **2** (1.36 g, 7.68 mmol) was suspended in anhydrous acetonitrile (250 mL) under an argon atmosphere. To this suspension was added dropwise DBU (1.16 mL, 7.68 mmol) and the mixture was then stirred for 1 hour at room temperature after which it became a clear, deep red solution. To this solution was added 2-α-chlorodeoxy-3,5-di-O-*p*-chlorobenzoyl-β-D-ribose (compound **8**; 3.96 g, 9.21 mmol) at 0°C. The reaction was stirred at 0°C for 1 hour and then at room temperature overnight. The solvent was removed on a rotary evaporator and the residue purified by flash chromatography using a gradient of toluene/ethyl acetate (4:1 to 2:1) to give the product as 2.76 g (63%) as a light yellow solid, comprising a mixture of epimers **9** and **10**, in a ratio of 3:1, respectively, as estimated from the NMR spectrum. R_f 0.47 in toluene:ethyl acetate (1:1). ^{1}H NMR (d^{6}-DMSO, 360 MHz) δ 2.28 (s, 3 x 0.25 H, CH_3-C(6), *α*-epimer), 2.38 (s, 3 x 0.75 H, CH_3-C(6), *β*-epimer), 2.99-2.85 (m, 2 x 0.25 H, HC(2'), *α*-epimer), 2.54 (m, 0.75H, HC(2'), *β*-epimer), 3.17 (m, 0.75H, HC(2'), *β*-epimer), 4.45-4.57 (m, 2H, HC(5')), 4.70 (m, 0.75H, HC(4'), *β*-epimer), 5.08 (m, 0.25H, HC(4'), α-epimer), 5.57 (m, 0.25, HC(3'), *α*-

epimer), 5.93 (m, 0.75H, HC(3'), *β*-epimer), 7.13 (dd, *J* = 7.1 Hz, *J* = 13.9 Hz, 0.25H, HC(1'), *α*-epimer), 7.34 (dd, *J* = 4.1 Hz, *J* = 9.2 Hz, 0.75 H, HC(1'), *β*-epimer), 7.50-7.61 (m, 4H), 7.68-7.77 (br, 2H, NH_2), 7.92-8.03 (m, 4H), 8.22 (s, 0.75H, H-C(2), *β*-epimer), 8.23 (s, 0.25H, H-C(2), *α*-epimer); MS (*m/z*): 615.0 $(M+2Na-H)^+$, 591.8 $(M+Na)^+$.

4-Amino-6-methyl-8-(2-deoxy-β-D-ribofuranosyl)-8*H*-pteridin-7-one (11). The mixture of compounds **9** and **10** (2.40g, 4.22 mmol) was added to a solution of sodium (96 mg, 4.17 mmol) in anhydrous methanol (96 mL). This suspension was stirred at room temperature for 3 hours. After neutralization with acetic acid to pH = 7, the suspension was concentrated to 40 mL and kept at 4^oC for 3 hour. The precipitate was collected, washed with cold methanol (5 mL), and dried under high vacuum to give 630 mg (68%) of product **11** as a white solid. R_f 0.73 in DCM:methanol (5:1). ^1H NMR (d^6-DMSO, 360 MHz) δ 1.99 (m, 1H, HC(2')), 2.36 (s, 3H, CH_3-C(6)), 2.86 (m, 1H, HC(2')), 3.51 (m, 1H, HC(5')), 3.65 (m, 1H, HC(5')), 3.76 (m, 1H, HC(4')), 4.42 (m, 1H, HC(3')), 4.72 (t, *J* = 5.6 Hz, 1H, HO-C(5')), 5.16 (d, *J* = 4.7 Hz, 1H, HO-C(3')), 7.15 (t, *J* = 7.1 Hz, 1H, HC(1')), 7.50 (br, 1H, -HN), 7.70 (br, 1H, -HN), 8.20 (s, 1H, HC(2)); MS (*m/z*): 316.0 $(M+Na)^+$, 294.0 $(M+H)^+$.

4-Amino-6-methyl-8-{5-[*bis*-(4-methoxyphenyl)phenylmethoxymethyl]-4-hydroxy-tetrahydrofuran-2-yl}-8*H*-pteridin-7-one (12). Compound **11** (530 mg, 1.80 mmol) was dissolved in anhydrous pyridine (10 mL) under an argon atmosphere. To this solution was added 4,4'-dimethoxytrityl chloride (732 mg, 2.16 mmol) and the resulting solution was stirred for 12 hours at room temperature. After evaporation, the residue was dissolved in DCM (150 mL), washed with saturated sodium bicarbonate (2 x 20 mL), and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent, the residue was purified by column chromatography on silica gel using a gradient of toluene/ethyl acetate (2:1 to 1:2) to give 995 mg (93%) of product **12** as a white solid. R_f 0.29 in toluene:ethyl acetate (1:2). ^1H NMR (d^6-DMSO, 360 MHz) δ 2.09 (m, 1H, HC(2')), 2.29 (s, 3H, CH_3-C(6)), 2.77 (m, 1H, HC(2')), 3.12 (dd, *J* = 3.2 Hz, *J* = 9.7 Hz, 1H, HC(5')), 3.31 (m, 1H, HC(5')), 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.92 (m, 1H, HC(4')), 4.42 (m, 1H, HC(3')), 5.16 (d, *J* = 5.2 Hz, 1H, HO-C(3')), 6.73-6.81 (m, 4H, trityl), 7.11-7.26 (m, 8H, trityl, HC(1')), 7.33-7.36 (m, 2H, trityl), 7.49 (br, 1H, -HN), 7.67 (br, 1H, -HN), 8.10 (s, 1H, HC(2)); MS (m/z): 618.0 $(M+Na)^+$.

3-N-Diisopropylamino(2-cyanoethyl)-phosphoramido-5-{2-[*bis*-(4-methoxyphenyl) phenyl methoxymethyl]tetrahydrofuranyl}-4-amino-6-methyl-7-oxo-7*H*-pteridin-7-one (4). To a solution of compound **12** (5.60 g, 9.41 mmol) in anhydrous DCM (134 mL) was added 2-cyanoethyl tetraisopropylphosphorodiamidite (4.62 mL, 14.1 mmol) and 1-H-tetrazole (329 mg, 4.7 mmol) under an argon atmosphere. The solution was stirred at room temperature for 12 hours, then diluted with DCM (750 mL), washed with saturated sodium bicarbonate (2 x 50 mL) and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent the residue was purified by silica gel using a gradient of toluene:ethyl acetate (4:1 to 1:1) to give 7.07 g (94%) of product **4** as a white solid. R_f 0.50 in toluene:ethyl acetate (1:2). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 0.92 (d, J = 6.7 Hz, 6H, $CH(CH_3)_2$), 1.07 (d, J = 6.7 Hz, 6H, $CH(CH_3)_2$), 2.29 (s, 3H, CH_3-C(6)), 2.34 (m, 1H, HC(2')), 2.72 (t, J = 5.8 Hz, 2H), 2.86 (m, 1H, HC(2')), 3.14 (dd, J = 2.5 Hz, J = 9.7 Hz, 1H, HC(5')), 3.31 (m, 1H, HC(5')), 3.39-3.50 (m, 4H), 3.69 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.07 (m, 1H, HC(4')), 4.68 (m, 1H, HC(3')), 6.72-6.79 (m, 4H, trityl), 7.13-7.23 (m, 8H, trityl, HC(1')), 7.32-7.34 (m, 2H, trityl), 7.51 (br, 1H, -HN), 7.69 (br, 1H, -HN), 8.10 (s, 1H, HC(2)); MS (*m/z*): 818.2 $(M+Na)^+$.

6-Amino-2-methylsulfanyl-3*H*-pyrimidin-4-one (14). To a 10% solution of sodium hydroxide (400 mL) was added 6-amino-2-mercapto-3H-pyrimidin-4-one (100 g, 0.62 mol) followed by the addition of dimethylsulfate (76 mL, 0.80 mol) over 15 minutes. The mixture was allowed to stand for 15 minutes at room temperature and the precipitate formed was collected by filtration. The moist solid was recrystallized from 95% ethanol (800 mL) to give 62.0 g (64%) of **14** as a white solid. R_f 0.63 in DCM:methanol (5:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 3.34 (s, 3H), 4.90 (s, 1H), 6.45 (br, 2H), 11.4 (br, 1H); MS (m/z): 180.1 $(M+Na)^+$, 157.9 $(M+H)^+$.

6-Amino-3-methyl-2-methylsulfanyl-3*H*-pyrimidin-4-one (15). To a solution of 1 N potassium hydroxide (403 mL) was added compound **14** (62.0 g, 0.39 mol) followed by dropwise addition of dimethyl sulfate (42 mL, 0.44 mol) at room temperature. The white precipitate was filtered immediately once the pH of the solution decreased to <7. The filtrate was neutralized with 10% sodium hydroxide and evaporated to dryness. The residue was washed with cold water and the precipitate filtered and combined with the solid already obtained. The

combined solids were suspended with 200 mL 5% ammonium hydroxide, and the precipitate filtered and washed with ether to give 43.9 g (65%) of **15** as a white solid. R_f 0.79 in DCM:methanol (5:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 2.49 (s, 3H), 3.27 (s, 3H), 4.93 (s, 1H), 6.42 (br, 2H); MS (m/z): 194.1 $(M+Na)^+$, 172.1 $(M+H)^+$.

6-Amino-3-methyl-2-methylsulfanyl-5-nitroso-3*H*-pyrimidin-4-one (16). To a suspension of compound **15** (43.9 g, 0.26 mol) in 30% acetic acid (395 mL) was added dropwise a solution of sodium nitrite (19.5 g, 0.28 mol) in H_2O (39 mL). The mixture was stirred for one additional hour at room temperature and then cooled at 4°C overnight. The precipitate was collected and washed with water and acetone and dried at 100°C to give 38.1 g (74%) of **16** as a blue solid. R_f 0.76 in dichloromethane:methanol (5:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 2.61 (s, 3H), 3.48 (s, 3H), 8.96 (br, 1H), 11.02 (br, 1H); MS (*m/z*): 223.1 $(M+Na)^+$, 201.1 $(M+H)^+$.

5,6-Diamino-3-methyl-2-methylsulfanyl-3*H*-pyrimidin-4-one (17). Compound **16** (22.0 g, 0.11 mol) was added to a 20% aqueous ammonium sulfide solution (220 mL) and the mixture was heated under for 30 minutes. After cooling, the precipitate was collected, washed with ethanol and dried in a desiccator to give 14.9 g (63%) of **17** as a yellow powder. R_f 0.68 in DCM:methanol (5:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 2.48 (s, 3H), 3.35 (s, 3H), 5.68 (br, 4H); MS (*m/z*): 209.3 $(M+Na)^+$, 187.1 $(M+H)^+$.

(4-Amino-1-methyl-2-methylsulfanyl-6-oxo-1,6-dihydro-pyrimidin-5-ylimino)-acetic acid ethyl ester (18). Compound **17** (14.8 g, 79.6 mmol) was dissolved in warm water (80°C, 2.5 L) and the solution was then cooled to room temperature. To this solution was added ethyl glyoxylate-hemiethylacetal (14.8 g, 100 mmol). After stirring for 1 hour, the precipitate was filtered and washed with ethanol. Recrystallization from ethanol (450 mL) gave 16.5 g (77%) of **18** as a bright yellow solid. R_f 0.83 in DCM:methanol (10:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 1.28 (t, J = 7.0 Hz, 3H), 2.58 (s, 3H), 3.34 (s, 3H), 4.22 (q, J = 7.0 Hz, 2H), 6.74 (br, 1H), 7.49 (br, 1H), 9.02 (s, 1H); MS (*m/z*): 269.0 $(M-H)^-$.

3-Methyl-2-methylsulfanyl-3*H*,8*H*-pteridine-4,7-dione sodium salt (19). Compound **18** (14.44 g, 53.5 mmol) was suspended in ethanol (200 mL), a solution of 0.5 N sodium

bicarbonate (400 mL) was added and the mixture was heated under reflux for 30 minutes. The resulting solution was cooled overnight and the precipitate collected, washed with methanol, and dried at 100°C under vacuum to give 9.95 g (76%) of **19** a light yellow powder. ^{1}H NMR (d^6-DMSO, 360 MHz) δ 2.55 (s, 3H), 3.39 (s, 3H), 7.37 (s, 1H); MS (*m/z*): 247.1 $(M+Na)^+$.

8-(2-deoxy-3,5-di-O-*p*-chlorobenzoyl-D-ribofuranosyl)-3-methyl-2-methylsulfanyl-3*H*,8*H*-pteridine-4,7-dione (20, 21). To a suspension of **19** (7.46 g, 30.3 mmol) in anhydrous DCM (600 mL) was added compound **8** (14.3 g, 33.3 mmol) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was washed twice with 5% sodium bicarbonate (300 mL) and the aqueous layer was re-extracted with DCM (300 mL). The combined organic solutions were dried over sodium sulfate. After evaporation of the solvent, the residue was purified by column chromatography using a gradient of toluene:ethyl acetate (4:1 to 2:1) on silica gel to give 11.21 g (60% yield) of product as a light yellow solid, comprising a mixture of isomers **20** and **21**, in a ratio of 3:1, respectively, as estimated from the ^{1}H NMR spectrum. Recrystallization from toluene:ethyl acetate (400 mL, 1:1) gave pure **20** (5.79 g). R_f 0.32 in toluene/ethyl acetate solution (1:1). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.53 (m, 1H, HC(2')), 2.68 (s, 3H, -SMe), 3.35 (m, 1H, HC(2')), 3.61 (s, 3H, -NMe), 4.56 (m, 1H, HC(4')), 4.69 (dd, J = 6.9 Hz, J = 11.5 Hz, 1H, HC(5')), 4.78 (dd, J = 4.6 Hz, J = 11.6 Hz, 1H, HC(5')), 5.98 (m, 1H, HC(3')), 7.34 (d, J = 8.5 Hz, 2H, -ClBz), 7.41 (d, J = 8.5 Hz, 2H, -ClBz), 7.44 (m, 1H, HC(1')), 7.95 (d, J = 8.4 Hz, 2H, -ClBz), 7.97 (d, J = 8.3 Hz, 2H, -ClBz), 8.04 (s, 1H, HC(6)); MS (*m/z*): 638.7 $(M+Na)^+$.

2-Amino-8-(2-deoxy-β-D-ribofuranosyl)-3-methyl-3*H*,8*H*-pteridine-4,7-dione (22). A solution of **20** (5.78 g, 9.38 mmol) in anhydrous acetonitrile (200 mL) was added to a pre-saturated ammoniacal methanol solution (200 mL). The mixture was stirred at room temperature for 48 hours, during which time ammonia gas was bubbled through the reaction for 30 minute periods approximately 12, 24 and 36 hours after the initiation of the reaction. The solvents were then evaporated to dryness and the residue evaporated twice more with methanol. Purification by flash chromatography using a gradient of DCM:methanol (10:1 to 5:1) gave 2.68 g (92%) of **22** as a white solid. R_f 0.25 in CH_2Cl_2/MeOH (5:1) solution. ^{1}H NMR (d^6-DMSO, 360 MHz) δ 1.92 (m, 1H, HC(2')), 2.89 (m, 1H, HC(2')), 3.30 (s, 3H, -NMe), 3.49 (m, 1H, HC(5')), 3.64 (m, 1H,

HC(5’)), 3.71 (m, 1H, HC(4’)), 4.42 (m, 1H, HC(3’)), 4.65 (m, 1H, HO-C(5’)), 5.14 (m, 1H, HO-C(3’)), 7.11 (m,1H, HC(1’)), 7.53 (s, 1H, HC(6)), 7.86 (br, 2H, $-NH_2$); MS (*m/z*): 308 $(M-H)^-$.

2-Amino-8-{5-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-2-deoxy-β-D-ribofuranosyl}-3-methyl-3*H*,8*H*-pteridine-4,7-dione (23). Intermediate **22** (0.56 g, 1.81 mmol) and 4,4'-dimethoxytrityl chloride (0.74 g, 2.17 mmol) were treated as described in the procedure for compound **11** to provide 1.03 g (93%) of **23** as a white solid. R_f 0.60 in DCM:methanol (7:1). ^{1}H NMR (d^6-DMSO, 360 MHz) δ 2.03 (m, 1H, HC(2’)), 2.78 (m, 1H, HC(2’)), 3.10 (m, 1H, HC(5’)), 3.28 (s, 3H, -NMe), 3.35 (m, 1H, HC(5’)), 3.70 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.88 (m, 1H, HC(4’)), 4.41 (m, 1H, HC(3’)), 5.12 (d, 1H, J = 5.1 Hz, HO-C(3’)), 6.76 (d, 2H, J = 8.4 Hz, trityl), 6.81 (d, 2H, J = 8.5 Hz, trityl), 7.24-7.14 (m, 8H, trityl, HC(1’)), 7.36 (d, 2H, J = 7.6 Hz, trityl), 7.61 (s, 1H, HC(6)), 7.78 (br, 2H, $-NH_2$); MS (*m/z*): 634.0 $(M+Na)^+$, 610.3 $(M-Na)^-$.

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