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Synthesis of homochiral tetrahydropteridines

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A R T I C L E I N F O

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Dedicated to the memory of Professor Sandy McKillop

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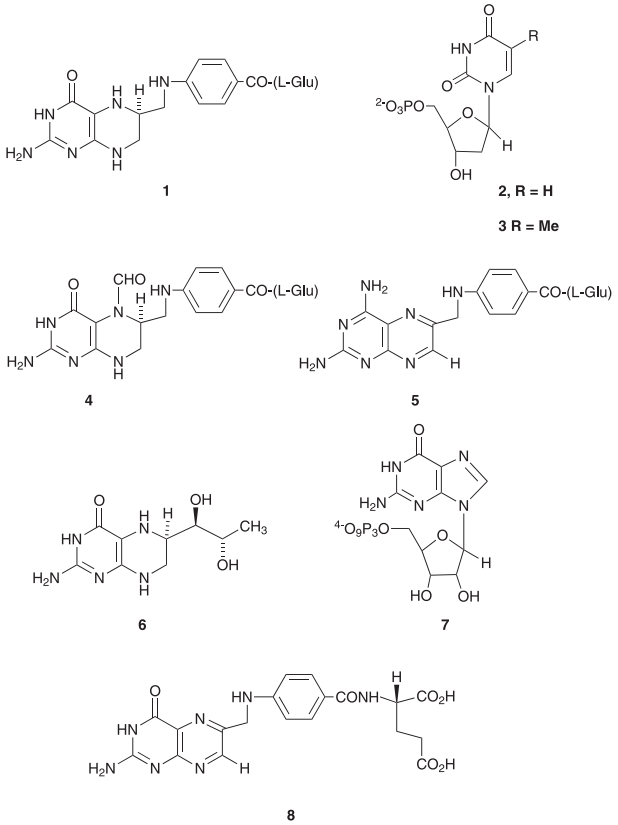
A synthesis of protected homochiral tetrahydropteridines from (2*S*)-malic acid has been developed. This presents methodology for the synthesis of reduced pteridine coenzymes and pharmaceuticals.

1. Introduction

The reduced pteridine coenzyme, tetrahydrofolic acid **1**, is important for mediating enzyme-catalysed one-carbon transfer reactions.[1] Its involvement in the one-carbon transfer catalysed by thymidylate synthase (EC 2.1.1.45), which converts deoxyuridine monophosphate **2** into thymidine monophosphate **3** in a process requiring the enzyme dihydrofolate reductase (EC 1.5.1.3) for coenzyme regeneration, makes it important in the design of anticancer chemotherapeutics. The cancer rescue agent folinic acid **4**, which allows larger doses of the drug methotrexate **5** to be used in medicine is a one-carbon adduct of tetrahydrofolic acid.

The coenzyme **1** and the related cofactor tetrahydrobiopterin **6**, which mediates enzyme-catalysed aromatic amino acid hydroxylation are biosynthesised from guanosine triphosphate (GTP) **7** in several enzyme-catalysed steps by microorganisms. Mammals, however, cannot synthesise tetrahydrofolate **1** by this route and require to take the vitamin folic acid **8** in their diet, reducing it to the coenzyme using dihydrofolate reductase. This makes the enzymes involved in the microbiological synthesis targets for antibacterial drugs.



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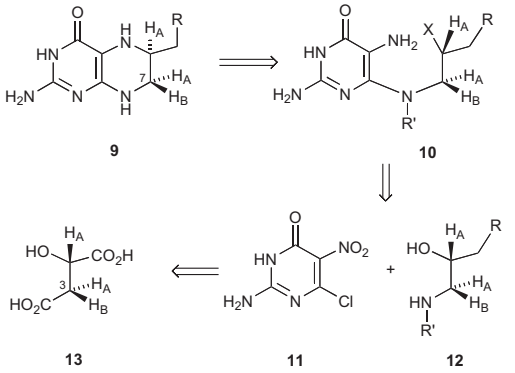
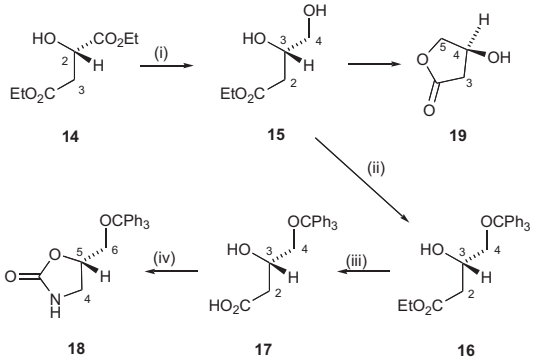
Most homochiral reduced pteridines have so far been accessed by semi-synthetic or biological methods and we now wish to report a totally synthetic method, which will allow access to a variety of targets containing the natural stereochemistry at C-6. Our retrosynthetic plan, suggested in Scheme 1, requires inversion of stereochemistry in the step **9**⇒**10** to obtain the appropriate stereochemistry at C-6, and so (2*S*)-malic acid **13** was chosen as starting material. Since we had already developed methods for obtaining large quantities of samples of (2*S*)-malic acid, **13**, H_A=^{2}H and **13**, H_B=^{2}H, which are deuteriated stereospecifically at C-3,[2] the synthesis will also allow for the preparation of samples of the co-enzymes **1** and **6**, which are stereospecifically labelled at C-7 for studies of the Amadori rearrangement involved in their biosynthesis.



Scheme 1.

2. Results and discussion

Our first tasks were selectively to convert the α-carboxyl group of (2*S*)-malic acid **13** into a potential side chain and to convert the β-carboxyl group into an amine. Conversion of the α-carboxylate into a hydroxymethyl group has been achieved by Saito et al.[3] who selectively reduced the α-ester of diethyl (2*S*)-malate **14** in good yield and we used this reaction to obtain the diol **15** in 87% yield as shown in Scheme 2.



Scheme 2. Reagents and conditions: (i) Ref. 3; (ii) Ph_3CCl, pyridine, rt 1 h, then 50 °C, 4 h (95%); (iii) 1 N aq NaOH, THF, rt, 36 h (93%); (iv) (a) $ClCO_2^iBu$, Et_3N, THF, −30 °C, 1 h, (b) NaN_3, H_2O, 0 °C, 1.5 h, (c) toluene, 60 °C, 1 h, then reflux, 30 min (30%).

This compound spontaneously formed the lactone **19** on standing and so it was immediately converted into the trityl derivative **16** in 95% yield by reaction with triphenylmethyl chloride in pyridine. Hydrolysis using aqueous sodium hydroxide in tetrahydrofuran gave the acid **17**. This was converted into the mixed anhydride with *iso*-butyl chloroformate and subsequent reaction with sodium azide gave the corresponding azide. Heating resulted in Curtius rearrangement and cyclisation of the intermediate isocyanate to give the oxazolidinone **18**. Although we had prepared a useful synthetic intermediate for our target compound **12**, problems were encountered in scaling up and so the alternative route shown in Scheme 3 was developed.

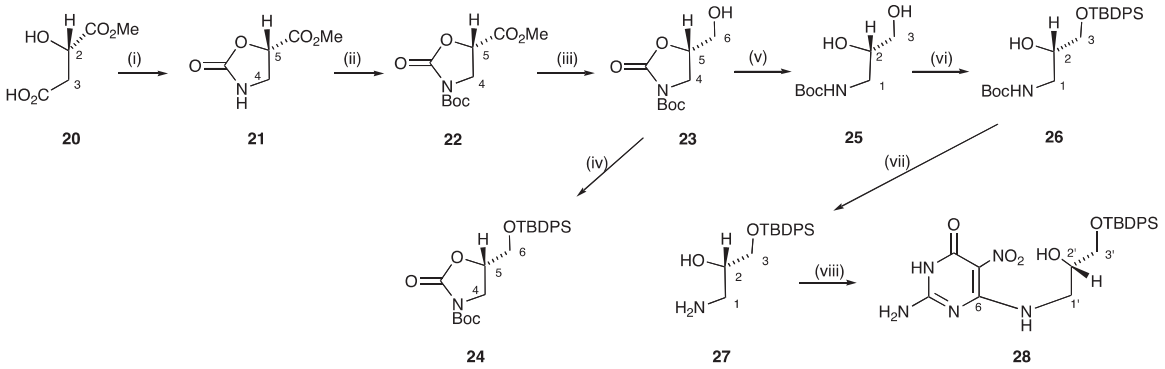
α-Methyl (2*S*)-malate **20**, prepared using the method of Miller,[4] was reacted with diphenylphosphoryl azide and triethylamine. Curtius rearrangement of the resultant azide with spontaneous cyclisation of the intermediate isocyanate gave the oxazolidinone **21** in 74% yield. This was converted into the urethane **22** in 95% yield using di-*tert*-butyl dicarbonate, triethylamine and dimethylaminopyridine in dioxane and the ester was reduced using sodium borohydride in tetrahydrofuran at −15 °C to afford the alcohol **23**. Although this was converted into the *tert*-butyldiphenylsilyl ether **24** using *tert*-butyldiphenylsilyl chloride, DMAP and triethylamine in dichloromethane, various attempts to hydrolyse this directly to the compound **26** proved fruitless. The alcohol **23**, however, underwent cleavage using caesium carbonate in methanol at room temperature to afford the diol **25** and this was converted into the silyl ether **26** in 87% yield on reaction with *tert*-butyldiphenylsilyl chloride, triethylamine and DMAP in dichloromethane at room temperature. The urethane protecting group was now removed using trifluoroacetic acid and the resultant amine **27** was reacted in triethylamine and methanol with 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11**, prepared by the method of Wood,[5] to give the adduct **28**.

Various unsuccessful attempts were made to convert the product **28** to an amino-mesylate analogue of compound **10** (X=OMs), which might be induced to cyclise to the required reduced pteridine. Arguing that the free 6-amino moiety of **28** might form an aziridine intermediate, and encouraged by the fact that a benzylated analogue had been shown to cyclise,[6] we converted the amine **27** into the benzyl derivative **29** in 64% yield by reaction with benzaldehyde and triethylamine in ethanol followed by in situ reduction with sodium borohydride as shown in Scheme 4. Reaction with 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11** then gave the product **30** in good yield.

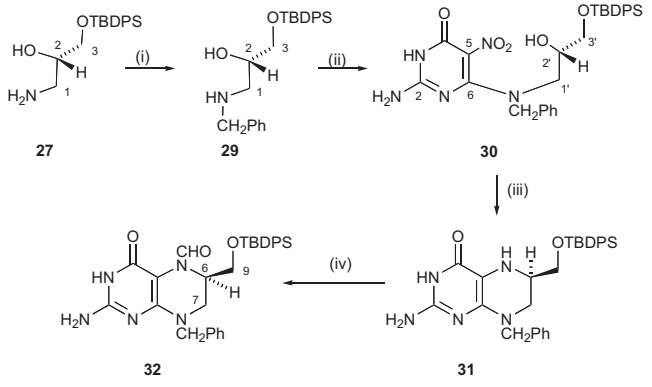
The pyrimidine **30** was now converted into the triflate using triflic anhydride and pyridine and this was hydrogenated in tetrahydrofuran containing catalytic quantity of 10% palladium on carbon. The product displayed *m*/*z* (FAB, 3-NBA) 526, which was in keeping with $[M+H]^+$ for the desired compound **31** but the ^{1}H NMR spectrum was ill-resolved and the product showed a tendency to oxidise. We therefore repeated the ring-closure reaction but immediately treated the product **31** with freshly prepared[7] formic acetic anhydride. The product was purified by extensive HPLC in 19% yield and had the spectroscopic characteristics of the desired compound **32** containing a small amount of bis formylated material.

When the methoxymethylene protected compound **37** was prepared from the oxazolidinone **21** as outlined in Scheme 5 and described in the Experimental section, mesylation followed by hydrogenation using 10% palladium on charcoal in methanol gave a compound, which was reacted with freshly prepared[7] formic acetic anhydride in pyridine.

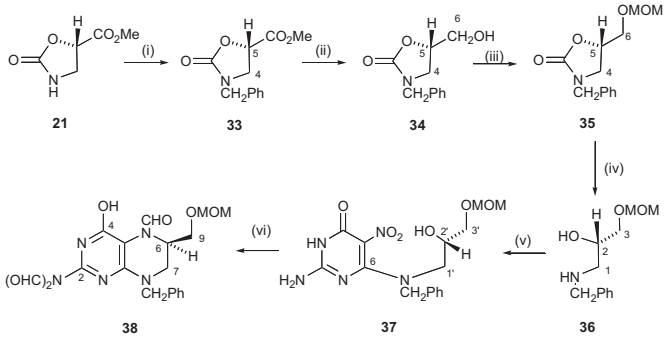
The spectra of the product indicated that it was the cyclised triformyltetrahydropteridine **38**, which interestingly, appeared to exist as the 4-phenol tautomer rather than the more usual 3,4-amide. In NOE experiments, summarised in Fig. 1, irradiation of the multiplet at δ 4.86 ppm for H-6 caused enhancement of the singlet at δ 8.70 ppm for the 5-formyl proton and of the peaks due to H-7 and H-9. Irradiation of the broad singlet at δ 12.1 ppm also caused enhancement of the singlet at δ 8.70 ppm for the 5-formyl proton. Irradiation of the singlet at δ 8.60 ppm for one 4-*N*-formyl



Scheme 3. Reagents and conditions: (i) $(PhO)_2P(O)N_3$, Et_3N, C_6H_6, reflux, 1 h (74%); (ii) (^{t}BuOCO)$_2$O, Et_3N, DMAP, dioxane, rt, 1 h (95%); (iii) $NaBH_4$, THF, −15 °C, 20 min, (83%); (iv) ^{t}Bu(Ph)$_2$SiCl, Et_3N, DMAP, CH_2Cl_2, rt 1 day (65%); (v) Cs_2CO_3, MeOH, rt, 18 h (86%); (vi) ^{t}Bu(Ph)$_2$SiCl, Et_3N, DMAP, CH_2Cl_2, rt overnight (87%); (vii) F_3CCO_2H, 20 min 0 °C (quant); (viii) **11**, Et_3N, MeOH, 60 °C, then reflux 2 h, then 4 °C, overnight (72%).



Scheme 4. Reagents and conditions: (i) PhCHO, Et_3N, EtOH, reflux, 1 h, $NaBH_4$, rt, 45 min (64%); (ii) **11**, Et_3N, MeOH, 60 °C, then reflux 4 h, then 4 °C, overnight (76%); (iii) (a) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, −30 °C, then 0 °C, 1 h (b) H_2, 10% Pd–C, THF, rt, overnight, (iv) HCO_2COCH_3, pyridine, rt, overnight (19%).



Scheme 5. Reagents and conditions: (i) (a) LDA, THF, HMPA, −78 °C, then $PhCH_2Br$, −78 °C, 2 h, rt, 2 h (51%); (ii) $NaBH_4$, THF, −15 °C, 15 min then MeOH (89%); (iii) P_2O_5, $(MeO)_2CH_2$, $CHCl_3$, rt, 1.5 h (79%); (iv) 2 M aq NaOH, MeOH, reflux, 12 h (90%); (v) **11**, MeOH, Et_3N, 60 °C, reflux, 7 h (80%); (vi) (a) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min, (b) H_2, MeOH, 10% Pd–C, rt, 1.5 h, (c) HCO_2COCH_3, pyridine, 0 °C, then rt, overnight (33%).

proton caused enhancement of the singlet at δ 8.88 ppm for the other 4-*N*-formyl proton.

3. Conclusions

We have succeeded in developing a synthesis of protected homochiral tetrahydropteridines with the stereochemistry of natural coenzymes at C-6 from (2*S*)-malic acid. This will be of use in synthesis of reduced pteridine coenzymes and pharmaceuticals.

4. Experimental

4.1. General

Melting points were determined on a Kofler hot-stage apparatus. Optical rotations (given in units of 10^{-1} deg cm^2 g^{-1}) were recorded on a Perkin–Elmer PE241 polarimeter using a 1 dm pathlength micro cell. IR spectra were recorded on a Perkin–Elmer 1710 Fourier transform instrument and UV spectra on an ATI Unicam UV2-100 Fourier transform scanning spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AC-P 250 (250 MHz), DPX 300 (300 MHz), WM 360 (360 MHz) and AMX 500 (500 MHz) Fourier transform instruments. ^{13}C NMR spectra were recorded on a Bruker DPX 300 (75.48 MHz) Fourier transform instrument. INEPT and DEPT experiments were used to help assign ^{13}C resonances where necessary. Residual undeuteriated solvent peaks were used as internal references in the NMR spectra. *J* values are given in Hertz. Mass spectra were recorded on Kratos MS80RF and Fisons Instruments VG Auto Spec double focussing spectrometers by Dr. A. Abdul-Sada. Accurate mass measurements were carried out by the EPSRC National Mass Spectrometry Service Centre, Swansea. Microanalyses were performed at Glaxo-Wellcome, Stevenage and by Medac Ltd. Column chromatography was performed using Merck Kieselgel 60 (230–400 mesh)—Art 9385 and Fluka Silica gel 60 (220–440 mesh). HPLC was performed using a Waters Symmetry C_{18}, 3.5 μm, 4.6×150 mm column (analytical) and a Waters Symmetry Prep C_{18}, 7 μm, 19×150 mm column (preparative) on a Waters Millenium HPLC fitted with a Waters 600 controller, Waters 996 Photodiode Array detector, Waters 717 plus autosampler and Waters in-line degasser. Petroleum ether refers to that fraction of hexanes bp 60–80 °C.

4.2. Ethyl (3*S*)-3,4-dihydroxybutanoate (15)

This was prepared using the method of Saito et al.[3] from diethyl (2*S*)-malate **14** in 87% yield as a clear oil; *m*/*z* [+ve FAB (3-NBA)] 149 ([M+H]$^+$); ν_{max} (film)/cm^{-1} 3399 (br, OH) and 1735 (ester); δ_H

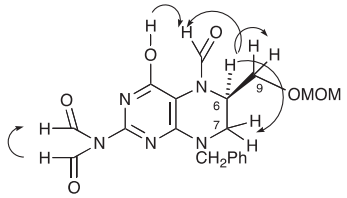


Fig. 1. NOE experiments on the reduced pteridine **38**.

(360 MHz, C^2HCl_3) 1.28 (3H, t, *J* 7.15, CH_3), 2.48 (1H, dd, $J_{2B,2A}$ 16.4, $J_{2B,3}$ 4.3, H-2B), 2.55 (1H, dd, $J_{2A,2B}$ 16.4, $J_{2A,3}$ 8.3, H-2A), 3.52, (1H, dd, $J_{4B,4A}$ 11.3, $J_{4B,3}$ 6.2, H-4B), 3.68 (1H, dd, $J_{4A,4B}$ 11.3, $J_{4A,3}$ 3.5, H-4A), 4.15 (1H, m, H-3) and 4.18 (2H, q, *J* 7.2, OCH_2). This compound decomposed to give (4*S*)-4-hydroxydihydrofuran-2(3*H*)-one **19** upon prolonged exposure to silica gel or storage and so was freshly prepared as required. (4*S*)-4-Hydroxydihydrofuran-2(3*H*)-one **19** was isolated by column chromatography as a clear oil; $[\alpha]_D^{26}$ −69.2 (*c* 1, MeOH) (lit[8] $[\alpha]_D^{31}$ −83.2 (*c* 0.41, EtOH)); *m*/*z* [+ve FAB (3-NBA)] 103 ($[M+H]^+$) and 205 ($[2M+H]^+$); ν_{max} (film)/cm^{-1} 3414 (br, OH) and 1771 (lactone); δ_H (300 MHz, $C^2H_3O^2H$) 2.37 (1H, d, $J_{3B,3A}$ 17.8, H-3*B*), 2.83 (1H, dd, $J_{3A,3B}$ 17.8, $J_{3A,4}$ 5.8, H-3*A*), 4.23 (1H, d, $J_{5B,5A}$ 10.0, H-5B), 4.43 (1H, dd, $J_{5A,5B}$ 10.0, $J_{5A,4}$ 4.2, H-5A) and 4.75 (1H, m, H-4); δ_C (75.48 MHz, $C^2H_3O^2H$) 37.48 (C-3), 67.31 (C-4), 76.72 (C-5) and 178.12 (lactone).

4.3. Ethyl (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoate (16)

Triphenylmethyl chloride (752 mg, 2.69 mmol), dried under high vacuum and stored overnight in a vacuum desiccator, was added to a solution of freshly prepared ethyl (3*S*)-3,4-dihydroxybutanoate **15** (200 mg, 1.35 mmol) in dry pyridine (2 mL) under nitrogen. The resulting yellow solution was stirred at room temperature for 1 h and heated at 50 °C with stirring for a further 1 h. Pyridine (1 mL) was added and the reaction was stirred at 50 °C for 4 h. Ethyl acetate (20 mL) was added and the mixture was washed with water (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was azeotroped with toluene (2×10 mL) to give a yellow crystalline solid. Column chromatography on silica gel using ethyl acetate/petroleum ether (1:4) as eluent gave ethyl (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoate **16** as a yellow oil, which crystallised on standing (501 mg, 95%); mp 92–94.5 °C; $[\alpha]_D^{26}$ −5.3 (*c* 1, $CHCl_3$); (found: C, 77.0; H, 6.8, $C_{25}H_{26}O_4$ requires C, 76.9; H, 6.7%); *m*/*z* [+ve FAB (3-NBA)] 413 ($[M+Na]^+$); ν_{max} (film)/cm^{-1} 3438 (br, OH) and 1730 (ester); δ_H (300 MHz, C^2HCl_3) 1.30 (3H, t, *J* 7.1, CH_3), 2.57 (1H, dd, $J_{2B,2A}$ 16.1, $J_{2B,3}$ 7.7, H-2B), 2.64 (1H, dd, $J_{2A,2B}$ 16.1, $J_{2A,3}$ 4.6, H-2A), 3.08 (1H, br s, OH, exch. with ^2H_2O), 3.23 (2H, d, $J_{4,3}$ 5.4, H-4), 4.20 (2H, q, *J* 7.1, OCH_2), 4.29 (1H, m, H-3) and 7.24–7.77 (15H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 16.13 (CH_3), 40.51 (C-2), 62.69 (C-4), 68.52 (OCH_2), 69.58 (C-3), 88.69 ($OCPh_3$), 129.1–145.71 (Ar) and 174.28 (ester).

4.4. (3*S*)-3-Hydroxy-4-*O*-triphenylmethyloxybutanoic acid (17)

1 N Aqueous sodium hydroxide (2 mL) was added to a solution of ethyl (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoate **16** (464 mg, 1.2 mmol) in tetrahydrofuran (15 mL) and the mixture was stirred at room temperature for 36 h. The sodium salt of the product, (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoic acid **17**, was extracted into water (2×15 mL) and the water layer was washed with ethyl acetate (2×10 mL). Ethyl acetate (30 mL) was added to the water layer and compressed air was passed through the mixture to ensure thorough mixing of the layers. 1 N Aqueous hydrochloric acid was added dropwise to the mixture until the pH was 4–5. A white precipitate formed and dissolved in the ethyl acetate. The layers were separated and the ethyl acetate layer was washed with water (2×15 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to yield (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoic acid **17** as a clear glass (398 mg, 93%); mp 85–89 °C; $[\alpha]_D^{26}$ −8.3 (*c* 1.0, $CHCl_3$); (found C, 74.1; H, 5.9, $C_{23}H_{22}O_4 \cdot 0.5H_2O$ requires C, 74.4; H, 6.2%); *m*/*z* [+ve FAB (3-NBA)] 385 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 3431 (br, OH) and 1709 (acid); δ_H (360 MHz, C^2HCl_3) 2.56 (1H, dd, $J_{2B,2A}$ 16.4, $J_{2B,3}$ 7.8, H-2B), 2.63 (1H, dd, $J_{2A,2B}$ 16.4, $J_{2A,3}$ 4.6, H-2A), 3.17 (1H, dd, $J_{4B,4A}$ 9.5, $J_{4B,3}$ 5.8, H-4B), 3.25 (1H, dd, $J_{4A,4B}$ 9.5, $J_{4A,3}$ 4.9, H-4A), 4.24 (1H, m, H-3) and 7.24–7.56 (15H, m, aromatic); δ_C (75.48 MHz, C^2HCl_3) 37.0 (C-2), 65.45 (C-4), 66.46 (C-3), 85.74 ($OCPh_3$), 126.1–127.6 (Ar), 142.61 (acid) and 145.78 (Ar).

4.5. (5*S*)-5-(Triphenylmethyloxymethyl)-oxazolidin-2-one (18)

Triethylamine (1.95 mL, 14 mmol) was added to a solution of (3*S*)-3-hydroxy-4-*O*-triphenylmethyloxybutanoic acid **17** (2.54 g, 7 mmol) in tetrahydrofuran (40 mL) under nitrogen. The mixture was cooled to −30 °C and *iso*-butyl chloroformate (0.94 mL, 7.14 mmol) was added dropwise. A precipitate slowly formed and the reaction was left for 1 h. The suspension was filtered onto tetrahydrofuran (100 mL) at −30 °C. An ice-cooled solution of sodium azide (547 mg, 8.4 mmol) in water (25 mL) was added and the temperature was allowed to warm to 0 °C over 1 h and stirred at 0 °C for a further 30 min. The precipitate was removed by filtration and the solvents were removed under reduced pressure giving a white residue. Chloroform (100 mL) was added, forming a suspension, which was washed with water (4×50 mL) and brine (50 mL). The chloroform layer was dried ($MgSO_4$) and the solvent was removed in vacuo to give a foam, which was dissolved in toluene (80 mL) and heated to 60 °C with stirring for 1 h. The reaction was heated at reflux with stirring for a further 30 min and cooled. The solvent was removed under reduced pressure to give the crude product as a foam. Column chromatography on silica gel using ethyl acetate/petroleum ether (1:1) as eluent gave (5*S*)-5-(triphenylmethyloxymethyl)-oxazolidin-2-one **18** as a white solid (760 mg, 30%); mp 198–200 °C; $[\alpha]_D^{26}$ +13.9 (*c* 0.85, $CHCl_3$); (found: C, 76.65; H, 5.9; N, 3.7, $C_{23}H_{21}NO_3$ requires: C, 76.9; H, 5.9; N, 3.9%); *m*/*z* [+ve FAB (3-NBA)] 360 ($[M+H]^+$) and 382 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 3415 (br, NH), 1772 and 1720 (urethane and amide); δ_H (360 MHz, C^2HCl_3) 3.24 (1H, dd, $J_{4B,4A}$ 10.4, $J_{4B,5}$ 4.5, H-4B), 3.40 (1H, dd, $J_{4A,4B}$ 10.4, $J_{4A,5}$ 4.3, H-4A), 3.46 (1H, m, H-6B), 3.62 (1H, m, H-6A), 4.75 (1H, m, H-5), 5.18 (1H, s, NH, exch. with ^2H_2O) and 7.23–7.56 (15H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 41.56 (C-4), 63.12 (C-6), 74.38 (C-5), 85.80 ($OCPh_3$), 126.2–142.37 (Ar) and 157 (urethane).

4.6. Methyl (5*S*)-oxazolidin-2-one-5-carboxylate (21)

Triethylamine (7.05 mL, 51 mmol) was added to a suspension of α-methyl (2*S*)-malate **20**[4] (5.0 g, 34 mmol) in benzene (180 mL) under nitrogen at room temperature, dissolving the suspension. Diphenylphosphoryl azide (8.32 mL, 38 mmol) was added dropwise and the resulting mixture was heated at reflux with stirring for 1 h and cooled to room temperature. The solvents were removed in vacuo to yield the crude product as a red oil. Column chromatography on silica gel using ethyl acetate/petroleum ether (2:1) as eluent gave methyl (5*S*)-oxazolidin-2-one-5-carboxylate **21** as a yellow solid (3.62 g, 74%). A small sample was recrystallised from ethyl acetate; mp 98–99 °C; $[\alpha]_D^{26}$ −15.7 (*c* 1.0, MeOH); *m*/*z* [+ve FAB (3-NBA)] 146 ($[M+H]^+$), 291 ($[2M+H]^+$), 436 ($[3M+H]^+$) and 581 ($[4M+H]^+$); ν_{max} (KBr)/cm^{-1} 3277 (NH), 1763 (ester) and 1736 (urethane); δ_H (300 MHz, C^2HCl_3) 3.64 (1H, dd, $J_{4B,4A}$ 9.3, $J_{4B,5}$ 5.5, H-4B), 3.78 (3H, s, OCH_3), 3.84 (1H, t, $J_{4A,4B}$=$J_{4A,5}$=9.3, H-4A), 4.98 (1H, dd, $J_{5,4A}$ 9.3, $J_{5,4B}$ 5.5, H-5) and 6.26 (1H, br s, NH, exch. with MeO^2H); δ_C (75.48 MHz, C^2HCl_3) 45.43 (C-4), 54.78 (OCH_3), 74.37 (C-5), 160.83 (urethane) and 171.18 (ester).

4.7. Methyl (5*S*)-3-*tert*-butoxycarbonyloxazolidin-2-one-5-carboxylate (22)

A solution of di-*tert*-butyl dicarbonate (7.14 g, 33 mmol) in 1,4-dioxane (8 mL) was added to a solution of methyl (5*S*)-oxazolidin-

2-one-5-carboxylate **21** (3.17 g, 22 mmol) in 1,4-dioxane (80 mL), triethylamine (4.56 mL, 33 mmol) and dimethylaminopyridine (533 mg, 4.3 mmol) under nitrogen. The reaction was stirred at room temperature for 1 h with evolution of CO_2. The solvents were removed in vacuo and the residue was dissolved in ethyl acetate (75 mL). The ethyl acetate was washed with water (2×50 mL) and brine (30 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to yield the product as a red oil, which crystallised on standing (5.09 g, 95%). A small sample was purified further by column chromatography on silica gel using ethyl acetate/petroleum ether (2:3) as eluent to yield an analytical sample of methyl (5*S*)-3-*tert*-butoxycarbonyloxazolidin-2-one-5-carboxylate **22** as an orange oil, which crystallised on standing; mp 75–76 °C; $[\alpha]_D^{26}$ +24.5 (*c* 1.0, $CHCl_3$); (found C, 49.2; H, 6.2; N, 5.7, $C_{10}H_{15}NO_6$ requires C, 49.0; H, 6.2; N, 5.7%); *m*/*z* [+ve FAB (3-NBA)] 246 ($[M+H]^+$) and 268 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 1807 (bisurethane), 1755 (ester) and 1719 (imide); δ_H (300 MHz, C^2HCl_3) 1.51 (9H, s, $C(CH_3)_3$), 3.86 (3H, s, OCH_3), 4.03 (1H, dd, $J_{4B,4A}$ 10.7, $J_{4B,5}$ 5.3, H-4B)), 4.18 (1H, dd, $J_{4A,4B}$ 10.1, $J_{4A,5}$ 9.6 H-4A)) and 4.92 (1H, dd, $J_{5,4A}$ 9.6, $J_{5,4B}$ 5.3, H-5)); δ_C (75.48 MHz, C^2HCl_3) 28.33 ($C(CH_3)_3$), 46.53 (C-4), 53.67 (OCH_3), 69.30 (C-5), 84.92 ($OC(CH_3)_3$), 149.26 and 150.93 (urethane) and 168.96 (ester).

4.8. (5*S*)-3-*tert*-Butoxycarbonyl-5-hydroxymethyloxazolidin-2-one (23)

A solution of methyl (5*S*)-3-*tert*-butoxycarbonyloxazolidin-2-one-5-carboxylate **22** (5.0 g, 20.3 mmol) in tetrahydrofuran (100 mL) was cooled in an ice/salt water bath to −15 °C under nitrogen and stirred for 15 min. Sodium borohydride (1.16 g, 30.7 mmol) was added in one portion and the mixture was stirred at −15 °C for 20 min. Methanol (40 mL) was added causing effervescence. The solution was allowed to warm to room temperature with stirring over 1 h. The solvents were removed in vacuo and the residue was partitioned between ethyl acetate (75 mL) and brine (50 mL). The brine layer was extracted with ethyl acetate (25 mL) and the combined ethyl acetate layers were washed with brine (25 mL). The ethyl acetate was removed under reduced pressure to afford (5*S*)-3-*tert*-butoxycarbonyl-5-hydroxymethyloxazolidin-2-one **23** as a red oil, which crystallised on standing (3.66 g, 83%). A sample was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (3:1 then 1:1) to yield a white solid; mp 83–85 °C; $[\alpha]_D^{26}$ +44.6 (*c* 1.0, $CHCl_3$); (found C, 49.6; H, 7.2; N, 6.3, $C_9H_{15}NO_5$ requires C, 49.8; H, 7.0, N 6.45%); *m*/*z* [+ve FAB (3-NBA)] 218 ($[M+H]^+$) and 240 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 3449 (OH), 1787 and 1717 (urethane); δ_H (300 MHz, C^2HCl_3) 1.47 (9H, s, $C(CH_3)_3$), 2.68 (1H, br s, OH, exch. with MeO^2H), 3.62 (1H, dd, $J_{4B,4A}$ 12.7, $J_{4B,5}$ 3.9, H-4B), 3.63 (3H, m, H-4A, H-6A and H-6B) and 4.54 (1H, m, H-5); δ_C (75.48 MHz, C^2HCl_3) 28.36 ($C(CH_3)_3$), 45.11 (C-4), 62.90 (C-6), 73.50 (C-5), 84.39 ($OC(CH_3)_3$), 149.79 and 152.77 (urethane).

4.9. (5*S*)-3-*tert*-Butoxycarbonyl-5-(*O*-*tert*-butyldiphenylsilyloxymethyl)-oxazolidin-2-one (24)

A solution of (5*S*)-3-*tert*-butoxycarbonyl-5-hydroxymethyl oxazolidin-2-one **23** (820 mg, 3.8 mmol) in dichloromethane (10 mL) was cooled in an ice/water bath under a nitrogen atmosphere. Triethylamine (0.58 mL, 4.1 mmol) was added followed by dimethylaminopyridine (18 mg, 0.15 mmol) and *tert*-butyldiphenylsilyl chloride (1.08 mL, 4.1 mmol). The mixture was stirred at room temperature for 1 day and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed with water (2×15 mL) and brine (10 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to yield the crude product as an oil. Column chromatography on silica gel using ethyl acetate/petroleum ether (1:4) as eluent afforded (5*S*)-3-*tert*-butoxycarbonyl-5-(*O*-*tert*-butyldiphenylsilyloxymethyl)-oxazolidin-2-one **24** as a clear oil, which crystallised on standing (1.13 g, 65%); mp 80–83 °C; $[\alpha]_D^{26}$ +31.4 (*c* 1.0, $CHCl_3$); *m*/*z* (FAB, PEGNa/NOBA) found 478.2009, $[C_{25}H_{33}NO_5Si+Na]$ requires 478.2026; *m*/*z* [+ve FAB (3-NBA)] 456 ($[M+H]^+$) and 478 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 1805 and 1722 (urethane and imide); δ_H (300 MHz, C^2HCl_3) 0.96 (9H, s, $SiC(CH_3)_3$), 1.48 (9H, s, $OC(CH_3)_3$), 3.59 (1H, dd, $J_{4B,4A}$ 11.5, $J_{4B,5}$ 2.8, H-4B), 3.81 (1H, dd, $J_{4A,4B}$ 11.5, $J_{4A,5}$ 3.1, H-4A), 3.87 (2H, d, $J_{6,5}$ 7.0, H-6), 4.46 (1H, m, H-5) and 7.28–7.61 (10H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 19.59 ($SiC(CH_3)_3$), 27.09 ($SiC(CH_3)_3$), 28.41 ($OC(CH_3)_3$), 45.43 (C-4), 64.65 (C-6), 72.81 (C-5), 84.03 ($OC(CH_3)_3$), 128.26–136.05 (Ar), 149.97 and 152.32 (urethane).

4.10. (2*S*)-*N*-*tert*-Butoxycarbonyl-2,3-dihydroxypropylamine (25)

Caesium carbonate (901 mg, 2.8 mmol) was added to a solution of (5*S*)-3-*tert*-butoxycarbonyl-5-hydroxymethyloxazolidin-2-one **23** (2.73 g, 12.5 mmol) in methanol (150 mL) under nitrogen and the mixture was stirred at room temperature for 18 h. The solution was neutralised by addition of 5% aqueous citric acid (ca. 8 mL). The methanol was removed under reduced pressure and the residue was dissolved in ethyl acetate (50 mL). The ethyl acetate was washed with brine (3×30 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to yield (2*S*)-*N*-*tert*-butoxycarbonyl-2,3-dihydroxypropylamine **25** as a yellow oil (2.06 g, 86%); $[\alpha]_D^{27}$ +8.9 (*c* 1.0, $CHCl_3$); *m*/*z* [+ve FAB (3-NBA)] 192 ($[M+H]^+$), 214 ($[M+Na]^+$), 383 ($[2M+H]^+$) and 405 ($[2M+Na]^+$); ν_{max} (film)/cm^{-1} 3332 (br, OH, NH) and 1688 (urethane); δ_H (300 MHz, C^2HCl_3) 1.37 (9H, s, $C(CH_3)_3$), 2.63 (2H, br s, OH, exch. with ^2H_2O), 3.17 (1H, dd, $J_{1B,1A}$ 14.4, $J_{1B,2}$ 5.5, H-1B), 3.22 (1H, dd, $J_{1A,1B}$ 14.4, $J_{1A,2}$ 5.1, H-1A), 3.50 (1H, dd, $J_{3B,3A}$ 11.6, $J_{3B,2}$ 5.0, H-3B), 3.55 (1H, dd, $J_{3A,3B}$ 11.6, $J_{3A,2}$ 4.3, H-3A), 3.68 (1H, m, H-2) and 4.89 (1H, br s, NH, exch. with ^2H_2O); δ_C (75.48 MHz, C^2HCl_3) 28.77 ($C(CH_3)_3$), 43.16 (C-1), 64.03 (C-3), 71.64 (C-2), 80.36 ($OC(CH_3)_3$) and 157.72 (urethane).

4.11. (2*S*)-*N*-*tert*-Butoxycarbonyl-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine (26)

Triethylamine (3.2 mL, 23 mmol) and dimethylaminopyridine (101 mg, 0.8 mmol) were added to an ice-cold solution of (2*S*)-*N*-*tert*-butoxycarbonyl-2,3-dihydroxypropylamine **25** (1.99 g, 10.3 mmol) in dichloromethane (30 mL) under a nitrogen atmosphere. *tert*-Butyldiphenylsilyl chloride (2.97 mL, 11.4 mmol) was added dropwise to the mixture and the solution was stirred overnight. The solution became yellow and a precipitate formed. The organic solution was washed with water (2×30 mL) and brine (20 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to yield the crude product as a yellow oil. Column chromatography on silica gel using ethyl acetate/petroleum ether (1:3) as eluent gave (2*S*)-*N*-*tert*-butoxycarbonyl-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **26** as a very pale yellow oil (3.86 g, 87%); $[\alpha]_D^{26}$ −9.0 (*c* 1.0, $CHCl_3$); *m*/*z* (FAB, PEGH/NOBA) found 429.233654, $C_{24}H_{35}NO_4Si$ requires 429.233537; found 430.238639, $[C_{24}H_{35}NO_4Si+H]^+$ requires 430.241362; ν_{max} (film)/cm^{-1} 3428 (br, OH, NH) and 1694 (urethane); δ_H (300 MHz, C^2HCl_3) 0.98 (9H, s, $SiC(CH_3)_3$), 1.34 (9H, s, $OC(CH_3)_3$), 2.80 (1H, br s, OH, exch. with ^2H_2O), 3.05 (1H, dd, $J_{1B,1A}$ 14.0, $J_{1B,2}$ 6.7, H-1B), 3.27 (1H, dd, $J_{1A,1B}$ 14.0, $J_{1A,2}$ 3.3, H-1A), 3.52 (1H, dd, $J_{3B,3A}$ 10.3, $J_{3B,2}$ 6.1, H-3B), 3.59 (1H, dd, $J_{3A,3B}$ 10.3, $J_{3A,2}$ 4.8, H-3A), 3.71 (1H, m, H-2), 4.80 (1H, br s, NH, exch. with ^2H_2O) and 7.26–7.60 (10H, ArH); δ_C (75.48 MHz, C^2HCl_3) 19.65 ($SiC(CH_3)_3$), 27.29 ($SiC(CH_3)_3$), 28.78 ($C(CH_3)_3$), 43.5 (C-1), 65.89 (C-3), 71.73 (C-2), 79.99 ($OC(CH_3)_3$), 128.27–135.95 (Ar) and 157.0 (urethane).

4.12. (2*S*)-3-(*O*-*tert*-Butyldiphenylsilyloxy)-2-hydroxypropyl amine (27)

Trifluoroacetic acid (90 mL) was cooled in an ice/water bath under a nitrogen atmosphere and added to (2*S*)-*N*-*tert*-butoxycarbonyl-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **26** (2.18 g, 5.08 mmol). The solution was stirred for 20 min at 0 °C and the solvent was removed in vacuo to yield the trifluoroacetate of (2*S*)-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **27** as a pale green solid that was wet with trifluoroacetic acid. This was azeotroped with ethyl acetate (50 mL) and diethyl ether (50 mL). The crystalline solid was placed under high vacuum but the final solid still contained trifluoroacetic acid (2.54 g). A small sample was treated with triethylamine and extracted into ethyl acetate. The ethyl acetate was washed with brine, dried ($MgSO_4$) and the solvent was removed in vacuo to afford an analytical sample of (2*S*)-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **27** as a clear oil, free from trifluoroacetic acid; $[\alpha]_D^{26}$ −5.4 (*c* 1.0, $CHCl_3$); *m*/*z* (FAB, PEGH/NOBA) found 330.187426, $[C_{19}H_{27}NO_2Si+H]^+$ requires 330.188933; *m*/*z* [+ve FAB (3-NBA)] 330 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 3363 (br, OH, NH); δ_H (300 MHz, C^2HCl_3) 0.95 (9H, s, $SiC(CH_3)_3$), 2.68 (2H, m, H-1), 3.48 (2H, d, $J_{3,2}$ 5.2, H-3), 3.63 (1H, br s, H-2), 4.07 (3H, br, NH and OH, exch. with ^2H_2O), 7.25–7.64 (10H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 17.34 ($SiC(CH_3)_3$), 24.97 ($SiC(CH_3)_3$), 43.4 (C-1), 63.94 (C-3), 69.3 (C-2) and 125.76–133.94 (Ar).

4.13. 2-Amino-6-((2′*S*)-3′-*O*-*tert*-butyldiphenylsilyloxy-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidinone (28)

Triethylamine was added to a solution of freshly prepared[5] 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11** (870 mg, 4.5 mmol) in methanol (82 mL) at 60 °C under nitrogen until the pH was 9–10. Triethylamine was also added to a solution of (2*S*)-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **27** (2.025 g, 6.15 mmol) in methanol (5 mL) until the pH was 7. This solution was added to the hot pyrimidine solution. The pH of the resulting mixture was adjusted to 9–10 using triethylamine and the mixture was heated at reflux with stirring for 2 h and cooled to room temperature. The solvents were removed in vacuo until about a third of the original volume remained. The resulting suspension was allowed to stand at 4 °C overnight. The product was collected by filtration and washed with cold diethyl ether. The product was dried in a vacuum dessicator to yield 2-amino-6-((2′*S*)-3′-*O*-*tert*-butyldiphenylsilyloxy-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidinone **28** as a yellow solid (1.43 g, 66%). A second crop of product was collected (132 mg, 6%); mp 255–261 °C; $[\alpha]_D^{29}$ +26.0 (*c* 0.75, $CHCl_3$); (found: C, 56.1; H, 6.2; N, 14.3, $C_{23}H_{29}N_5O_5Si\cdot 0.5H_2O$ requires; C, 56.1; H, 6.1; N, 14.2%); *m*/*z* [+ve FAB (3-NBA)] 484 ($[M+H]^+$); ν_{max} (KBr)/cm^{-1} 3308 (br, OH, NH) and 1685 (amide); λ_{max} (MeOH)/nm 212 and 236 (shoulder), 283 and 332; λ_{max} (OH^-, MeOH) 209 and 344; δ_H (500 MHz, C^2HCl_3) 1.11 (9H, s, $SiC(CH_3)_3$), 2.77 (overlap with water, br s, OH), 3.52 (1H, m, H-1′*B*), 3.75 (2H, d, $J_{3',2'}$ 5.2, H-3′), 3.91 (1H, m, H-1′*A*), 4.03 (1H, s, H-2′), 5.29 (1H, br, NH), 7.38–7.69 (10H, m, ArH), 8.24 (1H, br s, 2-NH_2), 9.76 (1H, t, $J_{6NH,1'}$ 5.0, 6-NH) and 10.63 (1H, br s, NH); in a saturation transfer experiment irradiation of 2-NH at δ 8.24 ppm affected the signal at δ 5.29 ppm thus allowing us to define the signals due to the 2-NH_2 group; δ_C (75.48 MHz, C^2HCl_3) 18.69 ($SiC(CH_3)_3$), 26.27 ($SiC(CH_3)_3$), 44.03 (C-1′), 64.79 (C-3′), 73.10 (C-2′), 125.25 (C-5), 127.09–134.96 (Ar), 153.20 (C-2), 157.69 (C-4) and 159.18 (C-6).

4.14. (2*S*)-*N*-Benzyl-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine (29)

Triethylamine was added to a solution of (2*S*)-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **27** (1.23 g, 3.7 mmol) in ethanol (8 mL) under nitrogen until the pH of the solution was 8. Benzaldehyde (310 μL, 3.0 mmol) was added and the pH was readjusted to 8–9 using triethylamine. The mixture was heated at reflux for 1 h, allowed to cool to room temperature and further cooled in an ice/water bath. Sodium borohydride (324 mg, 8.6 mmol) was added very slowly and the reaction was allowed to warm to room temperature and stirred until effervescence stopped (about 45 min). The solvents were removed in vacuo and the residue was dissolved in dichloromethane (15 mL). The solution was washed with water (2×10 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to yield the crude product as a waxy solid. Column chromatography on silica gel using ethyl acetate/petroleum ether (1:1 then 2:1) as eluent gave (2*S*)-*N*-benzyl-3-(*O*-*tert*-butyldiphenylsilyloxy)-2-hydroxypropylamine **29** as a white solid (806 mg, 64%); mp 88.5–90 °C; $[\alpha]_D^{26}$ −6.8 (*c* 1.0, $CHCl_3$); (found: C, 74.4; H, 8.0; N, 3.3, $C_{26}H_{33}NO_2Si$ requires: C, 74.4; H, 7.9; N, 3.3%); *m*/*z* [+ve FAB (3-NBA)] 420 ($[M+H]^+$) and 362 ($[M-C(CH_3)_3]^+$); ν_{max} (KBr)/cm^{-1} 3422 and 3324 (OH and NH); δ_H (250 MHz, $C_6^2H_6$) 1.12 (9H, s, $SiC(CH_3)_3$), 2.50 (1H, dd, $J_{1B,1A}$ 11.9, $J_{1B,2}$ 6.5, H-1B), 2.57 (1H, dd, $J_{1A,1B}$ 11.9, $J_{1A,2}$ 4.3, H-1A), 3.45 (1H, d, $J_{B,A}$ 13.3, NCH_2Ph), 3.52 (1H, d, $J_{A,B}$ 13.3, NCH_2Ph), 3.67 (1H, d, $J_{3B,2}$ 1.4, H-3B), 3.69 (1H, s, H-3A), 3.74 (1H, m, H-2), 7.04–7.26 and 7.71–7.78 (15H, m, ArH); δ_C (75.48 MHz, $C_6^2H_6$) 19.64 ($SiC(CH_3)_3$), 27.24 ($SiC(CH_3)_3$), 51.85 (C-1), 54.20 (NCH_2Ph), 67.23 (C-3), 70.79 (C-2) and 127.27–140.99 (Ar).

4.15. 2-Amino-6-((2′*S*)-*N*-benzyl-3′-*O*-*tert*-butyldiphenylsilyloxy-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidinone (30)

Triethylamine was added to a solution of freshly prepared[5] 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11** (343 mg, 1.8 mmol) in methanol (40 mL) at 60 °C under nitrogen until the pH was 9–10. A solution of *N*-benzyl-3-*O*-*tert*-butyldiphenylsilyloxy-(2*S*)-hydroxypropanolamine **29** (755 mg, 1.8 mmol) in methanol (6 mL) was added, the pH of the mixture being 9–10. The solution was heated at reflux with stirring for 4 h. The reaction was cooled to room temperature and the solvents were removed in vacuo to yield the crude product as a yellow foam (1.27 g). This was combined with the crude product from a further reaction (200 mg). Column chromatography on silica gel using a gradient solvent system from ethyl acetate/petroleum ether (2:1) to ethanol/ethyl acetate (3:7) as eluent afforded 2-amino-6-((2′*S*)-*N*-benzyl-3′-*O*-*tert*-butyldiphenylsilyloxy-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidinone **30** as a yellow foam (790 mg, 76% total). A second purification on the mixed fraction afforded a second crop (40 mg); 86.2–87.3 °C; $[\alpha]_D^{26}$ +2.2 (*c* 1.0, $CHCl_3$); *m*/*z* (FAB, PEGH/NOBA) found 574.250170, $[C_{30}H_{35}N_5O_5Si+H]^+$ requires 574.248573; ν_{max} (KBr)/cm^{-1} 3438 (br, OH, NH) and 1673 (amide); λ_{max} (MeOH)/nm 218, 254, 283 and 358 (ε 38,439, 17,509, 7228 and 4590); λ_{max} (OH^-) 216, 251 and 366; δ_H (300 MHz, [$^2H_6$]-DMSO) 0.77 (9H, s, $SiC(CH_3)_3$), 2.95 (1H, dd, $J_{1'B,1'A}$ 13.6, $J_{1'B,2'}$ 8.1, H-1′*B*), 3.25–3.42 (3H, m, H-1′*A*, H-3′), 3.76 (1H, m, H-2′), 4.62 (1H, d, $J_{B,A}$ 15.8, NCH_2Ph), 4.84 (1H, d, $J_{A,B}$ 15.8, NCH_2Ph), 4.89 (1H, d, $J_{OH,2'}$ 5.7, 2′-OH, exch. with ^2H_2O), 7.10–7.50 (15H, m, ArH) and 10.56 (1H, br s, NH, exch. with ^2H_2O); δ_C (75.48 MHz, C^2HCl_3) 21.43 ($SiC(CH_3)_3$), 29.05 ($SiC(CH_3)_3$), 54.4 (C-1′), 63.6 (NCH_2Ph), 67.81 (C-3′), 71.7 (C-2′), 115.36 (C-5), 130.01–138.00 (Ar), 154.99 (C-2), 161.22 (C-4) and 164.6 (C-6).

4.16. (6*R*)-(2-Amino-8-benzyl-5-formyl-4(3*H*)-oxo-6-(*O*-*tert*-butyldiphenylsilyloxymethyl)-5,6,7,8-tetrahydropteridine (32)

Pyridine (42 μL, 520 μmol) was added to a solution of 2-amino-6-((2′*S*)-*N*-benzyl-3′-*O*-*tert*-butyldiphenylsilyloxy-2-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidinone **30** (100 mg, 174 μmol) in dichloromethane (1.25 mL) under argon. The resulting

solution was cooled to −30 °C and trifluoromethanesulfonic anhydride (38 μL, 227 μmol) was added. The solution was allowed to warm to 0 °C over 1 h. Tetrahydrofuran (25 mL) was added to the reaction and the flask was evacuated and refilled with argon several times. 10% Palladium on carbon (500 mg) was added and the mixture was stirred overnight under hydrogen at room temperature and pressure. The UV spectrum of the crude reaction mixture showed λ_{max} 205 and 293 (very small). The solvents were removed in vacuo and the flask was refilled with argon. Pyridine (0.8 mL) was added dropwise to the resulting residue under an argon atmosphere. This was cooled in an ice/water bath and freshly prepared[7] cold formic acetic anhydride (10 mL) was added dropwise. The mixture was allowed to warm to room temperature. Effervescence continued for 3 h. The reaction was stirred overnight at room temperature. The solvents were removed in vacuo. Dichloromethane (10 mL) was added and the solution was filtered. The filtrate was washed with water (2×10 mL) and brine (5 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to yield the crude product as an orange oil (70 mg). HPLC on a C_{18} preparative column (19 mm×150 mm) using 44% aqueous ammonium formate (0.1%, pH 7.3) in acetonitrile as eluent at a flow rate of 5 mL min^{-1} gave samples, which eluted at 26 min, λ_{max} 200 and 252 nm, and 42 min, λ_{max} 200 and 257 nm. These were separately azeotroped with dichloromethane and placed under high vacuum (0.1 mmHg) with mild heating to remove the ammonium formate by sublimation. This gave two samples of (6*R*)-(2-amino-8-benzyl-5-formyl-4(3*H*)-oxo-6-(*O*-*tert*-butyldiphenylsilyloxymethyl)-5,6,7,8-tetrahydropteridine **32** (10 mg and 9 mg, respectively, 19% total) as oils; $[\alpha]_D^{26}$ +103.2 (*c* 0.68, $CHCl_3$); *m*/*z* (FAB, PEGH/NOBA) found 554.257376, $[C_{31}H_{35}N_5O_3Si+H]^+$ requires 554.258744; *m*/*z* [+ve FAB (3-NBA)] 1107 ($[2M+H]^+$), 576 ($M+Na]^+$) and 554 ($[M+H]^+$), with ions for a diformyl by-product at *m*/*z* 582 ($[M+H]^+$) and 604 ($[M+Na]^+$); ν_{max} (thin film)/cm^{-1} 3269 (NH), 1694 (w), 1628 and 1564 (amide); λ_{max} (MeOH)/nm 207, 255 and 272 (ε 111,074, 22,425 and 23,532); λ_{max} (H^+) 208 and 272; λ_{max} (OH^-) 210 and 274; δ_H (500 MHz, C^2HCl_3+$C^2H_3O^2H$, 223 K) 0.88 (9H, s, $SiC(CH_3)_3$), 3.4–3.8 (4H, m, H-7 and H-9), 4.40 (1H, d, $J_{B,A}$ 15.5, NCH_2Ph), 4.49 (1H, m, H-6), 4.97 (1H, d, $J_{A,B}$ 15.5, NCH_2Ph), 7.07–7.54 (15H, ArH) and 8.13 (1H, s, NCHO). A minor by-product with very close resemblance to the major product was clearly present with a signal at 7.99 ppm (0.4H, s, NCHO), which was not due to a rotamer, since saturation transfer was not be observed with the singlet at 8.13 ppm. Correlation spectroscopy (COSY) was used to assign the signals from 3.4 to 4.99 ppm. This showed the coupling of the signals from the major product. The mass spectrum was recorded again after 3 weeks in deuteriochloroform and displayed no $[M+H]^+$ ion at *m*/*z* 582 for the diformylated by-product, suggesting loss the second formyl group after storage in the acidic solvent.

4.17. Methyl (5*S*)-3-benzyloxazolidin-2-one-5-carboxylate (33)

Butyl lithium (10.8 mL of a 1.6 M solution, 17.28 mmol) was added to a solution of diisopropylamine (2.4 mL, 17.2 mmol) in tetrahydrofuran (9 mL) at −78 °C. The solution was allowed to warm to 0 °C and stirred for 15 min before being transferred via cannula to a solution of methyl (5*S*)-oxazolidin-2-one-5-carboxylate **21** (2.240 g, 15.45 mmol) in tetrahydrofuran (80 mL) at −78 °C. Hexamethylphosphoramide (10.8 mL) was added and the solution was warmed to −30 °C at which it was maintained for 30 min. The solution was cooled to −78 °C and a solution of benzyl bromide (2.4 mL, 20.18 mmol) in tetrahydrofuran (9 mL) was added. The solution was stirred at −78 °C for 2 h, allowed to warm to room temperature and stirred for 2 h. The solvent was removed in vacuo to yield an orange oil. Flash chromatography on silica gel using ethyl acetate/petroleum ether (2:3) as eluent yielded methyl (5*S*)-3-benzyloxazolidin-2-one-5-carboxylate **33** (1.844 g, 51%) as a yellow oil; $[\alpha]_D^{23}$ +11.6 (*c* 1, $CHCl_3$); *m*/*z* (ammonia CI) found 236.0925, $[C_{12}H_{13}NO_4+H]^+$ requires 236.0923; *m*/*z* [FAB, 3-NBA] 236 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 1746 (br, C=O); δ_H (300 MHz, C^2HCl_3) 3.49 (1H, dd, $J_{4A,4B}$ 9.4, $J_{4A,5}$ 5.3, H-4A), 3.68 (1H, t, $J_{4B,4A}$=$J_{4B,5}$=9.4, H-4B), 3.81 (3H, s, OCH_3), 4.44 (2H, AB, J_{AB} 14.9, NCH_2Ph), 4.90 (1H, dd, $J_{5,4A}$ 5.3, $J_{5,4B}$ 9.4, H-5) and 7.25–7.40 (5H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 47.7 (C-4), 49.3 (NCH_2Ph), 54.0 (OCH_3), 70.7 (C-5), 129.1, 130.0 and 136.1 (Ar), 157.7 (urethane) and 170.3 (ester).

4.18. (5*S*)-3-Benzyl-5-hydroxymethyloxazolidin-2-one (34)

Sodium borohydride (423 mg, 11.18 mmol) was added to a solution of methyl (5*S*)-3-benzyloxazolidin-2-one-5-carboxylate **33** (1.766 g, 7.52 mmol) in tetrahydrofuran (37 mL) at −15 °C. After 15 min methanol (15 mL) was added and the solution was allowed to warm to room temperature. When TLC indicated that no starting material remained, the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (30 mL) and washed with saturated brine (10 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (4:1) as eluent afforded (5*S*)-3-benzyl-5-hydroxymethyloxazolidin-2-one **34** (1.386 g, 89%) as a white solid; mp 70–71 °C; $[\alpha]_D^{23}$ +17.9 (*c* 1.06, $CHCl_3$); (found C, 63.5; H, 6.3; N, 6.7. $C_{11}H_{13}NO_3$ requires C, 63.8; H, 6.3; N, 6.8%); *m*/*z* [FAB, 3-NBA] 208 ($[M+H]^+$); ν_{max} (KBr)/cm^{-1} 3338 (OH) and 1723 (br, OCON); δ_H (300 MHz, C^2HCl_3) 2.20–2.36 (1H, br s, OH), 3.34 (2H, ABX, $J_{4A,4B}$ 9.6, $J_{4A,5}$ 7.2, $J_{4B,5}$ 8.4, H-4), 3.70 (2H, ABX, $J_{6A,6B}$ 9.6, $J_{6A,5}$ 4.8, $J_{6B,5}$ 2.4 H-6), 4.36 (2H, AB, J_{AB} 13.2, NCH_2Ph), 4.52 (1H, dddd, $J_{5,4A}$ 7.2, $J_{5,4B}$ 8.4, $J_{5,6A}$ 4.8, $J_{5,6B}$ 2.4, H-5) and 7.16–7.36 (5H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 45.5 (C-4), 48.7 (NCH_2Ph), 63.4 (C-6), 74.1 (C-5), 128.4, 129.3 and 135.9 (Ar) and 158.5 (C-2).

4.19. (5*S*)-3-Benzyl-5-(*O*-methoxymethoxymethyl)-oxazolidin-2-one (35)

Phosphorus pentoxide (5.0 g, 35.2 mmol) was added to a solution of (5*S*)-3-benzyl-5-hydroxymethyloxazolidin-2-one **34** (1.908 g, 9.2 mmol) and dimethoxymethane (25 mL, 282 mmol) in chloroform (25 mL). The mixture was stirred at room temperature for 1.5 h and decanted into ice-cool saturated aqueous sodium carbonate (50 mL). The resulting mixture was extracted with ethyl acetate (3×25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (7:3) as eluent afforded (5*S*)-3-benzyl-5-(*O*-methoxymethoxymethyl)-oxazolidin-2-one **35** (1.819 g, 79%) as a colourless oil; $[\alpha]_D^{23}$ +17.2 (*c* 1, $CHCl_3$); *m*/*z* (ammonia CI) found 252.1235, $[C_{13}H_{17}NO_4+H]^+$ requires 252.1236; *m*/*z* [FAB, (3-NBA)] 252 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 1746 (OCON); δ_H (300 MHz, C^2HCl_3) 3.26–3.36 (1H, m, H-4A), 3.31 (3H, s, OCH_3), 3.35–3.51 (1H, m, H-4B), 3.60–3.71 (2H, m, H-6), 4.44 (2H, m, CH_2Ph), 4.60–4.68 (1H, m, H-5), 4.62 (2H, s, OCH_2O) and 7.28–7.39 (5H, m, ArH); δ_C (75.48 MHz, C^2HCl_3) 45.8 (C-4), 48.2 (NCH_2Ph), 55.4 (OCH_3), 67.9 (C-6), 71.6 (C-5), 96.7 (OCH_2O), 128.1, 128.8, and 135.7 (Ar) and 157.8 (C-2).

4.20. (2*S*)-1-Benzylamino-3-(methoxymethoxy)-propan-2-ol (36)

A solution of (5*S*)-3-benzyl-5-(*O*-methoxymethoxymethyl)-oxazolidin-2-one **35** (531 mg, 2.116 mmol) in methanol (10 mL) was heated at reflux for 12 h with aqueous sodium hydroxide (2 M, 10 mL, 20 mmol). The methanol was removed in vacuo and the residue was extracted with ethyl acetate (3×25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. Flash chromatography on silica gel using ethyl acetate as

eluent afforded (2*S*)-1-benzylamino-3-(methoxymethyoxy)-propan-2-ol **36** as a colourless oil (427 mg, 90%); $[\alpha]_D^{23}$ −1.5 (*c* 1, $CHCl_3$); *m*/*z* (ammonia CI) found 226.1442, $[C_{12}H_{19}NO_3+H]^+$ requires 226.1443; *m*/*z* [FAB, 3-NBA] 226 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 3315 (OH and NH); δ_H (300 MHz, C^2HCl_3) 2.62–2.80 (2H, m, H-1), 2.82 (1H, br s, OH), 3.35 (3H, s, OCH_3), 3.49–3.62 (2H, m, H-3), 3.80 (2H, m, NCH_2Ph), 3.85–3.91 (1H, m, H-2), 4.62 (2H, s, OCH_2O) and 7.23–7.25 (6H, m, ArH and NH); δ_C (75.48 MHz, C^2HCl_3) 51.8 (C-1), 54.2 (NCH_2Ph), 55.7 (OCH_3), 69.4 (C-2), 71.5 (C-3), 97.3 (OCH_2O), and 125.5, 128.6, 128.9 and 140.3 (Ar).

4.21. 2-Amino-6-((2′*S*)-*N*-benzyl-3′-(*O*-methoxymethylhydroxy)-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidone (37)

2-Amino-6-chloro-5-nitro-pyrimidin-4-(3*H*)-one **11**[5] (190 mg, 1.0 mmol) was dissolved in methanol (22 mL) at 60 °C. The pH was adjusted to 9 by addition of triethylamine and a solution of (2*S*)-1-benzylamino-3-(methoxymethoxy)-propan-2-ol **36** (223 mg, 0.991 mmol) in methanol (3 mL) was added. The pH was adjusted to 9. The solution was heated at reflux for 7 h. The solvent was removed in vacuo to afford a yellow foam. Flash chromatography on silica gel using ethanol/ethyl acetate (1:9) as eluent yielded 2-amino-6-((2′*S*)-*N*-benzyl-3′-(*O*-methoxymethylhydroxy)-2′-hydroxypropylamino)-5-nitro-4-(3*H*)-pyrimidone **37** (302 mg, 80%) as a yellow solid; mp 79–80 °C; $[\alpha]_D^{23}$ +33.6 (*c* 1, $CHCl_3$); *m*/*z* (ammonia CI) found 380.1569, $[C_{16}H_{21}N_5O_6+H]^+$ requires 380.1570; *m*/*z* [EI+] 379 ($[M]^+$); ν_{max} (KBr)/cm^{-1} 3320 (OH), 3219 (NH) and 1677 (C=O); λ_{max} (MeOH)/nm 357, 285 and 253 (ε 4822, 6979 and 15,330); λ_{max} (H^+) 357, 285 and 253; λ_{max} (OH^-) 368 and 250; δ_H (300 MHz, $(C^2H_3)_2CO$) 3.10 (3H, s, OCH_3), 3.22–3.42 (4H, m, H-3′ and H-1′), 3.91–4.00 (1H, m, H-2′), 4.10 (1H, br s, OH), 4.42 (2H, s, OCH_2O), 4.54–4.88 (2H, m, NCH_2Ph), 7.14–7.22 (5H, m, ArH) and 10.64 (1H, br s, NH); δ_C (75.48 MHz, $(C^2H_3)_2CO$, 324 K) 53.4 (C-1′), 54.8 (NCH_2Ph), 55.4 (OCH_3), 69.3 (C-2′), 71.2 (C-3′), 97.6 (OCH_2O), 114.7 (C-5), 128.3, 128.8, 129.4 and 138.0 (Ar), 154.1 (C-6), 159.7 (C-2) and 162.4 (C-4).

4.22. (6*R*)-2-(*N*,*N*-Diformylamino)-8-benzyl-5-formyl-4-(3*H*)-oxo-6-(methyloxymethyloxymethyl)-5,6,7,8-tetrahydropteridine (38)

Methanesulfonyl chloride (0.028 mL, 0.362 mmol) was added to a solution of 2-amino-6-((2′*S*)-*N*-benzyl-3′-(*O*-methoxymethylhydroxy)-propylamino)-5-nitro-4-(3*H*)-pyrimidone **37** (100 mg, 0.264 mmol) and triethylamine (0.048 mL, 0.345 mmol) in dichloromethane (2.5 mL) at 0 °C. After 10 min TLC (1:9, ethanol/ethyl acetate) indicated no starting material remaining and a single higher running product. Methanol (20 mL) was added, followed by 10% palladium on charcoal (400 mg). The mixture was stirred under an atmosphere of hydrogen for 1.5 h and the solvent was removed in vacuo. Pyridine (0.8 mL) was added and the mixture was cooled in an ice bath. Freshly prepared[7] formic acetic anhydride (10 mL) was added. When the effervescence ceased, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with dichloromethane and filtered through Celite®. The solvent was removed in vacuo (high vacuum). Flash chromatography on silica gel using ethanol/ethyl acetate (1:9) as eluent afforded (6*R*)-2-(*N*,*N*-diformylamino)-8-benzyl-5-formyl-4-(3*H*)-oxo-6-(methyloxymethyloxymethyl)-5,6,7,8-tetrahydropteridine **38** (36 mg, 33%) as a white solid; mp 84–86 °C; $[\alpha]_D^{23}$ +2.0 (*c* 0.91, $CHCl_3$); *m*/*z* [FAB, (3-NBA)] 416 ($[M+H]^+$); *m*/*z* (EI^+) 415 $[M]^+$; ν_{max} (KBr)/cm^{-1} 3424 (OH), 1678 (C=O) and 1638 (C=O); λ_{max} (MeOH)/nm 274 and 250 (ε 2084 and 2460); λ_{max} (H^+) 294 and 252; λ_{max} (OH^-) 278 and 228; δ_H (500 MHz, C^2HCl_3) 3.30 (3H, s, OCH_3), 3.71 (1H, dd, $J_{9A,9B}$ 9.8, $J_{9A,6}$ 3.5, H-9A), 3.78 (1H, dd, $J_{7A,7B}$ 10.5, $J_{7A,6}$ 2.6, H-7A), 3.89 (1H, t, $J_{9B,9A}$=$J_{9B,6}$=9.8, H-9B), 4.23 (1H, dd, $J_{7B,7A}$ 10.5, $J_{7B,6}$ 4.4, H-7B), 4.46 (2H, AB, J_{AB} 16.2, NCH_2Ph), 4.62 (2H, AB, J_{AB} 6.5, OCH_2O), 4.86 (1H, dddd, $J_{6,7A}$ 4.4, $J_{6,7B}$ 2.6, $J_{6,9A}$ 9.8, $J_{6,9B}$ 3.5, H-6), 7.18–7.42 (5H, m, ArH), 8.60 (1H, s, CHO), 8.70 (1H, s, CHO), 8.88 (1H, s, CHO) and 12.10 (1H br s, OH); δ_C (C^2HCl_3) 50.9 (C-7), 53.3 (NCH_2Ph), 55.7 (C-6), 56.1 (OCH_3), 66.0 (C-9), 97.1 (OCH_2O), 125.4 (C-4a), 126.9, 129.0, 129.8 and 134.5 (Ar), 150.7 (C-8a) 152.5 (C-2), 153.8 (C-4), 163.7 (CHO), 164.0 (CHO) and 175.4 (CHO).

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