Synthesis of the organic ligand of the molybdenum cofactor, in protected form

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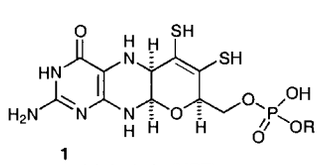
The organic ligand of the cofactor of the oxomolybdoenzymes has been synthesised in the protected and masked form, **5**. The key steps in the conversion of the previously prepared **3**, a protected 5-(2-amino-4-oxopteridin-6-yl)-4-(1,2-dihydroxyethyl)-1,3-dithiol-2-one were: formation of the pyran ring by reaction with a chloroformate giving a protected 8-amino-4-hydroxymethyl-6-(alkyloxycarbonyl)-5a,6,9,10-tetrahydro-[1,3]dithiolo[4′,5′:4,5]pyrano-[3,2-*g*]pteridine-2,10-dione, **10**, cyanoborohydride reduction of the 11,11a-imine, protection at N-11, and finally conversion of the alcohol into a protected phosphate giving **5**.

Introduction

Molybdenum and tungsten are notable in being the only 4d- and 5d-transition elements that are known to be essential for the normal metabolism of biological systems. Molybdoenzymes are found in all forms of life from bacteria, through higher plants and animals, to man.[1,2] Recently, evidence for the involvement of tungsten in biological systems has been obtained and there are some striking parallels between the nature and function of molybdenum and tungsten centres in enzymes.[3] With the exception of the nitrogenases, each molybdoenzyme and virtually all of the tungstoenzymes, catalyse a conversion, the net effect of which is to transfer an oxygen atom to or from the substrate, as in eqn. (1). Examples of

$$X + H_2O \rightleftharpoons XO + 2H^+ + 2e^- \qquad (1)$$

molybdoenzymes include: xanthine dehydrogenases; aldehyde oxidases; sulfite oxidases; nitrate reductases; and DMSO reductases. In each case, the catalytic process involves a mononuclear metal centre that proceeds from Mo^{VI} to Mo^{IV} (oxidases), or *vice versa* (reductases), with Mo^{V} intervening as the catalytically active state is regenerated. In each of these molybdo- and tungsto-enzymes, the metal is bound to one or two molecules of a special cofactor. The nature of this entity has been established, following an extensive series of biochemical investigations by Rajagopalan *et al.*,[4] and several protein crystallographic studies.[5] The cofactors are molybdenum/tungsten complexes in which the dianion (ene-1,2-dithiolate) of 'molybdopterin' – (MPT) **1**, a novel biochemical species – is one of the ligands to the metal.



1
molybdopterin (MPT)

MPT is a tricyclic pyranopteridine with the pyran ring carrying an ene-1,2-dithiol (or dithiolene) and the side chain is a phosphate group. In all of the native enzymes so far structurally characterised, the pteridine is at the dihydro oxidation level and the dithiolene group acts as a bidentate ligand to bind Mo (or W); in some bacterial enzymes the phosphate group is covalently linked to a dinucleotide. We have postulated that MPT plays a significant role in the operation of the molybdo- and tungsto-enzymes.[1,6,7] The dithiolene ligation undoubtedly supports the metal through the redox changes necessary for catalytic action. The metal-based redox behaviour may be complemented by a $2H^+/2e^-$ redox change that derives from the pyran ring opening and closing.[7]

We have developed a strategy[1,8,11] for the synthesis of MPT, to enable this important moiety to be isolated and characterised independently of a biochemical source. If successful, this approach should enable MPT complexes of Mo and W (and other metals) to be synthesised, characterised and their properties determined for comparison with those of the catalytic centres of the molybdo- and tungsto-enzymes. It is not envisaged that free MPT, with an unprotected dithiolene group (as in **1**), will be sufficiently stable to be isolated and characterised. Therefore, our strategy has been to carry the dithiolene functionality in protected/masked form, during the modifications and extensions of the carbon framework. For this purpose a 1,3-dithiol-2-one group has been employed and we have demonstrated that this functionality can be released in the presence of a suitable metal centre to form dithiolene complexes.[9–13]

We have described the synthesis of the key quinoxaline[14] and pteridine[15] 1,3-dithiole-containing intermediates **2** and **3**. The most efficient route to these compounds that we have developed involves the coupling of 2-iodoquinoxaline or the protected 6-iodopteridine **7b**, respectively, with the tin compound **4**, using copper thiophene-2-carboxylate as a stoichiometric mediator.[16] Compound **4** provides a C_4-side-chain that carries a protected dithiolene group and the two alcoholic groups in a protected form, one of which is destined to form the pyran ring and the other to be linked to the phosphate group. We have also detailed[13] our method for the construction of the third ring – the pyran ring – fused to a quinoxaline or pteridine.

Herein, we describe an alternative and much more efficient synthesis of the iodopterin[17] **7b**. As reported previously,[15] **7b** has been coupled to **4** and then, employing the procedure for constructing a pyran ring on a quinoxaline ring,[13] we have achieved the synthesis of **5** – MPT in a protected and masked form – and the protected/masked dephospho-proligand **13**.

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Results and discussion

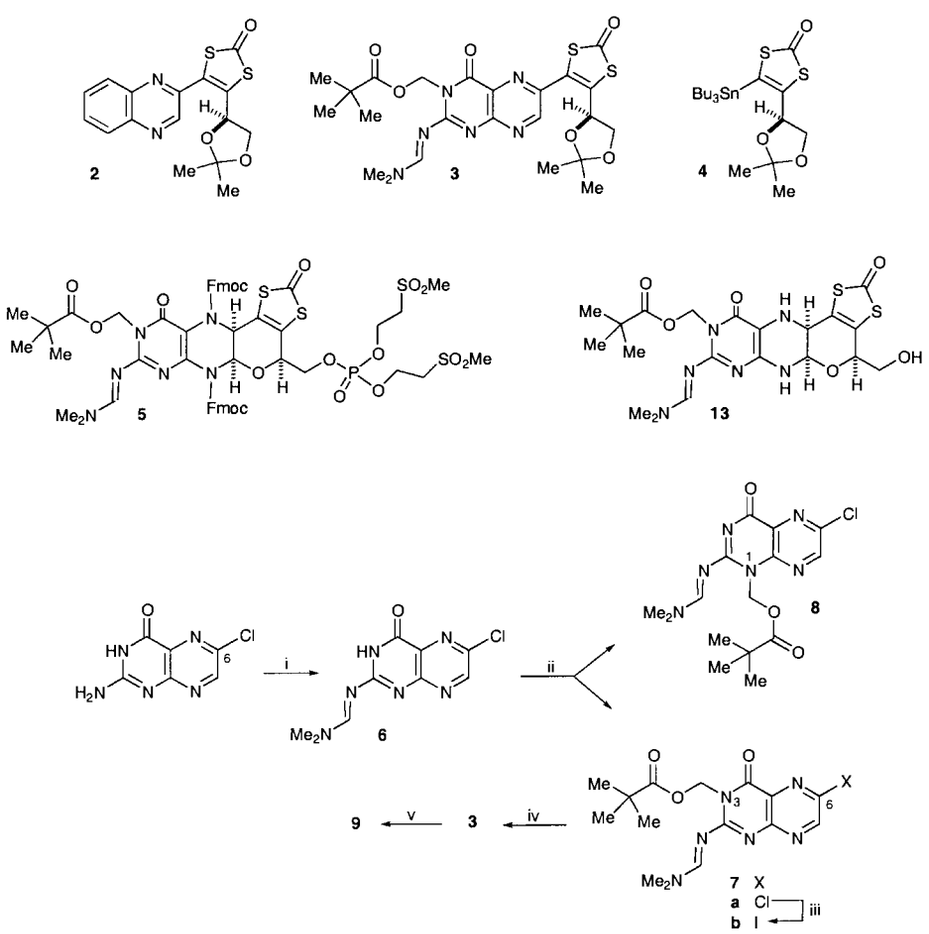
Our published routes[15] to protected 6-iodopterin **7b** were laborious and inefficient. The improved route to this key coupling partner is based on a report by Taylor[18] concerning pterin 8-oxide. This *N*-oxide, easily prepared from pterin, was converted by a combination of acetyl chloride and trifluoroacetic acid into 6-chloropterin. The regiochemistry of this unusual azine *N*-oxide rearrangement has now been verified by X-ray crystal structure determinations[19,20] on derivatives (see below).

After protecting the 2-amino group in 6-chloropterin by reaction with Bredereck's reagent (t-BuO(Me$_2$N)$_2$CH), giving **6**, and the 3-NH by treatment of **6** with base and chloromethyl pivaloate, the chloride **7a** was obtained. The chlorine was replaced by iodine in a two-step, one-pot sequence involving palladium(0)-catalysed conversion to a 6-stannane and then *ipso* displacement by reaction with iodine producing **7b**. In the process of introducing the pivaloyloxymethyl (POM) protection, a substantial quantity of the *N*-1-POM derivative **8** was produced. Both POM derivatives formed crystals suitable for X-ray analysis,[19,20] thus confirming the location of the chlorine at C-6. The use of diazabicycloundecane (DBU) as base, instead of potassium carbonate, during the N-1/N-3 protection improved both the efficiency of *N*-alkylation and the ratio of the **7a** : **8** isomers. The coupling of **7b** and **4** using copper thiophene-2-carboxylate (CuTC) now proceeds in a 60% yield (Experimental) giving **3**, hydrolysis of the acetal function then producing diol **9** (Scheme 1).

Following the methodology worked out in the quinoxaline series,[13] the diol was exposed to 9*H*-fluoren-9-ylmethyl chloroformate (FmocCl) leading to ring closure and the formation of pyrano-pteridines **10** and **11**. The two isomers **10** and **11** were readily separated by chromatography, and the stereochemistry of the *cis* isomer **10**, unambiguously settled by the observation of a positive NOE effect between the protons at C-4 and at C-5a (Scheme 2). Although the ratio of the desired **10** to undesired **11** stereoisomers was more favourable in reaction at 25 °C, the process was very slow and during the *ca.* 7 days required for complete consumption of starting materials, side-reactions led to a complex product mixture. The conditions eventually settled on – reaction at 35 °C – gave a considerably better overall yield, though only a ratio of 2 : 1 for the two products. The undesired isomer **11** was recycled by treatment with diethylamine, removal of the Fmoc group leading to rapid ring opening (N–C–O cleavage) and the formation of **9** in essentially quantitative yield.

Cyanoborohydride reduction of the *cis*-isomer **10** gave **12** a single diastereomer, meaning that the reduction was stereospecific, as in the corresponding reaction for quinoxalines.[13] The structure **12** as indicated in Scheme 2 is as anticipated and this was confirmed by the observed coupling constant between H-5a and H-11a of 1.5 Hz, leading to the conclusion that the ring junction is *cis*.

In an experiment to examine the stability of the tricyclic pyran-containing system, the Fmoc protection was removed from **12** leading to **13**, in which the pyran ring remained. However, it is important to record the relative fragility of the N–C–O system in this compound. Although **13** could be isolated by chromatography over basic alumina, and the material thus obtained fully characterised by spectroscopic means, **13** was found to be labile to acidic conditions. For example, attempted chromatography over silica, in the presence of air, resulted in the isolation of the aromatic pteridine-diol **9**, presumably *via* initial pyran-ring opening and then easy aerial oxidation.



Scheme 1 *Reagents and conditions*: i, t-BuO(Me$_2$N)$_2$CH, DMF, rt (93%); ii, ClCH$_2$OCOC(Me)$_3$, K$_2$CO$_3$, NaI, DMF, 75 °C (20% **8**, 28%, **7a**); iii, Me$_3$SnSnMe$_3$, Pd(OAc)$_2$, Ph$_3$P, 1,4-dioxane, 100 °C then I$_2$, CH$_2$Cl$_2$, rt (86%); iv, **4**, CuTC, NMP, 0 °C ⟶ rt (60%); v, TFA, CH$_2$Cl$_2$, 0 °C ⟶ rt (95%).

Since the introduction of a (protected) phosphate onto the primary alcoholic group in **12** was to entail an oxidative step, and in the light of prior knowledge of the sensitivity to oxidising conditions even of *N*-4-acyl-1,2,3,4-tetrahydroquinoxalines[10] – even aerial oxidation in solution slowly reintroduces a 1,2-double bond – we judged it advisable to protect the remaining secondary amine. Reaction with FmocCl gave **14** now ready for the introduction of a (protected) phosphate unit. Treatment of the alcohol with *N,N*-diisopropyl-bis[2-(methylsulfonyl)-ethyl]phosphoramidite[21] in the presence of tetrazole resulted in the clean formation of a new product which was oxidised, without isolation, by the addition of *tert*-butyl hydroperoxide, the protected phosphate **5** being obtained in 79% yield for the two steps (Scheme 2).

The extension of our synthetic strategy reported herein has provided a route to **5**, the first time that the framework of MPT has been assembled chemically, albeit in a protected and masked form. In the future, this work will be extended, especially to characterise the chemical properties and physical characteristics of the MPT framework, alone and complexed to Mo, W (and other metal) centres. This work will require removal of the protecting groups, all of which were chosen so that they could be removed under basic conditions, such that the fragile N–C–O unit will remain intact (see preceding paper). These studies should provide an improved chemical basis for understanding the nature and reactivity of the catalytic centres of the molybdo- and tungsto-enzymes.

Our investigations have demonstrated the acid-sensitive nature of the tricyclic pyranopterin ring system and we comment that in retrospect, it is not surprising that the early degradative work did not lead to the isolation of any products which retained the pyran ring system.[4] That we have been able to isolate and handle the tricyclic products **5** and **13**, albeit in heavily protected forms, does lend further credence to the involvement of such tricyclic species in the operation of the enzyme, but at the same time suggests that proton-catalysed N–C–O cleavage processes could well contribute, as we have suggested,[6,7] to the role of the cofactor in enzyme catalysis.

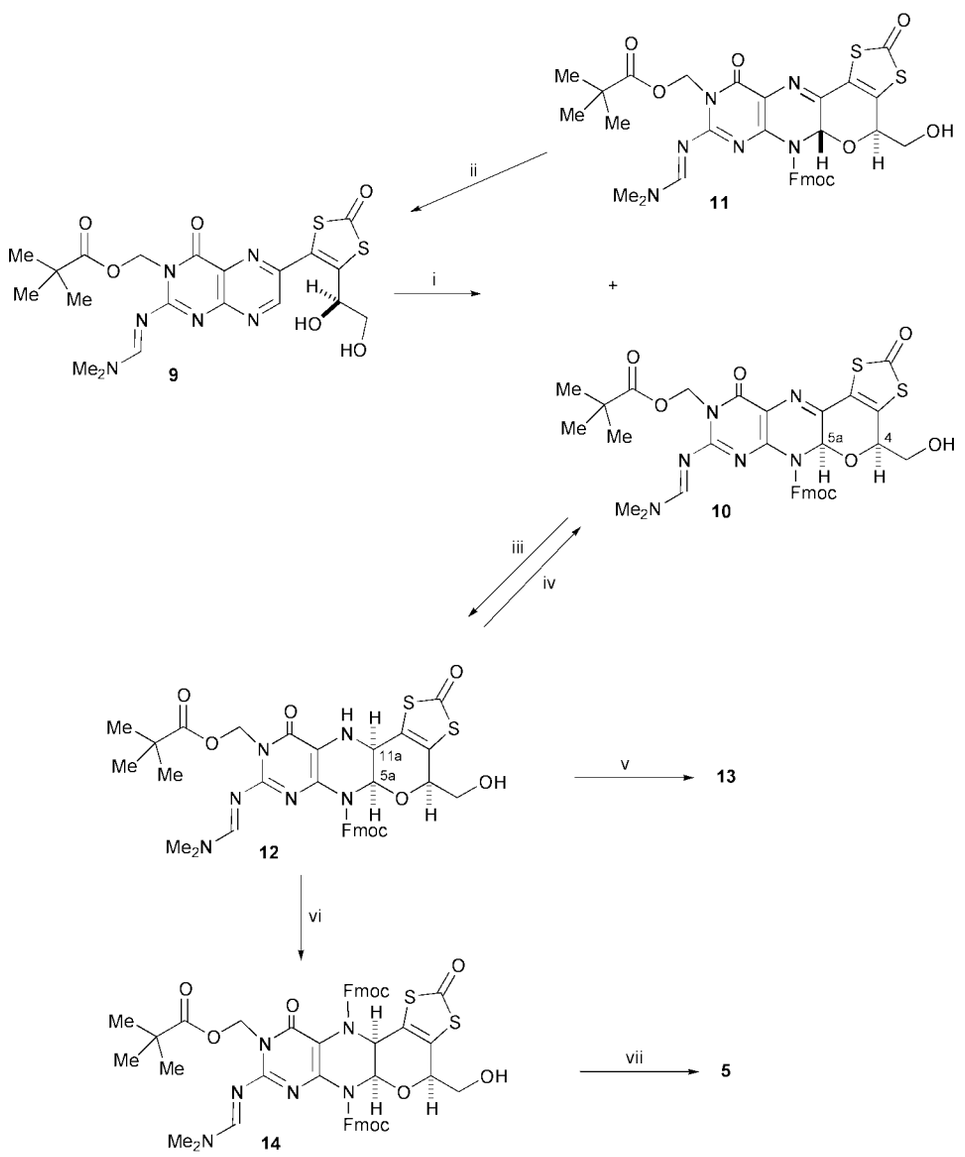
Experimental

General

See preceding paper.[13]

6-Chloro-2-{[(dimethylamino)methylene]amino}pteridin-4-one 6

To a stirred solution of 6-chloropterin[18] (11.5 g, 0.058 mol) in DMF (25 ml) at 25 °C was added *t*-BuO(Me$_2$N)$_2$CH (Bredereck's reagent) (14.2 ml, 65.3 mmol) in one portion.



Scheme 2 *Reagents and conditions*: i, FmocCl, NaHCO$_3$, H$_2$O, 1,4-dioxane, 35 °C (**10**, 52%; **11**, 32%); ii, Et$_2$NH, rt (~100%); iii, NaB(CN)H$_3$, AcOH, MeOH, CH$_2$Cl$_2$, 0 °C (92%); iv, in solution, O$_2$; v, Et$_2$NH, THF, H$_2$O, rt (85%); vi, FmocCl, NaHCO$_3$, 1,4-dioxane, H$_2$O, 35 °C (92%); vii, *i*-Pr$_2$NP(O(CH$_2$)$_2$SO$_2$Me)$_2$, tetrazole, MeCN, rt then *t*-BuO$_2$H (79%).

After 24 h, Et_2O (100 ml) was added and the resulting precipitate collected by filtration. The precipitate was washed with Et_2O then dried by heating (100 °C) under vacuum to yield *6-chloro-2-{[(dimethylamino)methylene]amino}pteridin-4-one* **6** (13.6 g, 93%) as a fine yellow powder, mp 218 °C (dec); λ_{max} ($CHCl_3$)/nm 362, 312, 244; ν_{max}(film)/cm^{-1} 3425, 1690, 1632; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.09 (1H, br s, N*H*), 8.88 (1H, s, C*H*), 8.79 (1H, s, C*H*), 3.29 (3H, s, NCH_3), 3.12 (3H, s, NCH_3); *m*/*z* (CI) 253 (MH^+, 10%), 150 (22), 133 (100); found M^+ 253.606. $C_9H_9ClN_6O$ requires *M* 253.604.

6-Chloro-2-{[(dimethylamino)methylene]amino}-3-(2,2-dimethylpropanoyloxymethyl)pteridin-4-one 7a and 6-chloro-2-{[(dimethylamino)methylene]amino}-1-(2,2-dimethylpropanoyloxymethyl)pteridin-4-one 8

Method 1. To a stirred solution of **6** (12 g, 33 mmol), K_2CO_3 (6.89 g, 49.8 mmol), NaI (1.42 g, 9.5 mmol) in DMF (46 ml), was added chloromethyl 2,2-dimethylpropanoate (10.25 ml, 70.4 mmol) and the resulting solution heated to 75 °C for 0.5 h. The solution was then stirred for a further 2 h at room temperature, the solvent was evaporated *in vacuo* and the residue taken up in CH_2Cl_2 (100 ml) and H_2O (75 ml). The resulting mixture was filtered through a pad of Celite, the organic layer separated and the aq. layer was then re-extracted with CH_2Cl_2 (2 × 100 ml). The combined organic extracts were washed with brine (100 ml), dried ($MgSO_4$) and the solvent evaporated. Purification by flash chromatography (silica, 3 ⟶ 5% MeOH in CH_2Cl_2) yielded first *N-3 protected pteridine* **7a** (4.88 g, 28%) as light yellow solid, mp 208 °C (dec); λ_{max} ($CHCl_3$)/nm 318, 246; ν_{max} (film)/cm^{-1} 2974, 1731, 1701, 1634; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (1H, s, C*H*), 8.63 (1H, s, C*H*), 6.29 (2H, s, CH_2), 3.21 (3H, s, NCH_3), 3.12 (3H, s, NCH_3), 1.11 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 159.2, 153.6, 150.3, 143.8, 65.9, 41.7, 38.8, 35.6, 26.9; *m*/*z* (CI) 367 (MH^+, 100), 266 (10), 253 (8); found C, 49.2; H, 5.6; N, 23.0%; M^+ 366.1211. $C_{15}H_{19}ClN_6O_3$ requires C, 49.1; H, 5.2; N, 22.9%; *M* 366.1207: and from later fractions *N-1 protected pteridine* **8** (3.46 g, 20%) as a dark yellow solid mp 216 °C (dec); λ_{max} ($CHCl_3$)/nm 342, 328, 278, 242; ν_{max} (film)/cm^{-1} 3425, 2973, 1729, 1649; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (1H, s, C*H*), 8.57 (1H, s, C*H*), 6.54 (2H, s, CH_2), 3.24 (3H, s, NCH_3), 3.18 (3H, s, NCH_3), 1.12 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.3, 159.2, 146.4, 145.5, 66.1, 65.9, 41.9, 38.8, 35.8, 26.9, 26.8; *m*/*z* (CI) 384 (MNH_4^+, 5%), 367 (MH^+, 100%), 253 (10); found C, 49.3; H, 5.6; N, 22.9%; M^+ 366.1203. $C_{15}H_{19}ClN_6O_3$ requires C, 49.1; H, 5.2; N, 22.9%; *M* 366.1207.

Method 2. To a stirred solution of **6** (500 mg, 1.98 mmol) and NaI (60 mg, 0.4 mmol) in dry CH_2Cl_2 (20 ml) was added DBU (386 µl, 2.58 mmol) dropwise *via* syringe over 0.25 h. The resulting solution was cooled to 0 °C and chloromethyl 2,2-dimethylpropanoate (429 µl, 2.98 mmol) was added *via* syringe over a period of 0.5 h and the mixture was then allowed to warm to room temperature then stirred for 24 h. Further portions of DBU (193 µl, 0.99 mmol) and chloromethyl 2,2-dimethylpropanoate (215 µl, 1.49 mmol) were added and the solution stirred for a further 6 h. The reaction mixture was processed as described above giving **7a** (520 mg, 72%) and **8** (80 mg, 11%) as yellow solids with spectroscopic characteristics identical to those described above.

2-{[(Dimethylamino)methylene]amino}-3-(2,2-dimethylpropanoyloxymethyl)-6-iodopteridin-4-one 7b

$Pd(OAc)_2$ (79 mg, 0.35 mmol) was added to a stirred solution of **7a** (6.47 g, 17.7 mmol), hexamethylditin (10.0 g, 26.5 mmol), and Ph_3P (464 mg, 1.77 mmol) in dry 1,4-dioxane (25 ml). The resulting solution was heated to 100 °C for 2.75 h. The solvent was evaporated, the crude stannylated pteridine dissolved in CH_2Cl_2 (200 ml) and iodine (15.7 g, 0.062 mol) was added with efficient stirring. The reaction was closely monitored until no stannylated material remained (~3 h). The organic phase was decanted from residual solid and washed with aq. 1 M Na_2SO_3 (2 × 200 ml). The combined aq. phases were re-extracted with CH_2Cl_2 (100 ml), the combined organic extracts were washed with brine (50 ml), dried and concentrated. Purification by flash chromatography (silica, 2 ⟶ 3% MeOH in CH_2Cl_2) provided the title compound as a yellow solid (6.92 g, 86%), mp 179 °C; λ_{max} ($CHCl_3$)/nm 324, 252; ν_{max} (film)/cm^{-1} 2972, 1728, 1702, 1633; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (1H, s, C*H*), 8.92 (1H, s, C*H*), 6.39 (2H, s, CH_2), 3.27 (3H, s, NCH_3), 3.18 (3H, s, NCH_3), 1.18 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 157.9, 154.0, 132.0, 128.4, 110.4, 65.9, 41.7, 38.8, 35.6, 27.0; *m*/*z* (CI) 476 (MNH_4^+, 5%), 459 (MH^+, 20), 333 (100), 279 (60); found C, 39.5; H, 4.0; N, 18.0; I, 26.8%, M^+ 458.0572. $C_{15}H_{19}IN_6O_3$ requires C, 39.3; H, 4.2; N, 18.3; I, 27.7%, *M* 458.0565.

5-(2-{[(Dimethylamino)methylene]amino}-3-(2,2-dimethylpropanoyloxymethyl)-4-oxopteridin-6-yl)-4-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-1,3-dithiol-2-one 3

To a solution of iodopterin **7b** (3.0 g, 6.55 mmol) in CH_2Cl_2 (15 ml) and NMP (20 ml) cooled to 0 °C was added copper thiophene-2-carboxylate (2.56 g, 9.83 mol) and the resulting solution stirred for 3 min. A solution of **4** [14] (4.35 g, 8.56 mol) dissolved in CH_2Cl_2–NMP (1 : 1, 14 ml), was added *via* syringe, in two portions, the second after 10 min. After 1 h the mixture was allowed to warm to room temperature and stirred for a further 2 h. The solution was diluted with CH_2Cl_2 (100 ml) then filtered through a pad of alumina eluting with CH_2Cl_2 (250 ml) then 2% MeOH in CH_2Cl_2 (3 × 250 ml). The combined fractions were concentrated and purified by flash chromatography (silica, 1 ⟶ 3% MeOH in CH_2Cl_2) providing the *coupled product* **3** (2.14 g, 60%) as a yellow solid, mp 227 °C (dec); λ_{max} ($CHCl_3$)/nm 398, 342, 270, 240; ν_{max} (film)/cm^{-1} 2979, 1733, 1706, 1634; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (1H, s, C*H*), 8.75 (1H, s, C*H*), 6.40 (2H, s, CH_2), 5.77 (1H, dd, *J* = 5.6, 7.1 Hz, C*H*), 5.09 (1H, dd, *J* = 7.1, 9.1 Hz, one of CH_2), 4.12 (1H, dd, *J* = 5.4, 9.1 Hz, one of CH_2), 3.30 (3H, s, NCH_3), 3.22 (3H, s, NCH_3), 1.60 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.60 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.31, 160.9, 159.3, 157.7, 153.4, 148.7, 141.7, 141.3, 129.1, 121.9, 111.0, 74.8, 71.2, 65.8, 41.7, 38.8, 35.7, 27.0, 26.0, 24.6; *m*/*z* (CI) 566 (MNH_4^+, 5%), 549 (MH^+, 100), 491 (20), 388 (5); found MH^+ 549.1582. $C_{23}H_{28}N_6O_6S_2$ requires *M* + H 549.1590.

5-(2-{[(Dimethylamino)methylene]amino}-3-(2,2-dimethylpropanoyloxymethyl)-4-oxopteridin-6-yl)-4-[(1*R*)-1,2-dihydroxyethyl]-1,3-dithiol-2-one 9

To a stirred solution of **3** (1.5 g, 2.74 mmol) in dry CH_2Cl_2 (100 ml) at 0 °C was added TFA (40 ml) dropwise *via* syringe over 0.25 h. The solution was then warmed to room temperature and stirred for a further 1 h. The solvent was evaporated *in vacuo* and the residue azeotroped with Et_2O (3 × 100 ml) to remove residual TFA and then purified by flash chromatography (silica, 5% MeOH in CH_2Cl_2) giving an oil which, on trituration with Et_2O gave the *diol* **9** (1.32 g, 95%) as a fine yellow powder, mp 194–195 °C; λ_{max} ($CHCl_3$)/nm 248, 342, 398; ν_{max} (film)/cm^{-1} 3392, 2968, 2932, 2870, 1704, 1637; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (1H, s, C*H*), 8.75 (1H, s, C*H*), 6.30 (2H, s, CH_2), 5.35 (1H, unresolved, C*H*), 4.15 (1H, unresolved, one of CH_2), 3.74 (1H, dd, *J* = 6.8, 11.5 Hz, one of CH_2), 3.22 (3H, s, NCH_3), 3.13 (3H, s, NCH_3), 1.60 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.3, 177.2, 161.6, 159.5, 157.6, 153.1, 149.3, 141.6, 141.5, 128.3, 121.9, 72.8, 67.7, 65.9, 41.8, 38.8, 35.80, 27.0; *m*/*z* (CI) 509 (MH^+, 100%), 219 (50), 100 (87); found C, 47.4; H, 4.5; N, 16.3; S, 12.3%; M^+ 508.1206. $C_{20}H_{24}N_6O_6S_2$ requires C, 47.2; H, 4.8; N, 16.5; S, 12.6%; *M* 508.1199.

(4*R*,5a*R*)-8-{[(Dimethylamino)methylene]amino}-4-hydroxymethyl-9-(2,2-dimethylpropanoyloxymethyl)-6-(9*H*-fluoren-9-ylmethyloxycarbonyl)-5a,6,9,10-tetrahydro-4*H*-[1,3]dithiolo-[4′,5′:4,5]pyrano[3,2-*g*]pteridine-2,10-dione 10 and (4*R*,5a*S*)-8-{[(dimethylamino)methylene]amino}-4-hydroxymethyl-9-(2,2-dimethylpropanoyloxymethyl)-6-(9*H*-fluoren-9-ylmethyl-oxycarbonyl)-5a,6,9,10-tetrahydro-4*H*-[1,3]dithiolo[4′,5′:4,5]-pyrano[3,2-*g*]pteridine-2,10-dione 11

A stirred solution of diol **9** (280 mg, 0.55 mmol), 9*H*-fluoren-9-ylmethyl chloroformate (7.13 g, 27.6 mmol) and solid $NaHCO_3$ (2 g) in 1,4-dioxane–H_2O (9.8 : 0.2 ml) was heated to 35 °C for 16 h. The reaction mixture was poured into petroleum ether (200 ml) and the resulting mixture was passed through a pad of silica and washed through with a large excess of petroleum ether in order to remove the excess chloroformate reagent. The filtrate was discarded and the product removed from the silica by elution with 10% MeOH in CH_2Cl_2. The solvent was evaporated then the residue purified by flash chromatography (silica, 0 ⟶ 2% MeOH in CH_2Cl_2) giving firstly *trans-pyrano-pteridine* **11** (130 mg, 32%) then *cis-pyrano-pteridine* **10** (210 mg, 52%) as yellow solids. Pyrano-pteridine **10** had mp 168 °C; λ_{max} ($CHCl_3$)/nm 268, 412; ν_{max} (film)/cm^{-1} 3374, 1758, 1733, 1663, 1632; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (1H, s, C*H*NMe_2), 7.25–7.83 (8H, m, Ar*H*), 6.28 (2H, s, NCH_2O), 5.78 (1H, s, H-5a), 5.0 (1H, dd, J = 5.9, 10.8 Hz, one of NCO_2CH_2CH), 4.62 (1H, dd, J = 6.2, 10.8 Hz, one of NCO_2CH_2CH), 4.38 (2H, m, NCO_2CH_2CH and H-4), 3.70 (2H, m, CH_2OH), 3.04 (3H, s, NCH_3), 2.88 (3H, s, NCH_3), 2.03 (1H, t, J = 6.3 Hz, O*H*), 1.18 (9H, s, *t*-Bu); *m*/*z* (ES+) 730 (MH^+, 100%); found C, 57.5; H, 4.6; N, 11.5; S, 8.7%; *M* 508.1206. $C_{35}H_{34}N_6O_8S_2$ requires C, 57.5; H, 4.7; N, 11.5; S, 8.7%; *M* − (9*H*-fluoren-9-ylmethyloxycarbonyl) 508.1199. Pyrano-pteridine **11** had mp 209 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (1H, s, C*H*NMe_2), 7.82–7.30 (8H, m, Ar*H*), 6.60 (1H, s, H-5a), 6.20 (2H, s, NCH_2O), 4.9 (2H, m, NCO_2CH_2CH), 4.36 (2H, m, NCO_2CH_2CH and H-4a), 3.40 (2H, m, CH_2OH), 3.04 (3H, s, NCH_3), 2.54 (3H, s, NCH_3), 1.12 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.3, 177.4, 159.7, 159.2, 156.3, 152.0, 146.1, 143.1, 142.0, 141.2, 136.4, 128.0, 127.4, 126.2, 125.0, 120.0, 108.4, 79.3, 76.6, 68.9, 68.0, 65.6, 46.8, 41.5, 38.9, 35.6, 27.2; *m*/*z* (ES+) 730 (MH^+, 100%), 676 (15), 508 (30), 490 (38), 472 (70), 449 (55), 227 (30); found C, 57.3; H, 4.6; N, 11.3; S, 8.5%; $C_{35}H_{34}N_6O_8S_2$ requires C, 57.5; H, 4.7; N, 11.5; S, 8.7%.

(4*R*,5a*R*,11a*R*)-8-{[(Dimethylamino)methylene]amino}-4-hydroxymethyl-9-(2,2-dimethylpropanoyloxymethyl)-6-(9*H*-fluoren-9-ylmethyloxycarbonyl)-5a,6,9,10,11,11a-hexahydro-4*H*-[1,3]dithiolo[4′,5′:4,5]pyrano[3,2-*g*]pteridine-2,10-dione 12

To a stirred solution of **10** (276 mg, 0.378 mmol) in CH_2Cl_2 (30 ml) and MeOH (10 ml) at 0 °C was added AcOH (91 μl, 1.89 mmol) and after 5 min, $NaB(CN)H_3$ (95 mg, 1.51 mmol) and the resulting mixture stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 (80 ml) then sat. aq. $NaHCO_3$ (50 ml) followed by addition of solid $NaHCO_3$ until a neutral pH was attained. The aq. layer was separated and washed with CH_2Cl_2 (2 × 30 ml). The combined organic layers were washed with brine (50 ml), dried, concentrated and the residue purified by flash chromatography (silica, 2 ⟶ 4% MeOH in CH_2Cl_2) to provide the *pyrano-pteridine* **12** as a yellow solid (266 mg, 92%); mp 150 °C; ν_{max} (film)/cm^{-1} 3331, 2968, 1728, 1630, 1534; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (1H, s, C*H*NMe_2), 7.27–7.80 (8H, m, Ar*H*), 6.27 (1H, d, J = 9.1 Hz, one of NCH_2OCOBu^t), 6.23 (1H, d, J = 9.1 Hz, one of NCH_2OCOBu^t), 5.15 (1H, d, J = 1.9 Hz, H-5a), 5.08 (1H, dd, J = 5.1, 10.9 Hz, one of NCO_2CH_2CH), 4.70 (1H, dd, J = 5.0, 10.9 Hz, one of NCO_2CH_2CH), 4.30 (2H, m, NCO_2CH_2CH and H-4a), 4.12 (1H, br s, NH), 3.65 (3H, m, CH_2OH and H-11a), 3.01 (3H, s, NCH_3), 2.94 (3H, s, NCH_3), 1.18 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.2, 177.4, 158.4, 158.3, 157.2, 151.4, 143.1, 141.2, 134.7, 128.4, 128.0, 127.9, 127.5, 127.3, 124.9, 124.1, 120.1, 110.8, 78.0, 77.3, 77.0, 67.5, 64.0, 47.5, 47.2, 40.9, 38.8, 34.9, 27.0; *m*/*z* (ES+) 755 (M + Na^+, 35%), 733 (MH^+, 100%), 564 (45), 525 (42), 415 (40), 351 (43), 157 (55).

(4*R*,5a*R*,11a*R*)-8-{[(Dimethylamino)methylene]amino}-4-hydroxymethyl-9-(2,2-dimethylpropanoyloxymethyl)-5a,6,9,10,11,11a-hexahydro-4*H*-[1,3]dithiolo[4′,5′:4,5]pyrano-[3,2-*g*]pteridine-2,10-dione 13

To a stirred solution of **12** (100 mg, 0.027 mmol) in THF–H_2O (14 : 1 ml) was added Et_2NH (3.3 ml). After 2 h the reaction mixture was diluted with 1 : 1 CH_2Cl_2–Et_2O (50 ml), dried (Na_2SO_4) and then concentrated *in vacuo*. Purification (alumina, 2 ⟶ 4% MeOH in CH_2Cl_2) provided the *diamine* **13** (59 mg, 85%) as a yellow solid, mp 197 °C, ν_{max} (film)/cm^{-1} 3384, 2923, 1726, 1631; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (1H, s, C*H*NMe_2), 6.12 (1H, d, J = 9.4 Hz, one of NCH_2OCOBu^t), 6.08 (1H, d, J = 9.4 Hz, one of NCH_2OCOBu^t), 5.90 (1H, br s, N-6-H), 5.12 (1H, br d, J = 4.5 Hz, H-5a), 4.68 (1H, br t, J = 5.0 Hz, H-4), 3.70 (4H, m, CH_2OH + H-11a + N-11-H), 3.05 (3H, s, NCH_3), 2.94 (3H, s, NCH_3), 1.06 (9H, s, C$(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.5, 177.4, 157.5, 157.1, 153.4, 145.6, 129.1, 125.3, 102.3, 79.2, 76.1, 65.6, 64.5, 47.6, 41.2, 38.9, 35.2, 27.2; *m*/*z* (CI) 511 (MH^+, 25%); found M^+ 510.1341. $C_{20}H_{26}N_6O_6S_2$ requires *M* 510.1355.

(4*R*,5a*R*,11a*R*)-8-{[(Dimethylamino)methylene]amino}-4-hydroxymethyl-9-(2,2-dimethylpropanoyloxymethyl)-6,11-bis(9*H*-fluoren-9-ylmethyloxycarbonyl)-5a,6,9,10,11,11a-hexahydro-4*H*-[1,3]dithiolo[4′,5′:4,5]pyrano[3,2-*g*]pteridine-2,10-dione 14

A stirred solution of pteridine **12** (250 mg, 0.34 mmol), 9*H*-fluoren-9-ylmethyl chloroformate (4.41 g, 17.1 mmol, 50 eq.) and solid $NaHCO_3$ (1.0 g) in 1,4-dioxane–H_2O (5.9 : 0.1 ml) was heated to 35 °C for 16 h. The reaction mixture was diluted with EtOAc (50 ml) and the solution dried then diluted with petroleum ether (60–80, 450 ml) and then passed through a pad of silica and washed through with a large excess of 10% EtOAc in petroleum ether in order to remove the excess chloroformate reagent. The filtrate was discarded and the product removed from the silica by thorough elution with 70% EtOAc in petroleum ether. The solvent was evaporated and purification of the residue by flash chromatography (silica, 50 ⟶ 60 ⟶ 70% EtOAc in petroleum ether) provided **14** (299 mg, 92%) as a yellow solid; mp 143–144 °C; ν_{max} (film)/cm^{-1} 3449, 2956, 1725, 1689, 1637, 1511; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.5 (1H, s, C*H*NMe_2), 7.82–7.2 (16H, m, Ar*H*), 6.35 (1H, d, J = 9 Hz, one of NCH_2O), 6.26 (1H, d, J = 9 Hz, one of NCH_2O), 6.08 (1H, br, H-5a), 5.38 (1H, br, H-11a), 4.62 (4H, m, 2 × NCO_2CH_2CH), 4.58 (1H, br, H-4a), 4.34 (3H, m, 2 × NCO_2CH_2CH and H-4a), 3.78 (2H, br m, CH_2OH), 3.12 (3H, s, NCH_3), 2.90 (3H, s, NCH_3), 2.30 (1H, br, O*H*), 1.10 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 177.4, 158.8, 158.3, 156.8, 152.7, 143.0, 141.3, 128.7, 128.0, 127.8, 127.4, 127.2, 125.3, 125.1, 107.2, 83.1, 74.1, 69.2, 68.6, 65.7, 64.3, 54.2, 46.0, 41.2, 41.0, 38.7, 27.1; *m*/*z* (ES+) 977 (M + Na, 100%), 936 (20), 711 (50); found C, 63.1; H, 4.6; N, 8.6; S, 6.6%; $C_{50}H_{46}N_6O_{10}S_2$ requires C, 62.9; H, 4.9; N, 8.8; S, 6.7%.

***N,N*-Diisopropyl-bis[2-(methylsulfonyl)ethyl]phosphoramidite**

To a stirred solution of dichlorophosphorus diisopropylamine[22] (8.14 g, 0.040 mol, 7.43 ml) in MeCN (80 ml) at 0 °C was added diisopropylethylamine (10.47 g, 0.081 mol, 14.11 ml), followed by dropwise addition of 2-(methylsulfonyl)-ethanol (10 g, 0.081 mol) dissolved in MeCN (50 ml). The mixture was allowed to warm to rt, stirred for 18 h, then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2

(200 ml), washed with sat. aq. Na_2CO_3 solution, H_2O and then dried. Evaporation of the solvent and purification by flash chromatography (0.5% Et_3N, 50% EtOAc in petroleum ether) produced the phosphoramidite (6.03 g, 40%) as a white solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (4H, m, 2 × CH_2SO$_2$CH$_3$), 3.62 (2H, m, 2 × CH(CH$_3$)$_2$), 3.30 (4H, m, 2 × CH_2CH$_2$SO$_2$CH$_3$), 3.02 (6H, m, 2 × SO$_2$CH_3), 1.22 (12H, d, J = 6.9 Hz, 2 × CH(CH_3)$_2$); *m*/*z* (CI) 378 (MH^+, 80%), 294 (20), 277 (35), 272 (40).

(4*R*,5a*R*,11a*R*)-8-{[(Dimethylamino)methylene]amino}-9-[(2,2-dimethyl-1-oxopropoxy)methyl]-6,11-bis(9*H*-fluoren-9-ylmethyloxycarbonyl)-2,10-dioxo-5a,6,9,10,11,11a-hexahydro-4*H*-[1,3]dithiolo[4′,5′:4,5]pyrano[3,2-*g*]pteridin-4-ylmethyl bis(2-methylsulfonylethyl) phosphate 5

To a stirred solution of pteridine **14** (77 mg, 0.084 mmol), *N,N*-diisopropyl-bis[2-(methylsulfonyl)ethyl]phosphoramidite (48 mg, 0.126 mmol) in MeCN (10 ml) was added tetrazole (9 mg, 0.126 mmol). After 2 h, when TLC analysis indicated the complete disappearance of pteridine **14**, *t*-BuOOH (0.1 ml) was added dropwise. After 1 h at rt the reaction mixture was partitioned between CH_2Cl_2 (20 ml) and H_2O (20 ml), the layers were separated and the aq. layer re-extracted with CH_2Cl_2 (2 × 10 ml). The combined organic layers were washed with brine (5 ml), dried and the solvent evaporated. Purification by flash chromatography (silica, 0 ⟶ 2 ⟶ 4% MeOH in CH_2Cl_2) provided **5** (55 mg, 79%) as an off-white solid, mp 151–152 °C; ν_{max} (film)/cm^{-1} 2963, 1723, 1688, 1634, 1509; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.4 (1H, s, CHNMe$_2$), 7.74–7.04 (16H, m, ArH), 6.23 (1H, d, J = 8.9 Hz, one of NCH_2O), 6.15 (1H, d, J = 8.9 Hz, one of NCH_2O), 6.0 (1H, br, H-5a), 5.20 (1H, br, H-11a), 4.60 (4H, m, 2 × NCO$_2$-CH_2CH), 4.22 (4H, m, 2 × CH$_2$SO$_2$CH$_3$), 4.38 (5H, m, 2 × NCO$_2$CH$_2$CH, H-4a and CH_2OH), 3.22 (4H, m, 2 × CH_2CH$_2$SO$_2$CH$_3$), 2.98 (3H, s, NCH_3), 2.75 (3H, s, NCH_3), 1.18 (9H, s, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.6, 177.2, 164.0, 159.1, 156.9, 152.7, 151.1, 141.2, 137.7, 128.4, 128.1, 127.9, 127.3, 127.0, 126.5, 125.3, 119.9, 83.1, 72.3, 68.1, 65.9, 61.5, 54.7, 49.3, 47.1, 42.8, 41.5, 36.6, 29.8, 27.0; *m*/*z* (ES+) 1247 (MH^+, 100%), 1207 (10), 606 (8), 418 (10), 271 (20), 204 (30).

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