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Synthesis of N3- and 2-NH_2-substituted 6,7-diphenylpterins and their use as intermediates for the preparation of oligonucleotide conjugates designed to target photooxidative damage on single-stranded DNA representing the *bcr–abl* chimeric gene

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Two 17-mer oligodeoxynucleotide-5′-linked-(6,7-diphenylpterin) conjugates, **2** and **3**, were prepared as photosensitisers for targeting photooxidative damage to a 34-mer DNA oligodeoxynucleotide (ODN) fragment **1** representing the chimeric *bcr–abl* gene that is implicated in the pathogenesis of chronic myeloid leukaemia (CML). The base sequence in the 17-mer was $^{3'}$ G G T A G T T A T T C C T T C T T $^{5'}$. In the first of these ODN conjugates (**2**) the pterin was attached at its N3 atom, *via* a –$(CH_2)_3$OPO(OH)– linker, to the 5′-OH group of the ODN. Conjugate **2** was prepared from 2-amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10**, using phosphoramidite methodology. Starting material **10** was prepared from 5-amino-7-methylthiofurazano[3,4-*d*]pyrimidine **4** *via* an unusual highly resonance stabilised cation **8**, incorporating the rare 2*H*,6*H*-pyrimido[6,1-*b*][1,3]oxazine ring system. In the characterisation of **10** two pteridine phosphazenes, **15** and **29**, were obtained, as well as new products containing two uncommon tricyclic ring systems, namely pyrimido[2,1-*b*]pteridine (**20** and **24**) and pyrimido[1,2-*c*]pteridine (**27**). In the second ODN conjugate **3** the linker was –$(CH_2)_5$CONH$(CH_2)_6$OPO(OH)– and was attached to the 2-amino group of the pterin. In the preparation of **3**, the *N*-hydroxysuccinimide ester **37** of 2-(5-carboxypentylamino)-6,7-diphenyl-4(3*H*)-pteridinone **36** was condensed with the hexylamino-modified 17-mer. Excitation of **36** with near UV light in the presence of the single-stranded target 34-mer, $^{5'}$T G A C C A T C A A T A A G^{14} G A A G^{18} A A G^{21} C C C T T C A G C G G C C $^{3'}$ **1** caused oxidative damage at guanine bases, leading to alkali-labile sites which were monitored by polyacrylamide gel electrophoresis. Cleavage was observed at all guanine sites with a marked preference for cleavage at G14. In contrast, excitation of ODN–pteridine conjugate **2** in the presence of **1** caused oxidation of the latter predominantly at G18, with a smaller extent of cleavage at G15 and G14 (in the double-stranded portion) and G21. These results contrast with our previous observation of specific cleavage at G21 with ruthenium polypyridyl sensitisers, and suggest that a different mechanism, probably one involving Type 1 photochemical electron transfer, is operative. Much lower yields were found with the ODN–pteridine conjugate **3**, perhaps as a consequence of the longer linker between the ODN and the pteridine in this case.

Introduction

Compounds containing the pteridine ring system are ubiquitous in nature, often playing an essential role in the biochemical processes of both plants and animals, including humans, and their chemistry has been widely explored.[1,2] It has been known for a long time that pteridines occur in photosensitive organs of both vertebrates and invertebrates,[3,4] and some of the less well known biological functions of pteridines depend upon their photochemical properties. For example, pteridines have a role in the photoreception and metabolism of plants,[5–8] and have been shown to be involved in the light-gathering chromophore of DNA photolyase.[9–11] There are also several reports in the literature of pteridines causing photochemical damage on DNA or its nucleobases. Thus, Chahidi *et al.* showed that the pterin chromophore was efficiently quenched by guanosine, the observed formation of the semi-reduced pterin molecule indicating that its excited state had oxidised guanine,[3] and Ito and Kawanishi investigated the photooxidation properties of a number of pteridine derivatives targeted to single-stranded and double-stranded DNA sequences.[12] These and other results showed that pteridines are excellent photosensitising molecules for the photooxidation of guanine in DNA primarily by a Type I mechanism, although they may also produce $^1O_2$.[13,14] More recently Hirakawa *et al.* have shown that a pteridine produced by photohydrolysis of methotrexate, a widely used pteridine antineoplastic agent, induces guanine-specific damage in DNA through a photo-induced electron transfer mechanism,[15] and the highly fluorescent nature of many pteridines led Hawkins *et al.* to prepare a series of pteridine nucleoside analogues, which were incorporated into nucleic acids for use as fluorescent probes.[16] Lastly, Bannwarth and his co-workers have described the linking of several types of pteridine molecules to oligonucleotides.[17,18]

In the past few years considerable interest has been evinced in the targeting of chemical reactions to particular sequences in nucleic acids, since such procedures may have applications both as sensors and in therapy.[19,20] One approach is to use modified oligonucleotides which are complementary to a particular sequence in the target (*e.g.* anti-sense or anti-gene methodologies), for it is found that formation of conjugates can enhance stability, control delivery to the cell and/or direct a reactive chemical species to the desired target.[20,21] The selectivity of such reactions can be further enhanced by using photochemical methods, and a range of photosensitiser–oligonucleotide conjugates has

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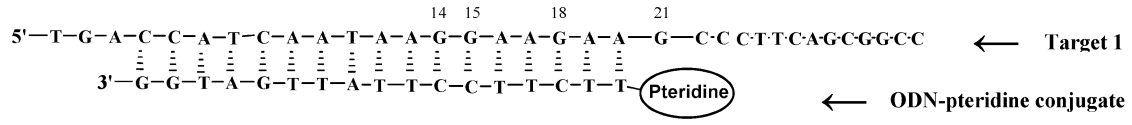


Fig. 1 17-mer ODN–pteridine conjugate hybridised to the target 34-mer **1**.

been reported.[22,23] For example, we have already targeted a sequence using a non-intercalating ruthenium complex coordinated to a complementary oligonucleotide sequence.[24] The target oligonucleotide corresponded to a chimeric nucleic acid sequence generated by the fusion of the breakpoint cluster region gene (*bcr*) and the abelson proto-oncogene (*abl*) found in chronic myeloid leukaemia cells. Binding of this ruthenium sensitiser to the 5′-end of the complementary sequence with subsequent visible light irradiation, led to photooxidation on the target strand with remarkable specificity (at G21).

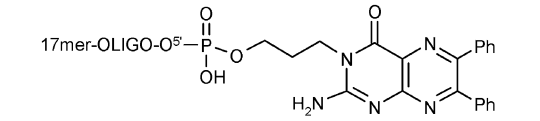
In order to extend this study to a different class of photosensitisers we have investigated the preparation of pteridine-coupled oligodeoxynucleotides, and have explored their use in targeting a specific sequence in a single-stranded nucleic acid. The target **1** (see Fig. 1) was the same synthetic 34-mer oligonucleotide used in our earlier ruthenium work.[24] Its base sequence acted as a model for the mRNA sequence transcribed from the *bcr–abl* fusion gene, which is present on the Philadelphia chromosome in CML cells. The 17-mer oligonucleotide attached to the pteridine incorporated the complementary base sequence (Fig. 1). In most of the pteridine–nucleotide conjugates prepared up to now by other workers the pteridine has been joined to the nucleotide *via* its 2-amino group, through a linker chain. In the present work we describe the formation of a new resonance-stabilised heterocyclic cation **8** incorporating the rare 2*H*,6*H*-pyrimido[6,1-*b*][1,3]oxazine ring system, which offers a route to pteridines carrying a side chain attached to N-3. We report some new chemistry of these side chain pteridines, leading up to the attachment of 6,7-diphenylpterin at its N-3 position, *via* a linker to the 17-mer oligodeoxynucleotide. 6,7-Diphenylpterin was chosen as the pteridine photosensitiser because it absorbs strongly in the near UV range, thus allowing its efficient selective excitation.

The resulting oligonucleotide–pteridine conjugate **2** was designed to hybridise to the target 34-mer **1** so that the pteridine moiety, acting as a photosensitiser, would be held close to the target guanine residue at position 21. This is illustrated in Fig. 1. The linker between the pteridine and the oligonucleotide in **2** is only three carbon atoms long. Accordingly, a second oligonucleotide–pteridine conjugate **3** was also prepared, incorporating a much longer-chain linker. This enabled us to study the effect of the linker chain length on the specificity of targeting to the DNA.

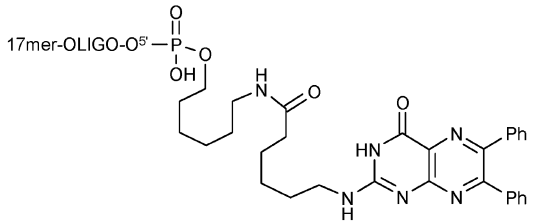
Results and discussion

N-3 side chain pteridines[25]

The key starting material for the synthesis of **2** was 2-amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone, **10**. Preparation of **10** started from 5-amino-7-methylthiofurazano[3,4-*d*]pyrimidine **4**, which upon treatment with 3-chloropropanol in presence of bromine[25,26] gave 5-amino-7-(3-chloropropoxy) furazano[3,4-*d*]pyrimidine **5** (see Scheme 1). The proton NMR spectrum of **5** is notable for the low field signal of the $-OCH_2-$ group at δ 4.67, reflecting the known very low electron density at position 7 of furazano[3,4-*d*]pyrimidines.[26,27] The furazan ring in furazano[3,4-*d*] pyrimidines is also known to be cleaved hydrogenolytically,[28] and hydrogenolysis of **5** occurred readily with palladised charcoal and hydrogen. The subsequent fate of the initially formed air-labile diaminopyrimidine **6** was found, however, to depend critically on the reaction conditions. Thus hydrogenolysis of **5** in either a methanol or tetrahydrofuran solution, followed by evaporation of the solvent, gave **6** as an unstable solid. This on dissolving in dry tetrahydrofuran containing benzil gave 2-amino-4-(3-chloropropoxy)-6,7-diphenylpteridine **9**, which could also be obtained by direct hydrogenolysis of **5** in tetrahydrofuran followed by addition of benzil, without isolation of intermediate **6**. The proton NMR spectrum of a solution of 2,5,6-triamino-4-(3-chloropropoxy)pyrimidine **6** dissolved in trideuteroacetonitrile showed signals due to the three methylene groups, together with broad overlapping signals due to the three amino groups. When D_2O was added to this solution, however, thus making the medium more polar, a reaction occurred that could be followed in the sample tube by proton NMR. The new product **8** still showed three methylene group signals in its proton NMR spectrum, but at chemical shifts different from those in **6**. Furthermore, an aqueous solution of it contained chloride ions (precipated with silver chloride). It is believed that with addition of water, the more polar medium encourages an intramolecular cyclisation of **6** to give the highly resonance-stabilized multidentate bicyclic cation **8**. The 2*H*,6*H*-pyrimido[6,1-*b*][1,3]oxazine ring system of **8** is rare, and only a few examples of it are known,[29,30,31] although some time ago we described[26] the formation of an analogous cation **7**, which incorporates the 1,3-oxazolo[2,3-*c*]pyrimidine ring system, and Lipkin and Lovett have reported ^{18}O mechanistic studies on another related bicyclic cation.[32,33] The UV spectrum of **8** was very similar to that of the previously reported cation **7**. No product was detected corresponding to cyclisation of the ω-chlorine atom of the side-chain of **6** with the exocyclic 5-amino nitrogen atom.

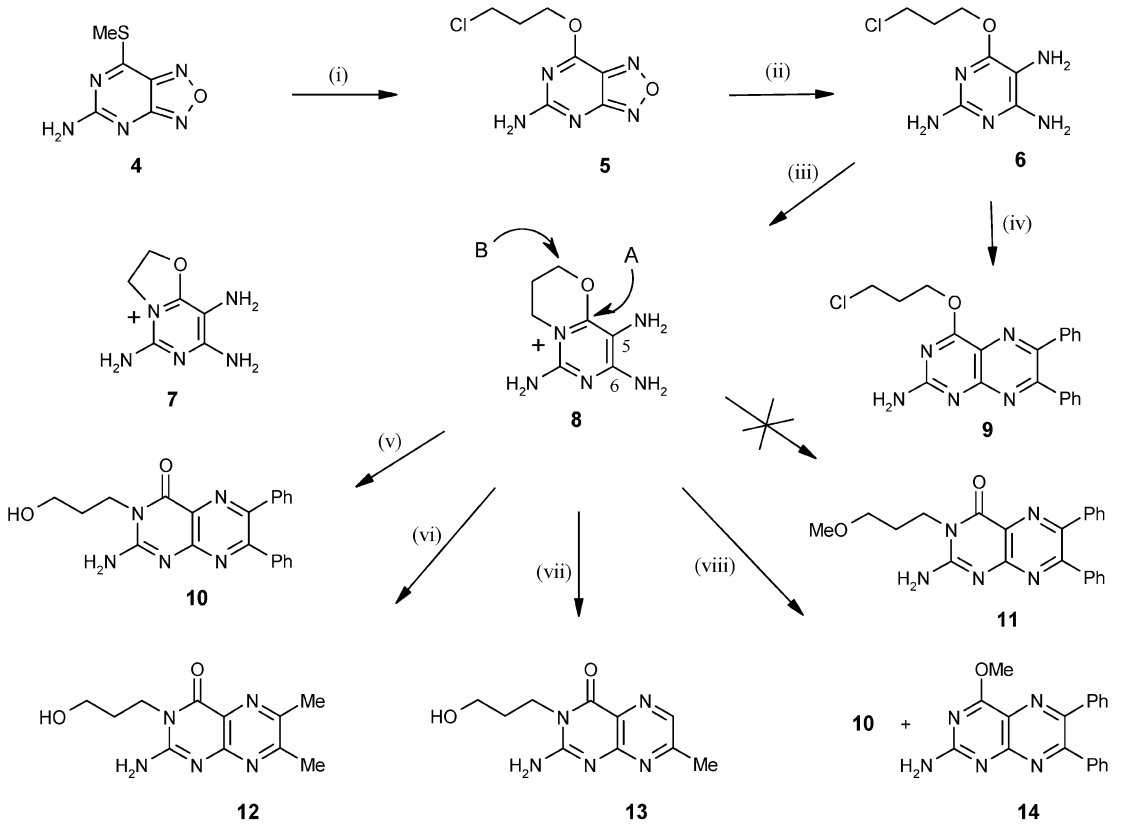


2



3

Cation **8** is susceptible to attack by nucleophiles at several different positions in the molecule. An aqueous solution of it was formed directly by hydrogenolysis of **5** with palladium and hydrogen in water, and addition of potassium hydroxide and benzil to this solution gave the desired 3-(3-hydroxypropyl)-6,7-diphenylpterin **10** in 66% yield. A similar reaction with butane-2,3-dione gave the 6,7-dimethylpterin **12** (74%). Interestingly, reaction with the unsymmetrical 1,2-dicarbonyl compound, pyruvaldehyde, proceeded with a high degree of regiospecificity, and gave only one of the two possible regioisomers in 95% purity, as shown by NMR. While the spectra do not permit an unambiguous assignment of regioisomers, we take the product to be the 7-methylpterin **13**, since the 5-amino group in cation **8** should be much more nucleophilic than the 6-amino group, which is involved in resonance delocalisation of the positive charge (pyrimidine numbering). The 5-amino group would thus be expected to react preferentially with the more electrophilic aldehyde group of the pyruvaldehyde, leading to **13**, rather than its 6-methyl regioisomer. A solid sample of the resonance stabilised cation **8** was prepared by hydrogenation of **5** in water

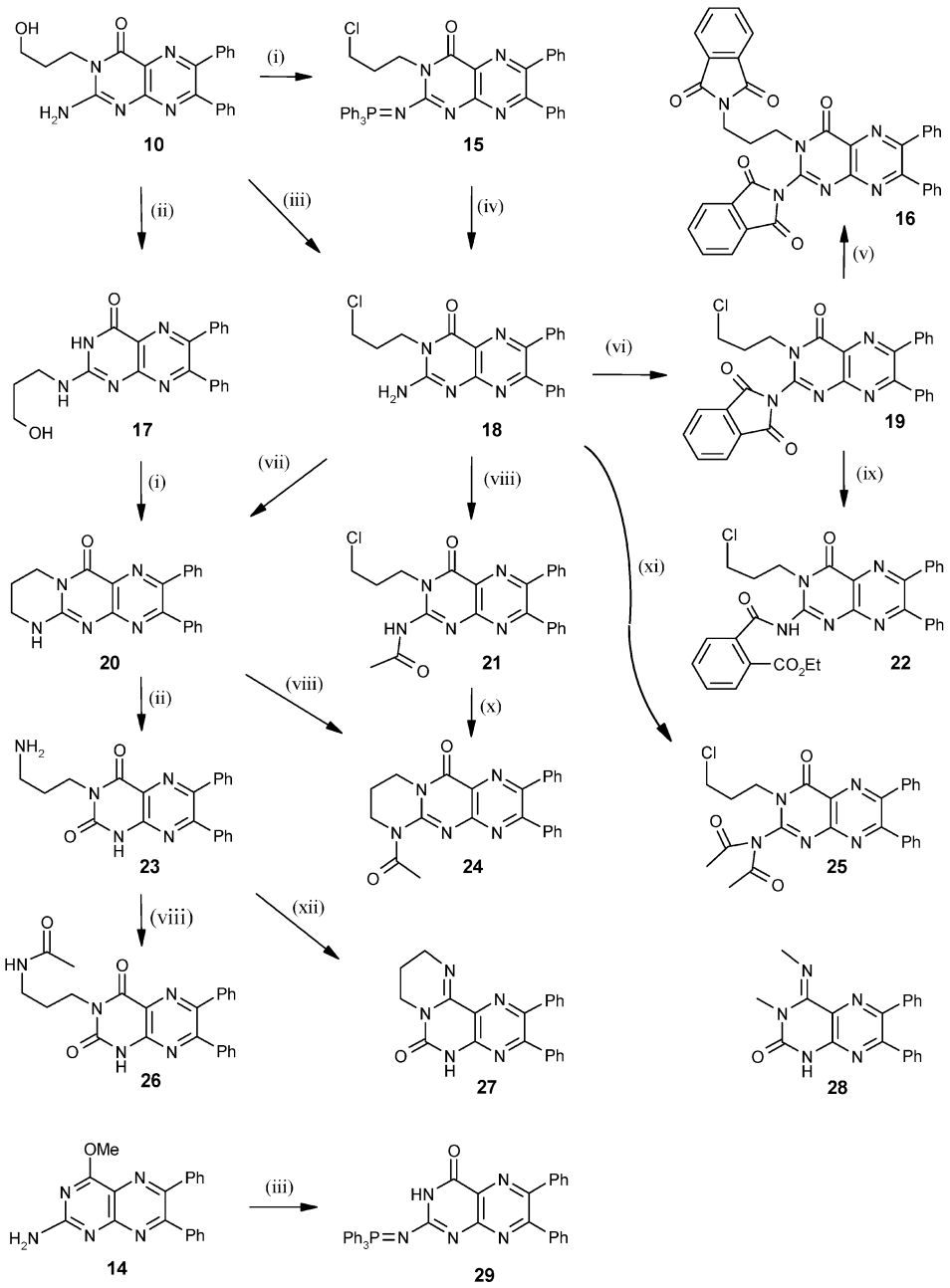


Scheme 1 (i) 3-Chloropropanol, Br_2; (ii) H_2, Pd in tetrahydrofuran or H_2O; (iii) H_2O; (iv) benzil, tetrahydrofuran; (v) benzil, KOH, H_2O, MeOH, 40 °C; (vi) biacetyl; (vii) pyruvaldehyde; (viii) benzil, MeOH, NaOMe.

followed by evaporation of solvent. When this sample of **8** was refluxed in methanol containing sodium and benzil the main product obtained was pteridine **10**, together with a small amount of **14**. No N^3-(3-methoxypropyl) product **11** was obtained, suggesting that the oxazine ring in **8** (or in the corresponding oxazinopteridine) is attacked nucleophilically by methoxide ion at position A rather that at position B. This conclusion is supported by similar results reported earlier for the analogous resonance stabilised ion **7**.[26] Addition of sodium and benzil to a methanol solution of triaminopyrimidine **6** led to formation of the same two pteridines, **10** and **14**. Under these conditions cyclisation of **6** to cation **8** is not so favorable, and the main product obtained here was the 4-methoxypteridine **14**, which could have been formed by direct methoxide ion substitution of the chloropropoxy side chain in either pyrimidine **6** or pteridine **9**. Again no N^3-(3-methoxypropyl) product **11** was isolated.

The structure of compound **10**, which was required for preparation of its oligonucleotide conjugate **2**, follows from its spectroscopic properties, and also from the conversion of it into a variety of new derivatives, some of them of novel structure. These chemical interactions are described below and are summarised in Scheme 2. Treatment of **10** with triphenylphosphine in dry tetrachloromethane containing tetrahydrofuran gave 66% of the expected chloropropyl pterin **18**. In contrast, if the reaction was carried out in pure tetrachloromethane the main product obtained was the pteridine phosphazene **15** (47%), together with **18** (39%). Very little work has been reported on pteridine phosphazenes, and the formation of **15** occurs here under very mild conditions without any specific deprotonation steps. Its proposed structure is consistent with its observed spectroscopic and chemical properties, including proton NMR spectra which showed the presence of five phenyl groups, ^{31}P NMR spectra which showed the presence of a phosphorus atom, and elemental analysis and high resolution mass spectra which corresponded to the required formula of $C_{39}H_{31}ClN_5OP$. In contrast to the extreme insolubility of most pteridines, phosphazene **15** is readily soluble in dichloromethane and ethyl acetate. It is stable in refluxing aqueous ethanol, but suffers cleavage of the phosphazene group in refluxing ethanolic hydrochloric acid, to give the chloropropyl pterin **18**. Another pteridine phosphazene **29**, which is readily soluble in acetone and in dichloromethane, was prepared in 60% yield by treatment of the 2-amino-4-methoxy pteridine **14** with triphenylphosphine in tetrahydrofuran and tetrachloromethane.

Treatment of **18** with sodium hydroxide in aqueous ethanol led to a smooth intramolecular cyclisation to give **20**, which incorporates the little known pyrimido[2,1-*b*]pteridine ring system. Although first described many years ago by Angier and Curran[34] very little has since been published on this ring system.[35,36] The same tricyclic compound **20** could also be obtained from the 3-hydroxypropylpteridine **10**, by conversion of the latter to its 2-hydroxypropylamino isomer **17** using a base catalysed Dimroth rearrangement, and then heating **17** with triphenylphosphine in tetrachloromethane. Acetylation of **20** with acetic anhydride in pyridine gave its acetyl derivative **24**, which was identical with the product obtained from **18** by acetylation of the latter in acetic anhydride and pyridine to give **21** followed by base catalysed cyclisation of **21** to **24**. Interestingly, acetylation of **18** in acetic anhydride at room temperature led to the unusual 2-diacetylaminopteridine **25**. An attempt to obtain a phthalimido derivative of **25** by nucleophilic substitution of its chlorine atom using potassium phthalimide, led instead to intramolecular cyclisation, giving again **24**. A monophthalimido derivative of **18**, namely **19**, could be obtained by treatment of **18** with phthalic anhydride, and it was found to be possible to convert **19** into its diphthalimido derivative **16** using potassium phthalimide in hexamethylphosphoramide. An effort to convert **16** into 2-amino-3-(3-aminopropyl)-6,7-diphenylpterin by treatment of it with hydrazine (Gabriel synthesis) led only to the tricyclic cyclised compound **20**, however. The phthalimido ring of **19** could be partially hydrolysed in high yield to the phthalic acid derivative **22** using dilute

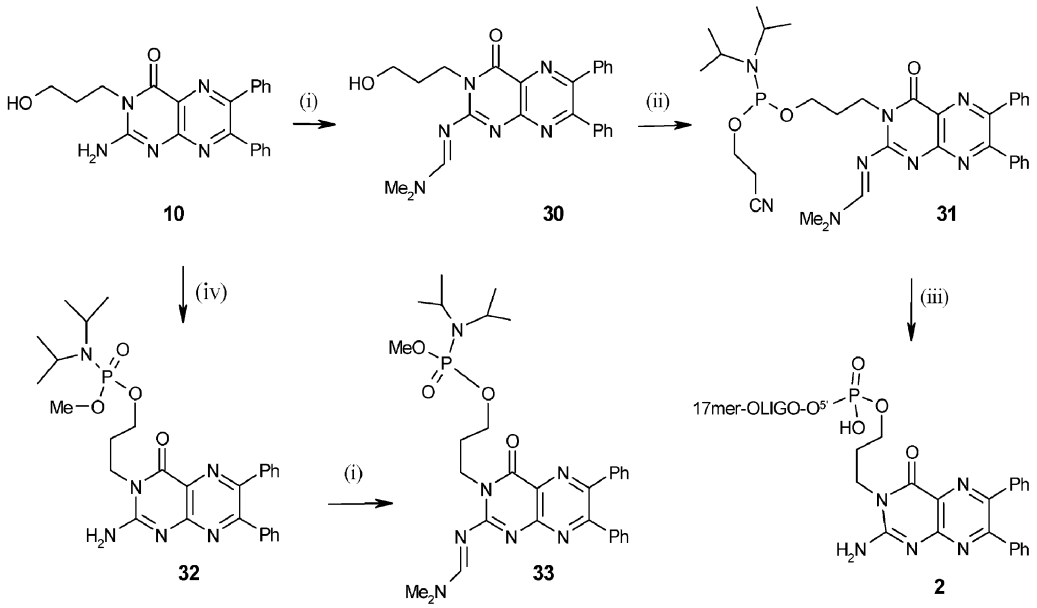


Scheme 2 (i) PPh_3–CCl_4; (ii) KOH–H_2O–MeOH; (iii) PPh_3–CCl_4, tetrahydrofuran; (iv) HCl–EtOH; (v) potassium phthalimide–HMPA;(vi) phthalic anhydride–pyridine; (vii) NaOH–H_2O–EtOH; (viii) Ac_2O–pyridine; (ix) NaOH–H_2O–EtOH–CH_2Cl_2; (x) NaOH–H_2O–tetrahydrofuran; (xi) Ac_2O, 20 °C; (xii) MeOH, reflux.

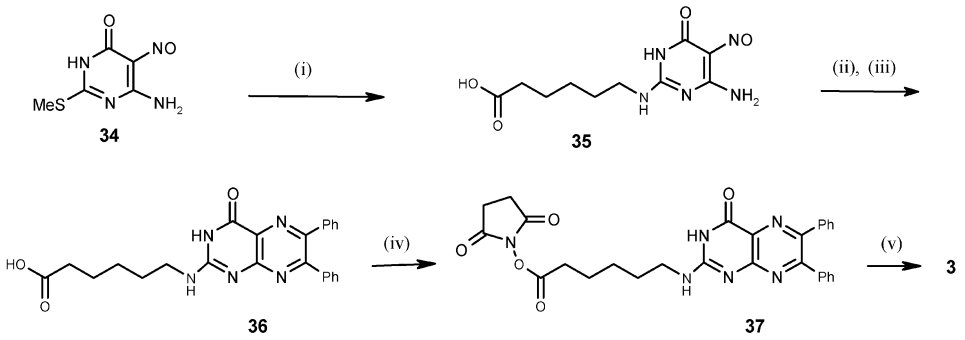
sodium hydroxide in aqueous ethanol–dichloromethane. The tricyclic compound **20** underwent ring opening when treated with concentrated potassium hydroxide in aqueous methanol, to give the unstable 3-(3-aminopropyl)lumazine **23**. When heated in methanol solution **23** gave another unstable product, believed to be the tricyclic compound 2,3-diphenyl-5,8,9,10-tetrahydropyrimido[1,2-*c*]pteridin-6-one **27**. The pyrimido[1,2-*c*]pteridine ring system in **27** is also rare.[37] The new compound **27** was too unstable to allow isolation of it in analytically pure form. Evidence for the proposed structure **27**, however, comes from its high resolution mass spectrum, and also from its UV spectra in neutral acid and alkaline solutions, all of which correspond closely to the UV spectra reported for a closely related pteridine analog **28**.[38] The unstable 3-(3-aminopropyl)lumazine **23** was characterised by conversion of it into its acetyl derivative **26**.

Pteridine–oligonucleotide conjugate (2)

Compound **10** incorporates a pteridine moiety, designed as the photosensitiser for inducing a lesion in an adjacent DNA chain. Compound **10** also incorporates a side chain terminating in a hydroxyl group, which feature allowed attachment of the pteridine moiety to an oligonucleotide *via* the side chain "linker" (see Scheme 3). This attachment was achieved by first protecting the 2-amino group of **10** as its formamidine, **30**, and then treating the latter with either 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylaminophosphorodiamidite and tetrazole, or with chloro-2-cyanoethyl-*N*,*N*-diisopropylaminophosphine. The latter, more reactive, phosphitylating reagent gave the cleaner reaction and offered the method of choice for the preparation of phosphoramidite **31**. While not very stable, **31** could be purified by column chromatography on neutral alumina, and the pure material could be used for further work for up to a week after its preparation. It was characterised by ^{31}P and ^{1}H NMR spectra, the former showing a single peak at δ 148.4, characteristic of trivalent phosphorus. Interestingly, if compound **10** were allowed to react with chloro(*N*,*N*-diisopropylamino)methoxyphosphine without prior protection of the 2-amino group, the product isolated was **32**, in which the



Scheme 3 (i) $CH(OMe)_2NMe_2$; (ii) $NCCH_2CH_2O\text{-}P(NPr^i_2)Cl$; (iii) DNA synthesiser; (iv) $MeOP(NPr^i_2)Cl$.



Scheme 4 (i) $HO_2C(CH_2)_5NH_2$; (ii) Pd-charcoal, H_2; (iii) benzil; (iv) $BF_4^-Me_2N^+{=}C(NMe_2)ON(COCH_2)_2$; (v) 17-mer-OLIGO-$O^{5'}$-$P(O)(OH)O(CH_2)_6NH_2$.

phosphorus atom has undergone oxidation to the pentavalent state. A similar oxidation was observed by Frier *et al.* in attempts to form phosphoramidites of flavin derivatives.[39] Compound **32** could be converted to its corresponding formamidine **33**. The phosphorus(V) oxidation state in **32** and **33** was easily demonstrated by the high field chemical shift of the phosphorus atoms in their ^{31}P NMR spectra, at δ 14.5 and δ 22.4 respectively.

The pteridine phosphoramidite **31** was attached to the synthetic oligonucleotide using an Applied Biosystems 391 automated DNA synthesiser. The routine deprotection steps during this procedure also removed the formamidine protecting group. The product was purified by a combination of precipitation, electroelution and preparative PAGE techniques, and it was characterised as **2** by PAGE and UV spectroscopy and by mass spectrometry. A negative ion electrospray mass spectrum of the product exhibited a series of peaks representing multiply charged negative ions. Deconvolution of this series gave a molecular weight of 5592.7 for the synthesised conjugate. Structure **2** has a molecular weight of 5592.8. The purified compound **2** was used in photosensitisation experiments with a 34-mer oligonucleotide target.

Pteridine–oligonucleotide conjugate (3)

Conjugate **3** differs from **2** in two main respects. Firstly, the linker chain is attached at position 2 of the pteridine ring system instead of at position 3, and secondly, the linker chain is 14 atoms long, as opposed to 3 atoms long in **2**. These structural differences are expected to affect the efficiency of the photochemical reaction when the conjugates are hybridised with the target oligonucleotide (see below). Finally, in the synthesis of conjugate **3**, the pteridine moiety was attached to the 17-mer oligonucleotide using a succinimide–ester coupling method, instead of a phosphoramidite method.

Synthesis of the 17-mer ODN–pteridine conjugate **3** is outlined in Scheme 4. The 5-carboxypentylamino side chain of pyrimidine **35** was introduced by nucleophilic substitution of the 2-methylthio group in the nitrosopyrimidine **34**, using a modification of a procedure described by Sugimoto *et al.*[40] Compound **35** was then converted into the diphenylpteridine **36** by reduction followed by condensation with benzil. The carboxyl group of **36** was then activated as its succinimide ester **37** using a method developed by Bannwarth *et al.*[41,42] involving treatment of **36** with *o*-(*N*-succinimidyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate. The *N*-hydroxy-succinimide activated ester **37** was allowed to react with the aminohexyl-modified 17-mer oligonucleotide, giving the required oligonucleotide pteridine conjugate **3**.

Photolysis of free pteridine 36 with single stranded ODN 1

In order to assess whether a pteridine–ODN conjugate can direct photo-specific damage to guanine bases in a complementary target in a fashion similar to that which we have observed with ruthenium conjugates,[24] we have studied the photoproducts generated by the pteridine-conjugates **2** and **3** with a single-stranded complementary 34-mer DNA **1** (see Fig. 1). Initially it was important to assess the effectiveness of the free pteridine in bringing about photosensitized damage on the 34-mer target **1**, and control experiments were set up, involving the UV

irradiation ($\lambda > 330$ nm) of the 34-mer target in the presence of an equimolar amount of **36**. The sites of cleavage were identified by comparing with Maxam and Gilbert sequencing experiments[43] in which treatment of the target 34-mer oligonucleotide with formic acid followed by hot piperidine cleaves the target at every purine base. The results of the subsequent PAGE electrophoresis experiments are given in Fig. 2.

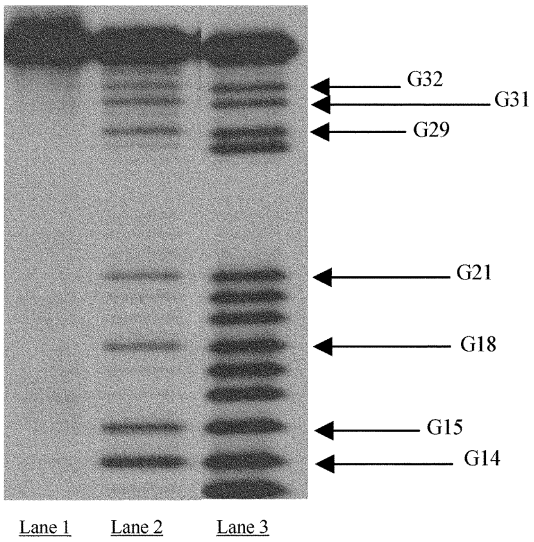


Fig. 2 Irradiation of 34-mer target **1** in presence of pteridine **36** in 10 mM phosphate–100 mM NaCl solution (pH 6.9). *Lane 1*: 7.5 min UV irradiation without piperidine treatment. *Lane 2*: 7.5 min irradiation followed by piperidine treatment. *Lane 3*: 34-mer treated with formic acid followed by piperidine.

From lane 2 it is clear that when pteridine **36** was excited in the presence of the target 34-mer **1** in aerated solution, subsequent treatment with hot piperidine induced strand breaks specifically at guanine bases in **1**. In contrast, irradiation without subsequent piperidine treatment resulted in no cleavage of **1** (lane 1). This is consistent with pteridine **36** causing oxidative damage at guanine sites on the 34-mer **1**, but producing lesions which do not lead to cleavage of the phosphodiester backbone of DNA until the sample is heated under alkaline conditions. (Blank experiments also confirmed that the unsensitised 34-mer **1** is stable under these conditions.) An interesting feature noticeable in Fig. 2 is that cleavage did not occur at all guanine sites equally, there being a significant preference for cleavage at G14, which is the 5′G of the G14–G15 doublet in the target. Such preferential reaction has been recognized for Type 1 processes on double stranded DNA and has been attributed to the fact that the HOMO for GG doublets is localised on the 5′G, making this base the most easily oxidised site.[43] The fact that this pattern occurred for the single stranded 34-mer target **1** may be attributed to the reaction occurring in a hairpinned structure, in which the G14–G15 doublet is now in a double stranded region, as shown in Fig. 3.

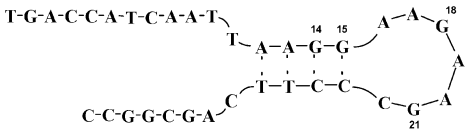


Fig. 3 The 34-mer target ODN **1** showing hairpinning, with G14 and G15 in a double stranded region.

Our experiments do not allow us to identify the degraded guanine product which leads to cleavage of the DNA strand by alkali treatment. While it is known that Type I processes with 2′-deoxyguanosine yield the alkali-labile products 2,2-diamino-4-[(2′-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5(2*H*)-oxazolone and its precursor 2-amino-5-[(2′-deoxy-β-D-*erythro*-pentofuranosyl)-amino]-4*H*-imidazol-4-one,[45] in double-stranded DNA, the dominant product should be 8-oxo-7,8-dihydroguanine. Although this latter compound does not react further in hot piperidine, it is itself very readily photo-oxidised yielding products such as the above mentioned oxazolone and imidazolone, spiroiminodihydantoin, guanidinohydantoin derivatives and oxaluric acid.[46]

Photolysis of ODN–pteridine conjugate 2 with single stranded ODN 1

The possibility of ODN–pteridine conjugates **2** and **3** being used for site selective photoreaction was explored by allowing them to hybridise with their complementary target 34-mer **1** (by warming to about 85 °C and allowing to cool slowly), exposing to UV light ($\lambda > 330$ nm), and then examining by gel electrophoresis after treatment with piperidine.

Initial experiments were carried out with conjugate **3**. Irradiation for extended periods (>2 hours) formed alkali-labile sites. These occurred exclusively at guanine residues, with most cleavage at G18.[47] Similar results were observed with conjugate **2**, but in this case the yields were much higher and this system was therefore studied in more detail. The phosphoimagery trace (Fig. 4) revealed that cleavage of the 34-mer occurred only at guanine bases, but the cleavage occurred preferentially at bases different from those damaged by free sensitiser **36**. The major cleavage site is G18 which is in the double-stranded region and probably close to the pteridine-sensitiser moiety if it has intercalated into the base-pair. The next strongest band is that due to cleavage at G21 and this is expected to be located close to the pteridine photosensitiser especially if the photosensitiser does not intercalate (see Fig. 1). Damage at G14 and G15 is also found but the yield is much lower. As pteridines are known to undergo photo-induced electron transfer with guanine, leading to the guanine radical cation (Type 1 process),[12] it is possible that the resultant migration of the oxidised centre through the base-stack from G18 of the double-stranded portion of the hybrid could explain the extent of cleavage at G14 and G15.[44]

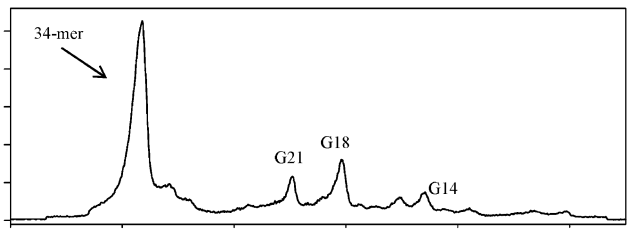


Fig. 4 Densitometry plot of phosphorimage of gel from 30 min irradiation experiment of duplex between 34-mer target **1** and ODN–pteridine conjugate **2** (1 : 10 stoichiometry).

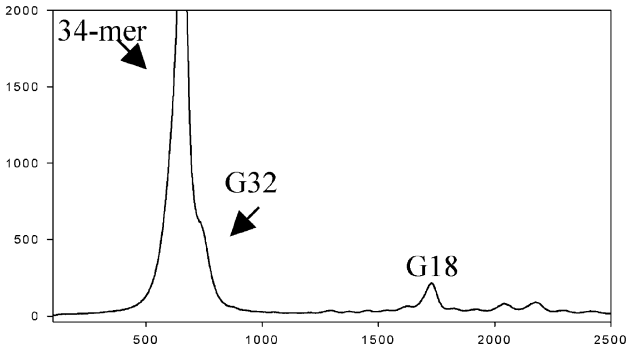
In the above experiment with 34-mer **1** and pteridine conjugate **2**, reaction on the single-stranded portion was weak, except for at G21. An interesting effect, however, was observed when the sequence of the 34-mer target **1** was changed to that of **38**. This change involved replacing G21 by a C and replacing C23 by a G, in effect moving the original G21 two bases further away from the double stranded region (see Fig. 5). When the the duplex between ODN–pteridine sensitiser **2** and variant target **38** was irradiated and then treated with piperidine, the cleavage pattern (G18 >> G14 > G15) in the double-stranded region was closely similar to that found with target **1**, the original 34-mer target. In the single-stranded region little or no cleavage at G23 was observed. A poorly resolved strong band was found, however, corresponding to cleavage at G32, which is also in the single-stranded region although seemingly remote from the pteridine photosensitising molecule (see Fig. 6A). As with our earlier work using a ruthenium conjugate,[24] this cleavage is believed

$^{5'}$T G A C C A T C A A T A A **G^{14} G^{15}** A A **G^{18}** A A **G^{21}** C C C T T C A G C G **G^{32}** C C $^{3'}$ **1**

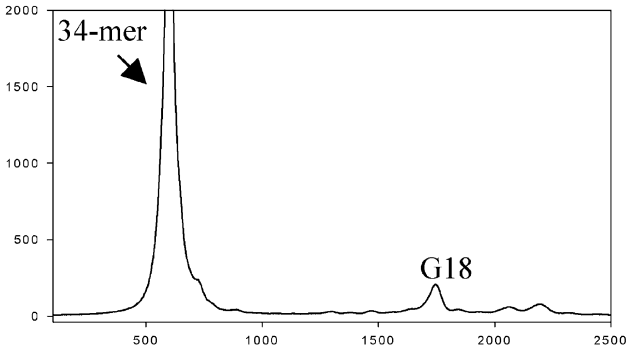
$^{5'}$T G A C C A T C A A T A A **G^{14} G^{15}** A A **G^{18}** A A C C **G^{23}** C T T C A G C G **G^{32}** C C $^{3'}$ **38**

$^{5'}$T G A C C A T C A A T A A **G^{14} G^{15}** A A **G^{18}** A A T A **G^{23}** T T T C A G C G **G^{32}** C C $^{3'}$ **39**

Fig. 5 34-mer Target **1**, hairpinning variant **38** and non-hairpinning variant **39**.



A. Cleavage of Variant **38**



B. Cleavage of Variant **39**

Fig. 6 Densitometry plots from 30 min irradiation of ODN–pteridine conjugate **2** with (**A**) variant **38** (hairpinning) and (**B**) variant **39** (non-hairpinning).

to be due to the hairpinning shown in Fig. 7, which brings G32 into close proximity with the pteridine. In support of this contention, when another variant **39** (designed so as to eliminate the possibility of hairpinning of the type shown in Fig. 7) was used instead of **38** the band corresponding to cleavage at G32 was greatly reduced (Fig. 6B). These results are entirely consistent with the main reaction of pteridine conjugate **2** taking place when the photosensitising pteridine is intimately associated with the double-stranded region, possibly by intercalation. Reaction with guanines outside this segment in the single-stranded region requires the pteridine and guanine to be in close proximity, as would be required for a Type 1 process.

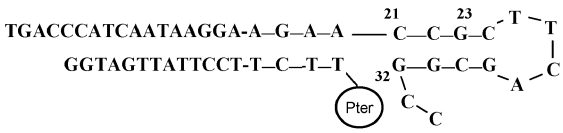


Fig. 7 Interaction of pteridine–ODN-duplex, with hairpinned variant **38**.

Conclusion

Two approaches to the functionalisation of 6,7-diphenylpterins and their conjugation to oligonucleotides to generate sequence-targetted photoactivable reagents have been assessed. The first exploits an unusual resonance-stabilised cation **8**, which we have shown to be a particularly valuable synthetic intermediate. The ODN–conjugate **2** synthesised from this precursor is effective at targeting photo-oxidative damage to its complementary sequence. Alkali treatment causes cleavage at guanine, the pattern being consistent with a Type I process, where the excited state of the diphenylpterin abstracts an electron from guanine, following which the hole may migrate through the base-pair stack of the double helix. In the other ODN–conjugate **3** the oligonucleotide was attached *via* a linking chain to the 2-amino group of the diphenylpterin. This conjugate, however, was found to be much less active, probably due to the much greater length of the linker chain compared to that in **2**.

Experimental

Thin layer chromatography was carried out on Merck Kieselgel 60 F_{254} 0.2 mm silica gel plates. Flash chromatography was carried out on Merck Kieselgel 60 (mesh 230–400 ASTM) silica gel. Tetrahydrofuran was dried over calcium chloride, distilled from lithium aluminium hydride, and then re-distilled from sodium wire and benzophenone. Melting points are uncorrected. Infrared spectra were recorded in Nujol mulls on Perkin Elmer 298, 599 or 883 spectrophotometers. Ultraviolet spectra were recorded on Pye Unicam PU 8800, Pye Unicam SP8-200 or Perkin Elmer 402 UV/visible spectrophotometers. Nuclear magnetic resonance spectra were recorded on Bruker 80, 300 or 400 instruments. Chemical shifts δ_H and δ_C, of ^{1}H and ^{13}C respectively, are reported in ppm relative to tetramethylsilane as an internal standard. Chemical shifts of ^{31}P are relative to 85% H_3PO_4. Coupling constants are quoted in Hertz. Elemental analyses were carried out at either the Microanalytical Laboratory, National University of Ireland, Dublin; or the Microanalytical Laboratory, University College London. Electron ionisation mass spectrometry was carried out by Dr Peter Bladon and Mr Mohammed Imran at the Department of Pure and Applied Chemistry, University of Strathclyde, using an AEI (Kratos) M59 spectrometer, modified to the MS 902 specification. Electrospray mass spectrometry was carried out using a Micromass electrospray TOF instrument.

Pteridinone–oligodeoxynucleotide conjugate 2

The 17-mer oligonucleotide component of **2** was first assembled (1 μmol scale) on an Applied Biosystems 391 automated DNA synthesiser using standard phosphoramidite chemistry. Following deprotection of the 5′-dimethoxytrityl group, a solution comprising (3-(2-*N*,*N*-dimethylaminomethyleneamino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)propoxy)(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphoramidite **31** (17 mg, 0.027 mmol) dissolved in dry acetonitrile (365 μl) was introduced onto the synthesis column. The coupling reaction was allowed to proceed for 120 s and the synthetic cycle then completed. The oligonucleotide conjugate thus formed was cleaved from the solid support with concentrated ammonia and the solution further heated at 55 °C for 3 h to remove base protecting groups. After evaporation, the residual product was purified by electrophoresis on 12% polyacrylamide gels containing 7 M urea. Bands corresponding to the pteridinone conjugate were detected by UV shadowing, excised, and transferred to a Biotrap electroelution apparatus (Schleicher and Schuell). After running for 2 h at 2–3 W, the purified conjugate **2** was recovered

from the anodic well in TRIS–borate buffer. It was precipitated twice from sodium acetate solution with cold ethanol, then eluted in water from a NAP™10 column (Sephadex G-25 DNA grade resin, Pharmacia) to give 3.0 A_{260} units of **2**. On negative ion electrospray mass spectrometry, the synthetic conjugate gave rise to a series of negative ions whose deconvolution predicted a molecular weight of 5592.7; structure **2** requires 5592.8.

Pteridinone–oligodeoxynucleotide conjugate 3

The 5′-aminohexyl modified 17-mer reagent was prepared (1 μmol scale) on the automated DNA synthesiser by terminal addition of 5′-AminoModifier phosphoramidite (Glen Research) to the 17-mer. It was purified using a reverse phase cartridge (PolyPak, Transgenomics) and converted to its lithium salt by precipitation from LiCl solution with ethanol. A solution of 2-(6-succinimidocarboxypentylamino)-6,7-diphenyl-4(3*H*)-pteridinone **37** (15 mg, 0.029 mmol) in anhydrous DMF (150 μl) was added to an Eppendorf tube containing 16.0 A_{260} units of the aminohexyl modified 17-mer dissolved in 100 mM sodium–borate buffer, pH 8.8 (100 μl). The mixture was vortexed and heated at 40 °C for 1 h. The reaction mixture was then passed through a NAP™10 column and eluted with water (2 × 1 cm^3). The fractions were pooled, concentrated, and then purified by successive denaturing polyacrylamide gel electrophoresis and electroelution steps as described for conjugate **2** above. The recovered solution of **3** in TRIS–borate buffer was desalted and concentrated by repeated extraction with 1-butanol prior to ethanol precipitation from sodium acetate solution. When dissolved in water, the pale yellow pellet afforded 4.7 A_{260} units of product **3**. Deconvolution of its negative ion electrospray mass spectrum gave a predicted molecular weight of 5749.2; structure **3** requires 5748.1.

5-Amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine 5

5-Amino-7-(methylthio)furazano[3,4-*d*]pyrimidine[26] **4** (1.88 g, 10.3 mmol) was suspended in 3-chloropropanol (24 cm^3) and heated to 55 °C in a flask with a drying tube and dropping funnel attached. A 30% w/v solution of bromine in 3-chloropropanol was added dropwise, keeping the temperature at 55 °C. Each successive addition was made when the bromine colour from the previous addition had disappeared, and addition was continued until the solution maintained a permanent bromine colour. The reaction mixture was cooled to room temperature and poured with vigorous stirring on to ethyl acetate (500 cm^3) containing sodium hydrogen carbonate (8 g). The mixture was stirred for 10 min, saturated aq sodium chloride (40 cm^3) was added, and the mixture stirred for a further 10 min. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give a yellow oil. Diethyl ether (20 cm^3) was added and the solution stored at −5 °C overnight. 5-Amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine, **5** was obtained as a yellow solid (1.59 g, 69%), mp 178–80 °C (found (tungstic oxide): C, 36.48; H, 3.45; Cl, 15.73; N, 30.42. Calc. for $C_7H_8ClN_5O$: C, 36.61; H, 3.51; Cl, 15.44; N, 30.50%); λ_{max}(EtOH)/nm 214 (log ε/dm^3 mol^{-1} cm^{-1} 4.21), 255 (3.68) and 339 (3.55); ν_{max}/cm^{-1} 3410, 870, 840 and 785; δ_H (400 MHz; d_6-DMSO) 2.32 (2 H, m, $CH_2CH_2CH_2$), 3.82 (2 H, t, *J* 6.5, $ClCH_2$), 4.67 (2 H, t, *J* 6.5, CH_2O), 7.84 (2 H, d, NH_2).

2-Amino-4-(3-chloropropoxy)-6,7-diphenylpteridine 9

A suspension of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]-pyrimidine **5** (205 mg, 0.89 mmol), 10% palladised carbon (40 mg) in tetrahydrofuran (20 cm^3) was deaerated with nitrogen and then stirred for 36 h at room temperature under an atmosphere of hydrogen. The reaction flask was flushed with nitrogen, benzil (500 mg, 2.38 mmol) was added, and the mixture was then refluxed under nitrogen for 18 h. The reaction mixture was cooled and filtered, and the filtrate evaporated to dryness. The residue was extracted with hexane to remove excess benzil and was then purified by flash column chromatography using chloroform–ethanol (15 : 1) as eluant to give 2-amino-4- (3-chloropropoxy)-6,7-diphenylpteridine **9** (74 mg, 21%), mp >278 °C (decomp.) (from $CHCl_3$/EtOH) (found: C, 64.41; H, 4.44; Cl, 8.87; N, 17.82. Calc. for $C_{21}H_{18}ClN_5O$: C, 64.37; H, 4.63; Cl, 9.05; N, 17.87%); λ_{max}(EtOH)/nm 226 (log ε/dm^3 mol^{-1} cm^{-1} 4.47), 240 (4.43), 288 (4.31) and 388 (4.14); ν_{max}/cm^{-1} 3460, 3260, 3080, 1630, 1590, 1530, 1240, 1170, 1150, 1090, 1005, 825, 790, 765, 725 and 700; δ_H (80 MHz; d_6-DMSO) 2.32 (2 H, m, CH_2CH_2Cl), 3.83 (2 H, t, *J* 6.5, CH_2Cl), 4.64 (2 H, t, *J* 6.2, OCH_2-), 7.34 and 7.38 (12 H, 2 × s, NH_2 and 6,7-diPh).

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone 10

A suspension of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine **5** (200 mg, 0.87 mmol) and 10% palladised carbon (50 mg) in deaerated water (18.5 cm^3) was stirred vigorously under hydrogen at atmospheric pressure until hydrogen uptake ceased. The catalyst was removed by vacuum filtration under an argon atmosphere. A solution of potassium hydroxide (123 mg, 2.19 mmol) and benzil (387 mg, 1.92 mmo1) in deaerated methanol (18.5 cm^3) was added and the resulting suspension was stirred at 40 °C for 1.5 h. The suspension was neutralised with dilute HCl and the solvent evaporated to give a yellow residue which was extracted with methanol. Evaporation of the methanol extract gave a solid, which on flash chromatography using chloroform–ethanol (5 : 1) as eluant, gave 2-amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** as a yellow solid (216 mg, 67%), mp 264 °C (from EtOH/H_2O) (found: C, 66.14; H, 5.09; N, 18.21. Calc. for $C_{21}H_{19}N_5O_2$(0.5 H_2O): C, 65.96; H, 5.27; N, 18.31%); λ_{max}(EtOH)/nm 226 (log ε/dm^3 mol^{-1} cm^{-1} 4.38), 297.5 (4.36) and 382 (4.05); ν_{max}/cm^{-1} 3372, 3130, 1692, 1669, 1543 and 1528; δ_H (300 MHz; d_6-DMSO) 1.83 (2 H, m, CH_2CH_2OH), 3.52 (2 H, m, CH_20H), 4.09 (2 H, t, *J* 7.0, CH_2N), 4.68 (l H, t, *J* 5.0, O*H*), 7.29–7.43 (10 H, m, 6,7-diPh), 7.54 (2 H, br s, NH_2).

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone 10, and 2-amino-4-methoxy-6,7-diphenylpteridine 14, from 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine 5

A. (By hydrogenation of **5** in water and isolation of the intermediate resonance stabilised cation **8**). A suspension of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]-pyrimidine **5** (110 mg, 0.48 mmol) and 10% palladised carbon in deaerated water was stirred overnight under hydrogen at room temperature and pressure. The catalyst was filtered off under nitrogen and the filtrate evaporated to dryness at room temperature under reduced pressure using an oil pump. The resulting solid **8** was dissolved in deaerated methanol (15 cm^3) under nitrogen. Sodium (15 mg, 0.65 mmol) and benzil (190 mg, 1.10 mmol) were added, and the solution was refluxed under nitrogen for 3 h. The mixture was allowed to cool, neutralised with dilute methanolic sulfuric acid, filtered, and evaporated to dryness under reduced pressure. Flash chromatography of the residue on silica using chloroform–ethanol (gradient from 50 : 1–7 : 1) as eluant yielded 2-amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (64 mg, 35%), identical in properties with the sample prepared as described above, and 2-amino-4-methoxy-6,7-diphenylpteridine **14** (5 mg, 3%).[26]

B. (By hydrogenation of **5** in methanol). A mixture of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine **5** (200 mg, 0.87 mmol) and 10% palladised carbon (48 mg) in methanol (30 cm^3) was stirred at room temperature under an atmosphere of hydrogen for 2 h. The flask was transferred to an atmosphere of nitrogen, and sodium (30 mg, 1.3 mmol) was added. When the sodium had dissolved, benzil (380 mg, 1.81 mmol) was added and the mixture refluxed with stirring under nitrogen for 3 h. After cooling, the mixture was neutralised

with dilute methanolic sulfuric acid, filtered, and evaporated to dryness under a reduced pressure. Flash column chromatography of the residue using chloroform–ethanol (50 : 1–100 : 15) as eluant yielded 2-amino-4-methoxy-6,7-diphenylpteridine **14** (90 mg, 31%)[26], and **10** (66 mg, 20%), identical with that prepared as described above.

2-Amino-3-(3-hydroxypropyl)-6,7-dimethyl-4(3*H*)-pteridinone 12

A mixture of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine **5** (100 mg, 0.43 mmol), 10% palladised carbon (36 mg) and deaerated water (20 cm^3) was stirred overnight at room temperature under an atmosphere of hydrogen. The catalyst was filtered off under nitrogen by means of a filter stick and the filtrate heated to 70 °C under nitrogen. Biacetyl (0.25 g, 2.9 mmol) was added and the mixture stirred and heated to 70 °C for a further 2 h. Tetrahydrofuran (20 cm^3) was added at room temperature with stirring, and examination after 1 h by TLC (chloroform–ethanol, 15 : 2) showed complete conversion to a polar fluorescent material (*R*f 0.2). The solution was evaporated to dryness and flash chromatography of the residue using chloroform–ethanol (50 : 1–100 : 15) as eluant yielded 2-amino-3-(3-hydroxypropyl)-6,7-dimethyl-4(3*H*)-pteridinone **12** (80 mg, 74%), mp >220 °C (decomp.) (from EtOH) (found: C, 53.07; H, 5.96; N, 27.86. Calc. for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10%); λ_{max} (EtOH 95%)/nm 225 infl (log ε/dm^3 mol^{-1} cm^{-1} 3.97), 243 (4.07), 279 (4.07) and 354 (3.74); ν_{max}/cm^{-1} (nujol) 3560 (NH_2), 3380 (OH), 1720 and 1680 (C=O and C=N); δ_H (80 MHz; d_6-DMSO) 1.78 (2 H, m, CH_2CH_2OH), 2.504 and 2.496 (6 H, 2 s, 2 × CH_3), 3.49 (2 H, m, CH_2OH), 4.04 (2 H, t, *J* 7.0, NCH_2), 4.72 (1 H, t, *J* 5.0, O*H*) and 7.31 (2 H, s, NH_2).

2-Amino-3-(3-hydroxypropyl)-7-methyl-4(3*H*)-pteridinone 13

A mixture of 5-amino-7-(3-chloropropoxy)furazano[3,4-*d*]pyrimidine **5** (100 mg, 0.43 mmol), 10% palladised carbon (36 mg) and deaerated water (25 cm^3) was stirred overnight at room temperature under an atmosphere of hydrogen. The catalyst was filtered off under a nitrogen atmosphere by means of a filter stick. The filtrate was cooled in ice and pyruvaldehyde (27% aq solution, 1 cm^3, 3.8 mmol) added under nitrogen. The mixture was stirred for 2 h at 0–5 °C, when examination by TLC (chloroform–ethanol, 15 : 2) showed a fluorescent spot of very low *R*f. The mixture was concentrated *in vacuo* with gentle warming to about one fifth of its volume, tetrahydrofuran (25 cm^3) added under nitrogen, and the mixture stirred at room temperature for a further 1.5 h. Examination by TLC (chloroform–ethanol, 15 : 2) showed a new polar product (*R*f 0.2) and evaporation of the mixture to dryness under reduced pressure followed by flash chromatography using chloroform–ethanol (20 : 1–2 : 1) as eluant yielded **13** (70 mg, 69%), mp >220 °C (decomp.) (from ethanol) (found: C, 50.69; H, 5.30; N, 29.61. Calc. for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77%); λ_{max} (EtOH)/nm 243 (log ε/dm^3 mol^{-1} cm^{-1} 4.17), 280 (4.08) and 353 (3.82); ν_{max}/cm^{-1} 3430, 3300, 1675, 1640 and 1540; δ_H (300 MHz; d_6-DMSO) 1.78 (2 H, m, CH_2CH_2OH), 2.52 (3 H, s, CH_3), 3.48 (2 H, m, CH_2OH), 4.02 (2 H, t, *J* 7.2, NCH_2), 4.67 (l H, t, *J* 5.0, O*H*), 7.44 (2 H, br s, NH_2) and 8.27 (l H, s, 6*H*); δ_C (75.5 MHz, d_6-DMSO) + DEPT 21.87 (CH_3), 30.03 (CH_2), 38.66 (CH_2), 58.03 (CH_2), 125.29 (C), 139.27 (CH), 154.08 (C), 154.87 (C), 159.32 (C) and 160.67 (C).

3-(3-Chloropropyl)-6,7-diphenyl-2-triphenylphosphazino-4(3*H*)-pteridinone 15 and 2-amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone 18

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (107 mg, 0.28 mmol), triphenylphosphine (481 mg, 1.84 mmol) and tetrachloromethane (27 cm^3) were refluxed with stirring under nitrogen for 15 h. After cooling, the reaction mixture was evaporated under reduced pressure and the residue taken up in chloroform, washed with water, dried ($MgSO_4$) and evaporated. Flash chromatography using chloroform as eluant separated the products from polar impurities. Chromatography of the products on a second column, using progressively more polar solvent mixtures as eluant (ethyl acetate–hexane, 1 : 5–1 : 1; ethyl acetate–chloroform, 1 : 1; chloroform; chloroform–ethanol, 10 : 1) allowed the isolation of two products, 2-amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (43 mg, 39%), identical with the product obtained from **10** under different conditions as described below, and 3-(3-chloropropyl)-6,7-diphenyl-2-triphenylphosphazino-4(3*H*)-pteridinone **15** (85 mg, 46%), mp 191–93 °C (from ethyl acetate–hexane) (found (using tungstic oxide): C, 71.76; H, 4.92; Cl, 5.78; N, 10.58. Calc. for $C_{39}H_{31}C1N_5OP$: C, 71.83; H, 4.79; Cl, 5.44; N, 10.74%); ν_{max}/cm^{-1} 3070, 1700 (C=O), 1530, 1515, 1190, 1115, 990 and 725; λ_{max} (EtOH)/nm 261 (log ε/dm^3 mol^{-1} cm^{-1} 4.29), 314 (4.51) and 390 (4.07); λ_{max} (1,4-dioxane)/nm 229 (log ε/dm^3 mol^{-1} cm^{-1} 4.60), 320 (4.44) and 381 (4.09); δ_H (300 MHz; $CDCl_3$) 2.38–2.48 (2 H, m, CH_2CH_2Cl), 3.77 (2 H, t, *J* 6.4, CH_2Cl), 4.73 (2 H, t, *J* 7.2, NCH_2) and 7.18–8.00 (25 H, m, Ph_3P and 6,7-di-Ph); δ_C (75.5 MHz, $CDCl_3$) 31.06, 41.94, 43.33, 126.98, 127.08, 127.88, 127.98, 128.34, 128.60, 128.76, 128.86, 129.91, 130.00, 132.52, 133.53, 133.66, 133.90, 147.61, 153.32, 157.30 and 162.81; δ_P (121.5 MHz, $CDCl_3$) 20.4 (s); *m*/*z* (EI) 651.1958 (M^+, 27%, $C_{39}H_{31}C1N_5OP$ requires 651.1955), 574.1778 (0.4, $M^+ - C_3H_6Cl$), 276.0919 (3, $C_{18}H_{15}NP$), 262.0914 (100, $C_{18}H_{15}P$), 185.0533 (21, $C_{12}H_{10}P$), 108.0133 (32, C_6H_5P) and 77.0398 (19, C_6H_5).

6,7-Diphenyl-2-phthalimido-3-(3-phthalimidopropyl)-4(3*H*)-pteridinone 16

3-(3-Chloropropyl)-6,7-diphenyl-2-phthalimido-4(3*H*)-pteridinone **19** (50 mg, 0.96 mmol) and potassium phthalimide (34 mg, 0.184 mmol) in hexamethylphosphoramide were heated under nitrogen for 40 min. The mixture was cooled and acidified with dilute hydrochloric acid (0.5 cm^3), and was then added to ethyl acetate, washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. Chromatography on a short column using dichloromethane–ethanol (150 : 1) as eluant removed some highly polar impurities, and crystallisation of the crude product thus obtained from dichloromethane/hexane/ethyl acetate afforded pure 6,7-diphenyl-2-phthalimido-3-(3-phthalimidopropyl)-4(3*H*)-pteridinone **16** as white crystals (15 mg, 22%), mp 286–290 °C (found: C, 68.16; H, 4.38; N, 11.65. Calc. for $C_{37}H_{24}N_6O_5$/EtOAc: C, 68.33; H, 4.47; N, 11.66%); λ_{max} (1,4-dioxane)/nm 218 (log ε/dm^3 mol^{-1} cm^{-1} 4.64), 265 (4.36) and 350 (4.17); ν_{max}/cm^{-1} 3059, 1791, 1766, 1733, 1710, 1592, 1531, 1407, 1246, 1186, 1102, 1024, 959, 881, 720 and 703; δ_H (300 MHz; $CDCl_3$) 1.26 (3 H, t, *J* 7.1, EtOAc), 2.04 (3H, s, EtOAc), 2.22 (2 H, m, $CH_2CH_2CH_2$), 3.76 (2 H, t, *J* 6.2, CH_2-phth), 4.12 (2 H, q, *J* 7.2, EtOAc), 4.24 (2 H, t, *J* 8.0, CH_2-pteridine), 7.26–7.60 (10 H, m, 6,7-diPh), 7.66 (4 H, s, phth) and 7.78–7.88 (4 H, m, phth); δ_C (75.5 MHz, $CDCl_3$) 14.18 (EtOAc), 21.03 (EtOAc), 27.87, 35.32, 43.58, 60.37 (EtOAc), 123.26, 124.58, 128.23, 128.38, 129.47, 129.74, 130.02, 130.18, 131.42, 131.73, 133.91, 133.25, 137.12, 137.53, 144.15, 150.53, 154.69, 158.74, 160.68, 165.36, 168.00 and 171.13 (EtOAc); *m*/*z* (EI) 632.1796 (M^+, 65%), 486.1575 (16, $M^+ - N(CO)_2C_6H_4$), 472.1459 (18, $M^+ - CH_2N(CO)_2C_6H_4$), 444.1176 (14, $M^+ - (CH_2)_3N(CO)_2C_6H_4$) and 77.0394 (12, C_6H_5).

2-(3-Hydroxypropylamino)-6,7-diphenyl-4(3*H*)-pteridinone 17

A solution of 2-amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (940 mg, 2.46 mmol) and potassium hydroxide (33 g, 0.59 mol) in methanol–water (1 : 1) (300 cm^3) was refluxed for 3 h and allowed to cool. The mixture was neutralised with dilute aqueous sulfuric acid and stored at 5 °C overnight.

The resulting solid material was filtered off and extracted with methanol, the extracts were combined and concentrated under reduced pressure, when 2-(3-hydroxypropylamino)-6,7-diphenyl-4(3*H*)-pteridinone **17** crystallised out (600 mg, 65%), mp 275–76 °C (from ethanol) (found: C, 67.22: H, 4.94; N, 18.48. Calc. for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.75%); λ_{max} (EtOH)/nm 224 (log ε/dm^3 mol^{-1} cm^{-1} 4.36), 250 (4.25), 299 (4.38) and 381 (4.05); λ_{max} (aq KOH 0.1M)/nm 219 (log ε/dm^3 mol^{-1} cm^{-1} 4.32) 279 (4.36) and 389 (4.09); δ_H (300 MHz; d_6-DMSO) 1.74 (2 H, m, –C*H*$_2$CH$_2$OH), 3.52 (4 H, m, CH$_2$CH$_2$C*H*$_2$OH), 6.33 (2 H, 2 × br s, D$_2$O exchangeable, –CH$_2$N*H* and –O*H*), 7.34 and 7.38 (10 H, 2 × s, 6,7-diPh) and 11.36 (1 H, br s, D$_2$O exchangeable, CON*H*).

2-Amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone 18

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (214 mg, 0.56 mmol) and triphenylphosphine (1.74 g, 6.63 mmol) were dissolved in dry tetrahydrofuran (60 cm^3) with heating under nitrogen. Tetrachloromethane (60 cm^3) was added and the solution heated to reflux for 30 min. The reaction mixture was cooled and then evaporated under reduced pressure. Flash chromatography of the residue, using successively as eluant ethyl acetate, ethyl acetate–ethanol (15 : 1), chloroform, and finally chloroform–ethanol (100 : 1), gave 2-amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (144 mg, 66%), mp >300 °C (decomp) (found: C, 64.16; H, 4.87, Cl, 8.78; N, 17.48. Calc. for $C_{21}H_{18}ClN_5O$: C, 64.37; H, 4.63; Cl, 9.05; N, 17.87%); λ_{max} (EtOH)/nm 225 (log ε/dm^3 mol^{-1} cm^{-1} 4.40) 245 infl (4.28), 297 (4.37) and 382 (4.05); ν_{max}/cm^{-1} 3490, 3300, 3050, 1710 (C=O), 1655, 1550, 1195 and 695; δ_H (300 MHz; d_6-DMSO) 2.35 (2 H, m, C*H*$_2$CH$_2$Cl), 3.75 (2 H, t, *J* 5.7, C*H*$_2$Cl), 4.29 (2 H, t, *J* 7.3, NC*H*$_2$), 5.64 (2 H, br s, N*H*$_2$) and 7.29–7.57 (10 H, m, 6,7-diPh).

3-(3-Chloropropyl)-6,7-diphenyl-2-phthalimido-4(3*H*)-pteridinone 19

2-Amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (100 mg, 0.26 mmol), phthalic anhydride (1.6 g, 10.8 mmol) and dry pyridine (5 cm^3) were heated to 50 °C with stirring under nitrogen for 5 h. The reaction mixture was cooled, added to ethyl acetate (150 cm^3) and washed with water (6 × 50 cm^3). The organic layer was dried ($MgSO_4$) filtered, and evaporated under reduced pressure. Flash chromatography of the residue, using as eluant dichloromethane followed by dichloromethane–ethanol (200 : 1), yielded 3-(3-chloropropyl)-6,7-diphenyl-2-phthalimido-4(3*H*)-pteridinone **19** (92 mg, 69%), mp 269–70 °C (from dichloromethane–hexane) (found: C, 67.08; H, 3.88; Cl, 6.87; N, 13.28. Calc. for $C_{29}H_{20}C1N_5O_3$: C, 66.73; H, 3.86; Cl, 6.79; N, 13.42%); λ_{max} (1,4-dioxane)/nm 265 (log ε/dm^3 mol^{-1} cm^{-1} 4.35), 350 (4.18); ν_{max}/cm^{-1} 1795, 1734, 1707, 1594, 1538, 1406, 1365, 1322, 1244, 1225, 1107, 955, 883, 857, 793, 722 and 697; δ_H (300 MHz; $CDCl_3$) 2.28 (2 H, m, C*H*$_2$CH$_2$Cl), 3.59 (2 H, t, *J* 5.8, C*H*$_2$Cl), 4.36 (2 H, t, *J* 7.2, NC*H*$_2$), 7.22–7.62 (10 H, m, 6,7-di-Ph), 7.88 (2 H, dd, *J* 5.6 and 3.2, phth) and 8.02 (2 H, dd, *J* 5.6 and 3.2, phth); δ_C (75.5 MHz, $CDCl_3$) + DEPT 30.92 (CH_2), 42.04 (CH_2), 43.08 (CH2), 124.7 (CH), 128.28 (CH), 128.42 (CH), 129.54 (CH), 129.70 (C), 130.02 (CH), 130.20 (CH), 131.62 (C), 135.40 (CH), 137.11 (C), 137.55 (C), 144.24 (C), 150.69 (C), 154.74 (C), 158.86 (C), 160.99 (C) and 165.61 (C).

2,3-Diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridine-11-one 20

A. 2-Amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (50 mg, 0.13 mmol) was dissolved in ethanol (20 cm^3) with heating and the solution was then cooled to room temperature. Sodium hydroxide (0.1 g, 2.6 mmol) was dissolved in water and this solution added to the ethanolic pteridine solution. After 30 min, water (10 cm^3) was added to the reaction mixture, followed by evaporation of most of the ethanol under reduced pressure. The aqueous residue was extracted with chloroform and the extract washed with water, dried ($MgSO_4$), and evaporated under reduced pressure, to give 2,3-diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridine-11-one **20** (35 mg, 78%), mp >310 °C (decomp.) (from chloroform–ethyl acetate) (found: C, 70.53; H, 4.81; N, 19.40. Calc. for $C_{21}H_{17}N_5O$: C, 70.97; H, 4.82; N, 19.71%); λ_{max} (EtOH)/nm 226 (log ε/dm^3 mol^{-1} cm^{-1} 4.35), 250 (4.27), 301 (4.38) and 390 (4.01); ν_{max}/cm^{-1} 1687, 1639, 1546, 1527, 1316, 1287, 1245, 1188, 1069, 972, 789, 778 and 699; δ_H (300 MHz; $CDCl_3$) 2.12 (2 H, m, CH$_2$C*H*$_2$CH$_2$), 3.64 (2 H, t, *J* 5.4, C*H*$_2$), 4.18 (2 H, t, *J* 5.9, C*H*$_2$), 7.23–7.51 (10 H, m, 2,3-diPh) and 9.01 (1 H, br s, N*H*); δ_C (75.5 MHz, $CDCl_3$) 2.05, 39.39, 40.53, 125.82, 128.00, 128.09, 128.29, 129.94, 138.42, 138.56, 148.44, 152.45, 153.79, 158.06 and 161.02.

B. 2-(3-Hydroxypropylamino)-6,7-diphenyl-4(3*H*)-pteridinone **17** (210 mg, 0.56 mmol), triphenylphosphine (1.1 g, 4.2 mmol) and tetrachloromethane (80 cm^3) were refluxed together with stirring under nitrogen for 12 h. The reaction mixture was cooled and evaporated under reduced pressure. Flash chromatography, eluting with chloroform followed by chloroform–ethanol (50 : 1) afforded 2,3-diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridine-11-one **20** (39 mg, 20%), identical in properties with that prepared as described above.

2-Acetamido-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone 21

2-Amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (100 mg, 0.26 mmol) was dissolved in dry pyridine (8 cm^3) in a closed flask. Acetic anhydride (27 µl, 29 mg, 0.28 mmol) was added and the reaction mixture was stirred in the closed flask at room temperature for 70 h. The pyridine was then evaporated under reduced pressure. Flash chromatography of the residue with chloroform–ethanol (200 : 1) as eluant yielded a product that was rechromatographed on a second column using ethyl acetate–hexane (2 : 5) as eluant. Fractions containing the first eluted product were combined and evaporated, to afford **21** (36 mg, 33%), mp 178 °C (decomp.) (from acetone–hexane) (found: C, 64.07; H, 4.65; Cl, 8.22; N, 16.32. Calc. for $C_{23}H_{20}ClN_5O_2$: C, 63.67; H, 4.65, Cl, 8.17; N, 16.14%); λ_{max} (1,4-dioxane)/nm 234 (log ε/dm^3 mol^{-1} cm^{-1} 4.33), 273 (4.37) and 368 (4.23); ν_{max}/cm^{-1} 3413 (NH), 3061, 1716 (C=O), 1593, 1546, 1280, 1252, 1196, 1121, 1010, 978, 774 and 695; δ_H (300 MHz; $CDC1_3$) 2.28 (2 H, m, C*H*$_2$CH$_2$Cl), 2.33 (3 H, s, COC*H*$_3$), 3.67 (2 H, t, *J* 6.6, C*H*$_2$Cl), 4.50 (2 H, t, *J* 7.0, NC*H*$_2$) and 7.27–7.53 (10 H, m, 6,7-diPh).

3-(3-Chloropropyl)-2-(2-ethoxycarbonylbenzamido)-6,7-diphenyl-4(3*H*)-pteridinone 22

3-(3-Chloropropyl)-6,7-diphenyl-2-phthalimido-4(3*H*)-pteridinone **19** (55 mg, 0.11 mmol) was dissolved in dichloromethane (2.0 cm^3). Ethanol (10 cm^3) followed by dilute aqueous sodium hydroxide (0.2 cm^3) were added and the mixture was stirred for 2 min. Purification was effected by preliminary chromatography of the crude reaction mixture on a short silica column, eluting with dichloromethane–ethanol (10 : 1), followed by flash chromatography eluting with dichloromethane–ethanol (100 : 1), to give 3-(3-chloropropyl)-2-(2-ethoxycarbonylbenzamido)-6,7-diphenyl-4(3*H*)-pteridinone **22** (54 mg, 91%), mp 180–81 °C (from acetone–hexane) (found: C, 65.65; H, 4.69; Cl, 6.12; N, 12.35. Calc. for $C_{31}H_{26}ClN_5O_4$: C, 65.55; H, 4.61; Cl, 6.24; N, 12.33%); λ_{max} (1,4-dioxane)/nm 306 (log ε/dm^3 mol^{-1} cm^{-1} 4.46) and 370 (4.38); ν_{max}/cm^{-1} 1727 (C=O), 1712 (C=O), 1581, 1548, 1357, 1313, 1287, 1259, 1193, 1120, 1035, 746 and 699; δ_H (300 MHz; $CDCl_3$) 1.37 (3 H, t, *J* 7.2, C*H*$_3$), 2.32 (2 H, m, C*H*$_2$CH$_2$Cl), 3.69 (2 H, t, *J* 6.5, C*H*$_2$Cl), 4.41 (2 H, q, *J* 7.2, C*H*$_2$CH$_3$), 4.58 (2 H, t, *J* 7.1, NC*H*$_2$), 7.29–7.63 (13 H, m,

6,7-diPh and Ar) and 8.13–8.17 (1 H, m, Ar); δ_C (75.5 MHz, $CDCl_3$) + DEPT 14.09 (CH_3), 30.38 (CH_2), 41.01 (CH_2), 42.36 (CH_2), 61.42 (CH_2), 125.42 (C), 128.05 (CH), 128.32 (CH), 128.35 (CH), 129.19 (CH), 129.70 (CH), 129.75 (CH), 129.92 (CH), 130.14 (CH), 130.32 (CH), 131.06 (CH), 133.68 (C), 136.52 (C), 136.95 (C), 137.09 (C), 143.54 (C), 152.02 (C), 152.93 (C), 157.85 (C), 159.52 (C), 169.33 (C) and 179.70 (C).

6-Acetyl-2,3-diphenyl-6,7,8,9-tetrahydropyrimido[2,1-b]pteridine-11-one 24

A. 2,3-Diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridin-11-one **20** (63 mg, 0.18 mmol) was added to dry pyridine (10 cm^3) followed by acetic anhydride (1.0 cm^3). The mixture was heated with stirring to 45 °C until the pteridine was fully dissolved and was then stirred for 12 h at room temperature, after which the pyridine was evaporated under a reduced pressure. Flash chromatography of the residue, using chloroform as eluant, followed by further chromatography on a second column eluting with hexane–acetone (4 : 1), afforded 6-acetyl-2,3-diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridine-11-one **24** (29 mg, 41%), mp >134 °C (decomp.) (found: C, 69.27; H, 4.59; N, 17.35. Calc. for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.82; N, 17.62%); λ_{max} (EtOH)/nm 255 (log ε/dm^3 mol^{-1} cm^{-1} 4.27), 304 (4.29) and 366 (4.13); ν_{max}/cm^{-1} 3060, 1691 (C=O), 1583, 1531, 1412, 1294, 1261, 1211, 1153, 1036, 973, 774 and 695; δ_H (300 MHz; CD_3COCD_3) 2.25–2.34 (2 H, m, $CH_2CH_2CH_2$), 2.65 (3 H, s, $COCH_3$), 3.92 (2 H, t, *J* 6.7, CH_2), 4.21 (2 H, t, *J* 5.8, CH_2) and 7.32–7.65 (10 H, m, 6,7-diPh); δ_C (75.5 MHz, CD_3COCD_3) 32.24, 36.09, 50.72, 53.25, 138.60, 138.65, 139.18, 139.88, 140.36, 140.46, 148.96, 149.1, 161.4, 161.6, 162.2, 168.3, 170.1 and 182.4.

B. 2-Acetamido-3-(3-chloropropyl)-6,7-diphenyl-4-(3*H*)-pteridinone **21** (16 mg, 0.037 mmol) was dissolved in tetrahydrofuran (10 cm^3), and dilute aqueous sodium hydroxide (0.25 cm^3) was then added to the solution. The mixture was stirred at room temperature for 2.5 h, and was then evaporated under a reduced pressure. Flash chromatography of the residue, using dichloromethane as eluant followed by a dichloromethane–ethanol gradient (150 : 1–75 : 1) afforded 6-acetyl-2,3-diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridine-11-one **24** (7 mg, 49%), identical in properties to the sample prepared as described above.

3-(3-Chloropropyl)-2-diacetylamino-6,7-diphenyl-4(3*H*)-pteridinone 25

2-Amino-3-(3-chloropropyl)-6,7-diphenyl-4(3*H*)-pteridinone **18** (99 mg, 0.25 mmol), dry pyridine (7 cm^3) and acetic anhydride (5 cm^3, 5.4 g, 0.053 mol) were stirred together at room temperature for 5 h. The mixture was added to ethyl acetate (100 cm^3) and the solution washed with water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography, eluting first with chloroform and then with chloroform–ethanol (15 : 1), gave 3-(3-chloropropyl)-2-diacetylamino-6,7-diphenyl-4(3*H*)-pteridinone **25** (86 mg, 72%), mp >164 °C (decomp.) (from acetone–hexane) (found: C, 63.04; H, 4.60; Cl, 8.00; N, 14.72. Calc. for $C_{25}H_{22}ClN_5O_3$: C, 63.09; H, 4.66; Cl, 7.45; N, 14.72%); λ_{max} (l,4-dioxane)/nm 236 (log ε/dm^3 mol^{-1} cm^{-1} 4.37), 265 (4.33) and 351 (4.17); ν_{max}/cm^{-1} 3060, 1740 (C=O), 1705 (C=O), 1595, 1530, 1400, 1240, 1215, 1045, 980, 955, 800, 790 and 700; δ_H (300 MHz; $CDCl_3$) 2.29 (2 H, m, $ClCH_2CH_2$), 2.47 (6 H, s, 2 × $COCH_3$), 3.67 (2 H, t, *J* 5.9, $ClCH_2$), 4.21 (2 H, t, *J* 7.8, CH_2N) and 7.25–7.64 (10 H, m, 6,7-diPh).

3-(3-Acetamidopropyl)-6,7-diphenyl-2,4(1*H*,3*H*)-pteridinedione 26

2,3-Diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridin-11-one **20** (7 3 mg, 0 21 mmol), methanol (15 cm^3), water (8.0 cm^3) and potassium hydroxide (3.0 g) were placed in a flask and refluxed for 14 h. The reaction mixture was cooled and then neutralised with dilute aqueous hydrochloric acid. Most of the methanol was evaporated under reduced pressure. The aqueous residue was extracted with chloroform, and the extract dried ($MgSO_4$), filtered, and concentrated under reduced pressure to a volume of about 30 cm^3 to give a solution of crude **23**. To this solution were added acetic anhydride (0.5 cm^3) and pyridine (0.5 cm^3), and the mixture was stirred under nitrogen at room temperature for 1 h. It was then washed with water, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography of the residue on silica, using dichloromethane–ethanol (100 : 1) as eluant, gave **26** (29 mg, 32%), mp 215–19 °C (from dichloromethane–hexane) (found: C, 63.45; H, 5.12; N, 15.76. Calc. for $C_{23}H_{21}N_5O_3$ 0.25CH_2Cl_2: C, 63.95; H, 4.96; N, 16.04%); λ_{max} (MeOH)/nm 226 (log ε/dm^3 mol^{-1} cm^{-1} 4.46), 269 (4.20) and 363 (4.19); ν_{max}/cm^{-1} 3319, 3080, 1734 (C=O), 1661 (C=O), 1560, 1243, 752, 720 and 697; δ_H (300 MHz; $CDCl_3$) 1.96 (2 H, quintet, *J* 6.1, $CH_2CH_2CH_2$), 2.02 (3 H, s, $COCH_3$), 3.27 (2 H, q, *J* 6.1, CH_2NH), 4.21 (2 H, t, *J* 6.2, $CH_2CH_2CH_2NH$), 5.29 (0.5 H, s, 0.25 × CH_2Cl_2), 6.41(l H, t, *J* 5.9, CH_2 N*H*), 7.25–7.47 (10 H, m, 6,7-diPh) and 8.99 (l H, br s, pteridine-N*H*); δ_C (75.5 MHz; $CDCl_3$) + DEPT 23.4 (CH_3), 27.7 (CH_2), 36.0 (CH_2), 39.0 (CH_2), 124.1 (C), 128.4 (CH), 129.1 (CH), 129.8 (CH), 129.9 (CH), 130.3 (CH), 136.8 (C), 137.3 (C), 144.9 (C), 149.8 (C), 151.0 (C), 157.2 (C), 161.1 (C) and 170.4 (C).

2,3-Diphenyl-5,8,9,10-tetrahydropyrimido[1,2-c]pteridine-6-one 27

2,3-Diphenyl-6,7,8,9-tetrahydropyrimido[2,1-*b*]pteridin-11-one **20** (116 mg, 0.33 mmol), methanol (20 cm^3), water (10 cm^3) and potassium hydroxide (4.0 g) were heated together under reflux for 5.5 h. The mixture was cooled and then neutralised with dilute aqueous hydrochloric acid. Most of the methanol was evaporated under a reduced pressure and the aqueous residue was extracted with chloroform. The extract was dried ($MgSO_4$) and evaporated under a reduced pressure, to give a mixture of **23** and **27**. Flash chromatography, eluting first with dichloromethane–ethanol (15 : 1, 15 : 2 and 5 : 1) and then with methanol, yielded **27**, together with the more polar **23**. The latter on refluxing in methanol solution for a further 3 h, followed by workup and chromatography gave more **27**. The combined yield of 2,3-diphenyl-5,8,9,10-tetrahydropyrimido[1,2-*c*]pteridine-6-one **27** was 55 mg (47%), λ_{max} (EtOH)/nm 297, 383 and 406; λ_{max} (0.1 M aq KOH)/nm 301 and 399, λ_{max} (0.1 M aq HCl)/nm 278 and 379; ν_{max}/cm^{-1} 3120, 1700 (C=O), 1640, 1560, 1280, 1190, 1100, 1035, 980, 810, 770, 730 and 700; δ_H (300 MHz; $CDCl_3$) 2.03 (2 H, m, $CH_2CH_2CH_2$), 3.82 (2 H, t, *J* 5.6, CH_2N), 4.00 (2 H, t, *J* 5.9, CH_2N) and 7.2–7.4 (10 H, m, 6,7-diPh); δ_C (75.5 MHz, $CDCl_3$) + DEPT 20.03 (CH_2), 41.37 (CH_2), 44.94 (CH_2), 126.35 (C), 128.21 (CH), 128.27 (CH), 128.54 (CH), 129.51 (CH), 129.81 (CH), 129.85 (CH), 137.37 (C), 137.94 (C), 144.21 (C), 144.91 (C), 149.28 (C), 149.40 (C) and 154.48 (C); *m*/*z* (EI) 355 (100%, M^+), 299 (2, $M^+ - C_3H_6N$) and 77 (7, C_6H_5).

6,7-Diphenyl-2-triphenylphosphazino-4(3*H*)-pteridinone 29

2-Amino-4-methoxy-6,7-diphenylpteridine **14** (50 mg, 0.15 mmol) and triphenylphosphine (800 mg, 3.05 mmol) were dissolved in a mixture of tetrachloromethane (20 cm^3) and tetrahydrofuran (5 cm^3) and the solution refluxed for 1.2 h, after which time complete reaction was indicated by TLC. (No reaction occurred in the absence of tetrahydrofuran, even after 6 h refluxing). The reaction mixture was cooled, evaporated under a reduced pressure, and purified by flash chromatography, using ethyl acetate–hexane (1 : 1–3 : 1) as eluant, giving 6,7-diphenyl-2-triphenylphosphazino-4(3*H*)-pteridinone **29** (52 mg, 60%), mp 189–91 °C (from acetone–hexane) (found: C, 74.31; H, 4.63; N, 11.64. Calc. for $C_{36}H_{26}N_5OP$(0.5 H_2O): C, 73.96; H, 4.65; N, 11.98%); λ_{max} (EtOH)/nm 260 (log ε/dm^3 $mol^{-1}cm^{-1}$

4.31), 314 (4.54) and 385 (412); ν_{max}/cm^{-1} 3050, 1690 (C=O), 1580, 1560, 1525, 1175, 1115, 995 and 720; δ_H (300 MHz; $CDCl_3$) 7.17–7.94 (25 H, m, Ph_3P and 6,7-diPh) and 9.18 (1 H, br s, N*H*); δ_P (121.5 MHz; $CDCl_3$; H_3PO_4) 19.28.

2-(*N*,*N*-Dimethylaminomethyleneamino)-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone 30

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (102 mg, 0.278 mmol) was suspended in dry tetrahydrofuran (36 cm^3) under a nitrogen atmosphere. *N*,*N*-Dimethylformamide dimethylacetal (39 µl, 0.295 mmol) was added *via* a syringe and the reaction was stirred for 16 h. Evaporation of solvent gave a yellow oil which was purified by flash chromatography, eluting with dichloromethane–ethanol (150 : 3–150 : 4) to give 2-(*N*,*N*-dimethylaminomethyleneamino)-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **30** as a yellow solid (73 mg, 62%); δ_H (400.13 MHz; d_6-DMSO) 2.07 (2 H, m, C*H*$_2$CH$_2$OH), 3.22 (3 H, s, NC*H*$_3$), 3.27 (3 H, s, NC*H*$_3$), 3.79 (1H, s, O*H*), 3.59 (2 H, t, *J* 6.0, C*H*$_2$OH), 4.55 (2 H, t, *J* 7.0, NC*H*$_2$), 7.26–7.57 (10 H, m, 6,7-diPh), 9.03 (1 H, s, NC*H*N); δ_C (100.6 MHz, d_6-DMSO) + DEPT 159.5 (N*C*HN), 30.9, 39.9, 58.24 (3 × *C*H$_2$), 35.6 and 41.68 (2 × N*C*H$_3$).

(3-(2-*N*,*N*-Dimethylaminomethyleneamino-3,4-dihydro-4-oxo-6,7diphenyl-3-pteridinyl)propoxy) (2-cyanoethoxy) (*N*,*N*-diisopropylamino)phosphoramidite 31

A. 2-(*N*,*N*-Dimethylaminomethyleneamino)-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **30** (30 mg, 0.07 mmol) was dried by performing three coevaporations with dry dichloromethane (1 cm^3). 1(*H*)-tetrazole (2.5 mg, 0.036 mmol) dissolved in dry acetonitrile (1 cm^3) was added and the solvent was removed by vacuum pump. Dry dichloromethane (1 cm^3) was added and the solution was stirred for 30 min under an atmosphere of nitrogen. 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (46.7 µl, 0.14 mmol) was added by micropipette and the reaction was stirred in an icebath in the dark for 30 min. TLC (alumina plates; dichloromethane–diisopropylethylamine, 25 : 1) showed the absence of starting material. The solvent was removed by vacuum pump with the flask wrapped in tin foil. Ethyl acetate (5 cm^3) and dichloromethane (5 cm^3) were added, followed by aqueous sodium hydrogen carbonate solution 5%, 10 cm^3), when the organic layer was extracted and dried (Na_2SO_4). Removal of solvent *in vacuo* gave (3-(2-*N*,*N*-dimethylamino methyleneamino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)-propoxy) (2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphoramid -ite **31** as a yellow solid, which was stored at −5 °C in the dark. δ_H (400 MHz; $CDCl_3$) 1.18 (12 H, m, 2 × CH(C*H*$_3$)$_2$), 2.06 (2 H, m, CH$_2$C*H*$_2$CH$_2$), 2.75 (2 H, t, *J* 6.1, C*H*$_2$CN), 3.28 (3 H, s, NC*H*$_3$), 3.39 (3 H, s, NC*H*$_3$), 3.55–3.70 (4 H, m, 2 × POC*H*$_2$), 3.75–3.9 (2 H, m, 2 × C*H*(CH$_3$)$_2$), 4.68 (2 H, t, *J* 6.9, NC*H*$_2$), 7.3–7.7 (10 H, m, 6.7-diPh), 8.93 (1 H, s, N=C*H*N); δ_P (161.9 MHz, $CDCl_3$), 148.4.

B. 2-(*N*,*N*-Dimethylaminomethyleneamino)-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **30** (25 mg, 0.058 mmol) was dissolved in dry dichloromethane (2 cm^3) in a two necked flask fitted with a septum and a gas tap, and under an atmosphere of nitrogen. Diisopropylethylamine (225 µl, 1.25 mmol) was added to the flask, followed by 2-cyanoethyl *N*,*N*-diisopropyl-chlorophosphoramidite (37.5 µl, 0.167 mmol) *via* a micropipette. The reaction was stirred at room temperature in the dark for 30 min and the solvent was removed by vacuum pump. Ethyl acetate (5 cm^3) and dichloromethane (5 cm^3) were added, followed by aqueous sodium hydrogen carbonate (5%, 10 cm^3). The organic layer was extracted and dried (Na_2SO_4), and the solvent then removed by vacuum pump. The crude product thus obtained was stored at −5 °C in the dark. The product was purified on a column of neutral alumina, eluting with dichloromethane–diisopropylethylamine, (25 : 1), giving (3-(2-*N*,*N*-dimethylaminomethyleneamino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)-propoxy)(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine **31** (17.2 mg, 46.6%) as a yellow solid. δ_H (400.13 MHz; d_6-acetone) 1.18 (12 H, m, 2 × NCH(C*H*$_3$)$_2$), 2.12 (2 H, m, C*H*$_2$CH$_2$N), 2.76 (2 H, t, *J* 6.1, C*H*$_2$CN), 3.29 (3 H, s, NC*H*$_3$), 3.40 (3 H, s, NC*H*$_3$), 3.65 (2 H, m, 2 × C*H*(CH$_3$)$_2$), 3.85 (4 H, m, 2 × POC*H*$_2$), 4.48 (2 H, t, *J* 6.9, NC*H*$_2$), 7.34–7.54 (10 H, m, 6,7-diPh, 8.94 (1 H, s, N=C*H*N); δ_P (161 MHz, d_6-acetone) 148.4.

(3-(2-Amino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)-propoxy)diisopropylaminomethoxyphosphine oxide 32

2-Amino-3-(3-hydroxypropyl)-6,7-diphenyl-4(3*H*)-pteridinone **10** (25 mg, 0.065 mmol) was dissolved in dry tetrahydrofuran (3 cm^3) under nitrogen, and diisopropylethylamine (100 µl, 74 mg, 0.057 mmol) was added to the solution. Chloro(diisopropylamino)methoxyphosphine (20 µl, 20 mg, 0.10 mmol) was added to the mixture in several portions over a period of 7 min. The reaction mixture was stirred at room temperature for 30 min, when more chloro(diisopropylamino)methoxyphosphine (8 µl, 8 mg, 0.04 mmol) was added. After stirring for a further 30 min, the mixture was added to ethyl acetate (30 cm^3). The solution was washed with saturated aqueous potassium hydrogen carbonate, and the organic layer dried ($NaSO_4$) and evaporated under reduced pressure. Flash chromatography of the residue, eluting with a gradient of chloroform–ethanol (150 : 2–150 : 3), yielded 3-(2-amino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)-propoxy(diisopropylamino)methoxyphosphine oxide **32** (8.8 mg, 24%), mp 123–25 °C (from acetone–hexane) (found: C, 61.10; H, 6.63; N, 15.13. Calc. for $C_{28}H_{35}N_6O_4P$: C, 61.08; H, 6.41; N, 15.26%); λ_{max} (EtOH)/nm 242 inflection (log ε/dm^3 mol^{-1} cm^{-1} 4.42), 298 (4.50) and 382 (4.19); ν_{max}/cm^{-1} 3391, 3297, 3059, 1712, 1679, 1568, 1541, 1523, 1246, 1235, 1190, 1026, 1016, 838, 792 and 694; δ_H (200 MHz; d_6-DMSO; relative to CD_2HSOCD_3) 1.13 (12 H, d, *J* 6.6, 2 × CH(C*H*$_3$)$_2$), 1.96 (2 H, m, NCH$_2$C*H*$_2$), 3.52 (3 H, d, *J* 11.2, POC*H*$_3$), 3.97 (2 H, m, POC*H*$_2$), 4.12 (2 H, t, *J* 6.5, NC*H*$_2$), 4.24–4.40 (2 H, m, 2 × C*H*(CH$_3$)$_2$), 7.31–7.36 (10 H, m, 6,7-diPh) and 7.65 (2 H, br s, N*H*$_2$); δ_C (75.5 MHz, $CDCl_3$) 22.74, 22.53, 28.11, 40.47, 46.24, 52.67, 65.17, 125.64, 127.97, 128.09, 128.35, 129.37, 129.89, 130.18, 130.10, 138.51, 149.03, 153.40, 154.13, 158.51 and 161.31; δ_P (161 MHz; d_6-DMSO) 14.48.

(3-(2-*N*,*N*-Dimethylaminomethyleneamino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)propoxy)diisopropylaminomethoxyphosphine oxide 33

(3-(2-Amino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl) propoxy)diisopropylaminomethoxyphosphine oxide **32** (14 mg, 0.025 mmol) was dissolved in dry tetrahydrofuran (5 cm^3), *N*,*N*-dimethylformamide dimethyl acetal (7 µl, 6.3 mg, 0.053 mmol) was added, and the solution was heated at 50 °C under nitrogen for 2 h. The solution was cooled and evaporated under a reduced pressure, when flash chromatography of the residue on silica, eluting with a gradient of dichloromethane–ethanol (100 : 2–100 : 2.5) afforded (3-(2-*N*,*N*-dimethylaminomethyleneamino-3,4-dihydro-4-oxo-6,7-diphenyl-3-pteridinyl)propoxy)diisopropylaminomethoxyphosphine oxide **33** (10 mg, 65%), mp 128–29 °C (from acetone–hexane) (found: C, 60.02; H, 6.62; N, 15.58. Calc. for $C_{31}H_{40}N_7O_4P{\cdot}H_2O$: C, 59.70; H, 6.79; N, 15.72%); λ_{max} (EtOH)/nm 320 (log ε/dm^3 mol^{-1} cm^{-1} 4.56), and 382 (4.28); ν_{max}/cm^{-1} 1683, 1634, 1528, 1485, 1449, 1426, 1343, 1246, 1191, 1110, 1023, 1008, 887, 794, 770 and 695; δ_H (300 MHz; $CDCl_3$) 1.21 (12 H, d, *J* 6.8, 2 × CH(C*H*$_3$)$_2$), 2.14–2.20 (2 H, m, NCH$_2$C*H*$_2$), 3.20 (3 H, s, NC*H*$_3$), 3.23 (3 H, s, NC*H*$_3$), 3.37–3.48 (2 H, m, 2 × C*H*(CH$_3$)$_2$, 3.65 (3 H, d, *J* 11.2, POC*H*$_3$), 4.03–4.17 (2 H, m, POC*H*$_2$), 4.51 (2 H, t, *J* 7.4, NC*H*$_2$), 7.22–7.53 (10 H, m, 6,7-diPh), 8.98 (1H, s, N=C*H*); δ_P (121.5 MHz; $CDCl_3$) 22.41.

2-(6-Carboxypentylamino)-6-amino-5-nitroso-4-(3*H*) pyrimidinone 35

6-Amino-2-methylthio-5-nitroso-4-(3*H*)-pyrimidinone **34** (500 mg, 2.7 mmol) and 6-aminohexanoic acid (1.0 g, 7.6 mmol) were suspended in water (20 cm^3). The suspension was refluxed for 1 h (with a hydrogen disulfide scrubber attached to the condenser), during which time the blue suspension changed to a red solution. The reaction was cooled in an ice bath, when 2-(6-carboxypentylamino)-6-amino-5-nitroso-4-(3*H*) pyrimidinone **35** separated as an orange solid (300 mg, 45%), δ_H (400.13 MHz; d_6-DMSO) 1.3 (2 H, m, CH_2), 1.52 (4 H, m, 2 × CH_2), 2.21 (2 H, t, *J* 7.0, CH_2), 7.53–8.4 (2 H, s, NH_2); δ_H (400.13 MHz; NaOD; Me_4Si) 1.31 (2 H, br s, CH_2), 1.52 (4 H, br s, 2 × CH_2), 2.14 (2 H, t, *J* 7.0, CH_2), 3.31 (2 H, t, *J* 6.5, CH_2); δ_C (100.6 MHz; d_6-DMSO) + DEPT 24.5 (CH_2), 26.2 (CH_2), 28.8 (CH_2), 34.0 (CH_2), 40.7 (CH_2), 141.7 (pyrimidine C), 152.3 (pyrimidine C), 154.5 (pyrimidine C), 161.6 (pyrimidine C) and 175.0 (C=O).

2-(6-Carboxypentylamino)-6,7-diphenyl-4-(-3*H*) pteridinone 36

2-(6-Carboxypentylamino)-6-amino-5-nitroso-4-(3*H*) pyrimidinone **35** (100 mg, 0.317 mmol) was dissolved in aqueous sodium hydroxide (2 M, 10 cm^3), palladised carbon (10%, 60 mg) was added, and the mixture stirred vigorously under hydrogen at atmospheric pressure until hydrogen uptake ceased. The mixture was filtered under vacuum into a solution of benzil, (78 mg, 0.32 mmol) in ethanol (10 cm^3). The solution was refluxed until TLC (dichloromethane–methanol, 15 : 2) showed that no benzil remained. The solution was cooled to room temperature, neutralised to pH 7 with dilute hydrochloric acid and evaporated to dryness. The solid was extracted with methanol and the product was purified by column chromatography, eluting initially with dichloromethane followed by an increasing gradient of methanol. Pure fractions were pooled and the solvent evaporated to give 2-(6-carboxypentylamino)-6,7-diphenyl-4-(-3*H*) pteridinone **36** as a yellow solid (115 mg, 73%), δ_H (400.13 MHz; d_6-DMSO) 1.3 (2 H, m, CH_2), 1.52 (4 H, m, 2 × CH_2), 2.21 (2 H, t *J* 7.0, CH_2), 3.37 (2 H, t, *J* 6.5, CH_2), 4.5 (2 H, br s, 2 × NH), 7.29–7.4 (10 H, m, 6,7-diPh); δ_C (100.6 MHz; d_6-DMSO) + DEPT 25.1 (CH_2), 26.5 (CH_2), 29.0 (CH_2), 35.3 (CH_2), 40.7 (CH_2), 128.2 (C), 128.7 (C), 129.0 (C), 129.2 (C), 129.8 (C), 174.1 (C=O); *m*/*z* (ES^-), 428 ($M - H^+$).

2-(6-Succinimidocarboxypentylamino)-6,7-diphenyl-4-(-3*H*)-pteridinone 37

2-(6-Carboxypentylamino)-6,7-diphenyl-4-(3*H*)-pteridinone **36** (86 mg, 0.2 mmol) was suspended in anhydrous *N*,*N*-dimethylformamide (2.5 cm^3). To the suspension were added diisopropylethylamine (61µl) and *N*,*N*,*N*′,*N*′-tetramethyl (succinimido)uronium tetrafluoroborate (72 mg, 0.22 mmol) and the mixture was stirred in the dark for 20 h under a nitrogen atmosphere, when TLC (dichloromethane–methanol, 15 : 2) showed that no starting material remained. The brown reaction mixture was evaporated to dryness under vacuum and purified by flash chromatography, eluting initially with dichloromethane, followed by an increasing gradient of ethanol. Pure fractions were pooled and the solvent evaporated to give 2-(6-*N*-hydroxysuccinimidocarboxypentylamino)-6,7-diphenyl-4-(3*H*)-pteridinone **37** as a yellow solid (58 mg, 55%); δ_H(400.13 MHz; $CDCl_3$), 1.4 (2 H, m, CH_2), 1.6 (4 H, m, 2 × CH_2), 2.6 (2 H, t *J* 7.2, CH_2), 2.81 (4 H, br s, 2 × CH_2), 3.4 (2 H, t, *J* 6.7 CH_2), 17.3–7.4 (10 H, m, 6,7-diPh); δ_C (100.6 MHz; $CDCl_3$) + DEPT, 24.4 (CH_2), 25.8 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 30.5 (CH_2), 40.4 (CH_2), 128.2 (C), 128.7 (C), 129.0 (C), 129.2 (C), 129.8 (C), 168.9 (C=O), 170.2 (C=O); *m*/*z* (ES^+) 527 ($M + H^+$).

Radiolabelling experiments

The 34-base oligodeoxynucleotides (ODNs) were synthesised without a phosphate group at their 5′ termini and were labelled by the transfer of the $\gamma^{32}P$ from $\gamma^{32}P$[ATP] using the enzyme bacteriophage T4 polynucleotide kinase (PNK). The ODN (25 pmol), radioactive isotope ($\gamma^{32}P$[ATP], 2 µl (specific activity 5000 Ci/mmol ⇒ 4pmol)), PNK buffer (10 × PNK buffer, 2 µl) and water (9 µl) were placed in a sterile Eppendorf tube and vortexed. The enzyme (PNK enzyme, 2 µl, 20 units) was then added and the tube was tapped rather than vortexed to mix the contents, since vortexing is known to denature the enzyme. The reaction was incubated at 37 °C for 40 min, followed by 10 min at 68 °C (which inactivates the enzyme). 3M Ammonium acetate (100 µl) and cold 98% ethanol (400 µl) were added to the reaction mixture which was then vortexed and placed at −20 °C for 20 min. The reaction tube was then centrifuged at 12 000 g for 20 min and the supernatant was removed. Cold 98% ethanol (1 cm^3) was added and the mixture was vortexed and stored at −20 °C for 20 min. The reaction tube was then centrifuged at 12 000 g for 20 min. Following removal of the supernatant, the pellet was dried at room temperature and stored in sterile water.

General procedure for irradiation experiments

To an Eppendorf tube were added 1 µl of the photoactive conjugate, 1 µl of the radiolabelled target and 8 µl of 12.5 mM phosphate buffer/125 mM sodium chloride (pH 6.9). The tube was vortexed to equilibrate the contents and centrifuged at 5000 rpm. The tube was then heated to 90 °C for 5 min and cooled slowly to room temperature to allow hybridisation to occur. The tube was then placed in an ice water bath and irradiated with light of wavelength >330 nm (high pressure mercury lamp radiation passed through pyrex glass filter). After irradiation, the sample was dried by vacuum centrifuge and 1 M piperidine (15 µl) added. After vortexing and centrifuging, the sample was heated at 90 °C for 30 min and dried by vacuum centrifuge. The sample was washed by adding water (10 µl) and then vortexing and centrifuging. The sample was dried by vacuum centrifuge. Loading dye (5 µl; 1% bromophenol blue, 1% xylene cyanol in an 80% aqueous formamide solution) was then added to the sample prior to sequencing gel electrophoresis.

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