Design and Synthesis of Transition-state Analogues for a Cationic Cyclisation

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Transition-state analogues based upon the 6-(hydroxymethyl)-13-azagona-1,3,5(10),8-tetraene structure (*e.g.*, **40**) have been designed and synthesized as part of a programme to elicit antibodies capable of catalysing cationic cyclisations. Methodology for conjugating such analogues to proteins has also been developed.

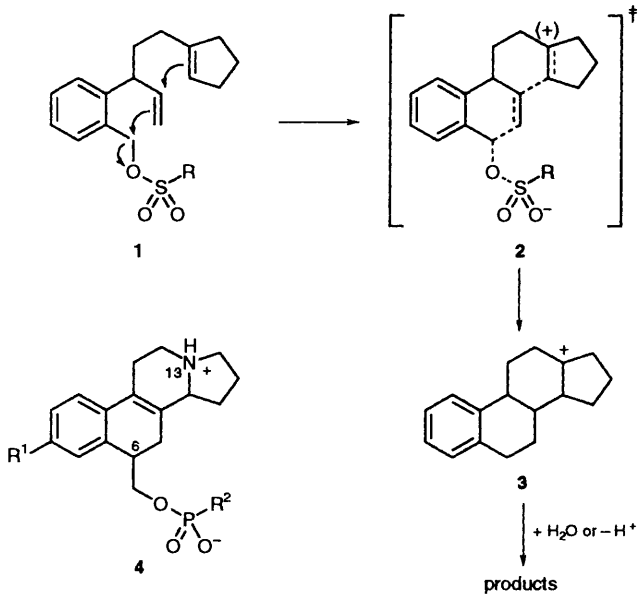
Cationic cyclisations are a fundamental type of reaction, implicated in the biosynthesis of the vast number of isoprenoid natural products. Terpene cyclases, for example, use cationic cyclisations to convert acyclic terpenoid precursors, such as farnesyl pyrophosphate, into an array of different carbon skeletons which pervade the biochemistry of plants and micro-organisms.[1] A number of cyclisations may occur, as well as rearrangements such as methyl migrations and hydride shifts.[2]

Although the mechanisms of terpene cyclases have been probed extensively by classical feeding experiments with variously labelled precursors, the enzymes themselves have proven to be difficult to isolate and purify. Each terpene cyclase is believed to hold its substrate in the precise conformation for reaction, to facilitate the departure of the allylic pyrophosphate leaving group, and to stabilise the cationic, high-energy intermediates which result from carbon–carbon bond-forming reactions. However, to date, very little is known about the nature of their active sites and the precise mechanisms by which these enzymes control the conformations of substrates and intermediates and also successfully manipulate the carbocations involved.

We hope to use a different approach, involving catalytic antibodies, to explore how cationic cyclisations can be promoted and controlled by a protein. Antibodies which can catalyse particular reactions have been generated by eliciting an immune response to suitably designed analogues of the transition state.[3] This approach has been particularly successful in ester-hydrolysis reactions and a number of other types of reaction have been catalysed such as eliminations, opening of epoxides and metal-insertion reactions. Carbon–carbon bond formation has been seen in pericyclic Diels–Alder and Claisen rearrangement reactions but no example of a cationic cyclisation catalysed by an antibody has yet been reported.

The cationic cyclisation that we planned to develop antibodies to catalyse is **1**→**3** (Scheme 1). In this reaction a benzylic sulfonate is the leaving group (*cf.* allylic pyrophosphate in terpene cyclases), the cation is captured by two successive C=C double bonds to give the cation **3**, which would be quenched either by attack of water to give a tertiary alcohol or by loss of a proton to give an alkene. This reaction was chosen because (i) it involves two cationic cyclisation reactions, (ii) two new chiral centres are formed and any one monoclonal antibody should be stereospecific, though different antibodies may give different stereochemistries and (iii) it gives products having a tetracyclic steroid skeleton closely related to estrogen.

The transition state **2** of the chosen reaction has developing positive charge on C-13 of the steroid skeleton and developing negative charge on the leaving group. The analogues of this transition state were therefore chosen to have the overall steroid skeleton, with a protonated nitrogen atom at position 13 to mimic the positive charge and a phosph(on)ate side-chain, which mimics the growing negative charge on the sulfonate



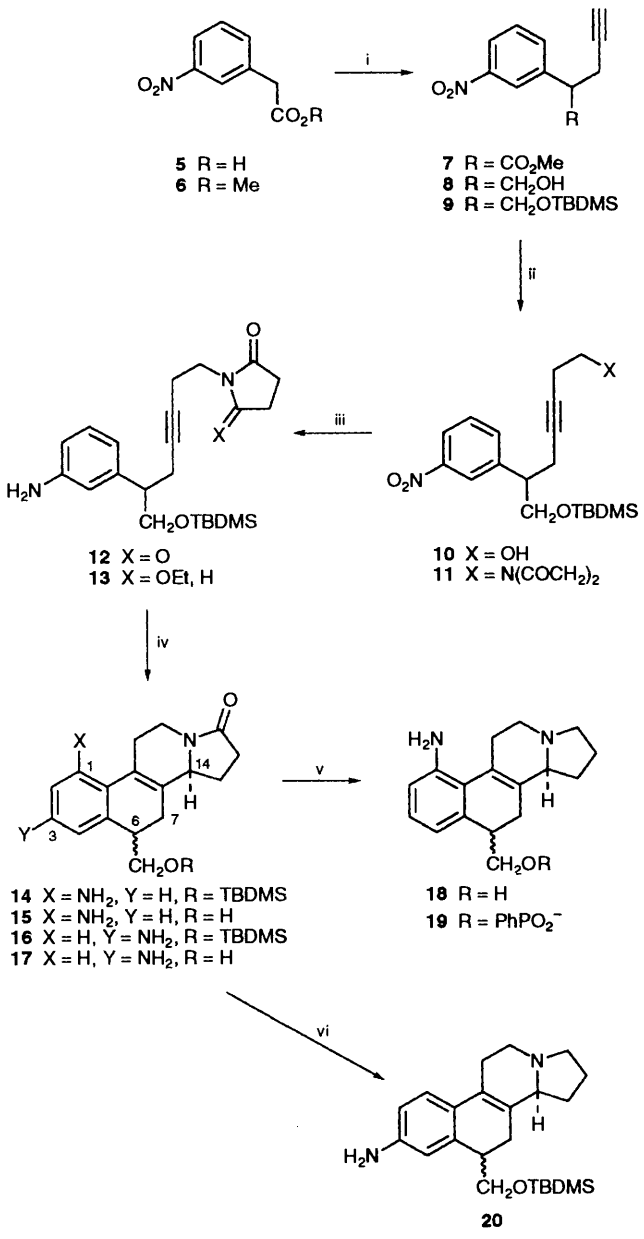
Scheme 1

leaving group, *i.e.* general structure **4**. The phosph(on)ate group is separated from C-6 of the steroid skeleton by an extra methylene group so as to mimic the lengthening of the C-6-to-oxygen bond which occurs during the reaction. One risk in this strategy is that an anionic centre on the antibody, induced by the cationic site in the transition state analogue, may adventitiously trap the cationic intermediate formed during the desired cyclisation reaction. This is a problem inherent in cationic cyclisations, be they enzyme- or antibody-catalysed. Terpene cyclase enzymes clearly manage to avoid this problem and we would hope to identify antibodies that do not suffer from the problem either. However, antibodies that do get alkylated would also be of interest as this would occur only for antibodies that are capable of promoting formation of cations.

In addition to mimicking the transition state, the analogue must also be linked to a protein in order to generate a suitable immune response. We intended to achieve such conjugation either *via* an anilide side-chain (R^1 = NHCO-linker) or *via* the anionic side-chain (R^2 = linker). This paper describes the synthesis of transition state analogues **4** designed for attachment of linkers at either position and also the coupling of one of the analogues to a protein *via* the latter type of linker, R^2.

Results and Discussion

Synthesis of the First Transition-state Analogue.—Our initial aim was to synthesize a transition-state analogue that has an amino group on the aromatic ring to serve as the point of



Scheme 2 *Reagents and yields:* i, MeOH, H_2SO_4 (94%); then LDA, $HC{\equiv}CCH_2Br$ (76%); ii, $LiAlH_4$ (89%); then Bu^tMe_2SiCl (TBDMSCl), imidazole (98%); then LDA, ethylene oxide (40%); iii, succinimide, DEAD, Ph_3P (91%); then Fe, AcOH (82%); iv, $NaBH_4$, EtOH, HCl (67%); then $SnCl_4$ [29% (**14**) + 17% (**15**) + 10% (**16**) + 16% (**17**)]; v, Red-Al (77%); then $PhPOCl_2$, triazole, pyridine (50%); vi, $LiAlH_4$ (81%)

attachment for the link to the protein. Our approach to the required transition-state analogues is shown in Scheme 2. We envisaged assembly of the basic 13-azasteroid skeleton in analogy with the synthesis of 13-azagona-1,3,5(10),8-tetraen-17-one by Dijkink and Speckamp,[4] with the addition of the aromatic nitrogen substituent and the hydroxymethyl group at C-6. These modifications would be used to link the analogue to a protein and to add a phenylphosphonate side-chain, respectively.

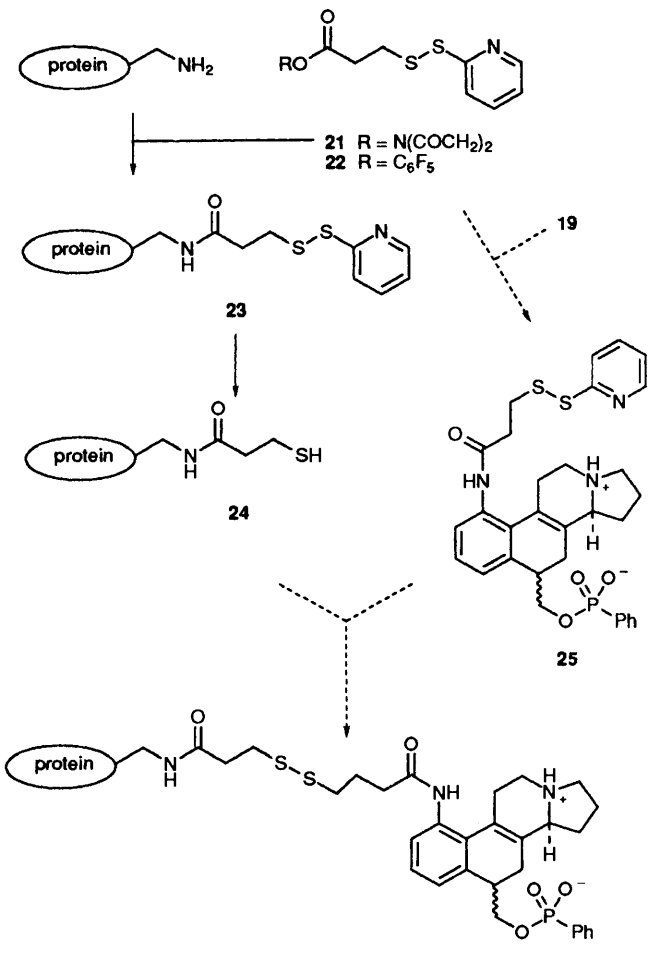
The synthesis of the first transition-state analogue started with esterification of (3-nitrophenyl)acetic acid **5** in acidic methanol and alkylation of the lithium enolate of the ester **6** with prop-2-ynyl bromide to give pent-4-ynoate ester **7** in 76% yield. Reduction of the ester with lithium aluminium hydride and protection of the derived alcohol **8** as a *tert*-butyldimethylsilyl (TBDMS) ether[5] gave compound **9** (87%). Deprotonation of the acetylene, using lithium diisopropylamide (LDA), followed by alkylation with ethylene oxide gave the desired alcohol **10** in only 40% yield (based upon unrecovered starting material). This low yield was seemingly due to the competing reactivity of the aromatic nitro group which led to dimerisation, as evidenced by the ^1H NMR spectrum of the major by-product. A Mitsunobu reaction[6] allowed replacement of the hydroxy group of compound **10** by a succinimidyl group to give imide **11** in 91% yield. The nitro group was then reduced, since its electron-withdrawing properties would be deleterious to the intended acid-catalysed cyclisation step, which involves electrophilic attack on the aromatic ring. The reduction to aniline **12** was achieved in 82% yield by heating compound **11** with iron powder in ethanolic acetic acid.[7] Selective reduction of the imide **12** was then carried out using sodium boranuide (sodium borohydride) in ethanol and acid[4] to give the ethoxy lactam **13**.

A number of different acid catalysts for the cyclisation reaction were tested and tin(IV) chloride in methylene dichloride gave the cleanest results. In all cases, however, a mixture of products was obtained. Alkylation of the aniline ring had occurred both *ortho* and *para* to the amino group, providing the cyclised product (72% total yield) with the nitrogen substituent at C-1 or C-3 in a ratio of ~2:1. In addition, each product was generated as a mixture of diastereoisomers and the silyl ether was partially cleaved under the reaction conditions, which could be tailored to favour either the silyl ethers, **14** and **16**, or the free alcohols, **15** and **17**. Since the ^1H NMR spectra of the diastereoisomeric mixtures were difficult to assign fully, compound **14** (~3:1 mixture of diastereoisomers) was further purified by HPLC, and this allowed the major diastereoisomer to be identified as 6β-**14** by decoupling and NOE experiments—in particular, NOEs were observed from both 6-H and 14-H to the *same* hydrogen on C-7. The origin of the selectivity shown in this reaction is not apparent.

Reduction of lactam **15** with sodium bis(2-methoxyethoxy)-aluminium hydride (Red-Al) gave amine **18**, which was treated with a mild phosphorylating reagent, generated from phenylphosphonic dichloride and triazole, to give the transition-state analogue **19**. With this in hand, we turned our attention to coupling of the analogue to a suitable protein *via* its aromatic amino group.

The conjugation strategy (Scheme 3) was to utilise *N*-succinimidyl 3-(2-pyridyldisulfanyl)propanoate **21** (SPDP)[8] as a heterobifunctional linker. The carrier protein was to be derivatised with SPDP and the disulfide **23** reduced with dithiothreitol (DTT) to give the modified protein **24** (Scheme 3). A disulfide-exchange reaction with phosphonate **25**, the conjugate of compound **19** and SPDP, should give the derivatised carrier protein **26**.

The first step in this coupling strategy was to derivatise the transition-state analogue with SPDP **21**. In model reactions, aniline itself reacted successfully with SPDP in pyridine at 50 °C to give the corresponding anilide, *N*-phenyl-3-(2-pyridyldisulfanyl)propanamide. However, the same conditions applied to compound **19** caused it to decompose rapidly. We reasoned that a more reactive heterobifunctional linker might circumvent the decomposition, and so the pentafluorophenyl ester analogue **22** was synthesized. This new linker derivatised aniline rapidly at −15 °C in pyridine, to give the anilide as before. Unfortunately, treatment of compound **19** with this pentafluorophenyl ester resulted only in a similar decomposition to that observed with SPDP, to yield an unidentified, highly polar, fluorescent material. It was thought that an amino group at C-3, as in the aniline **16**, should be much less sterically hindered than at C-1, as in compound **19**. Therefore the lactam group in compound **16** was reduced with lithium aluminium hydride to give the amine **20**. Unfortunately, treatment of compound **20**



Scheme 3

with the same pentafluorophenyl ester **22**, either in pyridine or in methylene dichloride with triethylamine, only resulted in decomposition of the aniline. It seems that these electron-rich anilines were prone to some unknown side-reaction which was occurring more rapidly than the desired coupling reactions.

It would have been possible to study the attachment of other linkers to the anilines **18** and **20** but at this stage we decided instead to synthesize a slightly different transition-state analogue. This decision was taken for a number of reasons: first, it seemed probable that the aniline group of compound **18** would be too hindered for efficient derivatisation, whereas the aniline **20** was not available in sufficient quantity because its precursor **16** was only a minor product from the cyclisation of compound **13**; secondly, the intended substrate **1** for the antibody-catalysed reaction does not have any substituent on the aromatic ring and so it would be more appropriate if the transition-state analogue had no substituent there either; thirdly, the leaving group should be allowed to leave from the antibody-combining site during the course of the catalysed reaction and this should be possible for antibodies raised against a transition-state analogue **4** which is attached to the carrier protein *via* its anionic side-chain, thus ensuring that the approach of the antibody to the protein-bound analogue can only be from the opposite side. Consequently, a new transition state analogue **40**, which incorporates these features, was designed.

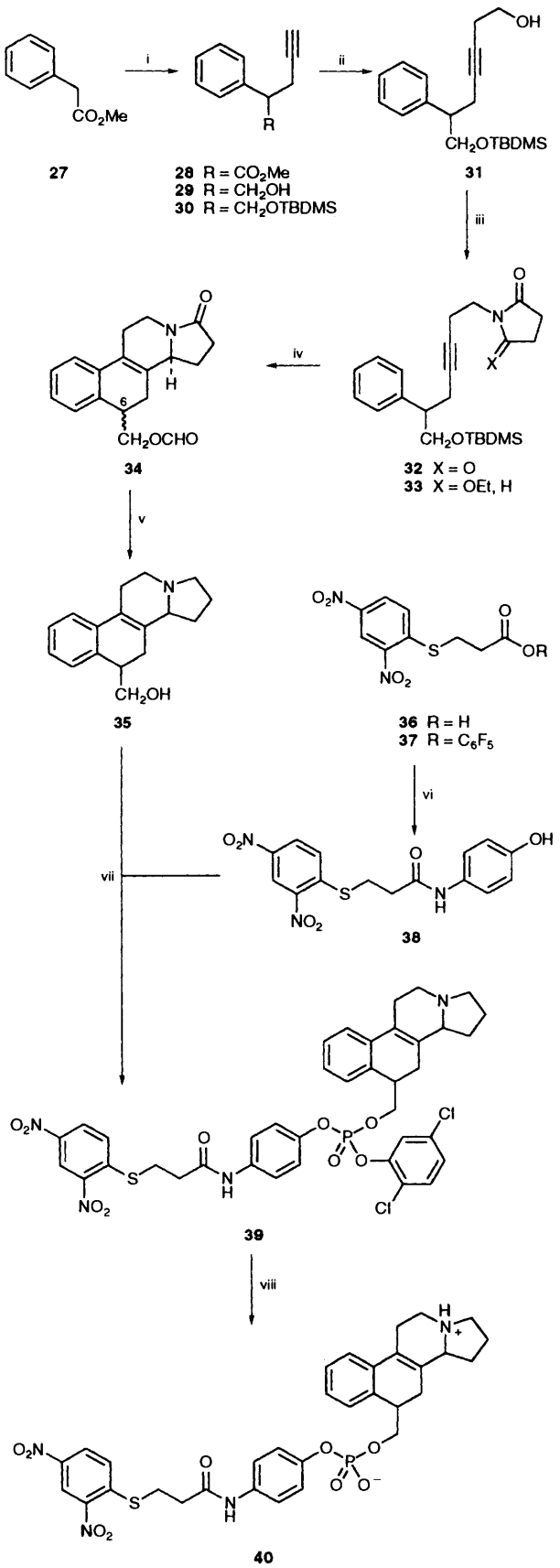
Synthesis of the Second Transition-state Analogue.—It was decided that coupling of the transition-state analogue to a carrier protein would be achieved *via* a thiol in the anionic side-chain, since thiols are versatile in cross-linking reactions such as those with maleimide,[9] dipyridyl disulfide[8] and active halogen[10] derivatives. An aromatic phosphate would be used to mimic the anionic leaving group, since this would allow the use of oligonucleotide synthesis methodology to incorporate the side-chain. These considerations, in addition to removal of the aromatic amino group, led to structure **40** as our next target compound (Scheme 4). Synthesis of the required alcohol **35** should be straightforward, by analogy with the synthesis of anilines **18** and **20** described above. The thiol group of the linker would be protected as a 2,4-dinitrophenyl sulfide, cleavage of which is reported to be quite mild,[11] and the phenol **38** should be obtained *via* reaction of 4-aminophenol with a suitable acylating agent, such as the ester **37**.

The assembly of the complete transition-state analogue is detailed in Scheme 4. The first three steps used the same methodology as in Scheme 2, and provided the acetylene **30** in good yield. However, the alkylation of this acetylene was still not straightforward, even in the absence of the aromatic nitro group. Although treatment of compound **30** with butyllithium at 5 °C gave essentially quantitative lithiation at the acetylenic position, as judged by quenching with deuterium oxide, the lithiated species was quite unreactive, giving only a modest yield of the desired alcohol **31** even after treatment with 50 mole equivalents of ethylene oxide. The Mitsunobu reaction[6] between alcohol **31** and succinimide and reduction of the resulting imide **32** to the ethoxy lactam **33** with sodium boranuide and hydrochloric acid in ethanol went smoothly. As before, the cyclisation step was attempted with a variety of acid catalysts and formic acid was found to be most effective; it provided the formate ester **34** as a diastereoisomeric mixture (~3:2, from ^{1}H NMR evidence). This formate ester was reductively removed by Red-Al in tetrahydrofuran (THF) and concurrently the lactam was reduced to the tertiary amine **35**. Flash column chromatography on silica cleanly separated the two diastereoisomers of compound **35**, and thus allowed both diastereoisomers of the transition-state analogue **40** to be synthesized separately.

The phenol **38**, required for linking to a protein, was synthesized in excellent yield by protection of 3-sulfanylpropanoic acid with Sanger's reagent (2,4-dinitrofluorobenzene) to give the dinitrophenyl sulfide **36**,[12] generation of pentafluorophenyl ester **37** by coupling with pentafluorophenol using 1,3-dicyclohexylcarbodiimide (DCC), and treatment of this activated ester with an equimolar amount of 4-aminophenol. The acylation occurred cleanly on the amino rather than the hydroxy group, as judged by the ^{1}H NMR spectrum of the product **38** and its carbonyl absorption band at 1650 cm^{-1}.

The final assembly of compound **40** was achieved, in analogy with known oligonucleotide synthesis methods,[13] by sequential addition of the phenol **38** and the alcohol **35** to the phosphorylating reagent 2,5-dichlorophenyl bis(benzotriazol-1-yl) phosphate,[13] followed by partial purification on a column of silica gel to give an impure sample of the 2,5-dichlorophenyl phosphate ester **39**. Removal of the dichlorophenyl group was effected by treatment with pyridine-2-aldoxime and 1,1,3,3-tetramethylguanidine, to give each diastereoisomer, in turn, of the transition-state analogue **40**.

Attachment of the Transition-state Analogue **40** *to a Protein.*—In order to conjugate compound **40** to a carrier protein, the 2,4-dinitrophenyl (DNP) group must be removed and the free thiol allowed to react with a suitably derivatised protein, such as the disulfanylpyridyl-derivatised amide **23**. Although thiolysis of DNP sulfides is reported to proceed at pH 8.0 and 22 °C in 1 h,[11] we found little evidence of such cleavage under these conditions, even in the presence of 100-fold excess of thiol, and



Scheme 4 *Reagents* (*and yields*): i, LDA, $HC{\equiv}CCH_2Br$ (67%); ii, $LiAlH_4$ (76%); then TBDMSCl, imidazole (100%); then BuLi, ethylene oxide (42%); iii, succinimide, DEAD, Ph_3P (95%); iv, $NaBH_4$, EtOH, HCl (85%); then HCO_2H (87%); v, Red-Al (82%); vi, C_6F_5OH, DCC (89%); then $HOC_6H_4NH_2$, pyridine (90%); vii, 1-hydroxybenzotriazole, $(C_6H_3Cl_2)OP(O)Cl_2$; viii, pyridine-2-aldoxime, $(Me_2N)_2C{=}NH$.

it was necessary to increase the pH of the buffer to 9.5 in order to accelerate thiolysis. Our methodology for the deprotection/conjugation sequence was to incubate the DNP-protected transition-state analogue **40** in the presence of ethane-1,2-dithiol (50 mol equiv.) at pH 9.5. After deprotection, the buffer was adjusted to pH 7.5 and extracted with diethyl ether to remove the excess of ethanedithiol. The remaining aqueous layer was combined with a solution of the protein–SPDP conjugate[8] under an inert atmosphere and the release of pyridine-2-thione was followed by the change in absorbance at 343 nm until the reaction had reached completion. Both isomers of compound **40** were separately conjugated to the immunogenic tuberculin purified protein derivative (PPD) in this way.

In conclusion, the synthesis of transition-state analogues for a cationic cyclisation is described. The final such analogue has been conjugated to suitable proteins to allow immunisation and production of monoclonal antibodies and these biological studies are ongoing. These experiments should help to define the relationship between hapten design and the catalytic potency of antibodies raised to that hapten and may illuminate the mechanisms of action of the terpene cyclases.

Experimental

General Directions.—M.p.s were determined on a Kofler hot-stage apparatus, and are uncorrected; electronic spectra were recorded with a Kontron Instruments Uvikon 860 spectrophotometer on solutions in methanol; IR spectra were determined using a Perkin-Elmer 1310 spectrometer or 1710 Fourier Transform spectrometer on solutions in chloroform unless otherwise stated. Proton NMR spectra were recorded on Varian EM-390, Bruker WM250 or Bruker AM400 spectrometers, operating at 90, 250 and 400 MHz respectively, with tetramethylsilane (TMS) or the solvent peak as standard. Chemical shifts are quoted on the δ-scale relative to TMS as $\delta = 0$ and coupling constants are given in Hz. Proton-decoupled ^{13}C NMR spectra were recorded on Bruker AM400 spectrometers at 100 MHz. Where deuteriochloroform was used as the solvent, it was passed down a column of dried, basic alumina directly before use. Mass spectra were recorded on A.E.I. MS30, MS90 or MS50 machines.

Analytical TLC or preparative TLC (PLC) was performed on plates coated with Merck Kieselgel 60 F_{254}. Silica used for flash column chromatography[14] was Merck Kieselgel 60 (230–400 mesh). Organic solutions were usually dried over anhydrous magnesium sulfate or sodium sulfate prior to evaporation. All solvents were redistilled before use. Solvents and reagents for anhydrous reactions were dried by conventional methods[15] and such reactions were performed under a small positive pressure of argon. Tuberculin purified protein derivative (PPD) was obtained from Cambridge Research Biochemicals.

Methyl (3-*Nitrophenyl*)*acetate* **6**.—A solution of (3-nitrophenyl)acetic acid **5** (2.91 g, 16.1 mmol) in methanol (200 cm^3) containing sulfuric acid (0.5 cm^3) was heated at reflux for 2.5 h, then was evaporated to ~70 cm^3 under reduced pressure, poured into water (100 cm^3), and extracted with methylene dichloride (3 × 80 cm^3). The combined organic extracts were washed with brine (100 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to give the methyl ester[16] **6** as waxy needles (2.95 g, 94%), m.p. 28–30 °C (Found: M^+, 195.0532. Calc. for $C_9H_9NO_4$: M, 195.0532); λ_{max}/nm 261; ν_{max}/cm^{-1} 1740, 1520 and 1350; δ_H(250 MHz; $CDCl_3$) 3.72 (3 H, s, OMe), 3.74 (2 H, s, $ArCH_2CO_2Me$), 7.50 (1 H, td, *J* 7 and 1, 5-H), 7.62 (1 H, d, *J* 7, 6-H) and 8.11–8.16 (2 H, m, 2- and 4-H); *m*/*z* 195 (M^+) and 136 ($M - CO_2CH_3$).

Methyl 2-(3-*Nitrophenyl*)*pent*-4-*ynoate* **7**.—Butyllithium (1.4 mol dm^{-3} solution in hexane; 13.9 cm^3, 19.5 mmol) was added to a stirred solution of diisopropylamine (3.4 cm^3, 24 mmol) in dry THF (300 cm^3) at −78 °C. The mixture was warmed to −10 °C for 10 min, then was recooled to −78 °C. A solution of the ester **6** (2.92 g, 15.0 mmol) in THF (60 cm^3) was added dropwise, at such a rate that the temperature did not rise above −60 °C. After 10 min, freshly distilled prop-2-ynyl bromide (2.7 cm^3, 30.3 mmol) was added dropwise. The mixture was stirred at −78 °C for 70 min, then was allowed to warm to −20 °C and stirred for 20 min, then was recooled to −78 °C and, after a further 40 min, quenched with a solution of sulfuric acid (2.4 g) in water (300 cm^3). This mixture was extracted with diethyl ether (3 × 100 cm^3) and the combined extracts were washed successively with saturated aq. ammonium chloride (100 cm^3) and brine (100 cm^3), and were dried ($MgSO_4$). Evaporation under reduced pressure gave an oil (3.4 g), which was purified by flash column chromatography on silica, and elution with light petroleum (distillation range 60–80 °C)–ethyl acetate (5:2), to yield the *alkyne* **7** as an oil (2.64 g, 76%) (Found: M^+, 233.0682. $C_{12}H_{11}NO_4$ requires M, 233.0688); λ_{max}/nm 260; ν_{max}/cm^{-1} 3305, 2120, 1730, 1600, 1580, 1520 and 1345; δ_H(250 MHz; $CDCl_3$) 1.96 (1 H, t, *J* 3, C≡CH), 2.73 (1 H, ddd, *J* 17, 8 and 3) and 2.95 (1 H, ddd, *J* 17, 7 and 3; together $CH_2C{\equiv}CH$), 3.71 (3 H, s, OMe), 3.91 (1 H, dd, *J* 8 and 7, $CHCO_2Me$), 7.52 (1 H, t, *J* 8, 5-H), 7.66 (1 H, dt, *J* 8 and 1, 6-H) and 8.14–8.20 (2 H, m, 2- and 4-H); *m*/*z* 233 (M^+) and 202 (M − OCH_3).

2-(3-*Nitrophenyl*)*pent*-4-*yn*-1-*ol* **8**.—A solution of the ester **7** (2.75 g, 11.8 mmol) in dry THF (25 cm^3) was added to a stirred suspension of lithium aluminium hydride (920 mg, 41.3 mmol) in THF (200 cm^3) at −78 °C. After 65 min, the mixture was allowed to warm to −40 °C for 20 min, then was quenched with wet diethyl ether, followed by water. The resulting mixture was acidified with dil. sulfuric acid and extracted with diethyl ether (3 × 100 cm^3). The combined extracts were washed with brine (100 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica, and elution with light petroleum (60–80 °C)–ethyl acetate (4:3), to yield the *alcohol* **8** as an oil (2.15 g, 89%) (Found: M^+, 205.0738. $C_{11}H_{11}NO_3$ requires M, 205.0739); λ_{max}/nm 263; ν_{max}/cm^{-1} 3595, 3300, 2120, 1600, 1575, 1520 and 1345; δ_H(250 MHz; CD_2Cl_2) 1.67 (1 H, br s, CH_2OH), 2.03 (1 H, t, *J* 3, C≡CH), 2.60 (1 H, ddd, *J* 17, 8 and 3) and 2.71 (1 H, ddd, *J* 17, 7 and 3; together $CH_2C{\equiv}CH$), 3.16 (1 H, tt, *J* 7 and 6, $CHCH_2OH$), 3.92 (2 H, d, *J* 6, CH_2OH), 7.53 (1 H, t, *J* 8, 5-H), 7.66 (1 H, dt, *J* 8 and 1, 6-H) and 8.09–8.17 (2 H, m, 2- and 4-H); *m*/*z* 205 (M^+) and 188 (M − OH).

5-(tert-*Butyldimethylsiloxy*)-4-(3-*nitrophenyl*)*pent*-1-*yne* **9**.—A solution of the alcohol **8** (1.61 g, 7.85 mmol), TBDMSCl (1.78 g, 11.8 mmol) and imidazole (1.33 g, 19.6 mmol) in dry dimethylformamide (20 cm^3) was stirred for 16 h at room temperature and was then poured into a mixture of water (80 cm^3) and ethyl acetate (80 cm^3). The aqueous phase was extracted with ethyl acetate (2 × 40 cm^3) and the combined extracts were washed successively with a solution of sulfuric acid (0.96 g) in water (100 cm^3) and then brine (50 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica, and elution with light petroleum (60–80 °C)–ethyl acetate (2:1), to yield the silyl ether **9** as an oil (2.45 g, 98%) [Found: (M^+ − CH_3), 304.1371. $C_{16}H_{22}NO_3Si$ requires *m*/*z*, 304.1369]; λ_{max}/nm 264; ν_{max}/cm^{-1} 3280, 2100, 1515 and 1340; δ_H(400 MHz; $CDCl_3$) −0.02 (6 H, s, $OSiMe_2$), 0.84 (9 H, s, $OSiBu^t$), 1.94 (1 H, t, *J* 3, C≡CH), 2.56 (1 H, ddd, *J* 17, 8 and 3) and 2.72 (1 H, ddd, *J* 17, 6 and 3; together $CH_2C{\equiv}CH$), 3.09 (1 H, qn, *J* 7, $CHCH_2OSi$), 3.81 (1 H, dd, *J* 10 and 6) and 3.87 (1 H, dd, *J* 10 and 5; together CH_2OSi), 7.47 (1 H, t, *J* 8, 5-H), 7.59 (1 H, dt, *J* 8 and 1, 6-H), 8.10 (1 H, dt, *J* 8 and 1, 4-H) and 8.17 (1 H, t, *J* 1, 2-H); *m*/*z* 304 (M − CH_3), 289 (M − C_2H_6) and 262 (M − C_4H_9).

7-(tert-*Butyldimethylsiloxy*)-6-(3-*nitrophenyl*)*hept*-3-*yn*-1-*ol* **10**.—Butyllithium (1.4 mol dm^{-3} solution in hexane; 4.1 cm^3, 5.7 mmol) was added to a stirred solution of diisopropylamine (0.98 cm^3, 6.9 mmol) in dry THF (16 cm^3) under argon at −78 °C. The mixture was warmed to −10 °C for 10 min, then was recooled to −78 °C. A solution of compound **9** (1.38 g, 4.33 mmol) in THF (24 cm^3) was added slowly, *via* a cannula. After 30 min, ethylene oxide (~5 cm^3, large excess) was added. The cooling bath was removed after a further 30 min and the mixture was stirred overnight and was then quenched with a solution of sulfuric acid (0.6 g) in water (150 cm^3). The resulting mixture was extracted successively with diethyl ether (3 × 80 cm^3) and ethyl acetate (100 cm^3). The combined extracts were washed successively with saturated aq. ammonium chloride (100 cm^3) and brine (100 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The resulting oil was purified initially by elution through a plug of silica (CH_2Cl_2) and then by flash column chromatography on silica, and elution with light petroleum (60–80 °C)–ethyl acetate (3:2), to give the alcohol **10** as an oil (440 mg, 28%) [Found: (M − C_4H_8), 306.1170. $C_{15}H_{20}NO_4Si$ requires *m*/*z*, 306.1162]; λ_{max}/nm 265; ν_{max}/cm^{-1} 3550, 1510 and 1340; δ_H(400 MHz; $CDCl_3$) 0.01 (6 H, s, $OSiMe_2$), 0.87 (9 H, s, $OSiBu^t$), 2.39 (2 H, tt, *J* 6 and 2, $C{\equiv}CCH_2CH_2OH$), 2.58 (1 H, ddt, *J* 17, 8 and 2) and 2.71 (1 H, ddt, *J* 17, 6 and 2; together $CH_2C{\equiv}CCH_2CH_2$), 3.08 (1 H, m, $ArCHCH_2OSi$), 3.63 (2 H, t, *J* 6, CH_2OH), 3.86 (2 H, m, CH_2OSi), 7.50 (1 H, t, *J* 8, 5-H), 7.61 (1 H, d, *J* 8, 6-H), 8.13 (1 H, d, *J* 8, 4-H), and 8.17 (1 H, s, 2-H); *m*/*z* 348 (M − CH_3), 306 (M − C_4H_9) and 276 (M − C_4H_9 − C_2H_6).

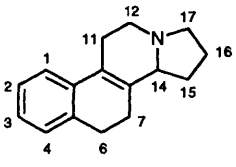
Purification of the less polar compounds by flash column chromatography, and elution with light petroleum (60–80 °C)–ethyl acetate (8:1), reclaimed some starting material **9** (416 mg). The yield of product **10** based on unrecovered starting material is therefore 40%.

N-[7-tert-*Butyldimethylsiloxy*)-6-(3-*nitrophenyl*)*hept*-3-*yn*-*yl*]*succinimide* **11**.—A solution of diethyl azodicarboxylate (DEAD) (160 mg, 0.920 mmol) in dry THF (5 cm^3) was added to a stirred, ice-cooled solution of the alcohol **10** (331 mg, 0.912 mmol), succinimide (119 mg, 1.20 mmol) and triphenylphosphine (241 mg, 0.922 mmol) in THF (5 cm^3). The ice-bath was removed after 15 min and the mixture was stirred at room temperature for 3 h, then was quenched with water (25 cm^3) and extracted with methylene dichloride (3 × 25 cm^3). The combined extracts were washed with brine (25 cm^3) and dried ($MgSO_4$). Evaporation under reduced pressure, followed by purification by PLC, with dichloromethane–1% methanol as developing solvent, yielded the *succinimide* **11** as an oil (368 mg, 91%) (Found: M^+, 444.2086. $C_{23}H_{32}N_2O_5Si$ requires M, 444.2081); λ_{max}/nm 212 and 247; ν_{max}/cm^{-1} 1690; δ_H(400 MHz; $CDCl_3$) −0.05 (6 H, s, $OSiMe_2$), 0.82 (9 H, s, $OSiBu^t$), 2.38 (2 H, t, *J* 7, $C{\equiv}CCH_2CH_2N$), 2.42–2.67 (2 H, m, $ArCHCH_2C{\equiv}C$), 2.65 [4 H, s, $N(COCH_2)_2$], 3.00 (1 H, m, $ArCHCH_2OSi$), 3.56 (2 H, t, *J* 7, CH_2N), 3.78 (2 H, m, CH_2OSi), 7.45 (1 H, t, *J* 8, 5-H), 7.56 (1 H, d, *J* 8, 6-H), 8.08 (1 H, d, *J* 8, 4-H) and 8.13 (1 H, s, 2-H); *m*/*z* 444 (M^+), 429 (M − CH_3) and 387 (M − C_4H_9).

N-[6-(3-*Aminophenyl*)-7-(tert-*butyldimethylsiloxy*)*hept*-3-*yn*-*yl*]*succinimide* **12**.—A stirred mixture of the nitro compound **11** (368 mg, 0.828 mmol), iron powder (730 mg, 13.0 mmol) and acetic acid (1.04 g, 17.3 mmol) in absolute ethanol (22 cm^3) was heated at reflux under argon for 2 h and was then quenched with

cold water (20 cm^3). The resultant mixture was extracted with methylene dichloride (3 × 25 cm^3), and the combined extracts were washed successively with water (2 × 25 cm^3) and brine (25 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by PLC, with methylene dichloride–4% methanol as solvent, to yield the *aniline* **12** as an oil (281 mg, 82%) (Found: M^+, 414.2340. $C_{23}H_{34}N_2O_3Si$ requires M, 414.2339); λ_{max}/nm 212 and 236; ν_{max}/cm^{-1} 3445, 3360, 1695 and 1605; δ_H(250 MHz; $CDCl_3$) −0.02 (6 H, s, $OSiMe_2$), 0.85 (9 H, s, $OSiBu^t$), 2.36–2.49 (3 H, m, $CHHC{\equiv}CCH_2CH_2N$), 2.54–2.77 (1 H, m, $ArCHCHHC{\equiv}C$), 2.62 [4 H, s, $N(COCH_2)_2$], 2.83 (1 H, m, $ArCHCH_2OSi$), 3.57 (2 H, t, *J* 7, CH_2N), 3.62–3.78 (2 H, m, CH_2OSi), 6.78–6.83 (3 H, m, 2-, 4- and 6-H) and 7.14 (1 H, dd, *J* 8 and 7, 5-H); *m/z* 414 (M^+), 399 (M − CH_3), 357 (M − C_4H_9) and 283 (M − $OSiC_6H_{15}$).

N-[6-(3-*Aminophenyl*)-7-(tert-*butyldimethylsiloxy*)*hept*-3-*ynyl*]-5-*ethoxypyrrolidin*-2-*one* **13**.—To a stirred solution of the succinimide **12** (279 mg, 0.673 mmol) in absolute ethanol (14 cm^3) at 5 °C was added sodium boranuide (sodium borohydride) (175 mg, 4.61 mmol). While the temperature was kept at 0–5 °C, hydrochloric acid (0.168 mol dm^{-3} solution in ethanol; 3 drops) was added at 15 min intervals for a period of 4 h. The excess of sodium boranuide was destroyed by addition of hydrochloric acid (2.0 mol dm^{-3} solution in ethanol) at 0–5 °C until the solution reached pH 2–3. After being stirred for a further 1 h at 0–5 °C, the mixture was poured into dil. aq. sodium hydrogen carbonate (50 cm^3) and extracted with methylene dichloride (3 × 50 cm^3). The combined extracts were washed with brine (50 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. Purification of the residual oil by flash column chromatography on silica, and elution with methylene dichloride–3% methanol, yielded the *ethoxy lactam* **13** as an oil (200 mg, 67%) (Found: M^+, 444.2823. $C_{25}H_{40}N_2O_3Si$ requires M, 444.2808); λ_{max}/nm 244 and 287; ν_{max}/cm^{-1} 2240 and 1670; δ_H(250 MHz; $CDCl_3$) 0.01 (6 H, s, $OSiMe_2$), 0.88 (9 H, s, $OSiBu^t$), 1.18 (3 H, t, *J* 7, OCH_2Me), 1.82–2.85 (11 H), 3.09–3.58 (4 H, m, CH_2N and OCH_2Me), 3.65–3.80 (2 H, m, CH_2OSi), 4.96 [1 H, m, $NCH(OEt)$], 6.52–6.77 (3 H, m, 2-, 4- and 6-H) and 7.06 (1 H, t, *J* 8, 5-H); *m/z* 444 (M^+), 387 (M − C_4H_9) and 341 (M − C_4H_9 − C_2H_5OH).



The numbering system used for the 13-azagonatetraene skeleton

1-*Amino*-6-(tert-*butyldimethylsiloxymethyl*)-13-*azagona*-1,3,5(10),8-*tetraen*-17-*one* **14**.—To a stirred solution of ethoxy lactam **13** (27 mg, 0.061 mmol) in dry methylene dichloride (2 cm^3) was added tin(IV) chloride (0.014 cm^3, 0.12 mmol). The reaction mixture was stirred at room temperature for 18 h, then was quenched with saturated aq. sodium hydrogen carbonate (5 cm^3) and extracted with methylene dichloride (3 × 5 cm^3). The combined extracts were washed with dil. aq. sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by PLC, with methylene dichloride–10% methanol as solvent, to yield the *azasteroid* **14** as an oil (7.1 mg, 29%), which was a mixture of two diastereoisomers (~3:1 from ^1H NMR spectroscopy). This mixture was further purified by HPLC on a semi-preparative silica column, with ethyl acetate–10% hexane–0.5% triethylamine as eluent (R_f 0.59), to yield the major, 6β-diastereoisomer (Found: M^+, 398.2392. $C_{23}H_{34}N_2O_2Si$ requires M, 398.2390); λ_{max}/nm 277; ν_{max}/cm^{-1} 3360 and 1655; δ_H(400 MHz; $CDCl_3$) 0.07 and 0.09 (each 3 H, s, $OSiMe_2$), 0.91 (9 H, s, $OSiBu^t$), 1.65 (1 H, m, 15-H_A), 1.95 (1 H, t, *J* 15, 7-H_{ax}), 2.10 (1 H, dd, *J* 15 and 5, 7-H_{eq}), 2.39 (1 H, m, 15-H_B), 2.41–2.57 (2 H, m, 16-H_2), 2.61–2.76 (2 H, m, 11-H_2), 2.79 (1 H, m, 6-H), 2.87 (1 H, td, *J* 12 and 3, 12-H_{ax}), 3.65 (2 H, br s, $ArNH_2$), 3.71 (1 H, dd, *J* 10 and 8) and 4.09 (1 H, dd, *J* 10 and 5; together 6-CH_2O), 4.26 (1 H, ddd, *J* 13, 5 and 2, 12-H_{eq}), 4.37 (1 H, t, *J* 8, 14-H), 6.57 (1 H, d, *J* 8, 2-H), 6.70 (1 H, d, *J* 8, 4-H) and 6.99 (1 H, t, *J* 8, 3-H); *m/z* 398 (M^+), 341 (M − C_4H_9) and 266 (M − $HOSiC_6H_{15}$).

Purification of the reaction mixture by PLC also yielded the following products as diastereoisomeric mixtures:

1-*Amino*-6-(*hydroxymethyl*)-13-*azagona*-1,3,5(10),8-*tetraen*-17-*one* **15** (R_f 0.39) as an oil (3.0 mg, 17%) (Found: M^+, 284.1533. $C_{17}H_{20}N_2O_2$ requires M, 284.1525); λ_{max}/nm 275; ν_{max}/cm^{-1} 3340 and 1655; δ_H(400 MHz; $CDCl_3$) (major isomer) 1.47–1.69 (1 H, m, 15-H_A), 1.50–1.68 (2 H, m), 2.05 (1 H, dd, *J* 15 and 5, 7-H_{eq}), 2.16 (1 H, t, *J* 15, 7-H_{ax}), 2.30–2.64 (4 H, m), 2.70–3.04 (2 H, m), 3.98–4.09 (2 H, m, 6-CH_2O), 4.27 (1 H, dd, *J* 12 and 4, 12-H_{eq}), 4.38 (1 H, t, *J* 8, 14-H), 6.56–6.64 (1 H, m, 2-H), 6.73 (1 H, d, *J* 8, 4-H) and 7.02 (1 H, t, *J* 8, 3-H); (minor isomer) the same except: 2.03–2.22 (2 H, m, 7-H_2), 3.55 (1 H, dd, *J* 10 and 8, 18-H_A), 3.63 (1 H, dd, *J* 10 and 6, 18-H_B), 4.14 (1 H, dd, *J* 14 and 6, 12-H_{eq}), 4.24 (1 H, t, *J* 8, 14-H), 6.56–6.64 (2 H, m, 2- and 4-H) and 6.97 (1 H, t, *J* 8, 3-H); *m/z* 284 (M^+) and 283 (M − H).

3-*Amino*-6-(tert-*butyldimethylsiloxymethyl*)-13-*azagona*-1,3,5(10),8-*tetraen*-17-*one* **16** (R_f 0.66) as an oil (2.4 mg, 10%) (Found: M^+, 398.2415. $C_{23}H_{34}N_2O_2Si$ requires M, 398.2390); δ_H(250 MHz; $CDCl_3$) (major isomer) −0.02 and 0.01 (each 3 H, s, $OSiMe_2$), 0.88 (9 H, s, $OSiBu^t$), 1.60–1.79 (1 H, m), 2.21–2.66 (7 H, m), 2.76–2.97 (2 H, m), 3.39–3.88 (2 H, m), 3.45 (1 H, dd, *J* 10 and 8, 6-CH_AO), 3.57 (1 H, dd, *J* 10 and 6, 6-CH_BO), 4.07–4.26 (1 H, m), 4.35 (1 H, dd, *J* 14 and 6, 12-H_{eq}), 6.48–6.64 (2 H, m, 2- and 4-H) and 7.03 (1 H, d, *J* 9, 1-H); (minor isomer) the same except: 3.39–3.88 (4 H, m, incl. 6-CH_2O), 4.07–4.26 (2 H, m, incl. 12-H_{eq}) and 6.96 (1 H, d, *J* 9, 1-H); *m/z* 398 (M^+), 341 (M − C_4H_9) and 266 (M − $HOSiC_6H_{15}$).

3-*Amino*-6-(*hydroxymethyl*)-13-*azagona*-1,3,5(10),8-*tetraen*-17-*one* **17** (R_f 0.33) as an oil (2.8 mg, 16%) (Found: M^+, 284.1524. $C_{17}H_{20}N_2O_2$ requires M, 284.1525); δ_H(400 MHz; $CDCl_3$) (major isomer) 1.51–1.67 (2 H, m), 2.15–2.58 (6 H, m), 2.82–2.93 (2 H, m), 3.48–3.82 (4 H, m), 4.16–4.23 (1 H, m, 14-H), 4.33 (1 H, dd, *J* 13 and 6, 12-H_{eq}), 6.48–6.59 (2 H, m, 2- and 4-H) and 6.95 (1 H, d, *J* 8, 1-H); (minor isomer) the same except: 7.03 (1 H, d, *J* 8, 1-H); *m/z* 284 (M^+) and 283 (M − H).

[1-*Amino*-13-*azagona*-1,3,5(10),8-*tetraen*-6-*yl*]*methanol* **18**.—To a stirred solution of the lactam **15** (7.5 mg, 0.026 mmol) in dry THF (3 cm^3) was added Red-Al (0.6 mol dm^{-3} solution in toluene; 0.22 cm^3, 0.132 mmol). The resultant mixture was stirred at room temperature for 90 min, then was quenched with dil. aq. sodium carbonate and extracted with ethyl acetate (3 × 8 cm^3). The combined extracts were evaporated under reduced pressure, and the residue was purified by PLC, with methylene dichloride–20% methanol–3% triethylamine as solvent, to yield the amine **18** as an oil (5.5 mg, 77%), which was a mixture of diastereoisomers (~2:1 from ^1H NMR spectroscopy); λ_{max}/nm 267 and 318; ν_{max}/cm^{-1} 3685, 3600 and 1605; δ_H(400 MHz; $CDCl_3$) (major isomer) 1.68–2.25 (5 H, m), 2.32–2.43 (1 H, m), 2.59–3.02 (5 H, m), 3.13–3.29 (2 H, m), 3.49–3.80 (5 H, m), 6.56 (1 H, d, *J* 8, 2-H), 6.62 (1 H, d, *J* 8, 4-H) and 6.96 (1 H, t, *J* 8, 3-H); (minor isomer) the same except: 1.46–1.58 (2 H, m), 1.68–2.25 (3 H, m) and 6.95 (1 H, t, *J* 8, 3-H); *m/z* (FD) 270 (M^+); (FAB) 271 (MH^+).

[1-*Amino*-13-*azagona*-1,3,5(10),8-*tetraen*-6-*yl*]*methyl Hydrogen Phenylphosphonate* **19**.—The amine **18** (1 mg, 3.7 μmol) was

dissolved in dry pyridine and the solution was then evaporated; this process was carried out three times and the residue was again dissolved in pyridine (0.4 cm^3). Triazole (2 mg, 29 μmol) was dissolved in pyridine and the pyridine was evaporated off; this process was carried out twice, and the residue was again dissolved in pyridine (0.4 cm^3). To the triazole solution was added phenylphosphonic dichloride (1 mm^3, 7.5 μmol) and the resultant mixture was stirred for 30 min and was then added to the solution of the alcohol **18**. The resulting mixture was stirred for 20 min and then triethylamine (3 mm^3) and water (2 mm^3) were added. The mixture was stirred for 10 min and then was evaporated to dryness under reduced pressure. Purification by PLC, with methylene dichloride–40% methanol–5% triethylamine as solvent, gave the phosphonate **19** (~0.75 mg, 50%); δ_H(400 MHz; CD_3OD) 1.52–3.75 (16 H, m), 6.50 and 6.61 (each 1 H, dd, *J* 8 and 1, 2- and 4-H), 6.85 (1 H, t, *J* 8, 3-H) and 7.30–7.39 and 7.67–7.73 (together 5 H, m, $PhPO_3$); *m/z* (FAB) 411 (MH^+) and 433 (MNa^+).

6-(tert-*Butyldimethylsiloxymethyl*)-13-*azagona*-1,3,5(10),8-*tetraen*-3-*amine* **20**.—To a stirred solution of the lactam **16** (5 mg, 0.013 mmol) in dry THF (2 cm^3) was added lithium aluminium hydride (2 mg, 0.05 mmol). The resultant mixture was stirred at room temperature for 2 h, then was quenched with water followed by saturated aq. sodium hydrogen carbonate and extracted with ethyl acetate (3 × 7 cm^3). The combined extracts were evaporated under reduced pressure and the residue was purified by PLC, with methylene dichloride–10% methanol–1% triethylamine as solvent, to yield the amine **20** as an oil (3.9 mg, 81%), as a mixture of diastereoisomers (~3:1 from ^1H NMR spectroscopy); δ_H(250 MHz; $CDCl_3$) (major isomer) −0.04 to −0.02 (6 H, m, $OSiMe_2$), 0.88 (9 H, s, $OSiBu^t$), 1.48–1.97 (3 H, m), 2.02–2.11 (1 H, m), 2.23–2.41 (3 H, m), 2.46–2.57 (1 H, m), 2.60–2.85 (3 H, m), 2.90–3.00 (2 H, m), 3.24 (1 H, t, *J* 8, 14-H), 3.45–3.52 (2 H, m, 6-CH_2O), 3.60 (2 H, br s, $ArNH_2$), 6.48–6.55 (2 H, m, 2- and 4-H) and 7.01 (1 H, d, *J* 8, 1-H); (minor isomer) the same except: 2.15–2.21 (1 H, m), 2.23–2.41 (2 H, m), 2.90–3.00 (1 H, m), 3.10–3.17 (1 H, m), 3.29 (1 H, t, *J* 8, 14-H) and 6.98 (1 H, d, *J* 8, 1-H).

Pentafluorophenyl 3-(2-*Pyridyldisulfanyl*)*propanoate* **22**.—To a stirred solution of 3-(2-pyridyldisulfanyl)propanoic acid[8] (320 mg, 1.49 mmol) and pentafluorophenol (300 mg, 1.63 mmol) in dry methylene dichloride (10 cm^3) was added DCC (337 mg, 1.63 mmol). The reaction mixture was stirred for 17 h at room temperature, then the precipitated dicyclohexylurea was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by PLC, with methylene dichloride–3% methanol as solvent, to yield the pentafluorophenyl ester **22** as an oil (363 mg, 64%); δ_H(400 MHz; $CDCl_3$) 3.14 (4 H, s, $SCH_2CH_2CO_2H$), 7.12 (1 H, ddd, *J* 7, 5 and 2, 5-H), 7.62–7.68 (2 H, m, 3- and 4-H) and 8.49 (1 H, dd, *J* 5 and 1, 6-H).

Methyl 2-*Phenylpent*-4-*ynoate* **28**.—To a stirred solution of diisopropylamine (26.3 cm^3, 0.188 mol) in dry THF (600 cm^3) at −78 °C was added butyllithium (1.4 mol dm^{-3} solution in hexane; 118 cm^3, 0.165 mol). The resultant mixture was allowed to warm to 0 °C for 5 min, then was recooled to −78 °C. A solution of methyl phenylacetate **27** (21.5 cm^3, 0.150 mol) in THF (150 cm^3) was added slowly, such that the temperature of the reaction mixture did not rise above −50 °C. After a further 30 min, freshly distilled prop-2-ynyl bromide (14.0 cm^3, 0.157 mol) was added, and the reaction mixture was allowed to warm to −20 °C for 15 min before being quenched with a solution of sulfuric acid (9.2 g) in water (600 cm^3). The THF layer was removed, and the aqueous phase was extracted with methylene dichloride (2 × 600 cm^3). The combined extracts were washed successively with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by flash column chromatography on silica, with light petroleum (60–80 °C)–diethyl ether (6:1) as eluent, gave the ester[17] **28** as an oil (18.9 g, 67%) (Found: M^+, 188.0840. Calc. for $C_{12}H_{12}O_2$: M, 188.0837); λ_{max}/nm 206; ν_{max}/cm^{-1} 3300, 2120 and 1725; δ_H(400 MHz; $CDCl_3$) 1.95 (1 H, t, *J* 3, C≡CH), 2.63 (1 H, ddd, *J* 17, 7 and 3) and 2.93 (1 H, ddd, *J* 17, 8 and 3; together $CH_2C{\equiv}C$), 3.69 (3 H, s, OMe), 3.81 (1 H, dd, *J* 8 and 7, $CHCO_2$) and 7.27–7.37 (5 H, m, Ph); *m/z* 188 (M^+), 173 (M − CH_3), 149 (M − C_3H_3) and 129 (M − $C_2H_3O_2$).

2-*Phenylpent*-4-*yn*-1-*ol* **29**.—A solution of the ester **28** (9.40 g, 50 mmol) in dry THF (100 cm^3) was added to a stirred suspension of lithium aluminium hydride (2.85 g, 75 mmol) in THF (200 cm^3) at 0 °C. The reaction mixture was stirred for 30 min and then was quenched successively with wet diethyl ether, water, and a solution of sulfuric acid (15 g) in water (400 cm^3). This mixture was extracted with methylene dichloride (3 × 300 cm^3) and the combined organic extracts were washed successively with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica, with light petroleum (60–80 °C)–diethyl ether (1:1) as eluent, to yield the *alcohol* **29** as an oil (6.11 g, 76%) (Found: M^+, 160.0897. $C_{11}H_{12}O$ requires M, 160.0888); λ_{max}/nm 213 and 257; ν_{max}/cm^{-1} 3580, 3290 and 2110; δ_H(250 MHz; CD_2Cl_2) 2.03 (1 H, t, *J* 3, C≡CH), 2.55 (1 H, ddd, *J* 17, 8 and 3) and 2.67 (1 H, ddd, *J* 17, 7 and 3; together $CH_2C{\equiv}C$), 3.02 (1 H, qn, *J* 7, PhC*H*), 3.81 and 3.85 (each 1 H, dd, *J* 11 and 6; together CH_2OH) and 7.24–7.41 (5 H, m, Ph); *m/z* 160 (M^+), 142 (M − H_2O) and 129 (M − CH_3O).

5-(tert-*Butyldimethylsiloxy*)-4-*phenylpent*-1-*yne* **30**.—The alcohol **29** (11.31 g, 70.7 mmol) was stirred in dry DMF (75 cm^3) with TBDMSCl (15.83 g, 105 mmol) and imidazole (11.99 g, 176 mmol) for 20 min at room temperature. The reaction mixture was poured into a mixture of water (200 cm^3) and ethyl acetate (200 cm^3) and the aqueous layer was extracted further with ethyl acetate (2 × 200 cm^3). The combined extracts were washed successively with water and saturated aq. ammonium chloride, dried ($MgSO_4$), and concentrated under reduced pressure. Flash chromatography on a short silica column, and elution with light petroleum (60–80 °C)–diethyl ether (1:1), yielded the silyl ether **30** as an oil (19.38 g, 100%) [Found: (M^+ − C_4H_9), 217.1045. $C_{13}H_{17}OSi$ requires *m/z*, 217.1049]; λ_{max}/nm 214 and 257; ν_{max}/cm^{-1} 3290, 2120 and 1595; δ_H(400 MHz; $CDCl_3$) 0.01 (6 H, s, $OSiMe_2$), 0.88 (9 H, s, $OSiBu^t$), 1.92 (1 H, t, *J* 3, C≡CH), 2.53 (1 H, ddd, *J* 17, 8 and 3) and 2.74 (1 H, ddd, *J* 17, 6 and 3; together $CH_2C{\equiv}C$), 2.98 (1 H, qn, *J* 7, PhC*H*), 3.78 (1 H, dd, *J* 10 and 7) and 3.83 (1 H, dd, *J* 10 and 5; together CH_2OSi) and 7.21–7.33 (5 H, m, Ph); *m/z* 217 (M − C_4H_9).

7-(tert-*Butyldimethylsiloxy*)-6-*phenylhept*-3-*yn*-1-*ol* **31**.—To a stirred solution of the silyl ether **30** (13.37 g, 48.8 mmol) in dry THF (400 cm^3) at −78 °C was added butyllithium (1.4 mol dm^{-3} solution in hexane; 37 cm^3, 51.8 mmol). The resultant mixture was stirred for 1 h at 5 °C. Ethylene oxide (100 cm^3, 2.5 mol), dried over anhydrous $CaSO_4$, was then added *via* a cannula. The reaction mixture was stirred at 5–10 °C for 7 h and then at room temperature for 18 h. Argon was bubbled through the solution for 1 h to ensure the removal of unchanged ethylene oxide and a solution of sulfuric acid (3.0 g) in water (400 cm^3) was then added to quench the reaction. The THF layer was removed and the aqueous phase extracted further with ethyl acetate (2 × 200 cm^3). The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. Partial purification by flash column chromatography

on silica, with light petroleum (60–80 °C)–diethyl ether (2:1) as eluent, gave the starting silyl ether **30** (2.31 g recovery) and impure alcohol **31**. A second column, eluted with methylene dichloride–0.5% methanol, yielded the pure alcohol **31** as an oil (5.36 g, 42% based on unrecovered starting material) [Found: ($M^+ - C_4H_9$), 261.1310. $C_{15}H_{21}O_2Si$ requires *m/z*, 261.1311]; λ_{max}/nm 210; ν_{max}/cm^{-1} 3560 and 1600; δ_H(250 MHz; $CDCl_3$) −0.02 (6 H, s, $OSiMe_2$), 0.86 (9 H, s, $OSiBu^t$), 2.32 (2 H, tt, *J* 6 and 2, CH_2CH_2OH), 2.49 (1 H, ddt, *J* 17, 8 and 2) and 2.69 (1 H, ddt, *J* 17, 6 and 2; together $PhCHCH_2$), 2.94 (1 H, qn, *J* 7, PhC*H*), 3.53 (2 H, t, *J* 6, CH_2OH), 3.74 (1 H, dd, *J* 10 and 7) and 3.80 (1 H, dd, *J* 10 and 5; together CH_2OSi) and 7.19–7.34 (5 H, m, Ph); *m/z* 261 ($M - C_4H_9$) and 243 ($M - C_4H_9 - H_2O$).

N-[7-(tert-*Butyldimethylsiloxy*)-6-*phenylhept*-3-*ynyl*]*succinimide* **32**.—To a stirred solution of the alcohol **31** (2.04 g, 6.42 mmol), succinimide (0.83 g, 8.34 mmol) and triphenylphosphine (1.77 g, 6.74 mmol) in dry THF (35 cm^3) at 0 °C was added a solution of DEAD (1.06 cm^3, 6.74 mmol) in THF (35 cm^3). The reaction mixture was stirred at room temperature for 2.5 h, then was quenched with water (200 cm^3) and extracted with methylene dichloride (3 × 100 cm^3). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. Flash column chromatography on silica, and elution with methylene dichloride–0.5% methanol, yielded the succinimide **32** as a waxy crystalline solid, m.p. 55–56 °C (2.42 g, 95%) [Found: ($M^+ - CH_3$), 384.2022. $C_{22}H_{30}NO_3Si$ requires *m/z*, 384.1995]; λ_{max}/nm 263; ν_{max}/cm^{-1} 1775 and 1700; δ_H(400 MHz; $CDCl_3$) −0.05 (6 H, s, $OSiMe_2$), 0.84 (9 H, s, $OSiBu^t$), 2.39–2.46 (3 H, m, $CHC{\equiv}CCH_2CH_2N$), 2.59 [4 H, s, $N(COCH_2)_2$], 2.59–2.63 (1 H, m, $CHC{\equiv}CCH_2CH_2N$), 2.88 (1 H, qn, *J* 7, PhC*H*), 3.57 (2 H, t, *J* 7, CH_2N), 3.71 (1 H, dd, *J* 10 and 7) and 3.76 (1 H, dd, *J* 10 and 5; together CH_2OSi) and 7.19–7.30 (5 H, m, Ph); *m/z* 384 ($M - CH_3$), 342 ($M - C_4H_9$) and 268 ($M - OSiC_6H_{15}$).

N-[7-(tert-*Butyldimethylsiloxy*)-6-*phenylhept*-3-*ynyl*]-5-*ethoxypyrrolidin*-2-*one* **33**.—To a stirred solution of the succinimide **32** (2.95 g, 7.39 mmol) in absolute ethanol (145 cm^3) at 5 °C was added sodium boranuide (sodium borohydride) (1.90 g, 50.2 mmol). The reaction mixture was stirred at 0–5 °C for 4 h, during which time hydrochloric acid in ethanol (1.90 mol dm^{-3}; 3 drops) was added at 15 min intervals, then the mixture was acidified to pH 3 by the addition of hydrochloric acid in ethanol, stirred for 15 min, then was poured into dil. aq. sodium hydrogen carbonate and extracted with methylene dichloride (3 × 200 cm^3). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash column chromatography on silica, and elution with a gradient of methylene dichloride–1 to 3% methanol, gave the *ethoxy lactam* **33** as an oil (2.68 g, 85%) (Found: M^+, 429.2677. $C_{25}H_{39}NO_3Si$ requires *M*, 429.2699); λ_{max}/nm 257; ν_{max}/ cm^{-1} 1675; δ_H(400 MHz; $CDCl_3$) −0.05 (6 H, s, $OSiMe_2$), 0.84 (9 H, s, $OSiBu^t$), 1.18 (3 H, m, OCH_2Me), 1.83–1.91, 1.94–2.05, 2.18–2.49 and 2.62–2.70 (8 H, m, $CH_2C{\equiv}CCH_2CH_2NCOCH_2CH_2$), 2.85–2.93 (1 H, m, PhC*H*), 3.13 (1 H, ddd, *J* 13, 7 and 6, CH*H*N), 3.38 (2 H, m, OCH_2Me), 3.53 (1 H, dt, *J* 13 and 7, C*H*HN), 3.71 (1 H, dd, *J* 10 and 7) and 3.76 (1 H, dd, *J* 10 and 5; together CH_2OSi), 4.96 (1 H, m, NC*H*OEt) and 7.18–7.30 (5 H, m, Ph); *m/z* 429 (M^+), 428 ($M - H$), 414 ($M - CH_3$) and 372 ($M - C_4H_9$).

[17-*Oxo*-13-*azagona*-1,3,5(10),8-*tetraen*-6-*yl*]*methylFormate* **34**.—A solution of ethoxy lactam **33** (1.19 g, 2.77 mmol) in formic acid (60 cm^3) was stirred at room temperature for 22 h and was then concentrated under reduced pressure. A solution of the residue in methylene dichloride (150 cm^3) was washed with dil. aq. sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure. Flash column chromatography on silica, and elution with methylene dichloride–3% methanol, yielded the *formate ester* **34** as a foam (720 mg, 87%), which was a mixture of two diastereoisomers (~3:2 from ^1H NMR spectroscopy) (Found: M^+, 297.1366. $C_{18}H_{19}NO_3$ requires M, 297.1365); λ_{max}/nm 262; ν_{max}/cm^{-1} 2910, 1715 and 1670; δ_H(400 MHz; $CDCl_3$) (major isomer) 1.51–1.65 (1 H, m, 15-H_A), 2.15–2.67, 2.89–2.99 and 3.15–3.25 (9 H, m), 4.07–4.44 (4 H, m, 12-H_{eq}, 14-H and 6-CH_2O), 7.17–7.34 (4 H, m, ArH) and 8.10 (1 H, s, HCO_2); (minor isomer) the same except: 8.08 (1 H, s, HCO_2CH_2); *m/z* 297 (M^+) and 251 ($M - HCO_2H$).

[13-*Azagona*-1,3,5(10),8-*tetraen*-6-*yl*]*methanol* **35**.—To a stirred solution of the formate ester **34** (700 mg, 2.36 mmol) in dry THF (50 cm^3) at 0 °C was added Red-Al (1.0 mol dm^{-3} solution in toluene; 18.9 cm^3, 18.9 mmol). The ice-bath was removed after 20 min and the mixture was stirred for 1 h at room temperature before being quenched with dil. aq. sodium hydrogen carbonate (200 cm^3) and extracted with ethyl acetate (3 × 100 cm^3). The combined extracts were evaporated under reduced pressure. Flash column chromatography on silica, and elution with methylene dichloride–5% methanol–3% triethylamine, yielded both diastereoisomers of the amine **35**.

Isomer 1 (R_f 0.18 with the above eluent) (210 mg, 35%) was recrystallised from methylene dichloride–hexane, m.p. 130–133 °C (Found: M^+, 255.1615. $C_{17}H_{21}NO$ requires *M*, 255.1623); λ_{max}/nm 264; ν_{max}/cm^{-1} 3360; δ_H(400 MHz; $CDCl_3$) 1.52–1.62 (1 H, m, 15-H_A), 1.76–1.89 (2 H, m, 16-H_2), 2.07–2.16, 2.17–2.28, 2.43–2.52 and 2.60–2.71 (together 5 H, m, 7- and 11-H_2 and 15-H_B), 2.79–2.98 and 3.05–3.13 (together 5 H, m, 6-H and 12- and 17-H_2), 3.45–3.56 (3 H, m, 14-H and 6-CH_2O) and 7.10–7.25 (4 H, m, ArH); *m/z* 255 (M^+), 254 ($M - H$) and 224 ($M - CH_2OH$).

Isomer 2 (R_f 0.26 with the above eluent) (284 mg, 47%) was an oil (Found: M^+, 255.1600); λ_{max}/nm 217 and 268; ν_{max}/cm^{-1} 3320; δ_H(400 MHz; $CDCl_3$–trifluoroacetic acid) 1.78–1.89 (1 H, m, 15-H_A), 1.95–2.04 (2 H, m, 16-H_2), 2.30–2.54 (4 H, m, 7-H_2, 11-H_A and 15-H_B), 2.76–2.86 (1 H, m, 11-H_B), 2.89–2.97 (1 H, m, 6-H), 3.00–3.09, 3.19–3.26 and 3.41–3.50 (together 4 H, m, 12- and 17-H_2), 3.52 (1 H, dd, *J* 11 and 8) and 3.60 (1 H, dd, *J* 11 and 6, 6-CH_2O), 3.87 (1 H, t, *J* 8, 14-H) and 7.12–7.25 (4 H, m, ArH); *m/z* 255 (M^+), 254 ($M - H$) and 224 ($M - CH_2OH$).

3-(2,4-*Dinitrophenylsulfanyl*)*propanoic Acid* **36**.—The reaction of 3-sulfanylpropanoic acid with 2,4-dinitrofluorobenzene was carried out in a similar manner to the published procedure[18] and afforded the acid **36** as pale yellow needles, m.p. 155–157 °C [from ethanol–light petroleum (60–80 °C); lit.,[18] 160 °C (from water)] (Found: M^+, 272.0101. Calc. for $C_9H_8N_2O_6S$: *M*, 272.0103); λ_{max}/nm 329; ν_{max}/cm^{-1} 3200–2800br, 1725, 1600 and 1350; δ_H(400 MHz; CD_3OD) 2.77 (2 H, t, *J* 7, CH_2CO_2), 3.39 (2 H, t, *J* 7, SCH_2), 7.84 (1 H, d, *J* 9, 6-H), 8.46 (1 H, dd, *J* 9 and 2, 5-H) and 9.00 (1 H, d, *J* 2, 3-H); *m/z* 272 (M^+).

Pentafluorophenyl 3-(2,4-*dinitrophenylsulfanyl*)*propanoate* **37**.—The acid **36** (390 mg, 1.43 mmol), pentafluorophenol (290 mg, 2.58 mmol) and DCC (325 mg, 1.58 mmol) were stirred in a mixture of dry methylene dichloride (40 cm^3) and dry DMF (10 cm^3) at room temperature for 2 h. The mixture was then evaporated to dryness, the residue was dissolved in diethyl ether, and the solution was filtered to remove the dicyclohexylurea. The diethyl ether was evaporated off under reduced pressure and the residue was purified by flash chromatography on a short silica column, and eluted with methylene dichloride, to yield the pentafluorophenyl ester **37** as a pale yellow solid (561 mg, 89%); λ_{max}/nm 269 and 326; ν_{max}/cm^{-1} 1780, 1580,

1510 and 1340; δ_H(250 MHz; CD_2Cl_2) 3.20 (2 H, t, *J* 7, CH_2CO_2), 3.49 (2 H, t, *J* 7, SCH_2), 7.65 (1 H, d, *J* 9, 6-H), 8.44 (1 H, dd, *J* 9 and 2, 5-H) and 9.08 (1 H, d, *J* 2, 3-H); *m/z* (FD) 438 (M^+).

N-(4-*Hydroxyphenyl*)-3-(2,4-*dinitrophenylsulfanyl*)*propanamide* **38**.—A solution of pentafluorophenyl ester **37** (111 mg, 0.253 mmol) in pyridine (2 cm^3) was added to a stirred solution of 4-aminophenol (27.6 mg, 0.253 mmol) in dry pyridine (2 cm^3). The resultant mixture was evaporated to dryness after 90 min and the residue was washed thoroughly with methylene dichloride (3 × 8 cm^3). The residual yellow solid (83 mg, 90%) was recrystallised from methanol–light petroleum (60–80 °C) to give the *phenol* **38** as yellow rhombic crystals, m.p. 228–230 °C (Found: M^+, 363.0554. $C_{15}H_{13}N_3O_6S$ requires M, 363.0525); λ_{max}/nm 250 and 329; ν_{max}(Nujol)/cm^{-1} 3600–3200, 1650, 1585, 1540 and 1500; δ_H(400 MHz; CD_3COCD_3) 2.85 (2 H, t, *J* 7, SCH_2CH_2), 3.54 (2 H, t, *J* 7, SCH_2CH_2), 6.76 (2 H, d, *J* 9, 3′- and 5′-H), 7.45 (2 H, d, *J* 9, 2′- and 6′-H), 8.04 (1 H, d, *J* 9, 6-H), 8.17* (1 H, s, OH), 8.50 (1 H, dd, *J* 9 and 2, 5-H), 8.97 (1 H, d, *J* 2, 3-H) and 9.12* (1 H, br s, CONH) (* exchanges with D_2O); *m/z* 363 (M^+).

[13-*Azagona*-1,3,5(10),8-*tetraen*-6-*yl*]*methyl* 4-[3-(2,4-*Dinitrophenylsulfanyl*)*propanamido*]*phenyl Hydrogen Phosphate* **40**.—1-Hydroxybenzotriazole (438 mg, 3.24 mmol) was dried by dissolution in toluene–ethanol and evaporation to dryness (the process carried out three times) and was then dissolved in a mixture of dry pyridine (0.26 cm^3, 3.24 mmol) and dry 1,4-dioxane (6.5 cm^3). A solution of 2,5-dichlorophenyl dichlorophosphate (453 mg, 1.62 mmol) in 1,4-dioxane (1.3 cm^3) was added and the resultant mixture was stirred at room temperature for 1 h and was then filtered anhydrously. This solution of bis(benzotriazol-1-yl) 2,5-dichlorophenyl phosphate (0.2 mol dm^{-3} in 1,4-dioxane) was stored under argon at −20 °C until used.

The solution of bis(benzotriazo-1-yl) 2,5-dichlorophenyl phosphate (1.1 cm^3, 0.22 mmol) was added to the phenol **38** (70 mg, 0.193 mmol), followed by dry pyridine (0.025 cm^3, 0.31 mmol). The resultant solution was stirred for 40 min, then a solution of alcohol **35** isomer 1 (64 mg, 0.25 mmol) was added in pyridine (1.0 cm^3). The reaction mixture was stirred for a further 1 h, then was applied directly to a silica column and purified by flash column chromatography, with methylene dichloride–4% methanol–3% triethylamine as eluent. Product-containing fractions were combined, and evaporated under reduced pressure, with addition and re-evaporation of toluene three times, to give a crude sample of the 2,5-dichlorophenyl phosphate ester **39** isomer 1 (122 mg).

A solution of this crude product in dry 1,4-dioxane–acetonitrile (1:1; 5 cm^3), with pyridine-2-aldoxime (221 mg, 1.81 mmol) and 1,1,3,3-tetramethylguanidine (0.198 cm^3, 1.58 mmol) added, was stirred at room temperature for 20 h and was then concentrated to a volume of ~3 cm^3 under reduced pressure. This residue was purified by flash column chromatography on silica, and eluted with a gradient of methylene dichloride–12 to 15% methanol–5% triethylamine. Product-containing fractions were combined, and concentrated under reduced pressure, with addition and re-evaporation of toluene three times, to give the *phosphate diester* **40** isomer 1 as a yellow gum (54 mg) (Found: MH^+, 681.1784. $C_{32}H_{34}N_4O_9PS$ requires *m/z*, 681.1784); λ_{max}/nm 250 and 325; δ_H(400 MHz; CD_3OD) 1.84–1.93 (1 H, m, 15-H_A), 2.03–2.13 (2 H, m, 16-H_2), 2.27–2.58 and 2.87–2.96 (5 H, m, 7- and 11-H_2 and 15-H_B), 2.85 (2 H, t, *J* 7, SCH_2CH_2), 2.98–3.60 (7 H, m, 6-H, 12- and 17-H_2, and SCH_2CH_2), 3.74–3.86 (2 H, m, 6-CH_2O), 3.93 (1 H, t, *J* 8, 14-H), 7.12 and 7.45 (each 2 H, d, *J* 9, OC_6H_4N), 7.15–7.30 (4 H, m, 1-, 2-, 3- and 4-H), 7.91 (1 H, d, *J* 9, 6″-H), 8.45 (1 H, dd, *J* 9 and 2, 5″-H) and 8.97 (1 H, d, *J* 2, 3″-H); δ_C(100 MHz; CD_3OD) (all s except where J_{CP} is indicated) 21.8, 22.6, 27.6, 28.6, 29.1, 35.6, 39.5 (d, *J* 8), 46.7, 54.0, 63.6, 67.9 (d, *J* 7), 121.5 (d, *J* 4), 122.4, 122.6, 124.0, 126.1, 126.8, 128.4, 128.7, 129.1, 129.2, 129.6, 134.2, 135.0, 135.9, 145.6, 146.6, 146.9, 150.8 (d, *J* 5) and 171.2; *m/z* (FAB) 681 (weak, MH^+).

An analogous procedure, utilising alcohol **35** isomer 2, yielded the *phosphate diester* **40** isomer 2 as a yellow powder, m.p. 193–195 °C (Found: MH^+, 681.1784); λ_{max}/nm 249 and 328; δ_H(400 MHz; CD_3OD) 1.70–1.80 (1 H, m, 15-H_A), 1.84–2.05 (2 H, m, 16-H_2), 2.17–2.52 (4 H, m, 7-H_2, 11-H_A and 15-H_B), 2.65–2.75 (1 H, m, 11-H_B), 2.82 (2 H, t, *J* 7, SCH_2CH_2), 2.98–3.12 and 3.19–3.28 (together 5 H, m, 6-H and 12- and 17-H_2), 3.48 (2 H, t, *J* 7, SCH_2CH_2), 3.61 (1 H, t, *J* 8, 14-H), 3.77 and 3.87 (each 1 H, dt, *J* 10 and 6, 6-CH_2O), 7.08 and 7.43 (each 2 H, d, *J* 9, OC_6H_4N), 7.10–7.24 (4 H, m, 1-, 2-, 3- and 4-H), 7.89 (1 H, d, *J* 9, 6″-H), 8.44 (1 H, dd, *J* 9 and 2, 5″-H) and 8.97 (1 H, d, *J* 2, 3″-H); δ_C(100 MHz; CD_3OD) 21.5, 22.9, 28.1, 29.1, 30.1, 35.7, 39.5 (d, *J* 8), 47.1, 55.0, 64.0, 67.8 (d, *J* 5), 121.4 (d, *J* 5), 122.2, 122.3, 123.5, 125.7, 126.3, 128.4, 129.1, 129.2, 129.4, 130.0, 135.0, 135.3, 135.7, 145.5, 146.6, 146.9, 150.9 (d, *J* 7) and 170.9; *m/z* (FAB) 681 (weak, MH^+).

Procedure for Protein-conjugation Reactions.—Tuberculin purified protein derivative (PPD) was dissolved in sodium phosphate buffer (0.1 mol dm^{-3}; pH 7.5, 1.0 cm^3) containing sodium chloride (0.1 mol dm^{-3}), and the mixture was centrifuged. The supernatant was chromatographed on a Sephadex G-25 gel filtration column, with the same buffer as eluent, to remove any low-molecular mass material. The protein fraction was adjusted to a volume of 2.2 cm^3 with the same buffer and a mixture of SPDP (2.5 mg, 8 μmol) in absolute ethanol (0.3 cm^3) was added dropwise. The reaction mixture was stood at room temperature for 2 h and excess of reagent was then removed by gel filtration on Sephadex G-25, with the same buffer as eluent. The protein concentration could be verified from the absorbance of PPD at 260 nm, which is 2.6 for a solution of concentration 1 mg cm^{-3}.[19] The modified protein was stored at 4 °C. The content of bis-2-pyridyl disulfide units in the modified PPD was determined by reducing an aliquot of the derivatised protein with DTT and measuring the absorbance change at 343 nm, which corresponds to release of pyridine-2-thione[8] (ε_{343} = 8080 dm^3 mol^{-1} cm^{-1}).[20]

The 2,4-dinitrophenyl-protected transition state analogue **40** (1.4 μmol) was dissolved in DMF (0.2 cm^3) under argon in a Schlenk tube. To this solution were added sodium phosphate buffer (0.1 mol dm^{-3}; pH 9.5; 1.0 cm^3) and ethane-1,2-dithiol (6 mm^3, 70 μmol). The reaction mixture was stirred vigorously at room temperature for 18 h and then sodium phosphate buffer (1.0 mol dm^{-3}; pH 7.5; 0.3 cm^3) was added. The ethane-1,2-dithiol was extracted with diethyl ether (3 × 1 cm^3) and residual ether was removed by a stream of argon. The resultant solution was adjusted to a volume of 3.0 cm^3 with sodium phosphate buffer (0.1 mol dm^{-3}; pH 7.5) containing sodium chloride (0.1 mol dm^{-3}) and the mixture was transferred to a sealed UV cell under argon. To this was added the PPD–SPDP derivative (containing 1.5 μmol of 2-pyridyl disulfide units) and the conjugation reaction was monitored by following the release of pyridine-2-thione at 343 nm.

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