1 PERKIN

Regiocontrolled synthesis of the macrocyclic polyamine alkaloid (±)-lunarine, a time-dependent inhibitor of trypanothione reductase

Chris J. Hamilton,[a] Alan H. Fairlamb[b] and Ian M. Eggleston*[a]

[a] *Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, Carnelley Building, University of Dundee, Dundee, UK DD1 4HN*
[b] *Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, Wellcome Trust Biocentre, University of Dundee, Dundee, UK DD1 5EH*

Received (in Cambridge, UK) 9th November 2001, Accepted 26th February 2002
First published as an Advance Article on the web 3rd April 2002

A regiocontrolled synthesis of the macrocyclic polyamine alkaloid (±)-lunarine is described. The key steps involve the preparation of the differentially functionalised *cis*-3-oxo-8-bromo-9b-cyano-1,2,3,4,4a,9b-hexahydrobenzofuranyl tricyclic scaffold **14** which, following further elaboration, is coupled to the selectively protected acrylamidospermidine† **5** *via* a Heck coupling reaction to give the pre-cyclised lunarine derivative **23**.

Introduction

The parasitic protozoa *Trypanosoma* and *Leishmania* cause a number of severely infectious diseases in man and domestic livestock in tropical countries. These include the often-fatal South American Chagas' disease and African sleeping sickness (caused by *Trypanosoma cruzi* and *Trypanosoma brucei* respectively), and kala-azar, oriental sore and espundia (caused by *Leishmania* spp.). The parasites are transmitted by blood-sucking insects and currently there are no vaccines against the resulting diseases, while existing drug treatments are either ineffective or rather toxic.

A potential means of controlling trypanosomes and leishmania has become apparent through the identification of a unique feature in their metabolism. They both possess the metabolite trypanothione (N^1,N^8-bis(glutathionyl)spermidine), which performs many of the protective and anti-oxidant roles which are associated with glutathione in mammalian cells (Fig. 1a).[1] Furthermore, the parasites do not contain glutathione reductase but instead use the analogous enzyme trypanothione reductase (TryR) to maintain trypanothione in the reduced form which plays a major role in the defence against reactive oxygen species generated by host cells. Disabling the function of TryR in *Leishmania*[2] and *T. brucei*[3] has been shown to markedly increase the parasites' sensitivity to oxidative stress.

For some time a variety of molecules have been known to be inhibitors of TryR, most notably polyamine derivatives[4a–c] such as the naturally occurring bis(tetrahydrocinnamoyl)spermine, kukoamine A.[4a] More recently, the availability of the X-ray structure of TryR has allowed the structure-based identification and development of several interesting small molecule inhibitors.[4d–g] In the course of one such study the macrocyclic polyamine alkaloid lunarine **1** was predicted to be a potential lead inhibitor (Fig. 1b).[5] This was subsequently confirmed using a sample of naturally occurring lunarine **1**, but surprisingly this proved not to be a simple competitive inhibitor, instead showing time-dependent inhibition kinetics. It was proposed that the origin of this time-dependent inhibition stemmed from the covalent modification of TryR through the Michael addition of an active site of cysteine onto either C-10 or C-25 of the lunarine macrocycle.[5] In view of this, it

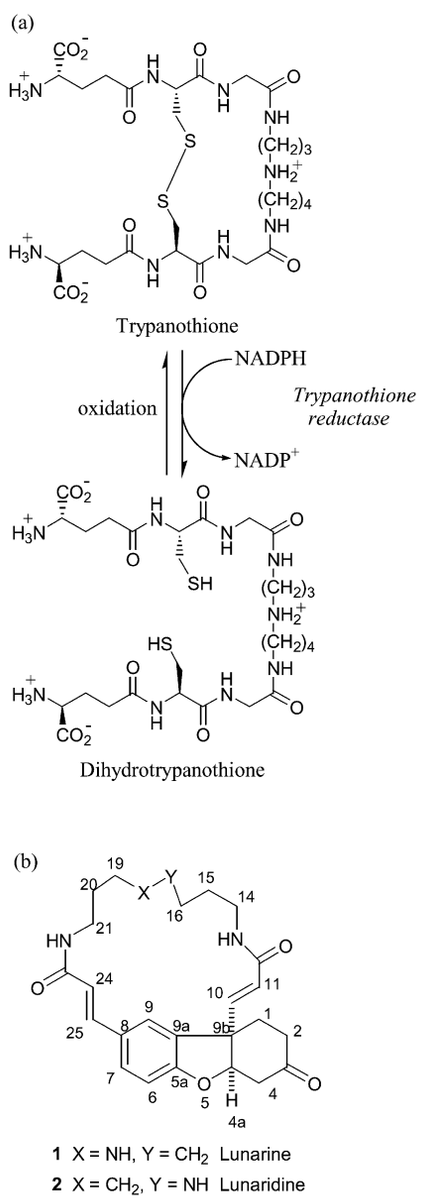


Fig. 1

† The IUPAC name for spermidine is *N*-(3-aminopropyl)butane-1,4-diamine.

DOI: 10.1039/b110149h

was essential to obtain adequate supplies of lunarine for more detailed enzyme inhibition studies. Reported herein is a regioselective synthesis of (±)-lunarine **1** that allows access to various other *Lunaria* alkaloids and their analogues.

Results and discussion

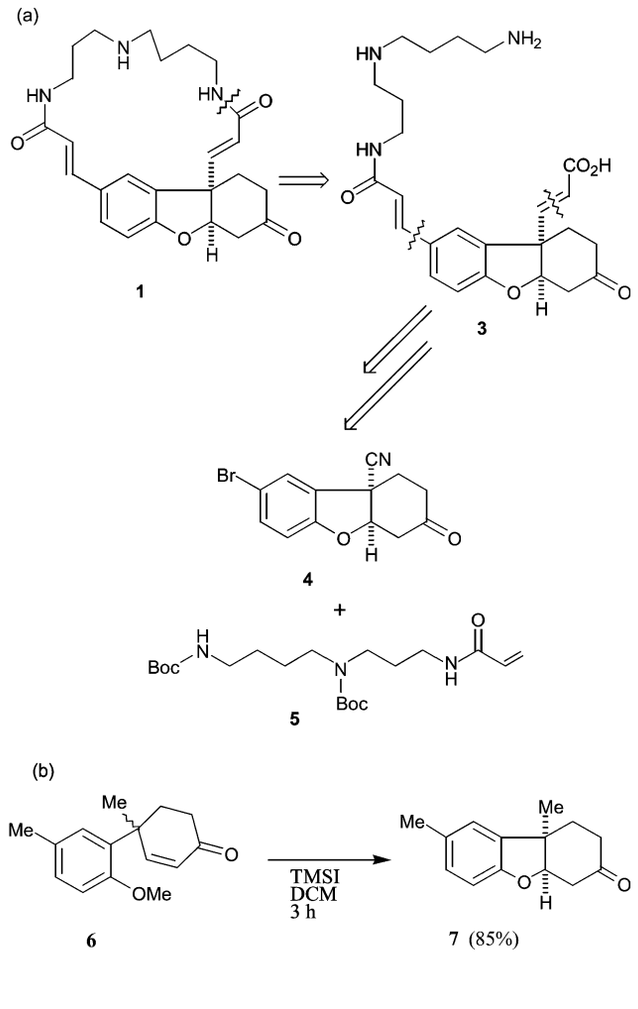
The biosynthesis of lunarine has recently attracted some attention with respect to the formation of the tricyclic "Pummerer ketone" moiety. Zenk and co-workers[6] have demonstrated that *in vitro* lunarine is formed by the stereoselective phenolic oxidative coupling of N^1,N^{10}-bis(*p*-coumaryl)spermidine, but although such a synthetic strategy is conceptually attractive, it has not so far been successfully realised.[7] Similarly, although oxidative phenolic coupling has been employed in the preparation of a key intermediate towards the Pummerer ketone unit in earlier, non-regioselective, syntheses of lunarine **1** and its regioisomer **2**,[7,8] in our hands such an approach proved to be highly inefficient. As these syntheses also did not allow differential elaboration of the two side-chains at C-8 and C-9b (desirable for the preparation of simplified acyclic analogues), we were prompted to examine other routes which would, *inter alia*, permit the regiocontrolled synthesis of lunarine and derivatives. Our retrosynthetic approach is outlined in Scheme 1a. We aimed to achieve the regioselective synthesis of lunarine *via* an intramolecular macrolactamisation of a "pre-cyclised" adduct such as **3**. This, in turn, could be prepared from the Heck coupling of an aryl bromide precursor **4** and the N^1-acrylamidospermidine derivative **5**. The differentially functionalised tricyclic scaffold **4** should also then allow access to a range of valuable analogues (*eg.* dihydrolunarine derivatives with selective saturation of one of the α,β-unsaturated amide moieties found in lunarine).

The key step in the preparation of **4** is an intramolecular Michael addition upon an α,β-unsaturated ketone, elaborated *via* Diels–Alder addition upon a suitable phenolic dienophile partner. The former step has some precedent in a recent synthesis of the prototypical tricyclic Pummerer ketone scaffold **7** (Scheme 1b)[9] that was first described in 1925.[10]

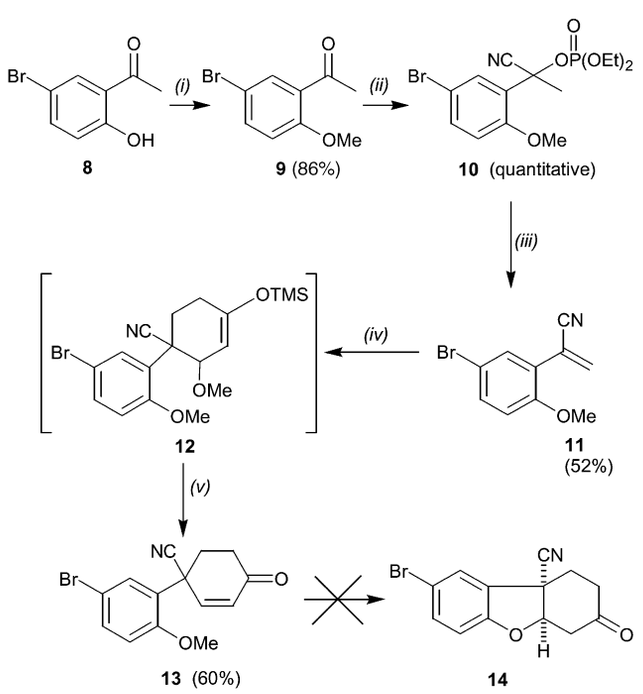
The pre-cyclisation adduct **13** was prepared as outlined in Scheme 2. 5-Bromo-2-methoxyacetophenone **9** was quantitatively converted to cyano phosphate **10** by treatment with diethyl cyanophosphonate in the presence of a catalytic amount of LDA.[11] As significant product loss occurred during chromatographic purification (60% isolated yield), crude **10** (following work-up) was treated directly with boron trifluoride–diethyl etherate to give the acrylonitrile adduct **11** in 52% overall yield.[11b] The dienophile **11** readily underwent Diels–Alder cycloaddition with Danishefsky's diene,[12] to give the Diels–Alder adduct **12** which when treated with catalytic trimethylsilyl triflate‡ (TMSOTf)[13] gave the desired cyclohexenone **13**. Catalytic TMSOTf was used *in lieu* of dilute acid hydrolysis to avoid the predominant formation of the β-methoxy ketone by-product, which was encountered when **12** was treated with 1 mM aqueous HCl.

Whilst the methyl ether proved to be an excellent protecting group in the preceding series of reactions, its removal from **13** prior to intramolecular cyclisation to produce **14** proved to be very problematic. Treatment of **13** with trimethylsilyl iodide, under the same reaction conditions used for **6**,[9] failed to remove the methyl group. Other deprotection procedures, using lithium salts[14] or various boron trihalides,[15] were also tried without success. Deprotection methods were limited to electrophilic reagents as nucleophilic reagents, such as cyanide or thiolate anions, would preferentially react with the enone moiety.

Allyl protection was now chosen as an alternative as this has been used in similar intramolecular cyclisation procedures to produce the tricyclic scaffold of the *Amaryllidaceae* alkaloid



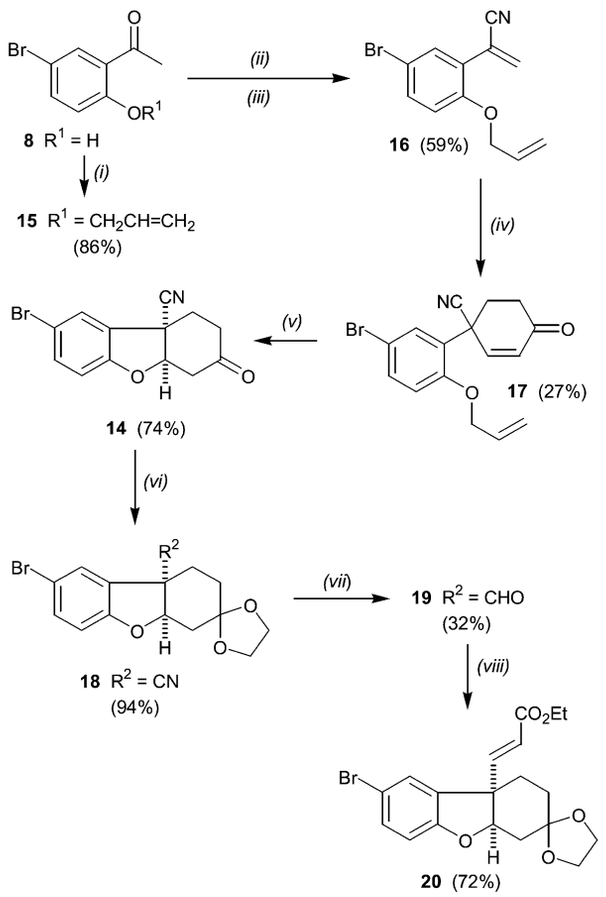
Scheme 1



Scheme 2 (i) K_2CO_3, MeI, acetone, reflux, 3 h; (ii) LDA (cat.), $(EtO)_2P(O)CN$, THF, −10 °C, 2.5 h; (iii) BF_3OEt_2, CH_2Cl_2, 15 min; (iv) 1-methoxy-3-(trimethylsilyloxy)buta-1,3-diene, PhMe–CH_2Cl_2, reflux, 5 h; (v) TMSOTf, collidine, acetone, −78 °C, 10 min.

‡ The IUPAC name for triflate is trifluoromethanesulfonate.

lycoramine.[16] The new dienophile **16** was prepared from the *o*-allyloxy acetophenone **15** in 59% yield without isolation of the cyano phosphate intermediate (Scheme 3).[11] The *o*-allyloxy



Scheme 3 (i) NaH, allyl bromide, DMF, 0–20 °C, 12 h; (ii) LDA (cat.), $(EtO)_2P(O)CN$, THF, −10 °C, 2 days; (iii) BF_3OEt_2, CH_2Cl_2, 10 min; (iv) 1-methoxy-3-(trimethylsilyloxy)buta-1,3-diene, CH_2Cl_2–PhMe reflux, 6.5 h, then TMSOTf, collidine, acetone, −78 °C, 20 min; (v) $(PPh_3)_4Pd(0)$, morpholine, THF, 30 min; (vi) $(TMSOCH_2)_2$, TMSOTf, CH_2Cl_2, −78 to 0 °C, 4.5 h; (vii) DIBAL-H, CH_2Cl_2, −10 °C, 20 min; (viii) NaH, triethyl phosphonoacetate, THF, 2 h.

substituent in **15** appeared to significantly retard the rate of reaction with diethyl cyanophosphonate to give the intermediate cyano phosphate derivative. This reaction took 2 days to reach completion when using 1.5 equivalents of diethyl cyanophosphonate compared with the 2.5 hours that were required for the preparation of the *o*-methoxy derivative **10**. Larger excesses of diethyl cyanophosphonate have been used to accelerate such sluggish reactions[11] and in this example the reaction time was reduced to 5 hours by increasing the diethyl cyanophosphonate quantity to just 2.5 equivalents. At room temperature, compound **16** gradually degraded over several days to give an insoluble resin, and since storage at −20 °C failed to retard this process, **16** was promptly converted after purification to the more stable cyclohexenone **17**.[12,13] For this reaction, the dienophile **16** proved to be soluble in dichloromethane but insoluble in higher boiling point solvents like toluene. The low boiling point of dichloromethane made it an unsuitable solvent for this thermally driven reaction, so a co-solvent mixture of dichloromethane and toluene was initially used. The low yield (27%) obtained at this stage was therefore due to the difficulty in achieving an appropriate balance between reagent solubility and reaction temperature. Because **16** was also soluble in the higher boiling point solvent chloroform, this was used in the scale-up preparations (see below) although the reaction had to be maintained at reflux for 21 hours in order to drive it to completion.

Treatment of **17** with either rhodium trichloride,[16] palladium/carbon in the presence of toluene-*p*-sulfonic acid[17] or potassium *tert*-butoxide followed by acidic work-up[18] failed to effect the clean removal of the allyl ether group. However, on reaction of **17** with a catalytic amount of tetrakis(triphenylphosphine)-palladium(0) in the presence of an excess of morpholine at room temperature[19] the allyl ether was cleanly removed followed by intramolecular cyclisation to give the racemic fused tricyclic product **14** in 74% yield. An original concern with this procedure was that under these Heck-type reaction conditions the palladium catalyst would be inactivated by preferential complexation with the aryl bromide functionality of **17**. However, at room temperature the palladium catalysed isomerisation of the allyl ether proved to be a more favourable reaction. Compound **14** was recrystallised from dichloromethane and an X-ray structure was obtained in order to verify the relative stereochemistry across the fused ring junction (Fig. 2). As

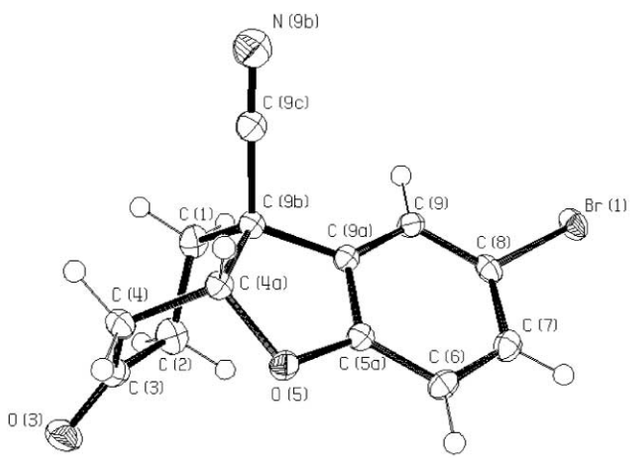
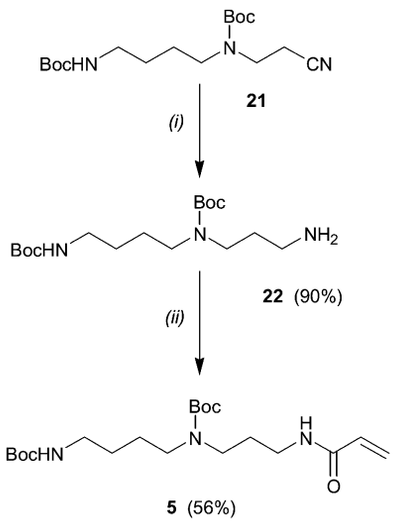


Fig. 2 Molecular structure of **14** showing 50% probability ellipsoids

anticipated, the crystal structure of **14** shows the desired *cis* stereochemistry of the nitrile and H-4a at the fused ring junction. It is interesting to note that the cyclohexanone component sits in a skew-boat conformation[20] comparable to that of lunarine **1**;[21] a factor which we believe plays an important part in determining the effectiveness of some other *Lunaria* alkaloid derivatives as time-dependent inhibitors of TryR. It should be noted that during scale-up preparations it was possible to complete the 4-step preparation of compound **17**, from **8**, on a multi-gram scale without the need for intermediate purification (other than standard work-up procedures). In this manner the overall yield of **17** was increased to 58% (compared to 14% when purification was carried out at each step).

Standard acid catalysed preparation of the 1,3-dioxolane **18** using ethylene glycol under Dean–Stark conditions gave very poor yields, but the trimethylsilyl triflate catalysed reaction of **14** with 1,2-bis(trimethylsilyloxy)ethane[22] proved to be extremely effective giving **18** in 94% yield. Conversion of nitrile **18** to the aldehyde **19** was effected in modest but acceptable yield (32%) using DIBAL-H. This step, which was not optimised, was followed by Wadsworth–Emmons reaction with triethyl phosphonoacetate[23] to give the α,β-unsaturated ethyl ester **20**, in 72% yield. As outlined above, compound **20**, with its readily differentiable aryl bromide and carboxy functions, is not only a key intermediate for the present synthesis of lunarine **1**, but also for the preparation of various cyclic and acyclic lunarine analogues where the acrylamido 'side chains' of the tricyclic core have been selectively modified. The synthesis of such compounds and their use as mechanistic probes to determine the nature of TryR inhibition by lunarine **1** will be reported elsewhere.[27]

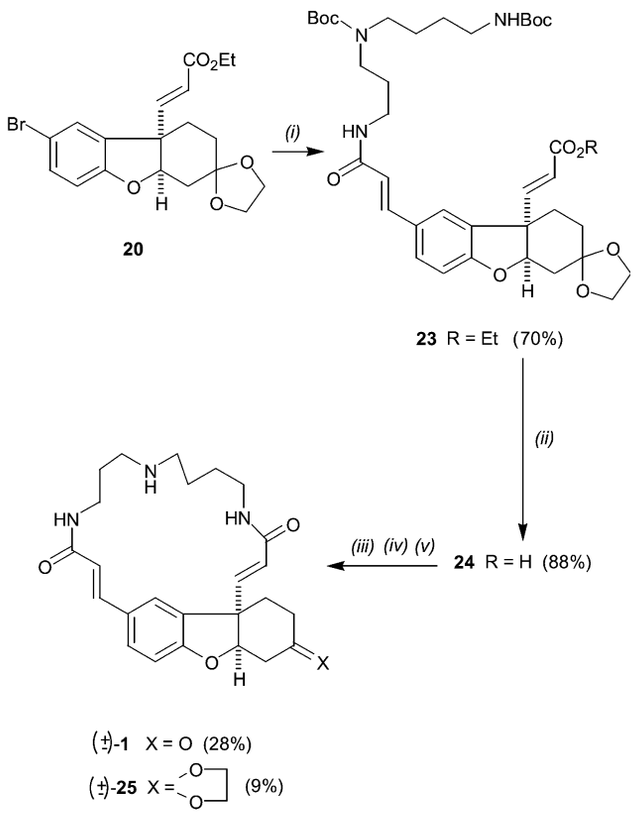
The selectively protected spermidine precursor **21** was prepared according to the method of Humora and Quick (Scheme 4).[24] These authors used lithium aluminium hydride to reduce



Scheme 4 (i) Raney nickel (cat.), NaOH, EtOH, H_2 (40 psi), 24 h; (ii) acryloyl chloride, DMAP, Et_3N, CH_2Cl_2, 16 h.

the nitrile **21** to the primary amine **22**, but in our hands this method gave very poor results. Hydrogenation of **21** in the presence of Raney nickel under basic conditions was much more satisfactory and gave excellent recovery of essentially pure product **22** following a simple work-up.[25] Reaction of **22** with acryloyl chloride in dichloromethane then gave the fully protected acrylamidospermidine **5** in 50% overall yield from **21**.

The final functionalisation of the tricyclic scaffold **20** *via* a Heck coupling with the acrylamidospermidine fragment **5** proceeded smoothly within three hours at 60 °C to give **23** in 70% yield (Scheme 5). In this reaction, tri-*o*-tolylphosphine was used as a palladium ligand to minimise the occurrence of unwanted side-reactions that are sometimes encountered when electron-rich *para*-substituted aryl bromides are employed in Heck coupling procedures.[26] Saponification of **23** then gave the free acid **24** in 88% yield which was the desired "precyclised" lunarine intermediate. The dioxolane group was retained until this stage to prevent the β-phenoxyketone functionality from undergoing a base-induced retro-Michael reaction, followed by 1,4-addition of the resultant phenoxide anion onto the α,β-unsaturated ester moiety in **23**.[8] Macrolactamisation of **24** was achieved by first reacting the free acid with pentafluorophenol in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) to give the activated pentafluorophenyl ester. Following work-up, and without further purification, the *N*-Boc protecting groups were removed using 4 M HCl in anhydrous dioxane. Excess HCl was removed from the resulting amine salt by repeated coevaporation with dichloromethane followed by drying of the solid under high vacuum for several hours. Intramolecular cyclisation was then induced by treating this salt with a large excess of *N*,*N*-diisopropylethylamine (DIPEA) in dichloromethane under high dilution conditions (concentration of the pre-cyclised product was 0.9 mM). Within 16 hours the cyclisation was deemed complete (by TLC) and following purification, by chromatography, racemic lunarine **1** was isolated in 28% yield. A small quantity of the 1,3-dioxolane derivative **25** was also recovered (9%). The relative ease of formation of this 20-membered macrocycle is presumably due to the rigidity of the tricyclic scaffold which helps maintain a pre-cyclised conformation which is amenable to macrolactamisation. Lunarine **1** was indistinguishable (^1H, ^{13}C NMR) from an authentic sample of natural (+)-lunarine.



Scheme 5 (i) $Pd(OAc)_2$, **5**, Et_3N, (*o*-Tol)$_3$P, DMF, 60 °C, 11 h; (ii) LiOH, EtOH (aq), 8 h; (iii) C_6F_5OH, EDC, DMAP (cat.), CH_2Cl_2, 15 h; (iv) 4 M HCl in dioxane; (v) DIPEA, CH_2Cl_2, 21 h.

In conclusion, the first regiocontrolled synthesis of racemic lunarine **1** has been achieved. The strategy employed is now being used for the preparation of a range of lunarine derivatives and may also be employed for the asymmetric synthesis of such compounds. Racemic lunarine **1** and the dioxolane **26** are now being evaluated against TryR as part of our ongoing studies on the anti-trypanosomal properties of the *Lunaria* alkaloids. These results will be reported elsewhere.[27]

Experimental

General

Melting points were recorded on an Electrothermal IA9000 series digital melting point apparatus, using open capillaries, and are quoted uncorrected. NMR Spectra: ^1H-NMR spectra were recorded on a Bruker Avance DPX 300 FT-spectrometer at 300 MHz, ^{13}C-NMR were recorded on the same spectrometer at 75 MHz. All NMR spectra were taken at 300 K, except where specified. Chemical shifts (δ) are expressed in ppm and coupling constants (J) are given in Hz. Mass spectra were recorded on a VC 70-S double focusing mass spectrometer in fast atom bombardment (FAB) and electron impact (EI) modes. Electrospray mass spectra were carried out on a VG Quattro triple quadrupole mass spectrometer. Elemental analyses were carried out by Medac Ltd, Brunel Science Centre, Surrey, UK. Flash Chromatography was performed on columns of silica gel (Fluorochem, Silica Gel 60; 40–63 μ). Petroleum ether(bp 40–60 °C, referred to as petrol) was distilled through a Vigreux column prior to use. Anhydrous acetone was prepared by stirring over B_2O_3 for 12 h prior to distillation and storage under nitrogen. Collidine was distilled from CaH_2 and stored over 3 Å molecular sieves at −18 °C. Triethylamine was distilled from CaH_2 and stored under nitrogen at room temperature. All other solvents were dried using standard procedures.

N-(3-acrylamidopropyl)-N,N-bis(*tert*-butoxycarbonyl)butane-1,4-diamine (5)

A solution of **22** (570 mg, 1.65 mmol), triethylamine (1.8 cm^3, 12.91 mmol) and DMAP (2 mg, 0.017 mmol) in anhydrous dichloromethane (8 cm^3), at 0 °C, was treated with a solution of acryloyl chloride (0.15 cm^3, 1.85 mmol) in dichloromethane (2 cm^3), added dropwise. The reaction mixture was slowly allowed to warm to room temperature whilst stirring overnight. It was then washed with 5% aqueous citric acid and brine, dried (Na_2SO_4) and the solvent was evaporated to give an oil. This was purified by chromatography (petrol–EtOAc (2 : 8) eluent) to give **5** as a viscous colourless oil (367 mg, 56%). ν_{max}(NaCl)/cm^{-1} 3480–3200 (NH), 3075 (CH=CH_2), 1724–1625 (C=O), 989 (CH_2=CH, def); δ_H (300 MHz, $CDCl_3$) 1.44 (9H, s, 3 × CH_3), 1.46 (9H, s, 3 × CH_3), 1.49–1.62 (2H, m, CH_2), 1.64–1.80 (4H, br, 2 × CH_2), 3.09–3.22 (4H, br, 2 × CH_2), 3.22–3.40 (4H, br, 2 × CH_2), 4.58 (1H, br, N^8–H), 5.62 (1H, d, *J* 10.2, =CH_{trans}), 6.14 (1H, dd, *J* 16.8, 10.2, =CH_{cis}), 6.28 (1H, d, *J* 16.8, C*H*=CH_2), 7.05 (1H, br, N^1–H); δ_C (75 MHz, $CDCl_3$) 25.45 (C-6), 27.16, 27.40 (C-7) and (C-2), 28.17 (CH_3), 35.56 (C-1), 39.80 (C-8), 43.24 (C-3), 46.42 (C-5), 78.73 (C), 79.48 (C), 125.40 (=CH_2), 131.21 (*C*H=CH_2), 155.91, 156.16 (2 × carbamate C=O), 165.50 (amide C=O); *m*/*z* (FAB) 400 (27%, M^+), 300 (37, M + H − ^{t}BuOCO), 244 (53, M − (^{t}BuOCO + CH_2CH_2CO).

5-Bromo-2-methoxyacetophenone (9)

A mixture of 5-bromo-2-hydroxyacetophenone (6.06 g, 28.0 mmol), potassium carbonate (6.94 g, 70.0 mmol) and methyl iodide (7 cm^3, 113 mmol) in acetone (100 cm^3, pre-dried over 4 Å molecular sieves) was heated under reflux for 3 h. The reaction mixture was filtered and the solvents were evaporated to give an oil. This was re-dissolved in ether and then washed with water, 0.1 M NaOH and then water once more. The organics were dried ($MgSO_4$), the solvents evaporated and the crude material purified by chromatography (petrol–EtOAc eluent 9 : 1) to give **9** as an off-white solid (5.54 g, 86%). δ_H (300 MHz, $CDCl_3$) 2.59 (3H, s, CH_3), 3.90 (3H, s, CH_3O), 6.86 (1H, d, *J* 8.8, H-3), 7.53 (1H, dd, *J* 8.8, 2.6, H-4), 7.82 (1H, d, *J* 2.6, H-6); δ_C (75 MHz, $CDCl_3$) 32.11 (CH_3), 56.23 (CH_3O), 113.50 (C-5), 113.95 (C-3), 130.03 (C-1), 133.33 (C-6), 136.47 (C-4), 158.33 (C-2), 198.62 (C=O); [Found: (EI) 227.97873, $C_9H_9BrO_2$ requires 227.97859] *m*/*z* 227 (30%, M^+), 213 (100, M − CH_3).

2-(5-Bromo-2-methoxyphenyl)acrylonitrile (11)

A solution of freshly made LDA (0.351 mmol) in THF (4 cm^3) at −15 °C was added dropwise to a solution of **9** (1.61 g, 7.03 mmol) in THF (10 cm^3), pre-cooled to −15 °C, to give a pale yellow solution. This was stirred at −15 °C for 15 min before the dropwise addition of neat diethyl cyanophosphonate (1.38 g, 8.43 mmol) which was then stirred at −15 to 0 °C for 2.5 h. The reaction mixture was quenched (H_2O) and then extracted with ethyl acetate. The combined organics were dried ($MgSO_4$) and the solvent was evaporated to give the cyano phosphate intermediate **10** as a light brown oil (2.98 g, quantitative yield). This was dissolved in dichloromethane (20 cm^3) and boron trifluoride–diethyl etherate (2.99 g, 21.06 mmol) was added dropwise and the reaction was stirred at room temperature for 15 min. The reaction mixture was diluted with dichloromethane (30 cm^3) and then washed with saturated aqueous $NaHCO_3$ followed by water. The organics were dried (Na_2SO_4), the solvents evaporated and the crude product purified by chromatography (CH_2Cl_2 eluent) to give **11** as a pale yellow oil (872 mg, 52%) that slowly solidified. Mp (CH_2Cl_2) 163–164 °C; δ_H (300 MHz, $CDCl_3$) 3.91 (3H, s, OCH_3), 6.26 (1H, s, =CH_{trans}), 6.41 (1H, s, =CH_{cis}), 6.85 (1H, d, *J* 8.8, H-3), 7.48 (1H, dd, *J* 8.7, 2.5, H-4), 7.53 (1H, d, *J* 2.5, H-5); δ_C (75 MHz, $CDCl_3$) 56.36 (OCH_3), 113.33 (C-5), 113.56 (C-3), 118.12 (CN), 119.65 (*C*=CH_2), 124.46 (C-1), 132.54 (C-6), 133.93 (C-4), 134.30 (=CH_2), 156.65 (C-2); [Found: (EI) 236.97760, $C_{10}H_8BrNO$ requires 236.97893] *m*/*z* 237 (38%, M^+), 222 (20, M − CH_3), 211 (9, M − CN), 143 (100, M − CH_3 − Br).

4-(2-Methoxy-5-bromophenyl)-4-cyanocyclohex-2-en-1-one (13)

A mixture of **11** (859 mg, 3.61 mmol) and 1-methoxy-3-(trimethylsilyloxy)buta-1,3-diene (1.05 cm^3, 4.69 mmol) in a co-solvent mixture of anhydrous toluene (13 cm^3) and dichloromethane (10 cm^3) was heated to reflux under argon for 5 h. The reaction was cooled to −78 °C and anhydrous acetone (1.3 cm^3, 18.05 mmol) was added. A solution of TMSOTf (100 µl, 0.55 mmol) and anhydrous collidine (9 µl, 0.07 mmol) in dichloromethane (1.4 cm^3) was then added *via* a cannula to give a deep red solution, which was stirred at −78 °C for 10 min. The reaction mixture was quenched with brine and was extracted with dichloromethane. The combined organics were dried ($MgSO_4$) and the solvents evaporated to give a brown oily solid. The crude product was purified by chromatography (CH_2Cl_2 eluent) to give **13** as a white solid (663 mg, 60%). δ_H (300 MHz, $CDCl_3$) 2.45–2.85 (4H, m, 2 × CH_2), 3.91 (3H, s, OCH_3), 6.28 (1H, d, *J* 10.0, =CH-2), 6.89 (1H, d, *J* 8.7, H-3′), 6.95 (1H, dd, *J* 10.0, 0.9, =CH-3), 7.47 (1H, d, *J* 2.4, H-6′), 7.51 (1H, dd, *J* 8.7, 2.4, H-4′); δ_C (75 MHz, $CDCl_3$) 33.22 (C-5), 35.18 (C-6), 40.52 (C-4), 56.34 (OCH_3), 113.70 (C-Br), 114.42 (C-3′), 119.17 (CN), 126.79 (C-1′), 130.93 (C-2), 131.39 (C-4′), 133.72 (C-6′), 146.02 (C-3), 156.46 (C-2′), 196.74 (C=O); [Found: (EI) 305.0046, $C_{14}H_{12}BrNO_2$ requires 305.0051] *m*/*z* 305 (75%, M^+), 198 (95), 127 (100).

***cis*-3-Oxo-8-bromo-9b-cyano-1,2,3,4,4a,9b-hexahydrodibenzo-[*b*,*d*]furan (14)**

Tetrakis(triphenylphosphine)palladium(0) (2.30 g, 1.99 mmol) was added, in one portion, to a solution of **17** (9.97 g, 30.01 mmol) and anhydrous morpholine (23 cm^3, 263.7 mmol) in anhydrous THF (250 cm^3) under argon. The reaction mixture was stirred at room temperature for 30 min. Most of the solvent was then removed and the residue was diluted in ethyl acetate (100 cm^3). The organics were washed with 0.1 M HCl (× 2), followed by water, dried ($MgSO_4$) and the solvent was evaporated to give a brown oily solid (*ca*. 15 g). The crude product was first passed through a thin bed of silica (CH_2Cl_2 eluent) to give approximately 10 g of a brown solid, which was further purified by chromatography (CH_2Cl_2 eluent). Mixed fractions were purified by an additional chromatographic step to give **14** as an off-white solid (6.48 g, 74%). Mp (CH_2Cl_2) 163–164 °C; ν_{max}($CDCl_3$)/cm^{-1} 2255 (CN), 1732 (C=O); δ_H(300 MHz, $CDCl_3$) 1.95–2.67 (4H, m, 2 × CH_2), 2.92 (1H, dd, *J* 17.3, 3.4, *cis*-H-4), 3.03 (1H, dd, *J* 17.3, 3.4, *trans*-H-4), 5.43 (1H, t, *J* 3.4, H-4a), 6.75 (1H, d, *J* 8.6, H-6), 7.41 (1H, dd, *J* 8.6, 2.1, H-7), 7.52 (1H, d, *J* 2.0, H-9); δ_C (75 MHz, $CDCl_3$) 32.39 (C-1), 34.78 (C-2), 40.99 (C-4), 43.04 (C-9b), 85.00 (C-4a), 112.79 (C-6), 114.65 (C-Br), 120.53 (CN), 127.65 (C-9), 127.81 (C-9a), 134.72 (C-7), 158.19 (C-5a), 204.78 (C=O); [Found: (EI) 290.98595, $C_{13}H_{10}BrNO_2$ requires 291.00949] *m*/*z* 291 (100%, M^+), 265 (27, M − CN), 221 (45, M − C_4H_6O).

5-Bromo-2-allyloxyacetophenone (15)

A solution of 5-bromo-2-hydroxyacetophenone (8.78 g, 40.83 mmol) in DMF (10 cm^3) was added dropwise to a stirred suspension of sodium hydride (2.12 g, 53.1 mmol of a 60% dispersion in mineral oil, pre-washed with petrol) in anhydrous DMF (70 cm^3), cooled to 0 °C. This was stirred at room temperature for 2 h to give a yellow–brown solution. After cooling to 0 °C allyl bromide (9.79 g, 80.98 mmol, freshly distilled from K_2CO_3) was added dropwise and the reaction was left to stir at room temperature overnight. The reaction mixture was quenched (H_2O, 100 cm^3) and was extracted with diethyl ether

(2 × 100 cm^3). The organics were then washed with 0.1 M NaOH (3 × 60 cm^3), followed by water (2 × 30 cm^3), dried ($MgSO_4$) and the solvent was evaporated. The crude material was recrystallised from a minimum amount of hot petrol to give **15** as a white solid (8.94 g, 86%). Mp (petrol) 55–57 °C (lit.,[28] 58 °C); $\nu_{max}(CDCl_3)/cm^{-1}$ 1677 (C=O), 1598 (C=C); δ_H (300 MHz, $CDCl_3$) 2.65 (3H, s, CH_3), 4.63 (2H, d, *J* 5.2, OCH_2), 5.34 (1H, dd, *J* 10.5, 1.0, CH=CHH_{cis}), 5.43 (1H, dd, *J* 17.3, 1.0, CH=CHH_{trans}), 6.00–6.13 (1H, m, C*H*=CH_2), 6.84 (1H, d, *J* 8.8, H-3), 7.52 (1H, dd, *J* 8.7, 2.6, H-4), 7.84 (1H, d, *J* 2.6, H-6); δ_C (75 MHz, $CDCl_3$) 32.29 (CH_3), 70.12 (OCH_2), 113.72 (C-Br), 115.12 (C-3), 119.05 (=CH_2), 130.38 (C-1), 132.53 (*C*H=CH_2), 133.42 (C-6), 136.38 (C-4), 157.30 (C-2), 198.73 (C=O); [Found: (EI) 253.99568, $C_{11}H_{11}BrO_2$ requires 253.99424] *m*/*z* 254 (35%, M^+), 199 (100, M − 55), 132 (28, M − CH_3CO − Br).

2-(5-Bromo-2-allyloxyphenyl)acrylonitrile (16)

A solution of **15** (7.02 g, 27.52 mmol) in THF (10 cm^3), pre-cooled to −15 °C, was added dropwise to a solution of freshly prepared LDA (1.38 mmol) in THF (40 cm^3) at −15 °C to give a pale yellow solution. After stirring at −15 °C for 20 min, neat diethyl cyanophosphonate (5.8 cm^3, 38.23 mmol) was added. The mixture was left at 0 °C for 1 h and then at room temperature for 2 days after which most of the starting material had been consumed. The reaction mixture was quenched (H_2O) and then extracted with ethyl acetate. The combined organics were dried ($MgSO_4$) and the solvent evaporated to give a light brown oil (11.44 g). This was dissolved in dichloromethane (80 cm^3) and boron trifluoride–diethyl etherate (10 cm^3, 81.62 mmol) was added dropwise producing a dark red–brown solution which was stirred for 10 min at room temperature. The reaction mixture was diluted with dichloromethane (100 cm^3) and then washed with water. The organics were dried ($MgSO_4$), the solvents evaporated and the crude oil purified by chromatography (CH_2Cl_2 eluent) to give **16** as a pale yellow oil (4.28 g, 59%). Once purified, **16** started to degrade into an insoluble resin within 24 h when stored at −20 °C. Due to its instability, it was promptly used following purification. δ_H (300 MHz, $CDCl_3$) 4.60–4.63 (2H, m, OCH_2), 5.33 (1H, dd, *J* 10.6, 1.3, CH=CHH_{cis}), 5.45 (1H, dd, *J* 17.3, 1.4, CH=CHH_{trans}), 6.00–6.13 (1H, m, C*H*=CH_2), 6.24 (1H, s, =CHH_{trans}), 6.40 (1H, s, =CHH_{cis}), 6.82 (1H, d, *J* 8.8, H-3), 7.43 (1H, dd, *J* 8.8, 2.4, H-4), 7.51 (1H, d, *J* 2.4, H-6); δ_C (75 MHz, $CDCl_3$) 70.17 (CH_2), 113.50 (CBr), 114.78 (C-3), 118.14 (CN), 118.86 (CH=*C*H_2), 119.70 (C), 124.77 (C-1), 132.48, 132.62 (C-4 and C-6), 133.86 (*C*H=CH_2), 134.39 (=CH_2), 155.57 (C-2); [Found: (EI) 262.99483, $C_{12}H_{10}BrNO$ requires 262.99458] *m*/*z* 263 (16%, M^+), 222 (62, M − CH_2CH=CH_2), 143 (100, M − CH_2CH=CH_2 − Br).

4-(2-Allyloxy-5-bromophenyl)-4-cyanocyclohex-2-ene-1-one (17)

A mixture of **16** (273 mg, 1.034 mmol) and 1-methoxy-3-(trimethylsilyloxy)buta-1,3-diene (0.3 cm^3, 1.49 mmol) in a 1 : 1 co-solvent mixture of anhydrous dichloromethane–toluene (4 cm^3) was heated to reflux under argon for 6.5 h. The solution was transferred to another round bottomed flask *via* a syringe to separate undissolved and unconsumed starting material. This was cooled to −78 °C and anhydrous acetone (0.37 cm^3, 5.04 mmol) was added. A solution of TMSOTf (30 μl, 0.166 mmol) and 0.1 M anhydrous collidine in dichloromethane (0.5 cm^3) were then added, *via* a cannula, to give a dark red–brown solution, which was stirred at −78 °C for 20 min. The reaction mixture was quenched with water and extracted with dichloromethane. The combined organics were dried ($MgSO_4$) and the solvent was evaporated to give a purple–brown oil. The crude product was purified by chromatography (CH_2Cl_2 eluent) to give **17** as a white solid (93 mg, 27%). The EI mass spectrum of this compound did not show the expected molecular ion at *m*/*z* = 331. The dominant fragment was the $[M - OH]^+$ ion at *m*/*z* = 314 whose formula was confirmed by accurate mass analysis. Although accurate mass analysis of the molecular ion was not possible, the empirical formula was confirmed by satisfactory elemental analysis. (Found: C, 57.82; H, 4.25; N, 4.21. $C_{16}H_{14}NO_2Br$ requires C, 57.85; H, 4.28; N, 4.14%) $\nu_{max}(CDCl_3)/cm^{-1}$ 2260 (CN), 1697 (C=O); δ_H(300 MHz, $CDCl_3$) 2.40–2.83 (4H, 2 × CH_2), 4.62 (2H, dd, *J* 4.2, 0.9, OCH_2), 5.31 (1H, dd, *J* 10.5, 0.8, CH=CHH_{cis}), 5.41 (1H, dd, *J* 17.3, 1.1, CH=CHH_{trans}), 5.95–6.10 (1H, m, C*H*=CH_2), 6.25 (1H, d, *J* 10.0, H-2′), 6.87 (1H, d, *J* 9.4, H-3), 6.95 (1H, d, *J* 10.0, H-3′), 7.42–7.49 (2H, m, H-4, H-6); δ_C (75 MHz, $CDCl_3$) 33.16 (C-5′), 35.16 (C-6′), 40.54 (C-4′), 70.28 (OCH_2), 113.76 (C-Br), 115.45 (C-3), 119.03 (=CH_2), 119.21 (CN), 126.83 (C-1), 131.00, 131.46, 132.24, 133.63 (C-2′, C-4, C-6, *C*H=CH_2), 146.05 (C-3′), 155.41 (C-2), 196.65 (C=O); [Found: (EI) 314.01825, $C_{16}H_{13}BrNO$ requires 314.01798] *m*/*z* 314 (100%, $[M - OH]^+$), 304 (29, M − HCN), 290 (22, M − CH_2=$CHCH_2$).

Scale-up preparation of 4-(2-allyloxy-5-bromophenyl)-4-cyanocyclohex-2-ene-1-one (17)

To a stirred suspension of sodium hydride (2.40 g, 60 mmol of a 60% dispersion in mineral oil, pre-washed with petrol) in anhydrous DMF (70 cm^3), cooled to 0 °C, was added a solution of 5-bromo-2-hydroxyacetophenone (9.73 g, 45.25 mmol) in DMF (10 cm^3). This was stirred at room temperature for 2 h to give a yellow–brown solution. After cooling to 0 °C, allyl bromide (7.8 cm^3, 90.50 mmol, freshly distilled from K_2CO_3) was added dropwise and then left to stir at room temperature for 16 h. The reaction mixture was quenched with water (100 cm^3) and extracted with diethyl ether (2 × 100 cm^3). The organics were washed with 0.1 M NaOH (3 × 60 cm^3), followed by water (2 × 30 cm^3), dried ($MgSO_4$) and the solvents evaporated to give crude **15** as a a pale yellow solid (10.89 g). An additional 2.39 g of previously made **15** was added to this prior to the subsequent chemical steps.

A solution of crude **15** (13.28 g, 52.06 mmol) in THF (20 cm^3), pre-cooled to −15 °C, was added dropwise to a solution of freshly made LDA (2.56 mmol) in THF (40 cm^3) at −15 °C to give a brown solution. This was stirred at −15 °C for 10 min before the dropwise addition of neat diethyl cyanophosphonate (20.0 cm^3, 131.2 mmol) which was stirred at 0 °C for 5 h. The reaction mixture was quenched (H_2O) and then extracted with ethyl acetate. The combined organics were dried ($MgSO_4$) and the solvent was evaporated to give a brown oil (28.3 g). This was dissolved in dichloromethane (100 cm^3) and boron trifluoride–diethyl etherate (18 cm^3, 147.9 mmol) was added dropwise producing a dark brown solution that was stirred for 5 min at room temperature. The reaction mixture was diluted with dichloromethane (100 cm^3), an excess of saturated $NaHCO_3$ was added and the mixture was stirred vigorously until the effervescence had stopped. The organic layer was separated, washed (saturated $NaHCO_3$ followed by water), dried ($MgSO_4$) and the solvents were evaporated to give crude **16** as an oil (15.75 g).

A mixture of **16** (52.1 mmol) and 1-methoxy-3-(trimethylsilyloxy)buta-1,3-diene (14 cm^3, 69.6 mmol) in anhydrous chloroform (60 cm^3) was heated to reflux under argon for 21 h. The reaction was cooled to −78 °C and anhydrous acetone (18.0 cm^3, 245.1 mmol) was added. A solution of TMSOTf (2.01 cm^3, 11.05 mmol) and anhydrous collidine (0.43 cm^3, 3.45 mmol) in dichloromethane (15 cm^3) was then added, *via* a cannula, to give a deep purple solution, which was stirred at −78 °C for 20 min. The reaction mixture was quenched with water and extracted with dichloromethane. The combined organics were dried ($MgSO_4$) and the solvent was evaporated to give a brown oily solid. The crude product was purified by chromatography (petrol–EtOAc (8 : 2) eluent) to give **17** as a white solid (10.09 g, 58% overall yield from 5-bromo-2-hydroxyacetophenone).

cis-3,3-(Ethylenedioxy)-8-bromo-9b-cyano-1,2,3,4,4a,9b-hexahydrodibenzo[*b*,*d*]furan (18)

A solution of **17** (6.20 g, 21.22 mmol) in dichloromethane (80 cm^3), was pre-cooled to −78 °C and added *via* a cannula to a solution of trimethylsilyl triflate (0.280 cm^3, 1.55 mmol) and 1,2-bis(trimethylsilyloxy)ethane (6.46 cm^3, 26.36 mmol) in dichloromethane (20 cm^3), cooled to −78 °C. This was allowed to slowly warm to room temperature, whilst stirring over 8 h. The reaction mixture was quenched with pyridine (1 cm^3), poured into saturated $NaHCO_3$ and extracted with dichloromethane. The combined organics were washed with brine, dried ($MgSO_4$), and the solvent evaporated to give a yellow solid. This was purified by chromatography (petrol–EtOAc (3 : 1) eluent) to give **18** as a white solid (6.96 g, 94%). Mp (EtOAc) 126–130 °C; δ_H (300 MHz, $CDCl_3$) 1.67–2.35 (6H, m, 3 × CH_2), 3.90–4.06 (4H, m, OCH_2CH_2O), 4.98 (1H, t, *J* 4.4, H-4a), 6.81 (1H, d, *J* 8.5, H-6), 7.36 (1H, dd, *J* 8.6, 2.2, H-7), 7.45 (1H, d, *J* 2.1, H-9); δ_C (75 MHz, $CDCl_3$) 30.69 (C-1), 31.93 (C-2), 35.37 (C-4), 43.23 (C-9b), 64.67, 65.35 (2 × OCH_2CH_2O), 86.01 (C-4a), 106.43 (C-3), 113.54 (C-6), 114.14 (C-Br), 120.24 (CN), 126.72 (C-9), 130.87 (C-9a), 133.67 (C-7), 157.56 (C-5a); [Found: (EI) 335.01816, $C_{15}H_{14}BrNO_3$ requires 335.01571] *m*/*z* 335, 337 (27%, 1 : 1, M^+), 236, 238 (8, 1 : 1, M − 99), 99 (100, M − 236).

cis-3,3-(Ethylenedioxy)-8-bromo-9b-formyl-1,2,3,4,4a,9b-hexahydrodibenzo[*b*,*d*]furan (19)

A solution of **18** (6.93 g, 20.61 mmol) in anhydrous dichloromethane (200 cm^3) was cooled to −10 °C and a 1.5 M solution of DIBAL-H in toluene (24 cm^3, 36 mmol) was added dropwise to give a pale yellow solution which was stirred at −10 °C for 20 min. The reaction mixture was quenched by the slow addition of water (200 cm^3) whilst stirring vigorously. The aqueous layer was extracted with dichloromethane, the combined organics dried ($MgSO_4$) and the solvent evaporated to give a sticky white foam. This was purified by chromatography (CH_2Cl_2 eluent) to give **19** as a pale yellow oil (2.25 g, 32% yield). ν_{max}(NaCl)/cm^{-1} 1724 (C=O); δ_H (300 MHz, $CDCl_3$) 1.62–2.30 (6H, 3 × CH_2), 3.86–4.02 (4H, m, –OCH_2CH_2O–), 5.22 (1H, t, *J* 6.7, H-4a), 6.78 (1H, d, *J* 8.5, H-6), 7.22 (1H, d, *J* 2.0, H-6), 7.33 (1H, dd, *J* 8.5, 2.1, H-7), 9.57 (1H, s, CHO); δ_C (75 MHz, $CDCl_3$) 24.93 (C-1), 30.58 (C-2), 36.70 (C-4), 59.53 (C-9b), 64.66, 65.01, (OCH_2CH_2O), 82.85 (C-4a), 107.47 (C-3), 113.12 (C-6), 113.54 (C-Br), 127.33 (C-9), 129.45 (C-9a), 133.28 (C-7), 158.88 (C-5a), 197.52 (C=O); [Found: (EI) 338.01730, $C_{15}H_{15}BrO_4$ requires 338.01537] *m*/*z* 338 (23%, M^+), 309 (68, M − CHO), 115 (100, cyclic ethylene ketal fragmentation $C_5H_6O_3^+$).

cis-3,3-(Ethylenedioxy)-8-bromo-9b-[(*E*)-2-ethoxycarbonylvinyl]-1,2,3,4,4a,9b-hexahydrodibenzo[*b*,*d*]furan (20)

A suspension of sodium hydride (21.50 mmol) in THF (25 cm^3), was treated with triethyl phosphonoacetate (4.3 cm^3, 21.67 mmol), added dropwise. This was stirred under argon at room temperature for 60 min until hydrogen gas evolution had ceased. A solution of **19** (2.19 g, 6.47 mmol) in THF (15 cm^3) was then added dropwise and the mixture was left to stir at room temperature. The reaction was complete after 2 h (as determined by ^{1}H NMR of a reaction sample following a mini work-up). The reaction mixture was quenched with water and extracted with ether. The combined organics were dried ($MgSO_4$) and the solvent evaporated to give an oil. This was purified by chromatography [eluting with CH_2Cl_2 and then CH_2Cl_2–EtOAc (19 : 1)] to give the **20** as a colourless gum (2.08 g, 81%). ν_{max}(NaCl)/cm^{-1} 1720 (C=O), 1649 (C=C); δ_H(300 MHz, $CDCl_3$) 1.30 (3H, t, *J* 7.2, CH_3), 1.55–2.10 (6H, m, 3 × CH_2), 3.83–4.01 (4H, m, –OCH_2CH_2O–), 4.20 (2H, q, *J* 7.2, OCH_2), 4.74 (1H, t, *J* 5.5, H-4a), 5.80 (1H, d, *J* 15.9, =CH-11), 6.77 (1H, d, *J* 8.4, H-6), 7.09 (1H, d, *J* 15.9, =CH-10), 7.14 (1H, d, *J* 1.9, H-9), 7.28 (1H, dd, *J* 8.5, 2.0, H-7); δ_C (75 MHz, $CDCl_3$) 14.62 (CH_3), 29.40 (C-1), 30.67 (C-2), 36.46 (C-4), 50.23 (C-9b), 61.11 (OCH_2), 64.57, 65.10 (OCH_2CH_2O), 87.77 (C-4a), 107.61 (C-3), 113.12 (C-6), 113.41 (C-Br), 122.09 (=CH-11), 126.97 (C-9), 132.31 (C-7), 134.82 (C-9a), 150.09 (=CH-10), 158.21 (C-5a), 166.56 (C=O); [Found: (EI) 426.0916, $C_{19}H_{25}BrNO_5$ requires 426.09161] *m*/*z* 431 (100%, M + Na^+), 426 (30, M + NH_4^+).

N-(3-aminopropyl)-*N*,*N*'-bis(*tert*-butoxycarbonyl)butane-1,4-diamine (22)

A mixture of **21** (957 mg, 2.80 mmol) and Raney nickel (250 mg) in 10 M ethanolic sodium hydroxide (10 cm^3) were stirred at room temperature under a hydrogen atmosphere (40 psi) for 24 h. The nickel residues were filtered off and most of the ethanol was removed from the filtrate before diluting with water and extracting with dichloromethane. The organics were dried ($MgSO_4$) and the solvent evaporated to give **22** (872 mg, 90%) as a colourless oil. δ_H (300 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 1.41 (9H, s, 3 × CH_3), 142–1.58 (4H, m, 2 × CH_2), 160–1.69 (2H, m, CH_2), 230–2.61 (2H, br, NH_2), 2.67 (2H, t, *J* 6.6, CH_2NH_2), 3.00–3.31 (6H, m, 3 × CH_2), 4.57–4.81 (1H, br, amide-NH); δ_C (75 MHz, $CDCl_3$) 25.49 (C-6), 27.15 (C-7), 28.16 (CH_3), 28.20 (CH_3), 30.88 (C-2), 38.55 (C-1), 39.95 (C-8), 43.63 (C-3), 46.32 (C-5), 78.91 (C), 79.31 (C), 156.03 (C=O); *m*/*z* (ESI) 346 (100%, M + H^+).

cis-3,3-(ethylenedioxy)-8-((*E*)-2-{3-[*N*-(*tert*-butoxycarbonyl)-*N*-(4-*tert*-butoxycarbonylaminobutyl)amino]propylcarbamoyl}-vinyl)-9b-[(*E*)-ethoxycarbonylvinyl]hexahydrodibenzo[*b*,*d*]furan 23

A solution of **20** (800 mg, 1.906 mmol), **5** (870 mg, 2.178 mmol), palladium acetate (44 mg, 0.192 mmol), tri-*o*-tolylphosphine (170 mg, 0.559 mmol) and triethylamine (0.5 cm^3, 3.578 mmol) in DMF (6 cm^3) was stirred at 60 °C under argon overnight. The reaction mixture was then quenched with water and extracted four times with diethyl ether. The combined organics were dried (Na_2SO_4) and the solvent was evaporated to give a brown gum. Purification by chromatography [petrol–EtOAc (2 : 8) eluent] failed to separate the product from the unconsumed starting material **5** so product fractions were re-chromatographed on a finer grade of silica (Merck silica gel 60, Merck No. 1.07729) (CH_2Cl_2–MeOH (98 : 2) eluent) to give **23** as a foamy white solid (975 mg, 70%). ν_{max}(CH_2Cl_2)/cm^{-1} 3500–3200 (br, NH), 1713, 1699, 1694 C=O, overlapping); δ_H (300 MHz, $CDCl_3$) 1.22 (3H, t, *J* 7.1, CH_3), 1.37 (9H, s, 3 × CH_3), 140 (9H, s, 3 × CH_3), 1.24–1.70 (8H, m, 4 × CH_2), 1.93–2.12 (4H, m, 2 × CH_2), 2.94–3.38 (8H, m, 4 × CH_2), 3.80–3.96 (4H, m, –OCH_2CH_2O–), 4.13 (2H, q, *J* 7.1, CH_2), 4.71 (1H, t, *J* 5.5, H-4a), 4.80–4.90 (1H, br, carbamate NH), 5.74 (1H, d, *J* 15.9, =CH-11), 6.30 (1H, d, *J* 15.6, =CH-14), 6.79 (1H, d, *J* 8.3, H-6), 6.99 (1H, d, *J* 15.9, =CH-10), 7.03–7.12 (1H, br, amide NH), 7.18, (1H, s, H-9), 7.27 (1H, d, *J* 8.3, H-7), 7.49 (1H, d, *J* 15.6, =CH-13); δ_C (75 MHz, $CDCl_3$) 13.98 (CH_3), 25.48, 27.19, 27.53 (3 × CH_2), 28.19 (CH_3), 28.74 (C-1), 30.06 (C-2), 35.60 (CH_2), 35.91 (C-4), 39.81, 43.16, 46.42 (3 × CH_2), 49.24 (C-9b), 60.40 (CO_2CH_2), 63.90, 64.40 (OCH_2CH_2O), 78.81 (C), 79.53 (C), 87.09 (C-4a), 107.02 (C-3), 111.00 (C-6), 118.88 (C-14), 121.23 (C-11), 122.22 (C-9), 128.53 (C-8), 129.93 (C-7), 132.58 (C-9a), 139.74 (C-13), 149.92 (C-10), 155.88 (C=O), 156.28 (C-5a), 159.75 (C=O), 166.00 (C=O); *m*/*z* (ESI) 729 (45%, M + H^+), 629 (40, M − ^{t}BuOCO), 529 (80, M − 2^{t}BuOCO), 383 (100, M − $NH(CH_2)_3N(Boc)(CH_2)_4NH(Boc)$).

cis-3,3-(ethylenedioxy)-8-((*E*)-2-{3-[*N*-(*tert*-butoxycarbonyl)-*N*-(4-*tert*-butoxycarbonylaminobutyl)amino]propylcarbamoyl}-vinyl)-9b-[(*E*)-carboxyvinyl]hexahydrodibenzo[*b*,*d*]furan (24)

A solution of **23** (304 mg, 0.418 mmol) and lithium hydroxide

(400 mg, 9.13 mmol) in 60% aqueous ethanol (10 cm^3) was stirred for 8 h at room temperature. 5% Aqueous citric acid was then added dropwise until a milky emulsion formed at pH 7. The emulsion was extracted with ethyl acetate and the combined extracts were washed with H_2O, dried ($MgSO_4$) and the solvent was evaporated to give **24** as a white foamy solid (257 mg, 88%). δ_H (300 MHz, $CDCl_3$, 323 K) 1.43 (9H, s, 3 × CH_3), 146 (9H, s, 3 × CH_3), 1.48–2.18 (12H, m, 6 × CH_2), 3.04–3.42 (8H, m, 4 × CH_2), 3.81–4.02 (4H, m, OCH_2CH_2O), 4.76 (1H, t, *J* 5.3, H-4a), 5.81 (1H, d, *J* 15.9, =CH-11), 6.31 (1H, d, *J* 15.6, =CH-14), 6.82 (1H, d, *J* 8.3, H-6), 7.09 (1H, d, *J* 15.9, =CH-10), 7.21 (1H, s, H-9), 7.32 (1H, d, *J* 7.3, H-7), 7.54 (1H, d, *J* 15.6, =CH-13); δ_C (75 MHz, $CDCl_3$) 25.61, 27.28, 27.62 (3 × CH_2), 28.33 (CH_3, ^{t}Bu), 28.96 (C-1), 30.20 (C-2), 35.79 (C-4), 35.91 (CH_2), 39.99, 43.42, 46.60 (3 × CH_2), 49.41 (C-9b), 64.04, 64.59 (OCH_2CH_2O), 79.18 (C), 79.87 (C), 87.24 (C-4a), 107.22 (C-3), 111.16 (C-6), 118.74 (C-14), 121.34 (C-11), 122.55 (C-9), 128.65 (C-8), 130.04 (C-7), 132.73 (C-9a), 140.31 (C-13), 151.13 (C-10), 156.09 (C=O), 156.49 (C=O), 159.94 (C-5a), 166.59 (C=O), 169.07 (C=O); *m*/*z* (ESI) 722 (52%, M + Na^+), 700 (50, M + H^+), 600 (100, M + H − ^{t}BuOCO).

(±)-Lunarine (1) and (±)-3,3-(ethylenedioxy)lunarine (25)

A solution of **24** (200 mg, 0.286 mmol), in dichloromethane (8 cm^3) was cooled to 0 °C and then treated with a solution of EDC (63 mg, 0.329 mmol), DMAP (2 mg, 0.016 mmol) and pentafluorophenol (63 mg, 0.343 mmol) in dichloromethane (1 cm^3). This was left to stir at room temperature overnight. The reaction mixture was then washed with three portions of saturated aqueous $NaHCO_3$, followed by brine, dried ($MgSO_4$) and the solvent was evaporated to give the crude pentafluorophenol ester as a pale brown solid. The *tert*-butoxycarbonyl groups were then removed by dissolving the pentafluorophenol ester in a 4 M solution of HCl in anhydrous dioxane (10 cm^3), which was left to stir at room temperature for 20 min. The solvent was then evaporated off and the residue co-evaporated several times with dichloromethane to give a pale yellow–brown foam. This was re-dissolved in dichloromethane (10 cm^3) before slowly being added dropwise, over 20 min, to a solution of DIPEA (1.5 cm^3, 8.61 mmol) in dichloromethane (300 cm^3) and the mixture was left to stir at room temperature overnight. The reaction mixture was then concentrated to 50 cm^3, washed with two portions of saturated aqueous $NaHCO_3$ followed by brine, dried (Na_2SO_4) and the solvent was then evaporated to give a solid. This was purified by chromatography (eluting with a 5–12% gradient of ethanol (saturated with ammonia) in chloroform) to give **1** as a white solid (35 mg, 28%). Some of the uncleaved dioxolane **25** was also isolated as a white solid (15 mg, 11%). **1**: δ_H (300 MHz, d_6-DMSO) 1.42–1.71 (7H, m), 2.04–2.15 (1H, m), 2.28–2.53 (5H, m), 2.70–2.82 (2H, m), 3.03–3.23 (3H, m), 3.44–3.55 (2H, m), 5.25 (1H, d, *J* 2.9, H-4a), 6.23 (1H, d, *J* 15.5, =CH-24), 6.37 (1H, d, *J* 15.8, =CH-11), 6.88 (1H, d, *J* 8.1, H-6), 6.93 (1H, *J* 15.8, =CH-10), 7.36 (1H, d, *J* 8.3, H-7), 7.40 (1H, s, H-9), 7.43 (1H, d, *J* 15.5, =CH-25), 8.01–8.08 (1H, br, amide-NH), 9.04–9.11 (1H, br, amide-NH); δ_C (75 MHz, d_6-DMSO) 26.72, 26.12, 28.15 (C-1, C-15, C-16), 31.22 (C-20), 35.38 (C-2), 38.48, 41.31 (C-14, C-21), 49.52 (C-9b), 50.61 (C-4, C-17, C-19 overlapping), 88.31 (C-4a), 109.67 (C-6), 121.40 (C-24), 121.63 (C-9), 125–70 (C-11), 129.09 (C-8), 132.21 (C-7), 133.36 (C-9a), 138.17 (C-25), 145.29 (C-10), 160.36 (C-5a), 164.32 (amide-C=O), 164.83 (amide-C=O), 208.62 (ketone-C=O); *m*/*z* (ESI) 438 (100%, $[MH]^+$). **25**: δ_H (300 MHz, d_6-DMSO) 1.40–1.70 (9H, m), 1.80–2.05 (2H, m), 2.25–2.55 (6H, m), 2.65–2.75 (2H, m), 2.95–3.10 (1H, m), 3.75–3.95 (4H, m, OCH_2CH_2O), 4.76 (1H, br, H-4a), 6.15 (1H, d, *J* 15.5, =CH-24), 6.43 (1H, d, *J* 15.8, =CH-11), 6.71 (1H, d, *J* 15.8, =CH-10), 6.85 (1H, *J* 8.1, H-6), 7.23 (1H, d, *J* 1.7, H-9), 7.28 (1H, dd, *J* 8.1, 1.7, H-7), 7.36 (1H, *J* 15.5, =CH-25), 8.00–8.08 (1H, br, NH), 8.47–8.55 (1H, br, NH), 9.09–9.16 (1H, br, NH); δ_C (75 MHz, d_6-DMSO) 26.59, 27.14, 28.21 (C-1, C-15, C-16), 30.55, 30.73 (C-2, C-20), 34.84 (C-4), 38.47, 41.40 (C-14, C-21), 48.84 (C-9b), 50.66 (C-17, C-19 overlapping), 63.66, 64.38 (OCH_2CH_2O), 89.42 (C-4a), 107.06 (C-3), 110.23 (C-6), 119.93 (C-9), 120.68 (C-24), 126.67 (C-11), 128.30 (C-8), 131.87 (C-7), 136.84 (C-9a), 138.27 (C-25), 142.07 (C-10), 159.87 (C-5a), 164.31 (C=O), 164.83 (C=O); *m*/*z* (ESI) 482 (100%, M + H^+).

Crystal data § for *cis*-3-oxo-8-bromo-9b-cyano-1,2,3,4,4a,9b-hexahydrodibenzo[*b*,*d*]furan (14)

Crystals of **14** were grown from dichloromethane. $C_{13}H_{10}BrNO_2$, M = 292.13. Triclinic, a = 7.5226(15), b = 9.1659(18), c = 9.3917(19) Å, α = 84.65(3), β = 74.43(3), γ = 66.85(3)°, V = 573.5(2) $Å^3$, T = 150(2) K, space group $P\bar{1}$, Z = 2, D_x = 1.692 g cm^{-3} μ = 3.57 mm^{-1}, 6708 reflections collected, 3033 independent reflections, R_1 = .0452 [$I > 2\sigma(I)$], wR_2 (F^2) = 0.1231. Data processing was carried out using the DENZO,[29] COLLECT,[30] and SORTAV[31] and SHELX[32] software packages. Fig. 2 was produced using PLATON solution and refinement software.[33] The cyclohexanone ring C-1,2,3,4,4a,9b is in a skew boat conformation. Taking the plane C2,3,4a,9b the average deviation of those atoms from the plane is 0.13 Å; C-1, C-4 and the CN group on C-9b are all on one side of the plane, at −0.62, −0.53 and −1.84 (N-9b) Å respectively. C-9b and O-5 are at 0.002 Å from the plane of the benzene ring. C-4a is −0.330 Å from that plane. H-4a and the CN group are both axial.

Acknowledgements

We would like to thank Dr C. Poupat (Institute de Chemie des Substances Naturelles, CNRS, Gif-sur-Yvette, France) for the generous gift of an authentic sample of (+)-lunarine, Dr J. C. Barnes (School of Life Sciences, University of Dundee) for assistance with the crystallographic studies, Professor M. Hursthouse for data collection at the EPSRC X-Ray Crystallographic Service, University of Southampton and the UNDP/WORLD BANK/WHO (Special Programme for Research and Training in Tropical Diseases) for financial support for C. J. H.

§ CCDC reference number 174011. See http://www.rsc.org/suppdata/p1/b1/b110149h/ for crystallographic files in .cif or other electronic format.

References

1 A. H. Fairlamb and A. Cerami, *Ann. Rev. Microbiol.*, 1992, **46**, 695.
2 (*a*) C. Dumas, M. Ouellette, J. Tovar, M. Cunningham, A. H. Fairlamb, S. Tamar, M. Olivier and B. Papadopoulou, *EMBO J.*, 1997, **16**, 2590; (*b*) J. Tovar, M. L. Cunningham, A. C. Smith, S. L. Croft and A. H. Fairlamb, *Proc. Nat. Acad. Sci. USA*, 1998, **95**, 5311.
3 S. Krieger, W. Schwarz, M. R. Aryinayagam, A. H. Fairlamb, R. L. Krauth-Siegel and C. Clayton, *Mol. Microbiol.*, 2000, **35**, 542.
4 (*a*) J. A. Ponasik, C. Strickland, C. Faerman, S. Savvides, P. A. Karplus and B. Ganem, *Biochem. J.*, 1995, **311**, 371; (*b*) M. O'Sullivan, Q. B. Zhou, Z. L. Li, T. B. Durham, D. Rattendi, S. Lane and C. J. Bacchi, *Bioorg. Med. Chem.*, 1997, **7**, 2145; (*c*) B. Chitkul and M. Bradley, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2367; (*d*) D. Horvath, *J. Med. Chem.*, 1997, **40**, 2412; (*e*) C. Chen, H. Yin, J. Garforth, J. H. Mckie, R. Jaouhari, P. Speers, K. T. Douglas, P. J. Rock, V. Yardley, S. L. Croft and A. H. Fairlamb, *J. Med. Chem.*, 1998, **41**, 148; (*f*) C. H. Faerman, S. N. Savvides, C. Strickland, M. A. Breidenbach, J. A. Ponasik, B. Ganem, D. Ripoll, R. L. Krauth-Seigel and P. A. Karplus, *Bioorg. Med. Chem.*, 1996, **4**, 1247; (*g*) S. Girault, E. Davioud-Charvet, L. Salmon, A. Berecibar, M.-A. Debreu and C. Sergerhaert, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1175.
5 C. S. Bond, Y. Zhang, M. Berriman, M. L. Cunningham, A. H. Fairlamb and W. N. Hunter, *Structure*, 1999, **7**, 81.
6 S. Sagner, Z.-W. Shen, B. Deus-Neumann and M. H. Zenk, *Phytochemistry*, 1998, **47**, 375.
7 H.-P. Husson, C. Poupat, B. Rodriguez and P. Potier, *Tetrahedron*, 1973, **29**, 1405.

8 Y. Nagao, S. Takao, T. Miyasaka and E. Fujita, *J. Chem. Soc., Chem. Commun.*, 1981, 286.
9 J. M. Vierfond, A. Reynet, H. Moskowitz and C. Thal, *Synth. Commun.*, 1992, **22**, 1783.
10 (*a*) R. Pummerer, H. Puttfarcken and P. Schopflocher, *Chem. Ber.*, 1925, **58**, 1808; (*b*) D. H. Barton, A. M. Deflorin and O. E. Edwards, *J. Chem. Soc.*, 1956, 530.
11 (*a*) S. Harusawa, R. Yoneda, T. Kurihara, Y. Hameda and T. Shiori, *Chem. Pharm. Bull.*, 1983, **31**, 2932; (*b*) S. Harusawa, R. Yoneda, T. Kurihara, Y. Hameda and T. Shiori, *Tetrahedron Lett.*, 1984, **25**, 427.
12 S. Danishefsky, T. Kitahara, C. F. Yan and J. Morris, *J. Am. Chem. Soc.*, 1979, **44**, 6996.
13 P. E. Vornman, *J. Org. Chem.*, 1990, **55**, 3693.
14 A. M. Bernard, M. R. Ghiani, P. P. Piras and R. Rivoldini, *Synthesis*, 1989, 287.
15 (*a*) J. M. Lasinger and R. C. Ronald, *Synth. Commun.*, 1979, **9**, 341; (*b*) P. R. Brooks, M. C. Wirtz, M. G. Vetelino, D. M. Rescek, G. F. Woodworth, B. P. Morgan and J. M. Coe, *J. Org. Chem.*, 1999, **64**, 9719.
16 S. F. Martin and P. J. Garrison, *J. Org. Chem.*, 1982, **47**, 1513.
17 R. Boss and R. Schefford, *Angew. Chem., Int. Ed. Engl.*, 1976, **15**, 558.
18 J. Gigg and R. Gigg, *J. Chem. Soc. C*, 1968, 1903.
19 H. Kunz and H. Waldmann, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 71.
20 N. L. Allinger, H. M. Blatter, L. A. Freiberg and F. M. Karkowski, *J. Am. Chem. Soc.*, 1966, **88**, 2999.
21 (*a*) J. A. D. Jeffreys and G. Ferguson, *J. Chem. Soc. B*, 1970, **5**, 826; (*b*) C. Tamura and G. A. Sim, *J. Chem. Soc. B*, 1970, **5**, 991.
22 (*a*) T. Tsunoda, M. Suzuki and R. Noyori, *Tetrahedron Lett.*, 1980, **21**, 1357; (*b*) J. R. Hwu, L.-C. Leu, J. A. Robl, D. A. Anderson and J. M. Wetzel, *J. Org. Chem.*, 1987, **52**, 188.
23 W. S. Wadsworth, *Org. React.*, 1977, **25**, 73.
24 M. Humora and J. Quick, *J. Am. Chem. Soc.*, 1979, **44**, 1165.
25 (*a*) R. J. Bergeron and J. R. Garlich, *Synthesis*, 1984, 782; (*b*) R. J. Bergeron, J. R. Garlich and N. J. Stolowich, *J. Org. Chem.*, 1984, **49**, 2997.
26 R. F. Heck, *Org. React.*, 1979, **27**, 345.
27 C. J. Hamilton, A. Saravanamuthu, A. H. Fairlamb and I. M. Eggleston, in preparation.
28 A. B. Sen and S. S. Parmar, *J. Indian Chem. Soc.*, 1953, **30**, 59.
29 DENZO: Data collection and processing software; Z. Otwinowski and W. Minor, in *Methods in Enzymology*, ed. C. W. Carter Jr, and R. M. Sweet, Academic Press, New York, 1997, vol. 276, p. 307.
30 COLLECT: Data collection and processing user interface: Collect: Data collection software, R. Hooft, Nonius B. V., 1998.
31 SORTAV: Absorption correction software package: R. H. Blessing, *Acta Crystallogr. Sect. A*, 1995, **51**, 33–37; R. H. Blessing, *J. Appl. Cryst.*, 1997, **30**, 421.
32 PLATON: Multi-purpose crystallographic tool A. L. Spek, *Acta Crystallogr. Sect. A*, 1990, **46**, C34.
33 SHELX: Solution and refinement software: G. M. Sheldrick, SHELXL-97. Program for refinement of crystal structures, University of Gottingen, Germany, 1997.