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Design and preparation of sterol mimetics as potential antiparasitics

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ABSTRACT

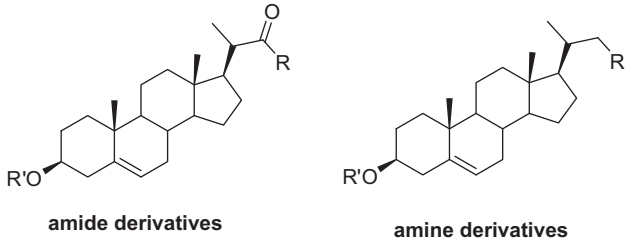
We have previously shown that azasterols have activity against *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* species, which are the causative agents of various neglected tropical diseases. In this paper, we discuss the replacement of the sterol core of the azasterols with sterol mimics. Various mimics were designed, and the structures were minimised to see if they could adopt a similar conformation to that of the azasterols. From this, two series of mimics were synthesised and then evaluated against the parasites. Compounds showed moderate activity.

1. Introduction

Parasitic infections caused by the trypanosomatid family, are a major health problem in many parts of the world, particularly in tropical countries. Protozoan parasites of this family give rise to human African trypanosomiasis (*Trypanosoma brucei* spp.), Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* spp.).[1] There is a need for the development of new drugs to treat these diseases, as the current drugs available are inadequate for a variety of different reasons, including costs, efficacy and side effects.

We have reported on azasterols as having activity against the parasites which cause these diseases.[2–7] Examples of the class of compounds that we have prepared are shown in Figure 1. The design of these molecules was predicated on the discovery that azasterols can inhibit the enzyme 24-sterol methyltransferase in yeast and various plants.[8–12] This enzyme is involved in the biosynthesis of ergosterol and other related 24-alkylated sterols that are found in yeast and plants and also in the trypanosomatid parasites. We have further developed these initial leads in our laboratories.[2–6] Subsequent work has shown that the mode of action of these compounds is not (only) through inhibition of sterol 24-methyltransferase, but includes other modes of action. One particular case is that blood stream form *T. brucei* is reported to scavenge cholesterol from the host rather than biosynthesise ergosterol; hence inhibition of sterol 24-methyltransferase should not have any effect on the growth of this life-stage form of *T. brucei*.[13,14] However some of the azasterols that we have investigated have a potent effect on the growth of *T. brucei*.[2–4] The most potent compound that was discovered was compound **A** which had an EC_{50} of 12 nM for blood stream form *T. brucei rhodesiense* (Fig. 2).[2,3] Interestingly, we discovered that sterol 24-methyltransferase is expressed in blood stream form *T. brucei*, although these azasterols were not inhibitors of this enzyme.[2,3]

The pharmacophore that we have discovered for the azasterols is shown in Figure 2: there should be an acetate group at the 3β-position and on the side chain, linked either via an amine and a chain length of 3 carbon atoms, a carboxy ester. We were interested to investigate if the sterol molecule was a rigid spacer or whether it had some particular role/interaction in the mode of action of these compounds. Therefore, we decided to investigate replacements of the sterol nucleus. By changing the sterol nucleus to a mimic, it might be possible to manipulate the physicochemical and pharmacokinetic properties of the molecules.



R = NHbutyl, piperidyl, $NHCH_2Ph$, $NH(CH_2)_nNH_2$ (n=2,4,6,8,10)
$NH(CH_2)_nCOOMe$, $NH(CH_2)_nCOOH$, (n= 1-7)

R' = H, Ac

Figure 1. Examples of azasterols that have been prepared in our research group.

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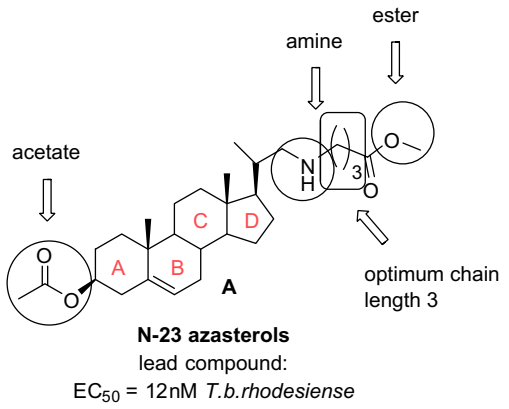


Figure 2. The pharmacophore for activity of the azasterols for anti-trypanosomal and anti-leishmanial activity.

Development of sterol mimics is an attractive field, particularly in the treatment of cancer and hormone regulation. A number of different sterol mimics have been reported; a selection are referenced.[15–24] In this paper, we report the design, synthesis and evaluation of some sterol mimics for our azasterols. In the approach that we have adopted, the B and C rings of the lead azasterol **A** were removed (Fig. 2) and replaced by spacers intended to mimic the planarity of the sterol nucleus and maintain the relative positions of the acetate in the 3β-position and the side chain at position 20 (Fig. 3).

2. Molecular modelling

In order to find a replacement for the sterol nucleus, that retained the other functional groups in the correct relevant orientations, a modelling approach was adopted. This involved designing new analogues, carrying out an energy minimisation and then superimposing this on the low energy conformation of the most potent azasterol **A**.

A selection of the new analogues designed is shown in Figure 4. These analogues were designed as follows:

- The 'A' ring was replaced by a phenyl ring. The acetate in the 3β-position was either attached directly to the sterol nucleus, or via methylene linker. The latter should have greater chemical stability, as phenoxide is a good leaving group.
- The 'B' and 'C' rings were replaced by amide or urea linkages.
- The 'normal' and 'inverse' amides were used.
- The 'D' ring was either retained or replaced by a phenyl ring.
- A slight variation in the linker to the side chain was allowed.
- In one case the 'B' ring was retained.

2.1. Conformational analysis and superimposition

Studies of conformational analysis were carried out on the structure of **A** and on those of the proposed derivatives, in order to predict whether the conformation of the designed mimics could be superimposed upon the conformation of the lead compound **A**. This assumes that the active conformation of the azasterols such as **A** is at an energetic minimum.

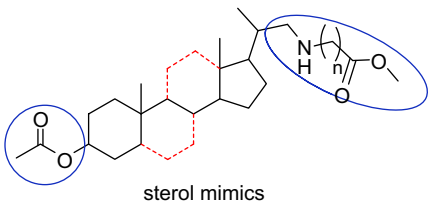


Figure 3. New analogues to mimic the azasterol compounds.

The lowest energy conformations were generated in vacuo using Moloc,[25] which uses the MAB force-field to calculate descriptions of molecular geometries and energies. The search for the minimized conformations was carried out using a stochastic run: with this method, the atomic positions were randomly varied, starting from the last minimized structure, in order to obtain a library of conformations and identify that which corresponds to the energetic minimum. The minimised structures obtained using Moloc were imported into Sybyl 7.2 and further minimised, correcting for the presence of water using a dielectric constant. The conformations thus obtained were superimposed with the similarly obtained conformation for azasterol **A**. Atoms superimposed are shown in Figure 5.

As expected, the urea derivatives **10** and **11** and the tricyclic mimic **9** were predicted to be planar. These superimposed well with the predicted low energy conformation of azasterol **A** (Fig. 6). One of the benzanilide derivatives **7** also gave a good superimposition with **A**, with the amide in the predicted *trans* (*E*) conformation (Fig. 6C). In this minimised conformation, the ester is shown as π-stacking with one of the phenyl rings; this probably is a consequence of the energy initially being calculated in vacuo. In an aqueous environment, the ester group would be solvated, which would probably disrupt this conformation.

Interestingly the other benzanilides gave a *cis* (*Z*) conformation about the amide bond (Fig. 6D). In this *cis* conformation the two phenyl rings were twisted slightly out of plane, presumably to prevent steric clashes between each other. The *cis* conformation was not planar and did not superimpose onto the planar structure **A**. The reason for this *cis* conformation is unknown. It may be an artefact, as this does not seem a reasonable conformation. One possibility is that a potential π-cation stacking interaction between the protonated amine and phenyl ring 'A' might be driving this conformation. To investigate this, the substituents on the mimics were removed and only the cores were minimised (Fig. 7). Similar results were obtained, with the benzanilides giving a *cis* conformation.

As a consequence of the modelling and the potential complexity of the synthetic chemistry, it was decided to proceed with synthesis of the benzanilide **7** and the phenyl-urea-pyrrolidine **11**. These were predicted to mimic the conformation of the sterol nucleus and the directionality of the side chains from the sterol nucleus, although not perfectly the conformations of the side chains.

3. Chemistry

The synthesis of benzanilide **7** and phenyl-urea-pyrrolidine **11** compounds was carried out. In accordance with previous studies, where the best activity was found for analogues with a side chain between 3 and 5 carbons, and to keep the main features of lead **A**, initially only derivatives with 3 or 5 carbons side chain were planned.

3.1. Benzanilide derivatives

The synthetic pathway followed for the synthesis of benzanilide derivatives is shown in Scheme 1. 4-(Hydroxymethyl)-benzoic acid was acetylated with acetic anhydride, introducing the acetyl group. This was then coupled to 4-aminophenyl ethanol. The hydroxyl group was silyl protected prior to the coupling reaction, to ensure coupling to the acid **15** was through the amine rather than the hydroxyl group. The coupling of acid **15** and amine **16** was carried out using TBTU and HOBT,[26] to give the required benzanilide **17**. The yield of this reaction was lowered by concomitant hydrolysis of

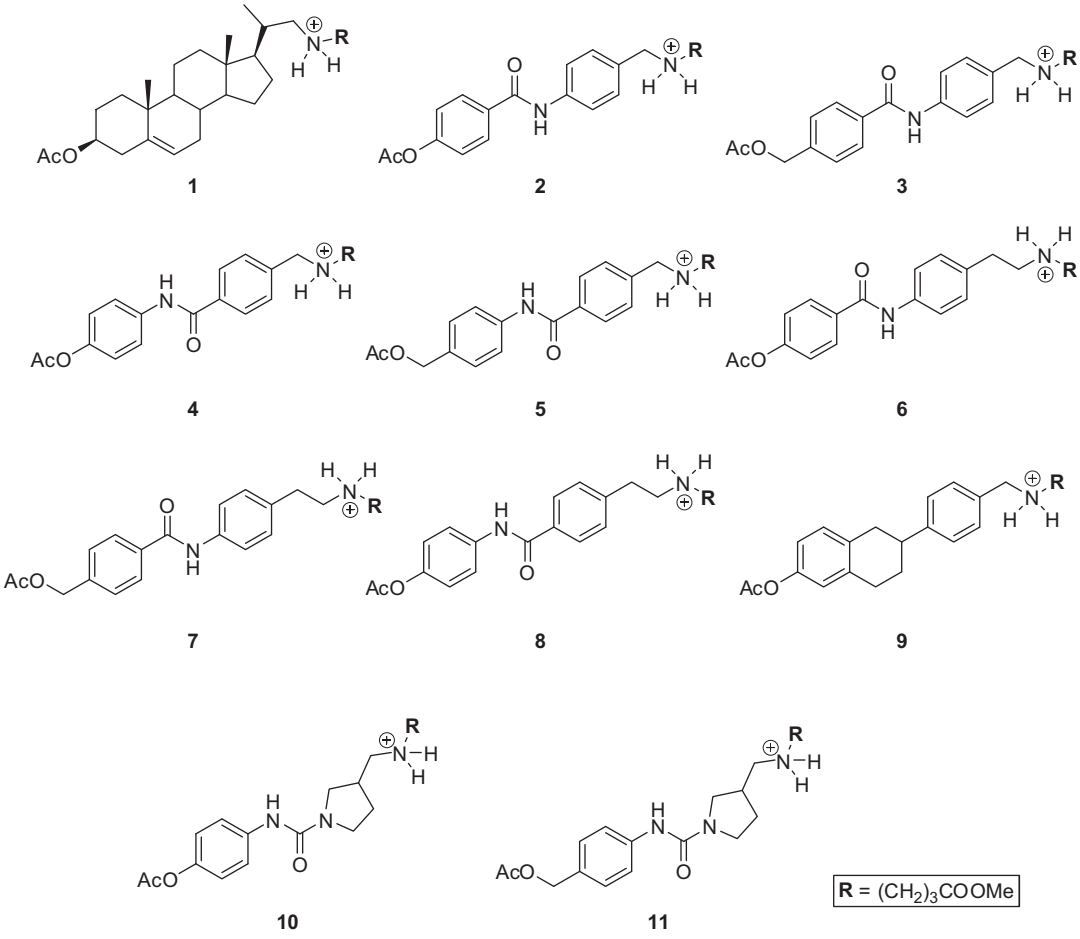


Figure 4. Key analogues minimised.

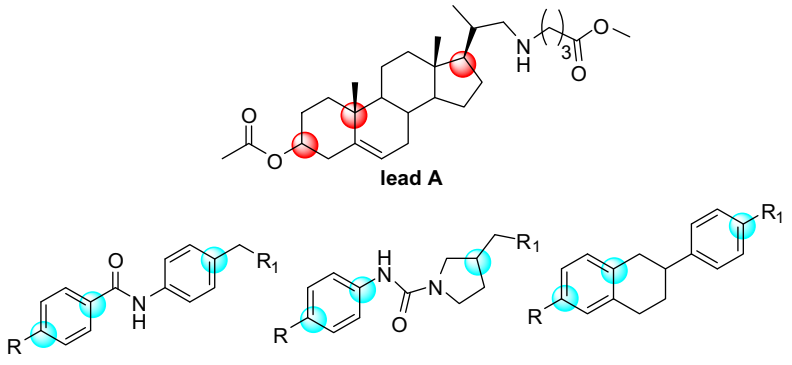


Figure 5. Points over-layed in the superimposition analysis.

the silyl group during coupling. The silyl group was then removed using TBAF.

The alcohol **18** was then oxidized to aldehyde, in order to attach the side chain through a reductive amination. Oxidation with the Dess–Martin periodinane, gave the best results for the reagents investigated. The aldehyde **19** was not purified, to avoid possible decomposition of the aldehyde, and was used crude for next step. The target molecule **7** was then prepared by reductive amination with methyl 4-aminobutyric acid **20**.

Interestingly, when the alcohol **18** was oxidised with PCC, the product recovered was the aldehyde **21**, in which loss of a methylene group was observed. Fernandes and Kumar[27] have also reported scission of the C–C bond during oxidation of a series of homobenzylic alcohols. We carried out reductive amination with this aldehyde and methyl 4-aminobutyric acid **20** to give the target molecule **3**.

In order to investigate the conformation of the product **3**, a NOESY spectrum was taken. This showed that compound **3** maintains a planar conformation, with the phenyl rings *trans* to each other. This was proved by lack of coupling between 8-H and 17-H, as it would happen in a *cis* conformation (Fig. 8); in this spectrum only the coupling of the amide proton with the 13-H/17-H was detected (see Supplementary data). The *trans*-conformation was not what was predicted from the modelling for this com-

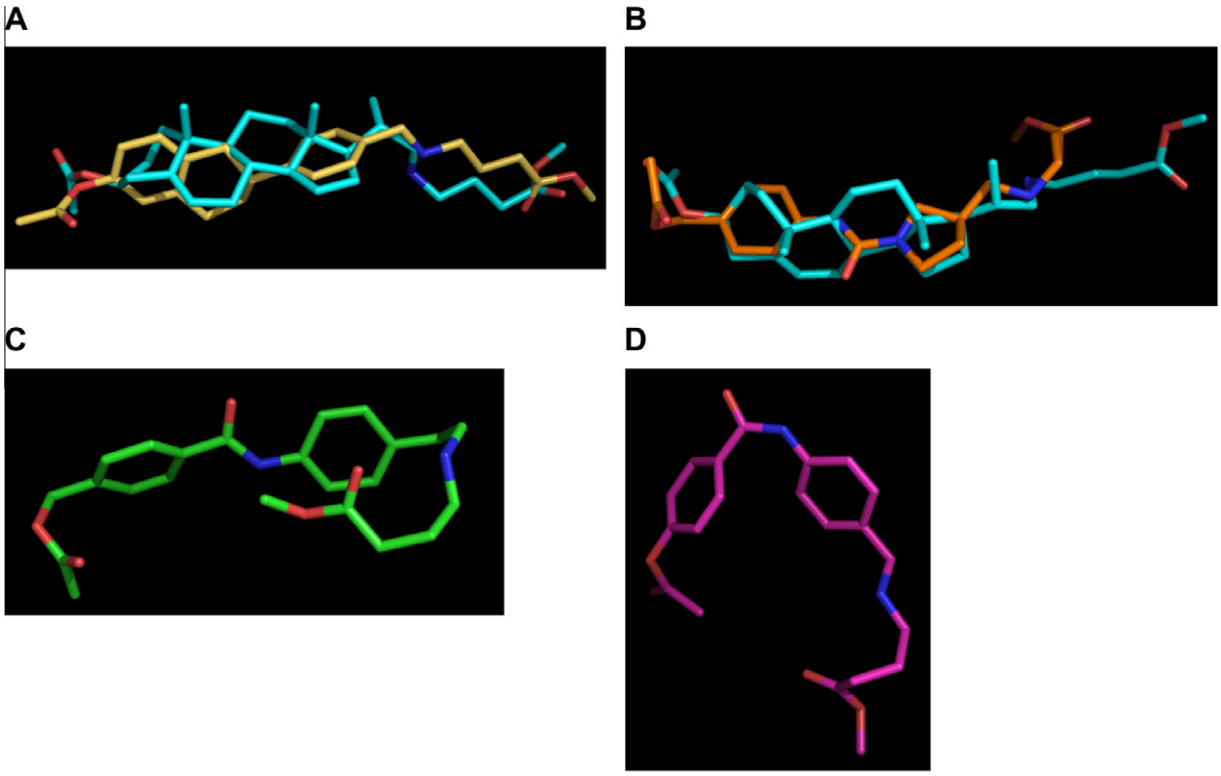
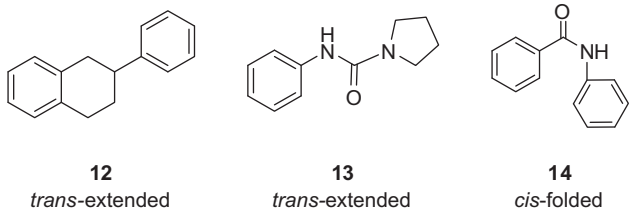


Figure 6. Minimised structures: (A) Compound **9** (yellow) superimposed with compound **A** (blue); (B) compound **11** (orange) over-layed with compound **A** (blue); (C) compound **7**; (D) compound **2**.



12 *trans*-extended **13** *trans*-extended **14** *cis*-folded

Figure 7. Core structures minimised.

pound, rather a *cis* conformation; however the *trans*-conformation was observed experimentally.

3.2. Phenyl-urea-pyrrolidine derivatives

The successful route for the preparation of the phenyl-urea-pyrrolidine analogues is outlined in Scheme 2. 4-Aminobenzyl alcohol **22** was acetylated with acetic anhydride to give the mono-acetylated derivative **23**; by careful control of conditions, it was possible to get selective acetylation of the hydroxyl group rather than the aniline. The urea **27** was then formed in two steps. Firstly the acetylated 4-aminobenzyl alcohol **23** was treated with *p*-nitrophenyl chloroformate to form the intermediate *p*-nitrophenyl carbamate **24**.[16,20] This was immediately reacted with pyrrolidine **26** (which was obtained by deprotection of the commercially available Boc-hydroxymethylpyrrolidine **25**) to give the required urea **27** in a yield of 57%.

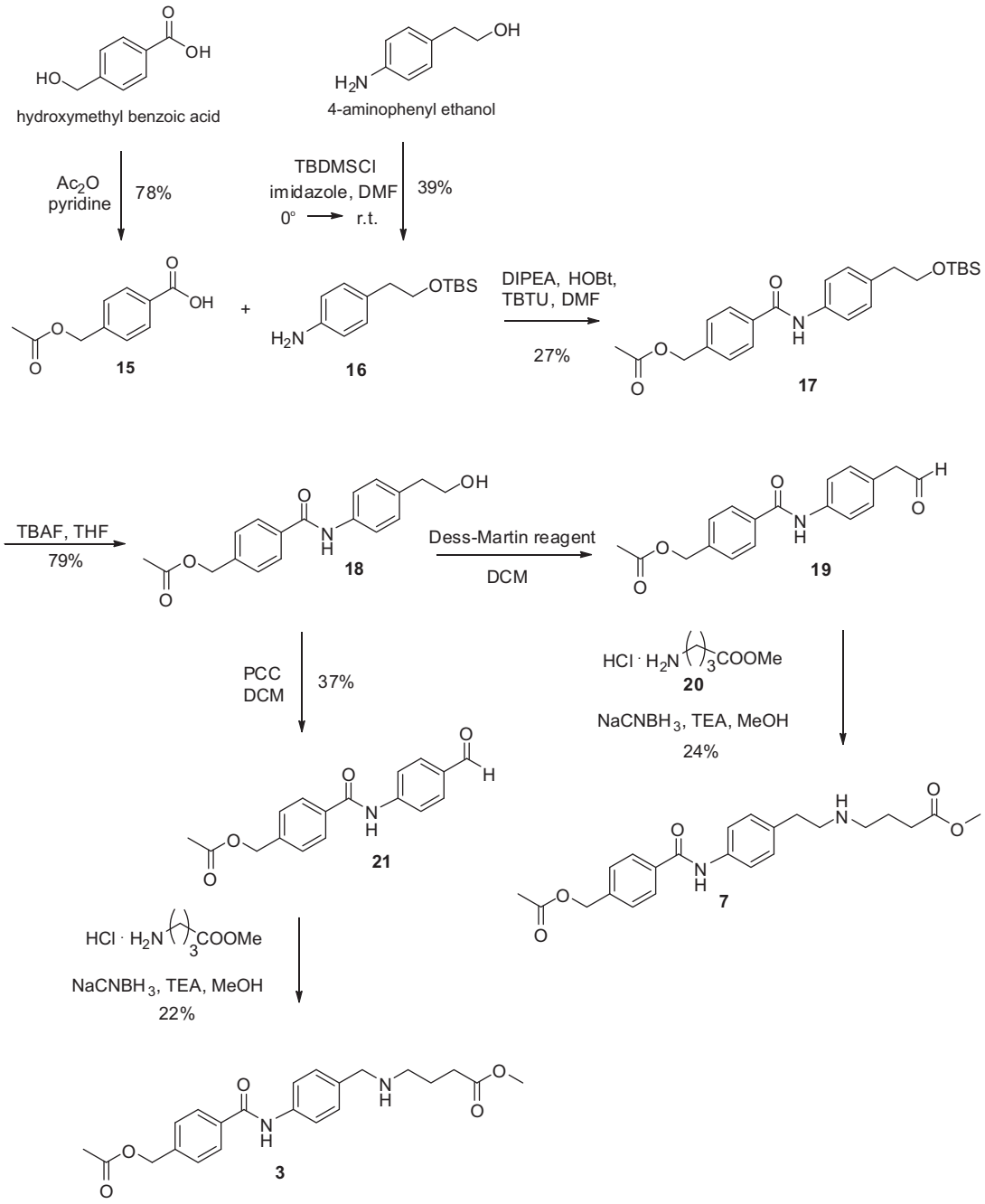
Oxidation of hydroxyl **27** with Dess–Martin periodinane gave the required aldehyde. This was then reductively aminated with either methyl 4-aminobutyric acid **20** to give the target molecule **11** with a 3 carbon linker, or with methyl 6-aminohexanoic acid **29** to give the target molecule **30** with a 5 carbon linker. In the case of **11**, the yield was lowered due to formation of the side product **31**, which occurred by lactamisation of the side chain. A similar reaction was observed previously for azasterol derivatives with a 3 carbon chain between the N and the ester group.

For these analogues, the NOESY experiment (see Supplementary data) confirmed what had been suggested by the modelling studies: no enhancement seemed to occur between the 8-H of the phenyl and the 13-H or 16-H of the pyrrolidine (Fig. 9), which supports the hypothesis that the ureas should retain a '*trans*' planar conformation, and hence be a good mimic of the sterol nucleus.

For derivatives **11**, **30** and **31** a characteristic doubling of peaks was observed in the ^{13}C NMR spectra for the carbons of the pyrrolidine. This was due to restricted rotation around the pyrrolidine ring. In order to prove this, the NMR experiment was performed at 25 °C and at 50 °C: the signals for the carbons belonging to the pyrrolidine ring and the side chain, which were split in two at room temperature, merged into one when the experiment was carried out at 50 °C, thus confirming the presence of rotational conformers for this kind of molecules.

4. Results and discussion

New benzanilide and phenyl-urea-pyrrolidine derivatives were synthesised as potential mimics of azasterol compounds which had showed activity against *trypanosomes* and *Leishmania* species.[2,3,7] For analogues **7** and **11** the modelling studies had suggested a '*trans*-extended' conformation, which should allow them to reproduce the structure and the features of the lead compound **A**. This was confirmed by NOESY spectra (see Supplementary data). Also for compound **30**, the homologue of **11**, the NOESY spectra suggested a *trans*-extended conformation, and no coupling was observed between the two rings. For analogue **3** the conformational analysis had suggested a folded conformation with the two phenyls *cis* to each other; however the NOESY experiment indicated that this compound had a planar conformation, with the two phe-



Scheme 1. Synthesis of the benzanilde analogues **3** and **7**.

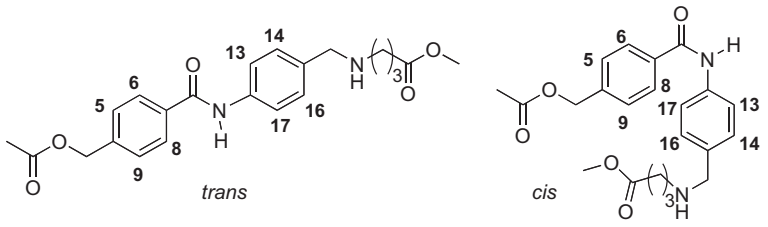
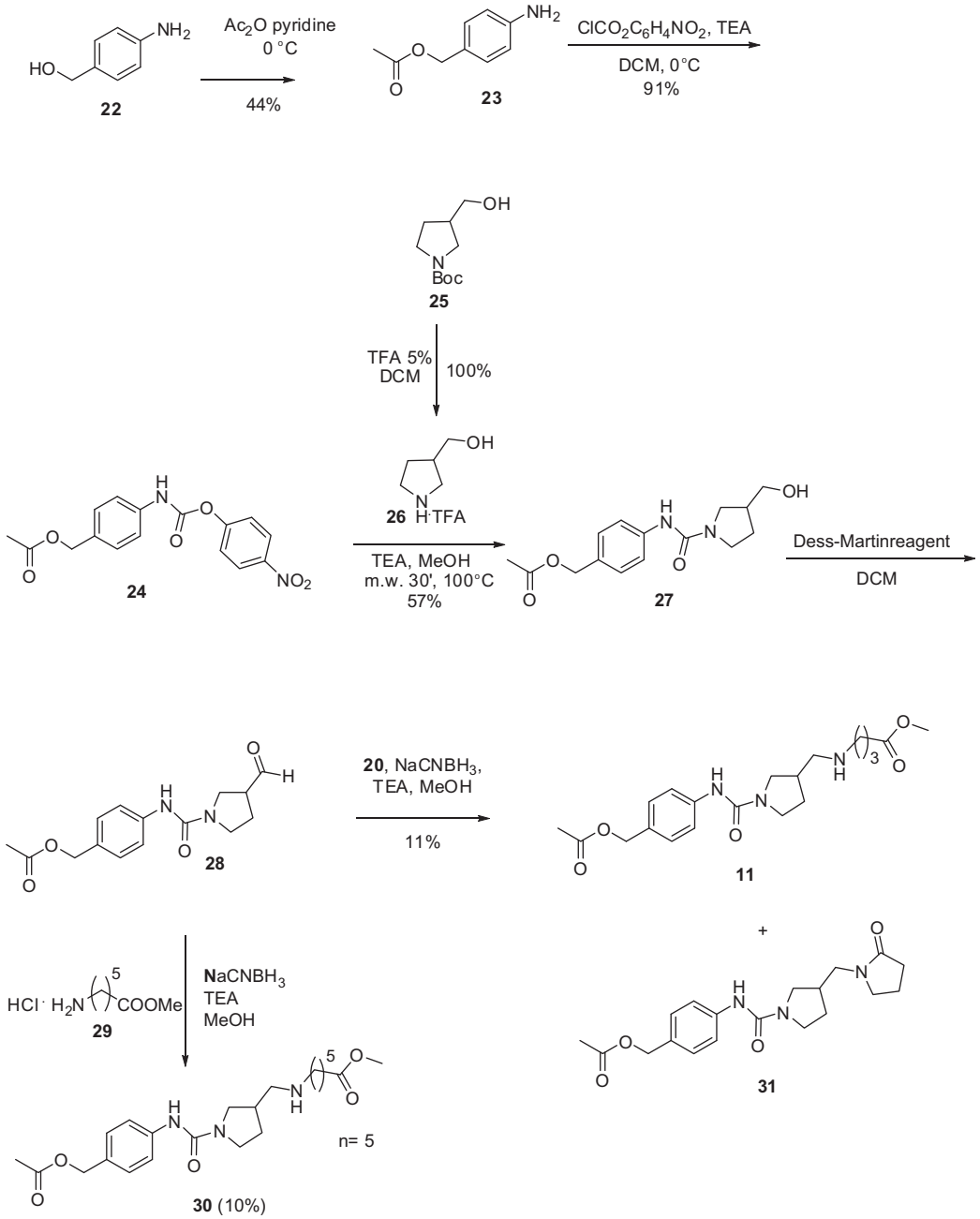


Figure 8. *trans* and *cis* conformation for benzanilide derivative **3**.

nyl rings *trans* to each other, giving a promising mimic of the lead **A**. This indicates that the modelling studies only give an indication of the conformations.

Compounds **3**, **27** and **31** were evaluated to gain further information on the SAR. The new derivatives were evaluated as growth inhibitors of the clinically relevant forms of the kinetoplastids: *T.*



Scheme 2. Synthesis of phenyl-urea-pyrrolidine analogues.

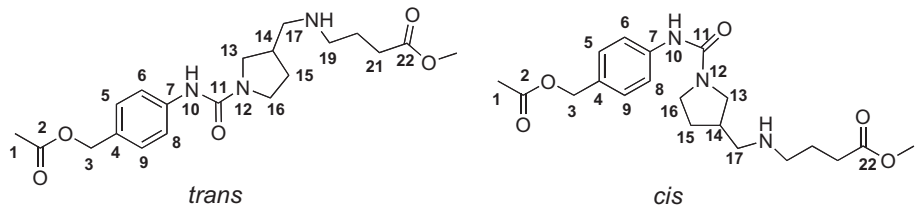
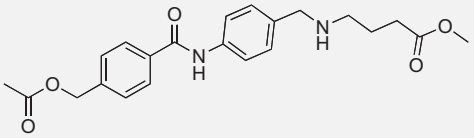
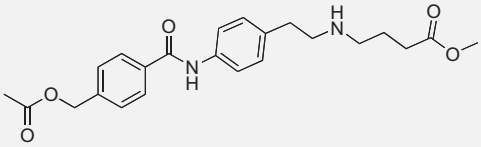
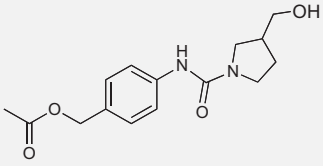
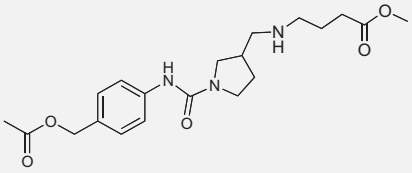
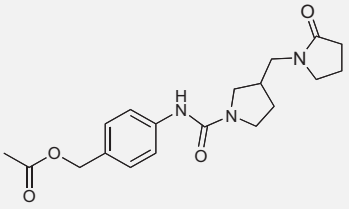
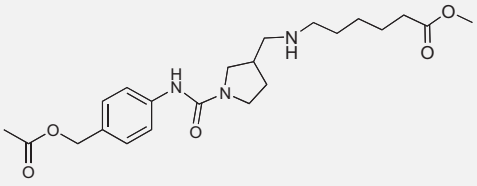


Figure 9. *trans* and *cis* conformation for the urea derivatives.

brucei rhodesiense blood stream forms; *T. cruzi* intracellular amastigotes; and *Leishmania donovani* axenic amastigotes. Compounds were also screened against L6-cells, which are rat myoblasts, as a measure of toxicity against mammalian cells. The data for this is shown in Table 1.

Unfortunately neither of the benzanilide compounds **3** or **7** showed potent antiparasitic activity. The only compound showing marginal activity against *L. donovani* was **3.** Similarly, the phenyl-urea-pyrrolidine compounds failed to give significant growth inhibition of the parasites.

Table 1
Biological results for benzanilide and phenyl-urea-pyrrolidine derivatives

		T. b. rhodesiense EC_{50} (μM)	*T. cruzi* EC_{50} (μM)	*L. donovani* EC_{50} (μM)	L6-cells TD_{50} (μM)
	Control drugs	Melarsoprol 0.01	Benznidazole 1.6	Miltefosine 0.31	Podophyl. 0.012
3		78	207	2.6	161
7		11.5	141	73	>218
27		52	>308	26	>308
11		40	>230	80	>230
31		43	181	30	>250
30		76	>215	41	>214

L6-cells are rat skeletal myoblasts and are used as an indication of toxicity to mammalian cells. Data for **A**, as reported by Gros et al.:[2] *T.b. rhodesiense*, EC_{50} 0.012 μM; *L. donovani*, EC_{50} 3.2 μM; Toxicity, L-6 cells, 19.2 μM. Controls are: *T.b. rhodesiense*, melarsoprol; *L. donovani*, miltefosine; L6-cells, podophyllotoxin.

Therefore neither the benzanilide nor the urea derivatives showed significant antiproliferative activity against the parasites, with general EC_{50} values higher than those of the lead compound **A**. This might be due to poor mimetic of the lead compound, although the modelling studies had suggested a planar conformation for these derivatives, which matched the sterol nucleus. Alternatively this might also suggest that the sterol nucleus has an important interaction with the molecular target, which does not take place with these analogues.

5. Conclusions

Using a combination of background literature, molecule modelling and chemical intuition, we developed two classes of sterol mimics. These were successfully synthesized, and NOESY spectra indicated that these mimics had similar steric properties to sterols. Unfortunately when derivatised in a similar pattern to the lead azasterol, none of these compounds gave antiparasitic activity of the same order as the lead compound **A**. This may indicate that the sterol nucleus itself may be important for the antiparasitic activity of these type of compounds, and that it is difficult to replace.

6. Experimental

6.1. General experimental details

When applicable, all glassware were oven-dried overnight and all reactions were carried out under Argon atmosphere. Sensitive liquids and solutions were transferred via syringe and were introduced into reaction vessels through rubber septa. Reagents were purchased from Aldrich or Fluka. All the reactions were carried out using dry solvents unless otherwise stated. All dry solvents, ethanol, methanol, dichloromethane, tetrahydrofuran, dimethylformamide were purchased from Aldrich or Fluka in sure sealed bottles. Analytical TLCs were performed on Silica Gel 60 F254 plates (Merck). Visualisation of spots was effected by one of the following techniques: (a) UV illumination, (b) immersion of the plate in a 3% solution of ninhydrin in ethanol followed by heating and (c) immersion of the plate in a 48 g L^{-1} solution of phospho-

molibdic acid in methanol followed by heating. Column chromatography was carried out on Silica Gel 60 (40–60 micron) purchased from Fluka.

NMR spectra were recorded on a Bruker Avance DPX 300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C or a Bruker Avance DPX 500 MHz spectrometer at 500 MHz for ^{1}H, 125 MHz for ^{13}C. Chemical shifts are reported downfield in parts per million and coupling constants (*J* values) are in Hertz. Melting points were determined with a GallenKamp melting point apparatus. Low-resolution mass measurements were performed on Applied Biosystem Mariner API-TOF. LC–MS and accurate mass measurements were performed in house with Agilent 1100 HPLC in series with Bruker MicroTof spectrometer or at EPSRC National Mass Spectrometry Service Centre in the Chemistry Department, University of Wales Swansea, Swansea, Wales, UK.

6.2. Biological in vitro assays

The in vitro assays against *T. b. rhodesiense* was carried out as described by Wenzler et al.[28] The assays for *T. cruzi*, *L. donovani* and L6-cells were performed following the procedure reported by Ganapaty et al.[29]

6.2.1. 4-(Acetoxymethyl) benzoic acid (15)

Ac_2O (1.02 mL, 10.84, 1.1 equiv) was added to a solution of commercially available hydroxymethyl benzoic acid (1.497 g, 9.84 mmol) in pyridine (10 mL), and the reaction was stirred overnight at room temperature. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (20 mL); the organic layer was washed with HCl 1 M (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure, to yield pure **15**, as a white solid (1.500 g, 78% yield); R_f = 0.53 ($CHCl_3$/MeOH: 80:20); LRMS, *m*/*z* (ES^+ mode): 217.1 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{10}H_{10}O_4$ (Theory): C, 61.85; H, 5.19. Found: C, 61.62; H, 5.11; mp = 124 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.18 (3H, s, 1-CH_3), 5.22 (2H, s, 3-CH_2), 7.49 (2H, d, *J* = 8.4, 5-CH and 9-CH), 8.14 (2H, d, *J* = 8.3, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0 (1-CH_3), 65.5 (3-CH_2), 127.8 (5-CH and 9-CH), 128.9 (7-C), 130.5 (6-CH and 8-CH), 142.0 (4-C), 170.8 (10-C=O), 171.0 (2-C=O).

6.2.2. (2-*p*-Aminophenylethoxy)-*tert*-butyldimethylsilane (16)

A solution of *tert*-butyldimethylsilyl chloride (2.703 g, 17.93 mmol, 1.2 equiv) and imidazole (1.267 g, 18.61 mmol, 1.3 equiv) in DMF (8 mL) was cooled at 0 °C under Argon, then a solution of commercially available 4-aminophenyl ethanol (2.000 g, 14.58 mmol) in DMF (8 mL) was slowly added. The reaction was allowed to warm to room temperature and stirred overnight. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (20 mL), the organic layer was washed with $NaHCO_3$ (3 × 20 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by column chromatography (Hexane/EtOAc 100:0→85:15) to afford **16** as a yellow oil (1.418 g, 39% yield); R_f = 0.33 (Hexane/EtOAc: 80:20); LRMS, *m*/*z* (ES^+ mode): 252.18 ($[M+H]^+$, 100%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.01 6H, s, 10-CH_3 and 11-CH_3), 0.88 (9H, s, 13-CH_3, 14-CH_3 and 15-CH_3), 2.72 (2H, t, *J* = 7.3, 8-CH_2), 3.56 (1H, br s, 2-NH), 3.74 (2H, t, *J* = 7.3, 9-CH_2), 6.62 (2H, d, *J* = 8.3, 3-CH and 7-CH), 6.99 (2H, d, *J* = 8.1, 4-CH and 6-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.3 10-CH_3 and 11-CH_3), 18.4 (12-C), 26.0 (13-CH_3, 14-CH_3 and 15-CH_3), 38.8 (8-CH_2), 65.0 (9-CH_2), 115.2 (3-CH and 7-CH), 129.1 (5-C), 129.9 (4-CH and 6-CH), 144.5 (2-C).

6.2.3. 4-4-2-(*tert*-Butyldimethylsilyloxy)ethylphenylcarbamoylbenzyl acetate (17)

To a solution of **15** (0.950 g, 4.89 mmol), HOBt (1.059 g, 0.78 mmol, 1.6 equiv) and TBTU (2.516 g, 7.83 mmol, 1.6 equiv) in DMF (12 mL), DIPEA (2.1 mL, 12.24 mmol, 2.5 equiv) was added and the reaction stirred and monitored by TLC. When the formation of the activated ester with HOBt was complete (after ~1 h), the amine **16** was added (1.418 g, 5.63 mmol, 1.15 equiv) and the reaction stirred overnight at room temperature. The mixture was then extracted with $CHCl_3$ (30 mL) and washed with H_2O (2 × 30 mL), the organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure. After purification by column chromatography ($CHCl_3$/MeOH 100:0→90:10), the product was collected as a yellow solid (0.473 g, 27% yield); R_f = 0.94 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 428.25 ($[M+H]^+$, 100%); Anal. Calcd for $C_{24}H_{33}NO_4Si$ (Theory): C, 67.41; H, 7.78; N, 3.28. Found: C, 67.36; H, 7.41; N, 4.23; mp = 83–86 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.01 6H, s, 20-CH_3 and 21-CH_3), 0.88 (9H, s, 23-CH_3, 24-CH_3 and 25-CH_3), 2.15 (3H, s, 1-CH_3), 2.82 (2H, t, *J* = 7.0, 18-CH_2), 3.81 (2H, t, *J* = 7.0, 19-CH_2), 5.18 (2H, s, 3-CH_2), 7.22 (2H, d, *J* = 8.5, 14-CH and 16-CH), 7.48 (2H, d, *J* = 8.3, 5-CH and 9-CH), 7.55 (2H, d, *J* = 8.1, 13-CH and 17-CH), 7.72 (1H, br s, 11-NH), 7.87 (2H, d, *J* = 8.3, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.4 20-CH_3 and 21-CH_3), 18.4 (22-C), 21.0 (1-CH_3), 26.0 (23-CH_3, 24-CH_3 and 25-CH_3), 39.0 (18-CH_2), 64.5 (19-CH_2), 65.5 (3-CH_2), 120.1 (13-CH and 17-CH), 127.3 (5-CH and 9-CH), 128.3 (6-CH and 8-CH), 129.8 (14-CH and 16-CH), 134.8 (7-C), 135.7 (12-C), 135.9 (15-C), 139.9 (4-C), 165.1 (10-C=O), 170.8 (2-C=O).

6.2.4. 4-4-(2-Hydroxyethyl)phenylcarbamoylbenzyl acetate (18)

To a solution of **17** (0.255 g, 0.60 mmol) in THF (8 mL) TBAF (0.59 mL, 0.60 mmol, 1 equiv) was added and the reaction was stirred at room temperature until the TLC showed complete disappearance of the starting material. The solvent was then removed under reduced pressure and the crude was purified by column chromatography (DCM/MeOH 100:0→85:15) to afford **18** as a white solid (0.148 g, 79% yield); R_f = 0.35 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 314.14 ($[M+H]^+$, 100%); Anal. Calcd for $C_{18}H_{19}NO_4$ (Theory): C, 68.99; H, 6.11; N, 4.47. Found: C, 68.80; H, 6.08; N, 4.82; mp = 163–164 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.14 (3H, s, 1-CH_3), 2.85 (2H, t, *J* = 7.1, 18-CH_2), 3.77 (2H, t, *J* = 7.1, 19-CH_2), 5.21 (2H, s, 3-CH_2), 7.26 (2H, d, *J* = 8.7, 14-CH and 16-CH), 7.53 (2H, d, *J* = 8.1, 5-CH and 9-CH), 7.63 (2H, d, *J* = 8.4, 13-CH and 17-CH), 7.95 (2H, d, *J* = 8.4, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 21.0 (1-CH_3), 38.7 (18-CH_2), 63.7 (19-CH_2), 65.5 (3-CH_2), 120.5 (13-CH and 17-CH), 127. 3 (5-CH and 9-CH), 128.3 (6-CH and 8-CH), 129.7 (14-CH and 16-CH), 134.7 (7-C), 134.9 (12-C), 136.3 (15-C), 140.0 (4-C), 165.2 (10-C=O), 170.8 (2-C=O).

6.2.5. 4-4-(Formylmethyl)phenylcarbamoylbenzyl acetate (19)

The Dess–Martin periodinane (0.406 g, 0.96 mmol, 2 equiv) and **18** (0.150 g, 0.48 mmol) were dissolved in DCM (15 mL) and the reaction was stirred overnight at room temperature. The mixture was then extracted with EtOAc (20 mL) and washed with $NaHCO_3$ (20 mL), $Na_2S_2O_3$ (25 mL) and brine (20 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford **19** as a brown sticky solid. The product was not purified but used as a crude for next step; R_f = 0.63 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 312.15 ($[M+H]^+$, 100%); mp = 126–127 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.12 (3H, s, 1-CH_3), 3.68 (2H, t, *J* = 2.3, 18-CH_2), 5.16 (2H, s, 3-CH_2), 7.22 (2H, d, *J* = 8.4, 14-CH and 16-CH), 7.46 (2H, d, *J* = 8.2, 5-CH and 9-CH), 7.64 (2H, d, *J* = 8.4, 13-CH and 17-CH), 7.86 (2H, d, *J* = 8.2, 6-CH and 8-CH), 9.69 (1H, t, *J* = 2.3, 19-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 21.0 (1-CH_3), 50.0 (18-CH_2), 65.5 (3-CH_2), 120.7 (13-CH and 17-CH), 127. 3 (5-CH and 9-CH), 128.0 (6-CH and 8-CH), 128.3 (14-CH and 16-CH), 130.4 (15-C), 134.6 (7-C), 137.2 (12-C), 140.1 (4-C), 166.2 (10-C=O), 165.3 (2-C=O), 199.3 (19-C=O).

6.2.6. 4-(4-Formylphenylcarbamoyl)benzyl acetate (21)

Pyridinium chlorochromate (0.460 g, 2.13 mmol, 2.1 equiv) and **18** (0.323 g, 1.03 mmol) were stirred in DCM (20 mL) overnight at room temperature, in presence of molecular sieves. The mixture was then poured onto a silica pad and eluted with 1.5 L of DCM, then with 1.5 L DCM/MeOH (90:10). The filtrate collected was concentrated under reduced pressure and the crude was purified by column chromatography ($CHCl_3$/MeOH in presence of TEA, 100:0→95:5) to obtain **21** as a yellow powder (0.113 g, 37% yield); R_f = 0.86 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 298.11 ($[M+H]^+$, 100%); Anal. Calcd for $C_{17}H_{15}NO_4{\cdot}0.15H_2O$ (Theory): C, 68.06; H, 5.14; N, 4.67. Found: C, 68.13; H, 5.21; N, 4.71; mp = 137 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 2.17 (3H, s, 1-CH_3), 5.20 (2H, s, 3-CH_2), 7.53 (2H, d, *J* = 8.4, 5-CH and 9-CH), 7.93 (6H, m, 6-CH, 8-CH, 13-CH, 14-CH, 16-CH and 17-CH), 9.97(1H, s, 18-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 21.0 (1-CH_3), 65.4 (3-CH_2), 119.6 (13-CH and 17-CH), 127. 4 (5-CH and 9-CH), 128.4 (6-CH and 8-CH), 131.3 (14-CH and 16-CH), 134.8 (15-C), 135.2 (7-C), 139.4 (12-C), 142.4 (4-C), 165.2 (10-C═O), 170.8 (2-C═O), 191.0 (18-C═O).

6.2.7. Methyl *N*-4-(4-acetoxymethylbenzamidyl)phenethyl-4-aminobutanoate (7)

TEA (0.078 ml, 0.56 mmol, 2.3 equiv) was added to a solution of methyl 4-aminobutanoate hydrochloride **20** $H_2N(CH_2)_3COOMeHCl$ (0.079 g, 0.51 mmol, 2.1 equiv) in MeOH (8 mL) and the solution stirred at room temperature. After 30 min, a solution of **19** (0.076 g, 0.24 mmol) in MeOH (2 mL) was added and the reaction stirred for a further 30 min. Finally $NaCNBH_3$ (0.44 mL of 1 M solution in THF, 0.44 mmol, 1.8 equiv) was added and the reaction stirred overnight at room temperature. The mixture was then diluted with H_2O (10 ml), extracted with $CHCl_3$ (15 mL), the organic layer was washed with a saturated solution of NaCl (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by column chromatography ($CHCl_3$/MeOH 100:0→90:10) to afford the product **7** as a white sticky compound (0.024 g, 24% yield); R_f = 0.53 (CH_2Cl_2/MeOH: 90:10); LC–MS, *m*/*z* (ES^+ mode): 413.18 ($[M+H]^+$, M = 9, 75%), 381.16 ($[M+H]^+$, M = lactam derivative, MW = 380.17, 16%); HRMS (ES^+ mode), Calcd for $C_{23}H_{29}N_2O_5$ ($M+H^+$): 413.2071. Found: 413.2061; ^1H NMR (500 MHz, $CDCl_3$): δ 2.02 2H, m, 22-CH_2), 2.07 (3H, s, 1-CH_3), 2.43 (2H, m, 18-CH_2), 2.48 (2H, m, 23-CH_2), 3.02 (2H, m, 21-CH_2), 3.26 (2H, m, 19-CH_2), 3.64 (3H, s, 25-CH_3), 5.09 (2H, s, 3-CH_2), 7.15 (2H, m, 14-CH and 16-CH), 7.44 (4H, m, 5-CH, 9-CH, 13-CH and17-CH), 7.82 (2H, d, *J* = 8.3, 6-CH and 8-CH), 8.21 (1H, br s, 11-NH); ^{13}C NMR (125 MHz, $CDCl_3$): 19.9 (1-CH_3), 28.7 (22-CH_2), 30.5 (23-CH_2), 43.6 (18-CH_2), 50.0 (19-CH_2), 51.2 (21-CH_2), 53.3 (25-CH_3), 64.4 (3-CH_2), 120.7 (13-CH and 17-CH), 126.6 (5-CH and 9-CH), 127.1 (6-CH and 8-CH), 128.5 (14-CH and 16-CH), 131.3 (7-C), 133.0 (15-C), 135.8 (12-C), 139.2 (4-C), 164.8 (10-C═O), 169.8 (2-C═O, 173.1 (24-C═O).

6.2.8. Methyl *N*-4-(4-acetoxymethylbenzamidyl)benzyl-4-amino butanoate (3)

TEA (0.122 ml, 0.88 mmol, 2.3 equiv) was added to a solution of methyl 4-aminobutanoate hydrochloride **20** $H_2N(CH_2)_3COOMe{\cdot}HCl$ (0.123 g, 0.80 mmol, 2.1 equiv) in MeOH (10 mL) and the solution stirred at room temperature. After 30 min, a solution of **21** (0.114 g, 0.38 mmol) in MeOH (2 mL) was added and the reaction stirred for a further 30 min. Finally $NaCNBH_3$ (0.69 mL of 1 M solution in THF, 0.69 mmol, 1.8 equiv) was added and the reaction stirred overnight at room temperature. The mixture was then diluted with H_2O (25 mL) and extracted with $CHCl_3$ (30 mL), the organic layer was washed with a saturated solution of NaCl (25 ml), dried over $MgSO_4$ and the solvent concentrated under reduced pressure. The crude was purified by column chromatography ($CHCl_3$/MeOH 100:0→90:10) to afford product **3** as a white oily compound (0.033 g, 22% yield); R_f = 0.6 ($CHCl_3$/MeOH: 80:20); LRMS, *m*/*z* (ES^+ mode): 399.19 ($[M+H]^+$, 100%); HRMS (ES^+ mode), Calculated for $C_{22}H_{27}N_2O_5$ ($M+H^+$): 399.1914. Found: 399.1920; ^1H NMR (500 MHz, $CDCl_3$): δ 1.87 2H, m, 21-CH_2), 2.16 (3H, s, 1-CH_3), 2.43 (2H, t, *J* = 7.3, 22-CH_2), 2.71 (2H, t, *J* = 7.3, 20-CH_2), 3.69 (3H, s, 24-CH_3), 3.80 (2H, s, 18-CH_3), 5.20 (2H, s, 3-CH_2), 7.35 (2H, d, *J* = 8.3, 14-CH and 16-CH), 7.50 (2H, d, *J* = 8.2, 5-CH and 9-CH), 7.62 (2H, d, *J* = 8.3, 13-CH and 17-CH), 7.87 (1H, br s, 11-NH), 7.90 (2H, d, *J* = 8.2, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 21.0 (1-CH_3), 29.7 (21-CH_2), 31.9 (22-CH_2), 46.1 (18-CH_2), 46.6 (20-CH_2), 51.7 (24-CH_3), 65.5 (3-CH_2), 120.5 (13-CH and 17-CH), 127. 3 (5-CH and 9-CH), 128.3 (6-CH and 8-CH), 129.0 (14-CH and 16-CH), 133.0 (15-C), 134.9 (7-C), 136.3 (12-C), 140.0 (4-C), 165.0 (10-C═O), 170.8 (2-C═O, 174.1(23-C═O).

6.2.9. 4-Aminobenzyl acetate (23)

Ac_2O (1.28 mL, 13.63 mmol, 1 equiv) was added to a solution of commercially available 4-aminobenzyl alcohol (2.015 g, 16.36 mmol, 1.2 equiv) in pyridine (10 mL) and the reaction was stirred at 0°C and monitored by TLC. When all the starting material had disappeared (26 h) the mixture was extracted with EtOAc (20 mL) and washed with H_2O (2 × 20 mL), then with HCl (15 mL) and then dried over MgSO. The solvent was removed under reduced pressure, to recover pure **23** as a white solid (1.182 g, 44% yield); R_f = 0.22 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 166.09 ($[M+H]^+$, 100%); Anal. Calcd for $C_9H_{11}NO_4$ (Theory): C, 65.44; H, 6.71; N, 8.48. Found: C, 66.33; H, 6.88; N, 8.77; mp = 144; ^1H NMR (500 MHz, $CDCl_3$): δ 2.21 (3H, s, 1-CH_3), 4.68 (2H, s, 3-CH_2), 7.20 (2H, br s, 10-NH_2), 7.35 (2H, d, *J* = 8.3, 6-CH and 8-CH), 7.52 (2H, d, *J* = 8.3, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.7 (1-CH_3), 65.0 (3-CH_2), 120.0 (6-CH and 8-CH), 127.9 (5-CH and 9-CH), 136.8 (4-C), 137.3 (7-C), 168.2 (2-C═O).

6.2.10. 4-(4-Nitrophenyloxycarbonylamino)benzyl acetate (24)

Commercially available *p*-nitrophenylchloroformate (0.648 g, 3.22 mmol, 1 equiv) and **23** (0.532 g, 3.22 mmol) were dissolved in CH_2Cl_2 (4 mL) at 0°C, then TEA (0.45 mL, 3.22 mmol, 1 equiv) was added and the reaction was allowed to warm to room temperature and stirred until the complete disappearance of starting material was observed by TLC and LC–MS. The precipitate formed was then filtered and the filtrate was extracted with CH_2Cl_2 (10 mL), washed with H_2O (2 × 10 mL) and the solvent was removed under reduced pressure to afford the product as a yellow solid (0.965 g, 91% yield). The product was not purified and only identified by ^1H NMR and LC–MS, then used as a crude for next step; R_f = 0.86 (CH_2Cl_2/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 331.12 ($[M+H]^+$, 100%); Anal. Calcd for $C_{16}H_{14}N_2O_6$ (Theory): C, 58.18; H, 4.27; N, 8.48. Found: C, 57.71; H, 4.45; N, 8.65; ^1H NMR (500 MHz, $CDCl_3$): δ 2.12 (3H, s, 1-CH_3), 5.18 (2H, s, 3-CH_2), 7.11 (1H, br s, 10-NH), 7.31 (2H, m, 13-CH and 17-CH), 7.34 (2H, d, *J* = 8.5, 5-CH and 9-CH), 7.48 (2H, d, *J* = 8.3, 6-CH and 8-CH), 8.20 (2H, dd, J_1 = 2.2, J_2 = 7.0, 14-CH and 16-CH).

6.2.11. (Pyrrolidin-3-yl)methanol trifluoroacetate (26)

TFA (0.5 mL, 1.6 equiv) was added to a solution of *N*-Boc-hydroxymethylpyrrolidine commercially available (0.852 g, 4.23 mmol) in DCM (4.5 mL) and the reaction stirred and monitored by TLC and LC–MS. When the removal of the Boc group was completed, all the solvent was removed under reduced pressure, leaving pure **26** as a pink oil (quantitative); R_f = 0.36 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES^+ mode): 102.10 ($[M-CF_3COO]^+$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.96 (1H, m, 4^a-CH_2), 2.36 (1H, m, 3-CH), 2.93 (1H, m, 4^b-CH_2), 3.22 (1H, m, 2^a-CH_2), 3.43 (1H, m, 2^b-CH_2), 3.54 (1H, m, 5^a-CH_2), 3.60 (1H, m, 5^b-CH_2), 4.41 (1H, dd, J_1 = 6.9, J_2 = 11.2, 6^a-CH_2), 4.49 (1H, dd, J_1 = 5.5, J_2 = 11.3, 6^b-CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.0

(4-CH_2), 36.6 (3-CH), 45.5 (5-CH_2), 47.5 (2-CH_2), 67.1 (6-CH_2); ^{19}F NMR (470 MHz, $CDCl_3$): δ −75.9 3F, CF_3COOH).

6.2.12. *N*-(4-Acetoxymethylphenyl)-3-hydroxymethylpyrrolidine-1-carboxamide (27)

TEA (0.613 mL, 4.42 mmol, 1.6 equiv) was added to **24** (0.935 g, 2.83 mmol) in MeOH (18 mL), then **26** (0.950 g, 4.41 mmol, 1.5 equiv) was added and the reaction stirred in the microwave for 30 min at 100°C. The precipitate formed was filtered and the filtrate was concentrated under reduced pressure. The crude was then extracted with CH_2Cl_2 (15 mL) and washed with H_2O (3 × 10 mL), then dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH 100:0→90:10) afforded **27** as light yellow oil (0.471 g, 57% yield); R_f = 0.30 (CH_2Cl_2/MeOH: 90:10); LRMS, *m*/*z* (ES$^+$ mode): 310.18 ([M+NH_4]$^+$, 100%); HRMS (ES$^+$ mode), Calcd for $C_{15}H_{21}N_2O_4$ (M+H$^+$): 293.1496. Found: 293.1505; ^{1}H NMR (500 MHz, MeOD): δ 1.70 (1H, m, 15^a-CH_2), 2.00 (1H, m, 14-CH), 2.12 (3H, s, 1-CH_3), 2.39 (1H, m, 15^b-CH_2), 3.17 (2H, m, 16-CH_2), 3.52 (4H, m, 13-CH_2 and 17-CH_2), 5.06 (2H, s, 3-CH_2), 7.32 (2H, d, *J* = 7.8, 5-CH and 9-CH), 7.54 (2H, d, *J* = 8.5, 6-CH and 8-CH); ^{13}C NMR (125 MHz, MeOD): δ 23.8 (1-CH_3), 28.3 and 29.1 (15-CH_2), 41.8 (14-CH), 42.6 (16-CH_2), 46.5 and 46.8 (13-CH_2), 64.3 (17-CH_2), 67.7 (3-CH_2), 121.0 (6-CH and 8-CH), 129.7 (5-CH and 9-CH), 133.9 (7-C), 139.8 (4-C), 156.8 (11-C═O), 171.7 (2-C═O).

6.2.13. *N*-(4-Acetoxymethylphenyl)-pyrrolidine-3-carbaldehyde-1-carboxamide (28)

The Dess–Martin periodinane (1.086 g, 2.56 mmol, 2 equiv) and **27** (0.374 g, 1.28 mmol) were dissolved in CH_2Cl_2 (30 mL) under Argon and the reaction was stirred overnight at room temperature. The mixture was then diluted with EtOAc (30 mL) and washed with $NaHCO_3$ (20 mL), $Na_2S_2O_3$ (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford **28** as a brown sticky solid (0.370 g, quantitative). The product was not purified but used as a crude for next step; R_f = 0.63 (CH_2Cl_2/MeOH: 90:10); LRMS, *m*/*z* (ES$^+$ mode): 318.17 ([M+NH_4]$^+$, 100%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.06 (1H, m, 15^a-CH_2), 2.11 (3H, s, 1-CH_3), 2.18 (1H, m, 15^b-CH_2), 2.99 (1H, m, 14-CH), 3.37 (2H, m, 16-CH_2), 3.50 (1H, m, 13^a-CH_2), 3.72 (1H, m, 13^b-CH_2), 5.02 (2H, s, 3-CH_2), 7.26 (2H, d, *J* = 6.3, 5-CH and 9-CH), 7.41 (2H, d, *J* = 7.4, 6-CH and 8-CH), 9.62 (1H, d, *J* = 2.9, 17-CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.7 (1-CH_3), 29.7 (15-CH_2), 44.9 (13-CH), 45.3 (16-CH_2), 49.6 (14-CH_2), 66.6 (3-CH_2), 119.9 (6-CH and 8-CH), 129.0 (5-CH and 9-CH), 133.1 (7-C), 137.7 (4-C), 158.1 (11-C═O), 168.5 (2-C═O), 196.8 (17-C═O).

6.2.14. *N*-(4-Acetoxymethylphenyl)-3-(*N*′-3-methoxycarbonylpropyl)-aminomethylpyrrolidine-1-carboxamide (11)

TEA (0.22 mL, 1.58 mmol, 2.3 equiv) was added to a solution of methyl 4-aminobutanoate hydrochloride **20** (0.222 g, 1.45 mmol, 2.1 equiv) in methanol (15 mL) and the solution was stirred at room temperature. After 30 min, a solution of **28** (0.200 g, 0.69 mmol) in MeOH (5 mL) was added and the reaction stirred for a further 30 min. Finally $NaCNBH_3$ (1.24 mL of 1.0 M solution in THF, 1.24 mmol, 1.8 equiv) was added and the reaction stirred overnight at room temperature. The mixture was then diluted with H_2O (20 mL) and extracted with $CHCl_3$ (20 mL), the organic layer was dried over $MgSO_4$, then concentrated under reduced pressure. The crude was purified by column chromatography ($CHCl_3$/MeOH 100:0→95:5) to afford product **11** as a white oily compound (0.029 g, 11% yield); R_f = 0.42 ($CHCl_3$/MeOH: 80:20); LRMS, *m*/*z* (ES$^+$ mode): 392.25 ([M+H]$^+$, 100%); HRMS (ES$^+$ mode), Calcd for $C_{20}H_{30}N_3O_5$ (M+H$^+$): 392.2180. Found: 392.2186; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52 (1H, m, 15^a-CH_2), 1.74 (2H, m, 20-CH_2), 1.95 (1H, m, 15^b-CH_2), 2.11 (3H, s, 1-CH_3), 2.31 (2H, m, 21-CH_2), 2.52 (1H, m, 14-CH), 2.58 (3H, m, 17^a-CH_2 and 19-CH_2), 2.99 (1H, m, 17^b-CH_2), 3.28 (1H, m, 16^a-CH_2), 3.48 (3H, m, 13-CH_2 and 16^b-CH_2), 3.60 (3H, s, 23-CH_3), 5.00 (2H, s, 3-CH_2), 7.25 (2H, d, *J* = 8.1 Hz, 5-CH and 9-CH), 7.29 (1H, br s, 10-NH), 7.41 (2H, d, *J* = 8.4, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 24.7 (1-CH_3), 24.9 (20-CH_2), 29.2 and 29.9 (15-CH_2), 31.8 (21-CH_2), 41.2 (14-CH), 49.2 (16-CH_2), 50.3 (19-CH_2), 50.3 (17-CH_2), 51.7 (23-CH_3), 52.3 and 52.4 (13-CH_2), 66.3 (3-CH_2), 119.9 (6-CH and 8-CH), 129.0 (5-CH and 9-CH), 132.9 (7-C), 137.6 (4-C), 154.9 (11-C═O), 158.1 (2-C═O), 174.1 (22-C═O).

The side product of this reaction, **31**, was also isolated pure as a white sticky solid (0.026 g, 10% yield).

6.2.15. *N*-(4-Acetoxymethylphenyl)-3-(2-oxopyrrolidin-1-yl) methylpyrrolidine-1-carboxamide (31)

R_f = 0.76 ($CHCl_3$/MeOH: 90:10); LRMS, *m*/*z* (ES$^+$ mode): 377.23 ([M+NH_4]$^+$, 100%; HRMS (ES$^+$ mode), Calcd for $C_{19}H_{26}N_3O_4$ (M+H$^+$): 360.1918. Found: 360.1924; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.55 (1H, m, 15^a-CH_2), 1.89 (1H, m 15^b-CH_2), 1.96 (2H, m, 20-CH_2), 2.09 (3H, s, 1-CH_3), 2.32 (2H, t, *J* = 8.1, 21-CH_2), 2.32 (1H, m, 14-CH), 3.04 (1H, m, 17^a-CH_2), 3.22 (1H, m, 17^b-CH_2), 3.43 (6H, 13-CH_2, 16-CH_2 and 19-CH_2), 4.99 (2H, s, 3-CH_2), 7.22 (2H, d, *J* = 8.3, 5-CH and 9-CH), 7.42 (2H, d, *J* = 7.5, 6-CH and 8-CH), 7.89 (1H, br s, 10-NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.1 (20-CH_2), 24.5 (1-CH_3), 28.9 and 29.4 (15-CH_2), 30.9 (21-CH_2), 36.7 and 37.7 (14-CH), 45.1 and 45.2 (17-CH_2), 45.6 (19-CH_2), 47.9 and 47.9 (16-CH_2), 49.5 and 49.9 (13-CH_2), 66.5 (3-CH_2), 119.9 (6-CH and 8-CH), 128.8 and 128.9 (5-CH and 9-CH), 132.6 (7-C), 137.9 (4-C), 154.9 (11-C═O), 168.7 (2-C═O), 175.4 (22-C═O).

6.2.16. *N*-(4-Acetoxymethylphenyl)-3-*N*′-5-methoxycarbonylpent-1-yl)-aminomethyl-pyrrolidine-1-carboxamide (30)

TEA (0.22 mL, 1.58 mmol, 2.3 equiv) was added to a solution of methyl 6-aminohexanoate hydrochloride **29** (0.262 g, 1.44 mmol, 2.1 equiv) in methanol (15 mL) and the solution stirred at room temperature. After 30 min a solution of **28** (0.200 g, 0.69 mmol) in MeOH (5 mL) was added and the reaction stirred for a further 30 min. Finally $NaCNBH_3$ (1.24 mL of 1.0 M solution in THF, 1.24 mmol, 1.8 equiv) was added and the reaction stirred overnight at room temperature. The mixture was then diluted with H_2O (20 mL) and extracted with $CHCl_3$ (20 mL), the organic layer was dried over $MgSO_4$, then concentrated under reduced pressure. The crude was purified by column chromatography ($CHCl_3$/MeOH 100:0→90:10) to afford product **30** as a white oily compound (0.029 g, 10% yield); R_f = 0.56 ($CHCl_3$/MeOH: 80:20); LRMS, *m*/*z* (ES$^+$ mode): 420.24 ([M+H]$^+$, 100%); HRMS (ES$^+$ mode), Calcd for $C_{22}H_{34}N_3O_5$ (M+H$^+$): 420.2493. Found: 420.2496; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (2H, m, 21-CH_2), 1.55 (5H, m, 15^a-CH_2, 20-CH_2 and 22-CH_2), 1.97 (1H, m, 15^b-CH_2), 2.10 (3H, s, 1-CH_3), 2.25 (2H, t, *J* = 7.4, 23-CH_2), 2.37 (1H, m, 14-CH), 2.62 (3H, m, 17^a-CH_2 and 19-CH_2), 3.01 (1H, m, 17^b-CH_2), 3.28 (1H, m, 16^a-CH_2), 3.49 (3H, m, 13-CH_2 and 16^b-CH_2), 3.59 (3H, s, 25-CH_3), 4.99 (2H, s, 3-CH_2), 7.24 (2H, m, 5-CH and 9-CH), 7.34 (1H, br s, 10-NH), 7.40 (2H, m, 6-CH and 8-CH); ^{13}C NMR (125 MHz, $CDCl_3$): 23.5 (1-CH_3), 25.5 (22-CH_2), 27.9 (21-CH_2), 28.7 and 28.9 (20-CH_2), 29.4 (15-CH_2), 32.8 (23-CH_2), 37.4 (14-CH), 44.2 and 44.6 (16-CH_2), 48.3 and 48.4 (19-CH_2), 48.9 (17-CH_2), 49.3 (13-CH_2), 50.5 (25-CH_3), 65.4 (3-CH_2), 119.0 (6-CH and 8-CH), 127.8 and 127.9 (5-CH and 9-CH), 131.9 (7-C), 136.6 (4-C), 153.8 (11-C═O), 166.7 (2-C═O), 173.1 (24-C═O).

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Supplementary data

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