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# The Synthesis of Cyclic Tetrapeptoid Analogues of the Antiprotozoal Natural Product Apicidin

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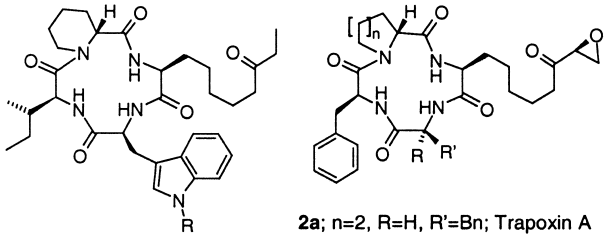
**Abstract**—A novel synthetic strategy is described which may be used to prepare analogues of the antimalarial, fungal metabolite apicidin. Compared to the natural product, one analogue shows potent and selective activity in vitro against the parasite *Trypanosoma brucei* and low mammalian cell toxicity. 

The antiparasitic agent apicidin **1**,[1] which was recently isolated from cultures of *Fusarium pallidoroseum*, belongs to a rare group of cyclic tetrapeptide (CTP) fungal metabolites that includes the cytostatic agents trapoxin **2**[2] and chlamydocin **3**[3] (Fig. 1). Yoshida et al. have already shown[2a,4] that trapoxin is a potent, irreversible inhibitor of HDAC (histone deacetylase) and that it induces hyperacetylation in a variety of mammalian cells.



**1a**; R=OMe; Apicidin **2a**; n=2, R=H, R'=Bn; Trapoxin A

**1b**; R=H; Apicidin A **2b**; n=1; R=H, R'=Bn; Trapoxin B

**3**; n=1, R=R'=Me; Chlamydocin

**Figure 1.** Selected cyclic tetrapeptide natural products.

In contrast, apicidin inhibits protozoal HDAC and is orally active against *Plasmodium berghei* malaria in mice.[1b] Recent studies have confirmed the importance of the non-proteinogenic amino acid (2*S*)-2-amino-8-oxo-decanoic acid (Aoda), which is believed to represent a reactive isostere of *N*-acetyl lysine, as a key pharmacophoric element and this has led to the discovery of new, semi-synthetic, mechanism based inhibitors of HDAC.[5]

A key step in the preparation of CTPs is the formation of the 12-membered macrocyclic core.[6] However, this process is known to be highly sequence-dependent. For example, studies on the preparation of chlamydocin,[3b] revealed that the choice of the correct linear precursor was pivotal to success. We have, therefore, sought improved methods to prepare CTP analogues which could find utility as biochemical reagents for affinity labelling, probes for the characterisation of transcriptional regulators, as well as antibiotic, antitumour and antiprotozoal agents. Recent studies in this laboratory[7] have revealed that the introduction of a single, reduced peptide bond surrogate into linear tetrapeptide frames has a pronounced influence on their propensity to undergo intramolecular cyclisation. Herein, we report the successful application of this approach to the preparation of novel apicidin analogues.

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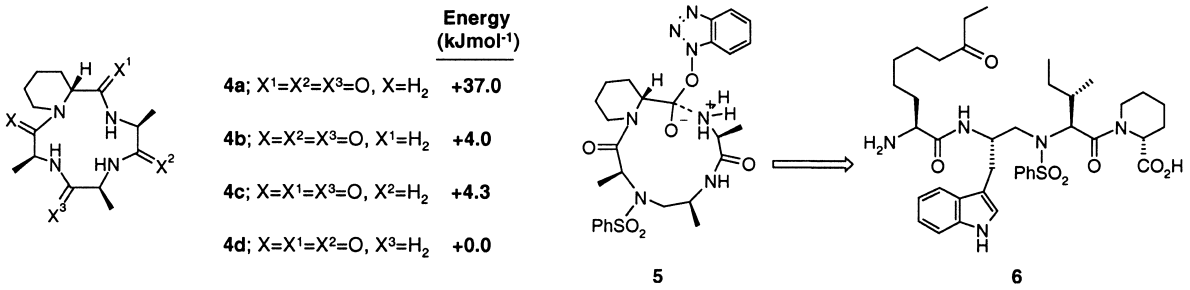
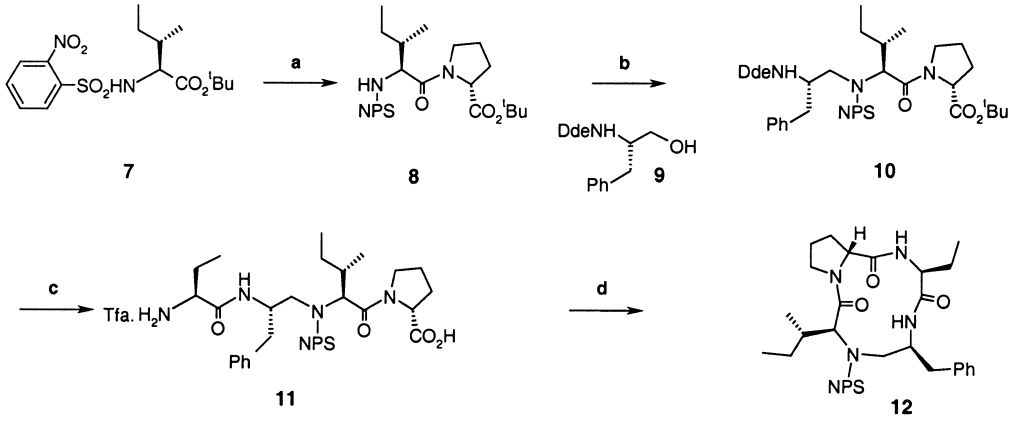



**Figure 2.** Conformational searches and calculation of lowest energy transition state energies for CTP analogues.

The strategy rests upon removing the partial double bond character of a selected amide bond by substituting a methylene group for the amidic carbonyl and protecting any resulting secondary amine as a sulfonamide. Conformational searches[8] were conducted on the four possible mono-desoxy derivatives of a truncated model peptide, *cyclo*[Ala-Ala-Ala-D-Pip], representing the apicidin core structure, to determine the optimal site for reduction (Fig. 2). This revealed that reduction of the secondary amides results in three isomers of similar total energies **4b–d**, whereas reduction of the tertiary amide, to give **4a**, is highly unfavourable. Calculations[7] on tetra-L-alanine derivatives predict that reducing either the second or third amide bond from the N-terminus is most effective in lowering the transition state energy for cyclisation. Linear precursors containing pipecolic acid as the N-terminal group were excluded in order to avoid tertiary amide bond formation as the ring-forming step. Transition state calculations[9] on the four mono-desoxy model structures satisfying all of these criteria, revealed highly unfavourable energies ($+23$ to $+35$ kJ mol$^{-1}$) for three of the precursors relative to the remaining structure **5** (Fig. 2). This model is consistent with a strategy involving reduction of the tryptophanyl carbonyl in apicidin and a cyclisation of the linear tetramer **6**.

Initial synthetic studies (Scheme 1) were undertaken on a simplified peptide in which the reactive indole nucleus was replaced with a phenyl group (Trp→Phe) and the Aoda side chain was truncated to an ethyl substituent (Aoda→Abu). In addition, D-pipecolic acid was replaced in the target structure with D-proline, since it is significantly less costly and is present in closely related structures, such as trapoxin B.[2] Acid hydrolysis of the *N*-sulfonyl amino ester **7** followed by coupling to D-proline *tert*-butyl ester gave the dipeptide **8**. The peptide bond surrogate was incorporated by reacting[10] the sulfonamide **8**, with Dde protected[11] phenylalaninol **9**, under Mitsunobu conditions, providing the trimer **10** in high yield. After selective removal[11] of the Dde protective group, routine peptide coupling to *N*-Boc aminobutyric acid, followed by acidolysis, gave the linear tetrameric precursors **11**. In the presence of the coupling agent TBTU and HOBt, peptide **11** (2 mg mL$^{-1}$) was completely consumed in less than an hour, forming a single product (by HPLC analysis), which was isolated in >80% yield and characterised as the desired cyclic tetramer **12**. In contrast the non-reduced analogue of **11** ($H_2$N-Abu-Phe-Leu-D-Pro-OH) gave 10% of the corresponding CTP, and 68% of cyclic octomer (COP) when subjected to the cyclisation under identical conditions. Under the high dilution conditions (0.2 mg mL$^{-1}$) commonly employed for the cyclisation of tetrapeptides this reaction gave an equimolar mixture of the CTP and COP in 84% yield after 3 days. The structural assignment of **12** was confirmed by a single crystal X-ray diffraction analysis[12] (Fig. 3) which reveals that the three amide bonds have the *trans* configuration and that the reduced peptide bond surrogate also adopts a pseudo-*trans* orientation. An intramolecular hydrogen bond, observed between the *N*–H of phenylalanine and the isoleucine carbonyl, is part of a type II″ β-turn, having the D-proline and Abu residues at the $i+1$ and $i+2$ positions.




**Scheme 1.** (a) (i) TFA, $CH_2Cl_2$ (1:1), rt, 1 h, 98%; (ii) H-D-Pro-O′Bu, PyBOP, HOBt, DIPEA, $CH_2Cl_2$, DMF (20:1), rt, 3 h, 97%; (b) $PPh_3$, DEAD, THF, 0 °C to rt, 6 h, 69–75%; (c) (i) 55% $N_2H_4{\cdot}H_2O$, MeOH, rt, 4 h; (ii) Boc-Abu-OH, TBTU, HOBt, DIPEA, $CH_2Cl_2$, DMF (20:1), rt, 18 h, 67–85% (2 steps); (iii) TFA, $CH_2Cl_2$ (1:1), rt, 3 h, 76–78%; (d) TBTU, HOBt, DIPEA, $CH_2Cl_2$, rt, 82%; NPS = (2-nitrophenyl)sulfonyl; Dde = *N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl].

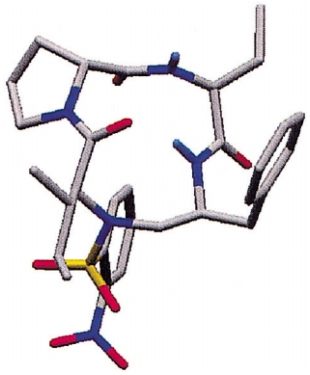
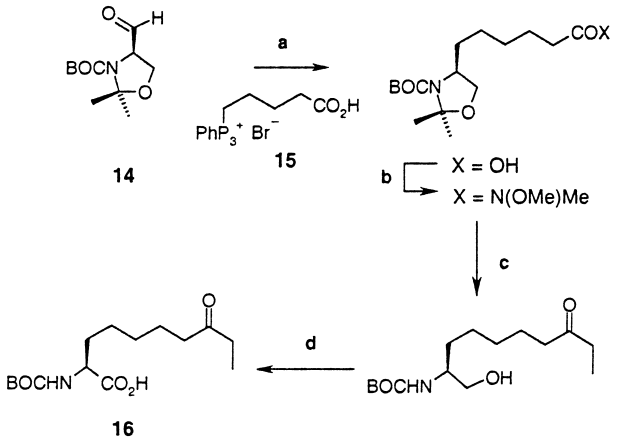


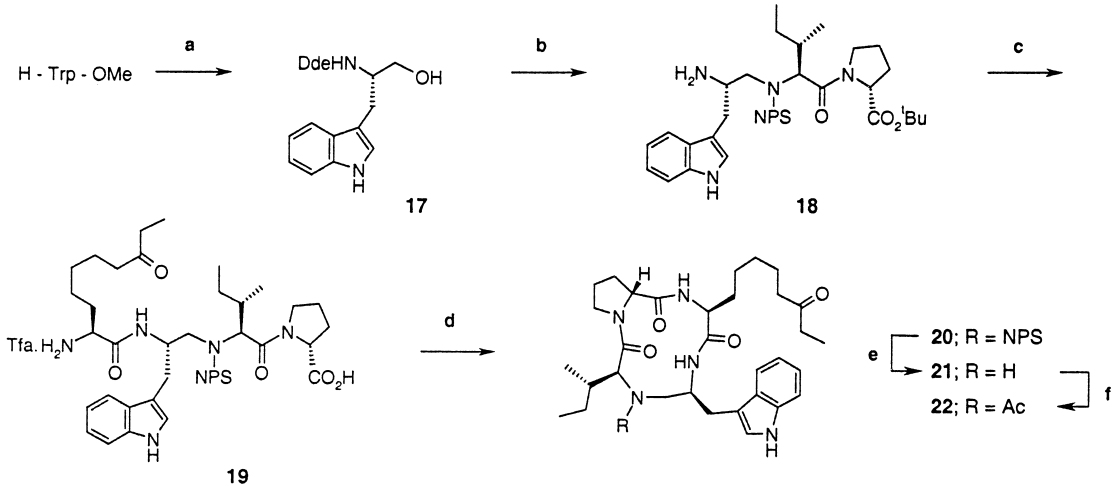
**Figure 3.** X-ray structure of the cyclic tetrapeptoid **12**.




**Scheme 2.** (a) (i) LiHMDS, **15**, THF, 15 °C to rt, 1 h, 88%; (ii) Pd–C (20% w/w, $H_2$, EtOH, rt, 1.5 h, quant.; (b) TBTU, $Et_3N$, MeNH(OMe)·HCl, $CH_2Cl_2$, rt, 2 h, 66%; (c) (i) EtMgBr, THF, $Et_2O$, 0 °C to rt, 6 h; (ii) Amberlyst-15 resin, MeOH, rt, 4 days, 89% (2 steps); (d) PDC, DMF, 4 Å molecular sieves, rt, 4 h, 65%.

The rapid, high yielding cyclisation of **11** confirmed the viability of our proposed synthetic strategy and we therefore turned our attention to the synthesis of novel apicidin analogues exploiting this methodology. The required Aoda derivative was prepared via homologation of the aldehyde **14**[13] as shown (Scheme 2). The remaining building block **17**, was made by reduction of tryptophan methyl ester and subsequent protection of the primary amine as the Dde derivative (Scheme 3). This β-amino alcohol coupled with the dipeptide **8**, under Mitsunobu conditions, to give the trimer **18**, after chemoselective cleavage of the Dde group with hydrazine. Acylation of the amine with the Aoda derivative **16**, and acidolysis of the *N*-Boc and *tert*-butyl ester protective groups afforded the linear tetramer **19**, the precursor for the key synthetic step. In the event, this compound cyclised rapidly, under standard peptide coupling conditions, to give our first target structure, the apicidin analogue **20**, in high yield. Nucleophilic cleavage[10] of the NPS group with phenylthiolate provided the secondary amine **21**, a useful intermediate for the introduction of additional diversity elements. The excellent orthogonality of the sulfonamide, and the recent development of an NPS based resin linker[14] suggest that this group has potential as a backbone anchor for peptide assembly, which would allow the ready transfer of this methodology to the solid phase. Finally the amine **21** was 'capped' with acetic anhydride to give the tertiary acetamide **22**.

Compounds **20**–**22** were screened in vitro against a panel[15] of parasitic organisms, using apicidin **1a** as the positive control (Table 1). This revealed a modified spectrum of antiprotozoal activity for the synthetic analogues compared to the natural product. Although the antimalarial activity for the CTP analogues was substantially reduced or lost, the sulfonamide **20**, in particular, retained significant potency against *Trypanosoma brucei,* the causative agent of African sleeping




**Scheme 3.** (a) (i) $NaBH_4$, EtOH, $H_2O$, 0 °C to reflux, 18 h, 88%; (ii) Dde-OH, EtOH, rt, 4 h, quant.; (b) (i) **8**, $PPh_3$, DEAD, THF, 0 °C to rt, 18 h, 57%; (ii) $N_2H_4{\cdot}H_2O$, (55% v/v in $H_2O$), MeOH, rt, 2.5 h; (c) (i) **16**, TBTU, HOBt, DIPEA, $CH_2Cl_2$, DMF (20:1), rt, 3 h, 65%; (ii) TFA, $CH_2Cl_2$ (1:1), thioanisole, 2 h, rt, 49%; (d) TBTU, HOBt, DIPEA, $CH_2Cl_2$, rt, 1 h, 64%; (e) PhSH, $K_2CO_3$, DMF, rt, 30 h, 50%; (f) $Ac_2O$, pyridine, $CH_2Cl_2$, rt, 40 h, 48%.

**Table 1.** Antiprotozoal activities of apicidin **1a**[a] and analogues **20–22**

| Parasite | $ED_{50}$ (mg $mL^{-1}$)[b] | | | |
|---|---|---|---|---|
| | **3a** | **20** | **21** | **22** |
| *Plasmodium falciparum* | 0.22±0.03 | >1.0 | >1.0 | >1.0 |
| *Leishmania donovani* | T[c] | >30 | >30 | >30 |
| *Trypanosoma cruzi* | T[c] | >30 | >30 | >30 |
| *Trypanosoma brucei* | 1.90±0.54 | 1.97±0.13 | 11.10±6.97 | 12.97±3.93 |
| Cytotoxicity against KB cells | 0.2±0.3 | 134.8±0.62 | 194.2±39.6 | 170.8±1.06 |

[a]Apicidin **1a** was obtained by Dr. Paul Stead from cultures of *Fusarium pallidoroseum* ATCC 7432 within our Biological Chemistry and Bioprocessing Unit.
[b]Values are the mean±SEM (95%) of a representative experiment for triplicate points at each drug concentration in a three fold dilution series.
[c]Toxicity to both mouse macrophage host cells and intracellular parasites prevented determination of antiprotozoal activity.

sickness. In addition, whilst apicidin showed clear signs of cellular toxicity in the assays at the higher doses, these effects were much less marked for the synthetic mimetics. The results from a preliminary cytotoxicity assay[16] (Table 1) appear to confirm these observations. The toxicity of the reduced analogues **20–22**, towards KB cells, (a human nasopharyngeal epithelial cell line) was observed to be in the order of 1000-fold lower than the natural product.

These encouraging preliminary results suggest that the methodology revealed above has the potential to provide apicidin analogues possessing antiparasitic activity with an improved mammalian toxicity profile. Further studies, utilising a solid-phase cyclitive cleavage approach,[17] are currently underway to prepare additional analogues which incorporate both alternative amino acids and variations in the key non-proteinogenic residue Aoda. These results will be the subject of a future publication.

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8. Macromodel 5.5 run on a Silicon Graphics, Indigo 2; Amber force field, GB/SA (water) solvation; Monte Carlo conformational search.
9. In the transition state model **5** (Fig. 2), the bond forming between the ammonium nitrogen and the carboxyl carbon was fixed at 1.8±0 Å (force constant = 900). All other parameters were free to optimise. The benzotriazole and phenylsulfonyl groups were included as indicated.
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12. Crystallographic data for **12** is deposited with the Cambridge Crystallographic Data Centre (Supplementary Publication No.: CCDC 119472) and can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, by fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk
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