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Solid-phase synthesis of apicidin A and a cyclic tetrapeptoid analogue

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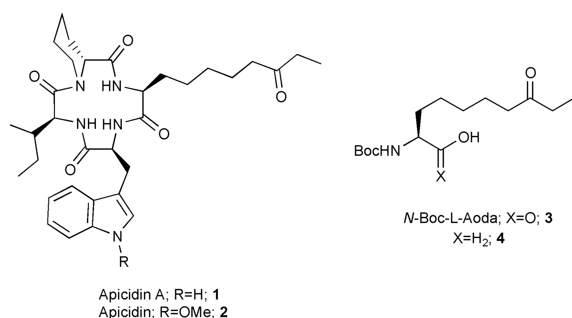
The solid-phase synthesis of the antiprotozoal cyclic tetrapeptide apicidin A is reported and its synthetic accessibility is contrasted with that of a structurally similar reduced cyclic tetrapeptoid analogue.

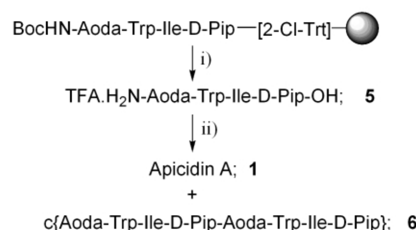
Apicidin A **1** and apicidin **2** are members of a rare class of cyclic tetrapeptide (CTP) fungal metabolites isolated from cultures of *Fusarium pallidoroseum* which are characterised by the presence of the non-proteinogenic α-amino acid (*S*)-2-amino-8-oxodecanoic acid (Aoda) **3**.[1] These natural products have been shown to exhibit broad spectrum cidal activity against members of the apicomplexan family of protozoa such as *Plasmodium* sp. and *Crytosporidinium parvum*, which are the causitive agents of malaria and cryptosporidosis respectively.[2] Their biological activity is attributable to inhibition of the zinc metallopeptidase histone deacetylase (HDAC).[3] The results of chemical modifications to the natural product apicidin itself with the objective of identifying *parasite-selective* and hence potentially non-toxic HDAC inhibitors have been disclosed by workers at Merck.[4]



Recently, a solution-phase synthesis of apicidin A **1** was reported by Liyuan and Singh.[5] Herein, we report a solid-phase synthesis of apicidin A **1** and describe an efficient approach to more readily accessible reduced cyclic tetrapeptoid analogues which may, therefore, represent a more viable platform upon which to base a drug discovery program.[6]

It is now well established that the correct choice of linear tetrapeptide precursor is pivotal in order to achieve a successful cyclisation to a desired CTP and that this process is typically in competition with that leading to the formation of the corresponding cyclic octapeptide (COP).[7] Therefore, from a consideration of both the work of Schreiber[8a] and Schmidt,[8b] and by modelling of the likely transition state leading to cyclisation, we envisaged an approach to apicidin and related analogues which invokes a cyclisation between an *N*-terminal Aoda and a *C*-terminal (*R*)-pipecolic acid.

Thus, *N*-Boc-L-Aoda **3** was prepared from the Garner aldehyde.[9] In an improvement to the previously reported route,[6] oxidation of the precursor **4** to furnish the desired carboxylic acid **3** was achieved in 74% isolated yield under the conditions described by Zhao *et al.*[10] ($NaClO_2$, NaClO, TEMPO). The requisite linear tetrapeptide **5** was then obtained in 39% overall yield by conventional Fmoc peptide synthesis on 2-chlorotrityl resin. Cyclisation of **5** was then examined in solution under a

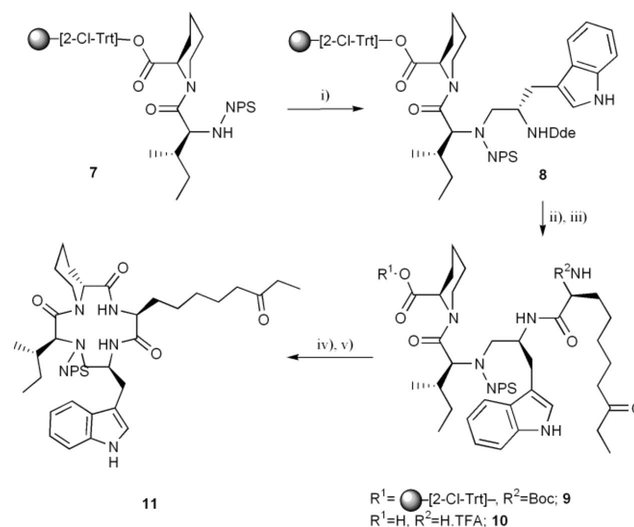


Scheme 1 i) TFA–H_2O–CH_2Cl_2 (49 : 1 : 50), rt; ii) activator, HOBt, $^iPr_2EtN$, solvent, concentration (Table 1), rt.

variety of conditions by varying coupling reagent [*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) or (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP)], solvent (DMF or CH_2Cl_2), and concentration (Scheme 1).

The results of this study (Table 1) clearly attest to the difficulty in obtaining a high yield of the desired CTP **1** in the presence of competing COP **6** formation. Nevertheless, it was possible to prepare apicidin A **1** under high dilution conditions (0.2 mM **5** in DMF; Table 1, entry 2) in 62% yield after purification by HPLC. This material was identical[11] to an authentic sample prepared by the catalytic hydrogenolysis of apicidin.[12]

In contrast, we have recently demonstrated by the solution-phase synthesis of a model compound that the incorporation of a reduced amidic linkage in conjunction with an *o*-nitrophenylsulfonamide (NPS) group as a ′backbone hinge′ into the tetrapeptide chain can greatly facilitate cyclisation leading to the corresponding cyclic tetrapeptoid analogues, without evidence for competing cyclic octapeptoid formation.[6] However, a solution-phase preparation of the desired linear tetra-



Scheme 2 i) *N*-Dde-tryptophanol, TBAD, PPh_3, CH_2Cl_2, rt; ii) N_2H_4 (55% in H_2O)–DMF (1 : 4), rt; iii) **3**, PyBOP, HOBt, $^iPr_2EtN$, CH_2Cl_2, DMF, rt; iv) TFA–H_2O–CH_2Cl_2 (49 : 1 : 50), rt; v) activator, HOBt, $^iPr_2EtN$, solvent, concentration (Table 1), rt. NPS = *o*-nitrophenylsulfonyl.

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Table 1 Cyclisation of linear tetramers **5** and **10** under various conditions

Entry	Starting tetramer	Conditions			Isolated yields[a]		HPLC ratio[b]
		Concentration	Activator	Solvent	Cyclic tetramer	Cyclic octamer	
1	**5**	0.2 mM	TBTU	DMF	56%	34%	67/33
2	**5**	0.2 mM	PyBOP	DMF	62%	23%	71/29
3	**5**	0.2 mM	TBTU	CH_2Cl_2	22%	50%	37/63
4	**5**	0.2 mM	PyBOP	CH_2Cl_2	33%	52%	35/65
5	**5**	2 mM	TBTU	DMF	34%	63%	31/69
6	**10**	2 mM	TBTU	CH_2Cl_2	90%	—	> 99/1[c]
7	**10**	2 mM	TBTU	DMF	88%	—	> 99/1[c]
8	**10**	20 mM	TBTU	DMF	83%	—	> 99/1[c]

[a] Yield of product after purification by preparative HPLC. [b] HPLC ratio of compounds in the crude mixture (215 nm). [c] The cyclic octamer could not be detected by LCMS analysis of the crude mixture.

peptoid proved to be laborious and thus unsuitable for the high-throughput synthesis of such compounds. To address this shortcoming, we have therefore developed an alternative solid-phase route (Scheme 2). Thus, Fmoc-D-pipecolic acid was immobilised on 2-chlorotrityl polystyrene resin, deprotected and coupled with *N*-Fmoc-isoleucine. The Fmoc protecting group was removed and the resulting terminal amine was sulfonylated with *o*-nitrophenylsulfonyl chloride to provide the sulfonamide **7**. Homologation with Dde-L-tryptophanol (Dde = 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) was then effected under Mitsunobu conditions to afford **8**. After some optimisation, it was established that a combination of di-*tert*-butyl azodicarboxylate (TBAD) and triphenylphosphine in dichloromethane gave the best results. Removal of the Dde protecting group and coupling of the resulting amine with *N*-Boc-L-Aoda **3** gave the resin bound tetrapeptoid **9**. Finally, treatment with TFA to effect both cleavage from the resin and concomitant removal of the Boc protecting group provided the linear tetrapeptoid **10** in 42% overall yield.

Cyclisation of **10** was then contrasted to that of the corresponding tetrapeptide **5** (Scheme 2). In all cases, no competing formation of the corresponding cyclic octapeptoid was observed and the cyclisation to afford **11** was found to be largely independent of both solvent (DMF or CH_2Cl_2) and concentration (Table 1, entries 1–5). Whereas at a 2 mM concentration (8.2 mg of **5** in 5.3 ml of DMF), the CTP **1** was obtained as an approximately 1 : 2 mixture with the cyclodimer (Table 1, entry 5), the corresponding cyclic tetrapeptoid **11** was obtained as the sole isolated product in 88% yield (Table 1, entry 7). In this latter case, even with a ten-fold increase in concentration to 20 mM (12.1 mg of **10** in 0.65 ml of DMF), the cyclic tetrapeptoid **11** was isolated in 83% yield (Table 1, entry 8). Notably, working at higher concentrations than before, the cyclisation was complete in less than 15 minutes and no trace of the corresponding cyclodimer could be detected by LCMS.

In summary, we have prepared the CTP natural product apicidin A **1** and a related cyclic tetrapeptoid analogue **11** bearing a reduced amidic linkage. Both linear precursors were efficiently assembled by solid-phase synthesis and their cyclisations were compared in solution under a range of conditions. The linear tetrapeptoid **10** undergoes a much more efficient cyclisation which is tolerant of a wide range of concentrations whilst still giving a high cyclisation yield with no observed propensity to form cyclic dimers. In contrast, the cyclisation of the linear tetrapeptide **5** afforded apicidin A **1** contaminated with the corresponding COP **6** even under high dilution conditions.

We are currently investigating cyclic tetrapeptoids of this type as novel HDAC inhibitors and these results will be reported in due course. In addition, we anticipate that cyclic tetrapeptoids will prove to be an accessible and useful scaffold on which to generate compound libraries as generic pharmacophoric probes.

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