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Synthetic routes to NEtXaa[4]-cyclosporin A derivatives as potential anti-HIV I drugs

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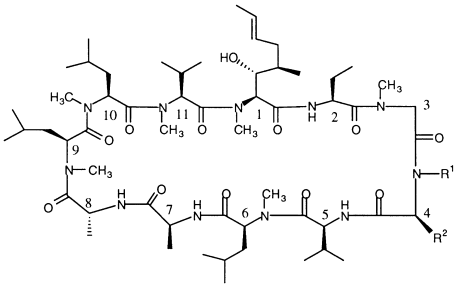
Abstract

An efficient synthesis in 10 steps and overall yields up to 27% of NEtXaa[4]-cyclosporin A derivatives (Xaa = Leu, Val, Ile, Thr) starting from cyclosporin A is described. Biological activities of the new analogues show promising results for the design of cyclosporin derivatives exhibiting non-immunosuppressive and anti-HIV activity.

Keywords: cyclosporin A derivatives; anti-HIV drug; *N*-ethyl amino acids.

Since the discovery of the immunosuppressive activity of cyclosporin A (CsA), considerable work has been devoted to the chemical synthesis of analogues of CsA.[1] More recently, the finding of a potential anti-HIV I activity of CsA evoked interest for the design of more selective cyclosporins active against HIV I but devoid of immunosuppressive activity.[2]

Based on previous observations, the synthesis of NEtXaa[4] derivatives of CsA (R^1 = Et, Fig. 1) on a preparative scale became particularly appealing. The *N*-methyl group at position 4 being



	R^1	Residue 4 Side chain R^2
1 (CsA)	-Me	Leu
5a	-Et	Leu
5b	-Et	Val
5c	-Et	Ile
5d	-Et	Thr

Figure 1. Structure of cyclosporin A (CsA) **1**, NEtLeu[4]-CsA **5a**, NEtVal[4]-CsA **5b**, NEtIle[4]-CsA **5c**, NEtThr[4]-CsA **5d**

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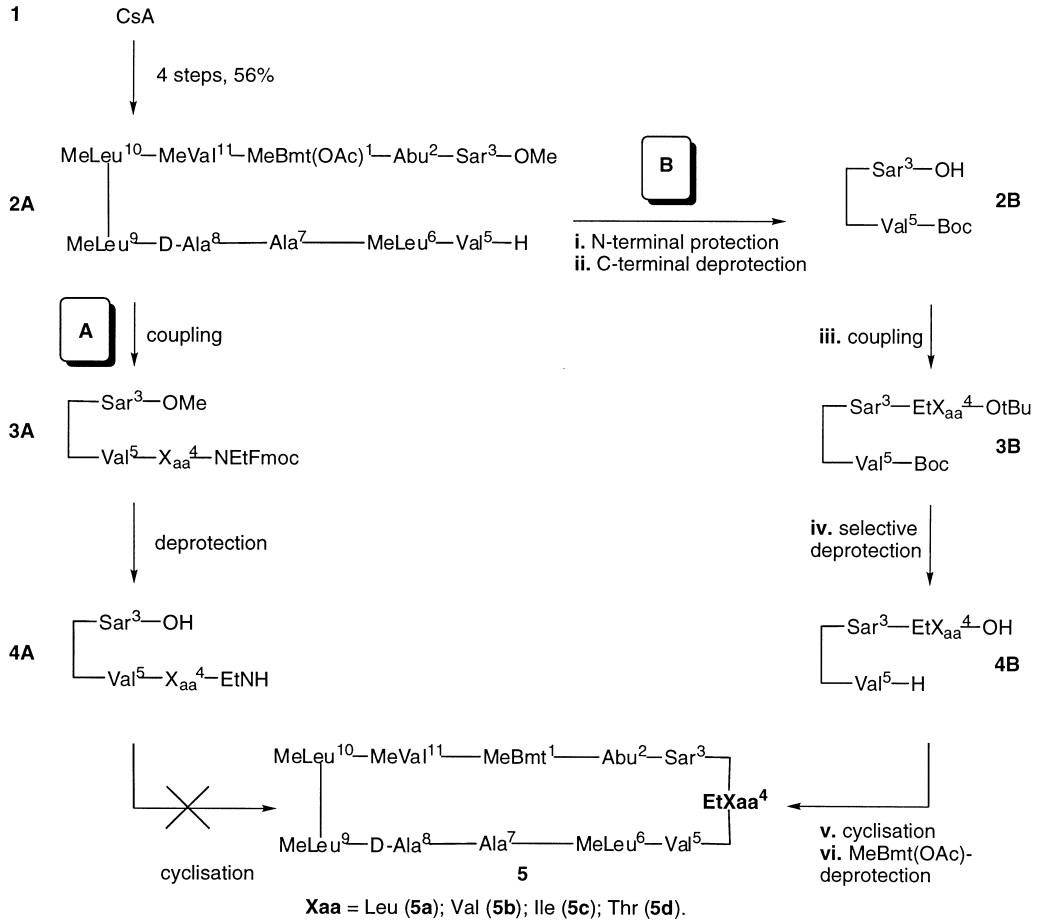

Scheme 1. Reaction scheme for the synthesis of NEtXaa[4]-CsA **5a–d**: (i) Boc_2O (2 equiv.), DIEA (3 equiv.), 0.02 molar in dioxane, 3 h, rt; (ii) $LiOH.H_2O$ (1.4 equiv.+1.4 equiv.), 0.018 molar in THF:H_2O (4:1), o.n., 0°C until rt; (iii) HATU (2.2 equiv.), DIEA (4.5 equiv.), H-NEt-Xaa-OtBu (2.5 equiv.), 0.02 molar in CH_2Cl_2, o.n., rt or isobutylchloroformiate (2 equiv.), *N*-methylmorpholine (2 equiv.), H-NEt-Xaa-OtBu (3 equiv.), 0.02 molar in CH_2Cl_2, 5 h–o.n., –25°C until rt; (iv) 0.01–0.03 molar in TFA:CH_2Cl_2 (1:5), 1 h, rt (0.01 molar in TFA, 2 h, 0°C for **3Bd**); (v) TFFH (3 equiv.), sym.-collidine (11 equiv.), 10^{-4} molar in CH_2Cl_2, 1–18 h, rt (PyAOP (5 equiv.), DMAP (3 equiv.), 10^{-3} molar in DMF, o.n. rt for **4Bc**); (vi) $NaOCH_3$ (2–4.3 equiv.), 0.025–0.05 molar in CH_3OH, 3–48 h, 0°C until rt ($NaOCH_3$ (4.3 equiv.), 0.07 molar in CH_3OH, 1 h at 0°C, 3 h at rt for **4Bd**). Numbering of amino acid residues of peptides following cyclosporin notation MeBmt-1 to MeVal-11[12]

involved in one of the main metabolic degradation pathways,[3] we guessed that the corresponding *N*-ethyl derivative, together with the appropriate choice of residue 4, may result in CsA analogues of increased anti-HIV and reduced immunosuppressive activity.[4]

A synthetic pathway for accessing Xaa[4] derivatives of CsA was published by Papageorgiou et al.[4,5] featuring a final cyclisation step between residues 3 and 4. The choice of this cyclisation was dictated by the presence of the achiral sarcosine at position 3 avoiding the risk of epimerisation during the activation step (Scheme 1, A). In applying this strategy for the synthesis of NEt[4] derivatives of CsA, Fmoc-protected *N*-ethyl-amino acid derivatives[6] were coupled to the linear

cyclosporin precursor **2A**[7] resulting in peptides **3A** which, after deprotection, gave the linear undecapeptides **4A** in yields of 30 to 50%. Due to the steric hindrance induced by the *N*-ethyl group, all efforts to cyclise intermediates **4A** proved unsuccessful even when applying powerful coupling reagents such as BOP-Cl,[8] PyBOP,[9] PyAOP,[10] TFFH[11] or alternative bases (DIEA, DBU, DMAP) and solvents (DCM, DMF). Under optimised conditions (TFFH/DIEA/DCM) only minor amounts of the target compounds were detected by analytical HPLC.

In order to bypass this critical step, an alternative strategy was developed in which the final cyclisation step was effected between residues 4 and 5 (Scheme 1 B). Here, the cyclisation reaction to Val[5] (*N*-non-alkylated) of the activated ester of protected *N*-ethyl-residues should proceed more smoothly to the expense of a potential risk for C-terminal epimerisation. According to Scheme 1B, the reactive site of peptide **2A** was inverted by Boc-protection of the *N*-terminal group and saponification at the C-terminus (product **2B**). Subsequent activation of the *N*-ethyl amino acid residue[13] with HATU[14] and coupling with **2B** gave the fully protected undecapeptide **3B** which, after deprotection of the N-terminal (Boc) and C-terminal (OtBu) groups, resulted in the linear precursor **4B**. For **3Bd**, the deprotection step was carried out in pure TFA for cleaving both the Boc and the side chain *tert*-butylether-protecting groups of the NEtThr. Cyclisation of **4B** proceeded to good yields within 1 h (Table 1) applying TFFH as a coupling agent in combination with sym.-collidine as a weak base without observable epimerisation. In contrast, cyclisation of **4Bc** using PyAOP as coupling agent resulted in partial epimerisation of residue 4. Finally, methanolysis of the acetate protecting group (step vi) produced the corresponding CsA analogues **5a–d** in overall yields of 16 to 46% (Table 1). According to this reaction scheme, analogue **5b** could be prepared on a 5 g scale in our laboratory.

Table 1
Characterisation and biological activities of CsA analogues **5a–d** (see Scheme 1)

Product	Overall yield % (from **2A**)	HPLC t_R	Mass m/z found $[M+H]^+$ (calcd)	CyP-A IC_{50} / IC_{50} CsA	IL2-RGA
NEtLeu[4]CsA (**5a**)	32	25.8 min	1217.2 (1215.9)	1.2	1.6
NEtVal[4]CsA (**5b**)*	46	23.1 min	1203.3 (1201.8)	0.67	>2500
NEtIle[4]CsA (**5c**)	30	25.1 min	1217.2 (1215.9)	0.83	~300
NEtThr[4]CsA (**5d**)	16	21.0 min	1204.5 (1203.8)	n.d.	n.d.

* mp 140-148°C, Rf (acetone/hexane 4/7) 0.4, $[\alpha]_D^{20}$ −177 (c=0.07, MeOH)

In vitro activities of cyclosporin derivatives **5a–d** were evaluated applying the IL-2 reporter gene assay (measuring immunosuppressive activity) and the binding affinity to CypA. The IL-2 reporter gene assay detects substances interfering with IL-2 gene activation along the T cell signalling pathway.[15] The binding to CypA was determined using the improved spectrophotometric assay described by Rich.[16] The results (Table 1) indicate that the synthetic cyclosporin *N*-ethyl[4] derivatives **5a–c** show comparable binding affinities but strongly reduced immunosuppressive activities compared to CsA. In particular, compound **5b** may serve as lead for the design of non-immunosuppressive cyclosporins with potential anti-HIV activity.

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