

Total synthesis of Cyclosporin O

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Introduction

Cyclosporines (Cs) are produced as fungal metabolites of *Cylindrocarpum lucidum booth* and *Tolyposcaldium inflatum gams*. They exhibit strong T-cell specific immunosuppression. Their potential anti-HIV activity has evoked interest for the design of selective cyclosporines against HIV. Cyclosporin O (CsO) exhibits marked immunosuppression but with considerably less nephrotoxicity than parent CsA. Structurally it differs from other Cs by not containing MeBmt at position 1. But the total synthesis of CsO is challenging [1] due to the presence of hindered *N*-Me amino acids and a difficult sequence containing four consecutive *N*-Me amino acids.

Results and discussion:

Different methods have been reported for solution as well as solid phase synthesis of CsO [2-5] based on fragment condensation approaches. We herein describe an epimerization free and efficient total synthesis of CsO by stepwise linear synthesis approach in solution phase employing Fmoc-amino acid derived acid chlorides and mediated by Zinc dust. The acid chlorides are one of the powerful modes of activation of carboxylic acids. They can be rapidly and inexpensively prepared. Their utility in the synthesis of sterically hindered dialkyl amino acids is known [6,7]. The couplings employing acid chlorides require a base to abstract the liberated HCl. Alternatively, Zn dust can be used for this purpose under non-Schotten-Baumann conditions [8-12] in organic solvents like CH₂Cl₂ or CHCl₃. Performing couplings in presence of Zn under

neutral conditions eliminates the base-catalyzed side reactions like formation of oxazolones and causes no racemization. Also, the duration of couplings can be extended to long periods. These advantages offered by the combined use of amino acid chlorides and zinc dust prompted us to explore them for the total synthesis of CsO.

Towards the first step, Fmoc-MeLeu⁶-Ala⁷-OBzl **1** was synthesized using Fmoc-MeLeu-Cl in presence of zinc dust (Scheme 2). To a mixture of Fmoc-MeLeu-Cl (1.2 mmol) and freshly activated zinc dust (1.5 mmol) in DCM (3 mL) was added a solution of H₂N-Ala-OBzl (1 mmol) in DCM (3 mL) and stirred at rt till the completion of the reaction. The progress of the reaction was monitored by TLC and IR. The formation of **1** was complete in 25 min and workup of the reaction mixture resulted in pure peptide in 93 % yield. The overall strategy involved in the synthesis of CsO is given in the Scheme 1.

For the deprotection of Fmoc group, the use of *tris*(2-aminoethyl)amine (TAEA) and diethyl amine (DEA) were tested. When TAEA is used in solution phase, aqueous work is required after deprotection to remove excess of TAEA and the dibenzofulvene (DBF) adduct. However in our studies, TAEA mediated deprotection of Fmoc group resulted in the loss of amino free peptide ester accounting for low yield of tripeptide during aqueous work up. On the contrary, DEA, being a low boiling solvent (b.p. 55 °C), can be completely removed by simple evaporation. The deprotection with DEA is complete in 30 min and the DBF adduct can be removed through column chromatography after coupling. The Fmoc group of **1** was deprotected using DEA/DCM.

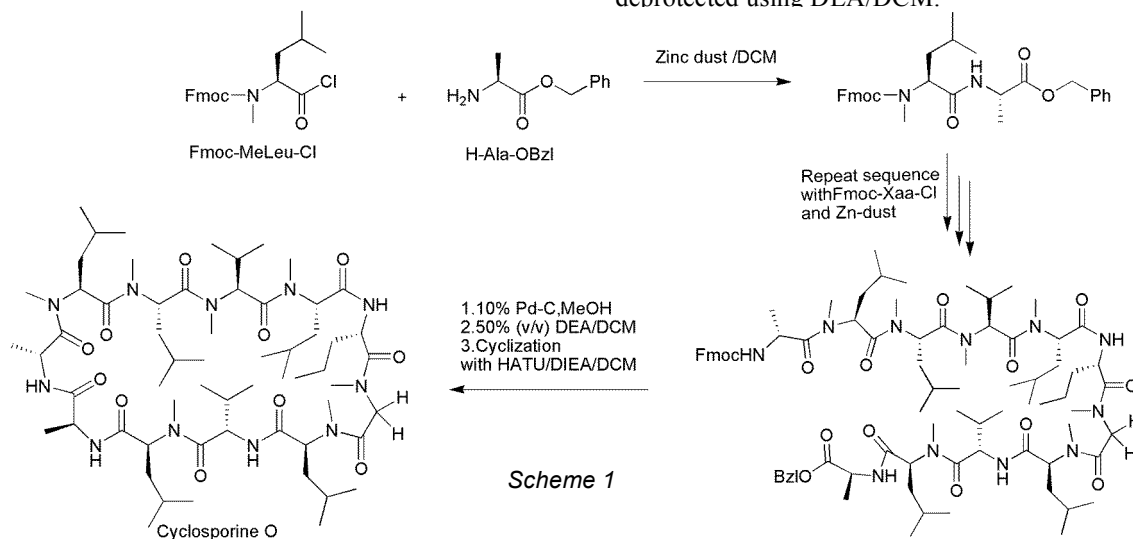
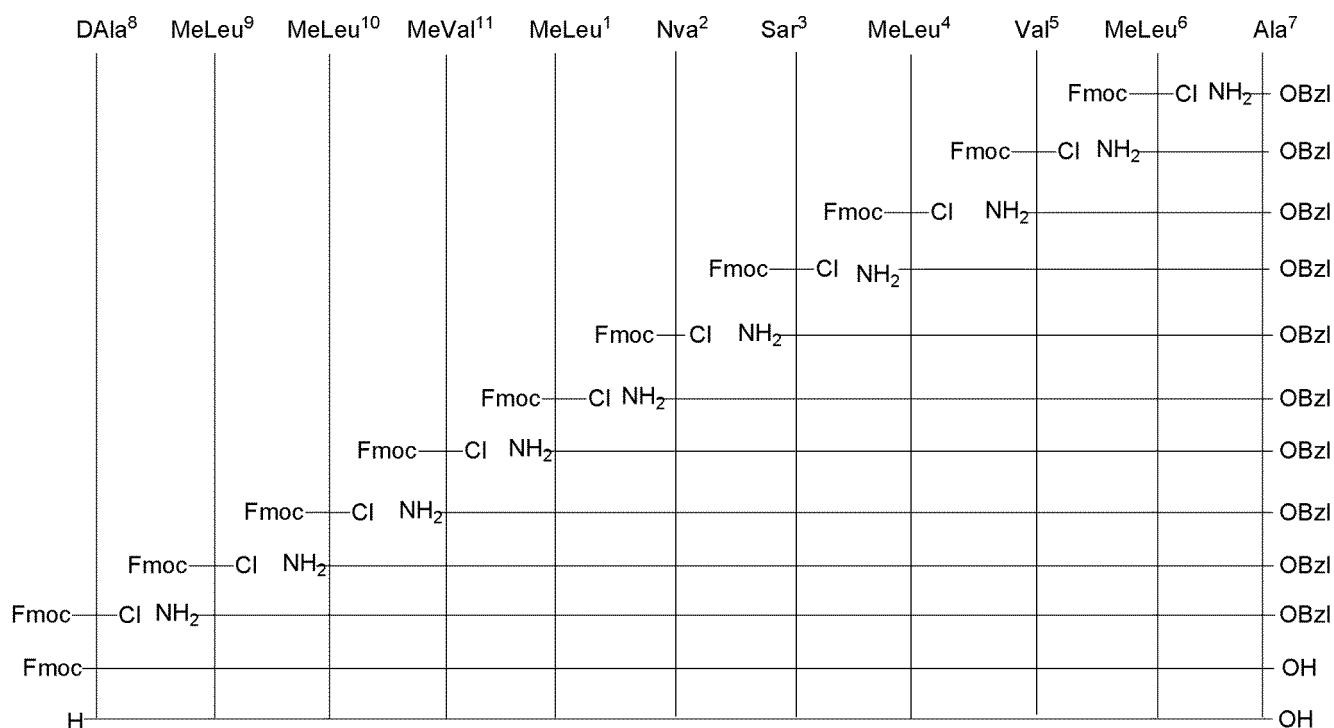


Table 1. A comparative study of coupling of Fmoc-Val-Cl to H-MeLeu-Ala-OBzl.

Coupling method	Solvent	base (equiv)	yield (%) ^a
Acidchloride/Zn dust	DCM	-	90
Acid chloride/base	DCM	DIEA (2)	68
BOP-Cl	DCM	DIEA (2)	60
PyBrOP	DCM	DIEA (2)	48
HBTU	THF	DIEA (2)	53

The resulting amino free dipeptide benzyl ester H₂N-MeLeu⁶-Ala⁷-OBzl was used directly without isolation and reacted with Fmoc-Val-Cl in presence of Zn dust to obtain the tripeptide Fmoc-Val⁵-MeLeu⁶-Ala⁷-OBzl **2**. A comparative study of coupling of Fmoc-Val-Cl to H-MeLeu-Ala-OBzl to make tripeptide Fmoc-Val-MeLeu-Ala-OBzl is given in the table 1. Subsequent deprotection and coupling of **2** with Fmoc-MeLeu-Cl resulted in the tetrapeptide Fmoc-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **3**. The pentapeptide Fmoc-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **4** was obtained by reacting two equiv of Fmoc-Sar-Cl with the amino free tetrapeptide. The hexapeptide Fmoc-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **5** and the heptapeptide

Fmoc-MeLeu¹-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **6** were obtained starting from their corresponding precursor amino free peptide benzyl esters. The incorporation of Fmoc-MeVal at the 11 position and Fmoc-MeLeu at 9 and 10 positions was crucial and was the most difficult aspect in the stepwise assembly. During the synthesis of the octapeptide Fmoc-MeVal¹¹-MeLeu¹-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **7**, the formation of peptide bond between Fmoc-MeVal-Cl and the heptapeptide was sluggish and the yield was only 45% after the first coupling. LC-MS of the crude **7** showed the presence of the free *N*-deprotected heptapeptide. Repetition of the coupling twice, each time employing Fmoc-MeVal-Cl (1.5 equiv) and zinc dust (1.6 equiv) and with a duration of 2.5 hr improved the yield of octapeptide to 79%. Thus, the use of triple coupling and longer duration of reaction time drastically enhanced the yield from 45% to 79%. A similar approach was adopted further for the incorporation of the remaining three residues. The nonapeptide, Fmoc-MeLeu¹⁰-MeVal¹¹-MeLeu¹-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **8**, the decapeptide Fmoc-MeLeu⁹-MeLeu¹⁰-MeVal¹¹-MeLeu¹-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **9** and the undecapeptide Fmoc-D-Ala-MeLeu⁹-MeLeu¹⁰-MeVal¹¹-MeLeu¹-Nva²-Sar³-MeLeu⁴-Val⁵-MeLeu⁶-Ala⁷-OBzl **10**



For coupling: acid chloride/zinc dust
For deprotection: 50% (v/v) DEA: DCM
For benzyl ester deprotection: 10% Pd-C / MeOH

Fig. 1. Linear synthesis of amino-free undecapeptide acid

Table 2. summary of coupling conditions employed during CsO synthesis

Acid chloride equiv used	No. of couplings	Coupling duration	Peptide obtained	Yield (%)
1.1	One	0.25 h	Di	93
1.1	One	0.40 h	Tri	90
1.1	One	0.25 h	Tetra	90
2.0	One	2.0 h	Penta	84
1.1	One	1.5 h	Hexa	91
1.1	One	1.5 h	Hepta	90
1.5	Three	2.5 h	Octa	79
1.5	Three	2.5 h	Nona	73
1.5	Three	2.5 h	Deca	76
1.5	Three	2.5 h	Undeca	82

were obtained in 73%, 76% and 82% yields respectively. The summary of coupling conditions employed during CsO synthesis is furnished in Table 2. The carboxyl group of the linear undecapeptide 10 was deprotected from its benzyl ester using 10% Pd on carbon by catalytic hydrogenation. The Fmoc group of resulting Fmoc linear undecapeptide acid was deprotected by DEA/DCM and the free undecapeptide was cyclised using *O*-(azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluroniumhexafluorophosphate (HATU)/DIEA to obtain CsO as a white solid in 85% yield (Fig. 1).

In conclusion, the stepwise linear synthesis of CsO was carried out employing Fmoc-amino acid chlorides and zinc dust under neutral conditions. The amino free peptide benzyl esters were used directly without isolation for coupling with next amino acid chloride. The assembly of the segment containing four consecutive *N*-methyl amino acids required triple coupling and extended coupling duration in order to enhance the yield and purity of the respective peptides. All the ten intermediate Fmoc-protected peptides starting from the dipeptide ester to the linear undecapeptide ester were isolated and characterized by ¹H NMR, mass spectroscopy and HPLC (Fig. 2). Deprotection of benzyl ester and Fmoc groups from the protected undecapeptide followed by the cyclization with HATU in DCM resulted in CsO as white solid. Starting from dipeptide 1 the final CsO was obtained in an overall of 16 % yield.

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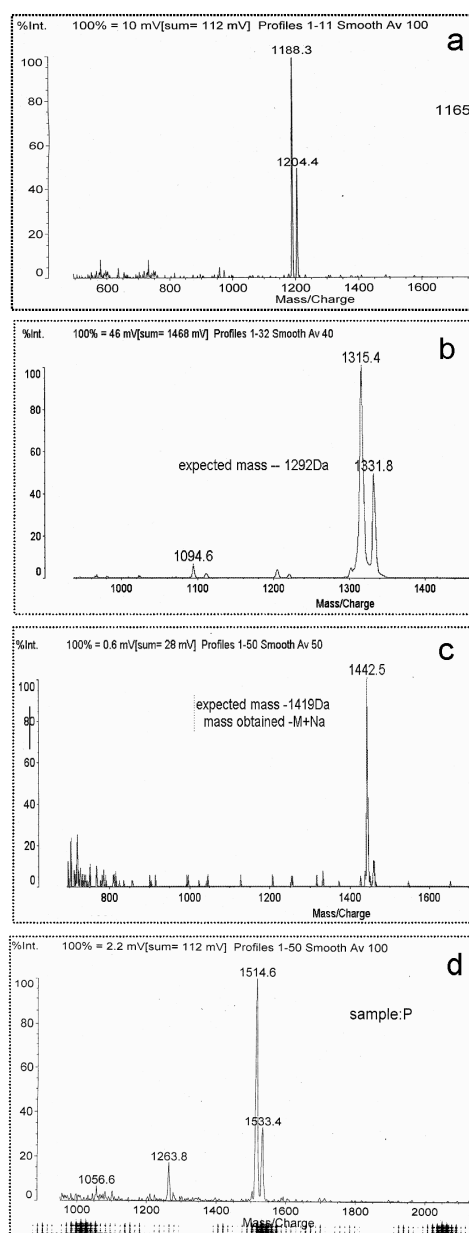


Fig. 2. MALDI-TOF spectra of key intermediate Fmoc-protected peptide fragments a) octapeptide 7, b) nonapeptide 8, c) decapapride 9, d) undecapeptide 10.