SYNTHESIS OF THE SOUTHERN TRIPEPTIDE (C₁–N₁₂) OF SANGLIFEHRINS USING ASYMMETRIC ORGANOCAatalYSIS

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GRAPHICAL ABSTRACT

Abstract The tripeptide southern region of the novel cyclophilin binding natural product macrolides, namely sanglifehrins, is synthesized involving asymmetric organocatalysis as chirality-inducing step. List’s asymmetric α-amination was used in the synthesis of the m-hydroxyphenylalanine part, whereas α-hydrazination was used for the piperazic ester part.

Keywords α-Amination; α-hydrazination; asymmetric organocatalysis; peptide coupling; tripeptide

INTRODUCTION

The immunosuppressive activity of cyclosporin A,[1a] FK 506, and rapamycin[1b] is attributed to immunophilin modulation, wherein the cyclosporin operates through cyclophilin binding.[1] Thus an exhaustive screening was initiated to identify novel natural products having cyclophilin binding properties. In this process, a novel class of macrolides has been isolated by Sanglier et al.[2] from actinomycete strains based on their affinity of binding to cyclophilin A, a cytosolic protein binding to cyclosporin. These macrolides are found to have 20-fold greater affinity to cyclophilin-A over the marketed drug cyclosporin A.
Further, Kallen et al.\cite{3} have provided mechanistic insights of this higher binding affinity through cocrystal studies and also observed that the piperazic acid moiety present in sanglifehrins is deeply buried in the hydrophobic cavity formed by the amino acids Phe\textsuperscript{60}, Met\textsuperscript{61}, Phe\textsuperscript{113}, and Leu\textsuperscript{122} of cyclophilin A.

The profound biological activities attributed to these macrolides\cite{4} along with unprecedented type of structure having macrocyclic lactone core with two uncommon amino acids, (S)-3-carboxy-piperazine and (S)-m-tyrosine, naturally attracted interest from the synthetic organic chemistry community. To date, two total syntheses\cite{5} and several partial syntheses\cite{6} of sanglifehrin A 1\textit{a} (Fig. 1) are in print. The piperazic acids are common constituents of monamycins\cite{7} whereas m-tyrosine has a profound role in probing the pathways in the central nervous system (CNS).\cite{8}

Our group has a long-term objective of evaluating the synthesis of natural products incorporating uncommon amino acids\cite{9} and studying their biological activity. Towards this goal, we identified the southern tripeptide core of sanglifehrin A as a powerful privileged fragment for evaluating as a key synthon toward SAR studies. Herein, the synthesis of the C\textsubscript{1}-N\textsubscript{12} fragment 2 of sanglifehrins is presented using asymmetric organocatalysis, wherein chirality is introduced via (R)-proline.

**RESULTS AND DISCUSSION**

The classical retrosynthetic analysis of tripeptide 2 produced two synthetic fragments 3 and 5, which in turn could be obtained from 6 and 7 through organocatalytic \(\alpha\)-amination and \(\alpha\)-hydrazination respectively (Scheme 1).

The synthesis of (S)-m-benzylxoy tyrosine methyl ester (3) is described in Scheme 2. The 3-(3-(benzyloxy)phenyl)propanal (6) was prepared following literature procedure from 3-hydroxybenzaldehyde.\cite{10} The aldehyde 6 underwent a very
Scheme 1. Retrosynthetic analysis of tripeptide 2.

Scheme 2. Synthesis of fragment 3.
highly enantioselective organocatalytic α-amination\textsuperscript{11} in the presence of (R)-proline (10 mol%) and di-tert-butyl azodicarboxylate to furnish hydrazino aldehyde 8, which was reduced to 8a using NaBH\textsubscript{4} for characterization. The ee of the product 8a was determined to be 93\%\textsuperscript{12}. The hydrazino aldehyde 8 was oxidized to carboxylic acid, which was esterified immediately as methyl ester 9. The catalytic hydrogenation of 9 furnished phenol 10 in 86\% yield. The vicinal Boc groups on hydrazine 10 were knocked down with trifluoroacetic acid (TFA), which followed Raney–Ni hydrogenation to generate the amino ester, which was protected as a Boc amide 11\textsuperscript{13}. All these three transformations were carried out without engaging any purification steps with an overall yield of 67\%. Rebenzylation of phenolic group in 11 was achieved in quantitative yields to furnish benzyl protected meta-tyrosine methyl ester 3, for which the spectral data was matched with that reported.\textsuperscript{14} A direct conversion of 9 to 11 was attempted, albeit in poor yields in the presence of Raney-Ni (Scheme 2).

(3S)-Methyl piperazate 15 was synthesized using organocatalytic α-hydrazination from 5-bromopentanal 7, following the procedure reported by Hamada et al.,\textsuperscript{15} except that (R)-proline (10 mol\%) and di(Boc)-hydrazine were used to synthesize (S)-enantiomer. Thus, 7 on treatment with di(Boc)-hydrazine and (R)-proline\textsuperscript{16} followed by NaBH\textsubscript{4} reduction furnished (S)-hydrazino alcohol 12. The optical purity of 12 was determined as its benzoate ester 12a, which was synthesized by treating 12 with BzCl and pyridine in 97\% yield. The ee of the product 12a was found to be 81\%.\textsuperscript{17} Exposure of 12 to TBSOTf and 2,6-lutidine followed by treatment with NaH provided piperazine derivative 13 in 95\% yield for two steps. The silyl group in 13 was cleaved with TBAF to furnish alcohol 14 (96\% yield),
which was oxidized to known methyl ester 5 in two steps (87% overall yield) and whose identity was fully confirmed. Exposure of 5 to TFA in CH₂Cl₂ furnished di-TFA salt of piperazic ester 15 (Scheme 3).

After establishing the synthetic procedures for the synthesis of uncommon amino acid derivatives 3 and 15, compound 3 was exposed to TFA in CH₂Cl₂, which was further coupled with N-Boc (S)-valine 4 using HOBT (1-hydroxybenzotriazole) and EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) to furnish the required dipeptide 16 in 89% yield with excellent diastereoselectivity. The major diastereomer of the dipeptide was separated through column chromatography and subjected to hydrolysis with LiOH to furnish dipeptide acid 17 in 87% yield. Further, the dipeptide acid 17 was coupled selectively at N(1) of piperazic ester[5a,5b] 15 using HATU (N,N,N',N'-tetramethyl-O-7-azabenzo-triazol-1-yluroniumhexafluorophosphate) and DIPEA to afford the fully and differentially protected desired tripeptide 2 (76% yield) in 4:1 diastereomeric ratio,[18] which was separated by silica-gel column chromatography. Spectral data are in agreement with the reported data[6g](Scheme 4).

**EXPERIMENTAL**

¹H and ¹³C NMR spectra were recorded in CDCl₃/dimethylsulfoxide (DMSO) with 300-, 400-, 500-, or 700-MHz spectrometers at ambient temperature. Chemical shifts were measured in parts per million (ppm) and coupling constants in hertz (Hz); shifts were measured relative to the signals for residual CHCl₃ (δ = 7.26 ppm), CDCl₃ (δ = 77.0 ppm), DMSO (δ = 2.50 ppm), and DMSO (δ = 39.43 ppm). Optical rotations were measured with an Anton-Paar MCP 200 digital polarimeter by using a 1-mL cell and a 1-dm path length. FT-IR spectra were recorded as KBr discs or neat. High-resolution mass spectra (HRMS) were recorded with an Agilent Technologies
6510 Q-TOF spectrometer. Technical-grade ethyl acetate and hexanes used for column chromatography were distilled prior to use.

**Synthesis and Characterization Data of Southern Tripeptide 2[^6g]**

HATU (48 mg, 0.127 mmol) and DIPEA (0.06 mL, 0.37 mmol) were added to a solution of compound 17 (0.050 g, 0.01 mmol) in dry DMF (3 mL) at room temperature and stirred for 10 min. To this reaction mixture, compound 15 (16 mg, 0.01 mmol) in dry DMF (2 mL) was added and stirred for 2 h. The reaction mixture was diluted with ethyl acetate (20 mL), washed with brine (10 mL), and dried over sodium sulfate. The combined organic layer was concentrated under vacuo and purified through silica-gel column chromatography (100–200 mesh) to furnish required diastereomer 2 as a liquid (48 mg, 76%). \([\alpha]_D^{20} = -22.4\ (c = 3.6, \text{MeOH})\). IR (KBr): \(\nu_{\text{max}} = 3347, 2950, 2923, 1699, 1440, 1219, 1115, 772\ \text{cm}^{-1}\). \(^1\)H NMR (DMSO, 500 MHz): \(\delta 7.66\ (d, J = 11.2 \text{ Hz}, 1\text{H}), 7.46–7.31\ (m, 5\text{H}), 7.16\ (m, 1\text{H}), 7.04–6.73\ (m, 3\text{H}), 6.58\ (d, J = 9.4 \text{ Hz}, 1\text{H}), 5.55–5.41\ (m, 1\text{H}), 5.07–4.98\ (m, 2\text{H}), 3.78\ (s, 3\text{H}), 3.76–3.72\ (m, 1\text{H}), 3.69–3.56\ (m, 1\text{H}), 3.15\ (br, 1\text{H}), 2.99–2.93\ (dd, J = 13.2, 3.5 \text{ Hz}, 1\text{H}), 2.73–2.65\ (dd, J = 8.3, 12.1 \text{ Hz}, 1\text{H}), 2.47–2.41\ (m, 1\text{H}), 1.91–1.83\ (m, 1\text{H}), 1.80–1.72\ (m, 1\text{H}), 1.70–1.47\ (m, 3\text{H}), 1.35\ (s, 9\text{H}), 0.76–0.72\ (m, 6\text{H})\ ppm. \(^13\)C NMR (DMSO, 125 MHz): \(\delta 171.4, 171.3, 170.5, 158.1, 155.2, 139.2, 137.0, 128.9, 128.3, 127.7, 127.5, 121.7, 115.7, 112.4, 78.0, 68.9, 59.9, 52.0, 51.6, 49.5, 40.4, 38.2, 30.3, 28.0, 22.0, 19.1, 19.0, 18.0\ ppm. HRMS (ESI): calcd. for C\(_{32}\)H\(_{45}\)N\(_4\)O\(_7\) [M+H]\(^+\) 597.3282; found 597.3281.

**CONCLUSION**

In conclusion, organocatalytic asymmetric \(\alpha\)-amination and hydrazination have been utilized to build the privileged tripeptide fragment of sanglifehrins.

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**SUPPLEMENTAL MATERIAL**

Full experimental details, \(^1\)H and \(^13\)C NMR, HPLC, and HRMS reports of all the compounds for this article can be accessed on the publisher’s website.

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18. Diastereomeric excess was determined by HPLC analysis. Column: Atlantis dc18, 150 × 4.6, 5U; mobile phase: 60% ACN in H2O (0.1% FA); detection: 210 nm; flow rate: 1.0 mL/min; major isomer tR 9.23 min, minor isomer tR 9.71 min.