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The total synthesis and stereochemical assignment of scytonemin A†

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The total synthesis of scytonemin A and its C-9 epimer, as well as elucidation of the absolute stereochemistry of natural scytonemin A is described.

In 1988, Moore and co-workers reported the isolation of an unusual cycloundecapeptide, scytonemin A (**1**, Fig. 1), from the cyanobacterium *Scytonema* sp. which possesses potent calcium antagonistic properties.[1] Previous work by our group on marine natural products[2] led to the assignment/revision of a number of marine natural products.[2a–f] Thus, we were encouraged to consider the synthesis of other natural products of uncertain stereochemistry. Here we report the first total synthesis and unambiguous assignment of absolute configuration of scytonemin A. Structurally, scytonemin A possesses a 34-membered macrocyclic peptide backbone which is composed of the unique, non-proteinogenic amino acid residues such as the D-(2*R*,3*S*)-threo-3-hydroxyleucine (HyLeu), L-(2*S*,3*S*)-*trans*-3-methylproline (MePro), L-(2*S*,3*R*,4*R*)-4-hydroxy-3-methylproline (HyMePro), and (2*S*,3*R*,5*S*)-3-amino-2,5,9-trihydroxy-10-phenyldecanoic acid (Ahda) as well as L-homoserine (Hse) and several D-amino acids. The relative and absolute stereochemistry of each amino acid residue was determined by a combination of Marfey analysis, circular dichroism spectra and NMR data. Although the absolute stereochemistry of C2, C3, and C5 in the Ahda moiety were assigned by a CD study of the corresponding degradation fragment, the absolute configuration of C9 in the Ahda remained unknown for the past two decades.[3] The final structural determination had to await the total synthesis of the two diastereomeric structures proposed for the natural product.

As shown in the retrosynthetic analysis plan (Fig. 1), we chose to construct the peptide macrocycle *via* an intramolecular coupling between D-serine and glycine, and a subsequent retro amide-bond formation to dissect the generated chain into two key fragments (**2**, **3**) of similar complexity. The relatively unhindered

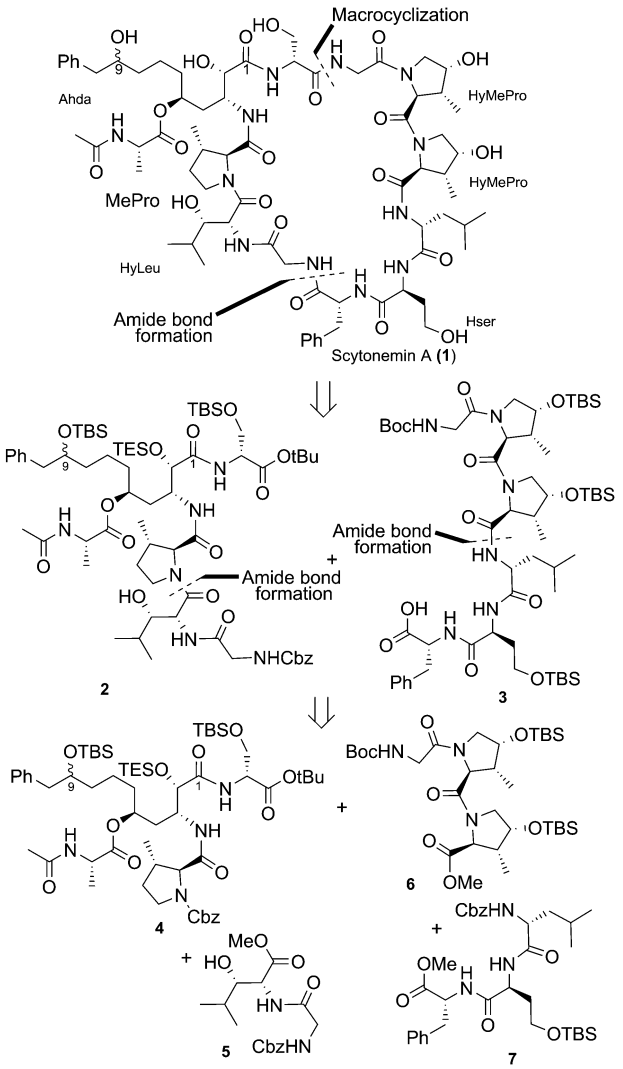

Fig. 1 Retrosynthetic analysis of scytonemin A (**1**).

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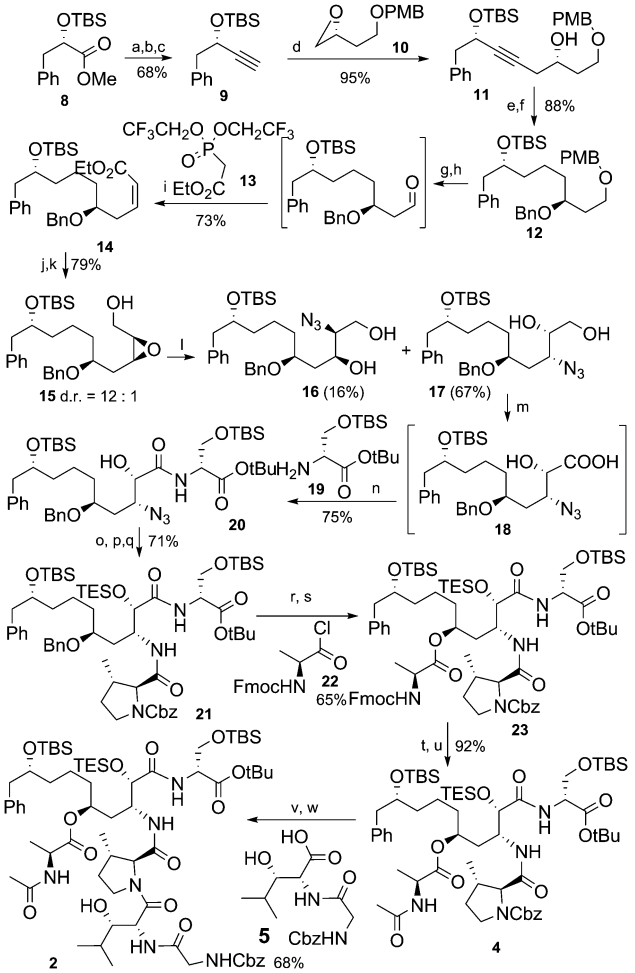
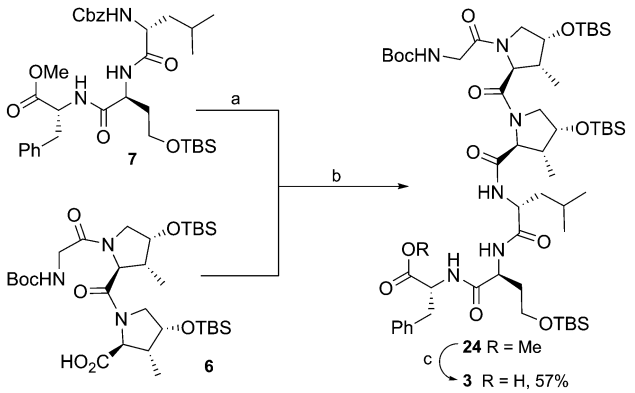
structure of the glycine nucleophile presented in both fragments may contribute to the efficiency of the segment coupling and macrocyclization. Our analysis was further guided by a hypothesis that the 4-hydroxy-3-methylproline residue located near the end of the socialization precursor may enforce a β-turn type conformation which would facilitate the final macrocyclization event.

The synthesis of fragment **2** commenced with the Dibal-H reduction of the known methyl ester **8**[4] in dichloromethane at −78 °C provided the corresponding aldehyde which was subjected to Corey–Fuchs' homologation conditions[5] using CBr_4 and PPh_3 in CH_2Cl_2 at 0 °C to afford the corresponding dibromo olefin (Scheme 1). This olefin was then converted into terminal alkyne **9** in a one-pot procedure. Regioselective opening of the known epoxide **10**[6] with the lithium anion derived from alkyne **9** by $BF_3{\cdot}OEt_2$-promoted alkylation[7] at −78 °C produced alcohol **11** in 95% yield. Catalytic hydrogenation of the internal alkyne provided the corresponding saturated alcohol, which was then protected as its benzyl ether in 88% yield. Selective removal of the PMB group from **12** in the presence of DDQ in CH_2Cl_2–H_2O (86% yield) followed by TCCA–TEMPO mediated oxidation[8] of the resulting primary alcohol readily provided the corresponding aldehyde which was subsequently subjected to a Still–Gennari modification[9] of the HWE olefination with phosphonate **13** to afford a crude α,β-unsaturated ester (*Z*/*E* 10 : 1) and the geometrically pure form of **14** was isolated in 73% yield. DIBAL-H reduction of **14** afforded the corresponding allylic alcohol, which was subjected to a Sharpless asymmetric epoxidation,[10] using (−)-DIPT, to provide epoxy alcohol **15** in 79% yield as an 12 : 1 diastereomeric mixture. The major and desired isomer was isolated in the diastereomerically pure form following chromatographic purification. Treatment of epoxide **15** with sodium azide in the presence of ammonium chloride furnished the desired azido diol **17**[11] as the major isomer, which could be isolated in 67% yield as the pure product after silica gel chromatography purification. The next challenge in the synthesis was the selective oxidation of the primary alcohol of diol **17**, to the corresponding α-hydroxy carboxylic acid. Thus, selective oxidation[12] of the primary hydroxy group of **17** with catalytic TEMPO, NaOCl and stoichiometric $NaClO_2$ and NaBr in the MeCN-buffer (pH 8–9) afforded the desired α-hydroxy acid **18** as the major product. PyAOP-mediated amide formation[13] of the crude acid **18** with bis-protected amine **19**, proceeded smoothly to afford the corresponding amide **20** in 75% isolated yield. The azide moiety of **20** was selectively reduced to the primary amine with hydrogen and Pd/C followed by a PyAOP-mediated condensation with Cbz-protected MeProline[14] and subsequent protection of the secondary hydroxy group as a TES ether to afford **21** in 71% yield over three steps. Hydrogenolytic debenzylation of **21** with various catalysts and solvents led to either recovery or degradation of the starting material. To our delight, oxidative debenzylation of **21** with excess DDQ[15] in wet dichloromethane was furnished smoothly and the corresponding alcohol was esterified with acid chloride **22**, in the presence of DMAP and NMM in toluene gave rise to the desired ester **23** in 65% yield over two steps. Fmoc-protected **23** was converted into amide **4** in 92% overall yield by a two-step sequence, including base-promoted removal of the Fmoc protecting group and acetylation of the resulting amine with acetic anhydride in pyridine in the presence of a catalytic amount of DMAP. Hydrogenolysis of the Cbz group of **4** followed by PyAOP-mediated condensation with dipeptide acid **5**[16] produced **2** in 68% yield over two steps.



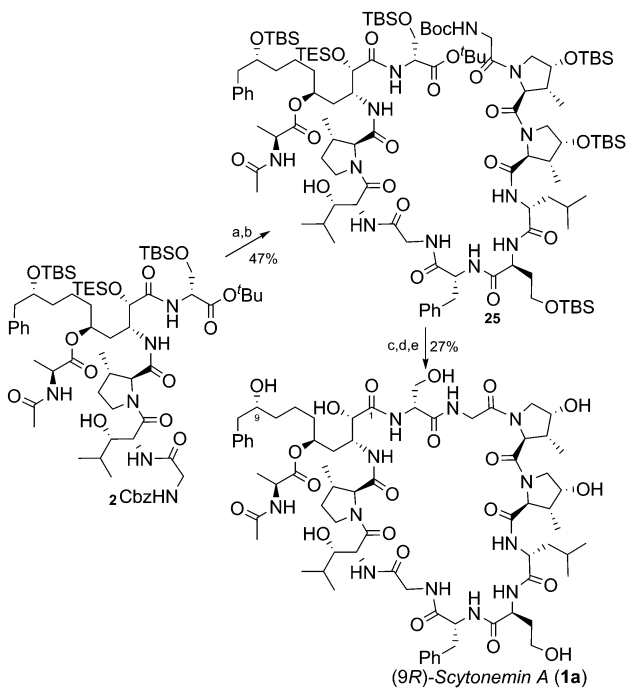
Scheme 1 *Reagents and conditions*: (a) DIBAL-H, DCM; (b) Ph_3P, CBr_4, DIPEA; (c) *n*-BuLi, THF; (d) LiHMDS, $BF_3{\cdot}Et_2O$, −78 °C, then **10**; (e) Pd/C, H_2, DIPEA; (f) NaH, BnBr, THF; (g) DDQ, DCM–H_2O (8 : 1); (h) TCCA, TEMPO, DCM; (i) **13**, KHMDS, 18-Crown-6, THF then aldehyde; (j) DIBAL, THF; (k) (−)-DIPT, $Ti(O\text{-}iPr)_4$, TBHP, 4A MS, DCM; (l) NaN_3, NH_4Cl, MeOH–H_2O, reflux; (m) TEMPO, $NaClO_2$, NaClO, NaBr, MeCN-buffer (pH 8–9); (n) **19**, PyAOP, DIPEA, DCM; (o) Pd/C, H_2, DIPEA; (p) Cbz-MeProline, PyAOP, DIPEA; (q) TESOTf, 2,6-lutidine; (r) DDQ (3 eq.) wet DCM; (s) **22**, DMAP, NMM, PhMe; (t) DEA, DCM; (u) Ac_2O, Pyridine, DMAP, DCM; (v) Pd/C, H_2, EA; (w) **5**, PyAOP, DIPEA, DCM.

Having the two required subunits (**2**, **3**) in hand, their assembly to (9*R*)-scytonemin A (**1a**) was undertaken. Investigations therefore began with the liberation of the N-terminus of pentapeptide fragment **2**, which proceeded smoothly upon exposure of **2** to



Scheme 2 *Reagents and conditions*: (a) Pd/C, H_2; (b) HATU, DIPEA, MeCN; (c) NaOH (0.5 M), THF.

hydrogen over palladium on charcoal to provide the corresponding free amine (Scheme 3). This amine was then condensed with acid **3** by the action of HATU as the coupling agent, affording the expected linear undecapeptide **25** in 47% yield. With **25** in hand, we anticipated that the execution of the synthesis would be completed in a straightforward manner since the remaining deprotection steps followed by macrolactamization had previously been employed for our total synthesis of largamide H[2k] and grassypeptolide.[2i] Surprisingly, our efforts to cleave both *tert*-butyl ester and the Boc-protecting group under various acidic conditions, including HCO_2H, TFA/CH_2Cl_2, $TFA/Et_3SiH/CH_2Cl_2$, $TMSOTf/lutidine/CH_2Cl_2$, were unsuccessful. A survey of the literature indicated that titanium tetrachloride could be employed as a mild and effective deprotective reagent for the hydrolysis of *tert*-butyl esters.[19] Consequently, we elected to use titanium tetrachloride for the deprotection of both



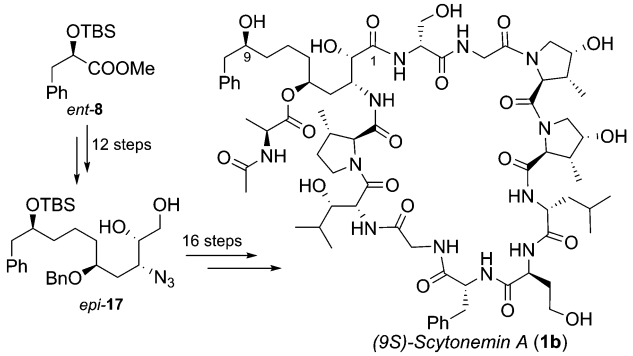
Scheme 3 *Reagents and conditions*: (a) Pd/C, H_2; (b) 3, HATU, NMM; (c) $TiCl_4$, DCM; (d) HATU, NMM, MeCN-DCM; (e) HCl, THF.

tert-butyl ester and Boc carbamate of **25**. In the event, exposure of **25** to 5 equivalents of titanium tetrachloride in dichloromethane resulted in deprotection at the two termini as well as additional cleavage of two silyl protecting groups. The resultant amino acid was activated by HATU in the presence of NMM to afford the corresponding macrolactam, which was immediately subjected to a global desilylation using concentrated HCl in THF to afford (9*R*)-isomer (**1a**) in 27% yield over three steps.

After the successful synthesis of **1a**, we turned to the synthesis of the (9*S*)-isomer (**1b**). This was readily achieved by employing *ent*-**8** as the starting material and following the same synthetic procedure as for **1a** (Scheme 4).

With both (9*R*)-isomer (**1a**) and (9*S*)-isomer (**1b**) in hand, we were able to compare their spectroscopic and physical properties with those of natural scytonemin A to establish the absolute configuration of the C-9 hydroxy group. Firstly, comparison of the optical rotations of the synthetic material with the (9*R*)-isomer (**1a**) $\{[\alpha]_D^{20} = +37$ ($c = 0.06$, MeOH)$\}$ and that of the (9*S*)-isomer (**1b**) $\{[\alpha]_D^{20} = +26$ ($c = 0.06$, MeOH)$\}$ with that reported for the natural product $\{[\alpha]_D^{20} = +38.8$ ($c = 0.04$, MeOH)$\}$ suggested that the natural product may bear a side chain with (*R*)-stereochemistry at C9. Secondly, comparison of the ^1H and ^{13}C NMR spectra of **1a** and **1b** with the spectroscopic data recorded for natural scytonemin A clearly showed that the (9*R*)-isomer (**1a**) was identical in all aspects, whereas the (9*S*)-isomer (**1b**) showed several significant differences, especially at C-9 carbon of the Ahda moiety (also see ESI†). Finally, co-injection of **1a** and **1b** as well as the authentic sample of the natural product on chiral HPLC revealed that the (9*R*)-isomer (**1a**) and the natural product were indistinguishable. Taken together, the excellent correlation of the ^1H and ^{13}C spectra of the synthetic (9*R*)-isomer (**1a**) with those of the natural product and the similar optical rotations of the natural material and synthetic (9*R*)-isomer (**1a**) enabled us to assign the stereogenic center at C9 of the Ahda moiety of naturally occurring scytonemin A as (*R*)-configured.

In summary, the first total synthesis of scytonemin A proceeded in 28 steps and gave 0.57% overall yield (longest linear sequence from the known and easily accessible methyl ester **8**). The route detailed herein is convergent and flexible, thereby allowing for the synthesis of the C9 epimer that facilitated the stereochemical assignment. Notable features of the synthesis include a regioselective epoxide-opening process, TEMPO-catalyzed selective oxidation



Scheme 4 The synthesis of (9*S*)-scytonemin A (**1b**).

of 1,2-diol to the corresponding hydroxy acid, DDQ-mediated deprotection of benzyl ether, $TiCl_4$-promoted deprotection of both *tert*-butyl ester and Boc carbamate at a late stage in the synthesis. These studies reinforce the vital role that total synthesis continues to play in determining the actual structures of promising natural products.

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