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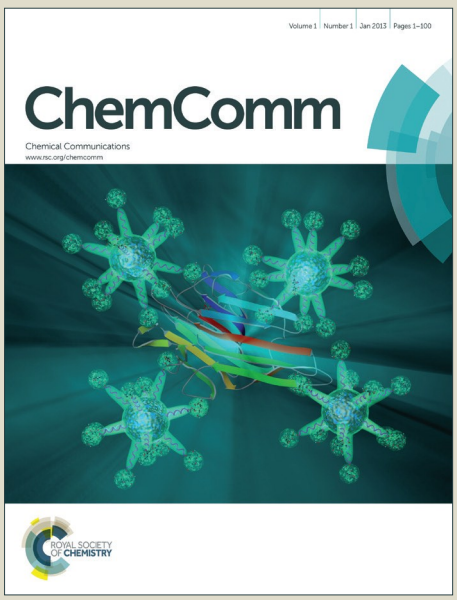

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Total Synthesis and Stereochemical Assignment of Scytonemin A

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Junyang Liu,[a,b] Lei Wang,[a] Juefei Zhang,[a] Zhengshuang Xu, *[a] and Tao Ye*[a,b]

Total synthesis of scytonemin A and its C-9 epimer, as well as elucidation of the absolute stereochemistry of nature scytonemin A are described.

In 1988, Moore and co-workers reported the isolation of an unusual cycloundecapeptide, scytonemin A (**1**, Figure 1), from the cyanobacterium *Scytonema sp.* which possesses potent calcium antagonistic properties.[1] Previous work in 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 acids residues such as the D-(2R,3S)-threo-3-hydroxyleucine (HyLeu), L-(2S,3S)-trans-3-methylproline (MePro), L-(2S,3R,4R)-4-hydroxy-3-methylproline (HyMePro), and (2S,3R,5S)-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 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 (Figure 1), we

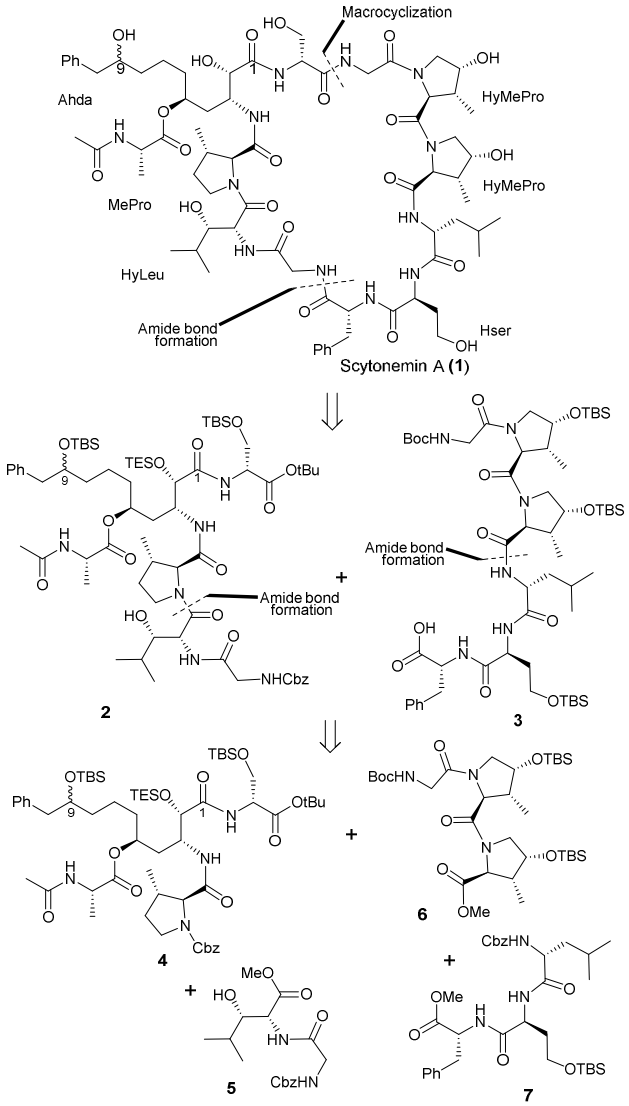

Figure 1. Retrosynthetic analysis of scytonemin A (**1**).

[a] *Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, University Town, Xili, Shenzhen, China, 518055. Email: yet@pkusz.edu.cn; xuzs@pkusz.edu.cn*
[b] *Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hong Kong.*
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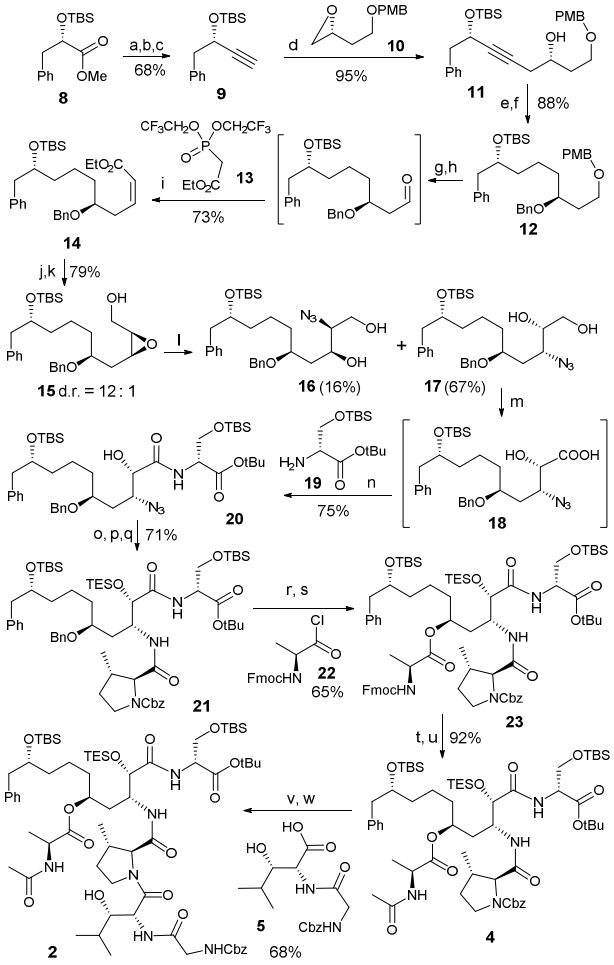
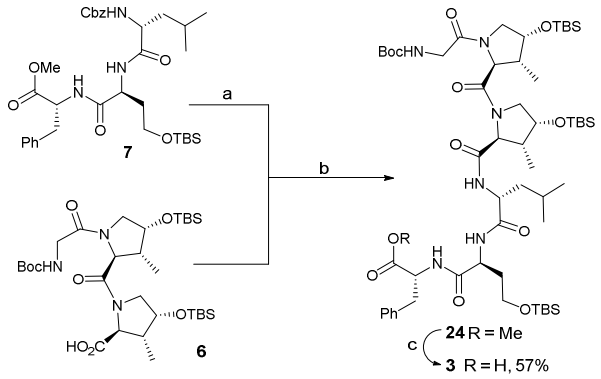
chose to construct the peptide macrocycle via an intramolecular coupling between D-Serine and Glycine, and a subsequent retro amide-bond formation to dissect the generated chain into two key fragments (**2**, **3**) of similar complexity. The relatively unhindered structure of the glycine nucleophile 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.



Scheme 1. Reagents and Conditions: a) DIBAL-H, DCM; (b) Ph_3P, CBr_4, DIPEA; (c) *n*-BuLi, THF; (d) LiHMDS, BF3·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) DMAP, NMM, PhMe; t) Pd/C, H_2, EA; u) **5**, PyAOP, DIPEA, DCM; v) DEA, DCM; w) Ac_2O, Pyridine, DMAP, DCM.

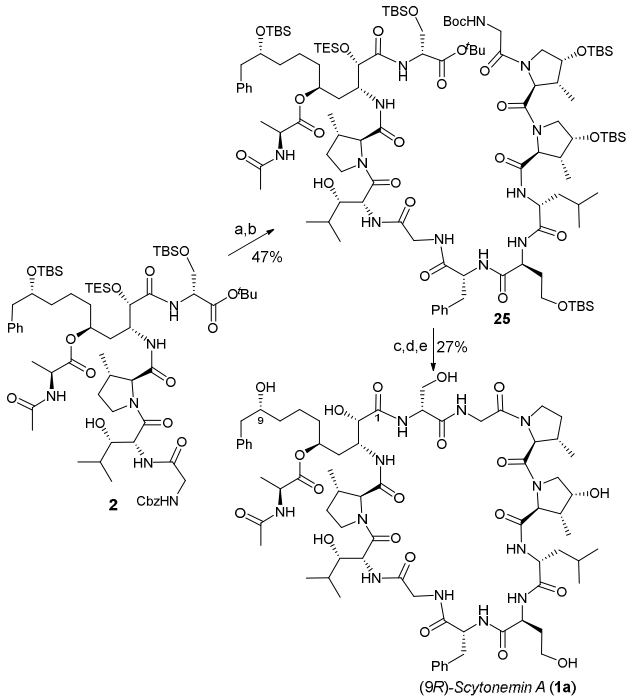
The synthesis of fragment **2** commenced with the Dibal-H reduction of the known methyl ester **8**[4] in dichloromethane at – 78 °C provided the corresponding aldehyde which was subjected to Corey-Fuchs' homologation conditions[5] using CBr_4 and PPh_3 in CH_2Cl_2 at 0 °C to afford the corresponding dibromo olefin. 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·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 submitted to a Still-Gennari modification[9] of the HWE olefination with phosphonate **13** to afford 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 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 a pure product after silica gel chromatograph 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 starting material. To our delight, oxidative debenzylation of **21** with excess DDQ[15] in wet dichloromethane 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 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 a PyAOP-mediated condensation with dipeptide acid **5**[16] produced **2** in 68% yield over two steps.



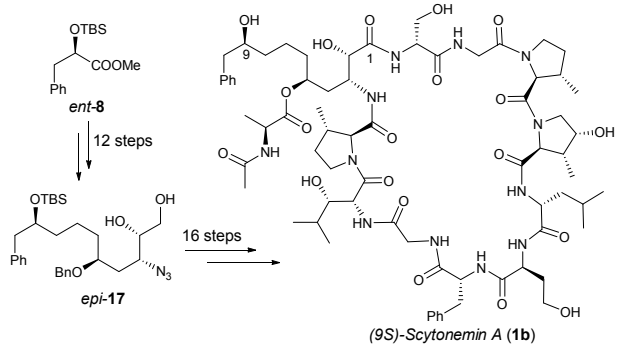
Scheme 2. Reagents and Conditions: a) Pd/C, H_2; b) HATU, DIPEA, MeCN; c) NaOH (0.5 M), THF.

The synthesis of hexapeptide **3** is shown in Scheme 2. Hydrogenolytical removal of the Cbz protecting group in **7**[17] afforded the corresponding amine which was then condensed with tripeptide acid **6**[18] by using HATU in the presence of Hünig's base gave rise to hexapeptide. Hydrolysis of the methyl ester was then carried out with sodium hydroxide to give free acid **3** in 57% yield over three steps.



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.

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 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 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 and 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 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 *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.



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

After 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 the 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 ^{1}H 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 Supplementary Information). 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 ^{1}H 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.

Conclusions

In summary, the first total synthesis of scytonemin A proceeded in 28 steps and 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 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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