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Total synthesis of the potent anti-inflammatory natural product solomonamide A along with structural revision and biological activity evaluation†

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Herein, we report the total synthesis of solomonamide A along with its structural revision for the first time. The natural product possesses very potent anti-inflammatory activity, and it contains a macrocyclic peptide having four consecutive stereocenters on an unnatural amino acid component. The key features in the present synthesis include the application of an Evans aldol reaction, ligand-free Heck macrocyclization and chemoselective oxidations. The challenging task of fixing the stereochemistry of OH at the C5-position was accomplished with the help of DFT calculations, applying a quantum-mechanical (QM)/NMR combined approach. Biological evaluation in a mouse paw edema model revealed that a low dose (0.3 mg kg^{-1}) of the synthesized solomonamide A showed 74% reduction at 6 h, which was comparable to a high dose (10 mg kg^{-1}) standard drug dexamethasone effect (75% at 6 h). Thus, we further confirmed the revised structure of solomonamide A.

The main compounds bearing peptides are the prominent group of naturally occurring mediators for biological processes. In this context, peptides have gained great interest as therapeutics during the past few years.[1] At present, almost 100 peptide-based drugs have reached the market for the benefit of patients and around 400 novel therapeutic peptides are under development at various stages.[2] In this peptide category, macrocyclic peptides occupy a specific region in the chemical space between small molecules and biologics and have recently generated significant interest for their potential to deliver molecules with the desired properties.[3] The limited conformational freedom conferred by cyclization enables cyclic peptides to span large surfaces while retaining the conformational restriction that yields high selectivity and affinity towards the concerned biological targets.[4] Marine sponges are an important source of structurally diverse bioactive secondary metabolites,[5] in particular, macrocycles. Two such macrocyclic peptides solomonamide A (**1**) and solomonamide B (**2**) were isolated by Zampella's group from the marine sponge *Theonella swinhoei*.[6] The challenging structural assignments of both the natural products were performed using various spectroscopic and computational techniques. Biologically, solomonamide A exhibited potent anti-inflammatory activity, causing a 60% paw edema reduction in the animal model at a very low dose (100 μg kg^{-1}). Therefore, the solomonamides have gained significant attention from our group[7] as well as other synthetic research groups, which include Chandrasekhar, Sarabia and Butler.[8] Very recently, we have accomplished the first total synthesis of the proposed structure of solomonamide B, which necessitated the revision of its structure, in particular, the stereochemistry at the C-3 and C-4 positions. Our next plan was to synthesize solomonamide A. It was proposed in the literature that solomonamide A was biogenetically derived from solomonamide B, which makes the structural revision of solomonamide A inevitable. Accordingly, the three stereocenters (2*S*, 3*S*, and 4*S*) present in solomonamide B should be present in solomonamide A, thus leaving one stereocenter (C5-hydroxy group) in the 4-amino(2′-amino-4′-hydroxyphenyl)-3,5-dihydroxy-2-methyl-6-oxohexanoic acid (ADMOA) fragment, whose absolute stereochemistry needs to be fixed. Keeping this in mind, we designed a structure of solomonamide A as shown in Fig. 1 towards stereochemical revision. The plan was to use the macrocycle **4**[7e] prepared in our group and subject it to dihydroxylation followed by chemoselective benzylic oxidation to afford **5**. Finally, serine coupling and subsequent removal of the protecting group can result in the target compound **1** with the desired stereochemistry. The key macrocyclic intermediate **4** required for the present task was prepared in sufficient quan-

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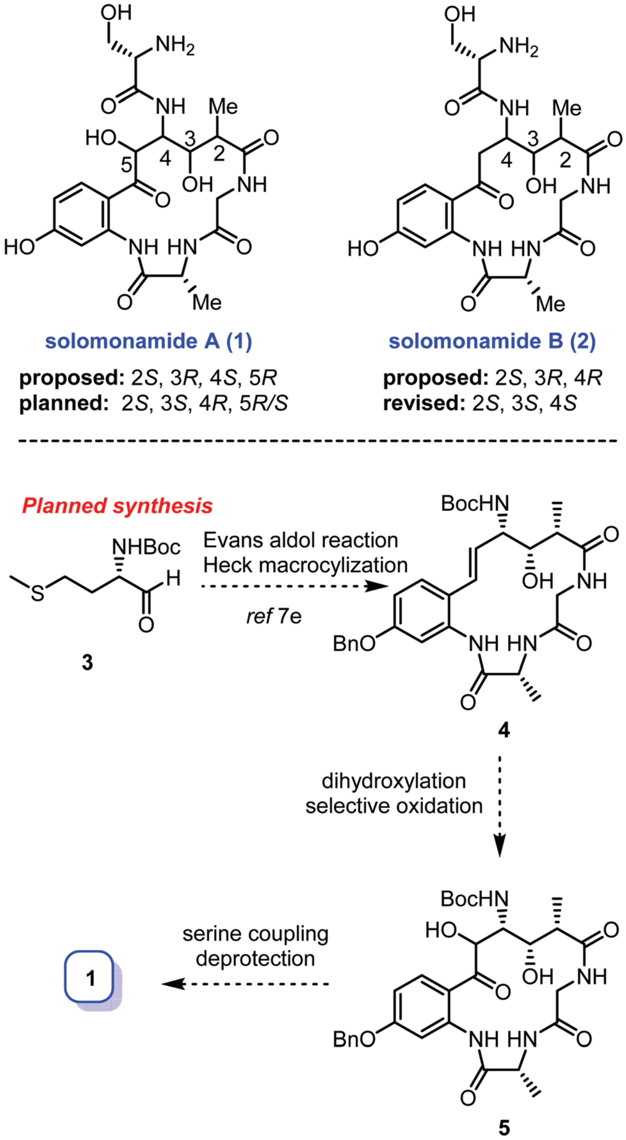
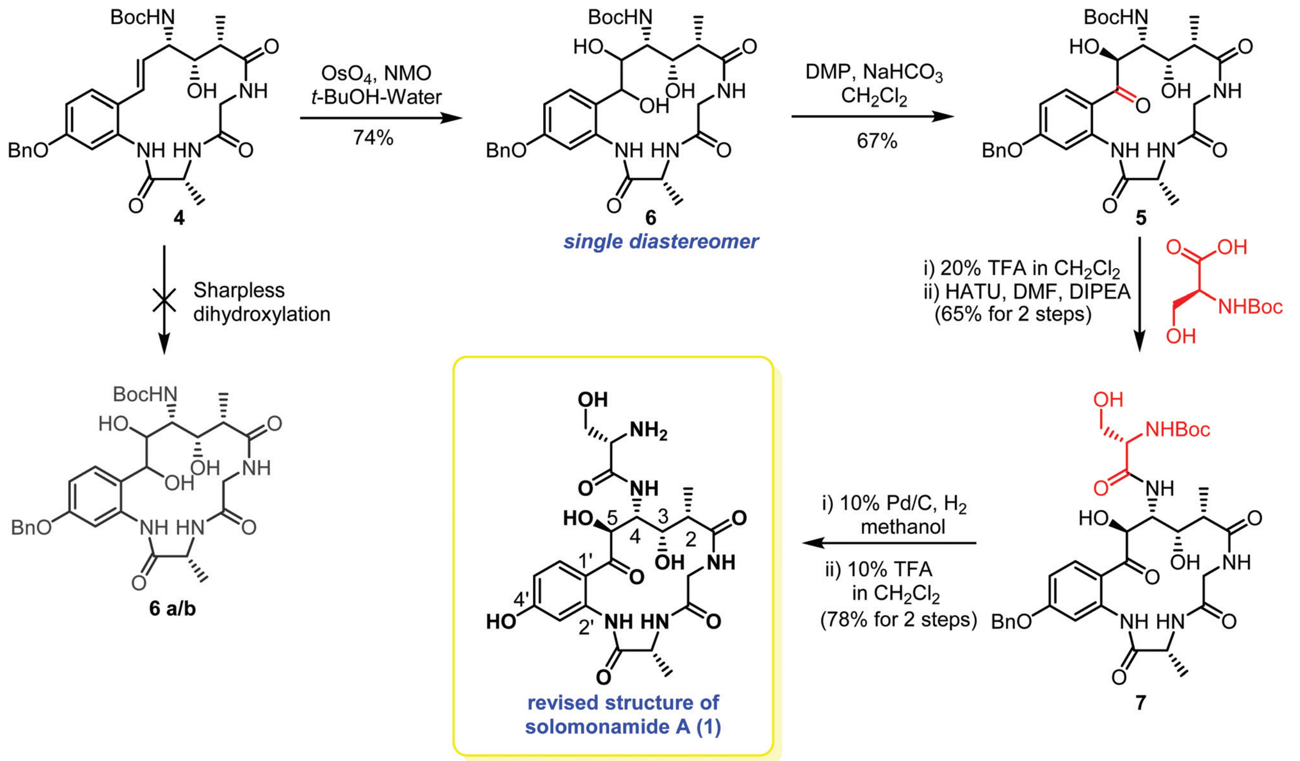


Fig. 1 Structure of solomonamides and the synthetic plan towards the synthesis of solomonamide A with the revised structure.

tities starting from L-methionine and using the ligand-free Heck macrocyclization as the key step. We attempted a Sharpless asymmetric dihydroxylation[9] on compound **4** to access both the diastereomeric triols (**6a**/**b**), as they will be playing a crucial role in the structural revision of the natural product (through spectral data comparison). But to our misfortune, we did not observe the formation of triols (**6a**/**b**) despite a few attempts. So, we relied on Upjohn dihydroxylation[10] for this conversion. Accordingly, macrocyclic intermediate **4** on treatment with 2.5% OsO_4 and NMO in a *t*-BuOH and water mixture furnished the triol **6** in 74% yield as a single diastereomer. The reason behind the formation of a single diastereomeric triol can possibly be explained by the rigid conformation through intramolecular H-bonding. The next task was to perform selective benzylic oxidation, and it was achieved using Dess–Martin periodinane (DMP) to afford α-hydroxy ketone **5** with a yield of 67%.[11] The obtained ketone **5** upon Boc deprotection followed by coupling with Boc NH-L-Ser-OH afforded compound **7** in 65% yield over two steps. Deprotection of the benzyl group in **7** with the help of 10% Pd/C under a blanket of a hydrogen atmosphere yielded the phenolic compound, which was further treated with 10% TFA in CH_2Cl_2 to remove the Boc protecting group to furnish the target compound **1** (Scheme 1). To our delight, the spectral data of compound **1** were in complete agreement with the reported data of the natural product. The comparison of the ^1H and ^{13}C NMR spectra of both the compounds is provided in the ESI.†[12] The magnitude of the optical rotation $[\alpha]_D^{26}$ +3.4 (*c* 0.52, CH_3OH) of the synthetic solomonamide A is slightly higher than that of the natural solomonamide A ($[\alpha]_D^{25}$ +2.3 (*c* 0.17, CH_3OH)). Thus, we have successfully accomplished the first total synthesis of solomonamide A. However, the fixing of the stereochemistry at the C5-hydroxy position was a challenging task as it was difficult for us to come up with an explanation during the Upjohn dihydroxylation step, which gave exclusive selectivity because of the conformational rigidity of the macrocycle **4**. To address this issue, we tried crystallization efforts on triol **6** and α-hydroxy ketone **5** and attempted to make derivatives, but all our attempts were unsuccessful (see the ESI†).

At this stage, a QM/NMR approach was employed to shed light on the C-5 configuration for solomonamide A (**1**). Briefly, this methodology is based on the comparison of the experimental NMR chemical shift data and the related values are calculated at the density functional theory (DFT) level for all the possible theoretical diastereoisomers of a case-study compound.[13] Specifically, the isomer showing the lowest mean absolute error (MAE) values represents the solution mostly compatible with the experimental data (see Computational details, ESI†). In this specific case, we firstly compared the experimental ^{13}C chemical shift data of solomonamide A (**1**) with the related values calculated for C-5*R* and C-5*S* isomers (compounds **1a** and **1b**, respectively, see Fig. 2), using the MPW1PW91/6-31g(d,p) level of theory (see Computational details, ESI†). The predicted ^{13}C chemical shift data, compared with the set of experimental values, allowed us to assign the *S* configuration at C-5 for **1**, since **1b** showed the lowest MAE value (see Table S1, ESI†). In order to corroborate the proposed absolute configuration of **1**, we also considered its precursor **5** that differs from **1** for the absence of an L-serine residue and must feature the same configuration at C-5, as reported in Scheme 1. Again, the related diastereoisomer of **5** featuring the *S* configuration at C-5 (compound **5b**, see Chart S1, ESI†) was showing a higher similarity between the experimental and calculated ^{13}C chemical shift data and, accordingly, the lower MAE values, compared to the related C-5*R* isomer (compound **5a**, see Chart S1 and Table S2, ESI†). Taken together, all these data allowed us to assign the 2*S*,3*S*,4*R*,5*S* absolute configuration for solomonamide A (**1**) (Fig. 2).

Having solomonamide A in hand for the first time, we became interested in evaluating its anti-inflammatory poten-



Scheme 1 Towards the synthesis of the revised structure of solomonamide A.

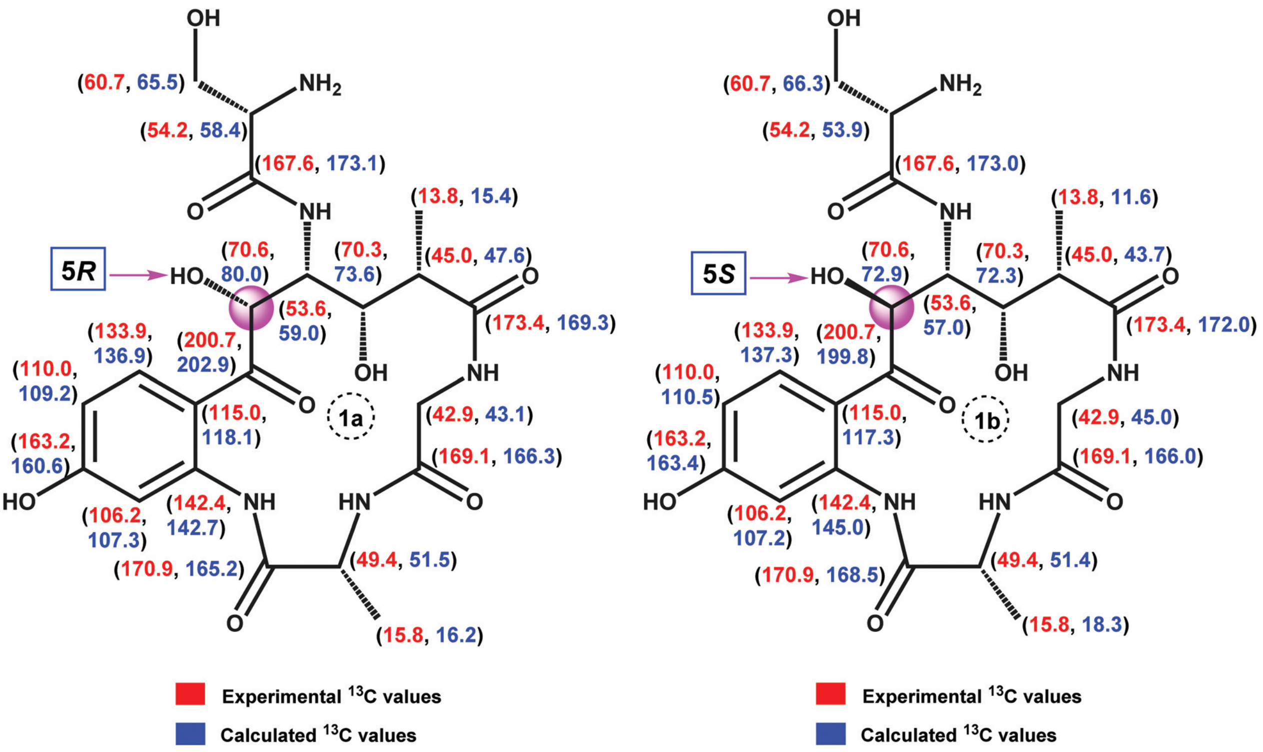


Fig. 2 Comparison of the predicted ^{13}C NMR values of **1a** and **1b** with the experimental values.

tial. Accordingly, we evaluated the synthesized solomonamide A in a carrageenan-induced mouse paw edema model and the reduction in inflammation was measured at different doses (0.03, 0.1 and 0.3 mg kg^{-1} i.p.) in comparison with carrageenan alone. Dexamethasone (10 mg kg^{-1}, i.p.) was used as a reference anti-inflammatory drug.[6] The synthesized solomonamide A showed a dose-dependent anti-inflammatory effect at 4 and 6 h. Lower doses of solomonamide A (0.1 and 0.03 mg kg^{-1}) showed comparatively low inhibition at 4 h (~48 and 44%, respectively) and 6 h (~61 and 60%, respectively), whereas a higher dose of solomonamide A (0.3 mg kg^{-1}) showed ~56% inhibition at 4 h and ~74% inhibition at 6 h, which were significantly higher and were also comparable to the dexamethasone effect (~74% and 75% inhibition at 4 h and 6 h, respectively). From these data, it is evident that the synthesized solomonamide A showed promising anti-inflammatory activity, which is in agreement with the natural solomonamide A (Fig. 3). In summary, we have accomplished the total synthesis of solomonamide A for the first time. Evans aldol, ligand-free Heck macrocyclization, stereoselective Upjohn dihydroxylation and chemoselective oxidation reactions are the key steps in our route. The synthesized solomonamide A was tested for its anti-inflammatory activity which is in agreement with the natural solomonamide A, thus underlining the utility of interdisciplinary approaches for structure determination. The synthetic strategy presented herein can also be potentially applicable in the generation of a library of compounds around solomonamide, in particular, with varying stereochemistries. SAR studies, lead optimization and mechanism of action are the future directions of this project.

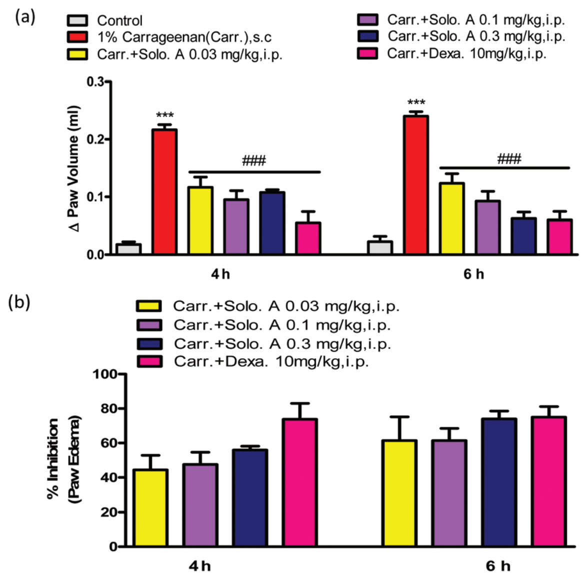


Fig. 3 Anti-inflammatory activity of solomonamide A (0.03, 0.1 and 0.3 mg kg^{-1}, i.p.) and dexamethasone (10 mg kg^{-1}, i.p.) in the carrageenan-induced paw edema model in mice (n = 6–10 animals per group). 50 μL of saline or 1% carrageenan in the treatment group or saline in the control group was administered subcutaneously in the mice hind paw. The results are shown as mean ± SEM: (a) paw volume in mL and (b) % inhibition in paw edema.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 (*a*) K. Fosgerau and T. Hoffmann, *Drug Discovery Today*, 2015, **20**, 122; (*b*) J. Zhao and X. Jiang, *Chin. Chem. Lett.*, 2018, **29**, 1079.

2 F. Albericio and H. G. Kruger, *Future Med. Chem.*, 2012, **4**, 1527.

3 (*a*) A. Mullard, *Nat. Rev. Drug Discovery*, 2012, **11**, 173; (*b*) S. Ghosh, *Chim. Oggi Chem. Today*, 2016, **34**, V–VII; (*c*) T. Passioura, T. Katoh, Y. Goto and H. Suga, *Annu. Rev. Biochem.*, 2014, **83**, 727.

4 (*a*) J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro and M. R. Prinsep, *Nat. Prod. Rep.*, 2017, **34**, 235; (*b*) D. J. Newman and G. M. Cragg, *Planta Med.*, 2016, **82**, 775.

5 (*a*) N. H. Shady, E. M. E. Hossary, M. A. Fouad, T. A. M. Gulder, M. S. Kamel and U. R. Abdelmohsen, *Molecules*, 2017, **22**, 781; (*b*) J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. Munro and M. R. Prinsep, *Nat. Prod. Rep.*, 2016, **33**, 382.

6 C. Festa, S. De Marino, V. Sepe, M. V. D’Auria, G. Bifulco, C. Débitus, M. Bucci, V. Vellecco and A. Zampella, *Org. Lett.*, 2011, **13**, 1532.

7 (*a*) K. Kashinath, N. Vasudevan and D. S. Reddy, *Org. Lett.*, 2012, **14**, 6222; (*b*) D. S. Reddy, K. Kashinath and N. Vasudevan, *US* 9751911B2, 2017; (*c*) N. Vasudevan, K. Kashinath and D. S. Reddy, *Org. Lett.*, 2014, **16**, 6148; (*d*) K. Kashinath, S. Dhara and D. S. Reddy, *Org. Lett.*, 2015, **17**, 2090; (*e*) K. Kashinath, G. R. Jachak, P. R. Athawale, U. K. Marelli, R. G. Gonnade and D. S. Reddy, *Org. Lett.*, 2016, **18**, 3178.

8 (*a*) N. Kavitha, V. P. Kumar and S. Chandrasekhar, *Tetrahedron Lett.*, 2013, **54**, 2128; (*b*) N. Kavitha and S. Chandrasekhar, *Org. Biomol. Chem.*, 2015, **13**, 6242; (*c*) L. Calvo, J. Hazlerig and S. C. Butler, 249th ACS National Meeting & Exposition, Denver, CO, United States, March 22–26, 2015; CHED-1117; (*d*) L. Calvo, J. Hazlerig and S. C. Butler, 247th ACS National Meeting & Exposition,

Dallas, TX, United States, March 16–20, 2014; CHED-1076; (*e*) I. Cheng-Sánchez, C. García-Ruiz and F. Sarabia, *Tetrahedron Lett.*, 2016, **57**, 3392; (*f*) I. Cheng-Sánchez, P. Carrillo, A. Sánchez-Ruiz, B. Martínez-Poveda, A., R. Quesada, M. Á. Medina, J. M. López-Romero and F. Sarabia, *J. Org. Chem.*, 2018, **83**, 5365.

9 E. N. Jacobsen, I. Marko, W. S. Mungall, G. Schroeder and K. B. Sharpless, *J. Am. Chem. Soc.*, 1988, **6**, 1968.

10 (*a*) H. C. Kolb, M. S. VanNieuwenhze and K. B. Sharpless, *Chem. Rev.*, 1994, **8**, 2483; (*b*) V. VanRheenen, R. C. Kelly and D. Y. Cha, *Tetrahedron Lett.*, 1976, **17**, 1973.

11 (*a*) K. Tatsuta, S. Takano, T. Sato and S. Nakano, *Chem. Lett.*, 2001, **2**, 172; (*b*) I. C. Choong, W. Lew, D. Lee, P. Pham, M. T. Burdett, J. W. Lam, C. Wiesmann, T. N. Luong, B. Fahr, W. L. DeLano, R. S. McDowell, D. A. Allen, D. A. Erlanson, E. M. Gordon and T. O'Brien, *J. Med. Chem.*, 2002, **45**, 5005.

12 We obtained the corrected ^{13}C NMR data from Prof. Zampella in a private communication (through email exchange), where six carbons have slightly different values as compared with the original report from her group (ref. 6).

13 (*a*) G. Bifulco, P. Dambruoso, L. Gomez-Paloma and R. Riccio, *Chem. Rev.*, 2007, **107**, 3744; (*b*) S. Nadmin, A. Plaza, G. Lauro, R. Garcia, G. Bifulco and R. Müller, *Org. Lett.*, 2014, **16**, 4130.