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Synthetic studies of stereocalpin A and its C5-epimer: total synthesis of 11-*epi*- and 5,11-di*epi*-stereocalpin A

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ABSTRACT

This Letter describes the synthetic studies of stereocalpin A and its C5-epimer. After various cyclization attempts, successful macrolactamization at 9-10 position was tried inorder to obtain stereocalpin A and its C5-epimer, which were accompanied by complete racemization and resulted in the synthesis of 11-*epi*- and 5,11-di*epi*-stereocalpin A. Highly functionalized octanoic acid motif of the depsipeptide was constructed by applying Paterson's aldol methodology, owing to its diversity in synthesizing various analogs of aliphatic acid.

Secondary metabolites produced by cyanobacteria are an important source of biologically active compounds. Many of these secondary metabolites are cyclic peptides and depsipeptides. Stereocalpin A is one such a depsipeptide, isolated by Oh et al. from the dry lichen *Ramalina terebrata* of Antarctica in 2008.[1] They proposed the structure of stereocalpin A to be **1** (Fig. 1), with the help of a detailed spectroscopic analysis. Initial biological studies against various solid tumor cell lines showed promising results. Stereocalpin A inhibits the protein tyrosine phosphatase 1B (PTP1B) in a dose-dependent manner (IC_{50} = 40 μM), thereby exhibiting cytotoxic and antidiabetic activities.[2] However, extensive biological studies were hampered by the limited availability of the natural product. Ghosh and Xu[3a] reported the first total synthesis of the proposed structure of stereocalpin A (**1**) and pointed the incorrect structural assignment of the original molecule. Very recently Huang et al. reported the synthetic studies of stereocalpin A.[3b] High biological importance and our desire to assign the correct structure of stereocalpin A led us to plan and devise a rationalized strategy for the synthesis of all its possible stereoisomers. As a manifestation of our strategy, we initially aimed at the synthesis of the proposed structure of stereocalpin A and its C5-epimer that eventually led to the synthesis of 11-*epi*-stereocalpin A (**2**) and 5,11-di-*epi*-stereocalpin A (**3**). The present communication describes our efforts in this direction.

Retrosynthetically (Scheme 1), we envisaged that either a macrolactonization or macrolactamization strategy could be adopted for making the macrocycles of target molecules, while the cyclization precursors could be obtained from the highly substituted octanoic acid moieties (**4 and 5**) and the dipeptide unit **9** simply via coupling protocols. For the synthesis of poly propionate units **4** and **5** we envisaged that the use of Paterson's *anti*-aldol reaction,[4] would provide all the possible diastereoisomers of the acid fragment **4** required for the structural reassignment and analogs preparation.

Thus our synthesis commenced from the known keto compound **8**[5] (Scheme 2), which was subjected to substrate-controlled and reagent-controlled Paterson's aldol reaction conditions,[4] consecutively with butyraldehyde. Thus compound **8** on condensation with butyraldehyde under substrate control Paterson's aldol conditions[4a,b] using dicyclohexyl boran chloride afforded β-keto alchol, which was reduced in situ with $LiBH_4$ to give the diol compound **10** in good yield and diastereoselectivity. Likewise, reagent-controlled Paterson aldol reaction[4c] of compound **8** with butyraldehyde using (−)-Ipc_2BOTf followed by hydroxyl directed keto reduction[6] furnished 1,3-*anti* diol compound **11** in 70% yield (dr 4:1). Subsequently benzylidene rearrangement[7] with DDQ afforded compounds **12** and **13**, which on protection with TBSCl furnished fully protected compounds **14** and **15**, respectively. Finally opening of the benzylidene with DIBAL-H gave the primary alcohols **6** and **7**.[8]

After synthesizing the alcohols **6** and **7**, we decided first to synthesize the C5-epimer of the proposed structure of stereocalpin A, as it might help us to assign the correct structure of stereocalpin and we thought of using a macrolactonization strategy for the target molecule. Accordingly alcohol **7** was oxidized with DMP[9] to

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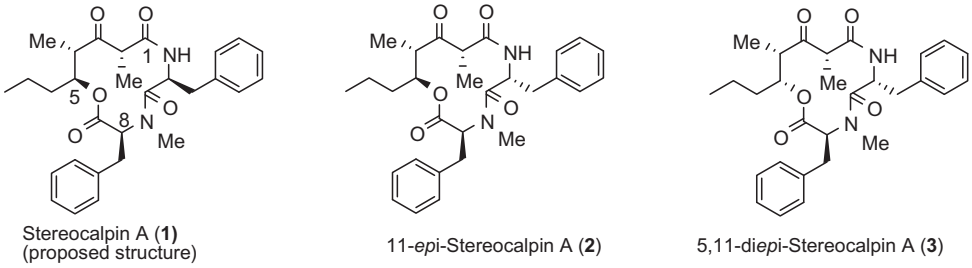
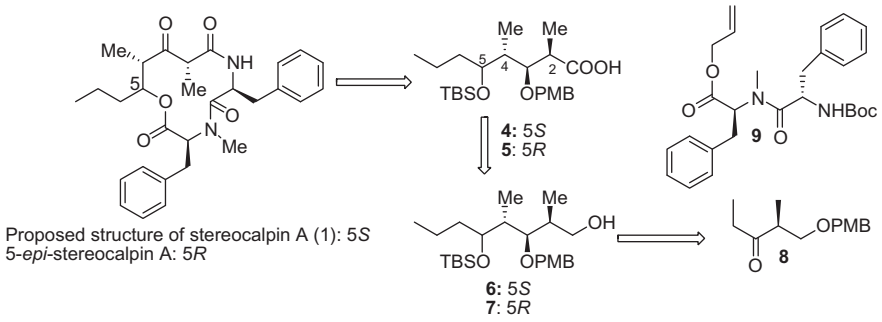
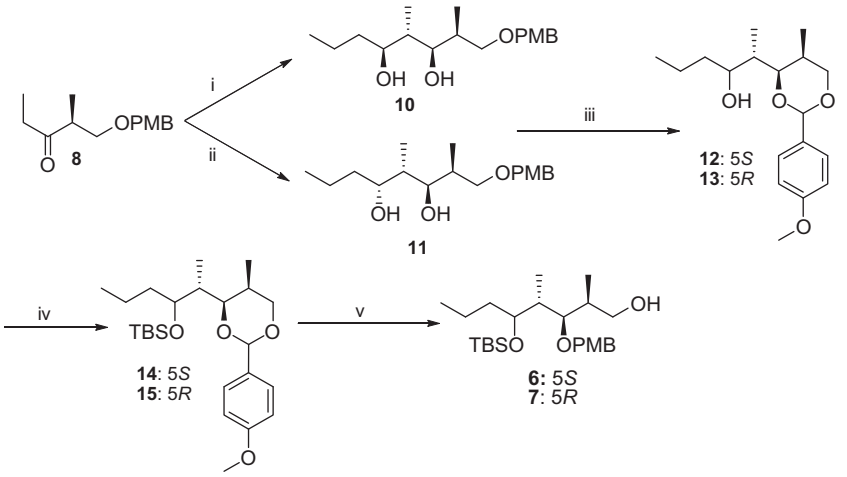


Figure 1.



Scheme 1. Retrosynthetic analysis.

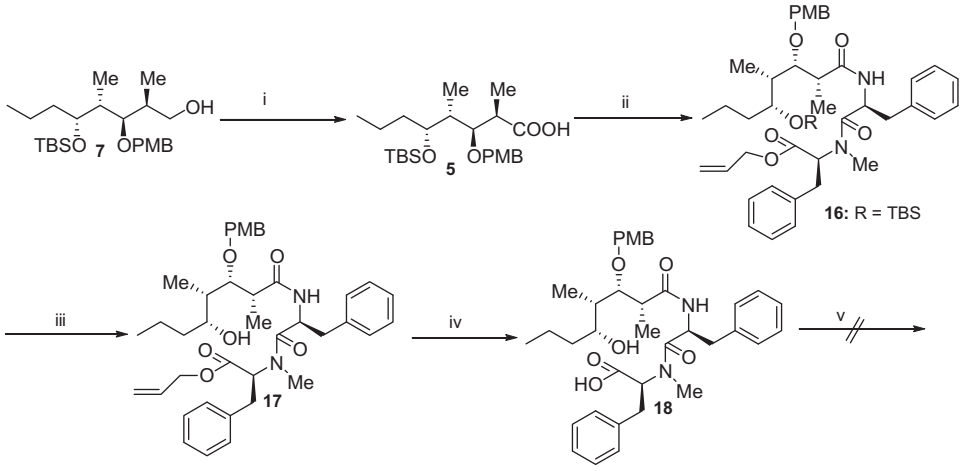


Scheme 2. Reagents and conditions: (i) $(c\text{-}C_6H_{11})_2BCl$, Et_3N, Et_2O, 0 °C, 2 h, then buteraldehyde, −78 °C to −20 °C, 14 h, $LiBH_4$, THF −78 °C, NaOH, H_2O_2, 75%, dr (85:15); (ii) (a) (−)-Ipc_2BOTf, iPr_2NEt, CH_2Cl_2, buteraldehyde, −78 °C to 0 °C; (b) $Me_4NBH(OAc)_3$ AcOH–CH_3CN (1:1), −20 °C, 19 h, 70%, dr (80:20); (iii) DDQ, 4 Å MS, −10 °C, 2 h, CH_2Cl_2, 85%; (iv) TBSOTf, 2,6 lutidine, CH_2Cl_2, rt, 45 min 96% (v) DIBAL-H, CH_2Cl_2, −40 °C to −10 °C, 2 h, 90%.

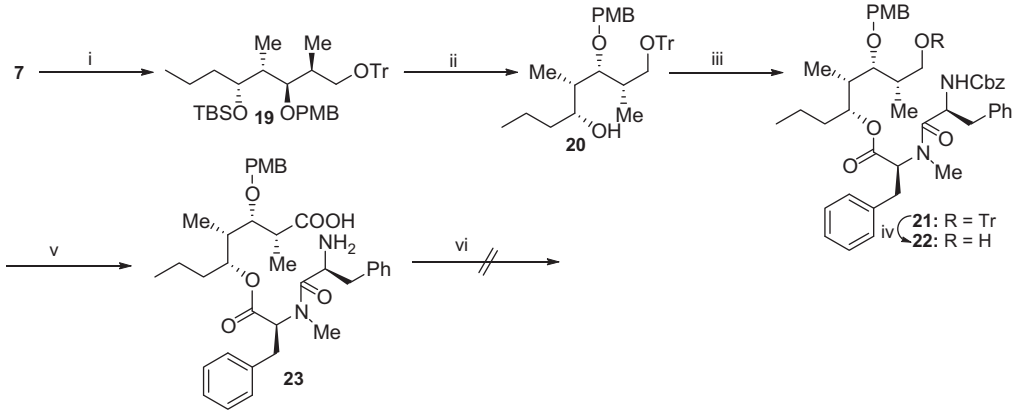
give an aldehyde, which on further oxidation with $NaClO_2$ afforded acid **5** in good yield. Coupling of the acid with dipeptide (*S*)-HCl·NH_2-Phe-(*S*)-MePhe-Oallyl using HATU as a coupling agent afforded compound **16** in 75% yield over three steps.[10] TBS deprotection of **16** with catalytic amount of CSA afforded the secondary alcohol, which on treatment with $Pd[PPh_3]_4$ furnished seco-acid, the macrolactonization precursor in good yield (Scheme 3). Seco-acid was then subjected to macrolactonization reaction under Yamaguchi conditions.[11] Unfortunately the Yamaguchi cyclization was not successful.

As an alternative, we surmised that a macrolactamization strategy between Phe-NH_2 and –COOH of the polypropionate unit (formation of N12–C1 bond) might provide the cyclized product (Scheme 4). To perform this primary alcohol **7** was protected as trityl ether to give fully protected compound **19**, which was desilylated with TBAF in THF to give compound **20** in 85% yield over two steps. Acylation of secondary hydroxyl group of compound **20** with the acid Cbz-(*S*)-Phe-(*S*)-Me Phe-OH under Yamaguchi esterification[11] protocol afforded compound **21**, which on detritylation with a catalytic amount of PTSA in MeOH furnished primary alcohol **22** in good yield. Two-step oxidation of primary alcohol **22** gave an acid, which on hydrogenolysis in the presence of catalytic amount of CH_3COONH_4 afforded amino acid **23**. The stage was set for the crucial macrolactamization, but unfortunately the macrolactamization reaction under various reagents and conditions again failed to provide the desired cyclized product.

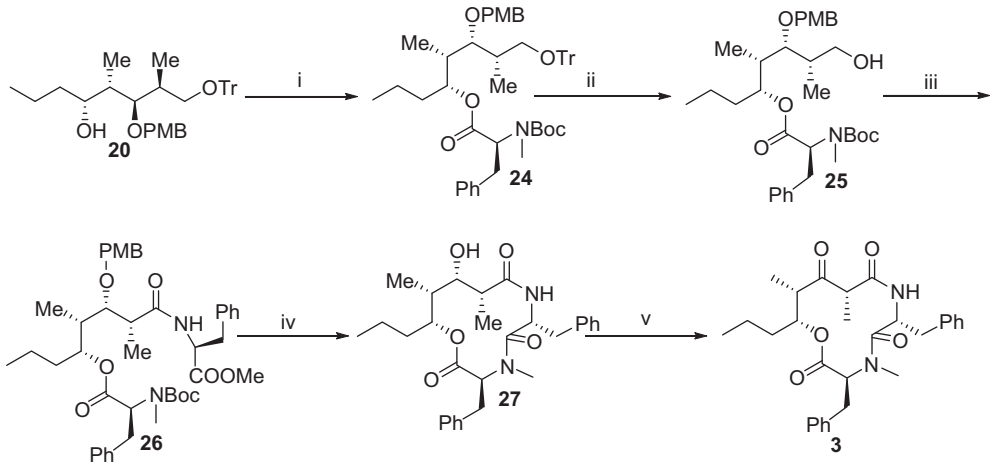
As a final resort we decided to perform the macrolactamization via Phe–Phe bond formation. Although Ghosh and Xu[3a] observed that the exclusive racemization had taken place at the C11-center during the cyclization for their substrate, we felt that by keeping C3–OH free, the steric hindrance can be reduced and racemization can be minimized. Accordingly acylation of the secondary hydroxyl



Scheme 3. Reagents and conditions: (i) (a) DMP, CH_2Cl_2, 0 °C to rt, 1 h; (b) $NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, H_2O, 2-methyl-2-butene, ^{t}BuOH, 1 h; (ii) HATU, *N*-ethyl piperidine, (*S*)-HCl·NH_2-Phe-(*S*)-Me Phe-Oallyl, CH_3CN, 0 °C to rt, 12 h, 75%; (iii) CSA, MeOH–CH_2Cl_2 (1:4), 0 °C, 1 h, 90%; (iv) Pd[PPh_3]$_4$, morpholine, THF, rt, overnight, 85%; (v) 2,4,6-trichlorobenzoyl chloride, DMAP, toluene, reflux, overnight.



Scheme 4. Reagents and conditions: (i) TrCl, Et_3N, CH_2Cl_2, 0 °C to rt, 95%; (ii) TBAF, THF, 0 °C to rt, 12 h, 90%; (iii) Cbz-(*S*)-Phe-(*S*)-Me Phe-OH, 2,4,6 Trichloro benzoyl chloride, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 7 h, 85%; (iv) PTSA, MeOH, rt, 30 min, 88%; (v) (a) DMP, CH_2Cl_2, 0 °C to rt, 45 min; (b) $NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, 1 h, H_2O, 2-methyl-2-butene, ^{t}BuOH; (c) H_2, $Pd(OH)_2$, CH_3COONH_4, MeOH, rt, 1h.



Scheme 5. Reagents and conditions: (i) Boc-(*S*)-Me Phe-OH, 2,4,6 Tri chloro benzoyl chloride, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 7 h, 75%; (ii) PTSA, MeOH, rt, 30 min, 88%; (iii) (a) DMP, CH_2Cl_2, 0 °C to rt, 1 h; (b) $NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, 1 h, H_2O, 2-methyl-2-butene, ^{t}BuOH; (c) (*S*)-HCl·NH_2-Phe-OMe, HATU, *N*-ethyl piperidine, CH_3CN, 0 °C to rt, 12 h, 75%; (iv) (a) LiOH, ^{t}BuOH–H_2O (2:1); (b) 50% TFA in CH_2Cl_2, 0 °C to rt, 2 h; (c) HATU, HOAt, DMF–CH_2Cl_2 (1.5: 7), DIPEA, 0 °C to rt 3 days, 40%; (v) DMP, CH_2Cl_2, 0 °C to rt, 1 h, 90%.

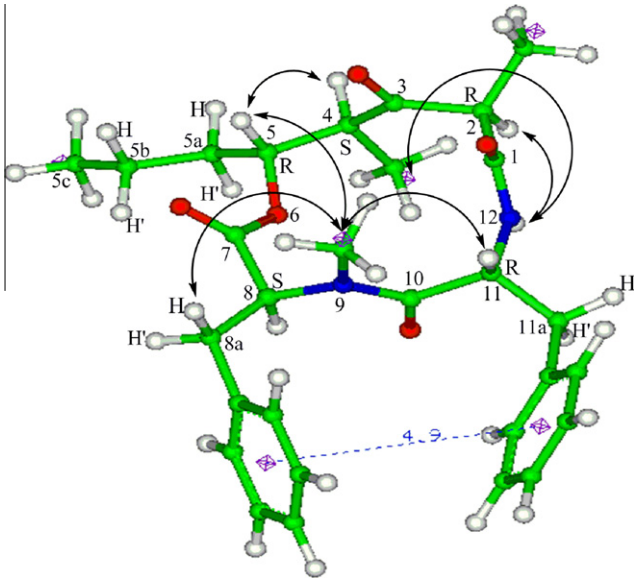


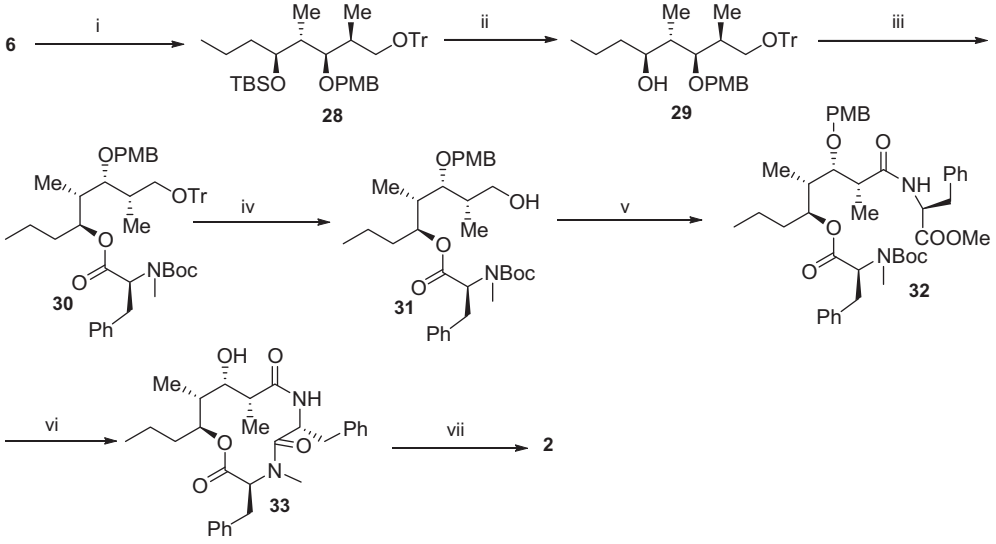
Figure 2. The energy minimized structure of '5,11-di*epi*-stereocalpin A(3)'. The arrows (↔) represent the important nOe correlations. The dotted line (- -) represent the spatial distance 4.9 Å between two phenyl groups.

group of **20** with Boc-(*S*)-Me Phe-OH under Yamaguchi conditions gave compound **24** (Scheme 5), which on detritylation with a catalytic amount PTSA in MeOH furnished primary alcohol **25** in 66% over two steps. Primary alcohol **25** was then oxidized to aldehyde with DMP to give an aldehyde, which on further oxidation followed by coupling of the resultant acid with (*S*)-HCl·NH_2-Phe-OMe using HATU gave compound **26** in 75% yield over three steps. Selective hydrolysis of the methyl ester with 1 N LiOH followed by Boc and PMB deprotection with TFA afforded amino acid with free hydroxyl group at C3 position. At this juncture we attempted BOP-Cl mediated cyclization as per the reported literature,[12] where it has been suggested that favored cyclizations with BOP-Cl occur when *N*-methyl aminoacid is present at the cyclization site. But even this attempt was unsuccessful. However, treatment of the amino acid with HATU and HOAt[13] under high dilution in CH_2Cl_2–DMF (7:1.5) afforded the cyclized compound **27**,[14] which on DMP oxidation gave a compound whose detailed NMR studies revealed that it is 5,11-di-*epi*-stereocalpin A,[15] but not the C5-epimer of the stereocalpin A.

Detailed 1D and 2D NMR experiments, using Double Quantum Filtered Correlation Spectroscopy (DQFCOSY) and Nuclear Overhauser Effect Spectroscopy (NOESY) techniques were carried out to confirm the structure of **3**. It was interesting to note from the coupling constants, that the side chains are rather rigid and contain predominance of single conformation around the C–C single bonds. For phenylalanine side chain at C8, the values of $^3J_{C8H-C8aH}$ and $^3J_{C8H-8aH'}$ are 11.0 and 5.8 Hz, respectively, implying predominance of a structure with *anti* arrangement of C8H and C8aH. Using the reported procedure,[16] the major rotamer about C8–C8a was found to be 'g^-' (N9-C8-C8a-C8aPh; $\chi \sim -60°$), with a population of about 72%. Similarly for the phenylalanine side chain at C11, the couplings $^3J_{C11H-C11aH}$ and $^3J_{C11H-C11aH'}$, have values of 5.0 and 10.0 Hz, which correspond to a major rotamer with an *anti* conformation for C11H–C11aH′. The major isomer is '*t*' (N12-C11-C11a-C11aPh; $\chi \sim 180°$), with a population of about 63%. This results in the two phenyl rings having a distance between the centroids of about 4.9 Å (Fig. 2) of each other, thus stabilizing the structure through π–π interactions.[17] Even the *n*-propyl side chain at the C5, interestingly show restrained C–C single bond rotations. The couplings, $^3J_{C5H-C5aH}$ = 3.5 Hz; $^3J_{C5H-C5aH'}$ = 10.0 Hz; $^3J_{C5aH-C5bH}$ = 6.2 Hz; $^3J_{C5aH-C5bH'}$ = 10.4 Hz; $^3J_{C5aH'-C5bH}$ = 10.0 Hz; $^3J_{C5aH'-C5bH'}$ = 5.0 Hz; clearly bring out this aspect. The major rotamer about C5–C5a is 'g^+' (O6-C5-C5a-C5b; $\chi 1 \sim 60°$), with a population of ∼63% and about C5a–C5b is '*t*' (C5-C5a-C5b-C5c; $\chi 2 \sim 180°$), with a population of ∼68%. It is evident from these NMR spectral studies that during the cyclization complete racemization had taken place at C11 center, which resulted in the formation of 5,11 di*epi*-stereocalpin(**3**).

Similarly when we attempted the synthesis of the proposed structure of stereocalpin A from compound **6** following the same set of reaction sequence as mentioned above, we ended up with 11-*epi*-stereocalpin A (**2**) (Scheme 6), whose spectral data[18] were in good agreement with the data reported by Ghosh and Xu.[3a]

In conclusion we have reported the synthetic studies of reported structure of stereocalpin A and its C5-epimer, which eventually resulted in the total synthesis of 11-*epi*- and 5,11-di-*epi*-stereocalpin A. We have observed that during cyclization



Scheme 6. Reagents and conditions: (i) TrCl, Et_3N, CH_2Cl_2, 0 °C to rt, 95%; (ii) TBAF, THF, 0 °C to rt, 12 h, 90%; (iii) Boc-(*S*)-Me Phe-OH, 2,4,6 tri chloro benzoyl chloride, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 7 h, 75%; (iv) PTSA, MeOH, rt, 30 min, 88%; (v) (a) DMP, CH_2Cl_2, 0 °C to rt, 1 h; (b)$NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, 1 h, H_2O, 2-methyl-2-butene, ^{t}BuOH; (c) (*S*)-HCl·NH_2-Phe-OMe, HATU, *N*-ethyl piperidine, CH_3CN, 0 °C to rt, 12 h, 75%; (vi) (a) LiOH, ^{t}BuOH–H_2O (2:1); (b) 50% TFA in CH_2Cl_2, 0 °C to rt, 2 h; (c) HATU, HOAt, DMF–CH_2Cl_2 (1.5:7), DIPEA, 0 °C to rt 3 days, 40%; (vii) DMP, CH_2Cl_2, 0 °C to rt, 1 h, 90%.

racemization had taken place at the C11 position of the molecule, which supports the observation of Ghosh and Xu. Here we have developed a simple and straight forward strategy for the synthesis of various stereoisomers of stereocalpin A by applying switchable Paterson aldol reaction. Currently we are trying to develop a different strategy to overcome the racemization, which might help us to assign the correct structure of stereocalpin A. We are also making various analogs of stereocalpin A by using present strategy for biological screening.

Acknowledgments

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14. Spectral data for compound **27**: R_f = 0.4 (SiO_2, 5% MeOH in chloroform). $[\alpha]_D^{29}$ −24.0 (*c* 0.55, $CHCl_3$);IR (neat): υ_{max} 3385, 2922, 2853, 1643, 1562, 1459, 1414, 1316, 1260, 1089, 1020, 799 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.25–6.92 (m, 10H), 6.60 (d, *J* = 10.2 Hz, 1H), 5.81 (dd, *J* = 9.8, 6.6 Hz, 1H), 5.23 (dt, *J* = 15.1, 4.9 Hz, 1H), 4.87 (d, *J* = 10.3 Hz, 1H), 3.66 (m, 1H), 3.32–2.98 (m, 4H), 2.82 (s, 3H), 2.71 (m, 1H), 2.35(m, 1H), 2.20–1.97 (m, 2H), 1.70–1.54 (m, 2H), 1.10 (d, *J* = 7.1 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.86 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.8, 173.9, 168.5, 136.3, 135.8, 129.5, 129.0, 128.8, 128.5, 126.7, 126.6, 80.0, 78.8, 55.5, 50.0, 48.0, 37.2, 36.8, 32.6, 30.5, 30.1, 19.8, 18.2, 13.7, 13.4; HRMS (ESIMS): calcd for $C_{29}H_{38}N_20_5Na$ $[M+Na]^+$:517.2678, found: 517.2679.
15. Spectral data of 5,11-di*epi*-stereocalpin A: R_f = 0.5 (SiO_2, 30% EtOAc in petroleum ether). $[\alpha]_D^{29}$ −60.5 (*c* 0.5, CH_2Cl_2); IR (neat): υ_{max} 3310, 2925, 2860, 1750, 1670, 1625, 1450, 1250, 1226, 1178, 960, 750, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.23–6.95 (m, 10H), 6.50 (d, *J* = 10.2 Hz, 1H), 5.72 (dd, *J* = 10.7, 5.6 Hz,1H), 5.21(dt, *J* = 10.2, 5.2 Hz, 1H), 4.92 (dt, *J* = 9.0, 3.7 Hz, 1H), 3.34–3.18 (m, 4H), 2.89 (dd, *J* = 15.4, 10.9 Hz, 1H), 2.82 (s, 3H), 2.77 (d, *J* = 5.3 Hz, 1H), 1.87–1.64 (m, 4H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.18 (d, *J* = 7.3 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$,75 MHz): δ 209.2, 172.6, 169.6, 167.6, 136.4, 136.0, 128.6, 128.5, 128.46, 128.41, 126.5, 126.4, 57.7, 56.1, 50.1, 42.4, 36.4, 32.6, 32.0, 30.4, 20.0, 16.4,14.0, 13.8, 13.7; HRMS (ESIMS): calcd for $C_{29}H_{37}N_2O_5$ $[M+H]^+$: 493.2702, found: 493.2708.
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18. Spectral data of 11-*epi*-stereocalpin A: R_f = 0.45 (SiO_2, 30% EtOAc in petroleum ether). $[\alpha]_D^{29}$ −67.0 (*c* 0.25, CH_2Cl_2); IR (neat): υ_{max} 3300, 2924, 2854, 1737, 1677, 1624, 1457, 1260, 1226, 1172, 962, 752, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): Contains two rotamers. δ 7.34–6.87 (m, 10H), 6.34 (d, *J* = 10.5 Hz, 0.6H), 5.64 (dd, *J* = 12.8, 4.4 Hz, 0.7H), 5.46 (d, *J* = 3.4 Hz, 0.3H), 5.22 (td, *J* = 10.5, 4.6 Hz, 0.7H), 5.15 (m, 0.2H), 4.99 (m, 0.7H), 4.60 (m, 0.4H), 3.40–3.49 (m, 2.5H), 3.29 (q, *J* = 7.0 Hz, 0.8H), 3.20 (dd, *J* = 14.0, 10.5 Hz, 0.7H), 2.99 (m, 0.5H), 2.93 (s, 0.8H), 2.77–2.83 (m, 1.4H), 2.76 (s, 2.4H), 2.31 (m, 0.5H), 1.88–2.04 (m, 1.1H), 1.53 (m, 1H), 1.46 (d, *J* = 7.0 Hz, 2H), 1.28 (d, *J* = 6.8 Hz, 0.8H), 1.22–1.4 (m, 3H), 1.05 (d, *J* = 7.0 Hz, 2.4H), 1.03 (d, *J* = 6.8 Hz, 0.8H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): M for major rotamer, m for minor rotamer, δ 211.1 (m), 207.6(M), 173.9(M), 171.1(m), 170.2(M), 169.0(m),167.7(m), 167.6(M), 136.4(M), 136.0(M), 135.8(m), 77.8(m), 76.9(M), 62.2(m), 58.9(m), 58.3(M), 58.0(m), 56.9(M), 50.6(M), 42.6(m), 41.7(M), 36.7(M), 34.6(m), 34.4(m), 34.1(M), 33.4(M), 31.9(M), 31.7(m), 31.1(m), 17.8(m), 16.5(M), 15.0(M), 14.8(m), 14.4(M), 14.0(m), 12.8(M), 12.5(m).HRMS (ESIMS): calcd for $C_{29}H_{37}N_2O_5$ $[M+H]^+$: 493.2702, found: 493.2706.