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Macrocyclization studies and total synthesis of cyclomarin C, an anti-inflammatory marine cyclopeptide

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Abstract—The studies on macrocyclization at possible sites toward the total synthesis of cyclomarin C are described. The results showed that both Trp and Phe derivatives involved in the target could not be the terminals of the final linear peptide precursors. Additionally, preparation of corresponding dipeptides with an *N*-methyl amide bond is not favorable in the synthesis of linear precursors. Site d was finally proved a proper site for the cyclopeptide formation, and the corresponding head-to-tail macrocyclization was achieved under mild conditions and gave repeatable and satisfactory yields.

1. Introduction

Cyclomarins A–C (**1**–**3**), three novel bioactive metabolites, were recently isolated and characterized from a marine bacterium collected in the vicinity of San Diego by Clardy et al.[1] The major constituent (95% of the total cyclomarin mixture), cyclomarin A displays significant anti-inflammatory activities both in vitro and in vivo assays, as well as anti-cancer activity. In the phorbol ester (PMA)-induced mouse ear edema assay, this compound shows 92% inhibition of edema at the standard testing dose of 50 μg/ear. In the same assay, cyclomarin A also shows promising in vivo activity (45% reduction in edema at 30 mg/kg ip administration), indicating it may be a potential drug candidate. Structurally, all three natural products are cyclic heptapeptides, and contain four noncoded amino acids in each. Their structures show remarkable resemblance to each other (Fig. 1). Cyclomarin A and C each contain three common amino acids (alanine, valine, and *N*-methylleucine), two uncommon amino acids (β-methoxyphenylalanine, *N*-methyl-5-hydroxyleucine), and two novel amino acids (2-amino-3,5-dimethylhex-4-enoic acids for both, *N′*-prenyl-β-hydroxytryptophan for cyclomarin C, and *N′*-(1,1-dimethyl-2,3- expoxypropyl)-β-hydroxytryptophan for cyclomarin A).

Cyclomarins have attracted much attention within the synthetic community due to their potent bioactivities and the unique structural complexicities. In the past several years, many efforts and achievements were reported about the elaboration of key amino acids,[2] while the first total synthesis of cyclomarin C was demonstrated by us very recently.[3] Due to its limited availability (1 mg, 3% of cyclomarins from nature), the biological activities of cyclomarin C could not be studied intensively. Therefore, the synthetic efforts toward cyclomarin C will definitely illustrate two sides of importance. In chemistry side, because of the structural similarity between cyclomarins, the methodologies and strategies developed during its total synthesis will benefit those efforts toward other members of cyclomarins. The other income will be on the biology side, because the total synthesis of cyclomarin C will be able to offer more quantity of sample to meet the requirements of biological studies, as well as possible derivatives. In our recent preliminary communication,[3] we reported the results on the total synthesis of cyclomarin C, including methods to

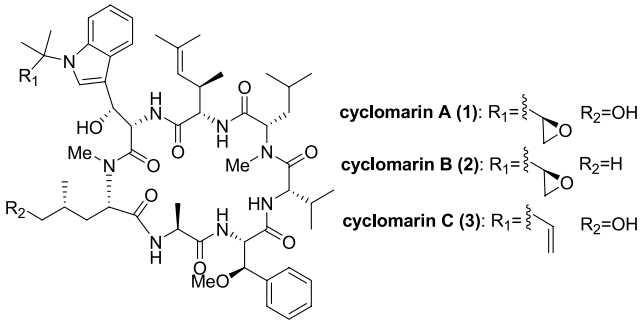

Figure 1. The chemical structures of cyclomarins A–C.

Keywords: Macrocyclization; Dipeptides; Cyclomarin C.
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elaborate those noncoded amino acids,[4] convergent assembly of linear peptide, and final successful macrocyclization at the optimized site. In this paper, we would like to address the details of our efforts to search a proper site for the final macrocyclization, which have played a key role in the total synthesis of cyclomarin C (**3**).

As mentioned, seven components (single amino acid derivatives) appear in cyclomarin C were synthesized by various fashions, and modified as the proper derivatives. With those fragments in hand, the following major task is how to link them together, affording the corresponding linear heptapeptide precursors for the final macrocyclization. In order to enhance the synthetic efficiency, two key points were initially considered in our retrosynthetic analysis for this stage of total synthesis. First, a convergent peptide-segment assembly strategy rather than a stepwise one should be more preferable. Second, because the noncoded amino acid, *N*′-prenyltryptophan, was highly sensitive to various acidic conditions, it would be favorable to incorporate this amino acid at the last stage of the synthesis of linear heptapeptide. Figure 2 illustrates all of our trials for a proper macrocyclization site and the corresponding linear precursors.

2. Synthesis and discussion

Disconnection of the amide bond at site a afforded the first route (see Fig. 2), in which Trp derivative **22** was incorporated at the last step of the linear precursor **4**, and a [3+3+1] peptide-segment-assembly strategy was adopted. The first tripeptide **13** was prepared by the coupling of noncoded amino acid derivative (amine) **12** and dipeptide (acid) **10**,[5] using EDCI-HOBt-based conditions (Scheme 1). Similarly, the other tripeptide **18** was synthesized starting from **14** via two successive coupling reactions. The active ester of *N*-Boc-Ala-OH generated in situ was treated with β-methoxyphenylalanine derivative (amine) **15** to afford dipeptide **16** (88%), whose *N*-Boc protecting group could be removed by 10% TFA in dichloromethane quantitatively. Another noncoded amino acid derivative (acid) **17** was activated by EDCI and HOBt and then allowed to react with the amine derived from dipeptide **16**, giving the desired tripeptide **18** in 80% yield.

Coupling between the above two tripetides was executed as Scheme 2. At first, methyl ester **18** and *N*-Boc derivative **13** were treated with LiOH in water–THF and 10% TFA in dichloromethane, respectively, affording the corresponding acid and amine. The amide bond formation between these two intermediates was achieved under EDCI and HOBt conditions, and the linear hexapeptide **20** was obtained in 63% yield. After removal of *N*-Boc functionality of **20**, the resulting amine reacted with tryptophan derivative **22** in the presence of EDCI and HOAt[6] to give heptapeptide **4** in reasonable yield. Treatment of heptapeptide **4** with 10% piperidine in dichloromethane followed by LiOH in THF–water afforded macrocyclization precursor **23** with both amino and carboxylic acid functionalities. It is noteworthy that the hydrolysis of methyl ester was a low-yield reaction and generated many undesired by-products. To make matters worse, macrocyclization of **23** turned out to be troublesome. A variety of condensation conditions were

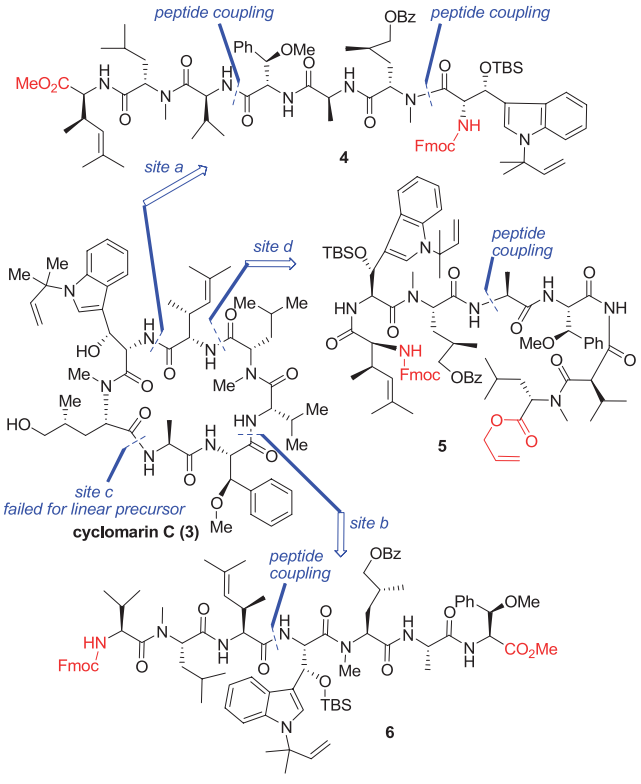
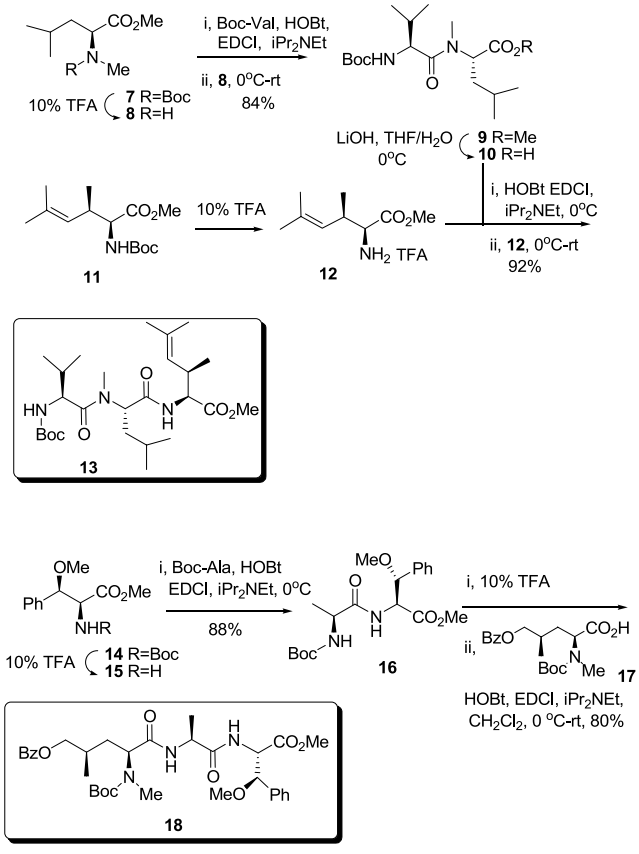
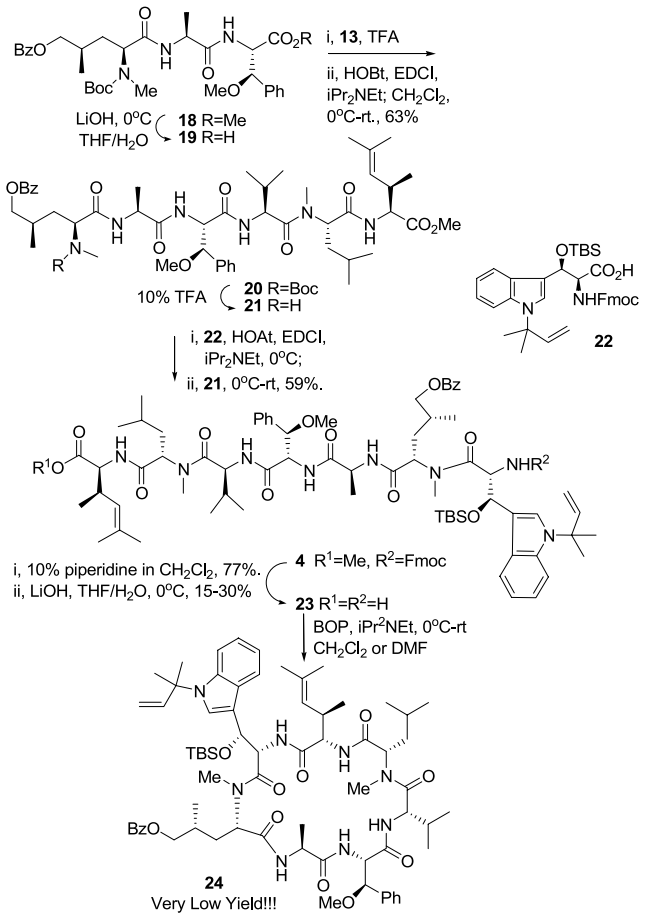


Figure 2. Outline of tested macrocyclization sites and their corresponding linear precursors.



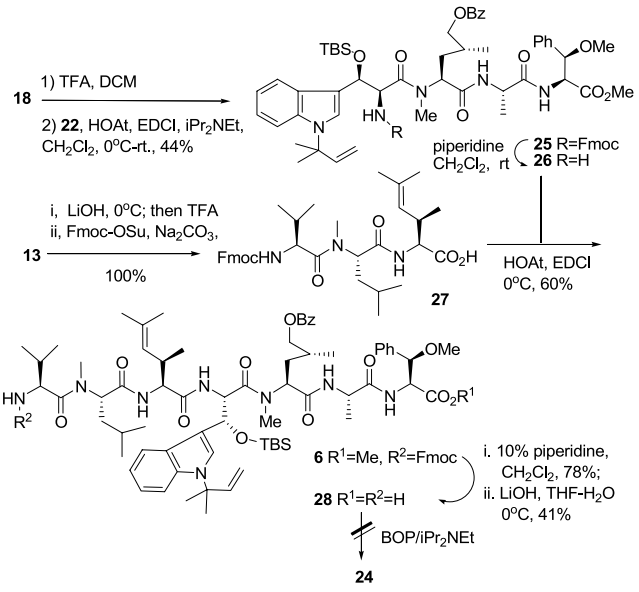
Scheme 1. Synthesis of two tripeptides, **13** and **18**.



Scheme 2. Coupling between tripeptides **13** and **18**, and trials for the macrocyclization.

examined for this cylcization, but none led to satisfactory results. For example, in the presence of BOP and diisopropylethylamine in DMF or dichloromethane, only trace of macrocycle **24** (~2–4%) was formed as detected by mass spectrum and the linear precursor was decomposed during the reaction (Scheme 2). Based on this failure and our experience on the preparation of **22**, we realized that not only the free form of **22** (nonprotected form) is unstable, the linear peptides with it as the terminal moiety, like **23**, were not stable either. So, in the alternative macrocyclization precursors, the unstable component **22** should be placed in the middle region of the final precursor (with two amide bond in both C and N terminals of this amino acid, as it is in natural product) for macrocyclization.

With the above considerations in mind, the second route (b) was designed and heptapeptide 6 was envisioned to be the cyclization precursor (Fig. 2), in which the β-hydroxyl Trp derivative **22** was at the middle of the molecule to avoid the involvement in final cyclization reaction. Synthesis of the linear peptide 6 adopted a [4+3] peptide assembly strategy (Scheme 3). Tripeptide **13** was saponified with LiOH in water, and then treated with 10% TFA in dichloromethane and FmocOSu in the presence of Na_2CO_3 sequentially to give peptide acid **27** quantitatively. The tetrapeptide segment (amine) **26** was prepared by coupling of tripeptide **18** derived amine and β-hydroxyl Trp derivative **22** (44%), and *N*-Fmoc removal by 10% piperidine in dichloro-

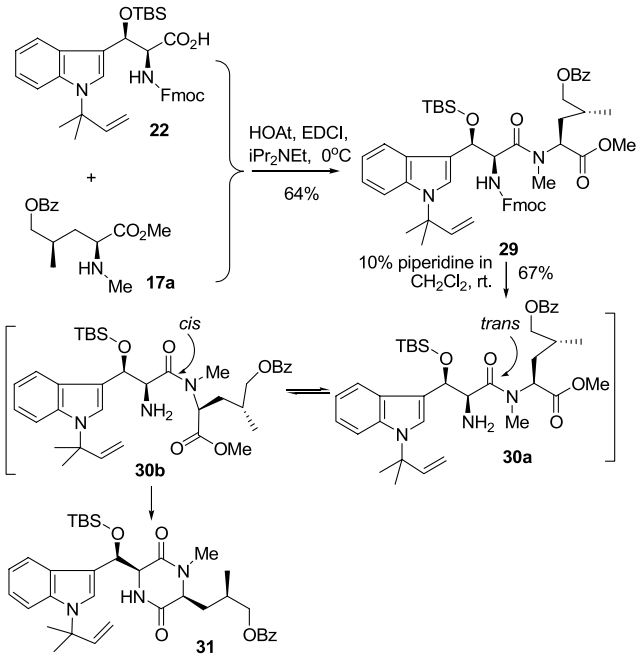


Scheme 3. Synthetic efforts via route b and its [4+3] peptide assembly strategy.

methane. Linkage of segments **26** with **27** was achieved through amide bond formation in the presence of EDCI and HOAt (60% yield) to give heptapeptide **6**, whose *N*-Fmoc group was removed smoothly (78%) using 10% piperidine in dichloromethane. Subsequent saponification of methyl ester gave 41% yield of precursor **28**. The lower yield of ester hydrolysis may be partially due to the elimination of C-terminal residue, β-methoxylphenylalanine methyl ester. To our disappointment, all efforts towards macrocyclization of precursor **28** again failed. Similar to route a, the last step resulted in complex mixtures under a variety of conditions. These results indicate β-methoxyphenylalanine residue is not a suitable C-terminal for the cyclization precursor.

The third approach to cyclomarin C (**3**) through bond disconnection at site c (Fig. 2) also encountered problems at the early stage of total synthesis, and failed to prepare the designed heptapeptide (Scheme 4). Dipeptide **29** with an *N*-methyl amide functionality was found to easily cyclize intramolecularly[7] when it was treated with 10% piperidine in dichloromethane, giving 2,5-piperazinedione derivative **31** (67%). An explanation for this result is the conformational equilibrium (*trans* and *cis*) of *N*-methyl amide bond, in which its *cis* conformation favors the observed cyclization. Because of the severe side reactions in preparing the dipeptides, further investigations were not explored.

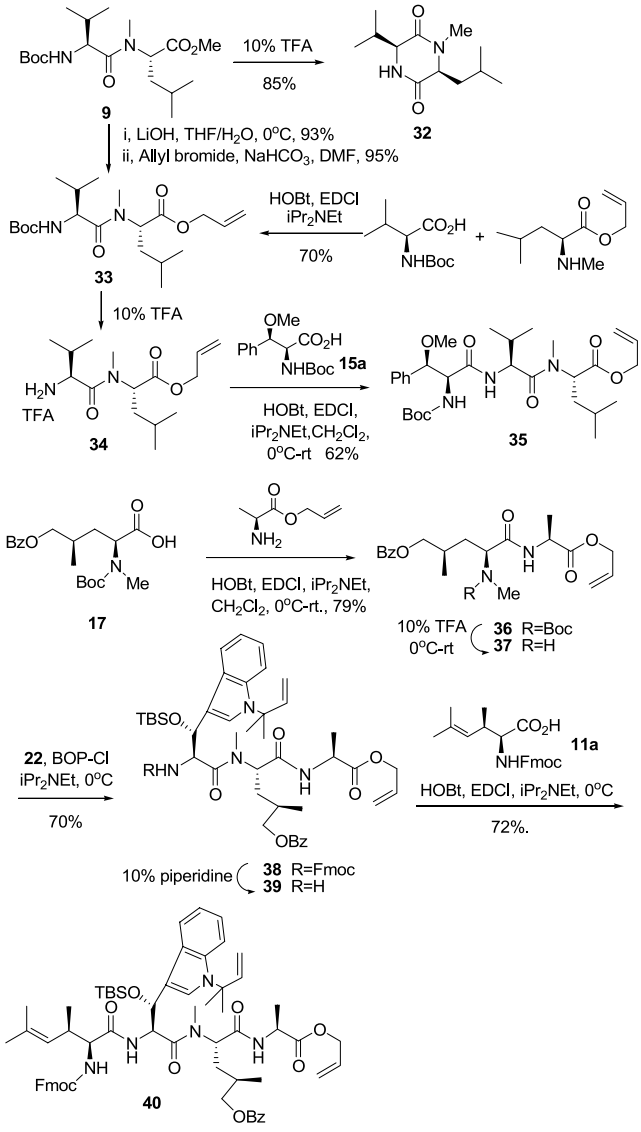
Analyzing the above unsatisfactory results, we concluded that the following situations should be avoided during the total synthesis: (1) the Trp and Phe derivatives involved should not be the terminal of the final linear peptide precursors; (2) preparations of dipeptides with an *N*-methyl amide bond is not favorable (see also the conversion of **9** to **32**, Scheme 5). With those principles, only limited sites could be chosen for the macrocylization. Path d was the final and successful one we tried to elaborate the whole molecule. In this route, cyclomarin C was disconnected at site d and a [4+3] peptide assembly strategy was employed (Fig. 2). In order to avoid the complexity caused by saponifications at



Scheme 4. 2,5-Piperazinedione derivative formation at the early stage of route c.

C-terminals, route d adapted allyl ester as the acid protecting form, because it could be easily removed by Pd(0) or Rh(I)-catalyzed isomerization protocols[8–9] in mild conditions (Scheme 5). Dipeptide **33** derived from *N*-Boc Val-OH and *N*-Me Leu(Oallyl) was treated with 10% TFA, giving the dipeptide TFA salt **34**. The *N*-Boc amino acid **15a** was then allowed to couple with **34** in the presence of EDCI and HOBt to afford tripeptide **35** (62%). Preparation of the other segment, tetrapeptide **40** was started from the coupling of *N*-Boc amino acid **17** and Ala(Oallyl) using EDCI and HOBt. The resultant dipeptide **36** was first treated with 10% TFA and then coupled with *N*-Fmoc amino acid **22** using BOPCl as the carboxyl activation reagent to afford tripeptide **38** (70%). The fourth amino acid derivative **11a** was finally incorporated into amino-liberated tripeptide **39**, affording tetrapeptide **40** (72%). Parallel removals of *N*-Boc protecting group of **35** (10% TFA in dichloromethane) and *O*-allyl ester of **40** ($Pd(PPh_3)_4$, PhNHMe, THF)[10] afforded the both precursors essential for the followed coupling to generate the desired heptapeptide **41** (Scheme 6).

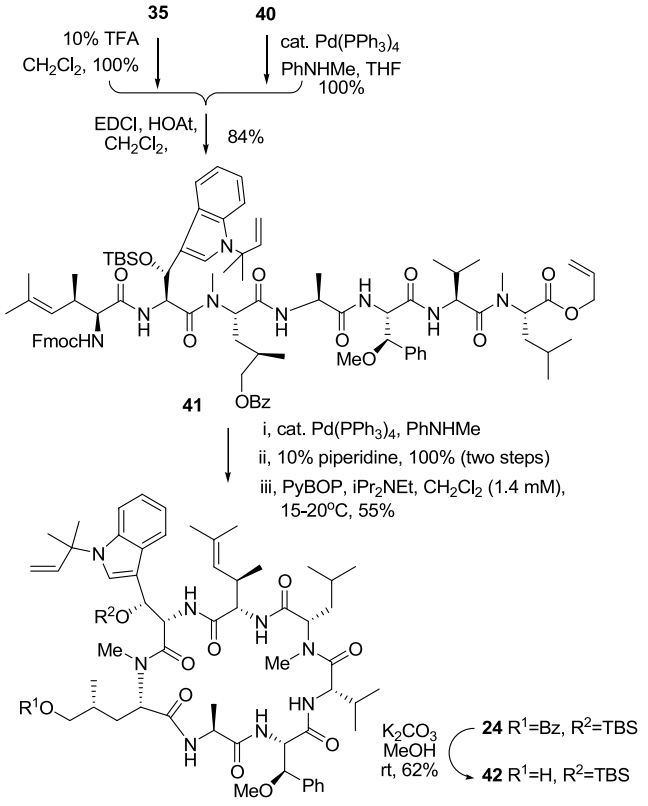
This coupling reaction was performed very well under the conditions of EDCI and HOAt.[11] It is noteworthy here that replacement of HOAt with HOBt resulted in much lower efficiency. Upon stable heptapeptide **41**, both the N- and C-terminal protections were similarly removed by the above methods, Pd(0)-catalyzed deprotection of allyl ester and 10% piperidine for *N*-Fmoc removal. Under the presence of PyBOP[12] and diisopropylethylamine, the resulting precursor was cyclized smoothly by head to tail in dichloromethane at a diluted concertration (1.4 mM), affording macrocycle **24** in 55% yield. Deprotection of benzoate at one of the side chains of **24** was achieved by K_2CO_3 in methanol at rt, giving **42** in 62% yield. Unfortunately, the followed removal of *O*-TBS ether existing in **42** using TBAF failed. No reactions were observed at rt, while decomposition happened when the temperature was raised



Scheme 5. Synthesis of tetrapeptide **40** and tripeptide **35**.

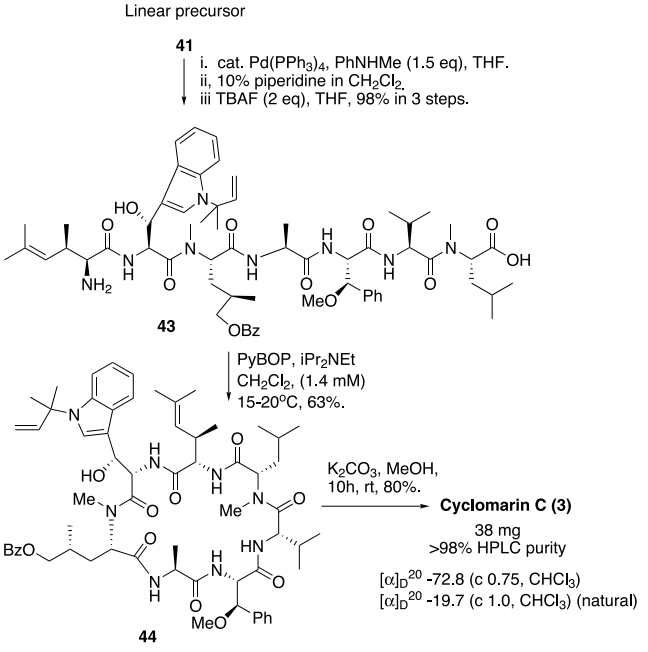
up to 60–70 °C. Spatially hindrance enhancement after macrocycle formation may be a reason for the difficulty to remove *O*-TBS ether of **42**. Therefore, we decided to remove *O*-TBS ether before the cyclization step, that is, at the linear stage (Scheme 7). The subtle change of the reaction sequence proved very successful. Removal of caps at C- and N-terminals of **41** followed by TBS-deprotection with TBAF gave a satisfactory yield of **43** (98%). Macrocylclization was again accomplished under similar conditions and afforded macrocycle **44** (63%). Finally, treatment of **44** with K_2CO_3 in methanol at rt afforded cyclomarin C (**3**, 38 mg, 80% yield), and reproducibility of this reaction was perfectly high.

The synthetic sample of **3** purified by silica gel chromatography showed high purity (>98%, by HPLC analysis using same conditions described in Ref. 1). However, significant difference was observed in comparison of specific optical rotation of synthetic sample ($[\alpha]_D^{20} = -72.8$ (*c* 0.75, $CHCl_3$)) with that reported for natural product ($[\alpha]_D^{20} = -19.7$ (*c* 1.0, $CHCl_3$)). Because of unavailability



Scheme 6. Macrocyclization of linear heptapeptide.

of natural product sample for a CD spectrum, peak-to-peak comparisons[4] of both ^{1}H NMR and ^{13}C NMR spectra were adapted. These results show the synthetic sample is exactly same as the natural one, while the reported ^{1}H NMR spectrum of natural product (in supporting information of Ref. 1) shows that it contains small amount of impurities.



Scheme 7. Completion of total synthesis of cyclomarin C.

3. Conclusion

In summary, our efforts to search for the suitable macrocylization sites and the first total synthesis of cyclomarin C were described in details. These studies showed that the Trp and Phe derivatives involved in the target could not be the terminals of the final linear peptide precursors, and preparations of the corresponding dipeptides with an *N*-methyl amide bond is not favorable. Site d finally proved to be a proper site for macrocyclization, and the head-to-tail macrocyle formation was realized under the dilute conditions at ambient temperature in repeatable and satisfactory yields. The first total synthesis of cyclomarin C was thus achieved based on these explorations. All these will be helpful for the synthesis of other members of cyclomarins. Further applications of the developed methods and strategies to cyclomarins A and B, as well as the evaluation of activity contribution caused by each amino acid component in cyclomarins are under investigation in our laboratory.

4. Experimental

4.1. General

Physical data of compounds in rout d are available in supporting information of Ref. 3.

4.1.1. Dipeptide 9. To a solution of *N*-Boc, *N*-methyl-L-Leu-OMe (3.8 g, 14.7 mmol) in dichloromethane (60 mL) cooled with ice-water bath was added dropwise TFA (9 mL). After stirred at room temperature for about 4.5 h, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (30 mL) and basified to pH 9 with sat. aq. $NaHCO_3$ solution at 0 °C. The aqueous phase was separated and extracted with ethyl acetate (20 mL×3). The organic layers were dried with anhydrous Na_2SO_4 and concentrated to give compound **8** (2.42 g) as a yellow oil, which was used in the next step without further purification.

To a solution of *N*-Boc-L-valine (3.25 g, 15 mmol) in dichloromethane (60 mL) at 0 °C were added successively HOBt (2.18 g, 16 mmol), EDCI (3.13 g, 16 mmol) and diisopropylethylamine (2.85 mL, 16 mmol). The mixture was stirred for 20 min, and then a solution of compound **8** (14.7 mmol) in dichloromethane (60 mL) was added. The resulting mixture was stirred at room temperature until starting material disappeared, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane (60 mL×3). The organic phases were combined, washed with sat. aq. NH_4Cl solution (50 mL×3) and brine (50 mL) and dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by silica gel chromatography (PE/EA, 5/1, v/v) to give compound **9** (4.42 g, 84%) as an oil.[5] $[\alpha]_D^{20} = -26.8$ (*c* 1.05, $CHCl_3$). IR: ν_{max} 1014, 1176, 1270, 1367, 1496, 1648, 1709, 1745, 2961, 3327 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–1.02 (m, 12H), 1.43 (s, 9H), 1.67–1.77 (m, 3H), 2.00 (m, 1H), 3.02 (s, 3H), 3.70 (s, 3H), 4.43 (dd, 1H, $J=6.9$, 9.3 Hz), 5.22 (d, 1H, $J=8.7$ Hz), 5.38

(dd, 1H, J=6.9, 9.0 Hz) ppm. EI-MS (m/z): 302 (M^+-56), 285 (M^+-100).

4.1.2. Tripeptide 13. To a solution of compound **9** (700 mg, 1.96 mmol) in THF/H_2O (10 mL, v/v 2:1) at 0 °C was added $LiOH\cdot H_2O$ (168 mg, 4 mmol). After 3 h, 4 N HCl was added to adjust the pH to 2–3. The mixture was extracted three times with ethyl acetate. The organic phases were dried with anhydrous Na_2SO_4 and then concentrated to give the crude product **10**.[5] The crude acid was dissolved in dichloromethane (5 mL) and cooled with ice-water bath. To it were added sequentially diisopropylethylamine (0.16 mL, 0.95 mmol), HOBt (83 mg, 0.61 mmol) and EDCI (119 mg, 0.61 mmol). After 15 min, a solution of **12** (0.47 mmol) in dichloromethane (2 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then allowed to warm to room temperature and stirred for additional 10 h, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The organic phases were combined, washed with sat. aq. NH_4Cl solution and brine (50 mL) and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 5/1, v/v) to give compound **13** (208 mg, 89%) as an oil. $[\alpha]_D^{20}=-56.7$ (c 1.1, $CHCl_3$). IR: ν_{max} 1015, 1174, 1367, 1518, 1630, 1685, 1743, 2933, 2964, 3315 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.82–1.02 (m, 15H), 1.43 (s, 9H), 1.52–1.70 (m, 3H), 1.65 (s, 6H), 1.92–2.05 (m, 1H), 2.75–2.85 (m, 1H), 3.01 (s, 3H), 3.71 (s, 3H), 4.39–4.45 (m, 2H), 4.86 (d, 1H, J=9.6 Hz), 5.15–5.22 (m, 2H), 6.34 (d, 1H, J=8.1 Hz) ppm. ESI-MS (m/z): 498.3 (MH^+).

4.1.3. Dipeptide 16. To a mixture of compound **15** (3.2 mmol) and *N*-Boc-L-Ala-OH (725 mg, 3.8 mmol) in dichloromethane (15 mL) at 0 °C were added sequentially diisopropylethylamine (0.75 mL, 4.18 mmol), HOBt (569 mg, 4.18 mmol) and EDCI (818 mg, 4.18 mmol). The reaction mixture was stirred at the same temperature for 2 h, additional 1 h at room temperature, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane (10 mL×3). The combined organic phase was washed with sat. aq. NH_4Cl solution (7 mL×3) and brine (7 mL), and dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by silica gel chromatography (PE/EA, 4/1, v/v) to give compound **16** (1.07 g, 88%) as an oil. $[\alpha]_D^{20}=-52.2$ (c 1.4, $CHCl_3$). IR: ν_{max} 716, 1098, 1178, 1555, 1676, 1749, 2933, 3271, 3364 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (m, 3H), 1.45 (s, 9H), 3.28 (s, 3H), 3.76 (s, 3H), 4.13 (m, 1H), 4.73–4.85 (m, 3H), 6.70 (d, 1H, J=8.7 Hz), 7.25–7.37 (m, 5H) ppm. ESI-MS (m/z): 403.2 ($M+Na^+$).

4.1.4. Tripeptide 18. To a solution of **17** (438 mg, 1.2 mmol) in dichloromethane (5 mL) at 0 °C were added successively HOBt (177 mg, 16 mmol), EDCI (254 mg, 1.3 mmol), and diisopropylethylamine (0.80 mL, 4.4 mmol) and then a solution of dipeptide **16** derived amine (1 mmol) in dichloromethane (5 mL). After stirred at 0 °C for 2 h and then room temperature 2 h, the reaction mixture was quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phase was washed with sat. aq. NH_4Cl solution and brine, and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (PE/EA, 2/1, v/v) to give compound **18** (500 mg, 80%) as white solid. IR: ν_{max} 714, 1142, 1274, 1509, 1721, 1752, 2977, 3322 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, 3H, J=6.6 Hz), 1.20 (d, 3H, J=7.2 Hz), 1.47 (s, 9H), 1.70–2.18 (m, 3H), 2.77 (s, 3H), 3.26 (s, 3H), 3.75 (s, 3H), 4.20–4.30 (m, 2H), 4.40–4.60 (m, 2H), 4.70–4.82 (m, 2H), 6.37–6.74 (m, 2H), 7.20–7.40 (m, 5H), 7.40–7.50 (m, 2H), 7.54–7.60 (m, 1H), 8.04 (d, J=7.8 Hz, m, 2H) ppm. ESI-MS (m/z): 628.3 (MH^+). Anal. Calcd for $C_{33}H_{45}N_3O_9$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.08; H, 7.24; N, 6.55.

4.1.5. Hexapeptide 20. Acid **19** (0.30 mmol) derived from ester **18** by saponification was dissolved in dichloromethane (3 mL) and cooled with ice-water bath. To it were added sequentially diisopropylethylamine (52 μL, 0.30 mmol), HOBt (49 mg, 0.36 mmol) and EDCI (70 mg, 0.36 mmol). After 15 min, a solution of an amine (0.30 mmol, prepared from **13** by treatment with TFA) in dichloromethane (3 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature overnight, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phase was washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 1/1, v/v) to give compound **20** (186 mg, 63%) as a solid. $[\alpha]_D^{20}=-75.9$ (c 0.7, $CHCl_3$). IR: ν_{max} 713, 1100, 1274, 1522, 1627, 1655, 1689, 1723, 1745, 2933, 2966, 3310 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.90–1.00 (m, 18H), 1.13 (d, 3H, J=6.3 Hz), 1.50 (s, 9H), 1.58–1.78 (m, 4H), 1.66 (s, 6H), 1.88–2.16 (m, 3H), 2.80 (s, 3H), 2.78–2.82 (m, 1H), 2.99 (s, 3H), 3.31 (s, 3H), 3.70 (s, 3H), 4.26 (d, 2H, J=4.5 Hz), 4.08–4.30 (m, 2H), 4.60–4.92 (m, 6H), 5.18–5.25 (m, 1H), 6.34 (d, 1H, J=9.3 Hz), 6.70–6.79 (m, 2H), 7.13–7.38 (m, 5H), 7.42–7.50 (m, 2H), 7.55–7.60 (m, 1H), 8.08 (d, 2H, J=7.8 Hz) ppm. HR-MS (ESI, m/z) for $C_{53}H_{80}N_6O_{12}Na^+$: 1015.5726, found: 1015.5719.

4.1.6. Heptapeptide 4. To a solution of acid **22** (94 mg, 102 μmol) in dichloromethane (2 mL) were added sequentially diisopropylethylamine (36 μL, 0.20 mmol), HOBt (28 mg, 0.20 mmol) and EDCI (40 mg, 0.20 mmol). After 15 min, a solution of compound **21** (70 μmol, prepared from **20** by treatment with TFA) in dichloromethane (2 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature overnight, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (CH_2Cl_2/MeOH, 100/1, v/v) to give compound **4** (62 mg, 59%) as a solid. IR: ν_{max} 742, 1099, 1211, 1453, 1529, 1631, 1686, 1722, 2928, 2960, 3310 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ −0.24 (m, 3H), 0.15 (s, 3H), 0.78–1.00 (m, 27H), 1.15 (d, 3H, J=6.9 Hz), 1.52–1.74 (m, 16H), 1.90–2.10 (m, 3H), 2.64 (s, 3H), 2.80 (m, 1H), 2.91 (s, 3H), 3.10 (s, 3H), 3.70 (s, 3H), 4.22–4.47 (m, 5H), 4.50–4.92 (m, 8H), 5.00–5.35 (m, 5H), 5.80–6.12 (m, 2H), 6.38 (d, 1H, J=8.7 Hz), 6.62 (d, 1H, J=7.2 Hz), 7.00–7.66 (m, 18H), 7.72–7.82 (m, 2H), 7.90 (d, 1H, J=9.0 Hz), 8.00 (t, 2H, J=7.5 Hz), 8.13

(m, 1H) ppm. HR-MS (ESI, *m/z*) for $C_{85}H_{114}N_8O_{14}SiNa^+$: 1521.8116, found: 1521.8107.

4.1.7. Compound 24. To a solution of **4** (100 mg, 67 μmol) in dichloromethane (5 mL) was added piperidine (0.5 mL) at 0 °C. The mixture was stirred for 2 h and then concentrated. The residue was purified by flash chromatography (PE/EA, 2:1 then CH_2Cl_2/MeOH, 20/1) to give the de-Fmoc product (66 mg, 77%). The product obtained above was dissolved in THF/H_2O (8 mL, 3:1) and cooled with ice-water bath. To it was added $LiOH\cdot H_2O$ (9 mg, 0.21 mmol), and the resulting mixture was stirred for 9 h, neutralized with 1 N HCl to pH 7 and then extracted with ethyl acetate. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (CH_2Cl_2/MeOH, 100/1, 40/1 and then 10/1) to give **23** (16 mg, 25%) as a solid. **23** (15 mg, 12 μmol) was dissolved in dichloromethane (10 mL), and was added slowly via syringe pump to a mixture of BOP (16 mg, 36 μmol) and diisopropylethylamine (7 μL, 40 μmol) in dichloromethane (10 mL) over 12 h. After complete addition, the reaction mixture was stirred for additional 4 h and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA 4/1, 2/1, 1/1) to give compound **24** (4 mg). ESIMS (*m/z*): 1263.7 (MH^+).

4.1.8. Heptapeptide 6. To a solution of compound **13** (30 mg, 60 μmol) in THF/H_2O (2.5 mL, v/v 4:1) at 0 °C was added $LiOH\cdot H_2O$ (5 mg, 120 μmol). After 4 h, 4 N HCl was added to adjust the pH to 3–4. The mixture was extracted three times with ethyl acetate. The organic phases were combined, dried with anhydrous Na_2SO_4, and then concentrated to give the crude product. The crude acid (29 mg) was dissolved in dichloromethane (2 mL) and cooled with ice-water bath. To it was added TFA (0.5 mL), and the resulting mixture was stirred for 2 h, and then concentrated. The residue was dissolved in THF (2 mL) and neutralized with sat. aq. $NaHCO_3$ at 0 °C, then solid sodium carbonate (13 mg, 120 μmol) and Fmoc-*O*su (24 mg, 63 μmol) were added. After 3 h, 0.5 N HCl was added to adjust the pH to 2–3. The aqueous phase was extracted with ethyl acetate (10 mL×3). The organic phases were combined, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 4/1, then CH_2Cl_2/MeOH, 20/1) to give dipeptide-acid **27** (38 mg, 100%).

4.1.9. Compound 25. The title compound was prepared according to the procedure similar to compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.31 to −0.23 (m, 3H), 0.14 (d, 3H, J=5.1 Hz), 0.81 (s, 9H), 0.85 (d, 3H, J=6.6 Hz), 1.10 (d, 3H, J=7.2 Hz), 1.50–1.80 (m, 9H), 2.63 (d, 2H, J=4.8 Hz), 2.80 (d, 1H, J=3.0 Hz), 3.10 (s, 2H), 3.21 (s, 1H), 3.60 (s, 2H), 3.70 (s, 1H), 4.10–4.38 (m, 6H), 4.60–4.76 (m, 5H), 5.00–5.32 (m, 2H), 5.81 (d, 1H, J=4.5 Hz), 5.98–6.18 (m, 1H), 6.65–6.78 (m, 2H), 7.00–8.04 (m, 18H) ppm.

To a solution of acid **27** (19 mg, 31 μmol) in dichloromethane (1 mL) were added sequentially diisopropylethylamine (6 μL, 34 μmol), HOAt (4 mg, 29 μmol) and EDCI (6 mg, 31 μmol). After 15 min, a solution of compound **26** (24 μmol, prepared from **25** by treatment with piperidine) in dichloromethane (1 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature for 5 h, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 2/1, v/v) to give compound **6** (23 mg, 60%) as a solid. ^{1}H NMR (300 MHz, d^6-DMSO): δ −0.38 (s, 3H), −0.07 (s, 3H), 0.64 (s, 9H), 0.89–0.98 (m, 15H), 1.12 (d, 3H, J=7.5 Hz), 1.24 (m, 3H), 1.41 (s, 3H), 1.52 (s, 3H), 1.60 (s, 3H), 1.63 (s, 3H), 1.40–1.70 (m, 4H), 1.92–2.10 (m, 3H), 2.80 (m, 1H), 2.93 (s, 3H), 3.10 (s, 3H), 3.34 (s, 3H), 3.50 (s, 3H), 3.85–4.34 (m, 8H), 4.45 (dd, 2H, J=4.5, 9.0 Hz), 4.66 (d, 1H, J=4.8 Hz), 4.82–5.10 (m, 6H), 5.38–5.50 (m, 1H), 6.10 (dd, 1H, J=11.1, 18.6 Hz), 6.90–7.04 (m, 3H), 7.20–7.54 (m, 10H), 7.54–7.80 (m, 7H), 7.80–8.00 (m, 6H), 8.40–8.52 (m, 1H) ppm. ESI-MS (*m/z*): 1520.8 ($M+NH_4^+$), 1521.7 ($M+Na^+$).

4.1.10. Dipeptide 29. The title compound was prepared according to the procedure similar to compound **6**: $[\alpha]_D^{20}$=−38.2 (*c* 1.2, $CHCl_3$). IR: ν_{max} 741, 838, 1071, 1274, 1452, 1721, 2929, 2955, 3310 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.27 (s, 3H), 0.04 (s, 3H), 0.84 (s, 9H), 0.98 (d, 3H, J=6.6 Hz), 1.60–1.70 (m, 1H), 1.70 (d, 6H, J=3.9 Hz), 1.80–1.92 (m, 1H), 1.98–2.10 (m, 1H), 2.71 (s, 3H), 3.46 (s, 3H), 4.04–4.38 (m, 5H), 5.10 (d, 1H, J=17.1 Hz), 5.14–5.25 (m, 4H), 5.79 (d, 1H, J=8.1 Hz), 6.15 (dd, 1H, J=10.2, 17.1 Hz), 7.02–7.14 (m, 2H), 7.22–7.62 (m, 11H), 7.71–7.82 (m, 3H), 8.01 (d, 2H, J=6.9 Hz) ppm. ESI-MS (*m/z*): 908.4 ($M+Na^+$), 754.3 (M^+-131). Anal. Calcd for $C_{52}H_{63}N_3O_8Si$: C, 70.48; H, 7.17; N, 4.74. Found: C, 70.62; H, 7.42; N, 4.49.

4.1.11. Compound 31. To a solution of **29** (22 mg, 25 μmol) in dichloromethane (2 mL) at 0 °C was added dropwise piperidine (0.2 mL). The mixture was stirred for 1 h, and then concentrated. The residue was purified by chromatography (PE/EA, 3/1 then CH_2Cl_2/MeOH, 50/1) to give compound **31** (11 mg, 67%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.16 (s, 3H), −0.06 (s, 3H), 0.89 (s, 9H), 1.10 (d, 3H, J=6.6 Hz), 1.62–1.70 (m, 1H), 1.72 (s, 3H), 1.76 (s, 3H), 2.30–2.42 (m, 1H), 2.58–2.69 (m, 1H), 3.02 (s, 3H), 3.88 (d, 1H, J=8.7 Hz), 4.12–4.20 (m, 2H), 4.33 (d, 1H, J=10.5 Hz), 5.13 (d, 1H, J=17.4 Hz), 5.23 (d, 1H, J=10.8 Hz), 5.64 (s, 1H), 5.76 (d, 1H, J=1.8 Hz), 6.12 (dd, 1H, J=10.8, 17.4 Hz), 7.06–7.19 (m, 2H), 7.30 (s, 1H), 7.40–7.63 (m, 5H), 8.05 (d, 2H, J=8.4 Hz) ppm. ESI-MS (*m/z*): 500.3 (M^+-131).

4.1.12. Cyclomarin C.[3] $[\alpha]_D^{20}$=−72.8 (*c* 0.8, $CHCl_3$); IR: ν_{max} 703, 742, 1095, 1376, 1458, 1509, 1649, 1686, 2873, 2962, 3310 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.62 (d, 3H, J=6.3 Hz), 0.65 (m, 1H), 0.73 (d, 3H, J=6.7 Hz), 0.82 (d, 3H, J=6.6 Hz), 0.88 (d, 3H, J=6.6 Hz), 0.93 (d, 3H, J=6.6 Hz), 1.05 (d, 3H, J=6.3 Hz), 1.05 (m, 1H), 1.25 (s, 3H), 1.31 (d, 3H, J=7.2 Hz),1.43 (m, 2H), 1.55 (s, 3H), 1.63 (m, 1H), 1.70 (s, 3H), 1.73 (s, 3H), 2.15–2.35 (m, 3H),

2.42 (br s, 1H), 2.72 (s, 3H), 2.82 (s, 3H), 3.10–3.30 (m, 2H), 3.35 (s, 3H), 4.07 (t, 1H, $J=9.8$ Hz), 4.30 (s, 1H), 4.40 (t, 1H, $J=8.7$ Hz), 4.56 (br s, 1H), 4.77 (d, 1H, $J=10.0$ Hz), 4.78–4.83 (m, 2H), 4.88 (t, 1H, $J=4.9$ Hz), 4.82–4.93 (m, 1H), 5.07 (d, 1H, $J=5.2$ Hz), 5.15 (d, 1H, $J=17.4$ Hz), 5.22 (d,1H, $J=10.7$ Hz), 5.31 (d, 1H, $J=3.0$ Hz), 6.07 (dd, 1H, $J=10.7$, 17.4 Hz), 6.72 (d, 1H, $J=2.9$ Hz), 7.05 (dd, 1H, $J=7.2$, 7.2 Hz), 7.12 (d, 1H, $J=5.3$ Hz), 7.15–7.21 (m, 2H), 7.22–7.30 (m, 4H), 7.33 (s, 1H), 7.48 (d, 1H, $J=$ 8.3 Hz), 7.55 (d, 1H, $J=7.8$ Hz), 7.95 (d, 1H, $J=7.7$ Hz), 8.05 (d, 1H, $J=9.3$ Hz), 8.17 (d, 1H, $J=10.3$ Hz) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.8, 18.5, 18.9, 19.3, 20.0, 20.8, 22.4, 23.5, 25.1, 25.7, 27.9, 27.9, 29.3, 29.5, 30.9, 33.2, 33.4, 35.6, 38.9, 50.6, 52.7, 55.3, 55.9, 57.7, 58.1, 58.6, 59.3, 59.3, 66.4, 68.5, 80.0, 111.4, 113.8, 114.4, 118.7, 119.5, 121.5, 123.1, 124.8, 126.8, 127.9, 128.3, 128.6, 128.7, 134.5, 135.1, 135.8, 143.7, 168.4, 168.8, 169.6, 170.6, 171.1, 171.6, 172.6 ppm. ESI-MS (*m*/*z*): 1049.4 ($M+Na^+$), 1009.5 (MH^+-H_2O).

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