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# Toward the total synthesis of a lagunamide B analogue

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ABSTRACT

Lagunamides, isolated from a marine cyanobacterium *Lyngbya majuscule* found in Singapore, showed very potent activities against *Plasmodium falciparum* and murine leukemia cell line (P388). Herein, a concise synthetic approach toward the total synthesis of a lagunamide B analogue is discussed. Macrolactonization, HWE-olefination, and modified Crimmin's aldol are some of the key reactions featured in this synthesis.



Lagunamides A and B (Fig. 1), cyclic depsipeptides,[1] were isolated from the marine cyanobacterium *Lyngbya majuscule* obtained from Pulau Hantu Besar, Singapore.[2] They displayed effective antimalarial properties, with $IC_{50}$ values of 0.19 and 0.91 μM, respectively, when tested against *Plasmodium falciparum*.[3] Lagunamides A and B also exhibited significant cytotoxic activities against P388 murine leukemia cell lines, with $IC_{50}$ value of 6.4 and 20.5 nM, respectively.[4] Further, these cyanobacterial compounds[5] showed moderate anti-swarming activities[4] when tested against *Pseudomonas aeruginosa*$^{PA01}$.[6]

Lagunamide A was first synthesized by Dai et al. and later by Lin and co-workers.[7] Till now there is no report on the total synthesis of lagunamide B. Structurally, lagunamide A comprises eleven stereogenic centers, whereas lagunamide B consists of ten such centers. Both have a novel polyketide moiety, one hydroxy acid unit, two L-amino acids, and three residues of *N*-methyl amino acids (D-Phe, Gly, and L-Ala) in a 26-membered macrocycle. Lagunamide A contains one normal ester group and one α,β-unsaturated ester. But lagunamide B consists of one α,β unsaturated ester and another allylic ester group. The challenges in the synthesis of lagunamide B are—(i) macrolactonization[8] versus lactamization[9] for successful ring closure,[10] (ii) proper protection of one ester group over another, and (iii) synthesis of the polyketide moiety. It is always challenging for a synthetic chemist to devise a strategic esterification protocol for a molecule with more than one ester group that would facilitate selective saponification eventually when required. Total synthesis of lagunamide A raised doubts over the stereochemical assignments of the molecule. According to Dai et al.,[7a] the molecule is having an L-isoleucine in place of L-alloisoleucine and C-39 stereochemistry would be of *R* configuration instead of *S*. This urges the revision of the assigned stereochemistry for lagunamide B as well, since according to Tan and co-workers,[4] both lagunamides A and B should have the same C-39 configuration. This could only be achieved by the total synthesis of the molecule. This prompted us to devise an efficient strategy that could be employed to prepare different isomers of the molecule and compare their data with the reported literature. The present Letter describes the synthesis of **1** (Fig. 1), one such analogue of

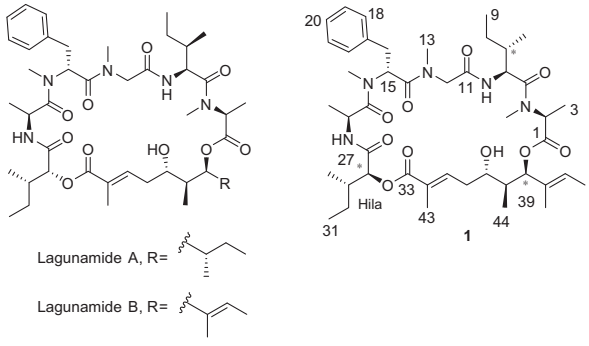


**Figure 1.**

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lagunamide B, having L-isoleucine as in lagunamide A,[7a] L-isoleucine derived Hila (2-hydroxyisoleucic acid), and retaining the C-39(*S*) stereochemistry as originally proposed.

The molecule contains five amide and two ester bonds. Each one of them could serve as a probable site for final cyclization. Though macrolactamization is much more favorable process over macrolactonization, it was preferred to go through the latter option due to the presence of two ester bonds. As the reactivities of the two esters in the molecule are quite different, our retrosynthetic strategy (Fig. 2) focused on the selective cleavage of one ester over the less reactive α,β-unsaturated ester and preparing the more reactive allyl ester in the last step of cyclization of precursor **2**. The hydroxyl groups of the polyketide fragment should be protected with orthogonal protecting groups so that before cyclization the allylic alcohol could be deprotected selectively to provide the necessary precursor molecule **2**. Fragment **2** could be assembled by the amide coupling of subtargets **3** and **4**.

The peptidic fragment **3** could easily be obtained by coupling of the smaller units, **5** and **6**. The polyketide part of the molecule, **4** was planned to be assembled by homologation through Horner–Wadsworth–Emmons (HWE) olefination of phosphonate **8** with the aldehyde obtained from the protected 3,5-dihydroxy-4,6-dimethyl-6-octanol **7**.

The synthesis of the peptide fragment commenced with the coupling of Boc-D-Phe-OH and HCl·H-Gly-OMe using EDCI[11] and HOBt[12] in $CH_2Cl_2$ in the presence of DIPEA to obtain dipeptide Boc-D-Phe-Gly-OMe at 86% yield (Scheme 1). Dipeptide was next treated with methyl iodide and silver oxide in DMF to give the *N*-methylated dipeptide at 79% yield which was then deprotected in the presence of TFA in $CH_2Cl_2$ and coupled with Boc-Ala-OH using HATU[13] and DIPEA in $CH_2Cl_2$ to generate tripeptide **5** in 62% yield. Saponification of **5** with LiOH afforded the corresponding acid **9** in quantitative yield.

The commercially available Boc-Ala-OMe was converted to *N*-methyl ester using methyl iodide and silver oxide in DMF in 74% yield (Scheme 2). The *N*-methylated compound was deprotected with TFA in $CH_2Cl_2$ and coupled with Boc-Ile-OH using HATU in $CH_2Cl_2$ in the presence of DIPEA to obtain dipeptide **6** in 71% yield. The dipeptide was then deprotected with TFA in $CH_2Cl_2$ to furnish **10** that was coupled with **9** to prepare **11** in 72% yield. Pentapeptide **11** (**3**, R = Me) was deprotected with TFA in $CH_2Cl_2$ to get the amine salt **12**.

The synthesis of the polyketide fragment began with a Crimmin's modified aldol reaction[14] between the chiral auxiliary

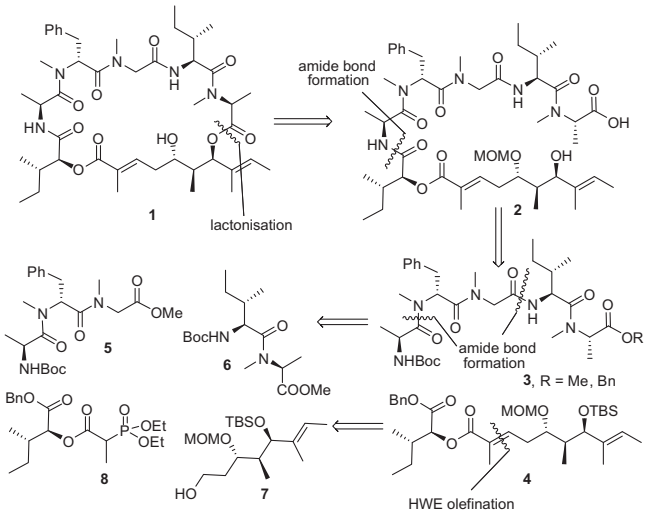
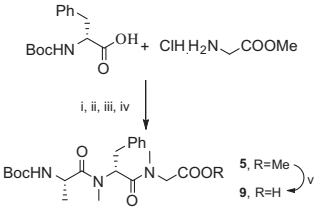
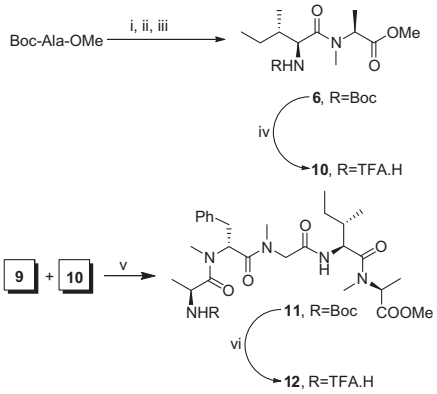


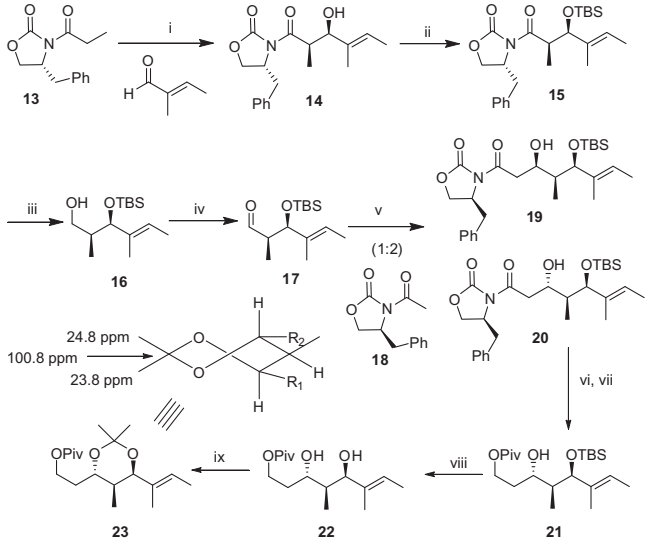

**Figure 2.**




**Scheme 1.** Reagents and conditions: (i) EDCI, HOBt, DIPEA, $CH_2Cl_2$, 8 h, 86%; (ii) $Ag_2O$, MeI, DMF, 6 h, 79%; (iii) TFA, $CH_2Cl_2$, 2 h, quant; (iv) Boc-Ala-OH, HATU, DIPEA, $CH_2Cl_2$, 8 h, 62%; (v) LiOH, THF/MeOH/$H_2O$, 1 h, quant.




**Scheme 2.** Reagents and conditions: (i) $Ag_2O$, MeI, DMF, 6 h, 74%; (ii) TFA, $CH_2Cl_2$, 2 h, quant; (iii) Boc-Ile-OH, HATU, DIPEA, $CH_2Cl_2$, 8 h, 71%; (iv) TFA, $CH_2Cl_2$, 2 h, quant; (v) EDCI, HOBt, DIPEA, $CH_2Cl_2$, 8 h, 72%; (vi) TFA, $CH_2Cl_2$, 2 h, quant.




**Scheme 3.** Reagents and conditions: (i) $TiCl_4$, DIPEA, 1-NMP, $CH_2Cl_2$, 0 °C, 2 h, 88% dr 97:3; (ii) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C, 30 min, 93%; (iii) $LiBH_4$, THF/MeOH (4:1), 0 °C, 1 h, 81%; (iv) py·$SO_3$, $Et_3N$, DMSO/DCM (1:0.8), 0 °C, 1 h, 93%; (v) $Bu_2BOTf$, DIPEA, $CH_2Cl_2$, 0 °C, 2 h, cooled to −78 °C followed by addition of **21** at −78 °C, 2 h, 63%; (vi) $LiBH_4$, THF/MeOH (4:1), 0 °C, 1 h, 82%; (vii) PivCl, py, $CH_2Cl_2$, 1 h, 63%; (viii) HF·py, THF, 2 h, 76%; (ix) 2,2-DMP, CSA, $CH_2Cl_2$, 1 h, 81%.

**13** and tiglic aldehyde (Scheme 3). Compound **13** was treated with $TiCl_4$, DIPEA, and 1-methyl-2-pyrolidinone in $CH_2Cl_2$ at 0 °C to generate the corresponding enolate followed by the addition of tiglic aldehyde at 0 °C to give compound **14** in 88% yield with a diastereoselective ratio 97:3. The free allylic hydroxyl group of **14** was treated with TBSOTf in the presence of 2,6-lutidine in $CH_2Cl_2$ to give TBS protected compound **15** in 93% yield. Reductive removal

of auxiliary of TBS protected compound **15** was carried out with lithium borohydride in THF/MeOH (4:1) at 0 °C to give the corresponding alcohol **16** at 81% yield. Alcohol **16** underwent facile oxidation with pyridine-sulfur trioxide complex and $Et_3N$ in a mixture of $CH_2Cl_2$ and DMSO to give the corresponding aldehyde **17** in 93% yield.

Chiral auxiliary **18** was treated with $^nBu_2BOTf$ and DIPEA at 0 °C and stirred for 2 h. After that the mixture was cooled to −78 °C and aldehyde **17** was added at the same temperature. The reaction was stirred for 2 h and worked up. This led to the formation of two isomers with major compound **20** and minor compound **19**, in 2:1 ratio, in 63% yield. To understand the stereochemistry of the newly generated center of the alcohol, major stereoisomer **20** was subjected to reductive removal of the chiral auxiliary with lithium borohydride followed by selective protection of the primary alcohol over secondary one with pivaloyl chloride to produce compound **21** at 63% yield. Next, silyl protection was removed with HF·py in THF to obtain **22** at 76% yield. Both the hydroxyls of **22** were then protected with the acetonide group to prepare **23** in 81% yield. $^{13}C$ NMR spectrum of the **23** (acetonide Me $\delta$ 23.8 and 24.8 ppm, ketal carbon $\delta$ 100.8 ppm) concluded the *anti* orientation of the 1,3-diol for the major isomer **20**.[15]

Major isomer **20** was then protected as MOM-ether, using MOMCl in the presence of DIPEA in $CH_2Cl_2$, in 81% yield (Scheme 4) followed by deprotection with lithium borohydride in THF/MeOH (4:1) at 0 °C to give the corresponding alcohol **7** at 85% yield. Alcohol **7** underwent facile oxidation with pyridine-sulfur trioxide complex and $Et_3N$ in a mixture of $CH_2Cl_2$ and DMSO to give the corresponding aldehyde **23** in 93% yield.

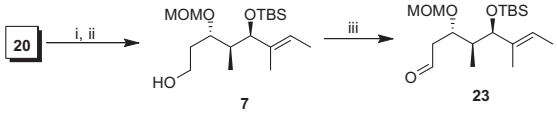
For phosphonate ester fragment **8**, L-Ile was treated with $NaNO_2$ solution in 1 M $H_2SO_4$ at 0 °C for 5 days (Scheme 5).[16] During the addition of $NaNO_2$ solution to the reaction mixture and during the reaction period, the internal temperature of the reaction was kept below 5 °C. It led to the formation α-hydroxy acid **24** with retention in configuration. Now, α-hydroxy acid was reacted with BnBr in the presence of DBU in ACN to give the benzyl protected α-hydroxy ester **25**.[17]

The α-hydroxy ester **25** was coupled with 2-(diethoxyphosphoryl)propanoic acid **26** using DIC and a catalytic amount of DMAP in $CH_2Cl_2$. The reaction yielded phosphonate compound **8** in 83% yield. Compound **8** was coupled with **23** in the presence of LiCl and DIPEA in ACN to give fragment **4** in 59% yield. Next, the α,β-unsaturated ester **4** was hydrogenated with $PdCl_2$ and $Et_3SiH$ in the presence of $Et_3N$ in $CH_2Cl_2$[18] to afford the free acid **27** which was coupled with **12** to give **28** in 34% yield. Compound **28** was treated with HF·py complex in THF to give the linear seco acid **29** in 63% yield. Compound **29** was treated with LiOH in THF/MeOH/$H_2O$ for selective deprotection of the methyl ester. But it led to complete degradation of the compound.

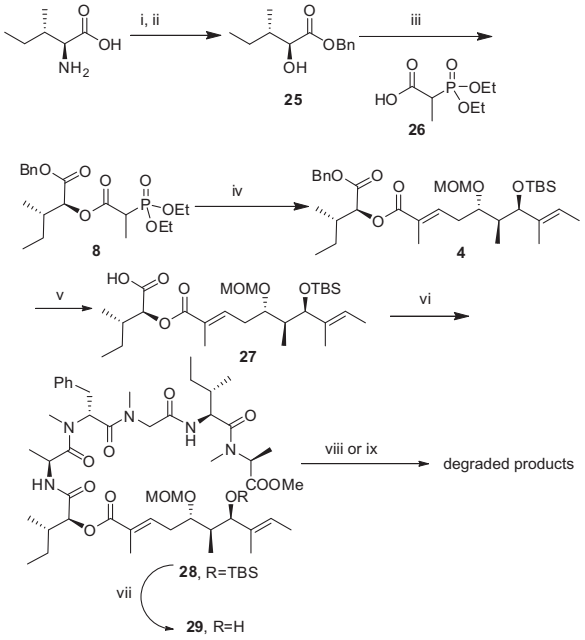
Subsequently, **29** was treated with $Me_3SnOH$ in DCE at 80 °C for 12 h to get the seco-acid **2**.[19] But even in this reaction, several spots were generated, as seen in the TLC, indicating degradation of the molecule.

To overcome these problems, it was decided to try an ester protecting group that could be hydrolyzed under mild conditions. Pentapeptide **11** was saponified with LiOH in THF/MeOH/$H_2O$ to prepare the corresponding acid and was treated with benzylchloroformate in $Et_3N$ and DMAP catalyst in $CH_2Cl_2$ to give the benzyl ester **30** in 78% yield (Scheme 6). Pentapeptide benzyl ester **30** was deprotected with TFA in $CH_2Cl_2$ to prepare amine salt **31** and coupled with **27** to give **32** in 35% yield.[20]

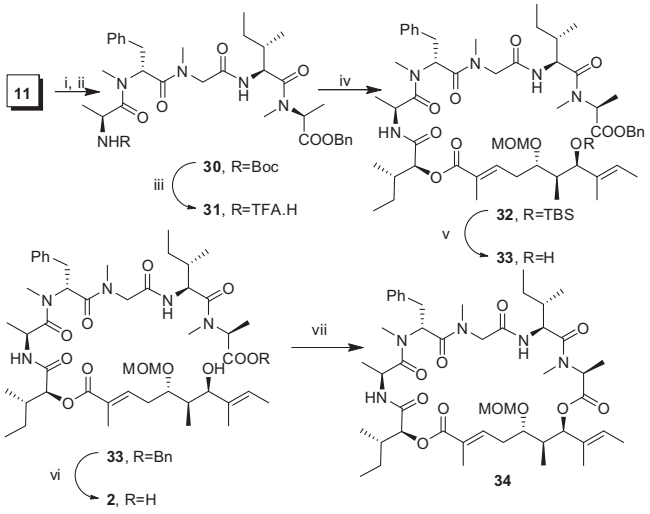
Compound **32** was treated with HF·py complex in THF to give the intermediate **33** in 61% yield.[21] Compound **33** carried a labile benzyl ester, in place of methyl ester, that was expected not to give any problem while deprotecting using the same conditions used above for **29**. As a result, compound **33** was treated with bases—(i) LiOH in THF/MeOH/$H_2O$, and (ii) $Me_3SnOH$ in DCE at 80 °C for 7 h—to furnish the target acid **37**. But in both cases, the degraded products were observed albeit in less quantities. As an alternative




**Scheme 4.** Reagents and conditions: (i) MOMCl, DIPEA, $CH_2Cl_2$, 0 °C, 1 h, 81%; (ii) $LiBH_4$, THF, 0 °C, 1 h, 85%; (iii) py·$SO_3$, $Et_3N$, DMSO/DCM (1:0.8), 0 °C, 1 h, 93%.




**Scheme 5.** Reagents and conditions: (i) Ref. 16; (ii) Ref. 17; (iii) DIC, DMAP, $CH_2Cl_2$, 83%; (iv) **23**, LiCl, DIPEA, ACN, 20 h, 59%; (v) $PdCl_2$, $Et_3N$, $Et_3SiH$, $CH_2Cl_2$, 15 min; (vi) **12**, EDCI, HOBt, DIPEA, $CH_2Cl_2$, 8 h, 34%; (vii) HF·py, THF, 2 h, 63%; (viii) LiOH, THF/MeOH/$H_2O$, 1 h, degraded products; (ix) $Me_3SnOH$, DCE, 80 °C, 12 h, degraded products.




**Scheme 6.** Reagents and conditions: (i) LiOH, THF/MeOH/$H_2O$, 1 h, quant; (ii) $PhCH_2OCOCl$, $Et_3N$, DMAP, $CH_2Cl_2$, 4 h, 78%; (iii) TFA, $CH_2Cl_2$, 2 h, quant; (iv) **27**, EDCI, HOBt, DIPEA, $CH_2Cl_2$, 8 h, 35%; (v) HF·py, THF, 2 h, 61%; (vi) $PdCl_2$, $Et_3N$, $Et_3SiH$, $CH_2Cl_2$, 15 min; (vii) MNBA, DIPEA, $CH_2Cl_2$, 48 h; low yield.

method, compound **33** was treated with $PdCl_2$ and $Et_3SiH$ in the presence of $Et_3N$ in $CH_2Cl_2$ to afford the seco acid **2**. The reaction was fairly clean with very negligible degraded products observed in the TLC.

Compound **2** was subjected to Mitsunobu cyclization with TPP and DEAD in THF.[22] But the expected product could not be isolated. ESI mass spectra of the crude and worked-up product did not show any peak at the desired region. Compound **2** was also tried to be cyclized with 2-chloro-*N*-methylpyridinium iodide[23] in the presence of DIPEA in ACN under highly diluted conditions, but again failed to provide the desired result.

Finally, success was achieved when compound **2** was subjected to the Shiina cyclization method[24] using 2-methyl-6-nitrobenzoic anhydride (MNBA) in the presence of DIPEA and a catalytic amount of DMAP in $CH_2Cl_2$ at high dilution that gave a product which was isolated and showed an ESI mass peak at *m*/*z* 906 $[M+Na]^+$. But the yield was very low. As a result, it was becoming very difficult to get the final compound in sufficient quantities for purification, spectral characterization, and further work. Work is now in progress to improve the yields of some of the steps, especially the macrocyclization step, and complete the total synthesis of the target molecule.

In conclusion, we have been able to devise a synthetic protocol for the synthesis of lagunamide B with a careful choice of an appropriate ester protecting group. At the end, its selective removal keeping the other ester intact allowed us to successfully construct the macrocyclic framework of this depsipeptide using lactonization strategy rather than lactamization approach.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.04.079.

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20. *Synthesis of* ***32****:* To a stirred solution of **4** (450 mg, 0.74 mmol) in $CH_2Cl_2$ (3 mL), $Et_3N$ (0.15 mL, 1.1 mmol), $PdCl_2$ (7 mg, 0.04 mmol), and $Et_3SiH$ (0.2 mL, 1.1 mmol) were sequentially added at 0 °C. After stirring for 15 min at 0 °C, the reaction mixture was quenched with saturated $NH_4Cl$ solution and diluted with EtOAc (60 mL), washed with 1 N HCl (25 mL), water (25 mL), brine (25 mL), dried with anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo to afford crude acid **27** as a yellow liquid. To a stirred solution of **30** (630 mg, 0.89 mmol) in $CH_2Cl_2$ (6 mL) at 0 °C, trifluoroacetic acid (3 mL) was added and stirring was continued for 2 h at room temperature. The reaction mixture was then concentrated in vacuo to get the trifluoroacetate salt **31**. To a stirred solution of **27** in dry $CH_2Cl_2$ (10 mL) at 0 °C, $HOBt{\cdot}H_2O$ (169 mg, 1.1 mmol) and EDCI (213 mg, 1.1 mmol) were sequentially added. After 10 min, **31**, dissolved in dry $CH_2Cl_2$ (5 mL), was added to the reaction mixture followed by the addition of DIPEA (0.4 mL, 2.2 mmol). After stirring for 8 h at room temperature, the reaction mixture was diluted with EtOAc (125 mL), washed with 1 N HCl (2 × 75 mL), saturated $NaHCO_3$ (2 × 75 mL), water (75 mL), brine (75 mL), dried (anhydrous $Na_2SO_4$), filtered, and concentrated in vacuo. Purification by silica gel column chromatography ($SiO_2$, 50% EtOAc in petroleum ether eluant) afforded compound **32** (286 mg, 35%) as pale yellow liquid. $R_f$ = 0.5 ($SiO_2$, 60% EtOAc in petroleum ether). $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.29–7.15 (m, 10H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.89 (m, 1H), 6.61 (d, *J* = 8.6 Hz, 1H), 5.75–5.65 (m, 1H), 5.38–5.31 (m, 2H), 5.17 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 5.01–4.97 (m, 1H), 4.87–4.71 (m, 1H), 4.63 (d, *J* = 7.0 Hz, 1H), 4.52 (d, *J* = 7.0 Hz, 1H), 3.69 (d, *J* = 9.2 Hz, 1H), 3.44–3.40 (m, 1H), 3.31 (s, 3H), 3.24–2.70 (m, 5H), 3.03 (s, 3H), 3.00 (s, 3H), 2.88 (s, 3H), 2.39–2.28 (m, 1H), 2.21–2.15 (m, 1H), 2.05–1.96 (m, 4H), 1.88–0.79 (m, 8H), 1.84 (s, 3H), 1.58 (d, *J* = 6.2 Hz, 3H), 1.57 (s, 3H), 1.40 (d, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.89–0.79 (m, 10H), 0.88 (s, 9H), 0.03 (s, 3H), −0.05 (s, 3H). MS (ESI): *m*/*z* (%): 1128 (100) $[M+Na]^+$, HRMS (ESI): calcd for $C_{60}H_{95}N_5O_{12}SiNa$ 1128.6639 $[M+Na]^+$, found 1128.6637.
21. *Synthesis of* ***33****:* To a stirred solution of **32** (286 mg, 0.26 mmol) in dry THF (3 mL), HF·pyridine complex (1 mL) at 0 °C was added. The reaction was monitored using TLC. After stirring for 2 h at 0 °C, the reaction mixture was quenched with aqueous saturated $NaHCO_3$ solution and diluted with EtOAc (125 mL), washed with 1 N HCl (50 mL), water (50 mL), brine (50 mL), dried (anhydrous $Na_2SO_4$), filtered and concentrated in vacuo. Purification by silica gel column chromatography ($SiO_2$, 50% EtOAc in petroleum ether eluant) afforded compound **33** (157 mg, 61%) as pale yellow liquid. $R_f$ = 0.5 ($SiO_2$, 70% EtOAc in petroleum ether). $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.33–7.20 (m, 10H), 6.88 (m, 2H), 6.67 (d, *J* = 8.9 Hz, 1H), 5.69–5.67 (m, 1H), 5.57–5.55 (m, 1H), 5.34–5.33 (m, 1H), 5.17 (d, *J* = 12.0 Hz, 1H), 5.10 (d, *J* = 12.1 Hz, 1H), 5.01 (m, 2H), 4.81–4.76 (m, 2H), 4.66 (s, 2H), 4.24–4.13 (m, 2H), 3.77–3.66 (m, 2H), 3.41–3.32 (m, 1H), 3.38 (s, 3H), 3.04 (s, 3H), 3.01 (s, 3H), 2.85 (s, 3H), 3.15–2.80 (m, 5H), 2.49 (m, 2H), 2.41–2.33 (m, 2H), 2.05–2.00 (m, 4H), 1.88 (s, 3H), 1.64 (d, *J* = 3.09 Hz, 3H), 1.56 (s, 3H), 1.41–1.06 (m, 6H), 1.06–0.73 (m, 12H). MS (ESI): *m*/*z* (%): 1014 (100) $[M+Na]^+$, HRMS (ESI): calcd for $C_{54}H_{82}N_5O_{12}$ 992.5954 $[M+H]^+$, found 992.5931.
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