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Total Syntheses and Biological Activities of Vinylamycin Analogs

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Abstract

Natural depsipeptide vinylamycin was reported to be an antibiotic previously. Herein, we report vinylamycin to be active against K562 leukemia cells (IC_{50} = 4.86 μM), and be unstable in plasma ($t_{1/2}$ = 0.54 h). A total of 24 vinylamycin analogs with modification of the OH group and chiral centers were generated via a combinatorial approach. The lead compound **1a** was subsequently characterized as having: no anti-microbial activity, significantly higher plasma stability ($t_{1/2}$ = 14.3 h), improved activity against K562 leukemia cells (IC_{50} = 0.64 μM), and up to 75% cell inhibition without significant toxicities in K562 cells xenograft zebrafish model. Furthermore, compound **1a** maintained its activity against the breast cancer cell line MCF-7 under hypoxic conditions. In comparison, the activity of paclitaxel in the same hypoxic *in vitro* model of MCF-7 cells was 92-fold lower. Therefore, the present results demonstrate that **1a** has great potential as an anticancer agent.

Introduction

Natural depsipeptides that contain 4-amino-2,4-pentadienoate moieties include rakicidins,[1] vinylamycin,[2] microtermolides,[3, 4] and BE-43547A$_1$ (Scheme 1).[5, 6] In particular, rakicidin A has exhibited unique activity against chronic myelogenous leukemia (CML) stem cells[7] and hypoxia-selective cytotoxicity in solid tumors.[8, 9] These observations led to the total synthesis and structural determination of rakicidin A,[10, 11] as well as an investigation of its structure-activity relationship (SAR).[12] Recently, Poulsen and co-works reported total synthesis of *ent*-BE-43547A$_1$ and

revealed it significant hypoxia-selectivity against PANC-1 cancer cell line.[6] Unfortunately, rakicidin A was found to be highly unstable at room temperature[10, 11] and the preparation of rakicidin A or its analogs involved more than 35 steps with an overall yield of 0.17%.[12]

Vinylamycin is a depsipeptide that was originally isolated as a metabolite of *Streptomyces sp*. and was subsequently identified as an antibiotic by Takeuchi and co-workers.[2] The two amino acids of vinylamycin were identified as D-valine (2*R*) and L-alanine (5*S*),[2] and only recently were the three consecutive chiral centers in the polyketide fragment determined to be 14*R*, 15*R*, and 16*R* *via* total syntheses in our group.[13] The total synthesis of vinylamycin was accomplished with an overall yield of 3.7%,[13] which is significantly higher than that for rakicidin A. However, similar to rakicidin A, vinylamycin was also found to be unstable. Therefore, 4-amino-2,4-pentadienoate (APD) containing depsipepetides serve as good starting point for drug development,[14] thus, a series of vinylamycin analogs were synthesized by using a combinatorial approach, and the activities of these analogs were subsequently tested.

Results and Discussion

Synthesis of analogs

Scheme 2 shows the retro-synthetic analysis that was used to obtain TBDPS-protected vinylamycins (compounds **1a-e**). This synthetic sequence was modified from our previously reported synthesis of vinylamycin.[13] To obtain the

precursor for the vinylamycin analogs (compound **2**), several fragments were synthesized individually: serinol derivative **3**, Fmoc-valine **4** (with two isomers), Fmoc-alanine (with two isomers) or Fmoc-glycine **5**, and polyketide fragment **6** (with three isomers).

The polyketide fragments were obtained by a highly diastereoselective aldol reaction between chiral aldehyde **9a**[15] and two camphorsultam derivatives, **8a** and **8b** (Scheme 3). The corresponding aldol products, **10a** and **10b**, were hydrolyzed in an aqueous THF solution in the presence of LiOH. The resulting carboxylic acids were then converted into allyl esters **6a** and **6b**. Allyl ester **6c** was synthesized as previously reported[13].

Direct esterification under previously optimized conditions between compounds **6a** and **6c** with Fmoc-valine **4a** and **4b** produced esters **12a**, **12b**, and **12c**(Scheme 4).[13] After deprotection of Fmoc in compounds **12a-c**, the resulting amines were coupled with compound **5a-d** using EDCI and HOBt. Deprotection of the resulting intermediates produced free carboxylic acids which were then subjected to coupling reactions with serinol derivative **3** to generate the PMB-protected precursor **13a-e**. After exchanging the PMB protecting group with TBDPS to obtain compounds **15a-e**, an additional two steps of deprotection were performed, and the intermediates were cyclized using HATU and DIPEA to provide compounds **2a-e**.[13] The TBS protection group in **2a-e** was selectively deprotected under acidic conditions[16, 17]. Mesylation of the resulting alcohol, followed by elimination with DBU, led to the production of O-TBDPS-*ent*-vinylamycin **1a,** (14*S*, 15*S*, 16*R*)-O-TBDPS-vinylamycin **1b**,

O-TBDPS-vinylamycin **1c**, O-TBDPS-*ent*-5-demethylvinylamycin **1d**, and O-TBDPS *ent*-5-demethyl-6-methyl vinylamycin **1e**.

A series of O-functionalized vinylamycin analogs with a 2*S*, 5*R*, 14*S*, 15*S*, 16*R* configuration were synthesized using a combinatorial chemistry strategy. Compound **13a** was used as the common intermediate for synthesis of a library of compounds since it could be easily prepared in gram-scale (Scheme 5). Deprotection of the two protection groups in **13a** followed by macrolactamization, cyclic compound **16** was delivered in good yield. However, selective PMB cleavage to obtain compound **17** proved to be problematic. In an aqueous DCM suspension in the presence of DDQ, both the TBS and PMB groups were removed within 30 min[18, 19], and an undesired compound **18** was produced. Lowering of the reaction temperature to –10 ºC did not improve the selectivity of the reaction. Therefore, alternative fragments **24a-b**, TBDPS-protected serinol derivatives, were used instead of TBS-protected derivatives. Briefly, aldehydes **21a-b** were prepared as previously described,[20] while compounds **23a-23b** were obtained through the Wittig reaction (Scheme 6). Compounds **23a-b** were then treated with TFA to afford serinol derivatives **24a-b**.

Removal of the allyl ester group of **25a-b** were followed by coupling with fragments **24a-b** to obtain cyclization of precursors **26a-b**. After further deprotection of the allyl group and Fmoc group, the resulting compounds were subjected directly to a macrolactamization step, and cyclic compounds **27a-b** were synthesized in good yield. This time, TBDPS was very stable under DDQ oxidation condition to provide compound **29,** and functionalization of the primary hydroxyl group was

straightforward. Thus, esterification of **29** delivered analogs **31a-h**. After all of the analogs were exposed to TBAF to produce primary alcohols (**32a-h**), the resulting hydroxyl groups were activated by MsCl and eliminated with DBU respectively, vinylamycin analogs **33a-h** were produced, and O-PMB vinylamycin analogs **28a-28b** were also obtained with successive deprotection of TBDPS and elimination (Scheme 7).

As shown in Scheme 8, TBS, TIPS and TES silyl ether vinylamycin analogs were synthesized. Briefly, compound **27a** was treated with TBAF/HOAc to obtain compound **34**, and mesylation of alcohol **34** afforded compound **35**. Deprotection of PMB in compound **35** with DDQ, followed by reaction with **36a-36c** and imidazole, and elimination by DBU successively, silyl ether analogs **37a-37c** were obtained.

Bn ether analogs were also synthesized (Scheme 9). Coupling of alcohol **6b** and acid **4a** under DIC and DMAP condition provided ester **38**. After deprotection of Fmoc in compounds **38**, the resulting amine was coupled with compound **5a** using EDCI and HOBt. Deprotection of the resulting intermediate produced free carboxylic acid, which was subjected to coupling reaction with serinol derivative **24a** to generate the cyclization precursor **39**. After further deprotection of the allyl group and Fmoc group, the resulting compound was subjected directly to a macrolactamization step, which provided us compound **40**. Compound **40** was exposed to TBAF to produce primary alcohols **41**, and the OH group was activated by MsCl and eliminated with DBU to produce analog **42**. PMB ether of micortermolide A **44** was obtained through activation of precursor **43** by MsCl and elimination with DBU.

Activities of the synthesized compounds against the CML cell line, K562

Twenty-four analogs were evaluated for their effects on viability of the CML cell line K562 (Table 1). In addition, imatinib was introduced as a positive control, and the natural product, vinylamycin, was included for comparison. The natural product vinylamycin exhibited moderate potency against the K562 cells (IC_{50} = 4.86 μM). Microtermoides A had no inhibitory activity on K562 cell line (IC_{50} > 50 μM), while all of the TBDPS analogs exhibited stronger activity. For example, 16-*epi*-O-TBDPS vinylamycin **1f** (IC_{50} = 1.78 μM) was 3 times more potent than vinylamycin, while O-TBDPS-vinylamycin **1c** and the N-methyl group removed analog **1d** exhibited 4-fold and 2-fold greater potency compared with vinylamycin (IC_{50} = 1.27 μM and 2.31 μM, respectively). The polyketide fragment analog with reversed chirality **1b**, O-TBDPS-*ent*-vinylamycin **1a**, and O-TBDPS *ent*-5-demethyl-6-methyl-vinylamycin **1e** exhibited even higher potency against the K562 cell line (IC_{50} = 0.88 μM, 0.64 μM, and 0.4 μM, respectively), while (14*S*, 15*S*, 16*R*)-O-PMB-vinylamycin **28b** (IC_{50} = 4.6 μM) demonstrated comparable activity with vinylamycin, and O-PMB-*ent*-vinylamycin **28a** (IC_{50} = 2.49 μM) was more potent than vinylamycin and **28b.** PMB analogs **28a** and **28b** were less potent than their corresponding TBDPS analogs **1a** and **1b**.

Regarding the ester analogs **33a-h**, most exhibited lower anti-leukemia activity compared with the TBDPS ether analogs. Specifically, 10-undecynoic acid (which formed ester **33a**) exhibited an IC_{50} of 4.41 μM, which was similar to the IC_{50} of

vinylamycin. The ester with a shorter side chain, compound **33b**, exhibited about two-fold weaker inhibition activity (IC_{50} = 8.00 μM) compared with **33a**. Interestingly, the anti-CML activity of the greasy fatty acid ester **33e** was abolished (> 50 μM). Thus, various of organic acids were employed to generate ester analogs. The anti-CML activity of compound **33c** which contains a tri-phenyl group (IC_{50} = 3.79 μM) was comparable to that of vinylamycin. As to the Boc group protected phenylalanine analog **33f**, its anti-CML activity exhibited a moderate decrease to IC_{50} = 5.95 μM. Compounds **33d** and **33g,** which included a diamantane group and a phosphonate side chain, respectively, exhibited similar potency. When Boc group as the protection group, i.e. compound **33h**, its inhibitory activity against K562 was also decreased to IC_{50} = 5.43 μM. The inhibitory activities of other silyl ether analogs with TBS, TIPS and TES were assayed. TBS ether analog (**37a**) and TES ether analog (**37c**) show the moderate decreased potency (IC_{50} = 2.31 and 1.54 μM, respectively) than TBDPS ether analog. TIPS ether exhibited the similar potency (IC_{50} = 1.05 μM) with TBDPS ether. O-Bn-*ent*-vinylamycin **42** exhibited similar inhibitory activity (IC_{50} = 3.06 μM) with O-PMB-*ent*-vinylamycin **28a**. O-PMB-Microtermolides A **44** showed weak inhibitory activity (IC_{50} = 13.69 μM), while Microtermoides A was basically inactive (IC_{50} > 50 μM). The potency of the OH group precursors of vinylamycin **32a**, **34** and **41** have much lower activities (17.88, 21.36 and 33.65 μM respectively) than vinylamycin.

Antimicrobial activity of the synthesized compounds against *Staphylococcus aureus*

The minimal inhibitory concentration (MIC) of the various synthesized compounds against *Staphylococcus aureus* were determined, with ciprofloxacin and vinylamycin included as positive controls (Table 2). None of the tested synthetic vinylamycin analogs exhibited antimicrobial activity (MIC > 64 μg/mL).

TBDPS analog 1a and ester analog 33a induce apoptosis in K562 cells

Compounds **1a** and **33a** were further analyzed in an apoptosis study by annexin V-FITC/PI double staining. Based on these results, it is hypothesized that the ability to induce apoptosis by **1a** and **33a** is the cause of the reduced proliferation induced by the compounds as seen from Figure 1.

Chemical stability

In general, vinylamycin exhibited poor chemical stability. In addition, vinylamycin was very sensitive to acids, bases, and temperature. However, a freeze-dried powder of pure vinylamycin that was stored at –20 ºC under an argon atmosphere showed minimal decomposition after 3 days. In contrast, all of the O-functionalized vinylamycin analogs were much more stable, and most could be stored at –20 ºC for at least one month without apparent decomposition. These results indicate that the introduction of an ester or silyl group for the free OH group significantly improves the chemical stability of vinylamycin.

Plasma stability

Plasma stability of vinylamycin and analogs **1a** and **1e** in the plasma of SD rat were tested. Vinylamycin had the shortest half-life (0.54 h) in plasma among the three compounds. In comparison, the plasma stability of analogs **1a** and **1e** were significantly improved, with half-lives of 14.3 h and 20.1 h, respectively. Thus, structural modifications at the free hydroxyl group appear to improve the plasma stability of vinylamycin.

Cytotoxicity of vinylamycin analogs under two oxygenation conditions and index of hypoxia selectivity

Hypoxia selectivity against the breast cancer cell line, MCF-7, was evaluated for compounds **1a**, **1b**, **1c**, **1d**, **1f,** and **28b** (Table 3). The inhibitory activities of gemcitabine and paclitaxel against MCF-7 cells under hypoxic conditions were 15.6-fold and 92-fold lower than that under normoxic conditions, respectively. In contrast, the inhibitory activities of the analogs under hypoxic conditions were essentially maintained, with selectivity index values ranging from 0.78–1.37. Taken together, these results indicate that these analogs of vinylamycin can inhibit breast cancer cells under hypoxic conditions. The inhibitory activities of rakicdin A, vinylamycin, and methyl ester of rakicidin A have higher hypoxia selectivity (selectivity index = 0.27, 0.74 and 0.38 respectively). Microtermolides A had no inhibitory activity under hypoxic conditions and normoxic conditions.

Safety and inhibitory activity in xenograft zebrafish model.

The safety and inhibitory activity of compound **1a** against K562 cells *in vivo* were assayed with xenografted zebrafish model (Table 4 and Figure 2)[21]. Compound **1a** was safe to zebrafish even at the saturated concentration of 50 μg/mL, which indicated that the toxicity of compound **1a** was not higher than imatinib. The inhibition of cancer cells of compound **1a** were 66%, 70% and 75% respectively in concentration of 5.6 μg/mL, 16.7 μg/mL and 50 μg/mL. It suggests that compound **1a** have comparable inhibitory activity as imatinib (71% inhibition in 50 μg/mL) *in vivo*.

Conclusions

Initially, vinylamycin was reported to be an antibiotic natural product.[2] Herein we report its activity against the growth of CML cell line K562, and its low stability in rat plasma. 24 vinylamycin analogs with modification of the OH group and chiral centers were synthesized via a highly efficient combinatorial approach, which was modified from our previously reported total synthesis of vinylamycin.[11] Biological assays that were performed for these analogs identified that the TBDPS ether analogs (compounds **1a-f**) were more potent than the PMB ether analog (compound **28**) and the ester analogs (**33a-g**) (Table 1). Among the TBDPS ether analogs, O-TBDPS-*ent*-vinylamycin (compound **1a**) did not exhibit anti-microbial activity (Table 2), yet was still able to induce apoptosis of K562 cells. Finally, compound **1a** maintained activity against the breast cancer cell MCF-7 under hypoxic conditions, while the activity of paclitaxel was found to decrease 92-fold under the hypoxia conditions (Table 3). These results are of particular interest given that tumor cells

under hypoxic conditions are generally resistant to conventional chemo/radiotherapy, and most compounds are less potent in hypoxic conditions[22-24]. These observations, in addition to the increased stability of compound **1a** at room temperature and in rat plasma, and good *in vivo* efficacy in K562 cells xenografted zebrafish models, suggest that compound **1a** is a promising anti-cancer agent that warrants further investigation.

EXPERIMENTAL SECTION

1. Chemistry. General. Unless otherwise mentioned, all reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions. The used solvents were purified and dried according to common procedures. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Purity testing was done by means of analytical HPLC on a Shimadzu LD-20A system with an ODS-C18 column (4.6 × 150 mm, 5 μm) eluted at 1 mL/min with Milli-Q water and CH_3CN. All tested compounds were > 95% pure. FTIR spectra were obtained with a Bruker Tensor 27 instrument. All IR samples were reported in wave numbers (cm^{-1}). NMR spectra were recorded with a 400 MHz or 600 MHz spectrometer using $CDCl_3$, DMSO-d_6 or CD_3OD. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants and integration.

(3S,6R,14S,15S,E)-14-(2-(tert-Butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetr

aone (1a)

To a solution of compound **2a** (0.10 g, 0.11 mmol) in MeOH (1.5 mL) was added a solution of camphor sulfonic acid in MeOH (0.40 mL, 2 M, 0.80 mmol) at - 10 ºC. The mixture was stirred at this temperature for 3 h, and then quenched with saturated aqueous $NaHCO_3$ (10 mL). The organic solvent was removed under reduced pressure and the residue was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel chromatography (CH_2Cl_2: MeOH = 100: 3 to 100: 5) to obtain a white solid.

To a solution of obtained solid (33 mg, 0.044 mmol) in THF (2 mL), triethyl amine (36 μL, 0.260 mmol) and methane sulfonyl chloride (10 μL, 0.13 mmol) were added at 0 ºC. After stirred for 30 min, the reaction solution was quenched by addition of water (0.1 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was dissolved in THF (2 mL). To the resulting solution was added DBU (0.11 g, 0.70 mmol) at 20 ºC. After stirred for 2 h, the reaction was quenched by addition of 1 % HCl (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (3 × 3 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 30: 1) to obtain **1a** (14 mg, 17% for three steps) as a white solid. $[\alpha]^{20}_D = -97.4$ (c = 0.11, DMSO); ν_{max} (KBr): 3293, 2930, 2861, 1736, 1673, 1522, 1464, 1522, 1256, 1106, 982, 893, 828 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.23

(d, J = 9.8 Hz, 1H), 7.66 – 7.56 (m, 5H), 7.44 (dq, J = 14.0, 7.1 Hz, 6H), 6.85 (d, J = 15.2 Hz, 1H), 6.20 (d, J = 15.2 Hz, 1H), 5.34 (s, 1H), 5.24 – 5.18 (m, 2H), 4.32 – 4.24 (m, 1H), 4.22 – 4.16 (m, 1H), 3.73 – 3.58 (m, 2H), 2.92 – 2.82 (m, 1H), 1.99 – 1.87 (m, 1H), 1.85 – 1.63 (m, 3H), 1.27 (m, 13H), 0.99 (s, 9H), 0.91 (d, J = 6.5 Hz, 3H), 0.84 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.0, 168.8, 166.1, 138.7, 137.5, 135.0, 133.1, 132.9, 129.8, 127.85, 118.9, 116.2, 75.9, 61.4, 57.5, 50.9, 44.7, 33.6, 33.4, 32.2, 31.9, 31.1, 28.8, 26.6, 22.0, 19.3, 18.6, 18.2, 18.1, 13.9, 13.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{42}H_{61}N_3NaO_6Si^+$, 754.4222, found 754.4218. HPLC purity: 97.2%.

(3R,6S,14S,15S,E)-14-(2-(tert-Butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (1b)

The titled compound **1b** was obtained following the general procedure described for **1a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 19% for three steps; white powder; $[\alpha]^{20}_D$ = − 24.1 (c = 0.1, DMSO); ν_{max} (KBr): 3273, 3051, 2927, 2860, 1735, 1671, 1622, 1514, 1464, 1370, 1198, 1105, 990, 821 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 9.1 Hz, 1H), 8.23 (s, 1H), 7.70 (d, J = 5.2 Hz, 1H), 7.62 (d, J = 7.6 Hz, 4H), 7.51 – 7.37 (m, 6H), 6.72 (d, J = 15.7 Hz, 1H), 6.09 (d, J = 15.8 Hz, 1H), 5.60 (s, 1H), 5.34 (s, 1H), 5.10 – 5.04 (m, 1H), 4.54 (dd, J = 9.8, 5.6 Hz, 1H), 4.10 – 4.02 (m, 1H), 3.70 – 3.59 (m, 2H), 3.11 – 3.00 (m, 1H), 1.97 (d, J = 6.3 Hz, 1H), 1.76 – 1.67 (m, 1H), 1.65 – 1.56 (m, 1H), 1.42 – 1.32 (m, 2H), 1.29 – 1.12 (m, 14H), 0.99 (s, 8H), 0.87 – 0.79 (m, 9H), 0.74 (d, J = 6.6 Hz, 3H). ^{13}C NMR

(100 MHz, DMSO-d_6) δ 173.4, 170.9, 170.4, 167.7, 137.3, 136.9, 135.0, 135.0, 132.9, 132.9, 129.9, 127.9, 127.8, 120.6, 113.6, 76.8, 60.8, 55.9, 51.2, 45.9, 34.7, 32.4, 31.5, 31.0, 30.3, 28.9, 26.6, 25.6, 22.0, 19.1, 18.7, 18.5, 17.3, 14.5, 13.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{42}H_{61}N_3NaO_6Si^+$, 754.4222, found 754.4219. HPLC purity: 97.9%.

(3R,6S,14R,15R,E)-14-(2-(tert-Butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-11-methylene-15-((S)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (1c)

The titled compound **1c** was obtained following the general procedure described for **19a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 25% for three steps; white powder; $[\alpha]^{20}_D$ = + 88.3 (c = 0.12, DMSO); ν_{max} (KBr): 3292, 2929, 2860, 1737, 1673, 1524, 1465, 1258, 1106, 983, 823 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 9.8 Hz, 1H), 7.66 – 7.56 (m, 5H), 7.44 (dq, J = 14.0, 7.1 Hz, 6H), 6.85 (d, J = 15.2 Hz, 1H), 6.20 (d, J = 15.2 Hz, 1H), 5.34 (s, 1H), 5.24 – 5.18 (m, 2H), 4.32 – 4.24 (m, 1H), 4.22 – 4.16 (m, 1H), 3.73 – 3.58 (m, 2H), 2.92 – 2.82 (m, 1H), 1.99 – 1.87 (m, 1H), 1.85 – 1.63 (m, 3H), 1.27 (m, 13H), 0.99 (s, 9H), 0.91 (d, J = 6.5 Hz, 3H), 0.84 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.0, 168.8, 166.1, 138.7, 137.5, 135.0, 133.1, 132.9, 129.8, 127.85, 118.9, 116.2, 75.9, 61.4, 57.5, 50.9, 44.7, 33.6, 33.4, 32.2, 31.9, 31.1, 28.8, 26.6, 22.0, 19.3, 18.6, 18.2, 18.1, 13.9, 13.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{42}H_{61}N_3NaO_6Si^+$, 754.4222, found 754.4216. HPLC purity: 96.7%

(3S,14S,15S,E)-14-(2-(tert-Butyldiphenylsilyloxy)ethyl)-3-isopropyl-11-methylene-1

5-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (1d)

The titled compound **1d** was obtained following the general procedure described for **1a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 20% for three steps; white powder; $[\alpha]^{20}_D = -52.3$ (c = 0.13, DMSO); ν_{max} (KBr): 3315, 2927, 2860, 1721, 1680, 1526, 1463, 1364, 1264, 1105, 983, 859, 826 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.22 (d, J = 9.7 Hz, 1H), 7.76 – 7.56 (m, 5H), 7.51 – 7.33 (m, 6H), 6.83 (d, J = 15.2 Hz, 1H), 6.17 (d, J = 15.2 Hz, 1H), 5.34 (s, 1H), 5.23 – 5.15 (m, 2H), 4.27 – 4.20 (m, 1H), 4.11 (dd, J = 17.4, 4.9 Hz, 1H), 3.73 – 3.49 (m, 3H), 3.01 – 2.82 (m, 1H), 1.92 (dd, J = 13.5, 6.6 Hz, 1H), 1.82 – 1.63 (m, 3H), 1.38 – 1.15 (m, 12H), 0.99 (s, 9H), 0.93 – 0.79 (m, 14H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 168.9, 168.8, 166.9, 138.3, 137.5, 135.0, 133.1, 132.9, 131.6, 129.8, 128.6, 127.9, 118.8, 116.1, 75.9, 67.4, 61.4, 57.5, 44.6, 44.2, 38.1, 33.6, 33.4, 32.3, 31.6, 31.1, 29.8, 28.8, 28.3, 26.6, 23.2, 22.4, 22.0, 19.3, 18.6, 18.3, 13.9, 13.2, 10.8. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{41}H_{59}N_3NaO_6Si^+$, 740.4065, found 740.4069. HPLC purity: 98.2%.

(3S,14S,15S,E)-14-(2-(tert-Butyldiphenylsilyloxy)ethyl)-3-isopropyl-7-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (1e)

The titled compound **1e** was obtained following the general procedure described for **1a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 18% for three steps; white powder; $[\alpha]^{20}_D = -156.7$ (c = 0.09, DMSO); ν_{max} (KBr): 2959, 2860, 1731, 1689, 1611, 1528, 1470, 1259, 1107, 982, 820 cm^{-1}; ^1H NMR (400 MHz,

DMSO-d_6) δ 8.93 (s, 1H), 8.27 (d, J = 9.6 Hz, 1H), 7.70 – 7.56 (m, 4H), 7.52 – 7.37 (m, 6H), 6.81 (d, J = 14.8 Hz, 1H), 6.40 (d, J = 14.8 Hz, 1H), 5.33 (s, 1H), 5.24 – 5.12 (m, 2H), 4.61 (d, J = 17.4 Hz, 1H), 4.20 (dd, J = 9.5, 7.7 Hz, 1H), 3.85 (d, J = 17.4 Hz, 1H), 3.73 – 3.55 (m, 2H), 3.36 (s, 2H), 3.00 (s, 3H), 1.99 – 1.83 (m, 1H), 1.81 – 1.73 (m, 2H), 1.70 – 1.64 (m, 1H), 1.52 (s, 1H), 1.35 – 1.10 (m, 16H), 0.99 (s, 9H), 0.94 – 0.83 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 168.7, 168.4, 165.7, 138.4, 137.7, 135.0, 133.1, 132.9, 129.8, 127.9, 118.8, 116.0, 76.1, 61.5, 57.7, 52.3, 45.4, 44.5, 36.6, 33.6, 33.4, 32.3, 31.7, 31.1, 28.8, 26.6, 22.0, 19.3, 18.7, 18.4, 13.9, 13.2, 8.7. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{42}H_{61}N_3NaO_6Si^+$, 754.4222, found 754.4214. HPLC purity: 95.7%.

(3S,6R,11R,14S,15S,E)-11-((tert-Butyldimethylsilyloxy)methyl)-14-(2-(tert-butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (2a)

The compound **15a** (0.370 g, 0.330 mmol) and $Pd(PPh_3)_4$ (76 mg, 0.0660 mmol) was dissolved in anhydrous THF (3.0 mL), and N-methyl aniline (72 μL, 0.660 mmol) was added. After stirred at room temperature for 1.5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid as pale yellow foam. The obtained acid was dissolved in CH_2Cl_2 (2 mL) and diethyl amine (1 mL), the reaction mixture was stirred at room temperature for 3 h, and then the solvent was removed under reduced pressure to afford the crude amino acid. The mixture was dissolved in THF (270 mL), and then DIPEA (0.75 mL, 4.30

mmol) and HATU (0.820 g, 2.20 mmol) was added successively at 0 ºC. After stirred at room temperature for 12 h, the solvent was removed under reduced pressure, and then the residue was dissolved in ethyl acetate (200 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4. The solution was filtrated and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 100: 1 to 100: 4) to obtain the cyclic peptide **2a** (0.171 g, 60 % for three steps) as a white powder. $[\alpha]^{20}_D = -36.7$ (*c* = 0.15, DMSO); ν_{max} (KBr): 3403, 2931, 2860, 1732, 1677, 1531, 1464, 1260, 1109, 986, 845 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.70 – 7.61 (m, 5H), 7.46 – 7.37 (m, 6H), 6.95 (d, *J* = 15.1 Hz, 1H), 6.05 (d, *J* = 15.1 Hz, 1H), 5.45 (d, *J* = 10.6 Hz, 1H), 4.65 (s, 1H), 4.42 – 4.32 (m, 2H), 3.83 – 3.68 (m, 2H), 3.56 – 3.46 (m, 2H), 2.89 – 2.72 (m, 1H), 2.11 – 1.96 (m, 1H), 1.81 (d, *J* = 6.5 Hz, 2H), 1.62 – 1.51 (m, 1H), 1.47 – 1.39 (m, 4H), 1.39 – 1.16 (m, 15H), 1.04 (s, 9H), 0.97 – 0.78 (m, 28H), 0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 174.7, 170.6, 169.1, 145.8, 136.8, 136.7, 134.8, 134.7, 131.1, 131.1, 129.0, 119.7, 77.5, 65.9, 62.7, 59.5, 53.7, 52.7, 46.5, 35.4, 35.2, 33.9, 33.6, 32.95, 30.67, 28.42, 27.5, 26.5, 23.7, 20.3, 20.0, 19.2, 19.0, 14.6, 13.8, -5.1, -5.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{48}H_{77}N_3NaO_7Si_2^+$, 886.5192, found 886.5196.

(3R,6S,11S,14S,15S,E)-11-((tert-Butyldimethylsilyloxy)methyl)-14-(2-(tert-butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (2b)

The titled compound **2b** was obtained following the general procedure described for

2a. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 1 to 100: 4); yield, 49% for three steps; white powder; $[\alpha]^{20}_D = -35.1$ (c = 0.1, DMSO); ν_{max}(KBr): 3286, 3064, 2927, 2859, 1735, 1665, 1521, 1463, 1435, 1365, 1268, 1233, 1105, 978, 821 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 4H), 7.45 – 7.33 (m, 6H), 6.48 (dd, J = 15.0, 9.7 Hz, 1H), 6.10 (d, J = 15.0 Hz, 1H), 5.05 (d, J = 8.1 Hz, 1H), 4.78 (d, J = 6.4 Hz, 1H), 4.59 (s, 1H), 4.41 (s, 1H), 4.28 (s, 1H), 3.80 – 3.58 (m, 4H), 2.38 – 1.96 (m, 2H), 1.38 – 1.19 (m, 14H), 1.03 (s, 9H), 0.94 (d, J = 6.6 Hz, 3H), 0.84 (d, J = 15.3 Hz, 20H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, acetone-d_6) δ 174.2, 172.2, 171.5, 171.3, 168.3, 142.2, 136.4, 136.3, 134.4, 134.2, 130.7, 128.7, 128.7, 123.0, 78.9, 65.7, 61.8, 57.3, 52.7, 46.3, 36.3, 33.8, 32.6, 32.5, 31.2, 27.3, 27.0, 26.3, 23.4, 19.8, 19.4, 18.8, 17.7, 15.5, 14.4, -5.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{48}H_{77}N_3NaO_7Si_2^+$, 886.5192, found 886.5197.

(3R,6S,11S,14R,15R,E)-11-((tert-Butyldimethylsilyloxy)methyl)-14-(2-(tert-butyldiphenylsilyloxy)ethyl)-3-isopropyl-6-methyl-15-((S)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (2c)

The titled compound **2c** was obtained following the general procedure described for **2a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 1 to 100: 4); yield, 52% for three steps; white powder; $[\alpha]^{20}_D = +31.3$ (c = 0.2, DMSO); ν_{max} (KBr): 3402, 2931, 2860, 1731, 1677, 1530, 1464, 1260, 1108, 986, 845 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.70 – 7.61 (m, 5H), 7.46 – 7.37 (m, 6H), 6.95 (d, J = 15.1 Hz, 1H), 6.05 (d, J = 15.1 Hz, 1H), 5.45 (d, J = 10.6 Hz, 1H), 4.65 (s, 1H), 4.42 – 4.32 (m, 2H), 3.83 – 3.68 (m, 2H), 3.56 – 3.46 (m, 2H), 2.89 – 2.72 (m, 1H), 2.11 – 1.96 (m, 1H), 1.81 (d,

J = 6.5 Hz, 2H), 1.62 – 1.51 (m, 1H), 1.47 – 1.39 (m, 4H), 1.39 – 1.16 (m, 15H), 1.04 (s, 9H), 0.97 – 0.78 (m, 28H), 0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 174.7, 170.6, 169.1, 145.8, 136.8, 136.7, 134.8, 134.7, 131.1, 131.1, 129.0, 119.7, 77.5, 65.9, 62.7, 59.5, 53.7, 52.7, 46.5, 35.4, 35.2, 33.9, 33.6, 32.95, 30.67, 28.42, 27.5, 26.5, 23.7, 20.3, 20.0, 19.2, 19.0, 14.6, 13.8, -5.1, -5.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{48}H_{77}N_3NaO_7Si_2^+$, 886.5192, found 886.5196.

(3S,11R,14S,15S,E)-11-((tert-Butyldimethylsilyloxy)methyl)-14-(2-(tert-butyldiphenylsilyloxy)ethyl)-3-isopropyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (2d)

The titled compound **2d** was obtained following the general procedure described for **2a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 1 to 100: 4); yield, 58% for three steps; white powder; $[\alpha]^{20}_D = -56.4$ (*c* = 0.09, DMSO); ν_{max} (KBr): 3320, 2929, 2864, 1733, 1673, 1452, 1256, 1054, 976, 914, 850 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 – 7.63 (m, 4H), 7.49 – 7.37 (m, 6H), 6.91 (dd, *J* = 15.1, 2.6 Hz, 1H), 6.00 (d, *J* = 15.1 Hz, 1H), 5.45 (d, *J* = 10.7 Hz, 1H), 4.66 (s, 1H), 4.45 (d, *J* = 6.7 Hz, 1H), 4.10 (d, *J* = 17.4 Hz, 1H), 3.86 – 3.72 (m, 3H), 3.66 – 3.45 (m, 2H), 2.78 (dd, *J* = 13.6, 9.1 Hz, 1H), 2.06 (dd, *J* = 13.4, 6.7 Hz, 1H), 1.82 (d, *J* = 7.0 Hz, 2H), 1.67 – 1.54 (m, 1H), 1.45 – 1.18 (m, 13H), 1.04 (s, 9H), 1.00 – 0.82 (m, 22H), 0.03 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.5, 171.0, 170.9, 170.2, 145.1, 136.8, 136.7, 134.8, 134.7, 133.9, 133.2, 133.1, 131.1, 131.1, 130.1, 130.0, 129.0, 119.8, 77.3, 66.0, 62.7, 59.7, 59.6, 53.7, 46.5, 45.5, 39.0, 35.4, 35.2, 34.1, 33.2, 33.0, 30.7, 28.5, 27.6, 26.6, 23.8, 20.2, 20.0, 19.3, 19.0, 14.6, 14.0, -5.0, -5.0. HRMS–MALDI (m/z): [M +

Na]$^+$ calcd for $C_{47}H_{75}N_3NaO_7Si_2^+$, 872.5036, found 872.5041.

(3S,11R,14S,15S,E)-11-((tert-Butyldimethylsilyloxy)methyl)-14-(2-(tert-butyldiphenylsilyloxy)ethyl)-3-isopropyl-7-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (2e)

The titled compound **2e** was obtained following the general procedure described for **2a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 1 to 100: 4); yield, 61% for three steps; white powder; $[\alpha]^{20}_D$ = − 43.1 (*c* = 0.13, DMSO); ν_{max} (KBr): 3351, 2933, 2860, 1705, 1664, 1545, 1467, 1427, 1391, 1276, 1220, 1110, 1082, 912, 839 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, *J* = 8.9 Hz, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 6.2 Hz, 3H), 7.52 – 7.33 (m, 6H), 6.69 (d, *J* = 14.9 Hz, 1H), 6.01 (d, *J* = 14.6 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 4.48 (s, 1H), 4.37 (d, *J* = 17.9 Hz, 1H), 4.19 (s, 1H), 3.85 (d, *J* = 18.0 Hz, 1H), 3.65 (d, *J* = 7.5 Hz, 2H), 3.41 (m, 2H), 2.96 (s, 3H), 2.70 (s, 1H), 1.89 (d, *J* = 6.2 Hz, 1H), 1.69 (s, 2H), 1.48 (s, 1H), 1.33 – 1.06 (m, 10H), 0.98 (s, 9H), 0.91 – 0.63 (m, 21H), – 0.02 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.1, 168.8, 168.0, 165.4, 142.2, 135.0, 135.0, 133.1, 129.1, 127.8, 119.0, 75.0, 64.4, 61.2, 57.3, 52.1, 51.2, 44.1, 36.5, 33.5, 32.3, 31.5, 31.1, 28.9, 26.6, 25.7, 22.0, 19.3, 18.6, 18.4, 17.9, 13.9, 13.2, -5.6. HRMS–MALDI (m/z): [M + Na]$^+$ calcd for $C_{48}H_{77}N_3NaO_7Si_2^+$, 886.5192, found 886.5199.

(2S,3S,4R)-Allyl-3-hydroxy-2-(2-(4-methoxybenzyloxy)ethyl)-4-methyldecanoate (6a)

To a solution of **10a** (4.00 g 7.10 mmol) in THF/H_2O (40 mL/13 mL) was added LiOH•H_2O (447 mg, 10.6 mmol) at room temperature. After being stirred for 4 h at

this temperature, the reaction mixture acidified to pH = 4.0 with aqueous 10% $NaHSO_4$ and extracted with ethyl acetate (2 × 100 mL). The solvent was evaporated, and the resulting mixture was directly used for the next step without further purification.

To a solution of crude acid **11a** in DMF (12.0 mL) was added K_2CO_3 (750 mg, 5.41 mmol) and allyl bromide (600 mg, 4.92 mmol) at room temperature. The mixture was stirred at room temperature for 10 h, and then H_2O (100 mL) was added, and the resultant mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified with column chromatography on silica gel (petroleum ether: ethyl acetate = 20:1) to afford compound **6a** (1.6 g, 56% for two steps) as a colorless oil. $[\alpha]^{20}_D = -35.7$ (c = 1.0, $CHCl_3$). ν_{max}(KBr): 3527, 2955, 2928, 2856, 1731, 1612, 1513, 1460, 1248, 1173, 1097, 1036, 987, 931, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.2 Hz, 2H), 6.89 (d, J = 8.2 Hz, 2H), 5.98 – 5.83 (m, 1H), 5.34 (d, J = 16.8 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.59 (qd, J = 13.1, 5.8 Hz, 2H), 4.43 (s, 2H), 3.82 (s, 3H), 3.61 – 3.39 (m, 3H), 2.89 – 2.77 (m, 1H), 2.45 (d, J = 8.2 Hz, 1H), 2.10 – 1.97 (m, 1H), 1.96 – 1.84 (m, 1H), 1.58 – 1.49 (m, 1H), 1.47 – 1.38 (m, 1H), 1.38 – 1.12 (m, 11H), 0.96 – 0.85 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 159.2, 132.0, 130.4, 129.3, 118.6, 113.7, 77.4, 77.1, 76.7, 75.6, 72.7, 67.5, 65.3, 55.3, 45.6, 36.4, 33.6, 31.9, 30.0, 29.5, 27.0, 22.7, 14.1, 13.9.HRMS–ESI(m/z): $[M + Na]^+$ calcd for $C_{24}H_{38}NaO_5^+$, 429.2611, found 429.2619.

(2S,3S,4R)-Allyl-2-(2-(benzyloxy)ethyl)-3-hydroxy-4-methyldecanoate (6b)

The titled compound **6b** was obtained following the procedure described for **6a**. Flash column chromatography (petroleum ether: ethyl acetate = 20: 1 to 15: 1); yield: 81%; colorless oil; $[\alpha]^{20}_{D} = -38.2$ (c = 1.3, $CHCl_3$). ν_{max}(KBr): 3523, 2955, 2926, 2857, 1726, 1457, 1369, 1171, 1102, 987, 931 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 5.96 – 5.81 (m, 1H), 5.31 (d, J = 17.2 Hz, 1H), 5.22 (d, J = 10.4 Hz, 1H), 4.64 – 4.51 (m, 2H), 4.48 (s, 2H), 3.60 – 3.41 (m, 3H), 2.89 – 2.80 (m, 1H), 2.42 (d, J = 8.2 Hz, 1H), 2.10 – 1.98 (m, 1H), 1.95 – 1.81 (m, 1H), 1.53 (dt, J = 12.2, 6.3 Hz, 1H), 1.46 – 1.37 (m, 1H), 1.36 – 1.12 (m, 10H), 0.93 – 0.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 138.4, 132.1, 128.5, 127.8, 127.7, 118.8, 75.8, 73.2, 68.0, 65.4, 45.8, 36.6, 33.7, 32.0, 30.2, 29.6, 27.1, 22.8, 14.2, 14.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{23}H_{36}NaO_4^+$, 399.2506, found 399.2510.

(2S,3S,4R)-1-((6R,7R)-8,8-Dimethyl-2,2-dioxidohexahydro-1H-3,6-methanobenzo[c]isothiazol-1-yl)-3-hydroxy-2-(2-((4-methoxybenzyl)oxy)ethyl)-4-methyldecan-1-one (10a)

To a solution of compound **7a** (4.00 g, 9.50 mmol) in CH_2Cl_2 (16 mL) was added triethylamine (2.02 mL, 14.2 mmol) and TBSOTf (3.05 mL, 13.3 mmol). The reaction mixture was stirred at room temperature for 18 h. The resulting solution was directly used for the next step.

To a solution of aldehyde **9a** (14.0 mL, 14.0 mmol) in CH_2Cl_2 (40 mL) was added $TiCl_4$ (1 M in CH_2Cl_2, 17.4 mL, 17.4 mmol) dropwise at - 78 ºC. After 5 min, the solution of **8a** above was added to the reaction mixture. The reaction was stirred at - 78 ºC for 3 h before saturated aqueous $NaHCO_3$ (200 mL) was added. The aqueous

phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20:1 to 8:1) to obtain **10a** (4.2 g, 78%) as a colorless oil. $[\alpha]^{20}_D$ = − 35.7 (*c* = 2.3, $CHCl_3$). ν_{max} (KBr): 3527, 2955, 2928, 2856, 1731, 1612, 1513, 1460, 1248, 1173, 1097, 1036, 987, 931, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 11.0 Hz, 1H), 4.34 (d, *J* = 11.0 Hz, 1H), 3.79 (s, 3H), 3.71 (s, 1H), 3.63 (t, *J* = 9.3 Hz, 1H), 3.55 – 3.46 (m, 3H), 3.39 (d, *J* = 13.8 Hz, 1H), 3.32 (s, 1H), 2.27 (d, *J* = 11.0 Hz, 2H), 2.15 (dt, *J* = 15.6, 7.9 Hz, 1H), 2.02 (dd, *J* = 13.8, 7.9 Hz, 1H), 1.92 – 1.79 (m, 3H), 1.78 – 1.67 (m, 2H), 1.62 (s, 1H), 1.46 – 1.36 (m, 1H), 1.34 – 1.19 (m, 12H), 1.16 (s, 3H), 0.94 (s, 3H), 0.86 (t, *J* = 6.3 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 159.2, 131.0, 129.6, 113.8, 72.6, 68.3, 65.8, 55.4, 53.4, 48.3, 47.8, 47.0, 44.8, 38.6, 35.5, 34.2, 33.0, 32.1, 29.7, 29.2, 27.4, 26.5, 22.8, 20.9, 20.1, 14.3, 12.5. HRMS–ESI(m/z): $[M + Na]^+$ calcd for $C_{31}H_{49}NNaO_6S^+$, 586.3173, found 586.3169.

(2S,3S,4R)-2-(2-(Benzyloxy)ethyl)-1-((6R,7R)-8,8-dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)-3-hydroxy-4-methyldecan-1-one (10b)

The titled compound **10b** was obtained following the procedure described for **10a**. Flash column chromatography (petroleum ether: ethyl acetate = 20: 1 to 10: 1); yield: 67%; colorless oil; $[\alpha]^{20}_D$ = − 46.1 (*c* = 1.0, $CHCl_3$). ν_{max}(KBr): 3463, 2923, 2858, 1671, 1460, 1328, 1115, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.09 (m, 5H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.36 (d, *J* = 11.5 Hz, 1H), 3.65 (s, 1H), 3.62 – 3.53 (m, 1H),

3.48 (t, J = 6.5 Hz, 2H), 3.43 (d, J = 13.8 Hz, 1H), 3.35 – 3.17 (m, 2H), 2.28 – 2.17 (m, 2H), 2.15 – 2.03 (m, 1H), 1.96 (dd, J = 13.8, 7.9 Hz, 1H), 1.86 – 1.74 (m, 3H), 1.72 – 1.63 (m, 1H), 1.61 – 1.51 (m, 1H), 1.37 (s, 1H), 1.28 – 1.14 (m, 11H), 1.10 (s, 3H), 0.88 (s, 3H), 0.81 (t, J = 7.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 138.8, 128.3, 127.9, 127.5, 72.9, 68.5, 65.7, 53.3, 48.2, 47.8, 47.0, 44.7, 38.5, 35.5, 34.1, 33.0, 32.0, 29.6, 29.1, 27.4, 26.5, 22.8, 20.8, 20.0, 14.2, 12.4. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{30}H_{47}NNaO_5S^+$, 556.3067, found, 556.3070.

(2S,3S,4R)-Allyl-3-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-2-(2-(4-methoxybenzyloxy)ethyl)-4-methyldecanoate (12a)

To a solution of acid **4a** (2.99 g, 8.80 mmol) and **6a** (1.79 g, 4.40 mmol) in CH_2Cl_2 (15 mL) was added DMAP (215 mg, 1.76 mmol) and DIC (2.70 mL, 17.6 mmol) under argon atmosphere at 20 ºC. The reaction mixture was stirred for 18 h, and diluted with CH_2Cl_2 (50 mL) then quenched with H_2O (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). And the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 20:1) to obtain compound **12a** (3.10 g, 96%) as colorless oil. $[\alpha]^{20}_D$ = − 82.9 (c = 1.0, $CHCl_3$). IR (KBr) ν_{max}: 3443, 3377, 2952, 2931, 2860, 1740, 1734, 1612, 1512, 1463, 1366, 1248, 1173, 1092, 1038, 986, 931, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 7.5 Hz, 2H), 7.58 – 7.51 (m, 2H), 7.32 (t, J = 7.3 Hz, 2H), 7.23 (td, J = 7.3, 2.8 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 5.87 – 5.70 (m, 1H), 5.30 (d, J = 9.3 Hz, 1H), 5.22 (d, J = 16.9 Hz, 1H), 5.14 (d, J = 10.4 Hz, 1H), 5.11 – 5.07 (m, 1H),

4.44 (d, *J* = 5.6 Hz, 1H), 4.30 (d, *J* = 5.5 Hz, 1H), 4.23 (dd, *J* = 9.3, 4.4 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 1H), 3.71 (s, 3H), 3.44 – 3.36 (m, 1H), 3.32 (m, 1H), 2.98 – 2.90 (m, 1H), 2.15 – 2.05 (m, 1H), 1.93 – 1.81 (m, 1H), 1.70 (s, 2H), 1.36 – 1.26 (m, 1H), 1.25 – 1.10 (m, 9H), 1.06 (d, *J* = 6.5 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.85 – 0.75 (m, 11H).^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.3, 159.2, 156.3, 144.0, 143.8, 141.3, 132.0, 130.3, 129.3, 127.7, 127.1, 125.2, 120.0, 118.6, 113.8, 78.0, 72.7, 67.1, 65.4, 59.3, 55.3, 53.5, 47.2, 44.8, 42.2, 34.8, 33.4, 31.8, 31.0, 29.4, 26.9, 23.5, 22.6, 19.5, 17.2, 14.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{44}H_{57}NNaO_8^+$, 750.3976, found 750.3973.

(2S,3S,4R)-Allyl-3-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-2-(2-(4-methoxybenzyloxy)ethyl)-4-methyldecanoate (12b)

The titled compound **12b** was obtained following the procedure described for **12a**. Flash column chromatography (petroleum ether: ethyl acetate = 9:1); yield: 92%; colorless oil; $[\alpha]^{20}_D$ = − 43.1 (*c* = 1.0, $CHCl_3$). ν_{max}(KBr): 3443, 3377, 2952, 2931, 2860, 1740, 1734, 1612, 1512, 1463, 1366, 1248, 1173, 1092, 1038, 986, 931, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 7.23 (t, *J* = 7.2 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.83 – 5.66 (m, 1H), 5.24 – 5.17 (m, 1H), 5.17 – 5.10 (m, 2H), 5.08 (d, *J*= 10.3 Hz, 1H), 4.41 (dd, *J* = 13.4, 5.7 Hz, 2H), 4.33 – 4.20 (m, 5H), 4.14 (t, *J* = 7.0 Hz, 1H), 3.69 (s, 3H), 3.42 – 3.26 (m, 2H), 2.96 – 2.79 (m, 1H), 2.13 (dt, *J* = 20.3, 6.8 Hz, 1H), 1.92 – 1.81 (m, 1H), 1.72 – 1.62 (m, 2H), 1.34 (s, 1H), 1.27 – 1.09 (m, 10H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.85 – 0.69 (m, 11H).^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6,

171.3, 159.2, 156.2, 144.0, 143.9, 141.3, 132.0, 130.3, 129.3, 127.7, 127.1, 125.2, 125.1, 120.0, 118.7, 113.8, 77.9, 72.8, 67.3, 67.0, 65.5, 59.1, 55.3, 47.2, 45.3, 34.5, 33.7, 31.8, 31.1, 29.4, 29.2, 27.1, 27.0, 22.7, 19.5, 17.1, 14.1, 13.8. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{44}H_{57}NNaO_8^+$, 750.3976, found 750.3973.

(R,E)-Allyl-4-((5R,8S,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybenzyloxy)ethyl)-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-(tert-butyldimethylsilyloxy)pent-2-enoate (13a)

The ester **12a** (3.63 g, 4.99 mmol) was dissolved in CH_2Cl_2 (30 mL) and diethylamine (15 mL) was added. After 2 h, the solvent was removed and the residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 10:1 to 2:1) to afford the amino ester as colorless oil.

The obtained amine (1.61 g, 3.20 mmol) and Fmoc amino acid **5a** (1.20 g, 3.84 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL). And HOBt (520 mg, 3.84 mmol), EDCI (740 mg, 3.84 mmol), Et_3N (535 μL, 3.84 mmol) was added successively. The reaction mixture was stirred for 18 h and the solvent was removed. The residue was dissolved in CH_2Cl_2 (100 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate = 10: 1 to 5:1) to obtain the amide as a colorless oil.

To a solution of obtained amide (2.30 g, 2.88 mmol) in anhydrous THF (30 mL), $Pd(PPh_3)_4$ (666 mg, 0.576 mmol) and N-methylaniline (625 μL, 5.76 mmol) were added. The reaction mixture was stirred for 1 h at room temperature, and diluted with

ethyl acetate (200 mL). The organic phase was washed by 1% HCl (2 × 60 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid.

The obtained acid above and amine **3a** (1.05 g, 1.38 mmol) was dissolved in anhydrous CH_2Cl_2 (14 mL), and TEA (230 μL, 1.66 mmol), HOBt (224 mg, 1.66 mmol) and EDCI (318 mg, 1.66 mmol) were added successively. The reaction mixture was stirred for 18 h and the solvent was removed. The residue was diluted with CH_2Cl_2 (100 mL) and washed successively with 1 % HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to obtain compound **13a** (1.04 g, 20 % for 4 steps) as a colorless oil. $[\alpha]^{20}_D = -8.5$ (c = 0.8, $CHCl_3$); ν_{max}(KBr): 3322, 2931, 2860, 1726, 1665, 1515, 1364, 1181, 1105, 1039, 989, 935, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.1 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.27 (dd, J = 13.1, 5.8 Hz, 2H), 7.19 (d, J = 7.8 Hz, 2H), 6.92 – 6.79 (m, 4H), 6.23 (d, J = 7.8 Hz, 1H), 6.09 (d, J = 7.7 Hz, 1H), 5.96 – 5.80 (m, 2H), 5.28 (t, J = 14.1 Hz, 1H), 5.19 (d, J = 10.2 Hz, 1H), 5.08 (t, J = 5.4 Hz, 1H), 4.70 – 4.61 (m, 1H), 4.57 (d, J = 5.4 Hz, 2H), 4.48 – 4.26 (m, 6H), 4.20 (t, J = 7.0 Hz, 1H), 3.76 (s, 2H), 3.69 – 3.63 (m, 1H), 3.63 – 3.57 (m, 1H), 3.51 – 3.44 (m, 1H), 3.43 – 3.35 (m, 1H), 2.79 (dd, J = 13.8, 6.0 Hz, 1H), 2.13 – 2.01 (m, 1H), 1.83 – 1.66 (m, 3H), 1.44 – 1.34 (m, 5H), 1.34 – 1.16 (m, 13H), 1.18 – 1.06 (m, 2H), 0.97 – 0.78 (m, 28H), 0.03 (s, 6H). ^{13}C

NMR (100 MHz, $CDCl_3$) δ 171.3, 170.7, 169.2, 164.4, 158.2, 155.1, 144.9, 142.8, 140.3, 131.0, 129.0, 128.3, 126.7, 126.1, 124.1, 124.1, 121.0, 118.9, 117.2, 112.8, 71.6, 66.1, 65.8, 64.1, 63.3, 57.1, 54.2, 50.6, 49.5, 46.1, 44.8, 33.8, 32.7, 30.7, 30.1, 28.6, 28.5, 25.7, 24.8, 21.6, 18.3, 17.8, 17.3, 16.7, 13.4, 13.1, -6.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{58}H_{83}N_3NaO_{11}Si^+$, 1048.5689, found 1048.5695.

(S,E)-Allyl-4-((5S,8R,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybenzyloxy)ethyl)-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-(tert-butyldimethylsilyloxy)pent-2-enoate (13b)

The titled compound **13b** was obtained following the general procedure described for **13a**. Flash column chromatography (petroleum ether: ethyl acetate = 5:1); yield, 21% for 4 steps; colorless oil;$[\alpha]^{20}_D$ = − 23.8 (*c* = 1.74, $CHCl_3$); ν_{max}(KBr): 3322, 3041, 2931, 2860, 2741, 1727, 1665, 1614, 1515, 1460, 1364, 1515, 1460, 1364, 1302, 1251, 1181, 1105, 1039, 989, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.1 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.31 (dd, *J* = 13.8, 6.7 Hz, 2H), 7.07 – 6.88 (m, 4H), 6.86 – 6.65 (m, 3H), 6.11 (d, *J* = 15.7 Hz, 1H), 5.97 – 5.83 (m, 2H), 5.27 (dd, *J* = 17.3, 1.3 Hz, 1H), 5.19 (dd, *J* = 10.4, 1.0 Hz, 1H), 4.97 (s, 1H), 4.76 (s, 1H), 4.66 – 4.59 (m, 2H), 4.55 – 4.45 (m, 2H), 4.33 – 4.08 (m, 5H), 3.79 – 3.68 (m, 5H), 3.38 – 3.30 (m, 1H), 3.27 – 3.19 (m, 1H), 2.19 – 2.08 (m, 1H), 2.03 (s, 1H), 1.42 (s, 4H), 1.31 – 1.20 (m, 14H), 0.99 – 0.83 (m, 30H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 171.4, 171.3, 165.8, 159.2, 156.7, 146.6, 143.9, 141.4, 132.2, 129.6, 127.8, 127.6, 127.1, 127.1, 125.0, 124.9, 121.5, 120.0, 119.9, 118.1, 113.6, 78.5, 72.9, 67.6, 65.0, 64.4, 58.3, 55.2, 51.7, 47.1, 46.4, 38.6, 34.8, 33.4, 31.8,

30.1, 29.7, 29.5, 26.9, 26.6, 25.9, 25.8, 22.6, 19.3, 18.3, 18.0, 14.1, -5.4. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{58}H_{83}N_3NaO_{11}Si^+$, 1048.5689, found 1048.5694.

(R,E)-Allyl-4-((8S,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybenzyloxy)ethyl)-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-(tert-butyldimethylsilyloxy)pent-2-enoate (13d)

The titled compound **13d** was obtained following the general procedure described for **13a**. Flash column chromatography (petroleum ether: ethyl acetate = 5:1); yield, 28% for 4 steps; colorless oil; $[\alpha]^{20}_D = -29.4$ (c = 1.32, $CHCl_3$); ν_{max}(KBr): 3323, 3042, 2931, 2860, 2741, 1727, 1665, 1614, 1515, 1460, 1364, 1515, 1461, 1364, 1302, 1251, 1182, 1105, 1039, 987, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 6.94 – 6.81 (m, 3H), 6.74 (d, J = 8.8 Hz, 1H), 6.24 (d, J = 8.1 Hz, 1H), 6.05 – 5.81 (m, 3H), 5.29 (dd, J = 17.2, 1.3 Hz, 1H), 5.21 (d, J = 10.4 Hz, 1H), 5.11 (t, J = 5.6 Hz, 1H), 4.71 – 4.58 (m, 3H), 4.50 (dd, J = 8.9, 5.7 Hz, 1H), 4.45 – 4.32 (m, 4H), 4.22 (t, J = 7.1 Hz, 1H), 4.15 – 3.99 (m, 1H), 3.85 (dd, J = 16.9, 5.1 Hz, 1H), 3.78 (s, 3H), 3.67 (dd, J = 10.1, 3.5 Hz, 1H), 3.61 (dd, J = 10.2, 4.6 Hz, 1H), 3.52 – 3.45 (m, 1H), 3.45 – 3.37 (m, 1H), 2.84 – 2.75 (m, 1H), 2.14 – 2.02 (m, 2H), 1.85 – 1.70 (m, 4H), 1.39 – 1.05 (m, 14H), 0.98 – 0.79 (m, 26H), 0.04 (d, J = 2.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 169.2, 168.0, 164.5, 158.3, 144.8, 142.8, 140.3, 131.0, 129.0, 128.3, 126.7, 126.1, 124.1, 124.1, 121.0, 119.0, 117.3, 112.8, 76.6, 71.6, 66.2, 65.8, 64.2, 63.3, 57.0, 54.2, 50.6, 46.1, 44.7, 43.5, 33.7, 32.7, 30.8, 30.0, 28.5, 25.8, 24.8, 21.6, 18.3, 17.3, 16.7, 13.3, 13.1, -6.5. HRMS–MALDI (m/z):

$[M + Na]^+$ calcd for $C_{57}H_{81}N_3NaO_{11}Si^+$, 1034.5533, found 1034.5527.

(R,E)-Allyl-4-((8S,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybenzyloxy)ethyl)-4-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-(tert-butyldimethylsilyloxy)pent-2-enoate (13e)

The titled compound **13e** was obtained following the general procedure described for **13a**. Flash column chromatography (petroleum ether: ethyl acetate = 5:1); yield, 23% for 4 steps; colorless oil; $[\alpha]^{20}_D$ = − 37.6 (c = 1.06, $CHCl_3$);ν_{max}(KBr): 3324, 3043, 2931, 2860, 2744, 1727, 1662, 1613, 1515, 1460, 1364, 1515, 1461, 1364, 1302, 1251, 1182, 1105, 1038, 985, 839 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 6.7 Hz, 2H), 7.58 (s, 2H), 7.44 – 7.36 (m, 2H), 7.34 – 7.27 (m, 2H), 7.21 (d, J = 8.4 Hz, 2H), 6.91 (dd, J = 15.8, 5.6 Hz, 1H), 6.85 (d, J = 8.5 Hz, 2H), 6.02 – 5.85 (m, 2H), 5.31 (d, J = 17.2 Hz, 1H), 5.25 – 5.13 (m, 2H), 4.71 – 4.60 (m, 3H), 4.45 – 4.32 (m, 4H), 4.25 (s, 1H), 3.99 (s, 2H), 3.78 (s, 3H), 3.69 – 3.63 (m, 1H), 3.61 – 3.55 (m, 1H), 3.44 (d, J = 21.6 Hz, 2H), 3.03 (s, 3H), 2.76 (d, J = 6.5 Hz, 1H), 1.76 (s, 3H), 1.23 (d, J = 14.7 Hz, 11H), 0.94 – 0.71 (m, 24H), 0.04 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 170.5, 168.8, 165.5, 159.3, 145.9, 143.9, 143.8, 141.3, 132.1, 130.2, 129.3, 127.8, 127.1, 125.1, 122.0, 120.0, 118.3, 113.8, 72.6, 68.2, 65.2, 64.5, 57.3, 55.3, 47.2, 34.5, 33.7, 31.8, 30.9, 29.4, 26.9, 25.9, 22.6, 19.5, 18.3, 17.2, 14.1, -5.4. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{58}H_{83}N_3NaO_{11}Si^+$, 1048.5689, found 1048.5691.

(R,E)-Allyl-5-(tert-butyldimethylsilyloxy)-4-((5R,8S,11S,12S)-12-(2-(tert-butyldiphenylsilyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)pent-2-enoate (15a)

To a solution of compound **13a** (0.600 g, 0.58 mmol) in CH_2Cl_2/H_2O (5.4 mL/0.6 mL) was added DDQ (0.160 g, 0.700 mmol). The mixture was stirred at room temperature for 1.5 h, and diluted with CH_2Cl_2 (100 mL). The organic phase was washed with saturated $NaHCO_3$ (3 × 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 4: 1) to obtain a colorless oil.

The colorless oil was resolved in CH_2Cl_2 (4 mL), and then DMAP (5.0 mg, 0.04 mmol), imidazole (0.160 g, 2.30 mmol) and TBDPSCl (0.3 mL, 1.20 mmol) were added successively. The mixture was stirred at room temperature for 5 h and quenched with CH_3OH (0.2 mL). The mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1% HCl, saturated aqueous $NaHCO_3$, brine successively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to obtain compound **15a** (0.410 g, 61% for two steps) as a colorless oil. $[\alpha]^{20}_{D}$ = − 9.1 (*c* = 0.43, $CHCl_3$); ν_{max}(KBr): 3321, 2932, 2860, 1727, 1673, 1511, 1464, 1360, 1253, 1182, 1107, 991, 938, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.68 – 7.54 (m, 6H), 7.46 – 7.33 (m, 8H), 7.29 (d, *J* = 7.4 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.14 (d, *J* = 8.2 Hz, 1H), 6.05 – 5.79 (m, 3H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.20 (d, *J* = 10.3 Hz, 1H), 5.06 (t, *J* = 5.5 Hz, 1H), 4.68 (s, 1H), 4.58 (d, *J* = 5.6 Hz, 2H), 4.47 (dd, *J* = 8.8, 5.9 Hz, 1H), 4.44 – 4.34 (m, 2H), 4.33 – 4.25 (m, 1H), 4.20 (t, *J* = 7.1 Hz, 1H), 3.83 – 3.71 (m, 1H), 3.69 – 3.53 (m, 3H), 2.98 – 2.88 (m, 1H), 2.13 – 1.99 (m, 1H), 1.83 – 1.72 (m, 2H), 1.68 – 1.58 (m, 1H), 1.45 –1.19 (m, 14H), 1.05 (s,

9H), 0.96 – 0.82 (m, 23H), 0.03 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.6, 170.3, 165.5, 156.0, 145.8, 143.9, 141.3, 135.5, 135.5, 133.4, 133.3, 132.0, 129.9, 129.9, 127.8, 127.8, 127.7, 127.1, 125.2, 125.1, 122.2, 120.0, 118.2, 77.8, 67.1, 65.2, 64.3, 60.9, 58.1, 51.7, 50.6, 47.2, 45.0, 35.0, 33.8, 32.6, 31.8, 31.1, 29.6, 26.95, 26.67, 25.83, 22.7, 19.4, 19.2, 19.0, 18.2, 17.7, 14.6, 14.1, -5.4, -5.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{66}H_{93}N_3NaO_{10}Si_2^+$, 1166.6292, found 1166.6287.

(S,E)-Allyl-5-(tert-butyldimethylsilyloxy)-4-((5S,8R,11S,12S)-12-(2-(tert-butyldiphenylsilyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)pent-2-enoate (15b)

The titled compound **15b** was obtained following the general procedure described for **15a**. Flash column chromatography (petroleum ether: ethyl acetate = 5: 1); yield, 62% for two steps; colorless oil; $[\alpha]^{20}_D$ = − 20.3(c = 5.0, $CHCl_3$); ν_{max}(KBr): 3322, 3041, 2931, 2860, 2741, 1726, 1665, 1515, 1460, 1364, 1515, 1464, 1302, 1251, 1182, 1105, 1039, 979, 839 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 7.5 Hz, 2H), 7.63 – 7.52 (m, 6H), 7.40 – 7.30 (m, 8H), 7.23 (d, J = 8.0 Hz, 2H), 6.94 (dd, J = 15.7, 5.3 Hz, 1H), 6.71 (d, J = 9.0 Hz, 1H), 6.37 (d, J = 8.4 Hz, 1H), 6.04 (d, J = 15.7 Hz, 1H), 5.91 – 5.76 (m, 1H), 5.69 (d, J = 7.4 Hz, 1H), 5.28 – 5.19 (m, 2H), 5.14 (d, J = 10.4 Hz, 1H), 5.04 (s, 1H), 4.69 (s, 1H), 4.55 (d, J = 5.6 Hz, 2H), 4.39 (dd, J = 8.6, 5.1 Hz, 1H), 4.35 – 4.26 (m, 3H), 4.16 (t, J = 7.1 Hz, 1H), 3.74 – 3.55 (m, 4H), 3.03 – 2.89 (m, 1H), 2.25 – 2.10 (m, 1H), 1.75 – 1.64 (m, 2H), 1.41 – 1.31 (m, 4H), 1.27 – 1.16 (m, 10H), 1.01 (s, 9H), 0.93 (d, J = 6.1 Hz, 3H), 0.88 – 0.79 (m, 20H), 0.03 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 171.2, 165.7, 146.5, 143.8, 141.3, 135.6, 135.5, 133.6,

133.2, 132.2, 129.8, 127.8, 127.1, 125.1, 121.9, 119.9, 118.1, 78.4, 65.1, 64.5, 62.8, 61.0, 57.6, 51.6, 47.1, 45.6, 33.8, 31.8, 30.8, 29.5, 22.7, 19.5, 19.2, 18.3, 17.5, 14.1, -5.4, -5.4. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{66}H_{93}N_3NaO_{10}Si_2^+$, 1166.6292, found 1166.6285.

(S,E)-Allyl-5-(tert-butyldimethylsilyloxy)-4-((5S,8R,11R,12R)-12-(2-(tert-butyldiphenylsilyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-5-methyl-11-((S)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)pent-2-enoate (15c)

The titled compound **15c** was obtained following the general procedure described for **15a**. Flash column chromatography (petroleum ether: ethyl acetate = 5: 1); yield, 64% for two steps; colorless oil; $[\alpha]^{20}_D$ = + 10.9 (*c* = 0.59, $CHCl_3$); ν_{max}(KBr): 3320, 2932, 2860, 1727, 1673, 1511, 1464, 1360, 1252, 1182, 1107, 991, 937, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.68 – 7.54 (m, 6H), 7.46 – 7.33 (m, 8H), 7.29 (d, *J* = 7.4 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.14 (d, *J* = 8.2 Hz, 1H), 6.05 – 5.79 (m, 3H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.20 (d, *J* = 10.3 Hz, 1H), 5.06 (t, *J* = 5.5 Hz, 1H), 4.68 (s, 1H), 4.58 (d, *J* = 5.6 Hz, 2H), 4.47 (dd, *J* = 8.8, 5.9 Hz, 1H), 4.44 – 4.34 (m, 2H), 4.33 – 4.25 (m, 1H), 4.20 (t, *J* = 7.1 Hz, 1H), 3.83 – 3.71 (m, 1H), 3.69 – 3.53 (m, 3H), 2.98 – 2.88 (m, 1H), 2.13 – 1.99 (m, 1H), 1.83 – 1.72 (m, 2H), 1.68 – 1.58 (m, 1H), 1.45 –1.19 (m, 14H), 1.05 (s, 9H), 0.96 – 0.82 (m, 23H), 0.03 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.6, 170.3, 165.5, 156.0, 145.8, 143.9, 141.3, 135.5, 135.5, 133.4, 133.3, 132.0, 129.9, 129.9, 127.8, 127.8, 127.7, 127.1, 125.2, 125.1, 122.2, 120.0, 118.2, 77.8, 67.1, 65.2, 64.3, 60.9, 58.1, 51.7, 50.6, 47.2, 45.0, 35.0, 33.8, 32.6, 31.8, 31.1, 29.6, 26.95, 26.67, 25.83, 22.7, 19.4, 19.2, 19.0, 18.2,

17.7, 14.6, 14.1, -5.4, -5.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{66}H_{93}N_3NaO_{10}Si_2^+$, 1166.6292, found 1166.6293.

(R,E)-Allyl-5-(tert-butyldimethylsilyloxy)-4-((8S,11S,12S)-12-(2-(tert-butyldiphenylsilyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)pent-2-enoate (15d)

The titled compound **15d** was obtained following the general procedure described for **15a**. Flash column chromatography (petroleum ether: ethyl acetate = 5: 1); yield, 68% for two steps; colorless oil; $[\alpha]^{20}_D = -25.9$ (c = 0.18, $CHCl_3$); ν_{max}(KBr):3324, 2932, 2860, 1730, 1679, 1523, 1467 1365, 1259, 1180, 1109, 994, 937, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 7.5 Hz, 2H), 7.62 – 7.50 (m, 6H), 7.41 – 7.29 (m, 8H), 7.23 (d, J = 10.0 Hz, 2H), 6.88 – 6.81 (m, 1H), 6.72 (d, J = 8.5 Hz, 1H), 6.08 (d, J = 7.9 Hz, 1H), 5.96 – 5.76 (m, 2H), 5.24 (d, J = 17.1 Hz, 1H), 5.16 (d, J = 10.4 Hz, 1H), 5.03 (s, 1H), 4.68 – 4.53 (m, 3H), 4.49 – 4.43 (m, 1H), 4.40 – 4.25 (m, 2H), 4.17 (t, J = 7.0 Hz, 1H), 4.10 – 3.94 (m, 1H), 3.83 (dd, J = 16.6, 4.3 Hz, 1H), 3.74 – 3.50 (m, 4H), 2.88 (s, 1H), 2.03 (dd, J = 14.2, 7.6 Hz, 1H), 1.78 – 1.68 (m, 3H), 1.59 (s, 1H), 1.21 (s, 10H), 1.01 (s, 9H), 0.91 – 0.77 (m, 22H), -0.02 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.2, 168.0, 164.5, 155.5, 144.8, 142.8, 140.3, 134.4, 134.4, 132.4, 131.0, 128.8, 128.8, 126.8, 126.7, 126.7, 126.0, 124.1, 124.1, 121.1, 119.0, 117.2, 76.9, 66.2, 64.2, 63.3, 59.9, 57.0, 50.6, 46.1, 43.9, 43.5, 33.9, 32.7, 31.5, 30.8, 30.0, 28.6, 25.9, 25.7, 24.8, 21.6, 18.3, 18.2, 17.2, 16.7, 13.5, 13.1, -6.5, -6.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{65}H_{91}N_3NaO_{10}Si_2^+$, 1152.6135, found 1152.6138.

(R,E)-Allyl-5-(tert-butyldimethylsilyloxy)-4-((8S,11S,12S)-12-(2-(tert-butyldiphenyls

ilyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-4-methyl-11-((R)-octan-2-yl)-3,6,9-tri oxo-2,10-dioxa-4,7-diazatridecanamido)pent-2-enoate (15e)

The titled compound **15e** was obtained following the general procedure described for **17a**. Flash column chromatography (petroleum ether: ethyl acetate = 5: 1); yield, 63% for two steps; colorless oil; $[\alpha]^{20}_{D} = -13.7$ (c = 0.23, $CHCl_3$); ν_{max} (KBr):3325, 2932, 2860 , 1730, 1679, 1524, 1467 1364, 1259, 1180, 1108, 995, 937, 836 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 2H), 7.61 (s, 6H), 7.47 – 7.28 (m, 10H), 6.93 (dd, J = 15.8, 5.6 Hz, 1H), 6.60 (d, J = 25.4 Hz, 1H), 6.19 – 5.83 (m, 3H), 5.31 (d, J = 17.1 Hz, 1H), 5.23 (d, J = 10.5 Hz, 1H), 4.64 (d, J = 5.6 Hz, 3H), 4.51 (s, 1H), 4.39 (s, 2H), 4.26 (s, 1H), 3.99 (s, 2H), 3.79 – 3.58 (m, 3H), 3.51 (dd, J = 9.8, 5.5 Hz, 1H), 3.03 (s, 3H), 2.86 (s, 1H), 2.12 (s, 1H), 1.70 (d, J = 37.0 Hz, 4H), 1.24 (d, J = 11.8 Hz, 11H), 1.05 (s, 9H), 0.89 – 0.85 (m, 16H), 0.02 (d, J = 4.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.5, 164.5, 144.9, 142.8, 142.8, 140.3, 134.5, 134.4, 132.3, 131.0, 128.8, 126.8, 126.7, 126.1, 124.0, 121.0, 119.0, 117.3, 76.8, 67.1, 64.2, 63.4, 56.3, 50.6, 46.1, 33.6, 25.9, 24.8, 18.5, 18.2, 17.2, 16.1, 13.1, -6.5, -6.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{66}H_{93}N_3NaO_{10}Si_2^+$, 1166.6292, found 1166.6285.

(R,E)-Allyl 4-amino-5-((tert-butyldiphenylsilyl)oxy)pent-2-enoate(24a)

To a suspension of compound **22** (33.2 g, 75.1 mmol) in THF (600 mL) was added *t*-BuOK (7.80 g, 69.1 mmol) at 0 ºC. The mixture was stirred at this temperature for 0.5 h, and then a solution of aldehyde **21** (24.7 g, 57.8 mmol) in THF (50 mL) was added dropwise. The mixture was stirred at room temperature for 2 h and quenched by saturated aqueous NH_4Cl (300 mL). The solvent was removed under vaccum. The

residue was dissolved with ethyl acetate (500 mL) and washed with brine (200 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified through a pad of silica gel and used next step directly without further purification.

To a solution of compound **23** in DCM (540 mL), was added trifluoroacetic acid (180 mL) dropwise at 0 ºC. The mixture was warmed to room temperature and kept stirred for 1.5 h until the reaction completed. The mixture was quenched by saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 0 to 100: 2) to obtain compound **24** (19.5 g, 82% over two steps) as colorless oil. $[\alpha]^{20}_D$ = + 6.1 (*c* = 0.74, $CHCl_3$); ν_{max} (KBr): 3016, 2931, 2858, 1724, 1428, 1427, 1112, 988, 863 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.62 (m, 4H), 7.49 – 7.34 (m, 6H), 6.97 (dd, *J* = 15.7, 5.5 Hz, 1H), 6.05 (dd, *J* = 15.7, 1.3 Hz, 1H), 5.99 – 5.85 (m, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.24 (d, *J* = 10.4 Hz, 1H), 4.65 (dd, *J* = 5.6, 1.2 Hz, 2H), 3.74 – 3.61 (m, 2H), 3.55 (dd, *J* = 9.3, 6.4 Hz, 1H), 1.63 – 1.48 (m, 2H), 1.07 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 149.3, 135.6, 135.6, 133.2, 133.1, 132.2, 129.9, 127.8, 121.1, 118.2, 67.8, 65.1, 54.5, 26.9, 19.3. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{24}H_{31}NNaO_3Si^+$, 432.1965, found 432.1960.

(R,E)-Allyl-4-((5R,8S,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybenzyloxy)ethyl)-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-(tert-butyldiphenylsilyloxy)pent-2-enoate (26a)

The ester **12a** (3.07 g, 4.21 mmol) was dissolved in CH_2Cl_2 (30 mL) and diethylamine (15 mL) was added. After 2 h, the solvent was removed and the residue was purified by column chromatography (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the amino ester as a colorless oil.

The obtained amine (1.35 g, 2.67 mmol) and Fmoc amino acid **5b** (0.996 g, 3.20 mmol) were dissolved in anhydrous CH_2Cl_2 (4 mL), and HOBt (432 mg, 3.20 mmol), EDCI (613 mg, 3.20 mmol), Et_3N (450 μL, 3.20 mmol) were added successively. The reaction mixture was stirred for 18 h, and the solvent was removed. The residue was dissolved in ethyl acetate (100 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether: ethyl acetate = 4: 1) to obtain the amide as a colorless oil.

To a solution of obtained amide **25a** (1.00 g, 1.25 mmol) in anhydrous THF (12 mL), $Pd(PPh_3)_4$ (290 mg, 0.25mmol) and N-methyl aniline (270 μL, 2.50 mmol) were added. The reaction mixture was stirred for 1h at room temperature, and diluted with ethyl acetate (100 mL). The organic phase was washed by 1% HCl (2 × 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid.

The obtained acid above and amine **24a** (512 mg, 1.25 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL). TEA (174 μL, 1.25 mmol), HOBt (170 mg, 1.25 mmol) and EDCI (240 mg, 1.25 mmol) were added successively. The reaction mixture was

stirred for 18 h and the solvent was removed. The residue was dissolved in ethyl acetate (100 mL) and washed successively with 1 % HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to obtain compound **26a** (1.31 g, 27% for 4 steps) as a colorless oil. $[\alpha]^{20}_{D} = -90.6$ (c = 1.5, $CHCl_3$); ν_{max} (KBr): 3338, 3061, 2932, 2860, 1728, 1672, 1515, 1462, 1363, 1304, 1247, 1183, 1109, 1038, 990, 938, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H), 7.66 – 7.57 (m, 6H), 7.46 – 7.34 (m, 8H), 7.28 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 6.93 – 6.75 (m, 4H), 6.21 (d, J = 8.0 Hz, 1H), 6.05 (d, J = 7.8 Hz, 1H), 5.94 – 5.81 (m, 2H), 5.29 (dd, J = 17.2, 1.4 Hz, 1H), 5.21 (dd, J = 10.4, 0.9 Hz, 1H), 5.10 (t, J = 5.8 Hz, 1H), 4.72 (s, 1H), 4.60 (d, J = 5.6 Hz, 2H), 4.42 (m, 3H), 4.34 – 4.25 (m, 3H), 4.20 (t, J = 7.2 Hz, 1H), 3.75 (s, 3H), 3.67 (d, J = 4.2 Hz, 2H), 3.49 – 3.42 (m, 1H), 3.42 – 3.32 (m, 1H), 2.81 – 2.73 (m, 1H), 2.13 – 2.03 (m, 1H), 1.81 – 1.72 (m, 3H), 1.44 – 1.37 (m, 4H), 1.29 – 1.18 (m, 8H), 1.06 (d, J = 7.1 Hz, 9H), 0.95 – 0.79 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 171.7, 170.3, 165.4, 159.2, 156.1, 155.4, 145.9, 143.9, 141.3, 141.3, 135.6, 135.6, 132.7, 132.5, 132.0, 130.1, 130.0, 129.3, 127.0, 127.7, 127.1, 125.2, 125.1, 122.1, 120.0, 118.3, 113.8, 72.6, 66.8, 65.2, 58.1, 55.2, 51.7, 50.5, 47.2, 45.8, 43.6, 34.8, 33.8, 31.8, 31.1, 29.6, 26.9, 26.7, 22.63, 19.4, 19.3, 18.8, 17.7, 15.7, 14.4, 14.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{68}H_{87}N_3NaO_{11}Si^+$, 1172.6002, found 1172.5994.

(S,E)-Allyl-4-((5S,8R,11S,12S)-1-(9H-fluoren-9-yl)-8-isopropyl-12-(2-(4-methoxybe

nzyloxy)ethyl)-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatrideca namido)-5-(tert-butyldiphenylsilyloxy)pent-2-enoate (26b)

The titled compound was obtained following the general procedure described for **26a**. Flash column chromatography eluent (petroleum ether: ethyl acetate = 5: 1); yield, 25% for 4 steps; colorless oil; $[\alpha]^{20}_{D} = -41.7$ (c = 1.1, $CHCl_3$); ν_{max} (KBr): 3337, 3062, 2930, 2860, 1728, 1670, 1515, 1460, 1365, 1304, 1248, 1182, 1109, 1038, 990, 937, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.70 – 7.57 (m, 6H), 7.49 – 7.29 (m, 10H), 7.09 (dd, J = 15.7, 4.4 Hz, 1H), 6.90 (dd, J = 29.1, 8.7 Hz, 2H), 6.78 (d, J = 8.0 Hz, 1H), 6.63 (d, J = 8.1 Hz, 1H), 6.16 (d, J = 15.9 Hz, 1H), 5.99 – 5.81 (m, 2H), 5.29 (dd, J = 17.2, 1.4 Hz, 1H), 5.20 (dd, J = 10.4, 1.1 Hz, 1H), 4.90 (s, 2H), 4.62 (d, J = 5.3 Hz, 2H), 4.58 – 4.44 (m, 2H), 4.24 – 4.14 (m, 2H), 4.13 – 3.94 (m, 3H), 3.87 – 3.76 (m, 2H), 3.73 (s, 3H), 3.35 – 3.21 (m, 1H), 3.21 – 3.09 (m, 1H), 2.64 (d, J = 10.7 Hz, 1H), 2.19 – 2.02 (m, 1H), 1.77 (s, 3H), 1.57 – 1.47 (m, 1H), 1.45 – 1.36 (m, 3H), 1.35 – 1.22 (m, 11H), 1.08 (m, 9H), 0.99 – 0.76 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.5, 170.1, 164.7, 158.1, 145.6, 142.9, 140.4, 134.5, 132.0, 131.2, 128.8, 126.8, 126.6, 126.1, 126.0, 123.9, 123.7, 120.5, 119.0, 118.9, 117.1, 112.5, 77.5, 71.8, 66.8, 65.4, 64.2, 64.0, 57.6, 54.2, 50.9, 48.4, 46.1, 45.5, 33.9, 32.3, 30.8, 28.7, 28.5, 28.4, 25.8, 21.6, 18.3, 18.2, 17.2, 15.0, 14.1, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{68}H_{87}N_3NaO_{11}Si^+$, 1172.6002, found 1172.5996.

(3S,6R,11R,14S,15S,E)-11-((tert-Butyldiphenylsilyloxy)methyl)-3-isopropyl-14-(2-(4 -methoxybenzyloxy)ethyl)-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopen tadec-9-ene-2,5,8,13-tetraone (27a)

The compound **26a** (1.25 g, 1.10 mmol) and $Pd(PPh_3)_4$ (254 mg, 0.220 mmol) were dissolved in anhydrous THF (11 mL), and N-methyl aniline (238 μL, 2.20 mmol) was added. After stirred at room temperature for 1.5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid as pale yellow foam. The obtained acid was dissolved in CH_2Cl_2 (6 mL) and diethylamine (3 mL), the reaction mixture was stirred at room temperature for 3 h, and then the solvent was removed under reduced pressure to afford the crude amino acid. The mixture was dissolved in THF (1000 mL), and then DIPEA (2.85 mL, 16.3 mmol) and HATU (3.10 g, 8.16 mmol) were added successively at 0 ºC. After stirred at room temperature for 12 h, the solvent was removed under reduced pressure, and then the residue was dissolved in ethyl acetate (500 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4. The solution was filtrated and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 4) to obtain the cyclic peptide **27a** (702 mg, 73% for three steps) as a white powder. $[\alpha]^{20}_D = -41.1$ (c = 0.2, DMSO); ν_{max} (KBr): 3276, 2943, 2912, 2852, 1736, 1664, 1512, 1468, 1373, 1258, 1194, 1082, 1025, 947, 809 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 9.6 Hz, 1H), 7.72 (d, J = 6.2 Hz, 1H), 7.67 – 7.60 (m, 4H), 7.51 – 7.38 (m, 6H), 7.11 (d, J = 8.4 Hz, 3H), 6.83 (d, J = 8.5 Hz, 2H), 6.65 (dd, J = 15.7, 3.2 Hz, 1H), 6.11 (d, J = 15.4 Hz, 1H), 5.03 – 4.89 (m, 2H), 4.62 (dd, J = 9.7, 4.2 Hz, 1H), 4.19 (s, 2H), 3.99 – 3.92 (m, 1H), 3.72 (s, 3H), 3.68 – 3.57 (m, 2H), 2.82 (s, 1H), 2.41 – 2.28

(m, 1H), 2.00 – 1.86 (m, 1H), 1.65 – 1.43 (m, 4H), 1.34 – 1.15 (m, 14H), 1.11 (s, 4H), 1.05 – 0.94 (m, 12H), 0.89 – 0.75 (m, 12H), 0.64 (d, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 171.1, 170.3, 167.2, 158.6, 140.1, 135.1, 135.0, 132.6, 130.0, 129.0, 127.9, 127.9, 113.5, 77.5, 71.7, 67.0, 65.7, 55.6, 55.0, 51.2, 50.7, 45.8, 35.0, 32.0, 31.2, 31.1, 29.8, 28.9, 28.1, 26.5, 25.6, 22.1, 19.0, 18.7, 18.6, 17.5, 15.0, 13.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{50}H_{71}N_3NaO_8Si^+$, 892.4903, found 892.4909.

(3R,6S,11S,14S,15S,E)-11-((tert-Butyldiphenylsilyloxy)methyl)-3-isopropyl-14-(2-(4-methoxybenzyloxy)ethyl)-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (27b)

The titled compound **27b** was obtained following the general procedure described for **27a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 1 to 100: 4); yield, 68% for three steps; white powder; $[\alpha]^{20}_D = -85.3$ (c = 0.2, DMSO); ν_{max} (KBr): 3275, 2942, 2912, 2852, 1736, 1659, 1516, 1463, 1370, 1297, 1258, 1194, 1083, 1025, 943, 805 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 9.7 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 6.6 Hz, 5H), 7.52 (d, J = 5.8 Hz, 1H), 7.49 – 7.38 (m, 7H), 7.08 (d, J = 8.4 Hz, 2H), 6.87 (dd, J = 15.1, 2.3 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 6.05 (d, J = 15.1 Hz, 1H), 5.18 (d, J = 10.3 Hz, 1H), 4.76 (d, J = 6.3 Hz, 1H), 4.41 – 4.23 (m, 2H), 4.16 (s, 2H), 3.71 (s, 3H), 3.67 – 3.54 (m, 2H), 3.35 – 3.23 (m, 2H), 2.73 (t, J = 8.1 Hz, 1H), 2.06 (dd, J = 12.8, 6.4 Hz, 1H), 1.74 – 1.50 (m, 4H), 1.38 – 1.14 (m, 15H), 1.01 (s, 9H), 0.90 – 0.79 (m, 10H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 171.6, 169.6, 165.8, 158.8, 143.1, 135.3, 132.7, 130.3, 130.1, 129.1, 128.1, 119.4,

113.6, 74.5, 71.7, 67.0, 65.7, 56.7, 55.1, 51.2, 50.7, 45.3, 32.1, 31.3, 30.5, 29.2, 28.7, 26.7, 22.7, 22.2, 19.2, 18.9, 18.7, 17.6, 14.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{50}H_{71}N_3NaO_8Si^+$, 892.4903, found 892.4907.

(3S,6R,14S,15S,E)-3-Isopropyl-14-(2-((4-methoxybenzyl)oxy)ethyl)-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (28a)

To a solution of compound **22a** (200 mg, 0.230 mmol) in THF (2 mL) was added HOAc (40.0 μL, 0.690 mmol) and TBAF (218 mg, 0.690 mmol). The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (30 mL). The organic phase was washed with water (3 × 3 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (CH_2Cl_2: MeOH = 100: 3 to 100: 5) to obtain a white solid (100 mg).

To a solution of obtained solid (24.0 mg, 0.040 mmol) in THF (2 mL), then triethyl amine (34 μL, 0.24 mmol) and methanesulfonyl chloride (9.4 μL, 0.12 mmol) were added at 0 ºC. After stirred for 30 min, the reaction solution was quenched by addition of water (0.1 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was dissolved in THF (2 mL). To the resulting solution was added DBU (0.11 g, 0.70 mmol) at 20 ºC. After stirred for 2 h, the reaction was quenched by addition of 1 % HCl (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (3 × 3 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography

on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 3) to obtain **23** (12 mg, 34% for three steps) as a white solid. $[\alpha]^{20}_D$ = − 151.0 (*c* = 0.25, DMSO). ν_{max}(KBr): 3292, 2959, 2929, 2860, 1734, 1672, 1616, 1518, 1461, 1374, 1251, 1092, 1036, 982, 901, 854, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.15 (d, *J* = 9.8 Hz, 1H), 7.60 (d, *J* = 5.0 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 6.97 – 6.69 (m, 3H), 6.31 (d, *J* = 15.2 Hz, 1H), 5.43 (s, 1H), 5.37 (s, 1H), 5.21 (d, *J* = 10.1 Hz, 1H), 4.51 – 4.27 (m, 3H), 4.18 (t, *J* = 8.7 Hz, 1H), 3.72 (s, 3H), 3.10 – 2.93 (m, 1H), 1.90 (dd, *J* = 13.4, 6.8 Hz, 1H), 1.80 – 1.61 (m, 3H), 1.42 – 1.12 (m, 12H), 1.10 – 1.02 (m, 1H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.89 – 0.78 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.4, 168.7, 166.3, 158.7, 138.7, 137.6, 130.2, 129.3, 119.0, 115.9, 113.6, 76.4, 71.7, 66.7, 57.7, 55.0, 50.9, 45.1, 33.6, 33.5, 32.0, 31.2, 29.3, 28.9, 26.7, 22.1, 19.4, 18.3, 18.1, 14.0, 13.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{34}H_{51}N_3NaO_7^+$, 636.3619, found, 636.3622. HPLC purity: 96.1%.

(3R,6S,14S,15S,E)-3-Isopropyl-14-(2-(4-methoxybenzyloxy)ethyl)-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (28b)

The titled compound **28b** was obtained following the procedure described for **28a**. Flash column chromatography (CH_2Cl_2: MeOH = 100: 1 to 100: 3); yield: 41% for 3 steps; white solid; $[\alpha]^{20}_D$ = − 38.1 (*c* = 0.08, DMSO); ν_{max} (KBr): 3279, 2960, 2925, 2855, 1733, 1664, 1518, 1461, 1370, 1297, 1258, 1196, 1086, 1025, 964, 804 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.24 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 15.8 Hz, 1H), 6.06 (d, *J* = 15.8 Hz, 1H), 5.61 (s, 1H), 5.33 (d, *J* = 6.2 Hz, 1H),

5.22 (t, J = 6.2 Hz, 1H), 4.54 (d, J = 6.8 Hz, 1H), 4.41 (s, 2H), 4.14 (q, J = 7.1 Hz, 1H), 3.77 (s, 3H), 3.57 – 3.41 (m, 2H), 3.06 – 2.93 (m, 1H), 2.27 – 2.16 (m, 1H), 2.01 – 1.88 (m, 1H), 1.85 – 1.75 (m, 2H), 1.41 (t, J = 6.6 Hz, 4H), 1.34 – 1.20 (m, 11H), 1.04 – 0.85 (m, 14H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.9, 174.3, 171.9, 171.6, 160.9, 139.7, 138.8, 131.5, 130.7, 121.3, 117.2, 115.6, 114.8, 78.9, 73.9, 68.1, 58.6, 55.7, 53.3, 49.7, 49.5, 49.3, 49.0, 48.8, 48.6, 48.4, 47.8, 36.0, 34.7, 32.9, 32.0, 31.2, 30.6, 27.8, 23.7, 20.0, 19.0, 18.2, 14.7, 14.5. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{34}H_{51}N_3NaO_7^+$, 636.3619, found 636.3611. HPLC purity: 98.2%.

(3S,6R,11R,14S,15S,E)-11-((tert-Butyldiphenylsilyloxy)methyl)-14-(2-hydroxyethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (29)

To a solution of compound **27a** (0.55 g, 0.64 mmol) in CH_2Cl_2/H_2O (6 mL/2 mL) was added DDQ (0.18g, 0.77 mmol). The mixture was stirred at room temperature for 1.5 h, and then diluted with ethyl acetate (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (5 × 10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 4) to obtain compound **29** (0.33 g, 68%) as a white powder. $[\alpha]^{20}_D$ = −60.2 (c = 0.3, DMSO); ν_{max} (KBr): 3284, 2959, 2930, 2858, 1742, 1667, 1519, 1460, 1366, 1211, 1080, 1039, 981, 907 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 9.9 Hz, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.62 (d, J = 6.8 Hz, 4H), 7.54 – 7.38 (m, 7H), 6.83 (dd, J = 15.1, 2.5 Hz, 1H), 6.06 (d, J = 15.0 Hz, 1H), 5.32 (d, J = 10.5 Hz, 1H), 4.71 (d, J = 3.6 Hz, 1H), 4.47 (s, 1H), 4.33 – 4.19 (m, 2H), 3.67 – 3.49 (m, 2H), 2.89 –

2.78 (m, 1H), 1.93 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.78 (d, *J* = 6.3 Hz, 1H), 1.65 – 1.45 (m, 2H), 1.42 – 1.16 (m, 14H), 1.11 – 0.92 (m, 14H), 0.90 – 0.79 (m, 10H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 172.1, 169.3, 166.2, 143.3, 135.6, 135.5, 133.1, 133.0, 130.4, 128.4, 128.4, 119.9, 76.4, 66.0, 58.7, 57.9, 51.4, 51.2, 44.9, 34.0, 33.9, 33.0, 32.4, 31.6, 29.4, 27.2, 27.1, 22.5, 19.9, 19.3, 18.8, 18.7, 14.4, 13.6. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{42}H_{63}N_3NaO_7Si^+$, 772.4327, found 772.4330.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethylundec-10-ynoate (32a)

To a solution of compound **29** (80 mg, 0.11 mmol) in DCM (0.5 mL) were added acid **30a** (20 mg, 0.17 mmol), EDCI (36 mg, 0.19 mmol) and DMAP (13 mg, 0.11 mmol). The mixture was stirred at room temperature and diluted with ethyl acetate (30 mL). The organic phase was washed with 1% HCl, saturated aqueous $NaHCO_3$ and brine successively, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100:1 to 100: 3) to obtain a white solid.

To a solution of obtained solid in THF (1 mL) were added HOAc (7.0 μL, 0.12 mmol) and TBAF (38 mg, 0.12 mmol). The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (30 mL). The organic phase was washed with H_2O (10 × 3 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 3 to 100: 5) to obtain compound **32a** (56.5 mg, 85% for two steps) as a white powder. $[\alpha]^{20}_D = -254.1$ (*c* =

0.1, DMSO); ν_{max} (KBr): 3307, 2935, 2860, 2363, 1731, 1680, 1615, 1455, 1242, 1028, 975, 848 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.36 (d, J = 10.0 Hz, 1H), 7.93 (d, J = 9.0 Hz, 1H), 6.97 (dd, J = 15.1, 2.4 Hz, 1H), 6.11 (dd, J = 15.1, 1.4 Hz, 1H), 5.54 (d, J = 10.7 Hz, 1H), 4.72 (s, 1H), 4.47 – 4.41 (m, 1H), 4.37 (q, J = 6.8 Hz, 1H), 4.14 (t, J = 6.6 Hz, 2H), 3.64 – 3.53 (m, 2H), 2.94 (td, J = 10.1, 3.9 Hz, 1H), 2.34 (t, J = 7.4 Hz, 2H), 2.19 – 2.12 (m, 3H), 2.11 – 2.01 (m, 1H), 1.98 – 1.74 (m, 3H), 1.67 – 1.56 (m, 2H), 1.54 – 1.25 (m, 25H), 1.21 – 1.11 (m, 1H), 1.08 – 0.81 (m, 14H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.4, 175.1, 175.1, 174.2, 170.5, 169.1, 146.3, 119.9, 85.1, 77.7, 69.6, 64.7, 62.9, 59.5, 53.8, 52.8, 46.8, 35.5, 35.3, 35.1, 33.8, 33.0, 30.7, 30.4, 30.3, 30.1, 30.0, 29.8, 29.8, 28.6, 26.0, 23.8, 20.3, 19.1, 19.0, 14.6, 13.7. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{37}H_{61}N_3NaO_8^+$, 698.4351, found 698.4359. HPLC purity: 98.2%.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl hex-5-ynoate (32b)

The titled compound **32b** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 79% for two steps; white powder; $[\alpha]^{20}_D$ = − 66.7 (c = 0.3, DMSO); ν_{max} (KBr): 3304, 2959, 2931, 2871, 2366, 1733, 1672, 1440, 1250, 1059, 976, 890 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 6.97 (d, J = 15.1 Hz, 1H), 6.16 (d, J = 15.1 Hz, 1H), 5.52 (d, J = 10.6 Hz, 1H), 4.72 (s, 1H), 4.50 – 4.38 (m, 2H), 4.15 (t, J = 6.4 Hz, 2H), 3.63 – 3.54 (m, 2H), 3.28 – 3.21 (m, 4H), 3.02 (t, J = 9.4 Hz, 1H), 2.48 (t, J = 7.3 Hz, 2H), 2.27 –

2.22 (m, 3H), 2.10 – 2.02 (m, 1H), 1.94 (d, J = 7.3 Hz, 1H), 1.82 (m, 4H), 1.71 – 1.63 (m, 4H), 1.48 – 1.24 (m, 19H), 1.17 (dd, J = 17.1, 9.0 Hz, 1H), 1.07 – 0.94 (m, 16H), 0.93 – 0.86 (m, 4H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.1, 174.8, 174.2, 170.5, 169.2, 146.0, 120.1, 84.1, 77.9, 70.3, 64.7, 63.0, 59.6, 59.5, 53.7, 52.7, 46.7, 35.5, 35.4, 33.8, 33.8, 32.9, 30.6, 30.0, 28.5, 25.0, 24.9, 23.7, 20.8, 20.3, 19.0, 19.0, 18.5, 14.5, 14.0, 13.8. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{32}H_{51}N_3NaO_8^+$, 628.3568, found 628.2375.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl-3,3,3-trip henylpropanoate (32c)

The titled compound **32c** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 81% for two steps; white powder; $[\alpha]^{20}_D$ = − 40.8 (c = 0.5, DMSO); ν_{max} (KBr): 3323, 3051, 2923, 2857, 1728, 1678, 1532, 1461, 1385, 1236, 1109, 1080, 982, 913, 820, 803 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 – 7.14 (m, 15H), 6.97 (d, J = 15.2 Hz, 1H), 6.08 (d, J = 15.1 Hz, 1H), 5.46 (d, J = 10.5 Hz, 1H), 4.68 (s, 1H), 4.43 (d, J = 7.2 Hz, 1H), 4.36 (q, J = 6.7 Hz, 1H), 3.92 – 3.68 (m, 4H), 3.53 (d, J = 4.5 Hz, 2H), 2.77 (dd, J = 14.0, 9.7 Hz, 1H), 2.05 (dq, J = 13.4, 6.6 Hz, 1H), 1.69 (d, J = 5.9 Hz, 1H), 1.50 – 1.24 (m, 16H), 1.19 – 1.05 (m, 1H), 0.93 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 174.1, 172.7, 170.6, 169.3, 148.2, 146.5, 130.7, 129.0, 127.5, 120.0, 77.8, 64.8, 62.9, 59.7, 57.3, 53.8, 52.9, 47.3, 46.7, 35.7, 35.4, 33.9, 33.2, 30.9, 29.6, 28.8, 28.2, 24.0, 20.5, 19.2, 14.8, 13.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for

$C_{47}H_{61}N_3NaO_8^+$, 818.4351, found 818.4355.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyladamantane-1-carboxylate (32d)

The titled compound **32d** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 73% for two steps; white powder; $[\alpha]^{20}_D = -36.7$ (c = 0.12, DMSO); ν_{max} (KBr): 3314, 2913, 2856, 1730, 1672, 1534, 1455, 1237, 1078, 975, 909, 847 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.35 (d, J = 10.0 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 6.97 (dd, J = 15.1, 2.3 Hz, 1H), 6.11 (d, J = 15.1 Hz, 1H), 5.56 (d, J = 10.7 Hz, 1H), 4.72 (d, J = 2.7 Hz, 1H), 4.44 (dd, J = 12.0, 4.9 Hz, 1H), 4.36 (q, J = 6.6 Hz, 1H), 4.19 – 4.03 (m, 2H), 3.64 (dd, J = 10.8, 5.0 Hz, 1H), 3.57 (dd, J = 10.9, 6.0 Hz, 1H), 3.30 (s, 1H), 2.98 – 2.88 (m, 1H), 2.12 – 1.97 (m, 5H), 1.89 (d, J = 19.5 Hz, 10H), 1.76 (q, J = 12.5 Hz, 8H), 1.43 (d, J = 7.1 Hz, 7H), 1.29 (s, 9H), 1.22 – 1.10 (m, 2H), 1.05 (d, J = 6.7 Hz, 4H), 0.97 (t, J = 7.1 Hz, 6H), 0.93 – 0.85 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD) δ 179.2, 175.2, 174.2, 170.5, 169.1, 146.3, 119.9, 77.7, 64.6, 62.9, 59.6, 53.9, 52.8, 46.9, 42.0, 40.0, 37.6, 35.5, 35.4, 33.8, 33.0, 30.7, 30.0, 29.5, 28.6, 23.8, 20.3, 19.0, 14.6, 13.7. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{37}H_{59}N_3NaO_8^+$, 696.4194, found 696.4188.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl propionate (32e)

The titled compound **32e** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 83% for two steps; white powder; $[\alpha]^{20}_D = -36.4$ (c = 0.11, DMSO); ν_{max} (KBr): 3310, 2925, 2855, 1737, 1890, 1651, 1615, 1456, 1244, 1058, 975, 910, 887, 847 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.97 (d, J = 15.1 Hz, 1H), 6.11 (d, J = 15.0 Hz, 1H), 5.55 (d, J = 10.7 Hz, 1H), 4.72 (s, 1H), 4.44 (d, J = 7.1 Hz, 1H), 4.40 – 4.34 (m, 1H), 4.14 (t, J = 5.7 Hz, 2H), 3.65 – 3.53 (m, 2H), 2.99 – 2.90 (m, 1H), 2.39 – 2.27 (m, 2H), 2.06 (dd, J = 13.1, 6.5 Hz, 1H), 1.97 – 1.79 (m, 3H), 1.60 (d, J = 6.2 Hz, 2H), 1.49 – 1.26 (m, 41H), 1.07 – 0.88 (m, 16H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.4, 175.0, 174.1, 170.5, 169.1, 146.2, 119.9, 77.7, 64.6, 62.9, 59.5, 53.7, 52.8, 46.8, 35.5, 35.3, 35.1, 33.7, 33.1, 33.0, 30.9, 30.7, 30.6, 30.5, 30.3, 30.0, 28.6, 26.1, 23.8, 23.8, 20.3, 19.0, 14.6, 13.7. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{44}H_{79}N_3NaO_8^+$, 800.5759, found 800.5755.

(S)-2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl-2-(tert-butoxycarbonylamino)-3-phenylpropanoate (32f)

The titled compound **32f** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 78% for two steps; white powder; $[\alpha]^{20}_D = -88.0$ (c = 0.1, DMSO); ν_{max} (KBr): 3326, 2962, 2932, 2868, 1738, 1677, 1433, 1363, 1247, 1171, 1059, 975, 912, 853 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.37 (d, J = 10.0 Hz, 1H), 7.83 (d, J = 8.9 Hz, 1H), 7.33 – 7.18 (m, 5H), 6.99 (dd, J = 15.1, 2.4 Hz, 1H), 6.12 (d, J = 15.1 Hz, 1H), 5.51 (d, J =

10.7 Hz, 1H), 4.79 – 4.71 (m, 1H), 4.46 (t, *J* = 7.0 Hz, 1H), 4.41 – 4.28 (m, 2H), 4.23 – 4.12 (m, 1H), 4.09 – 4.00 (m, 1H), 3.60 (dd, *J* = 10.7, 5.2 Hz, 1H), 3.53 (dd, *J* = 10.6, 6.4 Hz, 1H), 3.06 (dd, *J* = 13.6, 6.7 Hz, 1H), 3.01 – 2.84 (m, 2H), 2.13 – 2.01 (m, 1H), 1.83 – 1.65 (m, 3H), 1.50 – 1.26 (m, 23H), 1.21 – 1.12 (m, 1H), 1.06 – 0.85 (m, 14H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.3, 175.2, 174.3, 174.1, 170.7, 169.2, 158.2, 146.3, 138.5, 130.5, 129.7, 128.1, 120.0, 81.0, 77.8, 64.9, 63.6, 59.7, 59.6 57.1, 54.0, 53.0, 46.8, 38.9, 35.6, 35.5, 33.9, 33.2, 30.9, 29.9, 29.0, 28.8, 23.9, 20.5, 19.2, 14.7, 13.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{40}H_{62}N_4NaO_{10}^+$, 781.4358, found 781.4361.

2-((3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl-2-(diethoxyphosphoryl)acetate (32g)

The titled compound **32g** was obtained following the general procedure described for **32a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield, 79% for two steps; white powder; $[\alpha]^{20}_D = -84.6$ (*c* = 0.13, DMSO); ν_{max} (KBr): 3307, 2960, 2930, 2866, 1736, 1672, 1539, 1440, 1249, 1028, 973, 911 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.55 (d, *J* = 9.9 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 6.97 (dd, *J* = 15.1, 2.9 Hz, 1H), 6.17 (dd, *J* = 15.2, 2.1 Hz, 1H), 5.51 (t, *J* = 7.4 Hz, 1H), 4.79 – 4.70 (m, 1H), 4.56 – 4.39 (m, 2H), 4.36 – 4.26 (m, 1H), 4.24 – 4.06 (m, 5H), 3.57 (d, *J* = 5.4 Hz, 2H), 3.34 – 3.06 (m, 3H), 2.16 – 1.98 (m, 2H), 1.91 – 1.78 (m, 1H), 1.77 – 1.66 (m, 1H), 1.53 – 1.25 (m, 20H), 1.22 – 1.10 (m, 1H), 1.06 – 0.84 (m, 14H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.3, 174.4, 170.4, 169.2, 166.9, 146.2, 120.0, 78.1, 64.8, 63.9,

59.5, 53.7, 52.6, 46.1, 35.5, 35.4, 34.6, 34.0, 33.3, 33.0, 30.7, 29.3, 28.6, 23.8, 20.3, 19.0, 18.9, 16.7, 16.7, 14.5, 13.8. ^{31}P NMR (162 MHz, CD_3OD) δ 22.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for$C_{32}H_{56}N_3NaO_{11}P^+$, 712.3545, found 712.3551.

tert-Butyl(2-((3S,6R,11R,14S,15S,E)-11-(hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl) carbonate (32h)

The titled compound **32a** was obtained following the procedure described for **32h**. Flash column chromatography (CH_2Cl_2: MeOH = 100: 3 to 100: 5); yield: 72%; white powder; $[\alpha]^{20}_D = -45.1$ (c = 0.58, DMSO). ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (d, J = 10.0 Hz, 1H), 7.93 (d, J = 9.0 Hz, 1H), 6.98 (dd, J = 15.1, 2.6 Hz, 1H), 6.11 (dd, J = 15.1, 1.8 Hz, 1H), 5.52 (d, J = 10.7 Hz, 1H), 4.79 – 4.68 (m, 1H), 4.50 – 4.42 (m, 1H), 4.41 – 4.30 (m, 1H), 4.20 – 4.02 (m, 2H), 3.65 – 3.49 (m, 2H), 2.94 (td, J = 10.3, 3.7 Hz, 1H), 2.06 (dq, J = 13.5, 6.7 Hz, 1H), 1.99 – 1.90 (m, 1H), 1.89 – 1.73 (m, 2H), 1.54 – 1.40 (m, 14H), 1.40 – 1.25 (m, 13H), 1.22 – 1.11 (m, 1H), 1.04 (d, J = 6.8 Hz, 3H), 1.01 – 0.93 (m, 6H), 0.93 – 0.86 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.2, 170.4, 169.1, 154.9, 146.1, 119.8, 83.0, 77.8, 65.3, 64.6, 59.5, 59.4, 53.7, 53.6, 52.8, 52.7, 46.7, 46.7, 35.4, 35.3, 33.7, 33.1, 32.9, 30.7, 30.6, 30.0, 28.4, 28.0, 23.7, 20.2, 18.9, 14.4, 13.6.

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl-undec-10-ynoate (33a)

To a solution of compound **32a** (49 mg, 0.080 mmol) in THF (2 mL), then

triethylamine (67 μL, 0.48 mmol) and methanesulfonyl chloride (19 μL, 0.24 mmol) were added at 0 ºC. After stirred for 30 min, the reaction solution was quenched by addition of water (0.1 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was dissolved in THF (3 mL). To the resulting solution was added DBU (0.11 g, 0.70 mmol) at 20 ºC. After stirred for 2 h, the reaction was quenched by addition of 1 % HCl (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (3 × 3 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 30: 1) to obtain **33a** (25 mg, 53% for two steps) as a white solid. $[\alpha]^{20}_D = -106.7$ (c = 0.18, DMSO); ν_{max} (KBr): 3308, 2928, 2858, 1736, 1670, 1529, 1461, 1368, 1258, 1094, 1027, 989, 905, 862, 804 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.24 (d, J = 9.8 Hz, 1H), 7.60 (d, J = 5.2 Hz, 1H), 6.88 (d, J = 15.1 Hz, 1H), 6.26 (d, J = 15.1 Hz, 1H), 5.50 (s, 1H), 5.42 (s, 1H), 5.23 (d, J = 10.2 Hz, 1H), 4.32 (d, J = 5.4 Hz, 1H), 4.24 – 4.18 (m, 1H), 4.05 – 3.94 (m, 2H), 2.98 (s, 1H), 2.73 (s, 1H), 2.27 (t, J = 7.4 Hz, 2H), 2.13 (t, J = 7.0 Hz, 2H), 1.97 – 1.89 (m, 1H), 1.83 – 1.73 (m, 3H), 1.55 – 1.47 (m, 2H), 1.45 – 1.38 (m, 2H), 1.36 – 1.17 (m, 23H), 1.11 – 1.03 (m, 1H), 0.96 (d, J = 6.6 Hz, 3H), 0.88 – 0.83 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.8, 172.7, 170.9, 168.7, 166.2, 138.7, 137.6, 119.0, 116.1, 84.5, 76.0, 71.1, 61.5, 57.5, 50.9, 45.1, 33.5, 33.4, 33.4, 31.9, 31.1, 28.8, 28.6, 28.4, 28.3, 28.0, 27.9, 26.7, 24.3, 22.0, 19.4, 18.2, 18.1, 17.6, 13.9, 13.0. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{37}H_{59}N_3NaO_7^+$, 680.4245, found 680.4241.

HPLC purity: 95.8%.

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8, 13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl hex-5-ynoate (33b)

The titled compound **33b** was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 49% for two steps; white powder; $[\alpha]^{20}_D = -61.5$ (c = 0.13, DMSO); ν_{max} (KBr): 3308, 2957, 2926, 2860, 1734, 1862, 1662, 1622, 1528, 1460, 1368, 1260, 1095, 1024, 804 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.30 (d, J = 9.8 Hz, 1H), 7.58 (d, J = 5.3 Hz, 1H), 6.85 (d, J = 15.2 Hz, 1H), 6.36 (d, J = 15.0 Hz, 1H), 5.52 (s, 1H), 5.40 (s, 1H), 5.21 (d, J = 10.2 Hz, 1H), 4.49 – 4.35 (m, 1H), 4.20 (dd, J = 9.4, 8.1 Hz, 1H), 4.07 – 3.94 (m, 2H), 3.13 – 3.03 (m, 1H), 2.80 (t, J = 2.6 Hz, 1H), 2.39 (t, J = 7.4 Hz, 2H), 2.18 (td, J = 7.0, 2.5 Hz, 2H), 1.96 – 1.86 (m, 1H), 1.84 – 1.73 (m, 3H), 1.73 – 1.63 (m, 2H), 1.38 – 1.15 (m, 14H), 0.96 (d, J = 6.7 Hz, 3H), 0.89 – 0.79 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 172.4, 171.1, 168.8, 166.3, 138.6, 137.6, 119.2, 116.0, 83.7, 76.1, 71.8, 61.8, 57.6, 50.9, 45.0, 33.6, 33.5, 32.3, 32.0, 31.2, 28.9, 28.1, 26.7, 23.4, 22.0, 19.4, 18.3, 18.2, 17.1, 14.0, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{32}H_{49}N_3NaO_7^+$, 610.3463, found 610.3467. HPLC purity: 98.3%.

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8, 13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl 3,3,3-triphenylpropanoate (33c)

The titled compound was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 52% for two

steps; white powder; $[\alpha]^{20}_{D}$ = − 114.1 (*c* = 0.17, DMSO); ν_{max} (KBr): 3305, 2960, 2929, 1738, 1674, 1018, 1522, 1454, 1256, 1199, 1147, 1083, 982, 912, 862 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.96 (s, 1H), 8.22 (d, *J* = 9.9 Hz, 1H), 7.60 (d, *J* = 5.1 Hz, 1H), 7.30 – 7.24 (m, 6H), 7.22 – 7.15 (m, 9H), 6.88 (d, *J* = 15.2 Hz, 1H), 6.24 (d, *J* = 15.2 Hz, 1H), 5.43 (s, 1H), 5.41 (s, 1H), 5.15 (d, *J* = 10.2 Hz, 1H), 4.32 (s, 1H), 4.24 – 4.17 (m, 1H), 3.82 – 3.75 (m, 2H), 3.74 – 3.68 (m, 1H), 3.68 – 3.61 (m, 1H), 2.87 (t, *J* = 9.8 Hz, 1H), 1.92 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.64 – 1.57 (m, 1H), 1.47 – 1.39 (m, 1H), 1.39 – 1.15 (m, 16H), 1.11 (s, 1H), 1.10 – 1.04 (m, 1H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.87 – 0.82 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 170.7, 170.1, 168.7, 166.2, 146.4, 138.7, 137.5, 128.9, 127.6, 126.0, 119.0, 116.2, 75.8, 61.2, 57.5, 55.3, 50.9, 45.2, 44.8, 33.4, 33.4, 31.9, 31.3, 31.1, 28.8, 27.7, 26.7, 22.0, 19.4, 18.2, 18.1, 13.9, 13.0. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{47}H_{59}N_3NaO_7^+$, 800.4245, found 800.4250. HPLC purity: 98.5%

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8, 13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl adamantane-1-carboxylate (33d)

The titled compound was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 53% for two steps; white powder; $[\alpha]^{20}_{D}$ = − 76.9 (*c* = 0.13, DMSO); ν_{max} (KBr): 3398, 2919, 2856, 1732, 1688, 1631, 1509, 1459, 1368, 1239, 1078, 1025, 980, 805 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.36 (d, *J* = 9.8 Hz, 1H), 7.59 (d, *J* = 5.4 Hz, 1H), 6.87 (d, *J* = 15.1 Hz, 1H), 6.36 (d, *J* = 15.2 Hz, 1H), 5.52 (s, 1H), 5.42 (s, 1H), 5.23 (d,

J = 9.7 Hz, 1H), 4.60 – 4.34 (m, 1H), 4.32 – 4.14 (m, 1H), 3.97 (t, *J* = 6.7 Hz, 2H), 3.17 – 3.01 (m, 1H), 2.10 – 1.87 (m, 4H), 1.84 – 1.77 (m, 7H), 1.72 – 1.57 (m, 6H), 1.47 – 1.18 (m, 12H), 1.16 – 1.06 (m, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.85 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 176.4, 172.8, 171.0, 168.7, 166.2, 138.6, 137.8, 119.2, 116.0, 76.2, 66.9, 61.5, 57.6, 50.8, 45.0, 40.0, 39.8, 39.7, 39.6, 39.4, 39.3, 39.2, 38.3, 35.9, 33.7, 33.5, 31.9, 31.3, 31.1, 28.8, 28.2, 27.3, 26.7, 22.0, 19.3, 18.2, 18.1, 13.8, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{37}H_{57}N_3NaO_7^+$, 678.4089, found 678.4093. HPLC purity: 98.9%.

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8, 13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl stearate (33e)

The titled compound **33e** was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 47% for two steps; white powder; $[\alpha]^{20}_D$ = − 100.0 (*c* = 0.10, DMSO); ν_{max} (KBr): 3349, 2924, 2855, 1732, 1688, 1664, 1623, 1529, 1460, 1253, 1199, 979, 915, 863 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.24 (d, *J* = 9.5 Hz, 1H), 7.59 (d, *J* = 5.0 Hz, 1H), 6.89 (d, *J* = 15.1 Hz, 1H), 6.26 (d, *J* = 15.0 Hz, 1H), 5.49 (s, 1H), 5.42 (s, 1H), 5.24 (d, *J* = 10.0 Hz, 1H), 4.35 – 4.30 (m, 1H), 4.27 – 4.17 (m, 1H), 4.00 (s, 2H), 2.97 (s, 1H), 2.26 (t, *J* = 7.1 Hz, 2H), 2.00 – 1.86 (m, 1H), 1.78 (s, 3H), 1.51 (s, 2H), 1.39 – 1.14 (m, 42H), 1.13 – 1.02 (m, 2H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.85 (t, *J* = 6.6 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 171.0, 168.9, 166.3, 138.8, 137.8, 119.2, 116.3, 76.1, 61.6, 57.7, 51.1, 45.2, 33.8, 33.6, 33.6, 32.1, 31.5, 31.4, 29.2, 29.2, 29.1, 29.0, 28.9, 28.9, 28.6, 28.3, 26.9, 24.5, 22.3, 22.2, 19.5, 18.4, 18.3, 14.0, 14.0, 13.2.

HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{44}H_{77}N_3NaO_7^+$, 782.5654, found 782.5649. HPLC purity: 99.9%.

(S)-2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl-2-(tert-butoxycarbonylamino)-3-phenylpropanoate (33f)

The titled compound **33f** was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 42% for two steps; white powder; $[\alpha]^{20}_D = -170.0$ (c = 0.08, DMSO); ν_{max} (KBr): 3316, 2963, 2930, 2864, 1724, 1692, 1521, 1365, 1259, 1174, 1022, 988, 862, 805 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.26 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 4.8 Hz, 1H), 7.33 – 7.19 (m, 5H), 6.89 (d, J = 15.1 Hz, 1H), 6.28 (d, J = 14.9 Hz, 1H), 5.49 (s, 1H), 5.44 (s, 1H), 5.24 (d, J = 10.0 Hz, 1H), 4.35 (s, 1H), 4.25 – 4.15 (m, 2H), 4.05 – 3.95 (m, 2H), 3.03 – 2.97 (m, 2H), 2.91 – 2.85 (m, 1H), 1.93 (dq, J = 13.5, 6.7 Hz, 1H), 1.76 – 1.65 (m, 3H), 1.38 – 1.17 (m, 21H), 1.13 – 1.04 (m, 2H), 0.97 (d, J = 6.4 Hz, 3H), 0.89 – 0.80 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 171.9, 170.8, 168.7, 166.1, 155.4, 138.7, 137.7, 137.5, 129.0, 128.1, 126.4, 119.0, 116.5, 78.3, 75.9, 62.0, 57.5, 55.2, 50.9, 44.8, 36.4, 33.6, 33.4, 31.9, 31.3, 31.1, 28.8, 28.1, 26.7, 22.0, 19.3, 18.2, 18.2, 13.9, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{40}H_{60}N_4NaO_9^+$, 763.4253, found 763.4244. HPLC purity: 99.3%.

2-((3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl 2-(diethoxyphosphoryl)acetate (33g)

The titled compound **33g** was obtained following the general procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield, 51% for two steps; white powder; $[\alpha]^{20}_D = -136.0$ (c = 0.12, DMSO); ν_{max} (KBr): 3276, 2961, 2929, 2863, 1738, 1684, 1620, 1529, 1462, 1375, 1255, 1028, 977, 862 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.23 (d, J = 9.9 Hz, 1H), 7.55 (d, J = 5.2 Hz, 1H), 6.88 (d, J = 15.1 Hz, 1H), 6.23 (d, J = 15.1 Hz, 1H), 5.49 (s, 1H), 5.42 (s, 1H), 5.25 (d, J = 10.2 Hz, 1H), 4.41 – 4.25 (m, 1H), 4.25 – 4.18 (m, 1H), 4.14 – 3.97 (m, 6H), 3.22 – 3.08 (m, 2H), 2.99 (td, J = 10.3, 3.7 Hz, 1H), 1.94 (dq, J = 13.7, 6.8 Hz, 1H), 1.87 – 1.71 (m, 3H), 1.41 – 1.17 (m, 18H), 1.14 – 1.05 (m, 1H), 0.97 (d, J = 6.7 Hz, 3H), 0.90 – 0.79 (m, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.6, 170.7, 168.8, 166.2, 165.6, 165.5, 138.6, 137.6, 118.9, 116.1, 76.0, 62.3, 62.1, 62.1, 62.0, 57.5, 51.0, 44.8, 33.8, 33.6, 33.4, 32.9, 31.8, 31.1, 28.8, 27.9, 26.6, 22.0, 19.3, 18.2, 18.1, 16.1, 16.0, 13.8, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{32}H_{54}N_3NaO_{10}P^+$, 694.3439, found 694.3448. HPLC purity: 99.9%.

tert-Butyl(2-((3S,6R,14S,15S,E)-3-isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-14-yl)ethyl) carbonate (33h)

The titled compound **33h** was obtained following the procedure described for **33a**. Flash column chromatography eluent (CH_2Cl_2: MeOH = 30: 1); yield: 53%; white powder; $[\alpha]^{20}_D = -107.3$ (c = 0.13, DMSO). ν_{max} (KBr): 3287, 2953, 2931, 2864, 1739, 1676, 1524, 1462, 1371, 1257, 1166, 1101, 985, 860 ,800 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.16 (s, 1H), 7.57 (d, J = 5.4 Hz, 1H), 6.85 (d, J =

15.2 Hz, 1H), 6.34 (dd, *J* = 14.9, 7.5 Hz, 1H), 5.55 (s, 1H), 5.41 (s, 1H), 5.21 (d, *J* = 10.3 Hz, 1H), 4.35 (s, 1H), 4.19 (dd, *J* = 9.7, 7.8 Hz, 1H), 4.06 – 3.90 (m, 2H), 3.04 (s, 1H), 2.00 – 1.86 (m, 1H), 1.82 – 1.69 (m, 3H), 1.39 (s, 9H), 1.33 – 1.19 (m, 12H), 1.09 – 1.03 (m, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.89 – 0.79 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.0, 168.7, 166.3, 152.8, 138.6, 137.4, 119.0, 116.1, 81.4, 76.1, 64.4, 57.6, 50.9, 45.0, 33.5, 33.4, 32.0, 31.1, 28.8, 28.1, 27.3, 26.6, 22.0, 19.4, 18.3, 18.1, 13.9, 13.1. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{31}H_{51}N_3NaO_8^+$, 616.3568, found, 616.3570. HPLC purity: 98.0%.

(3S,6R,11R,14S,15S,E)-11-(Hydroxymethyl)-3-isopropyl-14-(2-((4-methoxybenzyl)oxy)ethyl)-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (34)

To a solution of compound **27a** (400 mg, 0.46 mmol) in THF (4 mL) were added HOAc (79 μL, 1.38 mmol) and TBAF (435 mg, 1.38 mmol). The mixture was stirred at room temperature for 12 h, and then diluted with ethyl acetate (100 mL). The organic phase was washed with H_2O (3 × 30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 3 to 100: 7) to obtain compound **34** (206 mg, 71%) as a white powder. $[\alpha]^{20}_D$ = − 64.9 (*c* = 0.31, DMSO); ν_{max} (KBr): 3299, 2930, 2864, 1733, 1675, 1520, 1460, 1364, 1250, 1085, 1040, 979, 824 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 9.9 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 5.9 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.78 (dd, *J* = 15.0, 2.6 Hz, 1H), 5.98 (dd, *J* = 15.0, 1.8 Hz, 1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.98 (t, *J* = 5.4 Hz, 1H), 4.59 – 4.47 (m,

1H), 4.38 (d, *J* = 11.5 Hz, 1H), 4.32 (d, *J* = 11.5 Hz, 1H), 4.27 – 4.16 (m, 2H), 3.73 (s, 3H), 3.45 – 3.36 (m, 4H), 2.83 (td, *J* = 10.2, 3.8 Hz, 1H), 2.00 – 1.84 (m, 1H), 1.79 – 1.66 (m, 2H), 1.64 – 1.49 (m, 1H), 1.36 – 1.07 (m, 13H), 1.07 – 0.98 (m, 1H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.88 – 0.78 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 171.7, 169.3, 166.3, 159.2, 144.4, 130.9, 129.7, 119.4, 114.0, 76.2, 72.1, 67.1, 63.7, 57.9, 55.5, 52.0, 51.1, 45.0, 34.0, 33.9, 32.3, 31.6, 29.9, 29.4, 27.2, 22.5, 19.9, 18.8, 18.7, 14.4, 13.7. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{34}H_{53}N_3NaO_8^+$, 654.3725, found, 654.3728. HPLC purity: 98.0%.

((3S,6R,11R,14S,15S,E)-3-Isopropyl-14-(2-((4-methoxybenzyl)oxy)ethyl)-6-methyl-15-((R)-Octan-2-yl)-2,5,8,13-tetraoxo-1-oxa-4,7,12-triazacyclopentadec-9-en-11-yl)methyl methanesulfonate (35)

To a solution of compound **34** (160 mg, 0.25 mmol) in THF (5 mL), then triethylamine (138 μL, 1 mmol) and methanesulfonyl chloride (57.5 mg, 0.5 mmol) were added at 0 ºC. After stirred for 30 min, the reaction solution was quenched by addition of water (0.1 mL) and diluted with ethyl acetate (70 mL). The organic phase was washed with water (3 × 15 mL) and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 2 to 100: 5) to obtain compound **35** (140 mg, 79%) as a white powder. $[\alpha]^{20}_D$ = − 36.3 (*c* = 0.13, DMSO); ν_{max} (KBr): 3369, 3296, 2958, 2932, 2857, 1731, 1672, 1539, 1460, 1344, 1248, 1174, 1096, 1037, 971, 917, 842 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 – 8.10 (m, 2H), 7.57 (d, *J* = 5.6 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 6.69 (d, *J* = 15.4 Hz, 1H), 6.06 (d, *J* = 15.1 Hz, 1H), 5.29 (d, *J* = 10.6 Hz, 1H), 4.89 (s, 1H),

4.43 – 4.32 (m, 2H), 4.31 – 4.17 (m, 3H), 4.15 – 4.07 (m, 1H), 3.73 (s, 3H), 3.19 (s, 3H), 2.94 – 2.81 (m, 1H), 2.00 – 1.86 (m, 1H), 1.82 – 1.57 (m, 3H), 1.40 – 1.09 (m, 13H), 1.03 (d, J = 9.3 Hz, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 – 0.75 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 171.7, 168.9, 165.6, 158.7, 140.8, 130.5, 129.2, 120.9, 113.6, 75.7, 71.5, 70.9, 66.7, 57.4, 55.0, 50.8, 48.5, 44.5, 36.5, 33.6, 33.5, 31.9, 31.2, 29.4, 28.9, 26.8, 22.1, 19.5, 18.4, 18.2, 14.0, 13.2.

(3S,6R,14S,15S,E)-14-(2-((tert-Butyldimethylsilyl)oxy)ethyl)-3-isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (37a)

To a solution of compound **35** (133 mg, 0.19 mmol) in CH_2Cl_2/H_2O (6 mL/2 mL) was added DDQ (53 mg, 0.23 mmol). The mixture was stirred at room temperature for 1.5 h, and then diluted with ethyl acetate (50 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 3 to 100: 7) to obtain alcohol compound. To a solution of obtained compound (35 mg, 0.06 mmol) in DCM (1.5 mL), imidazole (24.5 mg, 0.36 mmol) and compound **36a** (27 mg, 0.18 mmol) were added. The mixture was stirred at room temperature for 3 h. The mixture was diluted with ethyl acetate (30 mL) and washed with 1% HCl aqueous solution (2 × 3 mL) and saturated aqueous $NaHCO_3$ (2 × 3 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was used next directly without further purification. The elimination precursor was dissolved in THF (2 mL), and DBU (36 μL, 0.24 mmol) was added. The mixture was stirred at room temperature for

2 h and diluted by ethyl acetate (30 mL). The organic phase was washed with 1% HCl aqueous solution (2 × 3 mL) and saturated aqueous $NaHCO_3$ (2 × 3 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 3) to obtained compound **37a** (18 mg, 48% for 3 steps) as a white powder $[\alpha]^{20}_D = -153.7$ (*c* = 0.1, DMSO); ν_{max} (KBr): 3296, 2931, 2858, 1736, 1621, 1524, 1465, 1383, 1255, 1099, 981, 889, 840 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.16 (d, *J* = 9.8 Hz, 1H), 7.59 (d, *J* = 5.4 Hz, 1H), 6.88 (d, *J* = 15.0 Hz, 1H), 6.22 (d, *J* = 15.0 Hz, 1H), 5.44 (s, 1H), 5.42 (s, 1H), 5.22 (d, *J* = 10.2 Hz, 1H), 4.34 – 4.24 (m, 1H), 4.21 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.58 (t, *J* = 7.0 Hz, 2H), 2.95 – 2.83 (m, 1H), 1.98 – 1.87 (m, 1H), 1.84 – 1.59 (m, 3H), 1.40 – 1.15 (m, 12H), 1.14 – 1.03 (m, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.88 – 0.80 (m, 16H), 0.02 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 171.6, 169.3, 166.6, 139.2, 138.2, 119.4, 116.5, 76.6, 61.0, 58.0, 51.4, 45.4, 34.1, 33.9, 32.9, 32.4, 31.6, 29.3, 27.1, 26.3, 22.5, 19.9, 18.7, 18.6, 18.5, 14.4, 13.6, -4.8, -4.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{32}H_{57}N_3NaO_6Si^+$, 630.3909, found, 630.3910. HPLC purity: 95.7%.

(3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-14-(2-((triisopropylsilyl)oxy)ethyl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (37b)

The titled compound **37b** was obtained following the procedure described for **37a**. Flash column chromatography (CH_2Cl_2: MeOH = 100:1 to 100:3); yield: 48%; white powder; $[\alpha]^{20}_D = -137.2$ (*c* = 0.12, DMSO). ν_{max}(KBr): 3317, 2959, 2936, 2866, 1738,

1673, 1623, 1526, 1464, 1381, 1259, 1105, 988, 881, 801 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.19 (d, J = 9.7 Hz, 1H), 7.59 (d, J = 5.3 Hz, 1H), 6.89 (d, J = 15.1 Hz, 1H), 6.19 (d, J = 15.1 Hz, 1H), 5.43 (s, 1H), 5.40 (s, 1H), 5.23 (d, J = 10.0 Hz, 1H), 4.34 – 4.24 (m, 1H), 4.21 (dd, J = 9.7, 7.6 Hz, 1H), 3.77 – 3.55 (m, 2H), 2.95 – 2.82 (m, 1H), 1.99 – 1.86 (m, 1H), 1.80 – 1.60 (m, 3H), 1.38 – 1.15 (m, 12H), 1.10 – 0.92 (m, 23H), 0.90 – 0.77 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.1, 168.8, 166.1, 138.8, 137.7, 118.9, 116.1, 76.0, 60.8, 57.5, 50.9, 44.8, 33.6, 33.4, 32.6, 31.9, 31.1, 28.8, 26.6, 22.0, 19.4, 18.2, 18.2, 17.8, 13.9, 13.2, 11.4. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{35}H_{63}N_3NaO_6Si^+$, 672.4378, found, 672.4383. HPLC purity: 96.8%.

(3S,6R,14S,15S,E)-3-Isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-14-(2-((triethylsilyl)oxy)ethyl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (37c)

The titled compound **37c** was obtained following the procedure described for **37a**. Flash column chromatography (CH_2Cl_2: MeOH = 100:1 to 100:3); yield: 35%; white powder; $[\alpha]^{20}_D$ = − 101.8 (c = 0.08, DMSO). ν_{max}(KBr): 3269, 2956, 2879, 1738, 1672, 1621, 1526, 1462, 1257, 1098, 989, 801 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.19 (d, J = 9.7 Hz, 1H), 7.59 (d, J = 5.4 Hz, 1H), 6.87 (d, J = 15.1 Hz, 1H), 6.27 (d, J = 15.1 Hz, 1H), 5.44 (s, 1H), 5.42 (s, 1H), 5.22 (d, J = 10.4 Hz, 1H), 4.33 (s, 1H), 4.20 (dd, J = 9.5, 7.8 Hz, 1H), 3.57 (t, J = 6.7 Hz, 2H), 2.94 (s, 1H), 1.99 – 1.86 (m, 1H), 1.81 – 1.60 (m, 3H), 1.39 – 1.15 (m, 12H), 1.12 – 1.02 (m, 1H), 0.99 – 0.71 (m, 18H), 0.54 (q, J = 7.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.3,

168.8, 166.2, 138.7, 137.8, 119.0, 115.9, 76.1, 60.0, 57.6, 50.9, 44.9, 33.6, 33.4, 32.5, 31.9, 31.1, 28.8, 26.7, 22.0, 19.4, 18.3, 18.1, 13.9, 13.1, 6.7, 3.9. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{32}H_{57}N_3NaO_6Si^+$, 630.3909, found, 630.3912. HPLC purity: 95.4%.

(2S,3S,4R)-Allyl-3-(((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoyl)oxy)-2-(2-(benzyloxy)ethyl)-4-methylnonanoate (38)

To a solution of acid **4a** (5.20 g, 15.3 mmol) and **6b** (2.89 g, 7.67 mmol) in CH_2Cl_2 (40 mL) were added DMAP (375 mg, 3.07 mmol) and DIC (4.75 mL, 30.7 mmol) under argon atmosphere at 20 ºC. The reaction mixture was stirred for 18 h, and diluted with CH_2Cl_2 (50 mL) then quenched with H_2O (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). And the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1) to obtain compound **38** (4.52 g, 84%) as a colorless oil. $[\alpha]^{20}_D$ = − 63.9 (*c* = 1.0, $CHCl_3$).ν_{max}(KBr): 3679, 3030, 2960, 2861, 1735, 1514, 1457, 1384, 1234, 1110, 1031, 988, 932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.66 – 7.58 (m, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.27 (m, 7H), 5.92 – 5.79 (m, 1H), 5.37 (d, *J* = 9.3 Hz, 1H), 5.30 (d, *J* = 16.2 Hz, 1H), 5.24 – 5.13 (m, 2H), 4.56 – 4.44 (m, 4H), 4.42 – 4.34 (m, 2H), 4.30 (dd, *J* = 9.3, 4.5 Hz, 1H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.56 – 3.47 (m, 1H), 3.46 – 3.38 (m, 1H), 3.08 – 2.98 (m, 1H), 2.23 – 2.12 (m, 1H), 2.02 – 1.90 (m, 1H), 1.85 – 1.72 (m, 2H), 1.59 (s, 1H), 1.43 (s, 1H), 1.37 – 1.18 (m, 12H), 1.03 – 0.95 (m, 3H), 0.93 – 0.80 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5,

172.6, 171.4, 156.4, 144.1, 144.0, 141.4, 138.3, 132.1, 128.5, 127.8, 127.8, 127.8, 127.2, 125.3, 120.1, 118.8, 78.2, 73.2, 67.6, 67.2, 65.5, 59.4, 47.3, 44.9, 34.9, 33.5, 31.9, 31.1, 29.5, 27.0, 22.8, 19.6, 17.3, 14.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{43}H_{55}NNaO_7^+$, 720.3871, found, 720.3875.

(R,E)-Allyl 4-((5R,8S,11S,12S)-12-(2-(benzyloxy)ethyl)-1-(9H-fluoren-9-yl)-8-isopropyl-5-methyl-11-((R)-octan-2-yl)-3,6,9-trioxo-2,10-dioxa-4,7-diazatridecanamido)-5-((tert-butyldiphenylsilyl)oxy)pent-2-enoate (39)

The ester **38** (4.5 g, 6.44 mmol) was dissolved in CH_2Cl_2 (40 mL) and diethylamine (20 mL) were added. After 2 h, the solvent was removed and the residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 10:1 to 2:1) to afford the amino ester as a colorless oil.

The obtained amine (1.74 g, 3.67 mmol) and Fmoc amino acid **5a** (1.37 g, 4.4 mmol) were dissolved in anhydrous CH_2Cl_2 (40 mL). And HOBt (594 mg, 4.4 mmol), EDCI (844 mg, 4.4 mmol), Et_3N (600 μL, 4.4 mmol) were added successively. The reaction mixture was stirred for 18 h and the solvent was removed. The residue was dissolved in CH_2Cl_2 (100 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate = 10: 1 to 5: 1) to obtain the amide as a colorless oil.

To a solution of obtained amide (2.74 g, 3.56 mmol) in anhydrous THF (36 mL), $Pd(PPh_3)_4$ (823 mg, 0.712 mmol) and N-methylaniline (772 μL, 7.12 mmol) were added. The reaction mixture was stirred for 1 h at room temperature, and diluted with

ethyl acetate (200 mL). The organic phase was washed by 1% HCl (2 × 60 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid.

The obtained acid above and amine **24a** (1.52 g, 3.72 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL), and DIEA (256 μL, 1.55 mmol), HOBt (502 mg, 3.72 mmol) and EDCI (713 mg, 3.72 mmol) were added successively. The reaction mixture was stirred for 18 h and the solvent was removed. The residue was diluted with CH_2Cl_2 (100 mL) and washed successively with 1 % HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and filtrated. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to obtain compound **39** (1.28 g, 31% for 4 steps) as a colorless oil. $[\alpha]^{20}_D = -6.9$ (c = 0.25, $CHCl_3$); ν_{max}(KBr): 3321, 2958, 2855, 1725, 1673, 1514, 1457, 1364, 1244, 1183, 1108, 989, 936, 859cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.67 – 7.52 (m, 8H), 7.46 – 7.35 (m, 10H), 7.25 (dt, J = 17.1, 7.4 Hz, 8H), 6.93 – 6.80 (m, 2H), 6.23 (d, J = 8.1 Hz, 1H), 6.07 (d, J = 7.9 Hz, 1H), 5.97 – 5.81 (m, 2H), 5.29 (dd, J = 17.2, 1.4 Hz, 1H), 5.21 (d, J = 10.4 Hz, 1H), 5.10 (dd, J = 13.6, 7.9 Hz, 1H), 4.72 (s, 1H), 4.59 (d, J = 5.6 Hz, 2H), 4.50 – 4.26 (m, 7H), 4.19 (, J = 14.7, 7.5 Hz, 1H), 3.66 (d, J = 4.1 Hz, 2H), 3.50 – 3.45 (m, 1H), 3.43 – 3.34 (m, 1H), 2.83 – 2.74 (m, 1H), 2.13 – 2.02 (m, 1H), 1.85 – 1.70 (m, 3H), 1.43 – 1.18 (m, 17H), 1.15 – 1.02 (m, 12H), 0.95 – 0.66 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.8, 170.4, 165.5, 156.2, 145.9, 144.0, 143.9, 141.4,

138.1, 135.7, 135.7, 132.8, 132.5, 132.1, 130.1, 128.5, 128.5, 128.0, 127.8, 127.2, 127.1, 125.3, 125.2, 122.2, 120.1, 118.4, 73.0, 67.2, 65.3, 65.2, 58.1, 51.8, 50.6, 47.2, 45.8, 34.9, 33.8, 31.9, 31.1, 29.6, 26.9, 26.8, 22.7, 19.5, 19.3, 18.9, 17.8, 14.5, 14.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{67}H_{85}N_3NaO_{10}Si^+$, 1142.5896, found 1142.5896.

(3S,6R,11R,14S,15S,E)-14-(2-(Benzyloxy)ethyl)-11-(((tert-butyldiphenylsilyl)oxy)methyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (40)

The compound **39** (1.19 g, 1.06 mmol) and $Pd(PPh_3)_4$ (243 mg, 0.21 mmol) were dissolved in anhydrous THF (10 mL), and N-methyl aniline (230 μL, 2.12 mmol) was added. After stirred at room temperature for 1.5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20: 1 to 1: 1) to afford the acid as pale yellow foam. The obtained acid was dissolved in CH_2Cl_2 (6 mL) and diethylamine (3 mL), the reaction mixture was stirred at room temperature for 3 h, and then the solvent was removed under reduced pressure to afford the crude amino acid. The mixture was dissolved in THF (850 mL), and then DIPEA (2.4 mL, 13.6 mmol) and HATU (2.59 g, 6.81 mmol) were added successively at 0 ºC. After stirred at room temperature for 12 h, the solvent was removed under reduced pressure, and then the residue was dissolved in ethyl acetate (500 mL) and washed successively with 1% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4. The solution was filtrated and concentrated under reduced pressure. The residue was purified by

column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 3) to obtain the cyclic peptide **40** (413 mg, 46% for three steps) as a white powder. $[\alpha]^{20}_D = -41.5$ (c = 0.12, DMSO); ν_{max} (KBr): 3322, 2933, 2858, 1726, 1673, 1514, 1467, 1364, 1244, 1183, 1108, 989, 936, 849cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, J = 9.8 Hz, 1H), 7.87 (d, J = 9.1 Hz, 1H), 7.70 – 7.58 (m, 4H), 7.53 (d, J = 5.7 Hz, 1H), 7.49 – 7.33 (m, 6H), 7.29 – 7.07 (m, 5H), 6.81 (dd, J = 15.1, 2.6 Hz, 1H), 6.04 (d, J = 15.1 Hz, 1H), 5.32 (d, J = 10.5 Hz, 1H), 4.71 (d, J = 6.8 Hz, 1H), 4.36 – 4.12 (m, 4H), 3.63 – 3.48 (m, 2H), 3.30 (dd, J = 13.1, 6.5 Hz, 2H), 2.83 (t, J = 7.8 Hz, 1H), 2.01 – 1.87 (m, 1H), 1.81 – 1.54 (m, 3H), 1.41 – 1.14 (m, 13H), 1.07 – 0.96 (m, 10H), 0.93 (d, J = 6.7 Hz, 3H), 0.88 – 0.72 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 171.4, 168.8, 165.8, 142.6, 138.3, 135.1, 135.1, 132.6, 132.6, 130.0, 128.1, 128.0, 127.3, 127.3, 119.5, 75.7, 71.9, 67.0, 65.6, 57.4, 51.1, 50.7, 44.6, 33.6, 33.4, 31.9, 31.2, 29.3, 28.9, 26.7, 26.6, 22.1, 19.5, 18.8, 18.4, 18.2, 14.0, 13.2. HRMS–MALDI (m/z): [M + Na]$^+$ calcd for $C_{67}H_{85}N_3NaO_{10}Si^+$, 1142.5896, found, 1142.5896.

(3S,6R,11R,14S,15S,E)-14-(2-(Benzyloxy)ethyl)-11-(hydroxymethyl)-3-isopropyl-6-methyl-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (41)

To a solution of compound **40** in THF (4 mL) were added HOAc (65 μL, 1.14 mmol) and TBAF (360 mg, 1.14 mmol). The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (60 mL). The organic phase was washed with H_2O (20 × 3 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 3 to 100: 5) to obtain

compound **41**(165 mg, 72%) as a white powder. $[\alpha]^{20}_{D}$ = − 49.7 (*c* = 0.31, DMSO); ν_{max} (KBr): 3305, 2959, 2930, 2859, 1734, 1675, 1534, 1459, 1387, 1256, 1081, 979, 912, 850 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, *J* = 9.0 Hz, 1H), 7.43 – 7.19 (m, 5H), 6.96 (d, *J* = 15.2 Hz, 1H), 6.10 (d, *J* = 15.2 Hz, 1H), 5.54 (d, *J* = 10.7 Hz, 1H), 4.72 (s, 1H), 4.62 – 4.41 (m, 3H), 4.36 (d, *J* = 6.8 Hz, 1H), 3.65 – 3.45 (m, 4H), 2.97 (t, *J* = 9.4 Hz, 1H), 2.06 (dd, *J* = 13.0, 6.4 Hz, 1H), 1.87 (s, 2H), 1.75 (s, 1H), 1.50 – 1.21 (m, 13H), 1.18 – 1.10 (m, 1H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.96 (t, *J* = 7.3 Hz, 6H), 0.92 – 0.86 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.6, 170.5, 169.1, 146.2, 139.6, 129.4, 129.1, 128.8, 119.8, 78.1, 74.0, 68.3, 64.7, 59.4, 53.6, 52.7, 46.8, 35.4, 35.3, 33.7, 32.9, 30.9, 30.6, 28.5, 23.7, 20.2, 18.9, 18.9, 14.4, 13.6. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{33}H_{51}N_3NaO_7^+$, 624.3619, found, 624.3622. HPLC purity: 98.2%.

(3S,6R,14S,15S,E)-14-(2-(benzyloxy)ethyl)-3-isopropyl-6-methyl-11-methylene-15-((R)-octan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (42)

To a solution of compound **41** (40 mg, 0.066 mmol) in THF (3 mL), then triethyl amine (55 μL, 0.40 mmol) and methanesulfonyl chloride (23 μL, 0.20 mmol) were added at 0 ºC. After stirred for 30 min, the reaction solution was quenched by addition of water (0.1 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was dissolved in THF (3 mL). To the resulting solution was added DBU (105 μL, 0.70 mmol) at 20 ºC. After stirred for 2 h, the reaction was quenched by addition of 1 % HCl (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed

with saturated aqueous $NaHCO_3$ (3 × 3 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 100: 1 to 100: 3) to obtain **42** (21 mg, 52% for two steps) as a white solid. $[\alpha]^{20}_D = -161.8$ (*c* = 0.11, DMSO); ν_{max} (KBr): 3257, 2959, 2929, 2861, 1734, 1673, 1622, 1520, 1459, 1379, 1257, 1199, 1105, 983, 900, 862, 805 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.12 (d, *J* = 9.7 Hz, 1H), 7.60 (d, *J* = 4.6 Hz, 1H), 7.41 – 7.16 (m, 5H), 6.86 (d, *J* = 15.3 Hz, 1H), 6.29 (d, *J* = 15.0 Hz, 1H), 5.44 (s, 1H), 5.37 (s, 1H), 5.22 (d, *J* = 10.1 Hz, 1H), 4.50 – 4.39 (m, 2H), 4.38 – 4.24 (m, 1H), 4.19 (t, *J* = 8.5 Hz, 1H), 3.41 (s, 2H), 3.00 (s, 1H), 1.91 (dd, *J* = 13.3, 6.4 Hz, 1H), 1.75 (s, 3H), 1.35 – 1.16 (m, 12H), 1.07 (s, 1H), 0.96 (d, *J* = 6.3 Hz, 3H), 0.92 – 0.76 (m, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.3, 168.7, 166.3, 138.7, 138.4, 137.6, 128.2, 127.5, 127.4, 118.9, 115.9, 76.3, 72.1, 67.1, 57.6, 51.0, 45.1, 33.6, 33.5, 32.0, 31.2, 29.3, 28.9, 26.7, 22.0, 19.4, 18.3, 18.1, 14.0, 13.2. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{33}H_{49}N_3NaO_6^+$, 606.3514, found, 606.3518. HPLC purity: 99.2%.

(3R,6S,14R,15R,E)-3-isopropyl-14-(2-((4-methoxybenzyl)oxy)ethyl)-6-methyl-11-methylene-15-((S)-pentan-2-yl)-1-oxa-4,7,12-triazacyclopentadec-9-ene-2,5,8,13-tetraone (44)

The titled compound **44** was obtained following the procedure described for **42**. Flash column chromatography (CH_2Cl_2: MeOH = 100:1 to 100: 3); yield: 43% for 2 steps; white powder; $[\alpha]^{20}_D = +127.9$ (*c* = 0.09, DMSO). ν_{max} (KBr): 3305, 2960, 2928, 2863, 1731, 1676, 1612, 1514, 1259, 1097, 1028, 805 cm^{-1}; ^1H NMR (400 MHz,

CD_3OD) δ 7.24 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 15.0 Hz, 1H), 6.86 (d, J = 8.3 Hz, 1H), 6.22 (d, J = 15.1 Hz, 1H), 5.52 – 5.40 (m, 1H), 4.49 – 4.34 (m, 2H), 3.56 – 3.43 (m, 2H), 3.00 (td, J = 10.2, 3.8 Hz, 1H), 2.13 – 2.02 (m, 1H), 1.96 – 1.78 (m, 3H), 1.48 – 1.24 (m, 9H), 1.20 – 1.09 (m, 1H), 1.04 (d, J = 6.8 Hz, 3H), 0.95 (t, J = 6.2 Hz, 6H), 0.91 – 0.82 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 174.3, 170.5, 169.5, 160.9, 141.8, 138.7, 131.4, 130.8, 120.0, 118.7, 114.8, 78.4, 73.9, 68.0, 59.5, 55.7, 53.0, 47.5, 37.5, 35.1, 33.7, 31.0, 21.5, 20.2, 18.9, 18.8, 14.5, 13.6. HRMS–MALDI (m/z): $[M + Na]^+$ calcd for $C_{31}H_{45}N_3NaO_7^+$, 594.3150, found, 594.3152. HPLC purity: 99.3%.

2. Experimental Procedure for the Cytotoxicity Assay. The human leukemia cell line K562 was purchased from American Type Culture Collection (ATCC, Rockville, MD). K562 cells were maintained in RPMI medium 1640 with 10% FBS at 37 ºC in a humidified atmosphere of 5% CO_2. Briefly, 1×10^4 exponentially growing cancer cells were seeded into 96-well culture plates. Then serially diluted compounds were added. After 72 hours, MTT was added into each well with a final concentration of 0.5 mg/mL. Then cells were incubated at 37 ºC, 5% CO_2 for 4 hours. The culture medium was then removed and formazan crystals were diluted in DMSO. The absorbance was measured at 570 nm. The IC_{50} values were calculated by Graphed prism 5 software.

3. Evaluation hypoxia selectivity cyctoxicity of vinylamycin analogs. The human breast cancer cell line MCF-7 was purchased from American Type Culture Collection (ATCC, Rockville, MD). Human breast cancer cell line MCF-7

was seeded in duplicated 96-well microplates at a density of 2×10^4 cells/100 μL medium and preincubated for 24 h to ensure complete adherence to the substratum. To these microcultures serially diluted sample solutions were added, and were separately incubated in either a normoxic (20% O_2) or hypoxic condition (1% O_2). Hypoxic conditions were achieved using a MIC-101 modular incubator chamber (Billups Rosenberg, Del Mar, CA, USA) equipped with an oxygen indicator. After 72 h of incubation, cytotoxicity at both the oxygenation conditions was compared by the MTT method under normoxic conditions.

4. Apoptosis induction assay of compound 1a and 33a in K562. K562 cells at a concentration of 1×10^5 cells/well were seeded in a 12-well plate. An annexin V-FITC/PI double-staining apoptosis assay was performed on these cells. The cells were treated with tested compounds for 24 h and 48 h and then collected cells washed twice with PBS buffer. We resuspended K562 cells in 1× binding buffer with 1×10^6 cells/mL and transferred 100 μL of 1× binding buffer with 1×10^5 cells/mL to a 5 mL fluorescence-activated cell sorting (FACS) tube. The following steps were performed in the dark. To the cells suspension was added 5 μL of annexin V-FITC and 10 μL of PI which was incubated for 15 min. Then added 400 μL of 1× binding buffer, samples were analyzed by flow cytometry within 1 h.

5. Experiment Procedure for the Antibacterial Activity Test. Minimum inhibitory concentration (MIC) was determined by the microdilution method in 96-well plates. *S. aureus* strains were cultured at 37 ℃ for 18 h in MH culture medium. Then *S. aureus* strains were diluted 50-fold in fresh MH culture medium,

and incubated at 37 °C for 2.5 to 3.0 h until the culture medium reached OD_{600} 0.8 – 1.0 (0.8×10^9 CFU/mL to 1×10^9 CFU/mL). Bacteria were serially diluted ten-fold with MH culture medium, 100 μL of bacteria into 900 μL of MH culture medium, until the concentration of the bacteria reached around 1×10^4 CFU/mL. To each well of the 96-well plate, aliquots of 150 μL the diluted bacteria were added. The tested compounds were serially diluted two-fold in 150 μL of bacteria solution, varying from 64 μg/mL to 0.0625 μg/mL. After incubation at 37 °C for 24 h, the MICs were determined according visual turbidimetry method.

6. Rat Plasma Stability Assay. ACN was purchased from Fisher Scientific and used without further purification. Rat plasma was collected in SD rat and diluted to 50% in pure water. Analytical HPLC was performed on a Shimadzu LD-20A HPLC using a Venusil MP C18(2) C18 column (5 μm, 4.6 mm × 250 mm) and H_2O/ACN as eluents. A solution of 1 mg/mL of each detected compound was prepared in ACN, and 40 μL aliquots were mixed with 1 mL of prewarmed (37 °C) plasma. At selected time points (0, 30, 60, 120, 240, 480, 1440 min), samples (100 μL) were collected and mixed with a solution of internal standard in ACN (250 μL) to precipitate plasma proteins which were deleted by centrifugation at 12000 rpm for 15 min. The supernatant was analyzed by HPLC using Venusil MP C18(2) C18 column (5 μm, 4.6 mm × 250 mm) and a gradient of ACN/H_2O (45% for vinylamycin; 95% for compound **1a** and **1e**) at a flow rate of 1 mL/min. The content of the test compound was determined by the internal standard method. Internal standard compound was Carbamazepine, n-EtOTBDPS and MeOTBDPS respectively.

7. Inhibitory activity in xenograft zebrafish model. The zebrafish of wild-type AB strain were randomly assigned to 6-well plates (30 fish per well) and treated with compound **1a** and imatinib at different concentration. The state of zebrafish was observed, and MTC was determined. The CM-Dil-labeled human chronic myeloid leukemia (K562) cells were transplanted into the zebrafish yolk of 2 dpf wild-type AB strain by microinjection. About 200 cells were transplanted into the zebrafish to establish the CML tumor transplantation model. Zebrafish injected with K562 cells were placed at 35 °C to 3 dpf. The zebrafish were randomly assigned to 6-well plates and were administered with compound **1a** at concentrations of 0, 5.6, 16.7 and 50 μg/mL, respectively. The positive control group was added 50 μg/mL imatinib. Every group had 30 zebrafish per well (3 mL per well) and administrated one time during experiment. The zebrafish were incubated at 35 °C for 2 days, and 10 zebrafish were randomly selected to observe, photographed, and preserved in a fluorescence microscope. The images were collected using Nikon NIS-Elements D 3.10 image analysis software to calculate the fluorescence intensity of cancer cells, respectively, to evaluate the fluorescence intensity of compound **1a** on zebrafish human chronic myeloid leukemia (K562) transplant tumor inhibition. The cancer inhibition calculated as following formulation: Inhibition(%) = (1-S(sample)/S(blank control))× 100%.

ASSOCIATED CONTENT

Supporting information

This material is available free of charge via the Internet at http://pubs.acs.org/.

Copies of $^{1}H/^{13}C$ NMR spectra, inhibitory curves of tested compound against K562 cell line and inhibitory curves of hypoxia selectivity of tested compounds. (PDF)

SMILES strings for vinylamycin, microtermolides A, compounds **1a-f**, **28a**, **28b**, **32a**, **33a-h**, **34**, **37a-c**, **41**, **42** and **44**. K562 IC_{50} values for vinylamycin, microtermolides A, compounds **1a-f**, **28a**, **28b**, **32a**, **33a-h**, **34**, **37a-c**, **41**, **42** and **44**. *Staphylococcus aureus* MIC values for vinylamycin, compounds **1a**, **1e** and **33a**. MCF-7 IC_{50} values under normoxia and hypoxia for vinylamycin, microtermolides A, compounds **1a-d**, **1f** and **28b**. (CSV)

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NOTES

The authors declare no competing financial interest.

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ABBREVIATIONS USED

EDCI, 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochlide; HOBt, 1-hydroxybenzotriazole; TBDPS, *tert*-butyldiphenylsilyl; DIPEA, *N*,*N*-diisopropylethylamine; MsCl, methanesulfonyl chloride; HATU, o-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIC, N,N-diisopropylcarbodiimide; TES, triethylsilyl; MTC, maximum tolerated concentration; ACN, acetonitrile.

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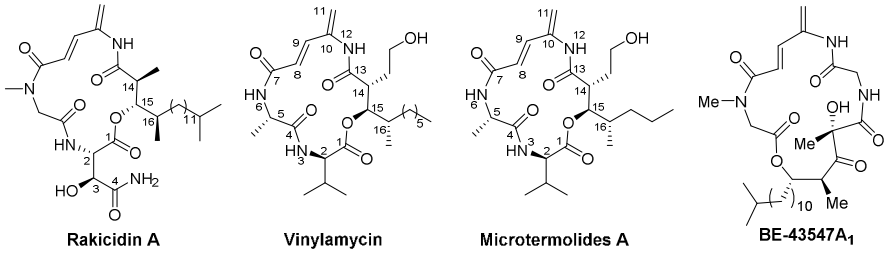
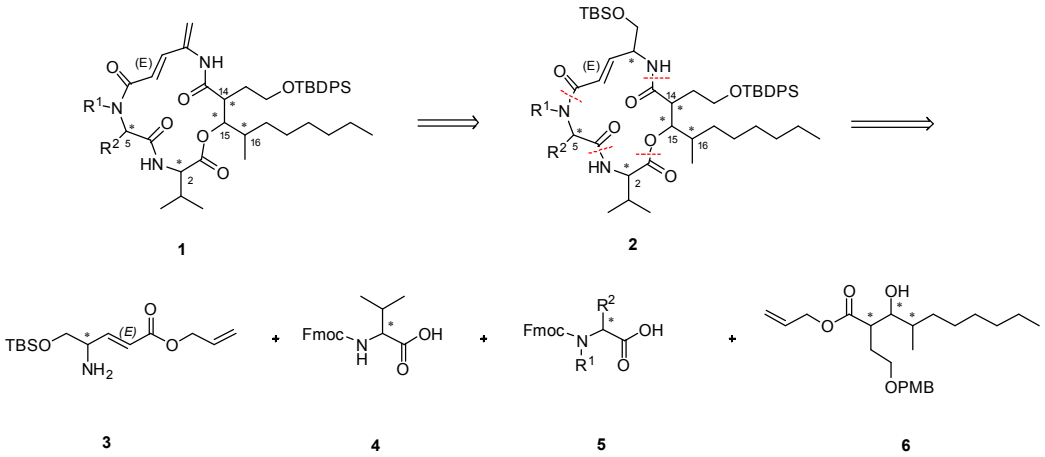
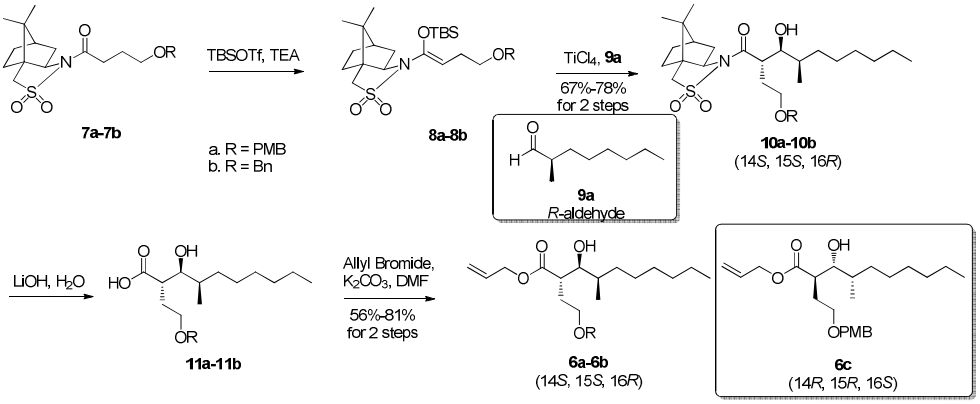


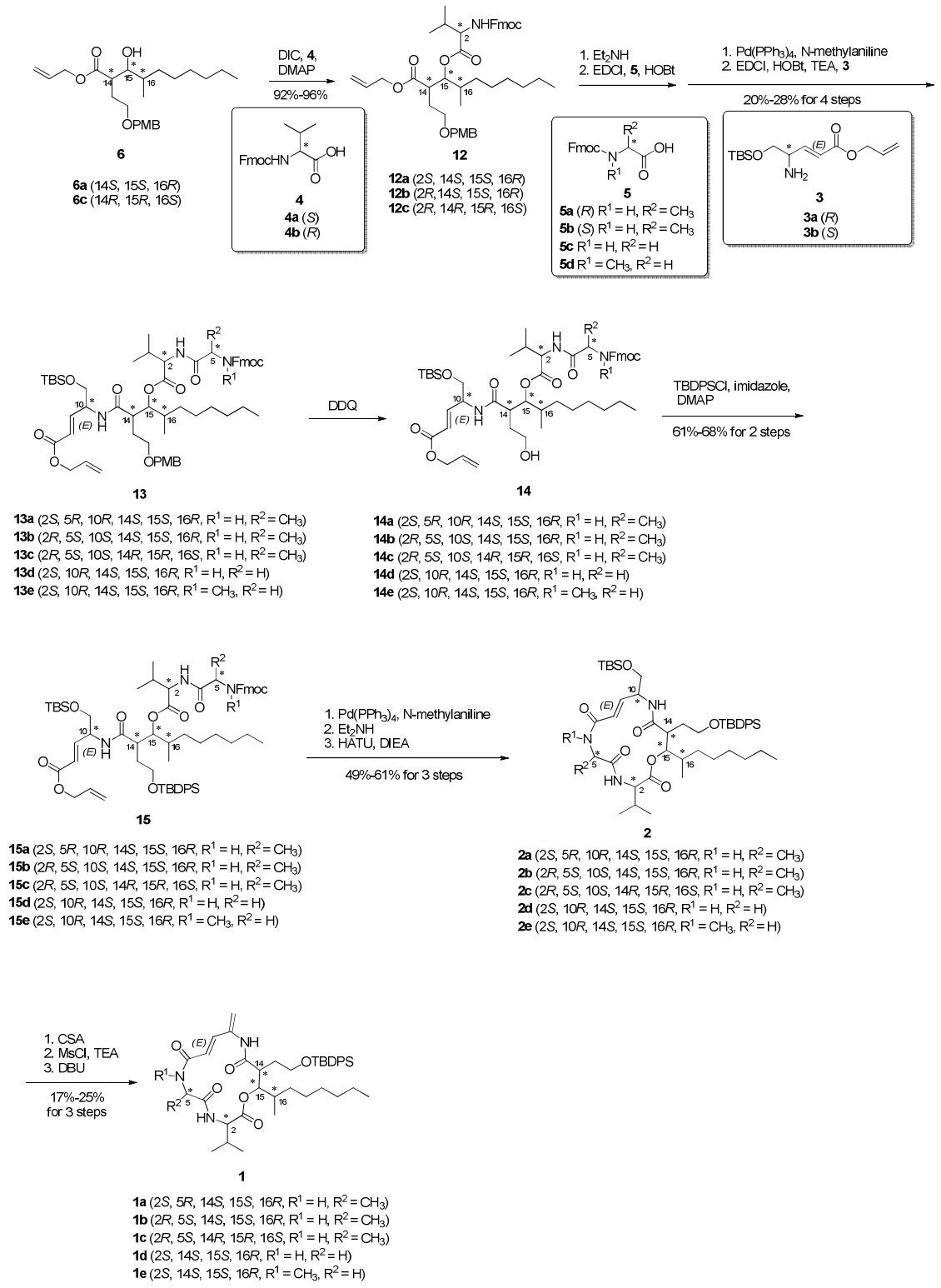
Scheme 1. Structure of rakicidin A, vinylamycin, microtermolide A, and BE-43547A$_1$.



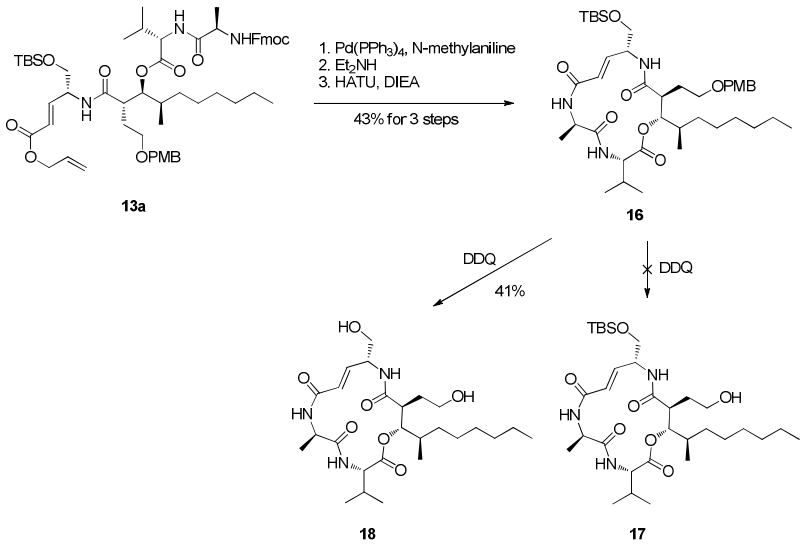
Scheme 2. Retrosynthetic analysis of O-TBDPS-vinylamycins.



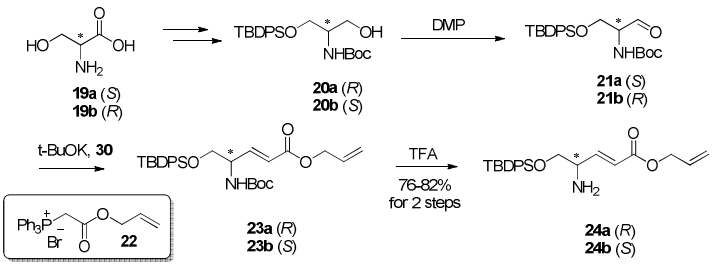
Scheme 3. Synthesis of the polyketide fragment of the vinylamycin analogs.



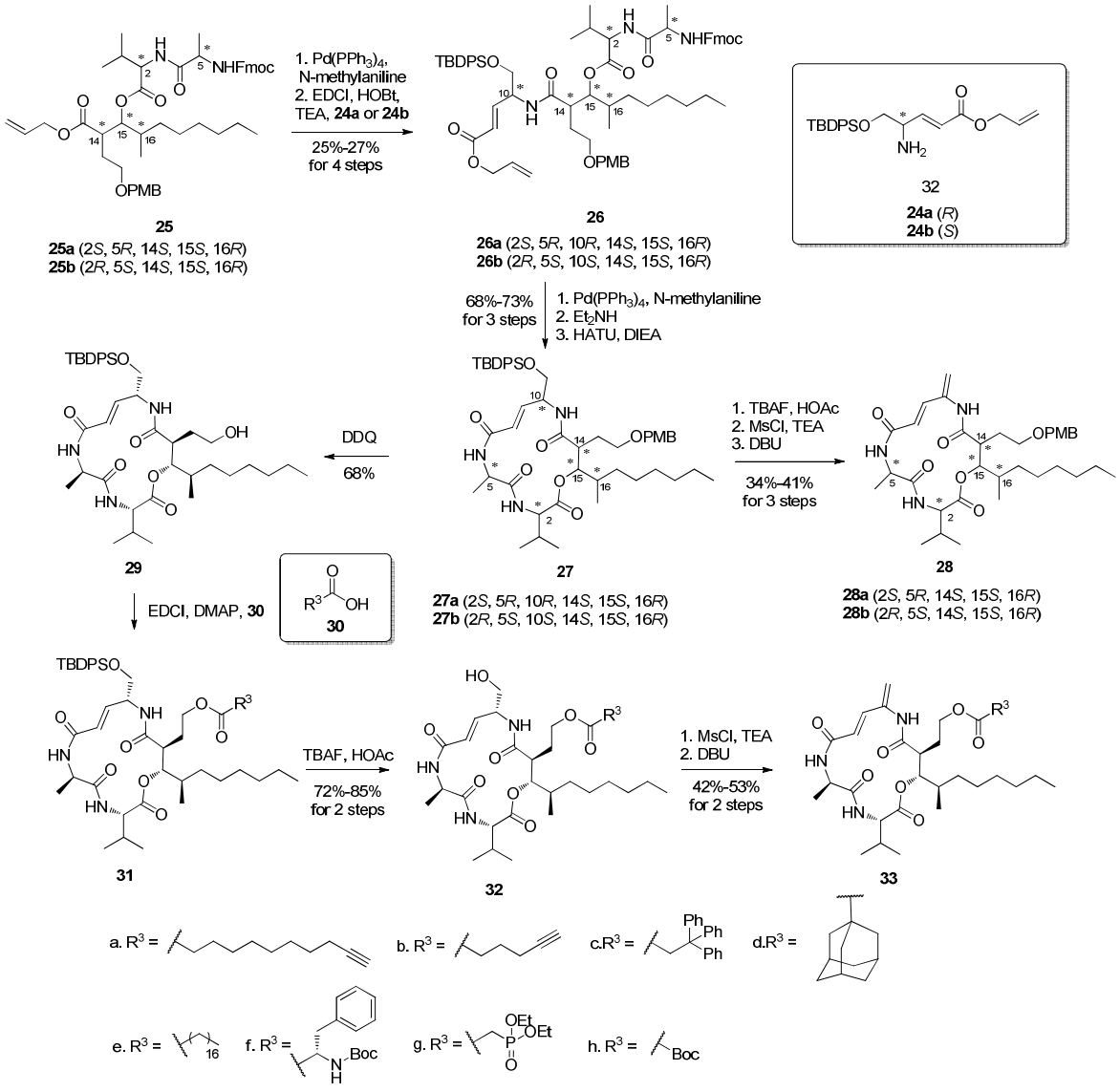
Scheme 4. Synthesis of O-TBDPS vinylamycin analogs with different configurations.



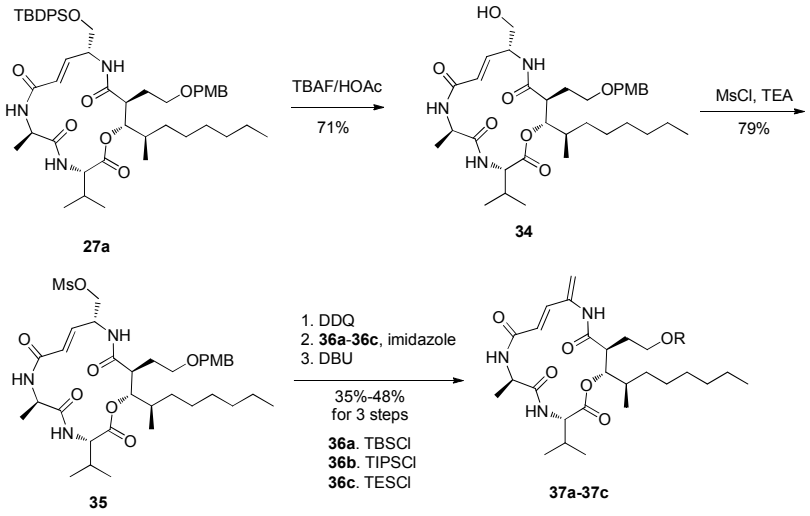
Scheme 5. Synthesis of intermediates for construction of a library of vinylamycin analogs.



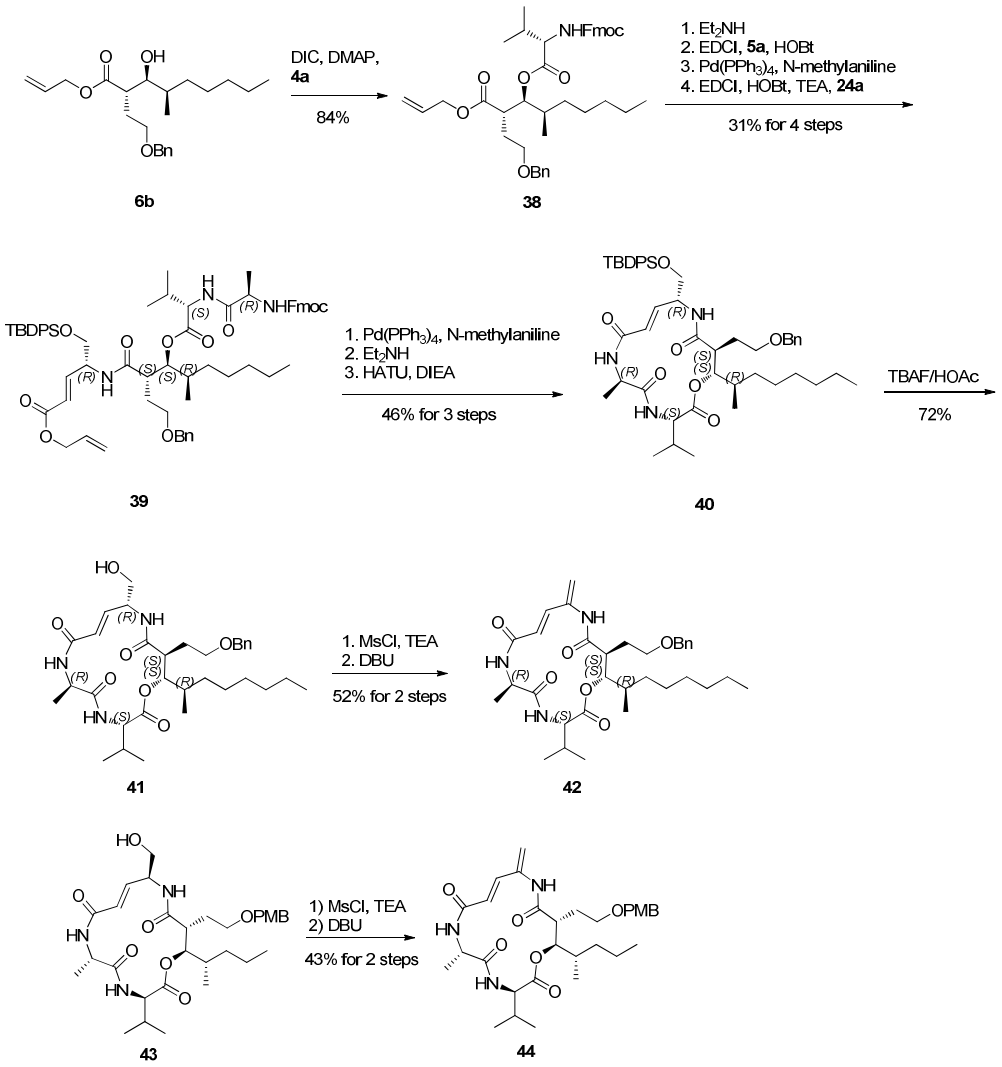
Scheme 6. Synthesis of a TBDPS-protected serinol fragment.



Scheme 7. Synthesis of vinylamycin ester analogs and O-PMB-vinylamycin analogs.

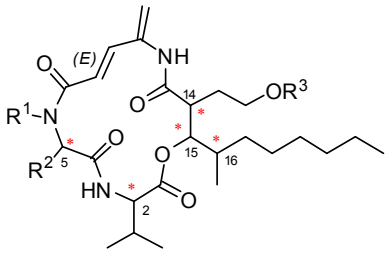


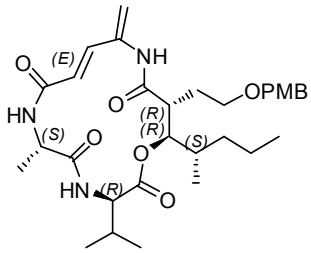
Scheme 8. Synthesis of vinylamycin silyl ether analogs.



Scheme 9. Synthesis of Bn ether of vinylamycin and PMB ether of microtermolide A.

Table 1. Inhibitory activity of vinylamycin analogs against K562 cells[a,b]





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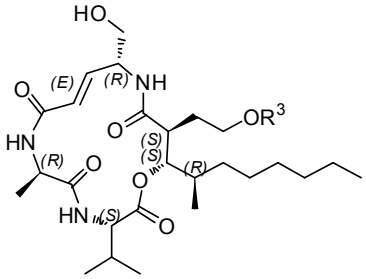
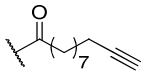
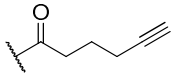
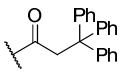
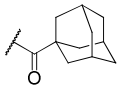
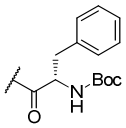
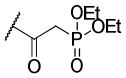
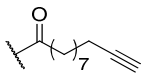


32a, **34** and **41**

Compound	Configuration	R^1	R^2	R^3	IC_{50} (μM)[c]
Vinylamycin	2*R*, 5*S*, 14*R*, 15*R*, 16*S*	H	CH_3	H	4.86 ± 0.52
Microtermolides A	2*R*, 5*S*, 14*R*, 15*R*, 16*S*	H	CH_3	H	> 50
1a	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3	TBDPS	0.64 ± 0.07
1b	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	TBDPS	0.88 ± 0.09
1c	2*R*, 5*S*, 14*R*, 15*R*, 16*S*	H	CH_3	TBDPS	1.27 ± 0.14
1d	2*S*, 14*S*, 15*S*, 16*R*	H	H	TBDPS	2.31 ± 0.19
1e	2*S*, 14*S*, 15*S*, 16*R*	CH_3	H	TBDPS	0.4 ± 0.07
1f	2*R*, 5*S*, 14*R*, 15*R*, 16*R*	H	CH_3	TBDPS	1.78 ± 0.33
28a	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3	PMB	2.49±0.14
28b	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	PMB	4.6 ± 0.39
33a	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		4.41 ± 0.28
33b	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		8.00 ± 0.69
33c	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		3.79 ± 0.22
33d	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		7.72 ± 0.69

33e	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		> 50
33f	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		5.95 ± 0.32
33g	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		7.90 ± 0.43
33h	2*S*, 5*R*, 14*S*, 15*S*, 16*R*	H	CH_3		5.43 ± 0.95
37a	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	TBS	2.31 ± 0.25
37b	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	TIPS	1.05 ± 0.14
37c	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	TES	1.54 ± 0.11
42	2*R*, 5*S*, 14*S*, 15*S*, 16*R*	H	CH_3	Bn	3.06 ± 0.32
44					13.69 ± 1.85
32a					17.88 ± 1.98
34				PMB	21.36±1.25
41				Bn	35.65 ± 9.4
Imatinib mesylate					0.31 ± 0.05

[a]All values are the mean of three independent experiments and reported as Mean ± SD. [b]K562: cultured chronic myeloid leukemia cell line. [c]IC_{50}: 50% cytotoxic concentration.

TBDPS: tert-butyldiphenylsilyl; PMB: p-methoxybenzyl.

Table 2. Antimicrobial activity of vinylamycin analogs against *Staphylococcus aureus*

Compound	MIC (μg/mL)
Ciprofloxacin	0.5
Vinylamycin	8
1a	> 64
1e	> 64
33a	> 64

MIC: minimal inhibitory concentration.

Table 3. Hypoxia selectivity of vinylamycin analogs[a]

Compound	MCF-7 cell line		
	Normoxia (μM)	Hypoxia (μM)	Selectivity Index[b]
Rakicidin A	0.30 ± 0.03	0.08 ± 0.002	0.27
Vinylamycin	12.33 ± 1.55	9.17 ± 1.37	0.74
Microtermolides A	>20	>20	N.A.[c]
Methyl ester of Rakicidin A	0.32 ± 0.11	0.12 ± 0.006	0.38
1a	6.26 ± 0.52	6.81 ± 1.32	1.09
1b	3.75 ± 0.84	2.93 ± 0.11	0.78
1c	5.11 ± 0.10	7.02 ± 1.61	1.37
1d	3.49 ± 0.22	4.69 ± 0.35	1.34
1f	2.98 ± 0.46	2.69 ± 0.48	0.90
28b	10.08 ± 0.22	7.98 ± 0.71	0.79
Gemcitabine	0.57 ± 0.15	8.90 ± 1.41	15.6
Paclitaxel	0.037 ± 0.05	3.40 ± 1.21	91.9

[a]All values are the mean of three independent experiments and reported as Mean ± SD. [b]Index of Hypoxia selectivity was calculated from: IC_{50}(hypoxia) / IC_{50}(normoxia). [C]N.A.: Not available.

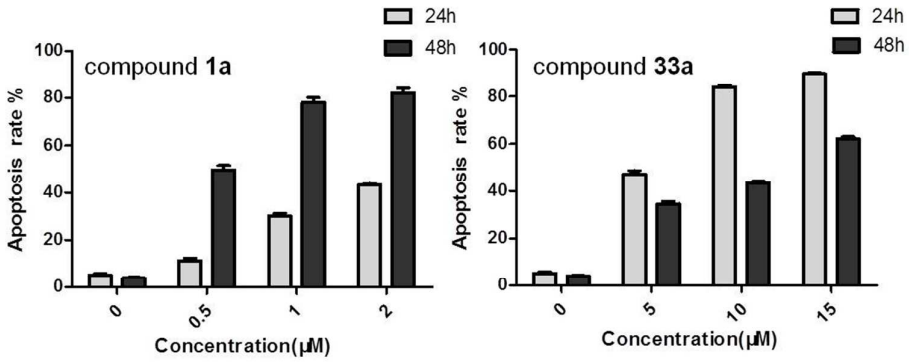


Figure 1. Apoptosis induced by TBDPS analog **1a** and ester analog **33a** at various concentrations in K562 cells after 24 h (gray bars) and 48 h (black bars) of treatment. All values are the mean of three independent experiments and the error is SD.

Table 4. Safety evaluated *in vivo* by zebrafish model[a]

concentration	compound **1a**		imatinib	
(μg/mL)	death number	mortality rate	death number	mortality rate
0.8	0	0	0	0
4	0	0	0	0
20	0	0	0	0
50	0	0	0	0
100	insoluble	N.A.[b]	30	100

[a]Every group contains 30 zebrafish. [b]N.A.: Not available.

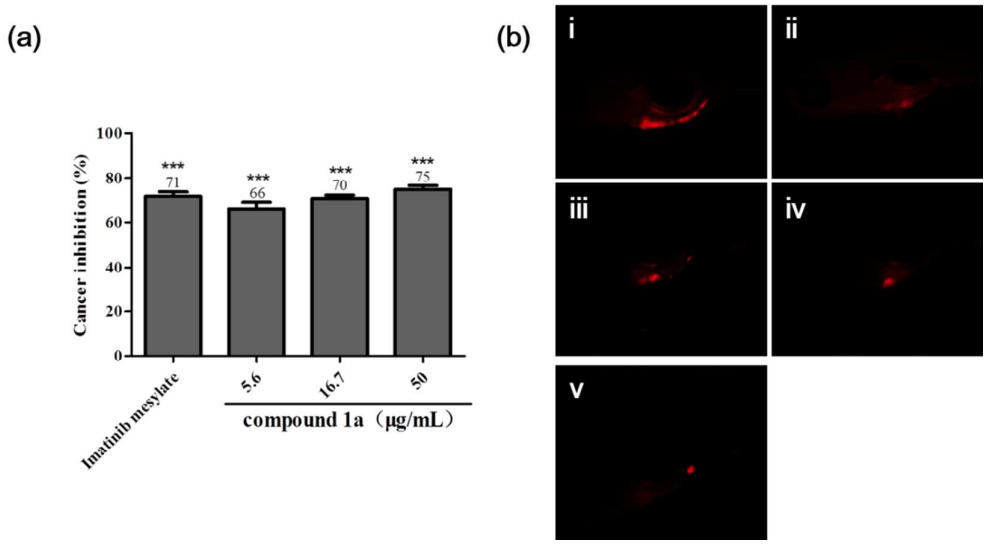


Figure 2. In vivo inhibitory activity of K562 cells xenografted zebrafish. Zebrafish were xenografted with fluorescently labeled K562 cells in the perivitelline space. Effects of drug treatment on K562 xenografts in zebrafish administrated with 0.1% DMSO aqueous solution control or detected compound for 3 d. (a) cancer inhibitory of 50 μg/mL imatinib mesylate, 5.6 μg/mL, 16.7 μg/mL and 50 μg/mL compound **1a** (n = 10, *** $p < 0.001$ *vs* control). (b) fluorescent images of zebrafish (i) 0.1% DMSO control, (ii) 50 μg/mL Imatinib mesylate, (iii) 5.6 μg/mL compound **1a**, (iv) 16.7 μg/mL compound **1a** and (v) 50 μg/mL compound **1a**.

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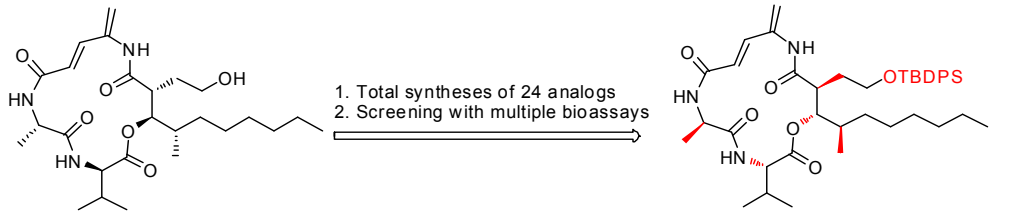

Vinylamycin

1. Anti-microbial activity
2. Inhibition of cancer cell growth
3. Stability in plasma: $t_{1/2}$ = 0.54 h

1a TBDPS-ent-Vinylamycin

1. No anti-microbial activity
2. Improved inhibition of cancer cell growth
3. Good *in vivo* efficacy in xenograft zebrafish model with K562 cells.
4. Superior stability in plasma: $t_{1/2}$ = 14.3 h
5. Maintains activity at hypoxia condition