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Antineoplastic Agents. 600. From the South Pacific Ocean to the Silstatins

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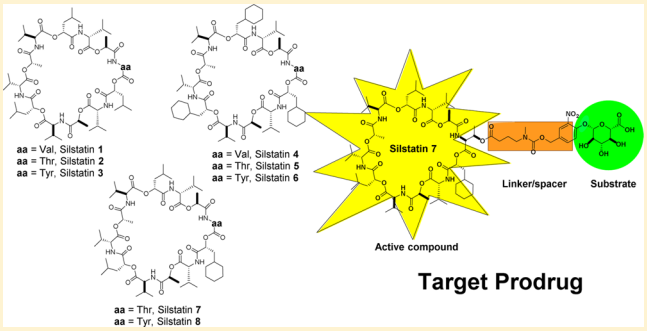
Supporting Information

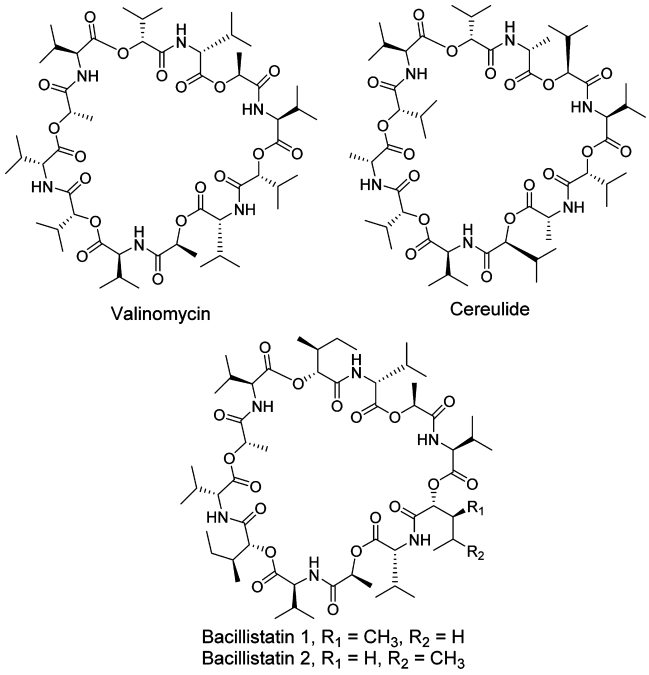
ABSTRACT: The recent advances in the development of antibody and other drug conjugates for targeted cancer treatment have further increased the need for powerful cancer cell growth inhibitors. Toward that objective we have extended our earlier discovery of the remarkable anticancer bacillistatins 1 and 2 from *Bacillus silvestris* to SAR and other structural modifications such as availability of a free hydroxy group for antibody−drug conjugate (ADC) and other prodrug linkage. That direction has resulted in seven structural modifications designated silstatins 1−8 (**7a**, **8a**, **8b**, **14a**, **15a**, **15b**, **18a**, and **18b**), where the exceptional cancer cell growth inhibition of some of them are in the range GI_{50} 10^{-3}−10^{-4} μM/mL. Silstatin 7 (**18a**) was converted to a glucuronic conjugate (**28**) that displayed an impressive reduction in toxicity during transport.



In 2009 we reported the isolation and structures of two very potent cancer cell growth inhibitors, cyclodepsipeptides designated bacillistatins 1 and 2[1,2] from *Bacillus silvestris* carried by a South Pacific (Chile) crab. Subsequently, we completed the total synthesis of bacillistatin 2.[2] Meanwhile, the promise of marine-derived microorganisms as productive sources of new anticancer and antiproliferative drugs continues to expand. Recent examples include discoveries of marine microorganisms containing small-molecule cancer cell growth inhibitors,[3a−h] antibiotics from bacteria,[4a−f] antibiotics from fungi,[5a−f] various inhibitors from cyanobacteria,[6a−c] and the increasing potential of deep-sea microorganisms.[7a−d] Structurally, the bacillistatins are similar to valinomycin, a well-known antibiotic and cytotoxic cyclodepsipeptide that acts as a carrier-type potassium ionophore. Another cyclic depsipeptide that resembles the structures of valinomycin and bacillistatins is cereulide, a toxin isolated in 1995 from *Bacillus cereus*.[8]

The cancer cell growth inhibition values for these molecules are on the order of 10^{-4}−10^{-5} μg/mL, which makes them good candidates as therapeutic agents for cancer treatment, especially when linked to monoclonal antibodies. That is a powerful technique to reduce side effects and increase selectivity as well as other targeting systems that allow the release of the drug, from a nontoxic precursor (prodrug), in the solid tumor microenvironment or inside the cancer cells. One of the major obstacles for this approach with the bacillistatins is the lack of easily derivatizable groups (e.g., $-NH_2$, $-SH$, $-OH$, $-COOH$, etc.) necessary for conjugation of these K^+ ionophores with antibodies/carriers.

Therefore, we undertook the synthesis and biological evaluation of a series of structural modifications of the bacillistatins containing hydroxy groups that could serve as coupling handles. Subsequent results showed that managing the overall lipophilicity of the modified bacillistatins containing a polar group (hydroxy) can lead to compounds with high cancer cell growth inhibition. Also it can provide useful bacillistatin conjugates such as the glucuronide prodrug of structure **18a**.



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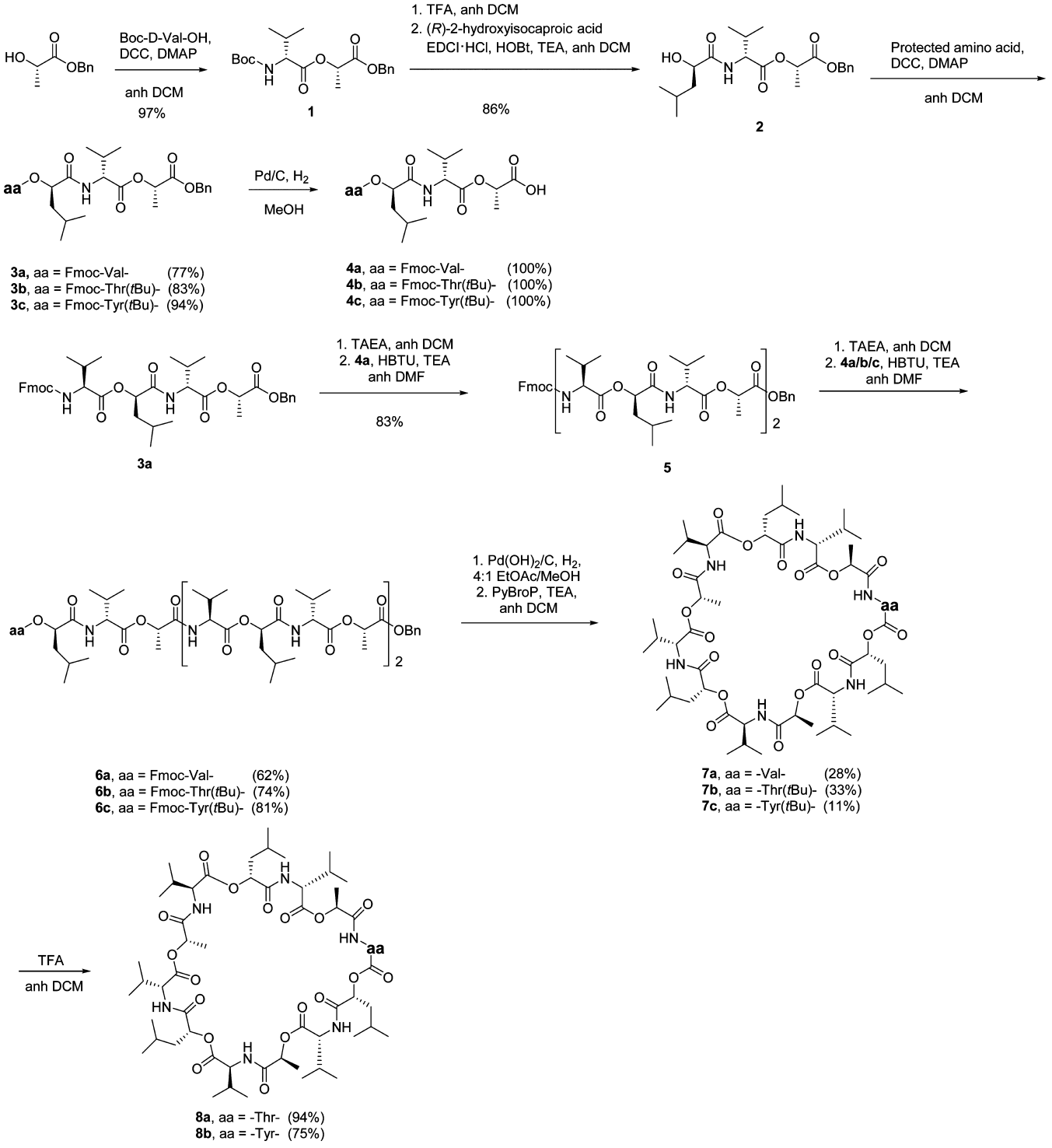
Scheme 1. Synthesis of Silstatins 1, 2, and 3 (7a, 8a, and 8b)



RESULTS AND DISCUSSION

The structure of bacillistatin 1 consists of six amino acids (three with L configuration and three with D configuration) and six α-hydroxy acids (three with L configuration and three with D configuration). These 12 units are symmetrically arranged along the structure, thus forming three principal fragments (with four units each) that are attached head to tail and cyclized. Using this analysis we proceeded to synthesize fragments containing four units that consisted of two amino acids, one with L and one with the D configuration, and two α-hydroxy acids, one with L and one with the D configuration in alternating positions. The L amino acid was used as the N terminus of each fragment to produce each fragment sequence as L-(amino acid)-D-(α-hydroxy acid)-D-Val-L-lactic acid.

The first series of new structures (**7a**, **8a**, and **8b**, named silstatins 1, 2, and 3, respectively) was synthesized as shown in Scheme 1. First, compound **1** was prepared by coupling benzyl lactate with Boc-D-Val-OH using dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP) in high yield. Compound **1** was N-deprotected using

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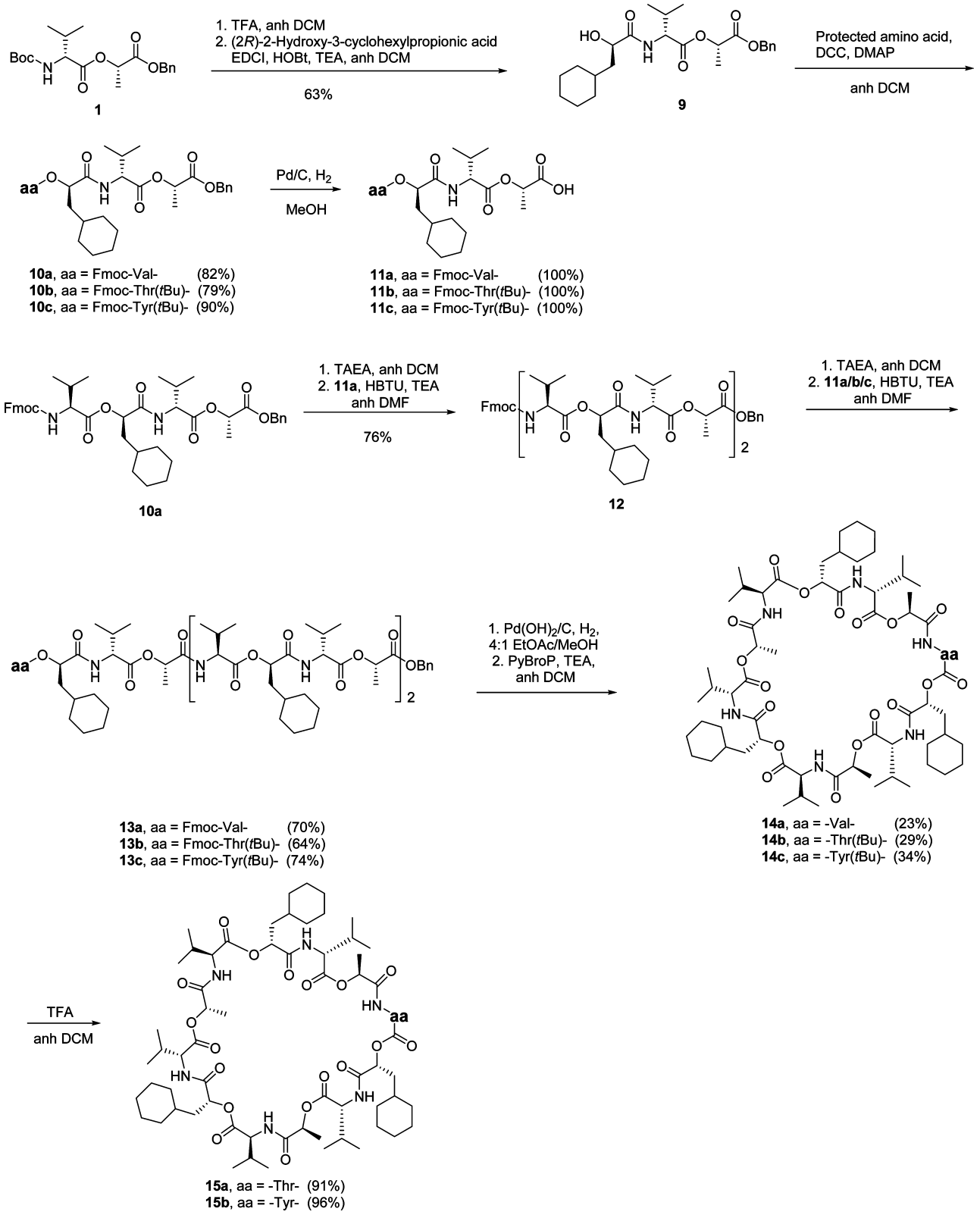
Scheme 2. Synthesis of Silstatins 4, 5, and 6 (14a, 15a, and 15b)



trifluoroacetic acid (TFA) and next coupled to (*R*)-2-hydroxyisocaproic acid (Hica) using *N*-(3-(dimethylamino)-propyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt), and triethylamine (TEA) to afford alcohol **2** in high yield. Next, compounds **3a**, **3b**, and **3c** were prepared by coupling alcohol **2** with the corresponding protected amino acid in high yields using DCC and DMAP. Compounds **3a**, **3b**, and **3c** were subjected to hydrogenolysis to obtain compounds **4a**, **4b**, and **4c** in quantitative yields and were used as obtained. Next, the fragments were attached head to tail before cyclization. Compound **3a** was N-deprotected using 2,2′,2″-triaminotriethylamine (TAEA) and then coupled

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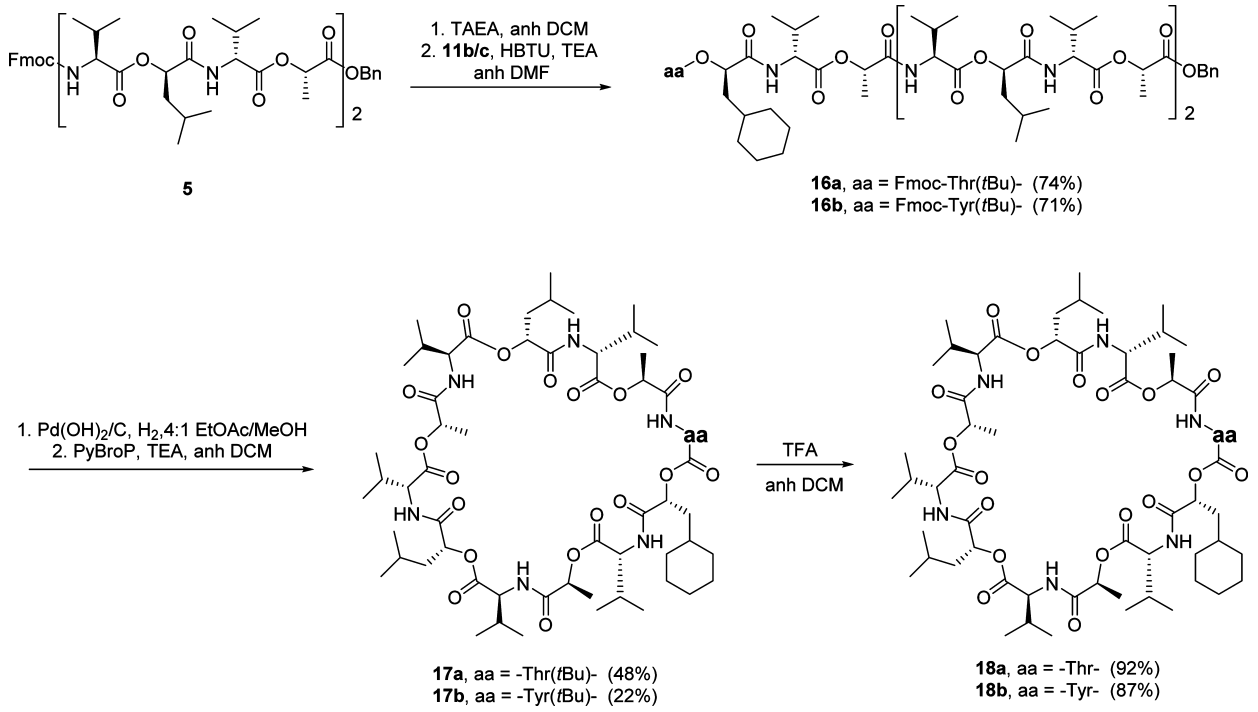
Scheme 3. Synthesis of silstatins 7 and 8 (18a and 18b)



to **4a** using *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)-uronium hexafluorophosphate (HBTU) and TEA to afford compound **5** in high yield. Compound **5** was treated in the same way as for **3a** and then coupled to the corresponding fragment (**4a**, **4b**, and **4c**) to afford compounds **6a**, **6b**, and **6c**. The Fmoc and benzyl protecting groups on **6a**, **6b**, and **6c** were removed in one step using $Pd(OH)_2$-on-carbon and hydrogen. The crude intermediates were cyclized using bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP) and TEA to afford compounds **7a**, **7b**, and **7c** in low yields. Compounds **7b** and **7c** were next treated with TFA to remove the *tert*-butyl protecting group from threonine and tyrosine side chains, obtaining compounds **8a** and **8b** in high yields, respectively.

For the synthesis of silstatins 4, 5, and 6 (**14a**, **15a**, and **15b**) (Scheme 2), the (*R*)-2-hydroxyisocaproic acid was replaced with (*R*)-2-hydroxy-3-cyclohexylpropionic acid (Hcha), which was prepared from D-cyclohexylalanine following a literature procedure.[9] The strategy and the series of reactions were the same as for the preparation of **7a**, **8a**, and **8b**, obtaining similar yields in each step.

The last SAR series consisted of a combination of fragments used in the synthesis of the previous analogues (*vide supra*). Intermediate **5** was N-deprotected using TAEA and then coupled to **11b** and **11c** using HBTU and TEA to afford compounds **16a** and **16b** in good yields. Compounds **16a** and **16b** were deprotected and cyclized using the same reagents as for the previous analogues to obtain **17a** and **17b**. Silstatins **7** and **8** (**18a** and **18b**) were prepared by TFA treatment of **17a** and **17b** in high yields (Scheme 3).

Compound **18a** was later coupled to glucuronic acid through a self-immolative linker (Scheme 4). The starting material, methyl 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucuronate, was prepared from glucuronolactone following Bollenback et al.'s strategy.[10] Compound **20** was prepared according to Duimstra et al.'s procedure.[11] Compound **25** was synthesized following Grinda et al.'s strategy starting from crude **20**.[12] Compound **25** was treated with 4-(methylamino)butyric acid and potassium carbonate to obtain compound **26**, which was partially separated and then coupled to compound **18a** using DCC and DMAP to afford compound **27** in moderate yield over two steps. Using 4-(methylamino)butyric acid as a spacer allowed us to link the drug to the glucuronic acid moiety through a carbamate and ester bond, which are more stable than a benzylic carbonate that would have formed otherwise.[13] The allyl protecting groups of compound **27** were finally deprotected using $Pd(PPh_3)_4$ in the presence of triethylammonium formate formed *in situ* by mixing TEA and formic acid to afford glucuronide prodrug **28**.

The mechanism by which the drug is liberated follows the same pattern of glucuronide prodrugs already published (Scheme 5).[14] Briefly, the glucuronic acid is cleaved by the action of β-glucuronidase, and then the nitrobenzyl group cleaves, forming carbon dioxide and 2-nitroquinone methide, which reacts with H_2O to form 4-hydroxy-3-nitrobenzyl alcohol. The 4-(methylamino)butyric ester formed self-cleaves to release the drug (**18a**) and *N*-methylpyrrolidone.[14c]

The growth inhibition properties of the silstatins and some intermediates was evaluated against a minipanel of cancer cell lines (Table 1). An overall observation pointed to small modifications on the structure of the bacillistatin, while keeping the overall lipophilicity as shown for silstatin 1 (**7a**), which did not affect its high potency. However, the introduction of polar groups (hydroxy) somewhat decreased activity, as shown for silstatins 2 and 3 (**8a** and **8b**). We observed that the only difference between valinomycin and bacillistatins 1 and 2 is an α-hydroxy acid that repeats three times along the structure. The structure of valinomycin contains (*R*)-2-hydroxyisovaleric acid, and the bacillistatins 1 and 2 contain (*R*)-2-hydroxyisocaproic acid and (2*R*,3*S*)-2-hydroxy-3-methylpentanoic acid, which are slightly more lipophilic than the 2-hydroxyisovaleric acid present in valinomycin. Following the same logic we introduced an even more lipophilic residue on the same position. However,

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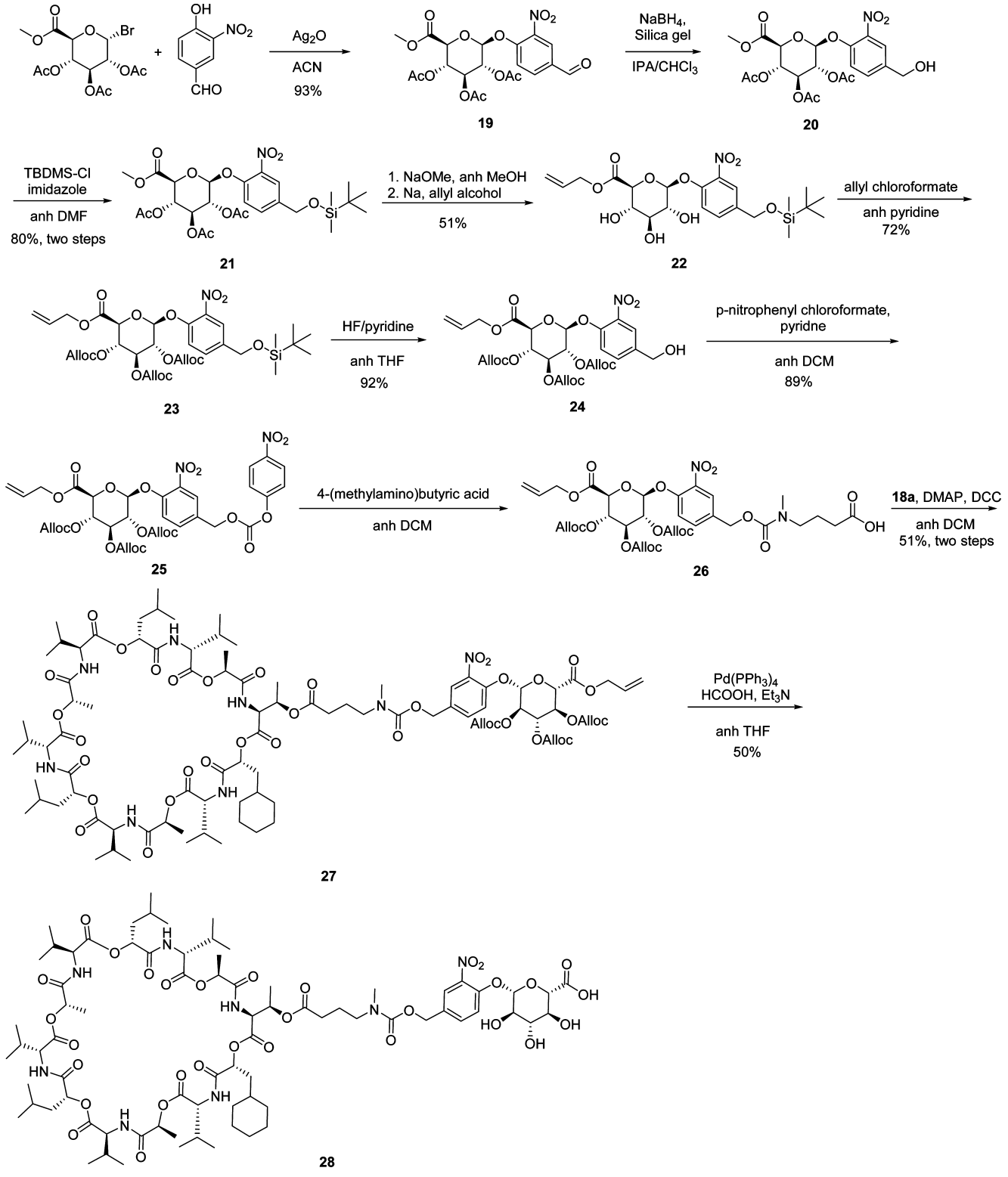
Scheme 4. Synthesis of Glucuronide Prodrug 28



introduction of an α-hydroxy acid with a more lipophilic side chain in place of the three (*R*)-2-hydroxyisocaproic acid units in silstatin 1 caused a marked reduction of cancer cell growth inhibition capability, as seen in silstatins 4 and 6 (**14a** and **15b**). In contrast, silstatin 5 (**15a**) showed very high inhibitory activity.

In order to maximize the activity displayed versus lipophilicity, only one (*R*)-2-hydroxyisocaproic acid residue was interchanged with (2*R*)-2-hydroxy-3-cyclohexylpropionic acid, thereby rescuing the activity as cancer cell growth inhibitors of the structures containing a hydroxy group, as shown by silstatins 7 and 8 (**18a** and **18b**), although without reaching the activity shown by silstatin 1 (**7a**) or the bacillistatins.

The position where the hydroxy group is introduced plays a big role in the cancer cell growth inhibition. Recently, it was observed that introduction of a hydroxy group on one of the L-

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Scheme 5. Drug (18a) Liberation

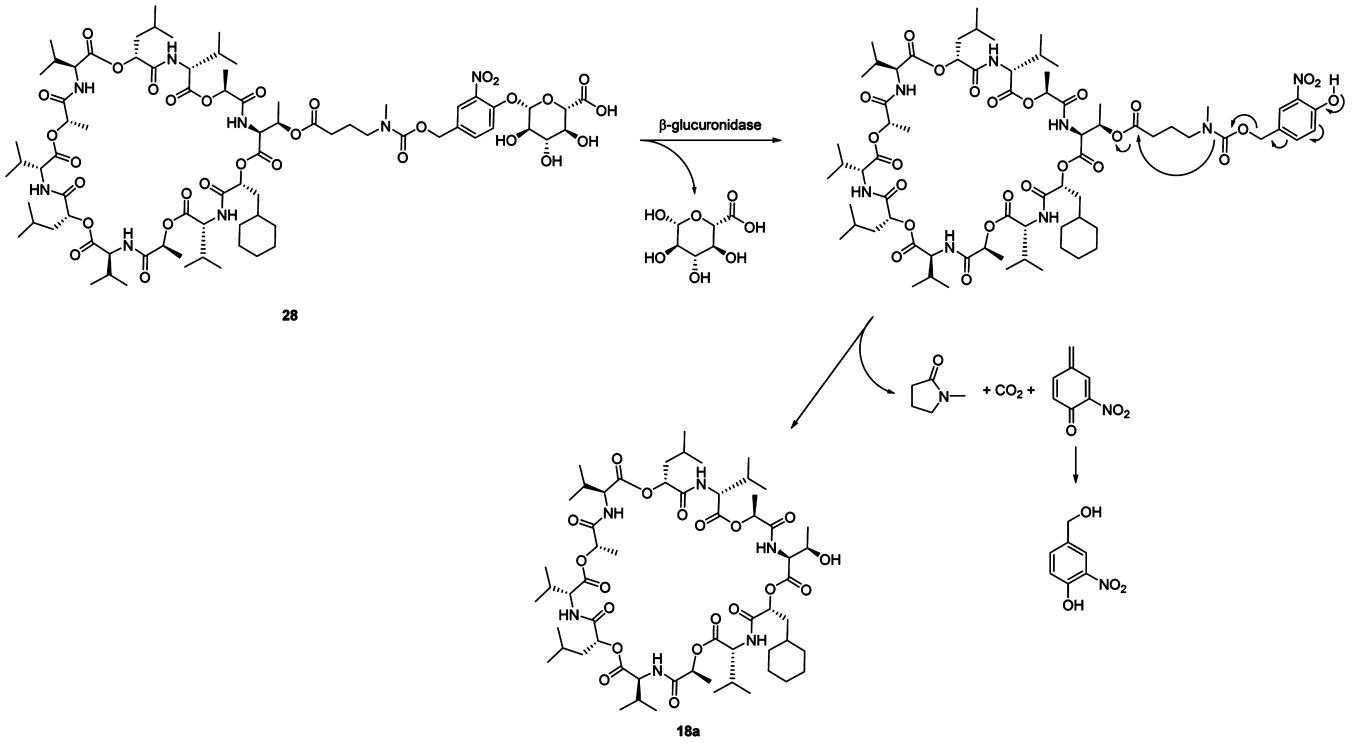

Table 1. Growth Inhibition of Human Cancer Cell Lines (GI_{50} μg/mL [nM])

	cell line[a]					
compound	BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
bacillistatin 1[1]	0.00095 [0.82]	0.00061 [0.53]	0.00045 [0.39]	0.0023 [1.2]	0.00087 [0.75]	0.0015 [1.3]
bacillistatin 2[1]	0.00034 [0.29]	0.00031 [0.27]	0.0018 [1.6]	0.00045 [0.39]	0.00026 [0.23]	0.00086 [0.75]
silstatin 1 (**7a**)	0.0008 [0.69]	0.00011 [0.095]	0.00021 [0.18]	0.0007 [0.61]	0.00016 [0.14]	0.00042 [0.36]
7c	0.030 [24]	0.0042 [3.3]	0.0025 [2.0]	0.029 [23]	0.0048 [3.8]	0.011 [8.6]
silstatin 2 (**8a**)	0.0040 [3.5]	0.0022 [1.9]	0.0031 [2.7]	0.0032 [2.8]	0.0033 [2.9]	0.0060 [5.2]
silstatin 3 (**8b**)	0.0050 [4.1]	0.0016 [1.3]	0.0040 [3.3]	0.0037 [3.0]	0.0011 [0.90]	0.0051 [4.2]
silstatin 4 (**14a**)	0.5 [392]	0.18 [141]	0.081 [63.6]	0.4 [314]	0.18 [141]	0.22 [173]
14b	0.18 [135]	0.075 [56.3]	0.075 [56.3]	0.32 [240]	0.08 [60]	0.12 [90]
14c	>1 [>717]	>1 [>717]	>1 [>717]	>1 [>717]	>1 [>717]	>1 [>717]
silstatin 5 (**15a**)	0.03 [24]	0.0031 [2.4]	0.0044 [3.4]	0.021 [16]	0.0044 [3.4]	0.005 [3.9]
silstatin 6 (**15b**)	0.53 [396]	0.12 [89.7]	0.05 [37.4]	0.32 [239]	0.09 [67.3]	0.22 [164]
17a	0.023 [18]	0.001 [0.79]	0.004 [3.2]	0.009 [7.2]	0.0024 [1.9]	0.007 [5.6]
17b	0.09 [68]	0.032 [24]	0.06 [46]	0.12 [91]	0.052 [40]	0.04 [30]
silstatin 7 (**18a**)	0.0038 [3.2]	0.0014 [1.5]	0.0031 [2.6]	0.0032 [3.3]	0.0015 [1.7]	0.003 [2.5]
silstatin 8 (**18b**)	0.006 [4.8]	0.0011 [0.88]	0.003 [2.4]	0.004 [3.2]	0.0021 [1.7]	0.0054 [4.3]
28	0.20 [120]	0.031 [18.6]	0.05 [30.0]	0.07 [42.0]	0.04 [24.0]	0.13 [78.1]

[a]Cancer cell lines in order: pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145).

valine or D-valine residues of valinomycin did not prominently affect its activity. However, when the hydroxy group was introduced on the (*R*)-2-hydroxyisovaleric acid, the activity as cancer cell growth inhibitor was decreased dramatically.[15] For that reason we introduced a threonine or a tyrosine residue in place of one of the L-valine residues present in bacillistatin 1 and bacillistatin 2. Another aspect that might be important, and needs to be assessed, is whether the position of the (2*R*)-2-hydroxy-3-cyclohexylpropionic acid residue needs to be adjacent to the hydroxy-containing residue (compounds **18a** and **18b**) or not. Future analogues in which the D-valine residue is replaced with a hydroxy-containing residue would also be of interest.

The structural characteristics of valinomycin provide the capacity to act as a potassium ion carrier. Valinomycin is lipophilic enough to solubilize in the lipid membrane and polar enough to reach the surface of the membrane in order to coordinate with the potassium ion.[16] The structural resemblance of the bacillistatins to valinomycin is such that it is believed that bacillistatins have the ability to transport potassium ions through the membranes in the same manner as valinomycin. Thus, the absolute configuration, the order of the residues (D and L configurations), and the overall lipophilicity along the structure are vital for high activity.

To demonstrate the utility of the new silstatins' feasibility for forming prodrugs and linkers to monoclonals, prodrug **28** was

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synthesized and evaluated against the same minipanel of cancer cell lines (Table 1). The use of glucuronide prodrugs has been validated by *in vivo* studies, showing superior therapeutic efficacy compared to the parent drug due to a difference in levels of β-glucuronidase in tissues. More importantly, β-glucuronidase is also observed in the tumor microenvironment.[17] Importantly, Papot and colleagues have completed a detailed study developing a glucuronide prodrug derived from the powerful anticancer drug auristatin E as its des-methyl counterpart. The conclusion was that the *in vivo* data for that combination had promise for selective treatment of cancer.[17d]

In turn, **28** proved to be a prodrug that should have greatly reduced toxicity during transport to the tumor, where a glucuronidase would release silstatin 7. Evidence of that expected result was obtained as follows. The cancer cell growth inhibition activity for prodrug **28** was found to be decreased 16−52 times with respect to the parent silstatin 7. Also, prodrug **28** was tested against two normal cell lines and compared to the parent drug (**18a**) (Table 2), and, as presumed, the prodrug **28** normal cell growth inhibition activity was decreased 62.5 and 12.5 times with respect to the parent silstatin 7 drug (**18a**) in the MCF-10A and CRL-2221 cell lines, respectively.

Table 2. Growth Inhibition of Human Normal Cell Lines (GI_{50} μg/mL [nM])

	cell line[a]	
compound	MCF-10A	CRL-2221
silstatin 7 (**18a**)	0.004 [3.35]	0.004 [3.35]
28	0.25 [150]	0.05 [30.0]

[a]Breast (MCF-10A); prostate (CRL-2221).

The quite high activity of prodrug **28** displayed in cancer and normal cell lines might be attributed to the fact that the prodrug could be crossing the cell membrane and releasing the drug (**18a**). Recently, a doxorubicin glucuronide prodrug capable of binding albumin through a maleamide moiety was synthesized, and the effects of this capability led to a relatively nontoxic prodrug with higher accumulation in the tumor (more selective) as compared to the glucuronide prodrug without the maleamide moiety.[18]

CONCLUSION

In summary, a series of bacillistatin structural modifications have been synthesized that can be bonded to linkers for transport to the cancer targets. In general, we observed an overall lipophilicity range at which these carrier-type potassium ionophores perform better as antiproliferative agents. Illustrative is the hydroxy linker site of bacillistatin modification **18a**, which can now be linked to other and more efficient targeting moieties especially monoclonal antibodies.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points are uncorrected and were determined with a Fisher−Johns melting point apparatus. Optical rotations were measured by use of a Perkin−Elmer 241 polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA 400 instrument with deuterated solvents; ^{1}H NMR chemical shifts were recorded relative to residual $CHCl_3$ at 7.26 ppm or MeOH 3.31 ppm; ^{13}C NMR chemical shifts were reported relative to residual $CHCl_3$ at 77.16 ppm or MeOH at 49.00 ppm. High-resolution mass spectra were obtained in the Arizona State University CLAS High Resolution Mass Spectrometry Laboratory.

The reactions were carried out under an atmosphere of nitrogen unless specified otherwise. Column chromatography was conducted using silica gel (E. Merck 60 Å, 230−400 mesh), applying a low-pressure stream of nitrogen. Analytical thin-layer chromatography separations were carried out on glass plates coated with silica gel (Analtech, GHLF uniplates). The TLC chromatograms were visualized using UV (short wave) lamp irradiation or by immersing the plates in ceric ammonium molybdate (CAM) staining solution followed by heating with a heat gun. Reagents and anhydrous solvents were purchased from Sigma−Aldrich Chemical Co. and Alfa-Aesar Inc. and were used as received. Degussa type E101 NE/W was employed for 20% palladium hydroxide on carbon.

Boc-D-Val-Lac-OBn (1). To a stirred solution containing Boc-D-Val (1.00 g, 4.60 mmol) and benzyl L-lactate (638 mg, 3.54 mmol) in 20 mL of anhydrous dichloromethane (DCM) at 0 °C was added DMAP (86 mg, 0.71 mmol) followed by DCC (840 mg, 4.07 mmol). The reaction mixture was stirred at 23 °C for 4 h. The mixture was filtered to remove most of the dicyclohexylurea (DCU), and the filtrate was diluted with 80 mL of DCM. The organic solution was washed with 50 mL of 0.3 N HCl, 50 mL of saturated aqueous $NaHCO_3$, and 50 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in 50 mL of cold CH_3CN, and the formed precipitate was filtered. The filtrate was concentrated under reduced pressure to afford **1** as a colorless oil: yield 97% (1.32 g, 3.47 mmol); $[\alpha]^{24}_D$ −3.43 (*c* 0.18, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (5H, m), 5.06 (4H, m), 4.22 (1H, m), 2.11 (1H, m), 1.42 (3H, d, *J* = 7.2 Hz), 1.37 (9H, s), 0.90 (3H, d, *J* = 6.8 Hz), and 0.83 (3H, d, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.3, 169.9, 155.4, 135.1, 128.4, 128.5, 128.0, 79.5, 69.0, 66.9, 58.5, 31.0, 28.2, 18.8, 17.4, and 16.8; HRMS (APCI) *m*/*z* 380.2067 [M + H]$^+$ (calcd for $C_{20}H_{30}NO_6$, 380.2073).

D-Hica-D-Val-Lac-OBn (2). To a stirred solution containing ester **1** (4.12 g, 10.9 mmol) in 60 mL of anhydrous DCM was added TFA (15 mL). The reaction mixture was stirred at 23 °C for 5 h and then concentrated under reduced pressure. The residue was dissolved in 140 mL of DCM and washed with two 100 mL portions of saturated aqueous $NaHCO_3$ and 100 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in 60 mL of anhydrous DCM and cooled to 0 °C. Next, (*R*)-2-hydroxyisocaproic acid (1.44 g, 10.9 mmol) was added followed by HOBt (2.22 g, 16.4 mmol), TEA (2.27 mL, 1.66 g, 16.4 mmol), and EDCI hydrochloride (3.14 g, 16.4 mmol). The reaction mixture was stirred at 23 °C for 18 h. The solution was diluted with 140 mL of DCM and washed with 100 mL of 0.3 N HCl, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was separated by chromatography on a silica gel column. Elution with 1:2 EtOAc/hexanes gave **2** as a colorless oil: yield 86% (3.68 g, 9.35 mmol); TLC R_f 0.40 (1:2 EtOAc/hexanes); $[\alpha]^{24}_D$ +12.8 (*c* 0.18, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (5H, m), 6.93 (1H, d, *J* = 9.2 Hz), 5.14 (3H, m), 4.64 (1H, dd, *J* = 9.2 Hz, 4.8 Hz), 4.14 (1H, m), 2.66 (1H, d, *J* = 4.8 Hz), 2.21 (1H, m), 1.84 (1H, m), 1.65 (1H, m), 1.53 (4H, m), and 0.93 (12H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 174.4, 171.2, 170.2, 135.3, 128.8, 128.6, 128.3, 71.1, 69.5, 67.3, 56.7, 44.0, 31.4, 24.7, 23.6, 21.5, 19.1, 17.7, and 17.1; HRMS (APCI) *m*/*z* 394.2239 [M + H]$^+$ (calcd for $C_{21}H_{32}NO_6$, 394.2230).

Fmoc-Val-D-Hica-D-Val-Lac-OBn (3a). To a stirred solution containing unit **2** (3.58 g, 9.10 mmol), Fmoc-Val (3.70 g, 10.9 mmol), and DMAP (334 mg, 2.73 mmol) in 100 mL of anhydrous DCM at 0 °C was added DCC (2.06 g, 10.0 mmol). The reaction mixture was stirred at 23 °C for 2 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. The crude product was separated by chromatography on a silica gel column. Elution with 1:4 EtOAc/hexanes gave **3a** as a colorless solid: yield 77% (5.0 g, 6.98 mmol); TLC R_f 0.40 (1:4 EtOAc/hexanes); mp 43−47 °C; $[\alpha]^{24}_D$ +6.59 (*c* 0.43, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, *J* = 7.2 Hz), 7.58 (2H, d, *J* = 7.6 Hz), 7.39 (2H, m), 7.33 (7H, m), 6.76

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(1H, d, J = 8.4 Hz), 5.39 (1H, d, J = 8.8 Hz), 5.24 (1H, m), 5.09 (3H, m), 4.55 (1H, m), 4.41 (1H, m), 4.25 (3H, m), 2.28 (1H, m), 2.15 (1H, m), 1.74 (3H, m), 1.42 (3H, d, J = 6.8 Hz), and 0.95 (18H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.6, 170.6, 170.1, 170.0, 156.5, 144.0, 143.8, 141.4, 135.3, 128.6, 128.4, 128.2, 127.8, 127.1, 125.2, 120.1, 120.0, 73.6, 69.3, 67.4, 67.1, 59.8, 57.4, 47.2, 40.8, 30.8, 30.7, 24.5, 23.3, 21.5, 19.2, 19.1, 18.0 and 16.8; HRMS (APCI) m/z 715.3586 $[M + H]^+$ (calcd for $C_{41}H_{51}N_2O_9$, 715.3594).

Fmoc-Thr(tBu)-D-Hica-D-Val-Lac-OBn (**3b**). Using the strategy followed for the preparation and purification of **3a**, unit **2** (492 mg, 1.25 mmol) was coupled to Fmoc-Thr(*t*Bu) (596 mg, 1.50 mmol) using DMAP (46 mg, 0.38 mmol) and DCC (285 mg, 1.38 mmol) in 15 mL of anhydrous DCM. After separation by column chromatography **3b** was obtained as a colorless solid. Yield: 83% (800 mg, 1.04 mmol); TLC R_f 0.40 (1:3 EtOAc/hexanes); mp 40−44 °C; $[\alpha]^{24}_D$ +10.8 (c 0.42, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.58 (2H, t, J = 7.2 Hz), 7.38 (2H, t, J = 7.6 Hz), 7.29 (7H, m), 6.77 (1H, d, J = 8.4 Hz), 5.69 (1H, d, J = 8.4 Hz), 5.18 (1H, m), 5.09 (3H, m), 4.55 (1H, dd, J = 8.8 Hz, 5.6 Hz), 4.35 (3H, m), 4.21 (2H, m), 2.27 (1H, m), 1.79 (3H, m), 1.45 (3H, d, J = 6.8 Hz), 1.23 (3H, d, J = 6.4 Hz), 1.18 (9H, s) and 0.95 (12H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.51, 170.50, 169.99, 169.72, 156.57, 144.03, 143.75, 141.32, 135.22, 128.60, 128.43, 128.18, 127.75, 127.10, 125.23, 120.01, 74.44, 73.87, 69.28, 67.42, 67.13, 67.07, 60.06, 57.23, 47.16, 40.89, 30.95, 28.55, 24.32, 23.08, 21.92, 20.53, 18.99, 17.93, and 16.85; HRMS (APCI) m/z 773.4015 $[M + H]^+$ (calcd for $C_{44}H_{57}N_2O_{10}$, 773.4013).

Fmoc-Tyr(tBu)-D-Hica-D-Val-Lac-OBn (**3c**). Using the procedure followed for the preparation and purification of **3a**, unit **2** (492 mg, 1.25 mmol) was coupled to Fmoc-Tyr(*t*Bu) (584 mg, 1.27 mmol) using DMAP (39 mg, 0.32 mmol) and DCC (241 mg, 1.17 mmol) in 15 mL of anhydrous DCM. After separation by column chromatography **3c** was obtained as a colorless solid: yield 94% (830 mg, 0.99 mmol); TLC R_f 0.40 (1:3 EtOAc/hexanes); mp 45−50 °C; $[\alpha]^{24}_D$ +6.04 (c 0.27, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.55 (2H, m), 7.38 (2H, t, J = 7.6 Hz), 7.26 (7H, m), 7.08 (2H, d, J = 8.4 Hz), 6.95 (1H, d, J = 8.8 Hz), 6.90 (2H, d, J = 8.4 Hz), 5.55 (1H, d, J = 7.2 Hz), 5.22 (1H, m), 5.07 (3H, m), 4.55 (2H, m), 4.35 (1H, m), 4.28 (1H, m), 4.15 (1H, t, J = 7.2 Hz), 3.13 (1H, m), 3.04 (1H, m), 2.31 (1H, m), 1.71 (2H, m), 1.50 (1H, m), 1.45 (3H, d, J = 7.2 Hz), 1.31 (9H, s), 0.97 (6H, m), and 0.87 (6H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.19, 170.51, 170.11, 170.06, 156.01, 154.68, 143.81, 143.67, 141.26, 135.18, 130.17, 129.66, 128.54, 128.36, 128.11, 127.74, 127.05, 125.07, 124.12, 119.97, 78.34, 73.66, 69.16, 67.31, 67.01, 57.40, 55.67, 47.01, 40.70, 36.93, 30.58, 28.84, 24.37, 23.14, 21.55, 19.05, 18.10, and 16.79; HRMS (APCI) m/z 835.4176 $[M + H]^+$ (calcd for $C_{49}H_{59}N_2O_{10}$, 835.4169).

Fmoc-[Val-D-Hica-D-Val-Lac]$_2$-OBn (**5**). To a stirred solution containing compound **3a** (300 mg, 0.42 mmol) in 10 mL of anhydrous DCM was added 2,2′,2″-triaminotriethylamine (633 μL, 614 mg, 4.20 mmol). The reaction mixture was stirred at 23 °C for 2 h. The solution was diluted with 10 mL of DCM and washed with two 10 mL portions of phosphate buffer (25.3 g of K_2HPO_4/12.3 g of KH_2PO_4 in 250 mL of H_2O) and 10 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in 5 mL of anhydrous DMF, and unit **4a** (262 mg, 0.42 mmol) was added followed by TEA (58 μL, 42 mg, 0.42 mmol) and HBTU (319 mg, 0.84 mmol). The solution was stirred at 23 °C for 16 h, then diluted with 50 mL of EtOAc, and washed with 50 mL of brine. The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The mixture was separated by chromatography on a silica gel column. Elution with 1:4 EtOAc/hexanes gave **5** as a colorless solid: yield 83% (384 mg, 0.35 mmol); TLC R_f 0.20 (1:4 EtOAc/hexanes); mp 59−64 °C; $[\alpha]^{24}_D$ +6.27 (c 0.26, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.2 Hz), 7.58 (2H, dd, J = 13.2 Hz, 7.6 Hz), 7.39 (3H, m), 7.33 (7H, m), 7.20 (1H, d, J = 7.2 Hz), 7.16 (1H, d, J = 8.8 Hz), 5.51 (1H, d, J = 7.6 Hz), 5.27 (2H, m), 5.23 (1H, m), 5.09 (3H, m), 4.42 (2H, m), 4.33 (1H, m), 4.20 (2H, m), 4.11 (1H, t, J = 7.6 Hz), 4.04 (1H, t, J = 7.6 Hz), 2.27 (3H, m), 2.15 (1H, m), 1.74 (6H, m), 1.41 (6H, d, J = 6.4 Hz), and 0.95 (36H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.99, 171.04, 170.91, 170.85, 170.52, 170.41, 170.28, 170.21, 156.89, 143.87, 143.62, 141.28, 135.29, 128.48, 128.24, 128.12, 127.72, 127.02, 125.02, 119.97, 73.15, 73.07, 70.43, 69.12, 67.46, 66.92, 60.40, 59.02, 57.80, 47.03, 40.68, 40.44, 34.64, 31.56, 30.38, 30.08, 29.72, 29.42, 25.25, 24.41, 23.19, 22.62, 21.42, 21.37, 19.17, 19.06, 19.01, 18.89, 18.79, 18.55, 18.32, 17.42, and 16.75; HRMS (APCI) m/z 1099.586 $[M + H]^+$ (calcd for $C_{60}H_{82}N_4O_{15}$, 1099.585).

Fmoc-[Val-D-Hica-D-Val-Lac]$_3$-OBn (**6a**). By means of the strategy followed for the preparation and purification of **5**, unit **5** (345 mg, 0.31 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (467 μL, 453 mg, 3.10 mmol) in 10 mL of DCM and then coupled to unit **4a** (194 mg, 0.31 mmol) using HBTU (235 mg, 0.62 mmol) and TEA (43 μL, 31 mg, 0.42 mmol) in 5 mL of anhydrous DMF. After separation by column chromatography **6a** was obtained as a colorless solid: yield 62% (284 mg, 0.19 mmol); TLC R_f 0.35 (1:2 EtOAc/hexanes); mp 59−63 °C; $[\alpha]^{24}_D$ +3.56 (c 0.23, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.66 (2H, m), 7.58 (2H, dd, J = 14.8 Hz, 7.2 Hz), 7.51 (1H, d, J = 6.0 Hz), 7.39 (3H, m), 7.33 (8H, m), 5.74 (1H, d, J = 7.2 Hz), 5.34 (1H, m), 5.20 (4H, m), 5.10 (3H, m), 4.38 (3H, m), 4.22 (1H, t, J = 7.2 Hz), 4.13 (2H, m), 4.04 (1H, t, J = 6.4 Hz), 3.95 (2H, m), 2.27 (5H, m), 1.99 (1H, m), 1.74 (9H, m), 1.41 (9H, d, J = 6.8 Hz), and 0.95 (54H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.54, 171.69, 171.55, 171.40, 170.96, 170.70, 170.68, 170.59, 170.58, 170.41, 170.38, 170.36, 157.20, 144.16, 143.78, 141.44, 135.49, 128.60, 128.35, 127.85, 127.19, 125.25, 120.10, 73.35, 73.10, 72.68, 70.54, 70.17, 69.21, 67.64, 67.04, 60.77, 60.04, 59.82, 59.66, 59.08, 58.16, 47.19, 40.94, 40.59, 40.51, 34.79, 31.71, 30.42, 30.08, 29.77, 29.47, 29.29, 29.18, 25.40, 24.57, 24.53, 24.50, 23.38, 23.35, 22.77, 21.68, 21.49, 21.40, 19.48, 19.35, 19.30, 19.26, 19.19, 19.16, 19.06, 18.88, 18.64, 17.56, 17.35, and 16.91; HRFTMS (ESI) m/z 1505.788 $[M + Na]^+$ (calcd for $C_{79}H_{114}N_6O_{21}Na$, 1505.793).

Fmoc-Thr(tBu)-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$-OBn (**6b**). With the procedure followed for the preparation and purification of **5**, unit **5** (300 mg, 0.27 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (407 μL, 395 mg, 2.70 mmol) in 12 mL of DCM and then coupled to unit **4b** (184 mg, 0.27 mmol) using HBTU (205 mg, 0.54 mmol) and TEA (37 μL, 27 mg, 0.27 mmol) in 5 mL of anhydrous DMF. After separation by column chromatography **6b** was obtained as a colorless solid: yield 74% (308 mg, 0.20 mmol); TLC R_f 0.35 (1:2 EtOAc/hexanes); mp 62−65 °C; $[\alpha]^{24}_D$ +13.0 (c 0.10, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, d, J = 7.6 Hz), 7.70 (1H, d, J = 6.0 Hz), 7.56 (4H, m), 7.51 (2H, t, J = 7.6 Hz), 7.33 (8H, m), 7.17 (1H, d, J = 6.4 Hz), 5.68 (1H, d, J = 7.2 Hz), 5.23 (5H, m), 5.10 (3H, m), 4.35 (3H, m), 4.22 (1H, t, J = 7.2 Hz), 4.04 (6H, m), 2.27 (5H, m), 1.74 (9H, m), 1.41 (9H, m), 1.22 (3H, d, J = 5.6 Hz), 1.18 (9H, s), and 0.95 (48H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.34, 171.27, 171.25, 170.89, 170.83, 170.81, 170.63, 170.59, 170.45, 170.44, 170.29, 170.27, 156.80, 143.84, 143.58, 141.25, 135.35, 128.46, 128.21, 128.14, 127.72, 127.04, 125.05, 119.98, 74.62, 73.22, 72.99, 72.54, 70.19, 70.08, 69.07, 67.50, 66.88, 66.83, 60.19, 59.91, 59.80, 59.46, 59.26, 58.05, 47.03, 40.79, 40.33, 34.62, 30.23, 29.65, 29.57, 29.33, 28.51, 28.46, 25.23, 24.36, 24.33, 24.21, 23.22, 23.02, 21.71, 21.40, 21.33, 20.54, 19.34, 19.22, 19.19, 19.15, 19.11, 19.09, 19.04, 18.97, 18.93, 18.52, 17.25, 17.12, and 16.74; HRFTMS (ESI) m/z 1563.833 $[M + Na]^+$ (calcd for $C_{82}H_{120}N_6O_{22}Na$, 1563.835).

Fmoc-Tyr(tBu)-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$-OBn (**6c**). Employing the strategy followed for the preparation and purification of **5**, unit **5** (630 mg, 0.57 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (850 μL, 834 mg, 5.70 mmol) in 20 mL of DCM and then coupled to unit **4c** (425 mg, 0.57 mmol) using HBTU (434 mg, 1.14 mmol) and TEA (79 μL, 58 mg, 0.57 mmol) in 5 mL of anhydrous DMF. After separation by column chromatography **6c** was obtained as a colorless solid: yield 81% (744 mg, 0.46 mmol); TLC R_f 0.35 (1:2 EtOAc/hexanes); mp 62−65 °C; $[\alpha]^{24}_D$ +5.71 (c 0.28, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (1H, d, J = 7.6 Hz), 7.74 (2H, d, J = 7.6 Hz), 7.67 (1H, m), 7.62 (1H, d, J = 6.4 Hz), 7.52 (2H, m), 7.44 (1H, m), 7.37 (3H, m), 7.30 (7H, m), 7.06 (2H, d, J = 8.0 Hz), 6.89 (2H, d, J = 8.4 Hz), 5.73 (1H, d, J = 6.4 Hz), 5.18 (8H, m), 4.35 (4H, m), 4.19 (1H, t, J = 6.8 Hz), 4.06 (3H, m), 3.95 (1H, t, J

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= 6.4 Hz), 3.03 (2H, m), 2.25 (5H, m), 1.74 (8H, m), 1.59 (1H, m), 1.41 (9H, m), 1.30 (9H, s), and 0.95 (48H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.92, 171.59, 171.45, 171.42, 171.21, 170.97, 170.88, 170.72, 170.64, 170.53, 170.44, 170.43, 156.72, 154.89, 144.01, 143.74, 141.36, 135.51, 130.70, 129.76, 128.61, 128.35, 128.31, 127.89, 127.17, 125.21, 124.24, 120.13, 80.72, 73.45, 73.14, 72.74, 70.46, 70.28, 69.26, 67.68, 67.05, 59.95, 59.92, 59.70, 59.24, 58.24, 56.19, 47.14, 40.93, 40.52, 36.61, 36.59, 30.40, 29.83, 29.79, 29.55, 29.47, 29.38, 29.26, 29.19, 28.99, 24.53, 24.51, 24.34, 23.39, 23.23, 21.65, 21.49, 19.46, 19.43, 19.31, 19.30, 19.27, 19.21, 19.12, 19.09, 18.68, 17.49, 17.44 and 16.91; HRFTMS (ESI) m/z 1625.844 $[M + Na]^+$ (calcd for $C_{87}H_{122}N_6O_{22}Na$, 1625.850).

*Cyclo-[Val-D-Hica-D-Val-Lac]$_3$ (**7a**, silstatin 1).* To a stirred solution containing depsipeptide **6a** (250 mg, 0.17 mmol) in 5 mL of 4:1 EtOAc/MeOH was added 20% palladium hydroxide-on-carbon (125 mg). The mixture was stirred under a hydrogen atmosphere (1 atm) at 23 °C for 7 h and then filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was dissolved in 125 mL of anhydrous DCM. TEA (24 μL, 17 mg, 0.17 mmol) was added followed by PyBroP (238 mg, 0.51 mmol), and the reaction mixture was stirred at 23 °C for 24 h. The solution was filtered through a silica gel plug, and the filtrate was concentrated under reduced pressure. The residue was separated by chromatography on a silica gel column. Elution with 1:4 EtOAc/hexanes gave **7a** (silstatin 1) as a colorless solid: yield 28% (54 mg, 47 μmol); TLC R_f 0.42 (1:4 EtOAc/hexanes); mp 137−138 °C; $[\alpha]^{24}_D$ +30.9 (c 0.11, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (3H, d, J = 7.6 Hz), 7.73 (3H, d, J = 6.4 Hz), 5.21 (3H, m), 5.13 (3H, m), 4.06 (3H, dd, J = 9.6 Hz, 8.0 Hz), 3.93 (3H, dd, J = 9.6 Hz, 6.4 Hz), 2.23 (6H, m), 1.74 (9H, m), 1.44 (9H, d, J = 7.2 Hz), 1.04 (18H, m), and 0.95 (36H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.4, 172.0, 171.6, 170.4, 73.5, 70.6, 60.3, 59.1, 40.8, 28.7, 28.5, 24.6, 23.4, 21.4, 19.8, 19.6, 19.3, 19.2, and 17.2; HRMS (APCI) m/z 1153.685 $[M + H]^+$ (calcd for $C_{57}H_{97}N_6O_{18}$, 1153.686).

*Cyclo-{Thr(tBu)-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**7b**).* By the method followed for the preparation and purification of **7a**, depsipeptide **6b** (300 mg, 0.20 mmol) was deprotected using 20% palladium hydroxide-on-carbon (300 mg) in 10 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (373 mg, 0.80 mmol) and TEA (111 μL, 81 mg, 0.80 mmol) in 200 mL of anhydrous DCM. After separation by column chromatography **7b** was obtained as a colorless solid: yield 33% (81 mg, 67 μmol); TLC R_f 0.5 (1:3 EtOAc/hexanes); mp 75−78 °C; $[\alpha]^{24}_D$ +23.3 (c 0.06, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, d, J = 8.0 Hz), 7.74 (1H, d, J = 7.2 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.49 (1H, d, J = 8.0 Hz), 7.45 (1H, d, J = 5.6 Hz), 7.37 (1H, d, J = 4.8 Hz), 5.20 (6H, m), 4.29 (1H, t, J = 8.4 Hz), 4.17 (2H, m), 4.05 (3H, m), 3.85 (1H, dd, J = 10 Hz, 6.0 Hz), 2.23 (5H, m), 1.74 (9H, m), 1.41 (9H, m), 1.20 (9H, s), 1.17 (3H, d, J = 6.4 Hz), 1.10 (18H, m), and 0.90 (30H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.33, 172.32, 172.14, 172.12, 171.81, 171.80, 171.13, 171.12, 170.35, 170.27, 170.19, 169.88, 74.76, 73.88, 73.37, 73.11, 70.91, 70.84, 70.64, 66.54, 60.82, 59.81, 59.12, 59.08, 58.53, 57.72, 40.96, 40.77, 40.71, 34.77, 29.64, 29.23, 28.78, 28.54, 28.53, 28.46, 25.38, 24.58, 24.55, 23.51, 23.50, 23.45, 21.47, 21.32, 21.20, 19.92, 19.86, 19.54, 19.53, 19.41, 19.38, 19.32, 19.04, 18.72, 18.45, 17.54, 17.13, and 17.05; HRMS (APCI) m/z 1211.723 $[M + H]^+$ (calcd for $C_{60}H_{103}N_6O_{19}$, 1211.728).

*Cyclo-{Tyr(tBu)-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**7c**).* Using the strategy followed for the preparation and purification of **7a**, depsipeptide **6c** (700 mg, 0.44 mmol) was deprotected using 20% palladium hydroxide-on-carbon (300 mg) in 10 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (373 mg, 0.80 mmol) and TEA (111 μL, 81 mg, 0.80 mmol) in 200 mL of anhydrous DCM. After separation by column chromatography **7c** was obtained as a colorless solid: yield 11% (66 mg, 52 μmol); TLC R_f 0.20 (1:4 EtOAc/hexanes); mp 64−67 °C; $[\alpha]^{24}_D$ +25.7 (c 0.14, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94 (1H, d, J = 7.2 Hz), 7.85 (2H, m), 7.76 (1H, d, J = 8.0 Hz), 7.68 (1H, d, J = 7.2 Hz), 7.65 (1H, d, J = 6.0 Hz), 7.09 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.4 Hz), 5.20 (4H, m), 5.05 (2H, m), 4.53 (q, J = 7.6 Hz, 1H), 4.13 (1H, t, J = 8.8 Hz), 4.02 (1H, dd, J = 9.6 Hz, 7.6 Hz), 3.95 (1H, dd, J = 9.6 Hz, 7.2 Hz), 3.89 (2H, dd, J = 10 Hz, 6.0 Hz), 3.10 (2H, m), 2.23 (5H, m), 1.74 (7H, m), 1.55 (2H, t, J = 6.8 Hz), 1.42 (6H, d, J = 6.8 Hz), 1.36 (3H, d, J = 6.8 Hz), 1.30 (9H, s), 1.05 (18H, m), 0.92 (24H, m), 0.75 (3H, d, J = 6.8 Hz), and 0.71 (3H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.49, 172.37, 172.10, 172.00, 171.71, 171.69, 171.53, 171.43, 170.59, 170.52, 170.27, 170.03, 154.31, 131.01, 129.66, 123.91, 78.11, 73.61, 73.18, 70.71, 70.50, 70.07, 60.41, 60.37, 59.39, 59.28, 58.39, 54.57, 40.73, 40.63, 40.61, 40.48, 35.27, 30.32, 29.65, 28.80, 28.58, 28.53, 28.46, 28.40, 24.45, 24.16, 23.71, 23.31, 22.88, 21.80, 21.24, 21.08, 19.73, 19.46, 19.35, 19.33, 19.17, 19.14, 19.11, 19.06, 19.04, 18.84, 17.19, 17.09, and 16.81; HRMS (APCI) m/z 1273.744 $[M + H]^+$ (calcd for $C_{65}H_{105}N_6O_{19}$, 1273.743).

*Cyclo-{Thr-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**8a**, silstatin 2).* To a stirred solution containing cyclodepsipeptide **7b** (70 mg, 58 μmol) in 1 mL of anhydrous DCM was added TFA (250 μL). The reaction mixture was stirred at 23 °C for 2 h. The solution was concentrated under reduced pressure, and the residue was separated by chromatography on a silica gel column. Elution with 1:3 EtOAc/hexanes gave **8a** (silstatin **2**) as a colorless solid: yield 94% (63 mg, 55 μmol); TLC R_f 0.25 (1:3 EtOAc/hexanes); mp 75−78 °C; $[\alpha]^{24}_D$ +25.9 (c 0.19, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (1H, d, J = 7.2 Hz), 7.91 (1H, d, J = 7.2 Hz), 7.81 (3H, m), 7.57 (1H, d, J = 6.4 Hz), 5.28 (2H, m), 5.17 (3H, m), 5.05 (1H, m), 4.87 (1H, br s), 4.14 (1H, t, J = 9.2 Hz), 3.95 (6H, m), 2.23 (4H, m), 2.12 (1H, m), 1.74 (9H, m), 1.41 (9H, m), 1.20 (3H, d, J = 5.6 Hz), 1.10 (18H, m), and 0.90 (30H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.51, 172.82, 172.39, 172.32, 172.20, 171.92, 171.53, 171.20, 171.00, 170.92, 170.29, 169.97, 74.49, 73.29, 73.16, 70.76, 70.70, 70.42, 66.19, 60.84, 60.47, 60.14, 59.89, 59.60, 58.52, 40.84, 40.70, 40.62, 28.72, 28.67, 28.55, 28.49, 28.21, 24.67, 24.57, 23.49, 23.37, 23.35, 23.33, 21.53, 21.36, 21.35, 19.70, 19.60, 19.53, 19.48, 19.47, 19.46, 19.45, 19.41, 19.37, 19.35, 18.98, 17.53, 17.10, and 17.03; HRMS (APCI) m/z 1155.664 $[M + H]^+$ (calcd for $C_{56}H_{95}N_6O_{19}$, 1155.665).

*Cyclo-{Tyr-D-Hica-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**8b**, silstatin 3).* Using the strategy followed for the preparation and purification of **8a**, cyclodepsipeptide **7c** (7 mg, 5.5 μmol) was deprotected with TFA (250 μL) in 1 mL of anhydrous DCM. After separation by column chromatography **8b** (silstatin 3) was obtained as a colorless solid: yield 75% (5 mg, 4.1 μmol); TLC R_f 0.25 (1:3 EtOAc/hexanes); mp 65−68 °C; $[\alpha]^{24}_D$ +35.0 (c 0.10, EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (6H, m), 7.04 (2H, d, J = 8.4 Hz), 6.73 (2H, d, J = 8.4 Hz), 5.20 (5H, m), 4.96 (1H, t, J = 6.0 Hz), 4.43 (1H, q, J = 7.6 Hz), 4.10 (3H, m), 3.95 (2H, m), 3.10 (2H, m), 2.23 (5H, m), 1.65 (9H, m), 1.42 (6H, d, J = 6.8 Hz), 1.36 (3H, d, J = 7.2 Hz), 1.04 (18H, m), 0.92 (24H, m), 0.71 (3H, d, J = 6.4 Hz), and 0.71 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.50, 172.38, 172.23, 172.04, 171.98, 171.94, 171.81, 171.53, 171.43, 170.36, 170.28, 170.20, 155.19, 130.57, 127.82, 115.78, 73.90, 73.58, 73.43, 70.73, 70.64, 60.57, 60.22, 59.01, 58.81, 58.80, 55.29, 40.94, 40.80, 40.68, 35.44, 29.85, 29.81, 29.08, 28.79, 28.73, 28.63, 24.68, 24.64, 24.28, 23.49, 23.20, 22.84, 21.66, 21.41, 21.31, 19.83, 19.71, 19.59, 19.55, 19.47, 19.38, 19.37, 19.12, 18.98, 18.83, 17.42, 17.18, and 17.04; HRMS (APCI) m/z 1217.681 $[M + H]^+$ (calcd for $C_{61}H_{97}N_6O_{19}$, 1217.681).

*D-Hcha-D-Val-Lac-OBn (**9**).* Using the strategy followed for the preparation and purification of **2**, unit **1** (2.52 g, 6.64 mmol) was deprotected with TFA (7.5 mL) in 30 mL of anhydrous DCM and then coupled to (2*R*)-2-hydroxy-3-cyclohexylpropionic acid (1.23 g, 7.14 mmol) using HOBt (1.35 g, 9.96 mmol), TEA (1.38 mL, 1.10 g, 9.96 mmol), and EDCI hydrochloride (1.91 g, 9.96 mmol) in 30 mL of anhydrous DCM. After separation by column chromatography **9** was obtained as a colorless oil (solidifies upon standing): yield 63% (1.80 g, 4.15 mmol); TLC R_f 0.30 (1:3 EtOAc/hexanes); mp 55−57 °C; $[\alpha]^{24}_D$ +19.1 (c 0.32, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (5H, m), 6.90 (1H, d, J = 9.2 Hz), 5.18 (2H, m), 4.64 (1H, dd, J = 9.2 Hz, 4.8 Hz), 4.14 (1H, m), 2.62 (1H, br s), 2.21 (1H, m), 1.78 (1H, d, J = 12.8 Hz), 1.66 (5H, m), 1.50 (5H, m), 1.20 (3H, m), and 0.93 (8H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 174.5, 171.1, 170.1, 135.3, 128.8, 128.6, 128.3, 70.5, 69.5, 67.3, 56.7, 42.7, 34.3, 34.1, 32.3, 31.4,

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26.6, 26.4, 26.2, 19.1, 17.7, and 17.1; HRMS (APCI), m/z 434.2542 $[M + H]^+$ (calcd for $C_{24}H_{36}NO_6$, 434.2543).

Fmoc-Val-D-Hcha-D-Val-Lac-OBn (**10a**). By the procedure followed for the preparation and purification of **3a**, unit **9** (1.80 g, 4.15 mmol) was coupled to Fmoc-Val (1.55 g, 4.57 mmol) using DCC (943 mg, 4.57 mmol) and DMAP (152 mg, 1.25 mmol) in 50 mL of anhydrous DCM. After separation by column chromatography **10a** was obtained as a colorless solid: yield 82% (2.56 g, 3.39 mmol); TLC R_f 0.25 (1:4 EtOAc/hexanes); mp 49−53 °C; $[\alpha]^{24}_D$ +0.45 (c 0.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.57 (2H, d, J = 7.6 Hz), 7.39 (2H, m) 7.33 (7H, m), 6.74 (1H, d, J = 8.8 Hz), 5.34 (1H, d, J = 8.4 Hz), 5.28 (1H, m), 5.09 (3H, m), 4.55 (1H, m), 4.41 (1H, m), 4.25 (2H, m), 4.18 (1H, m), 2.28 (1H, m), 2.15 (1H, m), 1.74 (7H, m), 1.41 (4H, m), 1.16 (3H, m), and 0.95 (14H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.63, 170.59, 170.13, 170.08, 156.50, 144.02, 143.81, 141.40, 135.29, 128.67, 128.49, 128.26, 127.84, 127.17, 125.20, 120.09, 73.02, 69.32, 67.44, 67.16, 59.84, 57.38, 47.21, 39.43, 33.94, 33.89, 32.16, 30.87, 30.82, 26.41, 26.34, 26.07, 19.22, 19.11, 18.09, 18.06, and 16.89; HRMS (APCI) m/z 755.3901 $[M + H]^+$ (calcd for $C_{44}H_{55}N_2O_9$, 755.3907).

Fmoc-Thr(tBu)-D-Hcha-D-Val-Lac-OBn (**10b**). With the method followed for the preparation and purification of **3a**, unit **9** (1.80 g, 4.15 mmol) was coupled to Fmoc-Thr(O*t*Bu) (381 mg, 0.96 mmol) using DCC (198 mg, 0.96 mmol) and DMAP (32 mg, 0.26 mmol) in 10 mL of anhydrous DCM. After separation by column chromatography **10b** was obtained as a colorless solid: yield 79% (561 mg, 0.69 mmol); TLC R_f 0.27 (1:4 EtOAc/hexanes); mp 50−54 °C; $[\alpha]^{24}_D$ +2.97 (c 0.37, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.57 (2H, t, J = 7.6 Hz), 7.39 (2H, t, J = 7.2 Hz), 7.33 (7H, m), 6.79 (1H, d, J = 8.8 Hz), 5.70 (1H, d, J = 8.0 Hz), 5.22 (1H, t, J = 6.8 Hz), 5.09 (3H, m), 4.55 (1H, dd, J = 8.4 Hz, 5.2 Hz), 4.35 (3H, m), 4.20 (3H, m), 2.28 (1H, m), 1.74 (7H, m), 1.41 (4H, m), 1.16 (15H, m), and 0.95 (8H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.50, 170.48, 169.95, 169.78, 156.51, 143.99, 143.71, 141.28, 135.19, 128.56, 128.38, 128.13, 127.72, 127.06, 125.20, 119.97, 74.40, 73.37, 69.25, 67.39, 67.10, 67.02, 60.05, 57.21, 47.12, 39.43, 33.70, 33.49, 32.53, 30.91, 28.54, 26.33, 26.14, 25.96, 20.52, 18.94, 17.91, and 16.82; HRMS (APCI) m/z 813.4324 $[M + H]^+$ (calcd for $C_{47}H_{61}N_2O_{10}$, 813.4326).

Fmoc-Tyr(tBu)-D-Hcha-D-Val-Lac-OBn (**10c**). Using the strategy followed for the preparation and purification of **3a**, unit **9** (376 mg, 0.87 mmol) was coupled to Fmoc-Tyr(O*t*Bu) (441 mg, 0.96 mmol) using DCC (198 mg, 0.96 mmol) and DMAP (32 mg, 0.26 mmol) in 10 mL of anhydrous DCM. After separation by column chromatography **10b** was obtained as a colorless solid: yield 90% (688 mg, 0.79 mmol); TLC R_f 0.25 (1:4 EtOAc/hexanes); mp 52−56 °C; $[\alpha]^{24}_D$ +11.2 (c 0.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.6 Hz), 7.57 (2H, t, J = 7.6 Hz), 7.39 (2H, t, J = 7.2 Hz), 7.33 (7H, m), 7.06 (2H, d, J = 8.4 Hz), 6.90 (2H, d, J = 8.4 Hz), 6.83 (1H, d, J = 8.4 Hz), 5.45 (1H, d, J = 7.6 Hz), 5.23 (1H, t, J = 6.4 Hz), 5.09 (3H, m), 4.55 (2H, m), 4.35 (1H, m), 4.26 (1H, m), 4.15 (1H, m), 3.17 (1H, m), 3.02 (1H, m), 2.28 (1H, m), 1.74 (7H, m), 1.42 (4H, m), 1.31 (12H, m), 1.15 (3H, m), and 0.95 (8H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.1, 170.6, 170.2, 170.1, 156.0, 154.7, 143.7, 141.3, 135.2, 130.3, 129.7, 128.6, 128.4, 128.2, 127.8, 127.1, 125.1, 124.2, 120.0, 78.5, 73.3, 69.3, 67.3, 67.1, 57.3, 55.5, 47.1, 39.4, 36.9, 33.8, 33.7, 32.4, 30.8, 28.9, 26.4, 26.1, 26.0, 19.1, 18.0 and 16.9; HRMS (APCI) m/z 875.4487 $[M + H]^+$ (calcd for $C_{52}H_{63}N_2O_{10}$, 875.4482).

Fmoc-[Val-D-Hcha-D-Val-Lac]$_2$-OBn (**12**). Using the experimental strategy followed for the preparation and purification of **5**, unit **10a** (300 mg, 0.40 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (605 μL, 588 mg, 4.00 mmol) in 10 mL of DCM and then coupled to unit **11a** (266 mg, 0.40 mmol) using HBTU (303 mg, 0.80 mmol) and TEA (55 μL, 40 mg, 0.40 mmol) in 5 mL of anhydrous DMF. After separation by column chromatography **12** was obtained as a colorless solid: yield 76% (360 mg, 0.31 mmol); TLC R_f 0.28 (1:4 EtOAc/hexanes); mp 60−65 °C; $[\alpha]^{24}_D$ +17.1 (c 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, J = 7.2 Hz), 7.58 (2H, dd, J = 14 Hz, 7.6 Hz), 7.39 (3H, m) 7.33 (7H, m), 7.21 (1H, d, J = 7.2 Hz), 7.16 (1H, d, J = 8.4 Hz), 5.50 (1H, d, J = 7.6 Hz), 5.26 (3H, m), 5.09 (3H, m), 4.42 (2H, m), 4.33 (1H, m), 4.22 (2H, m), 4.11 (1H, t, J = 7.6 Hz), 4.02 (1H, t, J = 7.6 Hz), 2.27 (3H, m), 2.15 (1H, m), 1.74 (14H, m), 1.41 (8H, m), 1.16 (6H, m), and 0.95 (28H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.17, 171.33, 171.06, 171.01, 170.66, 170.54, 170.52, 170.35, 157.04, 144.01, 143.75, 141.41, 135.44, 128.60, 128.36, 128.25, 127.85, 127.19, 125.16, 120.10, 72.65, 72.58, 70.52, 69.24, 67.61, 67.04, 60.58, 59.18, 57.98, 47.16, 39.32, 39.13, 34.77, 33.94, 33.83, 33.81, 32.18, 32.11, 31.69, 30.49, 30.22, 29.85, 29.49, 26.47, 26.42, 26.39, 26.34, 26.10, 26.05, 25.39, 19.21, 19.16, 19.03, 18.94, 18.74, 18.47, 17.56, and 16.88; HRMS (APCI) m/z 1179.645 $[M + H]^+$ (calcd for $C_{66}H_{91}N_4O_{15}$, 1179.648).

Fmoc-[Val-D-Hcha-D-Val-Lac]$_3$-OBn (**13a**). Again using the procedure followed for the preparation and purification of **5**, unit **12** (320 mg, 0.27 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (281 μL, 273 mg, 2.16 mmol) in 6 mL of DCM and then coupled to unit **11a** (179 mg, 0.27 mmol) using HBTU (205 mg, 0.54 mmol) and TEA (37 μL, 27 mg, 0.27 mmol) in 5 mL of anhydrous DMF. After separation by column chromatography **13a** was obtained as a colorless solid: yield 70% (304 mg, 0.19 mmol); TLC R_f 0.30 (1:4 EtOAc/hexanes); mp 60−64 °C; $[\alpha]^{24}_D$ +20.9 (c 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, d, J = 7.2 Hz), 7.66 (7.6 Hz, 2H, J = 4.4 Hz, 2H), 7.58 (3H, d, J = 15.6 Hz), 7.51 (1H, d, J = 6.0 Hz), 7.36 (3H, m), 7.29 (8H, m), 5.79 (1H, d, J = 7.2 Hz), 5.24 (5H, m), 5.10 (3H, m), 4.38 (3H, m), 4.21 (1H, t, J = 7.2 Hz), 4.13 (2H, m), 4.02 (1H, m), 3.93 (2H, m), 2.27 (5H, m), 1.99 (1H, m), 1.74 (21H, m), 1.41 (12H, m), and 1.02 (51H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.44, 171.71, 171.39, 171.38, 171.29, 170.88, 170.68, 170.52, 170.46, 170.42, 170.26, 170.18, 157.07, 144.03, 143.63, 141.27, 135.35, 128.43, 128.17, 128.13, 127.69, 127.02, 124.99, 119.94, 72.57, 72.29, 71.86, 70.35, 69.94, 69.04, 67.49, 66.86, 60.67, 59.95, 59.66, 59.64, 58.92, 58.08, 47.03, 39.31, 38.96, 34.63, 33.85, 33.81, 33.69, 33.66, 33.61, 32.15, 32.03, 31.94, 31.86, 30.20, 29.92, 29.54, 29.26, 29.15, 29.02, 28.97, 26.33, 26.31, 26.26, 26.21, 25.95, 25.92, 25.24, 20.67, 19.35, 19.19, 19.15, 19.14, 19.11, 19.06, 19.02, 18.96, 18.91, 18.79, 18.54, 17.42, 17.15, and 16.74; HRFTMS (ESI) m/z 1625.885 $[M + Na]^+$ (calcd for $C_{88}H_{126}N_6O_{21}Na$, 1625.887).

Fmoc-Thr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$-OBn (**13b**). By the procedure followed for the preparation and purification of **5**, unit **12** (500 mg, 0.42 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (633 μL, 614 mg, 4.2 mmol) in 15 mL of DCM and then coupled to unit **11b** (306 mg, 0.42 mmol) using HBTU (319 mg, 0.84 mmol) and TEA (58 μL, 42 mg, 0.42 mmol) in 7 mL of anhydrous DMF. After separation by column chromatography **13b** was obtained as a colorless solid: yield 64% (448 mg, 0.23 mmol); TLC R_f 0.40 (1:2 EtOAc/hexanes); mp 62−66 °C; $[\alpha]^{24}_D$ +10.6 (c 0.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, d, J = 7.2 Hz), 7.66 (4H, m), 7.36 (3H, m), 7.29 (8H, m), 7.14 (1H, d, J = 6.4 Hz), 5.66 (1H, d, J = 7.2 Hz), 5.24 (5H, m), 5.10 (3H, m), 4.38 (3H, m), 4.22 (1H, t, J = 7.2 Hz), 4.13 (4H, m), 4.02 (1H, m), 3.93 (1H, m), 2.27 (4H, m), 2.08 (1H, m), 1.74 (21H, m), 1.41 (12H, m), 1.21 (21H, m) and 1.02 (36H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.45, 171.44, 171.41, 171.01, 170.98, 170.84, 170.79, 170.56, 170.54, 170.42, 170.41, 156.89, 143.98, 143.73, 141.39, 135.51, 130.57, 128.59, 128.33, 127.86, 127.18, 125.20, 120.11, 74.76, 72.86, 72.48, 72.01, 70.30, 70.14, 69.19, 67.64, 66.99, 60.36, 60.08, 59.98, 59.69, 59.39, 58.25, 47.17, 39.42, 39.05, 39.00, 34.76, 33.97, 33.80, 33.77, 33.71, 33.55, 32.50, 32.16, 32.08, 31.68, 30.30, 29.79, 29.75, 29.69, 29.48, 29.43, 28.63, 26.47, 26.45, 26.40, 26.38, 26.35, 26.23, 26.07, 26.03, 26.00, 25.38, 20.73, 19.47, 19.37, 19.27, 19.24, 19.18, 19.08, 19.05, 18.69, 17.33, 17.23, and 16.87; HRFTMS (ESI) m/z 1683.927 $[M + Na]^+$ (calcd for $C_{91}H_{132}N_6O_{22}Na$, 1683.929).

Fmoc-Tyr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$-OBn (**13c**). By employing the procedure followed for the preparation and purification of **5**, unit **12** (500 mg, 0.42 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (633 μL, 614 mg, 4.2 mmol) in 15 mL of DCM and then condensed to unit **11c** (329 mg, 0.42 mmol) using HBTU (319 mg, 0.84 mmol) and TEA (58 μL, 42 mg, 0.42 mmol) in 7 mL of anhydrous DMF. Following separation by column chromatography **13c** was obtained as a colorless solid: yield 74% (534 mg, 0.31 mmol); TLC R_f 0.40 (1:2 EtOAc/hexanes); mp 61−65

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°C; $[\alpha]^{24}_{D}$ +14.3 (*c* 0.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, d, *J* = 7.6 Hz), 7.69 (1H, d, *J* = 6.0 Hz), 7.65 (1H, d, *J* = 6.0 Hz), 7.53 (3H, m), 7.38 (4H, m), 7.29 (7H, m), 7.06 (2H, d, *J* = 8.4 Hz), 6.90 (2H, d, *J* = 8.4 Hz), 5.74 (1H, d, *J* = 6.4 Hz), 5.26 (5H, m), 5.09 (3H, m), 4.38 (4H, m), 4.20 (1H, m), 4.11 (2H, m), 4.00 (1H, m), 3.94 (1H, m), 3.09 (2H, m), 2.28 (5H, m), 1.65 (21H, m), 1.41 (12H, m), 1.21 (18H, m), and 1.02 (36H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.67, 171.54, 171.63, 171.52, 171.45, 171.02, 170.93, 170.82, 170.59, 170.47, 170.42, 170.40, 156.64, 154.77, 143.92, 143.69, 141.37, 135.47, 130.19, 129.69, 128.54, 128.25, 127.82, 127.17, 125.15, 124.22, 120.06, 78.42, 72.95, 72.45, 72.03, 70.36, 70.15, 69.20, 67.58, 66.98, 59.95, 59.93, 59.73, 59.21, 58.26, 56.03, 47.09, 39.39, 39.08, 39.04, 39.03, 36.46, 34.74, 33.96, 33.95, 33.75, 33.70, 33.67, 33.63, 32.46, 32.18, 32.05, 31.66, 30.27, 29.69, 29.36, 29.27, 29.13, 28.94, 26.45, 26.39, 26.36, 26.06, 25.92, 25.35, 20.78, 19.41, 19.33, 19.30, 19.25, 19.24, 19.18, 19.05, 18.69, 17.38, 17.34, 16.83, 14.19, and 11.51; HRFTMS (ESI) *m*/*z* 1745.941 $[M + Na]^+$ (calcd for $C_{96}H_{134}N_6O_{22}Na$, 1745.944).

Cyclo-[Val-D-Hcha-D-Val-Lac]$_3$ (***14a**, silstatin 4*). Using the strategy followed for the preparation and purification of **7a**, depsipeptide **13a** (193 mg, 0.12 mmol) was deprotected using 20% palladium hydroxide-on-carbon (100 mg) in 8 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (224 mg, 0.48 mmol) and TEA (35 μL, 24 mg, 0.24 mmol) in 120 mL of anhydrous DCM. After separation by column chromatography silstatin 4 (**14a**) was obtained as a colorless solid: yield 23% (35 mg, 27 μmol); TLC R_f 0.5 (1:4 EtOAc/hexanes); mp 52−56 °C; $[\alpha]^{24}_{D}$ +35.0 (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (3H, d, *J* = 8.0 Hz), 7.73 (3H, d, *J* = 6.4 Hz), 5.23 (3H, m), 5.17 (8.0 Hz, dd), 3.93 (3H, dd, *J* = 9.6 Hz, 6.0 Hz), 2.23 (6H, m), 1.74 (21H, m), 1.46 (12H, m), and 1.02 (51H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.4, 172.1, 171.5, 170.4, 72.9, 70.6, 60.3, 59.1, 39.4, 34.0, 33.9, 32.1, 28.7, 28.6, 26.6, 26.4, 26.1, 19.8, 19.6, 19.4, 19.2, and 17.3; HRMS (APCI) *m*/*z* 1273.779 $[M + H]^+$ (calcd for $C_{66}H_{109}N_6O_{18}$, 1273.780).

Cyclo-{Thr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$} (***14b**). Using the strategy followed for the preparation and purification of **7a**, depsipeptide **13b** (530 mg, 0.26 mmol) was deprotected using 20% palladium hydroxide-on-carbon (200 mg) in 10 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (485 mg, 1.04 mmol) and TEA (144 μL, 105 mg, 1.04 mmol) in 260 mL of anhydrous DCM. After separation by column chromatography **14b** was obtained as a colorless solid: yield 29% (100 mg, 75 μmol); TLC R_f 0.4 (1:4 EtOAc/hexanes); mp 61−65 °C; $[\alpha]^{24}_{D}$ +27.1 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (1H, d, *J* = 8.4 Hz), 7.69 (1H, d, *J* = 7.2 Hz), 7.62 (1H, d, *J* = 8.4 Hz), 7.42 (1H, d, *J* = 8.0 Hz), 7.40 (1H, d, *J* = 6.0 Hz), 7.29 (1H, d, *J* = 5.2 Hz), 5.23 (5H, m), 5.13 (1H, dd, *J* = 9.2 Hz, 3.6 Hz), 4.29 (1H, t, *J* = 8.0 Hz), 4.23 (1H, t, *J* = 8.0 Hz), 4.05 (4H, m), 3.85 (1H, dd, *J* = 10 Hz, 5.6 Hz), 2.23 (5H, m), 1.74 (21H, m), 1.41 (12H, m), 1.21 (21H, m), and 1.02 (36H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.29, 172.16, 172.08, 172.01, 171.78, 171.65, 171.17, 171.02, 170.19, 170.13, 169.94, 169.93, 74.84, 73.29, 72.77, 72.47, 71.04, 70.81, 70.70, 66.64, 60.82, 59.76, 59.10, 58.94, 58.23, 57.81, 39.50, 39.47, 39.43, 39.21, 36.77, 34.79, 34.13, 34.09, 34.03, 33.90, 33.83, 33.80, 32.22, 31.93, 31.83, 29.91, 29.35, 28.98, 28.68, 28.64, 28.59, 28.57, 28.52, 26.52, 26.42, 26.35, 26.09, 24.82, 20.15, 19.91, 19.53, 19.51, 19.42, 19.36, 19.29, 18.95, 18.58, 18.46, 17.50, 17.21, and 17.13; HRFTMS (ESI) *m*/*z* 1353.805 $[M + Na]^+$ (calcd for $C_{69}H_{114}N_6O_{19}Na$, 1353.803).

Cyclo-{Tyr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$} (***14c**). By means of the strategy followed for the preparation and purification of **7a**, depsipeptide **13c** (502 mg, 0.29 mmol) was deprotected using 20% palladium hydroxide-on-carbon (200 mg) in 8 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (541 mg, 1.16 mmol) and TEA (160 μL, 118 mg, 1.17 mmol) in 290 mL of anhydrous DCM. After separation by column chromatography cyclodepsipeptide **14c** was obtained as a colorless solid: yield 34% (145 mg, 0.10 mmol); TLC R_f 0.4 (1:4 EtOAc/hexanes); mp 68−72 °C; $[\alpha]^{24}_{D}$ +31.3 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, d, *J* = 7.2 Hz), 7.93 (1H, d, *J* = 6.0 Hz), 7.85 (1H, d, *J* = 6.8 Hz), 7.75 (1H, d, *J* = 8.4 Hz), 7.63 (1H, d, *J* = 6.8 Hz), 7.58 (1H, d, *J* = 5.6 Hz), 7.11 (2H, d, *J* = 8.4 Hz), 6.88 (2H, d, *J* = 8.0 Hz), 5.23 (3H, m), 5.13 (3H, m), 4.57 (q, *J* = 7.2 Hz, 1H, *J* = 9.2 Hz, 4.0 Hz, dd), 4.07 (3H, dd, *J* = 9.2 Hz), 4.16 (1H, t, *J* = 8.8 Hz), 3.99 (1H, dd, *J* = 9.6 Hz, 7.2 Hz), 3.88 (3H, m), 3.11 (2H, m), 2.23 (5H, m), and 1.90−0.80 (87H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.79, 172.76, 172.42, 172.15, 171.92, 171.75, 171.62, 171.55, 170.77, 170.45, 170.34, 170.10, 154.34, 131.42, 129.84, 124.13, 78.28, 73.37, 73.20, 72.54, 70.98, 70.71, 70.03, 60.76, 60.63, 59.69, 59.64, 58.33, 54.29, 39.50, 39.44, 39.25, 39.24, 35.29, 34.06, 33.89, 33.85, 33.69, 33.46, 32.88, 31.95, 31.85, 29.80, 29.02, 28.96, 28.76, 28.69, 28.67, 28.64, 28.61, 28.50, 26.52, 26.45, 26.39, 26.10, 26.07, 26.02, 19.96, 19.64, 19.49, 19.47, 19.40, 19.35, 19.32, 19.25, 18.92, 17.41, 17.27, 16.86, and 14.22; HRFTMS (ESI) *m*/*z* 1415.822 $[M + Na]^+$ (calcd for $C_{74}H_{116}N_6O_{19}Na$, 1415.819).

Cyclo-{Thr-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$} (***15a**, silstatin 5*). By the experimental route followed for the preparation and isolation of **8a**, cyclodepsipeptide **14b** (80 mg, 60 μmol) was deprotected with TFA (250 μL) in 2 mL of anhydrous DCM. Isolation by column chromatography yielded cyclodepsipeptide **15a** (silstatin 5) as a colorless solid: yield 91% (70 mg, 55 μmol); TLC R_f 0.27 (1:3 EtOAc/hexanes); mp 58−63 °C; $[\alpha]^{24}_{D}$ +32.0 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (1H, d, *J* = 6.8 Hz), 7.88 (1H, d, *J* = 7.2 Hz), 7.76 (3H, m), 7.57 (1H, d, *J* = 6.0 Hz), 5.20 (5H, m), 5.05 (1H, dd, *J* = 9.2 Hz, 4.4 Hz), 4.12 (1H, t, *J* = 9.2 Hz), 4.03 (4H, m), 3.88 (2H, m), 2.23 (4H, m), 2.11 (1H, m), 1.70 (21H, m), 1.41 (12H, m), and 1.21−0.90 (48H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.36, 172.42, 172.26, 172.01, 171.96, 171.56, 171.36, 170.91, 170.61, 170.60, 170.28, 169.84, 73.80, 72.45, 72.34, 70.65, 70.60, 70.23, 66.01, 60.45, 60.36, 60.11, 59.60, 59.24, 58.37, 39.33, 39.07, 39.02, 34.62, 33.86, 33.75, 33.70, 33.65, 32.02, 31.96, 31.79, 28.61, 28.56, 28.47, 28.35, 28.11, 26.37, 26.35, 26.27, 26.22, 26.15, 25.97, 25.95, 25.93, 25.23, 19.62, 19.41, 19.40, 19.36, 19.34, 19.31, 19.30, 19.28, 19.27, 19.17, 19.15, 18.83, 17.35, 16.92, and 16.85; HRMS (APCI) *m*/*z* 1275.757 $[M + H]^+$ (calcd for $C_{65}H_{107}N_6O_{19}$, 1275.759).

Cyclo-{Tyr-D-Hcha-D-Val-Lac-[Val-D-Hcha-D-Val-Lac]$_2$} (***15b**, silstatin 6*). Again by the procedure followed for the preparation and purification of **8a**, cyclodepsipeptide **14c** (130 mg, 93 μmol) was deprotected with TFA (250 μL) in 2 mL of anhydrous DCM. After separation by column chromatography cyclodepsipeptide **15b** (silstatin 6) was obtained as a colorless solid: yield 96% (120 mg, 90 μmol); TLC R_f 0.28 (1:3 EtOAc/hexanes); mp 76−80 °C; $[\alpha]^{24}_{D}$ +26.2 (*c* 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, d, *J* = 7.2 Hz), 7.81 (1H, d, *J* = 5.6 Hz), 7.77 (1H, d, *J* = 8.4 Hz), 7.69 (1H, d, *J* = 6.4 Hz), 7.63 (1H, d, *J* = 6.0 Hz), 7.59 (1H, d, *J* = 7.2 Hz), 7.02 (2H, d, *J* = 8.8 Hz), 6.73 (2H, d, *J* = 8.4 Hz), 5.23 (3H, m), 5.10 (3H, m), 4.50 (q, *J* = 8.0 Hz, 1H), 4.15 (1H, t, *J* = 8.8 Hz), 3.99 (2H, m), 3.88 (2H, m), 3.11 (2H, m), 2.23 (5H, m), and 1.90−0.80 (78H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.65, 172.62, 172.32, 172.15, 171.92, 171.79, 171.76, 171.43, 170.84, 170.53, 170.35, 170.09, 155.46, 130.45, 127.60, 115.75, 73.49, 73.10, 72.57, 70.90, 70.76, 70.28, 60.75, 60.36, 59.17, 59.15, 58.56, 54.59, 39.45, 39.42, 39.40, 39.25, 39.24, 35.30, 34.79, 34.07, 33.88, 33.63, 33.57, 32.57, 31.92, 31.84, 31.70, 28.95, 28.91, 28.75, 28.74, 28.69, 26.51, 26.41, 26.12, 26.07, 25.96, 25.40, 22.77, 19.91, 19.79, 19.60, 19.46, 19.40, 19.30, 19.28, 19.27, 19.11, 18.97, 18.94, 17.39, 17.06, and 16.99; HRFTMS (ESI) *m*/*z* 1359.755 $[M + Na]^+$ (calcd for $C_{70}H_{108}N_6O_{19}Na$, 1359.756).

Fmoc-Thr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$-OBn (***16a**). Applying the method followed for the preparation and isolation of **5**, unit **5** (264 mg, 0.24 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (362 μL, 351 mg, 2.4 mmol) in 15 mL of DCM and then coupled to unit **11b** (170 mg, 0.24 mmol) using HBTU (182 mg, 0.48 mmol) and TEA (33 μL, 24 mg, 0.24 mmol) in 5 mL of anhydrous DMF. Separation by column chromatography provided **16a**, which was obtained as a colorless solid: yield 74% (281 mg, 0.18 mmol); TLC R_f 0.50 (1:2 EtOAc/hexanes); mp 56−60 °C; $[\alpha]^{24}_{D}$ +7.62 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, d, *J* = 7.2 Hz), 7.70 (1H, d, *J* = 6.0 Hz), 7.55 (4H, m), 7.39 (2H, m) 7.29 (8H, m), 7.14 (1H, d, *J* = 6.4 Hz), 5.65 (1H, d, *J* = 7.2 Hz), 5.24 (5H, m), 5.10 (3H, m), 4.38 (3H, m), 4.22 (1H, t, *J* = 7.2 Hz), 4.13 (4H, m), 3.98 (1H, m), 3.92 (1H, m), 2.27 (5H, m), 1.74 (13H, m), 1.41 (10H, m), 1.09 (15H, m), and 0.98 (44H, m); ^{13}C NMR ($CDCl_3$, 101

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MHz) δ 171.45, 171.38, 171.34, 171.03, 170.96, 170.91, 170.76, 170.69, 170.53, 170.48, 170.37, 170.36, 156.88, 143.95, 143.71, 141.38, 135.48, 128.58, 128.32, 128.26, 127.84, 127.16, 125.17, 120.09, 74.75, 73.10, 72.82, 72.62, 70.27, 70.18, 69.17, 67.63, 66.98, 66.94, 64.43, 60.35, 60.07, 59.97, 59.61, 59.42, 58.17, 47.15, 40.90, 40.43, 39.04, 33.80, 33.53, 32.47, 30.34, 29.76, 29.64, 29.44, 29.43, 28.61, 26.38, 26.21, 25.98, 24.48, 24.44, 23.33, 21.51, 21.44, 20.71, 19.47, 19.37, 19.31, 19.26, 19.23, 19.20, 19.15, 19.08, 19.06, 18.63, 17.36, 17.23, and 16.85; HRFTMS (ESI) *m/z* 1603.867 $[M + Na]^+$ (calcd for $C_{85}H_{124}N_6O_{22}Na$, 1603.866).

*Fmoc-Tyr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$-OBn (**16b**).* By following the experimental procedures used for the synthesis and purification of **5**, that unit (**5**) (221 mg, 0.20 mmol) was deprotected with 2,2′,2″-triaminotriethylamine (302 μL, 293 mg, 2.0 mmol) in 15 mL of DCM and then coupled to unit **11c** (158 mg, 0.20 mmol) using HBTU (152 mg, 0.40 mmol) and TEA (28 μL, 20 mg, 0.20 mmol) in 5 mL of anhydrous DMF. Isolation by column chromatography afforded **16b** as a colorless solid in 71% yield (232 mg, 0.14 mmol): TLC R_f 0.45 (1:2 EtOAc/hexanes); mp 58−62 °C; $[\alpha]^{24}_D$ +15 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (3H, m), 7.65 (1H, d, *J* = 7.2 Hz), 7.55 (3H, m), 7.30 (11H, m), 7.06 (2H, d, *J* = 8.0 Hz), 6.89 (2H, d, *J* = 8.4 Hz), 5.79 (1H, d, *J* = 6.8 Hz), 5.24 (5H, m), 5.08 (3H, m), 4.38 (3H, m), 4.05 (5H, m), 3.03 (2H, m), 2.25 (5H, m), 1.70 (13H, m), 1.41 (10H, m), 1.32 (9H, s), 1.09 (3H, m), and 0.98 (44H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.68, 171.57, 171.54, 171.42, 171.38, 170.93, 170.72, 170.58, 170.45, 170.43, 170.42, 170.38, 156.64, 154.73, 143.89, 143.67, 141.35, 135.42, 129.67, 128.54, 128.29, 128.23, 127.81, 127.15, 125.13, 124.21, 120.05, 78.43, 73.08, 72.92, 72.66, 70.22, 69.21, 67.55, 66.99, 59.91, 59.89, 59.87, 59.86, 59.60, 59.21, 58.18, 56.01, 47.07, 40.86, 40.47, 39.07, 36.44, 34.72, 33.66, 33.61, 32.43, 31.64, 30.33, 29.73, 29.40, 29.36, 29.28, 29.11, 28.92, 26.37, 26.04, 25.90, 25.34, 24.46, 24.44, 23.32, 23.31, 21.52, 21.41, 19.39, 19.30, 19.26, 19.23, 19.19, 19.14, 19.05, 18.62, 17.37, and 16.83; HRFTMS (ESI) *m/z* 1665.877 $[M + Na]^+$ (calcd for $C_{90}H_{126}N_6O_{22}Na$, 1665.882).

*Cyclo-{Thr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**17a**).* By employing the route followed for obtaining **7a**, depsipeptide **16a** (269 mg, 0.17 mmol) was deprotected using 20% palladium hydroxide-on-carbon (200 mg) in 8 mL of 4:1 EtOAc/MeOH and then cyclized employing PyBroP (317 mg, 0.68 mmol) and TEA (94 μL, 69 mg, 0.68 mmol) in 170 mL of anhydrous DCM. Separation by column chromatography provided **17a** as a colorless solid in 48% yield (102 mg, 81 μmol); TLC R_f 0.3 (1:4 EtOAc/hexanes); mp 58−62 °C; $[\alpha]^{24}_D$ +17.3 (*c* 0.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (1H, d, *J* = 8.0 Hz), 7.71 (1H, d, *J* = 7.2 Hz), 7.62 (1H, d, *J* = 8.4 Hz), 7.43 (2H, m), 7.33 (1H, d, *J* = 4.8 Hz), 5.18 (6H, m), 4.28 (1H, t, *J* = 8.0 Hz), 4.22 (1H, t, *J* = 8.0 Hz), 4.05 (4H, m), 3.85 (1H, dd, *J* = 10 Hz, 5.6 Hz), 2.23 (5H, m), 1.74 (13H, m), 1.43 (10H, m), and 1.25−0.9 (59H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.31, 172.21, 172.06, 171.97, 171.69, 171.20, 171.09, 170.24, 170.22, 170.14, 169.99, 169.94, 74.80, 73.41, 73.28, 73.12, 70.99, 70.79, 70.68, 66.61, 60.79, 59.77, 59.12, 58.97, 58.27, 57.84, 40.94, 40.69, 39.46, 34.03, 33.77, 32.20, 29.83, 29.80, 29.29, 28.91, 28.65, 28.56, 28.49, 26.49, 26.33, 26.07, 24.60, 24.55, 23.50, 21.29, 21.18, 20.12, 19.87, 19.53, 19.51, 19.40, 19.37, 19.33, 19.29, 18.96, 18.60, 18.47, 17.48, 17.19, and 17.12; HRMS (APCI) *m/z* 1251.760 $[M + H]^+$ (calcd for $C_{63}H_{107}N_6O_{19}$, 1251.759).

*Cyclo-{Tyr(tBu)-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**17b**).* Using the preceding method followed for the synthesis and isolation of **7a**, depsipeptide **16b** (220 mg, 0.13 mmol) was deprotected using 20% palladium hydroxide-on-carbon (200 mg) in 8 mL of 4:1 EtOAc/MeOH and then cyclized with PyBroP (250 mg, 0.54 mmol) and TEA (74 μL, 54 mg, 0.54 mmol) in 134 mL of anhydrous DCM. Isolation by column chromatography yielded **17b** as a colorless solid: yield 22% (38 mg, 29 μmol); TLC R_f 0.3 (1:4 EtOAc/hexanes); mp 55−59 °C; $[\alpha]^{24}_D$ +24 (*c* 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (1H, d, *J* = 7.2 Hz), 7.94 (1H, d, *J* = 5.6 Hz), 7.85 (1H, d, *J* = 7.2 Hz), 7.74 (1H, d, *J* = 8.8 Hz), 7.63 (1H, d, *J* = 6.8 Hz), 7.59 (1H, d, *J* = 5.6 Hz), 7.12 (2H, d, *J* = 8.4 Hz), 6.88 (2H, d, *J* = 8.4 Hz), 5.23 (5H, m), 5.13 (1H, m), 4.58 (q, *J* = 7.6 Hz, 1H), 4.18 (1H, t, *J* = 8.8 Hz), 4.01 (1H, dd, *J* = 10 Hz, 7.6 Hz), 3.88 (3H, m), 3.11 (2H, m), 2.23 (5H, m), and 1.90−0.80 (73H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.64, 172.53, 172.29, 172.02, 171.78, 171.60, 171.52, 171.31, 170.57, 170.35, 170.10, 169.92, 154.19, 131.28, 129.68, 123.97, 78.15, 73.78, 73.18, 73.08, 70.81, 70.58, 69.86, 60.55, 59.58, 59.47, 58.13, 54.12, 40.73, 40.58, 39.29, 35.10, 35.09, 33.51, 33.33, 32.73, 29.66, 28.82, 28.62, 28.56, 28.49, 28.45, 28.33, 26.35, 25.91, 25.86, 24.46, 24.45, 23.35, 23.32, 21.15, 21.03, 19.82, 19.80, 19.50, 19.35, 19.31, 19.27, 19.18, 19.16, 18.75, 17.27, 17.15, and 16.68; HRMS (APCI) *m/z* 1313.777 $[M + H]^+$ (calcd for $C_{68}H_{109}N_6O_{19}$, 1313.775).

*Cyclo-{Thr-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**18a**, silstatin 7).* Using the procedure used for the synthesis and isolation of **8a**, cyclodepsipeptide **17a** (80 mg, 64 μmol) was deprotected with TFA (250 μL) in 1 mL of anhydrous DCM. After separation by column chromatography **18a** (silstatin 7) was obtained as a colorless solid: yield 92% (70 mg, 59 μmol); TLC R_f 0.27 (1:3 EtOAc/hexanes); mp 75−79 °C; $[\alpha]^{24}_D$ +19 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, d, *J* = 6.8 Hz), 7.88 (1H, d, *J* = 7.2 Hz), 7.76 (3H, m), 7.57 (1H, d, *J* = 6.0 Hz), 5.20 (5H, m), 5.05 (1H, dd, *J* = 8.8 Hz, 4.0 Hz), 4.75 (1H, br s), 4.12 (1H, t, *J* = 8.8 Hz), 3.95 (6H, m), 2.23 (4H, m), 2.11 (1H, m), 1.74 (13H, m), 1.43 (10H, m), and 1.25−0.9 (50H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.4, 172.65, 172.30, 172.23, 172.13, 171.75, 171.57, 171.07, 170.94, 170.80, 170.40, 170.00, 73.91, 73.31, 73.19, 70.79, 70.72, 70.43, 66.16, 60.69, 60.48, 60.21, 59.86, 59.44, 58.59, 40.82, 40.73, 39.16, 34.00, 33.87, 32.12, 28.73, 28.68, 28.62, 28.52, 28.26, 26.50, 26.30, 26.10, 24.59, 24.58, 23.46, 23.34, 21.54, 21.34, 19.76, 19.58, 19.53, 19.49, 19.48, 19.47, 19.46, 19.45, 19.42, 19.32, 19.02, 17.50, and 17.05; HRMS (APCI) *m/z* 1195.696 $[M + H]^+$ (calcd for $C_{59}H_{99}N_6O_{19}$, 1195.697).

*Cyclo-{Tyr-D-Hcha-D-Val-Lac-[Val-D-Hica-D-Val-Lac]$_2$} (**18b**, silstatin 8).* By using the general procedure for the preparation and isolation of **8a**, cyclodepsipeptide **17b** (30 mg, 23 μmol) was deprotected with TFA (250 μL) in 1 mL of anhydrous DCM. Separation by column chromatography led to **18b** (silstatin 8) as a colorless solid: yield 87% (25 mg, 20 μmol); TLC R_f 0.27 (1:3 EtOAc/hexanes); mp 78−82 °C; $[\alpha]^{24}_D$ +21.3 (*c* 0.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (3H, m), 7.67 (2H, m), 7.61 (1H, d, *J* = 7.2 Hz), 7.05 (2H, d, *J* = 8.0 Hz), 6.74 (2H, d, *J* = 8.0 Hz), 6.03 (1H, br s), 5.23 (3H, m), 5.09 (3H, m), 4.48 (q, *J* = 7.4 Hz, 1H), 4.14 (1H, t, *J* = 8.8 Hz), 4.05 (2H, m), 3.93 (2H, m), 3.05 (2H, m), 2.23 (5H, m), 1.77 (7H, m), 1.60 (7H, m), 1.45 (6H, d, *J* = 6.8 Hz), 1.36 (3H, d, *J* = 6.8 Hz), and 1.11−0.80 (47H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.60, 172.44, 172.34, 172.22, 171.88, 171.78, 171.69, 171.49, 170.99, 170.45, 170.36, 170.14, 155.25, 130.52, 127.80, 115.81, 73.75, 73.54, 73.26, 70.86, 70.72, 70.38, 60.75, 60.31, 59.04, 59.01, 58.69, 54.69, 40.91, 40.74, 39.41, 35.32, 33.64, 33.62, 32.54, 29.85, 29.04, 28.91, 28.75, 28.72, 28.67, 26.53, 26.09, 25.97, 24.66, 24.63, 23.50, 23.49, 21.33, 21.21, 19.91, 19.76, 19.62, 19.50, 19.42, 19.36, 19.35, 19.07, 18.99, 18.89, 17.44, and 17.06; HRMS (APCI) *m/z* 1257.713 $[M + H]^+$ (calcd for $C_{64}H_{101}N_6O_{19}$, 1257.712).

*Methyl 1-(4-formyl-2-nitrophenyl)-2,3,4-tri-O-acetyl-β-D-glucuronate (**19**).* Employing the strategy presented by Duimstra et al.,[11] 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucuronate (2.32 g, 5.84 mmol) was coupled to 4-hydroxy-3-nitrobenzaldehyde (1.66 g, 9.93 mmol) using Ag_2O (6.14 g, 26.5 mmol) in 20 mL of anhydrous CH_3CN. Yield 93% (2.62 g, 5.42 mmol); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.98 (1H, s), 8.32 (1H, d, *J* = 1.6 Hz), 8.10 (1H, dd, *J* = 8.0 Hz, 1.6 Hz), 7.52 (1H, d, *J* = 8.8 Hz), 5.43 (2H, m), 5.31 (2H, m), 4.34 (1H, d, *J* = 8.8 Hz), 3.71 (3H, s), 2.13 (3H, s), 2.09 (3H, s), and 2.08 (3H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 188.7, 170.0, 169.4, 169.2, 166.8, 153.4, 141.2, 134.4, 131.6, 126.8, 118.9, 98.7, 72.8, 70.3, 69.9, 68.2, 53.2, 20.7, and 20.7.

*Methyl 1-(4-(tert-butyldimethylsilyloxy)methyl-2-nitrophenyl)-2,3,4-tri-O-acetyl-β-D-glucuronate (**21**).* By means of the synthesis presented by Duimstra et al.,[11] glucuronate **19** (2.60 g, 5.38 mmol) was reduced with sodium borohydride (305 mg, 8.07 mmol) in the presence of silica (1.08 g) in 65 mL of 1:5 2-propanol/$CHCl_3$ and then silyl protected using TBDMS-Cl (1.22 g, 8.07 mmol) and imidazole (549 mg, 8.07 mmol) in 30 mL of anhydrous DCM. After separation by column chromatography **21** was obtained as a colorless

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solid: yield 80% (2.58 g, 4.30 mmol); TLC R_f 0.25 (1:2 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (1H, d, J = 2.4 Hz), 7.45 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.32 (1H, d, J = 8.4 Hz), 5.31 (3H, m), 5.17 (1H, d, J = 7.2 Hz), 4.72 (2H, s), 4.19 (1H, d, J = 9.2 Hz), 3.75 (3H, s), 2.13 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 0.94 (9H, s), and 0.11 (6H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.16, 169.44, 169.43, 166.87, 147.92, 141.45, 138.24, 131.19, 122.56, 120.35, 100.23, 72.75, 71.37, 70.39, 68.97, 63.53, 53.18, 26.01, 20.74, 20.71, 20.65, and −5.18.

*Allyl 1-(4-(tert-butyldimethylsilyloxy)methyl-2-nitrophenyl)-β-D-glucuronate (**22**).* By employing the procedure recorded by Grinda et al.,[12] glucuronate **21** (1.58 g, 2.63 mmol) was deacetylated with 0.5 N sodium methoxide in MeOH (5.26 mL, 2.63 mmol) in 50 mL of anhydrous MeOH and then transesterified with a sodium allylate solution (prepared by dissolving sodium (12 mg, 0.53 mmol) in 5 mL of allyl alcohol). After separation by column chromatography **22** was obtained as a colorless solid: yield 51% (671 mg, 1.34 mmol); TLC R_f 0.25 (1:1 acetone/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (1H, d, J = 2.4 Hz), 7.42 (1H, dd, J = 8.4 Hz, 2.0 Hz), 7.29 (1H, d, J = 8.4 Hz), 5.87 (1H, m), 5.32 (1H, m), 5.20 (1H, m), 5.02 (1H, d, J = 7.2 Hz), 4.81 (1H, br s), 4.66 (4H, m), 4.39 (1H, br s), 4.28 (1H, br s), 4.11 (1H, d, J = 9.6 Hz), 3.92 (1H, m), 3.78 (1H, m), 0.91 (9H, s), and 0.07 (6H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 168.4, 149.1, 140.3, 137.0, 131.8, 131.4, 122.9, 119.1, 118.8, 102.3, 75.1, 74.8, 72.9, 71.0, 66.6, 63.5, 26.0, 18.5, and −5.2.

*Allyl 1-(4-(tert-butyldimethylsilyloxy)methyl-2-nitrophenyl)-2,3,4-tri-O-allyloxycarbonyl-β-D-glucuronate (**23**).* Following the experimental procedure recommended by Grinda et al.,[12] the free hydroxy groups of glucuronate **22** (200 mg, 0.40 mmol) were protected using allyl chloroformate (1.28 mL, 1.45 g, 12.0 mmol) in 2 mL of anhydrous pyridine, and following separation by column chromatography **23** was obtained as a colorless oil: yield 72% (216 mg, 0.29 mmol); TLC R_f 0.4 (1:3 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (1H, d, J = 2.4 Hz), 7.45 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.29 (1H, d, J = 8.4 Hz), 5.87 (4H, m), 5.33 (3H, m), 5.20 (7H, m), 4.68 (4H, m), 4.59 (6H, m), 4.31 (1H, m), 0.91 (9H, s), and 0.07 (6H, s); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.73, 154.03, 153.55, 153.54, 147.96, 140.99, 137.99, 131.31, 131.22, 131.15, 131.04, 130.97, 122.68, 119.38, 119.35, 119.32, 119.27, 119.05, 99.83, 75.15, 74.07, 72.49, 72.26, 69.54, 69.33, 69.18, 66.94, 63.42, 25.94, 18.41, and −5.24.

*Allyl 1-(4-hydroxymethyl-2-nitrophenyl)-2,3,4-tri-O-allyloxycarbonyl-β-D-glucuronate (**24**).* Again following the procedure presented by Grinda et al.,[12] compound **23** (409 mg, 0.54 mmol) was deprotected using a 7:3 HF/pyridine solution (2.05 mL) in 10 mL of anhydrous tetrahydrofuran (THF). Column chromatography using EtOAc/hexanes gave **24** as a colorless oil: yield 92% (316 mg, 0.50 mmol); TLC R_f 0.20 (1:3 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (1H, d, J = 2.0 Hz), 7.45 (1H, dd, J = 8.4 Hz, 2.0 Hz), 7.29 (1H, d, J = 8.4 Hz), 5.87 (4H, m), 5.33 (4H, m), 5.20 (8H, m), 4.67 (10H, m), and 4.35 (1H, d, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.80, 154.04, 153.58, 153.56, 148.16, 140.75, 137.52, 132.11, 131.24, 131.10, 131.00, 130.92, 123.33, 119.44, 119.37, 119.34, 119.12, 119.09, 99.54, 75.15, 74.07, 72.45, 72.07, 69.61, 69.36, 69.22, 67.01, and 63.25.

*Allyl 1-(4-(O-4-nitrophenyloxycarbonyl)methyl-2-nitrophenyl)-2,3,4-tri-O-allyloxycarbonyl-β-D-glucuronate (**25**).* Following the procedure presented by Grinda et al.,[12] compound **24** (316 mg, 0.50 mmol) was activated using 4-nitrophenyl chloroformate (202 mg, 1.00 mmol) and pyridine (101 μL, 99 mg, 1.25 mmol) in 10 mL of anhydrous dichloromethane. After separation by column chromatography **25** was obtained as a colorless oil: yield 89% (356 mg, 0.44 mmol); TLC R_f 0.4 (2:3 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (2H, d, J = 8.8 Hz), 7.87 (1H, d, J = 2.0 Hz), 7.58 (1H, dd, J = 8.4 Hz, 2.0 Hz), 7.29 (3H, m), 5.84 (4H, m), 5.33 (14H, m), 4.65 (8H, m), and 4.35 (1H, d, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.70, 155.36, 154.04, 153.55, 153.51, 152.39, 149.50, 145.60, 140.87, 134.20, 131.20, 131.11, 130.99, 130.90, 130.36, 125.68, 125.44, 121.86, 119.52, 119.45, 119.42, 119.22, 119.13, 99.35, 74.89, 73.93, 72.31, 72.30, 69.65, 69.40, 69.27, 68.93, and 67.05.

*Allyl-Protected Glucuronide Prodrug **27**.* To a stirred solution containing 4-(methylamino)butyric acid (38 mg, 0.32 mmol) in 1 mL of anhydrous DMF was added potassium carbonate (88 mg, 0.64 mmol) followed by compound **25** (256 mg, 0.32 mmol). The reaction mixture was stirred at 23 °C for 30 min, diluted with 60 mL of EtOAc, washed with 20 mL of 6% aqueous citric acid and 25 mL of brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was partially separated using a short silica gel plug to remove the 4-nitrophenol and afford intermediate **26** as a colorless oil: crude yield 80% (200 mg). Next, intermediate **26** (130 mg, 0.17 mmol) was dissolved in 3 mL of anhydrous DCM, and compound **18a** (167 mg, 0.14 mmol) was added followed by DMAP (5 mg, 0.04 mmol) and DCC (31 mg, 0.15 mmol). The reaction mixture was stirred at 23 °C for 16 h. The solvent was separated by filtration, and the filtrate was concentrated under reduced pressure. The crude product was separated by chromatography on a silica gel column. Elution with 3:2 hexanes/EtOAc gave the allyl-protected prodrug **27** as a colorless solid: yield 51% over two steps (176 mg, 90 μmol); TLC R_f 0.25 (2:3 EtOAc/hexanes); mp 76−79 °C; $[\alpha]^{24}_D$ +12.0 (c 0.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (1H, m), 7.77 (6H, m), 7.53 (1H, dd, J = 8.8 Hz, 2.0 Hz), 7.33 (1H, d, J = 8.8 Hz), 5.84 (4H, m), 5.25 (16H, m), 5.09 (4H, m), 4.65 (8H, m), 4.69 (8H, m), 4.48 (1H, dd, J = 8.4 Hz, 6.4 Hz), 4.33 (1H, d, J = 8.8 Hz), 4.08 (5H, m), 3.90 (1H, m), 3.47 (2H, m), 3.32 (3H, m), 2.92 (3H, s), 2.26 (5H, m), 1.77 (15H, m), 1.36 (13H, m), 1.08 (20H, m), and 0.94 (27H, m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.32, 172.26, 172.06, 172.03, 172.01, 171.84, 171.69, 171.57, 171.49, 170.46, 170.28, 170.08, 165.54, 156.71, 153.89, 153.41, 153.40, 148.68, 140.87, 133.38, 131.12, 131.00, 130.88, 130.79, 124.74, 119.89, 119.34, 119.26, 119.24, 119.21, 118.97, 99.54, 74.87, 73.83, 73.40, 73.27, 73.15, 72.27, 72.22, 70.47, 70.41, 70.37, 69.45, 69.22, 69.11, 68.50, 66.86, 65.17, 60.52, 59.88, 58.74, 58.63, 57.62, 49.10, 40.63, 40.57, 39.15, 34.62, 33.92, 33.73, 33.69, 32.12, 31.54, 28.62, 28.53, 28.49, 26.30, 26.16, 25.92, 25.58, 25.23, 24.90, 24.45, 24.41, 23.45, 23.23, 22.62, 22.61, 21.24, 20.95, 20.66, 19.71, 19.43, 19.39, 19.29, 19.24, 19.03, 18.92, 18.87, 17.21, 16.98, 16.88, and 14.07; HRFTMS (ESI), m/z 1979.8054 $[M + Na]^+$ (calcd for $C_{93}H_{136}N_8O_{37}Na$, 1979.8899).

*Glucuronide Prodrug **28**.* To a stirred solution containing protected prodrug **27** (24 mg, 12 μmol) in 1 mL of anhydrous THF was added a solution containing formic acid (1.4 μL, 1.7 mg, 36 μmol) and TEA (8.3 μL, 6.1 mg, 60 μmol) in 100 μL of anhydrous THF. The reaction mixture was stirred at 23 °C for 10 min; then $Pd(PPh_3)_4$ (1.4 mg, 1.2 μmol) was added, and the mixture was stirred at 23 °C for 2.5 h and then concentrated under reduced pressure. The crude product was separated by reversed-phase HPLC. Column: Phenomenex Luna C8(2), 250 × 10 mm, 5 μm. Flow rate: 3.5 mL/min. Solvents: (A) 50 mM NH_4OAc (pH = 3.5); (B) CH_3CN. Isocratic elution with 20% A in B from 0 to 10 min; next, gradient elution from 20% A in B to 1% A in B from min 10 to min 12; finally, isocratic elution with 1% A in B from min 12 to min 18. Retention time: 15.5 min. This provided a colorless solid: yield 50% (10 mg, 6 μmol); mp 133−136 °C; $[\alpha]^{24}_D$ +8.6 (c 0.04, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.84 (1H, m), 7.61 (1H, dd, J = 8.4 Hz, 1.6 Hz), 7.48 (1H, d, J = 8.8 Hz), 5.44 (1H, m), 5.12 (9H, m), 4.99 (1H, m), 4.39 (2H, d, J = 6.8 Hz), 4.25 (3H, m), 3.85 (1H, d, J = 9.6 Hz), 3.52 (3H, m), 3.36 (2H, m), 2.94 (3H, s), 2.30 (7H, m), 1.74 (15H, m), 1.41 (10H, m) 1.29 (6H, m), and 0.95 (44H, m); ^{13}C NMR (CD_3OD, 101 MHz) δ 175.10, 173.42, 173.40, 173.29, 173.16, 172.82, 172.77, 172.74, 172.12, 172.10, 172.01, 171.99, 171.97, 170.56, 157.75, 151.12, 141.73, 134.57, 132.65, 125.51, 119.06, 102.39, 77.66, 76.57, 74.87, 74.49, 74.44, 73.18, 71.95, 71.86, 71.79, 66.75, 60.00, 59.86, 59.80, 59.72, 57.24, 49.28, 41.97, 40.85, 40.83, 35.67, 34.99, 34.94, 34.90, 34.46, 33.37, 32.15, 32.12, 31.50, 31.40, 31.22, 31.07, 31.02, 27.43, 27.24, 27.04, 26.19, 25.70, 24.20, 23.75, 23.68, 23.61, 21.71, 21.60, 19.68, 19.63, 19.62, 19.61, 19.60, 19.28, 19.16, 18.93, 18.85, 18.78, 18.08, 18.03, and 17.19; HRFTMS (ESI) m/z 1687.7934 $[M + Na]^+$ (calcd for $C_{78}H_{120}N_8O_{31}Na$, 1687.7952).

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.[19] In summary, cells in a 5% fetal bovine serum/RPMI1640 medium were inoculated in 96-well plates and incubated for 24 h. Next, serial dilutions of the

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compounds were added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50}, or the drug concentration causing a 50% reduction in the net protein increase) was calcd from optical density data with Immunosoft software. Normal cells were treated in identical conditions. Normal human prostate CRL-2221 (PZ-HPV-7) was grown in MEM 10% FBS, and normal human breast MCF-10A in an MEGM kit.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**3c**, **5**−**8b**, **9**−**10c**, **12**−**15b**, **16a**−**18b**, **19**, **21**−**25**, **27**, and **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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