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Approaches for the Synthesis of Functionalized Cryptophycins

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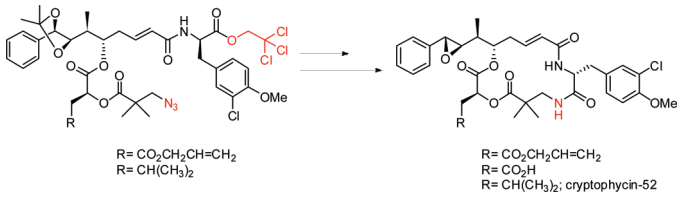

The first syntheses of bioactive cryptophycins functionalized at unit D were accomplished in a one-pot Staudinger reduction/cyclization step. An azido precursor for the lower part of the backbone was introduced to minimize protective group chemistry and enable a very convenient synthesis of cryptophycin-52 and unit D cryptophycin analogues containing an ester or a free carboxylic acid for bioconjugations. Both new cryptophycin derivatives show high biological activity in cytotoxicity assays.

Introduction

Cryptophycins are a family of macrocyclic depsipeptides. Cryptophycin-1 (**1**) was isolated 20 years ago from *Nostoc* sp. ATCC 53789.[1] Having isolated the same compound from a different cyanobacterium, Moore et al. made a first proposal regarding its stereochemistry in 1994,[2] while Kobayashi et al. contributed the first cryptophycin total synthesis.[3] The elucidation of the cryptophycin biosynthesis is still an ongoing challenge.[4]

Cryptophycin-1 and the synthetic analogue cryptophycin-52 (**2**) display extraordinary high cytotoxicity even against multi-drug-resistant cell lines. Cryptophycin-52 failed in clinical phase II studies due to lack of efficacy and high toxicity at the chosen doses.[5] Several elegant synthetic approaches, as well as extended SAR studies, have been performed for all four units of the cryptophycin backbone (Figure 1).[6]

Results and Discussion

We reasoned that adding a functionality such as an ester or a carboxylic acid to one of the units of the cryptophycin backbone might present an attachment point for conjugation and provide insight for the synthesis of future cryptophycin prodrugs. Unit D modifications of the cryptophycin backbone have not yet been investigated extensively, but the data obtained so far show a promising preservation of bioactivity.[7] Cryptophycins isolated from natural sources

(1) Schwartz, R. E.; Hirsch, C. F.; Sesin, D. F.; Flor, J. E.; Chartrain, M.; Fromtling, R. E.; Harris, G. H.; Salvatore, M. J.; Liesch, J. M.; Yudin, K. *J. Ind. Microbiol.* **1990**, *5*, 113–124.
(2) Golakoti, T.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994**, *116*, 4729–4737.
(3) Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2394–2396.
(4) (a) Beck, Z. Q.; Burr, D. A.; Sherman, D. H. *ChemBioChem* **2007**, *8*, 1373–1375. (b) Ding, Y.; Seufert, W. H.; Beck, Z. Q.; Sherman, D. H. *J. Am. Chem. Soc.* **2008**, *130*, 5492–5498.
(5) (a) D'Agostino, G.; Campo, J. D.; Mellado, B.; Izquierdo, M. A.; Minarik, T.; Cirri, L.; Marini, L.; Perez-Gracia, J. L.; Scambia, G. *Int. J. Gynecol. Cancer* **2006**, *16*, 71. (b) Edelman, M. J.; Gandara, D. R.; Hausner, P.; Israel, V; Thornton, D.; DeSanto, J.; Doyle, L. A. *Lung Cancer* **2003**, *39*, 197–199. (c) Liang, J.; Moore, R. E.; Moher, E. D.; Munroe, J. E.; Al-awar, R. S.; Hay, D. A.; Varie, D. L.; Zhang, T. Y.; Aikins, J. A.; Martinelli, M. J.; Shih, C.; Ray, J. E.; Gibson, L. L.; Vasudevan, V.; Polin, L.; White, K.; Kushner, J.; Simpson, C.; Pugh, S.; Corbett, T. H. *Invest. New Drugs* **2005**, *23*, 213–224.
(6) (a) Review: Eissler, S.; Stončius, A.; Nahrwold, M.; Sewald, N. *Synthesis* **2006**, *22*, 3747–3789. (b) Sammet, B.; Radzey, H.; Neumann, B.; Stammler, H.-G.; Sewald, N. *Synlett* **2009**, 417–420. (c) Eissler, S.; Bogner, T.; Nahrwold, M.; Sewald, N. *Chem.—Eur. J.* **2009**, *15*, 11273–11287. (d) Tripathy, N. K.; Georg, G. I. *Tetrahedron Lett.* **2004**, *45*, 5309–5311. (e) Seufert, W.; Beck, Z. Q.; Sherman, D. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 9298–9300. (f) Liu, W. L.; Zhang, J. C.; Jiang, F. Q.; Fu, L. *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 577–583.
(7) (a) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Le Husebo, T.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12030–12049. (b) Menon, K.; Alvarez, E.; Forler, P.; Phares, V.; Amsrud, T.; Shih, C.; Al-Awar, R.; Teicher, B. A. *Cancer Chemother. Pharmacol.* **2000**, *46*, 142–149.

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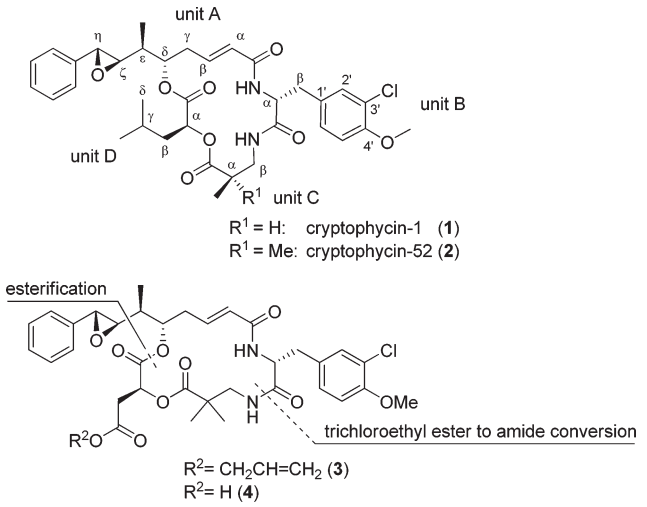
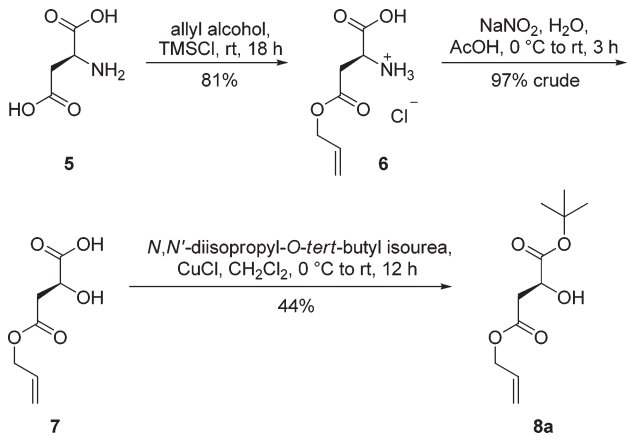


FIGURE 1. Structures of cryptophycins and the two novel unit D analogues **3** and **4**.

SCHEME 1. Synthesis of the Modified Unit D Precursor 8a

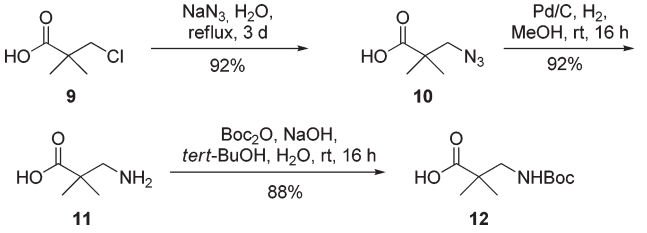


already contain one of four different α-hydroxy acids, thus some variation in that region is tolerated. Cryptophycins bearing the alternative *sec*-butyl, isopropyl, and *n*-propyl residue have a slightly decreased cytotoxicity.[7a] The bulky non-natural neopentyl residue shows equal effectiveness in vivo as the isobutyl group[7b] and even the inversion of the stereochemistry in unit D has no major effect on bioactivity.[8]

After analysis of possible protective group strategies, the allyl ester precursor **3** was chosen for the synthesis of carboxy cryptophycin **4** (Figure 1). The first step of the synthesis of a modified unit D building block was the known allylation of the side chain carboxy acid of L-aspartate (**5**), yielding its allyl ester **6** in 81% yield (Scheme 1).[9]

The amine **6** was then converted to the corresponding alcohol **7** with retention of configuration in 97% yield using a standard aqueous acidic diazotization protocol.[10] The crude α-hydroxy carboxylic acid **7** was directly used for an esterification mediated by *N*,*N*′-diisopropyl-*O*-*tert*-butyl isourea (*t*-BuOH preactivated by DIC). The *tert*-butyl ester

SCHEME 2. Synthesis of Unit C Precursor 12



8a was obtained in 44% yield.[11] The stereochemical purity of **8a** was confirmed by synthesis of the corresponding esters from both enantiomers of Mosher's acid. Comparison of ^{1}H and ^{13}C NMR spectra of both Mosher esters proved clearly that no diastereomeric mixtures were present (see Supporting Information for details).

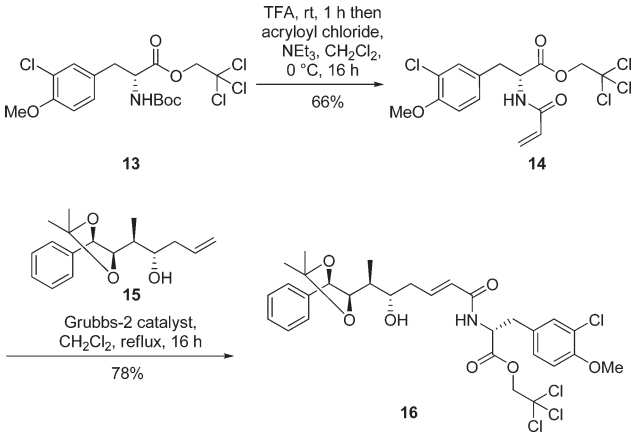
We recently introduced β-azidopivalic acid (**10**) as a unit C precursor for the synthesis of a bioactive cryptophycin-52 triazole analogue.[12] β-Azidopivalic acid can be synthesized in one step from commercially available β-chloropivalic acid (**9**) (Scheme 2). Furthermore, it can easily be reduced by catalytic hydrogenation to β-aminopivalic acid (**11**) for the synthesis of standard unit C precursors such as **12**.[13] Depending on the cyclization strategy, unit C precursors are normally based on such N-protected β-aminopivalic acid derivatives, which are most often synthesized in a four-step procedure from methyl or ethyl cyanoacetate.[13b]

The azido group can be considered as a protected amino function.[14] Therefore, to reduce the number of protective group operations, we decided to use β-azidopivalic acid (**10**) directly as a novel unit C precursor for the synthesis of cryptophycin-52 (**2**) and of cryptophycin unit D analogues **3** and **4**. For the synthesis of the cryptophycin backbone and the introduction of the epoxide moiety, previously known cryptophycin chemistry was developed further. A modular and convergent approach was envisaged to connect a novel unit CD fragment to the known unit AB fragment **16** (Scheme 3).[15] After initially constructing the ester between the AB and CD fragment of cryptophycin, intramolecular aminolysis of the trichloroethyl ester[16] eventually affects macrolactamization (Figure 1).

Boc-protected amino acid ester **13**[17] was deprotected with TFA and acrylated, affording acryl amide **14** in 66% yield. A metathesis reaction between **14** and 1.2 equiv of unit A precursor **15**[18] offers a novel access to unit AB precursor **16** in high yields. Azidopivalic acid (**10**) was converted to its acid chloride and esterified with *tert*-butyl esters **8a** or the leucine-derived (*S*)-*tert*-butyl 2-hydroxy-4-methylpentanoate (**8b**)[19]

(8) Buck, S. B.; Huff, J. K.; Himes, R. H.; Georg, G. I. *J. Med. Chem.* **2004**, *47*, 3697–3699.
(9) Belshaw, P. J.; Mzengeza, S.; Lajoie, G. A. *Synth. Commun.* **1990**, *20*, 3157–3160.
(10) Deechongkit, S.; You, S.-L.; Kelly, J. W. *Org. Lett.* **2004**, *6*, 497–500.
(11) Schultz, M.; Kunz, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1205–1220.
(12) Nahrwold, M.; Bogner, T.; Eissler, S.; Verma, S.; Sewald, N. *Org. Lett.* **2010**, *12*, 1064–1067.
(13) (a) McCubbin, J. A.; Maddess, M. L.; Lautens, M. *Org. Lett.* **2006**, *8*, 2993–2996. (b) Aikins, J. A.; Briggs, B. S.; Zhang, T. Y.; Zmijewski, M. J. U.S. Patent 6,376,230 B1, 2002.
(14) Jost, M.; Greie, J. C.; Stemmer, N.; Wilking, S. D.; Altendorf, K.; Sewald, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 4267–4269.
(15) Li, L.-H.; Tius, M. A. *Org. Lett.* **2002**, *4*, 1637–1640.
(16) Fray, A. H. *Tetrahedron: Asymmetry* **1998**, *9*, 2777–2781.
(17) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479–2490.
(18) Eissler, S.; Nahrwold, M.; Neumann, B.; Stammler, H.-G.; Sewald, N. *Org. Lett.* **2007**, *9*, 817–819.
(19) (a) Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1987**, *52*, 4978–4984. (b) Yang, D.; Li, B.; Ng, F.-F.; Yan, Y.-L.; Qu, J.; Wu, Y.-D. *J. Org. Chem.* **2001**, *66*, 7303–7312.

SCHEME 3. Novel Access to Unit AB Precursor 16

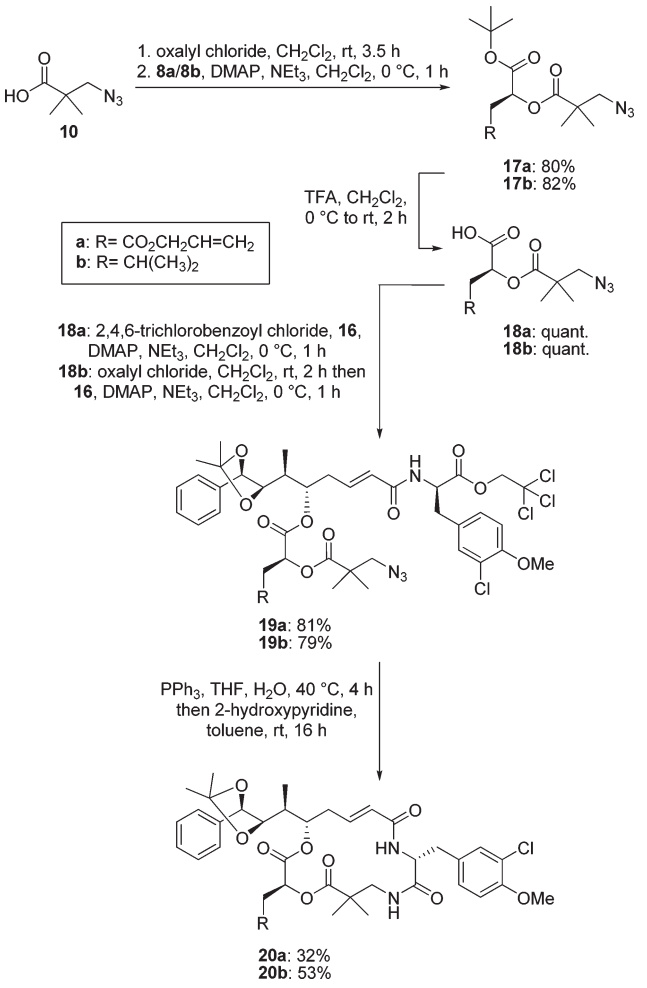


to give the unit CD precursors **17a/b** in high yields (Scheme 4). Subsequent deprotection with TFA furnished the crude carboxylic acids **18a/b** in good yields. Coupling of the unit AB precursor **16** and the unit CD precursors **18a/b** yielded the corresponding esters **19a/b** in 81 and 79% yield. In the case of the more functionalized unit D precursor **18a**, a milder esterification under Yamaguchi conditions was chosen as a precaution. Due to the presence of the double bond in the molecule, the azides **19a/b** could not simply be reduced to the free amines by catalytic hydrogenation. In addition, reduction of the azide with the Vilarrasa reagent[20] was not considered because the α,β-unsaturated amide within unit A is known to form Michael adducts with thiols.[21]

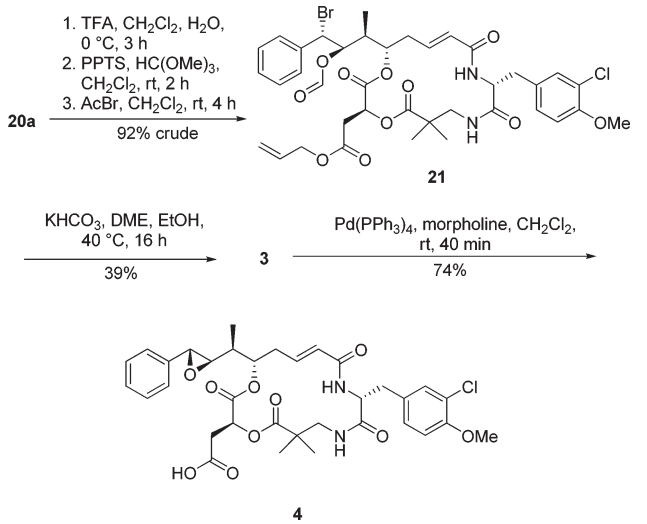
Therefore, we developed a one-pot Staudinger reduction/cyclization protocol to reduce the *seco*-CDAB azide precursors **19a/b** to the corresponding free amines with triphenylphosphine in aqueous THF. This is followed by cyclization of the molecule upon intramolecular substitution of the trichloroethyl ester[16] mediated by 2-hydroxypyridine to obtain macrocycles **20a/b**. In the case of *seco*-cryptophycin-52 precursor **19b**, this reaction sequence yielded 53% of the recently reported cryptophycin-52 precursor **20b**.[6c] The lower yield (32%) of the allyl ester **20a** is probably due to its higher sensitivity to basic ester cleavage.

Conversion of the cyclic acetonides **20a/b** to the epoxides is based on a four-step reaction sequence developed by Sharpless et al.,[22] which has been applied to cryptophycins before.[6c,15,23] The acetonide **20a** was cleaved by treatment with TFA in aqueous CH_2Cl_2 (Scheme 5). The cyclic orthoester of the *syn*-diol was obtained by treatment with trimethyl orthoformate. Opening the orthoester with acetyl bromide gave the corresponding bromohydrin formate **21**. Recently, a procedure was published for the cryptophycin-52 synthesis from the corresponding bromohydrin formyl ester applying potassium carbonate in an emulsion of 1,2-dimethoxyethane/ethylene glycol.[6c] However, in the case of **20a**, these reaction conditions led to complete decomposition. Instead, an alternative protocol making use of a suspension of $KHCO_3$

SCHEME 4. Construction of the Cryptophycin Backbone Using a Novel One-Pot Staudinger Reduction/Cyclization Step



SCHEME 5. Acetonide−Epoxide Conversion of the Base Labile Substrate 20a



in DME/EtOH was successfully modified and reproducibly provided epoxide **3** in 39% yield.[24] The final deprotection of the allyl ester **3** was performed using standard

(20) Bartra, M.; Urpí, F.; Vilarrasa, J. *Tetrahedron Lett.* **1987**, *28*, 5941–5944.
(21) Martinelli, M. J.; Vaidyanathan, R.; Khau, V. V.; Staszak, M. A. *Tetrahedron Lett.* **2002**, *43*, 3365–3367.
(22) Kolb, H. C.; Sharpless, K. B. *Tetrahedron* **1992**, *48*, 10515–10530.
(23) Mast, C. A.; Eissler, S.; Stončius, A.; Stammler, H.-G.; Neumann, B.; Sewald, N. *Chem.—Eur. J.* **2005**, *11*, 4667–4677.

morpholine/$Pd(PPh_3)_4$ conditions providing carboxy cryptophycin **4** in 74% yield.

The biological activity of **3** and **4** was determined in cell-based cytotoxicity assays using human cervix carcinoma KB-3-1 cells and the related multi-drug-resistant P-gp-expressing KB-V1 cells. P-glycoprotein (P-gp) represents an ATP-dependent efflux system that actively transports structurally diverse xenobiotics to the outside of the cell. For both cell lines, the reduction of resazurin to resorufin was used as a readout.[25] Cryptophycin-52 (**2**), the allyl ester **3**, and the free acid **4** exhibited IC_{50} values of 15.5, 14.5, and 88.6 pM against KB-3-1 and of 260.9 and 661.9 pM and 372.2 nM against the multi-drug-resistant KB-V1 cells, respectively. These results show that both cryptophycin analogues **3** and **4** modified at unit D show very high bioactivity against nonresistant tumor cells. In the case of the P-gp-expressing cells, allyl ester **3** also remains highly active. The greater than 1000-fold decrease in cytotoxicity of **4** compared to that of cryptophycin-52 can probably be attributed to the greater amphiphilicity of **4**, which renders it a better substrate for P-gp.[26]

Conclusions

In summary, new methods for the synthesis of cryptophycins and functionalized derivatives were developed. A one-pot Staudinger reduction/cyclization procedure significantly reduces the number of protective group operations in the synthesis. In addition, a new convenient metathesis approach for the AB fragment **16** is disclosed, and a very mild method for the hydrolysis of fragile cryptophycin bromohydrin formyl esters such as **21** is presented. The two novel functionalized cryptophycin derivatives **3** and **4** show high cytotoxicity and make the modification of unit D of cryptophycin an interesting possible target for the synthesis of cryptophycin bioconjugates.

Experimental Section

(*S*)-4-(Allyloxy)-2-hydroxy-4-oxobutanoic acid (7). According to a slightly modified literature procedure,[10] 2 N aqueous $NaNO_2$ solution (95 mL) is added at 0 °C within 15 min to a solution of the hydrochloride **6** (10.0 g, 47.7 mmol) in water (382 mL) and acetic acid (94 mL). The solution is allowed to reach rt and is stirred for overall 3 h under argon (caution: toxic gases!). After acidification to pH 2 with saturated aqueous $KHSO_4$, the aqueous solution is extracted with EtOAc (3 × 200 mL). The organic phase is washed with brine (50 mL) and dried over $MgSO_4$. After repeated coevaporation with toluene and removal of the solvent in vacuo, carboxylic acid **7** (8.02 g, 97%) is obtained as a yellow oil, which is used for the next step without further purification: $[\alpha]^{24}_D = -8.7$ (c = 1.5 in MeOH); ^{1}H NMR (250 MHz, $(CD_3)_2CO$) δ [ppm] = 2.72 (dd, J = 7.5, 16.0 Hz, 1H, $CHCH^AH^B$), 2.84 (dd, J = 4.6, 15.9 Hz, 1H, $CHCH^AH^B$), 4.49−4.67 (m, 3H, $CH^AH^BCH{=}CH_2$ and CHOH), 5.20 (m, 1H, $CH_2CH{=}CH^{cis}H^{trans}$), 5.33 (m, 1H, $CH_2CH{=}CH^{cis}H^{trans}$), 5.91 (m, 1H, $CH_2CH{=}CH^{cis}H^{trans}$); ^{13}C NMR (126 MHz, $(CD_3)_2CO$) δ [ppm] = 39.7 ($CHCH^AH^B$), 65.6 ($CH_2CH{=}CH_2$), 67.8 (CHOH), 117.9 ($CH_2CH{=}CH_2$), 133.5 ($CH_2CH{=}CH_2$), 170.6/174.5 (2× C=O); ESI-MS m/z 175.0 $[M + H]^+$; HR-EI calcd for $C_7H_{10}O_5$ $[M]^+$ 174.05282, found 174.05272; IR (neat, cm^{-1}) 2943, 2359, 1724, 1379, 1170.

(*S*)-4-Allyl 1-*tert*-butyl 2-hydroxysuccinate (8a). According to a literature procedure,[11] DIC (15.7 mL, 12.8 g, 101 mmol), *tert*-BuOH (12.5 mL, 131 mmol), and CuCl (220 mg, 2.22 mmol) are stirred overnight at rt for 5 days; 18.0 g of this suspension is dissolved in CH_2Cl_2 (60 mL). This mixture is added during 15 min to a solution of the hydroxy carboxylic acid **7** (5.00 g, 28.7 mmol) in CH_2Cl_2 (60 mL). The suspension is stirred for 1 h at 0 °C and overnight at rt. After filtering off the urea using filter paper and washing the solid with hexane (150 mL), the obtained suspension is filtered into a separatory funnel. The organic phase is washed with saturated aqueous $NaHCO_3$ (150 mL) and brine (30 mL). After drying over $MgSO_4$, the solvents are removed in vacuo. Purification by column chromatography (hexane/EtOAc 8:2) yields ester **8a** (2.94 g, 44%): R_f (hexane/EtOAc 4:1) = 0.44; $[\alpha]^{24}_D = -11$ (c = 1.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.49 (s, 9H, $(CH_3)_3$), 2.77 (dd, J = 5.8, 16.0 Hz, 1H, $CHCH^AH^B$), 2.84 (dd, J = 4.4, 16.1 Hz, 1H, $CHCH^AH^B$), 3.19 (d, J = 5.0 Hz, 1H, OH), 4.38 (m, 1H, CHOH), 4.62 (m, 2H, $CH^AH^BCH{=}CH_2$), 5.25 (dd, J = 10.7, 1.2 Hz, 1H, $CH_2CH{=}CH^{cis}H^{trans}$), 5.33 (dd, J = 17.2, 1.5 Hz, 1H, $CH_2CH{=}CH^{cis}H^{trans}$), 5.93 (m, 1H, $CH_2CH{=}CH^{cis}H^{trans}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 27.9 ($(CH_3)_3$), 39.0 ($CHOHCH_2$), 65.5 ($CH_2CH{=}CH_2$), 67.4 (CHOH), 83.1 ($C(CH_3)_3$), 118.6 ($CH_2CH{=}CH_2$), 131.8 ($CH_2CH{=}CH_2$), 170.1/172.6 (2 × C=O); ESI-MS m/z 253.0 $[M + Na]^+$, 482.3 $[2M + Na]^+$; HR-ESI calcd for $C_{11}H_{18}O_5Na^+$ 253.10464, found 253.10401; IR (neat, cm^{-1}) 3416, 2982, 2930, 1729, 1368. Anal. calcd (%) for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.37; H, 7.73.

3-Azido-2,2-dimethylpropanoic acid (10). According to a slightly modified literature procedure,[12] 3-chloropivalic acid (**9**) (3.00 g, 22.0 mmol) and sodium azide (4.77 g, 73.3 mmol) are refluxed in water (15 mL) for 3 days. After cooling to rt, the mixture is acidified to pH 2−3 by addition of concentrated aqueous hydrochloric acid (caution: toxic gases!). The aqueous phase is extracted with diethyl ether (3 × 100 mL). The combined organic phases are dried over $MgSO_4$ and concentrated in vacuo, yielding the azide **10** (2.88 g, 92%) as a yellow oil. The ^{1}H NMR data are in agreement with previously published specifications.[12]

3-Amino-2,2-dimethylpropanoic acid (11). According to a slightly modified literature procedure,[27] azide **10** (750 mg, 5.24 mmol) is dissolved in MeOH (107 mL); 10 % Pd/C (520 mg) is added, and the suspension is stirred for 3 h under hydrogen atmosphere (caution: hydrogenation rates may depend on catalyst activity and size of the solvent surface). Water (107 mL) is added, and stirring is continued for 30 min. Excess hydrogen is removed by flushing with argon, and the suspension is filtered through filter paper (caution: dry activated catalyst can start burning!). Further purification is achieved by filtering through syringe filters. After removal of the solvent in vacuo, the amino acid **11** (570 mg, 92%) is obtained as a colorless solid. The ^{1}H NMR data are in agreement with previously published specifications.[28]

3-(*tert*-Butoxycarbonylamino)-2,2-dimethylpropanoic acid (12). Amino acid **11** (200 mg, 1.71 mmol) is dissolved in 1 M aqueous NaOH (3.4 mL) and H_2O (1.6 mL). *tert*-BuOH (2.5 mL) and liquefied Boc_2O (430 μL, 1.88 mmol) are added. The viscous suspension is stirred overnight at rt; 1 M aqueous NaOH solution is added until a pH value of 13−14 is reached. The aqueous phase is first extracted with hexane (3 × 20 mL) then acidified with $KHSO_4$ (s) to pH 2−3 and extracted with EtOAc (3 × 25 mL). The combined organic phases are washed with 5% aqueous $KHSO_4$

(24) Eissler, S. Ph.D Thesis, Bielefeld University, 2004.
(25) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.
(26) Raub, T. J. *Mol. Pharmaceutics* **2006**, *3*, 3–25.
(27) Ma, D.-Y.; Wang, D.-X.; Zheng, Q.-Y.; Wang, M.-X. *Tetrahedron: Asymmetry* **2006**, *17*, 2366–2376.
(28) Okano, K.; Morimoto, T.; Sekiya, M. *Chem. Pharm. Bull.* **1985**, *33*, 2228–2234.

(1 × 30 mL). After drying over $MgSO_4$, the solvent is removed in vacuo, and the carboxylic acid **12** is obtained as a colorless solid (325 mg, 88%). The ^{1}H NMR data are in agreement with previously published specifications.[29]

(*R*)-2,2,2-Trichloroethyl 2-acrylamido-3-(3-chloro-4-methoxyphenyl)propanoate (14). Trichloroethyl ester **13**[17] (7.43 g, 16.1 mmol) is dissolved in TFA (16 mL), and the reaction mixture is stirred for 1 h at rt. Toluene (100 mL) is added, and the solvents are removed in vacuo. The residue is dissolved in CH_2Cl_2 (200 mL), and the organic solution is washed with saturated aqueous $NaHCO_3$ (200 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue is dried in high vacuum and then dissolved in CH_2Cl_2 (100 mL). The solution is cooled to 0 °C, and NEt_3 (6.7 mL, 4.9 g, 48 mmol) is added. To avoid polymerization of acrylated compounds, the following tasks were performed under reduced light: acryloyl chloride (2.0 mL, 2.2 g, 25 mmol) is added slowly over 15 min. The solution is stirred overnight at 0 °C in the dark and transferred to a separatory funnel with EtOAc (500 mL). The organic phase is washed with 5% aqueous $KHSO_4$ (100 mL). Phase separation can be improved by the addition of NaCl (s). The organic phase is washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL) and is dried over $MgSO_4$. In general, a few crystals of phenothiazine can be added to inhibit polymerization. The solvents are removed in vacuo. Purification by column chromatography (EtOAc/hexane 1:2→1:1) yields unit B precursor **14** (4.43 g, 66%) as a yellow oil: R_f (hexane/EtOAc 2:1) = 0.30; $[\alpha]^{24}_D = -54$ (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 3.12 (dd, J = 14.2, 6.2 Hz, 1H, C$^{\beta}$H^{A}H^{B}), 3.22 (dd, J = 14.3, 5.8 Hz, 1H, C$^{\beta}$H^{A}H^{B}), 3.87 (s, 3H, OCH$_3$), 4.74 (d, J = 11.9 Hz, 1H, CH^{A}H^{B}CCl$_3$), 4.80 (d, J = 11.9 Hz, 1H, CH^{A}H^{B}CCl$_3$), 5.08 (m, 1H, C$^{\alpha}$H), 5.71 (dd, J = 10.3, 1.0 Hz, 1H, CH=CH^{cis}H^{trans}), 6.07 (d, J = 7.7 Hz, 1H, NH), 6.12 (dd, J = 17.0, 10.3 Hz, 1H, CH=CH$_2$), 6.31 (dd, J = 17.0, 1.0 Hz, 1H, CH=CH^{cis}H^{trans}), 6.86 (d, J = 8.4 Hz, 1H, C$^{5'}$H), 7.04 (dd, J = 8.4, 2.1 Hz, 1H, C$^{6'}$H), 7.19 (d, J = 2.0 Hz, 1H, C$^{2'}$H); ^{13}C NMR (63 MHz, $CDCl_3$) δ [ppm] = 36.5 (C$^{\beta}$H$_2$), 53.1 (C$^{\alpha}$H), 56.2 (OCH$_3$), 74.8 (CH$_2$CCl$_3$), 94.2 (CCl$_3$), 112.3 (C$^{5'}$H), 122.7 (C$^{3'}$), 127.7 (CH=CH$_2$), 128.4 (C$^{6'}$), 128.5 (C$^{1'}$), 130.0 (CH=CH$_2$), 131.1 (C$^{2'}$H), 154.4 (C$^{4'}$), 165.1 (NC=O), 170.0 (CO$_2$); ESI-MS m/z 436.0 [M + Na]$^+$; HR-ESI calcd for $C_{15}H_{15}NO_4Cl_4Na^+$ [M + Na]$^+$ 435.96474, found 435.96468; IR (neat, cm^{-1}) 3270, 1756, 1256, 1160, 1064.

Unit AB Precursor 16. Unit B precursor **14** (375 mg, 0.843 mmol) and unit A precursor **15**[18] (300 mg, 1.01 mmol) are dissolved in CH_2Cl_2 (9 mL), and Grubbs-II catalyst (39 mg) is added. The solution is refluxed in the dark overnight. The solvent is removed in vacuo at 30 °C. The residue is purified by column chromatography (hexane/EtOAc 2:1→1:1). Trichloroethyl ester **16** is obtained as a yellow foam (435 mg, 78%): R_f (hexane/EtOAc 2:1) = 0.21. The ^{1}H NMR and ^{13}C NMR data are in agreement with previously published specifications.[15]

(*S*)-4-Allyl 1-*tert*-butyl 2-(3-azido-2,2-dimethylpropanoyloxy)-succinate (17a). 3-Azido-2,2-dimethylpropionic acid (**10**) (1.01 g, 7.07 mmol) and abs DMF (8 drops) in CH_2Cl_2 (10 mL) are cooled to 0 °C, and oxalyl chloride (0.68 mL, 1.01 g, 7.91 mmol) is added. The solution is stirred for 3.5 h at rt. All volatile components are removed in vacuo. DMAP (860 mg, 7.04 mmol) in CH_2Cl_2 (5 mL) is added to a solution of alcohol **8a** (730 mg, 3.15 mmol) and NEt_3 (0.99 mL, 0.72 g, 7.1 mmol) in CH_2Cl_2 (10 mL). The crude acid chloride is dissolved in CH_2Cl_2 (10 mL) and slowly added at 0 °C. The reaction mixture is stirred for 1 h at 0 °C and then poured into saturated aqueous $NaHCO_3$ (150 mL). The aqueous phase is extracted with Et_2O (3 × 50 mL). The combined organic phases are washed with brine (50 mL) and dried over $MgSO_4$. After removal of the solvent in vacuo, the crude product is purified by column chromatography (hexane/EtOAc 85:15). Ester **17a** is obtained as a yellow oil (889 mg, 80%): R_f (hexane/EtOAc 85:15) = 0.54; $[\alpha]^{24}_D = -20$ (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.25 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 1.26 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B})), 1.47 (s, 9H, uD-C(CH$_3$)$_3$), 2.88−2.94 (m, 2H, uD-C$^{\beta}$H$_2$), 3.42 (d, J = 12.0 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.46 (d, J = 12.0 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 4.57−4.67 (m, 2H, uD-OCH^{A}H^{B}CH=CH$_2$), 5.26 (d, J = 10.3 Hz, 1H, uD-CH$_2$CHCH^{cis}H^{trans}), 5.30−5.40 (m, 2H, uD-CH$_2$CHCH^{cis}H^{trans} and uD-C$^{\alpha}$H), 5.92 (m, 1H, uD-CH$_2$CHCH^{cis}H^{trans}); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 22.9 (uC-C$^{\alpha}$ (CH$_3$)^{A}(CH$_3$)^{B}), 27.9 (uD-C(CH$_3$)$_3$), 36.2 (uD-C$^{\beta}$H$_2$), 43.6 (uC-C$^{\alpha}$(CH$_3$)$_2$), 59.2 (uC-C$^{\beta}$H$_2$), 65.7 (uD-CH$_2$CH=CH$_2$), 68.9 (uD-C$^{\alpha}$H), 82.9 (uD-C(CH$_3$)$_3$), 118.7 (uD-CH$_2$CH=CH$_2$), 131.7 (uD-CH$_2$CH=CH$_2$), 167.5 (uD-CO$_2$C(CH$_3$)$_3$), 168.8 (uD-CO$_2$CH$_2$C=CH$_2$), 174.5 (uC-CO$_2$CH); ESI-MS m/z 378.2 [M + Na]$^+$; HR-ESI calcd for $C_{16}H_{25}N_3O_6Na^+$ [M + Na]$^+$ 378.16356, found 378.16293; IR (neat, cm^{-1}) 2979, 2102, 1735, 1368, 1132.

(*S*)-*tert*-Butyl 2-(3-azido-2,2-dimethylpropanoyloxy)-4-methylpentanoate (17b). 3-Azido-2,2-dimethylpropionic acid (**10**) (1.01 g, 7.07 mmol) and abs. DMF (8 drops) are dissolved in CH_2Cl_2 (10 mL). Oxalyl chloride (690 μL, 1.02 g, 7.91 mmol) is added over 10 min at 0 °C. The reaction mixture is stirred for 3.5 h at rt. All volatile materials are removed in vacuo. A solution of **8b** (593 mg, 3.15 mmol) in CH_2Cl_2 (10 mL) is cooled to 0 °C, and NEt_3 (990 mL, 720 mg, 7.10 mmol) and a solution of DMAP (860 mg, 7.04 mmol) in CH_2Cl_2 (5 mL) are added. The crude acid chloride in CH_2Cl_2 (10 mL) is added over 15 min, and the reaction mixture is stirred for 1 h at 0 °C. The reaction mixture is poured into saturated aqueous $NaHCO_3$ (150 mL), and the aqueous phase is extracted with Et_2O (3 × 50 mL). The combined organic phases are washed with brine (50 mL) and dried over $MgSO_4$. The solvent is removed under reduced pressure. The crude product is purified by column chromatography (hexane/EtOAc 9:1). Compound **17b** is obtained as a colorless oil (809 mg, 82%): R_f (hexane/EtOAc 9:1) = 0.5; $[\alpha]^{24}_D$ = −30 (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 0.93 (d, J = 6.5 Hz, 3H, uD-C$^{\gamma}$H(CH$_3$)^{A}(CH$_3$)^{B}), 0.97 (d, J = 6.5 Hz, 3H, uD-C$^{\gamma}$H(CH$_3$)^{A}(CH$_3$)^{B}), 1.27 (s, 6H, uC-C$^{\alpha}$(CH$_3$)$_2$), 1.47 (s, 9H, uD-C(CH$_3$)$_3$), 1.65 (m, 1H, uD-C$^{\gamma}$H(CH$_3$)$_2$), 1.70−1.84 (m, 2H, uD-C$^{\beta}$H$_2$), 3.44 (d, J = 11.3 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.47 (d, J = 11.9 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 4.91 (dd, J = 9.9, 3.8 Hz, 1H, uD-C$^{\alpha}$H); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 21.5 (uD-C$^{\gamma}$H-(CH$_3$)^{A}(CH$_3$)^{B}), 22.90 (uC-C$^{\alpha}$ (CH$_3$)^{A}(CH$_3$)^{B}), 22.94 (uC-C$^{\alpha}$-(CH$_3$)^{A}(CH$_3$)^{B}), 23.1 (uD-C$^{\gamma}$H(CH$_3$)^{A}(CH$_3$)^{B}), 24.8 (uD-C$^{\gamma}$H-(CH$_3$)^{A}(CH$_3$)^{B}), 28.0 (uD-C(CH$_3$)$_3$), 39.7 (uD-C$^{\beta}$H$_2$), 43.6 (uC-C$^{\alpha}$(CH$_3$)$_2$), 59.4 (uC-C$^{\beta}$H$_2$), 71.8 (uD-C$^{\alpha}$H), 82.0 (uD-C(CH$_3$)$_3$), 169.6 (uD-CO$_2$), 175.1 (uC-CO$_2$); ESI-MS m/z 336.2 [M + Na]$^+$; HR-ESI calcd for $C_{15}H_{27}N_3O_4Na^+$ [M + Na]$^+$ 336.18938, found 336.18921; IR (neat, cm^{-1}) 2960, 2101, 1733, 1472, 1125.

(*S*)-4-(Allyloxy)-2-(3-azido-2,2-dimethylpropanoyloxy)-4-oxobutanoic acid (18a). A solution of *tert*-butyl ester **17a** (505 mg, 1.42 mmol) in CH_2Cl_2 (11.8 mL) is cooled to 0 °C, and TFA (6.1 mL) is added within 20 min. The reaction mixture is stirred for 100 min at rt. After addition of toluene (30 mL), the solvents are removed in vacuo. The crude product is coevaporated with toluene several times. After drying in high vacuum, carboxylic acid **18a** is obtained as a yellow oil (436 mg, quant): R_f (hexane/EtOAc 7:3) = 0.4; $[\alpha]^{24}_D = -9.6$ (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.25 (s, 6H, uC-C$^{\alpha}$(CH$_3$)$_2$), 3.00 (d, J = 5.7 Hz, 2H, uD-C$^{\beta}$H$_2$), 3.44 (s, 2H, uC-C$^{\beta}$H$_2$), 4.57−4.67 (m, 2H, uD-CH^{A}H^{B}CH=CH$_2$), 5.27 (dd, J = 10.4, 1.1 Hz, 1H, uD-CH=CH^{cis}H^{trans}), 5.34 (dd, J = 17.2, 1.4 Hz, 1H, uD-CH=CH^{cis}H^{trans}), 5.54 (t, J = 6.1 Hz, 1H, uD-C$^{\alpha}$H), 5.90 (m, 1H, uD-CH=CH$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 22.7 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 35.9 (uD-C$^{\beta}$H$_2$), 43.6 (uC-C$^{\alpha}$CH$_2$), 59.2 (uC-C$^{\beta}$H$_2$), 66.0 (uD-CH$_2$CH=CH$_2$), 68.0 (uD-C$^{\alpha}$H), 119.0 (uD-CH=CH$_2$), 131.5 (uD-C=CH$_2$), 168.6 (uD-CO$_2$allyl), 174.0 (uD-CO$_2$H), 174.6 (uC-CO$_2$CH); ESI-MS m/z 301.0 [M + H]$^+$; HR-ESI calcd for

(29) Seebach, D.; Abele, S.; Sifferlen, T.; Hänggi, M.; Gruner, S.; Seiler, P. *Helv. Chim. Acta* **1998**, *81*, 2218–2243.

$C_{12}H_{17}N_3O_6Na^+$ $[M + Na]^+$ 322.10096, found 322.10116; IR (neat, cm^{-1}) 2979, 2103, 1732, 1131, 923.

(*S*)-2-(3-Azido-2,2-dimethylpropanoyloxy)-4-methylpentanoic acid (18b). A solution of *tert*-butyl ester **17b** (667 mg, 2.13 mmol) in CH_2Cl_2 (20 mL) is cooled to 0 °C. TFA (10.5 mL) is added within 15 min. The reaction mixture is allowed to reach rt and stirred for overall 3.5 h. The volatile material is removed in vacuo. The crude product is coevaporated with toluene several times. Carboxylic acid **18b** is obtained as a yellow oil (623 mg, quant): $[\alpha]^{24}_D = -25$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 0.95 (d, J = 6.5 Hz, 3H, uD-C$^\gamma$H(C$\underline{H}_3$)^A-(CH$_3$)^B), 0.98 (d, J = 6.5 Hz, 3H, uD-C$^\gamma$H(CH$_3$)^A(C$\underline{H}_3$)^B), 1.26 (s, 6H, uC-C$^\alpha$(C$\underline{H}_3$)$_2$), 1.72 (m, 1H, uD-C$^\beta$H^{A}H^B), 1.80 (m, 1H, uD-C$^\gamma$H), 1.88 (m, 1H, uD-C$^\beta$H^{A}H^B), 3.46 (s, 2H, uC-C$^\beta$H$_2$), 5.07 (dd, J = 9.8, 3.7 Hz, 1H, uD-C$^\alpha$H), 10.73 (s, 1H, uD-COOH); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 21.4 (uD-C$^\gamma$H(C$\underline{H}_3$)^A-(CH$_3$)^B), 22.7 (uC-C$^\alpha$(C$\underline{H}_3$)^A(CH$_3$)^B), 22.9 (uC-C$^\alpha$(C$\underline{H}_3$)^A-(C$\underline{H}_3$)^B), 23.0 (uD-C$^\gamma$H(C$\underline{H}_3$)^A(C$\underline{H}_3$)^B), 24.8 (uD-C$^\gamma$H(CH$_3$)^A-(C$\underline{H}_3$)^B), 39.5 (uD-C$^\beta$H$_2$), 43.6 (uC-C(CH$_3$)$_2$), 59.4 (uC-C$^\beta$H$_2$), 70.8 (uD-C$^\alpha$CH), 175.3 (uC-CO$_2$CH), 176.5 (uD-CO$_2$H); ESI-MS m/z 256.0 $[M - H^+]^-$; HR-ESI calcd for $C_{11}H_{18}N_3O_4$ $[M - H]^-$ 256.13028, found 256.13031; IR (neat, cm^{-1}) 2960, 2101, 1721, 1471, 1125.

***seco*-Depsipeptide 19a.** Unit CD precursor **18a** (85 mg, 0.28 mmol), unit AB precursor **16** (126 mg, 0.189 mmol), and DMAP (6 mg, 0.05 mmol) are dissolved in THF (2 mL), and the solution is cooled to 0 °C. NEt_3 (53 μL, 39 mg, 0.38 mmol) is added, followed by dropwise addition of 2,4,6-trichlorobenzoyl chloride (46 μL, 72 mg, 0.30 mmol) within 15 min. The reaction mixture is stirred for 1 h at 0 °C. After addition of 10% aqueous citric acid (3 mL), the mixture is extracted with EtOAc (3 × 30 mL) and the combined organic phases are washed with saturated aqueous $NaHCO_3$ (1 × 20 mL). After drying over $MgSO_4$, the crude product is purified by column chromatography (hexane/EtOAc 7:3). *seco*-Depsipeptide **19a** is obtained as a colorless resin (0.14 g, 81%): R_f (hexane/EtOAc 7:3) = 0.17; $[\alpha]^{24}_D = -34$ ($c = 0.91$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.09 (d, J = 7.0 Hz, 3H, uA-C$^\varepsilon$H$\underline{H}_3$), 1.17 (s, 3H, uC-C$^\alpha$(C$\underline{H}_3$)^A-(CH$_3$)^B), 1.18 (s, 3H, uC-C$^\alpha$(C$\underline{H}_3$)^A(CH$_3$)^B), 1.46 (s, 3H, uA-C(C$\underline{H}_3$)^A(CH$_3$)^B), 1.53 (s, 3H, uA-C(C$\underline{H}_3$)^A(CH$_3$)^B), 1.94 (m, 1H, uA-C$^\varepsilon$H), 2.28−2.45 (m, 2H, uA-C$^\gamma$H$_2$), 2.84−2.92 (m, 2H, uD-C$^\beta$H$_2$), 3.08 (dd, J = 14.2, 6.3 Hz, 1H, uB-C$^\beta$H^{A}H^B), 3.19 (dd, J = 14.2, 5.8 Hz, 1H, uB-C$^\beta$H^{A}H^B), 3.33 (d, J = 12.0 Hz, 1H, uC-C$^\beta$CH^{A}H^B), 3.40 (d, J = 12.0 Hz, 1H, uC-C$^\beta$CH^{A}H^B), 3.81 (dd, J = 8.8, 2.8 Hz, 1H, uA-C$^\zeta$H), 3.87 (s, 3H, uB-OCH$_3$), 4.53−4.65 (m, 2H, uD-CH^{A}H^{B}CH=CH$_2$), 4.68 (d, J = 8.8 Hz, 1H, uA-C$^\eta$H), 4.72 (d, J = 11.9 Hz, 1H, uB-CH^{A}H^{B}CCl$_3$), 4.78 (d, J = 11.9 Hz, 1H, uB-CH^{A}H^{B}CCl$_3$), 4.98−5.08 (m, 2H, uA-C$^\delta$H and uB-C$^\alpha$H), 5.27 (dd, J = 10.4, 1.1 Hz, 1H, uD-C=CH^{cis}H^{trans}), 5.31−5.39 (m, 2H, uD-C=CH^{cis}H^{trans} and uD-C$^\alpha$H), 5.56 (d, J = 15.6 Hz, 1H, uA-C$^\alpha$H), 5.91 (m, 1H, uD-CH=CH$_2$), 6.33 (d, J = 7.9 Hz, 1H, NH), 6.57 (dt, J = 15.6, 6.4 Hz, 1H, uA-C$^\beta$H), 6.86 (d, J = 8.4 Hz, 1H, uB-C$^{5'}$H), 7.04 (dd, J = 8.4, 2.1 Hz, 1H, uB-C$^{6'}$H), 7.18 (d, J = 2.1 Hz, 1H, uB-C$^{2'}$H), 7.29−7.49 (m, 5H, uA-C^{ar}H); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 9.6 (uA-C$^\varepsilon$HCH$_3$), 22.7 (2 × uC-C$^\beta$CH$_3$), 27.1 (uA-C(CH$_3$)^A(CH$_3$)^B), 27.2 (uA-C(CH$_3$)^A(CH$_3$)^B), 32.4 (uA-C$^\gamma$H$_2$), 35.6 (uD-C$^\beta$H$_2$), 35.9 (uA-C$^\varepsilon$H), 36.5 (uB-C$^\beta$H$_2$), 43.6 (uC-C$^\alpha$), 53.2 (uB-C$^\alpha$H), 56.1 (uB-OCH$_3$), 59.0 (uC-C$^\beta$H$_2$), 65.9 (uD-CH$_2$=CH), 68.6 (uD-C$^\alpha$H), 74.7 (uB-CH$_2$CCl$_3$), 75.8 (uA-C$^\delta$H), 80.6 (uA-C$^\eta$H), 82.2 (uA-C$^\zeta$H), 94.3 (uB-CCl$_3$), 109.1 (uA-C(CH$_3$)$_2$), 112.1 (uB-C$^{5'}$H), 118.9 (uD-CH=CH$_2$), 122.3 (uB-C$^{3'}$), 125.2 (uA-C$^\alpha$H), 127.0/128.61/128.62 (5 × uA-C^{ar}H), 128.74 (uB-C$^{1'}$), 128.82 (uB-C$^{6'}$H), 131.2 (uB-C$^{2'}$H), 131.6 (uD-CH=CH$_2$), 137.4 (uA-C^{ar}), 139.2 (uA-C$^\beta$H), 154.2 (uB-C$^{4'}$), 165.1 (uA-C=O), 167.9 (uD-C=O), 168.6 (uD-CO$_2$CH=CH$_2$), 170.0 (uB-C=O), 174.9 (uC-C=O); ESI-MS m/z 966.9 $[M + Na]^+$; HR-ESI calcd for $C_{42}H_{50}N_4O_{12}Cl_4Na^+$ $[M + Na]^+$ 965.20716, found 965.20486; IR (neat, cm^{-1}) 2981, 2359, 2103, 1736, 1502.

***seco*-Depsipeptide 19b.** Carboxylic acid **18b** (117 mg, 0.455 mmol) and DMF (one drop) are dissolved in CH_2Cl_2 (2 mL). Oxalyl chloride (48 μL, 71 mg, 0.56 mmol) is added over 10 min at 0 °C, and the reaction mixture is stirred for 3 min at 0 °C and for 2 h at rt. The solvent is removed under reduced pressure, and the crude acid chloride is dried in high vacuum. Unit AB precursor **16** (150 mg, 0.225 mmol), NEt_3 (72 μL, 52 mg, 0.52 mmol), and DMAP (63 mg, 0.52 mmol) are dissolved in CH_2Cl_2 (3 mL) and cooled to 0 °C. The crude acid chloride in CH_2Cl_2 (2 mL) is added over 10 min. After stirring for 1 h at 0 °C, saturated aqueous $NaHCO_3$ (20 mL) is added. The aqueous phase is extracted with Et_2O (4 × 30 mL). The combined organic phases are washed with 5% aqueous $KHSO_4$ (10 mL) and brine (10 mL). After drying over $MgSO_4$, the solvent is removed under vacuum and the crude product is purified by column chromatography (hexane/EtOAc 7:3). *seco*-Depsipeptide **19b** is obtained as a colorless solid (160 mg, 79%): R_f (hexane/EtOAc 7:3) = 0.3; $[\alpha]^{24}_D = -35$ ($c = 0.50$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 0.90 (d, J = 6.5 Hz, 3H, uD-C$^\gamma$H(C$\underline{H}_3$)^A(CH$_3$)^B), 0.96 (d, J = 6.3 Hz, 3H, uD-C$^\gamma$H(CH$_3$)^A(C$\underline{H}_3$)^B), 1.10 (d, J = 6.9 Hz, 3H, uA-C$^\varepsilon$H$\underline{H}_3$), 1.21 (s, 6H, uC-C(C$\underline{H}_3$)$_2$), 1.46 (s, 3H, uA-C(C$\underline{H}_3$)^A(CH$_3$)^B), 1.52 (s, 3H, uA-C(C$\underline{H}_3$)^A(CH$_3$)^B), 1.56−1.63 (m, 1H, uD-C$^\beta$H^{A}H^B), 1.70−1.83 (m, 2H, uD-C$^\gamma$H and uD-C$^\beta$H^{A}H^B), 1.96 (m, 1H, uA-C$^\varepsilon$HCH$_3$), 2.25−2.48 (m, 2H, uA-C$^\gamma$H$_2$), 3.07 (dd, J = 14.1, 6.7 Hz, 1H, uB-C$^\beta$H^{A}H^B), 3.19 (dd, J = 14.1, 5.7 Hz, 1H, uB-C$^\beta$H^{A}H^B), 3.32 (d, J = 12.1 Hz, 1H, uC-C$^\beta$H^{A}H^B), 3.42 (d, J = 12.1 Hz, 1H, uC-C$^\beta$H^{A}H^B), 3.80 (dd, J = 8.8, 3.0 Hz, 1H, uA-C$^\zeta$H), 3.86 (s, 3H, uB-OCH$_3$), 4.70 (d, J = 8.7 Hz, 1H, uA-C$^\eta$H), 4.74 (d, J = 12.0 Hz, 1H, uB-CH^{A}H^{B}CCl$_3$), 4.78 (d, J = 12.0 Hz, 1H, uB-CH^{A}H^{B}CCl$_3$), 4.87 (dd, J = 9.8, 3.5 Hz, 1H, uD-C$^\alpha$H), 4.95−5.08 (m, 2H, uB-C$^\alpha$H and uA-C$^\delta$H), 5.61 (d, J = 15.7 Hz, 1H, uA-C$^\alpha$H), 6.45 (d, J = 8.0 Hz, 1H, uB-NH), 6.57 (dt, J = 15.7, 6.3 Hz, 1H, uA-C$^\beta$H), 6.83 (d, J = 8.8 Hz, 1H, uB-C$^{5'}$H), 7.05 (dd, J = 8.4, 2.1 Hz, 1H, uB-C$^{6'}$H), 7.17 (d, J = 2.1 Hz, 1H, uB-C$^{2'}$H), 7.30−7.40 (m, 5H, uA-C^{ar}H); ^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm] = 9.5 (uA-C$^\varepsilon$HCH$_3$), 21.4 (uD-C$^\gamma$H(CH$_3$)^A(CH$_3$)^B), 22.72 (uC-C$^\beta$-(C$\underline{H}_3$)^A(CH$_3$)^B), 22.79 (uC-C$^\beta$(C$\underline{H}_3$)^A(C$\underline{H}_3$)^B), 23.2 (uD-C$^\gamma$-(C$\underline{H}_3$)^A(C$\underline{H}_3$)^B), 24.8 (uD-C$^\gamma$H), 27.1 (uA-C(C$\underline{H}_3$)^A(CH$_3$)^B), 27.2 (uA-C(CH$_3$)^A(C$\underline{H}_3$)^B), 32.1 (uA-C$^\gamma$H$_2$), 36.1 (uA-C$^\varepsilon$H), 36.6 (uB-C$^\beta$H$_2$), 39.3 (uD-C$^\beta$H$_2$), 43.6 (uC-C$^\beta$(CH$_3$)$_2$), 53.2 (uB-C$^\alpha$H), 56.1 (uB-OCH$_3$), 59.1 (uC-C$^\beta$H$_2$N$_3$), 71.5 (uD-C$^\alpha$H), 74.7 (uB-CH$_2$CCl$_3$), 75.1 (uA-C$^\delta$H), 80.6 (uA-C$^\eta$H), 82.3 (uA-C$^\zeta$H), 94.3 (uB-CCl$_3$), 109.0 (uA-C(CH$_3$)$_2$), 112.1 (uB-C$^{5'}$H), 122.2 (uB-C$^{3'}$), 125.0 (uA-C$^\alpha$H), 126.9/128.6/128.82 (5 × uA-C^{ar}H and uB-C$^{6'}$H), 128.86 (uB-C$^{1'}$) 131.3 (uB-C$^{2'}$H), 137.5 (uA-C^{ar}) 139.2 (uA-C$^\beta$H), 154.1 (uB-C$^{4'}$), 165.3 (uA-CONH), 169.9 (uD-CO$_2$), 170.0 (uB-CO$_2$), 175.8 (uC−CO$_2$); ESI-MS m/z 925.2 $[M + Na]^+$; HR-ESI calcd for $C_{41}H_{52}N_4O_{10}Cl_4$ $[M + H]^+$ 901.25103, found 901.25140; IR (neat, cm^{-1}) 2958, 2359, 2102, 1734, 1502.

***cyclo*-Depsipeptide 20a.** Azide **19a** (79 mg, 0.083 mmol) is dissolved in THF (970 μL). H_2O (57 μL) and PPh_3 (66 mg, 0.25 mmol) are added. After stirring for 10 min at rt, the reaction mixture is stirred for 4 h at 40 °C. Then H_2O (25 μL) is added, and the stirring is continued for 45 min at 40 °C. Toluene is added, and the solvents are removed in vacuo. After drying in high vacuum for a few hours, the residue is dissolved in toluene (4.3 mL) and 2-hydroxypyridine (16 mg, 0.17 mmol) is added. After stirring overnight at rt in the dark, the solvent is removed under reduced pressure. The residue is purified by column chromatography (hexane/EtOAc 3:7). Macrolactam **20a** is obtained as a colorless solid (21 mg, 32%): R_f (hexane/EtOAc 3:7) = 0.40; analytical HPLC 27.8 min; $[\alpha]^{24}_D = +9.5$ ($c = 0.93$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.10 (s, 3H, uC-C$^\alpha$(C$\underline{H}_3$)^A-(CH$_3$)^B), 1.13 (d, J = 6.9 Hz, 3H, uA-C$^\varepsilon$H$\underline{H}_3$), 1.20 (s, 3H,

uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 1.46 (s, 3H, uA-C(CH$_3$)^{A}(CH$_3$)^{B}), 1.51 (s, 3H, uA-C(CH$_3$)^{A}(CH$_3$)^{B}), 1.87 (m, 1H, uA-C$^{\epsilon}$H), 2.18−2.32 (m, 1H, uA-C$^{\gamma}$H), 2.45 (m, 1H, uA-C$^{\gamma}$H), 2.56 (dd, J = 16.3, 3.4 Hz, 1H, uD-C$^{\beta}$H^{A}H^{B}), 2.75 (dd, J = 16.3, 10.1 Hz, 1H, uD-C$^{\beta}$H^{A}H^{B}), 3.03 (dd, J = 14.4, 7.8 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.09−3.20 (m, 2H, uB-C$^{\beta}$H^{A}H^{B}), 3.36 (dd, J = 13.5, 8.2 Hz, 1H, uC-C$^{\beta}$H^{B}), 3.75 (dd, J = 8.8, 2.3 Hz, 1H, uA-C$^{\zeta}$H), 3.87 (s, 3H, uB-OCH$_3$), 4.55−4.61 (m, 2H, uD-CH^{A}H^{B}CH=CH$_2$), 4.66−4.77 (m, 2H, uA-C$^{\eta}$H and uB-C$^{\alpha}$H), 4.97−5.06 (m, 1H, uA-C$^{\delta}$H), 5.21 (dd, J = 10.1, 3.4 Hz, 1H, uD-C$^{\alpha}$H), 5.26 (d, J = 10.4 Hz, 1H, uD-CH$_2$CH=CH^{cis}H^{trans}), 5.30 (dd, J = 17.2, 1.2 Hz, 1H, uD-CH$_2$CH=CH^{cis}H^{trans}), 5.61 (bs, 1H, NH), 5.68 (d, J = 15.8 Hz, 1H, uA-C$^{\alpha}$H), 5.87 (m, 1H, uD-CH$_2$CH=CH$_2$), 6.50−6.65 (m, 1H, uA-C$^{\beta}$H), 6.84 (d, J = 8.4 Hz, 1H, uB-C$^{5'}$H), 7.06 (dd, J = 8.4, 1.9 Hz, 1H, uB-C$^{6'}$H), 7.10 (m, 1H, NH), 7.20 (d, J = 1.9 Hz, 1H, uB-C$^{2'}$H), 7.30−7.42 (m, 5H, uA-C^{ar}H); ^{13}C NMR (126 MHz, CDCl$_3$) δ [ppm] = 9.6 (uA-C$^{\epsilon}$HCH$_3$), 22.7 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 27.0 (uA-C(CH$_3$)^{A}(CH$_3$)^{B}), 27.3 (uA-C(CH$_3$)^{A}(CH$_3$)^{B}), 35.2/35.3/35.5 (uD-C$^{\beta}$H$_2$, uB-C$^{\beta}$H$_2$ and uA-C$^{\gamma}$H$_2$), 36.8 (uA-C$^{\epsilon}$H), 42.8 (uC-C$^{\alpha}$), 46.4 (uC-C$^{\beta}$H$_2$), 54.4 (uB-C$^{\alpha}$H), 56.2 (uB-OCH$_3$), 65.9 (uD-CH$_2$CH=CH$_2$), 68.7 (uD-C$^{\alpha}$H), 76.8 (uA-C$^{\delta}$H), 80.3 (uA-C$^{\eta}$H), 82.7 (uA-C$^{\zeta}$H), 109.2 (uA-C(CH$_3$)$_2$), 112.3 (uB-C$^{5'}$H), 118.9 (uD-CH$_2$CH=CH$_2$), 122.5 (uB-C$^{3'}$), 124.6 (uA-C$^{\alpha}$H), 126.7/128.3/128.7 (5 × uA-C^{ar}H), 128.8 (uB-C$^{6'}$H), 129.6 (uB-C$^{1'}$), 130.9 (uB-C$^{2'}$H), 131.5 (uD-CH$_2$CH=CH$_2$), 137.4 (uA-C^{ar}), 142.1 (uA-C$^{\beta}$H), 154.1 (uB-C$^{4'}$), 165.1 (uA-C=O), 168.2 (uD-CO$_2$-uA), 168.5 (uD-CO$_2$CH$_2$CH=CH$_2$), 170.3 (uB-C=O), 177.1 (uC−C=O); ESI-MS m/z 791.1 [M + Na]$^+$; HR-ESI calcd for $C_{40}H_{49}N_2O_{11}ClNa^+$ [M + Na]$^+$ 791.29171, found 791.29229; IR (neat, cm^{-1}) 2921, 2360, 2341, 1736, 1502.

***cyclo*-Depsipeptide 20b.** Azide **19b** (92 mg, 0.10 mmol) is dissolved in THF (1.2 mL) and H$_2$O (70 μL). PPh$_3$ (82 mg, 0.31 mmol) is added. The reaction mixture is stirred for 10 min at rt and for 4 h at 40 °C. H$_2$O (30 μL) is added, and stirring is continued for 40 min at 40 °C. Toluene is added, and the solvents are removed in vacuo. The residue is dried in high vacuum for several hours and subsequently dissolved in toluene (5.3 mL). 2-Hydroxypyridine (20 mg, 0.21 mmol) is added, and the reaction mixture is stirred overnight at rt in the dark. After removal of the solvent in vacuo, the crude product is purified by column chromatography (hexane/EtOAc 3:7). Macrolactam **20b** is obtained as colorless foam (39 mg, 53%): R_f (hexane/EtOAc 3:7) = 0.4. Anal. Calcd (%) for $C_{39}H_{51}ClN_2O_9$: C, 64.41; H, 7.07; N, 3.85. Found: C, 64.39; H, 7.16; N, 3.78. The ^{1}H NMR and ^{13}C NMR data are in agreement with previously published specifications.[6c]

Bromohydrin Formate 21. Acetonide **20a** (69 mg, 0.090 mmol) is dissolved in CH$_2$Cl$_2$ (0.9 mL) and cooled to 0 °C. TFA (0.9 mL) and water (5 drops) are added, and the reaction mixture is stirred for 3 h at 0 °C. After removal of the solvent in vacuo at rt, the residue is dissolved in EtOAc (50 mL) and washed with saturated aqueous NaHCO$_3$ (75 mL). The aqueous phase is extracted with EtOAc (3 × 25 mL). The combined organic phases are dried over MgSO$_4$ and concentrated in vacuo. The crude diol (analytical HPLC 23.2 min) is first dried in high vacuum and later for 2 h as a mixture with PPTS (56 mg, 0.23 mmol). CH$_2$Cl$_2$ (2.7 mL) and trimethylorthoformate (0.9 mL) are added to the dried mixture, and the reaction mixture is stirred for 2 h at rt. The suspension is filtered through a small silica gel column (1.2 cm i.d. × 5 cm), and the column is further eluted with EtOAc/CH$_2$Cl$_2$ (1:1; 300 mL). After removal of the solvents in vacuo, the crude cyclic orthoformates (two diastereomers are formed, analytical HPLC 24.3 and 24.5 min) are dried in high vacuum. The crude products are dissolved in CH$_2$Cl$_2$ (1.3 mL). AcBr (0.45 mL, 0.5 M solution in CH$_2$Cl$_2$) is added, and the solution is stirred for 4 h at rt. The reaction mixture is diluted with CH$_2$Cl$_2$ (10 mL), cooled to 0 °C, and poured into ice-cold half-saturated aqueous NaHCO$_3$ (50 mL). The reaction flask is washed with CH$_2$Cl$_2$ (12 mL). The aqueous phase is washed with CH$_2$Cl$_2$ (3 × 20 mL), and the combined organic phases are dried over MgSO$_4$, concentrated in vacuo, and dried in high vacuum. The crude bromoformate **21** (67 mg, 92%) is obtained as a yellow solid and is used without further purification for transformation to the epoxide: analytical HPLC 27.5 min; $[\alpha]^{24}_D$ = +64 (c = 1.1 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ [ppm] = 1.09 (d, J = 7.0 Hz, 3H, uA-C$^{\epsilon}$CH$_3$), 1.14 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 1.23 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 2.45 (m, 1H, uA-C$^{\gamma}$H^{A}H^{B}), 2.67 (m, 1H, uA-C$^{\gamma}$H^{A}H^{B}), 2.79 (m, 1H, uA-C$^{\epsilon}$H), 2.92−3.10 (m, 3H, uD-C$^{\beta}$H^{A}H^{B} and uB-C$^{\beta}$H^{A}H^{B}), 3.07−3.25 (m, 2H, uB-C$^{\beta}$H^{A}H^{B} and uC-C$^{\beta}$H^{A}H^{B}), 3.38 (dd, J = 13.5, 8.3 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.88 (s, 3H, uB-OCH$_3$), 4.60−4.75 (m, 3H, uD-CH^{A}H^{B}CH=CH$_2$ and uB-C$^{\alpha}$H), 4.82−4.93 (m, 2H, uA-C$^{\delta}$H and uA-C$^{\eta}$H), 5.26 (dd, J = 10.4, 0.9 Hz, 1H, uD-CH=CH^{cis}H^{trans}), 5.33 (dd, J = 17.2, 1.3 Hz, 1H, uD-CH=CH^{cis}H^{trans}), 5.40 (dd, J = 9.5, 4.0 Hz, 1H, uD-C$^{\alpha}$H), 5.74 (m, 2H, uB-C$^{\alpha}$NH and uA-C$^{\zeta}$H), 5.80 (d, J = 16.1 Hz, 1H, uA-C$^{\alpha}$H), 5.91 (m, 1H, uD-CH=CH$_2$), 6.72 (ddd, J = 15.0, 10.5, 4.4 Hz, 1H, uA-C$^{\beta}$H), 6.86 (d, J = 8.5 Hz, 1H, uB-C$^{5'}$H), 7.10 (dd, J = 8.4, 2.0 Hz, 1H, uB-C$^{6'}$H), 7.17−7.24 (m, 2H, uB-C$^{2'}$H and uC-C$^{\beta}$NH), 7.26−7.50 (m, 5H, C^{ar}H), 7.59 (s, 1H, H-C=O); ^{13}C NMR (126 MHz, CDCl$_3$) δ [ppm] = 9.9 (uA-C$^{\epsilon}$CH$_3$), 22.7 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 35.3/35.7 (uD-C$^{\beta}$H$_2$ and uB-C$^{\beta}$H$_2$), 36.8 (uA-C$^{\gamma}$H$_2$), 38.5 (uA-C$^{\epsilon}$CH$_3$), 42.9 (uB-C$^{\alpha}$H), 46.4 (uC-C$^{\beta}$H$_2$), 51.5 (uA-C$^{\eta}$H), 54.7 (uB-C$^{\alpha}$H), 56.2 (uB-OMe), 65.9 (uD-CH$_2$CH=CH$_2$), 68.8 (uD-C$^{\alpha}$H), 72.6 (uA-C$^{\zeta}$H), 75.2 (uA-C$^{\delta}$H), 112.3 (uB-C$^{5'}$H), 118.8 (uD-CHCH$_2$), 122.5 (uB-C$^{3'}$), 125.0 (uA-C$^{\alpha}$H), 128.3/128.5/128.6/129.1 (5 × uA-C^{ar}H and uB-C$^{6'}$H), 129.7 (uB-C$^{1'}$), 130.9 (uB-C$^{2'}$H), 131.6 (uD-CH$_2$CH=CH$_2$), 137.4 (uA-C^{ar}), 141.4 (uA-C$^{\beta}$H), 154.0 (uB-C$^{4'}$), 158.7 (H-C=O), 165.0 (uA-C$^{\alpha}$C=O), 168.5 (uD-CO$_2$-CH$_2$CH=CH$_2$), 168.9 (uD-CO$_2$CH), 170.4 (uB-C=O), 177.0 (uC-C=O); ESI-MS m/z 840.9 [M + Na]$^+$; HR-ESI calcd for $C_{38}H_{44}N_2O_{11}BrClNa$ [M + Na]$^+$ 841.17092, found 841.17222; IR (neat, cm^{-1}) 2927, 2360, 1723, 1502, 1139.

Allylester Functionalized Cryptophycin-52 Analogue 3. Bromoformate **21** (31 mg, 0.038 mmol) and KHCO$_3$ (64 mg, 0.64 mmol) are dried in high vacuum in a pear shape flask (5 mL) for several hours. DME (640 μL) and EtOH (210 μL) are added. The suspension is stirred for 2 h at 40 °C in the sealed flask and then is placed in an ultrasonic bath for 30 min. After 5 h reaction time at 40 °C, KHCO$_3$ (54 mg, 0.54 mmol) is added and the mixture is stirred overnight at 40 °C. After 22.5 h, the reaction flask is placed again in an ultrasonic bath for 30 min. After additional 3 h, DME (300 μL), EtOH (100 μL), and dry KHCO$_3$ (20 mg, 0.20 mmol) are added and the suspension is stirred for further 16 h at 40 °C. The suspension is filtered through a small silica gel column (1.2 cm i.d. × 5 cm), and the column is further eluted with EtOAc/DCM (1:1; 200 mL) and EtOAc (200 mL). After removal of the solvents in vacuo, the yellow residue is dissolved in acetonitrile and purified by preparative HPLC. The product (preparative HPLC 37.6 min) elutes before the starting material (preparative HPLC 39.0 min). To avoid hydrolysis, the product fractions are immediately combined and freeze-dried overnight. Cryptophycin analogue **3** (11 mg, 39%) is obtained as a colorless solid: R_f (EtOAc) = 0.70; analytical HPLC 26.2 min; $[\alpha]^{RT}_D$ = +26 (c = 0.89 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ [ppm] = 1.10 (s, 3H, uC-C$^{\alpha}$(CH$_3$)$_2$), 1.15 (d, J = 6.8 Hz, 3H, uA-C$^{\epsilon}$CH$_3$), 1.19 (s, 3H, uC-C$^{\alpha}$(CH$_3$)$_2$), 1.79 (dd, J = 13.9, 6.9 Hz, 1H, uA-C$^{\epsilon}$H), 2.28 (dd, J = 16.4, 3.1 Hz, 1H, uA-C$^{\gamma}$H^{A}), 2.46 (m, 1H, uA-C$^{\gamma}$H^{B}), 2.57−2.67 (m, 2H, uD-C$^{\beta}$H^{A}H^{B}), 2.87 (dd, J = 7.8, 1.8 Hz, 1H, uA-C$^{\zeta}$H), 3.00−3.20 (m, 3H, uB-C$^{\beta}$H^{A}H^{B} and uC-C$^{\beta}$H^{A}H^{B}), 3.42 (dd, J = 13.5, 8.8 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.67 (d, J = 1.6 Hz, 1H, uA-C$^{\eta}$H), 3.88 (s, 3H, uB-OCH$_3$), 4.48−4.62 (m, 2H, uD-CH^{A}H^{B}CH=CH$_2$), 4.73 (m, 1H, uB-C$^{\alpha}$H), 5.11−5.22 (m, 2H, uD-C$^{\alpha}$H and uA-C$^{\delta}$H), 5.26 (d, J = 10.4 Hz, 1H, uD-C=CH^{cis}H^{trans}), 5.30 (dd, J = 17.2, 1.1 Hz, 1H, uD-C=CH^{cis}H^{trans}), 5.50 (d, J = 7.7 Hz, 1H, NH), 5.74 (d, J = 15.1 Hz,

1H, uA-C$^{\alpha}$H), 5.87 (m, 1H, uD-CH=CH$_2$), 6.76 (ddd, J = 15.0, 10.8, 4.1 Hz, 1H, uA-C$^{\beta}$H), 6.85 (d, J = 8.4 Hz, 1H, uB-C$^{5'}$H), 7.05 (dd, J = 8.4, 2.0 Hz, 1H, uB-C$^{6'}$H), 7.12−7.17 (m, 1H, NH), 7.19 (d, J = 1.9 Hz, 1H, uB-C$^{2'}$H), 7.21−7.25 (m, 2H, uA-C^{ar}H), 7.28−7.37 (m, 3H, uA-C^{ar}H); ^{13}C NMR (151 MHz, $CDCl_3$) δ [ppm] = 13.8 (uA-C$^{\varepsilon}$HCH$_3$), 22.7 (uC-C$^{\alpha}$(CH$_3$)^{A}-(CH$_3$)^{B}), 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 35.3/35.4 (uD-C$^{\beta}$H$_2$ and uB-C$^{\beta}$H$_2$), 36.9 (uA-C$^{\gamma}$H$_2$), 40.8 (uA-C$^{\varepsilon}$H), 42.8 (uC-C$^{\alpha}$), 46.4 (uC-C$^{\beta}$H$_2$), 54.4 (uB-C$^{\alpha}$H), 56.2 (uB-OCH$_3$), 59.2 (uA-C$^{\eta}$H), 63.5 (uA-C$^{\zeta}$H), 65.8 (uD-CH$_2$CH=CH$_2$), 68.9 (uD-C$^{\alpha}$H), 76.6 (uA-C$^{\delta}$H), 112.4 (uB-C$^{5'}$H), 118.9 (uD-CH$_2$CH=CH$_2$), 122.6 (uB-C$^{3'}$), 124.9 (uA-C$^{\alpha}$H), 125.5/128.3/128.6/128.8 (5 × uA-C^{ar}H and uB-C$^{6'}$H), 129.4 (uB-C$^{1'}$), 130.9 (uB-C$^{2'}$H), 131.5 (uD-CH$_2$CH=CH$_2$), 136.7 (uA-C^{ar}), 141.6 (uA-C$^{\beta}$H), 154.2 (uB-C$^{4'}$), 164.9 (uA-C=O), 168.1 (uD-C=O), 168.9 (uD-CO$_2$-CH$_2$CH=CH$_2$), 170.4 (uB-C=O), 177.3 (uC-C=O); ESI-MS m/z 733.3 [M + Na]$^+$; HR-ESI calcd for $C_{37}H_{43}N_2O_{10}ClNa$ [M + Na]$^+$ 733.24984, found 733.25073; IR (neat, cm^{-1}) 2932, 1736, 1655, 1502, 1142.

Carboxylic Acid Functionalized Cryptophycin-52 Analogue 4. $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) is dissolved in CH_2Cl_2 (13.5 mL). An aliquot of 1.1 mL of the catalyst solution is added to the cryptophycin-52 analogue **3** (9 mg, 0.01 mmol), followed by morpholine (5 drops). The reaction mixture is stirred for 40 min at rt. The reaction mixture is transferred with CH_2Cl_2 (25 mL) into a separatory funnel and washed with 0.5% aqueous $KHSO_4$ (10 mL). The organic phase is dried over $MgSO_4$ and concentrated in vacuo. The residue is purified by column chromatography (CH_2Cl_2/EtOH/AcOH 96:4:1) and freeze-dried. Carboxylic acid **4** (6 mg, 74%) is obtained as a colorless solid: R_f (DCM/EtOH/AcOH 96:4:1) = 0.22; analytical HPLC 22.7 min; $[\alpha]^{24}_D$ = +36 (c = 1.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] = 1.11 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 1.15 (d, J = 6.8 Hz, 3H, uA-C$^{\varepsilon}$CH$_3$), 1.20 (s, 3H, uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 1.81 (dd, J = 13.7, 6.9 Hz, 1H, uA-C$^{\varepsilon}$H), 2.30−2.50 (m, 2H, uA-C$^{\gamma}$H$_2$), 2.58−2.69 (m, 2H, uD-C$^{\beta}$H), 2.88 (dd, J = 7.8, 1.7 Hz, 1H, uA-C$^{\zeta}$H), 3.01 (dd, J = 14.5, 7.6 Hz, 1H, uB-C$^{\beta}$H^{A}H^{B}), 3.06−3.19 (m, 2H, uB-C$^{\beta}$H^{A}H^{B} and uC-C$^{\beta}$H^{A}H^{B}), 3.38 (dd, J = 13.4, 8.6 Hz, 1H, uC-C$^{\beta}$H^{A}H^{B}), 3.68 (d, J = 1.5 Hz, 1H, uA-C$^{\eta}$H), 3.88 (s, 3H, uB-OCH$_3$), 4.73 (dd, J = 12.9, 7.5 Hz, 1H, uB-C$^{\alpha}$H), 5.10−5.25 (m, 2H, uD-C$^{\alpha}$H and uA-C$^{\delta}$H), 5.66 (d, J = 6.3 Hz, 1H, NH), 5.73 (d, J = 15.1 Hz, 1H, uA-C$^{\alpha}$H), 6.75 (ddd, J = 14.9, 10.8, 4.0 Hz, 1H, uA-C$^{\beta}$H), 6.85 (d, J = 8.4 Hz, 1H, uB-C$^{5'}$H), 7.05 (dd, J = 8.4, 1.8 Hz, 1H, uB-C$^{6'}$H), 7.12−7.20 (m, 2H, NH and uB-C$^{2'}$H), 7.21−7.25 (m, 2H, uA-C^{ar}H), 7.28−7.37 (m, 3H, uA-C^{ar}H); ^{13}C NMR (151 MHz, $CDCl_3$) δ [ppm] = 13.7 (uA-C$^{\varepsilon}$HCH$_3$), 22.7 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 22.8 (uC-C$^{\alpha}$(CH$_3$)^{A}(CH$_3$)^{B}), 35.0/35.3 (uD-C$^{\beta}$H$_2$ and uB-C$^{\beta}$H$_2$), 36.8 (uA-C$^{\gamma}$H$_2$), 40.7 (uA-C$^{\varepsilon}$H), 42.8 (uC-C$^{\alpha}$), 46.4 (uC-C$^{\beta}$H$_2$), 54.4 (uB-C$^{\alpha}$H), 56.2 (uB-OCH$_3$), 59.1 (uA-C$^{\eta}$H), 63.5 (uA-C$^{\zeta}$H), 68.7 (uD-C$^{\alpha}$H), 76.6 (uA-C$^{\delta}$H), 112.4 (uB-C$^{5'}$H), 122.6 (uB-C$^{3'}$), 125.0 (uA-C$^{\alpha}$H), 125.5/128.3/128.7/128.8 (5 × uA-C^{ar}H and uB-C$^{6'}$H), 129.4 (uB-C$^{1'}$), 130.9 (uB-C$^{2'}$H), 136.6 (uA-C^{ar})141.6 (uA-C$^{\beta}$H), 154.2 (uB-C$^{4'}$), 165.0 (uA-C=O), 168.8 (uD-C=O), 170.6 (uB-C=O), 171.2 (uD-COOH), 177.1 (uC-C=O); ESI-MS m/z 669.0 [M − H]$^-$; HR-ESI calcd for $C_{34}H_{39}N_2O_{10}ClNa^+$ [M + Na]$^+$ 693.21854, found 693.21851; IR (neat, cm^{-1}) 2932, 1728, 1652, 1504, 1144.

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Supporting Information Available: General information, ^{1}H and ^{13}C NMR spectra, and details on the cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.