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A Convenient Protocol for Selective Cleavage of 2-Hydroxy Acid Amides. Application to Semisynthesis of the Cyclic Heptapeptide Aza HUN-7293

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A two-step protocol for the first chemoselective cleavage of 2-hydroxy acid amides has been developed. Mesylation of the model substrate 2-(hydroxypropionylamino)-4-methylpentanoic acid methyl ester (**11**) followed by treatment with *N*-ethylthiourea (**13**) allows cleavage of 2-hydroxy acid amides under smooth conditions. Successful application of this methodology to the open-chain transesterification product **15** (methylester) of the cyclic heptadepsipeptide HUN-7293, a potent inhibitor of inducible cell adhesion molecule expression, delivered the corresponding hexapeptide **18** with unprotected *N*-terminus in 70−75% yield. This result demonstrates that the protocol developed even works in the presence of an ester and several methylated and unmethylated amide bonds. Finally, a sequence of ligation of methyl D-dehydroglutaminate (**20**) to the *C*-terminus of the saponification product **21**, followed by the degradation protocol and ring closure, allowed chemical "point mutation" at the DGCN site affording the aza analogue of HUN-7293 (**24**) in 15% overall yield. To the best of our knowledge this is the first report on chemoselective cleavage of 2-hydroxy acid amides.

Introduction

The cyclic heptadepsipeptide HUN-7293 (**1**) is a potent inhibitor of the expression of the adhesion molecules ICAM-1 and VCAM-1.[1] This cyclic heptadepsipeptide composed of six L-amino and one D-hydroxy acid (Figure 1) was first isolated from a fungal broth during a screen for potent inhibitors of inducible cell adhesion molecule expression in 1992. Independently, the identical natural product was isolated by a Japanese group from a different fungal strain screening for anti-HIV compounds.[2] Recently, the total synthesis of HUN-7293 has been achieved by D. Boger and co-workers in collaboration with Novartis chemists.[3]

To gain insight into the structural requirements for its unique biological activity, the three-dimensional structure of **1** has been determined, both in solution by NMR spectroscopy and in single crystals by X-ray diffraction.[4] These investigations revealed that the ester bond exists in the trans-configuration in solution and in the crystalline state. Therefore, we assumed that replacement of the ester moiety by an amide should be tolerated without changing the overall conformation of the molecule. From the viewpoint of a medicinal chemist

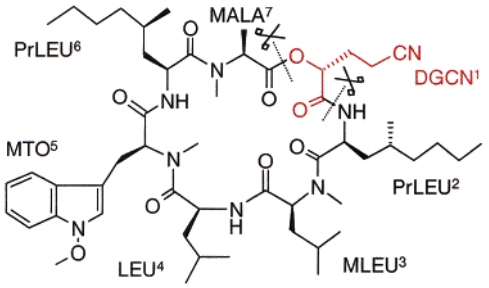

FIGURE 1. Structure of HUN-7293 (**1**).

there are three major reasons to move from a cyclic depsipeptide to a cyclic peptide: (a) an amide bond is more convenient to form than an ester bond; (b) an amide bond is chemically and metabolically more stable than an ester bond; and (c) the palette of commercially available optically pure amino acids is much broader than that for 2-hydroxy acids. So far, ester/amide exchange for such macrocyclic molecules has been realized only by total synthesis as demonstrated for Destruxin,[5] Leualacin,[6] and, recently by the Boger group, also HUN-7293.[7]

We considered the multitude of steps for the synthesis of the unnatural amino acids PrLEU and MTO and a series of critical coupling cycles as a hurdle for a potential

(1) Foster, C. A.; Dreyfuss, M.; Mandak, B.; Meingassner, J. G.; Naegeli, H.-U.; Nussbaumer, A.; Oberer, L.; Scheel, G.; Swoboda, E.-M. *J. Dermatol.* **1994**, *21*, 847.
(2) Itazaki, H.; Fujiwara, T.; Sato, A.; Kawamura, Y.; Matsumoto, K. Jpn Patent, Kokai Tokkyo Koho 07109299, 1995, Appl. 93/255592, Oct 13, 1993; *Chem. Abstr.* **1995**, *123*, 110270.
(3) Boger, D. L.; Keim, H.; Oberhauser, B.; Schreiner, E. P.; Foster, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 6197.
(4) Hommel, U.; Weber, H.-P.; Oberer, L.; Naegeli, H. U.; Oberhauser, B.; Foster, C. A. *FEBS Lett.* **1996**, *379*, 69.
(5) Cavelier, F.; Jacquier, R.; Mercadier, J.-L.; Verducci, J. *Tetrahedron* **1996**, *52*, 6173.
(6) Hu, M.-K.; Yang, F.-C.; Chou, C.-C.; Yen, M.-H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 563.
(7) Boger, D. L.; Chen, Y.; Foster, C. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1741.

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chemical development as a drug. Therefore, in contrast to Boger's total synthesis, we planned a semisynthetic approach starting from the readily available fermentation product. Our strategy envisaged chemical "point mutations" at the DGCN site involving cleavage of the lactone followed by degradation of the 2-hydroxy acid unit, incorporation of a new amino acid, and final ring closure forming an amide bond. Although in the past a series of cyclic depsipeptides showing interesting biological activities such as the antifungal aureobasidin A,[8] the antiinflammatory salinamides A and B,[9] the neurokinin antagonist Sch 217048,[10] the insecticide Destruxine,[11] and the calcium blocker leualacin[12] were investigated, no general methodology for the selective cleavage of 2-hydroxy acid amides, comparable to the Edman protocol for peptides, has been developed. We now report the first protocol for selective cleavage of 2-hydroxy acid amides in the presence of several methylated and unmethylated amides as well as an ester bond.

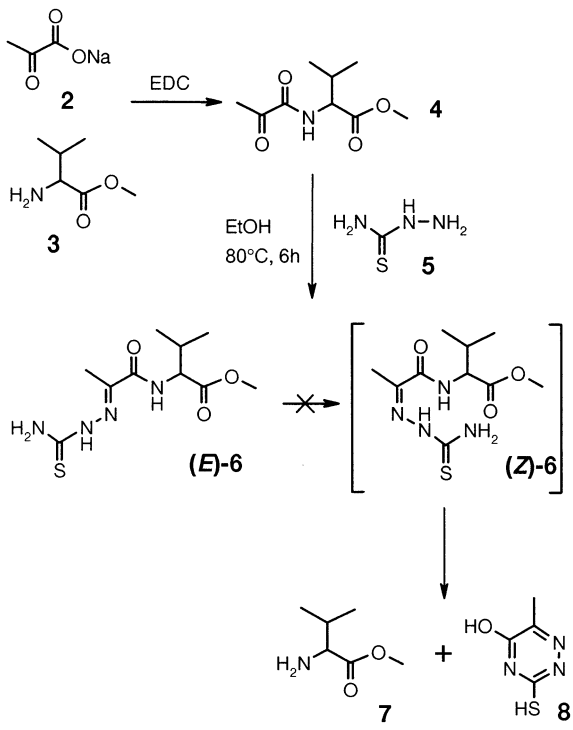
Results and Discussion

Model Reactions. On the basis of simple model substrates two potential approaches were evaluated. Method A is founded on a report[13] on the cyclization of the thiosemicarbazones of sodium pyruvic acid esters and subsequent ester cleavage. First, coupling of sodium pyruvate (**2**) and D,L-Val-OMe (**3**) in the presence of EDC afforded our model substrate *N*-(2-oxopropanoyl)leucine methyl ester (**4**). Indeed, heating of **4** in the presence of thiosemicarbazone **5** in EtOH at 80 °C resulted in the formation of the thiosemicarbazide (***E***)-**6** as well as cleavage products **7** and **8** (Scheme 1). This result indicated that under these conditions only the (*Z*)-isomer (***Z***)-**6** spontaneously cyclized, whereas isomerization of (***E***)-**6** to (***Z***)-**6**, necessary to obtain quantitative conversion, did not occur.

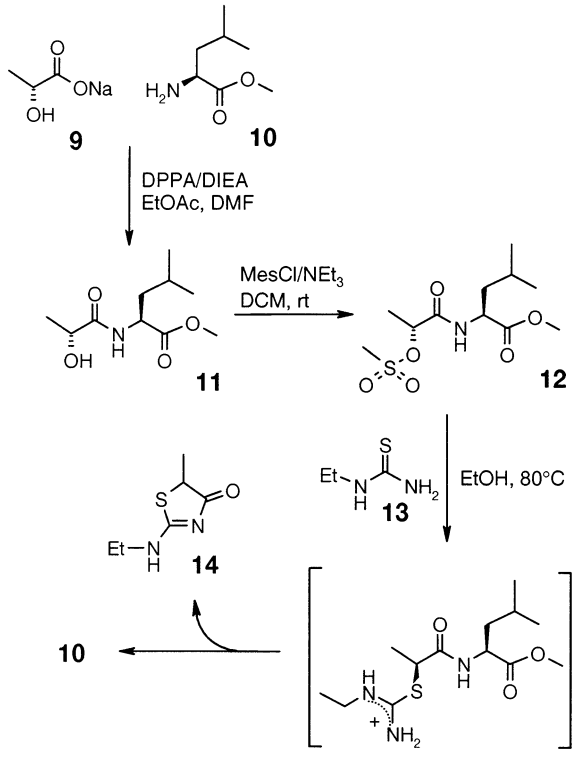
Method B takes advantage of a chemical principle developed for the selective cleavage of chloroacetate amides, wherein the chloride is first replaced by a thiourea moiety forming an intermediate thiouronium salt, which subsequently cyclizes and liberates the amine.[14] Coupling of sodium D-lactate (**9**) and D-Leu-OMe (**10**) provided the hydroxyl group of *N*-(*R*)-lactyl-(*S*)-leucine methylester (**11**), which was first transformed into the mesylate **12**. In a second step crude **12** was treated with *N*-ethylthiourea (**13**) in EtOH at 80 °C to first undergo nucleophilic substitution of the mesylate, forming analogously the intermediate thiouronium salt, and then to cyclize liberating **14** (43%) and the amine **10** in 49% yield (Scheme 2).

Application to HUN-7293. Both sequences were applied to the open-chained methylester **15**,[15] which is obtained from **1** by alkaline methanolysis as a 4:1 mixture of the diastereomeres arising from partial racemization (~20%) of MALA.[7] For method A, in the first step we oxidized **15** with Dess−Martin periodinane to afford ketone **16** in 89% yield. However, in contrast to

SCHEME 1

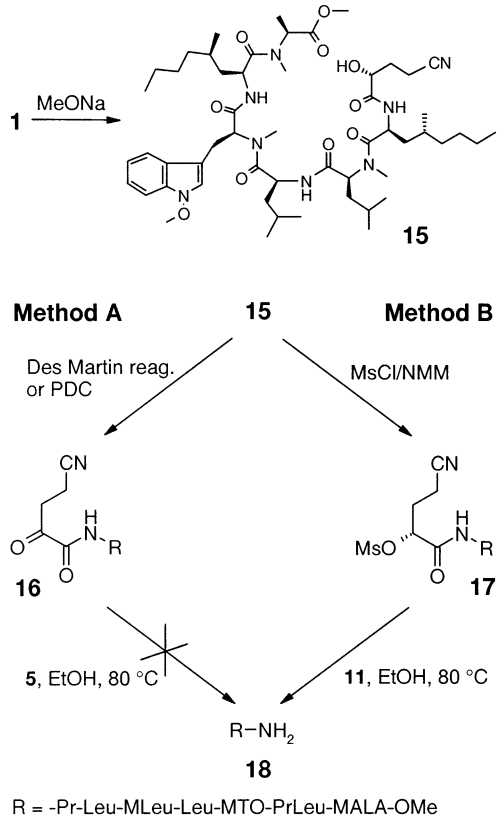


SCHEME 2



(8) Ikai, K.; Takesako, K.; Shiomi, K.; Moriguchi, M.; Umeda, Y.; Yamamoto, J.; Kato, I.; Naganawa, H. J. *Antibiotics* **1991**, *44*, 925.
(9) Trischman, J. A.; Tapiolas, D. M.; Jensen, P. R.; Dwight, R.; Fenical, W.; McKee, T. C.; Ireland, C. M.; Stout, T. J.; Clardy, J. *J. Am. Chem. Soc.* **1994**, *116*, 757.
(10) Hegde, V. R.; Puar, M. S.; Chan, T. M.; Dai, P.; Das, P. R.; Patel, M. *J. Org. Chem.* **1998**, *63*, 9584.
(11) Steiner, J. R.; Barnes, C. L. *Int. J. Pept. Protein Res.* **1988**, *31*, 212.
(12) Hamano, K.; Kinoshita, M.; Furuya, K.; Miyamoto, M.; Takamatsu, Y.; Hemmi, A.; Tanzawa, K. *J. Antibiot.* **1992**, *45* (8), C-4.
(13) Just, G.; Kim, S. *Can. J. Chem.* **1977**, *55*, 427.
(14) Steglich, W.; Batz, H.-G. *Angew. Chem.* **1971**, *83*, 83.
(15) Dreyfuss, M. M.; Foster, C. A.; Naegeli, H.-U.; Oberhauser, B. WO Patent 96,03430 A1, 1996.

SCHEME 3



the transformation of **4**, treatment of **16** with thiosemicarbazide (**5**) did not give detectable amounts of the desired amine. For method B we found our model reaction to be more successful. Mesylation of **15** in the presence of *N*-methylmorpholine (NMM) and subsequently heating of crude mesylate **17** with **13** in EtOH at 80 °C for 24 h (Scheme 3) yielded highly reproducibly the desired amine **18** in 70−75% yield.

In the course of our medicinal chemistry program **18** served as a central building block for the synthesis of a series of novel cyclic peptides related to HUN-7273 (**1**) accessed via coupling with *N*-protected amino acids and final ring closure forming an amide bond between MALA[7] and the amino acids newly introduced. Corresponding D-MALA isomers originating from racemization in the saponification steps were conveniently separated from the products with natural stereochemistry for MALA[7] by flash chromatography on silica gel, consistently eluting as less polar byproducts.

Synthesis of aza HUN-7293. For the semisynthesis of aza HUN-7293 (**24**) itself we first formed the bond between MALA[7] and the D-dehydro-Gln moiety followed by the degradation protocol and ring fusion between D-dehydro-Gln and PrLEU[2] (Scheme 4). Generally, this route was found to yield less of the corresponding D-MALA isomers as observed for that via **18**. The synthon for the amino counterpart of (2*R*)-hydroxy-4-cyanobutyric acid ((2*R*)-DGCN) methyl D-dehydroglutaminate **20** was obtained as the corresponding hydrochloride from the *N*-Boc derivative **19**[7] by esterification with CH_2N_2 in Et_2O and subsequent cleavage of the Boc-protecting group with hydrochloric acid in CH_2Cl_2. Starting from **1**, the lactone bond was selectively cleaved under alkaline conditions (LiOH, THF/H_2O), producing the lithium salt of the corresponding hydroxy acid **21** (98%), which was a 5:1 mixture of the 7-*S* and 7-*R* isomer occurring from partial racemization.[3] Amide bond formation between **20** and the open-chain hydroxy acid **21** was effected by treatment with HATU in DMF producing **22** (93%), the substrate for the degradation sequence. Mesylation of **22** followed by treatment with *N*-ethylthiourea (**13**) gave the desired heptapeptide **23** in 56% yield over both steps. Finally, saponification of the methyl ester and macrocyclization using BOP as the condensing agent in acetonitrile at a concentration of 6×10^{-4} M gave aza HUN-7293 (**24**) in 31% yield (15% from **1**). This result of the cyclization step is superior to the total synthesis route[7] via ring closure forming the MLEU[3]-LEU[4] amide bond (22% yield, 5:1 mixture together with its D-MLEU[3] isomer). Neither the product from a potential $C_2$1 epimerization in the course of the cyclization reaction nor the D-MALA[7] isomer were observed.

Comparative NMR spectroscopic investigations of HUN-7293 (**1**) and aza HUN-7293 (**24**) confirmed our working hypothesis that the exchange of 7-COO by 7-CONH does not significantly affect the overall conformation. In a $CDCl_3$ solution both compounds, **1** and **24**, exist as one main conformer (85%) besides traces of two others (about 10 and 5%). For **1** it was reported that conformations in $CDCl_3$/DMSO and MeOH/H_2O are very similar, suggesting that conformations observed in organic solvents can be assumed to also exist in aqueous solution.[4] The biological profile of **24** was found to be very similar to that observed for **1**; however, IC_{50} values for inhibition of the single adhesion molecules were found to be (10−20)-fold higher.[7]

Conclusion

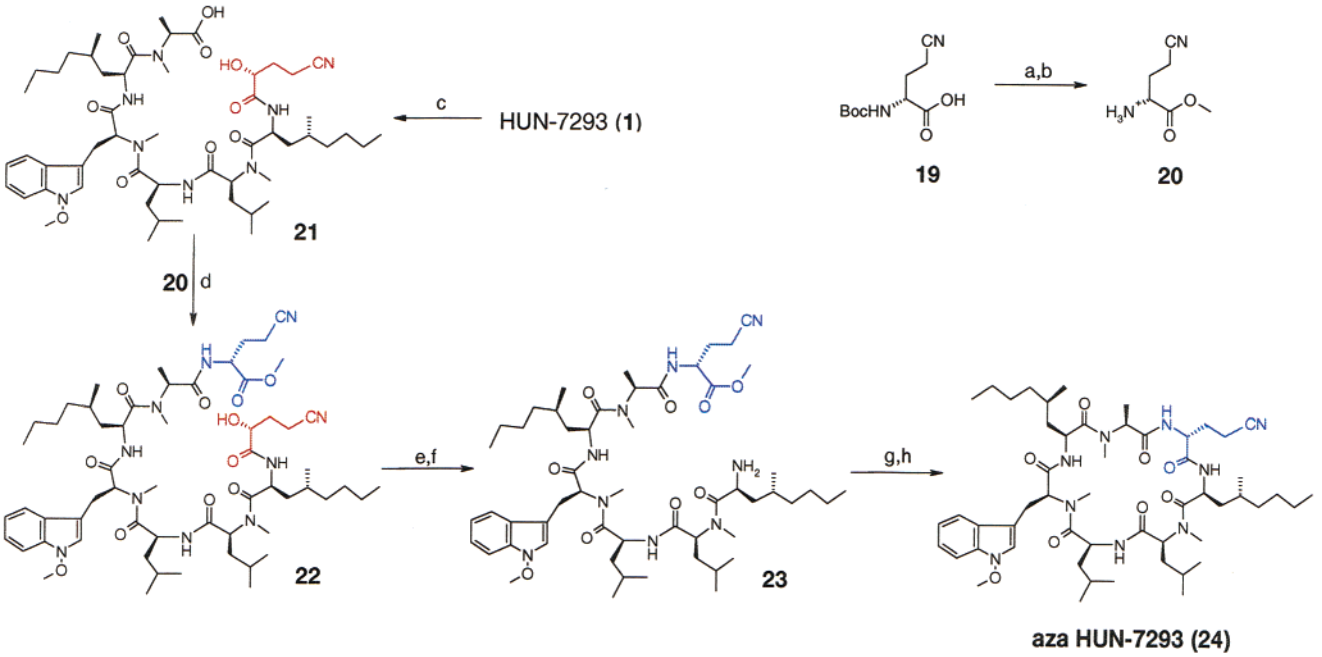
In conclusion, a two-step sequence for selective cleavage of 2-hydroxy acid amides in the presence of an ester and a series of methylated and unmethylated amide bonds has been developed. Evaluating two potential approaches, we found that transformation of the hydroxyl group into a leaving group via mesylation followed by treatment with *N*-ethylthiourea at elevated temperature gave the corresponding amine in good overall yield. Successful application of this methodology to the semisynthesis of aza HUN-7293 (**24**) demonstrated that degradation of the 2-hydroxy acid moiety of a cyclodepsipeptide followed by incorporation of an amino acid and final cyclization is an attractive strategy to achieve backbone ester/amide exchange by a chemical "point mutation" strategy without multistep total synthesis of the related cyclic peptide.

Experimental Section

General. All the reactions were carried out under an argon atmosphere. ^{1}H NMR was measured at 250, 400, or 500 MHz, and ^{13}C NMR was taken at 62.9, 100.6, or 125.8 MHz in the solvent indicated. High-resolution mass spectra were recorded in the ESI mode. Samples for analytics were additionally purified by size exclusion chromatography to remove traces of silica gel. Elemental analyses were performed by Solvias.

(*R*,*S*)-3-Methyl-2-(2-oxopropionylamino)butyric acid methyl ester 4: To a solution of sodium pyruvate (**2**) (1.1 g, 10 mmol), D,L-valine methylester hydrochloride (**3**) (1.68 g, 10 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

SCHEME 4[a]



[a] Reagents and conditions: (a) CH_2N_2, Et_2O, rt; (b) HCl, CH_2Cl_2, rt; (c) LiOH/THF, H_2O, rt; (d) HATU/DIEA, DMF, 0 °C; (e) Ms-Cl/NMM, CH_2Cl_2, rt; (f) **9**, EtOH, 80 °C; (g) LiOH, THF/H_2O; (h) BOP/DIEA, MeCN, rt.

hydrochloride (EDC; and 2.88 g, 10 mmol) in dry DMF (100 mL) at room temperature was added *N*-methylmorpholine (1.1 mL, 15 mmol) and the reaction mixture was stirred overnight. The solvent was evaporated and the residue obtained was dissolved in EtOAc and sequentially washed with 1 M aqueous HCl, aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated. Flash chromatography with EtOAc/c-hexane (1:1) afforded **4** (484 mg, 24%) as a pale yellow oil: ^{1}H NMR (225 MHz, $CDCl_3$) δ 7.36 (br s, 1H), 4.47 (dd, J = 9.2 and 5.0 Hz, 1H), 3.75 (s, 3H), 2.47 (s, 3H), 2.22 (m, 1H), 0.93 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 196.3, 171.3, 159.9, 57.3, 52.3, 31.3, 24.4, 18.9, 17.7; HRMS(ESI) calcd for $C_9H_{16}NO_4$ $[M + H]^+$ 202.1079, found 202.1075.

(*R*,*S*)-3-Methyl-2-(2-*E*-(*N*-thiocarbonylamino)hydrazono-propionylamino)butyric acid methyl ester (*E*)-6: A solution of **4** (90 mg, 0.45 mmol) and **5** (50 mg, 0.55 mmol) in ethanol (6 mL) was stirred for 6 h at 80 °C. The solvent was evaporated and the residue purified by chromatography with EtOAc/MeOH (0−20% MeOH), delivering 61 mg (50%) of ***E*-6** and 27 mg (42%) of **8** as white solids as well as 23 mg (39%) of Val-OMe **7** as a colorless oil. ***E*-6**: ^{1}H NMR (225 MHz, $CDCl_3$) δ 9.14 (br s, 1H), 7.94 (br s, 1H), 7.77 (d, J = 9.1, 1H), 7.45 (br s, 1H), 4.51 (dd, J = 9.1 and 6.6 Hz, 1H), 3.70 (s, 3H), 2.26 (m, 1 H), 2.11 (s, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 179.4, 173.0, 163.4, 142.8, 57.8, 52.1, 31.0, 19.1, 18.4, 10.9; HRMS(ESI) calcd for $C_{10}H_{19}N_4O_3S$ $[M + H]^+$ 275.1178, found 275.1179. **7** was found to be identical with a commercial sample.

(*S*)-2-((*R*)-2-Hydroxypropionylamino)-4-methylpentanoic acid methyl ester 11: To a solution of (*S*)-Leu-OMe (1.78 g, 9.8 mmol) and lithium (*R*)-lactate (**9**) (1.0 g, 9.8 mmol) in EtOAc/DMF (6:1, 60 mL) at 0 °C was added propanphosphonic acid anhydride (50% in EtOAc, 6 mL, 20 mmol) and DIEA (5.14 mL, 30 mmol). The reaction mixture was allowed to warm to room temperature overnight. The solvent was evaporated and the residue obtained was redissolved in EtOAc and sequentially washed with 1 M aqueous HCl, aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated. Flash chromatography with EtOAc/c-hexane (1:2) afforded 364 mg (17%) of **11** as a colorless oil. **11**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.90 (d, J = 8.2 Hz, 1H), 4.58 (dt, J = 8.6 and 5.0 Hz, 1H), 4.22 (dq, J = 6.8 and 5.2 Hz, 1H), 3.70 (s, 3H), 3.11 (d, J = 5.1 Hz; 1H), 1.67−1.49 (m, 3H), 1.40 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.2 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.8, 173.8, 68.8, 52.7, 50.8, 41.9, 25.3, 23.2, 22.2, 21.5; $[\alpha]^{20}_D$ −22.3 (*c* 1, MeOH); HRMS(ESI) calcd for $C_{10}H_{19}NO_4Na$ $[M + Na]^+$ 240.1212, found 240.1210.

Cleavage of 11 producing (*S*)-Leu-OMe 10 and 2-ethylamino-5-methyl-thiazol-4-one 14: To a solution of **7** (100 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added *N*-methylmorpholine (126 μL, 1.15 mmol) and Ms-Cl (89 μL,1.15 mmol) and the reaction was allowed to warm to room temperature within 3 h. Conversion was checked by TLC (SiO_2, EtOAc). The solvent was evaporated and the residue was redissolved in EtOAc and sequentially washed with 1 M aqueous HCl, aqueous $NaHCO_3$ solution, and brine. After drying over Na_2SO_4 the solvent was evaporated delivering crude **12**. A solution of **12** and **13** (240 mg, 2.3 mmol) in 3 mL of dry EtOH was heated at 80 °C for 24 h. The solvent was evaporated and the residue obtained purified by chromatography with 0−10% methanol in EtOAc affording 31 mg (43%) of **14** and 33 mg (49%) of the amine **10** as colorless foam. **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (q, J = 7.3 Hz, 1H), 3.38 (q, J = 7.3 Hz, 1H), 1.64 (d, J = 7.3 Hz, 3H), 1.41 (t, J = 7.3 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 189.44, 182.04, 49.74, 40.57, 19.43, 14.64. **10** was found to be identical with a sample obtained by treatment of an aqueous solution of the commercially available (*S*)-Leu-OMe hydrochloride with Na_2CO_3 and subsequent extraction with EtOAc.

***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-Hydroxy-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-*N*$^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alanine methyl ester 15:** To a solution of **1** (30 g, 30.6 mmol) in dry methanol (450 mL) was added sodium hydride (40 mg, 1.67 mmol) with stirring at room temperature until no starting material could be observed on TLC. Then the reaction mixture was neutralized with solid CO_2 and the solvent was evaporated. After the residual methanol was removed by codistillation with toluene the material was used for the next step.

***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-[*N*-[(2-oxo-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-$N^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alanine methyl ester 16:** A solution of **15** (200 mg, 0.2 mmol), Dess−Martin periodinane (102 mg, 0.24 mmol), and pyridine (33 μL, 0.4 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 6 h. Then a saturated $Na_2S_2O_3$ solution (1 mL) and a 5% aqueous $NaHCO_3$ solution (5 mL) were added and the mixture was stirred for an additional 30 min. After dilution with CH_2Cl_2 the organic layer was washed with 0.1 M aqueous HCl, saturated $NaHCO_3$ solution, and brine. Purification by size exclusion on Sephadex LH-20 with MeOH gave 180 mg (89%) of **16**. ^{1}H NMR (500 MHz, $CDCl_3$, only assigned signals of the major conformer) δ 8.40 (d, J = 8.0 Hz, 1 H), 7.55 (d, J = 7.9 Hz, 1 H), 7.48 (d, J = 8.9 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.36 (t, J = 7.3 Hz, 1H), 7.14 (t, J = 7.9 Hz, 1H), 7.00 (s, 1H), 6.28 (d, J = 6.3 Hz, 1H), 5.43 (q, J = 7.3 Hz, 1H), 5.02 (m, 2H), 4.90−4.78 (m, 2H), 4.16 (m, 1H), 4.05 (s, 3H), 3.74 (s, 3H), 3.40−3.23 (m, 3H), 3.16 (m, 1H), 2.98 (s, 3H), 2.95 (s, 3H), 2.94 (s, 3H), 2.64 (m, 1H), 1.47 (d, J = 7.4 Hz, 3H), 0.44 (d, J = 6.6 Hz, 3H), −0.09 (d, J = 6.6 Hz, 3H), −0.48 (ddd, J = 13.8, 10.9 and 2.9 Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, only assigned signals of the major conformer) δ 194.52, 173.39, 173.28, 172.74, 172.43, 170.33, 170.02, 159.19, 132.77, 124.13, 122.93, 122.55, 121.82, 120.15, 119.28, 118.65, 108.67, 107.15, 65.94, 61.55, 54.25, 52.53, 52.18, 48.39, 47.27, 40.53, 38.52, 37.77, 37.72, 33.64, 31.25, 31.15, 30.48, 29.57, 24.13, 23.83, 23.16, 14.79, 11.55; $[\alpha]^{20}_D$ −127.2 (*c* 1, MeOH). Anal. Calcd for $C_{54}H_{86}N_8O_{10}$: C, 64.39; H, 8.61; N, 11.12. Found: C, 64.12; H, 8.63; N, 11.13.

***N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-amino-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-$N^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-L-methyl-L-alanine methyl ester 18:** To a solution of **15** (31 g, 30.6 mmol) in CH_2Cl_2 (400 mL) at 0 °C was added *N*-methylmorpholine (11 mL, 100 mmol) and Ms-Cl (3.16 mL, 40 mmol) and the reaction was allowed to warm to room temperature within 3 h. Conversion was checked by TLC (SiO_2, EtOAc). For workup EtOAc/c-hexane (1:2, 200 mL) was added and the solution was washed with 1 M aqueous HCl, saturated $NaHCO_3$ solution, and brine. After drying over Na_2SO_4 the solvent was evaporated affording crude **14**. A solution of **14** (35 g) and **9** (25 g, 0.24 mol) in dry EtOH (150 mL) was heated at 80 °C for 24 h. For workup the solvent was evaporated and the residue obtained was taken up in toluene (1.5 L). The solution was extracted with 20% K_2HPO_4 solution (5 × 200 mL) and dried over Na_2SO_4. The solvent was removed and the residue obtained purified by chromatography with EtOAc/c-hexane/MeOH (6:12:1−2) affording 20.6 g (75%) of **18** as a yellow oil. ^{1}H NMR (500 MHz, CD_3OD, only assigned signals of the major conformer) δ 7.61 (d, J = 8.0 Hz, 1 H), 7.45 (d, J = 8.2 Hz, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.23 (s, 1H), 7.11 (t, J = 7.5 Hz, 1H), 5.19−5.13 (m, 2H), 5.00−4.92 (m, 2H), 4.38 (dt, J = 12.1 and 2.8 Hz, 1H), 4.07 (s, 3H), 3.79(dd, J = 9.2 and 6.7 Hz, 1H), 3.75 (s, 3H), 3.48 (dd, J = 15.1 and 3.2 Hz, 1H), 3.15 (s, 3H), 2.95 (s, 3H), 2.94 (s, 3H), 0.41 (d, J = 6.6 Hz, 3H), 0.06 (d, J = 6.6 Hz, 3H), −0.49 (ddd, J = 13.8, 10.9 and 2.9 Hz, 1H); ^{13}C NMR (125.8 MHz, CD_3OD, only assigned signals of the major conformer) δ 176.81, 174.92, 170.74, 170.60, 132.84, 124.02, 122.83, 122.68, 120.20, 118.81, 108.64, 107.02, 65.41, 61.73, 53.56, 51.73, 49.37, 49.06, 37.86, 31.32, 29.88, 24.03, 22.31, 18.96, 13.38; HRMS(ESI) calcd for $C_{49}H_{84}N_7O_8$ $[M + H]^+$ 898.6381, found 898.6380; $[\alpha]^{20}_D$ −121.9 (*c* 1, MeOH). Anal. Calcd for $C_{49}H_{83}N_7O_8$: C, 65.52; H, 9.31; N, 10.92. Found: C, 65.22; H, 9.34; N, 10.88.

***(R)*-2-Amino-4-cyanobutyric acid methyl ester hydrochloride 20:** To a solution of Boc-(D)-Gln-OH **19** (2.0 g, 8.1 mmol) in pyridine (30 mL) was added Ac_2O (0.92 mL, 9.7 mmol) with stirring at room temperature overnight. The solvent was evaporated and the residue was redissolved in EtOAc and washed with 1 M aqueous HCl and brine. After drying over Na_2SO_4 the solvent was removed. The crude product was dissolved in CH_2Cl_2 (50 mL) and a 1 M solution of diazomethane in Et_2O was added until the solution became slightly yellow. The solvent was again evaporated and the residue obtained was purified by chromatography with EtOAc/c-hexane (1:2) affording 1.52 g (77%) of (*R*)-2-(dimethylethyloxycarbonylamino)-4-cyanobutyric acid methyl ester as a colorless oil.

To a solution of (*R*)-2-(dimethylethyloxycarbonylamino)-4-cyanobutyric acid methyl ester (163 mg, 0.67 mmol) in dioxane (3 mL) was added 32% aqueous HCl (0.1 mL) and the solution was stirred at room temperature for 5 h. Evaporation of the solvent gave 100 mg (84%) of **20**: ^{1}H NMR (500 MHz, CD_3OD) δ 4.78 (br s, 3H), 4.19 (t, J = 6.5 Hz, 1H), 3.90 (s, 3H), 2.78 (m, 2H), 2.31 (m, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 160.34, 110.09, 44.59, 43.40, 18.16, 4.88; HRMS(ESI) calcd for $C_6H_{11}N_2O_2$ $[M + H]^+$ 143.0821, found 143.0820; $[\alpha]^{20}_D$ −32.6 (*c* 1, MeOH).

(2*R*)-*N*-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-[*N*-[(*R*)-2-Hydroxy-4-cyanobutanoyl]amino]-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-$N^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alaninyl]amino-4-cyanobutanoic acid methyl ester 22: To a solution of **20** (239 mg, 1.34 mmol), **21** (597 mg, 0.6 mmol), and HATU (570 mg, 1.5 mmol) in DMF (10 mL) at 0 °C was added DIEA (0.48 mL, 2.8 mmol) with stirring for 2 h. The solvent was evaporated and the residue was dissolved in EtOAc and sequentially washed with 1 M aqueous HCl, aqueous $NaHCO_3$ solution, and brine. After drying over anhydrous Na_2SO_4 the solvent was removed and the crude product purified by chromatography with EtOAc/c-hexane/MeOH (4:8:1). Yield: 625 mg (93%) as a colorless oil. ^{1}H NMR (500 MHz, d_6-DMSO, 410 K) δ 7.74 (br s, 1H), 7.61 (d, J = 8.0 Hz, 1 H), 7.48 (br s, 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.24 (s, 1H), 7.19 (t, J = 7.1 Hz, 1H), 7.06 (t, J = 7.1 Hz, 1H), 5.47 (d, J = 5.6 Hz, 1H), 5.25 (br s, 1H), 4.97 (m, 2H), 4.82 (m, 2H), 4.67 (br s, 1H), 4.43 (m, 1H), 4.03 (s, 3H), 4.02 (q, J = 6.8 Hz, 1H), 3.67 (s, 3H), 3.30 (dd, J = 15.3, 7.0 Hz, 1H), 3.01 (dd, J = 15.3, 8.6 Hz, 1H), 2.97 (br s, 3H), 2.92 (r bs, 3H), 2.47 (m, 2H), 2.13 (m, 1H), 2.00 (m, 2H), 1.78 (m, 1H), 1.70−1.15 (series of multiplets, 27H), 0.88 (m, 24H); HRMS(ESI) calcd for $C_{59}H_{94}N_{10}O_{11}Na$ $[M + Na]^+$ 1141.7001, found 1141.6997; $[\alpha]^{20}_D$ −116.2 (*c* 0.5, MeOH). Anal. Calcd for $C_{59}H_{94}N_{10}O_{11}$: C, 63.30; H, 8.46; N, 12.51. Found: C, 63.12; H, 8.41; N, 12.50.

(2*R*)-*N*-[*N*-[(2*S*,4*R*)-2-[[*N*-[*N*-[*N*-[(2*S*,4*R*)-2-Amino-4-methylheptanoyl]-*N*-methyl-L-leucinyl]-L-leucinyl]-$N^{1\prime}$-methoxy-*N*-methyl-L-tryptophanyl]amino]-4-methylheptanoyl]-*N*-methyl-L-alaninyl]amino-4-cyanobutanoic acid methyl ester 20: 19 (590 mg, 0.53 mmol) was transformed into **20** according to the procedure described for **18**, yielding 300 mg (56%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, d_6-DMSO, 410 K) δ 7.71 (br s, 1H), 7.58 (d, J = 8.0 Hz, 1 H), 7.35 (d, J = 8.2 Hz, 1H), 7.21 (s, 1H), 7.17 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 5.21 (br s, 1H), 4.91 (m, 1H), 4.87 (br s, 1H), 4.78 (m, 2H), 4.64 (br s, 1H), 4.39 (m, 1H), 4.00 (s, 3H), 3.65 (m, 1H), 3.64 (s, 3H), 3.28 (dd, J = 15.3, 7.0 Hz, 1H), 2.99 (dd, J = 15.3, 8.5 Hz, 1H), 2.95 (br s, 3H), 2.51 (t, J = 7.2 Hz, 2H), 2.10 (m, 1H), 1.98 (m, 1H), 1.76−1.12 (series of multiplets, 27H), 0.87 (m, 24H); HRMS (ESI) calcd for $C_{54}H_{90}N_9O_9$ $[M + H]^+$ 1008.6862, found 1008.6862; $[\alpha]^{20}_D$ −119.4 (*c* 0.33, MeOH). Anal. Calcd for $C_{54}H_{89}N_9O_9$: C, 64.32; H, 8.90; N, 12.50. Found: C, 64.03; H, 8.92; N, 12.46.

Aza-HUN-7293 (24): To a solution of **20** (300 mg, 0.3 mmol) was added a LiOH solution (0.5 M, 0.7 mL) with stirring overnight at room temperature. The solvent was removed and the residue obtained was dissolved in EtOAc and washed with brine. After drying over anhydrous Na_2SO_4 the solvent was evaporated. The crude betain and BOP (531 mg, 1.2 mmol) were dissolved in dry acetonitrile (500 mL), DIEA (0.21 mL, 1.2 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h. The solvent was removed and the residue dissolved in EtOAc (200 mL) and sequentially washed with 1 M HCl, saturated aqueous $NaHCO_3$ solution, and brine.

After drying over Na_2SO_4 the solvent was removed and the crude product purified by chromatography with EtOAc/cyclohexane/MeOH (7:14:1). Yield: 91 mg (31%). $[\alpha]^{20}_D$ −151.4 (*c* 0.5, MeOH).

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Supporting Information Available: Comparison of the ^1H and ^{13}C NMR spectra of **1** and **24**; copies of ^1H and ^{13}C NMR spectra of **2**, **(*E*)-4**, **7**, and **10**, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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