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Segment Solid-Phase Total Synthesis of the Anthelmintic Cyclooctadepsipeptides PF1022A and Emodepside

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Cyclodepsipeptides of the enniation, PF1022 and verticilide families represent a diverse class of highly interesting natural products with respect to their manifold biological activities. However, until now, no practicable solid-phase syntheses of these compounds have been accomplished, probably due to the problematic combination of *N*-methyl amino acids and hydroxycarboxylic acids. We report herein an efficient synthesis of the anthelmintic PF1022A and its commercial analogue emodepside on Kaiser and Wang resins. Our protocol provides the basis for the solid-phase synthesis of cyclodepsipeptide libraries with a high probability of anthelmintic, antibacterial or insecticidal activity.

Introduction

Nematode infections cause tremendous health problems in livestock and domestic animals. In humans they are a major cause of morbidity and loss of disability-adjusted life years.[1,2] Around two billion people are thought to have active nematode infections. According to the World Health Organization, parasitic worm infections are one of the most important classes of neglected tropical diseases.[3] Since the early 1960s three different types of broad-spectrum anthelmintics have become available, the benzimidazoles, the nicotinic acetylcholine receptor agonists and the macrocyclic lactones.[4] In particular, in the livestock industry, anthelmintic treatment has become problematic due to the growth in resistance against these commonly used anthelmintics. The 24-membered cyclooctadepsipeptide PF1022A (**1**), a metabolite of *Mycelia sterilia* (Rosselinia sp.) originally isolated from the leaves of *Camellia japonica*, has been established as a novel, resistance-breaking anthelmintic with low toxicity in animals (Figure 1).[5,6] A semi-synthetic analogue of PF1022A, emodepside (**2**), has been introduced into the market recently for the treatment of parasitic helminth infections in companion animals (Figure 1).[7]

A prerequisite for the development of a PF1022A-based anthelmintic against human worm infections is an efficient procedure that allows the synthesis of a large number of PF1022 analogues for biological screening. Despite several published total syntheses in solution and an in vitro synthesis approach, only a limited number of PF1022A derivatives have become available.[5,8–10] Remarkably, until now only a partial solid-phase synthesis of PF1022 analogues has been published, but not a total synthesis of PF1022A itself or its commercial analogue emodepside, although solid-phase synthesis is the method of choice not only for the preparation of peptides but also, with some limitations, of depsipeptides.[11,12]

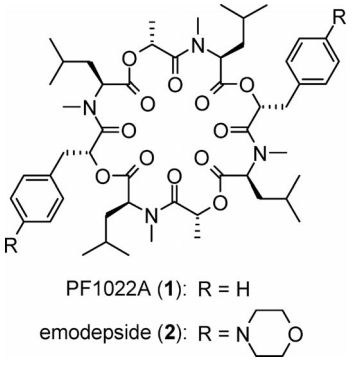

Figure 1. Structures of PF1022A (**1**) and emodepside (**2**).

However, solid-phase depsipeptide synthesis turns out to be considerably more difficult than conventional peptide couplings due to the generally lower state-of-the-art methodology in protecting-group chemistry and the lack of high-yielding coupling reagents for the formation of ester bonds. The solid-phase total synthesis of valinomycin by Kuisle et al. is one of the rare examples in which all the ester bonds were formed on the resin.[13] A more frequent strategy restricts the couplings on the resin to the formation of amide bonds by using depsipeptide building blocks prepared in solution.[14]

Also to be taken into account is the fact that couplings of *N*-methyl-substituted amino acids generally afford lower yields and increased racemization due to the steric hindrance caused by the bulky methyl groups.[15] That is especially true for syntheses on solid supports, as the reactivity

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of resin-bound secondary amines towards activated acids is even lower than in solution. Although this problem has been known for decades, there is no single method of choice. In solid-phase syntheses of the cyclopeptides cyclosporine and omphalotine A, triphosgene has been employed successfully to couple two adjacent *N*-methylvalines whereas in the solution synthesis of thiocoraline the more conventional HATU/HOAt (see the Exp. Sect. for the definitions of reagents) reagent afforded good yields.[16–18] Triazine-based coupling reagents introduced recently provide a new concept as they form a cyclic intermediate that tethers the two reacting centres and thus facilitates the coupling through an intramolecular pathway.[19]

An instructive example of the problems arising from *N*-methyl amino acids can be found in a paper by Lee describing the synthesis of conformationally restricted PF1022 analogues on Kaiser oxime. Starting with the Pro-Lac resin, already the removal of the first protecting group and coupling with Boc-MeLeu-PhLac-OH resulted in extensive formation of the Pro-Lac morpholinedione accompanied by immobilized Boc-MeLeu-PhLac and some tetradepsipeptide.[11,12] The problem of morpholinedione formation was "solved" by attaching a complete tetradepsipeptide to the resin, which reduces the on-resin chemistry to only two elongation steps. Also, from another aspect this synthesis cannot be regarded as a general route for the solid-phase synthesis of natural PF1022A or emodepside because all analogues contained a turn-inducing building block, which facilitates the macrocyclization reaction considerably.

We wish to report herein an efficient solid-phase synthesis of PF1022A and emodepside based on didepsipeptide coupling reactions. Following this strategy, the synthetic challenge is reduced to on-resin amide formation between an *N*-methyl amino acid and a hydroxycarboxylic acid. Due to the C_2 symmetry of PF1022A and emodepside, only two different didepsipeptides (Boc-L-MeLeu-D-Lac-OH and Boc-L-MeLeu-D-PhLac-OH or Boc-L-MeLeu-D-Lac-OH, Boc-L-MeLeu-D-morphPhLac-OH) are required. These are available in high yields by solution synthesis.

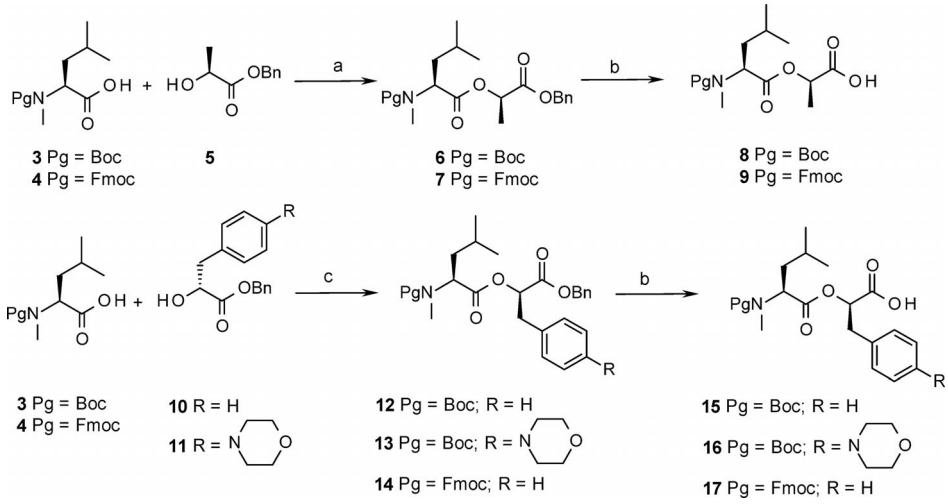
Results and Discussion

Synthesis of the Didepsipeptide Building Blocks

The didepsipeptides **8**, **9** and **15**–**17**, used as starting materials for the solid-phase synthesis, were prepared in good-to-excellent yields (Scheme 1) from Boc- or Fmoc-protected *N*-Me-Leu and the benzyl-protected lactates **5**, **10** and **11** following literature procedures.[20–23] Using the inexpensive natural (*S*)-lactic acid enantiomer, Mitsunobu conditions were applied to establish the correct *R* configuration during the coupling reaction of (*S*)-benzyl lactate (**5**) with *N*-Me-leucines **3** and **4** to afford the coupling products **6** and **7**. In the case of phenyllactic acid the *R* enantiomer was used directly due to its lower cost. Thus, coupling of the (*R*)-phenyllactic acids **10** and **11** with DCC afforded the didepsipeptides **12**–**14**. The benzyl protecting group was removed by hydrogenolysis with Pd/C or preferably with the Pearlman catalyst to give the corresponding acids in excellent yields.[24] No epimerizations were observed in either the coupling or the hydrogenolysis steps.

Solid-Phase Synthesis on Kaiser Resin

The Kaiser oxime was used as the resin of choice because it allows a cyclizative cleavage and can be reused without regeneration, an important prerequisite for solid-phase synthesis on a multigram scale.[25] However, piperazinedione formation is a notorious side-reaction of the Kaiser oxime because the oxime ester can be cleaved even with weak nucleophiles. Extensive studies have demonstrated the critical role of the first and second amino acid as well as the coupling conditions. Bulky amino acids such as Val, Leu or



Scheme 1. Preparation of the didepsipeptide segments in solution. Reagents and conditions: (a) TPP, DEAD, THF, 0 °C for 2 h, then room temp. for 1 h; (b) H_2, Pd/C (10%), EtOH, room temp., 4 h for **8** and **15**–**17**; H_2, $Pd(OH)_2$/C (20%), EtOH, room temp., 4 h for **9**; (c) DCC, HOBt, DMAP, DCM, 0 °C, 30 min, then room temp. for 24 h.

Phe in the first position and *N*-methyl amino acids in the second position, exactly the situation in the PF1022A synthesis, generally facilitate piperazinedione formation.[26–28]

As expected, the yields for the coupling of the didepsipeptides **8**, **15** and **16** to the Kaiser resin were strongly dependent on the didepsipeptide used as the first building block, the coupling reagents and the solvents (Table 1). The best yields, determined by picric acid titration after deprotection, were obtained with the didepsipeptide Boc-MeLeu-PhLac (**15**) by using HATU as the coupling reagent (Table 1, entry 6).[29,30] DIC, which was employed by Lee, gave in our experiments only poor yields (19%) with both didepsipeptides **8** and **15**.[11,12] In general, the didepsipeptide Boc-MeLeu-Lac (**8**) provided lower yields for the coupling to the resin (Table 1, entry 4) and tended to extensive diketopiperazine formation in the subsequent coupling to the tetradepsipeptide **21**. The yields for the coupling of the morpholino-substituted didepsipeptide **16** to the Kaiser oxime were similar.

During chain elongation to the tetradepsipeptide MeLeu-Lac-MeLeu-PhLac, cleavage of the didepsipeptide MeLeu-PhLac from the solid support with the formation of the corresponding dioxomorpholine became a major side-reaction. In addition, the liberated oxime resin reacted immediately with the second didepsipeptide Boc-MeLeu-Lac-OH to form a deletion sequence. However, the cleavage reaction was strongly dependent on the type of coupling reagent, the solvent and auxiliary base. Fortunately, HATU/DIEA, already used successfully for immobilizing the first building block (Boc-MeLeu-PhLac-OH), also proved the best coupling reagent for chain elongation to the tetradepsipeptide **24** (Table 2). In general, the use of HATU in the synthesis of both PF1022A and emodepside led to coupling yields in the range of 75–90%, which can be regarded as more than satisfactory for solid-phase couplings of *N*-methyl amino acids (Scheme 2). However, we were not able to suppress the cleavage of the *N*-terminal didepsipeptides completely.

Table 1. Yields for the coupling of didepsipeptides **8** and **15** to the Kaiser resin under different conditions.

Entry	Didepsipeptide (amount [equiv.])	Number of couplings	Conditions	Solvent	Yield [%]
1	**8** (2)	1	2 equiv. HATU, 2 equiv. HOBt, 5 equiv. DIEA	DMF	65
2	**8** (2)	1	3 equiv. DIC, 3 equiv. DMAP	DMF	19
3	**8** (3)	1	3 equiv. HATU, 3 equiv. HOBt, 8 equiv. DIEA	DMF	70
4	**8** (2)	1	2 equiv. HATU 3 equiv. DIPEA	DCM	62
5	**15** (1)	2	1 equiv. TBTU, 6 equiv. NMM	DMF	53
6	**15** (2)	1	2 equiv. HATU, 3 equiv. DIEA	DCM	87
7	**15** (1)	2	1 equiv. TBTU, 3 equiv. NMM	DMF	58
8	**15** (3)	1	3 equiv. HATU, 6 equiv. DIEA	DMF	48
9	**15** (3)	1	3 equiv. DIC, 3 equiv. HOBt, 6 equiv. DIEA	DMF	19

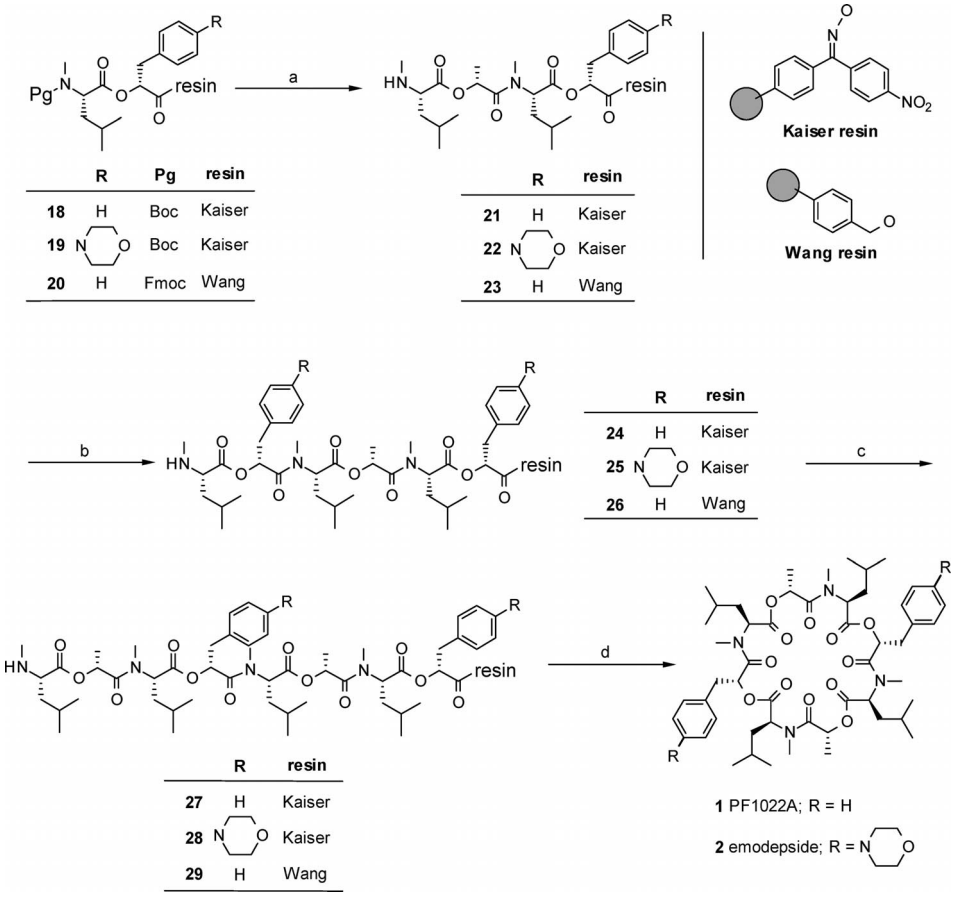
A particular feature that characterizes the Kaiser resin is the anchoring oxime ester bond, which, as a polymeric active ester, can be cleaved by solvolysis or aminolysis to afford peptide esters or amides.[31–33] The reactivity of the oxime ester towards nucleophiles also allows cyclizative cleavage, which releases the cyclodepsipeptide directly into solution without the need for an additional cyclization reaction. A limited loading of the resin (0.3 mmol/g resin) provides the high-dilution environment needed for the macrocyclization reaction. Cyclizative cleavages of cyclopeptides, but not of cyclodepsipeptides, have been accomplished under a variety of reaction conditions.[34–36] After some experimentation (Table 2) we found a weakly acidic solution of DIEA (2.2 equiv.) and HOAc (5.5 equiv.) in DCM best suited for the cyclizative cleavage of the linear octadepsipeptides **27** and **28** (Scheme 2), whereas neutral (ethyl acetate) or weakly basic (pyridine) reaction conditions afforded drastically reduced yields (10 and 27%, respectively).[27]

The unusually high yield (75%, based on the loading of the linear precursor **27**) of the ring-closing reaction to give PF1022A (**1**) can be attributed to a *cis*-amide bond between one leucine and one lactic acid residue. This positions the N- and C-terminus in close proximity and thus facilitates the macrocyclization reaction.[37] The raw PF1022A (**1**) had a purity of at least 80% and can be further purified by flash chromatography to afford an overall yield of 30%, which is around three times more than the yield of the solution synthesis.[37] Note, the macrocyclization yield declined significantly when Kaiser resins with loadings of 1 mmol/g resin or more were used, probably due to intermolecular coupling reactions with the resin.[34]

Table 2. Chain elongation to the tetradepsipeptide Boc-MeLeu-Lac-MeLeu-PhLac-resin (**24**).

Entry	Lac-Leu [equiv.]	Coupling reagent (amount [equiv.])	Base (amount [equiv.])	Solvent	Fragments[a]
1	2	TBTU (2)	NMM (6)	DMF	50% B, 45% AB
2	5	TBTU (5)	NMM (6.6)	DMSO	100% B
3	5	TBTU (5)	NMM (6.6)	nitrobenzene	100% B
4	5	TBTU (5)	NMM (6.6)	acetone	100% B
5	2	BTFFH (5)	DIEA (10)	DMF	80% B, 20% AB
6	3	HATU (2)	DIEA (6.5)	DMF	90% AB, 10% B
7	3	HATU (2)	DIEA (6.5)	DCM	70% AB, 30% B

[a] HPLC–MS of test cleavages with morpholine; B: morpholide of MeLeu-Lac; AB: morpholide of MeLeu-Lac-MeLeu-PhLac.



	R	Pg	resin
18	H	Boc	Kaiser
19	N O	Boc	Kaiser
20	H	Fmoc	Wang

	R	resin
21	H	Kaiser
22	N O	Kaiser
23	H	Wang

	R	resin
24	H	Kaiser
25	N O	Kaiser
26	H	Wang

	R	resin
27	H	Kaiser
28	N O	Kaiser
29	H	Wang

Scheme 2. Chain elongation steps and cyclization reactions. Reagents and conditions: (a) for **21**, **22**: i. 25% TFA/DCM, room temp., 30 min; ii. Boc-MeLeu-Lac-OH (**8**), HATU, DIEA, DMF, room temp., 16 h; iii. 25% TFA/DCM, room temp., 30 min; for **23**: i. 25% piperidine/THF, room temp., 30 min; ii. Fmoc-MeLeu-Lac-OH (**9**), HATU, DIEA, THF, room temp., 16 h; iii. 25% piperidine/THF, room temp., 30 min; (b) for **24**: i. Boc-MeLeu-PhLac-OH (**15**), HATU, DIEA, DMF, room temp., 16 h; ii. 25% TFA/DCM, room temp., 30 min; for **25**: i. Boc-MeLeu-morphPhLac-OH (**16**), HATU, DIEA, DMF, room temp., 16 h; ii. 25% TFA/DCM, room temp., 30 min; for **26**: i. Fmoc-MeLeu-PhLac-OH (**17**), HATU, DIEA, THF, room temp., 16 h; ii. 25% piperidine/THF, room temp., 30 min; (c) for **27**, **28**: i. Boc-MeLeu-Lac-OH (**8**), HATU, DIEA, DMF, room temp., 16 h; ii. 25% TFA/DCM, room temp., 30 min; for **29**: i. Fmoc-MeLeu-Lac-OH (**9**), HATU, DIEA, THF, room temp., 16 h; ii. 25% piperidine/THF, room temp., 30 min; (d) with Kaiser oxime: DIEA (2.2 equiv.), AcOH (5 equiv.), DCM, room temp., 16 h; with Wang resin: i. 50% TFA/DCM, room temp., 1 h; ii. BOPCl, DIEA, room temp., 48 h.

Remarkably, emodepside (**2**) was obtained in an even higher yield (40–45%) under the coupling and cyclizative cleavage conditions developed for PF1022A. The overall yield for emodepside complies with a coupling yield of more than 90% for each step, including the critical macrocyclization reaction.

Solid-Phase Synthesis on Wang Resin

A solid-phase synthesis on Wang resin with a complementary, base-labile protecting group strategy (Fmoc) provides access to PF1022 analogues that are not accessible by the Kaiser oxime route. In addition, the Wang resin has a higher loading and the anchor linkage is more stable, which reduces the formation of dioxomorpholine and premature cleavage from the resin during the chain-elongation steps. On the other hand, the more stable benzyl ester linkage renders a cyclizative cleavage unfeasible. This is, however, unproblematic in the particular case of PF1022A and related cyclodepsipeptides because excellent protocols for solution cyclizations exist.[37,38]

In contrast to the Kaiser oxime, a DIC/DMAP/HOBt coupling reagent combination was found to give the highest yields (93%) in the anchoring step, but the HATU/DIEA system proved best again for the amide couplings. However, the yields of the amide-forming reactions were generally lower on the Wang resin than on the Kaiser resin. Although the yield of the tetradepsipeptide Fmoc-MeLeu-Lac-MeLeu-PhLac was still in the range of 80%, increasing amounts of failure and deletion sequences accompanied by minor side-products resulting from cleavage of the ester bonds were identified in the couplings to hexadepsipeptide **26** and, in particular, to octadepsipeptide **29**, which was obtained in only around 50% yield from the hexadepsipeptide. Cleavage of the octadepsipeptide **29** from the resin with TFA gave a raw material with a linear octadepsipeptide content in the range of 40–50%. Subsequent solution cyclization (62% yield) of the crude octadepsipeptide with

BOPCl according to a literature procedure afforded PF1022A in an overall yield of 20–25% (Scheme 2) after chromatographic purification.[37]

Conclusion

We have developed an efficient solid-phase synthesis of the anthelmintic cyclooctadepsipeptide PF1022A and its commercial analogue emodepside by segment coupling reactions. After careful optimization of the oxime ester formation, the *N*-methylleucine couplings and the cyclizative cleavage, overall yields of 30–45% were obtained on Kaiser oxime. An analogous synthesis with complementary protecting group chemistry on Wang resin afforded somewhat lower yields in the range of 20–30%. Nevertheless, the standard conditions used for the Wang synthesis should allow unproblematic adaptation to an automated synthesizer, allowing the preparation of cyclodepsipeptide libraries for screening against human pathogenic worms and also for insecticidal and antibacterial activities.

Experimental Section

General: The starting materials (*R*)-3-phenyllactic acid, benzyl (*R*)-3-phenyllactate, *N*-Fmoc-*N*-methyl-L-leucine and *N*-Boc-*N*-methyl-L-leucine were either purchased or prepared by standard literature procedures.[21–23] All reactions except the hydrogenations were performed in dried solvents. Dichloromethane, DIEA and piperidine were heated at reflux for 1 h over calcium hydride and distilled. THF was heated at reflux for several hours over $LiAlH_4$ and then distilled. Toluene was dried by filtration through basic alumina. The following instruments were used: ^{1}H NMR: Bruker Avance 400, Bruker Avance III 600; ^{13}C NMR: Bruker Avance 400, Bruker Avance III 600. FTIR: Nicolet PROTÉGÉ 460 E.S.P. MS: Bruker micrOTOF; ESI-MS: Varian IT 500-MS Iontrap. LC: preparative low-pressure liquid chromatography (LPLC) was performed by using silica gel 60 μm (230–400 mesh, Macherey–Nagel), Büchi pump. TLC: silica gel 60 F_{254} (Merck). For preparative HPLC, a Spectra Physics Analytical Spectra System AS 3000 instrument with UV detection was used. The progress of the solid-phase reactions was monitored by using either the Bromophenol Blue colour test, determination of the resin-loading by UV analysis of the Fmoc group cleavage product or by test cleavages and HPLC-MS analysis.[39]

Abbreviations: ACN, acetonitrile; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; BOPCl, *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DIC, *N*,*N*′-diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uranium hexafluorophosphate; HOAc, acetic acid; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; MTBE, methyl *tert*-butyl ether; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TPP, triphenylphosphane.

Preparation of Building Blocks and Didepsipeptide Segments in Solution

Boc-L-MeLeu-D-Lac-OBn (6): Benzyl L-lactate (**5**; 3.60 g, 20.0 mmol), *N*-Boc-*N*-methyl-L-leucine (4.91 g, 20.0 mmol) and triphenylphosphane (6.30 g, 24.0 mmol) were dissolved in THF (12 mL). At 0 °C a solution of DEAD (3.78 mL, 24.0 mmol) in THF (12 mL) was added dropwise through a syringe pump during 2 h. The solution was stirred for 1 h at room temperature, diluted with ethyl acetate and washed twice with a saturated $NaHCO_3$ solution (18 mL each) and 0.5 M citric acid (18 mL each). The organic phase was dried with Na_2SO_4 and the solvents evaporated. Flash chromatography (cyclohexane/ethyl acetate, 8:2) of the residue afforded the protected didepsipeptide **6** (6.02 g, 74%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91–0.97 (m, 6 H, $C_\delta H_3$-Leu), 1.45 (s, 9 H, CH_3-*t*Bu), 1.48–1.54 (2 m, 4 H, C_γH-Leu, $C_\beta H_3$-Lac), 1.62–1.73 (m, 2 H, C_βH-Leu), 2.69–2.81 (m, 3 H, NCH_3), 4.68–4.80 (m, 1 H, C_αH-Leu), 4.90–5.04 (m, 1 H, C_αH-Lac), 5.09–5.24 (m, 2 H, CH_2-Bn), 7.29–7.39 (m, 5 H, Ar-H) ppm. ^{13}C NMR (CD_3CN): δ = 16.86, 23.19, 24.53, 24.80, 28.33, 29.81, 30.23, 37.28, 37.63, 55.82, 56.75, 67.02, 69.06, 79.81, 80.14, 128.23, 128.44, 128.59, 135.24, 156.31, 170.12, 171.89 ppm. IR (film): $\tilde{\nu}$ = 1693 (C=O), 1742 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 837 (5), 408 (31), 352 (34), 308 (100). HRMS (ESI): calcd. for $C_{22}H_{33}NNaO_6$ $[M + Na]^+$ 430.2200; found 430.2204.

Fmoc-L-MeLeu-D-Lac-OBn (7): Benzyl L-lactate (**5**; 7.00 g, 38.1 mmol), *N*-Fmoc-*N*-methyl-L-leucine (**4**; 13.99 g, 38.1 mmol) and triphenylphosphane (11.98 g, 45.7 mmol) were dissolved in THF (100 mL). A solution of DEAD (8.04 g, 45.7 mmol) in THF (50 mL) was added at 0 °C over 2 h. The reaction was stirred for 1 h at room temperature, then diluted with ethyl acetate and washed with a saturated $NaHCO_3$ solution (50 mL), 0.5 M citric acid (50 mL) and water (50 mL) twice each. Standard work-up and chromatographic purification (cyclohexane/ethyl acetate, 9:1) yielded the coupling product **7** as a viscous yellow oil (17.6 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 and 0.91 (2 d, *J* = 6.7 Hz, 3 H, $C_\delta H_3$-Leu), 0.96 (pseudo-t, *J* = 6.0 Hz, 3 H, $C_\delta H_3$-Leu), 1.47–1.74 (m, 6 H, $C_\beta H_2$-Leu, C_γH-Leu, $C_\beta H_3$-Lac), 2.85 and 2.87 (2 s, 3 H, NCH_3), 4.24 and 4.30 (2 m, 1 H, CH-Fmoc), 4.38 (m, 1 H, CH_2-Fmoc), 4.50 (m, 1 H, CH_2-Fmoc), 4.66 and 5.04 (2 m, 1 H, C_αH-Leu), 5.07 and 5.14 (2 m, 1 H, C_αH-Lac) 5.20 (m, 2 H, CH_2-Bn), 7.31 (m, 8 H, Ar-H), 7.57 (m, 3 H, Ar-H), 7.76 (m, 3 H, Ar-H), ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.9, 21.4, 23.3, 24.7, 30.7, 37.2, 47.3, 56.5, 67.1, 67.7, 69.2, 120.0, 124.8, 125.0, 125.1, 127.0, 127.7, 128.2, 128.5, 128.6, 141.3, 143.9, 144.1, 156.9, 170.0, 171.0 ppm. IR (KBr): $\tilde{\nu}$ = 1745 (C=O), 1704 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 308 (14), 530 (100), 547 (50). HRMS (ESI): calcd. for $C_{32}H_{35}NNaO_6$ $[M + Na]^+$ 552.2357; found 552.2356.

Boc-L-MeLeu-D-Lac-OH (8): A suspension of didepsipeptide **6** (4.08 g, 10.0 mmol) and Pd/C (10%, 0.5 g) in ethanol (60 mL) was hydrogenated (1 bar) overnight at room temperature. After that time the catalyst was filtered off and washed with ethanol. After evaporation of the combined organic phases Boc-L-MeLeu-D-Lac-OH (**8**; 3.17 g, 100%) was obtained as a light-grey oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89–0.97 (m, 6 H, $C_\delta H_3$-Leu), 1.45 (s, 9 H, CH_3-*t*Bu), 1.52 (d, *J* = 7.0 Hz, 3 H, $C_\beta H_3$-Lac), 1.54–1.63 (m, 1 H, C_γH-Leu), 1.62–1.79 (m, 2 H, C_βH-Leu), 2.80 (s, 3 H, NCH_3), 4.68–4.77 and 4.77–4.86 (2 m, 1 H, C_αH-Leu), 5.10 (q, *J* = 7.0 Hz, 1 H, C_αH-Lac), 8.39 (br. s, 1 H, COOH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.78, 16.84, 21.09, 21.44, 23.09, 23.16, 24.55, 24.84, 28.31, 29.93, 30.99, 37.38, 37.54, 68.64, 68.71, 80.44, 155.86, 156.31, 171.67, 174.49, 174.72 ppm. IR (film): $\tilde{\nu}$ = 1668 (C=O), 1697 (C=O), 1742 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 655 (8), 317 (4), 316 (23), 244 (100), 170 (41). HRMS (ESI): calcd. for $C_{15}H_{27}NNaO_6$ $[M + Na]^+$ 340.1731; found 340.1733.

Fmoc-L-MeLeu-D-Lac-OH (9): A suspension of benzyl ester **7** (19.71 g, 37.2 mmol) and $Pd(OH)_2$/C (20%, 1.05 g) in ethanol

(200 mL) was hydrogenated (1 bar H_2) at room temperature for 4 h. Then the catalyst was filtered and washed with ethanol. LPLC (cyclohexane/ethyl acetate, 8:2, + 0.1% acetic acid) of the crude product, obtained after evaporation of the solvent, gave Fmoc-L-MeLeu-D-Lac-OH (**9**) as a white foam (11.76 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.79 and 0.91 (2 d, J = 7.0 Hz, 2 H, $C_\delta H_3$-Leu), 0.94 (m, 4 H, $C_\delta H_3$-Leu), 1.51–1.78 (m, 6 H, $C_\beta H_2$-Leu, C_γH-Leu, $C_\beta H_3$-Lac), 2.87 and 2.89 (2 s, 3 H, NCH_3), 4.25 and 4.29 (2 m, 1 H, CH-Fmoc), 4.37 and 4.51 (2 m, 2 H, CH_2-Fmoc), 4.61 and 4.99 (2 m, 1 H, C_αH-Leu), 5.02 and 5.13 (2 m, 1 H, C_αH-Lac), 7.29 (m, 2 H, Ar-H), 7.40 (m, 2 H, Ar-H), 7.62 (m, 1 H, Ar-H), 7.79 (m, 2 H, Ar-H), 8.08 (br. s, 1 H, COOH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.2, 23.0, 24.6, 30.4, 37.2, 47.2, 56.5, 67.7, 68.9, 120.0, 124.8, 125.1, 125.1, 127.1, 127.6, 127.7, 128.5, 141.4, 143.8, 156.4, 157.2, 171.5, 175.1, 177.1 ppm. IR (KBr): $\tilde{\nu}$ = 3160 (O–H), 1746 (C=O), 1714 (C=O), 1667 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 179 (17), 218 (34), 440 (100), 457 (39). HRMS (ESI): calcd. for $C_{25}H_{29}NNaO_6$ [M + Na]$^+$ 462.1887; found 462.1888.

Benzyl (*R*)-2-Hydroxy-3-(4-morpholinophenyl)propanoate (11): A suspension of (*R*)-3-(4-morpholino)phenyllactic acid (4.02 g, 16.0 mmol), benzyl alcohol (5.19 g, 48.0 mmol) and *p*-toluenesulfonic acid (3.31 g, 19.2 mmol) in toluene (60 mL) was heated for 4 h at reflux until the theoretical amount of water had been collected in a Dean–Stark trap. The reaction was cooled to room temperature, diluted with methyl *tert*-butyl ether and washed with a saturated $NaHCO_3$ solution. The aqueous layer was extracted with methyl *tert*-butyl ether (3×). The combined organic phases were washed with a saturated NaCl solution and dried with Na_2SO_4. The solvent was removed in vacuo and the residue purified by LPLC (cyclohexane/ethyl acetate, 1:1, + 0.1% triethylamine) to afford benzyl ester **11** (4.22 g, 77%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.69 (s, 1 H, OH), 2.92 (dd, J = 6.0, 14.0 Hz, 1 H, $C_\beta H_2$), 3.05 (dd, J = 5.0, 14.0 Hz, 1 H, $C_\beta H_2$), 3.09–3.17 (m, 4 H, NCH_2-morpholine), 3.81–3.90 (m, 4 H, OCH_2-morpholine), 4.45 (m, 1 H, C_αH), 5.18 (s, 2 H, CH_2-Bn), 6.79 (d, J = 9 Hz, 2 H, Ar-H), 7.06 (d, J = 9.0 Hz, 2 H, Ar-H), 7.30–7.43 (m, 5 H, Ar-H-benzyl) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.61, 49.41, 66.92, 67.31, 71.40, 115.70, 127.37, 128.56, 128.63, 130.27, 135.12, 150.20, 174.05 ppm. IR: $\tilde{\nu}$ = 1716 (C=O), 3526 (OH) cm^{-1}. MS (ESI): *m*/*z* (%) = 705 (6), 342 (100). HRMS (ESI): calcd. for $C_{20}H_{24}NO_4$ [M + H]$^+$ 342.1700; found 342.1706.

Boc-L-MeLeu-D-PhLac-OBn (12): A solution of DCC (4.46 g, 21.6 mmol) in DCM (40 mL) was added dropwise at 0 °C over 20 min to a solution of benzyl 3-phenyllactate (**10**; 5.13 g, 20.0 mmol), *N*-Boc-*N*-methyl-L-leucine (4.91 g, 20.0 mmol), HOBt (2.92 g, 21.6 mmol) and DMAP (2.64 g, 21.6 mmol). The reaction was warmed to room temperature and stirred for another 24 h. Then the solvent was removed in vacuo and the residue was dissolved in ethyl acetate and filtered. The filtrate was washed twice with a saturated $NaHCO_3$ and NaCl solution, dried with Na_2SO_4 and the solvents evaporated. The residue was purified by LPLC (cyclohexane/ethyl acetate, 7:1) to yield the coupling product **12** (9.03 g, 93%) as a white crystalline solid. M.p. 50–51 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (d, J = 6.0 Hz, 6 H, $C_\delta H_3$-Leu), 1.43 (s, 9 H, CH_3-*t*Bu), 1.49–1.61 (m, 3 H, C_γH-Leu, C_βH-Leu), 2.61 and 2.66 (2 s, 3 H, NCH_3), 3.07–3.24 (m, 2 H, C_βH-PhLac), 4.61–4.99 (2 m, 1 H, C_αH-Leu), 5.04–5.19 (m, 2 H, OCH_2Ph), 5.27 and 5.29 (2 dd, J = 5.0 Hz, 1 H, C_αH-PhLac), 7.12–7.39 (2 m, 10 H, Ar-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.08, 21.31, 23.15, 24.45, 24.75, 28.30, 28.38, 29.78, 30.04, 37.28, 37.64, 55.64, 56.69, 67.13, 67.21, 73.26, 73.34, 127.04, 128.38, 128.43, 128.48, 128.57, 129.38, 129.55, 135.04, 135.54, 168.96, 171.74, 210.94 ppm. IR: $\tilde{\nu}$ = 1692 (C=O), 1740 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 501 (52), 484 (43), 384 (100). HRMS (ESI): calcd. for $C_{28}H_{37}NNaO_6$ [M + Na]$^+$ 506.2513; found 506.2520.

Boc-L-MeLeu-D-morphPhLac-OBn (13): MorphPhLac depsipeptide **13** was prepared following the protocol for compound **12**. After LPLC (cyclohexane/ethyl acetate, 1:1, + 0.1% TEA) a colourless oil was obtained in a yield of 92%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (d, J = 7.9 Hz, 6 H, $C_\delta H_3$-Leu), 1.44 and 1.49 (2 s, 9 H, CH_3-*t*Bu), 1.53–1.65 (m, 3 H, C_γH-Leu, C_βH-Leu), 2.64 and 2.66 (2 s, 3 H, NCH_3), 2.99–3.09 (m, 2 H, C_βH-PheLac), 3.08–3.13 (m, 4 H, NCH_2-morpholine), 3.82–3.87 (m, 4 H, OCH_2-morpholine), 4.71 and 4.99 (2 dd, J = 4.0, 5.0 Hz, 1 H, C_αH-PhLac), 5.12 (m, 2 H, CH_2-Bn), 5.17–5.26 (m, 1 H, C_αH-Leu), 6.79 (d, J = 8.0 Hz, 2 H, Ar-H), 7.06 (d, J = 8.0 Hz, 2 H, Ar-H), 7.20–7.37 (m, 5 H, Ar-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.09, 21.34, 23.18, 24.45, 24.74, 28.40, 29.79, 30.08, 36.44, 37.65, 49.35, 60.32, 66.89, 67.11, 73.57, 79.75, 115.62, 126.72, 128.39, 128.53, 130.20, 130.32, 135.00, 150.30, 169.00, 171.74 ppm. IR: $\tilde{\nu}$ = 1669 (C=O), 1752 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 591 (2), 570 (35), 569 (100), 513 (35). HRMS (ESI): calcd. for $C_{32}H_{44}N_2NaO_7$ [M + Na]$^+$ 591.3041; found 591.3052.

Fmoc-L-MeLeu-D-PhLac-OBn (14): DCC (6.38 g, 30.8 mmol) in DCM (100 mL), HOBt (4.71 g, 30.8 mmol) and DMAP (3.76 g, 30.8 mmol) were added to an ice-cold solution of benzyl D-3-phenyllactate (**10**; 7.30 g, 28.5 mmol) and *N*-Fmoc-*N*-methyl-L-leucine (**4**; 10.47 g, 28.5 mmol) in DCM (200 mL). After stirring for 24 h at room temperature, the solvent was evaporated and the residue was taken up in ethyl acetate (100 mL) and filtered. The solution was washed with a $NaHCO_3$ and a NaCl solution, dried with Na_2SO_4, filtered and the solvents evaporated. LPLC (cyclohexane/ethyl acetate, 8:2) afforded the coupling product **14** (2.156 g, 89%) as a white foam.[23] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.75 and 0.86 (2 d, J = 7.0 Hz, 2 H, $C_\delta H_3$-Leu), 0.94 (pseudo-t, J = 6.0 Hz, 4 H, $C_\delta H_3$-Leu), 1.45–1.64 (m, 3 H, $C_\beta H_2$-Leu, C_γH-Leu), 2.76 (s, 3 H, NCH_3), 3.15 and 3.20 (2 m, 2 H, $C_\beta H_2$-PhLac), 4.19 and 4.31 (m, 1 H, CH-Fmoc), 4.43–4.50 (m, 2 H, CH_2-Fmoc), 4.62 and 5.04 (m, 1 H, C_αH-Leu), 5.13–5.18 (m, 2 H, CH_2-Bn), 5.23 and 5.31 (m, 1 H, C_αH-PhLac), 7.14 (m, 2 H, Ar-H), 7.20 (m, 2 H, Ar-H), 7.29 (m, 3 H, Ar-H), 7.36 (m, 5 H, Ar-H), 7.45 (m, 2 H, Ar-H), 7.50 and 7.55 (d, J = 7.6 Hz, 1 H, Ar-H), 7.65 (t, J = 6.6 Hz, 1 H, Ar-H), 7.78 (d, J = 7.6 Hz, 1 H, Ar-H), 7.82 (d, J = 7.6 Hz, 1 H, Ar-H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 21.7, 23.0, 24.5, 30.1, 37.2, 37.6, 47.2, 56.4, 67.2, 67.7, 73.3, 120.0, 124.8, 125.0, 127.0, 127.6, 127.7, 128.4, 128.6, 129.0, 129.4, 135.0, 135.4, 141.4, 143.9, 144.1, 156.8, 171.2, 173.7 ppm. IR (KBr): $\tilde{\nu}$ = 1743 (C=O), 1701 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 606 (70), 623 (100), 628 (35). HRMS (ESI): calcd. for $C_{38}H_{39}NNaO_6$ [M + Na]$^+$ 628.2670; found 628.2671.

Boc-L-MeLeu-D-PhLac-OH (15): Hydrogenolysis of didepsipeptide **12** (3.87 g, 8.0 mmol) was performed in the same way as described for compound **17** (12 h, room temp., see below). The didepsipeptide acid **15** was obtained in quantitative yield as a colourless oil (3.15 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82–092 (d, J = 6.0 Hz, 6 H, $C_\delta H_3$-Leu), 1.43 and 1.45 (2 s, 9 H, CH_3-*t*Bu), 1.48–1.70 (m, 3 H, C_γH-Leu, C_βH-Leu), 2.73 and 2.75 (2 s, 3 H, NCH_3), 3.12 and 3.26 (2 dd, J = 4.0, 14.0 Hz, 2 H, $C_\beta H_2$-PhLac), 4.62–4.73 (m, 1 H, C_αH-Leu), 5.26 and 5.31 (2 dd, J = 4.8 Hz, 1 H, C_αH-PhLac), 7.18–7.34 (m, 5 H, Ar-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.11, 21.48, 23.02, 23.12, 24.46, 24.78, 28.29, 28.33, 29.97, 31.40, 37.18, 37.26, 37.50, 37.58, 56.71, 58.45, 72.99, 127.07, 128.43, 128.50, 129.30, 129.55, 135.70, 155.54, 155.66, 171.62, 173.01 ppm. IR (film): $\tilde{\nu}$ = 1671 (C=O), 1742 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 804 (21), 394 (20), 338 (38), 294 (100). HRMS (ESI): calcd. for $C_{21}H_{31}NNaO_6$ [M + Na]$^+$ 416.2044; found 416.2049.

Boc-L-MeLeu-D-morphPhLac-OH (16): Hydrogenolysis of didepsipeptide **13** was performed in the same way as described for compound **17** (12 h, room temp., see below). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87–0.94 (m, 6 H, $C_\delta H_3$-Leu), 1.44 (s, 9 H, CH_3-*t*Bu), 1.47–1.77 (m, 3 H, C_βH-Leu, C_γH-Leu), 2.74 and 2.83 (2 s, 3 H, NCH_3), 3.01–3.09 and 3.14–3.23 (2 m, 2 H, C_βH-PhLac), 3.10–3.15 (m, 4 H, NCH_2-morpholine), 3.82–3.87 (m, 4 H, OCH_2-morpholine), 4.37–4.47 and 4.63–4.74 (2 m, 1 H, C_αH-Leu), 5.18–5.25 and 5.25–5.33 (2 m, 1 H, C_αH-PhLac), 6.84 (d, *J* = 8.0 Hz, 2 H, Ar-H), 7.13 (d, *J* = 8.0 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.09, 21.34, 23.10, 23.18, 24.48, 24.79, 28.33, 29.99, 31.22, 36.39, 37.46, 37.63, 49.56, 56.56, 56.77, 58.40, 66.76, 73.42, 115.98, 127.62, 130.19, 130.31, 150.06, 156.46, 171.64, 172.60 ppm. IR (KBr): $\tilde{\nu}$ = 1613 (C=O), 1691 (C=O), 1783 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 957 (28), 480 (27), 479 (100), 423 (88). HRMS (ESI): calcd. for $C_{25}H_{39}N_2O_7$ $[M + H]^+$ 479.2752; found 479.2755.

Fmoc-L-MeLeu-D-PhLac-OH (17): A suspension of benzyl ester **14** (18.1 g, 30.0 mmol) and Pd/C (10%, 0.3 g) in ethanol (200 mL) was hydrogenated (1 bar) at room temperature for 2 h. Then the catalyst was filtered off and the solvent evaporated. LPLC (cyclohexane/ethyl acetate, 8:2, + 0.1% acetic acid) afforded Fmoc-L-MeLeu-D-Phlac-OH (**17**) as a white foam (11.20 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.75 and 0.87 (2 d, *J* = 7.0 Hz, 2 H, $C_\delta H_3$-Leu), 0.94 (pseudo-t, *J* = 7.0 Hz, 4 H, $C_\delta H_3$-Leu), 1.45–1.64 (m, 3 H, $C_\beta H_2$-Leu, C_γH-Leu), 2.81 (s, 3 H, NCH_3), 3.12 and 3.25 (2 m, 2 H, $C_\beta H_2$-PhLac), 4.16 and 4.30 (m, 1 H, CH-Fmoc), 4.48 (m, 2 H, CH_2-Fmoc), 4.59 and 4.93 (m, 1 H, C_αH-Leu), 5.20 and 5.32 (m, 1 H, C_αH-PhLac), 7.20–7.36 (m, 8 H, Ar-H), 7.43 (m, 2 H, Ar-H), 7.63 (m, 1 H, Ar-H), 7.80 (m, 2 H, Ar-H), 9.72 (br. s, 1 H, COOH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.0, 23.0, 24.5, 30.3, 37.2, 37.5, 47.2, 56.4, 67.7, 73.0, 120.0, 124.8, 125.1, 125.1, 127.1, 127.6, 127.7, 128.5, 128.6, 129.0, 129.4, 129.8, 135.5, 141.4, 143.8, 156.4, 157.1, 171.2, 173.7 ppm. IR (KBr): $\tilde{\nu}$ = 3156 (O–H), 1732 (C=O), 1668 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 294 (16), 516 (100), 533 (35), 538 (1). HRMS (ESI): calcd. for $C_{31}H_{33}NNaO_6$ $[M + Na]^+$ 538.2200; found 538.2201.

Solid-Phase Synthesis on Kaiser Oxime

Coupling of the First Segment to the Resin: Kaiser oxime (100 mg, 0.068 mmol) was suspended in DCM (1.0 mL) and agitated for 30 min. Then Boc-MeLeu-PhLac-OH (**15**; 53.5 mg, 0.136 mmol), HATU (51.7 mg, 0.136 mmol) dissolved in DCM (1 mL) and DIEA (29.0 μL, 0.204 mmol) were added. After shaking for 12 h the resin was filtered off, washed with DCM and DCM/EtOH (1:1) three times and dried in vacuo.

End-Capping: Acetic anhydride (0.064 mL, 0.68 mmol) and DIEA (58.0 μL, 0.34 mmol) were added to the resin (100 mg) suspended in DCM (0.5 mL). The mixture was shaken for 2 h. Then the resin was filtered off, washed three times alternating with MeOH (10 mL for each washing) and DCM (10 mL for each washing) and dried under high vacuum until a constant mass was reached.

Cleavage of the Boc Group: The Kaiser oxime was suspended in a TFA/DCM solution (25%, 1.3 mL/100 mg resin) and shaken for 30 min. The resin was filtered off and washed with DCM (3×), EtOH (1×), DCM (2×), EtOH (3×), DMF (3×) and DCM (1×).

Amide Coupling Reactions: A solution of the depsipeptide acid (3 equiv.) in DMF (1.0 mL), HATU (2 equiv.) and DIEA (6.5 equiv.) were added to a suspension of the resin (100 mg) in DMF (1.5 mL). After shaking for 12 h the resin was filtered off, washed with DMF (3 × 10 mL), DCM (3 × 10 mL), DMF (3 × 10 mL) and DCM (3 × 10 mL) and dried under high vacuum until a constant mass was reached.

Cyclizative Cleavage − PF1022A (1): A suspension of the resin-bound octadepsipeptide **27** (50 mg) in DCM (0.5 mL) containing DIEA (2.2 equiv.) and acetic acid (5.0 equiv.) was agitated for 12 h at room temperature. Then the resin was filtered off and washed several times with DCM. The filtrate and rinses were combined and the solvents evaporated. Preparative HPLC purification (ACN/H_2O gradient) of the residue afforded PF1022A (**1**; 8.1 mg) in a yield of 75% (based on the loading of the support with the linear precursor **27**) and a purity of 99.5%. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.67–0.98 (m, 21 H, CH_3-Leu, $C_\beta H_3$-Lac), 1.06–1.76 (m, 15 H, $C_\beta H_2$-Leu, C_γH-Leu), 1.28 (d, *J* = 6.7 Hz, 3 H, CH_3-Lac), 2.68, 2.77, 2.80, 2.85 and 2.91 (5 s, 12 H, NCH_3), 3.05 (m, 2 H, $C_\beta H_2$-PhLac), 4.41, 5.11 and 5.22 (3 m, 4 H, C_αH-Leu), 5.01, 5.32 and 5.42 (3 m, 2 H, C_αH-Lac), 5.52, 5.68 and 5.71 (3 m, 2 H, C_αH-PhLac), 7.21–7.33 (m, 10 H, Ar-H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 15.5, 16.3, 16.8, 20.4, 20.6, 20.8, 20.8, 20.9, 21.0, 23.0, 23.1, 23.2, 23.2, 23.4, 23.9, 24.2, 24.3, 24.3, 24.4, 28.9, 30.1, 30.1, 30.2, 30.3, 30.6, 35.7, 36.3, 36.5, 36.6, 36.7, 36.7, 36.8, 37.1, 37.3, 53.0, 53.2, 53.3, 56.4, 66.7, 67.5, 67.7, 70.1, 70.9, 71.0, 126.7, 126.7, 126.8, 128.1, 128.2, 128.2, 129.5, 135.1, 135.2, 135.9, 169.0, 169.0, 169.3, 169.5, 169.7, 170.2, 170.3, 170.7, 170.9 ppm. IR (KBr): $\tilde{\nu}$ = 1739 (C=O), 1660 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 949 (100), 966 (33). HRMS (ESI): calcd. for $C_{52}H_{76}N_4NaO_{12}$ $[M + Na]^+$ 971.5352; found 971.5351.

Cyclizative Cleavage − Emodepside (2): The cyclizative cleavage reaction was performed as described for PF1022A (**1**). After HPLC purification (ACN/H_2O), pale-yellow emodepside (**2**; 9.3 mg, 45% over all) was obtained, which was identical to a reference sample with respect to all the spectroscopic and biological data. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77–1.05 (m, 30 H, $C_\delta H_3$-Leu, $C_\beta H_3$-Lac), 1.20–1.83 (m, 18 H, $C_\beta H_2$-Leu, C_γH-Leu, CH_3-Lac), 2.73, 2.74, 2.80, 2.83 and 3.00 (5 s, 12 H, NCH_3), 2.85–3.07 (m, 4 H, $C_\beta H_2$-morphPhLac), 3.08–3.15 (m, 8 H, N-CH_2-morpholine), 3.81–3.88 (pseudo-t, 8 H, OCH_2morpholine), 4.47, 5.08, 5.19 5.34 and 5.38–5.53 (5 m, 6 H, C_αH-Leu, C_αH-morphPhLac), 5.54–5.67 (m, 2 H, C_αH-Lac), 6.77–6.86 (m, 4 H, Ar-H), 7.09–7.17 (m, 4 H, Ar-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.8, 17.1, 20.9, 21.1, 21.2, 23.4, 23.5, 23.5, 23.6, 24.2, 24.7, 24.9, 29.4, 30.5, 31.2, 36.2, 36.7, 37.2, 37.5, 38.1, 49.9, 54.0, 57.1, 66.5, 66.6, 66.9, 68.6, 70.8, 71.3, 116.1, 116.9, 130.4, 130.6, 141.7, 169.8, 170.2, 170.4, 170.6, 171.0, 171.2, 171.7 ppm. IR (KBr): $\tilde{\nu}$ = 1744 (C=O), 1665 (C=O), 1614 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 374 (28), 560 (85), 1119 (69), 1141 (100). HRMS (ESI): calcd. for $C_{60}H_{90}N_6NaO_{14}$ $[M + Na]^+$ 1141.6407; found 1141.6414.

Solid-Phase Synthesis on Wang Resin

Coupling of the First Segment to the Resin: Wang resin was suspended in THF (1.0 mL/100 mg resin) and agitated for 30 min to achieve a good swelling. The solvent was removed and a solution of didepsipeptide **17** (3 equiv.), DIC (3 equiv.), HOBt (3 equiv.) and DMAP (3 equiv.) in THF (1.0 mL/100 mg resin) was added. The suspension was agitated for 16 h at room temperature. Then the resin was washed three times with DCM (10 mL), acetone (10 mL) and DCM (10 mL) and dried under high vacuum until a constant mass was reached. The whole coupling and washing procedure was repeated once.

Cleavage of the Fmoc-Group: The resin was swollen in THF for 30 min. After removal of the solvent a solution of piperidine in THF (25%, 10 mL) was added and the suspension was shaken for 45 min at room temperature. Then the resin was drained and washed three times with DCM (10 mL), acetone (10 mL) and DCM (10 mL) and dried under high vacuum. Aliquots from the

combined rinses were used to determine the loading of the resin, as described in the General section.

Amide Couplings: A solution of the didepsipeptide acid (3 equiv.), HATU (2 equiv.) and DIEA (3 equiv.) in THF (1.0 mL/100 mg resin) were added to the swollen resin. The suspension was stirred for 16 h at room temperature. Then the solvent was filtered off and the resin washed three times with DCM (10 mL), acetone (10 mL) and DCM (10 mL). The whole coupling and washing procedure was repeated once.

H-L-MeLeu-D-Lac-L-MeLeu-D-PhLac-L-MeLeu-D-Lac-L-MeLeu-D-PhLac-OH (29): The octadepsipeptide-resin (712 mg) was treated with TFA/DCM (1:1; 10 mL/500 mg resin) and shaken for 1 h. The resin was filtered and washed eight times with DCM (10 mL each). The cleavage solution and the washings were combined and evaporated to yield a mixture of depsipeptides containing around 40% (114 mg) of octadepsipeptide **29** (23% overall yield). The crude material was used in the cyclization reaction without further purification.

Cyclooctadepsipeptide PF1022A (1): DIEA (65.3 mg, 0.504 mmol) and BOPCl (61.6 mg, 0.242 mmol) were added at 0 °C to a solution of the crude octadepsipeptide **29** (221.7 mg, corresponds to 0.092 mmol pure substance) in DCM. The reaction was stirred for 24 h and then the same amounts of BOPCl and DIEA were added and stirring was continued for another 24 h at room temperature. The solution was washed with a saturated $NaHCO_3$ solution, dried with Na_2SO_4 and the solvents evaporated. Chromatographic purification (toluene/2-propanol, 20:1) of the residue afforded PF1022A (87.8 mg, 100%) as a white solid, which was identical to a natural sample with respect to all the spectroscopic and biological data. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.68–0.98 (m, 21 H, $C_\delta H_3$-Leu, $C_\beta H_3$-Lac), 1.15–1.75 (m, 14 H, $C_\beta H_2$-Leu, C_γH-Leu), 1.28 (d, J = 6.8 Hz, 3 H, CH_3-Lac), 2.68, 2.77, 2.80, 2.85 and 2.91 (5 s, 12 H, NCH_3), 3.05 (m, 2 H, $C_\beta H_2$-PhLac), 4.41, 5.11 and 5.22 (3 m, 4 H, C_αH-Leu), 5.01, 5.32 and 5.42 (3 m, 2 H, C_αH-Lac), 5.52, 5.68 and 5.71 (3 m, 2 H, C_αH-PhLac), 7.23–7.32 (m, 10 H, Ar-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 15.5, 16.3, 16.8, 20.4, 20.6, 20.8, 20.8, 20.9, 21.0, 23.0, 23.1, 23.2, 23.2, 23.4, 23.9, 24.2, 24.3, 24.3, 24.4, 28.9, 30.1, 30.1, 30.2, 30.3, 30.6, 35.7, 36.3, 36.5, 36.6, 36.7, 36.7, 36.8, 37.1, 37.3, 53.0, 53.2, 53.3, 56.4, 66.7, 67.5, 67.7, 70.1, 70.9, 71.0, 126.7, 126.7, 126.8, 128.1, 128.2, 128.2, 129.5, 135.1, 135.2, 135.9, 169.0, 169.0, 169.3, 169.5, 169.7, 170.2, 170.3, 170.7, 170.9 ppm. IR (KBr): $\tilde{\nu}$ = 1739 (C=O), 1660 (C=O) cm^{-1}. MS (ESI): *m*/*z* (%) = 475 (4), 950 (1), 966 (100), 971 (23). HRMS (ESI): calcd. for $C_{52}H_{76}N_4NaO_{12}$ [M + Na]$^+$ 971.5352; found 971.5351.

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