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The Design and Synthesis of Dansyl-Containing Cyclic Pseudopeptides

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Six cyclic pseudopeptides with the general formula *cyclo*-(L-AA-D-Oxd)$_n$ [where AA is an α-amino acid, D-Oxd is *trans*-(4*R*,5*S*)-4-carboxy-5-methyloxazolidin-2-one and n = 3, 4] have been designed and efficiently synthesized in the liquid phase in good overall yields. The crucial step is the macrocyclization, which always occurs in satisfactory yields regardless of the cycle dimension and the nature of various functional groups in the side-chain. Moreover, a dansyl (dansyl = 5-dimethylamino-1-naphthylsulfonyl) unit was introduced into the side-chain with the aim of preparing a labelled cyclopeptide that can be easily located in a biological environment.

Introduction

Cyclic peptides have been extensively studied because they provide ideal scaffolds for exploring structure–activity relationships in ligand–receptor interactions[1] as cyclization favours the mimicking of turns and the formation of intramolecular hydrogen bonds, and increases membrane permeability by eliminating charged termini. Thus, many pharmacologically important natural products are constrained by macrocyclization, from the cyclic non-ribosomal peptides (NRP) tyrocidine A and cyclosporin to the polyketide (PK) antibiotic erythromycin, and the hybrid peptide/polyketide drugs rapamycin and epothilone.[2]

Furthermore several azole-based cyclic peptides have been isolated from marine organisms, fungi and algae. These molecules contain oxazole or triazole heterocyclic moieties, display interesting antitumour and antidrug resistance properties and are potential metal-ion chelators.[3] Thus, heterocycle-containing cyclic pseudopeptides are good candidates for mimicking the shape and pharmacological activity of these interesting natural products. For instance, systematic studies on the complexes formed between cyclic peptides and bivalent cations have been performed by Blout and co-workers with *cyclo*-(L-Pro-Gly)$_3$ and *cyclo*-(L-Pro-Gly)$_4$,[4] which are very flexible molecules.

As a part of a project directed towards the study of pseudopeptides containing pseudoproline moieties,[5] we wish to show here the synthesis of six cyclopeptides of different shape, all containing the D-Oxd unit [D-Oxd = *trans*-(4*R*,5*S*)-4-carboxy-5-methyloxazolidin-2-one unit] as an isoster for the D-proline unit alternating with a generic L-amino acid (Figure 1). Indeed, cyclic D,L-α-peptides are the archetypical members of a growing class of organic tubular macromolecular structures that have a plethora of promising potential applications,[6] so an efficient method for the preparation of these compounds is highly desirable.

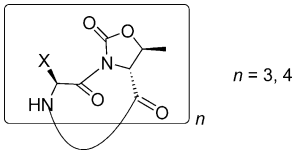

Figure 1. General chemical structure of the cyclic pseudopeptides reported in this study; X is a general side-chain.

The introduction of the Oxd moiety can be very useful in the preparation of rigid cyclopeptides because, as we have demonstrated, the Oxd moiety imparts rigidity to a pseudopeptide chain due to the presence of the oxazolidin-2-one endocyclic carbonyls, which force the Oxd carbonyl groups exclusively into the *trans* conformation.[5] This effect can be seen by analysis of the CH_α proton chemical shift of the nearby amino acid, which is always very deshielded with a chemical shift that is above 5 ppm, whereas the normal position is between 4 and 4.5 ppm (Figure 2).

Therefore the preparation of cyclic pseudopeptides with the general formula *cyclo*-(L-AA-D-Oxd)$_n$ (where AA is a generic α-amino acid and n = 3, 4; Figure 1) was devised. Our synthetic approach is quite general and is compatible with macrocycles of different sizes (n = 3, 4) and with several side-chains. Hence this general scaffold can be decorated with different chemical functions simply by changing the amino acid unit: as an example, we have prepared a cyclohexapeptide that contains the fluorescent dansyl (dansyl =

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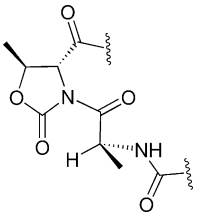


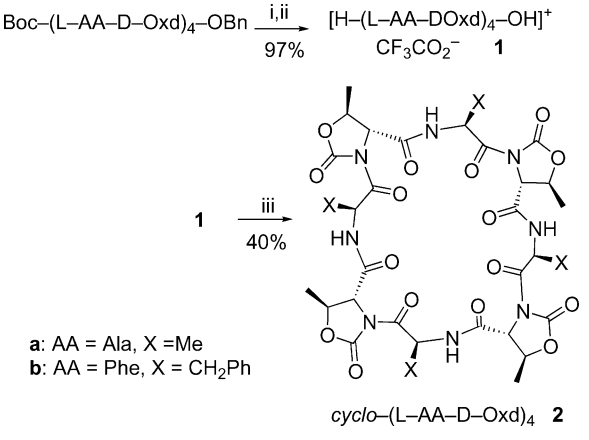
Figure 2. Preferred conformation for an L-AA-D-Oxd unit with the preferential *trans* conformation of the imide moiety, which accounts for the anomalous chemical shift of the amino acid CH_α proton.

DANS = 5-dimethylamino-1-naphthylsulfonyl) unit,[7] which can be used to locate the molecule in a biological environment.[8] Indeed, Kubik and co-workers recently demonstrated that the production of a cyclic structure containing fluorescent moieties allows the binding event of a synthetic receptor to be combined with the change of an easily measurable receptor property to produce a chemosensor.[9]

Results and Discussion

The synthesis of cyclic pseudopeptides always requires good reaction conditions to be found for the cyclization step, so we have tested this reaction both for the formation of cycles containing eight amino acid residues (24 atoms) and also for smaller cycles containing six amino acid units (18 atoms). The linear pseudopeptide preparations and the cyclizations were performed in the liquid phase.

First we synthesized the two cyclic pseudopeptides that contain eight amino acids units, **2a** and **2b** (Scheme 1). The synthesis of the open chain Boc-(L-Ala-D-Oxd)$_4$-OBn (not shown) has already been reported and was performed by the addition of one L-Ala-D-Oxd unit to the other.[5a] This approach allowed us to halve the coupling steps for the oligomer synthesis and is applicable to units containing any sort of amino acid, including β-amino acids.



Scheme 1. Synthesis of **2a**,**b**. Reagents and conditions: (i) H_2, Pd/C (10%), MeOH, room temp., 12 h; (ii) TFA (18 equiv.), dry CH_2Cl_2, room temp., 4 h; (iii) HATU (1.1. equiv.), TEA (3 equiv.), dry CH_3CN, room temp., 45 min.

The pseudopeptide Boc-(L-Ala-D-Oxd)$_4$-OBn was fully deprotected by hydrogenolysis with H_2 in the presence of Pd/C (10%) in methanol and then treated with trifluoroacetic acid (TFA) to obtain the amine **1a** as its trifluoroacetic salt. The reactions afforded the desired compound in 97% overall yield. Then the salt **1a** was cyclized to the corresponding cycle **2a**. For this reaction several coupling agents, such as *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU),[10] *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HCTU), were used in the presence of different bases, such as triethylamine (TEA), diisopropylethylamine (DIEA) or 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU), always using 1 equiv. of coupling agent and 3 equiv. of base. The solvent of choice was dry acetonitrile, which is polar enough to solubilize all the reagents. If wet acetonitrile was employed, the reaction yield diminished. Among all the combinations of coupling agents and bases tested, the best results were obtained by employing HATU and TEA to give the desired *cyclo*-(L-Ala-D-Oxd)$_4$ (**2a**).[11] Indeed, if HATU and DBU were used, the reaction yield was poor due to the immediate decomposition of the uronium agent. On the other hand, if HCTU and TEA were employed, the reaction was very slow and the yield poor due to their low reactivity. Finally, the concentration of the reagents was studied, and the best concentration of pseudopeptide to avoid intermolecular couplings was about 5 mM (Table 1).

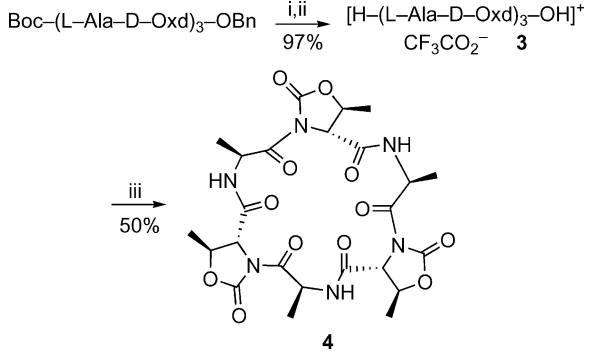
Table 1. Reaction yields of the cyclization reactions of H-(L-Ala-D-Oxd)$_4$-OH·CF_3CO_2H (**1a**) performed for 45 min at room temperature in dry acetonitrile.

Entry	Coupling reagent (1 equiv.)	Base (3 equiv.)	Conc. [mM]	% Yield
1	HBTU	DBU	10	10
2	HBTU	TEA	10	–
3	HCTU	TEA	10	5
4	HATU	DBU	10	–
5	HATU	DBU	5	10
6	HATU	DIEA	5	24
7	HATU	TEA	10	32
8	HATU	TEA	5	40

Furthermore the purification step proved to be complex because **2a** is very polar and water-soluble. Therefore the reaction mixture was concentrated to remove the volatiles and the residue was washed with small amounts of acetonitrile, purified with preparative HPLC and finally obtained in pure form in 40% yield. A similar approach allowed us to prepare *cyclo*-(L-Phe-D-Oxd)$_4$ (**2b**) in which L-alanine was replaced by L-phenylalanine in the pseudopeptide chain: the cyclization step afforded the desired compound **2b**, which was insoluble in several solvents. Thus, it was purified simply by washing the mixture several times with acetonitrile. A dilute solution of **2b** was analyzed by HPLC, which showed that the compound was pure and so its purification by preparative HPLC could be avoided.

Then the formation of the cycle **4** containing 18 atoms was tested by the cyclization of the salt [H-(L-Ala-D-Oxd)$_3$-

OH]$^+$·$CF_3CO_2^-$ (**3**), which was obtained by deprotection of Boc-(L-Ala-D-Oxd)$_3$-OBn following the previously reported protocol (Scheme 2). Also, in this case the cyclization step required some attention and the best reaction conditions had to be found. The best coupling reagents again turned out to be HATU and TEA in dry acetonitrile. The mixture was stirred at room temperature for 45 min and then concentrated. The residue was washed with small amounts of acetonitrile, purified by preparative HPLC and finally obtained in pure form in 50% yield.



Scheme 2. Synthesis of **4**. Reagents and conditions: (i) H_2, Pd/C (10%), MeOH, room temp., 12 h; (ii) TFA (18 equiv.), dry CH_2Cl_2, room temp., 4 h; (ii) HATU (1.1. equiv.), TEA (3 equiv.), dry CH_3CN, room temp., 45 min.

By using the same approach, three additional cyclic pseudopeptides were synthesized, replacing one L-Ala unit with an L-Lys unit that has been further derivatized (Figure 3). This variation was introduced with the aim of checking the stability of this class of molecule both in the presence of a polar moiety and in the presence of a large side-chain like the dansyl (DANS) unit.

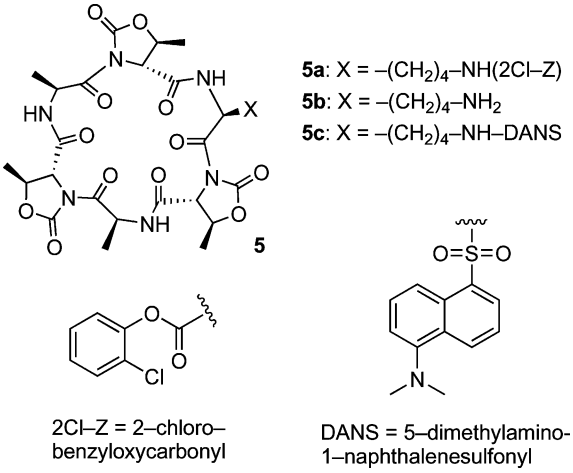
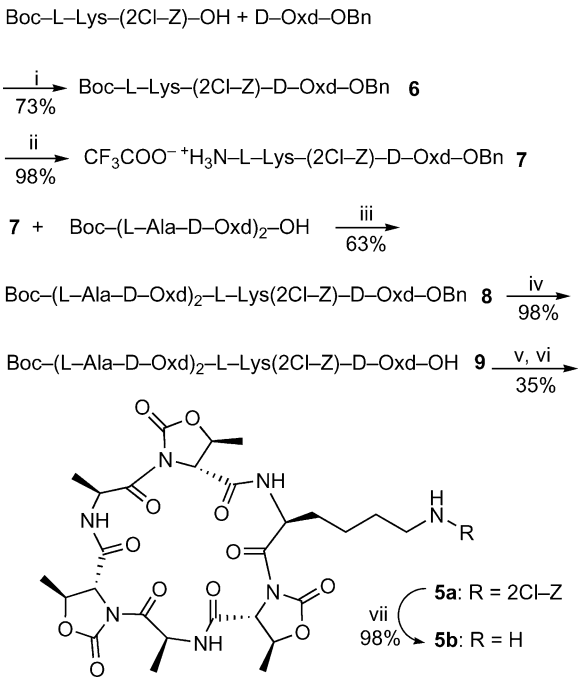


Figure 3. Chemical structure of the cyclic pseudopeptides **5a**–**c**.

To introduce a free lysine unit into the cyclic pseudopeptide, the side-chain of the lysine was initially protected with the 2-chlorobenzyloxycarbonyl (2-chlorobenzyloxycarbonyl = 2Cl-Z) group (Scheme 3). This compound is commercially available and was coupled with D-Oxd-OBn to obtain in 73% yield Boc-L-Lys(2Cl-Z)-D-Oxd-OBn (**6**), which was then deprotected at the C_α amino group to give **7**, which was then coupled with Boc-(L-Ala-D-Oxd)$_2$-OH.[5a] The linear pseudopeptide Boc-(L-Ala-D-Oxd)$_2$-L-Lys(2Cl-Z)-D-Oxd-OBn (**8**) was then deprotected at both ends to remove the Boc and benzyl moieties. The hydrogenolysis step to prepare the acid **9** required some attention as only the OBn group was to be cleaved with the 2Cl-Z group left untouched. The previously reported reaction conditions (H_2 and 10% Pd/C in methanol) resulted in the cleavage of both the OBn and 2Cl-Z groups. Thus, several reaction conditions were checked, including the use of other palladium catalysts such as PdO. The best results were obtained by replacing the methanol with ethyl acetate and stopping the reaction after 2 h: the more resistant 2Cl-Z group was untouched, whereas the OBn group was totally cleaved. The cyclization was performed under the previously reported conditions (HATU and TEA in dry acetonitrile at room temperature) and the cycle **5a** was obtained in pure form in 35% yield after purification by HPLC chromatography. Then *cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys-D-Oxd] (**5b**) containing the free lysine unit was prepared by hydrogenolysis of **5a** with H_2 on 10% Pd/C in methanol without affecting any other group of the cycle.



Scheme 3. Synthesis of **5b**. Reagents and conditions: (i) HBTU (1 equiv.), DBU (2 equiv.), dry CH_3CN, room temp., 45 min; (ii) TFA (18 equiv.), dry CH_2Cl_2, room temp., 3 h; (iii) HBTU (1 equiv.), TEA (3 equiv.), dry CH_3CN, room temp., 45 min; (iv) H_2, Pd/C (10%), AcOEt, room temp., 2 h; (v) TFA (18 equiv.) dry CH_2Cl_2, room temp., 4 h; (vi) HATU (1 equiv.), TEA (3 equiv.), room temp., 45 min; (vii) H_2, Pd/C (10%), MeOH, room temp., 12 h.

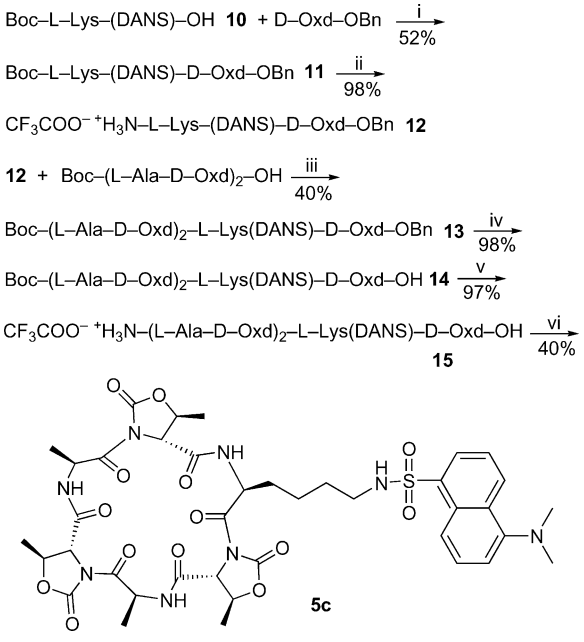
Finally, the cyclic pseudopeptide *cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys(DANS)-D-Oxd] (**5c**) was prepared by a similar strategy. In this case the dansyl group could be introduced into

the Boc-L-Lys-OH unit or into the cycle **5b**. We chose to introduce the dansyl unit at the beginning of the synthesis as it could be used to locate the products in the reaction mixtures. Thus, Boc-L-Lys-OH was treated with dansyl chloride in the presence of a base. To obtain the desired Boc-L-Lys(DANS)-OH (**8**) in a satisfactory yield, several reaction conditions were tested and are reported in Table 2. The best results were obtained with DBU (4 equiv.) in dichloromethane.[12] Any attempt to further increase the yield by using more equivalents of base or a longer reaction time failed.

Table 2. Reaction yields for the reactions of Boc-L-Lys-OH with dansyl chloride for 16 h at room temperature.

Entry	Equiv. of base	Solvent	% Yield
1	DIEA (4)/DMAP (0.5)	CH_2Cl_2	–
2	DIEA (4)/DMAP (0.5)	DMF	21
3	DBU (4)	CH_2Cl_2	73
4	DBU (4)	DMF	40
5	DBU (4)	CH_3CN	63

Then the cyclic pseudopeptide **5c** was prepared following the same reaction protocol as used for the preparation of **5a** (Scheme 4). All the reactions were performed with good-to-excellent yields and the final compound was purified by HPLC chromatography (from 100% H_2O to 100% CH_3CN in 5 min).



Scheme 4. Synthesis of **5c**. Reagents and conditions: (i) HBTU (1 equiv.), DBU (2 equiv.), dry CH_3CN, room temp., 3 h; (ii) TFA (18 equiv.), dry CH_2Cl_2, room temp., 3 h; (iii) HATU (1 equiv.), TEA (3 equiv.), dry CH_3CN, room temp., 2 h; (iv) H_2, Pd/C (10%), MeOH, room temp., 2 h; (v) TFA (18 equiv.), dry CH_2Cl_2, room temp., 3 h; (vi) HATU (1 equiv.), TEA (3 equiv.), room temp., 45 min.

Conclusions

Six cyclic pseudopeptides, two containing eight amino acid units and four containing six amino acid units, were prepared in the liquid phase in good overall yields. The cyclization step required some attention and the best reaction conditions were found using HATU and TEA as reagents in a 5 mM solution of dry acetonitrile at room temperature. All the pseudopeptides contained an alternating sequence of L-amino acid and D-Oxd, a proline analogue that imparts rigidity to the cyclic system. We have demonstrated that this synthetic method is compatible with different side-chains and cycle sizes so that it can be used to synthesize several molecules of the *cyclo*-(L-AA-D-Oxd)$_n$ (n = 3, 4) series in good overall yields.

Experimental Section

General: Routine NMR spectra were recorded with spectrometers at 400, 300 or 200 MHz (^{1}H NMR) and 100, 75 or 50 MHz (^{13}C NMR). Chemical shifts are reported in δ values relative to the solvent peak of $CHCl_3$, set at δ = 7.27 ppm. High quality ^{1}H NMR spectra were recorded with a Varian Inova 600 spectrometer. Measurements were carried out in $CDCl_3$, CD_3OD, [D_6]acetonitrile or D_2O. Proton signals were assigned by COSY spectra. Infrared spectra were recorded with an FTIR spectrometer. High quality infrared spectra (64 scans) were obtained at 2 cm^{-1} resolution using a 1 mm NaCl solution cell. All spectra were obtained in 3 mM solutions in dry CH_2Cl_2 at 297 K. All compounds were dried in vacuo and all the sample preparations were performed under nitrogen. Melting points were determined in open capillaries and are uncorrected. Purification of the six cyclic pseudopeptides **2a**, **2b**, **4**, **5a**, **5b** and **5c** was accomplished on an Agilent 1100 liquid chromatograph equipped with a variable-wavelength UV detector (deuterium lamp 190–600 nm) using a Zorbax Eclipse XDB-C18 PrepHT column (21.2 × 150 mm, 7 μm) (Agilent Technologies). Acetonitrile CHROMASOLV® for HPLC was purchased from Riedel-de Haën and was used as the eluting solvent (mixed with water).

General Method for the Hydrogenolysis: 10% palladium on charcoal (10 mg) was added to a stirred solution of Boc-(L-AA-D-Oxd)$_n$-OBn (0.10 mmol) in methanol (10 mL). The mixture was stirred under hydrogen for 12 h. Then the catalyst was filtered through a Celite pad and the mixture was concentrated. The product was obtained in pure form without any further purification.

General Method for the Hydrolysis of Boc-(L-AA-D-Oxd)$_n$-OH Compounds: A solution of Boc-(L-AA-D-Oxd)$_3$-OBn (0.1 mmol) and TFA (18 mmol, 1.39 mL) in dry dichloromethane (10 mL) was stirred for 4 h at room temperature, the volatiles were removed under reduced pressure and the product was obtained in pure form without any further purification.

General Method for the Cyclization of the Pseudopeptides: HATU (0.38 mmol, 144 mg) and then TEA (1.2 mmol, 0.16 mL) were added to a stirred solution of H-(L-AA-D-Oxd)$_n$-OH·CF_3CO_2H (0.38 mmol) in dry acetonitrile (80 mL, solution 5 mM) under an inert atmosphere at room temperature. The solution was stirred for 45 min under an inert atmosphere and then acetonitrile was removed under reduced pressure. The crude was washed with small portions of acetonitrile (2 × 2 mL) to remove all byproducts. The desired cycle was obtained as a white solid after HPLC chromatography (H_2O/acetonitrile, 80:20 → 70:30 as eluent).

H-(L-Ala-D-Oxd)$_4$-OH·CF$_3$CO$_2$H (1a): Yield 97% (90 mg). ^{1}H NMR (CD$_3$OD, 200 MHz): δ = 1.41–1.65 (m, 24 H, 8 Me), 4.63–5.10 (m, 8 H, 4 CHN, 4 CHO), 5.60–5.90 (m, 4 H, 4 CHN-Ala) ppm.

***cyclo*-(L-Ala-D-Oxd)$_4$ (2a):** Yield 40% (120 mg); m.p. 165 °C. $[\alpha]_D$ = –86.9 (c = 0.4, CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$, 3 mM): $\tilde{\nu}$ = 3385, 1784, 1709, 1660 cm^{-1}. ^{1}H NMR (CD$_3$CN, 600 MHz): δ = 1.43 (d, ^1J = 7.2 Hz, 12 H, 4 Me-Ala), 1.49 (d, ^1J = 6.0 Hz, 12 H, 4 Me-Oxd), 3.91 (d, ^1J = 6.0 Hz, 4 H, 4 CHN), 4.44 (dq, ^1J = 7.2 Hz, 4 H, 4 CHO), 4.56 (dq, ^1J = 6.0 Hz, 4 H, 4 CHN-Ala), 7.17 (br. s, 4 H, 4 NH) ppm. ^{13}C NMR (CD$_3$CN, 75 MHz): δ = 17.5, 20.9, 29.4, 30.2, 30.4, 30.9, 29.9, 49.6, 63.0, 63.5, 64.0, 75.9, 76.2, 153.0, 169.0, 173.3 ppm. C$_{32}$H$_{40}$N$_8$O$_{16}$ (792.26): calcd. C 48.48, H 5.09, N 14.14; found C 48.52, H 5.11, N 14.16.

H-(L-Phe-D-Oxd)$_4$-OH·CF$_3$CO$_2$H (1b): Yield 97% (108 mg); m.p. 215 °C. $[\alpha]_D$ = +43.6 (c = 0.1, MeOH). ^{1}H NMR (CDCl$_3$, 200 MHz): δ = 1.10–1.63 (m, 12 H, 4 Me), 2.81–3.43 (m, 8 H, 4 CH$_2$Ph), 4.01–4.83 (m, 8 H 4 CHO, 4 CHN), 5.60–6.53 (m, 4 H, 4 C*H*N-CH$_2$Ph), 7.18–7.61 (m, 20 H, 4 Ph) ppm.

***cyclo*-(L-Phe-D-Oxd)$_4$ (2b):** Yield 40% (67 mg); m.p. 230 °C. $[\alpha]_D$ = –73.2 (c = 0.1, MeOH). IR (CH$_2$Cl$_2$, 3 mM): $\tilde{\nu}$ = 3309, 1775, 1726, 1661 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 1.43 (m, 12 H, 4 Me), 2.70–3.81 (m, 12 H, 4 C*H*$_2$Ph, 4 CHN), 4.40–4.60 (m, 4 H, 4 CHO), 5.60 (m, 1 H, C*H*NCH$_2$Ph), 5.78–6.11 (m, 3 H, 3 C*H*NCH$_2$Ph), 7.07–7.40 (m, 20 H, 4 Ph), 8.38–8.65 (m, 4 H, 4 NH) ppm. ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 23.3, 37.4, 46.3, 87.6, 95.9, 126.8, 128.9, 153.3, 154.5, 156.1, 164.2, 166.7 ppm. C$_{56}$H$_{56}$N$_8$O$_{16}$ (1096.38): calcd. C 61.31, H 5.14, N 10.21; found C 61.35, H 5.16, N 10.23.

H-(L-Ala-D-Oxd)$_3$-OH·CF$_3$CO$_2$H (3): Yield 97% (70 mg). ^{1}H NMR (CD$_3$OD, 200 MHz): δ = 1.41–1.65 (m, 18 H, 3 CH$_3$-Ala, 3 CH$_3$-Oxd), 4.63–5.10 (m, 6 H 3 CHN-Oxd, 3 CHO-Oxd), 5.60–5.90 (m, 3 H, 3 CHN-Ala) ppm.

***cyclo*-(L-Ala-D-Oxd)$_3$ (4):** Yield 50% (110 mg); m.p. 271 °C (decomp.). $[\alpha]_D$ = –55 (c = 0.03, MeOH). IR (CH$_2$Cl$_2$, 3 mM): $\tilde{\nu}$ = 3440, 3284, 1789, 1662 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 1.29 (d, ^1J = 7.2 Hz, 9 H, 3 CH$_3$-Ala), 1.38 (d, ^1J = 6.6 Hz, 9 H, 3 CH$_3$-Oxd), 4.53 (d, ^1J = 3.0 Hz, 3 H, 3 CHN-Oxd), 4.64 (dq, ^1J = 9.3 Hz, 3 H, 3 CHO-Oxd), 5.28 (dq, ^1J = 6.0, 6.9 Hz, 1 H, 3 CHN-Ala), 8.76 (d, ^1J = 7.5 Hz, 3 H, 3 NH) ppm. ^{13}C NMR ([D$_6$]-DMSO, 100 MHz): δ = 16.7, 19.0, 20.7, 30.9, 45.8, 47.4, 61.2, 74.6, 78.7, 79.1, 79.6, 152.4, 167.6, 171.4 ppm. C$_{24}$H$_{30}$N$_6$O$_{12}$ (594.19): calcd. C 48.48, H 5.09, N 14.14; found C 48.45, H 5.03, N 14.19.

Boc-L-Lys(2Cl-Z)-D-Oxd-OBn (6): HBTU (1.25 mmol, 474 mg), then D-Oxd-OBn (1.25 mmol, 294 mg) and finally DBU (2.5 mmol, 0.37 mL) were added to a stirred solution of a commercial sample of Boc-L-Lys(2Cl-Z)-OH (1.25 mmol, 520 mg) in dry acetonitrile (10 mL) under an inert atmosphere. The solution was stirred for 45 min under an inert atmosphere and then acetonitrile was removed under reduced pressure and replaced with ethyl acetate (30 mL). The mixture was washed with brine (30 mL), aqueous HCl (3 × 20 mL) and 5% NaHCO$_3$ (20 mL), and then dried with Na$_2$SO$_4$, then ethyl acetate was eliminated under reduced pressure. The product was obtained in pure form after flash chromatography (eluent: cyclohexane/ethyl acetate 8.2) in 73% yield (0.56 g).

M.p. 98 °C. $[\alpha]_D$ = +13 (c = 0.03, MeOH). IR (CH$_2$Cl$_2$, 3 mM): $\tilde{\nu}$ = 3686, 3439, 2927, 2853, 1793, 1757, 1714, 1605, 1507 cm^{-1}. ^{1}H NMR (CDCl$_3$, 200 MHz): δ = 1.45 (m, 13 H, *t*Bu, 2 CH$_2$-Lys), 1.55 (d, ^1J = 6.2 Hz, 3 H, CH$_3$-Oxd), 1.79 (br. s, 2 H, CH$_2$-Lys), 3.23 (m, 2 H, CH$_2$-Lys), 4.47 (d, ^1J = 4 Hz, 1 H, CHN-Oxd), 4.58 (dq, ^1J = 4.4, 6.5 Hz, 1 H, CHO-Oxd), 5.01 (br. s, 1 H, CHN-Lys), 5.23 [m, 4 H, OC*H*$_2$Ph, OC*H*$_2$(2Cl-Z)], 5.44 (br. s, 1 H, NH), 7.28–7.40 (m, 9 H, Ph) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 21.3, 22.7, 28.4, 29.0, 33.0, 40.8, 53.1, 62.2, 63.8, 64.1, 68.2, 73.9, 80.1, 127.1, 128.5, 128.8, 129.3, 129.7, 134.8, 151.4, 156.5, 167.7, 173.5 ppm. C$_{30}$H$_{36}$ClN$_3$O$_9$ (617.21): calcd. C 58.30, H 5.87, N 6.80; found C 58.26, H 5.85, N 6.82.

CF$_3$COO$^-$·$^+$H$_3$N-L-Lys(2Cl-Z)-D-Oxd-OBn (7): A solution of Boc-L-Lys(2Cl-Z)-D-Oxd-OBn (**6**) (0.50 mmol, 316 mg) and TFA (9 mmol, 0.67 mL) in dry dichloromethane (8 mL) was stirred for 3 h at room temperature, then the volatiles were removed under reduced pressure and the product was obtained pure in 98% yield (93 mg) without any further purification. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 1.46–1.70 (m, 7 H, 2 CH$_2$-Lys, CH$_3$-Oxd), 2.05 (br. s, 2 H, CH$_2$-Lys), 3.24 (br. s, 2 H, CH$_2$-Lys), 4.56 (d, ^1J = 3.6 Hz, 1 H, CHN-Oxd), 4.65 (m, 1 H, CHO-Oxd), 5.16–5.34 [m, 6 H, CHN-Lys, OC*H*$_2$Ph, OC*H*$_2$(2Cl-Z), NH], 7.29–7.43 (m, 9 H, Ph), 8.1 (br. s, 1 H, COOH) ppm.

Boc-(L-Ala-D-Oxd)$_2$-L-Lys(2Cl-Z)-D-Oxd-OBn (8): A mixture of CF$_3$COO$^-$·$^+$H$_3$N-[L-Lys(2Cl-Z)-D-Oxd]-OBn (**7**) (0.50 mmol) and TEA (1.5 mmol, 0.41 mL) in dry acetonitrile (5 mL) was added to a stirred solution of Boc-(L-Ala-D-Oxd)$_2$-OH (0.50 mmol, 257 mg) and HBTU (0.50 mmol, 190 mg) in dry acetonitrile (10 mL) under an inert atmosphere at room temperature. The solution was stirred for 45 min under an inert atmosphere, then acetonitrile was removed under reduced pressure and replaced with ethyl acetate (30 mL). The mixture was washed with brine (30 mL), aqueous HCl (3 × 20 mL) and NaHCO$_3$ 5% (20 mL), and then dried with Na$_2$SO$_4$, then ethyl acetate was eliminated under reduced pressure. The product was obtained in pure form after flash chromatography (eluent: cyclohexane/ethyl acetate, 8:2) in 63% yield (0.32 g). M.p. 146 °C. $[\alpha]_D$ = +15.6 (c = 0.5, CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$, 3 mM): $\tilde{\nu}$ = 3440, 3356, 2930, 2848, 1790, 1721 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 1.30–1.60 (m, 28 H, *t*Bu, 2 CH$_3$-Ala, 3 CH$_3$-Oxd, 2 CH$_2$-Lys) 1.79 (m, 2 H, CH$_2$-Lys), 3.25 (m, 2 H, CH$_2$-Lys), 4.49 (d, ^1J = 5.7 Hz, 1 H, CHN-Oxd), 4.54 (d, ^1J = 5.4 Hz, 1 H, CHN-Oxd), 4.60–4.80 (m, 7 H, CHN-Oxd, 3 CHO-Oxd, CHN-Lys, 2 CHN-Ala), 5.18–5.35 [m, 5 H, OC*H*$_2$Ph, OC*H*$_2$(2Cl-Z), NH], 5.80 (br. s, 1 H, NH), 7.26–7.48 (m, 9 H, Ph) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 14.1, 15.8, 17.0, 21.0, 21.1, 22.2, 28.3, 29.1, 30.0, 31.2, 40.5, 49.3, 49.4, 51.7, 61.8, 62.3, 62.9, 63.6, 68.06, 73.88, 74.61, 75.2, 80.5, 126.8, 128.3, 128.5, 128.7, 129.1, 129.4, 129.5, 134.6, 134.7, 151.3, 151.6, 151.7, 156.3, 167.0, 167.4, 167.8, 171.8, 172.3, 174.4 ppm. C$_{46}$H$_{56}$ClN$_7$O$_{17}$ (1014.43): calcd. C 54.46, H 5.56, N 9.67; found C 54.48, H 5.56, N 9.66.

Boc-(L-Ala-D-Oxd)$_2$-L-Lys(2Cl-Z)-D-Oxd-OH (9): 10% palladium on charcoal (10 mg) was added to a stirred solution of Boc-(L-Ala-D-Oxd)$_2$-L-Lys(2Cl-Z)-D-Oxd-OBn (**8**) (0.10 mmol, 103 mg) in ethyl acetate (5 mL). The mixture was stirred under hydrogen for 2 h. Then the catalyst was filtered through a Celite pad and the mixture was concentrated. The product **6** was obtained in pure form in 98% yield (91 mg) without any further purification. M.p. 144 °C. $[\alpha]_D$ = –8.98 (c = 0.5, CH$_2$Cl$_2$). IR (Nujol): $\tilde{\nu}$ = 2956, 1793, 1713, 1677, 1536, 1296, 1259, 1213 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 1.26–1.60 (m, 28 H, *t*Bu, 2 CH$_3$-Ala, 3 CH$_3$-Oxd, 2 CH$_2$-Lys), 1.80 (m, 2 H, CH$_2$-Lys), 3.24 (br. s, 2 H, CH$_2$-Lys), 4.47–4.80 (m, 9 H, 3 CHN-Oxd, 3 CHO-Oxd, CHN-Lys, 2 CHN-Ala), 5.20–5.34 [m, 3 H, OC*H*$_2$(2Cl-Z), NH], 5.7 (br. s, 1 H, NH), 7.25–7.40 (m, 4 H, Ph) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 19.5, 21.4, 22.4, 24.5, 28.6, 30.0, 39.0, 40.8, 49.6, 60.7, 62.1, 64.0, 68.4, 72.7, 127.2, 128.6, 129.0, 129.5, 133.6, 134.4, 152.0, 156.9, 167.4, 168.3, 172.7 ppm. C$_{39}$H$_{50}$ClN$_7$O$_{17}$ (924.3): calcd. C 50.68, H 5.45, N 10.61; found C 50.66, H 5.42, N 10.57.

***cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys-(2Cl-Z)-D-Oxd] (5a):** Prepared according to the General Method for the Cyclization of the Pseudopeptides. Yield 35% (107 mg); m.p. 230 °C. $[\alpha]_D$ = +20 (c = 0.3, CH_2Cl_2). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3407, 3322, 3198, 1787, 1713, 1364, 1224 cm^{-1}. ^{1}H NMR (CD_3CN, 300 MHz): δ = 1.30–1.61 (m, 28 H, *t*Bu, 2 CH_3-Ala, 3 CH_3-Oxd, 2 CH_2-Lys), 1.79 (m, 2 H, CH_2-Lys), 3.17 (m, 2 H, CH_2-Lys), 4.42 (m, 2 H, 2 CHN-Oxd), 4.45 (d, ^1J = 3.6 Hz, 1 H, CHN-Oxd), 4.60–4.80 (m, 5 H, 3 CHO-Oxd, 2 CHN-Ala), 5.20 [s, 2 H, OCH_2(2Cl-Z)], 5.46 (m, 2 H, CHN-Lys, NH), 5.81 (br. s, 1 H, NH), 7.30–7.54 (m, 4 H, Ph) ppm. ^{13}C NMR (CD_3CN, 75 MHz): δ = 16.8, 20.02, 29.2, 40.5, 48.1, 52.0, 62.3, 63.4, 75.0, 75.2, 100.2, 127.5, 129.6, 167.7, 172.0 ppm. $C_{34}H_{40}ClN_7O_{14}$ (806.17): calcd. C 50.65, H 5.00, N 12.16; found C 50.68, H 5.04, N 12.17.

***cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys-D-Oxd] (5b):** 10% palladium on charcoal (2 mg) was added to a stirred solution of *cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys-(2Cl-Z)-D-Oxd] (**5a**) (0.020 mmol, 17 mg) in methanol (10 mL). The mixture was stirred under hydrogen for 12 h. Then the catalyst was filtered through a Celite pad and the mixture was concentrated. The product **1b** was obtained in pure form in 98% yield (13 mg) without any further purification. M.p. 205 °C. $[\alpha]_D$ = –120 (c = 0.4, CH_2Cl_2). IR (CH_2Cl_2, 3.0 mM): $\tilde{\nu}$ = 3682, 3623, 3401, 1798, 1781, 1670 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 0.76 (d, ^1J = 6.4 Hz, 3 H, CH_3-Ala), 0.90 (d, ^1J = 6.8 Hz, 3 H, CH_3-Ala), 1.12–1.25 (m, 4 H, 2 CH_2-Lys), 1.28–1.57 (m, 9 H, 3 CH_3-Oxd) 1.74 (m, 2 H, CH_2-Lys), 3.12 (m, 2 H, CH_2NH$_2$), 3.68 (m, 2 H, CHN-Oxd), 3.90–4.09 (m, 4 H, CHN-Oxd, 3 CHO-Oxd), 4.48–4.59 (m, 2 H, 2 CHN-Ala), 5.21 (m, 1 H, CHN-Lys), 7.48 (d, ^1J = 5.7 Hz, 1 H, NH-Lys), 8.85 (d, ^1J = 5.1 Hz, 1 H, NH-Ala), 8.95 (d, ^1J = 5.7 Hz, 1 H, NH-Ala) ppm. ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 14.7, 17.5, 18.7, 26.1, 29.2, 31.4, 47.1, 58.0, 59.8, 61.0, 75.5, 84.2, 101.2, 117.6, 128.2, 145.1, 173.2 ppm. $C_{27}H_{37}N_7O_{12}$ (651.62): calcd. C 49.77, H 5.72, N 15.05; found C 49.74, H 5.74, N 15.03.

Boc-L-Lys(DANS)-OH (10): Dansyl chloride (1.8 mmol, 493 mg) was added to a stirred solution of Boc-L-Lys-OH (1.8 mmol, 450 mg) and DBU (7.2 mmol, 1.1 mL) in dry dichloromethane . The mixture was stirred for 16 h at room temperature, then the volatiles were removed under reduced pressure and 1 N HCl was added to the residue to obtain pH = 3. The aqueous mixture was extracted with dichloromethane (3 × 30 mL) and then the combined organic layers were dried with Na_2SO_4 and removed under reduced pressure. The product **10** was obtained in pure form without any further purification in 73% yield (0.63 mg). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.26–1.54 (m, 13 H, *t*Bu, 2 CH_2-Lys), 1.80 (br. s, 2 H, CH_2-Lys), 2.95–3.26 (m, 8 H, 2 CH_3-DANS, CH_2-NH-Lys), 4.05 (br. s, 1 H, $NHSO_2$), 5.01 (m, 1 H, CHN-Lys), 7.42 (br. s, 1 H, NH), 7.54 (m, 2 H, DANS-H), 8.25 (d, ^1J = 7.1 Hz, 1 H, DANS-H), 8.31 (d, ^1J = 6.7 Hz, 1 H, DANS-H), 8.54 (br. s, 1 H, DANS-H), 8.70 (br. s, 1 H, DANS-H) ppm.

Boc-L-Lys(DANS)-D-Oxd-OBn (11): A mixture of D-Oxd-OBn (0.63 mmol, 263 mg) and DBU (1.26 mmol 0.19 mL) in dry acetonitrile (10 mL) was added to a stirred solution of Boc-L-Lys-(DANS)-OH (0.63 mmol, 304 mg) and HBTU (0.63 mmol. 239 mg) in dry acetonitrile (10 mL) under an inert atmosphere at room temperature. The solution was stirred for 3 h under an inert atmosphere and then acetonitrile was removed under reduced pressure and replaced with ethyl acetate (30 mL). The mixture was washed with brine (30 mL), aqueous HCl (3 × 20 mL) and 5% $NaHCO_3$ (20 mL), and then dried with Na_2SO_4, then ethyl acetate was eliminated under reduced pressure. The product was obtained in pure form after flash chromatography (eluent: cyclohexane/ethyl acetate, 7:3) in 52% yield (228 mg). M.p. 77 °C. $[\alpha]_D$ = +12 (c = 0.2, CH_2Cl_2). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3436, 3387, 1793, 1752, 1708 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.46 (m, 15 H, *t*Bu, 3 CH_2-Lys), 1.53 (d, ^1J = 6.6 Hz, 3 H, CH_3-Oxd), 2.95 (m, 8 H, 2 CH_3-DANS, CH_2-NH-Lys), 4.45 (d, ^1J = 3.9 Hz, 1 H, CHN-Oxd), 4.54 (m, 1 H, CHO-Oxd), 4.91 (br. s, 1 H, $NHSO_2$), 5.22 (s, 2 H, OCH_2Ph), 5.35 (m, 1 H, CHN-Lys), 7.21 (d, ^1J = 7.8 Hz, 1 H, DANS-H), 7.30–7.40 (m, 5 H, Ph), 7.56 (m, 2 H, 2 DANS-H), 8.23 (d, ^1J = 7.2 Hz, 1 H, DANS-H), 8.32 (d, ^1J = 8.1 Hz, 1 H, DANS-H), 8.58 (d, ^1J = 8.1 Hz, 1 H, DANS-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 12.7, 21.1, 22.1, 28.3, 42.8, 45.4, 68.0, 73.8, 80.7, 82.7, 90.3, 91.7, 97.1, 115.2, 1 23.2, 128.3, 128.7, 129.5, 134.8, 151.0, 173.1, 198.0, 216.7 ppm. $C_{35}H_{44}N_4O_9S$ (696.81): calcd. C 60.33, H 6.36, N 8.04; found C 60.35, H 6.32, N 8.01.

CF_3COO^{-}·$^{+}H_3$N-L-Lys(DANS)-D-Oxd-OBn (12): A solution of Boc-L-Lys(DANS)-D-Oxd-OBn (**11**) (0.25 mmol, 177 mg) and TFA (4.57 mmol, 0.34 mL) in dry dichloromethane (10 mL) was stirred for 3 h at room temperature, then the volatiles were removed under reduced pressure and the product was obtained in pure form in 98% yield (143 mg) without any further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.50 (s, 6 H, 3 CH_2 Lys), 1.54 (d, ^1J = 6.3 Hz, 3 H, CH_3 Oxd), 3.0 (br. s, 2 H, CH_2-NH-Lys), 3.46 (s, 6 H, 2 CH_3-DANS), 4.30–4.80 (m, 3 H, CHN-Oxd, CHO-Oxd, $NHSO_2$), 5.22 (m, 1 H, CHN Lys), 5.33 (s, 2 H, OCH_2Ph), 7.39 (m, 5 H, Ph), 7.80 (m, 2 H, DANS-H), 8.40 (m, 1 H, DANS-H), 8.50 (d, ^1J = 9 Hz, 1 H, DANS-H), 8.63 (d, ^1J = 8.7 Hz, 1 H, DANS-H), 8.91 (m, 1 H, DANS-H) ppm.

Boc-(L-Ala-D-Oxd)$_2$-L-Lys-(DANS)-D-Oxd-OBn (13): A mixture of CF_3COO^{-}·$^{+}H_3$N-L-Lys(DANS)-D-Oxd-OBn (0.25 mmol) and TEA (1.9 mmol, 0.15 mL) in dry acetonitrile (15 mL) was added to a stirred solution of Boc-(L-Ala-D-Oxd)$_2$-OH (0.25 mmol, 129 mg) and HATU (0.25 mmol, 95 mg) in dry acetonitrile (10 mL) under an inert atmosphere at room temperature. The solution was stirred for 2 h under an inert atmosphere, then acetonitrile was removed under reduced pressure and replaced with ethyl acetate (30 mL). The mixture was washed with brine (30 mL), aqueous HCl (3 × 20 mL) and 5% $NaHCO_3$ (20 mL), and then dried with Na_2SO_4, then ethyl acetate was eliminated under reduced pressure. The product was obtained in pure form after flash chromatography (eluent: cyclohexane/ethyl acetate, 8:2) in 40% yield (109 mg). M.p. 126 °C. $[\alpha]_D$ = +0.62 (c = 0.7, CH_2Cl_2). IR (CH_2Cl_2, 2.9 mM): $\tilde{\nu}$ = 3442, 3359, 2988, 2931, 1790, 1720, 1691 cm^{-1}. ^{1}H NMR (CD_3CN, 400 MHz): δ = 1.31 (m, 6 H, 2 CH_3-Ala), 1.40 (m, 18 H, 3 CH_2-Lys, *t*Bu, CH_3-D-Oxd), 1.46 (d, ^1J = 6.4 Hz, 3 H, CH_3-D-Oxd), 1.50 (d, ^1J = 6.4 Hz, 3 H, CH_3-D-Oxd), 2.90 (m, 2 H, CH_2-NH-Lys), 2.95 (s, 6 H, 2 CH_3-DANS), 4.39 (d, ^1J = 4.4 Hz, 1 H, CHN-Oxd), 4.43 (d, ^1J = 4.38 Hz, 1 H, CHN-Oxd), 4.54 (d, ^1J = 4 Hz, 1 H, CHN-Oxd), 4.56–4.74 (m, 3 H, CHO-Oxd), 5.14–5.29 (m, 4 H, 2 CHN-Ala, OCH_2Ph), 5.48 (m, 1 H, CHN-Lys), 6.0 (br. s, 1 H, $NHSO_2$), 7.40 (m, 5 H, Ph), 7.47 (m, 1 H, NH-Lys), 7.66 (m, 2 H, DANS-H), 8.20 (d, ^1J = 7.2 Hz, 1 H, DANS-H), 8.39 (m, 1 H, DANS-H), 8.55 (d, ^1J = 8.4 Hz, 1 H, DANS-H), 8.72 (d, ^1J = 4 Hz, 1 H, DANS-H) ppm. ^{13}C NMR (CD_3CN, 75 MHz): δ = 20.3, 22.4, 27.9, 28.6, 38.2, 42.8, 45.4, 48.8, 61.8, 62.9, 68.0, 74.6, 75.3, 116.4, 120.8, 124.4, 128.5, 128.5, 128.8, 129.3, 129.6, 135.6, 136.1, 152.2, 152.5, 168.0, 168.4, 172.0, 172.4, 174.1 ppm. $C_{51}H_{64}N_8O_{17}S$ (1093.16): calcd. C 56.03, H 5.90, N 10.25; found C 56.01, H 5.92, N 10.28.

Boc-(L-Ala-D-Oxd)$_2$-L-Lys(DANS)-D-Oxd-OH (14): Prepared according to the General Method for the Hydrogenolysis. Yield 98% (98 mg from 0.1 mmol of starting material); m.p. 162 °C. $[\alpha]_D$ = –5.73 (c = 0.7, CH_2Cl_2). IR (Nujol): $\tilde{\nu}$ = 3363, 2721, 2672, 2365,

2365, 2333, 1789, 1679, 1548 cm^{-1}. ^1H NMR (CD_3CN, 400 MHz): δ = 1.20–1.70 (m, 30 H, 2 CH_3-Ala, 3 CH_2-Lys, *t*Bu, 3 CH_3-Oxd), 2.50–3.40 (m, 8 H, CH_2-NH-Lys, 2 CH_3-DANS), 4.39–4.73 (m, 6 H, 3 CHN-Oxd, 3 CHO-Oxd), 5.26 (m, 2 H, 2 CHN-Ala), 5.43 (m, 2 H, CHN-Lys, NH), 5.88–5.98 (br. s, 1 H, $NHSO_2$), 7.28 (d, ^1J = 6.8 Hz, 1 H, DANS-H), 7.61 (m, 2 H, 2 DANS-H), 8.18 (d, ^1J = 6.8 Hz, 1 H, DANS-H), 8.29 (d, ^1J = 8.4 Hz, 1 H, DANS-H), 8.56 (d, ^1J = 8.4 Hz, 1 H, DANS-H) ppm. ^{13}C NMR (CD_3CN, 75 MHz): δ = 17.3, 20.1, 20.5, 22.4, 27.8, 38.1, 42.7, 45.0, 48.6, 48.8, 62.6, 75.5, 115.6, 117.6, 119.3, 123.7, 128.4, 129.2, 135.8, 152.1, 155.9 ppm. $C_{44}H_{58}N_8O_{17}S$ (1002.36): calcd. C 52.69, H 5.83, N 11.17; found C 52.73, H 5.88, N 11.20.

$CF_3COO^-{}^+H_3N$-(L-Ala-D-Oxd)$_2$-L-Lys(DANS)-D-Oxd-OH (15): Prepared according to the General Method for the Hydrolysis of Boc-(L-AA-D-Oxd)$_n$-OH Compounds. Yield 97% (99 mg). ^1H NMR (CD_3CN, 300 MHz): δ = 1.20–1.70 (m, 21 H, 2 CH_3-Ala, 3 CH_2-Lys, 3 CH_3-Oxd), 2.90 (m, 2 H, CH_2-NH-Lys), 3.25 (m, 6 H, 2 CH_3-DANS), 4.25–4.70 (m, 6 H, 3 CHN-Oxd, 3 CHO-Oxd), 5.26–5.50 (m, 3 H, 2 CHN-Ala, CHN-Lys), 6.10 (br. s, 1 H, $NHSO_2$), 7.80 (m, 3 H, DANS-H), 8.30 (m, 1 H, DANS-H), 8.50 (m, 1 H, DANS-H), 8.60 (m, 1 H, DANS-H) ppm.

***cyclo*-[(L-Ala-D-Oxd)$_2$-L-Lys(DANS)-D-Oxd] (5c):** Prepared according to the General Method for the Cyclization of the Pseudopeptides. Yield 40% (35 mg from 0.1 mmol of starting material); m.p. 204 °C. $[a]_D$ = –0.30 (*c* = 0.7, CH_2Cl_2). IR (CH_2Cl_2, 1.5 mM): $\tilde{\nu}$ = 3680, 3594, 1789, 1712, 1671, 1602 cm^{-1}. ^1H NMR ([D$_6$]acetone, 300 MHz): δ = 1.25–1.70 (m, 21 H, 2 CH_3-Ala, 3 CH_2-Lys, 3 CH_3-Oxd), 2.90 (m, 8 H, CH_2-NH-Lys, 2 CH_3-DANS), 4.57–4.78 (m, 3 H, 3 CHN-Oxd), 4.80–4.95 (m, 3 H, 3 CHO-Oxd), 5.59 (m, 3 H, 2 CHN-Ala, CHN-Lys), 6.80 (m, 1 H, $NHSO_2$), 7.31 (d, ^1J = 6.9 Hz, 1 H, DANS-H), 7.64 (m, 2 H, 2 DANS-H), 8.25 (d, ^1J = 7.2 Hz, 1 H, DANS-H), 8.42 (d, ^1J = 8.7 Hz, 1 H, DANS-H), 8.60 (d, ^1J = 8.7 Hz, 1 H, DANS-H) ppm. ^{13}C NMR (D$_6$]acetone, 75 MHz): δ = 17.3, 20.1, 22.6, 42.8, 45.0, 48.3, 52.0, 62.2, 74.5, 75.3, 115.5, 119.7, 123.6, 128.1, 129.0, 129.9, 130.1, 136.6, 152.2, 152.4 ppm. $C_{39}H_{48}N_8O_{14}S$ (884.91): calcd. C 52.93, H 5.47, N 12.66; found C 52.90, H 5.44, N 12.68.

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