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Stabilizing effects in oxazolidin-2-ones-containing pseudopeptides

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Abstract—Novel homo-oligomers of the Gly-L-Oxd moiety have been prepared and their preferential conformations analyzed by IR, ^{1}H NMR and CD spectroscopy, with the aim of determining whether these molecules were able to fold in ordered structures. In these homo-oligomers two stabilizing effects are active: besides the *trans* conformation of the imide group, the formation of C=O···H–N hydrogen bonds takes place and is very sensitive to the pseudopeptide size.

1. Introduction

The members of the δ-peptide family include isosteric replacements of dipeptide units, such as depsipeptides, thioesters or hetomethylene isoesters.[1] As such, these sequences have usually been introduced into polypeptide chains to create β-turn mimics.[2] However very little work has been done on δ-peptides though some pioneering work has been reported by Balaram et al.[3] and Toniolo et al.[4] on Xaa-Pro peptides. In contrast, the described δ-peptides mostly involve carbapeptoid backbones,[5] which are homo-oligomers of sugar amino acids and have been prepared from both furanose and pyranose residues. Furthermore oligomers of a new quinoline-derived δ-amino acids have recently been proposed as aromatic δ-peptides.[6]

Herein we report the synthesis and conformational analysis of L-pyroglutamic acid and *trans*-(4*S*,5*R*)-4-carboxy-5-methyl oxazolidin-2-one (L-Oxd) homo-oligomers,[7] up to the tetramer and pentamer level, respectively, and we demonstrate that these short oligomers fold in ordered structures similar to the polyproline II conformation, even with only two or three units. This peculiar property is due to the presence of a carbonyl on the ring, nearby to the nitrogen, so that an imide bond is formed. This latter group is characterized by a nitrogen atom connected to an *endo*-cyclic carbonyl and an *exo*-cyclic carbonyl, which tend to lie anti from one to the other and thus adopt the *trans* conformation. X-ray diffraction analysis of Boc-(L-pGlu)$_2$-OH (pGlu-OH = pyroglutamic acid) and high level DFT calculations proved a verification of this finding. On introduction of our pseudoprolines into small polypeptide chains,[8] we could demonstrate that the imide group always adopts the *trans* conformation, as was proven by ^{1}H NMR spectroscopy: thus the Xaa-Oxd moiety is a very rigid skeleton and can be considered as a single conformationally constrained δ-amino acid (Fig. 1). Moreover this skeleton can easily be modified, by simply changing the starting amino acids, which can belong to the D or L series.

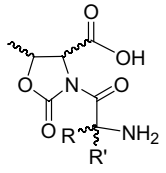

Figure 1. General structure of Xaa-Oxd, a conformationally constrained δ-amino acid. The preferential *trans* conformation of the imidic bond, which accounts for the anomalous chemical shift of the CH-α protons, is shown.

2. Results and discussion

Bearing this effect in mind, many possible Xaa-Oxd can be simply prepared by varying the amino acid or the oxazolidin-2-one moiety; for example, Oxd can be replaced by Oxac [(4*S*)-oxazolidineacetic acid, 2-oxo].[9] Furthermore by alternating an L-amino acid and a D-Oxd group completely different sequences[10] can be assembled. Herein, we report the results obtained for the synthesis and the conformational analysis of short

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pseudopeptides, which have the general formula Boc-(Gly-L-Oxd)$_n$-OBn and are the simplest oligomers that can be prepared.

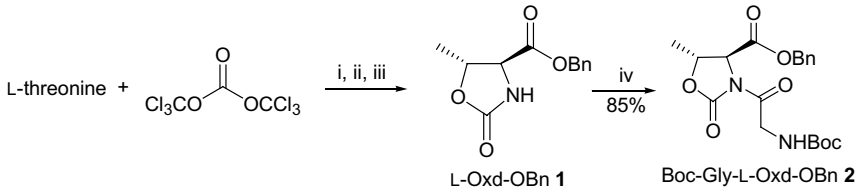
The monomer Boc-Gly-L-Oxd-OBn **2** was obtained by acylation of *trans*-(4*S*,5*R*)-4-carboxybenzyl-5-methyl oxazolidin-2-one (H-L-Oxd-OBn) **1**, which was achieved by a straightforward method from L-threonine[11] on a multigram scale (Scheme 1). The synthesis of the Boc-Gly-L-Oxd-OBn **2** unit however is not trivial. Previously,[8,9] we described the preparation of pentafluorophenyl esters, such as Boc-L-Oxd-OPfp or Boc-L-Val-OPfp under mild conditions,[12] and their coupling with H-L-Oxd-OBn **1** in the presence of diisopropylethylamine (DIEA) and 4-(dimethylamino)pyridine (DMAP) in DMF. This coupling requires two steps and, on some occasions, affords unsatisfactory results. As a result we utilized as the coupling reagent HBTU, which has recently been developed for the solid phase synthesis of polypeptides. This compound is an activated form of classical HOBt and, as Carpino et al.[13] has recently demonstrated, can exist in two forms, which are in equilibrium: the uronium form (more active) and the guanidinium form (less active). As the presence of triethylamine strongly favours the guanidinium form, it is essential to add the tertiary base always *after* the two reagents and *after* the HBTU in the formation of the imide bond.

Utilizing the same approach, the longer homo-oligomers were synthesized by liquid phase synthesis in satisfactory yields (Scheme 2).

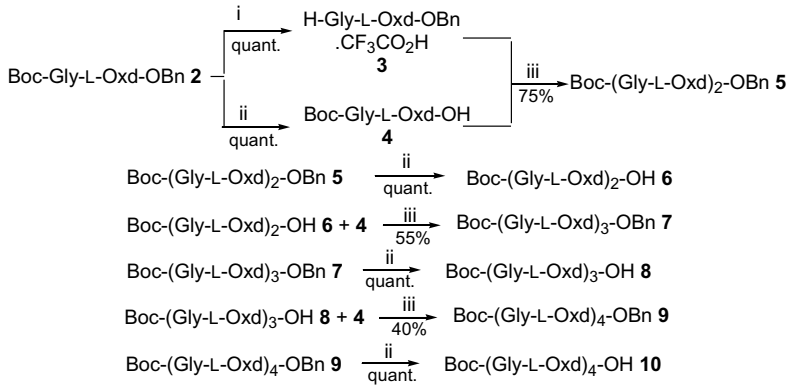
Then, in order to establish whether our molecules formed ordered structures, we analyzed their behaviour by IR, CD and ^{1}H NMR.

The IR absorption spectra of all the synthesized molecules were obtained as 3 mM solutions in methylene chloride: at this concentration the intramolecular aggregation is usually unimportant. Figure 2 shows the absorption bands of the NH stretching bonds (3500 and 3200 cm^{-1}) of the esters **2**, **5**, **7** and **9**: the non-hydrogen-bonded amide protons bands are above 3400 cm^{-1} while the hydrogen-bonded amide protons bands are below 3400 cm^{-1}.[14] Boc-Gly-L-Oxd-OBn **2** did not show any sign of forming a C=O···H–N hydrogen bond: this outcome is in agreement with the tendency to form a C=O···H–C hydrogen bond, which would clash with the formation of a C=O···H–N hydrogen bond. Better results were obtained with the longer chains, as there is a continuous shift of the NH stretching bands up to 3395 cm^{-1}; these results suggest that a cooperative effect takes place.

To further validate these results, the oligomers Boc-(Gly-L-Oxd)$_n$-OBn were analyzed by ^{1}H NMR spectroscopy. We extensively demonstrated[7,8] that the formation of a C=O···H–C bond can be identified simply by checking the Xaa α-hydrogen chemical shift, as the carbonyl proximity with the hydrogen involves a strong deshielding of the hydrogen chemical shift. Table 1 shows the most interesting chemical shift of Boc-Gly-L-Oxd-OBn **2** and of Boc-(Gly-L-Oxd)$_2$-OBn **5**, compared with Boc-Gly-L-Pro-NHMe.



Scheme 1. Reagents and conditions: (i) 1 M NaOH, DMF; (ii) Cs_2CO_3, H_2O; (iii) BnBr (1.1 equiv), DMF; (iv) (i) Boc-Gly-OH (1 equiv), HBTU (1 equiv), Et_3N (2 equiv), CH_3CN, 30 min, rt.



Scheme 2. Reagents and conditions: (i) TFA (18 equiv), CH_2Cl_2, 4 h, rt; (ii) H_2, Pd/C, MeOH, 2 h, rt; (iii) HBTU (1 equiv), Et_3N (3 equiv), CH_3CN, 30 min, rt.

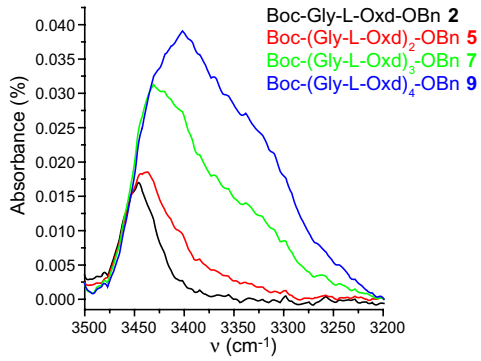


Figure 2. N–H stretch region FT-IR data for 3 mM samples of oligomers **2**, **5**, **7** and **9** in pure CH_2Cl_2 at room temperature, after subtraction of the spectrum of pure CH_2Cl_2.

Table 1. Selected values of chemical shifts of Boc-Gly-L-Pro-NHMe, **2** and **5** in 10^{-2} M solutions in $CDCl_3$ at 25 °C

Entry	Product	α-CH	α-CH
1	Boc-Gly-L-Pro-NHMe	3.89	—
2	Boc-Gly-L-Oxd-OBn **2**	4.37	—
		4.66	—
3	Boc-(Gly-L-Oxd)$_2$-OBn **5**	4.48	4.43
		4.84	4.60

The chemical shift of the Boc-Gly-L-Pro-NHMe α-hydrogens is reported in entry 1 and is below 4 ppm.[15] In contrast, entries 2 and 3 show that the chemical shifts of the Gly moieties α-hydrogens of Boc-Gly-L-Oxd-OBn **2** and of Boc-(Gly-L-Oxd)$_2$-OBn **5** are more deshielded by about 0.5–0.6 ppm. This implies that those molecules assume a preferential conformation with the two imide carbonyls in the *trans* conformation. The same effect is observed for the longer oligomers **7** and **9**, which show a complex ^{1}H NMR spectrum, but all the signals are between 4.20 and 4.80 ppm, thus we can confirm that all the imide groups assume a preferential conformation with the two carbonyls in *trans*, as expected.

From NOESY 1D experiments (Fig. 3), we can see that the Gly-NH hydrogen is near the α-hydrogen of the adjacent L-Oxd group.

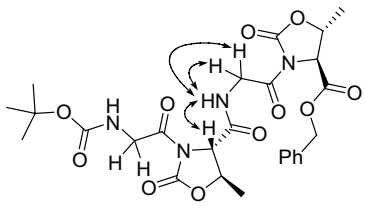


Figure 3. NOE enhancements of Boc-(Gly-L-Oxd)$_2$-OBn **5** obtained performing the NOESY 1D experiments on 10 mM solutions in $CDCl_3$ and utilizing a mixing time of 1.000 s.

The IR analysis suggests that there is a regular increase in the number of hydrogen bonds from the dimer **2** to the octamer **9**; this effect was also detected by investigation of the DMSO-d_6 dependence of the NH proton chemical shift.[16] This solvent has a strong hydrogen-bonding acceptor character and, if bound to a free NH proton, it would be expected to dramatically move its chemical shift downfield. The evaluation of inaccessible NH groups by ^{1}H NMR was performed by adding increasing amounts of DMSO-d_6 to 1 mM solutions in $CDCl_3$. The results are reported in Figure 4. While the variation of chemical shift on the increase of DMSO, d_6 percentage is very big for Boc-(Gly-L-Oxd)$_2$-OBn **5**, the results are a little better for Boc-(Gly-L-Oxd)$_3$-OBn **7**. The titration of octamer Boc-(Gly-L-Oxd)$_4$-OBn **9** proved to be in good agreement with the IR of the NH region, with a small variation of the chemical shift of the NH's. This outcome suggests that a secondary structure is forming, on prolonging the chain.

The oligomers Boc-(Gly-L-Oxd)$_n$-OH **3**, **6**, **8** and **10** were then investigated in MeOH by CD (Fig. 5). Although this technique is intrinsically a low resolution method,[17] it can furnish useful information on the formation of hydrogen bond, which drives to the formation of secondary structures.[18] The per-residue CD signal changes from **3** to **10** and shows a dramatic absorbance increase up to the octamer **10**, thus showing that an extra effect is present besides the chromophore absorption. This effect can be attributed to the formation of hydrogen bonds and of a secondary structure. This outcome is perfectly in agreement with the results obtained from the analysis of the IR and ^{1}H NMR spectra, which furnished evidence for the formation of hydrogen bonds.

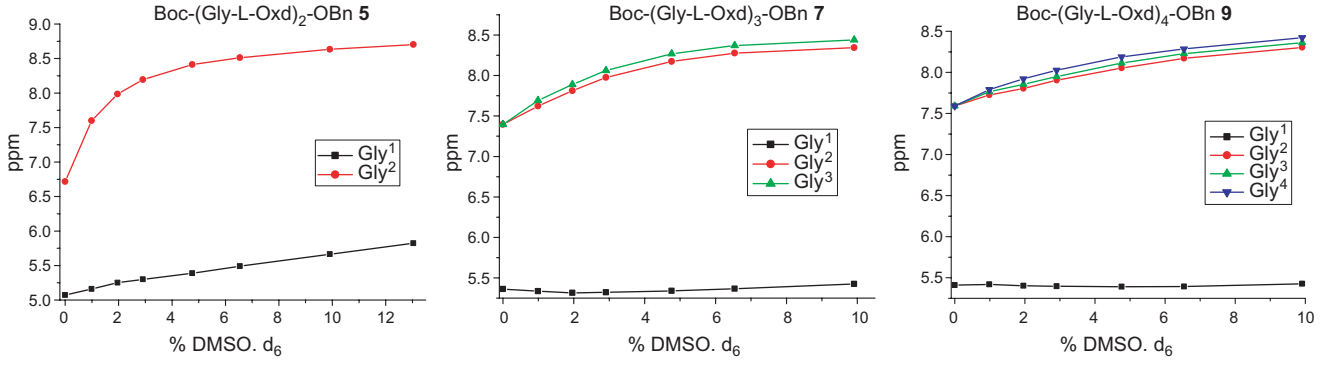


Figure 4. Variation of NH proton chemical shift (ppm) of **5**, **7** and **9** a function of increasing percentages of DMSO-d_6 to the $CDCl_3$ solution (v/v) (concentration: 1 mM).

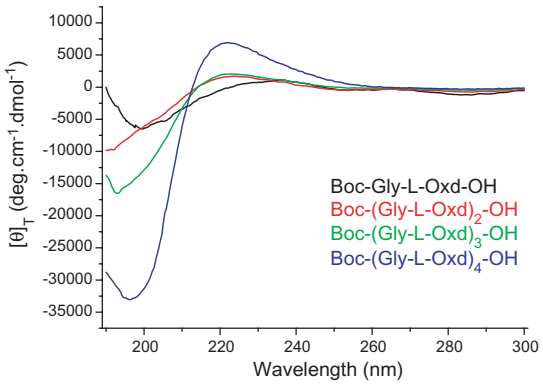


Figure 5. Normalized per-residue CD spectra of Boc-(Gly-L-Oxd)$_n$-OH ($n = 1, 2, 3, 4$) in methanol.

3. Conclusions

We have designed and prepared novel pseudopeptides with the general formula Boc-(Gly-L-Oxd)$_n$-OBn, where the monomer unit was obtained by reacting H-L-Oxd-OBn and Boc-Gly-OH. We have analyzed their preferred conformations by IR, ^{1}H NMR and CD analysis, with the aim of checking whether these molecules are able to fold in ordered structures. We observed that a C=O···H–N hydrogen bond was formed only in longer chains, while the *trans* conformation of the *endo*-cyclic and hexocyclic carbonyls of the Gly-L-Oxd unit is always present. Thus in these homo-oligomers two stabilizing effects of the chain are active: besides the *trans* conformation of the imide bond, which is a general effect and has never been contradicted, the formation of C=O···H–N hydrogen bonds takes place and is very sensitive to the pseudopeptide size. These results can be very useful for the design of secondary ordered structures: for instance PNA analogues[19] or especially designed pseudopeptides for ion recognition.

4. Experimental

Routine NMR spectra were recorded with spectrometers at 400, 300 or 200 MHz (^{1}H NMR) and at 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 7.27 ppm. Infrared spectra were recorded with an FT-IR spectrometer. Melting points were determined in open capillaries and are uncorrected.

High quality infrared spectra (64 scans) were obtained at 2 cm^{-1} resolution using a 1 mm NaCl solution cell and a Nicolet 210 FT-infrared spectrometer. 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 performed in a nitrogen atmosphere.

High quality ^{1}H NMR spectra were recorded with a Varian Inova 600. Measurements were carried out in $CDCl_3$ and in DMSO-d_6 using tetramethylsilane as the internal standard. Proton signals were assigned by COSY spectra. Data for conformational analysis are obtained with NOESY 1D spectra with typical mixing times of 1.0 s.

The CD spectra were obtained on a Jasco J-810 spectropolarimeter. Cylindrical fused quartz cells of 0.05 and 0.01 cm path length were used. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg × cm^2 × dmol^{-1}).

4.1. Boc-Gly-L-Oxd-OBn 2

To a stirred solution of L-Oxd-OBn **1** (4 mmol, 0.94 g), Boc-Gly-OH (4 mmol, 0.76 g) and HBTU (4 mmol, 1.52 g) in dry acetonitrile (20 mL) was added Et_3N (8 mmol, 1.16 mL) at room temperature. The mixture was stirred for 30 min, then acetonitrile removed under reduced pressure and replaced with ethyl acetate. The mixture was washed with brine, 1 M aqueous HCl (3 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulphate and concentrated in vacuo. Product **2** was obtained pure in 85% yield (1.33 g) as a waxy solid after silica gel chromatography (cyclohexane/ethyl acetate 8:2 as eluant). $[\alpha]_D^{20} = -31.2$ (c 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3443, 1792, 1752, 1712$ cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.44$ (s, 9H, *t*-Bu), 1.52 (d, 3H, $J = 6.3$ Hz, OCH–CH_3), 4.37 (dd, 1H, $J = 4.5$, 19.1 Hz, NC*H*HCO), 4.51 (d, 1H, $J = 4.8$ Hz, CHN), 4.56 (dq, 1H, $J = 4.8$, 6.3 Hz, CHO), 4.66 (dd, 1H, $J = 6.3$, 19.1 Hz, NCH*H*CO), 5.13 (br s, 1H, NH), 5.20 (s, 2H, OCH_2Ph), 7.29–7.38 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 200 MHz): $\delta = 21.0, 28.1, 44.6, 61.3, 67.9, 74.1, 79.8, 128.2, 128.6, 134.4, 151.7, 155.8, 167.4, 169.9$. $C_{19}H_{24}N_2O_7$ (392.40): calcd C 58.16, H 6.16, N 7.14; found C 58.19, H 6.13, N 7.10.

4.2. H-Gly-L-Oxd-OBn·CF_3CO_2H, 3

A solution of Boc-Gly-L-Oxd-OBn **2** (1 mmol, 0.39 g) and TFA (18 mmol, 1.39 mL) in dry methylene chloride (20 mL) was stirred for 4 h at room temperature, then the volatiles removed under reduced pressure and the product obtained pure in quantitative yield without any further purification. ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 1.51$ (d, 3H, $J = 5.8$ Hz, OCH–CH_3), 4.37–4.66 (m, 4H, NCH_2CO+CHN+CHO), 5.19 (AB, 2H, $J =$ 12.2 Hz, OCH_2Ph), 6.90–8.15 (br s, 4H, COOH+NH_3^+), 7.26–7.38 (m, 5H, Ph); NMR ($CDCl_3$, 200 MHz): $\delta = 20.6$, 43.2, 61.1, 68.7, 75.3, 128.5, 128.9, 129.0, 134.4, 152.1, 167.0, 167.3.

4.3. General method for the hydrogenolysis of the benzyl esters

To a solution of Boc-(Gly-L-Oxd)$_n$-OBn (1 mmol) in methanol (20 mL) was added 10% palladium on charcoal and the mixture stirred under hydrogen atmosphere for 1 h. The catalyst was then filtered on a Celite pad and the mixture concentrated. The corresponding acid

Boc-(Gly-L-Oxd)$_n$-OH was obtained pure in quantitative yield without any further purification.

4.4. General method for the homologation

To a stirred solution of Boc-(Gly-L-Oxd)$_n$-OH (1 mmol) and HBTU (1 mmol, 0.38 g) in dry acetonitrile (10 mL) was added a mixture of H-Gly-L-Oxd-OBn·CF_3CO_2H (1 mmol) and Et_3N (3 mmol, 0.44 mL) in dry acetonitrile (10 mL) at room temperature. The solution was stirred for 40 min, then acetonitrile removed under reduced pressure and was replaced with ethyl acetate. The mixture was washed with brine, 1 M aqueous HCl (3×30 mL) and with 5% aqueous $NaHCO_3$ (1×30 mL), dried over sodium sulphate and concentrated in vacuo. The product was obtained pure after silica gel chromatography (cyclohexane/ethyl acetate 8:2 as eluant).

4.5. Boc-Gly-L-Oxd-OH, 4

Mp = 52–53 °C; $[\alpha]_D^{20} = -15.5$ (*c* 1.0, MeOH); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3397, 1790, 1769, 1734, 1718, 1697\,cm^{-1}$; ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 1.47$ (s, 9H, *t*-Bu), 1.61 (d, 3H, $J = 6.2$ Hz, OCH–CH_3), 4.31–4.41 (m, 1H, NC*H*HCO), 4.51 (d, 1H, $J = 4.8$ Hz, CHN), 4.54–4.78 (m, 2H, CHO+NCH*H*CO), 5.19 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 200 MHz): $\delta = 21.8, 28.2, 44.7, 61.6, 74.7, 80.3, 152.3, 156.4, 170.2, 170.6$. $C_{12}H_{18}N_2O_7$ (302.28): calcd C 47.68, H 6.00, N 9.27; found C 47.71, H 6.01, N 9.29.

4.6. Boc-(Gly-L-Oxd)$_2$-OBn, 5

Yield: 75%. Mp = 146–148 °C; $[\alpha]_D^{20} = -35.5$ (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3430, 1793, 1753, 1713\,cm^{-1}$; ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 1.46$ (s, 9H, *t*-Bu), 1.55 (d, 3H, $J = 6.6$ Hz, OCH–CH_3, L-Oxd^1), 1.56 (d, 3H, $J = 6.6$ Hz, OCH–CH_3, L-Oxd^2), 4.43 (dd, 1H, $J = 4.2$, 16.2 Hz, NC*H*HCO, Gly^2), 4.44 (d, 1H, $J = 4.2$ Hz, CHN, L-Oxd^2), 4.48 (dd, 1H, $J = 4.8$, 18.6 Hz, NC*H*HCO, Gly^1), 4.50 (d, 1H, $J = 4.2$ Hz, CHN, L-Oxd^1), 4.60 (m, NC*H*HCO, Gly^2), 4.60 (dq, 1H, $J = 4.2$, 6.0 Hz, CHO, L-Oxd^2), 4.84 (dd, 1H, $J = 6.6$, 19.2 Hz, NCH*H*CO, Gly^1), 4.89 (dq, 1H, $J = 4.8$, 6.0 Hz, CHO, L-Oxd^1), 5.12 (s, 1H, NH), 5.24 (AB, 2H, $J = 12.0$ Hz, OCH_2Ph), 6.90 (br s, 1H, NH), 7.27–7.42 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 200 MHz): $\delta = 20.3, 20.9, 28.1, 43.7, 60.3, 61.2, 62.3, 68.1, 74.5, 75.3, 80.1, 128.2, 128.6, 134.5, 152.1, 152.5, 156.3, 167.7, 168.5, 168.8, 170.8$. $C_{26}H_{32}N_4O_{11}$ (576.55): calcd C 54.16, H 5.59, N 9.72; found C 54.11, H 5.55, N 9.68.

4.7. Boc-(Gly-L-Oxd)$_2$-OH, 6

Mp = 188–190 °C; $[\alpha]_D^{20} = -65.6$ (*c* 1.0, MeOH); IR (Nujol): $\nu = 3390, 3238, 1792, 1719, 1701\,cm^{-1}$; ^1H NMR (CD_3OD, 200 MHz): $\delta = 1.45$ (s, 9H, *t*-Bu), 1.54 (d, 3H, $J = 6.6$ Hz, OCH–CH_3), 1.55 (d, 3H, $J = 6.2$ Hz, OCH–CH_3), 4.40–4.45 (m, 2H, 2×NC*H*HCO), 4.50–4.58 (m, 2H, 2×NCH*H*CO), 4.61–4.83 (m, 4H, 2CHO+2CHN); ^{13}C NMR (CD_3OD, 200 MHz): $\delta = 20.8, 21.1, 28.7, 44.4, 45.4, 63.6, 76.5, 77.0, 80.7, 154.1, 154.4, 170.0, 171.1, 171.5$. $C_{19}H_{26}N_4O_{11}$ (486.43): calcd C 46.91, H 5.39, N 11.52; found C 46.95, H 5.42, N 11.55.

4.8. Boc-(Gly-L-Oxd)$_3$-OBn, 7

Yield: 55%. Mp = 128–129 °C; $[\alpha]_D^{20} = -49.5$ (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3423, 1793, 1753, 1719, 1693\,cm^{-1}$; ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 1.43$ (s, 9H, *t*-Bu), 1.53 (d, 3H, $J = 7.2$ Hz, OCH–CH_3), 1.55 (d, 6H, $J = 7.2$ Hz, 2×OCH–CH_3), 4.40–4.68 (m, 10H), 4.72–4.81 (m, 2H), 5.21 (AB, 2H, $J = 12.1$ Hz, OCH_2Ph), 5.37 (br s, 1H, NH), 7.20–7.40 (m, 7H, Ph+2NH); ^{13}C NMR ($CDCl_3$, 300 MHz): $\delta = 20.3, 20.5, 20.7, 20.9, 28.2, 43.6, 60.3, 61.3, 62.2, 68.1, 74.5, 75.5, 75.5, 80.0, 128.0, 128.2, 128.7, 134.5, 152.1, 152.7, 156.3, 167.7, 168.7, 169.3, 170.8, 171.1$. $C_{33}H_{40}N_6O_{15}$ (760.26): calcd C 52.10, H 5.30, N 11.05; found C 52.06, H 5.27, N 11.01.

4.9. Boc-(Gly-L-Oxd)$_3$-OH, 8

Mp = 189–191 °C; $[\alpha]_D^{20} = -93.8$ (*c* 0.3, MeOH); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3412, 3340, 1787, 1738, 1716, 1710\,cm^{-1}$; ^1H NMR (CD_3OD, 200 MHz): $\delta = 1.45$ (s, 9H, *t*-Bu), 1.53–1.59 (m, 9H, 3×OCH–CH_3), 4.30–4.83 (m, 12H, 3×CHN+3×CHO+3×NCH_2CO); ^{13}C NMR (CD_3OD, 200 MHz): $\delta = 20.7, 20.8, 21.2, 28.7, 44.5, 45.5, 63.6, 76.6, 77.0, 77.1, 80.7, 154.1, 154.3, 154.4, 158.6, 170.0, 170.1, 171.1, 171.2, 171.6$; $C_{26}H_{34}N_6O_{15}$ (670.58): calcd C 46.57, H 5.11, N 12.53; found C 46.60, H 5.09, N 12.56.

4.10. Boc-(Gly-L-Oxd)$_4$-OBn, 9

Yield: 40%. Mp = 216–219 °C; $[\alpha]_D^{20} = -48.6$ (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3×10^{-3} M): $\nu = 3390, 1792, 1718, 1693\,cm^{-1}$; ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 1.43$ (s, 9H, *t*-Bu), 1.55 (m, 12H, 4×OCH–CH_3), 4.25–4.82 (16H, 4×CHN+4×CHO+4×NCH_2CO), 5.21 (AB, 2H, $J = 12.0$ Hz, OCH_2Ph), 5.47 (br s, 1H, NH), 7.35–7.42 (m, 5H, Ph), 7.78 (br s, 3H, NH); ^{13}C NMR ($CDCl_3$, 300 MHz): $\delta = 20.5, 20.8, 28.3, 43.9, 52.0, 61.3, 62.3, 68.1, 74.5, 75.7, 80.1, 128.3, 128.7, 134.6, 152.2, 152.8, 156.2, 167.7, 168.7, 170.7$; $C_{40}H_{48}N_8O_{19}$ (944.85): calcd C 50.85, H 5.12, N 11.86; found C 50.82, H 5.07, N 11.81.

4.11. Boc-(Gly-L-Oxd)$_4$-OH, 10

Mp = 159–160 °C (dec); $[\alpha]_D^{20} = -55.2$ (*c* 0.5, MeOH); IR (Nujol): $\nu = 3562, 3321, 1789, 1752, 1719, 1683\,cm^{-1}$; ^1H NMR (CD_3OD, 400 MHz): $\delta = 1.44$ (s, 9H, *t*-Bu), 1.53–1.56 (m, 12H, 4×OCH–CH_3), 4.24–4.79 (16H, 4×CHN+4×CHO+4×NCH_2CO); ^{13}C NMR (CD_3OD, 400 MHz): $\delta = 20.7, 20.8, 21.2, 28.7, 44.5, 45.5, 62.8,$

63.5, 63.6, 76.5, 77.0, 77.1, 80.7, 154.1, 154.3, 154.4, 158.6, 170.0, 170.1, 171.1, 171.2, 171.6; $C_{33}H_{42}N_8O_{19}$ (854,73): calcd C, 46.37; H, 4.95; N, 13.11; found C, 46.33; H, 4.91; N, 13.14.

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