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Development of new calcium receptors based on oxazolidin-2-ones containing pseudopeptides

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With the aim of designing a new calcium receptor, the synthesis and the conformational analysis of a small library of dipeptides having the general formula Ac–Oxx–L-Xaa–OBn [Oxx = L-Oxd, (4*S*,5*R*)-4-methyl-5-carboxyoxazolidin-2-one; D-Oxd, (4*R*,5*S*)-4-methyl-5-carboxyoxazolidin-2-one; or D-Oxac (4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid] is reported. Ac–L-Oxd–L-Ala–OBn was identified as the most promising compound by MS–ESI analysis and this outcome was confirmed by photoluminescence spectroscopy.

Introduction

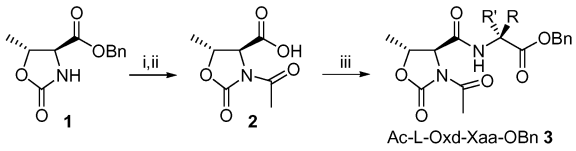
The presence of metal ions in a biological environment is of particular interest for diagnosis and the activity of several biologically active compounds. As an example, some calcium-dependent antibiotics (CDA) have been recently isolated and their activity has been tested: for instance, daptomycin is the first antibiotic of this class to have received approval for clinical use.[1] Thus the development of Ca^{2+} receptors is of general interest, eventually also for the design of new sensors that are extremely important in the study of biological matrixes.[2]

We have recently reported the synthesis of a small library of tetrapeptides containing the L-Oxd [(4*S*,5*R*)-4-methyl-5-carboxyoxazolidin-2-one], the D-Oxd [(4*R*,5*S*)-4-methyl-5-carboxyoxazolidin-2-one] and the D-Oxac [(4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid] moieties.[3] The introduction of these moieties into an oligopeptide chain preferentially induces rigid conformations, so that some selected compounds are able to form helixes or small β-turn secondary structures. Moreover, using mass spectrometry we noticed that most pseudopeptides containing the carboxyoxazolidin-2-one moieties are able to chelate water in the gas-phase,[4] probably owing to the presence of an additional carbonyl in the oxazolidin-2-one ring. It has been recently observed that the presence of cooperative effects between more than one functional group is crucial for the hydration of a polypeptide chain and it has been demonstrated that the gas-phase proteins should be viewed as tools to examine the interactions that are present in solution.[5] From the mass spectra analysis, the presence of a $[M + H_2O]^+$ ion was demonstrated by the presence of a main or an abundant peak for the molecules containing L-pGlu, L-Oxd or D-Oxd. As the same result was obtained when the tests were repeated, avoiding the contact with water, we gathered that the $M + H_2O$ peaks should be ascribed to the presence of a water molecule strongly bonded to the tetrapeptide between two or more functional groups.

As a consequence, we undertook a systematic analysis of some small dipeptides containing an oxazolidin-2-one moiety and an α-amino acid, having the general formula Ac–Oxx–L-Xaa–OBn **3–5** (where Oxx is L-Oxd, D-Oxd or D-Oxac). In order to analyze the behavior of these molecules both common α-amino acids, such as L-alanine (L-Ala) or L-valine (L-Val), and unusual α-amino acids, such as aminoisobutyric acid (Aib), L-isovaline (L-Iva), L-α-methyl–valine [L-(αMe)Val] or L-α-methyl–norvaline [L-(αMe)Nva], have been used. Moreover, we chose as protecting groups the acetyl for the NH moiety and the benzyl for the acid moiety. Preliminary results showed that the nature of the acid protecting group is unimportant and the benzyl was chosen only for convenience. In contrast, we noticed that the presence of the small acetyl moiety favors the chelation: indeed, a less intense $M + H_2O$ peak was obtained when the acetyl moiety was replaced with the pivaloyl group.

Results and discussion

In Scheme 1 the general method for the synthesis of the fully protected dipeptides Ac-L-Oxd–Xaa–OBn **3a–e** is reported, starting from the previously described H–L-Oxd–OBn.[6]



Compound	R	R'
Ac-L-Oxd-L-Ala-OBn **3a**	Me	H
Ac-L-Oxd-Aib-OBn **3b**	Me	Me
Ac-L-Oxd-L-Iva-OBn **3c**	Et	Me
Ac-L-Oxd-L-αMeNva-OBn **3d**	*n*-Pr	Me
Ac-L-Oxd-L-αMeVal-OBn **3e**	*i*-Pr	Me

Scheme 1 Reagents and conditions: (i) AcCl (1 eq.), DIEA (3 eq.), DMAP (0.5 eq.), dry DMF, rt, 16 h; (ii) Pd/C (5%), MeOH, rt, 2 h; (iii) HBTU (1 eq.), H–L-Ala–OBn·HCl (1 eq.), TEA (3 eq.), acetonitrile, rt, 40 min.

A similar approach was used to synthesize Ac–D-Oxd–L-Ala–OBn **4** and Ac–D-Oxac–L-Val–OBn **5** (Fig. 1).

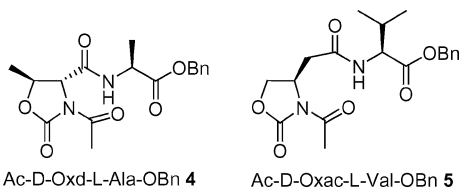


Fig. 1 Dipeptides containing the D-Oxd or the D-Oxac moiety.

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All these molecules have been analyzed by ^{1}H NMR, ^{13}C NMR and IR spectroscopy, and by MS–ESI spectrometry in the absence of calcium ions. The IR spectroscopy furnished information on the formation of NH···OC hydrogen bonds. The IR absorption spectra were obtained using 3 mM solutions in methylene chloride, as at this concentration the intramolecular aggregation is usually unimportant. The absorption bands between 3500 and 3250 cm^{-1} of compounds **3a–e**, **4** and **5** are reported in Fig. 2 in order to check the presence of non-hydrogen bonded amide protons bands (above 3400 cm^{-1}) or hydrogen bonded amide proton bands (below 3400 cm^{-1}).[7]

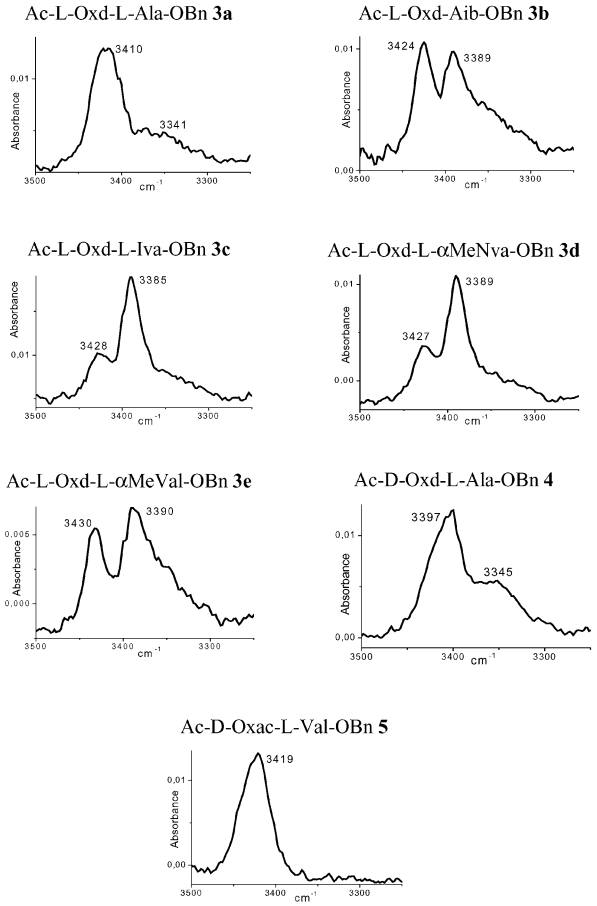


Fig. 2 IR spectra and significant NH stretching bands for compounds **3a–e**, **4** and **5**.

Significantly, we found many different results which account for different conformations, although the tested molecules are very similar. Indeed, the L-Ala and L-Val containing dipeptides (**3a**, **4** and **5**) show no aptitude to form NH···OC hydrogen bonds, while **3b**, **3c**, **3d** and **3e**, which contain α,α-disubstituted amino acids, tend to form NH···OC hydrogen bonds more easily.

In contrast, the ^{1}H NMR spectra of **3a–e**, **4** and **5** did not furnish clear-cut results as they show a very deshielded signal for the methyl group of the acetyl moiety, which always resonates at about 2.5 ppm (compared with 2.02 ppm of *N*-methyl acetamide). As we already observed,[3] this outcome should be attributed to the presence of the heterocycle carbonyl of the oxazolidin-2-one ring, which strongly deshields the hydrogen nearby, thus confirming that the imide bond always assumes a *trans*-conformation even in the presence of a very small side chain, like the acetyl group.

For this reason we choose to have a deeper insight into the conformation of Ac–L-Oxd–L-Ala–OBn **3a** (mostly non chelated NH) and of Ac–L-Oxd–L-Iva–OBn **3c** (mostly chelated NH). An evaluation of the NOESY-1D spectra ($CDCl_3$, 600 MHz) of the compounds **3a** and **3c** was consistent with the IR outcome but did not furnish any additional information. For these molecules we can foresee the conformations reported in Fig. 3.

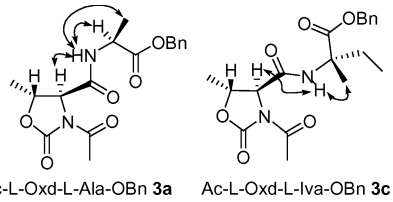


Fig. 3 Preferential conformation of **3a** and of **3c** suggested by IR and ^{1}H NMR data. Significant NOE enhancements of **3a** and **3c** obtained by performing the NOESY-1D experiments on 10 mM solutions in $CDCl_3$ (600 MHz) and using a mixing time of 1 s.

The preferential conformations of **3a** and **3c** have been further detected by investigation of the DMSO-d_6 dependence of NH proton chemical shift.[8] This solvent has a strong hydrogen bonding acceptor character and, if it binds to a free NH proton, it will 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 tetramer solutions in $CDCl_3$. The results for Ac–L-Oxd–L-Ala–OBn **3a** and Ac–L-Oxd–L-Iva–OBn **3c** are reported in Fig. 4 and show that the NH of **3a** is very sensitive to DMSO ($\Delta\delta = 1.80$ ppm), while the NH of **3c** undergoes a lower variation of chemical shift ($\Delta\delta = 1.06$ ppm), thus suggesting that this proton is at least partly hydrogen bonded: this result is in agreement with the IR outcome.

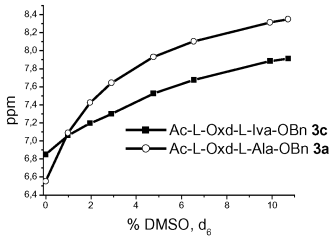


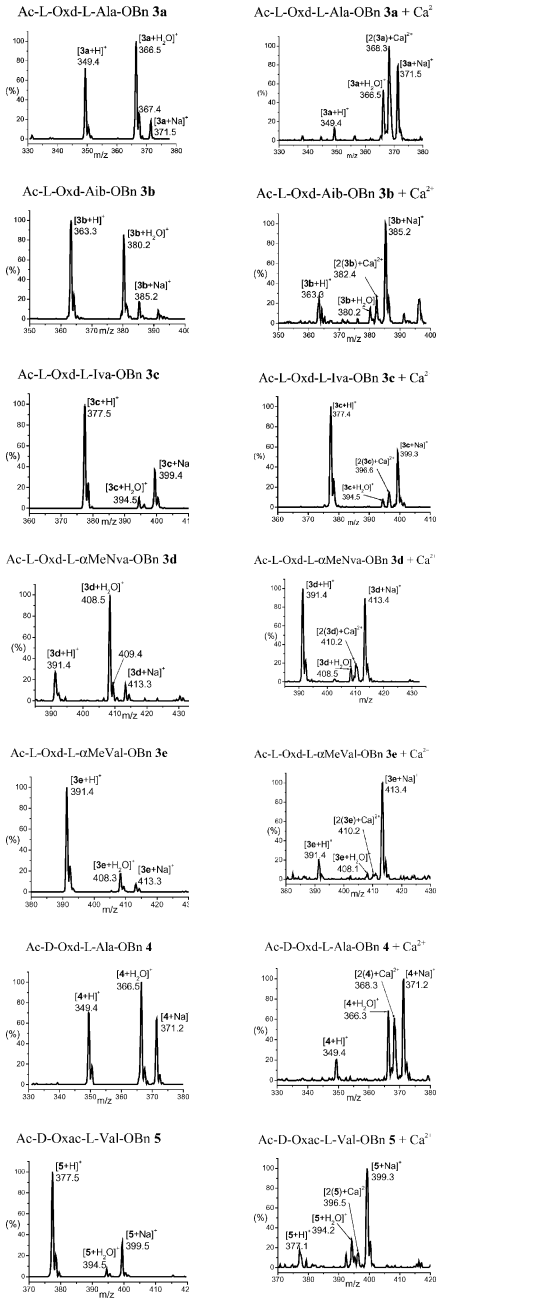
Fig. 4 Variation of NH proton chemical shift (ppm) of Ac–L-Oxd–L-Ala–OBn **3a** and Ac–L-Oxd–L-Iva–OBn **3c** as a function of increasing percentages of DMSO-d_6 to the $CDCl_3$ solution (v/v) (concentration: 1 mM).

As far as calcium complexation is concerned, we expected that a chelation in solution would be better obtained with molecules like **3a**, where the Xaa carbonyls are already displaced in the right position. Indeed, the chelation "skill" of **3a–e**, **4** and **5** has been evaluated by analysis of solutions containing protected dipeptides and calcium ions with a mass spectrometer equipped with an electrospray detector (MS–ESI). We prepared 10^{-3} M solutions of **3a–e**, **4** and **5** each in acetonitrile in the presence of 5 eq. of calcium tetrafluoborate and analyzed them with MS–ESI apparatus. Table 1 shows the results obtained from the MS–ESI analysis of 10^{-3} M solution of dipeptides **3a–e**, **4** and **5** (left) and 10^{-3} M solution of dipeptides **3a–e**, **4** and **5** with 5 eq. of calcium salt (right).

As Table 1 shows, all the spectra on the left side (pure compounds) contain peaks corresponding to M, M + 1, M + Na (M + 23) and M + H_2O (M + 18) in different proportions. The spectra on the right hand side (dipeptide + calcium ions) show the additional presence of a M + 20 peak, which can be attributed to an ion having the formula $(2M + Ca)^{2+}$, suggesting that two molecules of dipeptide can chelate only one calcium ion, although the calcium is in a large excess in the solution. Ions with the formula (M + Ca), producing signals of m/z (M + 40)/2, are totally absent.

The same results have been obtained utilizing 10^{-3} M solutions of **3a–e**, **4** and **5** in a 1 : 1 mixture of H_2O and acetonitrile. The

Table 1 Fragmentation peaks for the MS–ESI analysis of 10^{-3} M solutions of dipeptides **3a–e,4** and **5** and of mixtures of **3a–e**, **4**, **5** and calcium fluoborate. Only the area of the molecular weight is shown



peak at (M + 18) highlights the presence of water, which does not prevent some selected molecules from forming (2M + Ca)/2 ions.

The most intense peak at (2M + Ca)/2 was obtained in the MS–ESI spectrum of **3a** + Ca^{2+}. This outcome is in agreement with what we foresaw from the study of the IR and ^{1}H NMR spectra. Fig. 5 shows a possible chelation model (an hexacoordinate complex) which is in agreement with the experimental data. Indeed, this is perhaps the most common coordination number and the six ligands almost invariably lie at the vertices of an octahedron or a distorted octahedron.[9]

Moreover, this hypothesis is in agreement with the reported cyclo-(Pro–Gly)$_3$, that forms a 2 : 1 complex with Ca^{2+} in which the cation is sandwiched between the two peptide molecules.[10] The Gly carbonyls from each of the peptides are octahedrally coordinated to the cation with an average calcium oxygen coordination distance of 2.26 Å.

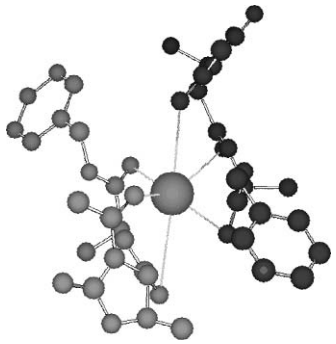


Fig. 5 Hypothetical structure of the complex **3a** + Ca^{2+}, which is in agreement with the IR, ^{1}H NMR and MS–ESI outcome.

A similar result was obtained with Ac–D-Oxd–L-Ala–OBn **4** and in this case also a M + 20 peak was observed, although less intense. This outcome shows that the L-Ala moiety is very important for the receptor design, possibly because more hindered side chains do not favour the useful conformation for the formation of the Ca^{2+} complex.

To prove this hypothesis, a mixture of **3a** and calcium fluoborate in CD_3CN [10^{-3} M solution of **3a**, with increasing amounts of $Ca(BF_4)_2$] was performed by ^{1}H NMR and IR spectroscopy, but no significant variations have been noticed in both sets of spectra. This outcome is not surprising, bearing in mind the results reported on the NMR analysis of the calcium binding effects of lipopetide antibiotic daptomycin.[11] The authors show that no variation of the chemical shifts in the ^{1}H NMR spectra between daptomycin and a mixture of daptomycin and Ca^{2+} has been obtained, but only a broadening of the resonance lines was observed. So, calcium binding induces aggregation of daptomycin but does not noticeably alter the conformation of the peptide.

Interestingly, the complexation process of **3a** with Ca^{2+} can be also monitored by photoluminescence spectroscopy. Fig. 6 shows that a new large and unstructured bond appears in the emission spectrum of Ac–L-Oxd–L-Ala–OBn **3a** between 400 and 600 nm when 0.5 eq. of Ca^{2+} was introduced in the 10^{-3} M solution of **3a** in acetonitrile. On the other hand, further additions of Ca^{2+} did not induce noticeable changes in the emission spectrum. This result corroborates the hypothesis of formation of a complex where the calcium ion is hexacoordinate with two molecules of **3a**. Further studies aiming to insert a suitable flourophore for Ca^{2+} detection *in vivo* are in progress in our laboratory.[12]

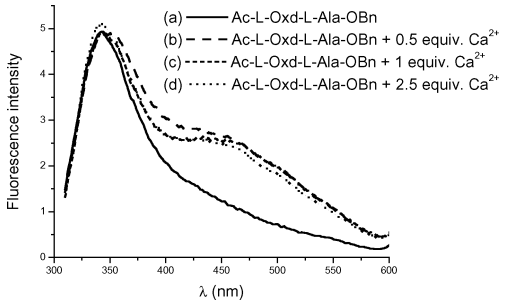


Fig. 6 Flourescence emission of (a) 1 mM Ac–L-Oxd–L-Ala–OBn **3a** in acetonitrile; (b) 1 mM Ac–L-Oxd–L-Ala–OBn **3a** + 0.5 eq. of $Ca(BF_4)_2$ in acetonitrile; (c) 1 mM Ac–L-Oxd–L-Ala–OBn **3a** + 1 eq. of $Ca(BF_4)_2$ in acetonitrile; (d) 1 mM Ac–L-Oxd–L-Ala–OBn **3a** + 2.5 eq. of $Ca(BF_4)_2$ in acetonitrile. The excitation was at 285 nm.

Conclusions

In conclusion, among a small library of dipeptides of the general formula Ac–Oxx–L-Xaa–OBn, Ac–L-Oxd–L-Ala OBn **3a** and Ac–D-Oxd–L-Ala–OBn **4** have the aptitude to chelate

calcium ions in acetonitrile solutions; probably as the presence of the carboxyoxazolidin-2-one moiety facilitates its chelation propensity due to the tendency of the imidic bond to always adopt the *trans*-conformation, which in turn forces the carbonyl group in the right direction. Indeed, as shown by ^{1}H NMR study on daptomicin,[11] the dipeptide conformation before calcium addition is very important.

Further studies on the preparation of oxazolidin-2-ones containing pseudopeptides and their use in the chelation of bivalent metal ions are currently ongoing. Above all, we will focus our attention on the development of pseudopeptides that are selective for similarly sized cations.

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.

H–L-Iva–OH, H–L-(αMe)Nva–OH and H–L-(αMe)Val–OH were prepared according to ref. 13, the others amino acids are commercially available. H–Xaa–OBn·CF_3CO_2H were obtained by deprotection of the corresponding fully protected Boc–Xaa–OBn with trifluoroacetic acid in dichloromethane, following standard procedures.

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 were performed in a nitrogen atmosphere.

High quality ^{1}H NMR spectra (600 MHz) were recorded with a Varian Inova 600. Measurements were carried out in $CDCl_3$ and in DMSO-d_6 using tetramethylsilane as an 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 sec.

Emission and excitation spectra were obtained with a Perkin Elmer LS 55 spectrofluorimeter. Corrections were made as previously reported.[14]

LC–MS Analysis. Acetonitrile and methanol for HPLC were purchased by Riedel-de Haën. All the samples were prepared by diluting 1 mg in 5 mL of a 1 : 1 mixture of H_2O and acetonitrile, in pure acetonitrile or in pure methanol. The samples were analyzed with a liquid chromatography Agilent Technologies HP1100 equipped with a Zorbax Eclipse® XDB-C8 Agilent and Technologies column (flow rate 0.5 mL min^{-1}) and provided with a diode-array UV detector (220 and 254 nm). The MSD1100 mass detector was utilized under the following conditions: mass range 100–2500 uma, positive scanning, energy of fragmentor 50 V, drying gas flow (nitrogen) 10.0 mL min^{-1}, nebulizer pressure 45 psig, drying gas temperature 350 °C, capillary voltage 4500 V.

(4*R*,5*S*)-3-Acetyl-5-methyl-2-oxo-oxazolidine-4-carboxylic acid 2

A solution of acetyl chloride (5.5 mmol, 0.4 mL) in DMF (0.5 mL) was added dropwise to a stirred solution of oxazolidin-2-one (**1**)[15] (5 mmol, 1.18 g), DIEA (15 mmol, 0.6 mL) and DMAP (2.5 mmol, 0.31 g) in dry DMF (3 mL). The mixture was stirred 16 h under nitrogen at rt, then was diluted with ethyl acetate (50 mL), washed with 1 N aqueous HCl (3 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The fully protected oxazolidin-2-one was obtained pure in 95% yield (1.32 g) as an oil after silica gel chromatography (cyclohexane–ethyl acetate, 8 : 2 as eluant).

To a solution of 3-acetyl-5-methyl-2-oxo-oxazolidine-4-carboxylic acid benzyl ester (4 mmol, 1.11 g) in ethyl acetate (20 mL) was added 10% palladium on charcoal (0.10 g) and the mixture was stirred in a Parr apparatus under 3 atm of hydrogen for 1 h. Then, the catalyst was filtered on a celite pad and the mixture was concentrated. The carboxylic acid (**2**) was obtained pure in quantitative yield (0.75 g) without any further purification.

Mp = 146–148 °C; $[\alpha]_D^{20}$ −16.2 (*c* 1.0, acetone); IR (nujol): ν = 3154, 1761, 1742, 1717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.58 (d, 3H, *J* = 6.3 Hz, Me–Oxd), 2.58 (s, 3H, CH_3CO), 4.50 (d, 1H, *J* = 4.5 Hz, CHN), 4.67 (dq, *J* = 4.5, 6.3 Hz, CHN), 4.80 (dq, *J* = 4.4, 6.4 Hz, CHO), 5.19 (AB, *J* = 12.4 Hz, OCH_2Ph), 6.55 (d, 1H, *J* = 6.5 Hz, NH), 7.80–7.98 (bs, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 21.2, 23.1, 61.4, 73.1, 152.0, 170.8, 172.2. Elemental analysis for $C_{17}H_{20}N_2O_6$ (348.4): calcd C 58.61, H 5.79, N 8.04%; found C 58.64, H 5.82, N 8.01%.

General method for the synthesis of ligands 3a–3e

To a stirred solution of Ac–L-Oxd–OH **2** (1 mmol, 187 mg) and HBTU (1 mmol, 0.38 g) in dry acetonitrile (10 mL) was added a mixture of H–Xaa–OBn·CF_3CO_2H (1 mmol) and Et_3N (3 mmol, 0.44 mL) in dry acetonitrile (10 mL) at rt. The solution was stirred 40 min, then acetonitrile was removed under a reduced pressure and was replaced with ethyl acetate. The mixture was washed with brine, 1 N aqueous HCl (3 × 30 mL) and with 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained pure after silica gel chromatography (cyclohexane–ethyl acetate, 9 : 1 to 7 : 3 as eluent) in quantitative yield.

Ac–L-Oxd–L-Ala–OBn 3a. Mp = 139–141 °C; $[\alpha]_D^{20}$ −12.0 (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3410, 3341, 1786, 1739, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.42 (d, 3H, *J* = 7.2 Hz, Me–Ala), 1.45 (d, 3H, *J* = 6.4 Hz, Me–Oxd), 2.54 (s, 3H, CH_3CO), 4.30 (d, 1H, *J* = 4.4 Hz, CHN), 4.62 (dq, *J* = 6.5, 7.2 Hz, CHN–Ala), 4.80 (dq, *J* = 4.4, 6.4 Hz, CHO), 5.19 (AB, *J* = 12.4 Hz, OCH_2Ph), 6.55 (d, 1H, *J* = 6.5 Hz, NH), 7.35–7.41 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 17.7, 20.5, 23.3, 48.6, 62.7, 67.3, 73.5, 128.1, 128.5, 128.6, 135.0, 152.4, 167.0, 171.1, 172.1. Elemental analysis for $C_{17}H_{20}N_2O_6$ (348.4): calcd C 58.61, H 5.79, N 8.04%; found C 58.64, H 5.82, N 8.01%.

Ac–L-Oxd–Aib–OBn 3b. Mp = 95–96 °C; $[\alpha]_D^{20}$ −11.0 (*c* 0.8, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3424, 3389, 1792, 1739, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.38 (d, 3H, *J* = 6.4 Hz, Me–Oxd), 1.53 (s, 3H, Me–Aib), 1.57 (s, 3H, Me–Aib), 2.48 (s, 3H, CH_3CO), 4.28 (d, 1H, *J* = 4.4 Hz, CHN), 4.60 (dq, *J* = 4.4, 6.4 Hz, CHO), 5.12 (AB, *J* = 12.0 Hz, OCH_2Ph), 6.93 (s, 1H, NH), 7.30–7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.5, 23.3, 24.1, 24.7, 56.9, 62.7, 67.4, 73.3, 128.1, 128.3, 128.5, 135.2, 152.5, 166.5, 170.9, 173.6. Elemental analysis for $C_{18}H_{22}N_2O_6$ (362.4): calcd C 59.66, H 6.12, N 7.73%; found C 59.63, H 6.14, N 7.77%.

Ac–L-Oxd–L-Iva–OBn 3c. Mp = 122–124 °C; $[\alpha]_D^{20}$ +1.0 (*c* 0.8, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3428, 3385, 1792, 1733, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.78 (t, 3H, *J* = 7.2 Hz, CH_2–CH_3), 1.48 (d, 3H, *J* = 6.6 Hz, Me–Oxd), 1.63 (s, 3H, Me–Iva), 1.86 (dq, 1H, *J* = 7.8, 13.8 Hz, CHH–CH_3), 2.29 (dq, 1H, *J* = 7.2, 13.8 Hz, CHH–CH_3), 2.57 (s, 3H, CH_3CO), 4.29 (d, 1H, *J* = 4.2 Hz, CHN), 4.72 (dq, *J* = 4.8, 6.6 Hz, CHO), 5.20 (AB, *J* = 12.0 Hz, OCH_2Ph), 6.87 (s, 1H, NH), 7.28–7.40 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 8.2, 20.7, 22.3, 23.3, 61.5, 63.1, 67.6, 73.4, 128.2, 128.5, 128.6, 135.1, 152.4, 166.2, 171.0, 173.7. Elemental analysis for $C_{19}H_{24}N_2O_6$ (376.4): calcd C 60.63, H 6.43, N 7.44%; found C 60.59, H 6.48, N 7.40%.

Ac–L-Oxd–L-αMeNva–OBn 3d. Waxy solid; $[\alpha]_D^{20}$ +5.5 (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3427, 3389, 1789, 1730, 1702 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.84 (t, 3H, *J* = 7.5 Hz, CH_2–CH_2–CH_3), 0.93–1.08 (m, 1H, CH_2–CHH–CH_3), 1.18–1.34 (m, 1H, CH_2–CHH–CH_3), 1.44 (d, 3H, *J* = 6.6 Hz, Me–Oxd), 1.61 (s, 3H, C–αMe), 1.75 (dd, 1H, *J* = 4.8, 13.2 Hz,

C*H*H–CH_2–CH_3), 2.16 (dd, 1H, J = 4.5, 13.2 Hz, CH*H*–CH_2–CH_3), 2.54 (s, 3H, CH_3CO), 4.29 (d, 1H, J = 4.2 Hz, CHN), 4.66 (dq, J = 4.2, 6.6 Hz, CHO), 5.17 (AB, J = 12.0 Hz, OC*H*$_2$Ph), 6.91 (s, 1H, NH), 7.31–7.42 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 13.8, 17.2, 20.7, 22.6, 23.3, 38.9, 60.9, 63.0, 67.6, 73.4, 128.2, 128.5, 128.6, 135.1, 152.4, 166.2, 171.0, 173.7. Elemental analysis for $C_{20}H_{26}N_2O_6$ (390.4): calcd C 61.53, H 6.71, N 7.18%; found C 61.54, H 6.75, N 7.15%.

Ac–L-Oxd–L-αMeVal–OBn 3e. Waxy solid; $[\alpha]_D^{20}$ −12.2 (c 0.9, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3430, 3390, 1786, 1737, 1701, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (d, 3H, J = 6.8 Hz, Me–*i*Pr), 0.94 (d, 3H, J = 6.8 Hz, Me–*i*Pr), 1.38 (d, 3H, J = 6.4 Hz, Me–Oxd), 1.58 (s, 3H, C–αMe), 2.15–2.29 (m, 1H, CH–*i*Pr), 2.54 (s, 3H, CH_3CO), 4.28 (d, 1H, J = 4.0 Hz, CHN), 4.68 (dq, J = 4.8, 6.4 Hz, CHO), 5.14 (AB, J = 12.0 Hz, OC*H*$_2$Ph), 6.78 (s, 1H, NH), 7.31–7.42 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 17.2, 18.5, 20.5, 23.4, 35.0, 62.9, 63.7, 67.3, 73.1, 128.4, 128.5, 128.6, 135.2, 152.4, 166.4, 171.3, 172.7. Elemental analysis for $C_{20}H_{26}N_2O_6$ (390.4): calcd C 61.53, H 6.71, N 7.18%; found C 61.50, H 6.77, N 7.10%.

Ac–D-Oxd–L-Ala–OBn 4. To a stirred solution of Ac–D-Oxd–OH (1 mmol, 187 mg) and HBTU (1 mmol, 0.38 g) in dry acetonitrile (10 mL) was added a mixture of H–L-Ala–OBn·CF_3CO_2H (1 mmol, 0.29 mg) and Et_3N (3 mmol, 0.44 mL) in dry acetonitrile (10 mL) at rt. The solution was stirred 40 min, then acetonitrile was removed under a reduced pressure and was replaced with ethyl acetate. The mixture was washed with brine, 1 N aqueous HCl (3 × 30 mL) and with 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained pure after silica gel chromatography (cyclohexane–ethyl acetate, 7 : 3 as eluent) in 93% yield. Mp = 86–87 °C; $[\alpha]_D^{20}$ −17.1 (c 0.9, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3397, 3345, 1790, 1743, 1697 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.43 (d, 3H, J = 7.2 Hz, Me–Ala), 1.49 (d, 3H, J = 6.3 Hz, Me–Oxd), 2.54 (s, 3H, CH_3CO), 4.41 (d, 1H, J = 3.9 Hz, CHN), 4.60 (dq, J = 6.9, 7.2 Hz, CHN–Ala), 4.85 (dq, J = 3.9, 6.3 Hz, CHO), 5.17 (AB, J = 12.0 Hz, OC*H*$_2$Ph), 6.92 (d, 1H, J = 6.9 Hz, NH), 7.33–7.41 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 17.5, 20.2, 23.0, 48.2, 62.2, 66.9, 73.6, 127.7, 128.1, 128.3, 134.9, 152.5, 167.3, 170.5, 172.0. Elemental analysis for $C_{17}H_{20}N_2O_6$ (348.4): calcd C 58.61, H 5.79, N 8.04%; found C 58.60, H 5.75, N 8.09%.

Ac–D-Oxac–L-Val–OBn 5. To a stirred solution of Ac–D-Oxac–OH[16] (1 mmol, 187 mg) and HBTU (1 mmol, 0.38 g) in dry acetonitrile (10 mL) was added a mixture of H–L-Val–OBn.CF_3CO_2H (1 mmol, 0.32 mg) and Et_3N (3 mmol, 0.44 mL) in dry acetonitrile (10 mL) at rt. The solution was stirred 40 min, then acetonitrile was removed under a reduced pressure and was replaced with ethyl acetate. The mixture was washed with brine, 1 N aqueous HCl (3 × 30 mL) and with 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained pure after silica gel chromatography (cyclohexane–ethyl acetate 7 : 3 as eluent) in 95% yield. Mp = 126–128 °C; $[\alpha]_D^{20}$ +72.6 (c 1.0, CH_2Cl_2); IR (CH_2Cl_2, 3 mM): ν = 3419, 1776, 1731, 1698, 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.84 (d, 3H, J = 6.8 Hz, Me–*i*Pr), 0.89 (d, 3H, J = 6.8 Hz, Me–*i*Pr), 2.05–2.15 (m, 1H, CH–*i*Pr), 2.51 (s, 3H, CH_3CO), 2.55 (dd, 1H, J = 9.6, 14.8 Hz, C*H*HCO), 3.05 (dd, 1H, J = 3.2, 15.2 Hz, CH*H*CO), 4.30 (dd, 1H, J = 3.6, 9.2 Hz, C*H*HO), 4.46 (t, 1H, J = 8.8 Hz, CH*H*O), 4.53 (dd, 1H, J = 4.8, 8.8 Hz, CHN), 4.65–4.78 (m, 1H, CHO), 5.16 (AB, 2 H, J = 12.0 Hz, OC*H*$_2$Ph), 6.33 (d, 1H, J = 8.4 Hz, NH), 7.30–7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 17.9, 19.2, 24.0, 31.3, 37.8, 51.6, 57.4, 67.4, 67.9, 128.6, 128.7, 128.8, 135.4, 153.9, 169.2, 171.0, 171.9. Elemental analysis for $C_{19}H_{24}N_2O_6$ (376.4): calcd C 60.63, H 6.43, N 7.44%; found C 60.67, H 6.40, N 7.42%.

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15 For the preparation of H–L-Oxd–OBn **1**, see ref. 6.

16 For the preparation of Ac–D-Oxac–OH, see ref. 3*d*.