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Structure-Property Relationship Study of *N*-(Hydroxy)Peptides for the Design of Self-Assembled Parallel *β*-Sheets.

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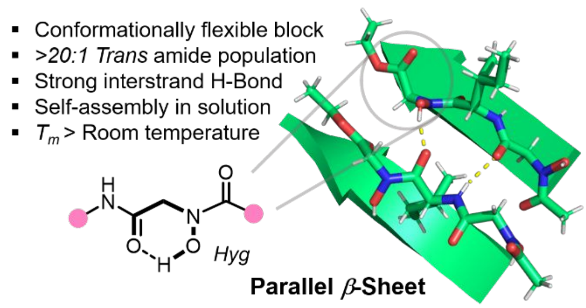
Supporting Information Placeholder

ABSTRACT: The design of novel and functional biomimetic foldamers remains a major challenge in creating mimics of native protein structures. Herein, we report the stabilization of a remarkably short *β*-sheet by incorporating *N*-(hydroxy)glycine (Hyg) residues into the backbone of peptides. These peptide−peptoid hybrids form unique parallel *β*-sheet structures by self-assembly upon hydrogenation. Our spectroscopic and crystallographic data suggest that the local conformational perturbations induced by *N*-(hydroxy)amides are outweighed by a network of strong interstrand hydrogen-bonds.

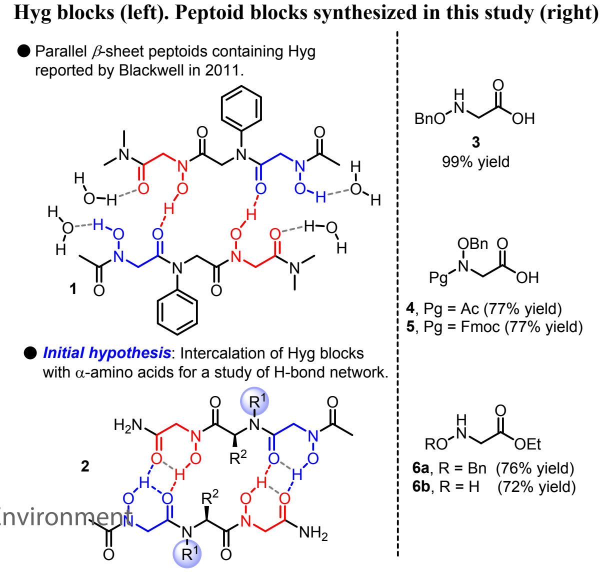


Introduction. In recent years, much interest has been devoted to the development of peptoids (*N*-substituted glycines)[1] due to their proteolytic stability and the ease to introduce various side chains without incorporating the asymmetric α-stereocenters typically found in peptides.[2] A major drawback in controlling these peptoid secondary structures is the lack of hydrogen bonding motifs and the high backbone flexibility of the central glycine methylene. Interestingly, one subclass of peptoid blocks, namely *N*-hydroxy-α-amino acids can be found in numerous bioactive natural products such as aurantimycins, polyoxypeptins, dentigerumycin and penicisulfuranols A−F,[3] and other bacterial siderophores.[4] Despite an intriguing potential arising from the secondary hydroxamate functional group as hydrogen-bond donor and acceptor, as well as metal chelator,[5,6] the notorious lack of stability of this motif (pH-dependent decarboxylation and possible dismutation) has hampered most synthetic studies.[7] Seemingly, only sparse examples of synthetic and structural studies have been reported on the propensity of *N*-(hydroxy)glycine (Hyg) to induce secondary interactions like *β*-turn[8], and sheet–like structures (*e.g.* **1**, Figure 1).[9] Blackwell's study demonstrated that Hyg residues positioned at hydrogen-bonded (inward) sites in peptoid sequences like **1** enabled the formation of anti-parallel *β*-sheets. Therefore, we hypothesized that by intercalating α-amino acids in such peptoids, sturdy *N*-(hydroxy)peptoid−peptide *β*-sheet hybrids (*e.g.* **2**, Figure 1) could potentially be generated. Given the important acidity of *N*-(hydroxyl)amides in comparison to typical amide groups,[10] we became interested in evaluating their hydrogen-bond donor/acceptor abilities to form *β*-sheets. *N*-(hydroxy)[9] and *N*-(alkoxy)peptoids[11] are known to preferentially adopt a *trans*-conformation, which is also appropriate to the design of novel *β*-sheet tertiary structures. Herein, we report the stabilizing effect of Hyg blocks inside a unique intra/inter-molecular hydrogen-bond network for the self-assembly of particularly short *β*-sheets (Figure 1).[12] This work provides a foundation to understand and rationalize the role of *N*-(hydroxy)amides as hydrogen-bond donor/acceptor motifs in the backbone of peptide−peptoid hybrids.

Results and Discussion.

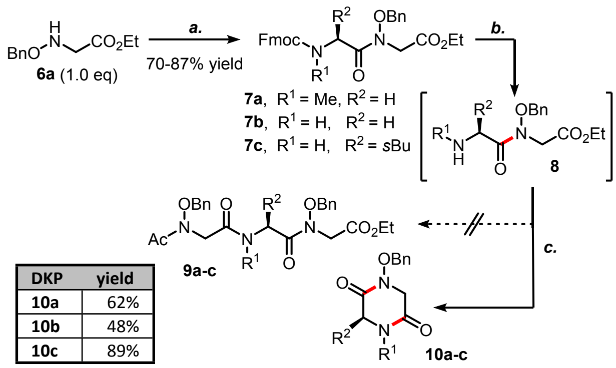
1. Synthesis of peptide-peptoid hybrids. The synthesis of *N*-hydroxy-α-amino acids in both racemic[13] and asymmetric[14] manners have been previously reported; However the insertion of these peptoid blocks into peptides has been fairly scarce.[15] In order to develop a robust synthesis of *N*-(hydroxy)peptoid–

Figure 1. Proposed novel *β*-sheet scaffold built by intercalation of Hyg blocks (left). Peptoid blocks synthesized in this study (right)



peptide hybrids, a series of *N*-(benzyloxy)glycine derivatives **3-6** was prepared with typical protecting groups for liquid- or solid-phase peptide synthesis (Figure 1). For the initial study of *N*-terminal elongation, dipeptide–peptoids **7a-c** were synthesized starting from *N*-(OBn)-Gly-OEt **6a** which was coupled with three different Fmoc-protected amino acids (Scheme 1). As for *N*-alkyl peptoid blocks, *N*-alkoxy residues

Scheme 1. *N*-terminal elongation toward tripeptide–peptoid 9a-c



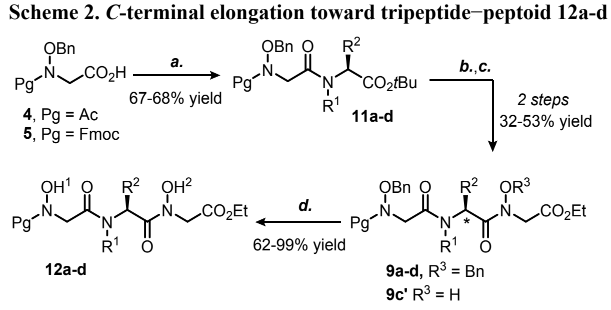
DKP	yield
10a	62%
10b	48%
10c	89%

Reaction conditions: ***a.*** Fmoc-AA-OH (4.0 eq.), HATU (4.0 eq.),HOAt (4.4 eq.), DIEA (4.0 eq.) in CH_2Cl_2/DMF (3:1); ***b.*** Piperidine or $NHEt_2$; ***c.*** **3** (4.0 eq), HATU (4.0 eq.), HOAt (4.4 eq.), DIEA (4.0 eq.) in CH_2Cl_2.

are known to have a low reactivity for amide bond formation, but a cocktail of HATU/HOAt reagents was found suitable to the synthesis of dipeptide–peptoids **7a-c** in 70-87% yields. Compounds **7a-c** were then engaged in a sequence of deprotection/coupling towards the tripeptide–peptoids **9a-c**. Unfortunately, under the large set of conditions tested,[16] *N*-terminal free dipeptide **8** proved difficult to isolate and the coupling towards **9a-c** failed. Instead, large amounts of degradation were observed along with the intramolecular cyclization into diketopiperazines (DKPs) **10a-c**. Although Hyg favors *trans* amide bonds thermodynamically, the low transition-state energy barrier previously reported for the *cis-trans* isomerization of this motif (~16 Kcal/mol)[7b] can explain the ease of forming DKPs. While *N*-(benzyloxy)peptoid oligomers are known to be exclusively in a most stable *trans* amide conformation,[11] the fast kinetics of *cis-trans* isomerization do not allow the *cis* rotamer to be observed on the NMR time scale. To obtain a direct evidence of the genuine *cis-trans* isomerization, we thought to force the equilibrium toward the *cis* rotamer by exploit the chelating nature of the Hyg residue. Dipeptide–peptoid **7c** was therefore hydrogenated to the corresponding Fmoc-Ile-Hyg-OEt dipeptide which was used as chelating ligand to form a gallium(III) complex (3:1 ligand/metal ratio).[17] In this complex, the strong NOESY (nuclear Overhauser effect spectroscopy) ^1H–^1H cross-peak signal between the Ile H_α and the Hyg methylene detected by NMR, supports the presence of a *cis N*-(hydroxy)amide rotamer. Taken together, this spectroscopic evidence of a *cis* rotamer and the facile DKP formation (**8** → **10**) further support that *N*-(hydroxy) and *N*-(benzyloxy)amides undergo fast *cis-trans* isomerization.

To circumvent the formation of DKP **10**, we turned our attention to a strategy of *C*-terminal elongation (Scheme 2). Dipeptides **11a-c** were synthesized by coupling the acetyl Hyg **4** with three different *t*butyl α-amino esters by activation with EDCi or HATU. When similar conditions were tested to couple Fmoc-Hyg **5** with Ile-O*t*Bu, an unexpected cleavage of the benzyl protecting group occurred. The coupling was therefore optimized with EEDQ to deliver dipeptide **11d** without epimerization.[18] A smooth deprotection with TFA lead quantitatively to all the corresponding free carboxylic acid of dipeptides **11a-d** which were directly coupled with **6a** in presence of HATU/HOAt to deliver tripeptide–peptoids **9a-d**. Unfortunately, **9c** was highly epimerized at the Ile α-stereocenter (2:3 dr). To improve the pivotal amide-bond formation, the coupling reaction was further evaluated with the unprotected block **6b**. In this case, the epimerization of tripeptide–peptoids **9c'** could not be detected by ^1H NMR, but the racemization was later confirmed by the presence of a mixture of Ile/allo-Ile residues in the crystal structure of **12c** (Figure 2). *N*-(hydroxy) and *N*-(benzyloxy)glycine blocks are less reactive than typical *N*-methylated amino acids which are already known to be poor nucleophiles in amide bond formation. Due to the oxygen electronegativity and the steric hindrance carried by the hydroxy- and benzyloxy-side chains during the approach to the activated carbonyl π* orbital, such peptoid ligation is particularly slow and difficult, leading ultimately to potential epimerization. To address this issue, several activating cocktails were evaluated for the difficult coupling of **6a**. To our delight, tripeptide–peptoid **9c** was prepared with ~17% of epimerization by using EEDQ as activating agent. Given the electron-withdrawing nature of the *N*-(benzyloxy) side chain, we hypothesized that the *N*-terminal acetyl of **11c** might be unusually basic and participates into the Ile epimerization (see Figure SI-1). Thus by switching to a Fmoc-protecting group in **11d**, the EEDQ-coupling was achieved successfully to produce **9d** in 21% yield with minimal epimerization (~ 3%).[16,18] To circumvent the low coupling yield, further optimizations were required. A cocktail of

Scheme 2. *C*-terminal elongation toward tripeptide–peptoid 12a-d



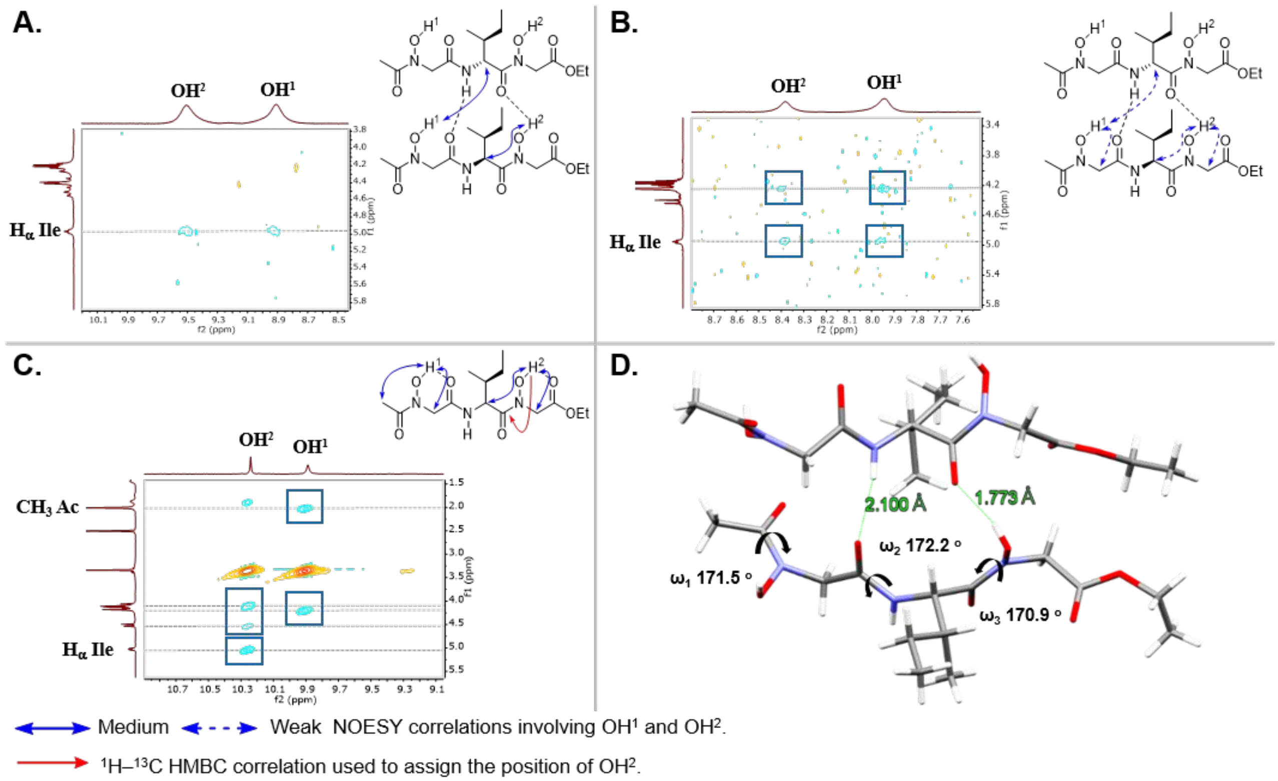
11a: R^1 = Me, R^2 = H, **9b**: R^1 = R^2 = H, **9c**: R^1 = H, R^2 = *s*Bu (*Pg = Ac*)
11d: R^1 = H, R^2 = *s*Bu (*Pg = Fmoc*)

Reaction conditions: ***a.*** from **4** (1.0 eq.), DIPEA (1.5 eq.), for **11a-b**: EDCi (1.2 eq.), HOBt (1.2 eq.) in CH_2Cl_2, for **11c**: H-AA-O*t*Bu (2.0 eq.), HATU (1.5 eq.), HOAt (1.5 eq.) in DMF; for **11d**: from **5** (1.0 eq.), EEDQ (1.2 eq.) in CH_2Cl_2; ***b.*** TFA / CH_2Cl_2 (1:1); ***c.*** for **9a-9c'**: **6a** or **6b** (2.0 eq.), HATU (2.0 eq.), HOAt (2.0 eq.), DIEA (2.0 eq.) in DMF, for **9d**: PivCl (1.2 eq.), lutidine (1.2 eq.) in CH_2Cl_2; ***d.*** Pd/C 10%mol, H_2 in EtOH.

pivaloyl chloride with lutidine (hindered base) provided tripeptide-peptoid hybrid **9d** in 50% yield with low epimerization (< 5%).[16] Finally, benzyl groups from all synthetic tripeptide–peptoids **9a-d**, **9c'** were removed under typical hydrogenation conditions to afford **12a-d** in 79% to 99% yields.

2. Structural analysis of peptide–peptoid hybrids by Nuclear Magnetic Resonance (NMR) spectroscopy. The synthesis of "turnless" *β*-sheets inspired by short protein sequences or by peptidomimetics remains a major challenge.[19] Indeed, motifs capable of enhancing non-bonded interstrand interactions to stabilize artificial short *β*-sheets are rare.[20] To

Figure 2. NOESY data of 12c in the region of the N-OH protons, A. in $CDCl_3$, B. in CD_3CN, and C. in DMSO-d_6. D. X-ray structure of 12c showing the solid-state conformation with interstrand hydrogen bonds represented by dashed lines in the parallel *β*-sheet.

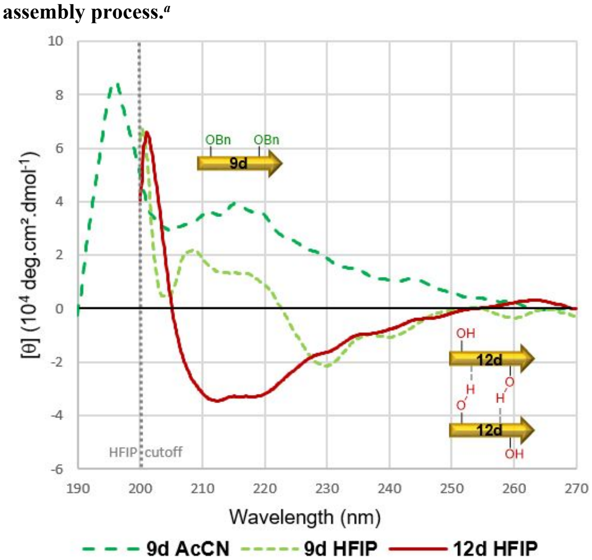


assess the presence of secondary and potential tertiary structures in the synthetic peptide–peptoid hybrids **9a-d**, **9c'** and **12a-d**, a careful solution NMR spectral analysis at 18 °C was performed (Figure 2A-C).[16,21] A combination of ^{1}H, ^{1}H–^{1}H NOESY and ^{1}H–^{13}C heteronuclear multiple-quantum correlation (HMBC) NMRs in solvents of various polarity ($CDCl_3$, CD_3CN, DMSO-d_6) facilitated the conformational and structural analysis of these peptoid–peptide hybrids.[16] The *cis-trans* equilibrium constants for both *N*-(benzyloxy) and *N*-(hydroxy)glycine residues in peptides **7a**, **11a** and **9a** were obtained with $K_{trans/cis}$ values >20 in each case, meaning that independently of the solvent, these amides have an exclusive thermodynamic preference for the *trans* geometry.[16,22] Interestingly, in all constructs **9**, **11-12**, the methylene protons ($H_\alpha/H_{\alpha'}$) of *N*-(hydroxy) or *N*-(benzyloxy)glycine blocks are always highly diastereotopic (doublets with geminal coupling constant of ~17 Hz) with a $\Delta\delta(H_\alpha/H_{\alpha'})$ of ~0.5 ppm, which typically characterize well-folded structures. Each hydroxyl proton OH^1 and OH^2 from Hyg(1) and Hyg(2) residues were easily assigned and differentiated based on distinct NOESY correlations as well as a clear HMBC correlation between OH^2 and the vicinal Ile carbonyl. Given the downfield chemical shifts of both OH^1 (δ 8.36-8.76 & 9.73-9.91 ppm) and OH^2 (δ 8.85-9.44 & 10.19-10.25 ppm) in $CDCl_3$ and DMSO-d_6 respectively, tripeptide–peptoid **9c'** and **12a-d**, hydroxyl hydrogens were determined to participate in inter- or intramolecular hydrogen bonds. A deshielding of ~0.5 ppm between δ OH^1 and OH^2 suggests that the OH^2 hydroxyl is more strongly hydrogen bonded and likely to participate in the *β*-sheet stabilization. As shown in Figure 2C, all NOESY cross-peaks obtained for **12c** in DMSO-d_6 can be attributed to interstrand interactions suggesting that no intermolecular assembly occurs in this solvent. In contrast, in CD_3CN and more markedly in $CDCl_3$ (Figure 2A-B), a stronger NOESY correlation between OH^1 on one strand and the Ile H_α on the second strand supports the formation of a *β*-sheet self-assembly. Indeed, in a pair of facing residues *i,j* on opposite strands from a parallel *β*-sheet (*e.g.* Ile/allo-Ile in **12c,d**) residue *i* is H-bonded to both residues *j-1* and *j+1*, which in this case both are Hyg residues.[23] As established by Abraham, the difference in chemical shits of exchangeable protons, or chemical shift deviation (CSD) due to solvent exposure $\Delta\delta = \delta(\text{DMSO-}d_6) - \delta(CDCl_3)$ can be used to assess the strength of intra- and intermolecular hydrogen bonds for OH and NH groups.[24] The $\Delta\delta(H_N)$ of 0.53 and 0.57 ppm in constructs **12c,d** suggest the presence of relatively strong hydrogen bonds likely arising from an intermolecular interaction between *β*-strands (see Table SI-8). Similarly, $\Delta\delta(OH^2)$ of 0.80 and 0.77 ppm in **12c** and **12d** respectively, supports the existence of hydrogen bonds from the Hyg(2) hydroxyls, while the larger $\Delta\delta(OH^1)$ of 1.13 and 1.11 ppm firmly indicate that OH^1 hydroxyls are more solvent exposed and not engaged in hydrogen bonding.[16] Collectively, the downfield shifts observed for the exchangeable protons (OH/H_N) as well as the small CSD values for both Ile H_N and Hyg(2) OH in constructs **12c,d** suggest that such hydrogens participate in strong interstrand hydrogen bonding. Furthermore, the self-assembly of **12c** was characterized by monitoring the chemical shift deviations of OH/H_N protons as a function of concentration in $CDCl_3$ (Figures SI-12/13).[16,21] In the range of concentrations studied, changes in chemical shifts values fitted well to a dimerization isotherm equation representing the assembly of strands into *β*-sheets.[20a,b] These results also suggest that upon hydrogenation of the *N*-

(benzyloxy)glycine residues, Hyg blocks play an important role in the self-assembly of constructs **12a-d** into short *β*-sheets.

3. X-ray analysis. The structural features of *β*-sheets revealed by the NMR study were further confirmed in the crystal structure of construct **12c** obtained by X-ray analysis (Figure 2D). In this crystal, the parallel *β*-sheets are packed in a head-to-head manner forming columns through a single and relatively weak hydrogen bond OH^1 ••• O=C (*N*-terminal acetyl). Certainly, the low-lying conformation observed in the crystal lattice reflects closely the major conformer of **12c** in solution ($CDCl_3$). Both H_N and OH^2 groups are shown to participate in intermolecular hydrogen bonding to stabilize a parallel *β*-sheet structure. Both Φ and ψ dihedral angles of Hyg(1/2) and Ile residues collected from the crystal structure are in agreement with an extended *β*-sheet arrangement (see Ramachandran plot, Figure SI-14). A notable feature of the parallel *β*-sheet is the nitrogen pyramidalization in each *N*-(hydroxy)amide (ω(OH^1) 171.5 °, and ω(OH^2) 170.9 °). A similar pyramidalization was previously reported by Kirshenbaum[11] and others[25] for *N*-alkoxy peptoids. This phenomenon is likely responsible for decreasing the nitrogen lone pair resonance in the *N*-(hydroxy)amide which in turn accentuates the basicity of the vicinal carbonyl. This stereoelectronic effect likely explains the unique hydrogen-bond acceptor nature of the *N*-(hydroxy)amide carbonyl responsible for the unusual parallel *β*-sheet alignment. In the original report from Blackwell, the central α-amino acid was instead a *N*-aryl peptoid block (*i.e.* construct **1** vs **2**, Figure 1). In such constructs, the central carbonyls were also good hydrogen-bond acceptors, but these peptoid blocks could not participate in intermolecular H-bond, which could justify the preference for an anti-parallel *β*-sheet direction. Our results on peptoid–peptide hybrids **12a-d** complement well Blackwell's study on peptoids in which Hyg residues were positioned at the H-bonded inward sites of antiparallel *β*-sheets.[9] Herein, we demonstrated that *N*-(hydroxy)amide groups can force intermolecular hydrogen bonds with Hyg at both H-bonded and non-H-bonded sites (inward and outward of strands) for stabilizing these novel *β*-sheets with a different parallel directionality.[26]

Figure 3. CD spectra comparison of 9d and 12d showing the self-assembly process.[a]

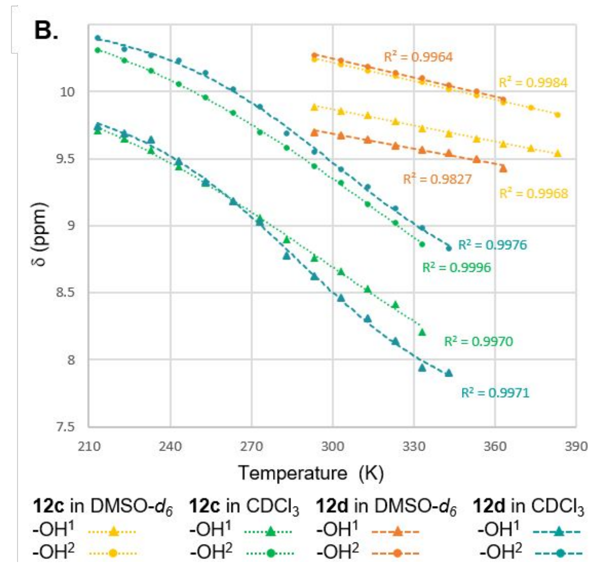


[a] CD spectra of **9d** in acetonitrile (90 mM) and HFIP (103 mM), and of **12d** in HFIP (116 mM) at 0 °C. Samples concentrations were accurately determined by UV-absorption of the fluorene (Fmoc) chromophore.

4. *β*-Sheet self-assembly and thermal stability. Secondary and tertiary peptidyl structures have well-established signatures

Figure 4. A. Temperature dependence of H_N and OH chemical shifts. B. Plots of hydroxyl protons (OH^1/OH^2) chemical shifts as a function of temperature in DMSO-d_6 and $CDCl_3$.

A.	T_C $CDCl_3$ Δδ/ΔT (ppb/K)			T_C DMSO-d_6 Δδ/ΔT (ppb/K)		
Constructs	*NH*	OH^1	OH^2	NH	OH^1	OH^2
9c'	-4.2	N/A	-13.6	-5.9	N/A	-4.6
***trans*-12a**	N/A	-14.2	-17.0	N/A	-6.1	-4.2
***cis*-12a**	N/A	-5.8	-15.8	N/A	-5.5	-4.5
12b	*Not soluble*			-5.3	-4.5	-4.2
12c	-8.2	-12.2	-12.7	-5.6	-4.0	-4.6
12d	-8.7	-15.6	-12.6	-5.5	-3.7	-4.7



in far-UV circular dichroism (CD), but the assignment of bands in peptoids is more challenging due to their important flexibility. *N*-alkoxy peptoid oligomers have been proposed by Kirshenbaum to adopt a polyproline II type (PPII) secondary structure that exhibit two maxima at 197 and 215 ± 5 nm.[27] The CD spectra obtained for tripeptide-peptoid hybrid **9d** present similar bands at 195/216 nm in CH_3CN and 200/218 nm in HFIP, suggesting that a similar PPII secondary structure formed (Figure 3). As shown by the superimposition of CD spectra in Figure 3, the hydrogenation of **9d** into **12d** triggers a dramatic change in the tripeptide–peptoid 3-dimensional structure. Typically, anti-parallel *β*-sheets are characterized by two bands, a positive maximum at 193-195 nm and a negative exciton at 213-218 nm, profoundly resembling the two bands for parallel *β*-sheets at 200-203 and 214-221 nm.[28] Therefore, the combination of a positive exciton at 201 nm and a large CD band overlay at 213-219 nm obtained for construct **12d** conclusively supports the formation of a parallel *β*-sheet. Furthermore, a positive band at 298 nm, characteristic of a π-π* transition was also observed in the near-UV CD spectrum of **12d** (Figure SI-15C).[29] This band arising from an interaction between the proximate fluorenyl π-systems of *N*-terminal Fmoc-groups also support the existence of a parallel arrangement between *β*-strands. Overall, the structural elucidation by CD from the Fmoc interaction at 298 nm and the exciton minima at 213/219 nm distinctively confirm a parallel β-sheet self-assembly of **12d** in solution.

To test the stability of these novel parallel β-sheets, variable temperature NMR spectra (VT-NMR) were recorded between -60 °C and 110 °C (Figure 4). As expected, spectra of tripeptide–peptoids **12c-d** in DMSO-d_6 revealed no evidence of tertiary structure as shown by the small temperature dependence coefficients (T_C, $\Delta\delta/T$ < -4.6 ppb/K) for H_N while both *N*-(hydroxy)amide hydrogens (OH^1/OH^2) might exist in relatively strong intramolecular hydrogen bond (-4.6 < T_C < -4.0 ppb/K).[30,31] The T_C representing the slopes of the best-fitted linear regression of OH^1 and OH^2 chemical shift drifts as a function of temperature were invariably low for all the constructs studied in DMSO-d_6 (Figure 4A and Table SI-7). It can be concluded that a strong intramolecular 6-membered ring hydrogen bond stabilizes each Hyg residue in DMSO-d_6 in single-stranded molecules (Figures SI-6/7).[16] In contrast, the significantly larger $T_C(OH^1)$ and $T_C(OH^2)$ values (-17.0 < T_C < -12.2 ppb/K) in $CDCl_3$ (Figure 4A) indicate that hydrogen bonds are weaker in apolar solvents. These counterintuitive results could be explained by the competition between a strong interstrand H-bond and the innate intramolecular H-bond of Hyg residues (as shown in DMSO-d_6). Competing intra- and intermolecular H-bonds result in a net weakening of the hydroxyls' hydrogen bond network. The results obtained for β-sheets **12c,d** are in line with the interactions reported earlier by Blackwell for a peptoid tetramer β-sheet (*e.g.* **1**, Figure 1). While examining the temperature dependency on the *N*-(hydroxy)amide chemical shifts in $CDCl_3$, it was found that both hydroxyl signals shifted in a non-linear fashion (Figure 4B). The sigmoidal dependence of *N*-(hydroxy)amide protons (OH^1/OH^2) chemical shifts as a function of temperature is distinctive of a β-sheet denaturation transition.[32] The melting curves observed for β-sheets **12c,d** indicate a rupture of folding at elevated temperatures. Thus melting temperatures (T_m) corresponding to the hydrogen-bond network cleavage within the parallel β-sheets of **12c,d** were estimated from the best-fitted curves of thermal transition to the Boltzmann equation.[16] $T_m(OH^1)$ of 23 and 14 ± 5 °C, and $T_m(OH^2)$ of 46 and 29 ± 5 °C were calculated for β-sheets of **12c** and **12d** respectively (Figure 4B, Table SI-6). In both molecules, the significantly higher melting temperatures (> 15 °C) associated with the H-bond rupture of hydroxyl OH^2 is a direct evidence that the interstrand hydrogen bond from Hyg(2) stabilizes the entire structure.

Conclusion. In conclusion, a coupling of the readily available *N*-(hydroxy)glycine peptoid blocks (Hyg) has been developed with low epimerization rate (3-5%) for *C*- and *N*-terminal insertion into peptide–peptoid hybrids. The synthetic constructs self-assembled in solution to form short parallel β-sheets which were characterized by a combination of NMR and CD experiments, and confirmed by the crystal structure of **12c**. A detailed examination of the major differences in NOESY cross-strand correlations, CSDs, T_C and T_m in various solvents clearly highlights the role of *N*-(hydroxy)amides from Hyg residues in the self-assembly and the stabilization of these tertiary structures.[8a] Our results demonstrated that *N*-(hydroxy)amides not only favor exclusively *trans* amide conformers *via* intramolecular H-bonds ($K_{trans/cis}$ >20), but are also amenable to strong interstrand hydrogen bonds in solvents of low polarity. Recent studies from Del Valle have shown that the insertion of *N*-(amino)[33] and *N*-(hydroxy) α-amino acids[15f] at specific non-hydrogen bonded sites (outward positions) can stabilize β-sheets within hairpins. In contrast, the present study establishes that a combination of *N*-(hydroxy)amides at hydrogen-bonded (inward) and non-hydrogen-bonded outward sites can be favorable due to a strong intramolecular and interstrand H-bond network. Given the typical difficulty to create stable β-sheets within short peptide constructs,[19,20] the ability of Hyg to improve hydrogen-bond donor/acceptor properties of proteinogenic backbone amides will certainly find numerous applications in foldamer chemistry. Ongoing studies in our laboratory will leverage the unique stereoelectronic properties of Hyg and other *N*-hydroxy α-amino acids to study the assembly of parallel and anti-parallel β-hairpins in water.[34]

EXPERIMENTAL SECTION

General information. All reagents used in the present paper were acquired from Alfa Aesar, Acros Organics, or Sigma Millipore. All bulk solvents were acquired from Fischer Scientific. Freshly distilled solvents were used in the reactions presented herein. Chloroform was dried over $CaCl_2$ overnight prior to distillation (B.P. 61 °C) and transferred under argon to a dark glass bottle with 3Å molecular sieves for storage. Tetrahydrofuran was purified by refluxing with and distilling from sodium with benzophenone and transferred under argon to a dark glass bottle for storage. Dichloromethane was dried over $CaCl_2$ overnight, prior to distillation (B.P. 40 °C) and transferred under argon to a dark glass bottle with 3Å molecular sieves for storage. Toluene was dried over $CaCl_2$, CaH_2, or $CaSO_4$ and molecular sieves and transferred under argon to a dark glass bottle for storage. Full procedures can be found in Purification of Laboratory Chemicals by Armarego, W. L.F.; and Chai C. L. L. editor (Sixth Edition). Reactions were performed in flame-dried glassware under a positive pressure of argon, unless the reaction occurs in water or aqueous solvent. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise noted. Analytical TLC was performed on 0.25 mm glass backed 60 Å F-254 TLC plates. Flash chromatography was performed using 230-400 mesh silica gels (Silicycle, Inc.). The plates were visualized by exposure to UV light (254 nm) and developed by a solution of phosphomolybdic acid in ethanol, or vanillin/sulfuric acid in ethanol, or ninhydrin in ethanol, or potassium permanganate in water/potassium carbonate/sodium hydroxide or cerium-ammonium-molybdate in water/sulfuric acid and heat. Melting points were determined using an MPA 160 digital melting point apparatus. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectra were performed on a Microflex LRF MALDI-TOF. High Resolution Mass Spectra (HRMS) were obtained from the University of Florida using an Agilent 6230 TOF instrument, using electrospray ionization (ESI). Infrared spectra were recorded on a Nicolet iS10 FT-IR spectrophotometer (Thermo scientific) with a SMART iTX ATR accessory. Optical rotation was measured on a JASCO P-2000 polarimeter.

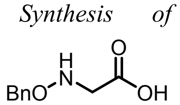
^1H NMR spectra were recorded on a Varian Mercury400 (400 MHz) spectrometer using Vnmrj 4.2 softwar, a Bruker AV-400 Ultrashield (400 MHz) spectrometer using Topspin 3.5 software or Varian Mercury500 (500 MHz) spectrometer using Vnmrj 4.2 software, and are reported in ppm using solvent as an internal standard (C_6D_6 at 7.16 ppm, $CDCl_3$ at 7.26 ppm, CD_3CN at 1.94 ppm, CD_3OD at 3.31 ppm, DMSO-d_6 at 2.50 ppm and 1,4-Dioxane-d_8 at 3.53 ppm). Data are reported as: (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet; coupling constant(s) in Hz, integration).

^{13}C NMR spectra were recorded on Bruker (100 MHz) spectrometer. Chemical shifts are reported in ppm, with solvent resonance employed as the internal standard (C_6D_6 at 128.1 ppm, $CDCl_3$ at 77.2 ppm, CD_3CN at 118.3 ppm and 1.3 ppm, CD_3OD at 49.0 ppm and DMSO-d_6 at 39.5 ppm). NOESY spectra were recorded at 291 K, in the solvents mentioned above, at concentrations of 5-10 mM, with a 300 ms mixing time.

Variable Temperature NMR were recorded on a Varian Mercury400 (400 MHz) spectrometer. The range of temperature studied was -60 °C to 70 °C in $CDCl_3$, -40 °C to 80 °C in CD_3CN, 0 °C to 100 °C in Dioxane-d_8, and 20 °C to 110 °C in DMSO-d_6. Spectra were recorded with 16 scans every 10 °C, with a 5 min stabilization at each temperature before data acquisition. A final spectrum was recorded afterwards at 20 °C to verify the absence of decomposition or transformation of the sample.

CD spectra were recorded on a JASCO J-810 Spectropolarimeter with a temperature controller module JASCO PFD-425S. The samples were prepared in a concentration range between 30 to 200 µM in CH_3CN or HFIP, and the sample concentrations were accurately determined by measuring the sample's absorbance using a JASCO V-670 spectrophotometer based off the UV absorption of the fluorenyl (Fmoc) group (ε_{290} = 6089 $M^{-1}.cm^{-1}$) at 290 nm. Concentrations were obtained based on the Beer law: c = A/(ε•*l*), with c the sample concentration, A the measured absorptivity, ε the molar absorptivity, and *l* the path-length of the cuvette. The raw CD data were recorded in mdeg with 8 scan from 185 nm to 270 nm for the far-UV and 270 to 350 nm for the near UV, every 0.1 nm at a speed of 100 nm per min. The CD spectra of the blank (pure solvent) was recorded and subtracted, while the baseline was set to 0 mdeg between 260 to 270 nm for the far-UV, and 330 to 340 nm for the near-UV. Spectra were smoothed and baseline corrected using the Spectragryph 1.2 software,[35] then converted into molar elipticity ($deg.cm^2.dmol^{-1}$). Products of functionalization **3**, **6a**, **6b**, **SI-1**, and **SI-2** have been reported previously and characterized in references [36d], [36c], [36b], [36e], and [36a] respectively.

Synthesis of N-(benzyloxy)glycine (3). *O*-benzyl hydroxylamine hydrochloride (5.00 g, 31.1 mmol, 1.0 eq.) and glyoxylic acid monohydrate (4.33 g, 47.0 mmol, 1.5 eq.) were solubilized in AcOH/water (1:1 v/v, 100 mL), and stirred at RT during 30 mins (completion monitored by TLC, oxime intermediate, R_f = 0.8, EtOAc/MeOH 1:1 v/v). The mixture was cool down to 0 °C with an ice bath and a solution of sodium cyanoborohydride (7.90 g, 125 mmol, 4.0 eq.) in AcOH/water (1:1 v/v, 50 mL) was added portion wise to avoid by-product formation. The mixture was stirred for 5 h at RT. The reaction completion was monitored by TLC until disappearance of the oxime intermediate. Water was then added and the crude reaction mixture was extracted with a mixture of *i*PrOH/$CHCl_3$ (5 X 20 mL). Combined organic layers were washed with saturated brine (3 X 20 mL), dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure (T < 35 °C) to afford **3** as a white solid (5.60 g, 30.9 mmol, 99% yield). R_f = 0.6 (100% EtOAc); **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_9H_{12}NO_3$, 182.0812; found, 182.0810 (- 1.1 ppm). N-(benzyloxy)glycine **3** was pure enough to be engaged directly in the second step.

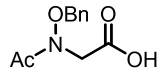


3

$C_9H_{11}NO_3$

MW 181.07 $g.mol^{-1}$

N-acetyl-N-(benzyloxy)glycine (4). Compound **3** (3.00 g, 16.6 mmol, 1.0 eq.) sodium carbonate (1.76 g, 16.6 mmol, 1.0 eq.) were solubilized in water (17 mL). Acetic anhydride (1.86 g, 18.2 mmol, 1.1 eq.) was then added dropwise resulting in a precipitation and a yellow coloration. The mixture was stirred for an additional 30 min at RT. An initial wash using diethyl ether (2 X 20 mL) was achieved directly from the crude reaction mixture, then the aqueous layer was acidified with a solution of 2.0 M HCl to pH ~ 3. The aqueous layer was extracted with dichloromethane (3 X 20 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvent evaporated under reduced pressure to afford product **4** in a pure form as a yellowish powder (2.86 g, 12.8 mmol, 77% yield). R_f = 0.7 (EtOAc/MeOH 7:3 v/v); **m.p.** 109.0-115.0 (± 0.6) °C; **IR** νmax: 2928, 1706, 1596, 1456, 1379, 1260, 964, 911, 847, 749 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ):9.97 (br, 1H), 7.37 (s, 5H), 4.87 (s, 2H), 4.31 (s, 2H), 2.15 (s, 3H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 175.0, 172.5, 134.2, 129.5 (2C), 129.2, 128.8 (2C), 77.5, 49.4, 20.2; **HRMS-ESI** (*m/z*): $[M+Na]^+$ calcd for $C_{11}H_{13}NO_4Na$, 246.0737; found, 246.0728 (-3.7 ppm).

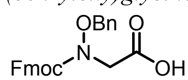


4

$C_{11}H_{13}NO_4$

MW 223.08 $g.mol^{-1}$

N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-(benzyloxy)glycine (5). Compound **3** (1.00 g, 5.52 mmol, 1.0 eq.) and sodium bicarbonate (928 mg, 11.0 mmol, 2.0 eq.) were solubilized in water (10 mL) by stirring 10 min at RT. The mixture was cooled down to 0 °C and (9H-fluoren-9-yl)methyl chloroformate (1.71 g, 6.63 mmol, 1.2 eq.) in dioxane (10 mL) was added to the mixture and stirred for 2 h at 0 °C. The dioxane was evaporated under reduced pressure, then a solution of saturated sodium bicarbonate was added (pH ~ 9) and the aqueous phase was washed with hexanes (20 mL). The resulting aqueous layer was acidified with a solution of HCl (2.0 M) to pH ~ 4 allowing for the product precipitation. The product was extracted with dichloromethane (3 X 20 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvent evaporated under reduced pressure. The crude product was purified by precipitation into petroleum ether at RT and then filtrated to afford product **5** in a pure form as a white powder (1.73 g, 4.29 mmol, 77% yield). R_f = 0.7 (100% EtOAc); **m.p.** 132.0-137.0 (± 0.6) °C; **IR** νmax : 3039, 2954, 1757, 1737, 1666, 1427, 1350, 1248, 1184, 1116, 872, 760, 733 cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD, δ): 7.77 (d, *J* = 7.5 Hz, 2H), 7.64 (d, *J* = 7.4 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.30 (m, 5H), 7.23 (m, 2H), 4.64 (s, 2H), 4.58 (d, *J* = 6.0 Hz, 2H), 4.25 (t, *J* = 6.0 Hz, 1H), 4.02 (s, 2H); **^{13}C{^{1}H} NMR** (100 MHz, DMSO-d_6, δ): 169.9, 157.3, 144.1 (2C), 141.4 (2C), 135.5, 129.9 (2C), 128.9, 128.7 (2C), 128.2 (2C), 127.6 (2C), 125.5 (2C), 120.6 (2C), 76.5, 67.3, 51.6, 47.1; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{22}NO_5$, 404.1492; found, 404.1485 (- 1.7 ppm).

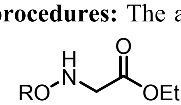


5

$C_{24}H_{21}NO_5$

MW 403.14 $g.mol^{-1}$

Substrates 6a and 6b were prepared by the following procedures: The appropriate alkoxyamine hydrochloride (1.0 mmol, 1.0 eq.), *p*-toluenesulfonic acid (0.1 mmol, 0.1 eq.) and a solution of ethyl glyoxylate (1.1 mmol, 1.1 eq.) in toluene (50% w/w) were mixed in EtOH (1.0 mL), and stirred at RT during 1 h until complete solubilization. The reaction was quenched with addition of a saturated $NaHCO_3$ solution (10

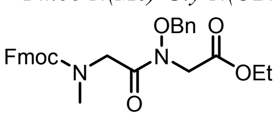


6a: R=Bn, $C_{11}H_{15}NO_3$
6b: R=H, $C_4H_9NO_3$

mL), and the product was extracted using dichloromethane (3 X 40 mL). The combined organic layers were washed with saturated brine (40 mL), dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure to afford the corresponding ethyl glyoxylate oxime, which were pure enough to be engaged directly. Ethyl glyoxylate oxime (1.0 mmol, 1.0 eq.) was dissolved in EtOH (0.10 mL). BH_3.pyr (1.5 mmol, 1.5 eq.) was added to the mixture which was cooled down to 0 °C. An ethanolic solution of HCl (6.0 M, 9.0 eq., freshly prepared by addition of AcCl into EtOH at 0 °C) was added dropwise over a period of 3 h at 0 °C. After the addition of the acid, the mixture was stirred at RT for 30 min (The reaction completion was monitored by TLC until disappearance of the ethyl glyoxylate oxime). Isolations of **6a** and **6b** were achieved as described below. Ethyl (benzyloxy)glycinate **6a:** The reaction was quenched with addition of an aqueous solution of NaOH (2.0 M) at 0 °C and the crude mixture was concentrated under reduced pressure to remove EtOH. The resulting oil was taken into dichloromethane and washed with water (3 X 40 mL). Combined organic layers were washed with saturated brine (3 X 20 mL), dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure to afford product **6a** as a yellowish oil (2.32 g, 11.1 mmol, 76% yield). R_f = 0.5 (hexanes/EtOAc 4:1 v/v); **IR** νmax: 3068, 2923, 2854, 1685, 1451, 1231, 1121, 1002, 956, 758, 738 cm^{-1}; **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ): 171.1, 137.8, 128.4 (3C), 127.9 (2C), 76.1, 61.1, 53.5, 14.2. Ethyl hydroxyglycinate **6b:** The crude mixture was concentrated under reduced pressure. The resulting oil was taken into dichloromethane and solid sodium carbonate was added to the solution at 0 °C. The mixture was then stirred overnight at room temperature. The solvent was filtrated and evaporated under reduced pressure to afford product **6b** as a yellowish oil (2.43 g, 20.4 mmol, 72% yield). R_f = 0.4 (hexanes/EtOAc 3:2 v/v); **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ): 171.1, 61.2, 54.9, 14.2.

General procedure A, for *N-O*-benzyl α-amino ester coupling with *N*-Fmoc α-amino acids: Carboxylic acid **6a** (4.0 mmol, 4.0 eq.) and the secondary amine **Fmoc-AA-OH** (1.0 mmol, 1.0 eq.) were solubilized in CH_2Cl_2 and DMF (3:1; 4.0 mL). HOAt (4.4 mmol, 4.4 eq.) and HATU (4.0 mmol, 4.0 eq.) were added and the solution was stirred 10 min at RT. DIPEA (4.0 mmol, 4.0 eq.) was added to the mixture which was stirred 4-15 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (5.0 mL) was added and the reaction mixture was washed successively with a citric acid solution (10 mL, 5% w/w), then saturated $NaHCO_3$ solution (10 mL) and saturated brine (10 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford the crude product **7a-c** which was purified by chromatography.

*Fmoc-N(Me)-Gly-N(OBn)-Ethyl-Glycinate (**7a**)* was prepared according to the general procedure A, using ethyl *N*-(benzyloxy)glycinate **6a** (50 mg, 0.24 mmol, 1.0 eq.) and **Fmoc-Sar-OH** (149 mg, 0.48 mmol, 2.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 9:1 v/v) to afford **7a** in a pure form as a white foam (104 mg, 0.21 mmol, 87% yield). R_f = 0.6 (hexanes/EtOAc 1:1 v/v); **m.p.** 42-45 °C; **IR** νmax: 2944, 1747, 1702, 1450, 1399, 1372, 1204, 1146, 992, 758, 739, 700 cm^{-1};



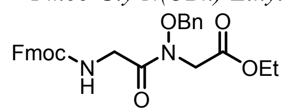
7a

$C_{29}H_{30}N_2O_6$

MW 502.21 g.mol^{-1}

Product **7a** was characterized as a mixture of *cis-trans* rotamers in a ratio 2:3 by ^1H – ^{13}C HSQC: **^1H NMR** (400 MHz, $CDCl_3$, δ) *trans* rotamer: 7.77 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.40 (m, 5H), 7.34 (m, 4H), 4.93 (s, 2H), 4.39 (d, *J* = 7.0 Hz, 2H), 4.29 (s, 2H), 4.26 (s, 2H), 4.24 (t, *J* = 3.3 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.02 (s, 3H), 1.28 (t, *J* = 6.7 Hz, 3H); *cis* rotamer: 7.72 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.40 (m, 5H), 7.34 (m, 4H), 7.16 (m, 2H), 4.73 (s, 2H), 4.43 (d, *J* = 6.6 Hz, 2H), 4.29 (s, 2H), 4.24 (t, *J* = 3.3 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.07 (s, 2H), 2.91 (s, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ) *trans* rotamer: 167.7 (2C), 157.0, 144.1 (2C), 141.3 (2C), 134.3, 129.6 (2C),129.2, 128.8 (2C), 127.6 (2C), 127.1 (2C), 125.2 (2C), 120.0 (2C), 77.3, 67.9, 61.7, 50.6, 49.9, 47.2, 35.6, 14.1; *cis* rotamer: 167.6 (2C), 156.4, 144.2 (2C), 141.3 (2C), 134.1, 129.6 (2C), 129.2, 128.8 (2C), 127.6 (2C), 127.1 (2C), 125.1 (2C), 119.9 (2C), 77.5, 67.3, 61.7, 50.3, 50.1, 47.3, 36.0, 14.1; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{31}N_2O_6$, 503.2177; found, 503.2161 (- 3.2 ppm).

*Fmoc-Gly-N(OBn)-Ethyl-Glycinate (**7b**)* was prepared according to the general procedure A, using ethyl N-(benzyloxy)glycinate **6a** (70 mg, 0.33 mmol, 1.0 eq.) and **Fmoc-Gly-OH** (398 mg, 1.34 mmol, 4.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 70:30 v/v) to afford **7b** in a pure form as a white foam (113 mg, 0.23 mmol, 70% yield). R_f = 0.2 (hexanes/EtOAc 70:30 v/v); **m.p.** 99-101 °C; **IR** νmax: 3376, 2953, 1745, 1691, 1532, 1400, 1277, 1226, 992, 741, 730, 694 cm^{-1}; **^1H NMR** (400 MHz, $CDCl_3$, δ): 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.40 (m, 7H), 7.32 (t, *J* = 7.3 Hz, 2H), 5.50 (br, 1H), 4.91 (s, 2H), 4.39 (d, *J* = 7.2 Hz, 2H), 4.28 (s, 2H), 4.22 (m, 5H), 1.27 (t, *J* = 7.1 Hz, 3H); **$^{13}C\{^1H\}$ NMR** (100 MHz, CD_3OD, δ): 173.1, 167.9, 157.7, 143.9 (2C), 141.2 (2C), 134.5, 129.4 (2C),128.8, 128.4 (2C), 127.4 (2C), 126.8 (2C), 124.9 (2C), 119.5 (2C), 76.9, 66.8, 61.3, 49.1, 47.1, 41.9, 13.0; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{29}N_2O_6$, 489.2020; found, 489.2013 (- 1.4 ppm).

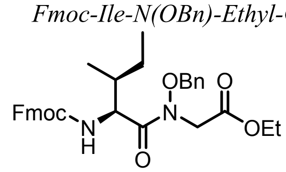


7b

$C_{28}H_{28}N_2O_6$

MW 488.19 g.mol^{-1}

*Fmoc-Ile-N(OBn)-Ethyl-Glycinate (**7c**)* was prepared according to the general procedure A, using ethyl N-(benzyloxy)glycinate **6a** (50 mg, 0.24 mmol, 1.0 eq.) and **Fmoc-Ile-OH** (298 mg, 0.96 mmol, 4.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 80:20 v/v) to afford **7c** in a pure form as a white foam (94 mg, 0.17 mmol, 72% yield). R_f = 0.4 (hexanes/EtOAc 70:30 v/v); **m.p.** 40-44 °C; **IR** νmax: 3057, 2964, 1716, 1660, 1506, 1449, 1264, 1201, 1021, 732, 700 cm^{-1}; **^1H NMR** (400 MHz, $CDCl_3$, δ): 7.47 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 6.5 Hz, 2H), 7.41 (m, 7H), 7.32 (t, *J* = 7.4 Hz, 2H), 5.42 (d, *J* = 9.8 Hz, 1H), 5.05 (dd, *J* = 32.3, 10.1 Hz, 2H), 4.86 (dd, *J* = 9.7, 6.0 Hz, 1H), 4.64 (d, *J* = 17.4 Hz, 1H), 4.40 (m, 2H), 4.25 (t, *J* = 7.2 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.99 (d, *J* = 17.5 Hz, 1H), 1.95 (m, 1H), 1.60 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.16 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H); **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ): 174.0, 167.3, 156.5, 144.0, 143.9, 141.3 (2C), 133.9, 129.6 (2C),129.2, 128.8 (2C), 127.7 (2C), 127.1 (2C), 125.2 (2C), 120.0 (2C), 77.9, 67.1, 61.6,



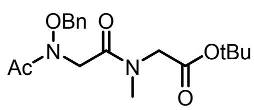
7c

$C_{32}H_{36}N_2O_6$

MW 544.26 g.mol^{-1}

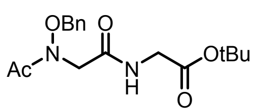
55.4, 48.6, 47.2, 37.3, 23.9, 15.7, 14.1, 11.2; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{32}H_{37}N_2O_6$, 545.2646; found, 545.2631 (- 2.8 ppm).

General procedure B, for the coupling of 4 with *t*-butyl α-amino ester residues: Carboxylic acid **4** (1.0 mmol, 1.0 eq.) and the **H-AA-O*t*Bu** (1.2 mmol, 1.2 eq.) were solubilized in CH_2Cl_2 (5.0 mL) under argon and cooled down to 0 °C. EDCi (1.2 mmol, 1.2 eq.) and HOBt (1.2 mmol, 1.2 eq.) were added to the mixture. DIPEA (2.0 mmol, 2.0 eq.) was added to the mixture which was stirred 15 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (5.0 mL) was added and the mixture was washed successively with a citric acid solution (10 mL, 5% w/w), then saturated $NaHCO_3$ solution (10 mL) and saturated brine (10 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford the crude product **11a-b** which was purified by chromatography.



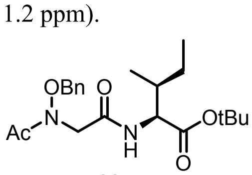
11a
$C_{18}H_{26}N_2O_5$
MW 350.18 g.mol^{-1}

Ac-N(OBn)-Gly-N(Me)-Tert-Butyl-Glycinate ***(11a)*** was prepared according to the general procedure B, using N-acetyl-N-(benzyloxy)glycine **4** (100 mg, 0.45 mmol, 1.0 eq.) and **H-Sar-O*t*Bu** (98 mg, 0.54 mmol, 1.2 eq.). The crude product was purified by chromatography (Ether/EtOAc 1:1 v/v) to afford **11a** in a pure form as an uncolored oil (106 mg, 0.30 mmol, 67% yield). $\boldsymbol{R_f}$ = 0.2 (hexanes/EtOAc 1:1 v/v); **IR** (dry film) νmax: 2980, 2937, 2865, 1737, 1658, 1454, 1367, 1227, 1154, 1119, 1055, 1032, 1013, 846, 747, 700 cm^{-1}; Product **11a** was characterized as a mixture of *cis-trans* rotamers in a ratio 2:5 by $^1H - ^{13}C$ HSQC: ^{1}**H NMR** (400 MHz, $CDCl_3$, δ) *trans* rotamer: 7.39 (m, 5H), 4.93 (s, 2H), 4.41 (s, 2H), 4.04 (s, 2H), 2.99 (s, 3H), 2.16 (s, 3H), 1.45 (s, 9H); *cis* rotamer: 7.39 (m, 5H), 4.93 (s, 2H), 4.33 (s, 2H), 3.91 (s, 2H), 2.98 (s, 3H), 2.14 (s, 3H), 1.48 (s, 9H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ) *trans* rotamer: 175.1, 168.0, 167.2, 134.9, 129.5 (2C),128.9, 128.7 (2C), 82.0, 77.3, 50.3, 50.0, 35.6, 28.1, 20.4; *cis* rotamer: 175.1, 167.7, 167.4, 134.8, 129.5 (2C),128.9, 128.7 (2C), 82.9, 77.2, 52.1, 49.8, 35.4, 28.0 (3C), 20.4; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{27}N_2O_5$, 351.1914; found, 351.1911 (- 0.9 ppm).



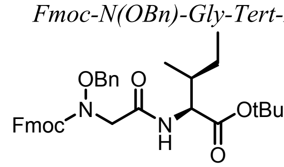
11b
$C_{17}H_{24}N_2O_5$
MW 336.17 g.mol^{-1}

Ac-N(OBn)-Gly-Tert-Butyl-Glycinate ***(11b)*** was prepared according to the general procedure B, using N-acetyl-N-(benzyloxy)glycine **4** (500 mg, 2.24 mmol, 1.0 eq.) and **H-Gly-O*t*Bu** (376 mg, 2.69 mmol, 1.2 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 1:1 v/v) to afford **11b** in a pure form as a yellowish oil (604 mg, 1.80 mmol, 67% yield). $\boldsymbol{R_f}$ = 0.3 (hexanes/EtOAc 1:1 v/v); **IR** (dry film) νmax: 3288, 2980, 2941, 2865, 1736, 1685, 1620, 1545, 1454, 1404, 1364, 1223, 1154, 1055, 1033, 1016, 961, 853, 753, 697, 641 cm^{-1}; ^{1}**H NMR** (400 MHz, $CDCl_3$, δ): 7.39 (s, 5H), 6.61 (br, 1H), 4.93 (s, 2H), 4.30 (s, 2H), 3.93 (d, J = 5.1 Hz, 2H), 2.14 (s, 3H), 1.46 (s, 9H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 173.8, 168.5, 167.8, 134.1, 129.5 (2C),129.2, 128.8 (2C), 82.4, 77.4, 51.9, 42.0, 28.0 (3C), 20.3; **HRMS-ESI** (*m/z*): $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_5$, 337.1758; found, 337.1754 (- 1.2 ppm).



11c
$C_{21}H_{32}N_2O_5$
MW 392.23 g.mol^{-1}

Ac-N(OBn)-Gly-Tert-Butyl-Isoleucinate ***(11c)***. N-acetyl-N-(benzyloxy)glycine **4** (700 mg, 3.14 mmol, 1.0 eq.), HATU (1.79 g, 4.71 mmol, 1.5 eq.) and HOAt (641 mg, 4.71 mmol, 1.5 eq.) were solubilized in DMF (8.0 mL) under argon. DIPEA (609 mg, 4.71 mmol, 1.5 eq.) was added to the mixture which was preactivated 5 min at RT. **H-Ile-O*t*Bu** (1.40 g, 6.28 mmol, 2.0 eq.) was then added and stirred 15 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (10 mL) was added and the mixture was washed successively with a citric acid solution (15 mL, 5% w/w), then saturated $NaHCO_3$ solution (15 mL) and saturated brine (15 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford the crude product **11c** in a pure form as a yellow oily solid (841.5 mg, 2.1 mmol, 68% yield). $\boldsymbol{R_f}$= 0.4 (hexanes/EtOAc 1:1 v/v); $[\alpha]_D^{18}$ -21.7 (*c* 1.00, MeOH); **IR** (dry film) νmax: 3358, 3276, 2962, 2932, 2873, 1735, 1720, 1689, 1650, 1556, 1536, 1448, 1412, 1391, 1365, 1287, 1257, 1151, 1133, 1079, 1028, 1009, 973, 948, 809, 737, 697 cm^{-1}; ^{1}**H NMR** (400 MHz, $CDCl_3$, δ): 7.38 (s, 5H), 6.66 (d, J = 8.1 Hz, 1H), 4.92 (s, 2H), 4.47 (dd, J = 8.5, 4.5 Hz, 1H), 4.34 (d, J = 16.5 Hz, 1H), 4.23 (d, J = 16.5 Hz, 1H), 2.14 (s, 3H), 1.87 (m, 1H), 1.58 (m, 1H), 1.45 (s, 9H), 1.15 (m, 1H), 0.92 (t, J = 7.4 Hz, 3H), 0.89 (d, J = 6.9 Hz, 3H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 173.8, 170.5, 167.4, 134.1, 129.4 (2C),129.1, 128.8 (2C), 82.1, 77.2, 56.9, 52.0, 38.1, 28.1 (3C), 25.3, 20.3, 15.3, 11.7; **HRMS-ESI** (*m/z*): $[M+Na]^+$ calcd for $C_{21}H_{32}N_2O_5Na$, 415.2203; found, 415.2204 (+ 0.2 ppm).

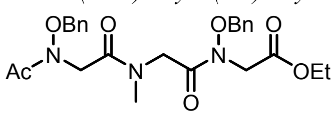


11d
$C_{34}H_{40}N_2O_6$
MW 572.29 g.mol^{-1}

Fmoc-N(OBn)-Gly-Tert-Butyl-Isoleucinate ***(11d)***. N-Fmoc-N-(benzyloxy)glycine **5** (500 mg, 1.24 mmol, 1.0 eq.) and EEDQ (460 mg, 1.86 mmol, 1.5 eq.) were solubilized in CH_2Cl_2 (3.0 mL) under argon. The mixture was preactivated 5 min at RT. **H-Ile-O*t*Bu** (832 mg, 3.72 mmol, 3.0 eq.) was then added and stirred 5 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (3.0 mL) was added and the mixture was washed successively with a citric acid solution (10 mL, 5% w/w), then saturated $NaHCO_3$ solution (10 mL) and saturated brine (10 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford the crude product **11d** in a pure form as a yellow oily solid (618 mg, 1.08 mmol, 87% yield). $\boldsymbol{R_f}$= 0.3 (hexanes/EtOAc 4:1 v/v); $[\alpha]_D^{18}$ -10.1 (*c* 1.00, MeOH); **IR** (dry film) νmax: 3343, 3067, 2965, 2936, 2880, 1725, 1685, 1523, 1451, 1414, 1392, 1368, 1340, 1249, 1216, 1143, 1092, 984, 912, 846, 757, 738, 698, 621 cm^{-1}; ^{1}**H NMR** (400 MHz, $CDCl_3$, δ): 7.75 (d, J = 7.5 Hz, 1H), 7.62 (m, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.32 (m, 7H), 6.55 (d, J = 8.4 Hz, 1H), 4.85 (s, 2H), 4.59 (dd, J = 6.6, 2.8 Hz, 2H), 4.48 (dd, J = 8.5, 4.5 Hz, 1H), 4.28 (t, J = 6.6 Hz, 1H), 4.14 (d, J = 17.2 Hz, 1H), 4.03 (d, J = 17.2 Hz, 1H), 1.84 (m, 1H), 1.42 (s, 9H), 1.40 (m, 1H), 1.11 (m, 1H), 0.86 (m, 6H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 170.5, 167.4, 157.5, 143.5 (2C), 141.4 (2C) 134.9, 129.4 (2C),128.7, 128.5 (2C), 127.8 (2C), 127.2 (2C), 125.1 (2C), 120.0 (2C), 82.1, 77.2, 68.3, 56.8, 54.3, 47.1, 38.2, 28.0 (3C), 25.3, 15.3, 11.7; **HRMS-ESI** (*m/z*): $[M+Na]^+$ calcd for $C_{34}H_{40}N_2O_6Na$, 595.2779; found, 595.2763 (- 2.7 ppm).

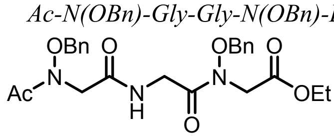
General procedure C, for the coupling of 6a or 6b with 11a-c: *t*-Butyl protected dipeptide **11a-c** (1.0 mmol, 1.0 eq.) was solubilized in dichloromethane (5.0 mL) and cooled down to 0 °C. Trifluoroacetic acid (5.0 mL) was added dropwise, the mixture was then stirred at 0 °C for 3-5 h. The reaction progress was monitored by TLC. The solvent and residual by-products were evaporated under reduced pressure to afford the desired unprotected acid dimer. The resulting product and the ethyl (benzyloxy)glycinate **6a** or ethyl hydroxyglycinate **6b** (2.0 mmol, 2.0 eq.) were solubilized in CH_2Cl_2 or DMF (5.0 mL). Then HOAt (2.0 mmol, 2.0 eq.) and HATU (2.0 mmol, 2.0 eq.) were added, and the solution was stirred 10 min at RT. DIPEA (2.0 mmol, 2.0 eq.) was added dropwise to the mixture which was stirred 15 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (5.0 mL) was added and the mixture was washed successively with a citric acid solution (10 mL, 5% w/w), then saturated $NaHCO_3$ solution (10 mL) and saturated brine (10 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford crude products **9a-d** which were further purified by chromatography.

Ac-N(OBn)-Gly-N(Me)-Gly-N(OBn)-Ethyl-Glycinate ***(9a)*** was prepared according to the general procedure C, using N-(benzyloxy)glycinate **6a** (740 mg, 3.54 mmol, 2.0 eq.) and peptide **11a** (520 mg, 1.77 mmol, 1.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 7:3 v/v) to afford **9a** as a white foam (277 mg, 0.57 mmol, 32% yield). $\boldsymbol{R_f}$ = 0.3 (hexanes/EtOAc 3:7 v/v); **m.p.** 47-49 °C; **IR** νmax: 2931, 1743, 1658, 1454, 1373, 1204, 1021, 973, 844, 733, 700 cm^{-1}; Product **9a** was characterized as a mixture of *cis-trans* rotamers in a ratio 1:2 by ^{1}H – ^{13}C HSQC: ^{1}**H NMR** (400 MHz, $CDCl_3$, δ) *trans* rotamer: 7.38 (m, 5H), 4.92 (s, 2H), 4.91 (s, 2H), 4.42 (s, 2H), 4.38 (s, 2H), 4.23 (s, 2H), 4.18 (m, 2H), 2.97 (s, 3H), 2.16 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H); *cis* rotamer: 7.38 (m, 5H), 4.88 (s, 2H), 4.84 (s, 2H), 4.33 (s, 2H), 4.18 (m, 4H), 3.98 (br, 2H), 2.80 (s, 3H), 2.10 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ) *trans* rotamer: 175.0, 172.3, 167.7, 167.5, 134.9, 134.2, 129.6 (2C), 129.5 (2C), 129.2, 129.0, 128.8 (2C), 128.7 (2C) 77.6 (2C), 61.6, 50.1 (2C), 49.6, 35.9, 20.4, 14.1; *cis* rotamer: 175.0, 173.3, 167.8, 167.4, 134.9, 134.1, 130.1 (2C), 129.6 (2C), 128.9, 128.8 (2C), 128.7, 128.5 (2C), 82.9, 77.0, 76.9, 61.9, 51.0, 50.1, 49.2, 35.4, 20.3, 14.1; **HRMS-ESI** (*m/z*): [M+H]$^+$ calcd for $C_{25}H_{32}N_3O_7$, 486.2235; found 486.2214 (- 4.3 ppm).



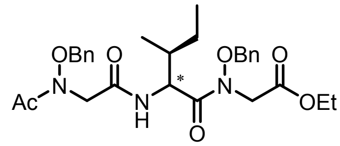
9a
$C_{25}H_{31}N_3O_7$
MW 485.22 g.mol^{-1}

Ac-N(OBn)-Gly-Gly-N(OBn)-Ethyl-Glycinate ***(9b)*** was prepared according to the general procedure C, using *N*-(benzyloxy)glycinate **6a** (242 mg, 1.16 mmol, 2.0 eq.) and peptide **11b** (171 mg, 0.58 mmol, 1.0 eq.). The crude product was purified by precipitation into petroleum ether and filtration to afford **9b** as a beige solid (127 mg, 0.27 mmol, 46% yield). $\boldsymbol{R_f}$ = 0.3 (hexanes/EtOAc 3:7 v/v); **m.p.** 91-96 °C; **IR** νmax: 3239, 2979, 2953, 1745, 1695, 1674, 1644 1544, 1455, 1403, 1372, 1240, 1207, 1054, 1033, 1007, 972, 737 cm^{-1}; ^{1}**H NMR** (400 MHz, $CDCl_3$, δ): 7.38 (s, 10H), 6.76 (br, 1H), 4.91 (d, J = 10.7 Hz, 2H), 4.29 (s, 2H), 4.26 (d, J = 4.6 Hz, 2H), 4.24 (s, 2H), 4.18 (q, J = 7.2 Hz, 2H), 2.15 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 174.1, 172.5, 167.8, 167.3, 134.2, 133.9, 129.5 (4C),129.3, 129.1, 128.9 (2C), 128.8 (2C), 77.8, 77.5, 61.8, 51.8, 50.0, 41.3, 20.4, 14.1; **HRMS-ESI** (*m/z*): [M+H]$^+$ Calcd for $C_{24}H_{30}N_3O_7$, 472.2078; found, 472.2061 (- 3.6 ppm).



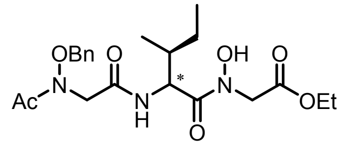
9b
$C_{24}H_{29}N_3O_7$
MW 471.20 g.mol^{-1}

Ac-N(OBn)-Gly-Ile-N(OBn)-Ethyl-Glycinate ***(9c)*** was prepared according to the general procedure C, using *N*-(benzyloxy)glycinate **6a** (363 mg, 1.74 mmol, 2.0 eq.) and peptide **11c** (292 mg, 0.87 mmol, 1.0 eq.). The crude product was purified by precipitation into petroleum ether and filtration to afford **9c** as an orange powder in a mixture of diastereoisomer (189 mg, 0.44 mmol, 41% yield, d.r. 3:2). $\boldsymbol{R_f}$ = 0.7 (hexanes/EtOAc 3:7 v/v); **m.p.** 117-120 °C; **IR** νmax: 3279, 2964, 2933, 1751, 1637, 1544, 1454, 1390, 1375, 1212, 1019, 972, 840, 750, 699 cm^{-1}; ^{1}**H NMR** (400 MHz, $CDCl_3$, δ) *(S, S) isomer*: 7.44 (m, 10H), 6.88 (d, J = 9.3 Hz, 1H), 5.10 (dd, J = 9.3, 6.7 Hz, 1H), 5.00 (m, 2H), 4.93 (s, 2H), 4.58 (d, J = 8.3 Hz, 1H), 4.33 (d, J = 3.5 Hz, 2H), 4.17 (m, 3H), 2.12 (s, 3H), 1.90 (m, 1H), 1.55 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.13 (m, 1H), 0.94 (d, J = 6.9 Hz, 3H), 0.86 (m, 3H); *(R, S) isomer*: 7.44 (m, 10H), 6.83 (d, J = 8.9 Hz, 1H), 5.19 (dd, J = 9.3, 4.3 Hz, 1H), 5.01 (s, 2H), 5.00 (m, 2H), 4.63 (d, J = 8.4 Hz, 1H), 4.33 (d, J = 3.5 Hz, 2H), 4.17 (m, 3H), 2.12 (s, 3H), 1.97 (m, 1H), 1.39 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.20 (m, 1H), 0.87 (m, 3H), 0.84 (m, 3H); ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ) *(S, S) isomer*: 173.7, 172.9, 167.7, 167.4, 135.0, 134.4, 129.7 (2C),129.6 (2C), 128.8, 128.7 (2C), 128.6 (2C), 77.2, 76.5, 61.3, 53.0, 50.2, 48.1, 36.9, 23.9, 19.8, 15.0, 13.5, 10.4; *(R, S) isomer*: 173.7, 173.2, 167.7, 167.6, 135.0, 134.3, 129.7 (2C),129.6 (2C), 129.0, 128.7 (2C), 128.6 (2C), 77.1, 76.5, 61.3, 51.9, 50.2, 48.2, 36.3, 26.4, 19.8, 15.0, 13.5, 11.1; **HRMS-ESI** (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{38}N_3O_7$, 528.2704; found, 528.2681 (- 4.4 ppm).



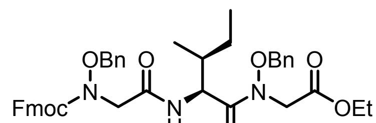
9c
$C_{28}H_{37}N_3O_7$
MW 527.26 g.mol^{-1}

Ac-N(OBn)-Gly-Ile-N(OH)-Ethyl-Glycinate ***(9c')*** was prepared according to the general procedure C, using N-hydroxyglycinate **6b** (283 mg, 2.38 mmol, 2.0 eq.) and peptide **11c** (400 mg, 1.19 mmol, 1.0 eq.). The crude product was purified by precipitation into petroleum ether and filtration to afford **9c'** as a light brown solid (231 mg, 0.53 mmol, 53% yield). $\boldsymbol{R_f}$ = 0.4 (hexanes/EtOAc 3:7 v/v); $[\alpha]_D^{18}$ -14.1 (*c* 1.00, MeOH); **m.p.** 118-120 °C; **IR** νmax: 3280, 2968, 2934, 1753, 1655, 1637, 1541, 1372, 1245, 1201, 1018, 737, 696, 621 cm^{-1}; ^{1}**H NMR** (400 MHz, CD_3CN, δ): 8.38 (s, 1H), 7.43 (m, 5H), 6.88 (d, J = 8.9 Hz, 1H), 4.97 (dd, J = 9.1, 6.5 Hz, 1H), 4.92 (s, 2H), 4.44 (d, J = 17.5 Hz, 1H), 4.29 (d, J = 2.1 Hz, 2H), 4.25 (d, J = 17.5 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 2.11 (s, 3H), 1.97 (m, 1H), 1.52 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.11 (m, 1H), 0.94 (d, J = 6.9 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}**C{^{1}H} NMR** (100 MHz, CD_3CN, δ): 173.8, 171.9, 167.9, 167.7, 134.9, 129.6 (2C),128.8, 128.6 (2C), 76.5, 61.2, 53.3, 50.1 (2C), 36.5, 23.8, 19.7, 15.1, 13.5, 10.6; **HRMS-ESI** (*m/z*): [M+H]$^+$ calcd for $C_{21}H_{32}N_3O_7$, 438.2235; found, 438.2214 (- 4.8 ppm).



9c'
$C_{21}H_{31}N_3O_7$
MW 437.22 g.mol^{-1}

Fmoc-N(OBn)-Gly-Ile-N(OBn)-Ethyl-

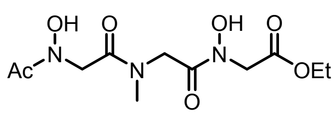


9d
$C_{41}H_{45}N_3O_8$
MW 707.32 g.mol^{-1}

Glycinate (9d). Peptide **11d** (190 mg, 0.37 mmol, 1.0 eq.), lutidine (0.03 mL, 0.44 mmol, 1.2 eq.) and **6a** (308 mg, 1.47 mmol, 4.0 eq.) were solubilized in CH_2Cl_2 (1.0 mL) under argon. ClCO*t*Bu (0.05 mL, 0.44 mmol, 1.2 eq.) was then added and stirred 15 h at RT. The reaction progress was monitored by TLC. CH_2Cl_2 (3.0 mL) was added and the mixture was washed successively with a citric acid solution (3 X 5.0 mL, 5% w/w), then saturated $NaHCO_3$ solution (3 X 5.0 mL) and saturated brine (5.0 mL). The organic layer was dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure to afford the crude product **9d** in a pure form as a yellowish oily gum (135 mg, 0.19 mmol, 51% yield). $\boldsymbol{R_f}$ = 0.65 (hexanes/EtOAc 6:4 v/v); $[\alpha]_{\mathbf{D}}^{\mathbf{18}}$ -7.8 (*c* 1.00, MeOH); **IR** (dry film) νmax: 3320, 2961, 2934, 2876, 1743, 1712, 1655, 1526, 1451, 1419, 1389, 1344, 1200, 1094, 1022, 979, 911, 854, 739, 698 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 7.75 (d, *J* = 7.5 Hz, 2H), 7.64 (dd, *J* = 7.3, 2.4 Hz, 2H), 7.37 (m, 14H), 6.59 (d, *J* = 9.4 Hz, 1H), 5.15 (dd, *J* = 9.4, 5.9 Hz, 1H), 5.03 (d, *J* = 10.1 Hz, 1H), 4.93 (d, *J* = 10.1 Hz, 1H), 4.86 (dd, *J* = 13.6, 10.1 Hz, 2H), 4.59 (m, 3H), 4.29 (m, 1H), 4.16 (m, 3H), 4.06 (d, *J* = 17.1 Hz, 1H), 3.90 (d, *J* = 17.5 Hz, 1H), 1.92 (m, 1H), 1.52 (m, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.03 (m, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ) 173.3, 167.8, 167.3, 157.7, 143.6 (2C), 141.4 (2C), 135.0, 133.9, 129.6 (2C),129.4 (2C), 129.1, 128.8, 128.7, 128.5 (2C), 127.8 (2C), 127.2 (2C), 125.2 (2C), 120.0 (2C), 77.9, 77.5, 68.3, 61.6, 54.3, 53.3, 48.5, 47.1, 37.2, 23.9, 15.7, 14.1, 11.1; **HRMS-ESI** (*m/z*): [M+Na]$^+$ calcd for $C_{31}H_{45}N_3O_8Na$, 730.3099; found, 730.3087 (- 1.6 ppm).

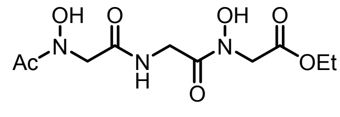
General procedure D, for the catalytic hydrogenation of *N*-(benzyloxy)-protecting groups: Peptides **9a-d** (1.0 mmol, 1.0 eq.) were solubilized in EtOH (10 mL) and Pd/C (0.10 mmol, 0.10 eq., 10% w/w) was added to the solution. For **9d**, the mixture was cooled down to 0 °C. The flask was placed under atmosphere of a balloon of $H_{2,gas}$ and the mixture was stirred for 1 h to 18 h (reaction progress monitored by TLC). The reaction mixture was filtered through a pad of cleaned Celite with silica (1% w/w), and the solvent evaporated to afford the crude product **12a-d** which were further purified by chromatography.

Ac-N(OH)-Gly-N(Me)-Gly-N(OH)-Ethyl-Glycinate ***(12a)*** was prepared according to the general procedure D, using peptide **9a** (63 mg, 0.13 mmol, 1.0 eq.). The crude product was purified by chromatography (Gradient of Hexanes/EtOAc 1:1 to EtOAc 100% and then, EtOAc/MeOH 19:1) to afford **12a** in a pure form as an uncolored oil (32 mg, 0.11 mmol, 81% yield). $\boldsymbol{R_f}$ = 0.10 (hexanes/EtOAc/AcOH 5:4:1); **m.p.** 118-121 °C; **IR** νmax : 3433, 3193, 2924, 2852, 1741, 1636, 1460, 1401, 1208, 1124, 1023, 822, 562 cm^{-1}; Product **12a** was characterized as a mixture of *cis-trans* rotamers in a ratio 2:3 by ^{1}H – ^{13}C HSQC: **^{1}H NMR** (400 MHz, DMSO-d_6, δ) *trans* rotamer: 10.18 (s, 1H), 9.76 (s, 1H), 4.42 (s, 2H), 4.29 (s, 2H), 4.28 (s, 2H), 4.13 (q, , *J* = 7.1 Hz, 2H), 2.93 (s, 3H), 2.02 (s, 3H), 1.20 (t, , *J* = 7.1 Hz, 3H); *cis* rotamer: 10.23 (s, 1H), 9.72 (s, 1H), 4.34 (s, 2H), 4.33 (s, 2H), 4.24 (s, 2H), 4.13 (q, , *J* = 7.1 Hz, 2H), 2.81 (s, 3H), 2.02 (s, 3H), 1.20 (t, , *J* = 7.1 Hz, 3H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ) *trans* rotamer: 172.8, 169.9, 169.6, 168.5, 61.9, 50.1, 49.5, 49.0, 36.4, 20.0, 14.1; *cis* rotamer: 172.8, 170.4, 169.4, 168.2, 61.9, 50.6, 50.5, 49.1, 35.4, 19.9, 14.1; **HRMS-ESI** (*m/z*): [M+Na]$^+$ calcd for $C_{11}H_{19}N_3O_7Na$, 328.1115; found, 328.1101 (- 4.2 ppm).



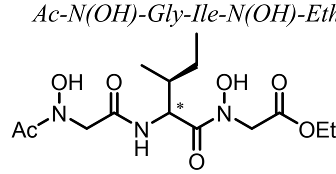
12a
$C_{11}H_{19}N_3O_7$
MW 305.12 g.mol^{-1}

Ac-N(OH)-Gly-Gly-N(OH)-Ethyl-Glycinate ***(12b)*** was prepared according to the general procedure D, using peptide **9b** (170 mg, 0.36 mmol, 1.0 eq.). The crude product was purified by chromatography (Gradient of Hexanes/EtOAc 1:1 to EtOAc 100% and then, EtOAc/MeOH 19:1) to afford **12b** in a pure form as a white solid (82 mg, 0.28 mmol, 79% yield). $\boldsymbol{R_f}$ = 0.05 (hexanes/EtOAc/AcOH 5:4:1); **m.p.** 140-142 °C; **IR** νmax: 3299, 3097, 2911, 2778, 2680, 1743, 1678, 1646, 1593, 1564, 1464, 1411, 1388, 1376, 1257, 1218, 1189, 1027, 823, 786, 729, 615, 541 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6, δ): 10.21 (s, 1H), 9.91 (s, 1H), 8.00 (br, 1H), 4.30 (s, 2H), 4.17 (s, 2H), 4.12 (q, , *J* = 7.1 Hz, 2H), 4.08 (d, , *J* = 5.6 Hz, 2H), 2.02 (s, 3H), 1.20 (t, , *J* = 7.1 Hz, 3H); **^{13}C{^{1}H} NMR** (100 MHz, DMSO-d_6, δ): 171.9, 170.9, 168.3, 168.0, 61.3, 51.4, 51.1, 40.4, 20.6, 14.5; **HRMS-ESI** (*m/z*): [M + Na]$^+$ calcd for $C_{10}H_{17}N_3O_7Na$, 314.0959; found, 314.0958 (+ 0.3 ppm).



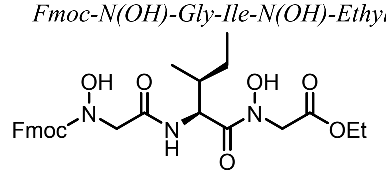
12b
$C_{10}H_{17}N_3O_7$
MW 291.11 g.mol^{-1}

Ac-N(OH)-Gly-Ile-N(OH)-Ethyl-Glycinate ***(12c)*** was prepared according to the general procedure D, using peptide **9c’** (300 mg, 0.69 mmol, 1.0 eq.). The crude product was purified by chromatography (Gradient of Hexanes/EtOAc 1:1 to EtOAc 100% and then, EtOAc/MeOH 19:1) to afford **12c** in a pure form as a yellow solid (239 mg, 0.69 mmol, 99% yield). $\boldsymbol{R_f}$ = 0.54 (hexanes/EtOAc/AcOH 5:4:1); $[\alpha]_{\mathbf{D}}^{\mathbf{18}}$ -14.2 (*c* 1.00, MeOH); **m.p.** 97-99 °C; **IR** νmax: 3276, 3210, 2965, 2-934, 1743, 1629, 1534, 1456, 1375, 1201, 1022, 634, 557 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6, δ): 10.24 (s, 1H), 9.89 (s, 1H), 7.82 (d, 1H), 5.02 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.52 (d, *J* = 17.3 Hz, 1H), 4.18 (s, 2H), 4.12 (q, *J* = 7.0 Hz, 2H), 4.08 (d, *J* = 17.4 Hz, 1H), 2.01 (s, 3H), 1.87 (m, 1H), 1.44 (m, 1H), 1.19 (t, *J* = 7.1 Hz, 3H), 1.05 (m, 1H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.83 (t, *J* = 7.3 Hz, 3H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 173.5, 172.2, 169.8, 168.0, 61.8, 53.6, 51.4, 50.0, 36.0, 24.4, 20.1, 15.4, 14.1, 11.0; **HRMS-ESI** (*m/z*): [M + H]$^+$ calcd for $C_{25}H_{31}N_2O_6$, 348.1765; found, 348.1766 (+ 0.3 ppm).



12c
$C_{14}H_{25}N_3O_7$
MW 347.17 g.mol^{-1}

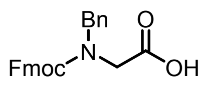
Fmoc-N(OH)-Gly-Ile-N(OH)-Ethyl-Glycinate ***(12d)*** was prepared according to the general procedure D, using peptide 9d (15 mg, 0.02 mmol, 1.0 eq.). The crude product was purified by chromatography (Gradient of Hexanes/EtOAc 4:1 to 2:6) to afford 12d in a pure form as a white powder (7 mg, 0.01 mmol, 62% yield). $\boldsymbol{R_f}$= 0.38 (hexanes/EtOAc 2:3 v/v); $[\alpha]_{\mathbf{D}}^{\mathbf{18}}$ -2.6 (*c* 0.50, MeOH); **m.p.** 164-169 °C; **IR** νmax: 3284, 2961, 2923, 2873, 1745, 1711, 1685, 1610, 1541, 1449, 1401, 1376, 1352, 1213, 1111, 1020, 974, 942, 738, 621 cm^{-1}; **^{1}H NMR** (400



12d
$C_{27}H_{33}N_3O_8$
MW 527.23 g.mol^{-1}

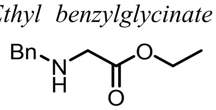
MHz, DMSO-d_6, δ) 10.26 (s, 1H), 9.69 (s, 1H), 7.89 (d, J = 7.8 Hz, 3H), 7.70 (d, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 5.06 (dd, J = 9.3, 5.9 Hz, 1H), 4.51 (d, J = 17.3 Hz, 1H), 4.26 (m, 3H), 4.13 (m, 5H), 1.88 (m, 1H), 1.45 (m, 1H), 1.18 (t, J = 7.1 Hz, 3H), 1.06 (m, 1H), 0.88 (d, J = 6.8 Hz, 3H), 0.82 (t, J = 7.2 Hz, 3H); **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ) 172.0, 169.9, 168.1, 158.1, 143.5 (2C), 141.3 (2C), 127.8 (2C),127.2 (2C), 125.2 (2C), 120.0 (2C), 68.9, 61.8, 54.4, 53.4, 49.9, 46.9, 35.8, 24.6, 15.4, 14.0, 10.9; **HRMS-ESI** (*m/z*): $[M+Na]^+$ calcd for $C_{27}H_{33}N_3O_8Na$, 550.2160; found, 550.2163 (+ 0.5 ppm).

N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-benzylglycine ***(SI-1)***. Benzylamine (200 mg, 1.87 mmol., 1.0 eq.) and glyoxylic acid monohydrate (344 mg, 3.73 mmol, 2.0 eq.) were solubilized in CH_2Cl_2 (9.0 mL) and stirred overnight at RT. The solvent was then evaporated and the *N*-Benzyl-*N*-formylglycine intermediate was characterized by ^1H NMR (400 MHz, DMSO-d_6, δ): 8.31 (s, 1H), 7.35 (m, 5H), 4.50 (s, 2H), 3.83 (s, 2H). The crude *N*-Benzyl-*N*-formylglycine was solubilized in a 1.0 M aqueous solution of HCl (9.0 mL) and stirred at reflux overnight. The water was then evaporated under reduced pressure to afford the desired *N*-benzylglycine hydrochloride (203 mg, 1.0 mmol, 54% yield) as a brown solid which was used without any purification for the second step. ***R$_f$*** = 0.7 (CH_2Cl_2/MeOH/AcOH 16:3:1 v/v); *N*-Benzylglycine hydrochloride (200 mg, 0.99 mmol, 1.0 eq.) was solubilized in dioxane (6.0 mL) and the solution was cooled down to 0 °C. A solution of sodium bicarbonate (200 mg, 2.44 mmol, 2.4 eq.) in water (2.0 mL) was then added and the mixture was stirred for 10 mins at 0 °C. a solution of (9H-fluoren-9-yl)methyl chloroformate (471 mg, 1.82 mmol, 1.8 eq.) in dioxane (2.0 mL) was added to the mixture which was slowly warm to RT and stirred overnight. Water was added and the aqueous layer (pH ~ 8) was washed with ethyl acetate (2 X 15 mL). The aqueous layer was acidified with a 5.0 M aqueous solution of HCl to pH ~ 3. The product was extracted with ethyl acetate (3 X 15 mL), the combined organic layers dried over Na_2SO_4, filtrated and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (isocratic solvent mixture of hexanes/EtOAc, 4:1 v/v) to afford product **SI-1** in a pure form as a white powder (107 mg, 0.28 mmol, 28% yield over 3 step). ***R$_f$*** = 0.7 (100% EtOAc); **m.p.** 110.0-113.0 (± 0.6) °C; **IR** νmax: 3068, 2923, 2854, 1685, 1451, 1231, 1121, 1002, 956, 758, 738 cm^{-1}; Product **SI-1** was characterized as a mixture of *cis-trans* rotamers in a 1:1 ratio by ^1H – ^{13}C HSQC: **^1H NMR** (400 MHz, CD_3OD, δ) *trans*-rotamer: 7.77 (d, J = 7.5 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.34 (t, J = 5.9 Hz, 2H), 7.23 (m, 5H), 6.96 (m, 2H), 4.53 (d, J = 5.8 Hz, 2H), 4.32 (s, 2H), 4.22 (t, J = 5.8 Hz, 1H), 3.85 (s, 2H); *cis*-rotamer: 7.73 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.37 (t, J = 5.8 Hz, 2H), 7.29 (m, 5H), 7.16 (m, 2H), 4.49 (s, 2H), 4.47 (d, J = 6.3 Hz, 2H), 4.22 (t, J = 5.8 Hz, 1H), 3.73 (s, 2H);



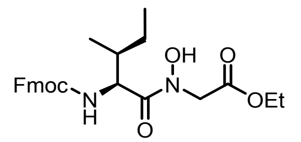
SI-1
$C_{24}H_{21}NO_4$
MW 387.15 g.mol^{-1}

Ethyl benzylglycinate ***(SI-2)***. Benzylamine (1.00 g, 9.33 mmol, 1.0 eq.) and a solution of ethyl glyoxylate (1.05 g, 10.3 mmol, 1.1 eq.) in toluene (50% w/w) were solubilized in dry ethanol (19.0 mL) and stirred for 1h at RT. A solution of sodium cyanoborohydride (1.18 g, 18.7 mmol, 2.0 eq.) in dry ethanol (3.0 mL) and acetic acid (0.05 mL, 0.93 mmol, 0.1 eq.) were added to the mixture which was stirred overnight at RT. The reaction completion was monitored by TLC (R_f = 0.35, EtOAc/ hexanes 1:1 v/v). The solvent was then evaporated and the crude was solubilized in dichloromethane (10 mL). The organic layer was washed with water (3 X 10 mL), dried over Na_2SO_4, filtrated, and the solvent evaporated under reduced pressure. The crude product was purified by chromatography (isocratic solvent mixture of hexanes/EtOAc, 7:3 v/v) to afford **SI-2** in a pure form as a yellow oil (542 mg, 2.81 mmol, 30% yield). ***R$_f$*** = 0.3 (EtOAc/hexanes 1:1 v/v); **IR** νmax: 3029, 2980, 2866, 1724, 1453, 1371, 1188, 1142, 1025, 736, 698 cm^{-1};



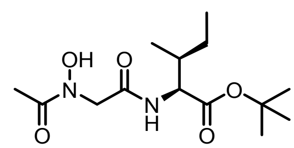
SI-2
$C_{11}H_{15}NO_2$
MW 193.11 g.mol^{-1}

Fmoc-Ile-N(OH)-Ethyl-Glycinate ***(SI-3)*** was prepared according to the general procedure D, using peptide **7c** (450 mg, 0.83 mmol, 1.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 7:3 v/v) to afford **SI-3** in a pure form as a white solid (339 mg, 0.75 mmol, 90% yield). ***R$_f$*** = 0.3 (hexanes/EtOAc 7:3 v/v); $[\alpha]_D^{18}$ -14.8 (*c* 1.00, MeOH); **m.p.** 147-149 °C; **IR** νmax: 3325, 3144, 2968, 1741, 1692, 1605, 1530, 1449, 1211, 1023, 758, 737, 646, 621 cm^{-1}; **^1H NMR** (400 MHz, $CDCl_3$, δ): 8.35 (br, 1H), 7.75 (d, J = 7.5 Hz, 2H), 7.56 (dd, J = 7.3, 2.9 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 5.53 (d, J = 9.1 Hz, 1H), 4.82 (t, J = 8.4 Hz, 1H), 4.45 (s, 2H), 4.37 (m, 2H), 4.20 (m, 3H), 1.94 (m, 1H), 1.61 (m, 1H), 1.25 (t, J = 7.1 Hz, 3H), 1.20 (m, 1H), 1.00 (d, J = 6.7 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H); **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$, δ): 172.5, 168.4, 157.2, 143.8, 143.7, 141.3 (2C), 127.7 (2C), 127.1 (2C), 125.1 (2C), 120.0 (2C), 67.5, 61.8, 55.4, 49.4, 47.1, 36.4, 24.4, 15.6, 14.1, 11.1; **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{31}N_2O_6$, 455.2177; found, 455.2157 (- 4.4 ppm).



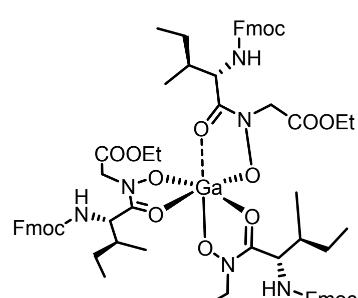
SI-3
$C_{25}H_{30}N_2O_6$
MW 454.21 g.mol^{-1}

Ac-N(OH)-Gly-Tert-Butyl-Isoleucinate ***(SI-4)*** was prepared according to the general procedure D, using peptide **11c** (450 g, 1.15 mmol, 1.0 eq.). The crude product was purified by chromatography (Hexanes/EtOAc 6:4 v/v) to afford **SI-4** in a pure form as an uncolored oil (344 g, 1.14 mmol, 99% yield). ***R$_f$*** = 0.2 (hexanes/EtOAc 6:4 v/v); $[\alpha]_D^{18}$ -15.1 (*c* 1.00, MeOH); **IR** (dry film) νmax: 3268, 2967, 2934, 1732, 1659, 1637, 1536, 1458, 1393, 1367, 1248, 1142, 1038, 989, 846, 776, 582 cm^{-1}; **^1H NMR** (400 MHz, DMSO-d_6, δ): 9.87 (s, 1H), 6.02 (d, J = 8.4 Hz, 1H), 4.17 (m, 3H), 2.01 (s, 3H), 1.74 (m, 1H), 1.41 (s, 3H), 1.37 (m, 1H), 1.18 (m, 1H), 0.86 (m, 6H); **$^{13}C\{^1H\}$ NMR** (100 MHz, DMSO-d_6, δ): 171.9, 170.9, 167.6, 81.2, 57.1, 51.2, 37.3, 28.1 (3C), 25.3, 20.6, 15.9, 11.8; **HRMS-ESI** (*m/z*): $[M+Na]^+$ calcd for $C_{14}H_{26}N_2O_5Na$, 325.1734; found, 325.1732 (- 0.6 ppm).



SI-4
$C_{14}H_{26}N_2O_5$
MW 302.18 g.mol^{-1}

Ga(Fmoc-Ile-N(O)-Ethyl-Glycinate)$_3$ ***(SI-5)***. Gallium sulfate (28 mg,



SI-5
$C_{75}H_{87}N_6O_{18}Ga$
MW 1428.53 g.mol^{-1}

0.07 mmol, 3.5 eq.) was solubilized in DMF (0.30 mL). A solution of NH_3 in 1,4-dioxane (0.5 M, 0.20 mL) was added to the mixture. A solution of dipeptide **SI-3** (10 mg, 0.02 mmol, 1.0 eq.) in DMF (0.30 mL) was added dropwise to the reaction mixture which was stirred overnight at RT. The resulting crude product was precipitated into cold water, filtrated and wash with water (3 X 1.0 mL). The solid was then solubilized in CH_2Cl_2, dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure to afford product **SI-5** as a white powder (29 mg, 0.02 mmol, 100% yield) **m.p.** 148-151 °C; **IR** νmax: 3298, 2964, 2928, 1753, 1709, 1589, 1509, 1450, 1219, 1197, 1024, 758, 736 cm^{-1}; The broadness of peaks in the ^{1}H NMR of product **SI-5** was suggest that several conformers or several ligand arrangements around the Ga center might co-exist, therefore, the following chemical shifts are representative of the major *cis*-conformation of the ligand **SI-5**: **^{1}H NMR** (400 MHz, CD_3CN, δ): 7.82 (m, 2H), 7.64 (m, 2H), 7.41 (m, 2H), 7.33 (m, 2H), 6.02 (d, *J* = 8.1 Hz, 1H), 4.87 (d, *J* = 17.9 Hz, 1H), 4.21 (m, 7H), 1.79 (m, 1H), 1.52 (m, 1H), 1.21 (m, 3H), 1.08 (m, 1H), 0.88 (m, 6H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, δ): 170.3, 164.7, 156.3, 143.7, 143.6, 141.3 (2C), 127.8 (2C), 127.1 (2C), 125.1 (2C), 120.0 (2C), 67.2, 61.8, 54.0, 53.1, 47.2, 36.9, 24.7, 15.4, 13.9, 10.9; **MALDI-TOF** (*m/z*): $[M + Na]^+$ calcd for $C_{75}H_{87}N_6O_{18}GaNa$, 1451.5225; found, 1453. 2538.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at…

CCDC 2009540 (for **12c**) contains the supplementary crystallographic data for this paper. These data collected at NSF's ChemMatCARS can be obtained free of charge from The Cambridge Crystallographic Data Centre. Tables of selected results from the *C*- and *N*-terminal coupling optimization and epimerization is reported. Complete experimental procedures and characterization data including ^{1}H, ^{13}C, NOESY and HMBC NMR spectra, as well as HPLC chromatograms for measuring levels of epimerization are available online.

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Author Contributions

This project was conceived by S.P.R. and the manuscript was written through contributions of S.P.R. and A.D.R.. All authors have given approval to the final version of the manuscript.
‡These two authors contributed equally to the work.

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