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Thionoglycine as a multifunctional spectroscopic reporter of screw-sense preference in helical foldamers†

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A single thionoglycine (glycine thioamide, $-HNCH_2C(=S)-$) residue inserted into a peptide foldamer provides both a pair of germinal protons for use as a ^1H NMR stereochemical probe and a chromophore giving rise to a well defined Cotton effect in CD. Comparison of the response of these two features to a local helically chiral environment validates them as independent methods for quantifying the conformational screw-sense preference of a helical oligomer, in this case a peptide made of repeated Aib units. The sign of the Cotton effect provides a measure of the sign of the screw-sense preference, while both the chemical shift separation of the anisochronous signals of the glycine CH_2 group and the magnitude of the Cotton effect give an estimate of the helicity excess of the oligomer. The thionoglycine unit is readily introduced synthetically by a thionation of a BocGlyAibOMe dipeptide.

Introduction

Foldamers are extended molecular structures with well defined global conformations.[1–4] Many foldamers are helical,[4,5] and such structures fall into two conceptual classes. Oligomeric helical foldamers built from *chiral* monomers typically prefer a single screw sense because the two screw-sense conformers are diastereoisomers, one being of higher energy than the other. In contrast the oligomers of *achiral* monomers, though they may adopt a helical conformation, must necessarily populate equally left (*M*) and right (*P*) handed screw-sense conformers. In some cases the screw sense isomers may interconvert slowly, even to the point of being separable,[6–8] while in others the racemisation rate is fast,[9] even on the NMR timescale.[10]

The equilibrium between these screw-sense conformers of achiral helical foldamers may be perturbed in a number of ways – by incorporation of a chiral residue in the achiral chain, for example, or by covalent or non-covalent interactions of chiral ligands with the body or terminus of the chain. We have used such strategies as a means of communicating information through foldamers by a mechanism analogous to that exploited by the G-protein coupled receptor, or for achieving remote stereocontrol over up to 60 bonds.[11]

Classically, screw-sense preference has been analysed by circular dichroism (CD), which is a particularly powerful method for assigning screw sense as left or right handed.[12] CD may also be quantitative provided a maximum value of molar ellipticity characteristic of a compound with a complete preference for a single screw sense may be determined.[13,14] However, in many cases this value is difficult to estimate accurately, and CD spectra may be difficult to interpret quantitatively if other absorption bands, for example from chiral residues, overlap with those characteristic of the helix itself.[15]

We have reported an alternative, practically simple method for quantifying screw-sense preference which relies on the behaviour of a pair of diastereotopic groups located within the foldamer chain under conditions where the rate of interconversion of the screw-sense conformers is either slow or fast on the NMR timescale.[16–19] At slow exchange, the diastereotopic groups appear as a pair of anisochronous signals by virtue of their location in the chiral environment of the helix. At fast exchange, the diastereotopic groups may still appear anisochronous, but only if the local chiral environment is maintained by the existence of an imbalance between the helical screw-sense conformers. We proposed that the ratio of the anisochronicity of the two signals at slow and fast exchange is directly proportional to this screw-sense preference, expressed as a 'helical excess'. Using the NMR signals arising from ^{13}C-labelled Me groups, we explored this hypothesis by using line shape analysis at a range of temperatures, and extracted from these data thermodynamic and kinetic parameters for screw-sense inversion.[17] Importantly, the value of the equilibrium constant *K* extracted by this method matched closely the value obtained simply from the quotient of the anisochronicities at fast and slow exchange.

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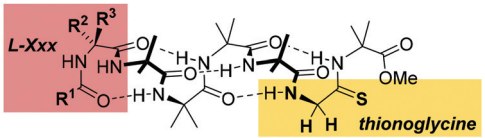
More convenient than relying on a specifically synthesised and incorporated ^{13}C- (or ^{19}F-)[19] labelled residue is the use of a pair of diastereotopic protons present in a methylene group – for example the protons of a glycine residue.[18] On the assumption that these diastereotopic signals behave in a comparable manner to those of the ^{13}C-labelled Aib residue, we have used the AB system arising from the coupled anisochronous signals of the CH_2 group of a glycine residue to quantify the different levels of screw-sense control from the N- and C-terminals of a peptide helix,[16] and to compare different controllers of helicity with one another.[18] We have also used the CH_2 groups of related C-terminal amino alcohol residues to assess the decay of conformational preference over extended foldamer lengths.[20]

However, while these studies gave values that were self-consistent,[19] we have thus far never demonstrated that the degree of screw-sense control measured by NMR may be correlated directly with the degree of screw-sense control observed by circular dichroism. In this paper we do this by making use of a thionated glycine residue (–NHCH$_2$(C=S)–) as a reporter of screw-sense preference. Among the possible peptide bond modifications, thioamides are one of the closest mimics of a peptide linkage. These 'endothiopeptides' have received much attention because of their interesting physical and chemical properties. For example: (1) the thioamide C–N bond undergoes reversible *cis*/*trans* photoisomerization upon irradiation at 260 nm;[21,22] (2) the S atom of a thioamide is a weaker hydrogen bond acceptor than the O atom of an amide, while the more acidic adjacent NH is a stronger hydrogen bond donor;[23] (3) endothiopeptides have been used in structure–activity relationship studies to alter biological activities;[24,25] (4) thioamides can act as isosteric peptide bond fluorescence quenchers.[26]

Thionoglycine contains both a chromophore suitable as a CD reporter in the form of a thioamide group and a pair of potentially diastereotopic ^{1}H nuclei for use as an NMR reporter, directly comparable with the ^{1}H NMR spectra of the related glycinamides.[18] By using a thioamide, we ensure that the Cotton effect arising from this chromophore is well separated in the CD spectrum from the bands associated with the amide carbonyls themselves. Typical applications of CD measure helicity in the foldamer *structure as a whole*, in contrast with our NMR methods, which measure conformational preference at a single site.[17] Here we use the thioamide function to achieve a localised CD response at a particular site in the foldamer chain.[27] The thionoglycine was located one residue from the C-terminus of the peptide chain, sufficiently far from any chiral residue for its chiral environment to be affected only by the local helicity of the chain, but still incorporated into the hydrogen-bonded network forming the helix.

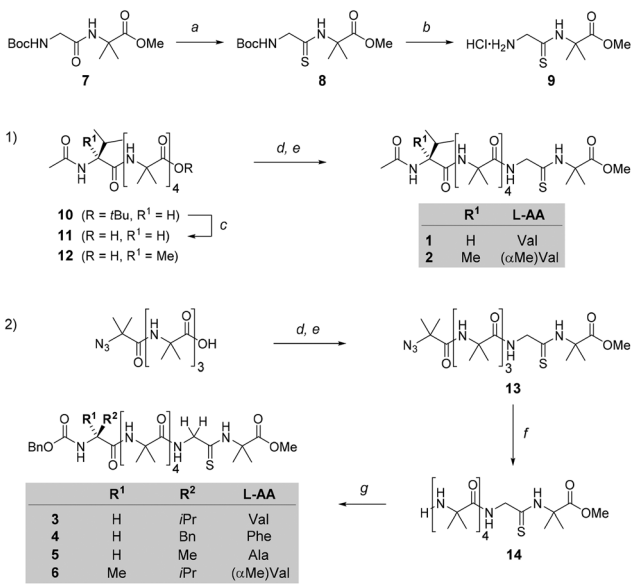
Design and synthesis of the endothiopeptides

Aib-rich, helical[28–30] endothiopeptides **1–6** bearing either an N-terminal Cbz group or an N-terminal acetamide (Fig. 1) were



compound	R^1	R^2	R^3	L-Xxx
1	Me	H	*i*Pr	AcVal
2	Me	Me	*i*Pr	Ac(αMe)Val
3	OBn	H	*i*Pr	CbzVal
4	OBn	H	Bn	CbzPhe
5	OBn	H	Me	CbzAla
6	OBn	Me	*i*Pr	Cbz(αMe)Val

Fig. 1 The endothiopeptides used in this study. A right-handed helix is shown for convenience only; see text for further explanation.



Scheme 1 Reagents and conditions: (a) Lawesson's reagent, THF, 60 °C; (b) HCl–Et$_2$O 2 M, HSCH$_2$CH$_2$OH, anisole; (c) TFA, CH$_2$Cl$_2$; (d) EDC, MeCN; (e) **9**, iPr$_2$NEt, MeCN; (f) PMe$_3$, THF, H$_2$O; (g) Cbz-L-AA-OH, HOAt, EDC, iPr$_2$NEt.

made by the route shown in Scheme 1. BocGlyAibOMe **7** was thionated regioselectively at the amide carbonyl group using Lawesson's reagent[31–33] to give the thioamide **8**. The Boc group of **8** was removed under acidic conditions[31,33] to provide a common precursor to **1–6**, HCl·H-Gly-ψ[CSNH]AibOMe **9** (Scheme 1),[34] which was coupled with a range of Aib-containing oligomers by opening of their C-terminal azlactones. Thus **11**, **12** and N$_3$Aib$_3$OH gave, respectively, **1**, **2** and **13**. Cbz-protected endothiopeptides **3–6** were obtained by Staudinger reduction of azide **13** followed by N-terminal capping with a range of protected L-amino acids (L-Xxx).

NMR and CD analysis

^{1}H NMR spectra of the endothiopeptides **1–6** in CD$_3$OD[35] at 23 °C showed characteristic AB signals for the methylene

Table 1 Measured anisochronicities ($\Delta\delta$) in the $CH_AH_BC(=S)NH$ methylene groups of oligomers **1–6**

Compound	L-Xxx	Marker[a]	$\Delta\delta$ Gly(CSNH)
1	AcVal	●	204
2	Ac(αMe)Val	■	195
3	CbzVal	○	135
4	CbzPhe	▼	190
5	CbzAla	▲	104
6	Cbz(αMe)Val	□	252

[a] See Fig. 2.

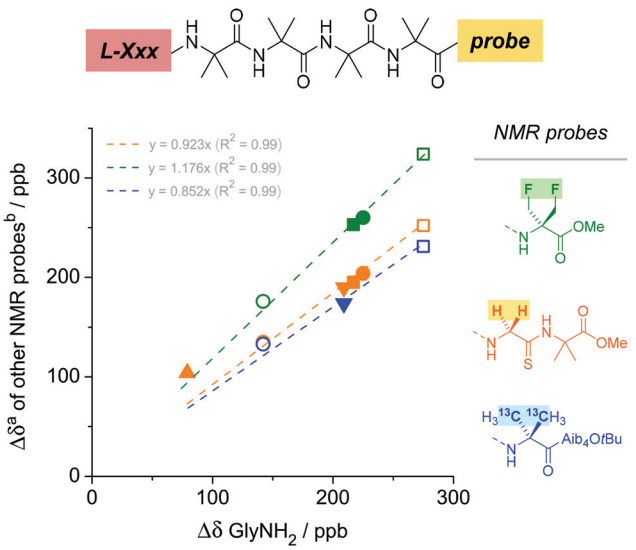


Fig. 2 Comparing the performance of the glycine thioamide as a ^1H NMR probe with other NMR reporters of helical screw-sense; [a] for ^{13}C Aib probe (blue), $\Delta\delta/10$; [b] measured anisochronicity of the signals from highlighted nuclei in the ^1H, ^{13}C and ^{19}F NMR spectrum.

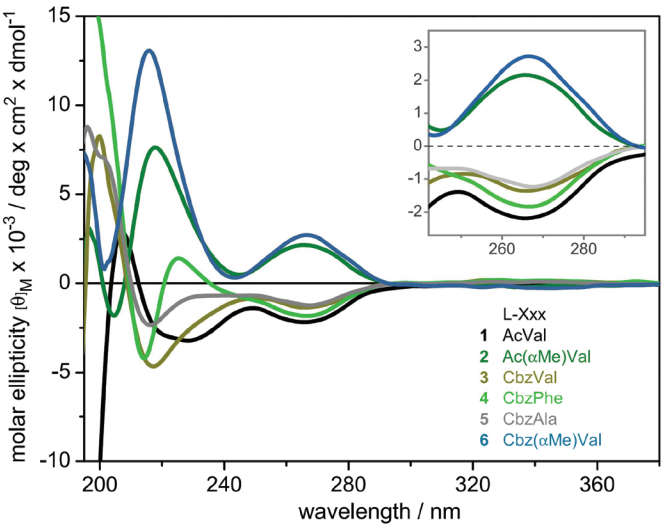


Fig. 3 CD spectra of the endothiopeptides **1–6** in methanol. Inset: the region of the spectrum arising from the $\pi-\pi^*$ transition of the thioamide chromophore.

protons, centred around 4.25 ppm. Table 1 shows the measured anisochronicity $\Delta\delta$[36] for each Aib-containing oligomer **1–6** XxxAib$_4$Gly-ψ[CSNH]AibOMe carrying an N-terminal controller of screw-sense. Plotting these values against the anisochronicities previously measured in non-thionated peptides XxxAib$_4$GlyNH$_2$,[18] XxxAib$_4$Aib**Aib$_4$O*t*-Bu,[17] and XxxAib$_4$FibOMe[19] bearing identical N-terminal protected chiral residues Xxx (Fig. 2) shows that the response of the thionoglycine probe is directly proportional to that of other NMR reporters of helical screw sense, validating its applicability in this context. This proportionality also strongly suggests that the thioamide-containing foldamers adopt, at least in their Aib$_4$ portion, the same 3_{10}-helical conformation as that adopted by related all-amide oligomers.[28–30]

CD spectra of the thionated peptides **1–6** were recorded in MeOH solution, in order to keep the experimental conditions consistent with those used in the NMR experiments. The thionated peptides display a distinct Cotton effect at about 268 nm, clearly separated from the rest of the peptide bond absorptions, which fall below 240 nm (Fig. 3). This strong band is assigned to the $\pi-\pi^*$ transition of the thioamide chromophore;[22,37–45] it is interesting that the $n-\pi^*$ band (usually lying at around 340 nm[32]) is hardly visible. The positions of these two bands match those observed in the UV spectra in the same solvent (see ESI†). Analysis of the far-UV portion of the CD spectrum, which usually reveals valuable information on helical conformations, is complicated by the contribution of the thioamide chromophore transitions at lower wavelengths.[12,46]

The ability to identify the contribution of bonding at a single residue to an overall circular dichroism spectrum is a valuable result of the thionation of the glycine peptide bond. Given the spatial separation of this thioamide chromophore from the nearest stereogenic centre, the sign of the thioamide $\pi-\pi^*$ absorption band centred at 268 nm (Fig. 3, inset) must result solely from the screw-sense preference experienced in the vicinity of the thionoglycine probe. Despite the common L-configuration of all the endothiopeptides at their N-terminal stereocontrolling residue, **2** and **6**, which are capped with a C^α-tetrasubstituted amino acid (Ac- or Cbz-(αMe)Val), show a positive Cotton effect, while **1** and **3–5**, which are capped with C^α-tertiary, proteinogenic amino acids (Ac- and Cbz-protected Val, Phe and Ala), show a negative Cotton effect. This observation is in agreement with the reported behaviour of peptides of the sequence L-XxxAib$_n$-, which adopt a left-handed (*M*) helical screw sense if Xxx is a *tertiary* L-amino acid and right-handed (*P*) helical screw sense if Xxx is a *quaternary* L-amino acid.[18,47] Previous studies of this behaviour by circular dichroism, initially identified by using an enantiomerically enriched ^{13}C isotopic label, had been frustrated by the lack of separation between the characteristic chromophores of the helical chain and those of the chiral N-terminal residue.

Circular dichroism is a powerful technique for identifying the sign of screw-sense preference (*M* or *P*), but NMR has been

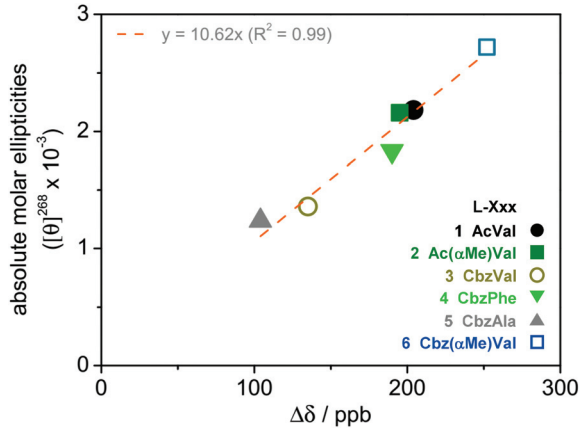


Fig. 4 Relationship between the measured absolute molar ellipticities at 268 nm and the h.e. values for peptides **1–6**.

of more use for quantifying the magnitude of the preference because (at least for ^{13}C NMR) maximum peak separation (corresponding to 100% helical excess) may be evaluated from low temperature NMR spectra, in which screw-sense conformers are in slow exchange.[48] However, the reported values of screw-sense preference induced by each controller determined by this method can now be correlated with the absolute molar ellipticities ($[\theta]_M$) measured at 268 nm (the CSNH π–π^* absorption) (Fig. 4). In confirmation that the sign and value of $[\theta]_M^{268}$ is a reporter of both sign and magnitude of screw-sense preference, these two values are proportional, with $[\theta]_M^{268} = 10.6 \times \Delta\delta$ (in ppb).[17]

As seen in earlier work on related compounds,[18] bulkier and C^{β}-branched amino acids induce a greater level of screw-sense control, while the role played by protecting groups (amide *vs.* carbamate) depends on whether the stereocontrolling residue is C^{α}-tri- or tetrasubstituted, probably because of the different type of β-turn[47] adopted at the N-terminus of the helix.

X-ray crystal structure analysis

Endothiopeptides **3** and **5**, having respectively Cbz-Val and Cbz-Ala at their N-terminus, formed crystals suitable for X-ray analysis (Fig. 5). Interestingly, the unit cell contains equal numbers of peptides in *M* and *P* screw-sense conformations, indicating that both helical conformers are of similar energy in the solid state as well as in solution. Furthermore, each helix is initiated by either one of the two types of β-turn (a distorted Type II or Type III) proposed[47] to account for the induction of screw-sense preference from the helix N-terminus. It is likely that the asymmetric environment experienced by the thioamide chromophore in solution results from the population of an unequal mixture of these two diastereoisomeric conformers.

‡X-ray data for **3** and **5** have been deposited with the Cambridge crystallographic data centre. Deposition numbers **3**: 969434; **5**: 969435.

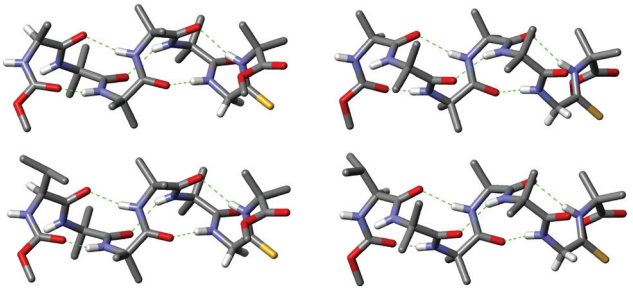


Fig. 5 X-ray crystal structures of endothiopeptides **3** (top) and **5** (bottom) in their *M* (left) and *P* (right) conformations as observed in the unit cell.‡ The *M* conformations display an N-terminal Type II β-turn while the *P* conformations display a Type III β-turn.[47] Cbz phenyl rings have been removed for clarity.

Conclusions

A thionoglycine residue inserted into a peptide provides a probe of the magnitude and sign of local helical screw sense, providing a qualitative and quantitative reporter of conformational preference. The versatile probe is readily introduced by selective thionation, and is responsive towards both magnetic and optical spectroscopies, with the helical excess values derived by NMR correlating closely those obtained by CD. The absolute measurement of a single thioamide chromophore contribution to the CD spectrum is made possible by virtue of its well-separated absorption wavelength, which may also permit its use in solvents that absorb in the far-UV region of the CD spectrum. Thioamides may prove useful in the future as tools for the conformational analysis of foldamers and peptide mimics embedded into membranes.

Experimental

BocGlyAibOMe[49] 7

Isobutyl chloroformate (0.74 mL, 5.7 mmol) was added dropwise to a cooled (−15 °C) solution of BocGlyOH (1 g, 5.7 mmol) and *N*-methylmorpholine (0.66 mL, 6 mmol) in dry THF (15 mL). The suspension was stirred at −15 °C for 15 min, and a mixture of H-AibOMe·HCl[50] (1.75 g, 11.4 mmol) and *N*-methylmorpholine (1.3 mL, 11.4 mmol) in dry THF (20 mL) was added. The mixture was warmed to room temperature and left stirring overnight. After solvent evaporation, the residue was taken up in EtOAc (100 mL) and washed with $KHSO_4$ 5% (3 × 10 mL), sat. $NaHCO_3$ (3 × 10 mL) and brine (10 mL). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude product was purified by column chromatography ($CHCl_3$–MeOH, 97 : 3) to give pure BocGlyAibOMe **7** (1.44 g, 92%) as a white solid. R_f 0.35 ($CHCl_3$–MeOH, 97 : 3); mp = 94–96 °C; IR (ATR, cm^{-1}): 3340, 3275, 1716, 1667, 1534, 1303, 1279, 1244, 1157; ^1H NMR (300 MHz, $CDCl_3$) δ_H 6.72 (s, 1H), 5.21 (s, 1H), 3.74 (d, J = 6.0 Hz, 1H), 3.73 (s, 3H), 1.54 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 168.9, 156.3, 80.5, 56.8, 52.9, 44.9,

44.8, 44.8, 28.5, 24.9; MS (ES^+, MeOH): 275 ($[M + H]^+$, 55%), 297 ($[M + Na]^+$, 100%); HRMS (ES^+, MeOH): Calcd for $C_{12}H_{22}N_2O_5Na$ $[M + Na]^+$ 297.1426, found 297.1437.

BocGly-ψ[CSNH]AibOMe 8

A stirred solution of BocGlyAibOMe **7** (1.410 g, 5.1 mmol) and Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione, 1.041 g, 2.55 mmol) in dry THF (15 mL) was heated to 60 °C for 4 hours. The mixture was cooled to room temperature and the volatiles evaporated under reduced pressure. After purification by column chromatography (EtOAc–PE 2 : 3) endothiopeptide BocGly-ψ[CSNH]-AibOMe **8** (0.87 g, 57%) was obtained as an off-white waxy solid. R_f 0.45 (EtOAc–PE, 2 : 3); mp = 96–98 °C; IR (ATR, cm^{-1}): 3324, 1741, 1667, 1520, 1427, 1409, 1286, 1256, 1153; ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.63 (s, 1H), 5.39 (s, 1H), 4.08 (d, J = 6.4 Hz, 2H), 3.71 (s, 3H), 1.67 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 199.0, 173.4, 156.8, 80.9, 60.0, 53.2, 52.7, 28.2, 23.9; MS (ES^+, MeOH): (292 $[M + H]^+$, 100%); HRMS (ES^+, MeOH): Calcd for $C_{12}H_{23}N_2O_4S$ $[M + H]^+$ 291.1373, found 291.1372.

HCl·H-Gly-ψ[CSNH]AibOMe 9

The method of Jensen *et al.* was used.[33] Anisole (1.1 mL, 1 mmol) and 2-mercaptoethanol (0.4 mL, 0.6 mmol) were added to a stirred solution of BocGly-ψ[CSNH]AibOMe **8** (565 mg, 1.95 mmol) in dry dioxane (4 mL), followed by HCl (2 M solution in diethyl ether, 10 mL, 20 mmol) at room temperature. The reaction was monitored by TLC. A precipitate was observed after 45 min. Stirring was continued for 2 h and the product was collected by filtration to give HCl·H-Gly-ψ[CSNH]-AibOMe as a white solid (241 mg, 65%). R_f 0.05 (EtOAc–PE, 2 : 3); mp = 206–207 °C; IR (ATR, cm^{-1}): 3223, 3045, 2928, 2864, 1741, 1542, 1438, 1428, 1352, 1276, 1181, 1153; ^1H NMR (500 MHz, MeOD) δ_H 3.83 (s, 2H), 3.66 (s, 3H), 1.63 (s, 6H); ^{13}C NMR (101 MHz, MeOD) δ_C 194.9, 174.6, 61.3, 52.9, 46.9, 24.4; MS (ES^+, MeOH): 191 ($[M + H]^+$, 100%); HRMS (ES^+, MeOH): Calcd for $C_7H_{15}N_2O_2S$ $[M + Na]^+$ 191.0849, found 191.0848.

AcValAib$_4$OH 11

To a solution of AcValAib$_4$O*t*Bu[47] (100 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) TFA was added (2 mL) dropwise. The progress of the reaction was followed by TLC. After reaction completion (4 h) the mixture was concentrated *in vacuo*. Et_2O was added to the residue (5 mL) and the solvent evaporated. This process was repeated 5 times. No further purification was required. AcValAib$_4$OH was obtained as a white solid. Yield: 86 mg, 96%. R_f 0.10 (CH_2Cl_2–MeOH, 95 : 5); mp = 242–245 °C (dec.). $[\alpha]_D^{25}$ = +19.2; IR (ATR, cm^{-1}): 3296, 1648, 1528, 1386, 1364, 1290, 1169; ^1H NMR (300 MHz, MeOD) δ_H 8.57 (s, 1H), 8.27 (d, J = 5.7 Hz, 1H), 7.70 (s, 1H), 7.65 (s, 1H), 7.51 (s, 1H), 3.90–3.81 (m, 1H), 2.08–1.98 (m, 1H), 2.00 (s, 3H), 1.53–1.39 (m, 24H), 1.04 (d, J = 6.7 Hz, 3H), 1.00 (d, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ_C 178.4, 177.0, 176.8, 176.8, 174.6, 174.5, 174.1, 62.0, 58.1, 58.0, 58.0, 57.9, 57.3, 31.0, 27.0–26.8 (overlapping signals), 26.5, 26.0, 25.9, 25.0, 24.9, 24.8, 24.7, 24.4, 24.4, 24.2, 24.1, 22.5, 19.7; MS (ES^+, MeOH): 500 ($[M + H]^+$, 40%), 522 ($[M + Na]^+$, 100%); HRMS (ES^+, MeOH): Calcd for $C_{23}H_{41}N_5O_7Na$ $[M + Na]^+$ 522.2904, found 522.2906.

AcValAib$_4$Gly-ψ[CSNH]AibOMe 1

To a mixture of AcValAib$_4$OH **11** (60 mg, 0.12 mmol) and HOAt (1-hydroxy-7-azabenzotriazole, 18 mg, 0.13 mmol) in a mixture of dry CH_2Cl_2 (3 mL) and dry DMF (0.4 mL), was added EDC·HCl (25 mg, 0.11 mmol). After stirring for 20 min, a solution of HCl·H-Gly-ψ[CSNH]AibOMe **9** (28 mg, 0.12 mmol) and *N*,*N*-diisopropylethylamine (0.061 mL, 0.35 mmol) in dry CH_2Cl_2 (2 mL) was added. The mixture was left stirring at room temperature for 6 days, after which the solvent was evaporated. The residue was taken up in EtOAc (10 mL) and washed with $KHSO_4$ 5% (3 × 2 mL), sat. $NaHCO_3$ (3 × 2 mL) and brine (2 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography ($CHCl_3$–MeOH, 95 : 5) to give pure AcValAib$_4$Gly-ψ[CSNH]AibOMe (45 mg, 52%) as a white solid. R_f 0.35 (CH_2Cl_2–MeOH, 95 : 5); mp = 116–117 °C; $[\alpha]_D^{25}$ = −29.6; IR (ATR, cm^{-1}): 3273, 1650, 1530, 1416, 1362, 1226, 1156; ^1H NMR (500 MHz, MeOD) δ_H 7.82 (s, 1H), 4.31 (d, A of AB, J = 17.7 Hz, 1H), 4.11 (d, B of AB, J = 17.7 Hz, 1H), 3.87 (d, J = 8.1 Hz, 1H), 3.63 (s, 3H), 2.06–1.98 (m, 1H), 2.00 (s, 3H), 1.68 (s, 3H), 1.67 (s, 3H), 1.53–1.34 (m, 24H), 1.04 (d, J = 6.7 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ_C 199.9, 177.9, 177.9, 177.9, 176.8, 175.5, 174.6, 174.1, 61.9, 61.4, 58.3, 58.1, 57.9, 57.9, 52.9, 52.3, 31.0, 30.9, 26.7, 26.6, 26.5, 26.4, 25.0–24.8 (overlapping signals), 24.3, 24.2, 22.5, 19.7, 19.6; MS (ES^+, MeOH): 694 ($[M + Na]^+$, 100%); HRMS (ES^+, MeOH) m/z calcd for $C_{30}H_{53}N_7O_8NaS$ $[M + Na]^+$ 694.3569, found 694.3555.

Ac(αMe)ValAib$_4$Gly-ψ[CSNH]AibOMe 2

To a mixture of Ac(αMe)ValAib$_4$OH[47] (74 mg, 0.14 mmol) and HOAt (21 mg, 0.15 mmol) in a mixture of dry CH_2Cl_2 (3 mL) and dry DMF (0.4 mL), EDC·HCl (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, 30 mg, 0.16 mmol) was added. After stirring for 20 min, a solution of HCl·H-Gly-ψ[CSNH]AibOMe **9** (33 mg, 0.14 mmol) and *N*,*N*-diisopropylethylamine (0.080 mL, 0.46 mmol) in dry CH_2Cl_2 (2 mL) was added. The mixture was left stirring at room temperature for 7 days, after which the solvent was evaporated. The residue was taken up in EtOAc (10 mL) and washed with $KHSO_4$ 5% (3 × 2 mL), sat. $NaHCO_3$ (3 × 2 mL) and brine (2 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography ($CHCl_3$–MeOH, 97 : 3 → 95 : 5) to give pure Ac(αMe)ValAib$_4$Gly-ψ[CSNH]AibOMe (54 mg, 55%) as a white solid. R_f 0.7 (CH_2Cl_2–MeOH, 9 : 1); mp = 191–193 °C; $[\alpha]_D^{25}$ = +31.2; IR (ATR, cm^{-1}): 3283, 1654, 1530, 1418, 1286; ^1H NMR (400 MHz, MeOD) δ_H 7.76 (s, 1H), 4.22 (d, A of AB, J = 17.7 Hz, 1H), 4.02 (d, B of AB, J = 17.7 Hz, 1H), 3.53 (s, 3H), 1.98–1.89 (m, 1H), 1.93 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H), 1.45–1.26 (m,

24H), 0.92 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ_C 199.8, 178.2, 177.8, 177.7, 177.1, 175.3, 175.1, 173.7, 63.9, 61.3, 58.2, 58.0, 57.9, 57.8, 52.7, 52.2, 36.2, 26.5, 26.4, 26.4, 24.9, 24.7, 24.6–24.2 (overlapping signals), 23.3, 18.6, 17.9, 17.7; MS (ES$^+$, MeOH): 708 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) m/z calcd for $C_{31}H_{55}N_7O_8NaS$ [M + Na]$^+$ 708.3725, found 708.3712.

N_3Aib_4Gly-ψ[CSNH]AibOMe 13

(1) *Azlactone formation*. N_3Aib_4OH (500 mg, 1.3 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and EDC (0.252 mL, 1.43 mmol) was added. The resulting solution was left stirring for 3 h at room temperature. The solvent was evaporated *in vacuo* and the residue was taken up in EtOAc (20 mL) and washed with $KHSO_4$ 5% (3 × 5 mL). The organic phase was dried ($MgSO_4$) and concentrated to give the crude azlactone product, which was used directly in the next step.

(2) *Azlactone coupling*. The crude azlactone was dissolved in dry CH_2Cl_2 (5 mL) and HCl·H-Gly-ψ[CSNH]AibOMe **9** (230 mg, 1.01 mmol) was added, together with *N*,*N*-diisopropylethylamine (0.232 mL, 1.33 mmol). The mixture was left stirring at room temperature for 7 days, after which the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc (10 mL) and washed with $KHSO_4$ 5% (3 × 2 mL), sat. $NaHCO_3$ (3 × 2 mL) and brine (2 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 97 : 3 → 95 : 5) to give pure N_3Aib_4Gly-ψ[CSNH]AibOMe (250 mg, 44%) as a colourless solid. R_f 0.75 (CH_2Cl_2–MeOH, 95 : 5); mp = 190–192 °C (dec.); IR (ATR, cm^{-1}): 3277, 2112, 1745, 1657, 1530, 1382, 1275, 1152; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.68 (s, 1H), 7.76 (t, J = 6.2 Hz, 1H), 7.36 (s, 1H), 6.93 (s, 1H), 6.28 (s, 1H), 4.31 (d, J = 6.2 Hz, 2H), 3.64 (s, 3H), 1.67 (s, 6H), 1.49 (s, 12H), 1.44 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 198.6, 175.0, 174.2, 173.9, 173.7, 173.3, 64.0, 60.3, 57.3, 57.0, 56.9, 52.4, 51.7, 25.5–25.4 (overlapping signals), 24.9, 24.4; MS (ES$^+$, MeOH): 579 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) m/z calcd for $C_{23}H_{40}N_8O_6NaS$ [M + Na]$^+$ 579.2689, found 579.2697.

H-Aib_4Gly-ψ[CSNH]AibOMe 14

N_3Aib_4Gly-ψ[CSNH]Aib-OMe **13** (112 mg, 0.2 mmol) was dissolved in distilled THF (2 mL). Water (0.018 mL, 1 mmol) was added, followed by PMe_3 (1 M solution in THF, 2 mL, 2 mmol). The reaction mixture was stirred overnight at room temperature. The volatiles were removed under vacuum and the residue purified by column chromatography (CH_2Cl_2–MeOH, 9 : 1 → 8 : 2) to give H-Aib_4Gly-ψ[CSNH]AibOMe (85 mg, 81%). R_f 0.10 (CH_2Cl_2–MeOH, 95 : 5); mp = 212–213 °C; IR (ATR, cm^{-1}): 3278, 1730, 1651, 1530, 1383, 1362, 1282, 1221, 1155; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.73 (s, 1H), 8.22 (s, 1H), 7.81 (t, J = 6.2 Hz, 1H), 7.69 (s, 1H), 6.36 (s, 1H), 4.31 (d, J = 6.2 Hz, 2H), 3.64 (s, 3H), 1.75 (brs, 2H), 1.68 (s, 6H), 1.50 (s, 6H), 1.42 (s, 6H), 1.39 (s, 6H), 1.32 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 198.7, 175.4, 174.8, 174.4, 174.1, 174.1, 60.4, 57.3, 56.7, 56.6, 55.5, 52.5, 51.8, 28.0, 25.5, 25.0, 24.4, 18.7, 17.7; MS (ES$^+$, MeOH): 553 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) m/z calcd for $C_{23}H_{42}N_6O_6NaS$ [M + Na]$^+$ 553.2784, found 553.2775.

CbzValAib$_4$Gly-ψ[CSNH]AibOMe 3

To a mixture of CbzValOH (31 mg, 0.12 mmol), HOAt (17 mg, 0.12 mmol) and H-Aib_4Gly-ψ[CSNH]AibOMe **14** (33 mg, 0.06 mmol) in dry CH_2Cl_2 (3 mL) was added EDC (0.022 mL, 0.12 mmol). The mixture was left stirring at room temperature for 3 days, and then the solvent was evaporated. The residue was taken up in EtOAc (5 mL) and washed with $KHSO_4$ 5% (3 × 1 mL), sat. $NaHCO_3$ (3 × 1 mL) and brine (1 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography (EtOAc–PE, 4 : 1) to give pure CbzValAib$_4$Gly-ψ[CSNH]-AibOMe (35 mg, 74%) as a white solid. R_f 0.65 (EtOAc–PE, 4 : 1); mp = 198–200 °C; $[\alpha]_D^{25}$ = −22.4; IR (ATR, cm^{-1}): 3279, 2983, 1651, 1529, 1284, 1226, 1157; ^{1}H NMR (500 MHz, MeOD) δ_H 7.47–7.23 (m, 5H), 5.11 (s, 2H), 4.28 (d, J = 17.7 Hz, 1H), 4.14 (d, J = 17.6 Hz, 1H), 3.75 (d, J = 7.4 Hz, 1H), 3.63 (s, 3H), 2.08–1.97 (m, 1H), 1.67 (s, 6H), 1.52–1.32 (m, 24H), 1.02 (d, J = 7.0 Hz, 3H), 1.00 (d, J = 6.9 Hz, 3H; ^{13}C NMR (126 MHz, MeOD) δ_C 199.8, 177.7, 177.7, 177.7, 176.6, 175.3, 174.6, 159.0, 138.2, 129.6, 129.1, 128.7, 67.7, 62.8, 61.3, 58.1, 58.0, 57.7, 57.7, 52.7, 52.2, 31.2, 26.2, 26.2, 26.0, 25.9, 25.1–24.7 (overlapping signals), 24.5, 24.3, 19.6, 19.4, 19.3 ppm; MS (ES$^+$, MeOH): 786 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) m/z calcd for $C_{36}H_{57}N_7O_9NaS$ [M + Na]$^+$ 786.3831, found 786.3817.

CbzPheAib$_4$Gly-ψ[CSNH]AibOMe 4

To a mixture of CbzPheOH (37 mg, 0.12 mmol), HOAt (17 mg, 0.12 mmol) and H-Aib_4Gly-ψ[CSNH]AibOMe **14** (33 mg, 0.06 mmol) in dry CH_2Cl_2 (3 mL) was added EDC (0.022 mL, 0.12 mmol), followed by *N*,*N*-diisopropylethylamine (0.05 mL, 0.03 mmol). The mixture was left stirring at room temperature for 3 days, and then the solvent was evaporated. The residue was taken up in EtOAc (5 mL) and washed with $KHSO_4$ 5% (3 × 1 mL), sat. $NaHCO_3$ (3 × 1 mL) and brine (1 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 95 : 5) to give pure CbzPheAib$_4$-Gly-ψ[CSNH]AibOMe (39 mg, 78%) as a white solid. R_f 0.5 (CH_2Cl_2–MeOH, 95 : 5); mp = 110–113 °C; $[\alpha]_D^{25}$ = −19.2; IR (ATR, cm^{-1}): 3291, 2984, 1657, 1529, 1383, 1226, 1155; ^{1}H NMR (500 MHz, MeOD) δ_H 7.90 (s, 1H), 7.81 (s, 2H), 7.39–7.16 (m, 1H), 5.10 (d, A of AB, J = 12.6 Hz, 1H), 5.06 (d, B of AB, 12.6 Hz, 1H), 4.30 (d, A of AB, J = 17.7 Hz, 1H), 4.23 (t, J = 7.8 Hz, 1H), 4.11 (d, B of AB, J = 17.7 Hz, 1H), 3.63 (s, 3H), 3.00 (ddd, J = 31.6, 13.6, 7.8 Hz, 2H), 1.67 (d, J = 2.5 Hz, 6H), 1.50 (s, 3H), 1.49 (s, 6H), 1.42 (s, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ_C 199.9, 177.9, 177.9, 177.8, 176.7, 175.5, 174.5, 158.7, 138.3, 138.2, 130.7, 129.7, 129.7, 129.2, 128.7, 128.1, 67.8, 61.4, 58.4, 58.3, 58.1, 57.9, 57.7, 52.8, 52.4, 38.4, 30.9, 26.6–26.3 (overlapping signals), 25.1, 24.9, 24.8, 24.3, 24.1; MS (ES$^+$, MeOH): 834

([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) *m*/*z* calcd for $C_{40}H_{57}N_7O_9NaS$ [M + Na]$^+$ 834.3831, found 834.3818.

CbzAlaAib$_4$Gly-ψ[CSNH]AibOMe 5

To a mixture of Cbz-Ala-OH (28 mg, 0.12 mmol), HOAt (17 mg, 0.12 mmol) and H-Aib$_4$Gly-ψ[CSNH]AibOMe **14** (33 mg, 0.06 mmol) in dry CH_2Cl_2 (3 mL) was added EDC (0.023 mL, 0.12 mmol), followed by *N*,*N*-diisopropylethylamine (0.05 mL, 0.03 mmol). The mixture was left stirring at room temperature for 3 days, and then the solvent was evaporated. The residue was taken up in EtOAc (5 mL) and washed with $KHSO_4$ 5% (3 × 1 mL), sat. $NaHCO_3$ (3 × 1 mL) and brine (1 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 97 : 3) to give pure CbzAlaAib$_4$-Gly-ψ[CSNH]AibOMe (35 mg, 77%) as a white solid. R_f 0.4 (CH_2Cl_2–MeOH, 97 : 3); mp = 204–205 °C; $[\alpha]_D^{25}$ = −13.2 IR (ATR, cm^{-1}) 3287, 2984, 1703, 1643, 1529, 1420, 1361, 1208, 1171; ^{1}H NMR (500 MHz, MeOD) δ_H 7.90 (s, 1H), 7.81 (s, 1H), 7.38–7.27 (m, 5H), 5.12 (d, A of AB, *J* = 12.7 Hz, 1H), 5.09 (d, B of AB, *J* = 12.7 Hz, 1H), 4.27 (d, A of AB, *J* = 17.7 Hz, 1H), 4.16 (d, B of AB, *J* = 17.7 Hz, 1H), 4.00 (q, *J* = 7.1 Hz, 1H), 3.63 (s, 3H), 1.67 (s, 6H), 1.50 (s, 6H), 1.47–1.36 (m, 18H), 1.34 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ_C 199.9, 177.9, 177.9, 177.8, 176.8, 176.1, 175.5, 158.8, 138.4, 129.7, 129.3, 128.8, 79.6, 67.8, 61.4, 58.2, 58.1, 57.9, 57.6, 52.8, 52.7, 52.4, 26.2, 25.9, 25.8, 25.4, 25.2–24.8 (overlapping signals), 24.7, 17.3; MS (ES$^+$, MeOH): 758 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) *m*/*z* calcd for $C_{34}H_{53}N_7O_9NaS$ [M + Na]$^+$ 758.3518, found 758.3505.

Cbz(αMe)ValAib$_4$Gly-ψ[CSNH]AibOMe 6

Acyl fluoride formation.[51] A solution of Cbz-(αMe)Val-OH (58 mg, 0.22 mmol) in dry CH_2Cl_2 (2 mL) was treated with pyridine (0.018 mL, 0.22 mmol) and TFFH (tetramethyl-fluoroformamidinium hexafluorophosphate, 87 mg, 0.33 mmol). The mixture was left stirring at room temperature for 4 h, after which ice-cold water was added (2 mL). The organic phase was separated and washed again with ice-cold water (2 mL), dried ($MgSO_4$) and concentrated to give crude Cbz-(αMe)Val-F as a colorless solid.

Acyl fluoride coupling. The freshly prepared amino acyl fluoride was dissolved in dry CH_2Cl_2 (2 mL) and the resulting solution was added to H-Aib$_4$Gly-ψ[CSNH]AibOMe **14** (39 mg, 0.073 mmol) in dry CH_2Cl_2 (3 mL). *N*,*N*-Diisopropylethylamine (0.134 mL, 0.77 mmol) was added and the mixture was left stirring at room temperature for 5 days, after which the solvent was evaporated. The residue was taken up in EtOAc (5 mL) and washed with $KHSO_4$ 5% (3 × 1 mL), sat. $NaHCO_3$ (3 × 1 mL) and brine (1 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 95 : 5) to give pure Cbz(αMe)ValAib$_4$Gly-ψ[CSNH]Aib-OMe (51 mg, 70%) as a white solid. R_f 0.25 (CH_2Cl_2–MeOH, 95 : 5); mp = 203–205 °C; $[\alpha]_D^{25}$ = +34.7; IR (ATR, cm^{-1}): 3281, 2983, 1707, 1651, 1530, 1454, 1382, 1361, 1287, 1163; ^{1}H NMR (400 MHz, MeOD) δ_H 7.91 (s, 1H), 7.83 (s, 1H), 7.43–7.27 (m, 5H), 5.20 (d, A of AB, *J* = 12.7 Hz, 1H), 5.07 (d, B of AB, *J* = 12.7 Hz, 1H), 4.34 (d, A of AB, *J* = 17.7 Hz, 1H), 4.09 (d, B of AB, *J* = 17.7 Hz, 1H), 3.63 (s, 3H), 2.02 (m, 1H), 1.68 (s, 3H), 1.67 (s, 3H), 1.53–1.33 (m, 24H), 1.27 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ_C 199.8, 178.2, 177.8, 177.7, 176.9, 175.9, 175.4, 158.2, 138.7, 129.6, 129.1, 128.7, 79.5, 73.4, 67.7, 63.9, 61.3, 58.2, 58.1, 58.0, 57.8, 57.7, 52.7, 52.2, 36.0, 26.7, 26.6, 24.8, 24.7–24.4 (overlapping signals), 23.9, 18.0, 17.7; MS (ES$^+$, MeOH): 800 ([M + Na]$^+$, 100%); HRMS (ES$^+$, MeOH) *m*/*z* calcd for $C_{37}H_{63}N_8O_9NaS$ [M + NH$_4$]$^+$ 795.4439, found 795.4433.

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