Synthesis of Conformationally Restricted Mimetics of γ-Turns and Incorporation into Desmopressin, an Analogue of the Peptide Hormone Vasopressin

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Abstract: Mimetics of tripeptides adopting inverse and classical γ-turn conformations have been designed and synthesized by an enantioselective approach and then incorporated in an analogue of the hormone vasopressin. In the mimetics one of the amide bonds of the γ-turn has been exchanged for a $\Psi[CH_2O]$ isostere and the hydrogen bond between residues i and $i+2$ of the turn has been replaced by a methylene bridge to give a six-membered, morpholin-3-one ring. The turn mimetics were assembled from three types of building blocks: azido epoxides, α-bromo acids and β-amino alcohols. Of these, the stereochemistry of the azido epoxide determines whether a classical or an inverse γ-turn is mimicked. The key azido epoxide building blocks were prepared in six steps and approximately 40% overall yield, whereas the α-bromo acids and β-amino alcohols were either commercially available or readily prepared from amino acids. The building-block approach allowed substantial variation of the side chains of the mimetics, together with complete stereocontrol, as well as use of uniform conditions for preparation of both classical and inverse γ-turn mimetics. Conformational studies based on ab initio calculations and ^{1}H NMR spectroscopy revealed that the minimum-energy conformations of the mimetics closely resembled inverse or classical γ-turns.

Keywords: azides · conformation analysis · γ-turn · hormones · peptidomimetics

Introduction

Turns, defined as regions where a peptide chain reverses its overall direction, constitute an important class of polypeptide secondary structure.[1] When direction reversal occurs over four residues in such a way that the carbonyl oxygen atom of the first residue (i) and the amide NH proton of the fourth residue ($i+3$) come close in space a β-turn is formed. In most cases this involves formation of an intramolecular hydrogen bond between residues i and $i+3$ to give a pseudo-ten-membered ring. Similarly, in γ-turns a hydrogen bond may be formed between the C=O of residue i and the NH of residue $i+2$, thereby generating a pseudo-seven-membered ring.

Turns may account for as many as one third of the residues of globular proteins.[1b] They are often located at the surface of proteins where they can undergo posttranslational modification and serve as recognition sites for interactions with receptors and antibodies.[1, 2] In addition, structural studies revealed that a large number of small peptides functioning as hormones or neurotransmitters, or having other regulatory roles in organisms, may adopt β- or γ-turn conformations. Examples include the hormones oxytocin,[3] which induces labor and lactation in mammals, vasopressin,[4] which regulates water readsorption in the kidneys, and LHRH,[5] which releases sex-specific hormones from the testes and ovaries. Furthermore, turns have been found in the endogenous morphinelike substance Leu-enkephalin,[6] as well as angiotensin II[7] and bradykinin,[8] which are involved in regulation of blood pressure. β-Turns are more frequent than γ-turns in these biologically active peptides, but vasopressin, Leu-enkephalin, angiotensin II and bradykinin have been found to populate γ-turn conformations.

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Peptides display a multitude of diverse and important biomedicinal activities, as illustrated by the above examples. Moreover, biological evaluation of libraries of peptides prepared by combinatorial chemistry continuously provides further peptide leads for drug development. Unfortunately, the poor pharmacokinetic properties of peptides, namely low uptake on oral administration, rapid enzymatic degradation and facile excretion, often restrict their use as drugs.[9] In addition, conformational flexibility may reduce the biological activity and receptor selectivity of peptides, and unfavourable solubility often imposes restrictions on their use under physiological conditions. To circumvent these problems large efforts have been devoted to the design and preparation of peptidomimetics, compounds that have chemical structures different from those of peptides but are nevertheless ligands at the same receptor as the parent peptide.[10]

Ideally, several requirements should be fulfilled by mimetics of peptide secondary structure, such as β- and γ-turns.[10b, c] First, the conformation of the mimetic should accurately resemble that of the peptide backbone in the turn. Since it is well-known that the side chains of biologically active peptides have crucial roles in receptor recognition it is also essential that these can be introduced at desired positions in the mimetic with correct stereochemistry. Finally, the synthetic route to the mimetic should be short and efficient. Peptidomimetics that meet the first two criteria can also be expected to suffer from fewer pharmacokinetic problems than peptide drug candidates because the replacement of amide bonds with isosteric groups[11] both decreases enzymatic degradation and improves uptake[12] across membranes.[13] Owing to their rigid nature, mimetics are also useful tools for establishing the bioactive conformation of peptides. Furthermore, since rigid analogues pay a lower entropy cost upon binding to a receptor, they should be more potent and more selective for a specific type of receptor.[10a, 14]

Herein we describe a novel approach which provides access to conformationally restricted mimetics of both inverse and classical γ-turns. In contrast to most,[7, 8, 15] but not all,[16] of the previous routes to γ-turn mimetics, our approach does not suffer from drawbacks such as lack of possibilities for introducing side chains at appropriate positions of the mimetic or to control their stereochemistry. To illustrate the scope of our approach, several γ-turn mimetic building blocks with different side chains were prepared. One of these was then incorporated into the drug desmopressin (**1**; [1-desamino-8-D-arginine]vasopressin DDAVP, Figure 1),[17] which is a synthetic analogue of the neurohypophyseal peptide hormone vasopressin (**2**).[18] Compared with vasopressin, desmopressin has a prolonged antidiuretic effect, but does not cause vasoconstriction and contraction of smooth muscles, and is therefore used for treatment of diabetes insipidus.[19] It is also useful in treatment of patients with mild haemophilia A, von Willebrand's disease and thrombocyte dysfunction prior to surgery.[20] The conformation of desmopressin, when bound to its cellular membrane receptor, has not been determined because of the difficulty of isolating intact receptor molecules. However, the conformations adopted by desmopressin in different solutions,[4c, 21] as well as when bound to the carrier protein neurophysin,[22] have recently been calculated based on NMR data. It was thus found that desmopressin populates different β-turn conformations when bound to neurophysin[22] and in solutions containing trifluoroethanol,[21] whereas an inverse γ-turn encompassing residues Phe3-Asn5 is formed in aqueous solution under physiological conditions.[4c]

Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-**D-Arg**-Gly-NH_2

1: Desmopressin

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-**Arg**-Gly-NH_2

2: Vasopressin

Figure 1. Structures of the drug desmopressin (**1**) and the hormone vasopressin (**2**). Desmopressin was developed[17] from vasopressin by structural modifications.

Results and Discussion

Design and molecular modelling of γ-turn mimetics: γ-Turns are stabilized by an intramolecular hydrogen bond between residues i and $i+2$ and are classified either as inverse or classical depending on the values of the backbone torsional angles Φ and Ψ for the second ($i+1$) residue of the turn (Figure 2).[1] γ-Turns have been termed open when no hydrogen bond is formed and the Φ, Ψ angles are within 30° of those given in Figure 2.[1] In inverse γ-turns, the side chain of the $i+1$ residue assumes a pseudoequatorial orientation. In contrast, it is pseudoaxial in classical γ-turns. The distance between the carbonyl carbon atom of residue i and the nitrogen atom of the amino group of residue $i+2$ in ideal γ-turns is 3.0 Å,[23] suggesting that the intramolecular hydrogen bond between these functionalities could be replaced by a methylene bridge. Simultaneous replacement of the amide bond between

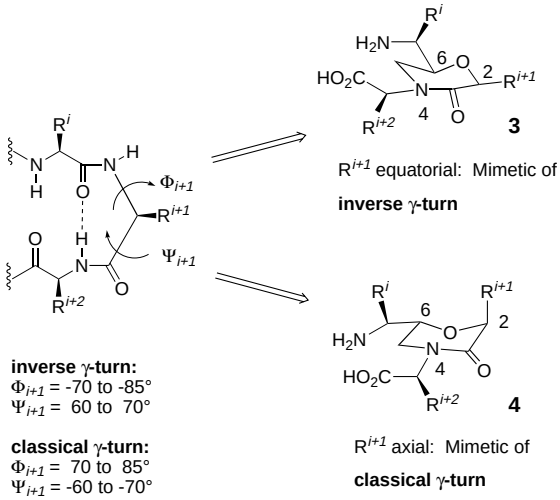

Figure 2. γ-Turns are divided into inverse and classical γ-turns depending on the Φ and Ψ torsional angles of the $i+1$ residue of the turn. Morpholin-3-ones having substituents in positions 2, 4, and 6 are able to mimic both inverse and classical γ-turns depending on the stereochemistry at C-6.

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residues i and $i+1$ with a methylene ether isostere revealed that morpholin-3-ones, with appropriate substituents in positions 2, 4 and 6, are γ-turn mimetics (cf. **3** and **4**, Figure 2). Moreover, construction of molecular models indicated that the morpholin-3-ones are able to mimic either inverse or classical γ-turns depending on the stereochemistry at C-6.

Ab initio calculations performed on the simplified inverse γ-turn mimetic **5**, which has three methyl group substituents on the morpholin-3-one ring (Figure 3A), revealed that it

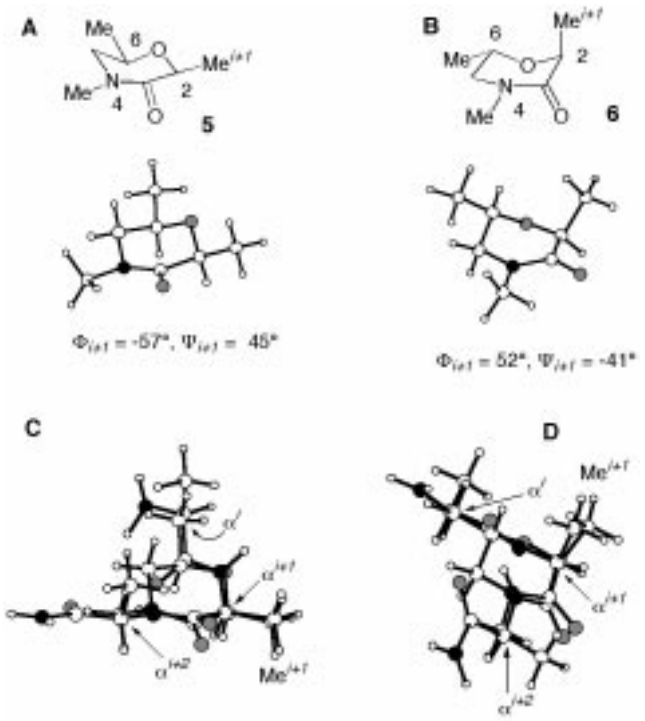
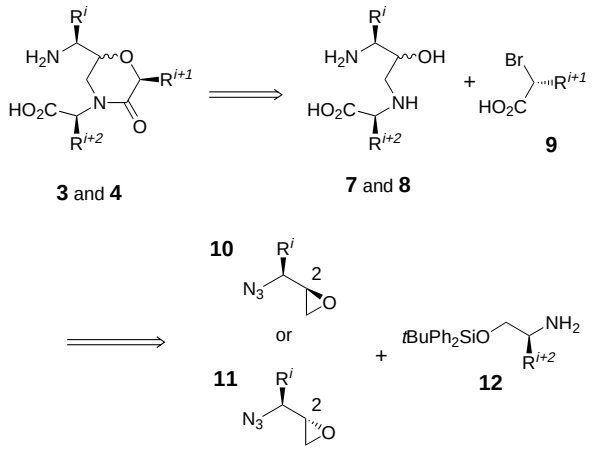


Figure 3. A) The substituent corresponding to the $i+1$ side chain in the minimum energy conformation of the simplified mimetic **5** occupies a pseudoequatorial orientation and **5** thereby mimics an inverse γ-turn; B) energy minimization of **6** revealed that the $i+1$ side chain is located in a pseudoaxial orientation and **6** thus mimics a classical γ-turn; C) and (D) superimpositions of mimetics **5** and **6** on Ala-Ala-Ala tripeptides oriented as an ideal inverse γ-turn ($\Phi = -77.5^\circ$ and $\Psi = 65^\circ$) and an ideal classical γ-turn ($\Phi = 77.5^\circ$ and $\Psi = -65^\circ$), respectively. Oxygen atoms are grey and nitrogen atoms black.

adopted a half-chair minimum-energy conformation with the C-2 substituent in a pseudoequatorial orientation, and that it thereby mimicked an inverse γ-turn.[24] Furthermore, the torsional angles for the $i+1$ residue of energy-minimized conformation of mimetic **5** ($\Phi = -57^\circ$, $\Psi = 45^\circ$) were found to be close to those of an inverse γ-turn (cf. values given in Figure 2). Consequently, superimposition of energy-minimized **5** on an Ala-Ala-Ala tripeptide oriented as an ideal inverse γ-turn revealed an excellent overlap with deviations of only 0.1–0.3 Å between the α-carbon atoms of the tripeptide and the corresponding atoms in the mimetic (Figure 3C). Ab initio calculations performed on the diastereomeric, classical γ-turn mimetic **6** showed that it adopted a minimum energy conformation with the substituent corresponding to the side chain of residue $i+1$ in a pseudoaxial orientation (Figure 3B). In this case a boatlike conformation with the $i+1$ substituent in an equatorial orientation was found to be a local energy minimum located 2 kcal mol^{-1} above the global minimum. In analogy with inverse γ-turn mimetic **5**, the torsional angles of mimetic **6** ($\Phi = 52^\circ$, $\Psi = -41^\circ$) were close to those of a classical γ-turn, and mimetic **6** superimposed well on a tripeptide oriented as a classical γ-turn (Figure 3D).

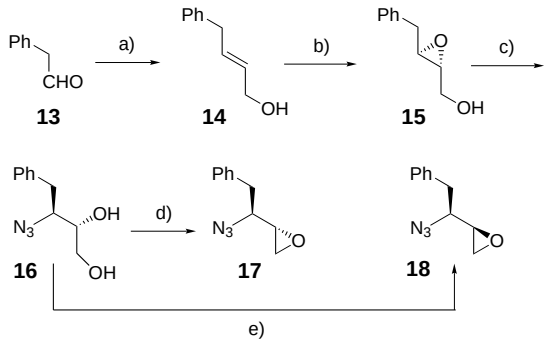
A retrosynthetic analysis suggested that bromo acids **9**, diastereomeric azido epoxides **10** and **11**, and protected β-amino alcohols **12** were suitable building blocks for synthesis of γ-turn mimetics **3** and **4** in enantiomerically pure form (Scheme 1). In these building blocks the azido group in **10** and



Scheme 1. Retrosynthetic analysis reveals that mimetics of inverse and classical γ-turns can be prepared from α-bromo acids **9**, azido epoxides **10** or **11**, and β-amino alcohols **12**.

11 serves as a precursor for the amino group of the first residue of the turn, while the silylated hydroxymethyl group in **12** corresponds to the carboxylic acid group of the third residue of the turn. The success of this approach relies on a route that provides ready access to both **10** and **11** in enantiomerically pure form, since the stereochemistry at C-2 of the epoxides determines which type of γ-turn, inverse or classical, will be prepared. Protected β-amino alcohols **12** may be prepared in a few steps from amino acids and then used for opening of **10** or **11** to give **7** or **8**. Acylation of **7** or **8** by (*R*)-2-bromo acids **9**, which are commercially available or prepared by diazotization of D-α-amino acids,[25] and intramolecular substitution of the bromine atom then complete the synthesis of γ-turn mimetics **3** and **4**. Because of our interest in replacing the Phe3-Gln4-Asn5 inverse γ-turn in desmopressin with turn mimetics, we focused on preparation of azido epoxides in which R^i is a benzyl group. Since glutamine at position 4 in desmopressin can be replaced by alanine, α-aminobutyric acid or valine with little decrease in or even enhanced activity,[26] we chose methyl, ethyl and isopropyl groups for the R^{i+1} position of the turn mimetics. Asparagine is required at position 5 for the activity of desmopressin and therefore amino alcohol **12** was prepared from asparagine.

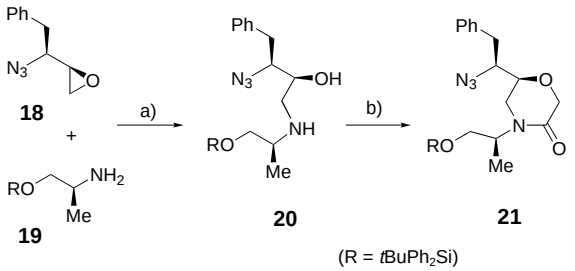
Synthesis of mimetics of inverse and classical γ-turns: The two key diastereomeric azido epoxides **17** and **18** were prepared by slight modifications of a published procedure (Scheme 2).[27] Wittig olefination of phenylacetaldehyde (**13**) and subsequent



Scheme 2. a) i) Ph_3PCHCO_2Et, CH_2Cl_2, RT, 2 h, 81 %; ii) DIBAL-H, CH_2Cl_2, −78 °C, 1 h, 81 %; b) $Ti(OiPr)_4$, *t*BuOOH, D-(−)-diethyl tartrate, CH_2Cl_2, −20 °C, 24 h, 87 %; c) $[Ti(OiPr)_2(N_3)_2]$, benzene, reflux, 30 min, 95 %; d) i) TsCl, DMAP (cat.), pyridine, 0 °C, 2 h, 92 %; ii) NaH, DMF, 0 °C, 1 h, 76 %; e) i) BzCl, collidine, CH_2Cl_2, −78 °C→RT, 18 h, then MsCl, 0 °C→RT, 24 h, 91 %; ii) NaOMe, THF, RT, 15 min, 80 %.

reduction with diisobutylaluminium hydride gave allylic alcohol **14**. This was subjected to Katsuki–Sharpless asymmetric epoxidation to give epoxide **15** (>95 % *ee* based on ^{1}H NMR analysis of a Mosher ester derivative),[28, 29] followed by regioselective nucleophilic opening of the epoxide with $Ti(OiPr)_2(N_3)_2$[30] to furnish azido diol **16**. Both azido epoxides were then prepared in enantiomerically pure form from this intermediate. Tosylation of the primary hydroxyl group of **16**, followed by sodium hydride induced intramolecular substitution, gave azido epoxide **17** in 38 % yield from **13**.[27, 31] The preparation of azido epoxide **18** (39 % yield from **13**) was accomplished by benzoylation of the primary hydroxyl group of **16**, mesylation of the secondary hydroxyl group and sodium methoxide mediated intramolecular ring closure.[27, 31]

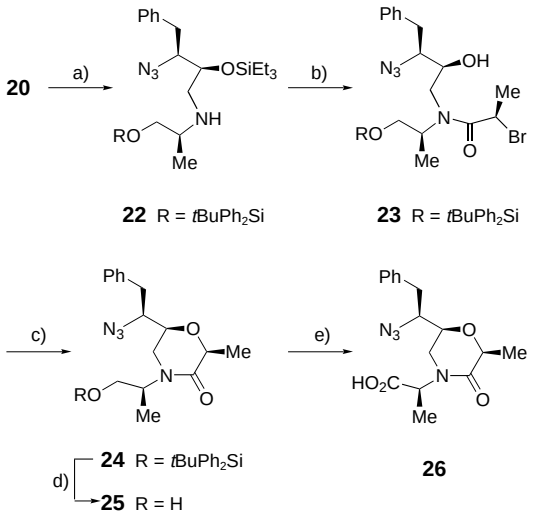
Mimetics of inverse γ-turns corresponding to the tripeptides Phe-Gly-Ala and Phe-Ala-Ala, which have nonreactive side chains, were first chosen as synthetic targets in order to establish suitable conditions for conversion of 3-azido epoxides **17** and **18** into γ-turn mimetics. Regioselective nucleophilic attack at the primary position of epoxide **18** with silylated (*S*)-(+)-2-amino-1-propanol (**19**) was achieved in refluxing EtOH to give amino alcohol **20** (68 %) (Scheme 3).[32] 2-Amino alcohols have previously been converted



Scheme 3. a) EtOH, reflux, 96 h, 68 %; b) NaH, THF, RT, 30 min, then add ethyl bromoacetate, RT→reflux, 5 h, 22 %.

into morpholin-3-ones by treatment with ethyl chloroacetate and sodium hydride as part of a synthetic route to $\Psi[CH_2O]$ dipeptide isosteres.[33] Attempted cyclization of **20** with the more reactive ethyl bromoacetate under basic conditions did indeed give morpholine-3-one **21**, which is a Phe-Gly-Ala inverse γ-turn mimetic, but only in a 22 % yield. Furthermore, this one-step procedure failed with more hindered α-halo esters such as esters of (*R*)-(+)-2-bromopropionic acid. Thus, preparation of γ-turn mimetics having side chains other than a hydrogen atom at the i+1 position of the turn was not possible by this route.

In efforts to improve the yield in the conversion of **20** to morpholin-3-ones, and to allow introduction of side chains at C-2 of the morpholinone ring, we turned our attention to a two-step acylation–intramolecular ring closure sequence (Scheme 4); a method which has been applied previously for



Scheme 4. a) Et_3SiCl, imidazole, CH_2Cl_2, 0 °C→RT, 4 h, 73 %; b) (*R*)-(+)-2-bromopropionic acid, diisopropylcarbodiimide, CH_2Cl_2, 0 °C, 4 h, then aqueous workup followed by 2 M aqueous HCl/THF (3:2), RT, 96 h, 67 %; c) KH, THF/DMF (3:1), 0 °C, 55 min, 86 %; d) TBAF, THF, RT, 2 h, 100 %; e) $NaIO_4$, $RuCl_3 \cdot H_2O$ (cat.), $H_2O/CCl_4/CH_3CN$ (3:2:2), RT, 2 h, 86 %.

preparation of stereochemically well-defined $\Psi[CH_2O]$ isosteres.[34] All attempts to acylate the amino group of **20** regioselectively in the presence of the hydroxyl group were unsuccessful. Therefore, the secondary hydroxyl group was first protected by silylation with triethylsilyl chloride to give **22** (73 %). Then the amino group was acylated with an excess of (*R*)-(+)-2-bromopropionic acid activated with diisopropylcarbodiimide. In spite of substantial efforts the desired amide could not be obtained completely free from diisopropylurea, the side-product of the coupling. However, removal of the triethylsilyl group by treatment with aqueous HCl allowed remaining diisopropylurea to be removed by flash column chromatography and hydroxyamide **23** was isolated in 67 % yield from **22**. Inclusion of additives during the coupling such as 1-hydroxybenzotriazole[35a] (HOBt) or 1-hydroxy-7-azabenzotriazole[35b] (HOAt), which are used to suppress racemization during synthesis of peptides, or use of other coupling reagents (1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDC) or the HOAt-derived HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate[36]) did not result in any further improvements in yield for these two steps. The key cyclization of **23** was then achieved by converting **23** into the corresponding alkoxide with potassium hydride in a mixture of THF and DMF at 0 °C. This resulted in a spontaneous intramolecular

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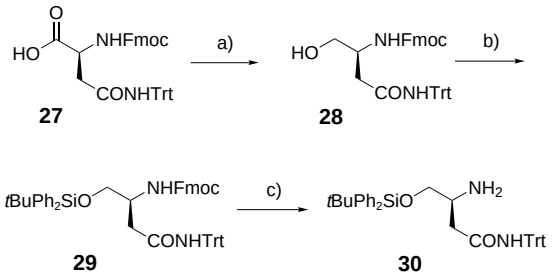
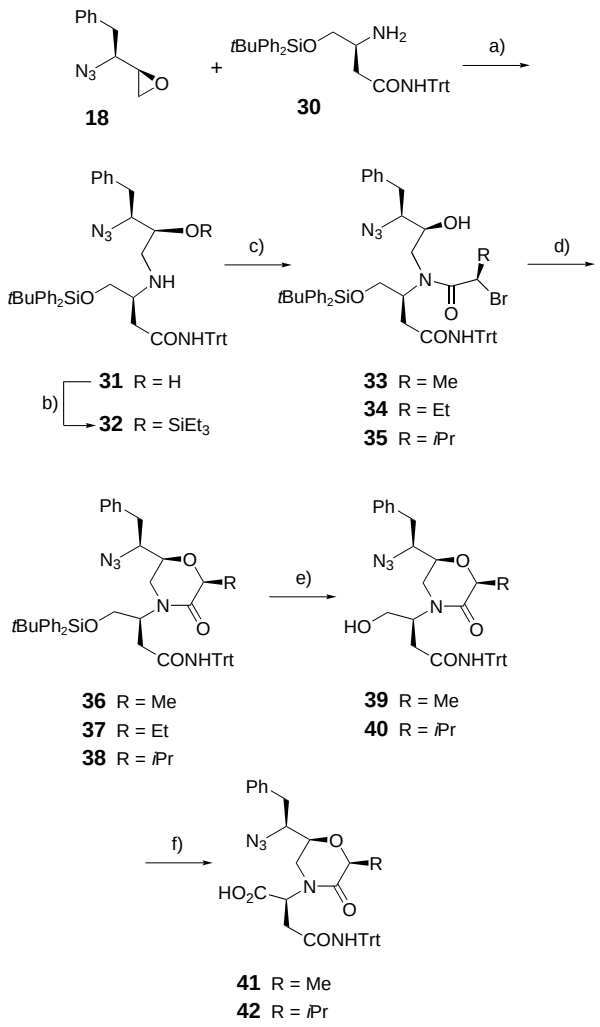
ring closure to give morpholin-3-one **24** in high yield (86%). Formation of side-products due to β-elimination or epimerization of the stereocentre α to the carbonyl group in **24** could not be detected in the ring-closure reaction (cf. discussion for compound **36** below). Finally, deprotection of the primary hydroxyl group in **24** with tetrabutylammonium fluoride (quantitative) and subsequent oxidation of **25** to the carboxylic acid stage with the biphasic $RuCl_3$–$NaIO_4$ system gave the desired inverse γ-turn mimetic **26** (86%),[37] which has side-chain functionalities corresponding to those of the tripeptide Phe-Ala-Ala.[38]

After establishing this synthetic approach to inverse γ-turn mimetics it was important to investigate whether it was compatible with incorporation of different side chains at positions *i*+1 and *i*+2 of the turn. As our aim was to prepare turn mimetics replacing the Phe3-Gln4-Asn5 segment of desmopressin (**1**), we chose Asn for the *i*+2 position, and the side chains of alanine, α-aminobutyric acid and valine were selected for the *i*+1 position. The required Asn building block was prepared from commercially available Fmoc-Asn(Trt)-OH (Trt = triphenylmethyl) by conversion into a mixed anhydride followed by reduction with sodium borohydride[39] and subsequent protection of alcohol **28** as a *tert*-butyldiphenylsilyl ether to give **29** (Scheme 5). Deprotection of the Fmoc



Scheme 5. a) *N*-methylmorpholine, isobutyl chloroformate, THF, −10 °C, 10 min, then $NaBH_4$, 15 min, followed by MeOH, 0 °C, 15 min, 70%; b) *tert*-butylchlorodiphenylsilane, imidazole, CH_2Cl_2, 0 °C→RT, 1.5 h, 77%; c) morpholine, RT, 1 h, 88%.

group with morpholine gave the asparaginol derivative **30** with a free amino group (52% yield over 3 steps). The triphenylmethyl group was chosen for protection of the side-chain amide functionality because of its compatibility with the conditions used in Fmoc solid-phase peptide synthesis. Of the required α-bromo acids that constitute building blocks for the *i*+1 residue of the turn, (*R*)-(+)-2-bromopropionic acid and (*R*)-(+)-2-bromo-3-methylbutyric acid are commercially available, whereas (*R*)-2-bromobutyric acid was prepared by diazotization of D-α-aminobutyric acid.[25] Three mimetics of inverse γ-turns corresponding to the sequences Phe-Ala-Asn, Phe-γAba-Asn and Phe-Val-Asn were then assembled from these building blocks by the sequence of transformations used in the preparation of Phe-Ala-Ala mimetic **26** (Scheme 6). Regioselective nucleophilic opening of azido epoxide **18** with protected asparaginol **30** was performed under the conditions employed in the reaction between **18** and alaninol **19**, and gave amino alcohol **31** in an almost identical yield (66%). The secondary hydroxyl group in **31** was then protected by silylation to give amine **32** (91%), which was acylated with (*R*)-(+)-2-

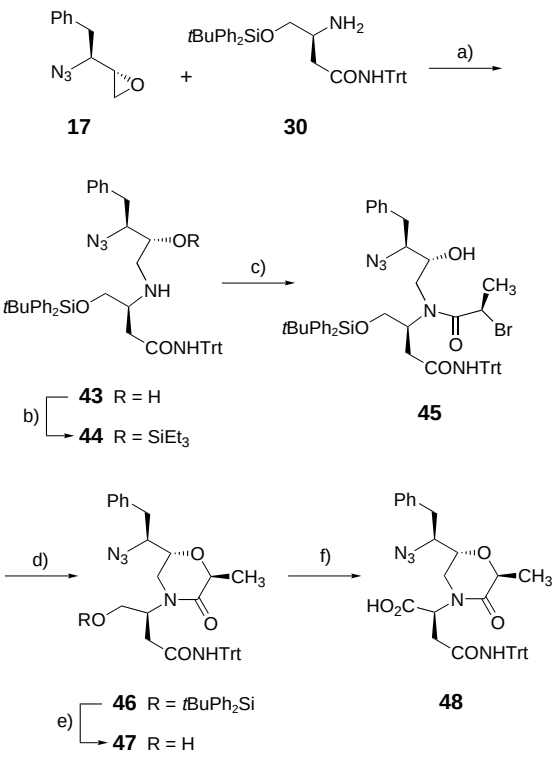


Scheme 6. a) EtOH, reflux, 96 h, 66%; b) Et_3SiCl, imidazole, CH_2Cl_2, 0 °C→RT, 4 h, 91%; c) (*R*)-(+)-2-bromopropionic acid *or* (*R*)-2-bromobutyric acid *or* (*R*)-(+)-2-bromo-3-methylbutyric acid, diisopropylcarbodiimide, CH_2Cl_2, 0 °C, 4–21 h, then aqueous workup followed by 2M aqueous HCl/THF (3:2), RT, 48–96 h, 67% for **33**, 31% for **34**, 40% for **35**; d) KH, THF/DMF (3:1), 0 °C, 40–60 min; 84% for **36**, 63% for **37**, 69% for **38**; e) TBAF, THF, RT, 3 h, 98% for both **39** and **40**; f) $NaIO_4$, $RuCl_3 \cdot H_2O$ (cat.), $H_2O/CCl_4/CH_3CN$ (3:2:2), RT, 3 h; 81% for **41**, 72% for **42**.

bromopropionic acid, (*R*)-2-bromobutyric acid and (*R*)-(+)-2-bromo-3-methylbutyric acid by means of diisopropylcarbodiimide as a coupling reagent. After removal of the triethylsilyl protective group the three hydroxyamides **33**, **34** and **35** were obtained in 67, 31 and 40% yields, respectively.[40] Potassium hydride induced cyclization of **33**–**35**, which is the key step of the synthetic route, furnished morpholin-3-ones **36**–**38** in 63–84% yields. Competing β-elimination was not observed in any of these cyclizations, including cyclization of sterically hindered **35**. Moreover, this base-induced cyclization was shown to proceed without epimerization of the stereocentre α to the carbonyl group in the morpholin-3-one ring (C-2). This was demonstrated by acylation of amine **32** with racemic 2-bromopropionic acid and subsequent cyclization, which gave **36** as an inseparable mixture with the corresponding C-2 epimer. The ^1H NMR spectrum of this mixture contained doublets at $\delta = 1.47$ and 1.38 derived from the methyl group in each of the two diastereomers. Of these

doublets, the one at $\delta = 1.47$ matched the sole doublet from the methyl group in **36**. Finally, the *tert*-butyldiphenylsilyl ether in **36** and **38** was cleaved and the resulting primary alcohols **39** and **40** were oxidized to give acids **41** and **42** (79% and 71% yields respectively, over two steps).

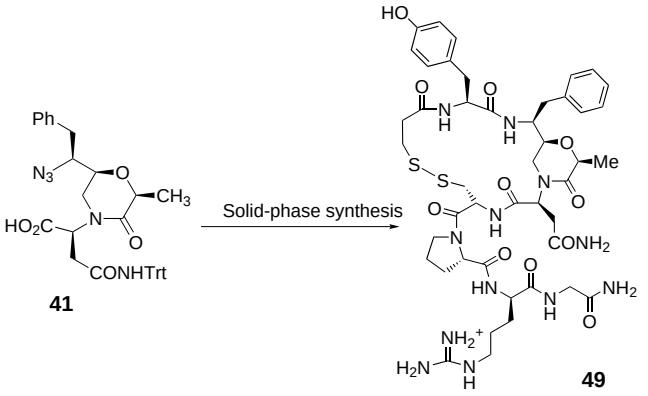
Mimetics of classical γ-turns can be prepared by an analogous route (Scheme 7). Thus, compound **48**, which is a mimetic of a classical Phe-Ala-Asn γ-turn, was prepared from azido



Scheme 7. a) EtOH, reflux, 43 h, 72%; b) Et_3SiCl, imidazole, CH_2Cl_2, 0 °C→RT, 1.5 h, 95%; c) (*R*)-(+)-2-bromopropionic acid, diisopropylcarbodiimide, CH_2Cl_2, 0 °C, 1 h, then aqueous workup followed by 2 M aqueous HCl/THF (3:2), RT, 96 h, 58%; d) KH, THF/DMF (3:1), 0 °C, 4 h, 84%; e) TBAF, THF, RT, 2 h, 100%; f) $NaIO_4$, $RuCl_3 \cdot H_2O$ (cat.), $H_2O/CCl_4/CH_3CN$ (3:2:2), RT, 3 h; 96%.

epoxide **17**, the asparagine building block **30** and (*R*)-(+)-2-bromopropionic acid by the same transformations as were used for synthesis of the inverse γ-turn mimetics. The yields of the steps leading to mimetic **48** closely resembled those observed in the synthesis of the diastereomeric inverse γ-turn mimetic **41**. This allowed mimetic **48** to be obtained in an overall yield of 32% from azido epoxide **17**. As for inverse γ-turn mimetics, the diastereomeric purity of morpholin-3-one **46** was probed in a control experiment starting from racemic 2-bromopropionic acid. Once again, ^{1}H NMR spectroscopy confirmed that the stereocentre α to the carbonyl group in the ring of **46** had not undergone detectable epimerization during the base-promoted cyclization of **45**.

Incorporation of an inverse γ-turn mimetic in desmopressin and preliminary conformational studies: The Phe-Ala-Asn inverse γ-turn mimetic **41** was incorporated in the desmopressin analogue **49** by means of solid-phase synthesis, thereby revealing that the γ-turn mimetics can be used as building blocks under standard conditions for Fmoc solid-phase peptide synthesis (Scheme 8). Synthesis of **49** was performed on a polystyrene resin grafted with polyethyleneglycol chains functionalized with the Rink linker.[41] N^{α}-Fmoc



Scheme 8. Incorporation of mimetic **41** in the desmopressin analogue **49** in 23% overall yield by solid-phase synthesis.

amino acids carrying standard side-chain protecting groups were coupled to the resin as benzotriazolyl (HOBt) esters,[35a] whereas mimetic **41** was activated as a more reactive HOAt ester.[35b] This allowed complete acylation of the resin-bound Cys-Pro-D-Arg-Gly tetrapeptide with only 1.3 equivalents of the valuable **41** relative to the capacity of the resin. After attachment of **41** to the solid phase the azido group was reduced by treatment with tin(II) chloride in the presence of thiophenol and triethylamine.[42] The reduction could be monitored conveniently by the disappearance of the N_3 stretch in the IR spectrum obtained from a few resin beads.[43] The azido group had thus served as a masked amino group throughout the synthesis of **41** and was unmasked at the latest possible stage of the synthesis. After completion of the synthesis, the peptide was cleaved from the resin, and the amino acid side chains were simultaneously deprotected by treatment with trifluoroacetic acid. Disulfide bond formation was effected by oxidation with iodine in methanol,[44] and purification by reversed-phase HPLC gave the desmopressin analogue **49** in 23% overall yield, based on the resin capacity. The structure of analogue **49** was confirmed by means of fast atom bombardment mass spectroscopy, amino acid analysis, and ^{1}H NMR spectroscopy (Table 1).[45]

The conformations of the inverse γ-turn mimetics **26**, **41** and **42** were investigated in chloroform solution by NOESY spectroscopy. For each of them a strong NOE was observed between the protons on C-2 and C-6 in the morpholin-3-one ring (Figure 4). This observation supported the prediction that the inverse γ-turn mimetics adopt the calculated half-chair conformation, with the substituents on the morpholin-3-one ring in pseudoequatorial or equatorial orientations and the C-2 and C-6 protons in pseudoaxial and axial positions (Figure 3 A). Furthermore, a strong NOE was observed between the C-2 and C-6 protons of the morpholin-3-one ring in an aqueous solution of the desmopressin analogue **49**. Thus, the conformation of the morpholin-3-one inverse γ-turn mimetic appears to be unaffected both by the incorporation in the macrocyclic peptide and by the solvent. In comparison,

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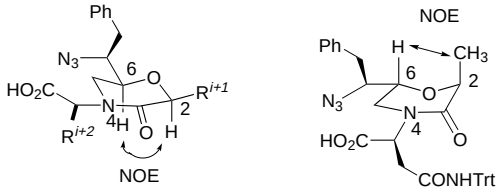
Table 1. ^{1}H NMR data (δ) for the desmopressin analogue **49** in aqueous solution.[a]

Residue	NH	H-α	H-β	H-γ	Others
Mpa[1]		2.61[b]	3.30, 2.82		
Tyr[2]	8.33	4.11	2.40[b]		6.72 and 6.67 (H arom)
Mimetic					
Phe[3]	7.72	4.24	2.97, 2.88		7.34–7.17 (H arom), 3.90 (CHO)
Ala[4]		4.29	1.43		
Asn[5]		4.28	2.90, 2.65		7.71 and 6.91 ($CONH_2$)
bridge					3.23 and 3.68 (CH_2N)
Cys[6]	8.11	4.63	3.14, 2.87		
Pro[7]		4.37	2.24, 1.83	1.99[b]	3.83 and 3.56 (H-δ)
D-Arg[8]	8.89	4.20	1.83, 1.70	1.58[b]	3.12 (H-δ),[b] 7.23 (NH-ε)
Gly[9]	8.33	3.86, 3.81			6.88 and 6.42 ($CONH_2$)

[a] Obtained at 600 MHz, 278 K, and pH = 6.70 (phosphate buffer) for an ≈8 mM solution of **49** in water containing 10% D_2O [HDO (δ = 4.98) as internal standard]. [b] Degeneracy has been assumed.



26 R^{i+1} = Me, R^{i+2} = Me
41 R^{i+1} = Me, R^{i+2} = $CH_2CONHTrt$
42 R^{i+1} = iPr, R^{i+2} = $CH_2CONHTrt$

48

Figure 4. NOEs which support the calculated conformations for inverse (**26**, **41**, and **42**) and classical (**48**) γ-turn mimetics.

the NOESY spectrum of the classical γ-turn mimetic **48** displayed a strong NOE between the methyl group at C-2 and the proton at C-6 of the six-membered ring (Figure 4). Again, this confirms predominant population of the half-chair calculated minimum-energy conformation, in which the methyl group is positioned pseudoaxially and the substituents at C-6 and N-4 occupy equatorial and pseudoequatorial orientations (Figure 3B).

Conclusions

We have developed an enantioselective synthetic approach to mimetics of both inverse and classical γ-turns and shown that the mimetics can be employed in solid-phase synthesis of peptides using the Fmoc strategy. The turn mimetics were assembled from three types of building blocks: azido epoxides, α-bromo acids and β-amino alcohols; of these, the stereochemistry of the azido epoxide determines if a classical or an inverse γ-turn is mimicked. The flexible synthetic approach allows introduction of different side chains in the turn mimetics with full stereochemical control. In addition, this approach may allow preparation of libraries of γ-turn mimetics, since i) the synthetic route to the key azido epoxides allows preparation of >10 g amounts in less than two weeks, and ii) α-bromo acids and β-amino alcohols are either commercially available or readily prepared from amino acids. Finally, conformational studies based on ab initio calculations and ^{1}H NMR spectroscopy revealed that the minimum-energy conformations of the mimetics closely resembled inverse or classical γ-turns.

Experimental Section

General methods and materials: All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 and THF were distilled from calcium hydride and sodium benzophenone, respectively. DMF was distilled and then dried over two portions of 3 Å molecular sieves. TLC was performed on silica gel 60 F_{254} (Merck) with detection by UV light and charring with phosphomolybdic acid in EtOH (26 mmol l^{-1}). Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon).

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-360, a Bruker DRX-400 or a Bruker ARX-500 spectrometer for solutions in $CDCl_3$ (residual $CHCl_3$ (δ_H = 7.26) or $CDCl_3$ (δ_C = 77.0) as internal standard) at 298 K. The NMR sample of the desmopressin analogue **49** was prepared by dissolving **49** (4 mg, 3.95 μmol) in 0.05 mL phosphate buffer (200 mM in D_2O, pD 6.41) and 0.45 mL of a 0.3 mM solution of NaN_3 in doubly distilled water (concentration of the sample: ca. 8 mM, pH 6.70). Spectra of **49** were recorded on a Bruker Avance DRX-600 spectrometer with HDO (δ_H = 4.98) as internal standard at 278 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra and proton resonances were assigned from appropriate combinations of COSY,[46a] TOCSY,[46b] ROESY[46c] and NOESY[46d–f] experiments. Ratios of diastereomeric or rotameric mixtures were determined by integration of ^{1}H NMR resonances. The index A refers to the major, B to the minor isomer.

IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. Optical rotations were measured with a Perkin–Elmer 343 polarimeter. Positive fast atom bombardment mass spectra (FAB MS) were recorded on a JEOL SX102 A mass spectrometer. Ions for FAB MS were produced by a beam of xenon atoms (6 keV) from a matrix of glycerol and thioglycerol. Combustion analyses were performed by F. Hambloch, Institute of Organic Chemistry, University of Göttingen (Germany).

Compounds **17**[27, 31] and **18**[27, 31] were prepared essentially as outlined in the cited references. Experimental procedures describing the syntheses of **17** and **18** are provided as Supporting Information. (*R*)-(+)-2-Bromopropionic acid and (*R*)-(+)-2-bromo-3-methylbutyric acid were purchased from Fluka (Switzerland) and Aldrich (USA), respectively, whereas (*R*)-(+)-2-bromobutyric acid was prepared by diazotization of (*R*)-2-aminobutyric acid as described previously.[25] (*S*)-Alaninol and Fmoc-Asn(Trt)-OH were purchased from Aldrich (USA) and Bachem (Switzerland), respectively.

Molecular modelling: Energy minimization of the simplified γ-turn mimetics **5** and **6** was performed by ab initio molecular orbital calculations with the Spartan RHF 4.1 program[47] using the STO-3G[48] basis sets. Superimposition of energy-minimized conformations of **5** and **6** on ideal inverse and classical γ-turns, respectively, was performed using the MacMimic 3 program.[49] In the superimpositions atoms C-6, C-2, and N-4 in the morpholinone ring of each mimetic were superimposed on the ***C***=O, ***C***-α and ***N***H atoms, respectively, of residues *i*, *i*+1, and *i*+2 of the corresponding γ-turn.

(*2S*)-1-(*tert*-Butyldiphenylsilyloxy)propyl-2-amine (19): Imidazole (1.216 g, 17.87 mmol) was added to (*S*)-alaninol (0.610 g, 8.12 mmol) in CH_2Cl_2 (40 mL) at room temperature. After cooling of the mixture to 0 °C, *tert*-butylchlorodiphenylsilane (3.99 mL, 4.23 g, 17.1 mmol) was added; the reaction mixture was stirred for 3 h, poured into satd. aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 2:1, then EtOH) of the residue yielded **19** (2.419 g, 95%): $[\alpha]_D^{20}$ = +3.3 (*c* = 1.9 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74–7.65 (m, 4H; Ph), 7.46–7.34 (m, 6H; Ph), 3.55 (ABX-type dd, *J* = 9.8 and 4.3 Hz, 1H; CH_2OSi), 3.36 (ABX-type dd, *J* = 9.8 and 7.5 Hz, 1H; CH_2OSi), 3.10–3.00 (m, 1H; C*H*NH_2), 2.17 (br s, 2H; NH_2), 1.06 (s, 9H; *t*Bu), 1.00 (d, *J* = 6.5 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.5 (2C), 129.6, 127.6, 70.4, 48.5, 26.8, 26.7, 19.2; IR (neat): $\tilde{\nu}$ = 1425 cm^{-1} (C–O); HR FAB MS calcd for $C_{19}H_{28}NOSi$ [*M*+H] 314.1940, found 314.1945; $C_{19}H_{27}NOSi$ (313.5): calcd C 72.79, H 8.68, N 4.47; found C 72.67, H 8.87, N 4.42.

(*2S*,*3S*)-3-Azido-1-[(*1S*)-2-(*tert*-butyldiphenylsilyloxy)-1-methylethylamino]-4-phenylbutan-2-ol (20): Azido epoxide **18**[27, 31] (0.151 g, 0.798 mmol) and the *O*-silylated alaninol **19** (0.238 g, 0.760 mmol) were refluxed in EtOH (5 mL) for 96 h. The solvent was evaporated and flash chromatography (heptane/ethyl acetate 1:1, then EtOH) of the residue yielded **20**

(0.260 g, 68 %): $[\alpha]_D^{20} = -1.0$ ($c = 0.4$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.68-7.62$ (m, 4H; Ph), 7.46–7.23 (m, 11H; Ph), 3.63–3.57 (m, 2H; C*H*$_2$OSi and C*H*OH), 3.50 (ABX-type dd, $J = 10.2$ and 6.4 Hz, 1H; C*H*$_2$OSi), 3.36–3.30 (m, 1H; C*H*N$_3$), 3.08 (ABX-type dd, $J = 13.6$ and 6.6 Hz, 1H; C*H*$_2$Ph), 3.00 (ABX-type dd, $J = 13.7$ and 8.4 Hz, 1H; C*H*$_2$Ph), 2.83–2.75 (m, 1H; C*H*$_2$NH), 2.74–2.61 (m, 3H; C*H*$_2$NH and O*H*), 1.06 (s, 9H; *t*Bu), 1.02 (d, $J = 6.5$, 3H; C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 137.5, 135.5 (2C), 133.3, 133.2, 129.7, 129.3, 128.6, 128.3, 127.7, 126.7, 70.1, 67.0, 65.7, 54.6, 49.3, 36.8, 26.8, 19.2, 17.1; IR (neat): $\tilde{\nu} = 3305$ (OH), 2105 cm^{-1} (N$_3$); HR FAB MS calcd for $C_{29}H_{39}N_4O_2Si$ [*M*+H] 503.2842, found 503.2841; $C_{29}H_{38}N_4O_2Si$ (502.7): calcd C 69.29, H 7.62, N 11.14; found C 69.31, H 7.33, N 11.35.

(6*S*)-6-[(1*S*)-1-Azido-2-phenylethyl]-4-[(1*S*)-2-(*tert*-butyldiphenylsilyloxy)-1-methylethyl]morpholin-3-one (21): Amino alcohol **20** (55 mg, 0.11 mmol) in THF (3 mL) was added to NaH (50 % dispersion in mineral oil, 4.8 mg, 0.12 mmol) and the suspension was stirred at room temperature for 30 min. After addition of ethyl bromoacetate (12.0 μL, 18.0 mg, 0.110 mmol) stirring was continued for 2 h at room temperature and finally under reflux for 3 h.[33a] The reaction was quenched with satd. aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were dried with Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 5:1 →1:1) of the residue yielded **21** (13 mg, 22 %): $[\alpha]_D^{20} = -30.1$ ($c = 1.7$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.58–7.53 (m, 4H; Ph), 7.46–7.39 (m, 2H; Ph), 7.38–7.18 (m, 9H; Ph), 4.81–4.72 (m, 1H; C*H*MeN), 4.38 (AB-type d, $J = 16.4$ Hz, 1H; C*H*$_2$CO), 4.10 (AB-type d, $J = 16.4$ Hz, 1H; C*H*$_2$CO), 3.70 (ABX-type dd, $J = 10.8$ and 4.5 Hz, 1H; C*H*$_2$OSi), 3.62–3.54 (m, 2H; C*H*$_2$OSi and C*H*O), 3.49 (ABX-type dd, $J = 11.0$ and 11.0 Hz, 1H; C*H*$_2$N), 3.31 (ddd, $J = 7.6$, 7.6 and 2.9 Hz, 1H; C*H*N$_3$), 3.14 (ABX-type dd, $J = 11.4$ and 2.3 Hz, 1H; C*H*$_2$N), 3.05 (ABX-type dd, $J = 13.4$ and 7.6 Hz, 1H; C*H*$_2$Ph), 2.98 (ABX-type dd, $J = 13.4$ and 7.7 Hz, 1H; C*H*$_2$Ph), 1.16 (d, $J = 7.1$ Hz, 3H; C*H*$_3$), 0.96 (s, 9H, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.0$, 136.5, 135.5 (2C), 133.0, 132.9, 129.9, 129.8, 129.1, 128.9, 127.8, 127.2, 73.4, 68.0, 65.0, 63.0, 49.3, 43.4, 36.0, 26.7, 19.1, 13.4; IR (KBr): $\tilde{\nu} = 2105$ (N$_3$), 1650 cm^{-1} (C=O); HR FAB MS calcd for $C_{31}H_{39}N_4O_3Si$ [*M*+H] 543.2791, found 543.2786; $C_{31}H_{38}N_4O_3Si$ (542.8): calcd C 68.60, H 7.06, N 10.32; found C 68.37, H 6.86, N 10.07.

(2*S*,3*S*)-*N*-[(1*S*)-2-(*tert*-Butyldiphenylsilyloxy)-1-methylethyl]-3-azido-4-phenyl-2-(triethylsilyloxy)butylamine (22): Imidazole (36 mg, 0.53 mmol) was added to amino alcohol **20** (0.115 g, 0.229 mmol) in CH_2Cl_2 (6 mL) at room temperature. The solution was cooled to 0 °C and chlorotriethylsilane (85 μL, 76 mg, 0.50 mmol) was added. After 4 h the reaction mixture was poured into satd. aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried with Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 10:1 →2:1) of the residue furnished **22** (0.103 g, 73 %): $[\alpha]_D^{20} = -1.9$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.73-7.68$ (m, 4H; Ph), 7.48–7.24 (m, 11H; Ph), 3.88–3.79 (m, 1H; C*H*OSi), 3.70–3.49 (m, 3H; C*H*$_2$OSi and C*H*N$_3$), 2.97 (ABX-type dd, $J = 13.9$ and 4.2 Hz, 1H; C*H*$_2$Ph), 2.93–2.70 [m, 4H; C*H*MeNH, C*H*$_2$NH and C*H*$_2$Ph (1H)], 1.09 (s, 9H; *t*Bu), 1.07–1.00 (m, 12H; SiCH$_2$C*H*$_3$ and C*H*$_3$), 0.70 (q, $J = 7.9$ Hz, 6H; SiC*H*$_2$CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 138.3$, 135.5, 133.5, 129.6 (2C), 129.1, 128.5, 127.6, 126.5, 67.9, 65.2, 54.8, 49.8, 36.6, 26.8, 19.2, 17.2, 6.9, 5.1; IR (neat): $\tilde{\nu} = 2105$ cm^{-1} (N$_3$); HR FAB MS calcd for $C_{35}H_{53}N_4O_2Si_2$ [*M*+H] 617.3707, found 617.3713; $C_{35}H_{52}N_4O_2Si_2$ (617.0): calcd C 68.13, H 8.49, N 9.08; found C 67.75, H 8.39, N 9.04.

(2*R*)-*N*-[(2*S*,3*S*)-3-Azido-2-hydroxy-4-phenylbutyl]-*N*-[(1*S*)-2-(*tert*-butyldiphenylsilyloxy)-1-methylethyl]-2-bromopropionamide (23): 1,3-Diisopropylcarbodiimide (DIC, 0.140 mL, 0.116 g, 0.917 mmol) was added to a solution of (*R*)-(+)-2-bromopropionic acid (97.3 μL, 0.160 g, 1.05 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, after which amine **22** (81 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) was added and stirring was continued for 4 h at 0 °C. Then diisopropylurea was filtered off and the filtrate was extracted with satd. aqueous $NaHCO_3$ (2 × 5 mL), 10 % citric acid (2 × 5 mL) and brine (5 mL). The organic solution was dried over Na_2SO_4 and concentrated. The residue was flash chromatographed (heptane/ethyl acetate 10:1) to give the desired triethylsilylated amide (0.136 g) contaminated by coeluting diisopropylurea. The silylated amide (0.136 g) was dissolved in a mixture of 2 M aqueous HCl (3 mL) and THF (2 mL) and stirred at room temperature for 96 h.[50] The reaction mixture was poured into satd. aqueous $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 10:1 →5:1) of the residue yielded **23** (56 mg, 67 % over 2 steps): $[\alpha]_D^{20} = -4.0$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.61-7.56$ (m, 4H; Ph), 7.50–7.36 (m, 6H; Ph), 7.34–7.22 (m, 5H; Ph), 4.55 (q, $J = 6.5$ Hz, 1H; C*H*Br), 4.12–4.02 (m, 2H; C*H*MeN and O*H*), 3.91–3.84 (m, 1H; C*H*OH), 3.59 (ABX-type dd, $J = 11.1$ and 4.6 Hz, 1H; C*H*$_2$OSi), 3.51–3.38 (m, 3H; C*H*$_2$OSi, C*H*$_2$N and C*H*N$_3$), 3.22 (ABX-type dd, $J = 14.4$ and 2.5 Hz, 1H; C*H*$_2$N), 3.07 (ABX-type dd, $J = 14.0$ and 4.9 Hz, 1H; C*H*$_2$Ph), 2.93 (ABX-type dd, $J = 13.9$ and 9.5 Hz, 1H; C*H*$_2$Ph), 1.75 (d, $J = 6.5$ Hz, 3H; CHBrC*H*$_3$), 1.12 (d, $J = 6.7$ Hz, 3H; C*H*$_3$), 1.04 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.5$, 137.7, 135.5, 135.4, 132.7, 132.4, 130.1 (2C), 129.2, 128.6, 127.9 (2C), 126.7, 72.8, 66.1, 64.8, 55.2, 46.1, 38.9, 36.4, 26.8, 21.6, 19.0, 14.6; IR (neat): $\tilde{\nu} = 3380$ (OH), 2105 (N$_3$), 1630 cm^{-1} (C=O); HR FAB MS calcd for $C_{32}H_{42}BrN_4O_3Si$ [*M*+H] 637.2210, found 637.2206; $C_{32}H_{41}BrN_4O_3Si$ (637.7): calcd C 60.27, H 6.48, N 8.79; found C 60.06, H 6.34, N 8.99.

(2*S*,6*S*)-6-[(1*S*)-1-Azido-2-phenylethyl]-4-[(1*S*)-2-(*tert*-butyldiphenylsilyloxy)-1-methylethyl]-2-methylmorpholin-3-one (24): Bromo alcohol **23** (72 mg, 0.11 mmol) in THF (0.5 mL) was added dropwise to KH (5.9 mg, 0.15 mmol) suspended in THF (1 mL) and DMF (0.3 mL) at 0 °C. After 55 min at 0 °C the reaction mixture was poured into satd. aqueous $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried with Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 10:1 →5:1) of the residue gave the morpholinone derivative **24** (54 mg, 86 %): $[\alpha]_D^{20} = -30.9$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.64-7.59$ (m, 4H; Ph), 7.50–7.44 (m, 2H; Ph), 7.43–7.23 (m, 9H; Ph), 4.82–4.72 (m, 1H; C*H*MeN), 4.23 (q, $J = 6.8$ Hz, 1H; OC*H*CH$_3$), 3.75 (ABX-type dd, $J = 10.7$ and 4.5 Hz, 1H; C*H*$_2$OSi), 3.71–3.61 (m, 2H; C*H*$_2$OSi and C*H*O), 3.55 (ABX-type dd, $J = 11.1$ and 11.1 Hz, 1H; C*H*$_2$N), 3.36 (ddd, $J = 7.6$, 7.6 and 3.2 Hz, 1H; C*H*N$_3$), 3.21 (ABX-type dd, $J = 11.5$ and 2.6 Hz, 1H; C*H*$_2$N), 3.10 (ABX-type dd, $J = 13.5$ and 7.5 Hz, 1H; C*H*$_2$Ph), 3.02 (ABX-type dd, $J = 13.5$ and 7.7 Hz, 1H; C*H*$_2$Ph), 1.59 (d, $J =$ 6.8 Hz, 3H; OCHC*H*$_3$), 1.20 (d, $J = 7.1$ Hz, 3H; C*H*$_3$), 1.02 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 169.2$, 136.8, 135.6, 133.1, 129.9, 129.2, 129.0, 127.9, 127.2, 74.7, 73.1, 65.2, 63.2, 49.7, 44.2, 36.2, 26.9, 19.2, 18.6, 13.6; IR (neat): $\tilde{\nu} = 2110$ (N$_3$), 1650 cm^{-1} (C=O); HR FAB MS calcd for $C_{32}H_{41}N_4O_3Si$ [*M*+H] 557.2948, found 557.2942.

(2*S*,6*S*)-6-[(1*S*)-1-Azido-2-phenylethyl]-4-[(1*S*)-2-hydroxy-1-methylethyl]-2-methylmorpholin-3-one (25): Tetrabutylammonium fluoride hydrate (32 mg, 10 μmol) was added to the protected alcohol **24** (52 mg, 93 μmol) in THF (3.5 mL) at room temperature. After stirring of the mixture for 2 h the solvent was evaporated. Flash chromatography (heptane/ethyl acetate 1:1, then EtOH) of the residue furnished **25** (30 mg, 100 %): $[\alpha]_D^{20} = -46.2$ ($c = 0.4$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.45-7.18$ (m, 5H; Ph), 4.68–4.57 (m, 1H; C*H*MeN), 4.21 (q, $J = 6.8$ Hz, 1H; OC*H*CH$_3$), 3.78 (ddd, $J = 10.7$, 3.1 and 3.1 Hz, 1H; C*H*O), 3.66 (ABX-type dd, $J = 11.6$ and 4.1 Hz, 1H; C*H*$_2$OH), 3.58–3.49 (m, 2H; C*H*$_2$OH and C*H*$_2$N), 3.46 (ddd, $J = 7.6$, 7.6 and 3.3 Hz, 1H; C*H*N$_3$), 3.20–3.01 [m, 3H; C*H*$_2$N (1H) and C*H*$_2$Ph (2H)], 1.54 (d, $J = 6.8$ Hz, 3H; OCHC*H*$_3$), 1.16 (d, $J = 7.1$ Hz, 3H; C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.1$, 136.6, 129.2, 128.9, 127.2, 74.6, 73.1, 64.5, 63.0, 51.7, 44.0, 36.2, 18.3, 13.2; IR (neat): $\tilde{\nu} = 3365$ (OH), 2110 (N$_3$), 1630 cm^{-1} (C=O); HR FAB MS calcd for $C_{16}H_{23}N_4O_3$ [*M*+H] 319.1770, found 319.1768; $C_{16}H_{22}N_4O_4$ (318.4): calcd C 60.17, H 7.26, N 17.54; found C 60.32, H 7.37, N 17.38.

(2*S*)-2-{(2*S*,6*S*)-6-[(1*S*)-1-Azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}propionic acid (26): A catalytic amount of $RuCl_3 \cdot H_2O$ (2.2 mol %) was added to a biphasic solution of alcohol **25** (30 mg, 94 μmol) and $NaIO_4$ (60 mg, 0.28 mmol) in CCl_4 (0.2 mL), acetonitrile (0.2 mL), and H_2O (0.3 mL). The black mixture was stirred vigorously for 2 h at room temperature. Then CH_2Cl_2 (5 mL) was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (4 × 3 mL). The combined organic extracts were dried with $MgSO_4$, filtered and concentrated. The resulting residue was triturated with diethyl ether (5 mL), filtered through a Celite pad and concentrated to yield the acid **26** (27 mg, 86 %): $[\alpha]_D^{20} = -41.5$ ($c = 0.4$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.21–7.36 (m, 5H; Ph), 5.13 (q, $J = 7.4$ Hz, 1H; C*H*MeN), 4.25 (q, $J =$ 6.8 Hz, 1H; OC*H*CH$_3$), 3.80 (ddd, $J = 10.7$, 3.2 and 3.2 Hz, 1H; C*H*O), 3.63 (ABX-type dd, $J = 10.9$ and 10.9 Hz, 1H; C*H*$_2$N), 3.45 (ddd, $J = 7.9$, 7.0 and 3.4 Hz, 1H; C*H*N$_3$), 3.12 (ABX-type dd, $J = 11.3$ and 2.7 Hz, 1H; C*H*$_2$N), 3.06 (ABX-type dd, $J = 13.8$ and 6.9 Hz, 1H; C*H*$_2$Ph), 3.02 (ABX-type dd, $J = 13.6$ and 8.0 Hz, 1H; C*H*$_2$Ph), 1.52 (d, $J = 6.8$ Hz, 3H; OCHC*H*$_3$), 1.46 (d, $J = 7.5$ Hz, 3H; C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.0$, 170.5, 136.5, 129.2, 128.9, 127.2, 74.6, 73.3, 62.9, 51.6, 45.4, 36.2,

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18.2, 13.7; IR (neat): $\tilde{\nu}=2110$ (N_3), 1730 (C=O), 1615 cm^{-1} (C=O); HR FAB MS calcd for $C_{16}H_{21}N_4O_4$ [M+H] 333.1563, found 333.1571; $C_{16}H_{20}N_4O_4$ (332.4): calcd C 57.82, H 6.07, N 16.86; found C 58.02, H 6.30, N 16.69.

***N*-Triphenylmethyl (3*S*)-3-(9-fluorenylmethoxycarbonylamino)-4-hydroxybutyramide (28).** *N*-Methylmorpholine (0.10 mL, 93 mg, 0.92 mmol) followed by isobutyl chloroformate (0.12 mL, 0.13 g, 0.92 mmol) were added to a stirred solution of Fmoc-Asn(Trt)-OH (**27**, 0.546 g, 0.915 mmol) in THF (5 mL) at $-10\,^{\circ}C$. After 10 min, $NaBH_4$ (0.103 g, 2.75 mmol) was added in one portion. After a further 15 min MeOH (10 mL) was added dropwise to the mixture over a period of 5 min at $0\,^{\circ}C$. The solution was stirred for an additional 10 min, and then neutralized with 2 M aqueous HCl (2 mL). The organic solvents were evaporated and the residue was extracted with EtOAc (3×10 mL). The combined organic phases were dried with Na_2SO_4 and the solvent was removed. Flash chromatography (toluene/EtOH 20:1) furnished alcohol **28** (0.373 g, 70 %) as a white solid: m.p. 168–170 °C; $[\alpha]_D^{20}=+21.6$ ($c=0.7$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.81-7.72$ (m, 2H; Ar), 7.60–7.53 (m, 2H; Ar), 7.44–7.37 (m, 2H; Ar), 7.32–7.14 (m, 17H; Ar), 5.87 (d, $J=7.6$ Hz, 1H; OCON*H*), 4.40–4.26 (m, 2H; CH_2OCO), 4.15 (t, $J=6.8$ Hz, 1H; fluorenyl-9-*H*), 3.96–3.85 (m, 1H; C*H*NFmoc), 3.71–3.48 (m, 3H; CH_2O*H*), 2.71 (ABX-type dd, $J=$ 14.5 and 5.8 Hz, 1H; CH_2CO), 2.60 (ABX-type dd, $J=14.4$ and 4.3 Hz, 1H; CH_2CO); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.7$, 156.4, 144.1, 143.7, 141.2, 128.5, 128.3, 127.9, 127.7, 127.1, 127.0, 125.0, 119.9, 70.8, 66.8, 64.1, 50.1, 47.0, 39.0; IR (neat): $\tilde{\nu}=3315$ (OH), 1705 cm^{-1} (C=O); HR FAB MS calcd for $C_{38}H_{35}N_2O_4$ [M+H] 583.2597, found 583.2591.

***N*-Triphenylmethyl (3*S*)-4-(*tert*-butyldiphenylsilyloxy)-3-(9-fluorenylmethoxycarbonylamino)butyramide (29)**: Imidazole (63 mg, 0.93 mmol) was added to alcohol **28** (0.271 g, 0.465 mmol) in CH_2Cl_2 (5 mL) at room temperature. After cooling to $0\,^{\circ}C$ *tert*-butylchlorodiphenylsilane (0.14 mL, 0.15 g, 0.62 mmol) was added and stirring was continued for 1.5 h. Then the reaction mixture was poured into brine (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic phases were dried with Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate 10:1 →1:1) of the residue yielded **29** (0.294 g, 77 %) as a white solid: m.p. 84–86 °C; $[\alpha]_D^{20}=-7.4$ ($c=0.6$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.78-7.72$ (m, 2H; Ar), 7.65–7.59 (m, 4H; Ar), 7.57–7.50 (m, 2H; Ar), 7.45–7.30 (m, 9H; Ar), 7.29–7.22 (m, 10H; Ar), 7.20–7.15 (m, 6H; Ar), 6.87 (s, 1H; N*H*Trt), 5.73 (d, $J=7.7$ Hz, 1H; OCON*H*), 4.29–4.22 and 4.15–4.06 (2m, 4H; CH_2OCO, fluorenyl-9-*H*, C*H*NFmoc), 3.84–3.77 (m, 1H; CH_2OSi), 3.72–3.64 (m, 1H; CH_2OSi), 2.87–2.78 (m, 1H; CH_2CO), 2.68–2.58 (m, 1H; CH_2CO), 1.05 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.0$, 155.9, 144.5, 143.9 (2C), 141.2, 135.4, 133.0, 129.9, 129.8, 128.6, 128.3, 128.0, 127.8 (2C), 127.7, 127.6, 127.1, 127.0, 125.2, 119.9, 70.8, 66.8, 64.5, 50.1, 47.1, 37.7, 26.9, 19.3; IR (neat): $\tilde{\nu}=1700$ cm^{-1} (C=O); $C_{54}H_{52}N_2O_4Si$ (821.1): calcd C 78.99, H 6.38, N 3.41; found C 79.22, H 6.58, N 3.32.

***N*-Triphenylmethyl (3*S*)-3-amino-4-(*tert*-butyldiphenylsilyloxy)butyramide (30)**: Compound **29** (0.294 g, 0.358 mmol) was dissolved in morpholine (5 mL) at room temperature. After 1 h the solution was coevaporated with toluene (3×5 mL). Flash chromatography (heptane/ethyl acetate 10:1 →1:1, then toluene/EtOH 1:1 →EtOH) of the residue gave **30** (0.189 g, 88 %) as a white solid: m.p. 125 °C; $[\alpha]_D^{20}=-7.4$ ($c=0.8$ in $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): $\delta=9.26$ (s, 1H; N*H*Trt), 7.66–7.62 (m, 3H; Ph), 7.49–7.34 (m, 5H; Ph), 7.32–7.20 (m, 17H; Ph), 3.60 (ABX-type dd, $J=10.1$ and 4.2 Hz, 1H; CH_2OSi), 3.49 (ABX-type dd, $J=10.0$ and 6.4 Hz, 1H; CH_2OSi), 3.31–3.21 (m, 1H; C*H*NH$_2$), 2.35–2.29 (m, 2H; CH_2CO), 1.08 (s, 9H; *t*Bu); ^{13}C NMR (90 MHz, $CDCl_3$): $\delta=170.7$, 144.9, 135.3, 132.9, 129.7, 128.5, 127.7, 127.6, 126.6, 70.1, 68.6, 50.4, 40.2, 26.8, 19.1; IR (KBr): $\tilde{\nu}=1655$ (C=O), 1105 cm^{-1} (C–O); $C_{39}H_{42}N_2O_2Si$ (598.9): calcd C 78.22, H 7.07, N 4.68; found C 78.52, H 7.32, N 4.53.

***N*-Triphenylmethyl (3*S*)-3-[(2*S*,3*S*)-3-azido-2-hydroxy-4-phenylbutylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (31)**: Opening of epoxide **18** (0.210 g, 1.11 mmol) with amine **30** (0.637 g, 1.06 mmol) as described for **20**, followed by concentration and flash chromatography (heptane/ethyl acetate 5:1 →1:1) of the residue, gave **31** (0.548 g, 66 %) as a white solid: m.p. 63–65 °C; $[\alpha]_D^{20}=-9.4$ ($c=0.2$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.13$ (s, 1H; N*H*Trt), 7.71–7.61 (m, 4H; Ph), 7.50–7.19 (m, 26H; Ph), 3.76 (ABX-type dd, $J=10.5$ and 4.2 Hz, 1H; CH_2OSi), 3.61 (ABX-type dd, $J=10.4$ and 4.6 Hz, 1H; CH_2OSi), 3.40 (ddd, $J=9.3$, 3.1 and 3.1 Hz, 1H; C*H*OH), 3.28 (ddd, $J=8.9$, 6.1 and 3.3 Hz, 1H; C*H*N$_3$), 3.01–2.84 (m, 3H; C*H*NH and CH_2Ph), 2.67 (ABX-type dd, $J=11.7$ and 9.4 Hz, 1H; CH_2NH), 2.57–2.40 (m, 3H; CH_2CO and CH_2NH), 1.10 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.9$, 144.8, 137.2, 135.5 (2C), 132.9 (2C), 129.9, 128.7, 128.6, 127.9, 127.8, 126.9, 126.8, 71.2, 70.2, 66.0, 63.9, 56.3, 49.9, 39.2, 37.0, 26.8, 19.2; IR (KBr): $\tilde{\nu}=3310$ (OH), 2100 (N_3), 1655 cm^{-1} (C=O); $C_{49}H_{53}N_5O_3Si$ (788.1): calcd C 74.68, H 6.78, N 8.89; found C 74.92, H 7.01, N 8.94.

***N*-Triphenylmethyl (3*S*)-3-[(2*S*,3*S*)-3-azido-4-phenyl-2-triethylsilyloxybutylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (32)**: Amino alcohol **31** was silylated (0.501 g, 0.637 mmol) in CH_2Cl_2 (20 mL) with chlorotriethylsilane (0.24 mL, 0.21 g, 1.4 mmol) in the presence of imidazole (0.100 g, 1.47 mmol), as described for **22**. After workup, flash chromatography (heptane/ethyl acetate 10:1 →1:1) of the residue furnished **32** (0.489 g, 91 %): m.p. 56–57 °C; $[\alpha]_D^{20}=-23.1$ ($c=0.2$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.44$ (s, 1H; N*H*Trt), 7.66–7.61 (m, 4H; Ph), 7.46–7.19 (m, 26H; Ph), 3.85 (ABX-type dd, $J=10.5$ and 3.2 Hz, 1H; CH_2OSi), 3.72 (ddd, $J=8.3$, 4.0 and 4.0 Hz, 1H; C*H*N$_3$), 3.49 (ABX-type dd, $J=10.5$ and 4.0 Hz, 1H; CH_2OSi), 3.39 (ddd, $J=11.1$, 3.6 and 3.6 Hz, 1H; C*H*OSi), 3.06–3.00 (m, 1H; C*H*NH), 3.00 (ABX-type dd, $J=14.1$ and 2.9 Hz, 1H; CH_2NH), 2.79 (ABX-type dd, $J=11.1$ and 8.5 Hz, 1H; CH_2Ph), 2.64 (ABX-type dd, $J=10.8$ and 4.2 Hz, 1H; CH_2Ph), 2.63 (ABX-type dd, $J=$ 14.1 and 10.8 Hz, 1H; CH_2NH), 2.54 (ABX-type dd, $J=16.2$ and 9.2 Hz, 1H; CH_2CO), 2.42 (ABX-type dd, $J=16.2$ and 2.8 Hz, 1H; CH_2CO), 2.10–1.87 (br s, 1H; N*H*), 1.07 (s, 9H; *t*Bu), 0.95 (t, $J=7.9$ Hz, 9H; SiCH$_2$C*H*$_3$), 0.55 (q, $J=8.0$ Hz, 6H; SiC*H*$_2$CH$_3$); ^{13}C NMR (90 MHz, $CDCl_3$): $\delta=171.3$, 145.1, 138.1, 135.6, 135.5, 133.1, 132.8, 129.9 (2C), 129.0, 128.8, 127.9, 127.8, 126.8 (2C), 74.1, 70.1, 66.5, 63.6, 56.8, 48.3, 39.8, 35.7, 26.9, 19.3, 7.0, 5.0; IR (KBr): $\tilde{\nu}=2105$ (N_3), 1680 (C=O), 1110 cm^{-1} (C–O); $C_{55}H_{67}N_5O_3Si_2$ (902.3): calcd C 73.21, H 7.48, N 7.76; found C 73.44, H 7.59, N 7.99.

***N*-Triphenylmethyl (3*S*)-3-{*N*′-[(2*S*,3*S*)-3-azido-2-hydroxy-4-phenylbutyl]-*N*′-[(1*R*)-1-bromoethylcarbonyl]amino}-4-(*tert*-butyldiphenylsilyloxy)butyramide (33)**: Acylation of amine **32** (0.666 g, 0.791 mmol) with (*R*)-(+)-2-bromopropionic acid (0.570 mL, 0.968 g, 6.33 mmol) and DIC (0.860 mL, 0.698 g, 5.54 mmol), followed by removal of the triethylsilyl group with 2 M aqueous HCl (15 mL) and THF (10 mL), as described for **23**, gave **33** (0.487 g, 67 % over 2 steps) after purification by flash chromatography (heptane/ethyl acetate 5:1). Compound **33** had: m.p. 80 °C; $[\alpha]_D^{20}=-38.1$ ($c=0.08$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 1:1 mixture of rotamers A and B): $\delta=7.65-7.51$ (m, 4H; Ph, A + B), 7.47–7.13 (m, 26H; Ph, A + B), 6.72 (s, 0.5H; N*H*Trt, A), 6.68 (s, 0.5H; N*H*Trt, B), 4.80 (q, $J=6.6$ Hz, 0.5H; C*H*Br, A), 4.58–4.44 [m, 1.5H; C*H*N (A + B, 1H) and C*H*Br (B, 0.5H)], 4.25–4.15 [m, 1.5H; CH_2OSi (A, 0.5H) and O*H* (A + B)], 3.99–3.92 (m, 0.5H; C*H*OH, A), 3.82–3.72 [m, 1H; CH_2OSi (A, 0.5H) and C*H*OH (B, 0.5H)], 3.69–3.62 (m, 0.5H; CH_2OSi, B), 3.53–3.34 [m, 1H; C*H*N$_3$ (A, 0.5H) and CH_2OSi (B, 0.5H), 3.33–3.22 [m, 1.5H; CH_2NH (A + B, 1H) and C*H*N$_3$ (B, 0.5H)], 3.18–3.14, 3.09–2.80, 2.75–2.68 and 2.40–2.28 [4m, 5H; CH_2NH (A + B, 1H), CH_2Ph (A + B, 2H) and CH_2CO (A + B, 2H)], 1.73 (d, $J=6.6$ Hz, 1.5H; CH_3, A), 1.72 (d, $J=$ 6.6 Hz, 1.5H; CH_3, B), 1.04 (s, 4.5H; *t*Bu, A), 1.00 (s, 4.5H; *t*Bu, B); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=172.4$, 170.8, 169.9, 168.1, 144.4, 144.3, 137.4 (2C), 135.6, 135.4, 132.5, 132.4, 132.2, 130.1, 130.0 (2C), 129.3, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 128.0 (2C), 127.9, 127.8, 127.2, 127.1, 126.9, 126.8, 71.7, 71.5, 70.9, 70.7, 66.2, 64.8, 64.0, 63.4, 56.3, 54.7, 47.6, 40.8, 39.7, 37.9, 37.1, 36.1, 35.9, 26.8, 21.8, 19.1, 19.0; IR (neat): $\tilde{\nu}=3360$ (OH), 2105 (N_3), 1650 cm^{-1} (C=O); HR FAB MS calcd for $C_{52}H_{57}BrN_5O_4Si$ [M+H] 922.3363, found 922.3366; $C_{52}H_{56}BrN_5O_4Si$ (923.0): calcd C 67.66, H 6.12, N 7.59; found C 68.01, H 6.39, N 7.51.

***N*-Triphenylmethyl (3*S*)-3-{*N*′-[(2*S*,3*S*)-3-azido-2-hydroxy-4-phenylbutyl]-*N*′-[(1*R*)-1-bromopropylcarbonyl]amino}-4-(*tert*-butyldiphenylsilyloxy)-butyramide (34)**: Acylation of amine **32** (0.111 g, 0.132 mmol) with (*R*)-2-bromobutyric acid[25] (0.176 g, 1.05 mmol) and DIC (0.143 mL, 0.117 g, 0.924 mmol), followed by removal of the triethylsilyl group using 2 M aqueous HCl (3 mL) and THF (2 mL), as described for **23**, gave **34** (38 mg, 31 % over 2 steps) after purification by flash chromatography (heptane/ethyl acetate 8:1 →3:1). Compound **34** had: m.p. 73–75 °C; $[\alpha]_D^{20}=-28.0$ ($c=0.6$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 7:3 mixture of rotamers A and B): $\delta=7.66-7.53$ (m, 4H; Ph, A + B), 7.48–7.13 (m, 26H; Ph, A + B), 6.69 (s; N*H*Trt, B), 6.67 (s; N*H*Trt, A), 4.65 (dd, $J=8.1$ and 5.5 Hz; C*H*Br, B), 4.61–4.54 (m; C*H*N), 4.28–4.17 [m; CH_2OSi and C*H*Br (B)], 4.00–3.92 (m; C*H*OH, A), 3.80–3.73 (m; C*H*OH, B), 3.73–3.70 (m; CH_2OSi), 3.65 (ABX-type dd, $J=10.0$ and 5.3 Hz; CH_2OSi),

3.56–3.46 (m; CH_2OSi), 3.45–3.39 (m; CHN$_3$, A), 3.38–3.23 [m; CH_2NH and CHN$_3$ (B)], 3.21–3.13 (m; CH_2NH), 3.08–2.56 (m; CH_2Ph and CH_2CO), 2.41–2.32 (m; CH_2CO), 2.08–1.90 (m, 2H; CH_2CH$_3$), 1.05 (s, 9H; *t*Bu, A + B), 1.04–1.00 (m, 3H; CH_3, A + B); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 172.1, 170.5, 169.9, 168.1, 144.4, 144.3, 137.5, 137.3, 135.6, 135.5 (2C), 135.4, 132.5, 132.4, 132.3, 132.2, 130.1, 130.0, 129.9 (2C), 129.3, 129.2, 128.7, 128.6, 128.5, 128.4, 128.0 (2C), 127.9 (2C), 127.8 (2C), 127.2, 127.1, 126.9, 126.8, 71.7, 71.6, 70.9, 70.7, 66.2, 64.6, 64.2, 63.6, 56.2, 48.0, 48.1, 47.8, 47.7, 37.8, 37.1, 36.0, 35.8, 29.0, 28.4, 27.0, 26.8, 19.0 (2C), 12.1, 11.9; IR (neat): $\tilde{\nu}$ = 3345 (OH), 2105 (N$_3$), 1650 cm^{-1} (C=O); HR FAB MS calcd for $C_{53}H_{59}BrN_5O_4Si$ [*M*+H] 936.3520, found 936.3530; $C_{53}H_{58}BrN_5O_4Si$ (937.1): calcd C 67.93, H 6.24, N 7.47; found C 67.89, H 6.25, N 7.51.

***N*-Triphenylmethyl (*3S*)-3-{*N'*-[(*2S*,*3S*)-3-azido-2-hydroxy-4-phenylbutyl]-*N'*-[(*1R*)-1-bromo-2-methylpropylcarbonyl]amino}-4-(*tert*-butyldiphenylsilyloxy)butyramide (35)**: Acylation of amine **32** (0.316 g, 0.375 mmol) with (*R*)-(+)-2-bromo-3-methylbutyric acid (0.543 g, 3.00 mmol) and DIC (0.406 mL, 0.331 g, 2.63 mmol), followed by removal of the triethylsilyl group using 2 M aqueous HCl (4.5 mL) and THF (3 mL), as described for **23**, gave **35** (0.143 g, 40% over 2 steps) after purification by flash chromatography (heptane/ethyl acetate 5:1). Compound **35** had: m.p. 78–80 °C; $[\alpha]_D^{20}$ = −35.1 (*c* = 1.0 in CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$, 4:1 mixture of rotamers A and B): δ = 7.65–7.52 (m, 4H; Ph, A + B), 7.47–7.12 (m, 26H; Ph, A + B), 6.67 (s, 0.2H; N*H*Trt, B), 6.65 (s, 0.8H; N*H*Trt, A), 4.61–4.53 [m, 0.4H; C*H*Br (B, 0.2H) and C*H*N (B, 0.2H)], 4.24 (br s, 1H; O*H*, A + B), 4.06–3.88 [m, 1H; C*H*Br (A, 0.8H) and C*H*OH (B, 0.2H)], 3.80–3.67 [m, 1H; C*H*OH (A, 0.8H) and CH_2OSi (B, 0.2H)], 3.64–3.50 (m, 1.8H; CH_2OSi, A + B), 3.44–3.20 [m, 4.6H; CH_2NH (A + B, 2H), CHN$_3$ (A + B, 1H), C*H*N (A, 0.8H) and CH_2CO (A, 0.8H)], 3.09–3.01 and 2.94–2.83 (2m, 2H; CH_2Ph, A + B), 2.75–2.68 (m, 0.2H; CH_2CO, B), 2.49–2.42 (m, 0.2H; CH_2CO, B), 2.36–2.28 (m, 0.8H; CH_2CO, A), 2.27–2.16 [m, 1H; CH(CH$_3$)$_2$, A + B], 1.04 (s, 9H; *t*Bu, A + B) 1.02–0.77 (m, 6H; CH(CH_3)$_2$, A + B); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.9, 170.6, 170.0, 168.1, 144.5, 144.4, 137.6, 137.4, 135.6 (2C), 135.5 (2C), 132.7, 132.5, 132.4, 130.1, 130.0, 129.3 (2C), 129.0, 128.7 (2C), 128.3, 128.0 (2C), 127.9, 127.8, 127.2, 127.1, 126.8, 72.2, 71.2, 71.0, 70.8, 66.3, 64.6, 64.3, 64.2, 56.3, 54.5, 53.6, 38.0, 37.1, 36.0, 35.9, 32.0, 31.8, 27.0, 26.9, 20.7, 20.3, 20.2, 19.1 (2C); IR (neat): $\tilde{\nu}$ = 3340 (OH), 2105 (N$_3$), 1645 cm^{-1} (C=O); HR FAB MS calcd for $C_{54}H_{61}BrN_5O_4Si$ [*M*+H] 950.3676, found 950.3657; $C_{54}H_{61}BrN_5O_4Si$ (951.1): calcd C 68.19, H 6.36, N 7.36; found C 67.97, H 6.68, N 7.16.

***N*-Triphenylmethyl (*3S*)-3-{(*2S*,*6S*)-6-[(*1S*)-1-azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-4-(*tert*-butyldiphenylsilyloxy)butyramide (36):** Intramolecular cyclization of bromo alcohol **33** (0.446 g, 0.483 mmol) with KH (44.6 mg, 1.11 mmol) as described for **24**, followed by workup and purification of the residue by flash chromatography (heptane/ethyl acetate 5:1 → 1:1) gave **36** (0.341 g, 84%): m.p. 75–78 °C; $[\alpha]_D^{20}$ = −18.6 (*c* = 0.1 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.64–7.58 (m, 4H; Ph), 7.45–7.13 (m, 26H; Ph), 6.76 (s, 1H; N*H*Trt), 4.09 (ABX-type dd, *J* = 9.3 and 9.3 Hz, 1H; CH_2OSi), 4.04 (q, *J* = 6.7 Hz, 1H; C*H*CH$_3$), 3.95-3.82 (m, 1H; C*H*N), 3.73 (ABX-type dd, *J* = 11.2 and 11.2 Hz, 1H; CH_2N), 3.68 (ABX-type dd, *J* = 10.0 and 5.1 Hz, 1H; CH_2OSi), 3.54–3.46 (m, 1H; C*H*O), 3.33–3.26 (m, 1H; CHN$_3$), 3.25–3.16 (m, 2H; CH_2N and CH_2CO), 2.99–2.88 (m, 2H; CH_2Ph), 2.50 (ABX-type dd, *J* = 15.3 and 4.1 Hz, 1H; CH_2CO), 1.47 (d, *J* = 6.6 Hz, 3H; CH_3), 1.04 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 170.2, 169.3, 144.5, 136.9, 135.5 (2C), 133.1, 132.9, 129.8, 129.2, 128.8, 128.6, 127.9, 127.8, 127.0, 74.3, 73.6, 70.4, 63.1, 62.9, 60.1, 51.6, 36.8, 36.1, 26.7, 19.1, 18.0; IR (KBr): $\tilde{\nu}$ = 2100 (N$_3$), 1640 (C=O), 1120 cm^{-1} (C−O); $C_{52}H_{55}N_5O_4Si$ (842.1): calcd C 74.17, H 6.58, N 8.32; found C 74.37, H 6.54, N 8.33.

***N*-Triphenylmethyl (*3S*)-3-{(*2S*,*6S*)-6-[(*1S*)-1-azido-2-phenylethyl]-2-ethylmorpholin-3-one-4-yl}-4-(*tert*-butyldiphenylsilyloxy)butyramide (37)**: Intramolecular cyclization of bromo alcohol **34** (38 mg, 41 μmol) with KH (3.7 mg, 93 μmol) as described for **24**, followed by workup and purification of the residue by flash chromatography (heptane/ethyl acetate 3:1), gave **37** (22 mg, 63%): m.p. 71–73 °C; $[\alpha]_D^{20}$ = −31.5 (*c* = 0.03 in CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.64–7.58 (m, 4H; Ph), 7.46–7.12 (m, 26H; Ph), 6.75 (s, 1H; N*H*Trt), 4.10 (ABX-type dd, *J* = 10.0 and 8.7 Hz, 1H; CH_2OSi), 4.04 (dd, *J* = 7.5 and 3.5 Hz, 1H; CHC$_2$H$_5$), 3.87–3.79 (m, 1H; C*H*N), 3.75 (ABX-type dd, *J* = 11.5 and 11.0 Hz, 1H; CH_2N), 3.67 (ABX-type dd, *J* = 10.2 and 5.1 Hz, 1H; CH_2OSi), 3.49 (ddd, *J* = 11.1, 3.4 and 3.4 Hz, 1H; C*H*O), 3.29–3.16 [m, 3H; CHN$_3$, CH_2N (1H) and CH_2CO (1H)], 3.00–2.88 (m, 2H; CH_2Ph), 2.49 (ABX-type dd, *J* = 15.1 and 4.6 Hz, 1H; CH_2CO), 2.07–1.96 (m, 1H; CH_2CH$_3$), 1.85–1.72 (m, 1H; CH_2CH$_3$), 1.09–0.99 (m, 12H; CH_3 and *t*Bu); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.7, 169.3, 144.5, 136.9, 135.5, 135.4, 133.0, 132.9, 129.8, 129.1, 128.8, 128.5, 127.9, 127.8 (2C), 127.0, 78.7, 73.5, 70.4, 63.1, 62.8, 60.9, 51.8, 36.9, 36.0, 26.7, 25.4, 19.1, 9.7; IR (KBr): $\tilde{\nu}$ = 2110 (N$_3$), 1685 (C=O), 1635 cm^{-1} (C=O); HR FAB MS calcd for $C_{53}H_{58}N_5O_4Si$ [*M*+H] 856.4258, found 856.4266; $C_{53}H_{58}N_5O_4Si$ (856.2): calcd C 74.35, H 6.71, N 8.18; found C 74.23, H 7.07, N 8.11.

***N*-Triphenylmethyl (*3S*)-3-{(*2S*,*6S*)-6-[(*1S*)-1-azido-2-phenylethyl]-2-isopropylmorpholin-3-one-4-yl}-4-(*tert*-butyldiphenylsilyloxy)butyramide (38)**: Intramolecular cyclization of bromo alcohol **35** (0.106 g, 0.115 mmol) with KH (13 mg, 0.27 mmol) as described for **24**, followed by workup and purification of the residue by flash chromatography (heptane/ethyl acetate 3:1) gave **38** (67 mg, 69%): m.p. 88–90 °C; $[\alpha]_D^{20}$ = −33.1 (*c* = 0.3 in CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.64–7.58 (m, 4H; Ph), 7.45–7.11 (m, 26H; Ph), 6.72 (s, 1H; N*H*Trt), 4.15–4.06 (m, 1H; CH_2OSi), 3.81 (d, *J* = 2.0 Hz, 1H; C*H*iPr), 3.78–3.67 [m, 3H; C*H*N, CH_2N (1H) and CH_2OSi (1H)], 3.50–3.42 (m, 1H; C*H*O), 3.31–3.13 [m, 3H; C*H*N$_3$, CH_2N (1H) and CH_2CO (1H)], 2.98–2.93 (m, 2H; CH_2Ph), 2.54–2.39 [m, 2H; CH(CH$_3$)$_2$ and CH_2CO], 1.13 (d, *J* = 7.1 Hz, 3H; CH_3), 1.03 (s, 9H; *t*Bu), 0.92 (d, *J* = 6.8 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.5, 169.3, 144.4, 136.9, 135.5, 135.4, 133.0, 132.9, 129.8, 129.1, 128.8, 128.5, 127.9, 127.8, 127.7, 127.0, 81.6, 73.3, 70.3, 63.2, 62.7, 37.0, 35.9, 30.6, 26.7, 19.4, 19.0, 15.8; IR (KBr): $\tilde{\nu}$ = 2105 (N$_3$), 1685 (C=O), 1635 cm^{-1} (C=O); HR FAB MS calcd for $C_{54}H_{60}N_5O_4Si$ [*M*+H] 870.4415, found 870.4404.

***N*-Triphenylmethyl (*3S*)-3-{(*2S*,*6S*)-6-[(*1S*)-1-azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-4-hydroxybutyramide (39)**: Deprotection of **36** (97 mg, 0.12 mmol) with tetrabutylammonium fluoride hydrate (33 mg, 0.13 mmol) as described for **25** followed by concentration and purification of the residue by flash chromatography (heptane/ethyl acetate 1:1, then EtOH) furnished **39** (68 mg, 98%): m.p. 93–94 °C; $[\alpha]_D^{20}$ = −35.7 (*c* = 0.2 in CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.45–7.24 (m, 20H; Ph), 4.11 (q, *J* = 6.8 Hz, 1H; C*H*CH$_3$), 4.05–3.93 (m, 1H; C*H*N), 3.82–3.63 [m, 3H; CH_2O (2H) and CH_2N (1H)], 3.58 (ddd, *J* = 10.8, 3.1 and 3.1 Hz, 1H; C*H*O), 3.48–3.38 (m, 1H; C*H*N$_3$), 3.32 (ABX-type dd, *J* = 11.4 and 2.5 Hz, 1H; CH_2N), 3.11 (ABX-type dd, *J* = 15.2 and 9.3 Hz, 1H; CH_2CO), 3.08–2.96 (m, 2H; CH_2Ph), 2.75 (ABX-type dd, *J* = 15.3 and 5.8 Hz, 1H; CH_2CO), 1.52 (d, *J* = 6.8 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 170.9, 169.5, 144.3, 136.6, 129.1, 128.7, 128.5, 127.8, 127.0, 126.9, 74.1, 73.4, 70.4, 63.0, 62.7, 59.0, 49.9, 36.1, 36.0, 18.0; IR (KBr): $\tilde{\nu}$ = 3410 and 3300 (N−H and O−H), 2110 (N$_3$), 1660 (C=O), 1635 cm^{-1} (C=O); HR FAB MS calcd for $C_{36}H_{37}N_5O_4$ [*M*+H] 604.2924, found 604.2931.

***N*-Triphenylmethyl (*3S*)-3-{(*2S*,*6S*)-6-[(*1S*)-1-azido-2-phenylethyl]-2-isopropylmorpholin-3-one-4-yl}-4-hydroxybutyramide (40)**: Deprotection of **38** (59 mg, 68 μmol) with tetrabutylammonium fluoride hydrate (23 mg, 88 μmol) as described for **25** followed by concentration and purification of the residue by flash chromatography (heptane/ethyl acetate 1:1, then EtOH) furnished **40** (42 mg, 98%): $[\alpha]_D^{20}$ = −46.6 (*c* = 0.2 in CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.40–7.14 (m, 20H; Ph), 7.07 (s, 1H; N*H*Trt), 4.18–3.99 (m, 1H; O*H*), 3.90 (d, *J* = 2.3 Hz, 1H; C*H*iPr), 3.83–3.71 (m, 3H; C*H*N and CH_2OH), 3.61 (ABX-type dd, *J* = 11.1 and 11.1 Hz, 1H; CH_2N), 3.45 (ddd, *J* = 10.7, 2.9 and 2.9 Hz, 1H; C*H*O), 3.25 (ABX-type dd, *J* = 11.7 and 2.6 Hz, 1H; CH_2N), 3.20 (ddd, *J* = 7.4, 7.4 and 3.1 Hz, 1H; C*H*N$_3$), 3.17–3.08 (m, 1H; CH_2CO), 3.04–2.95 (m, 2H; CH_2Ph), 2.84–2.76 (m, 1H; CH_2CO), 2.43 [septet of d, *J* = 6.9 and 2.4 Hz, 1H; CH(CH$_3$)$_2$], 1.14 (d, *J* = 7.0 Hz, 3H; CH_3), 0.92 (d, *J* = 6.8 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 170.6, 169.5, 144.3, 136.7, 129.1, 128.8, 128.5, 127.9, 127.0, 81.4, 73.2, 70.5, 63.9, 62.5, 60.4, 50.7, 36.1, 35.9, 30.9, 19.2, 15.6; IR (neat): $\tilde{\nu}$ = 3295 (OH), 2110 (N$_3$), 1630 cm^{-1} (C=O); HR FAB MS calcd for $C_{38}H_{42}N_5O_4$ [*M*+H] 632.3237, found 632.3244.

(*2S*)-2-{(*2S*,*6S*)-6-[(*1S*)-1-Azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-3-(*N*-triphenylmethylcarbamoyl)propionic acid (41): Oxidation of alcohol **39** (62 mg, 0.10 mmol) in a mixture of CCl$_4$ (0.4 mL), acetonitrile (0.4 mL), and H$_2$O (0.6 mL) with NaIO$_4$ (66 mg, 0.31 mmol) and a catalytic amount of RuCl$_3$·H$_2$O (2.2 mol%) as described for **26**, followed by purification, gave **41** (51 mg, 81%): m.p. 106–108 °C; $[\alpha]_D^{20}$ = −44.0 (*c* = 0.3 in CHCl$_3$); ^{1}H NMR (360 MHz, CDCl$_3$): δ = 8.84–8.15 (br s, 1H; COO*H*), 7.39–7.15 (m, 20H; Ph), 4.20 (q, *J* = 6.7 Hz, 1H; C*H*CH$_3$), 4.18–4.11 (m, 1H; C*H*N), 3.69 (ABX-type dd, *J* = 10.9 and 10.9 Hz, 1H; CH_2N), 3.50 (ddd, *J* = 10.6, 3.2 and 3.2 Hz, 1H; C*H*O), 3.38–3.25 (m, 2H; C*H*N$_3$ and CH_2N), 2.99–2.79 (m, 4H; CH_2Ph and CH_2CO), 1.50 (d, *J* = 6.8 Hz, 3H; CH_3); ^{13}C NMR (90 MHz, CDCl$_3$): δ = 172.6, 170.6, 169.5, 144.4, 136.8,

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129.1, 128.8, 128.7, 127.8, 127.0 (2C), 73.9, 70.5, 63.0, 58.6, 51.4, 36.2, 36.1, 29.6, 17.5; IR (KBr): $\tilde{\nu}$ = 3345 (COOH), 2115 (N_3), 1735 (C=O), 1675 (C=O), 1650 cm^{-1} (C=O); HR FAB MS calcd for $C_{36}H_{35}N_5NaO_5$ [*M*+Na] 640.2536, found 640.2523; $C_{36}H_{35}N_5O_5$ (617.7): calcd C 70.0, H 5.71, N 11.34; found C 69.93, H 5.83, N 11.46.

(2*S*)-2-{(2*S*,6*S*)-6-[(1*S*)-1-Azido-2-phenylethyl]-2-isopropylmorpholin-3-one-4-yl}-3-(*N*-triphenylmethylcarbamoyl)propionic acid (42): Oxidation of alcohol **40** (42 mg, 67 μmol) in a mixture of CCl_4 (0.4 mL), acetonitrile (0.4 mL), and H_2O (0.6 mL) with $NaIO_4$ (43 mg, 0.20 mmol) and a catalytic amount of $RuCl_3 \cdot H_2O$ (2.2 mol%) as described for **26**, followed by purification, gave **42** (31 mg, 72%): m.p. 118–119 °C; $[\alpha]_D^{20} = -50.3$ (c = 0.5 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.06 (m, 21H; Ph and NHTrt), 4.18–4.04 (m, 1H; CHN), 4.01–3.97 (m, 1H; CHiPr), 3.70 (ABX-type dd, J = 11.1 and 11.1 Hz, 1H; CH_2N), 3.53–3.44 (m, 1H; CHO), 3.31–3.23 (m, 1H; CH_2N), 3.22–3.13 (m, 1H; CHN$_3$), 2.97–2.84 [m, 3H; CH_2Ph (2H) and CH_2CO], 2.81–2.68 (m, 1H; CH_2CO), 2.51–2.37 [m, 1H; CH(CH$_3$)$_2$], 1.16 (d, J = 6.9 Hz, 3H; CH$_3$), 0.95 (d, J = 6.7 Hz, 3H; CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.0, 169.9, 169.5, 144.5, 137.0, 129.2, 129.1, 129.0, 128.8, 128.7, 128.3, 127.9, 127.8, 127.7, 127.1, 127.0 (2C), 81.4, 73.9, 70.5, 62.9, 58.9, 51.1, 36.2, 35.9, 30.8, 19.4, 15.6; IR (neat): $\tilde{\nu}$ = 3345 (COOH), 2110 (N_3), 1735 (C=O), 1685 (C=O), 1645 cm^{-1} (C=O); HR FAB MS calcd for $C_{38}H_{40}N_5O_5$ [*M*+H] 646.3029, found 646.3033; $C_{38}H_{39}N_5O_5$ (645.8): calcd C 70.68, H 6.09, N 10.85; found C 70.79, H 6.38, N 10.91.

***N*-Triphenylmethyl (3*S*)-3-[(2*R*,3*S*)-3-Azido-2-hydroxy-4-phenylbutylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (43)**: Opening of epoxide **17**[27, 31] (0.127 g, 0.671 mmol) with amine **30** (0.402 g, 0.671 mmol) as described for **20**, followed by concentration and flash chromatography (heptane/ethyl acetate 10:1 →1:1) of the residue gave **43** (0.382 g, 72%) as a white solid: m.p. 125–126 °C; $[\alpha]_D^{20} = -2.1$ (c = 0.9 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (s, 1H; NHTrt), 7.64–7.59 (m, 4H; Ph), 7.44–7.19 (m, 26H; Ph), 3.72 (ABX-type dd, J = 10.4 and 4.5 Hz, 1H; CH_2OSi), 3.63 (ABX-type dd, J = 10.5 and 5.0 Hz, 1H; CH_2OSi), 3.47 (ddd, J = 9.6, 5.6 and 4.0 Hz, 1H; CHN$_3$), 3.33 (ddd, J = 8.8, 5.8 and 3.0 Hz, 1H; CHOH), 3.05 (m, 1H; CHNH), 2.87 (ABX-type dd, J = 14.1 and 4.1 Hz, 1H; CH_2Ph), 2.72 (ABX-type dd, J = 12.5 and 3.0 Hz, 1H; CH_2NH), 2.66 (ABX-type dd, J = 14.0 and 9.4 Hz, 1H; CH_2Ph), 2.46 [m, 3H; CH_2NH (1H) and CH_2CO], 1.07 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 144.7, 137.5, 135.5 (2C), 133.0, 132.9, 129.9, 129.3, 128.7 (2C), 128.6, 127.9 (2C), 127.8, 127.0, 126.7, 72.2, 70.4, 66.5, 64.5, 56.6, 48.1, 39.0, 36.8, 26.9, 19.2; IR (KBr): $\tilde{\nu}$ = 3410 (OH), 3315 (NH), 2100 (N_3), 1655 (C=O), 1110 cm^{-1} (C–O); $C_{49}H_{53}N_5O_3Si$ (788.1): calcd C 74.68, H 6.78, N 8.89; found C 74.46, H, 6.94, N 8.81.

***N*-Triphenylmethyl (3*S*)-3-[(2*R*,3*S*)-3-azido-4-phenyl-2-triethylsilyloxybutylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (44)**: Silylation of amino alcohol **43** (0.144 g, 0.183 mmol) in CH_2Cl_2 (6 mL) with chlorotriethylsilane (68 μL, 61 mg, 1.4 mmol) in the presence of imidazole (29 mg, 0.42 mmol) was performed as described for **22**. After workup, flash chromatography (heptane/ethyl acetate 15:1 →3:1) of the residue furnished **44** (0.146 g, 95%) as a white solid: m.p. 47–49 °C; $[\alpha]_D^{20} = -5.0$ (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (s, 1H; NHTrt), 7.64–7.61 (m, 4H; Ph), 7.43–7.14 (m, 26H; Ph), 3.70–3.64 (m, 3H; CHOSi and CH_2OSi), 3.57 (m, 1H; CHN$_3$), 3.04 (m, 1H; CHNH), 2.85 (ABX-type dd, J = 14.1 and 3.5 Hz, 1H; CH_2Ph), 2.81 (ABX-type dd, J = 11.8 and 5.1 Hz, 1H; CH_2NH), 2.68 (ABX-type dd, J = 11.9 and 4.9 Hz, 1H; CH_2NH), 2.60 (ABX-type dd, J = 13.9 and 10.4 Hz, 1H; CH_2Ph), 2.38 (d, J = 5.5 Hz, 2H; CH_2CO), 1.06 (s, 9H; *t* Bu), 0.92 (t, J = 7.9 Hz, 9H; SiCH$_2$CH$_3$), 0.54 (q, J = 7.8 Hz, 6H; SiCH$_2$CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 144.9, 137.9, 135.5, 135.4, 133.0, 132.9, 129.8, 129.1, 128.7, 128.6 (2C), 127.8 (2C), 126.7, 126.6, 74.3, 70.2, 66.6, 64.9, 57.0, 48.7, 37.8, 36.6, 26.9, 19.2, 6.9, 4.8; IR (KBr): $\tilde{\nu}$ = 3315 (NH), 2100 (N_3), 1665 (C=O), 1110 cm^{-1} (C–O); $C_{55}H_{67}N_5O_3Si_2$ (902.3): calcd C 73.21, H 7.48, N 7.76; found C 73.64, H 7.67, N 7.75.

***N*-Triphenylmethyl (3*S*)-3-{*N*′-[(2*R*,3*S*)-3-azido-2-hydroxy-4-phenylbutyl]-*N*′-[(1*R*)-1-bromoethylcarbonyl]amino}-4-(*tert*-butyldiphenylsilyloxy)-butyramide (45)**: Acylation of amine **44** (0.400 g, 0.447 mmol) with (*R*)-(+)-2-bromopropionic acid (0.327 mL, 0.546 g, 3.57 mmol) and DIC (0.622 mL, 0.507 g, 3.13 mmol), followed by removal of the triethylsilyl group using 2 M aqueous HCl (3 mL) and THF (1.5 mL), as described for **23**, gave **45** (0.357 g, 58% over 2 steps) after purification by flash chromatography (heptane/ethyl acetate 15:1 →5:1). Compound **45** had: m.p. 76–78 °C; $[\alpha]_D^{20} = -38.0$ (c = 0.7 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 7:3 mixture of rotamers A and B): δ = 7.57 (m, 4H; Ph, A+B), 7.47–7.13 (m, 26H; Ph, A+B), 6.72 (s, 0.7H; NHTrt, A), 6.57 (s, 0.3H; NHTrt, B), 4.79 (q, 0.7H, J = 6.4 Hz; CHBr, A), 4.67 (q, 0.3H, J = 6.4 Hz; CHBr, B), 4.53–4.37 (m, 1H; CH_2OSi, A+B), 3.86–3.49 [3 m, 5.4H; CH_2OSi (1H), A+B; CHN$_3$ (1H), A+B; CHOH (1H), A+B; CHN (1H), A+B; CH_2N, (0.7H, 2 C), A], 3.33 [m, 1H; CH_2CO (0.7H), A; CH_2N (0.3H), B], 3.09 [m, 0.6H; CH_2N (0.3H), B; CH_2Ph (0.3H), B], 2.84 (ABX-type dd, J = 14.1 and 4.6 Hz, 0.7H; CH_2Ph, A), 2.70 (ABX-type dd, J = 14.1 and 9.3 Hz, 0.3H; CH_2Ph, B), 2.65 (ABX-type dd, J = 14.1 and 9.6 Hz, 0.7H; CH_2Ph, A), 2.56 (ABX-type dd, J = 14.8 and 3.5 Hz, 0.3H; CH_2CO, B), 2.34 (ABX-type dd, J = 15.3 and 3.1 Hz, 0.7H; CH_2CO, A), 2.17 (ABX-type dd, J = 14.8 and 9.8 Hz, 0.3H; CH_2CO, B), 1.83 (d, J = 6.5 Hz, 2.1H; CHCH_3, A), 1.72 (d, J = 6.5 Hz, 0.9H; CHCH_3, B), 1.04 (s, 6.3H; *t*Bu, A), 1.02 (s, 2.7H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.7, 171.2, 169.6, 167.2, 144.3, 144.2, 137.3, 137.2, 135.6, 135.5, 135.4, 135.3, 132.4 (2C), 130.2, 130.1, 130.0, 129.2, 129.2, 128.7 (2C), 128.6 (2C), 128.0 (2C), 127.9 (2C), 127.2, 127.1, 126.9, 126.8, 73.9, 70.9, 70.8, 69.3, 67.0, 66.4, 63.8, 62.6, 56.4, 46.6, 40.5, 39.1, 37.6, 37.1, 36.8, 26.9 (3C), 26.8 (3C), 21.6 (2C), 19.1, 18.9; IR (KBr): $\tilde{\nu}$ = 3415 (OH), 2110 (N_3), 1685 (C=O), 1655 (C=O), 1110 cm^{-1} (C–O); $C_{52}H_{56}BrN_5O_4Si$ (923.0): calcd C 67.66, H 6.12, N 7.59; found C 67.96, H 6.31, N 7.60.

***N*-Triphenylmethyl (3*S*)-3-{(2*S*,6*R*)-6-[(1*S*)-1-azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-4-(*tert*-butyldiphenylsilyloxy)butyramide (46)**: Intramolecular cyclization of bromo alcohol **45** (0.290 g, 0.315 mmol) with KH (28 mg, 0.72 mmol) as described for **24**, followed by workup and purification of the residue by flash chromatography (heptane/ethyl acetate 10:1 →3:1), gave **46** (0.219 g, 84%) as a white solid: m.p. 74–76 °C; $[\alpha]_D^{20} = +3.8$ (c = 0.7 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.63–7.61 (m, 4H; Ph), 7.43–7.22 (m, 20H; Ph), 7.19–7.16 (m, 6H; Ph), 6.93 (s, 1H; NHTrt), 4.36 (q, J = 6.0 Hz, 1H; CHCH$_3$), 4.16–4.00 (m, 2H; CHN and CH_2OSi), 3.81 (ddd, J = 10.0, 7.3 and 3.3 Hz, 1H; CHO), 3.70 (ABX-type dd, J = 9.9 and 8.2 Hz, 1H; CH_2OSi), 3.60 (ddd, J = 9.6, 6.5 and 3.2 Hz, 1H; CH N$_3$), 3.50 (ABX-type dd, J = 12.2 and 10.5 Hz, 1H; CH_2N), 3.35 (ABX-type dd, J = 12.3 and 3.2 Hz, 1H; CH_2N), 3.15 (m, 1H; CH_2CO), 3.03 (ABX-type dd, J = 14.1 and 3.2 Hz, 1H; CH_2Ph), 2.69 (ABX-type dd, J = 14.1 and 9.4 Hz, 1H; CH_2Ph), 2.45 (ABX-type dd, J = 14.9 and 4.2 Hz, 1H; CH_2CO), 1.40 (d, J = 7.0 Hz, 3H; CHCH_3), 1.04 (s, 9H; *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 169.1, 144.5, 136.9, 135.5, 135.4, 132.9 (2C), 129.8, 129.3, 128.6 (2C), 127.9 (2C), 127.8 (2C), 127.0, 126.9, 72.3, 70.6, 69.6, 64.9, 63.3, 59.8, 50.5, 36.8, 36.4, 26.8 (3C), 19.1, 17.2; IR (KBr): $\tilde{\nu}$ = 2110 (N_3), 1690 (C=O), 1640 (C=O), 1110 cm^{-1} (C–O); $C_{52}H_{55}N_5O_4Si$ (842.1): calcd C 74.17, H 6.58, N 8.32; found C 74.04, H 6.78, N 8.09.

***N*-Triphenylmethyl (3*S*)-3-{(2*S*,6*R*)-6-[(1*S*)-1-azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-4-hydroxybutyramide (47)**: Deprotection of **46** (0.200 g, 0.238 mmol) with tetrabutylammonium fluoride hydrate (72 mg, 0.27 mmol) as described for **25** followed by concentration and purification of the residue by flash chromatography (heptane/ethyl acetate 1:1, then EtOH) furnished **47** (0.144 g, 100%) as a white solid: m.p. 91–93 °C; $[\alpha]_D^{20} = -1.0$ (c = 0.8 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.19 (m, 20H; Ph), 4.34 (q, J = 6.8 Hz, 1H; CHCH$_3$), 4.03 (m, 1H; CHN), 3.75–3.57 (m, 4H; CHO and CHN$_3$ and CH_2O), 3.48 (ABX-type dd, J = 12.4 and 10.2 Hz, 1H; CH_2N), 3.30 (ABX-type dd, J = 12.4 and 3.0 Hz, 1H; CH_2N), 3.07 (ABX-type dd, J = 14.0 and 3.6 Hz, 1H; CH_2Ph), 3.03 (ABX-type dd, J = 15.0 and 9.5 Hz, 1H; CH_2CO), 2.73 (ABX-type dd, J = 14.0 and 9.1 Hz, 1H; CH_2Ph), 2.58 (ABX-type dd, J = 15.0 and 5.6 Hz, 1H; CH_2CO), 1.37 (d, J = 7.0 Hz, 3H; CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 169.2, 144.3, 136.6, 129.3, 128.6, 128.5 (2C), 127.8 (2C), 126.9, 126.8, 72.0, 70.4, 69.4, 64.5, 63.0, 59.4, 49.4, 36.7, 35.8, 17.3; IR (KBr): $\tilde{\nu}$ = 3415 (OH), 2110 (N_3), 1670 (C=O), 1640 cm^{-1} (C=O); HR FAB MS calcd for $C_{36}H_{37}N_5O_4$ [*M*+H] 604.2924, found 604.2925.

(2*S*)-2-{(2*S*, 6*R*)-6-[(1*S*)-1-azido-2-phenylethyl]-2-methylmorpholin-3-one-4-yl}-3-(*N*-triphenylmethylcarbamoyl)propionic acid (48): Oxidation of alcohol **47** (36 mg, 59 μmol) in a mixture of CCl_4 (0.4 mL), acetonitrile (0.4 mL), and H_2O (0.6 mL) with $NaIO_4$ (38 mg, 0.18 mmol) and a catalytic amount of $RuCl_3 \cdot H_2O$ (2.2 mol%) as described for **26**, followed by purification, gave **48** (35 mg, 96%): m.p. 103–105 °C; $[\alpha]_D^{20} = -13.8$ (c = 0.8 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.50 (br s, 1H; COOH), 7.36–7.18 (m, 20H; Ph), 4.53 (q, J = 7.0 Hz, 1H; CHCH$_3$), 4.23 (m, 1H; CHN), 3.84 (ddd, J = 9.8, 6.5 and 3.2 Hz, 1H; CHO), 3.56 (ddd, J = 9.8, 6.5 and 3.3 Hz, 1H; CHN$_3$), 3.41 (ABX-type dd, J = 12.5 and 10.1 Hz, 1H; CH_2N), 3.29 (ABX-type dd, J = 12.5 and 3.1 Hz, 1H; CH_2N), 3.06 (ABX-

type dd, $J = 14.1$ and 3.2 Hz, 1H; CH_2Ph), 2.89 (ABX-type dd, $J = 16.3$ and 5.7 Hz, 1H; CH_2CO), 2.82 (ABX-type dd, $J = 16.1$ and 8.3 Hz, 1H; CH_2CO), 2.69 (ABX-type dd, $J = 14.1$ and 9.6 Hz, 1H; CH_2Ph), 1.45 (d, $J =$ 6.9 Hz, 3H; CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.5$, 170.8, 169.4, 144.4, 137.7, 136.9, 129.2, 128.9, 128.7 (2C), 128.6, 128.5, 128.1, 127.8 (2C), 126.9, 126.8, 71.9, 70.6, 69.3, 64.5, 59.8, 51.7, 36.8, 35.8, 16.7; IR (KBr): $\tilde{\nu} =$ 3415 (COOH), 2110 (N_3), 1735 (C=O), 1685 (C=O), 1650 (C=O), 1260 cm^{-1} (C–O); $C_{36}H_{35}N_5O_5$ (617.7): calcd C 70.00, H 5.71, N 11.34; found C 69.83, H 5.62, N 11.23.

Procedure for solid-phase peptide synthesis of 49: Peptide **49** was synthesized with DMF as a solvent in a mechanically agitated reactor. A polystyrene resin grafted with aminated polyethyleneglycol chains (TentaGel S NH_2, Rapp Polymere, Germany; 0.309 g, 0.26 mequiv g^{-1}, 80 μmol) was used for the synthesis. The resin was functionalized with the linker *p*-[(*R*,*S*)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid[41] (Novabiochem, Switzerland). N^{α}-Fmoc amino acids (Bachem, Switzerland) with the following protective groups were used: 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) for D-Arg, triphenylmethyl (Trt) for Cys and 3-mercaptopropionic acid, and *tert*-butyl (*t*Bu) for Tyr. Reagent solutions and DMF for washing were added manually to the reactor.

The linker, the N^{α}-Fmoc amino acids and mercaptopropionic acid were coupled to the peptide resin as 1-benzotriazolyl (HOBt) esters.[35a] These were prepared, in situ, from the appropriate acid (0.32 mmol, 4 equiv), HOBt (65 mg, 0.48 mmol, 6 equiv), and DIC (48.5 μL, 0.314 mmol, 3.9 equiv) in DMF (1 mL), and added to the reactor after 60 min. The inverse γ-turn mimetic **41** (67 mg, 0.11 mmol, 1.3 equiv) was coupled in DMF (1 mL) containing HOAt[35b] (21 mg, 0.16 mmol, 2.0 equiv) and DIC (14.9 μL, 96.5 μmol, 1.2 equiv). Acylations were monitored by addition of bromophenol blue[51] (0.05% of the resin capacity) to the reactor and, additionally, by the ninhydrin test for the coupling of **41**.[52] N^{α}-Fmoc deprotection was performed by treatment with 20% piperidine in DMF (2×8 min). After incorporation of mimetic **41** into the peptide, the azido group[42, 53] was reduced by sequential addition of triethylamine (0.20 mL, 1.5 mmol), thiophenol (0.121 mL, 1.18 mmol) and $SnCl_2$ (49 mg, 0.26 mmol) to the suspended resin (0.317 g, 59 μmol) in THF (1 mL). After 19 h at room temperature the resin was washed with THF (5×1 mL) and CH_2Cl_2 (5×1 mL) and dried in vacuo. A positive ninhydrin test and the disappearance of the N_3 stretch in the IR spectrum (recorded on a few resin beads[43]) indicated successful reduction. The resin was then suspended in DMF (1 mL) and synthesis of **49** was continued as described above.

After completion of the synthesis, the resin was washed with CH_2Cl_2 ($5 \times$ 5 mL) and dried in vacuo to give 216 mg peptide resin. The peptide was then cleaved from the resin (133 mg resin, 26.5 μmol), and the amino acid side chains were deprotected by treatment with trifluoroacetic acid–water–thioanisole–ethanedithiol (87.5:5:2.5, 13.3 mL) for 2 h, followed by filtration. Acetic acid (6.5 mL) was added to the filtrate, the solution was concentrated, and acetic acid (6.5 mL) was added again followed by concentration. The residue was triturated with diethyl ether (6.5 mL) which gave a solid, crude peptide which was dissolved in acetic acid–water (1:9, 10 mL) and freeze-dried. Cyclization[44] was performed by alternating additions of portions of the crude peptide in acetic acid and 0.1M I_2 in methanol to 10% acetic acid in methanol (2 mL mg^{-1} cleaved resin). After the final addition of I_2, a light brown solution was obtained which was neutralized and decolourized by stirring with Dowex 1×8 anion exchange resin (Fluka, 50–100 mesh, converted from Cl^- to acetate form by washing with 1M aqueous NaOH, water, acetic acid, water, and methanol), filtered and concentrated. The residue was then dissolved in water and freeze-dried.

Peptide **49** was analyzed on a Beckman System Gold HPLC with a Kromasil C-8 column (100 Å, 5 μm, 4.6×250 mm) and a linear gradient of 0–80% of *B* in *A* over 60 min with a flow rate of 1.5 mL min^{-1} and detection at 214 nm (solvent systems: A, 0.1% aqueous trifluoroacetic acid and B, 0.1% trifluoroacetic acid in acetonitrile). Purification of crude **49** (33 mg, from 26.5 μmol resin) was performed with the same HPLC system on a Kromasil C-8 column (100 Å, 5 μm, 20×250 mm) with a flow rate of 11 mL min^{-1} (retention time: 26 min). This gave **49** (6.1 mg, 23%): FAB MS calcd for $C_{45}H_{63}N_{12}O_{11}S_2$ [*M*+H] 1011, found 1011. Amino acid analysis: Arg 1.03 (1), Cys 0.88 (1), Gly 1.05 (1), Pro 1.03 (1), Tyr 1.00 (1). ^{1}H NMR data for **49** are given in Table 1.

Supporting information: Supporting information for this article, available on the WWW under http://www.wiley-vch.de/home/chemistry/ or from the author, describes the synthetic procedures for the preparation of azido epoxides **17** and **18**; ^{13}C NMR spectra for compounds **24**, **28**, **38**, **39**, **40**, and **47**, ^{1}H NMR spectra, including COSY and TOCSY spectra, for compound **49**, are also included.

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