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Synthesis and Conformational Study of Model Peptides Containing *N*-Substituted 3-Aminoazetidine-3-carboxylic Acids

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Model peptides containing *N*-substituted 3-aminoazetidine-3-carboxylic acids have been synthesized to investigate the conformational features of these C^{α}-tetrasubstituted amino acids. The target peptides were designed and prepared by classical synthetic methods in solution, exploiting the convenient *N*-substituted 3-azidoazetidine-3-carboxylic acids as precursors. The conformational preferences of the newly synthesized peptides were investigated in solution by IR and NMR spectroscopy. It was observed that the 3-aminoazetidine-3-carboxylic acid moiety is likely a β-turn inducer. In addition, an interesting main-chain-to-side-chain hydrogen bond that forms a six-membered pseudo-cycle was detected. It connects the nitrogen (acceptor) of the azetidine ring to the amide NH (donor) of the immediately following residue. This unexpected hydrogen bond increases the number of conformational options offered by *N*-substituted 3-aminoazetidine-3-carboxylic acids when designing foldamers with new and predictable 3D structures.

Introduction

The secondary structure of an amino acid sequence is of essential importance for the biological activity of proteins.[1] This is one of the factors that nowadays sustains the growing field of "foldamers".[2] Short peptides based on proteinogenic amino acids are usually quite flexible. Therefore it is difficult to investigate their 3D structures and to assess structure–activity relationships. One way to restrict, induce, and rigidify the conformation of a peptide, as well as to reduce its biodegradation, is the use of non-proteinogenic C^{α}-tetrasubstituted α-amino acids in which the α-hydrogen atom of the α-amino acid is replaced by an alkyl (or aryl) group.[3]

By employing such amino acids, even short peptide sequences can be made to adopt stable secondary structures like β-turns,[4] 2.0_5-helices,[5] 3_{10}-helices, and/or α-helices.[6] Therefore interest in new C^{α}-tetrasubstituted α-amino acids has inreased in recent years resulting in numerous reports of such building blocks[7] that may be of great importance for the design of peptidomimetic drugs.[8]

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Small-size C^{α}-tetrasubstituted α-amino acids such as α-aminoisobutyric acid (Aib, **1**), 1-aminocyclopropane-1-carboxylic acid (Ac_3c, **2**), and 1-aminocyclobutane-1-carboxylic acid (Ac_4c, **3**; Figure 1) have found a great number of applications in the field of foldamers and beyond.

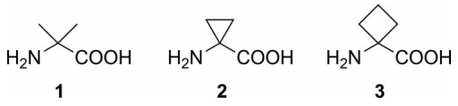


Figure 1. Chemical structures of Aib (**1**), Ac_3c (**2**), and Ac_4c (**3**).

Aib (**1**) and Ac_4c (**3**) are known to form short peptides with a conformational preference for β-turns and 3_{10}-helices.[3g,9] Aib, although not being a proteinogenic amino acid, has been found in meteorites[10] and is commonly occurring in peptides produced by microbial sources. Examples of such peptides are the cyclic peptide chlamydocin, which contains one Aib residue and displays antibiotic, neuroleptic, and anticancer properties,[11] and, in particular, the peptaibiotics,[12] in which Aib can represent up to 50% of the total number of residues. Owing to the peculiar stereochemical properties of Aib, the peptaibiotics form stable helices responsible for membrane disruption by different mechanisms[13] and enzyme recognition.[14] Remarkably, Aib, and likewise all C^{α}-tetrasubstituted α-amino acids, imparts an important proteolytic stability on a peptide.[15]

Probably due to their small size, Ac_3c and Ac_4c are partial GluN1 agonists, capable of binding instead of glycine.[16] The Ac_3c residue is present in the antitumor agents cytotrienins[17] and is incorporated into compounds that are antagonists or inverse agonists of the bradykinin B1-recep-

tor,[18] and Ac_4c is a structural component in an allosteric non-nucleoside inhibitor of the hepatitis-C virus (HCV).[19]

On the other hand, numerous applications have been proposed in the bio-organic and supramolecular fields. Just to mention a few, all three residues **1**–**3** have been exploited for the synthesis of naphthalenediimides, which are used in self-assembling naphthalenediimide (NDI)-based helical organic nanotubes,[20] and Aib (**1**) has been used for the synthesis of homo- and heterometallic clusters[21] and exploited as an electron-transfer[22] or long-range communication mediator.[23]

Thus, driven by the opportunities offered by the aforementioned class of amino acids, we started to explore heteroatom-containing constrained analogues, which form an important area of research in modern synthetic chemistry at the biological interface.[3h,8c,24] The four-membered-ring heteroatom-containing C^{α}-tetrasubstituted α-amino acids **4**–**6** (Figure 2; Azt, R = H) have already been synthesized,[25] however, the conformational bias induced by these residues on host peptides has not yet been investigated.

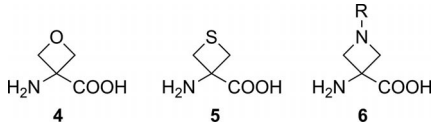


Figure 2. Chemical structures of known, heteroatom-containing Ac_4c analogues. Peptides containing Azt(R) (**6**: R = *t*Bu, **a**; R = *t*Amyl, **b**) are described in this work.

Having independently accomplished the synthesis of *N*-substituted 3-aminoazetidine-3-carboxylic acids **6** (R = *t*Bu, *t*Amyl),[25b] attempts were made to gain a deeper insight into their conformational features and applications. In addition to their potential use as foldamer building blocks, the 3-aminoazetidine-3-carboxylates **6** may be important for the synthesis of bioactive compounds, as exemplified for EGF receptor tyrosine kinase inhibitors,[26] CB_1 receptor antagonists,[27] modulators of the NMDA receptor complex,[25a] and inhibitors of bromodomain-containing proteins.[28] However, to the best of our knowledge, the conformational preferences of the residue **6** have not been studied up to now.

Results and Discussion

Synthesis

To investigate the conformational features of the azetidine-containing C^{α}-tetrasubstituted amino acids **6** (R = *t*Bu, *t*Amyl), and in particular to verify their ability to promote β-turns, the tripeptides Z-Azt(R)-Ala-Ala-OMe **7** and Z-Ala-Azt(R)-Ala-OMe **8** (Figure 3; R = *t*Bu, **a**; R = *t*Amyl, **b**) were synthesized by classical methods of peptide synthesis in solution, including the very convenient α-azido carboxylic acid intermediates.[29]

To this end, the ester group of the 3-azidoazetidine-3-carboxylic acid esters **9a**–**c**[25b,30] and **9d** (see the Exp. Sect.) was hydrolyzed in basic medium to quantitatively afford the hydrochlorides of the free azido acids (**10a**,**b**) upon acidification with aqueous HCl (Scheme 1). As these compounds are very hydrophilic, acid/base extraction was avoided. Instead, lyophilization had to be used and the crude products **10** were used in the next steps without further purification (purity >95%).

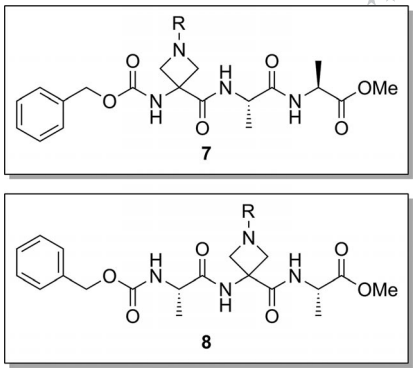
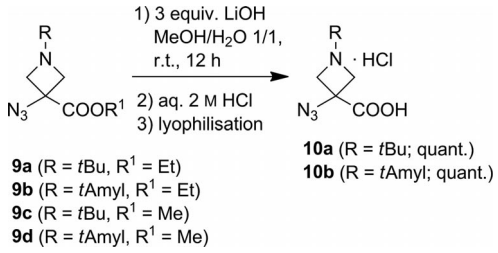
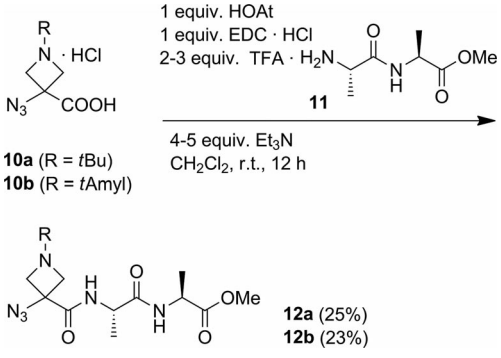


Figure 3. Chemical structures of the tripeptides Z-Azt(R)-Ala-Ala-OMe **7** and Z-Ala-Azt(R)-Ala-OMe **8** (R = *t*Bu, **a**; R = *t*Amyl, **b**).

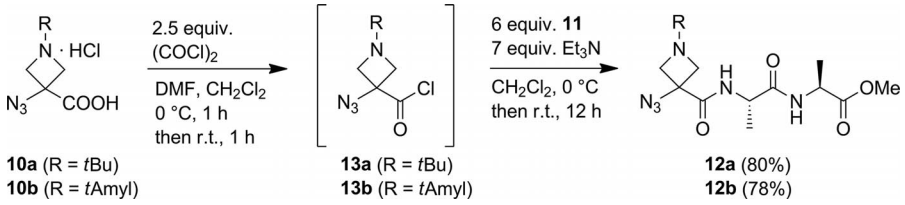


Scheme 1. Hydrolysis of the ester group of 3-azidoazetidine-3-carboxylic acid esters **9a**–**d**.

The synthesis of N_3-Azt(R)-Ala-Ala-OMe derivatives **12a**,**b** was then investigated. Starting from the known dipeptide Z-Ala-Ala-OMe,[31] both its free amine and its TFA salt **11** were prepared by catalytic hydrogenation, the latter to minimize the undesired cyclization to 3,6-dimethylpiperazine-2,5-dione during benzyloxycarbonyl deprotection. The azido acids **10a**,**b** were coupled with H-Ala-Ala-OMe (or its TFA salt **11**) by the HOAt/EDC activation method to afford the desired N_3-Azt(R)-Ala-Ala-OMe derivatives **12a**,**b**. However, under these conditions, only low yields (23–25%) of the target products were obtained (Scheme 2).



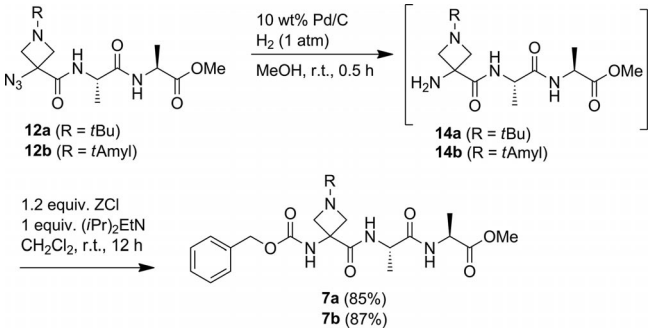
Scheme 2. Synthesis of N_3-Azt(R)-Ala-Ala-OMe derivatives **12a**,**b**.



Scheme 3. Improved synthesis of N_3-Azt(R)-Ala-Ala-OMe derivatives **12a**,**b**.

Subsequently, the possibility of using the corresponding acyl chlorides as more reactive intermediates for the coupling reaction was investigated. By treatment with oxalyl chloride,[32] azetidines **10a**,**b** were converted in situ into the corresponding acyl chlorides **13a**,**b**, which were immediately coupled with H-Ala-Ala-OMe TFA salt **11** under Schotten–Baumann-like conditions to obtain peptides **12a**,**b** in good yields (78–80%; Scheme 3).

Finally, reduction of the azido group of N_3-Azt(R)-Ala-Ala-OMe **12a**,**b** by catalytic hydrogenation and subsequent treatment of the free primary amine with benzyloxycarbonyl chloride afforded the target tripeptides Z-Azt(R)-Ala-Ala-OMe **7a**,**b** in good yields (85–87%; Scheme 4). The Z group was introduced to offer the tripeptide the possibility of forming a β-turn as this event requires a hydrogen-bond acceptor (the C=O of Z) positioned before Azt.



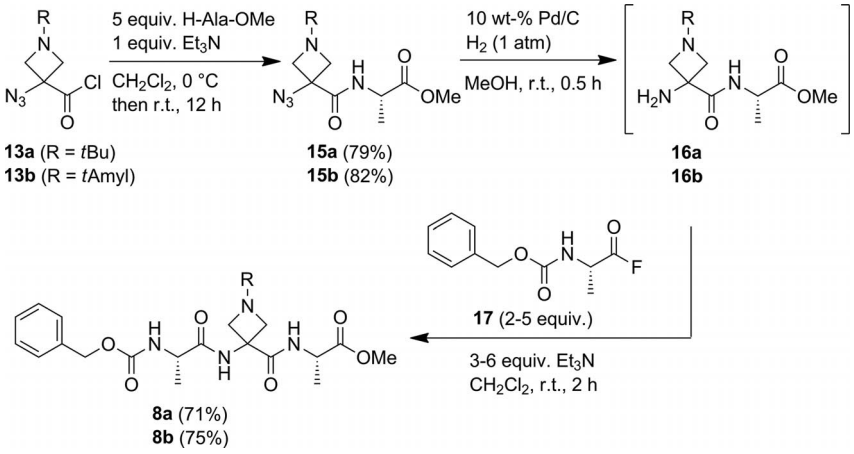
Scheme 4. Synthesis of Z-Azt(R)-Ala-Ala-OMe **7a**,**b**.

As the coupling method via acid chlorides proved to be the most efficient, it was also used for the synthesis of tripeptides **8**. In situ prepared acid chlorides **13a**,**b** were coupled with H-Ala-OMe to afford dipeptides **15a**,**b** in good yields (79–82%). Then catalytic hydrogenation was used to reduce the azido compounds **15a**,**b** to the amines **16a**,**b**, which were acylated with the freshly prepared Z-protected amino acid fluoride **17**[33] to give tripeptides Z-Ala-Azt(R)-Ala-OMe **8a**,**b** in good yields (71–75%; Scheme 5).

In summary, acyl halides, either chlorides or fluorides, appear to be the best choice for incorporating the Azt residues into peptides. However, despite their effectiveness, these activation procedures failed to give satisfactory yields in the preparation of the sterically hindered homo-Azt(R) sequences. To this end, we are currently exploring alternative synthetic strategies.

Solution Conformational Analysis

The conformational preferences of the newly synthesized peptides were investigated to assess whether Azt(R) **6**, a C^{α}-tetrasubstituted α-amino acid, has β-turn-inducing propensity as well as its optimal position in the β-turn itself. As, unfortunately, we were unable to grow single crystals suitable for X-ray diffraction analysis, the conformational preferences were investigated in $CDCl_3$ solution by IR and NMR spectroscopy. Circular dichroism did not provide useful information as the peptides are too short and the induced dichroism absorptions of the aromatic Z group overlap with those of the amide.



Scheme 5. Synthesis of Z-Ala-Azt(R)-Ala-OMe **8a**,**b**.

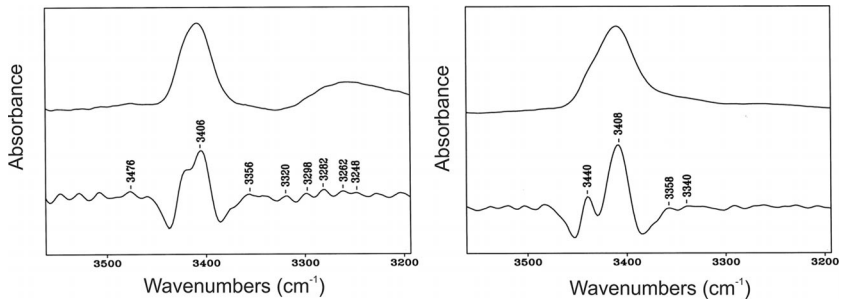


Figure 4. IR absorption spectra (top lines) of N_3-Azt(*t*Bu)-Ala-OMe (**15a**; left) and N_3-Azt(*t*Bu)-Ala-Ala-OMe (**12a**; right) in $CDCl_3$ solution at 1 mM peptide concentration. The bottom lines are the inverted second derivatives.

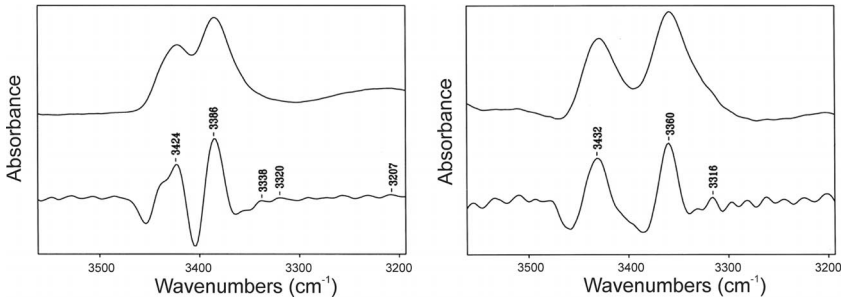


Figure 5. IR absorption spectra (top lines) of Z-Azt(*t*Bu)-Ala-Ala-OMe (**7a**; left) and Z-Ala-Azt(*t*Bu)-Ala-OMe (**8a**; right) in $CDCl_3$ solution at 1 mM peptide concentration. The bottom lines are the inverted second derivatives.

The two frequency intervals of the IR absorption spectrum richest in conformational information were analyzed, namely 3550–3200 cm^{-1} (Amide A band), related to the stretching vibrations of the N–H bonds belonging to the urethane and amide groups, and 1800–1600 cm^{-1} (Amide I band), related to the stretching vibrations of the C=O bonds of the ester, urethane, and amide groups. It is generally assumed that solvated NH groups (the so-called free NHs) in $CDCl_3$ resonate at wavenumbers higher than 3400 cm^{-1}, whereas hydrogen-bonded NH groups resonate at lower wavenumbers.

From the data obtained, at least two types of hydrogen bonds are deduced: One represented by the absorption at about 3360 cm^{-1}, typical of helix/β-turns, and a second one, indicative of a weaker hydrogen bond, at about 3400 cm^{-1}. Indeed, N_3-Azt(*t*Bu)-Ala-OMe (**15a**) and N_3-Azt(*t*Bu)-Ala-Ala-OMe (**12a**; Figure 4) display an absorption band at 3406 and 3408 cm^{-1}, respectively, that is, borderline between free (above 3430 cm^{-1}) and strongly hydrogen-bonded (below 3360 cm^{-1}) NHs. As neither peptide forms a β-turn, we must assume that a nonclassical, weak hydrogen bond is present.

The tripeptides Z-Azt(R)-Ala-Ala-OMe **7** and Z-Ala-Azt(R)-Ala-OMe **8** fold into a β-turn, stabilized by a hydrogen bond between the urethane C=O and the N–H of the *C*-terminal Ala residue. Indeed, an absorption band well below 3400 cm^{-1}, compatible with a β-turn, is observed in the IR spectra of all tripeptides **7** and **8**, as shown in Figure 5 for **7a** and **8a**. Interestingly, the hydrogen bond of **8a** appears to be stronger than that of **7a**. Thus, the position of Azt(R) in the tripeptide sequence is important, even if an influence from the weak hydrogen bond observed in the shorter peptides (unable to fold into a β-turn, Figure 4) cannot be excluded.

To ascertain whether the hydrogen bonds are intermolecular or intramolecular, the effect of dilution was studied for all peptides. As an example, Figure 6 shows the spectra of **8a** recorded at two concentrations (the 10-fold dilution is counterbalanced by a 10-fold increase in the cuvette pathlength). Only moderate dilution effects were observed for all peptides, from which it can safely be concluded that the observed hydrogen bonds are of the intramolecular type.

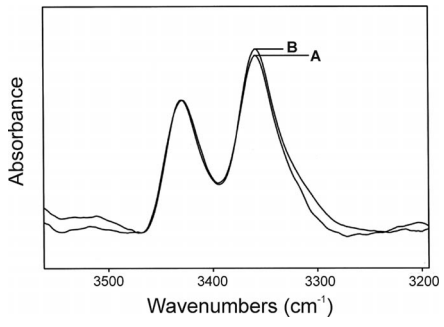


Figure 6. IR absorption spectra of Z-Ala-Azt(*t*Bu)-Ala-OMe (**8a**) at 1 mM (A) and 0.1 mM (B) peptide concentrations in $CDCl_3$ solution.

To summarize, the IR analysis indicates that the two Azt(R) residues investigated are indeed β-turn inducers. However, a weak hydrogen bond competing with that of the β-turn appears to interfere with this secondary structure.

The solution conformational analysis was extended to NMR spectroscopy. Complete assignment of the resonances was achieved by means of standard 2D NMR experiments. All peptides were subjected to a hydrogen-bond-revealing experiment that involves adding increasing amounts of deuteriated dimethyl sulfoxide ([D_6]DMSO), an excellent hydrogen-bond acceptor, to a $CDCl_3$ peptide solution. The amide protons exposed to the solvent form hydrogen bonds with DMSO, which usually cause a downfield shift of the amide N–H signals. On the other hand, amide protons that are already engaged in stable hydrogen bonds are shielded from the solvent and thus little affected by variation of the solvent composition. Unexpectedly, DMSO-insensitive NHs were also found in peptides unable to form β-turns, such as the dipeptides N_3-Azt(*t*Bu)-Ala-OMe (**15a**) and N_3-Azt(*t*Amyl)-Ala-OMe (**15b**; Figure 7). Besides their insensitivity to the addition of DMSO, the Ala NHs of dipeptides **15a** and **15b** resonate at unusually low fields. A reasonable explanation for these experimental data could be the presence of a six-membered pseudo-cycle stabilized by a hydrogen bond between the amide NH immediately following the Azt(R) residue and the trisubstituted nitrogen atom of the azetidine ring. This hypothesis is supported by a previous, similar finding observed in peptides containing *O*,*O*-isopropylidene-α-(hydroxymethyl)serine; a side-chain oxygen atom acts as hydrogen-bond acceptor for the amide N–H immediately following in the peptide chain, thus closing a six-membered pseudo-cycle.[34] Further support for our hypothesis of a side-chain-to-main-chain hydrogen bond comes from a recent report in which a trisubstituted azetidine ring nitrogen participates in a five-membered hydrogen-bonded pseudo-cycle.[35]

Also in the case of tripeptides N_3-Azt(*t*Bu)-Ala-Ala-OMe (**12a**) and N_3-Azt(*t*Amyl)-Ala-Ala-OMe (**12b**), unable to fold into a β-turn, an unexpected hydrogen bond is observed (Figure 7); the Ala^2 NH, that is, the NH of residue 2, is unaffected by the addition of DMSO, whereas the chemical shift of the Ala^3 NH is quite sensitive to the perturbing agent. Here too, a hydrogen bond between the nitrogen atom of the azetidine ring of Azt(R) and the amide NH of Ala^2 may exist. Moving to the Z-protected tripeptides **7** and **8**, in which β-turn formation is possible, data interpretation becomes complex. Indeed, the addition of DMSO causes an unexpected upfield shift for the amide NHs following the Azt(R) residues, that is, Ala^2 in **7** and Ala^3 in **8**. As an example, Figure 8 shows the behavior of tripeptides **7a** and **8a**, the IR absorption spectra of which are reported in Figure 5.

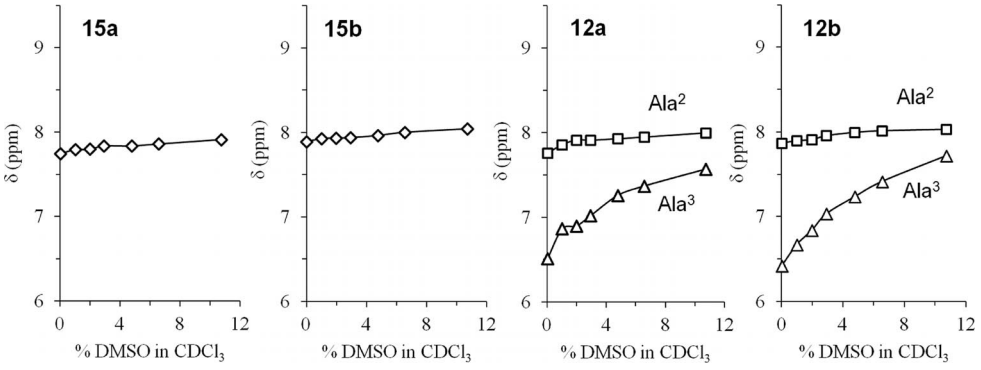


Figure 7. Plots of the NH chemical shifts in the ^{1}H NMR spectra of N_3-Azt(*t*Bu)-Ala-OMe (**15a**), N_3-Azt(*t*Amyl)-Ala-OMe (**15b**), N_3-Azt(*t*Bu)-Ala-Ala-OMe (**12a**), and N_3-Azt(*t*Amyl)-Ala-Ala-OMe (**12b**) as a function of increasing percentage of DMSO (vol.-%) in $CDCl_3$. Peptide concentration: 5 mM.

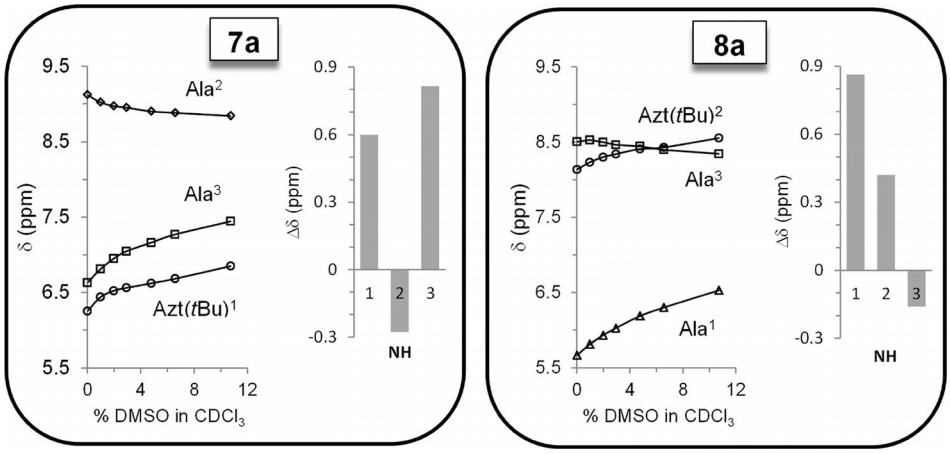


Figure 8. Plots of the ^{1}H NMR chemical shifts of NH in Z-Azt(*t*Bu)-Ala-Ala-OMe (**7a**) and Z-Ala-Azt(*t*Bu)-Ala-OMe (**8a**) as a function of increasing percentage of DMSO (vol.-%) in $CDCl_3$. Peptide concentration 5 mM.

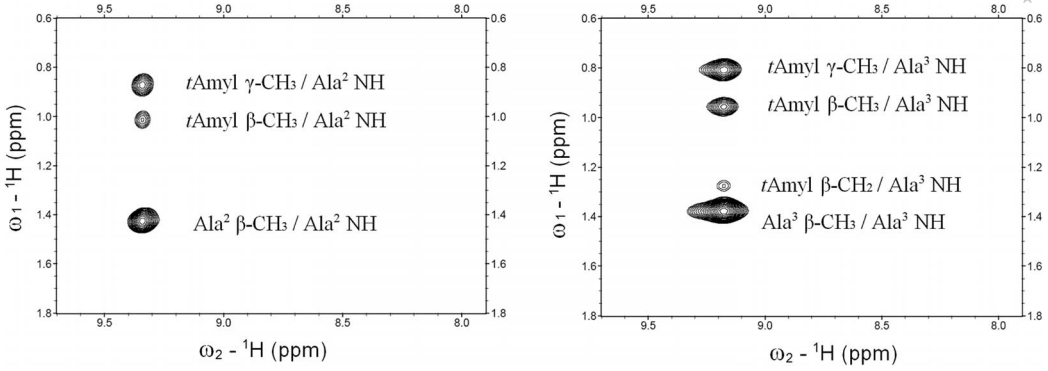


Figure 9. Sections of the NOESY spectra of Z-Azt(*t*Amyl)-Ala-Ala-OMe (**7b**; left) and Z-Ala-Azt(*t*Amyl)-Ala-OMe (**8b**; right) showing the proximity of the *tert*-amyl group to the amide proton of Ala^2 and Ala^3, respectively (400 MHz, 2 mM in $CDCl_3$ solution, 298K).

We ascribe the behavior observed in tripeptides **7** and **8** to an equilibrium mixture of two hydrogen-bond-stabilized major conformers: a β-turn involving the Z C=O and Ala^3 NH and a structure, as described above, characterized by a side-chain (azetidine N as acceptor)-to-main-chain (Ala NH donor) hydrogen bond. Confirmation of the presence of this latter structure, in addition to the above-mentioned low-field resonance of the amide NH following Azt(R), is offered by 2D NMR experiments: The *tert*-butyl or *tert*-amyl groups on the azetidine nitrogen (hydrogen-bond acceptor) are always close to the amide NH following the Azt(R) residue. See, for example, the two sections from the NOESY spectra of Z-Azt(*t*Amyl)-Ala-Ala-OMe (**7b**) and Z-Ala-Azt(*t*Amyl)-Ala-OMe (**8b**) reported in Figure 9.

It is also evident that the position (1 or 2) of Azt(R) in the tripeptide greatly affects the conformation(s) adopted. Indeed, the IR absorption spectra (Figure 5) show an important difference in the strength of the hydrogen bonds in the two tripeptides. In particular, when Azt(R) is at position 2, the IR pattern is closer to that of a β-turn conformation. This conclusion is in agreement with the findings of the DMSO experiments described above: The behavior of tripeptides **8** is more similar, compared with **7**, to that of a peptide folded into a β-turn because the NH of the residue at the third position is the least sensitive to DMSO perturbation (Figure 8). However, we must remember that in peptides **8** the NH at the third position following the Azt(R) may be involved in a side-chain-to-main-chain hydrogen bond as well.

The conformational differences between peptides **7** and **8** parallel those observed, although in the crystal state, for Ac_4c (**3**; Figure 1) tripeptides of the same type: Z-Ac_4c-Ala-Ala-OMe crystallizes into a type I β-turn whereas Z-Ala-Ac_4c-Ala-OMe prefers the type II β-turn.[9b] An inspection of molecular models reveals that the side-chain-to-main-chain hydrogen bond of our Azt(R) peptides is possible only when Azt(R) and the following residue occupy positions 1 and 2 of a type II (or II′) β-turn ($\phi_1 = -60°$, $\psi_1 = +120°$; $\phi_2 = +80°$, $\psi_2 = 0°$). Such a spatial arrangement may occur in peptides **7**, but is unlikely in peptides **8** in which Azt(R) occupies position 2 (not position 1) of the β-turn. Therefore we believe that in tripeptides **8** the contribution of β-turn conformers is dominant in the equilibrium mixture, whereas a higher population of the proposed side-chain-to-main-chain stabilized structure may be present in tripeptides **7**.

Conclusions

It can be safely concluded that the Azt(R) amino acids investigated herein are β-turn inducers, similarly to Ac_4c, the prototype of four-membered-ring α-amino acids. In addition, the trisubstituted nitrogen of the azetidine ring acts as a hydrogen-bond acceptor, most probably for the proton of the Azt(R) carboxyamide function. These two tendencies of the Azt(R) residues may increase the number of conformational options when designing foldamers with new and predictable 3D structures. However, it is evident that longer peptides, and in particular Azt(R) homopeptides, are needed to clarify the extent of the influence of the two tendencies on a 3D peptide structure. To this end, we are currently exploring improved synthetic strategies to access longer Azt(R) peptides.

Experimental Section

General Methods: Flame-dried glassware was used for all nonaqueous reactions. Commercially available solvents and reagents were purchased from common chemical suppliers and used without further purification, unless stated otherwise. The reaction mixtures were purified by flash chromatography, which was carried out by using a glass column filled with silica gel (Acros, particle size 0.035–0.070 mm, pore diameter ca. 6 nm). The reactions were monitored by initial TLC analysis on glass-backed silica plates (Merck Kieselgel, 60 F_{254}, precoated 0.25 mm) or aluminium-foil-backed silica sheets (Merck, Silica gel 60 F_{254} or Macherey–Nagel, Alugram® Sil G UV 254), which were developed by using standard visualization techniques or agents: UV fluorescence (254 and 366 nm) and coloring with permanganate solution and subsequent heating. The migration rate of the compounds is reported as the retention factor (R_f). Compounds with boiling points lower than 150 °C were removed by rotary evaporation (20–30 Torr) whereas a high-vacuum pump (0.01–2 Torr) was used for removal of high-boiling-point compounds. *N*-Ethyl,*N*′-[3-(dimethylamino)propyl]carbodiimide (EDC) and 7-aza-1-hydroxy-1,2,3-benzotriazole (HOAt)[36]

were from GL Biochem products. The L (= *S*) configuration of the amino acid Ala was used (for both Z-Ala-OH and the H-Ala-OMe HCl salt) and was obtained from Sigma–Aldrich as were all other chemicals. H-Ala-OMe and Z-Ala-Ala-OMe were synthesized according to published procedures.[37] For NMR analysis the compounds were diluted in $CDCl_3$, the chemical shifts quoted in parts per million (ppm), and referenced to tetramethylsilane (TMS, δ = 0 ppm) or the residual solvent peak. ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a JEOL Eclipse FT 300 NMR spectrometer, ^1H (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded with a Bruker DRX 400 spectrometer, and ^1H (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded with a Bruker AC 200 spectrometer at room temperature. Peak assignments were elucidated by using COSY, HSQC, HMQC, TOCSY, NOESY, DEPT-135, and DEPT-90 techniques when required. IR spectra of samples in neat form were recorded with a Perkin–Elmer Spectrum BX spectrometer with an ATR (attenuated total reflectance) accessory; only selected absorbances ($\tilde{\nu}_{max}$ [cm^{-1}]) are reported. IR absorption measurements on KBr pellets and in $CDCl_3$ solution were performed with a Perkin–Elmer model 1720 X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample shuttle device at a nominal resolution of 2 cm^{-1}, averaging 100 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with pathlengths of 0.1, 1, and 10 mm (with CaF_2 windows) were used. Absorption maxima, shoulders, and partially overlapping bands were detected with the inverse second derivative method. LC-MS was performed with Agilent 1100 Series VL (ES, 4000V) or SL (ES, 4000V) equipment by using electron-spray ionization at 4 (positive mode) or 3.5 kV (negative mode) and fragmentation at 70 eV with only molecular ions ($[MH]^+$) being reported and intensities quoted as a percentage of the base peak using either a LC-MS coupling or a direct inlet system. Mass spectra were recorded with an Agilent 1100 series mass spectrometer using either a direct inlet system (electron spray, 4000 V) or LC-MS coupling (UV detector) or a Mariner ESI-ToF mass spectrometer (Perseptive Biosystems). Prior to injection, samples were dissolved in a 1:1 mixture of water/MeOH containing 0.1% formic acid. Optical rotations were measured with a Perkin–Elmer model 241 polarimeter at the sodium D line wavelength using a cell with an optical pathlength of 10 cm. Concentrations are expressed in g/100 mL. $[\alpha]_D^{20}$ were calculated by using the formula $[\alpha]_D^{20} = \alpha/(cl)$ in which c is the concentration (in g/mL) and l is the optical path (in dm). Spectrophotometric-grade MeOH was used as solvent.

Synthetic Procedures

N_3-Azt(*t*Amyl)-OMe (9d): N_3-Azt(*t*Amyl)-OMe (**9d**) was prepared from Br-Azt(*t*Amyl)-OMe[25b,30] (157 mg, 2.42 mmol) in accordance with a literature procedure.[25b] Yellow oil; yield 92%. R_f = 0.41 (petroleum ether/EtOAc, 4:1). ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.85 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 0.91 [s, 6 H, $C(CH_3)_2$], 1.27 (q, J = 7.5 Hz, 2 H, CH_2CH_3), 3.32 [d, J = 8.5 Hz, 2 H, CH(H)NCH(H)], 3.71 [d, J = 8.5 Hz, 2 H, CH(H)NCH(H)], 3.84 (s, 3 H, OCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 8.6 (CH_2CH_3), 20.0 [$C(CH_3)_2$], 31.5 (CH_2CH_3), 53.2 (OCH_3), 54.4 (CH_2NCH_2), 54.7 (CCH_2CH_3), 59.7 (NCH_2C), 170.6 (C=O) ppm. IR (neat): $\tilde{\nu}$ = 2108 (N_3), 1737 (C=O) cm^{-1}. MS (ES, pos. mode): m/z (%) = 227 (100) $[M + H]^+$.

N_3-Azt(*t*Amyl)-Ala-Ala-OMe (12b)

Method A: LiOH·H_2O (26 mg, 0.63 mmol) was added to a solution of N_3-Azt(*t*Amyl)-OMe (**9d**; 50 mg, 0.21 mmol) in MeOH (2 mL) and water (2 mL) at 0 °C and the reaction mixture was stirred at room temperature for 12 h. Subsequently, the resulting reaction mixture was neutralized with aq. 2 M HCl solution. Evaporation of the solvent under reduced pressure and subsequent removal of water by lyophilization afforded crude N_3-Azt(*t*Amyl)-OH (**10b**), which was used in the next step without further purification or characterization.

Pd (21 mg, 10 wt.-% on carbon) and TFA (0.15 mL) were added to a solution of Z-Ala-Ala-OMe (214 mg, 0.69 mmol) in MeOH (15 mL). The reaction mixture was stirred under H_2 (1 atm) at room temperature for 30 min and then filtered through Celite®. The filter cake was washed with small portions of MeOH and the solvent was evaporated under reduced pressure. Excess TFA was removed by addition of water (1 mL) and subsequent lyophilization. The residual crude H-Ala-Ala-OMe·TFA (**11**) was characterized by ^1H NMR and used immediately in the next step without further purification.

The crude N_3-Azt(*t*Amyl)-OH (**10b**; 48 mg, 0.21 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C. Then HOAt (29 mg, 0.21 mmol) and EDC·HCl (40 mg, 0.21 mmol) were added and the resulting reaction mixture was stirred for 15 min. Then H-Ala-Ala-OMe·TFA (**11**; 169 mg, 0.63 mmol) and Et_3N (105 mg, 0.145 mL, 1.04 mmol) were added. The homogeneous mixture formed was warmed to room temperature and stirred for 12 h. Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc, 9:1–7:3) afforded pure compound **12b**.

Method B: Crude N_3-Azt(*t*Amyl)-OH (**10b**), prepared from **9d** (0.21 mmol) as described in Method A, was dissolved in anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C. Subsequently DMF (3 μL) and oxalyl chloride (66 mg, 0.52 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 1 h. Evaporation of the solvent under reduced pressure on a cold water bath afforded crude N_3-Azt(*t*Amyl)-Cl (**13b**), which was immediately dissolved without further purification in anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C. Then H-Ala-Ala-OMe·TFA (**11**; 347 mg, 1.20 mmol) and Et_3N (148 mg, 1.47 mmol) were added. The homogeneous mixture formed was warmed to room temperature and stirred for 12 h. Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc, 1:1) afforded pure compound **12b**.

Light-yellow oil; yield 23% (method A), 78% (method B). R_f = 0.30 (petroleum ether/EtOAc, 1:1). $[\alpha]_D^{20}$ = –51.2 (c = 0.5, MeOH). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.79 (t, J = 7.4 Hz, 3 H, CH_2CH_3), 0.87 [s, 6 H, $C(CH_3)_2$], 1.12–1.26 (m, 2 H, CH_2CH_3), 1.34 (d, J = 7.1 Hz, 3 H, $CHCH_3$), 1.35 (d, J = 6.8 Hz, 3 H, $CHCH_3$), 3.29 [d, J = 7.0 Hz, 2 H, CH(H)NCH(H)], 3.54 [d, J = 7.1 Hz, 1 H, CH(H)NCH(H)], 3.58 [d, J = 7.1 Hz, 1 H, CH(H)-NCH(H)], 3.68 (s, 3 H, OCH_3), 4.42–4.54 (m, 2 H, 2 $CHCH_3$), 6.80 (br. s, 1 H, NH), 7.91 (br. d, J = 7.6 Hz, 1 H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 8.5 (CH_2CH_3), 18.2 ($CHCH_3$), 18.5 ($CHCH_3$), 20.3 (CCH_3), 20.4 (CCH_3), 31.6 (CH_2CH_3), 48.2 ($CHCH_3$), 49.0 ($CHCH_3$), 52.6 (OCH_3), 54.6 [$C(CH_3)_2$], 55.6 (CH_2NCH_2), 59.8 (NCH_2C), 170.1 (C=O), 171.5 (C=O), 173.2 (C=O) ppm. IR (KBr): $\tilde{\nu}$ = 3314 (NH), 2116 (N_3), 1746 (C=O), 1657, 1520 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): m/z (%) = 369.2092 (100) $[M + H]^+$.

N_3-Azt(*t*Bu)-Ala-Ala-OMe (12a): This compound was prepared as described above for **12b** starting from **9c**. Light-yellow oil; yield 25% (method A), 80% (method B). R_f = 0.27 (petroleum ether/EtOAc, 1:1). $[\alpha]_D^{20}$ = –41.6 (c = 0.5, MeOH). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.93 [s, 9 H, $C(CH_3)_3$], 1.34 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 1.35 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 3.30 [d, J = 7.3 Hz, 2 H, CH(H)NCH(H)], 3.57, 3.61 [each d, each J = 7.3 Hz, each 1

H, C*H*(H)NC*H*(H)], 3.68 (s, 3 H, OCH_3), 4.41–4.53 (m, 2 H, 2 C*H*CH_3), 6.73 (d, *J* = 7.5 Hz, 1 H, NH), 7.77 (d, *J* = 7.6 Hz, 1 H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 18.2 (C*C*H_3), 18.5 (C*C*H_3), 24.2 [C(*C*H_3)$_3$], 48.2 (*C*HCH_3), 49.1 (*C*HCH_3), 52.3 [*C*(CH$_3$)$_3$], 52.6 (OCH_3), 55.6 (CH_2NCH_2), 59.5 (NCH_2C), 170.0 (C=O), 171.6 (C=O), 173.2 (C=O) ppm. IR (KBr): ṽ = 3309 (NH), 2114 (N_3), 1744 (C=O), 1654, 1516 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 355.2008 (100) [M + H]$^+$.

Z-Azt(*t*Bu)-Ala-Ala-OMe (7a): Pd (4 mg, 10 wt.-% on carbon) was added to a solution N_3-Azt(*t*Bu)-Ala-Ala-OMe (**12a**; 40 mg, 0.11 mmol) in MeOH (10 mL). The reaction mixture was stirred under H_2 (1 atm) at room temperature for 30 min, then filtered through Celite®, and the filter cake was washed with small portions of MeOH. The solvent was evaporated under reduced pressure. The residual crude H-Azt(*t*Bu)-Ala-Ala-OMe (**14a**; 37 mg, 0.11 mmol) was dissolved in anhydrous CH_2Cl_2 (4 mL) and cooled to 0 °C. Subsequently, benzyl chloroformate (23 mg, 0.14 mmol) and *N*,*N*-diisopropylethylamine (15 mg, 0.11 mmol) were added and the reaction mixture was stirred at room temperature for 12 h. Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) afforded pure compound **7a**. Light-yellow oil; yield 85%. R_f = 0.44 (CH_2Cl_2/MeOH, 19:1). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.99 [s, 9 H, C(CH$_3$)$_3$], 1.32 (d, *J* = 7.5 Hz, 3 H, CHC*H*$_3$), 1.35 (d, *J* = 6.8 Hz, 3 H, CHC*H*$_3$), 3.25 [s, 2 H, C*H*(H)NC*H*(H)], 3.66 (s, 3 H, OCH_3), 4.03 [s, 2 H, CH(*H*)NCH(*H*)], 4.38–4.54 (m, 2 H, 2 C*H*CH_3), 5.03 (s, 2 H, OCH_2), 6.23 (br. s, 1 H, NH), 6.68 (br. s, 1 H, NH), 7.20–7.32 (m, 5 H, 5 CH_{arom}), 9.13 (s, 1 H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 18.0 (CH*C*H_3), 18.4 (CH*C*H_3), 24.9 [C(*C*H_3)$_3$], 48.2 (*C*HCH_3), 49.2 (*C*HCH_3), 52.6 (OCH_3), 52.7 [*C*(CH$_3$)$_3$], 54.7 (CH_2NCH_2), 54.8 (NCH_2C), 66.6 (OCH_2), 128.1, 128.2 and 128.6 (5 CH_{arom}), 136.4 (C_{arom}), 155.3 (C=O), 171.7 (C=O), 173.2 (C=O), 173.4 (C=O) ppm. IR (KBr): ṽ = 3314 (NH), 1745 (C=O), 1725, 1655, 1521 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 463.2532 (100) [M + H]$^+$.

Z-Azt(*t*Amyl)-Ala-Ala-OMe (7b): This compound was prepared as described above for **7a** starting from **12b**. Light-yellow oil; yield 87%. R_f = 0.44 (CH_2Cl_2/MeOH, 19:1). $[\alpha]_D^{20}$ = –57.0 (*c* = 0.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.80 (t, *J* = 7.4 Hz, 3 H, CH_2CH_3), 0.94 [s, 6 H, C(C*H*$_3$)$_2$], 1.18–1.33 (m, 2 H, C*H*$_2$CH$_3$), 1.32 (d, *J* = 7.2 Hz, 3 H, CHC*H*$_3$), 1.35 (d, *J* = 7.0 Hz, 3 H, CHC*H*$_3$), 3.14 [d, *J* = 14.3 Hz, 2 H, C*H*(H)NC*H*(H)], 3.67 (s, 3 H, OCH_3), 4.09 [s, 2 H, CH(*H*)NCH(*H*)], 4.38–4.54 (m, 2 H, 2 C*H*CH_3), 5.03 (s, 2 H, CH_2O), 6.14 (br. s, 1 H, NH), 6.60 (d, *J* = 6.9 Hz, 1 H, NH), 7.20–7.32 (m, 5 H, 5 CH_{arom}), 9.26 (br. s, 1 H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 8.6 (CH_2CH_3), 17.9 (CH*C*H_3), 18.5 (CH*C*H_3), 21.1 (C*C*H_3), 21.3 (C*C*H_3), 31.9 (*C*H_2CH_3), 48.2 (*C*HCH_3), 49.2 (*C*HCH_3), 52.6 (OCH_3), 52.9 (*C*CH_2CH_3), 54.5 (CH_2NCH_2), 54.6 (NCH_2C), 66.6 (OCH_2), 128.1, 128.2, 128.7 (5 CH_{arom}), 136.4 (C_{arom}), 155.3 (C=O), 171.6 (C=O), 173.4 (C=O), 173.5 (C=O) ppm. IR (KBr): ṽ = 3315 (NH), 1744 (C=O), 1723, 1655, 1520 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 477.2608 (100) [M + H]$^+$.

N_3-Azt(*t*Bu)-Ala-OMe (15a): Crude N_3-Azt(*t*Bu)-OH (**10a**), which was prepared as described above for **10b** starting from **9a** (0.21 mmol), was dissolved in anhydrous CH_2Cl_2 (2 mL) and then cooled to 0 °C. Subsequently, DMF (3 μL) and oxalyl chloride (70 mg, 0.56 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h and also at room temperature for 1 h. Evaporation of the solvent under reduced pressure on a cold water bath afforded crude N_3-Azt(*t*Bu)-Cl (**13a**), which was used immediately without further purification or characterization.

N_3-Azt(*t*Bu)-Cl (**13a**) was dissolved in anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C. Then H-Ala-OMe (108 mg, 1.05 mmol) and Et_3N (22 mg, 0.21 mmol) were added. The homogeneous mixture formed was warmed to room temperature and stirred for 12 h. Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc, 1:1) afforded pure **15a**. Light-yellow oil; yield 79%. R_f = 0.21 (petroleum ether/EtOAc, 1:1). $[\alpha]_D^{20}$ = –32.4 (*c* = 0.5, MeOH) ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.01 [s, 9 H, C(CH$_3$)$_3$], 1.44 (d, *J* = 7.2 Hz, 3 H, CHC*H*$_3$), 3.37 [d, *J* = 7.2 Hz, 2 H, C*H*(H)NC*H*(H)], 3.65 [d, *J* = 7.2 Hz, 1 H, CH(*H*)NCH(H)], 3.69 [d, *J* = 7.1 Hz, 1 H, CH(H)NCH(*H*)], 3.76 (s, 3 H, OCH_3), 4.58 (quint., *J* = 7.2 Hz, 1 H, C*H*CH_3), 7.85 (d, *J* = 5.1 Hz, 1 H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 18.5 (CH*C*H_3), 24.3 [C(*C*H_3)$_3$], 47.8 (*C*HCH_3), 52.3 (OCH_3), 52.7 [*C*(CH$_3$)$_3$], 55.7, 55.8 (*C*H_2NCH_2), 59.6 (NCH_2C), 169.9 (C=O), 173.3 (C=O) ppm. IR (KBr): ṽ = 3341 (NH), 2116 (N_3), 1745 (C=O), 1682, 1520 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 284.1573 (100) [M + H]$^+$.

N_3-Azt(*t*Amyl)-Ala-OMe (15b): Compound **15b** was prepared as described above for **15a** starting from **9b**. Light-yellow oil; yield 82%. R_f = 0.51 (petroleum ether/EtOAc, 7:3). $[\alpha]_D^{20}$ = –46.4 (*c* = 0.5, MeOH).^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): δ = 0.80 (t, *J* = 7.4 Hz, 3 H, CH_2CH_3), 0.88 [s, 6 H, C(CH$_3$)$_2$], 1.13–1.27 (m, 2 H, C*H*$_2$CH$_3$), 1.37 (d, *J* = 7.2 Hz, 3 H, CHC*H*$_3$), 3.29 [d, *J* = 6.9 Hz, 2 H, C*H*(H)NC*H*(H)], 3.53 [d, *J* = 7.0 Hz, 1 H, CH(*H*)NCH(H)], 3.59 [d, *J* = 6.9 Hz, 1 H, CH(H)NCH(*H*)], 3.69 (s, 3 H, OCH_3), 4.52 (quint., *J* = 7.3 Hz, 1 H, C*H*CH_3), 7.91 (d, *J* = 7.6 Hz, 1 H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 7.3 (CH_2CH_3), 17.3 (CH*C*H_3), 19.3 [C(*C*H_3)$_2$], 30.5 (*C*H_2CH_3), 47.2 (*C*HCH_3), 51.5 (OCH_3), 53.6 (*C*CH_2CH_3), 54.6 (*C*H_2NCH_2), 58.6 (NCH_2C), 168.8 (C=O), 172.1 (C=O) ppm. IR (KBr): ṽ = 3335 (NH), 2118 (N_3), 1746 (C=O), 1684, 1520 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 298.1860 (100) [M + H$^+$].

Z-Ala-Azt(*t*Bu)-Ala-OMe (8a): Cyanuric fluoride (351 mg, 0.22 mL, 2.6 mmol) and pyridine (126 mg, 1.3 mmol) were added to a solution of Z-Ala-OH (290 mg, 1.3 mmol) in anhydrous CH_2Cl_2 (7 mL) at 0 °C under N_2 and stirred at room temperature for 3 h. The reaction mixture was poured into ice–water (10 mL) and extracted with CH_2Cl_2 (3× 10 mL). Drying ($MgSO_4$), filtration, and evaporation of the solvent under reduced pressure afforded crude Z-Ala-F (**17**; 219 mg, 0.97 mmol), which was used in the next step without further purification or characterization.

Pd (10 mg, 10 wt.-% on carbon) was added to a solution of N_3-Azt(*t*Bu)-Ala-OMe (**15a**; 55 mg, 0.19 mmol) in MeOH (10 mL). The reaction mixture was stirred under H_2 (1 bar) at room temperature for 30 min. Then it was filtered through Celite® and the filter cake was washed with small portions of MeOH. Evaporation of the solvent under reduced pressure afforded crude H-Azt(*t*Bu)-Ala-OMe (**16a**; 50 mg, 0.19 mmol), which was used immediately in the next step without further purification or characterization.

Z-Ala-F (**17**; 219 mg, 0.97 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. Then H-Azt(*t*Bu)-Ala-OMe (**16a**; 50 mg, 0.19 mmol) and Et_3N (118 mg, 1.17 mmol) were added. The homogeneous mixture was warmed to room temperature and stirred for 2 h. Then the volatiles were evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) to afford pure compound **8a**. Light-yellow oil; yield 71%. R_f = 0.30 (CH_2Cl_2/MeOH, 19:1). $[\alpha]_D^{20}$ = –57.2 (*c* = 0.5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.07 [s, 9 H, C(CH$_3$)$_3$], 1.34 (d, *J* = 7.3 Hz, 3 H, CHC*H*$_3$), 1.36 (d, *J* = 7.3 Hz, 3 H, CHC*H*$_3$), 3.67 (s, 3 H, OCH_3), 4.05 [d, *J* =

7.3 Hz, 2 H, C*H*(H)NC*H*(H)], 4.11 [d, *J* = 7.3 Hz, 2 H, CH(*H*)-NCH(*H*)], 4.46 (quint., *J* = 7.1 Hz, 1 H, C*H*CH$_3$), 4.94–5.09 (m, 3 H, OCH$_2$, C*H*CH$_3$), 5.54 (br. s, 1 H, NH), 7.13–7.32 (m, 5 H, 5 CH$_{arom}$), 7.81 (br. s, 1 H, NH), 8.62 (s, 1 H, NH) ppm. ^{13}C NMR (101 MHz, CDCl$_3$, 25 °C): δ = 17.8 (2 CH*C*H$_3$), 24.3 [C(*C*H$_3$)$_3$], 48.7 (*C*HCH$_3$), 51.3 (*C*H$_2$NCH$_2$), 52.5 (OCH$_3$), 52.9 [*C*(CH$_3$)$_3$], 54.0 (CH$_2$N*C*H$_2$), 67.1 (OCH$_2$), 68.8 (*C*HCH$_3$), 128.1, 128.3, 128.7 (5 CH$_{arom}$), 136.4 (C$_{arom}$), 156.3 (C=O), 173.1 (C=O), 173.2 (C=O), 173.3 (C=O) ppm. IR (KBr): ṽ = 3317 (NH), 1734 (C=O), 1673, 1528 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 463.2501 (100) [M + H]$^+$.

Z-Ala-Azt(*t*Amyl)-Ala-OMe (8b): This compound was obtained as described above for **8a** starting from **15b**. Light-yellow oil; yield 75%. R_f = 0.31 (CH$_2$Cl$_2$/MeOH, 19:1). [*a*]$_D^{20}$ = –42.0 (*c* = 0.5, MeOH). ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 0.88 (t, *J* = 7.4 Hz, 3 H, CH$_2$C*H*$_3$), 1.03 [s, 6 H, C(CH$_3$)$_2$], 1.35 (q, *J* = 7.3 Hz, 2 H, C*H*$_2$CH$_3$), 1.39 (d, *J* = 7.1 Hz, 3 H, CHC*H*$_3$), 1.45 (d, *J* = 7.2 Hz, 3 H, CHC*H*$_3$), 3.16–3.42 [m, 2 H, C*H*(H)NC*H*(H)], 3.75 (s, 3 H, OCH$_3$), 4.13–4.29 [m, 3 H, CH(*H*)NCH(*H*), C*H*CH$_3$], 4.57 (quint., *J* = 7.3 Hz, 1 H, C*H*CH$_3$), 5.07 [d, *J* = 12.3 Hz, 1 H, C*H*(H) O], 5.13 [d, *J* = 12.3 Hz, 1 H, CH(*H*)], 5.54 (br. s, 1 H, NH), 7.16–7.37 (m, 5 H, 5 CH$_{arom}$), 7.43 (br. s, 1 H, NH), 9.25 (br. s, 1 H, NH) ppm. ^{13}C NMR (101 MHz, CDCl$_3$, 25 °C): δ = 8.5 (CH$_2$*C*H$_3$), 18.2 (CH*C*H$_3$), 18.8 (CH*C*H$_3$), 21.0, 21.1 [C(*C*H$_3$)$_2$], 31.6 (*C*H$_2$CH$_3$), 48.4 (*C*HCH$_3$), 51.2 (*C*HCH$_3$), 52.5 (OCH$_3$), 53.0 (*C*CH$_2$CH$_3$), 53.9 (*C*H$_2$N*C*H$_2$), 55.3 (NCH$_2$*C*), 67.0 (OCH$_2$), 128.1, 128.2, 128.6 (5 CH$_{arom}$), 136.4 (C$_{arom}$), 156.0 (C=O), 172.6 (C=O), 173.1 (C=O) ppm. IR (KBr): ṽ = 3299 (NH), 1742 (C=O), 1717, 1666, 1525 [(C=O)NH] cm^{-1}. MS (ES, pos. mode): *m*/*z* (%) = 477.2608 (100) [M + H]$^+$.

Supporting Information (see footnote on the first page of this article): Mass, ^{1}H and ^{13}C NMR spectra.

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