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Atropisomerism in azadipeptides: evaluation of N^1-methylation and thioamide introduction

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ABSTRACT

Cbz-protected azadipeptides, designed as structurally reduced model compounds, were synthesized and investigated with respect to the occurrence of atropisomerism. Methylation at the carbamate nitrogen caused mixtures of *E* and *Z* isomers in Cbz-sarcosyl-azaglycine-amide (**9**) and Cbz-sarcosyl-methylazaalanine-amide (**10**). A formal *O*/*S* exchange led to Cbz-glycyl-azaglycine-thioamide (**11**) and Cbz-glycyl-methylazaalanine-thioamide (**12**), respectively. The $(MeN)_2$ fragment, present in **10** and **12** (but not **9** and **11**), serves as a chirality-inducing element owing to a restricted rotation around the N–N axis.

Azapeptides are peptides in which the $C_\alpha H$ unit of at least one amino acid in the peptide has been replaced by nitrogen. This replacement is an important element in the design of peptidomimetics.[1] Azapeptides can improve potency and selectivity and possess superior pharmacokinetics such as proteolytic resistance and higher bioavailability compared to their natural 'carbapeptide' counterparts. Recent examples for the manifold biological activities of azapeptides include enzyme inhibitory properties, such as inhibition of cysteine cathepsins,[2] caspases,[3] human neutrophil proteinase 3 (PR3),[4] and modulation of the insulin receptor tyrosine kinase (IRTK).[5] Other receptors have also been addressed by azapeptides, such as the cluster of differentiation 36 (CD36) receptor by analogues of the growth hormone releasing peptide-6 (GHRP-6),[6] and cyclic azapeptides were identified as potent antagonists of integrin receptors.[7] Moreover, successful attempts to convert azapeptides into activity-based probes (ABPs) and positron emission tomography (PET) tracers have been reported.[8]

Getting insights into the secondary structure of (aza)peptides is an important field of research to understand the respective protein–ligand interactions. The structural properties of azapeptides have been examined by theoretical techniques and experimental approaches, respectively.[9,10] Although it has been known for a long time that chirality enables distinct enantiomers to have different pharmacological effects, particular attention in drug discovery projects has been paid to atropisomerism only in the last decade.[11]

In a previous investigation it was demonstrated that the methylation of N^2 and N^3 of the structurally reduced model azadipeptide amide Cbz-Gly-azaGly-NH_2 (**1**) led to an *E* configuration of respective CO–N bonds in the resulting Cbz-Gly[ΨCONMe]-azaAla-NH_2 (**2**) and hence to atropisomerism due to a restricted rotation around the N–N axis (Fig. 1).[12] Vice versa, removal of the methyl groups of **2** gave rise to a regular *Z* configuration of the CO–N bonds and to a loss of chirality in **1**. N-methylation is a simple but meaningful manipulation of naturally occurring and synthetic peptides. It has a significant impact on the secondary structure of (aza)peptides and influences the *E*/*Z* equilibrium of peptide bonds.[13]

The *O*/*S*-exchange, has also been established as an important strategy in the design of peptidomimetics, which can lead to improved potency and stability.[14] Although the thioamide group is one of the closest mimics of an amide linkage, remarkably different properties result from the *O*/*S*-exchange, due to the better polarizability and lower electrophilicity of sulfur, the larger C–S bond (1.65 Å vs 1.20 Å), the higher acidity of the thioamide NH, and the stronger double bond character between carbon and nitrogen in case of the thio analogs with a somewhat higher rotational barrier.[14,15]

In the current letter, we report on the impact of N^1-methylation as well as the amide *O*/*S*-exchange in model azadipeptide amides on atropisomerism.[16,17] Axial chirality was diagnosed on the basis of ^1H NMR signals of diastereotopic Gly-methylene protons.[12]

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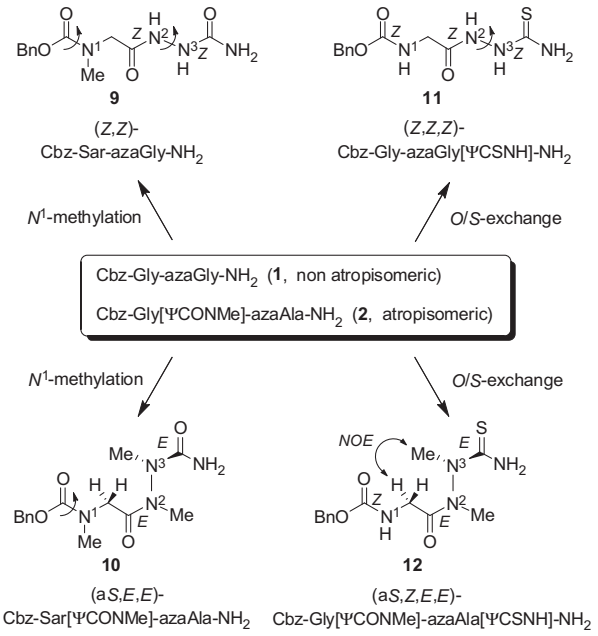
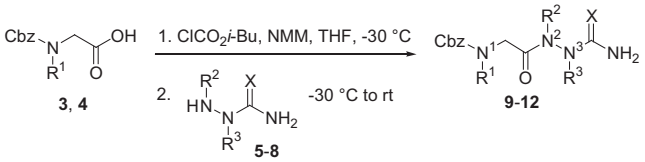


Figure 1. N^1-methylation and *O*/*S*-exchange of non-atropisomeric (**1**)[12] or atropisomeric (**2**)[12] azadipeptides.

To obtain the N^1-methylated compounds Cbz-Sar-azaGly-NH_2 (**9**) and Cbz-Sar[ΨCONMe]-azaAla-NH_2 (**10**), Cbz-Sar-OH (**3**)[18] was reacted with semicarbazide (**5**) and 1,2-dimethylsemicarbazide (**6**),[12] respectively, via the mixed anhydride method (Table 1). Azadipeptide thioamide Cbz-Gly-azaGly[ΨCSNH]-NH_2 (**11**) was prepared from Cbz-Gly-OH (**4**) and thiosemicarbazide (**7**) by applying the same coupling procedure. However, synthesis of Cbz-Gly[ΨCONMe]-azaAla[ΨCSNH]-NH_2 (**12**) required a different approach, in which 1,2-dimethylthiosemicarbazide (**8**), prepared from 1,2-dimethylhydrazine and potassium thiocyanate (see Supplementary material), was reacted with the acid chloride of **4**.

When inspecting the NMR spectra (500 MHz, DMSO-d_6) of the sarcosine-derived compound **9**, we observed a doubling of all signals in the ^1H and ^{13}C NMR spectra (see Supplementary material).

Table 1
Coupling of amino acids **3**, **4** with (thio)semicarbazides **5**–**8** to azadipeptide amides **9**–**12**



Compound	R^1	R^2/R^3	X	Yield (%)
3	Me			92
4	H			
5		H	O	
6		Me	O	
7		H	S	
8		Me	S	77
9	Me	H	O	42
10	Me	Me	O	72
11	H	H	S	44
12[a]	H	Me	S	31

[a] Coupling procedure applied: **3**, $(COCl)_2$, cat. DMF, CH_2Cl_2, rt, then DMAP, DIPEA, **6**, CH_2Cl_2, rt.

Thus, a mixture of *E*/*Z* isomers has been produced as a result of introducing a methyl group to the carbamate nitrogen N^1 of the achiral azadipeptide **1**. The *Z* configuration of the CO–NH bonds can be anticipated from previous investigations of related compounds.[12] However, raising the temperature to 343 K caused a coalescence of the respective signals, indicating that the *E*/*Z* isomers are not separable due to the known low rotational barrier of CN bonds of alkylated carbamates.[19]

This finding was confirmed by analyzing the NMR data of Cbz-Sar[ΨCONMe]-azaAla-NH_2 (**10**). Again, the doubling of all signals in ^1H and ^{13}C NMR spectra disappeared when recording the spectra at 343 K and single signals were obtained. However, a geminal coupling of the Gly-methylene protons was still apparent, indicating a restricted rotation around the N–N axis, which can be explained by an *E* configuration of the central peptide bond. The *E* configuration is adopted owing to methylation of the N^2 nitrogen in **10**.[12] This structural change results in steric and electronic repulsions between the N^3 substituents and the Gly-methylene protons as well as between the N^3 substituents and the N^2 methyl group. The $\Delta G^{\ddagger}_{298\ K}$ value for the barrier of rotation in **10** is expected to be in a similar range than that of **2**, that is, approximately 110 kJ mol^{-1}, because of the structural similarity of the *E*-configured azadipeptide amides **2** and **10**.

Turning our interest to azadipeptide thioamides, we observed a regular doublet for the Gly-methylene protons in the ^1H NMR spectrum of Cbz-Gly-azaGly[ΨCSNH]-NH_2 (**11**) due to coupling to the neighboring carbamate proton, which refers to achirality. In case of the N^2/N^3-bis-methylated analog Cbz-Gly[ΨCONMe]-azaAla[ΨCSNH]-NH_2 (**12**) an ABX spin system was observed. After assignment of the N^2 and the N^3 methyl groups by HMBC and HMQC techniques, an NOE experiment was performed. NOE correlations between the N^3 methyl group and one proton of the diastereotopic Gly-methylene fragment were detected (Fig. 1, for spectra, see Supplementary material). This indicated the adoption of an *E* configuration of the methylated CO–N bond in **12**, as it has also been reported for the oxo analog **2**.[12] X-ray crystallographic analysis gave further evidence for this finding (Fig. 2).

To get more insights into the stereochemical stability of **12** and to address a possible atropisomerism in **12**, the minimum energy conformations were computed at the non-local density functional level of theory and the rotational barrier around the N–N bond was calculated (see Supplementary material). Atropisomerism arises from a sterically hindered rotation about a single bond leading to two separable individual conformers.[16] Two rotational barriers were obtained for **12** and the energies of the corresponding transition states $\Delta G^{\ddagger}_{298K}$ are 122 and 135 kJ mol^{-1}. This demonstrates that

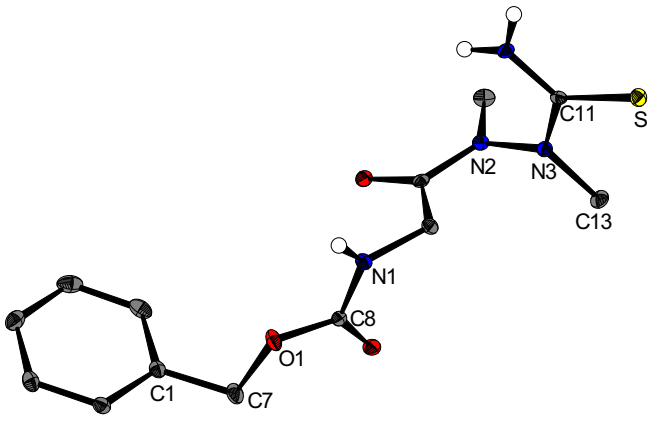


Figure 2. Molecular plot of (*Z*,*E*,*E*)-Cbz-Gly[ΨCONMe]-azaAla[ΨCSNH]-NH_2 (**12**). Displacement ellipsoids are drawn at 30% probability level.[20]

the novel azadipeptide thioamide **12** indeed forms two stable atropisomers.

In conclusion, four model azadipeptide (thio)amides **9**–**12** have been designed on the basis of N^1-methylation and *O*/*S* exchange of structurally reduced azadipeptide amides **1** and **2**. To experimentally elucidate structural features of **9**–**12**, diastereotopicity of the Gly-methylene protons was applied as a chiral reporter. The N^1 methylation in **9** and **10** leads to *E*/*Z* isomers detectable on the NMR time scale. In peptides **10** and **12**, a chirality-inducing element,that is, $(MeN)_2$, was successfully introduced with 1,2-dimethyl(thio)semicarbazide (**6**, **8**). The presence of this chirality-inducing element leads to a restricted rotation around the N–N bond and therefore to atropisomers.

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Supplementary data

Supplementary data (detailed synthetic procedures, analytical data, NMR spectra, an X-ray crystallographic file in CIF format, electronic structure calculations) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.06.074.

References and notes

1. For a review on azapeptides, see: Proulx, C.; Sabatino, D.; Hopewell, R.; Spiegel, J.; Ramos, Y. G.; Lubell, W. D. *Future Med. Chem.* **2011**, *3*, 1139.
2. (a) Wieczerzak, E.; Jankowska, E.; Rodziewicz-Motowidło, S.; Giełdoń, A.; Łągiewka, J.; Grzonka, Z.; Abrahamson, M.; Grubb, A.; Brömme, D. *J. Peptide Res.* **2006**, *66*, 1; (b) Löser, R.; Frizler, M.; Schilling, K.; Gütschow, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4331; (c) Loh, Y.; Shi, H.; Hu, M.; Yao, S. Q. *Chem. Commun.* **2010**, 8407; (d) Frizler, M.; Lohr, F.; Furtmann, N.; Kläs, J.; Gütschow, M. *J. Med. Chem.* **2011**, *54*, 396; (e) Ottersbach, P. A.; Schmitz, J.; Schnakenburg, G.; Gütschow, M. *Org. Lett.* **2013**, *15*, 448; (f) Schmitz, J.; Beckmann, A. M.; Dudic, A.; Li, T.; Sellier, R.; Bartz, U.; Gütschow, M. *ACS Med. Chem. Lett.* **2014**, *5*, 1076.
3. (a) Ekici, Ö. D.; Li, Z. Z.; Campbell, A. J.; James, K. E.; Asgian, J. L.; Mikolajczyk, J.; Salvesen, G. S.; Ganesan, R.; Jelakovic, S.; Grütter, M. G.; Powers, J. C. *J. Med. Chem.* **2006**, *49*, 5728; (b) Sexton, K. B.; Kato, D.; Fonovic, M.; Verhelst, S. H. L.; Bogyo, M. *Cell. Death Differ.* **2007**, *14*, 727.
4. Epinette, C.; Croix, C.; Jaquillard, L.; Marchand-Adam, S.; Kellenberger, C.; Lalmanach, G.; Cadene, M.; Viaud-Massuard, M. C.; Gauthier, F.; Korkmaz, B. *Biochem. Pharmacol.* **2012**, *83*, 788.
5. Kurian, L. A.; Silva, T. A.; Sabatino, D. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4176.
6. (a) Sabatino, D.; Proulx, C.; Pohankova, P.; Ong, H.; Lubell, W. D. *J. Am. Chem. Soc.* **2011**, *133*, 12493; (b) Proulx, C.; Picard, É.; Boeglin, D.; Pohankova, P.; Chemtob, S.; Ong, H.; Lubell, W. D. *J. Med. Chem.* **2012**, *55*, 6502.
7. Spiegel, J.; Mas-Moruno, C.; Kessler, H.; Lubell, W. D. *J. Org. Chem.* **2012**, *77*, 5271.
8. (a) Verhelst, S. H. L.; Witte, M. D.; Arastu-Kapur, S.; Fonovic, M.; Bogyo, M. *ChemBioChem* **2006**, *7*, 943; (b) Yang, P.-Y.; Wang, M.; Li, L.; Wu, H.; He, C. Y.; Yao, S. Q. *Chem. Eur. J.* **2012**, *18*, 6528; (c) Löser, R.; Bergmann, R.; Frizler, M.; Mosch, B.; Dombrowski, L.; Kuchar, M.; Steinbach, J.; Gütschow, M.; Pietzsch, J. *ChemMedChem* **2013**, *8*, 1330.
9. For examples, see: (a) Thormann, M.; Hofmann, H.-J. *J. Mol. Struct. (THEOCHEM)* **1999**, *469*, 63; (b) Lee, H.-J.; Song, J.-W.; Choi, Y.-S.; Park, H.-M.; Lee, K.-B. *J. Am. Chem. Soc.* **2002**, *124*, 11881.
10. Vanderesse, R.; Grand, V.; Limal, D.; Vicherat, A.; Marraud, M.; Didierjean, C.; Aubry, A. *J. Am. Chem. Soc.* **1998**, *120*, 9444.
11. (a) Clayden, J.; Moran, W. J.; Edwards, P. J.; LaPlante, S. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6398; (b) LaPlante, S. R.; Edwards, P. J.; Fader, L. D.; Jakalian, A.; Hucke, O. *ChemMedChem* **2011**, *6*, 505.
12. Ottersbach, P. A.; Schnakenburg, G.; Gütschow, M. *Chem. Commun.* **2012**, 5772.
13. For reviews on N-methylation of peptides, see: (a) Chatterjee, J.; Gilon, C.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331; (a) Chatterjee, J.; Rechenmacher, F.; Kessler, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 254.
14. For examples, see: (a) Neumann, U.; Gütschow, M. *Bioorg. Chem.* **1995**, *23*, 72; (b) Bach, A.; Eildal, J. N. N.; Stuhr-Hansen, N.; Deeskamp, R.; Gottschalk, M.; Pedersen, S. W.; Kristensen, A. S.; Strømgaard, K. *J. Med. Chem.* **2011**, *54*, 1333; (c) De Zotti, M.; Biondi, B.; Peggion, C.; De Poli, M.; Fathi, H.; Oancea, S.; Toniolo, C.; Formaggio, F. *Beilstein J. Org. Chem.* **2012**, *8*, 1161; (d) Goldberg, J. M.; Speight, L. C.; Fegley, M. W.; Petersson, E. J. *J. Am. Chem. Soc.* **2012**, *134*, 6088; (e) Huang, Y.; Jahreis, G.; Fischer, G.; Lücke, C. *Chem. Eur. J.* **2012**, *18*, 9841; (f) Ventosa-Andrés, P.; Valdivielso, A. M.; Pappos, I.; García-López, M. T.; Tsopanoglou, N. E.; Herranz, R. *Eur. J. Med. Chem.* **2012**, *58*, 98; (g) Laursen, J. S.; Engel-Andreasen, J.; Fristrup, P.; Harris, P.; Olsen, C. A. *J. Am. Chem. Soc.* **2013**, *135*, 2835; (h) Engel-Andreasen, J.; Wich, K.; Laursen, J. S.; Harris, P.; Olsen, C. A. *J. Org. Chem.* **2015**, *80*, 5415.
15. For a computational study on thioamide containing azapeptides, see: Lee, H.-J.; Kim, J. H.; Jung, H. J.; Kim, K.-Y.; Kim, E.-J.; Choi, Y.-S.; Yoon, C.-J. *J. Comput. Chem.* **2004**, *25*, 169.
16. Atropisomers exhibit rotational barriers higher than about 95 kJ mol^{-1} at room temperature: (a) Oki, M. *Top. Stereochem.* **1983**, *14*, 1; (a) Bringmann, G.; Price Mortimer, A. J.; Keller, P. A.; Gresser, M. J.; Garner, J.; Breuning, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 5384; (b) Wolf, C. *Dynamic Stereochemistry of Chiral Compounds*; RSC Publishing: Cambridge, UK, 2008.
17. For simplicity reasons, in the following, the terms *E*/*Z* are used with respect to the geometrical isomers at CO–N bonds and atropisomers with respect to a hindered rotation around the N–N bond.
18. Lai, M. Y. H.; Brimble, M. A.; Callis, D. J.; Harris, P. W. R.; Levi, M. S.; Sieg, F. *Bioorg. Med. Chem.* **2005**, *13*, 533.
19. (a) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 219; (b) Smith, B. D.; Goodenough-Lashua, D. M.; D'Souza, C. J. E.; Norton, K. J.; Schmidt, L. M.; Tung, J. C. *Tetrahedron Lett.* **2004**, *45*, 2747; (c) Pontes, R. M.; Basso, E. A.; dos Santos, F. P. *J. Org. Chem.* **2007**, *72*, 1901.
20. Crystallographic data for compound **12** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication, no CCDC 1401837. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or via www.ccdc.cam.ac.uk/data_request/cif).