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Triazolopeptides: chirospecific synthesis and *cis/trans* prolyl ratios of structural isomers

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Abstract—As *cis/trans* prolyl isomerization plays a crucial role in various biological processes, peptide mimics capable of modifying the *cis/trans* Xaa-Pro ratio are of particular interest. A practical approach toward proline derived triazolopeptides employing [3+2] azide–alkyne cycloadditions as the key reaction step and the analysis of their *cis/trans* prolyl ratios are reported. Structural investigations indicated the adjustability of both the cis-percentage and the conformational stability toward intramolecular H-bonding effects.

1. Introduction

Representing the only naturally occurring cyclic amino acid, proline displays properties distinct from those of open chain amino acids, when amide cis/trans isomerization deserves particular attention.[1,2] While engineering a number of mutant proteins to contain artificial proline analogs, Lummis et al. revealed very recently that cis/trans isomerization of a Pro in a hinge position of the 5-HT3 receptor elicits opening of an ion channel.[3] In fact, mutants bearing Pro mimetics with a high prevalence for the cis conformer proved constitutively active, while amino acids with very low cis/trans ratios rendered the receptor inactive. The importance of cis/trans isomerization for molecular recognition has also been shown by introduction of unnatural proline derivatives with an increased cis prevalence into a cyclic HIV-1 V3 loop analog. This loop has a common Gly-Pro-Gly-Arg motif, representing a type II β-turn, which is supposed to switch into a type VI β-turn, demanding a *cis*-proline peptide bond, as the key-step before getting the HIV-1 infective.[4] These findings underline the importance of molecular tools for a fine tuning of the *cis/trans* prolyl energy level ratio for the elucidation of biological systems.

Such an adjustment has been commonly accomplished by modifying the pyrrolidine moiety employing ring size variations,[5] sterically demanding substituents[6,7] as well as introduction of fluorine[8,9] or by introducing bridging elements to furnish Freidinger-type lactams.[10–13] Just few employing click chemistry, we herein report on the fine tuning of *cis/trans* prolyl ratios by bioisosteric replacement of the backbone amide group with a 1,2,3-triazole based moiety.[14,15]

In contrast to the established methods, this strategy leaves the geometric properties of the pyrrolidine moiety unmodified, which can be of particular interest if alterations on the sterical demand of the heterocyclic residue are considered unfavorable for the desired application.

[3+2] Azide–alkyne cycloadditions have been applied to various research areas, including drug discovery processes, bioconjugate chemistry, and solid phase organic synthesis.[16–19] Very recently, copper-promoted [3+2] cycloaddition reactions between amino acid derived building blocks gave rise to different '1,4' linked peptide mimetics that we call triazolopeptides. In principal, four types of triazolopeptides can be differentiated that we refer to as 1,4- and 1,5-triazolopeptides as well as 4,1- and 5,1-linked derivatives (Chart 1).

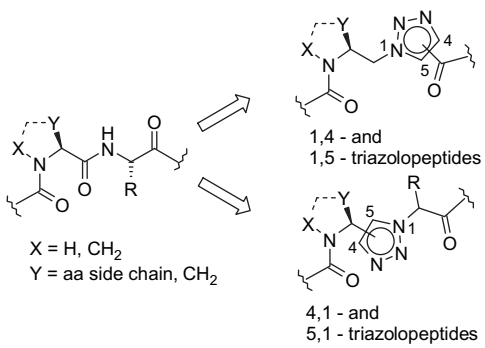

Chart 1.

Keywords: Triazolopeptide; *cis/trans* Prolyl isomerization; Click chemistry; Azide–alkyne cycloaddition.
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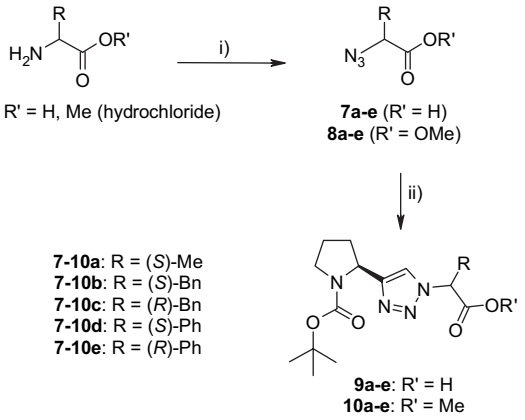
The sequence of the numbering that we use here corresponds to the substitution pattern of the triazole moiety, following the direction of the backbone (N to C terminal).[20–23]

2. Results and discussion

In this paper, we describe a divergent synthetic approach to all four subtypes and their impact on cis/trans isomerization when natural proline was employed as the common chiral building block. Taking advantage of previously described protocols,[24–27] commercially available *N*-Boc protected prolinol (**1**) was *O*-activated and subsequently reacted with sodium azide to give the azidomethylpyrrolidine **2** (Scheme 1). On the other hand, Swern oxidation of **1** with SO_3/pyridine and DMSO yielded the carbaldehyde **3** that could be transformed into the dibromo-substituted alkene **4** by Corey–Fuchs olefination. Subsequent treatment with 2 equiv of *n*-BuLi gave the pyrrolidinylacetylene **5**.

With the aim to understand the influence of the mode of triazole linkage on *cis*/*trans* prolyl ratios, we envisioned to transform the cyclization precursors **2** and **5** into the triazole-linked Pro-Gly mimetics **6a**,**b** and **6c**,**d**, respectively. In detail, azide **2** was reacted with ethyl propiolate when [3+2] azide–alkyne cycloadditions were carried out using both classical and Cu^I catalyzed conditions.[14,28] While Huisgen's 1,3-dipolar cycloaddition gave rise to an easily separable mixture of the 1,4- and 1,5-triazoles **6a** and **6b** in a 3:7 ratio, the copper assisted variant reported by Meldal and Sharpless exclusively yielded the protected 1,4-triazolopeptide **6a**. To approach to the 4,1- and 5,1-subtypes, the proline derived alkyne **5** was reacted with azidoacetic acid ethyl ester,[29] which was freshly prepared from ethyl bromoacetate and sodium azide. As expected, complete regiocontrol was observed under Cu^I catalysis resulting in the formation of the 4,1-substituted congener **6c** while heating in ethyl acetate afforded a 3:1 ratio of the regioisomers **6c** and **6d**.

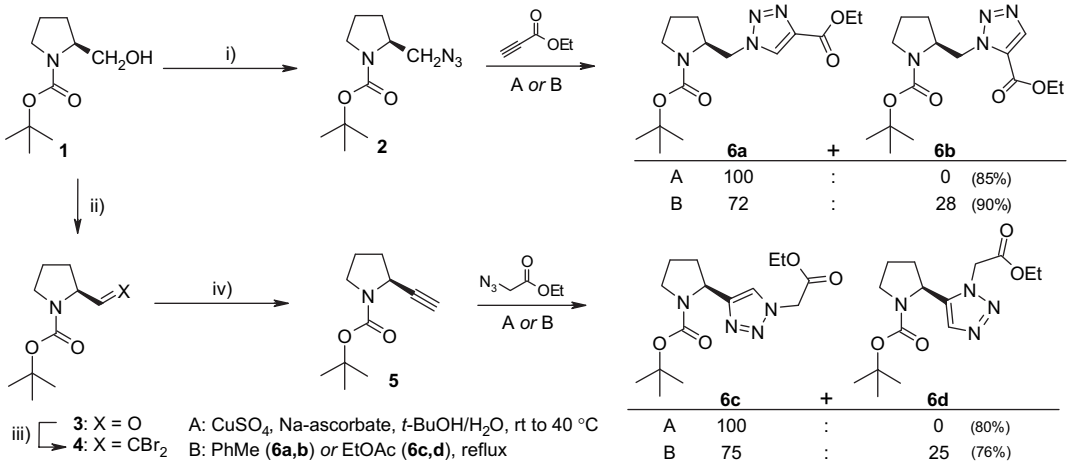
To evaluate a general access to triazole-linked Pro-Xaa mimetics, we intended an introduction of representative chiral α-amino acids into triazolopeptides of type 4,1. Therefore, (*S*)-alanine, (*S*)- and (*R*)-phenylalanine, and both enantiomers of phenylglycine as an amino acid, which is highly prone to racemization were reacted with trifluoromethylsulfonic azide and $CuSO_4$ to yield the respective azido acids **7a**–**e** (Scheme 2).[30] Employing analogous conditions, diazo transfer was also done starting from the corresponding methyl ester hydrochlorides to give rise to azido acid esters **8a**–**e**.



Scheme 2. (i) (1) **7a**–**e**: $CuSO_4 \cdot 5H_2O$, H_2O/MeOH, K_2CO_3, TfN_3, 12 h (62–86%); (2) **8a**–**e**:$CuSO_4 \cdot 5H_2O$, MeOH, DIPEA, TfN_3, 2.5–15 h, (71–91%); (ii) **5**, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, *t*-BuOH/H_2O, rt or 40 °C, 1–3 d (36–92%).

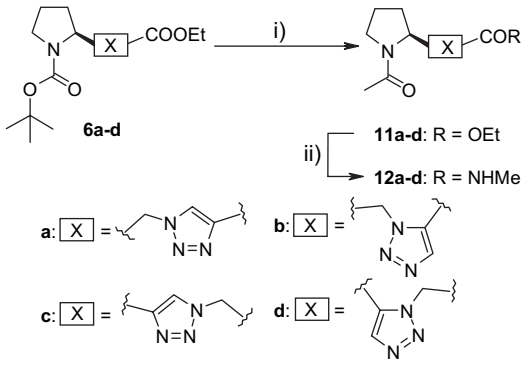
While the synthesis of building blocks **7a**–**e** and **8a**–**c** proceeded straightforward, the Phg derivatives **8d**,**e** displayed partial racemization, as detected by HPLC on a chiral column. However, pure enantiomers **8d** and **8e** could be prepared by esterification of **7d** and **7e**, respectively, using thionyl chloride in methanol.

Copper-promoted [3+2] azide–alkyne cycloaddition reactions of the azido acids **7a**–**e** and the azido esters **8a**–**e** with the central alkyne **5** proceeded smoothly resulting in the formation of the triazoles **9a**–**e** and **10a**–**e**, respectively. When starting from the phenylglycine derivatives **8d**,**e**, which are expectedly prone to epimerization,[31] room temperature proved to be necessary to diminish epimerization.



Scheme 1. (i) (1) MsCl, DIPEA, CH_2Cl_2, 0 °C to rt, 2.5 h (86%); (2) NaN_3, DMF, 70 °C, 24 h (83%); (ii) SO_3/pyridine, DIPEA, DMSO/CH_2Cl_2, −5 °C, 5 h (92% crude); (iii) PPh_3, CBr_4, CH_2Cl_2, −5 to 0 °C, 2 h (90%); (iv) *n*-BuLi, THF, −78 °C, 1 h 15 min (83%).

Exchange of the Boc protecting groups of the four isomeric triazolopeptides **6a–d** with acetyl groups gave rise to model Pro-Gly surrogates more ideally mimicking a *cis*/*trans* prolyl isomerization when treated with TFA and subsequent acetylation with acetyl chloride and DIPEA yielded *N*-acetyl analogs **11a–d** (Scheme 3).



Scheme 3. (i) (1) TFA, CH_2Cl_2, 0 °C, 35 min; (2) AcCl, DIPEA, CH_2Cl_2, rt, 19 h (23–88%; two steps); (ii) $MeNH_2$, EtOH, 0 °C, 2 h (18–86%).

To investigate the influence of intramolecular hydrogen bonding on the conformational behavior of our test set, the ethyl esters **11a–d** were converted to the *N*-methyl amides **12a–d** by treatment with methylamine.

The conformational properties of the model triazolopeptides **11a–d** and **12a–d** were examined by means of NMR spectroscopy in 2 mM $CDCl_3$ solution. The cis/trans ratios were determined on the basis of the triazole protons, which exhibited clearly separated signals for the two conformers in each case. NOESY experiments were done with the peptide mimetics **11a–d** and **12a** in order to unambiguously assign signal sets to particular isomers by comparing dipolar couplings of the acetyl methyl group with the C^2H or C^5H_2 protons of the pyrrolidine moiety. *N*-Acetylprolylglycine methyl ester and *N*-acetylprolylglycine *N*′-methyl amide were employed as reference peptides (Table 1).[32]

Table 1. NMR-derived *cis*/*trans* prolyl ratios of the model peptide surrogates **11a–d** and **12a–d** (2 mM, $CDCl_3$) compared to AcProGlyOMe and AcProGlyNHMe,[32] respectively (5% steps)

Cpd (type)	% cis	Cpd (type)	% cis
11a (1,4)	5	**12a** (1,4)	<1
11b (1,5)	30	**12b** (1,5)	15
11c (4,1)	30	**12c** (4,1)	30
11d (5,1)	10	**12d** (5,1)	5
AcProGlyOMe	10	AcProGlyNHMe	<1

NMR data indicated a 1:9 *cis*/*trans* prolyl ratio for the 5,1-triazolopeptide **11d**, which is very similar to *N*-acetylprolylglycine methyl ester.[32] Investigation of the 4,1-regioisomer **11c** showed a substantially higher cis-fraction of 30%. Thus, the homologization of the backbone led to an increase of the *cis*-isomer. Interestingly, the NMR spectra indicated an inverse behavior for the triazolopeptides **11a**,**b** when the 1,4-regioisomer with an elongated backbone displayed a low tendency to form a *cis* prolyl structure (**11a**: 5% cis) whereas the 1,5-isomer **11b** existed as 3:7 mixture of *cis*/*trans* isomers.

The additional amide NH function of *N*-acetylprolylglycine *N*′-methyl amide facilitates formation of an intramolecular hydrogen bond toward the acetyl carbonyl group. A β-turn can be adopted if the peptide bond between the amino acid in position *i* and proline in position *i*+1 displays trans geometry. As a consequence, almost exclusive formation of the *trans* prolyl isomer was observed.[32] Interestingly, substantial decrease of the cis-population was observed for the 1,5 and 5,1-triazolopeptides **12b**,**d** incorporating an NH that can form an intramolecular 10-membered ring. On the other hand the 1,4- and 4,1-linked isomers **12a**,**c** could only form an 11-membered ring, which is obviously less favored. As a consequence, introduction of an amide functionality did not significantly change the cis/trans ratio. FTIR spectroscopy of a 2 mM $CDCl_3$ solution clearly corroborated our observation revealing strong absorption bands at 3330–3350 cm^{-1} (besides absorptions at 3430–3455 cm^{-1}) for **12b**,**d** and the reference carboxamide clearly indicating an equilibrium between an intramolecular hydrogen bond and a non-associated conformation. Molecular origins for the distinct conformational behavior of our triazolopeptides might be attractive or repulsive dipolar interactions between the carbonyl group of the *N*-acetyl substituent and the triazole moiety.[33]

3. Conclusion

In conclusion, we applied [3+2] azide–alkyne cycloaddition reactions for the synthesis of four different types of triazolopeptides mimicking a Pro-Gly dipeptide. While 1,4- and 4,1-triazolopeptides were regioselectively accessible employing a Cu^I catalyzed protocol, the 1,5- and 5,1-linked subtypes resulted from separation of a mixture of regioisomers. A regiocontrolled approach toward 1,5- and 5,1-triazolopeptides employing ruthenium-catalyzed methodology appears very promising.[34] Structural studies indicated the adjustability of both the cis-percentage and the conformational stability toward intramolecular H-bonding effects. Investigations toward the application of triazolopeptides on the development of neurotensin analogs and TetR-based artificial transactivators are currently ongoing in our laboratory.

4. Experimental

4.1. General

Although we did not observe problems associated with a putatively explosive character of the used azides, the low weight azidoacetic acid ethyl ester was not distilled but used as a solution. Reagents and solvents were obtained from commercial sources unless stated otherwise, and were used as received. Unless otherwise noted, reactions were conducted without inert atmosphere. Evaporations of final product solutions were done under vacuo with a rotatory evaporator. Reaction temperatures were measured externally. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm), visualized by UV light, iodine and/or ninhydrin solution. Flash chromatography was carried out with 230–400 mesh silica gel and 0.8–1.0 bar nitrogen pressure. EIMS was carried out using EI ionization (70 eV) with solid inlet on a Finnigan MAT TSQ 70 or

a Jeol GCmate II spectrometer. HRMS was carried out with a resolution of $M/\Delta M = 5000$ relative to PFK on a Jeol GCmate II spectrometer. Standard NMR spectra were recorded at 300 K on a Bruker Avance 600 (^{1}H at 600 MHz, ^{13}C at 150 MHz) or a Bruker Avance 360 (^{1}H at 360 MHz, ^{13}C at 90 MHz). Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: 7.26, $CDCl_3$: 77.0). Elemental analyses were performed at the Institute of Organic Chemistry (Analytical Departments) of the Friedrich Alexander University, Erlangen-Nürnberg. Melting points were determined with a Büchi 510 apparatus and are uncorrected. HPLC analysis were run on a Agilent 1100 Series with a Diode Array Detector at 190, 230 and 250 nm. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. IR spectra were measured on a Jasco 410 FTIR spectrometer.

4.2. Azidoacetic acid ethyl ester

Bromoacetic acid ethyl ester (3.34 g, 2.22 mL, 20.0 mmol) and NaN_3 (2.60 g, 40.0 mmol) were dissolved in DMF (15 mL), heated to 60 °C, and stirred for 2 h. After cooling to room temperature, the solution was diluted with water (100 mL), extracted with ethyl acetate (4×10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL). This layer was re-extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with brine (50 mL), and the aqueous layer was re-extracted with ethyl acetate (2×10 mL). The combined organic layers were dried with Na_2SO_4, and the resulting solution was diluted to 100 mL, stored over molecular sieves under nitrogen, and was used for cycloaddition reactions without further purification.

4.3. General procedure for the diazo transfer on amino acid methyl ester hydrochlorides

The hydrochlorides of the amino acid methyl esters (approx. 16.0 mmol) were dissolved in methanol (12 mL). An aqueous solution of $CuSO_4 \cdot 5H_2O$ (10 g/L, approx. 0.01 equiv) and diisopropylethylamine (1.5 equiv) were added at room temperature, whereupon the color of the solution changed from turquoise to deep blue. Subsequently, a freshly prepared trifluoromethane sulfonic azide solution (1 M in CH_2Cl_2, 1.8 equiv) was added. After stirring for 15 h, the solvent was removed, and the crude product was purified either by Kugelrohr distillation under reduced pressure (**8a**) or by flash column chromatography (**8b**,**c**). TLC detection was done with a PPh_3/hexanes solution (1% m/v) followed by ninhydrin.

4.3.1. (*S*)-2-Azidopropionic acid methyl ester (8a). Following the general procedure, alanine methyl ester hydrochloride was converted to **8a** (90%) as a colorless oil. R_f 0.46 (hexanes/ethyl acetate 4:1); $[\alpha]_D^{25}$ 12.2 (*c* 1.0, $CHCl_3$); IR (Neat) 2958, 2138, 2108, 1748 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.49 (d, 3H, *J*=7.7 Hz, CH*CH*$_3$), 3.80 (s, 3H, OCH_3), 3.96 (q, 1H, *J*=7.7 Hz, *CH*CH$_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 16.8 (CH_3), 52.6 (OCH_3), 57.3 (CH), 171.4 (C=O).

4.3.2. (*S*)-2-Azido-3-phenylpropionic acid methyl ester (8b). Following the general procedure, (*S*)-phenylalanine methyl ester hydrochloride was converted to **8b** (91%) as a colorless oil. R_f 0.63 (CH_2Cl_2); $[\alpha]_D^{28}$ −47.8 (*c* 1.0, $CHCl_3$); IR (Neat) 3030, 2954, 2109, 1746 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.01 (dd, 1H, *J*=14.0, 8.7 Hz, CH_2), 3.18 (dd, 1H, *J*=14.0, 5.4 Hz, CH_2), 3.77 (s, 3H, OCH_3), 4.07 (dd, 1H, *J*=8.7, 5.4 Hz, CH), 7.19–7.36 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 37.6 (CH_2), 52.6 (OCH_3), 63.3 (CH), 127.3+128.7+129.2 (aryl CH), 135.9 (aryl *C*–C), 170.4 (C=O); EIMS 162 (M−HN_3), 118 (M−N_2−COOMe), 91 (Bn^+); M^+ not detected.

4.3.3. (*R*)-2-Azido-3-phenylpropionic acid methyl ester (8c). *Following the general procedure*, (*R*)-phenylalanine methyl ester hydrochloride was converted to **8c** (85%). $[\alpha]_D^{28}$ 48.5 (*c* 1.0, $CHCl_3$).

4.4. Esterification of phenylglycine derivates

4.4.1. (*S*)-2-Azido-2-phenylacetic acid methyl ester (8d). Methanol (10.0 mL) was cooled to −10 °C, whereupon thionyl chloride (1.09 mL, 15.0 mmol) was added dropwise over 5 min while stirring vigorously. Subsequently, a solution of **7d** (895 mg, 5.05 mmol) in methanol (10 mL) was added over 2 min and the mixture was allowed to warm to room temperature. After 6 h 50 min, ice (30 g) and water (80 mL) were added and the pH was adjusted to 8 with a NaH_2PO_4 buffer. The mixture was extracted with ether (4×20 mL), the combined organic layers were dried with $MgSO_4$, concentrated, and the crude product (862 mg, 89%) was used without further purification, as a HPLC analysis indicated sufficient purity. Colorless oil; R_f 0.46 (hexanes/ethyl acetate 4:1); TLC detection was done with a PPh_3/hexanes solution (1% m/v) followed by ninhydrin; HPLC: t_R 19.5 min, >99% ee (Chiralcel® OD, 4.6× 250 mm, hexanes/isopropanol 99:1, 0.5 mL/min, 254 nm detection); $[\alpha]_D^{22}$ 157.5 (*c* 1.0, $CHCl_3$); IR (Neat) 2955, 2844, 2105, 1746 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.78 (s, 3H, OCH_3), 4.98 (s, 1H, CH), 7.33–7.49 (m, 5H, ArH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 52.9 (OCH_3), 65.3 (CH), 127.6+129.1+129.3 (aryl CH), 133.8 (aryl C–C), 196.6 (C=O); EIMS 191 (M^+).

4.4.2. (*R*)-2-Azido-2-phenylacetic acid methyl ester (8e). Compound **7e** was reacted under the conditions described above, furnishing **8e** (88%) as a colorless oil. HPLC: t_R 17.9 min, >99% ee (Chiralcel® OD, 4.6×250 mm, hexanes/isopropanol 99:1, 0.5 mL/min, 254 nm detection); $[\alpha]_D^{22}$ −159.2 (*c* 1.0, $CHCl_3$).

4.5. (*S*)-1-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl-methyl)-(1,2,3)-triazole-4-carboxylic acid ethyl ester (6a) and (*S*)-1-(1-*tert*-Butoxycarbonylpyrrolidin-2-ylmethyl)-(1,2,3)-triazole-5-carboxylic acid ethyl ester (6b)

To a solution of **2** (3.92 g, 17.3 mmol) in toluene (60 mL) was added ethyl propiolate (5.26 mL, 51.9 mmol). The mixture was heated to reflux for 19 h, whereupon another portion of ethyl propiolate (1.75 mL, 17.3 mmol) was added and refluxing was continued for 5 h. After cooling to room temperature, the solvent was removed and the residue was purified by flash column chromatography (hexanes/ethyl acetate 9:1 increasing to 1:1), furnishing 3.68 g (66%) of **6a** and 1.40 g (25%) of **6b** as a waxy solid and viscous oil, respectively.

4.5.1. Compound 6a. Mp 86–88 °C, R_f 0.20 (hexanes/ethyl acetate 1:1); $[\alpha]_D^{22}$ −68.6 (*c* 1.0, $CHCl_3$); IR (Neat) 3132, 2978, 1739, 1723, 1693 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.410+1.415 (2×t, 3H, *J*=7.1 Hz, CH_2CH_3), 1.50 (s, 9H, Boc CH_3), 1.59–2.10 (br m, 4H, CH_2), 3.00–3.53+3.29 (br m+dd, 2H, *J*=14.2, 6.0 Hz, NCH_2), 4.12 (br s, 1H, CH), 4.36–4.85+4.42+4.52 +4.75 (br m+q+q+dd, 4H, *J*=7.1 Hz+7.1 Hz+12.8, 5.4 Hz, OCH_2+$CHCH_2$-triazole), 8.00+8.04 (br s+s, 1H, triazole H); EIMS 324 (M^+).

4.5.2. Compound 6b. R_f 0.36 (hexanes/ethyl acetate 1:1); $[\alpha]_D^{22}$ −8.8 (*c* 1.0, $CHCl_3$); IR (Neat) 3132, 2977, 1731, 1696 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.3–1.43+1.38 (br s+t, 12H, *J*=6.9 Hz, Boc CH_3+CH_2CH_3), 1.61–1.98 (br m, 4H, CH_2), 3.22–3.49 (br m, 2H, NCH_2), 4.28–4.57+4.38 (m+br q, 3H, *J*=6.9 Hz, CH+CH_2CH_3), 4.67–4.90+4.74 (m+dd, 2H, *J*=13.5, 5.6 Hz, $CHCH_2$-triazole), 8.09 (s, 1H, triazole H); EIMS 324 (M^+).

4.6. (*S*)-1-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl-methyl)-(1,2,3)-triazole-4-carboxylic acid ethyl ester (6a)

Compound **2** (226 mg, 1.00 mmol) and ethyl propiolate (122 μL, 1.20 mmol) were dissolved in *tert*-butanol. Aqueous solutions of $CuSO_4 \cdot 5H_2O$ (1 M, 20 μL) and sodium ascorbate (1 M, 100 μL) were added. After stirring at room temperature for 1 d, water (50 mL) was added and the mixture was extracted with ethyl acetate (3×20 mL). The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$, concentrated, and the residue was purified by flash column chromatography (hexanes/ethyl acetate 4:1), furnishing 276 mg (85%) of **6a**.

4.7. (*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid ethyl ester (6c) and (*S*)-2-[5-(1-*tert*-butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid ethyl ester (6d)

Compound **5** (195 mg, 1.00 mmol) was dissolved in a solution of azidoacetic acid ethyl ester (approx. 0.2 M in ethyl acetate, 7.5 mL, approx. 1.50 mmol) and heated to reflux for 48 h. After cooling to room temperature, the solvent was removed and the mixture of regioisomers was obtained by flash column chromatography (hexanes/ethyl acetate 4:1), furnishing 248 mg (76%) of a colorless oil that was used in the next step without further separation. R_f 0.15 (hexanes/ethyl acetate 1:1).

4.8. General procedure for cycloaddition reactions with 5 under catalytic conditions (6c, 9a–e, 10a–e)

Compound **5** (50–200 mg) and the corresponding azide (1.0–1.2 equiv; slight excesses with respect to **5** resulted in higher yields) were dissolved in *tert*-butanol (1–2 mL). After addition of aqueous solutions of $CuSO_4 \cdot 5H_2O$ (1 M, 0.02 equiv) and sodium ascorbate (1 M, 0.1 equiv), water (0.5–1 mL) was added. In cases where addition of water caused solubility issues, the latter was replaced by methanol. The mixture was stirred either at room temperature or at 40 °C until TLC indicated either stagnation or completion of the reaction, which was the case after 1–4 d. In case of **10d** and **10e**, maintaining room temperature and interrupting the reaction after 20 h were crucial for suppression of the epimerization rate. The mixtures were diluted with water (50 mL). In case of **9a–e**, the pH was adjusted to 2–3 by addition of aqueous HCl. The compounds were isolated by extraction with CH_2Cl_2 or ethyl acetate (4×20 mL), washed with brine (20 mL), re-extraction of the washing liquid (20 mL), drying of the combined organic layers with $NaSO_4$ or $MgSO_4$, evaporation, and flash column chromatography.

4.8.1. (*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid ethyl ester (6c). Compound **5** and azidoacetic acid ethyl ester in ethyl acetate were reacted at 40 °C following the general procedure (reaction time: 38 h). Flash column chromatography (hexanes/ethyl acetate 3:2) furnished **6c** (79%) as a colorless solid. Mp 75–76 °C; R_f 0.21 (hexanes/ethyl acetate 3:2); $[\alpha]_D^{29}$ −59.3 (*c* 1.0, methanol); IR (Neat) 3141, 2977, 2270, 1756, 1691 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.29 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 1.36+1.43 (2×br s, 9H, Boc CH_3), 1.83–2.56 (br m, 4H, CH_2), 3.30–3.63 (br m, 2H, NCH_2), 4.25 (q, 2H, *J*=7.1 Hz, CH_2CH3), 4.95–5.19 (br m, 3H, CH_2CO+NCH), 7.45+7.61 (br s+br s, 1H, triazole H); EIMS 324 (M^+).

4.8.2. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]propionic acid (9a). Compounds **5** and **7a** were reacted at 40 °C following the general procedure (reaction time: 48 h). Flash column chromatography (CH_2Cl_2/methanol/HCOOH 97:3:0.5) furnished **9a** (76%) as a colorless solid. Mp 58–62 °C; R_f 0.13 (CH_2Cl_2/methanol/HCOOH 95:5:0.5); $[\alpha]_D^{26}$ −47.5 (*c* 1.0, methanol); IR (Neat) 3481, 3142, 2977, 2881, 2484, 2249, 1743, 1687 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.16–1.56 (2×br s, 9H, Boc CH_3), 1.72–2.48+1.82 (br m+d, 7H, *J*=6.6 Hz, CH_2+$CHCH_3$), 3.35–3.63 (br m, 2H, NCH_2), 5.05 (dd, 1H, *J*=7.4, 2.4 Hz, NCH), 5.42 (br s, 1H, CH_3CH), 6.00 (br s, 1H, OH), 7.67 (br s, 1H, triazole H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 18.3+18.6 (CH_3), 23.4+24.4 (CH_2), 28.5 (Boc CH_3), 31.4+33.3 (CH_2), 46.5+47.0, 53.0+54.0, 58.4 (2×CH+CH_2), 80.5 (Boc C^q), 120.8+121.9, 149.1+150.9, 155.1 (2×triazole C+Boc C=O), 171.2 (COOH); EIMS 310 (M^+); Anal. Calcd for $C_{14}H_{22}N_4O_4 \cdot 0.25H_2O$: C, 53.41; H, 7.20; N, 17.79. Found: C, 53.41; H, 7.06; N, 17.89 (the percentage of H_2O was corroborated by ^1H NMR spectroscopy).

4.8.3. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-3-phenylpropionic acid (9b). Compounds **5** and **7b** were reacted at 40 °C following the general procedure (reaction time: 48 h). Flash column chromatography (CH_2Cl_2/methanol/HCOOH 97:3:0.5) furnished **9b** (73%) as a colorless solid. Mp 75–78 °C; R_f 0.18 (CH_2Cl_2/methanol/HCOOH 95:5:0.5); $[\alpha]_D^{26}$ −139.0 (*c* 1.0, methanol); IR (Neat) 3469, 3148, 2977, 2880, 2511, 2250, 1740, 1689, 1640 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.13–1.56 (2×br s, 9H, Boc CH_3), 1.72–2.40 (br m, 4H, CH_2), 3.30–3.59+3.53 (br m+dd, 2H, *J*=13.5, 5.6 Hz, NCH_2+$PhCH_2$), 5.01 (br d, 1H, *J*=5.5 Hz, NCH), 5.41–6.00 (br m, 2H, NCHCO+OH), 7.02 (br s, 2H, ArH), 7.15–7.23 (m, 3H, ArH), 7.58+7.69

(br s+br s, 1H, triazole H); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 23.1+24.5 (CH_2), 28.4+28.6 (Boc CH_3), 31.0+33.4, 39.0+39.1 (CH_2), 46.3+46.9, 52.8+54.1, 64.0+64.2 (NCH_2+2×NCH), 80.4+80.8 (Boc C^q), 121.6+122.9, 127.4+127.6, 128.8, 129.1, 135.2+135.3 (5×aryl C), 148.6+150.6, 154.9+155.3 (aryl C+Boc C=O), 169.7 (COOH); EIMS 386 (M^+); Anal. Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.50. Found: C, 61.93; H, 6.60; N, 14.40.

4.8.4. (2*R*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-3-phenylpropionic acid (9c). Compounds **5** and **7c** were reacted at 40 °C following the general procedure (reaction time: 48 h). Flash column chromatography (CH_2Cl_2/methanol/HCOOH 97:3:0.5) furnished **9c** (87%) as a colorless solid. Mp 72–75 °C; R_f 0.15 (CH_2Cl_2/methanol/HCOOH 95:5:0.5); $[\alpha]_D^{26}$ 14.7 (*c* 1.0, methanol); IR (Neat) 3474, 3138, 2978, 2880, 2512, 2249, 1740, 1689, 1640 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.23–1.48 (2×br s, 9H, Boc CH_3), 1.61–2.27 (br m, 4H, CH_2), 3.23–3.61+3.47+3.54 (br m+dd+dd, 4H, *J*=14.2, 8.3 Hz+14.2, 5.9 Hz, NCH_2+$PhCH_2$), 5.06 (dd, 1H, *J*=5.7, 3.9 Hz, NCH), 5.44–5.99 (br m, 2H, NCHCO+OH), 6.92–7.02 (br m, 2H, ArH), 7.12–7.26 (m, 3H, ArH), 7.48 (br s, 0.4H, triazole H), 7.93 (br s, 0.6H, triazole H); H/D exchange: 5.55 (br m, 1H, NCHCO); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 22.7+24.0 (CH_2), 28.6 (Boc CH_3), 32.2+33.3, 39.3+39.5 (CH_2), 46.3+47.0, 53.0+54.4, 64.1+64.3 (NCH_2+2×NCH), 80.7+80.9 (Boc C^q), 121.3+122.7, 127.5 (s), 128.7 (w), 128.9 (s), 129.2 (w), 135.3+135.3 (5×aryl C), 148.9+150.7, 155.0+155.8 (aryl C+Boc C=O), 169.9+170.0 (COOH); EIMS 386 (M^+); Anal. Calcd for $C_{20}H_{26}N_4O_4\cdot0.25H_2O$: C, 61.44; H, 6.83; N, 14.33. Found: C, 61.57; H, 6.62; N, 14.48 (the percentage of H_2O was corroborated by ^{1}H NMR spectroscopy).

4.8.5. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-2-phenylacetic acid (9d). Compounds **5** and **7d** were reacted at 40 °C following the general procedure (reaction time: 48 h). Flash column chromatography (CH_2Cl_2/methanol/HCOOH 97:3:0.5) furnished **9d** (92%) as a colorless solid. Mp 77–78 °C; R_f 0.21 (CH_2Cl_2/methanol/HCOOH 95:5:0.5); $[\alpha]_D^{26}$ 35.6 (*c* 1.0, methanol); IR (Neat) 3469, 3155, 2977, 2880, 2555, 2501, 2250, 1742, 1687, 1638 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$; rotamers were observed) δ 1.14–1.49 (2×br s, 9H, Boc CH_3), 1.68–1.95 (br m, 1.7H, CH_2), 2.01–2.29 (br m, 2H, CH_2), 2.34–2.49 (br m, 0.3H, CH_2), 3.36+3.38 (dd+dd, 2H, *J*=16.6, 5.5 Hz+16.6, 10.0 Hz, NCH_2), 4.99 (br d, 1H, *J*=5.7 Hz, NCH), 5.50–6.46 (br s, 1H, OH), 6.47–6.60 (br m, 1H, NCHCO), 7.32–7.48 (m, 5H, ArH), 7.79+7.86 (br s+br s, 1H, triazole H); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 23.4+24.5 (CH_2), 28.3+28.4 (Boc CH_3), 30.8+33.2, 46.4+46.8, 52.9+53.9, 66.3+66.4 (2×CH_2+2×NCH), 80.5+80.9 (Boc C^q), 121.5+122.4, 123.2, 128.1–128.4, 129.3–129.7, 133.8+134.0, 150.5, 154.9+155.4 (6×aryl C+Boc C=O), 169.0 (COOH); EIMS 372 (M^+); Anal. Calcd for $C_{19}H_{24}N_4O_4\cdot0.25H_2O$: C, 60.54; H, 6.55; N, 14.86; Found: C, 60.44; H, 6.40; N, 14.73 (the percentage of H_2O was corroborated by ^{1}H NMR spectroscopy).

4.8.6. (2*R*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-2-phenylacetic acid (9e). Compounds **5** and **7e** were reacted at 40 °C following the general procedure (reaction time: 48 h). Flash column chromatography (CH_2Cl_2/methanol/HCOOH 97:3:0.5) furnished **9e** (81%) as a colorless solid. Mp 74–76 °C; R_f 0.10 (CH_2Cl_2/methanol/HCOOH 95:5:0.5); $[\alpha]_D^{26}$ −117 (*c* 1.0, methanol); IR (Neat) 3155, 2977, 2880, 2555, 2487, 2249, 1738, 1690, 1642 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 0.93–1.50 (2×br s, 9H, Boc CH_3), 1.72–1.96 (br m, 1.8H, CH_2), 2.00–2.38 (br m, 2.2H, CH_2), 3.25–3.71+3.40 (br m+ddd, 2H, *J*=10.5, 8.0, 7.8 Hz, NCH_2), 5.03 (ddd, 1H, *J*=7.4, 2.8, 0.6 Hz, NCH), 5.96 (br s, 1H, OH), 5.55+6.62 (br s+br s, 1H, NCHCO), 7.23–7.49 (br m, 5H, ArH), 8.06 (s, 0.1H, triazole H), 8.25 (br s, 0.9H, triazole H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 23.1 (CH_2), 28.2+28.5 (Boc CH_3), 33.6, 46.7, 54.7, 64.2+66.0 (2×CH_2+2×NCH), 81.0 (Boc C^q), 121.8, 127.5, 129.3, 129.4, 135.0, 151.0, 156.0 (6×aryl C+Boc C=O), 169.0 (COOH); EIMS 372 (M^+); Anal. Calcd for $C_{19}H_{24}N_4O_4\cdot0.25H_2O$: C, 60.54; H, 6.55; N, 14.86. Found: C, 60.74; H, 6.45; N, 14.95 (the percentage of H_2O was corroborated by ^{1}H NMR spectroscopy).

4.8.7. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]propionic acid methyl ester (10a). Compounds **5** and **8a** were reacted at room temperature following the general procedure (reaction time: 4 d). Flash column chromatography (hexanes/ethyl acetate 8:2) furnished **10a** (36%) as a colorless resin. R_f 0.11 (hexanes/ethyl acetate 1:1); $[\alpha]_D^{27}$ −50.6 (*c* 1.0, methanol); IR (Neat) 3136, 2975, 2877, 1753, 1692 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.27–1.50 (2×br s, 9H, Boc CH_3), 1.72–2.60+1.81 (br m+d, 7H, *J*=7.3 Hz, CH_2+$CHCH_3$), 3.32–3.63 (br m, 2H, NCH_2), 3.75 (s, 3H, OCH_3), 5.03 (br s, 1H, NCH), 5.45 (br s, 1H, CH_3CH), 7.49+7.67 (br s+br s, 1H, triazole H); EIMS 324 (M^+); HRMS calculated for $C_{15}H_{24}N_4O_4$: 324.1798, found: 324.1798.

4.8.8. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-3-phenylpropionic acid methyl ester (10b). Compounds **5** and **8b** were reacted at room temperature following the general procedure (reaction time: 4 d). Flash column chromatography (hexanes/ethyl acetate 8:2) furnished **10b** (49%) as a colorless solid. Mp 69–72 °C; R_f 0.26 (hexanes/ethyl acetate 1:1); HPLC: t_R 32.8 min (silica gel 5 μm, 4.6×250 mm, diisopropyl ether/acetonitrile 94:6, 0.5 mL/min, 254 nm detection); $[\alpha]_D^{24}$ −112 (*c* 1.0, methanol); IR (Neat) 3136, 2976, 2876, 2246,1750, 1693 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.26–1.56 (2×br s, 9H, Boc CH_3),1.79–2.50 (br m, 4H, CH_2), 3.31–3.56+3.49 (br m+dd, 4H, *J*=14.1, 6.6 Hz, NCH_2+$PhCH_2$), 3.73 (s, 3H, OCH_3), 4.99 (br s, 1H, NCH), 5.54 (br s, 1H, NCHCO), 6.98–7.07 (m, 2H, ArH), 7.17–7.29 (m, 3H, ArH), 7.36 (br s, 0.6H, triazole H), 7.64 (br s, 0.4H, triazole H); EIMS 400 (M^+); Anal. Calcd for $C_{21}H_{28}N_4O_4$: C, 62.98; H, 7.05; N, 13.99. Found: C, 62.75; H, 7.06; N, 13.96.

4.8.9. (2*R*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-3-phenylpropionic acid methyl ester (10c). Compounds **5** and **8c** were reacted at room temperature following the general procedure (reaction time:

4 d). Flash column chromatography (hexanes/ethyl acetate 8:2) furnished **10c** (81%) as a colorless solid. Mp 97–99 °C; R_f 0.28 (hexanes/ethyl acetate 1:1); HPLC: t_R 34.4 min (silica gel 5 μm, 4.6×250 mm, diisopropyl ether/acetonitrile 94:6, 0.5 mL/min, 254 nm detection); $[\alpha]_D^{24}$ 10.8 (*c* 1.0, methanol); IR (Neat) 3138, 2975, 2877, 2251, 1751, 1692 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.18–1.58 (2×br s, 9H, Boc CH_3), 1.81–2.51 (br m, 4H, CH_2), 3.31–3.56+3.42+3.50 (br m+dd+dd, 4H, *J*=14.1, 8.9 Hz+14.1, 6.6 Hz, NCH_2+PhH_2), 3.72 (s, 3H, OCH_3), 4.99 (br s, 1H, NCH), 5.45 (br s, 1H, NCHCO), 6.99–7.07 (m, 2H, ArH), 7.17–7.28 (m, 3H, ArH), 7.40+7.54 (br s+br s, 1H, triazole H); EIMS 400 (M^+); Anal. Calcd for $C_{21}H_{28}N_4O_4$: C, 62.98; H, 7.05; N, 13.99. Found: C, 62.99; H, 7.07; N, 13.91.

4.8.10. (2*S*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-2-phenylacetic acid methyl ester (10d). Compounds **5** and **8d** were reacted at room temperature following the general procedure (reaction interrupted after 20 h). Flash column chromatography (hexanes/ethyl acetate 7:3) furnished **10d** (26%) as a colorless resin. R_f 0.26 (hexanes/ethyl acetate 1:1); HPLC: t_R 67.6 min, 56% de (silica gel 5 μm, 4.6×250 mm, diisopropyl ether/acetonitrile 99:1, 1.0 mL/min, 254 nm detection); $[\alpha]_D^{22}$ 19.5 (*c* 1.0, methanol); IR (Neat) 3144, 2976, 2875, 1754, 1692 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.02–1.51 (m, 9H, Boc CH_3), 1.80–2.53 (br m, 4H, CH_2), 3.27–3.60 (br m, 2H, NCH_2), 3.83 (s, 3H, OCH_3), 4.85–5.08 (br m, 1H, NCH), 6.53+6.56 (br s+br s, 1H, NCHCO), 7.29–7.76+7.58+7.67 (m+br s+br s, 6H, Ar–H+2×triazole H).

4.8.11. (2*R*,2′*S*)-2-[4-(1-*tert*-Butoxycarbonylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]-2-phenylacetic acid methyl ester (10e). Compounds **5** and **8e** were reacted at room temperature following the general procedure (reaction interrupted after 20 h). Flash column chromatography (hexanes/ethyl acetate 7:3) furnished **10e** (26%) as a colorless resin. R_f 0.26 (hexanes/ethyl acetate 1:1); HPLC: t_R 75.3 min, >98% de (silica gel 5 μm, 4.6×250 mm, diisopropyl ether/acetonitrile 99:1, 1.0 mL/min, 254 nm detection); $[\alpha]_D^{22}$ −108 (*c* 1.0, methanol); IR (Neat) 3144, 2976, 2877, 1754, 1692 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.02–1.51 (m, 9H, Boc CH_3), 1.80–2.53 (br m, 4H, CH_2), 3.27–3.60 (br m, 2H, NCH_2), 3.82+3.83 (2×s, 3H, OCH_3), 4.85–5.08 (br m, 1H, NCH), 6.53+6.56 (br s+br s, 1H, NCHCO), 7.29–7.76+7.58+7.67 (m+br s+br s, 6H, Ar–H+2×triazole H).

4.9. Exchange of the *N*-Boc group by an *N*-acetyl group

4.9.1. (*S*)-2-[4-(1-Acetylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid ethyl ester (11c). Compound **6c** (26.2 mg, 0.081 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and treated with TFA in CH_2Cl_2 (1:1, 1.0 mL) while stirring on an ice bath. After 35 min the mixture was evaporated to dryness. After addition of CH_2Cl_2 (2 mL), the evaporation was repeated and the residue was dried thoroughly. Then CH_2Cl_2 (1.0 mL) and DIPEA (30.0 μL, 0.181 mmol) were added and the mixture was cooled to 0 °C. After addition of acetyl chloride (10.0 μL, 0.123 mmol), the temperature was maintained for 12 min, whereupon the ice bath was removed and the mixture was stirred at room temperature for 17 h. Additional amounts of DIPEA (15.0 μL, 0.090 mmol) and acetyl chloride (15.0 μL, 0.185 mmol) were added. After 1 h 45 min, TLC indicated complete conversion. Aqueous HCl (2 N, 5 mL) was added and the mixture was extracted with CH_2Cl_2 (4×5 mL). The combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, evaporated, and the residue was purified by flash column chromatography (CH_2Cl_2/methanol 95:5), furnishing 18.9 mg (88%) of **11c** as a colorless solid. TLC detection: ninhydrin, 150 °C, 15 min. Mp 80–81 °C; R_f 0.27 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{26}$ −73.1 (*c* 2.0, $CHCl_3$); IR (Neat) 3137, 2270, 1751, 1636 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.29+1.30 (t+t, 3H, *J*=7.2 Hz, CH_2CH_3), 1.83–1.93 (m, 0.33H, $CH_{2,cis}$), 1.93–2.00+1.97 (m+s, 1.33H, $CH_{2,cis}$+acetyl $CH_{3,cis}$), 2.03–2.10+2.04 (m+s, 2.67H, $CH_{2,trans}$+acetyl $CH_{3,trans}$), 2.12–2.22 (m, 1H, CH_2), 2.39–2.42 (m, 1H, CH_2), 2.56–2.62 (m, 0.67H, $CH_{2,trans}$), 3.50 (ddd, 0.67H, *J*=9.5, 9.5, 7.1 Hz, $NCH_{2,trans}$), 3.57 (ddd, 0.33H, *J*=11.8, 10.0, 7.4 Hz, $NCH_{2,cis}$), 3.63 (ddd, 0.67H, *J*=9.5, 8.5, 2.9 Hz, $NCH_{2,trans}$), 3.68 (ddd, 0.33H, *J*=11.8, 8.6, 2.7 Hz, $NCH_{2,cis}$), 4.25+4.26 (q+q, 2H, *J*=7.2 Hz, CH_2CH_3), 5.04 (d, 0.67H, *J*=17.5 Hz, NCH_2CO_{trans}), 5.11 (d, 0.67H, *J*=17.5 Hz, NCH_2CO_{trans}), 5.13 (s, 0.67H, NCH_2CO_{cis}), 5.15 (dd, 0.33H, *J*=7.9, 1.3 Hz, NCH_{cis}), 5.28 (dd, 0.33H, *J*=7.9, 1.6 Hz, NCH_{cis}), 7.42 (s, 0.33H, triazole H_{cis}), 7.69 (s, 0.67H, triazole H_{trans}); ^{13}C NMR (90 MHz, $CHCl_3$; *cis*/*trans* rotamers were observed) δ 14.2 (CH_2CH_3), 22.3+25.1 (acetyl CH_3), 22.6+22.9, 30.2+34.2, 46.2+48.1 (3×CH_2), 50.9+51.1, 52.4+55.4, 62.4+62.7 (2×CH_2+CH), 122.2+124.6, 148.8+150.8 (2×triazole C), 166.2+166.5, 169.4+169.9 (2×C=O); EIMS 266 (M^+); HRMS: calculated for $C_{12}H_{18}N_4O_3$: 266.1379, found: 266.1378.

Starting from **6a**,**b**,**d**, **11a**,**b**,**d**, respectively, were prepared analogously (direct column chromatography, no extraction steps):

4.9.2. (*S*)-1-(1-Acetylpyrrolidin-2-ylmethyl)-(1,2,3)-triazole-4-carboxylic acid ethyl ester (11a). Reaction time: 3.5 h. Flash column chromatography (CH_2Cl_2/methanol 98:2) furnished **11a** (56%) as a colorless solid. TLC detection: ninhydrin, 150 °C, 15 min. Mp 71–72 °C; R_f 0.29 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{27}$ −64.4 (*c* 1.0, $CHCl_3$); IR (Neat) 2975, 1735, 1641 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; approx. 3% of a *cis* rotamer were visible; the *trans* rotamer is described) δ 1.40 (t, 3H, *J*=7.2 Hz, CH_2CH_3), 1.46–1.62 (m, 1H, CH_2), 1.76–1.90 (m, 1H, CH_2), 1.91–2.04 (m, 2H, CH_2), 2.09 (s, 3H, acetyl CH_3), 3.25 (ddd, 1H, *J*=9.9, 7.8, 4.4 Hz, NCH_2), 3.38 (ddd, 1H, *J*=9.9, 7.6, 7.6 Hz, NCH_2), 4.30–4.47+4.42 (m+s, 3H, *J*=7.2 Hz, NCH+CH_2CH_3), 4.63 (dd, 1H, *J*=13.7, 2.8 Hz, NCHCH_2-triazole), 4.75 (dd, 1H, *J*=13.7, 6.6 Hz, NCHCH_2-triazole), 8.07 (s, 1H, triazole H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.5 (CH_2CH_3), 23.1+23.9 (acetyl CH_3+CH_2), 28.1 (CH_2), 48.4, 51.4, 57.1, 61.5 (3×NCH_2+OCH_2), 128.4, 140.6 (2×triazole C), 160.8 (ester C=O), 170.5 (amide C=O); EIMS 266 (M^+); HRMS calculated for $C_{12}H_{18}N_4O_3$: 266.1379, found: 266.1379.

4.9.3. (*S*)-1-(1-Acetylpyrrolidin-2-ylmethyl)-(1,2,3)-triazole-5-carboxylic acid ethyl ester (11b). Reaction time:

1.5 h. Flash column chromatography (CH_2Cl_2/methanol 95:5) furnished **11b** (76%) as a colorless resin. TLC detection: ninhydrin, 150 °C, 15 min. R_f 0.56 (CH_2Cl_2/methanol 9:1); $[\alpha]_D^{27}$ −13.6 (*c* 1.0, $CHCl_3$); IR (Neat) 3122, 2976, 1728, 1648, 1639 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.40 (t, 3H, *J*=7.2 Hz, CH_2CH_3), 1.58–2.07+1.97+1.99 (m+s+s, 7H, CH_2+acetyl $CH_{3,cis}$+acetyl $CH_{3,trans}$), 3.33–3.63+3.38+3.44 (m+ddd+ddd, 2H, *J*=9.8, 9.1, 7.0 Hz+9.8, 8.5, 3.1 Hz), NCH_2CH_2), 4.39+4.40 (q+q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.68–4.76 (m, 1H, NCH), 4.86+4.90 (dd+dd, 2H, *J*=13.7, 5.7 Hz+13.7, 6.4 Hz, $NCHCH_2$-triazole), 8.09 (s, 0.74H, triazole H_{trans}), 8.12 (s, 0.26H, triazole H_{cis}); ^{13}C NMR (150 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 14.3 (CH_2CH_3), 21.7 (w), 21.75 (w), 22.8 (s), 23.9 (s) (acetyl CH_3+CH_2), 27.6+28.7 (CH_2), 45.8+47.8, 51.2+51.3, 55.8+58.1, 62.0+62.3 (3×NCH_2+OCH_2), 128.5+129.0, 137.9+138.0 (2×triazole C), 158.7+158.8 (ester C═O), 169.9+170.1 (amide C═O); EIMS 266 (M^+); HRMS calculated for $C_{12}H_{18}N_4O_3$: 266.1379, found: 266.1379.

4.9.4. (*S*)-2-[5-(1-Acetylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid ethyl ester (11d). The title compound was obtained from a mixture of **6c** and **6d** as obtained from thermal cycloaddition and was separated from **11c** by flash column chromatography (CH_2Cl_2/acetone 7:3) furnishing **11d** (31%) as a colorless resin. TLC detection: ninhydrin, 150 °C, 20 min. R_f 0.27 (CH_2Cl_2/acetone 1:1); $[\alpha]_D^{24}$ 37.2 (*c* 1.0, $CHCl_3$); IR (Neat) 3125, 1748, 1644 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.291+1.297 (t+t, 3H, *J*=7.2 Hz, CH_2CH_3), 1.84–2.44+1.94+2.01 (m+s+s, 7H, CH_2+acetyl $CH_{3,cis}$+acetyl $CH_{3,trans}$), 3.56 (ddd, 1H, *J*=10.0, 7.4, 7.4 Hz, NCH_2), 3.65 (ddd, 1H, *J*=10.0, 7.3, 4.3 Hz, NCH_2), 4.21+4.26 (dq+dq, 2H, *J*=11.0, 7.2 Hz, CH_2CH_3), 4.96 (d, 0.12H, *J*=17.6 Hz, NCH_2CO_{cis}), 5.03 (dd, 1H, *J*=7.4, 3.1 Hz, NCH), 5.29 (d, 0.12H, *J*=17.6 Hz, NCH_2CO_{cis}), 5.42 (d, 0.88H, *J*=17.7 Hz, NCH_2CO_{trans}), 5.69 (d, 0.88H, *J*=17.7 Hz, NCH_2CO_{trans}), 7.48 (s, 0.12H, triazole H_{cis}), 7.54 (s, 0.88H, triazole H_{trans}); ^{13}C NMR (90 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 14.2 (CH_2CH_3), 22.2 (w), 22.4 (w), 22.6 (s), 25.2 (s) (acetyl CH_3+CH_2), 32.3+33.3, 46.2+47.6, 49.4+49.8, 50.2+53.2, 62.3+62.9 (4×CH_2+CH), 131.0+132.6, 140.3 (2×triazole C), 167.5, 169.8 (2×C═O); EIMS 266 (M^+); HRMS: calculated for $C_{12}H_{18}N_4O_3$: 266.1379, found: 266.1379.

4.10. Methylaminolysis of ethyl esters 11a–d

4.10.1. (*S*)-2-[4-(1-Acetylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid *N*-methyl amide (12c). Compound **11c** (31.7 mg, 0.119 mmol) was dissolved in ethanol (1.0 mL) and cooled to 0 °C. After addition of methylamine solution (8 M in ethanol, 0.5 mL), the mixture was stirred for 2 h, whereupon TLC indicated complete conversion. The solvent was removed under reduced pressure, CH_2Cl_2 (2 mL) was added, evaporated to dryness and the residue was purified by flash column chromatography (CH_2Cl_2/methanol 95:5), furnishing 23.0 mg (77%) of **12c** as a colorless solid. TLC detection: ninhydrin, 150 °C, 25 min. Mp 109–112 °C; R_f 0.30 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{26}$ −68.5 (*c* 1.0, $CHCl_3$); IR (Neat) 3293, 3247, 3127, 3085, 1679, 1627 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.87–2.52+1.99+2.07 (m+s+s, 7H, CH_2+acetyl $CH_{3,cis}$+acetyl $CH_{3,trans}$), 2.79 (d, 2.25H, *J*=4.8 Hz, $NCH_{3,trans}$), 2.82 (d, 0.75H, *J*=4.8 Hz, $NCH_{3,cis}$), 3.52 (ddd, 1H, *J*=9.7, 9.4, 6.9 Hz, NCH_2), 3.66 (ddd, 1H, *J*=9.7, 8.0, 3.2 Hz, NCH_2), 4.90 (d, 0.75H, *J*=16.3 Hz, $NCH_2C{=}O_{trans}$), 4.96 (d, 0.75H, *J*=16.3 Hz, $NCH_2C{=}O_{trans}$), 4.90 (d, 0.50H, *J*=0.90 Hz, $NCH_2C{=}O_{cis}$), 5.14 (dd, 0.25H, *J*=7.7, 1.8 Hz, NCH_{cis}), 5.26 (dd, 0.75H, *J*=7.7, 2.4 Hz, NCH_{trans}), 6.34+6.46 (br s+br s, 1H, NH), 7.58 (s, 0.25H, triazole H_{cis}), 7.65 (s, 0.75H, triazole H_{trans}); ^{13}C NMR (150 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 22.4 (w), 22.8 (w), 22.9 (s), 24.9 (s), 26.5+26.6, 30.8+34.1 (2×CH_3+2×CH_2), 46.2+48.1, 52.6 (s), 53.0 (s), 53.1 (w), 55.1 (w) (2×NCH_2+NCH), 122.6+124.5, 149.3+150.5 (2×triazole C), 165.5+165.9, 169.7+170.0 (2×C═O); EIMS 251 (M^+); HRMS calculated for $C_{11}H_{17}N_5O_2$: 251.1382, found: 251.1382.

Starting from **11a**,**b**,**d**, **12a**,**b**,**d**, respectively, were prepared analogously:

4.10.2. (*S*)-1-(1-Acetylpyrrolidin-2-ylmethyl)-(1,2,3)-triazole-4-carboxylic acid *N*-methyl amide (12a). Reaction interrupted after 2 h 25 min. Flash column chromatography (CH_2Cl_2/methanol 95:5) furnished **12a** (19%; 54% of the starting material were recovered) as a colorless solid. TLC detection: ninhydrin, 150 °C, 25 min. Mp 150–151 °C; R_f 0.08 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{26}$ −88.4 (*c* 0.25, $CHCl_3$); IR (Neat) 3330, 2241, 1649, 1578 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.32–1.45 (m, 1H, CH_2), 1.74–2.07 (m, 4H, CH_2), 2.10 (s, 3H, acetyl CH_3), 3.01 (d, 3H, *J*=5.0 Hz, NCH_3), 3.24 (ddd, 1H, *J*=10.0, 7.9, 4.3 Hz, NCH_2), 3.37 (ddd, 1H, *J*=10.0, 7.8, 7.6 Hz, NCH_2), 4.31–4.44 (m, 1H, NCH), 4.61 (dd, 1H, *J*=13.9, 3.0 Hz, $CHCH_2$-triazole), 4.77 (dd, 1H, *J*=13.9, 6.1 Hz, $CHCH_2$-triazole), 7.11 (q, 1H, *J*=5.0 Hz, NH), 8.01 (s, 1H, triazole H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 23.1, 23.9, 25.9, 27.9 (2×CH_3+2×CH_2), 48.4, 51.5, 56.9 (2×NCH_2+NCH), 126.1, 143.7 (2×triazole C), 160.7, 170.5 (2×amide C═O); EIMS 251 (M^+); HRMS calculated for $C_{11}H_{17}N_5O_2$: 251.1382, found: 251.1382.

4.10.3. (*S*)-1-(1-Acetylpyrrolidin-2-ylmethyl)-(1,2,3)-triazole-5-carboxylic acid *N*-methyl amide (12b). Reaction time: 2.5 h. Flash column chromatography (CH_2Cl_2/methanol 95:5) furnished **12b** (64%) as a colorless solid. TLC detection: ninhydrin, 150 °C, 30 min. Mp 176–177 °C; R_f 0.04 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{26}$ 11.9 (*c* 1.0, $CHCl_3$); IR (Neat) 3282, 3120, 2970, 2242, 1670, 1652, 1626, 1627 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.79 (s, 0.66H, acetyl $CH_{3,cis}$), 1.92–2.06+1.98 (m+s, 6.34H, CH_2+acetyl $CH_{3,trans}$), 2.95+2.96 (d+d, 3H, *J*=4.6 Hz, NCH_3), 3.35–3.66 (m, 2H, NCH_2), 4.37–4.47 (m, 0.22H, NCH_{cis}), 4.59–4.81+4.73 (m+dd, 1.78H, *J*=13.3, 7.0 Hz, NCH_{trans}+$CHCH_2$-triazole), 4.87+4.91 (dd+dd, 1H, *J*=13.3, 5.7 Hz+13.3, 6.8 Hz, NCH_{trans}+NCH_{cis}), 7.06 (q, 0.22H, *J*=4.6 Hz, NH_{cis}), 7.54 (q, 0.78H, *J*=4.6 Hz, NH_{trans}), 7.99 (s, 0.22H, triazole H_{cis}), 8.03 (s, 0.78H, triazole H_{trans}); ^{13}C NMR (150 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 21.4 (w), 21.8 (w), 22.6 (s), 23.8 (s), 26.5+26.6, 28.0+28.8 (2×CH_3+2×CH_2), 45.8+47.7, 51.1+51.4, 56.3+58.4 (2×NCH_2+NCH), 131.7+132.2, 133.8+134.6 (2×triazole

C), 158.5+158.7, 170.1+170.5 (2×C=O); EIMS 251 (M^+); HRMS calculated for $C_{11}H_{17}N_5O_2$: 251.1382, found: 251.1383.

4.10.4. (*S*)-2-[5-(1-Acetylpyrrolidin-2-yl)-(1,2,3)-triazol-1-yl]acetic acid *N*-methyl amide (12d). Reaction time: 3.5 h. Flash column chromatography (CH_2Cl_2/methanol 9:1) furnished **12d** (86%) as a colorless solid. TLC detection: ninhydrin, 150 °C, 30 min. Mp 142–144 °C; R_f 0.33 (CH_2Cl_2/methanol 9:1); $[\alpha]_D^{22}$ −50.0 (*c* 0.5, $CHCl_3$); IR (Neat) 3294, 3119, 3086, 2951, 2881, 1684, 1627 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 1.90–2.42+1.92+2.06 (m+s+s, 7H, CH_2+acetyl $CH_{3,cis}$+acetyl $CH_{3,trans}$), 2.81+2.82 (d+d, 3H, J=4.8 Hz, NCH_3), 3.60 (ddd, 1H, J=10.0, 7.4, 7.4 Hz, NCH_2), 3.73 (ddd, 1H, J=10.0, 7.3, 4.8 Hz, NCH_2), 4.91 (d, 0.09H, J=16.5 Hz, $NCH_2C{=}O_{cis}$), 5.04 (d, 0.91H, J=16.5 Hz, $NCH_2C{=}O_{trans}$), 5.09 (dd, 1H, J=8.2, 3.6 Hz, NCH), 5.18 (d, 0.09H, J=16.5 Hz, $NCH_2C{=}O_{cis}$), 5.37 (d, 0.91H, J=16.5 Hz, $NCH_2C{=}O_{trans}$), 6.58 (br s, 0.09H, NH_{cis}), 6.75 (br s, 0.91H, NH_{trans}), 7.47+7.58 (s+s, 1H, triazole H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.7, 24.8, 26.6, 32.4 (2×CH_3+2×CH_2), 47.9, 51.0, 51.5 (2×NCH_2+NCH), 131.2, 140.5 (2×triazole C), 165.9, 169.9 (2×C=O); EIMS 251 (M^+); HRMS calculated for $C_{11}H_{17}N_5O_2$: 251.1382, found: 251.1382.

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Supplementary data

Copies of ^1H and ^{13}C NMR spectra of the target compounds **11a–d** and **12a–d**; copies of NOESY spectra of **11a–d** and **12a**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.07.007.

References and notes

1. Jabs, A.; Weiss, M. S.; Hilgenfeld, R. *J. Mol. Biol.* **1999**, *286*, 291–304.
2. Dugave, C.; Demange, L. *Chem. Rev.* **2003**, *103*, 2475–2532 and references cited therein.
3. Lummis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248–252.
4. Wittelsberger, A.; Keller, M.; Scarpellino, L.; Partiny, L.; Acha-Orbea, H.; Mutter, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 1111–1115; *Angew. Chem.* **2000**, *112*, 1153–1156.
5. Kern, D.; Schutkowski, M.; Drakenberg, T. *J. Am. Chem. Soc.* **1997**, *119*, 8403–8408.
6. Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312–3321.
7. An, S. S. A.; Lester, C. C.; Peng, J.-L.; Li, Y.-J.; Rothwarf, D. M.; Welker, E.; Thannhauser, T. W.; Zhang, L. S.; Tam, J. P.; Scheraga, H. A. *J. Am. Chem. Soc.* **1999**, *121*, 11558–11566.
8. Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777–778.
9. Renner, C.; Alefelder, S.; Bae, J. H.; Budisa, N.; Huber, R.; Moroder, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 923–925.
10. Gramberg, D.; Weber, C.; Beeli, R.; Inglis, J.; Bruns, C.; Robinson, J. A. *Helv. Chim. Acta* **1995**, *78*, 1588–1606.
11. Curran, T. P.; McEnaney, P. M. *Tetrahedron Lett.* **1995**, *36*, 191–194.
12. Kim, K.; Dumas, J.-P.; Germanas, J. P. *J. Org. Chem.* **1996**, *61*, 3138–3144.
13. Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem.* **2001**, *113*, 3465–3468; *Angew. Chem., Int. Ed.* **2001**, *40*, 3361–3364.
14. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; *Angew. Chem.* **2002**, *114*, 2708–2711.
15. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
16. Kolb, H. C.; Sharpless, K. B. *Drug Discov. Today* **2003**, *8*, 1128–1137.
17. Mocharla, V. P.; Colasson, B.; Lee, L. V.; Röper, S.; Sharpless, K. B.; Wong, C. H.; Kolb, H. C. *Angew. Chem.* **2005**, *44*, 116–120.
18. Bettinetti, L.; Löber, S.; Hübner, H.; Gmeiner, P. *J. Comb. Chem.* **2005**, *7*, 309–316.
19. Rodriguez Loaiza, P.; Löber, S.; Hübner, H.; Gmeiner, P. *J. Comb. Chem.* **2006**, *8*, 252–261.
20. Van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503–4506 and references cited therein.
21. Bock, V. D.; Perciaccante, R.; Jansen, T. P.; Hiemstra, H.; Van Maarseveen, J. H. *Org. Lett.* **2006**, *8*, 919–922.
22. Angelo, N. G.; Arora, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 17134–17135.
23. Zhang, Z.; Fan, E. *Tetrahedron Lett.* **2006**, *47*, 665–669.
24. Trybulski, E. J.; Kramss, R. H.; Mangano, R. M.; Rusinko, A. *J. Med. Chem.* **1990**, *33*, 3190–3198.
25. Cossy, J.; Cases, M.; Pardo, D. G. *Bull. Soc. Chim. Fr.* **1997**, *134*, 141–144.
26. Markidis, T.; Kokotos, G. *J. Org. Chem.* **2001**, *66*, 1919–1923.
27. Kimball, S. D.; Das, J.; Lau W. F.; Hall, S. E.; Han W. -C. U.S. Patent 5,583,146, 1996.
28. Norris, P.; Horton, D.; Levine, B. R. *Heterocycles* **1996**, *43*, 2643–2655.
29. Guanti, G.; Riva, R. *Tetrahedron: Asymmetry* **2001**, *12*, 1185–1200.
30. Lundquist, J. T., IV; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781–783.
31. Zabrocki, J.; Smith, G. D.; Dunbar, J. B., Jr.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 5875–5880.
32. Ishimoto, B.; Tonan, K.; Ikawa, S.-i. *Spectrochim. Acta, Part A* **1999**, *56*, 201–209.
33. Horne, W. S.; Yadav, M. K.; Stout, D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366–15367.
34. Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998–15999.