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Novel triazolopeptides: chirospecific synthesis and conformational studies of proline derived analogs

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ABSTRACT

Replacing the backbone amide function by a heterocyclic bioisostere, [3+2] azide–alkyne cycloaddition has been applied for the construction of biologically relevant peptidomimetics. Starting from amino-alkynoates, triazole formation was accomplished by addition of hydrazoic acid. NMR studies displayed that the newly developed 4,5-triazolopeptides, which incorporate a biomimetic triazole *NH*-function as polar constraint element, showed a substantially higher tendency to form a *cis*-prolyl-geometry than a comparable native peptide sequence.

1. Introduction

Poor bioavailability and in vivo enzymatic degradation substantially limit the potential of peptides as drugs and biomolecular tools.[1] Thus, the development of privileged molecular scaffolds efficiently mimicking biologically relevant motifs of peptides is of paramount importance in current drug discovery and chemical biology.[2] Structural constraints have been exploited to increase both binding and selectivity and to elucidate bioactive conformations.[3]

Replacing the amide function by a heterocyclic bioisostere, [3+2] azide–alkyne cycloaddition has been applied to the construction of backbone modifications including nonpeptidic foldamers,[4] macrocyclic peptide analogs,[5] and click chemistry derived molecular scaffolds that we termed triazolopeptides.[6] Four types of triazolopeptides were synthesized that we refer to as 1,4- and 1,5-triazolopeptides as well as 4,1- and 5,1-linked derivatives (Chart 1).[7] When mimicking a Pro-Gly dipeptide, structural studies indicated the adjustability of both the *cis*/*trans* prolyl ratio and the conformational stability toward intramolecular H-bonding effects. Different to the classical backbone amide function, the hitherto reported triazolopeptides are devoid of an *NH*-function presented in the natural lead compounds. As an extension of our previously described incorporation of polar constraint elements into type-II β-turn inducing peptidomimetics,[3k] we designed a novel class of 4,5-disubstituted triazolopeptides simulating the incorporation of a backbone *NH*-function. The aim of this project was to develop a novel class of biologically relevant backbone mimetic and to apply the novel constraint element for the synthesis of proline derived analogs with an increased *cis* prevalence.

Complementary to the use of terminal alkynes and alkyl azides, our plan of synthesis depended on the construction of amino-alkynoates that should be subjected to [3+2] cycloaddition reaction in presence of a hydrazoic acid equivalent.[8,9]

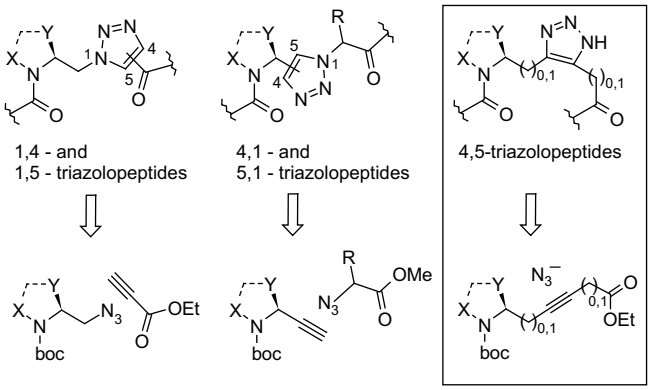

Chart 1.

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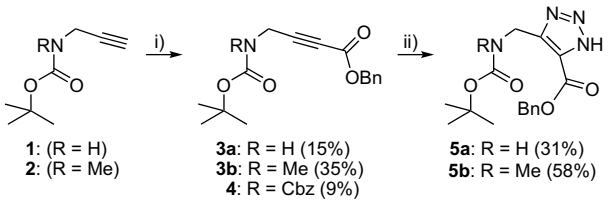
2. Results and discussion

Taking advantage of *N*-Boc protected alkynes as versatile precursors,[10] our initial investigations were directed to the synthesis of a glycine based model system of type **5**. Starting from suitable aminoalkynoates, triazole formation[9] was intended by addition of hydrazoic acid. To obtain 2-alkynoate derivatives of type **3**, we aimed to deprotonate the respective terminal alkynes followed by a reaction with chloroformate.[10,11]

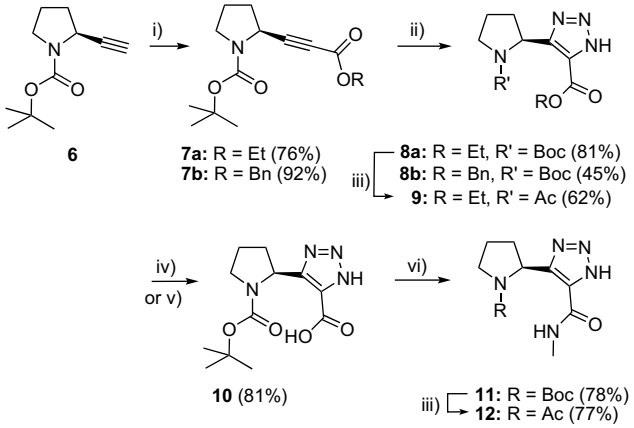
For the synthesis of the molecular scaffold **5**, we started from the Boc-protected propargylamines **1**[12] and **2**.[13]

Guided by a previously reported protocol,[10a] *n*-BuLi promoted deprotonation of **1** and subsequent acylation resulted in formation of the aminobutynoic acid **3a** (Scheme 1). The yield of **3a** was limited by formation of the *C*,*N*-bis-acylated byproduct **4**. As a consequence, treatment of the *N*-methyl analog **2** under identical conditions gave the alkynoate **3b** in more acceptable yield. Cycloaddition in presence of NaN_3/NH_4Cl[14,15] afforded the triazoles **5a**,**b** in 31% and 58% yields, respectively (Scheme 1).



Scheme 1. (i) (a) *n*-BuLi, THF, −78 °C, 30 min; (b) benzyl chloroformate, −78 °C to rt, 60–160 min; (ii) NaN_3, NH_4Cl, DMSO, rt, 9 h, 40 min.

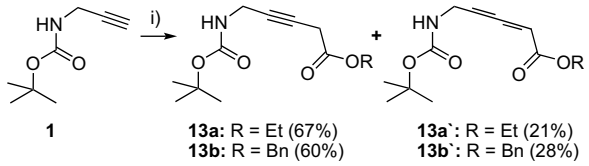
As *cis*/*trans* prolyl isomerization plays a crucial role in various biological processes[16] peptide mimics capable of modifying the *cis*/*trans* Xaa-Pro ratio are of particular interest. Thus, a 4,5-triazolopeptide simulating the sequences Ac-Pro-Gly-OEt and Ac-Pro-Gly-NHMe should be synthesized and subjected to conformational studies. Employing the pyrrolidinyl acetylene **6**, which can be obtained from *N*-Boc-proline[10d] as a chiral building block, lithiation and subsequent treatment with ethyl and benzyl chloroformate resulted in formation of the ethyl alkynoate **7a** and the benzyl alkynoate **7b**, respectively (Scheme 2). Upon cycloaddition in presence of hydrazoic acid, the triazoles **8a**,**b** were formed in 76–92% yield. Since aminolysis of **8a** to give the corresponding *N*-methylamide **11** failed we performed a hydrogenolytic debenzylation of **8b**. The thus formed carboxylic acid **10** was subjected to a promoted HATU coupling with methylamine to provide the



Scheme 2. (i) (a) *n*-BuLi, THF, −78 °C, 30 min; (b) ethyl or benzyl chloroformate, −78 °C to rt, 45–100 min; (ii) NaN_3, NH_4Cl, DMSO, 100 °C, 80–200 min; (iii) (a) TFA, CH_2Cl_2, 0 °C to rt, 50 min; (b) AcCl, DIPEA, CH_2Cl_2, 0 °C to rt, 16 h; (iv) H_2, $Pd(OH)_2/C$, EtOAc/MeOH 1:1, rt, 2 h; (v) LiOH, THF/H_2O 1:1 refl., 70 min; (vi) (a) HATU, DMF, DIPEA, 0 °C; (b) NH_2Me, EtOH, 0 °C to rt, 22 h.

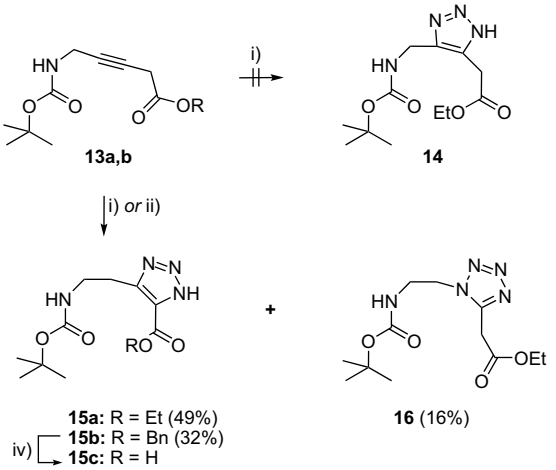
triazolopeptide **11** in 78% yield. Finally, the Boc groups of **8a** and **11** were exchanged by acetyl to give the model peptidomimetics **9** and **12**, respectively.

Whereas induction of an intramolecularly stabilized nine-membered ring should be investigated for the triazolopeptide of type **12**, insertion of an additional carbon atom should facilitate formation of a β-turn nucleating 10-membered ring. We were intrigued by the question whether the introduction of an acetic acid equivalent instead of a carboxylate synthon into the identical reaction sequence might provide this extra carbon and, thus, lead to a homologous backbone. In detail, we took advantage of a Cu(I) catalyzed coupling[17] of the terminal alkyne **1** with ethyl diazoacetate and benzyl diazoacetate[18] to afford the aminopentyne derivatives **13a** and **13b** in good yields (60–67%). It is worthy of note that considerable amounts of the isomeric allenes **13a**′ and **13b**′ could be identified as side products (Scheme 3).



Scheme 3. (i) CuI, ethyl or benzyl diazoacetate, CH_3CN, rt, 23–24 h.

To promote [3+2] azide–alkyne cycloaddition, the hardly separable mixture of **13a** and **13a**′ was subjected to NaN_3/NH_4Cl. Interestingly, two cycloaddition products were formed in a 3:1 ratio. However, none of these displayed a structure identical to the anticipated scaffold **14** (Scheme 4). Careful NMR spectroscopic elucidation including HMBC, HMQC, COSY, and NOESY experiments indicated the formation of the triazole carboxylate **15a** as the main product. Thus, an isomerization step was obvious. Whereas reaction of chromatographically purified **13a** gave identical products, NaN_3/NH_4Cl treatment of the allene **13a**′ in pure form didn't show any cycloaddition indicating that the alkyne–allene isomerization was not crucial to the formation of **15a**.[19] Isomerization of the triple bond to give the conjugated alkyne as an intermediate could be excluded since treatment of **13a** with NH_4Cl (excluding NaN_3) did not show any reaction.



Scheme 4. (i) NaN_3, NH_4Cl, DMF, 75 °C, 3 h; (ii) NaN_3, $N(C_4H_9)_4Br$, $H_2O/CHCl_3$, μ-wave, 75 W, rt to 55 °C, 2×5 min; (iii) NH_2Me, EtOH, rt, 2 h; (iv) H_2, $Pd(OH)_2/C$, EtOAc, rt, 2 h.

Elemental analysis and mass spectroscopy of the side product **16** clearly indicated the incorporation of one more nitrogen. Subsequent X-ray analysis unambiguously revealed formation of a tetrazole system as a key feature of the molecular scaffold **16** (Fig. 1).

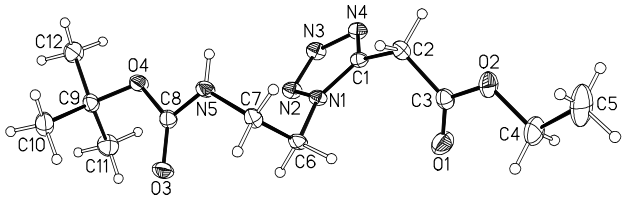
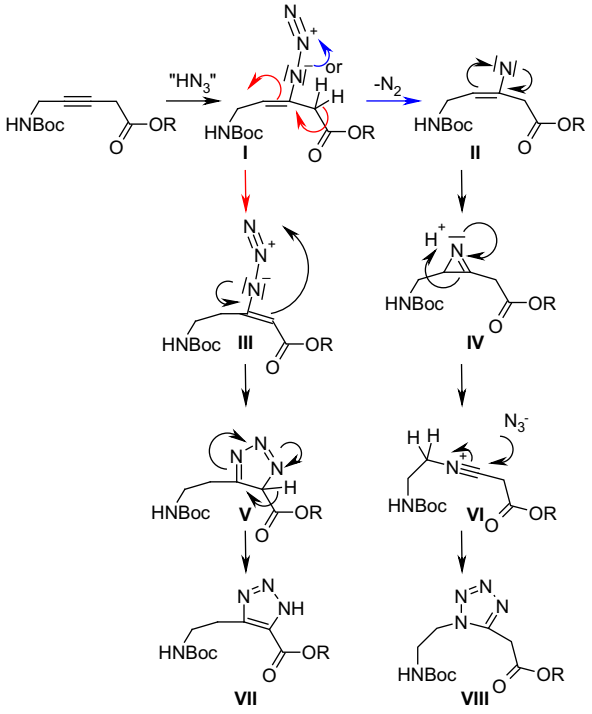


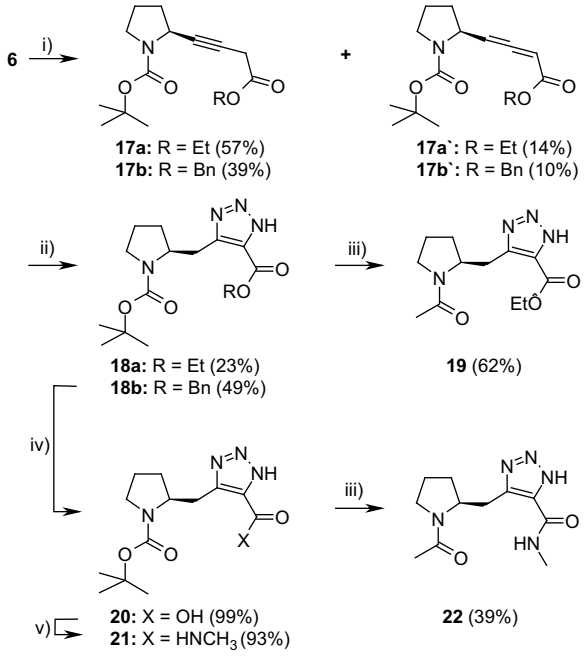
Figure 1. Molecular structure of **16** (thermal ellipsoid plot, 50% probability).

Modification of reaction conditions allowed to influence the triazole–tetrazole ratio significantly. Thus, microwave irradiation and the addition of acetic acid instead of NH_4Cl resulted in formation of **13a** and **14** in a 1:1 ratio. On the other hand, employment of aqueous sodium azide, chloroform, and phase transfer catalysis ($N(C_4H_9)_4Br$) led to selective triazole formation. This was exemplified for the preparation of the benzyl triazole carboxylate **15b** from the linear precursor **13b**/**13b′**. Hydrogenolytic debenzylation afforded the carboxylic acid **15c**. To the best of our knowledge, comparable rearrangements have not been described in the literature, yet. To explain a possible reaction mechanism of both triazole and tetrazole formation, we suggest the vinylazide derivative **I**, resulting from the addition of azide to the triple bond as a common intermediate (Scheme 5). Following the 'red arrow pathway', isomerization of the double bond leads to the conjugated tautomer **III**, which undergoes ring closure (**V**) and aromatization to give the triazole of type **VII**. Alternatively, after N_2-elimination ('blue arrow pathway'), the resulting nitrene **II** shows cyclization to give the azirine **IV**,[20] which—after acid promoted ring opening (**VI**)—furnishes the tetrazole derivative of type **VIII** (Scheme 5) via 1,3 dipolar cycloaddition with an azide anion.



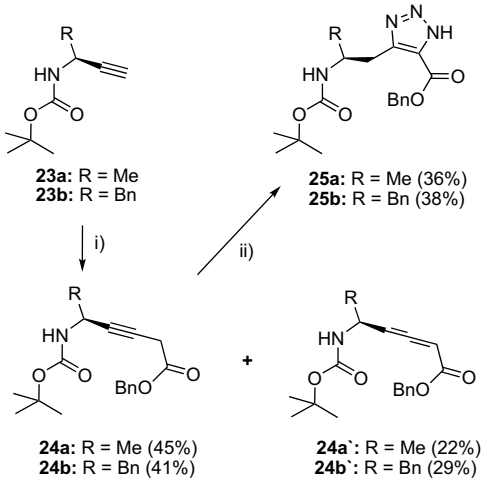
Scheme 5. Suggested rearrangement mechanism explaining the triazole and tetrazole pathway.

To demonstrate the general scope of the reaction sequence, the synthesis of proline, alanine, and phenylalanine analogs was intended. Initially, the proline derived alkyne **6** was reacted with ethyl and benzyl diazoacetate to furnish the pyrrolidinyl substituted alkynoates **17a** and **17b**, respectively, accompanied by approximately 10% of the allene derivatives **17a′** and **17b′**. Subsequently, the alkyne/allene mixtures were treated with NaN_3, NH_4Cl to give the triazoles **18a** and **18b**. Tetrazole formation could not be observed. Aminolysis of the ethyl ester **18a** to afford the model peptide surrogate **21** failed. Thus, we synthesized the carboxylic acid intermediate **20** by hydrogenolytic cleavage of **18b**. The carboxamide **21** was obtained by subsequent coupling with H_2NMe employing HATU. Finally, the Boc groups of **18a** and **21** were exchanged by acetyl to furnish the model peptidomimetics **19** and **22** (Scheme 6).



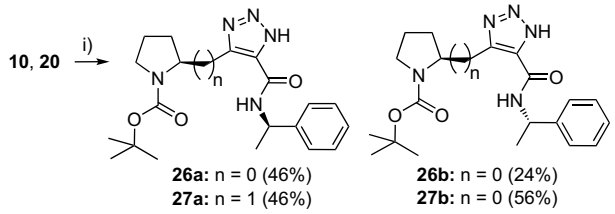
Scheme 6. (i) CuI, ethyl or benzyl diazoacetate, CH_3CN, rt, 4–21 h; (ii) NaN_3, NH_4Cl, DMSO or DMF, 100 °C, 1–3 h; (iii) (a) TFA, CH_2Cl_2, 0 °C to rt, 25–45 min; (b) AcCl, DIPEA, CH_2Cl_2, 0 °C to rt, 5 h; (iv) $Pd(OH)_2/C$, H_2, EtOAc, rt, 80 min; (v) (a) HATU, DIPEA, DMF, 0 °C; (b) NH_2Me, EtOH, rt, 22 h.

Alkyne analogues **23a**,**b** based on alanine and phenylalanine were synthesized[10b] and converted to the respective alkynoates **24**,**b** accompanied by 33% and 41% of the allenes **25a′**,**b′**. Triazole formation with NaN_3/NH_4Cl gave the triazolopeptides **25a** and **25b** in 23% and 49% yields (Scheme 7). Tetrazole formation was not observed.



Scheme 7. (i) CuI, ethyl or benzyl diazoacetate, CH_3CN, rt, 20 h; (ii) NaN_3, NH_4Cl, DMF, 105 °C, 95 min.

To examine the optical integrity of the synthesis, the proline derived representatives **10** and **20** were coupled with (*R*)-α-methylbenzylamine affording **26a** and **27a** and with (*S*)-α-methylbenzylamine yielding **26b** and **27b**, respectively (Scheme 8). Careful HPLC analysis indicated diastereomeric excess >96%.

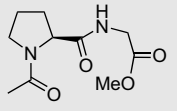
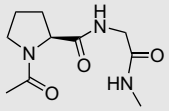
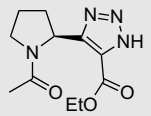
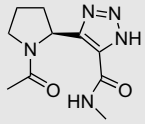
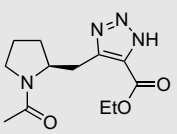
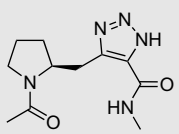


Scheme 8. (i) HATU (2.0 equiv), DMF, 0 °C to rt, (*R*)-α-methylbenzylamine or (*S*)-α-methylbenzylamine, 3 h 45 min to 10 h 30 min.

The importance of *cis*/*trans* isomerization for molecular recognition has been shown many times. For example, introduction of unnatural proline derivatives induced an increased *cis* prevalence for 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.[16d] These findings underline the importance of molecular tools for a fine tuning of the *cis*- versus *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,[21] sterically demanding substituents[22,23] as well as introduction of fluorine,[24,25] azide [26] or by introducing bridging elements to furnish Freidinger-type lactams.[27–29,3h]

Aiming to better understand the conformational properties, our newly developed 4,5-triazolopeptides, the Ac-Pro-Gly-NHMe surrogates **12** and **22**, were investigated by means of ^{1}H NMR spectroscopy (Table 1). Involving the ethyl ester analogs **9** and **19** into the study, we gave special attention to *cis*/*trans* prolyl ratios. *N*-Acetylprolylglycine methyl ester and *N*-acetylprolylglycine *N*′-methyl amide were used as reference peptides.[30] Using $CDCl_3$ as a solvent, two sets of signals were identified for the triazole carboxylate **9**. NOE measurements revealed a proximity of the acetyl CH_3 (2.14 ppm) and the pyrrolidine H-5 for one set of signals indicating *trans*-geometry. On the other hand, NOEs between the acetyl CH_3 (1.87 ppm) and H-2 (represented by the signal at 5.60 ppm) were diagnostic for the population representing the *cis*-isomer. Integration of baseline-separated signals allowed deducing a 40% portion of the *cis*-rotamer. Due to solubility problems in $CDCl_3$, addition of 5% DMSO-d_6 was necessary for the NMR investigations of the methylamide **12** when 45% of the *cis*-rotamer was determined. It is worthy to note, that a highly diluted sample in pure $CDCl_3$ revealed a very similar *cis*/*trans* ratio. NMR studies of the homologous triazole carboxylate **19** required the addition of 2.5% DMSO-d_6, a concentration that did not influence the *cis*/*trans* ratio of the structural analog **9**. The *cis*-population was identified by a nuclear Overhauser enhancement of the acetyl methyl signal at 2.11 ppm when irradiating at the H-2 resonance (4.24 ppm). Under these conditions a 40:60 *cis*/*trans* ratio was determined. For the methylamide **22**, one single set of signals was detected in pure $CDCl_3$, when an NOE between the acetyl methyl group and the pyrrolidine H-5 clearly proved exclusive trans conformation. Very interestingly, the *cis* portion significantly increased to 40%, when 2.5% DMSO-d_6 was added to the sample indicating that a β-turn like structure stabilized by an intramolecular H-bond might be responsible for the strong preference of the *trans*-geometry in pure $CDCl_3$. Compared to the native peptide sequence, the 4,5-triazolopeptides **9**, **12**, and **19** displayed a substantially higher tendency to form the *cis*-prolyl-geometry. For the methylamide **22**, this property seems to be overcompensated by an intramolecular H-bond putatively stabilizing a β-turn like conformation with *trans*-prolyl-geometry. Thus, the model peptide mimetic of type **12** obviously represents the molecular scaffold with a robust *cis*-proline peptide bond.

Table 1
NMR-derived prolyl *cis*-isomer ratios of the model peptide surrogates **9**, **12**, **19**, and **22** compared to Ac-Pro-Gly-OMe, Ac-Pro-Gly-NHMe,[30] respectively (5% steps)

	Cpd	cis		Cpd	cis
		10%			<1%
9		40%	**12**[a]		45%
19[a]		40%	**22**		<5%

[a] Addition of DMSO-d_6 was necessary to entirely dissolve the compounds **12** (5.0%) and **19** (2.5%). In order to obtain unambiguous resonance signals in the selective NOE measurements, samples were diluted to 5–10 mM concentrations.

3. Conclusion

In conclusion, [3+2] azide–alkyne cycloaddition has been applied for the construction of biologically relevant peptidomimetics. Starting from aminoalkynoates, triazole formation was accomplished by addition of hydrazoic acid. The newly developed molecular scaffolds include a biomimetic *NH* moiety, thus, complementing our previously described incorporation of polar lactam-bridged constraint elements. Whereas the recently described spirobarbiturates have been determined as type-II β-turn nucleating scaffolds incorporating a *trans*-proline peptide bond, NMR studies displayed that the newly developed 4,5-triazolopeptides showed a substantially higher tendency to form the *cis*-prolyl-geometry than a comparable native peptide sequence. Due to their artificial backbone, substantial resistance toward in vivo enzymatic is expected. Thus, 4,5-triazolopeptides represent a valuable alternative to previously reported *cis*-prolyl peptide analogs including pseudo-prolines.[16d] Complementing those, differential positions of the respective proximal side chains will provide a valuable opportunity for structure–activity relationship studies in medicinal chemistry.

4. Experimental

4.1. General

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 in vacuo with a rotary 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. If not otherwise stated, mass spectra were performed by EI ionization (70 eV) with solid inlet,

HRMS were obtained employing peak matching $M/\Delta M$=5000. ESI and APCI-MS were performed using a Bruker Esquire 2000 coupled with an Agilent 1100 analytic HPLC system. The NMR spectra were recorded 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) spectrometer, if not otherwise stated, in $CDCl_3$. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: 7.26, $CDCl_3$: 77.0) or to tetramethylsilane. NOEs were determined using the DPFGSE-NOE pulse sequence with a selective Gaussian shaped refocussing pulse of 50 ms length and a mixing time of 500 ms.[31] Elemental analyses were performed by the Institute of Organic Chemistry (Analytical Departments) of the Friedrich Alexander University Erlangen-Nürnberg and at Mikroanalytisches Laboratorium Beetz. Melting points were determined with a Büchi 510 apparatus and were uncorrected. HPLC analysis were run on Agilent 1100 Series with a Diode Array Detector at 210, 230, and 250 nm. Optical rotations were measured on a Perkin Elmer 241 polarimeter. IR spectra were measured on a Jasco 410 apparatus.

4.2. *N*-Boc-propargylamine (1)[12]

Di-*tert*-butyl-dicarbonate (20.462 g, 93.8 mmol) was dissolved in CH_2Cl_2 and cooled to 0 °C. Propargylamine (5.164 g, 93.8 mmol) was added over a period of 30 min, after warmed to room temperature and stirred for 12 h. The organic layer was extracted successively with HCl (2 N, 30 mL), NaOH (2 N, 30 mL) and $NaHCO_{3\ satd}$ (30 mL) and each time re-extracted with CH_2Cl_2 (20 mL). The combined organic layers then were dried over $MgSO_4$ and the solvent concentrated until an oily residue was observed. *n*-Pentane (100 mL) was added and after standing in the refrigerator for 12 h, the precipitate was collected by filtration. The filtrate was concentrated in order to collect another crop of **1** to afford 12.20 g (84%) as white needles. Mp 42 °C [lit.[12] mp 41–42 °C]; R_f 0.71 (petroleum ether/ethyl acetate 1:1); IR (neat) 3417, 3346, 3307, 2979, 2121, 1702 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; broadened signals were observed) δ 1.45 (s, 9H, Boc CH_3), 2.21 (t, 1H, J=2.3 Hz, C≡CH), 3.92 (dd, J=5.0, 2.3 Hz, 2H, NCH_2), 4.69 (br s, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.3 (Boc CH_3), 30.4 (NCH_2), 71.2 (C-3), 80.06 (Boc C^q), 80.12 (C-2), 155.2 (Boc C=O).

4.3. *N*-Boc-*N*-methylpropargylamine (2)[13]

Potassium hydride (875.7 mg, 21.84 mmol) was suspended in THF (10.0 mL) under nitrogen and **1** (3.104 g, 20.00 mmol) was added as a solution in THF (10.0 mL) at 0 °C. After 1 h, methyliodide (1.50 mL, 3.420 g, 24.10 mmol) was added over a period of 5 min and the solution was allowed to warm to room temperature. After 140 min the mixture was poured in $NaHCO_{3\ satd}$ (30 mL) and, subsequently, water (50 mL) and brine (20 mL) were added. After extraction with diethyl ether (4×20 mL), the combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography (petroleum ether/ethyl acetate 93:7) furnishing 3.01 g (89%) of **2** as a clear colorless oil. R_f 0.50 (petroleum ether/ethyl acetate 9:1); IR (neat) 3351, 3295, 3264, 2978, 2127, 1699 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.46 and 1.47 (2×s, 9H, Boc CH_3), 2.20 and 2.21 (2×t, each J=2.2 Hz, 1H, C≡CH), 2.91 (s, 3H, NCH_3), 4.03 (br s, 2H, NCH_2), signals were in accordance with Ref. 13, we also observed signals of the second rotamer; ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.4 (Boc CH_3), 33.4 (NCH_3), 37.7 (br, NCH_2), 71.5 (C-3), 79.2 (Boc C^q), 80.1 (C-2), 155.2 (Boc C=O); EIMS 113 (M^+−Isobutene).

4.4. Benzyl diazoacetate[18]

Glycine benzyl ester hydrochloride (3.820 g, 18.944 mmol) was dissolved in water (30.0 mL) and then CH_2Cl_2 (30.0 mL) was added. This biphasic mixture was cooled to 0 °C and $NaNO_2$ (1.570 g, 22.754 mmol), dissolved in water (5.0 mL), was added over a period of 2 min whereas the solution turned bright yellow. After stirring for 90 min at 0 °C, the organic layer was poured into $NaHCO_{3\ satd}$ (50 mL) via a funnel (*cave*: bubbling). The aqueous and $NaHCO_{3\ satd}$ layers were re-extracted with CH_2Cl_2 (each 20 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and evaporated. Purification by flash column chromatography (pentane/diethyl ether 8:2) furnished 2.83 g (85%) benzyl diazoacetate as a clear yellow oil. R_f 0.51 (pentane/diethyl ether 7:3); IR (neat) 3113, 2924, 2112, 1744, 1695, 1631 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 4.80 (br s, 0.85H, HC$^-$N$^+$N), 5.20 (s, 2H, OCH_2), 5.315+5.317 (2×s, 0.15H, HC=N$^+$=N$^-$$_{s\text{-cis}+s\text{-trans}}$), 7.30–7.41 (m, 5H, Ar–H) signals were in accordance with Ref. 18, furthermore, we observed signals of the second rotamer.

4.5. General procedure for synthesis of the 2-alkyne carboxylic acid esters (3a,b, 7a,b)

Over a period of 5 min, *n*-butyllithium in hexanes was added to a cooled (−78 °C) solution of the respective alkyne derivative in THF under nitrogen. After stirring for 10–20 min, the corresponding chloroformate was added and the mixture was then treated individually (see below). After complete conversion of starting materials, the mixture was quenched and extracted. After washing the organic layer with brine and drying over Na_2SO_4, the solvent was removed in vacuo and the crude product was purified by flash column chromatography.

4.5.1. *N-tert-Butoxycarbonyl-4-aminobut-2-ynic acid benzyl ester (**3a**) and N-benzyloxycarbonyl-N′-tert-butoxycarbonyl-4-aminobut-2-ynic acid benzyl ester (**4**)*

Compound **1** (310.4 mg, 2.0 mmol) in THF (15 mL) was reacted with *n*-BuLi (1.68 mL, 2.5 M solution, 4.2 mmol) for 10 min and then with benzyl chloroformate (313 μL, 2.2 mmol) for 1 h at −78 °C following the general procedure. Quenching: H_2O (20 ml), extraction: diethyl ether. Chromatography (pentanes/diethyl ether 9:1) furnished 88.6 mg (15%) of **3a** as a colorless solid and 72.1 mg (9%) of **4** as a colorless oil.

*4.5.1.1. Compound **3a**.* Mp 74–76 °C; R_f 0.30 (pentanes/diethyl ether 6:4); IR (neat) 3402, 3355, 2926, 2239, 1714 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.45 (s, 9H, Boc CH_3), 4.07 (d, J=5.0 Hz, 2H, HN–CH_2), 4.73 (br s, 1H, NH), 5.19 (s, 2H, OCH_2), 7.27–7.43 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 28.3 (Boc CH_3), 30.4 (NCH_2), 67.7 (OCH_2), 74.8 (C-2), 80.5 (Boc C^q), 84.3 (C-3), 128.5 (br) and 128.65 (br) (5×aryl CH), 134.7 (aryl C), 153.0 (ester C=O), 155.0 (Boc C=O); EIMS 233 (M^+-C_4H_8).

*4.5.1.2. Compound **4**.* R_f 0.44 (pentanes/diethyl ether 6:4); IR (neat) 2979, 2876, 2241, 1796, 1759, 1715 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.48 (s, 9H, Boc CH_3), 4.57 (s, 2H, NCH), 5.19 (s, 2H, $OCH_{2\ a}$), 5.25 (s, 2H, OCH_{2b}), 7.30–7.43 (m, 10H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 27.9 (Boc CH_3), 35.9 (NCH_2), 67.7 and 69.0 (2×OCH_2Ph, Cbz and ester), 69.0 (OCH_2), 74.6 (C≡*C*–C=O), 83.6 and 84.3 (Boc C^q and *C*≡C–C=O), 128.63 (10×aryl CH), 134.7 and 135.0 (2×aryl C), 150.6, 152.7 and 152.9 (2×carbamate C=O and ester C=O); EIMS 367 (M^+−C_4H_8), 276 [M−C_4H_8−/−C_7H_7]$^+$. Anal. Calcd for $C_{24}H_{25}NO_6$×0.25H_2O: C, 67.36; H, 6.01; N, 3.28. Found: C, 67.64; H, 6.27; N, 3.29.

4.5.2. *N-tert-Butoxycarbonyl-N-methyl-4-aminobut-2-ynoic acid benzyl ester (**3b**)*

Compound **2** (338.6 mg, 2.0 mmol) in THF (15 mL) was reacted with *n*-BuLi (0.84 mL, 2.5 M solution, 2.1 mmol) for 20 min and

then with benzyl chloroformate (313 μL, 2.2 mmol) for 165 min at −78 °C, then at −55 °C for 1 h and finally for 1 h at rt. Quenching: H_2O (20 mL) and brine (10 mL), extraction: diethyl ether. Flash column chromatography (hexanes/ethyl acetate 9:1) furnished 214.2 mg (35%) of **3c** as clear yellowish oil. R_f 0.45 (hexanes/diethyl ether 6:4); IR (neat) 2977, 2237, 1709 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.46 (s, 9H, Boc CH_3), 2.91 (s, 3H, NCH_3), 4.18 (br s, 2H, NCH_2), 5.20 (s, 2H, OCH_2), 7.31–7.41 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 28.3 (Boc CH_3), 33.8 (NCH_3), 37.7 and 38.6 (2×br, NCH_2), 67.7 (OCH_2), 75.3 (C-2), 80.7 (Boc C^q), 83.8 (C-3), 128.55, 128.63 and 128.64 (aryl CH), 134.7 (aryl C), 153.1, 154.7 (br) and 155.2 (br) (3×br, ester and Boc C═O); EIMS 303 (M^+). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.36; H, 7.00; N, 4.60.

4.5.3. (S)-1-tert-Butoxycarbonylpyrrolidin-2-yl-propynoic acid ethyl ester (**7a**)

Compound **6** (292.2 mg, 1.500 mmol) was reacted with *n*-BuLi (0.99 mL, 1.6 M solution, 1.6 mmol) for 5 min and then with ethyl chloroformate (290 μL, 3.0 mmol) at rt for 95 min following the general procedure. Quenching with MeOH (3 mL) and subsequent evaporation. Chromatography (hexanes/ethyl acetate 9:1) furnished 302.5 mg (76%) of **7a** as clear colorless oil. R_f 0.30 (hexanes/ethyl acetate 8:2); $[\alpha]_D^{24}$ +124.4 (*c* 1.0, $CHCl_3$); IR (neat) 2979, 2235, 1710, 1695 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; broadened signals were observed) δ 1.30 (t, *J*=7.1 Hz, 3H, OCH_2CH_3), 1.48 (s, 9H, Boc CH_3), 1.87–2.20 (m, 4H, H-3/H-4 pyrrolidine), 3.25–3.54 (m, 2H, H-5 pyrrolidine), 4.22 (br q, *J*=7.1 Hz, 2H, OCH_2CH_3), 4.50 (m, 1H, H-2 pyrrolidine); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 14.0 (ester CH_3), 23.8 and 24.6 (C-4 pyrrolidine), 28.4 (Boc CH_3), 32.4 and 33.0 (C-3 pyrrolidine), 45.7 and 46.0 (C-5 pyrrolidine), 47.8 and 48.0 (C-2 pyrrolidine), 61.9 (OCH_2), 73.9 (C≡C–C═O), 80.3 (br, Boc C^q), 87.6 (C≡C–C═O), 153.6 and 153.7 (2×br, Boc C═O), 153.8 and 152.9 (2×br, C═O ester); EIMS 267 (M^+). Anal. Calcd for $C_{14}H_{21}NO_4\times0.2H_2O$: C, 62.07; H, 7.96; N, 5.17. Found: C, 62.38; H, 7.95; N, 5.17.

4.5.4. (S)-1-tert-Butoxycarbonylpyrrolidin-2-yl-propynoic acid benzyl ester (**7b**)

Compound **6** (390.5 mg, 2.000 mmol) was reacted with *n*-BuLi (1.31 mL, 1.6 M solution, 2.1 mmol) for 10 min and then with benzyl chloroformate (427 μL, 3.0 mmol) for 50 min at −30 °C and then at rt for 5 min. Quenching: H_2O (10 mL), extraction: diethyl ether. Chromatography (pentanes/diethyl ether 8:2) furnished 608.8 mg (92%) of **7b** as clear colorless oil. R_f 0.33 (pentanes/diethyl ether 8:3); $[\alpha]_D^{24}$ −105.1 (*c* 1.0, MeOH); IR (neat) 2977, 2235, 1713, 1700 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; broadened signals were observed) δ 1.47 (s, 9H, Boc CH_3), 1.81–2.25 (m, 4H, H-3/H-4 pyrrolidine), 3.22–3.56 (m, 2H, H-5 pyrrolidine), 4.50 and 4.63 (2×br s, 1H, NCH), 5.19 (br s, 2H, OCH_2), 7.30–7.39 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 23.8 and 24.6 (C-4), 28.4 (Boc CH_3), 32.3 and 33.0 (C-3), 45.6 and 46.9 (NCH_2), 47.8 and 48.0 (NCH), 67.5 (OCH_2), 73.6 (C≡C–C═O), 80.1 and 80.3 (Boc C^q), 88.3 and 88.4 (C≡C–C═O), 128.4, 128.5 and 128.6 (aryl CH), 134.9 (aryl C), 153.4, 153.7 and 153.9 (ester, Boc C═O); EIMS 329 (M^+). Anal. Calcd for $C_{19}H_{23}NO_4\times0.25H_2O$: C, 68.35; H, 7.09; N, 4.19. Found: C, 68.40; H, 7.14; N, 4.24.

4.6. General procedure for synthesis of the alk-3-yne acid derivatives as a mixture with the allene isomers (13a,a′, 13b,b′, 17a,a′, 17b,b′, 24a,a′, and 24b,b′)

At rt, dried CuI was added to a solution of the respective alkyne in dry acetonitrile under nitrogen and, after stirring for 5 min, the according diazoacetate. In all cases, the mixture turned to a yellow-green color. After TLC indicated complete conversion of starting materials, the solvent was removed and the crude product was purified by flash column chromatography.

4.6.1. N-tert-Butoxycarbonyl-5-aminopent-2-yne acid ethyl ester (**13a**) and N-tert-butoxycarbonyl-5-aminopenta-2,3-dienoic acid ethyl ester (**13a′**)

Compound **1** (1.552 g, 10.0 mmol) in acetonitrile (20 mL) was reacted with CuI (286 mg, 1.5 mmol) and ethyl diazoacetate (1.104 mL, 10.5 mmol) for 24 h following the general procedure. Flash column chromatography (CH_2Cl_2/ethyl acetate 95:5) furnished 1.618 g (67%) of **13a** as colorless wax and 511 mg (21%) of **13a′** as yellowish oil, respectively.

4.6.1.1. Compound **13a**.

Mp 60–62 °C; R_f 0.46 (CH_2Cl_2/ethyl acetate 9:1); IR (neat) 3392, 3361, 2979, 2289, 1743, 1714, 1697 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.28 (t, 3H, *J*=7.1 Hz, OCH_2CH_3), 1.45 (s, 9H, Boc CH_3), 3.26 (t, *J*=2.2 Hz, 2H, CH_2C═O), 3.94 (br m, 2H, NCH_2), 4.19 (q, *J*=7.1 Hz, 2H, OCH_2), 4.69 (br s, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.1 (ester CH_3), 26.0 (CH_2C═O), 28.3 (Boc CH_3), 30.7 (NCH_2), 61.6 (OCH_2), 74.9 and 79.5 (C≡C), 79.9 (Boc C^q), 155.3 (Boc C═O), 168.2 (ester C═O); EIMS 241 (M^+). Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.80. Found: C, 59.73; H, 7.83; N, 5.85.

4.6.1.2. Compound **13a′**.

R_f 0.28 (CH_2Cl_2/ethyl acetate 9:1); IR (neat) 3361, 2979, 1965, 1714, 1701 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.28 and 1.39 (2×t, each *J*=7.1 Hz, 3H, OCH_2CH_3), 1.44 (s, 9H, Boc CH_3), 3.81–3.89 (br m, 2H, NCH_2), 4.07 and 4.20 (2×q, *J*=7.1 Hz, 2H, OCH_2), 4.72 (br s, 1H, NH), 5.67–5.73 (m, 2H, HC═C═CH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.3 (br, ester CH_3), 28.3 (Boc CH_3), 38.2 (NCH_2), 61.0 (OCH_2), 79.8 (Boc C^q), 90.6 (HC–C═O), 94.1 (CH_2HC), 155.5 (Boc C═O), 165.5 (ester C═O), 211.7 (HC═C═CH); EIMS 185 $[M-C_4H_8]^+$.

4.6.2. N-tert-Butoxycarbonyl-5-aminopent-2-yne acid benzyl ester (**13b**) and N-tert-butoxycarbonyl-5-aminopenta-2,3-dienoic acid benzyl ester (**13b′**)

Compound **1** (683.0 mg, 4.400 mmol) in acetonitrile (10 mL) was reacted with CuI (76.2 mg, 0.440 mmol) and benzyl diazoacetate (705.0 mg, 10.5 mmol) for 23 h following the general procedure. Flash column chromatography (hexanes/diethyl ether 9:1, separation was performed twice) furnished 454.4 mg (60%) of **13b** as ivory wax and 234 mg (28%) of **13b′** as yellowish oil, respectively.

4.6.2.1. Compound **13b**.

Mp 41 °C; R_f 0.55 (hexanes/diethyl ether 9:1); IR (neat) 3376, 2978, 2287, 1743, 1716, 1691 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.45 (s, 9H, Boc CH_3), 3.32 (t, *J*=2.2 Hz, 2H, CH_2C═O), 3.94 (br m, 2H, NCH_2), 4.66 (br s, 1H, NH), 5.17 (s, 2H, OCH_2), 7.30–7.42 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 26.2 (CH_2C═O), 28.5 (Boc CH_3), 30.9 (NCH_2), 67.5 and 67.8 (OCH_2), 75.0 and 79.9 (C≡C), 80.1 (Boc C^q), 128.5, 128.6, 128.7, 128.8, 128.9 (aryl CH), 135.4 (aryl C), 155.5 (Boc C═O), 168.3 (ester C═O); HRMS calcd for $C_{17}H_{21}NO_4$ 303.1471; found 303.1471.

4.6.2.2. Compound **13b′**.

R_f 0.47 (hexanes/diethyl ether 9:1); IR (neat) 3417, 3356, 2925, 1963, 1714, 1693 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.44 and 1.45 (2×s, 9H, Boc CH_3), 3.81–3.90 and 3.91–4.02 (2×br m, 2H, NCH_2), 4.12, 4.19 and 4.20 (3×q, *J*=7.1 Hz, 2H, OCH_2), 4.69 (br s, 1H, NH), 5.05 and 5.19 (2×s, 2H, OCH_2Ph), 5.29 and 5.96 (2×s, each 0.5H, HC═C═CH–C═O), 5.67–5.79 (m, 1H, HC═C═CH–C═O),

7.28–7.42 (m, 5H, Ch_2Ph); ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.3 (Boc CH_3), 30.9 (NCH_2), 38.2 (NCH_2), 66.7 (OCH_2), 79.8 (Boc C^q), 90.3 (HC–C=O), 94.3 (CH_2CH), 128.1, 128.3 and 128.6 (aryl CH), 135.8 (aryl C), 155.5 (Boc C=O), 165.4 (ester C=O), 212.1 (HC=C=CH); EIMS 303 (M^+). HRMS calcd for $C_{17}H_{21}NO_4$ 303.1471; found 303.1473.

4.6.3. (S)-2-(3-Ethoxycarbonylprop-1-ynyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**17a**) and a mixture of diastereomers of (S)-2-(3-ethoxycarbonylpropa-1,2-dienyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**17a′**)

Compound **6** (390.5 mg, 2.0 mmol) in acetonitrile (3 mL) was reacted with CuI (38.1 mg, 0.20 mmol) and ethyl diazoacetate (221 μL, 2.1 mmol) for 220 min following the general procedure. Flash column chromatography (*n*-pentanes/diethyl ether 7:3) furnished 402.6 mg (72%) of **17a**/**17a′** as yellowish oil. ^1H NMR integrals indicated about 10% of diastereomeric mixture of **17a′**. Separation of the three isomers/diastereomers failed. R_f 0.26 (pentanes/diethyl ether 7:3); IR (neat) 2977, 1962, 1779, 1746, 1698 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; mixture of isomers, all signals of the mixture of alkyne, diastereomeric allene derivatives and rotamers are given, peaks of common functional groups are not separately disclosed, but regarded as if derived from a single compound) δ 1.26 and 1.27 (2×t, J=7.2 Hz, 3H, OCH_2CH_3), 1.46 and 1.47 (2×s, 9H, Boc), 1.78–2.15 (br m, 3H, H-3/H-4 pyrrolidine), 3.16–3.51 (br m, 3.6H, H-2 pyrrolidine/CH_2C=O), 4.17 (q, J=7.2 Hz, 2H, OCH_2), 4.36–4.60 (m, 1H, NCH), 5.65–5.77 (m, 0.4H, HC=C=CH); EIMS 281 (M^+). Anal. Calcd for $C_{15}H_{23}NO_4\times2/3H_2O$: C, 61.41; H, 8.36; N, 4.77. Found: C, 61.09; H, 8.18; N, 4.69.

4.6.4. (S)-2-(3-Benzyloxycarbonylprop-1-ynyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**17b**) and a mixture of diastereomers of (S)-2-(3-benzyloxycarbonylpropa-1,2-dienyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**17b′**)

Compound **6** (249.0 mg, 1.280 mmol) in acetonitrile (5 mL) was reacted with CuI (25.5 mg, 0.134 mmol) and benzyl diazoacetate (248.0 mg, 0.141 mmol) for 21 h following the general procedure. Flash column chromatography (2×separation, hexanes/ethyl acetate 9:1) furnished 169.6 mg (39%, still containing ~10% of allene fraction as determined by ^1H NMR) of **17b** as clear colorless oil and 42.4 mg (10%) of **17b′** as yellowish oil, respectively.

4.6.4.1. Compound **17b** (including the signals of 10% allene derivative **17b′**).

R_f 0.33 (hexanes/ethyl acetate 7:3); IR (neat) 2976, 2111, 1748, 1697 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.44 and 1.46 (2×s, 9H, Boc CH_3), 1.74–1.92 and 1.92–2.13 (2×m, 4H, H-3/H-4 pyrrolidine), 3.18–3.51 and 3.31 (m and s, 3.8H, H-5 pyrrolidine and CH_2C=O), 4.36–4.60 (2×m, 1H, H-2 pyrrolidine), 5.08–5.26 and 5.16 (m and s, 2H, OCH_2/HC–C=O), 5.68–5.79 (m, 0.2H, HC=C=C), 7.28–7.40 (m, 5H, Ar–H); EIMS 343 (M^+). Anal. Calcd for $C_{20}H_{25}NO_4\times1/3H_2O$: C, 68.75; H, 7.40; N, 4.08. Found: C, 68.82; H, 7.48; N, 4.00.

4.6.4.2. Compound **17b′**.

R_f 0.34 (hexanes/ethyl acetate 7:3); IR (neat) 2976, 1961, 1720, 1695 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; mixture of diastereomers, peaks of common functional groups are not separately disclosed, but integrated as if derived from a single compound) δ 1.45 (br s, 9H, Boc CH_3), 1.74–2.08 (m, 4H, H-3/H-4 pyrrolidine), 3.17–3.41 (m, 2H, H-5 pyrrolidine), 4.35–4.48 and 4.48–4.59 (2×m, each 0.5H, NCH), 5.13 (d, J=12.4 Hz, 1H, OCH_2), 5.21 (d, J=12.4 Hz, 1H, OCH_2), 5.66–5.78 and 5.84–5.96 (2×m, 1.5 and 0.5H, HC=C=CH), 7.28–7.43 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; mixture of diastereomers, peaks of common functional groups are not separately disclosed, but regarded as if derived from a single compound) δ 23.0 and 23.8 (C-4 pyrrolidine), 28.5 (Boc CH_3), 30.2 and 31.5 (C-3 pyrrolidine), 45.9 and 46.2 (C-5 pyrrolidine), 54.6 and 55.0 (C-2 pyrrolidine), 66.6 (OCH_2), 79.7 (br, Boc C^q), 90.1 (br, CHC=O), 97.4 and 97.8 (HC=C=C), 128.1 (br), 128.2 (br) and 128.5 (br) (10×aryl CH), 135.9 (aryl C), 154.1 (br, Boc C=O), 165.4 (br, ester C=O), 211.3 (br, C=C=C); EIMS 287 $[M-C_4H_8]^+$.

4.6.5. (S)-5-tert-Butoxycarbonylaminohex-3-ynoic acid benzyl ester (**24a**) and a mixture of diastereomers of (S)-5-tert-butoxycarbonylaminohexa-2,3-dienoic acid benzyl ester (**24a′**)

Compound **23a** (507.7 mg, 3.000 mmol) in acetonitrile (10 mL) was reacted with CuI (61.0 mg, 0.320 mmol) and benzyl diazoacetate (640.5 mg, 3.637 mmol) for 20 h following the general procedure. Flash column chromatography (hexanes/ethyl acetate 95:5) furnished 641.5 mg (67%, still containing ~33% of allene fraction as determined by ^1H NMR) of a non separable mixture of **24a**/**24a′** as yellowish viscous oil. R_f 0.26 (hexanes/ethyl acetate 8:2); IR (neat) 3356, 2976, 1961, 1743, 1712, 1693 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; mixture of diastereomers/isomers, all signals of the mixture of alkyne, diastereomeric allene derivatives and rotamers are given, peaks of common functional groups are not separately disclosed, but regarded as if derived from a single compound) δ 1.25–1.29 (m, 1H, $CHCH_{3,allenes}$), 1.37 (d, J=6.9 Hz, 2H, $CHCH_{3,alkyne}$), 1.43 and 1.44 (2×s, 9H, Boc CH_3), 3.30 and 3.31 (2×s, 1.33H, CH_2C=O), 4.18–4.83 (br m, 2H, NH–CH and NH–CH), 5.160, 5.163, 5.17, 5.20 and 5.21 (d, s, 3×d, each J=12.5 Hz, 2H, OCH_2), 5.76–5.80 (m, 0.67H, HC=C=CH), 7.30–7.40 (m, 5H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; mixture of diastereomers/isomers) δ 15.3, 20.7 and 22.7, 26.0 (3×$CHCH_3$ and $CH_2C=O_{alkyne}$), 28.33 and 28.35 (Boc CH_3), 38.5 (NCH_{alkyne}), 44.3 ($NCH_{allenes}$), 65.8 and 66.6, 67.2 (OCH_2), 73.7 (C≡C–CH_2), 79.7 (Boc C^q), 84.2 (C≡C–CH_2), 90.7 and 91.1 (C=CH–C=O), 99.4 and 99.7 (HC=C=CH–C=O), 128.0, 128.1, 128.2, 128.4, 128.5, and 128.6 (aryl CH), 135.4 and 135.9 (aryl C), 154.6 (Boc $C=O_{alkyne}$), 154.86 and 154.90 (Boc $C=O_{allenes}$), 165.3 and 165.4 (ester $C=O_{allenes}$), 168.1 (ester $C=O_{alkyne}$), 211.3 and 211.5 (C=C=C); EIMS 317 (M^+).

4.6.6. (S)-5-tert-Butoxycarbonylamino-6-phenylhex-3-ynoic acid benzyl ester (**24b**) and a mixture of diastereomers of (S)-5-tert-butoxycarbonylamino-6-phenylhexa-2,3-dienoic acid benzyl ester (**24b′**)

Compound **23b** (202.0 mg, 0.823 mmol) was reacted in acetonitrile (5 mL) with CuI (21.5 mg, 0.113 mmol) and benzyl diazoacetate (183.8 mg, 1.043 mmol) for 20 h following the general procedure. Flash column chromatography (hexanes/ethyl acetate 95:5) furnished 227.8 mg (70%, still containing ~40% of the allene fraction as a mixture of diastereomers as determined by ^1H NMR) of a non separable mixture of **24b**/**24b′** as yellowish, viscous oil. R_f 0.24 (hexanes/ethyl acetate 8:2); IR (neat) 3411, 3356, 2976, 1963, 1743, 1712 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; all signals of the mixture of alkyne, diastereomeric allene derivatives and rotamers are given, peaks of common functional groups are not separately disclosed, but regarded as if derived from a single compound) δ 1.38, 1.39 and 1.42 (3×s, 9H, Boc CH_3), 2.79–3.01 (m, 2H, $CHCH_2$), 3.29 (br s, 1.2H, C≡C–CH_2), 4.50–4.71 (m, 2H, NH–CH), 5.16, 5.17 and 5.18 (3×s, 2H, OCH_2), 5.67–5.77 (m, 0.8H, HC=C=CH), 7.16–7.41 (m, 10H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$; all signals of the mixture of alkyne, diastereomeric allene derivatives and rotamers are given) δ 26.0 (C≡C–CH_2), 28.28 and 28.32 (Boc CH_3), 41.2, 41.5, 41.8, 42.0, 44.1 and 49.8 ($CHCH_2$ and $NCH_{allenes}$), 66.7 and 67.2 (OCH_2), 75.7 (C≡C–CH_2), 79.8 (Boc C^q), 82.5 (C≡C–CH_2), 90.9 and 91.3 (CH–C=O), 97.8 and 98.1 (HC=C=C), 126.7, 128.1, 128.2, 128.3, 128.4, 128.54, 128.56, 128.60, 129.5, 129.9 (30×aryl CH), 135.4, 135.8, 136.6, 136.8 and 136.9 (6×aryl C), 154.6, 154.8 and 155.0 (3×Boc C=O), 165.2 (ester $C=O_{allenes}$), 167.9 (ester $C=O_{alkyne}$), 211.0 and 211.3 (C=C=C); HRMS calcd for $C_{17}H_{21}NO_4$ 393.1940; found 393.1940.

4.7. General procedure for synthesis of the 1,2,3-triazoles (5a,b, 8a,b, 15a,b, 18a,b, 25a,b)

The alkynoates were dissolved in DMSO or DMF and NaN_3 and NH_4Cl were added under stirring at rt. The mixture was heated, if required, to complete the reaction. Work up was done in different ways: In case of **5a**, **b**, **15b**, and **18a**, the reaction mixture was diluted with water (50 mL), brine (5 mL), and HCl (2 N, 5 mL) and extracted with ethyl acetate (4×20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated. For **8a**,**b**, **15a**, **18b** and **25a**,**b**, water was added, the reaction mixture was frozen in liquid nitrogen and lyophilized. In all cases the dark residue was finally purified by flash column chromatography. For the latter work up, the residue was dissolved in CH_2Cl_2, silica gel was then added, and after evaporation the residue was used for flash chromatography. *Remark*: yields were not corrected with respect to the alkyne if alkyne/allene mixtures were utilized. Due to the formation of rotamers and the absence of protons, in some cases the ^{13}C signals for the triazole C-4 and C-5 could not be detected.

*4.7.1. 5-(N-tert-Butoxycarbonylaminomethyl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**5a**)*

Compound **3a** (76.9 mg, 0.266 mmol) in DMSO (3.5 mL) was reacted at rt with NaN_3 (86.4 mg, 1.33 mmol) and NH_4Cl (28.4 mg, 0.532 mmol) for 9 h following the general procedure. Flash column chromatography (hexanes/ethyl acetate 6:4) furnished 27.3 mg (31%) of **5a** as a yellowish resin. R_f 0.31 (hexanes/ethyl acetate 1:1); IR (neat) 3361, 3118, 2931, 1739, 1716, 1687 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.43 (s, 9H, Boc CH_3), 4.63 (d, 2H, J=5.9 Hz, NCH_2), 5.41 (s, 2H, OCH_2), 5.49 (br s, 1H, NH), 7.32–7.48 (m, 5H, Ar–H); ^{13}C NMR (150 MHz, $CDCl_3$; broadened signals were observed) δ 28.3 (Boc CH_3), 34.6 (NCH_2), 67.1 (OCH_2), 80.8 (Boc C^q), 128.54 and 128.56 (5×aryl CH), 135.3 (aryl C), 135.3 (br, triazole C-4), 144.1 (br, triazole C-5), both triazole carbons also evaluated by HBMC-NMR, 156.6 (Boc C=O), 161.3 (ester C=O); EIMS 332 (M^+). Anal. Calcd for $C_{16}H_{20}N_4O_4$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.98; H, 6.14; N, 16.81.

*4.7.2. 5-(N-tert-Butoxycarbonyl-N-methylaminomethyl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**5b**)*

Compound **3b** (100 mg, 0.330 mmol) was reacted at room temperature in DMSO (2.0 mL) with NaN_3 (214.5 mg, 3.30 mmol) and NH_4Cl (35.3 mg, 0.660 mmol) for 40 min following the general procedure. Upon addition of the reactants, the mixture turned blue and then quickly red. Flash column chromatography (hexanes/ethyl acetate 6:4) furnished 66.2 mg (58%) of **5b** as a yellowish resin. R_f 0.31 (hexanes/ethyl acetate 1:1); IR (neat) 3133, 2930, 1718, 1698, 1669 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.40 and 1.46 (2×br s, 9H, Boc CH_3), 2.86 and 2.91 (2×br s, 3H, NCH_3), 4.73 (s, 2H, NCH_2), 5.41 (s, 2H, OCH_2), 7.30–7.39 (m, 3H, Ar–H), 7.43–7.48 (m, 2H, Ar–H), 13.20 (br s, 1H, triazole–NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.5 and 29.8 (Boc CH_3), 35.6 (NCH_3), 41.8 and 44.1 (NCH_2), 67.1 (OCH_2), 81.2 (Boc C^q), 128.6, 128.7 and 128.8 (aryl CH), 135.5 (aryl C and br triazole C-4, evaluated by HBMC-NMR), 141.4 and 145.8 (br, triazole C-5), 156.2 and 157.3 (Boc C=O), 161.3 (ester C=O); EIMS 346 (M^+). Anal. Calcd for $C_{17}H_{22}N_4O_4$: C, 58.95; H, 6.40; N, 16.17. Found: C, 59.11; H, 6.51; N, 16.21.

*4.7.3. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-yl)-3H-1,2,3-triazole-4-carboxylic acid ethyl ester (**8a**)*

Compound **7a** (200.0 mg, 0.748 mmol) in DMSO (10.0 mL) was reacted with NaN_3 (243.2 mg, 3.74 mmol) and NH_4Cl (40.0 mg, 0.748 mmol) for 200 min at 100 °C following the general procedure. Flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 188.5 mg (81%) of **8a** as a colorless solid. Mp 176 °C; R_f 0.26 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D^{24}$ −18.8 (*c* 0.25, MeOH); IR (neat) 3127, 2978, 2249, 1735, 1718, 1700, 1660 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.21, 1.42 and 1.44 (3×br s, 12H, Boc CH_3 and OCH_2CH_3), 1.83–2.07 (br m, 3H, H-3/H-4 pyrrolidine), 2.29–2.49 (br m, 1H, H-3 or H-4 pyrrolidine), 3.39–3.76 (br m, 2H, H-5 pyrrolidine), 4.45 (br m, 2H, OCH_2), 5.46–5.54 (br m, 1H, H-2 pyrrolidine), 13.63 and 14.07 (2×br s, 1H, triazole–NH); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 14.2 (ester CH_3), 23.4 and 23.7 (C-4 pyrrolidine), 28.1 and 28.5 (Boc CH_3), 32.7 and 33.5 (C-3 pyrolidine), 46.7 and 47.1 (C-5 pyrrolidine), 52.9 and 53.4 (C-2 pyrolidine), 61.3 (OCH_2), 80.0 (Boc C^q), 134.7 (triazole C-5), 154.8 (Boc C=O), 161.4 (ester C=O), for the determination of the triazole C-4 the amount of substance was not sufficient; EIMS 310 (M^+). Anal. Calcd for $C_{14}H_{22}N_4O_4$: C, 54.18; H, 7.15; N, 18.05. Found: C, 54.26; H, 7.19; N, 18.08.

*4.7.4. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-yl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**8b**)*

Compound **7b** (329.4 mg, 1.000 mmol) in DMSO (5.0 mL) was reacted with NaN_3 (325.0 mg, 5.00 mmol) and NH_4Cl (53.5 mg, 1.000 mmol) in a sealed tube for 80 min at 100 °C following the general procedure. Flash column chromatography (CH_2Cl_2/acetone 9:1) furnished 167.1 mg (45%) of **8b** as a yellowish resin. R_f 0.49 (CH_2Cl_2/acetone 7:3); $[\alpha]_D^{24}$ −12.3 (*c* 1.0, $CHCl_3$); IR (neat) 3112, 2977, 2266, 1730, 1717, 1700, 1697, 1687, 1654 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.17 and 1.43 (2×s, 9H, Boc CH_3), 1.73–2.03 (br m, 3H, H-3/H-4 pyrrolidine), 2.19–2.41 (br m, 1H, H-3 or H-4 pyrrolidine), 3.37–3.57 (br m, 1H, H-5 pyrrolidine), 3.59–3.74 (br m, 1H, H-5 pyrrolidine), 5.24–5.53 (m, 3H, H-2 pyrrolidine/OCH_2), 7.29–7.39 (m, 3H, Ar–H), 7.39–7.48 (m, 2H, Ar–H), 13.53 (br s, 1H, triazole–NH); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 23.3 and 23.7 (C-4 pyrrolidine), 28.1, 28.5 and 29.6 (Boc CH_3), 32.8 and 33.5 (C-3 pyrrolidine), 46.7 and 47.0 (NCH_2), 52.8 and 53.2 (C-2 pyrrolidine), 66.8 (OCH_2), 80.0 and 80.3 (Boc C^q), 128.4, 128.5 and 128.6 (5CH), 134.2 (br, triazole C-4), 135.4 (aryl C), 150.1 (br, triazole C-5, signal is very weak), 154.7 (Boc C=O), 161.0 and 161.2 (ester C=O); EIMS 372 (M^+); HRMS calcd for $C_{19}H_{24}N_4O_4$ 372.1798; found 372.1797.

*4.7.5. 5-(N-tert-Butoxycarbonyl-2-aminoethyl)-3H-1,2,3-triazole-4-carboxylic acid ethyl ester (**15a**) and 2-[1-(2-tert-Butoxycarbonyl-aminoethyl)-1H-tetrazol-5-yl]-acetic acid ethyl ester (**16**)*

Compound **13a** (55.4 mg, 0.230 mmol) was reacted in DMF (3.5 mL) at 75 °C with NaN_3 (74.5 mg, 1.148 mmol) and NH_4Cl (12.7 mg, 0.295 mmol) for 3 h following the general procedure. Flash column chromatography (CH_2Cl_2/ethyl acetate 9:1) furnished 31.7 mg (49%) of **15a** as a colorless solid and 11.1 mg (16%) of **16** as colorless needles.

*4.7.5.1. Compound **15a**.* Mp 93–95 °C; R_f 0.25 (CH_2Cl_2/ethyl acetate 7:3); IR (neat) 3356, 3176, 3122, 2979, 1717, 1691 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 1.37 (t, J=7.2 Hz, 3H, OCH_2CH_3), 1.38 (s, 9H, Boc CH_3), 3.17–3.26 (m, 2H, NCH_2CH_2), 3.48–3.56 (m, 2H, NCH_2), 4.40 (q, J=7.1 Hz, 2H, OCH_2), 5.25 and 5.49 (2×br s, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.2 (ester CH_3), 25.5 (NCH_2CH_2), 28.3 (Boc CH_3), 39.2 and 40.4 (NCH_2), 61.3 (OCH_2), 80.0 and 80.7 (Boc C^q), 135.7 (triazole C-4), 144.0 (br, triazole C-5), both triazole carbons also confirmed by HBMC-NMR, 156.7 (Boc C=O), 161.7 (ester C=O); EIMS 284 (M^+).

*4.7.5.2. Compound **16**.* Mp 83–86 °C; R_f 0.49 (CH_2Cl_2/ethyl acetate 7:3); IR (neat) 3365, 2979, 1739, 1711 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.29 (t, 3H, J=7.2 Hz, OCH_2CH_3), 1.42 (s, 9H, Boc CH_3), 3.67 (dt, 2H, J=5.9, 5.7 Hz, NCH_2), 4.03 (s, 2H, CH_2C=O), 4.22 (q, 2H, J=7.2 Hz, OCH_2), 4.47 (t, 2H, J=5.7 Hz, NCH_2CH_2), 4.95 (br s, 1H, NH);

^{13}C NMR (90 MHz, $CDCl_3$) δ 14.0 (ester CH_3), 28.3 (Boc CH_3), 29.5 (CH_2C=O), 39.8 (NCH_2), 47.2 (NCH_2CH_2), 62.4 (OCH_2), 80.3 (Boc C^q), 149.7 (tetrazole C), 155.9 (Boc C=O), 166.6 (ester C=O); ESI-MS 322 $[M+Na]^+$, 300 $[M+H]^+$. Anal. Calcd for $C_{12}H_{21}N_5O_4$: C, 48.15; H, 7.07; N, 23.40. Found: C, 48.17; H, 7.12; N, 23.50.

4.7.6. 5-(2-tert-Butoxycarbonylaminoethyl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**15b**)

For the preparation of **15b**, we applied phase transfer conditions. Compound **13b** (105.2 mg, 0.347 mmol, contaminated with **13b′**), NaN_3 (227.6 mg, 3.0501 mmol) and tetrabutylammonium bromide (44.2 mg, 0.1371 mmol) were dissolved in $CHCl_3$/water (1:1, 4.0 mL) and three times subjected to microwave irradiation (constantly 75 W). Each time, the irradiation was stopped at a temperature of 55 °C. Then the separated aqueous layer was extracted with $CHCl_3$ (2×10 mL), the combined organic layers were washed with brine (10 mL) and dried over Na_2SO_4. After removal of the solvent, flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 38.5 mg (32%) of **15b** as a yellowish resin.

*4.7.6.1. Compound **15b**.* R_f 0.32 (CH_2Cl_2/MeOH 95:5); IR (neat) 3363, 3178, 3114, 2977, 2933, 2109, 1738, 1728, 1716, 1689, 1674 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.41 (s, 9H, Boc CH_3), 3.20 (br m, 2H, NCH_2CH_2), 3.51 (br m, 2H, NCH_2), 5.02 (br s, 1H, NH), 5.39 (s, 2H, OCH_2), 7.29–7.30 (m, 3H, Ar–H), 7.42–7.47 (m, 2H, Ar–H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 26.0 (NCH_2CH_2), 28.3 (Boc CH), 38.7 (NCH_2), 66.8 (OCH_2), 80.6 (Boc C^q), 128.4, 128.5 and 128.6 (aryl CH), 135.5 (aryl C), 135.6 (br, triazole C-4), 143.5 (v br, triazole C-5), 156.9 (Boc C=O), 161.4 (ester C=O); EIMS 346 (M^+). Anal. Calcd for $C_{17}H_{22}N_4O_4$: C, 58.95; H, 6.40; N, 16.17. Found: C, 58.47; H, 6.98; N, 15.88.

4.7.7. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-ylmethyl)-3H-1,2,3-triazole-4-carboxylic acid ethyl ester (**18a**)

Compound **17a** (102.5 mg, 0.3643 mmol) in DMSO (10.0 mL) was reacted in a sealed tube with NaN_3 (118.4 mg, 1.821 mmol) and NH_4Cl (19.5 mg, 0.364 mmol) for 3 h at 100 °C following the general procedure. Flash column chromatography (CH_2Cl_2/MeOH 9:1) furnished 26.4 mg (23%) of **18a** as a yellowish resin. R_f 0.17 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{22}$ +6.4 (*c* 1.0, $CHCl_3$); IR (neat) 3179, 3118, 2977, 2106, 1718, 1694, 1666 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; broadened signals were observed) δ 1.42 (t, *J*=7.2 Hz, 3H, OCH_2CH_3), 1.46 (s, 9H, Boc CH_3), 1.72–2.06 (br m, 3H, H-3/H-4 pyrrolidine), 3.18–3.52 (m, 4H, H-5 pyrrolidine and $CHCH_2$–triazole), 4.16–4.31 (m, 1H, H-2 pyrrolidine), 4.42 (q, *J*=7.2 Hz, 2H, OCH_2), 14.70 (br s, 1H, triazole–NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.3 (ester CH_3), 23.3 (C-4 pyrrolidine), 28.4 (Boc CH_3), 29.6 and 31.2 (C-3 pyrrolidine/CH_2–triazole), 46.6 (C-5 pyrrolidine), 56.4 (C-2 pyrrolidine), 61.0 (OCH_2), 81.0 (Boc C^q), 135.6 (br, triazole C-4), 141.3 (v br, triazole C-5), 155.9 (Boc C=O), 162.0 (ester C=O); EIMS 324 (M^+); HR-EIMS calcd for $C_{15}H_{24}N_4O_4$ 324.1798; found 324.1798.

4.7.8. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-ylmethyl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**18b**)

Compound **17b** (429.3 mg, 1.250 mmol) in DMF (5.0 mL) was reacted in a sealed tube with NaN_3 (406.3 mg, 6.250 mmol) and NH_4Cl (67.0 mg, 1.250 mmol) for 1 h at 100 °C following the general procedure. Flash column chromatography (hexanes/ethyl acetate 9:1) furnished 239.1 mg (49%) of **18b** as a yellowish resin. R_f 0.35 (hexanes/ethyl acetate 9:1); $[\alpha]_D^{24}$ +12.4 (*c* 0.5, $CHCl_3$); IR (neat) 3169, 3114, 2976, 2249, 2107, 1720, 1692, 1652 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 1.32 and 1.46 (2×s, 9H, Boc CH_3), 1.60–2.04 (br m, 4H, H-3/H-4 pyrrolidine), 3.05–3.47 (m, 4H, H-5 pyrrolidine and $CHCH_2$–triazole), 4.16–4.30 (m, 1H, H-2 pyrrolidine), 5.38 and 5.43 (2×d, each *J*=12.3 Hz, 2H, OCH_2), 7.27–7.39 (m, 3H, Ar–H), 7.44–7.49 (m, 2H, Ar–H), 14.80 (br s, 1H, triazole–NH); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers and broadened signals were observed) δ 22.6 and 23.3 (C-4 pyrrolidine), 28.4 (Boc CH_3), 29.4 and 30.9 (C-3 pyrrolidine/CH_2–triazole), 46.0 and 46.6 (C-5 pyrrolidine), 56.3 (C-2 pyrrolidine), 66.6 (OCH_2), 79.8 and 80.8 (Boc C^q), 128.3 and 128.6 (aryl CH), 135.4 (br, triazole C-4), 135.7 (aryl C), 141.3 (very weak signal, triazole C-5), 154.9 and 155.9 (Boc C=O), 161.4 and 161.8 (ester C=O); EIMS 386 (M^+). Anal. Calcd for $C_{20}H_{26}N_4O_4\times2/3H_2O$: C, 60.29; H, 6.91; N, 14.06. Found: C, 59.94; H, 6.66; N, 13.71.

4.7.9. 5-((S)-2-tert-Butoxycarbonylamino-propyl)-3H-1,2,3-triazole-4-carboxylic acid benzyl ester (**25a**)

The mixture of compounds **24a**/**a′** (246.7 mg, 0.777 mmol) in DMF (6.0 mL) was reacted in a sealed tube with NaN_3 (252.9 mg, 3.890 mmol) and NH_4Cl (40.5 mg, 0.757 mmol) for 95 min at 105 °C following the general procedure. Flash column chromatography (CH_2Cl_2/MeOH 98:2) furnished 102.0 mg (36%) of **25a** as a yellowish resin. R_f 0.46 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −9.5 (*c* 1.833, $CHCl_3$); IR (neat) 3363, 3178, 3114, 2976, 2252, 2103, 1716 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$; broadened signals were observed) δ 1.20 (d, 3H, *J*=4.5 Hz, $CHCH_3$), 1.37 (s, 9H, CH_3 Boc), 3.00–3.25 (m, 2H, CH_2–triazole), 3.96–4.13 (m, 1H, CH), 4.85 (br s, 1H, NH), 5.40 (s, 2H, OCH_2), 7.29–7.38 (m, 3H, Ar–H), 7.42–7.50 (m, 2H, Ar–H), 14.23 (br s, 1H, triazole–NH); ^{13}C NMR (90 MHz, $CDCl_3$; broadened signals were observed) δ 21.5 ($CHCH_3$), 28.3 (Boc CH_3), 32.3 (CH_2–triazole), 46.1 (NCH), 66.8 (OCH_2), 80.5 (Boc C^q), 128.4, 128.5 and 128.6 (5×aryl CH), 135.5 (aryl C), 135.9 (triazole C-4), 142.4 (br, triazole C-5), 156.3 (Boc C=O), 161.6 (ester C=O); EIMS 360 (M^+). Anal. Calcd for $C_{18}H_{24}N_4O_4\times1/8H_2O$: C, 59.61; H, 6.74; N, 15.45. Found: C, 59.87; H, 6.77; N, 15.01.

4.7.10. 5-((S)-2-tert-Butoxycarbonylamino-3-phenylpropyl)-3H-[1,2,3]triazole-4-carboxylic acid benzyl ester (**25b**)

The mixture of compounds **24b**,**b′** (128.8 mg, 0.327 mmol) in DMF (4.0 mL) was reacted in a sealed tube with NaN_3 (120.0 mg, 0.327 mmol) and NH_4Cl (18.5 mg, 0.346 mmol) for 95 min at 105 °C following the general procedure. Flash column chromatography (CH_2Cl_2/MeOH 98:2) furnished 102.0 mg (38%) of **25b** as a yellowish resin. R_f 0.50 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −1.3 (*c* 1.505, $CHCl_3$); IR (neat) 3368, 3172, 2929, 2251, 2104, 1714 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$; broadened signals were observed) δ 1.31 (br s, 9H, CH_3 Boc), 2.71–2.93 (m, 2H, CH_2Ph or CH_2–triazole), 3.04–3.28 (m, 2H, $CHCH_2$–Ph or CH_2–triazole), 4.11–4.31 (m, 1H, NCH), 4.84 (br s, 1H, NH), 5.36 (s, 2H, OCH_2), 7.10–7.17 (m, 2H, Ar–H), 7.18–7.24 (m, 1H, Ar–H), 7.24–7.30 (m, 2H, Ar–H), 7.30–7.39 (m, 3H, Ar–H), 7.41–7.46 (m, 2H, Ar–H), 13.95 (br s, 1H, triazole–NH); ^{13}C NMR (150 MHz, $CDCl_3$; broadened signals were observed) δ 28.2 (Boc CH_3), 30.1 (CH_2–triazole), 41.7 (CH_2–Ph), 50.9 (NCH), 66.9 (OCH_2), 80.5 (Boc C^q), 126.8, 128.4, 128.57, 128.63 and 129.3 (10×aryl CH), 135.4 (aryl C), 135.8 (br, triazole C-4), 137.0 (aryl C), 143.4 (br, triazole C-5), 156.4 (Boc C=O), 161.5 (ester C=O); EIMS 345 $[M-C_7H_7]^+$. Anal. Calcd for $C_{24}H_{28}N_4O_4$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.93; H, 6.54; N, 12.21.

4.8. General procedure for hydrogenolytic debenzylation of the benzyl esters (10, 15c, and 20)

The benzyl ester derivatives were dissolved in ethyl acetate or ethyl acetate/methanol (1:1), then $Pd(OH)_2$/C was added, then repetitive cycles of evaporation and exposition with H_2 were performed and stirring under H_2 atmosphere at room temperature was continued. After TLC indicated complete conversion of the starting materials, the mixture was filtered over Celite, concentrated in vacuo, and the crude product was purified by flash column chromatography.

4.8.1. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-yl)-3H-1,2,3-triazole-4-carboxylic acid (**10**)

Compound **8b** (73.8 mg, 0.198 mmol) in ethyl acetate/methanol 1:1 (6 mL) was reacted for 50 min following the general procedure with H_2 and $Pd(OH)_2/C$ (40 mg; ~20% Pd). Flash column chromatography (CH_2Cl_2/MeOH/formic acid 95:5:0.025) furnished 45.1 mg (81%) of **10** as a colorless solid. Mp 142–144 °C; R_f 0.49 (CH_2Cl_2/MeOH/formic acid 90:10:0.05); $[\alpha]_D^{24}$ −28.4 (*c* 0.25, MeOH); IR (neat) 3549, 3464, 3103, 2981, 1693, 1660, 1598 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$/DMSO-d_6=1:3; rotamers were observed) δ 1.14 and 1.37 (2×s, 9H, Boc CH_3), 1.70–2.03 and 2.14–2.38 (2×br m, 4H, H-3/H-4 pyrrolidine), 3.33–3.47 and 3.53–3.64 (2×m, 2H, H-5 pyrrolidine), 5.40 (dd, *J*=8.1, 3.5 Hz, 1H, H-2 pyrrolidine), 15.14 (br s, 1H, triazole NH); EIMS 282 (M^+).

4.8.2. 5-(2-tert-Butoxycarbonylaminoethyl)-3H-1,2,3-triazole-4-carboxylic acid (**15c**)

Compound **15b** (31.0 mg, 0.090 mmol) in ethyl acetate (2 mL) was reacted for 2 h following the general procedure with H_2 and $Pd(OH)_2/C$ (62 mg; ~20% Pd). Flash column chromatography (CH_2Cl_2/MeOH/formic acid 90:10:0.05) furnished 7.5 mg (33%) of **15c** as a colorless solid. Mp 155 °C (decomposition); R_f 0.18 (CH_2Cl_2/MeOH/formic acid 90:10:0.5); IR (neat) 3343, 3127, 2932, 1712, 1698 cm^{-1}; ^1H NMR (360 MHz, DMSO-d_6; rotamers were observed) δ 1.29 and 1.35 (2×br s, 9H, Boc CH_3), 2.99 (t, 2H, *J*=6.8 Hz, NCH_2CH_2), 3.22 (dt, *J*=6.8, 6.4 Hz, 2H, NCH_2), 6.50 and 6.89 (2×br s, 1H, *J*=6.4 Hz, CONH); EIMS 256 (M^+); HR-EIMS calcd for $C_{10}H_{16}N_4O_4$ 256.1172; found 256.1174.

4.8.3. 5-((S)-1-tert-Butoxycarbonylpyrrolidin-2-ylmethyl)-3H-1,2,3-triazole-4-carboxylic acid (**20**)

Compound **18b** (38.7 mg, 0.100 mmol) in ethyl acetate (2 mL) was reacted for 80 min following the general procedure with H_2 and $Pd(OH)_2/C$ (22 mg; ~20% Pd). Flash column chromatography (CH_2Cl_2/MeOH/formic acid 95:5:0.025) furnished 29.6 mg (99%) of **20** as a colorless solid. Mp 126–130 °C; R_f 0.38 (CH_2Cl_2/MeOH/formic acid 90:10:0.05); $[\alpha]_D^{24}$ +5.1 (*c* 1.0, MeOH); IR (neat) 3429, 3236, 3180, 3124, 2976, 1720, 1693, 1664, 1649 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.48 (br s, 9H, Boc CH_3), 1.70–2.21 (m, 4H, H-3/H-4 pyrrolidine), 3.25–3.55 (m, 4H, H-5 pyrrolidine and CH_2–triazole), 4.25–4.37 (m, 1H, H-2 pyrrolidine), 9.54 (br s, 2H, OH and triazole NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 23.2 (C-4 pyrrolidine), 28.4 (Boc CH_3), 29.0 (CH_2–triazole), 30.8 (C-3 pyrrolidine), 46.7 (C-5 pyrrolidine), 56.5 (C-2 pyrrolidine), 81.1 (Boc C^q), (triazole C-4), 135.2 (br, triazole C-5), 141.6 (br, triazole C-4), 156.0 (Boc C=O), 163.3 (acid C=O); HRMS calcd for $C_{13}H_{20}N_4O_4$ 296.1485; found 296.1484.

4.9. 5-(2-*tert*-Butoxycarbonylaminoethyl)-3*H*-1,2,3-]triazole-4-carboxylic acid (10)

A solution of **8a** (15.4 mg, 0.054 mmol) in THF/H_2O 1:1 (4.0 mL) was stirred at 0 °C and an aqueous solution of LiOH (2 N, 0.5 mL) was added. The mixture was allowed to warm to room temperature and was then refluxed for 70 min. After cooling to room temperature, HCl (2 N, 5.0 mL) and water (20 mL) were added and extraction with ethyl acetate (4×20 mL) was performed. The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was evaporated. Flash column chromatography (CH_2Cl_2/MeOH/formic acid 90:10:0.05) furnished 9.5 mg (69%) of **10** as a colorless solid. Analytical data see 4.8.1.

4.10. General procedure for amide formation via HATU coupling (11, 21, 26a,b, and 27a,b)

The acids and HATU were dissolved in dry DMF and cooled to 0 °C. Then base (1.1 equiv DIPEA) and subsequently the corresponding primary amine derivative was added dropwise (in case of **26a**,**b** and **27a**,**b** DIPEA was omitted). The mixture was allowed to warm to room temperature, and, after TLC had indicated complete conversion of the starting materials, the solvent was removed. In case of **11**, **21** **27a**,**b**, the residue was directly subjected to flash column chromatography. In case of **26a**,**b** the solid residue was partitioned between CH_2Cl_2 (10 ml) and HCl (2 N, 10 mL), the aqueous layer was extracted with CH_2Cl_2 (3×5 mL), the combined organic layers were washed with brine (10 mL), and dried over Na_2SO_4. After removal of the solvent, the residue was subjected to flash column chromatography.

4.10.1. (S)-2-(5-Methylcarbamoyl-1H-1,2,3-triazol-4-yl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**11**)

Compound **10** (27.7 mg, 0.098 mmol) in DMF (2.0 mL) was reacted with HATU (41.0 mg, 0.108 mmol)/DIPEA (18 μL, 0.108 mmol) and solution of methylamine in ethanol (8 M, 200 μL) following the general procedure. Reaction time: 22 h. Flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 22.7 mg (78%) of **11** as a colorless solid. Mp 118–120 °C; R_f 0.51 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −26.4 (*c* 0.25, MeOH); IR (neat) 3427, 3328, 3131, 2977, 2247, 1691, 1653 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.28 and 1.46 (2×br s, 9H, Boc CH_3), 1.78–2.64 (m, 4H, H-3/H-4 pyrrolidine), 3.02 (d, 3H, *J*=5.0 Hz, NCH_3), 3.48–3.61 (m, 1H, H-5 pyrrolidine), 3.62–3.68 (m, 2H, H-5 pyrrolidine), 5.36 and 5.51 (2×br s, 1H, H-2 pyrrolidine); ^{13}C NMR (90 MHz, $CDCl_3$) δ 23.6 (C-4), 25.8 (NCH), 28.3 (Boc CH_3), 33.8 (C-3), 47.1 (C-5), 52.9 (C-2), 80.5 (Boc C^q), 136.5 (br, triazole C-5), 145.4 (br) (triazole C-4), 155.2 (Boc C=O), 161.4 (amide C=O); EIMS 295 (M^+); HRMS calcd for $C_{13}H_{21}N_5O_3$ 295.1644; found 295.1645.

4.10.2. (S)-2-(5-Methylcarbamoyl-1H-1,2,3-triazol-4-ylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**21**)

Compound **20** (28.4 mg, 0.096 mmol) in DMF (2.0 mL) was reacted with HATU (40.7 mg, 0.107 mmol)/DIPEA (18 μL, 0.108 mmol) and a solution of methylamine in ethanol (8 M, 200 μL) following the general procedure. Reaction time: 22 h. Flash column chromatography (CH_2Cl_2/MeOH 98:2) furnished 27.4 mg (93%) of **21** as a colorless resin. R_f 0.59 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ +1.3 (*c* 1.0, MeOH); IR (neat) 3427, 3330, 3143, 3114, 2976, 2242, 1666, 1601 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; broadened signals were observed) δ 1.46 (s, 9H, Boc CH_3), 1.78–2.14 (m, 4H, H-3/H-4 pyrrolidine), 2.99 (d, *J*=5.0, 3H, NCH_3), 3.26 (dd, 1H, *J*=16.3, 7.2 Hz, $CHCH_2$–triazole), 3.31–3.44 (m, 2H, H-5 pyrrolidine), 3.44 (dd, *J*=16.3, 5.1 Hz, 1H, $CHCH_2$–triazole), 4.14–4.25 (m, 1H, H-2 pyrrolidine), 7.40 (br s, 1H, CONH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.4 (C-4 pyrrolidine), 25.7 (NCH_3), 28.5 (Boc CH_3), 29.7 and 31.5 (2×br, C-3 pyrrolidine/CH_2–triazole), 46.7 (C-5 pyrrolidine), 56.5 (C-5 pyrrolidine), 81.0 (Boc C^q), 137.7 (br, triazole), 156.2 (Boc C=O), 162.2 (amide C=O), for the determination of the other triazole carbon the amount of substance was insufficient; EIMS 309 (M^+); HRMS calcd for $C_{13}H_{21}N_5O_3$ 295.1644; found 295.1645.

4.10.3. (S)-2-(5-((R)-1-Phenylethylcarbamoyl)-1H-1,2,3-triazol-4-yl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**26a**)

Compound **10** (2.7 mg, 0.0096 mmol) in DMF (1.5 mL) was reacted with HATU (7.3 mg, 0.0192 mmol) and a 10% (*m*/*V*) solution of (*R*)-phenylethylamine in DMF (25 μL, 0.0192 mmol) following the general procedure stirring 4 h 30 min at 0 °C and 6 h at room temperature. Flash column chromatography (CH_2Cl_2/MeOH 97:3) furnished 1.7 mg (46%) of **26a** as a colorless resin. R_f 0.40 (CH_2Cl_2/MeOH 9:1); HPLC: t_R 24.2 min (Luna silica gel 5 μm, 4.6×250 mm, hexanes/2-propanol 99:1, 0.25 mL/min, 219/230/254 nm detection; 98.3% de); $[\alpha]_D^{23}$ −32.0 (*c* 0.100, $CHCl_3$); IR (neat) 3407, 3138, 2925,

1693, 1676, 1666, 1653 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.26 (br s, 9H, Boc CH_3), 1.59 (d, 3H, J=6.9 Hz, CHCH_3), 1.80–2.15 (m, 3H, H-3/H-4 pyrrolidine), 2.22–2.58 (m, 1H, H-3 or H-4 pyrrolidine), 3.45–3.57 (m, 1H, H-5 pyrrolidine), 3.57–3.67 (m, 1H, H-5 pyrrolidine), 5.27 (dq, J=6.9, 6.9 Hz, 1H, CHCH$_3$), 5.41–5.69 (br m, 1H, H-2 pyrrolidine), 7.20–7.56 (m, 6H, Ar–H/CONH); EIMS 385 (M^+); HRMS calcd for $C_{20}H_{27}N_5O_3$ 385.2114; found 385.2115.

4.10.4. (S)-2-(5-((S)-1-Phenylethylcarbamoyl)-1H-1,2,3-triazol-4-yl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**26b**)

Compound **10** (3.0 mg, 0.0106 mmol) in DMF (1.5 mL) was reacted with HATU (8.1 mg, 0.0212 mmol) and a 10% (m/V) solution of (*S*)-phenylethylamine in DMF (28 μL, 0.0212 mmol) following the general procedure stirring 4 h 30 min at 0 °C and 6 h at room temperature. Flash column chromatography (CH_2Cl_2/MeOH 97:3) furnished 1.0 mg (24%) of **26b** as a colorless resin. R_f 0.40 (CH_2Cl_2/MeOH 9:1); HPLC: t_R 21.6 min (silica gel 5 μm, 4.6×250 mm, hexanes/2-propanol 99:1, 0.25 mL/min, 219/230/254 nm detection; 96.4% de); $[\alpha]_D^{23}$ +33.0 (*c* 0.091, $CHCl_3$); IR (neat) 3407, 3301, 3138, 2925, 1693, 2252, 1695, 1680, 1666, 1654 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$; rotamers were observed) δ 1.26, 1.43 (2×br s, 9H, Boc CH_3), 1.59 (d, 3H, J=7.0 Hz, CHCH_3), 1.74–2.49 (br m, 4H, H-3/H-4 pyrrolidine), 3.27–3.71 (br m, 2H, H-5 pyrrolidine), 5.28 (dq, J=7.0, 7.0 Hz, 1H, CHCH$_3$), 5.20–5.63 (br m, 1H, H-2 pyrrolidine), 7.17–7.51 (m, 6H, Ar–H/NH); EIMS 385 (M^+); HRMS calcd for $C_{20}H_{27}N_5O_3$ 385.2114; found 385.2114.

4.10.5. (S)-2-(5-((R)-1-Phenylethylcarbamoyl)-1H-1,2,3-triazol-4-ylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**27a**)

Compound **20** (6.7 mg, 0.0226 mmol) in DMF (2.0 mL) was reacted with HATU (17.2 mg, 0.0452 mmol) and (*R*)-phenylethylamine (7 μL, 0.0507 mmol) following the general procedure stirring 4 h 50 min at room temperature. Flash column chromatography (CH_2Cl_2/acetone 9:1) furnished 4.1 mg (46%) of **27a** as a colorless resin. R_f 0.37 (CH_2Cl_2/acetone 85:15); HPLC: t_R 15.8 min (silica gel 5 μm, 4.6×250 mm, hexanes/2-propanol 92:8, 0.25 mL/min, 219/230/254 nm detection; 98.7% de); $[\alpha]_D^{26}$ −42.6 (*c* 0.1833, $CHCl_3$); IR (neat) 3409, 3126, 2925, 1689, 1668, 1657 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 1.48 (br s, 9H, Boc CH_3), 1.59 (d, J=7.0 Hz, 3H, CHCH_3), 1.80–2.04 (m, 4H, H-3/H-4 pyrrolidine), 3.23 (dd, J=17.0, 8.8 Hz, 1H, CH_2–triazole), 3.33 (ddd, J=11.0, 7.8, 3.1 Hz, 1H, H-5 pyrrolidine), 3.41–3.48 (m, 2H, H-5 pyrrolidine/CH_2–triazole), 4.18–4.24 (m, 1H, H-2 pyrrolidine), 5.25 (dq, J=7.0, 7.0 Hz, 1H, CHCH$_3$), 7.23–7.40 (m, 10H, Ar–H), 7.54 (br s, 1H, CONH); EIMS 399 (M^+); HRMS calcd for $C_{21}H_{29}N_5O_3$ 399.2270; found 399.2270.

4.10.6. (S)-2-(5-((S)-1-Phenylethylcarbamoyl)-1H-1,2,3-triazol-4-ylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**27b**)

Compound **20** (7.1 mg, 0.0478 mmol) in DMF (2.0 mL) was reacted with HATU (18.2 mg, 0.0212 mmol) and (*S*)-phenylethylamine (7 μL, 0.0507 mmol) following the general procedure stirring 3 h 45 min at room temperature. Flash column chromatography (CH_2Cl_2/acetone 9:1) furnished 5.4 mg (56%) of **27b** as a colorless solid. R_f 0.18 (CH_2Cl_2/MeOH 95:5); HPLC: t_R 14.4 min (silica gel 5 μm, 4.6×250 mm, hexanes/2-propanol 92:8, 0.25 mL/min, 219/230/254 nm detection; 97.8% de); $[\alpha]_D^{26}$ +13.6 (*c* 0.184, $CHCl_3$); IR (neat) 3411, 3302, 3138, 2976, 1691, 1666, 1656 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.47 (s, 9H, Boc CH_3), 1.59 (d, J=6.9 Hz, 3H, CHCH_3), 1.79–2.12 (m, 4H, H-3/H-4 pyrrolidine), 3.19 (dd, J=16.6, 7.7 Hz, 1H, CH_2–triazole), 3.27–3.37 (m, 1H, H-5 pyrrolidine), 3.37–3.52 (m, 2H, H-5 pyrrolidine/CH_2–triazole), 4.14–4.26 (m, 1H, H-2 pyrrolidine), 5.26 (dq, J=6.9, 6.9 Hz, 1H, CHCH$_3$), 7.21–7.42 (m, 5H, Ar–H), 7.51 (br s, 1H, CONH); EIMS 399 (M^+); HRMS calcd for $C_{21}H_{29}N_5O_3$ 399.2270; found 399.2270.

4.11. General procedure for the synthesis of the *N*-acetyl derivatives (9, 12, 19, 22)

The *N*-Boc protected compounds were dissolved in CH_2Cl_2 at 0 °C, trifluoroacetic acid (TFA) was added and the mixture was allowed to warm to rt. After 25–50 min, the solvent was removed and residual TFA was removed by redissolving in CH_2Cl_2 and subsequent evaporation. The residue was dissolved in CH_2Cl_2 and DIPEA and subsequently acetyl chloride was added at 0 °C. Stirring was continued until TLC (detection: ninhydrin solution at 150 °C for 15–25 min; in case of **19** HPLC–MS analysis) indicated complete conversion. The solvent was removed and the crude products were purified by flash column chromatography.

4.11.1. 5-((S)-1-Acetylpyrrolidin-2-yl)-1H-1,2,3-triazole-4-carboxylic acid ethyl ester (**9**)

A solution of compound **8a** (40.0 mg, 0.130 mmol) in CH_2Cl_2 (2.0 mL) was reacted with TFA (1.0 mL) for 50 min. Acetylation: DIPEA (48 μL, 0.299 mol) and acetyl chloride (17.0 μL, 0.236 mmol) in CH_2Cl_2 (1.0 mL) for 16 h. Flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 20.1 mg (62%) of **9** as a colorless solid. Mp 228 °C; R_f 0.29 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −57.2 (*c* 0.5, MeOH); IR (neat) 3437, 3153, 2960, 2885, 2785, 2717, 2615, 1734, 1606 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$; rotamers were observed, for the determination of the *cis/trans* ratio in $CDCl_3$ see Supplementary data) δ 1.40 and 1.44 (2×t, each J=7.2 Hz, 3H, OCH$_2$CH_3), 1.87 (s, 1.2H, acetyl CH_{3cis}), 1.89–2.08 (m, 3H, H-3/H-4 pyrrolidine), 2.14 (s, 1.8H, acetyl CH_{3trans}), 2.30–2.38 and 2.43–2.51 (m, 1H, H-3 or H-4 pyrrolidine), 3.56–3.68 (m, 1H, H-5 pyrrolidine), 3.82–3.93 (m, 1H, H-5 pyrrolidine), 4.35–4.48 (m, 2H, OCH_2), 5.60 (dd, J=8.0, 1.7 Hz, 0.4H, H-5 pyrrolidine$_{cis}$), 5.63 (dd, J=8.0, 1.7 Hz, 0.60H, H-5 pyrrolidine$_{trans}$); ^{13}C NMR (90 MHz, $CDCl_3$/DMSO-d_6=1:1; *cis/trans* rotamers were observed) δ 13.3 (ester CH_3), 21.1 (acetyl CH_{3cis} or C-4 pyrrolidine$_{cis}$), 21.2 (C-4 pyrrolidine$_{cis}$ or acetyl CH_{3cis}), 21.6 (acetyl CH_{3trans} or C-4$_{trans}$), 22.9 (C-4 pyrrolidine$_{trans}$ or acetyl CH_{3trans}), 31.4 and 33.1 (C-3 pyrrolidine), 45.4 and 46.9 (C-5 pyrrolidine), 51.5 and 53.1 (C-2 pyrrolidine), 59.7 and 60.1 (OCH_2), 132.6 and 132.8 (triazole C-4), 146.6 and 146.7 (triazole C-5), 160.1 and 160.2 (ester C=O), 168.2 and 168.4 (amide C=O); EIMS 252 (M^+); HRMS calcd for $C_{11}H_{16}N_4O_3$ 252.1222; found 252.1222.

4.11.2. 5-((S)-1-Acetylpyrrolidin-2-yl)-3H-1,2,3-triazole-4-carboxylic acid methylamide (**12**)

A solution of compound **11** (15.0 mg, 0.051 mmol) in CH_2Cl_2 (3.0 mL) was reacted with TFA (1.0 mL) for 50 min. Acetylation: DIPEA (20 μL, 0.122 mol) and acetyl chloride (17.0 μL, 0.236 mmol) in CH_2Cl_2 (2.0 mL) for 16 h. Flash column chromatography (CH_2Cl_2/MeOH 95:5 to 9:1) furnished 9.2 mg (77%) of **12** as a colorless solid. Mp 180–181 °C; R_f 0.24 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −44.4 (*c* 0.25, MeOH); IR (neat) 3427, 3369, 3311, 3139, 3091, 2927, 1660, 1606 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$/DMSO-d_6=95:5; *cis/trans* rotamers were observed) δ 1.77 (s, 1.35H, acetyl CH_{3cis}), 1.82–1.89 and 1.90–1.98 (2×m, 2H, H-4 or H-3 pyrrolidine), 2.00–2.06 and 2.07–2.18 (2×m, 1H, H-3 or H-4 pyrrolidine), 2.02 (s, 1.65H, acetyl CH_{3trans}), 2.90 and 2.91 (2×d, J=5.7 and 5.3 Hz, 3H, NCH_3), 3.44–3.55 (m, 1H, H-5 pyrrolidine), 3.70–3.79 (m, 1H, H-5 pyrrolidine), 5.47 (dd, J=7.8, 1.6 Hz, 0.55H, H-2 pyrrolidine$_{trans}$), 5.68 (dd, J=8.1, 2.1 Hz, 0.45H, H-2 pyrrolidine$_{cis}$), 7.15 (br s, 0.45H, CONH$_{cis}$), 7.88 (br s, 0.55H, CONH$_{trans}$); ^{13}C NMR (90 MHz, $CDCl_3$/DMSO-d_6 3:1; *cis/trans* rotamers were observed) δ 21.3, 21.7, 24.67 and 24.73 (acetyl CH_3/NCH_3/C-4 pyrrolidine), 31.6 and 33.3 (C-3 pyrrolidine), 45.5 and 47.1 (NCH_2–pyrrolidine), 51.3 and 52.9 (C-4 pyrrolidine), 135.3 and 135.8 (triazole C-4), 143.5 and 144.8 (v br, triazole C-5), 160.6 and 160.7 (amide C=O), 168.4 and 168.6 (acetyl C=O); EIMS 237 (M^+); HRMS calcd for $C_{11}H_{16}N_4O_3$ 237.1226; found 237.1226.

4.11.3. 5-((S)-1-Acetylpyrrolidin-2-ylmethyl)-3H-1,2,3-triazole-4-carboxylic acid ethyl ester (**19**)

A solution of compound **18a** (13.7 mg, 0.042 mmol) in CH_2Cl_2 (1.5 mL) was reacted with TFA (0.5 mL) for 45 min. Acetylation: DIPEA (9 μL, 0.084 mmol) and acetyl chloride (3 μL, 0.063 mmol) in CH_2Cl_2 (1.0 mL) for 5 h and after evaporation of the solvent again with DIPEA (18 μL, 0.170 mmol) and acetyl chloride (15 μL, 0.326 mmol) in CH_2Cl_2 (1.0 mL) for 3.5 h. Flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 4.4 mg (62%) of **19** as a colorless solid. Mp 129–131 °C; R_f 0.38 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{25}$ −4.0 (*c* 0.25, MeOH); IR (neat) 3103, 2980, 2276, 2102, 1732, 1717, 1652, 1634, 1608 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$/DMSO-d_6 97.5:2.5; *cis*/*trans* rotamers were observed) δ 1.39 (t, *J*=7.2 Hz, 3H, OCH_2CH_3), 1.71–2.01 (m, 4H, H-3/H-4 pyrrolidine), 2.06 (s, 1.8H, acetyl CH_{3trans}), 2.11 (s, 1.2H, acetyl CH_{3cis}), 3.06–3.12, 3.16–3.26, 3.34–3.49, 3.46–3.54 and 3.61–3.66 (5×m, 4H, CH_2–triazole/H-5 pyrrolidine), 4.21–4.28 (m, 0.4H, H-2 pyrrolidine$_{cis}$), 4.39 (q, 2H, *J*=7.2 Hz, OCH_2), 4.44–4.50 (m, 1H, H-2 pyrrolidine$_{trans}$); ^{13}C NMR (90 MHz, $CDCl_3$; *cis*/*trans* rotamers were observed) δ 14.3 (ester CH_3), 21.8 (acetyl CH_{3cis}), 22.8 (acetyl CH_{3trans}), 23.6 (C-4 pyrrolidine), 27.7 (CH_2–triazole), 29.9 and 30.3 (C-3 pyrrolidine), 45.6 and 48.0 (C-5 pyrrolidine), 56.8 and 57.8 (C-2 pyrrolidine), 61.0 (OCH_2), 135.7 (br, triazole C-5), 140.7 (br, triazole C-4), 162.2 (ester C═O), 170.0 and 171.2 (amide C═O); EIMS 266 (M^+); HRMS calcd for $C_{12}H_{18}N_4O_3$ 266.1379; found 266.1376.

4.11.4. 5-((S)-1-Acetylpyrrolidin-2-ylmethyl)-3H-1,2,3-triazole-4-carboxylic acid methylamide (**22**)

A solution of compound **21** (12.0 mg, 0.039 mmol) in CH_2Cl_2 (2.0 mL) was reacted with TFA (0.5 mL) for 25 min. Acetylation: DIPEA (13 μL, 0.079 mol) and acetyl chloride (5.0 μL, 0.071 mmol) in CH_2Cl_2 (1.0 mL) for 5 h, the same procedure was repeated for 4 h. Flash column chromatography (CH_2Cl_2/MeOH 95:5) furnished 3.8 mg (39%) of **22** as a colorless resin/solid. Mp 65–67 °C; R_f 0.41 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{24}$ +1.7 (*c* 0.33, MeOH); IR (neat) 3421, 3330, 3102, 2974, 2243, 1647, 1556, 1543 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$, only the trans rotamer was observed) δ 1.84–2.09 (m, 3H, H-3/H-4 pyrrolidine), 2.11–2.27 (m, 1H, H-3 or H-4 pyrrolidine), 2.17 (s, 3H, acetyl CH_3), 2.99 and 3.01 (2×d, each *J*=4.8 Hz, 3H, NCH_3), 3.28 (dd, *J*=15.1, 5.4 Hz, 1H, CH_2–triazole), 3.47 (ddd, *J*=10.1, 9.9, 7.7 Hz, 1H, H-5 pyrrolidine), 3.48–3.56 (m, 1H, CH_2–triazole), 3.59 (ddd, 10.1, 8.7, 2.6 Hz, 1H, H-5 pyrrolidine), 4.27–4.55 (m, 1H, H-2 pyrrolidine), 7.13 and 7.24 (2×br s, 1H, CONH), 15.18 (br s, 1H, triazole NH); ^{13}C NMR (90 MHz, $CDCl_3$) δ 22.8, 23.7, 25.6, 28.3 and 29.7 (acetyl CH_3, NCH_3, CH_2–triazole, C-4 pyrrolidine), 30.6 (C-3 pyrrolidine), 48.0 (C-5 pyrrolidine), 56.8 (C-2 pyrrolidine), 137.5 (br, triazole C-5), 162.2 (br, amide), for the determination of the triazole C-4 the amount of substance was insufficient; HRMS calcd for $C_{11}H_{17}N_5O_2$ 251.1382; found 251.1383.

4.12. X-ray crystal structure determination of 16

A colorless needle of approximately 0.30×0.08×0.07 mm in size was coated with protective perfluoropolyalkyl ether oil and mounted in the cold nitrogen gas stream of a Bruker-Nonius KappaCCD diffractometer. Data were collected at 100 K using Mo Kα radiation (graphite monochromator). Diffraction intensities were corrected for Lorentz and polarization effects. A semi-empirical absorption correction based on multiple measurements of reflections was applied (T_{min}=0.918, T_{max}=0.993).[32] The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2.[33] All non-hydrogen atoms were refined with anisotropic displacement parameters. The position of N5 bound hydrogen atom H5, which is involved in hydrogen bonding was derived from a difference Fourier synthesis while all other hydrogen atoms were placed in positions of optimized geometry and allowed to ride on their corresponding carbon atoms. The isotropic displacement parameters of all hydrogen atoms were tied to those of the adjacent carbon atoms by a factor of 1.5.

$C_{12}H_{21}N_5O_4$, triclinic, *P*-1 (no. 2), *a*=6.1775(3), *b*=10.8036(6), *c*=12.8540(9) Å, α=74.855(4)°, β=88.887(4)°, γ=76.713(4)°, *V*=805.15(8) $Å^3$, *Z*=2, ρ_{calcd}=1.235 Mg/m^3, μ(Mo Kα)=0.094 mm^{-1}, *F*(000)=320, 17,856 reflections collected, 3541 independent reflections, 2701 observed reflection [$I>2\sigma(I)$], $R_1[I>2\sigma(I)]$=0.0463, wR_2 (all data)=0.1140, GooF (all data)=1.147, $\Delta\rho_{max/min}$=0.252/−0.268 e $Å^{-3}$.

CCDC-724534 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Supplementary data

Copies of ^1H and ^{13}C NMR spectra of compounds **5a**,**b**; **8a**,**b**; **15a**,**b**; **16**; **18a**,**b**; **25**,**a**,**b**; **10**; **15c**, **20**; **11**; **21**; **27a**,**b**; **9**; **12**; **19**; **22** including selective NOE spectra (DPFGSE pulse sequence) for **9**; **12**; **19**; **22** as well as details of the X-ray crystal structure determination of **16** in CIF format. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.05.045.

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