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Bis(amino acid) derivatives of 1,4-diamino-2-butyne that adopt a C_2-symmetric turn conformation

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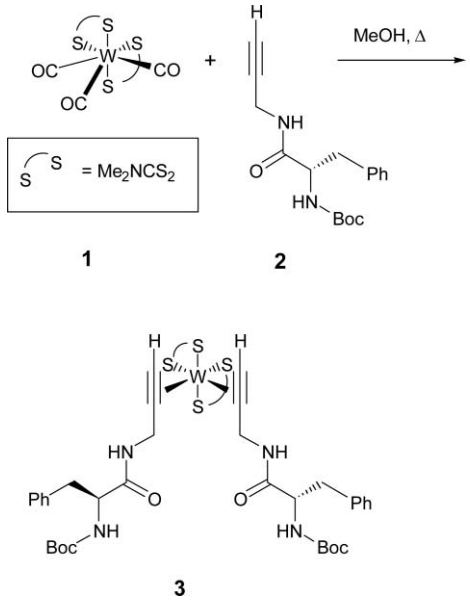
1,4-Diamino-2-butyne was prepared from 1,4-dichloro-2-butyne *via* 1,4-diazido-2-butyne. Bis(amino acid) derivatives of 1,4-diamino-2-butyne having the general structure (Boc-Xxx-NHCH$_2$C≡)$_2$ (Xxx = Ala, Phe and Met) were prepared and examined by ^{1}H NMR spectroscopy. Using chemical shift, coupling constant and DMSO titration data it is found that these compounds adopt a C_2-symmetric turn conformation featuring two intramolecular hydrogen bonds.

Introduction

Recent work in our laboratory has shown that **1** (W(CO)$_3$(dmtc)$_2$ [dmtc = dimethyldithiocarbamate]) will react with amino acid derivatives bearing an alkyne to produce bis(alkynylamino acid) tungsten complexes[1] and metallacyclic peptides.[2] For example, **1** readily reacts with the alkynylphenylalanine derivative **2** to produce the bis(alkyne) complex **3**. A novel feature of this work is the use of a π-ligand, like an alkyne, rather than a hard base ligand, for forming the organometallic-peptide complex; this area of research in bioorganometallic chemistry has not received much attention.[3] The attraction of the tungsten–alkyne coordination[4] is that the alkyne can be readily appended to the peptide, the alkyne is generally unreactive under typical peptide synthesis conditions, the alkynylpeptides readily react to form the bis(alkynylpeptide) complexes while maintaining the structual integrity of the peptide, and the resulting complexes are air-stable. A potential application of this chemistry is the construction of conformationally constrained peptides that adopt defined secondary structures.[5–8]



Scheme 1

Owing to the conformational flexibility of the alkyne ligands in these complexes, the conformation of **3** drawn in Scheme 1 is only one of three possible orientations open to this species.[1,9] As shown in Fig. 1, there is a conformation for **3** in which the two alkynes are aligned (*cis*) and two different conformations for **3** in which the two alkynes are not aligned (*trans*′ and *trans*″). Analysis of the ^{1}H NMR spectrum of **3** and similar species has shown that these complexes assume all three conformations in solution, and that interconversion between these conformers occurs slowly at room temperature.[1]

The presence of multiple conformers of **3** that slowly interconvert at room temperature means that the ^{1}H NMR spectrum of **3** contains multiple resonances for each proton. The multiple resonances in the spectrum makes it difficult to determine whether a species like **3**, when it is in the *cis* conformation, possesses hydrogen bonds between the two amino acids. One way to simplify the spectra of these complexes would be to eliminate the possibility of *cis*/*trans* isomerism. This goal can be achieved by complexation of a symmetrical bis(amino acid) alkyne species, like **4a–c**. Since **4a–c** are identical on each side of the alkyne, it is not possible to have *cis* and *trans* isomers.

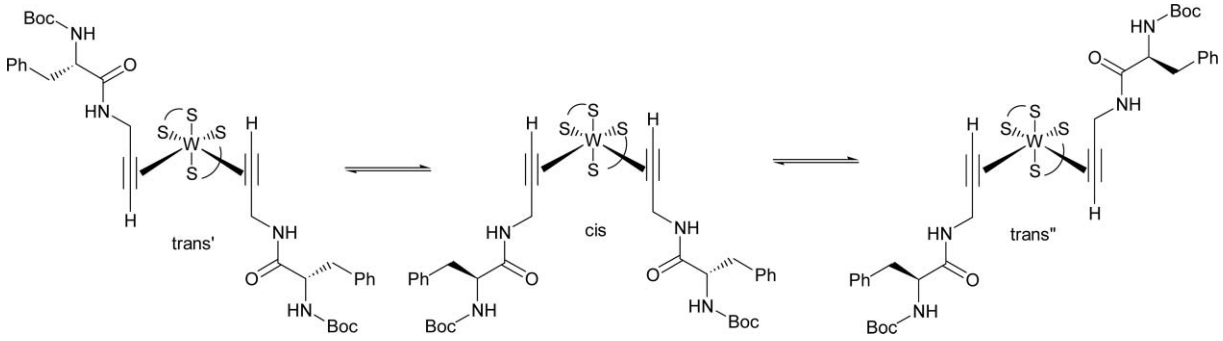


Fig. 1 The three conformational isomers adopted by **3**.

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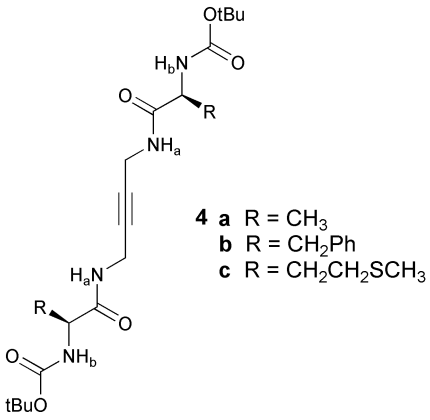
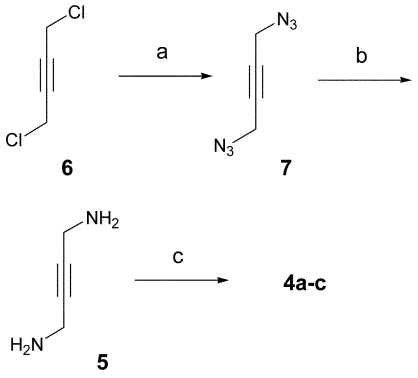


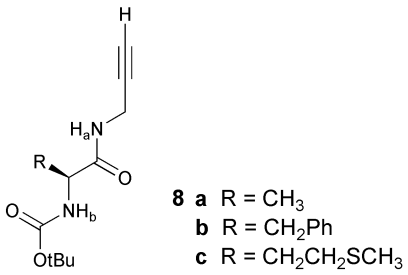
Prior to the study of tungsten bis(alkyne) complexes made from **4a–c**, the conformational behavior of **4a–c** was studied. In this paper is detailed the finding that **4a–c** adopt an ordered, C_2-symmetric turn conformation in solution featuring hydrogen bonding between the amino acid residues appended to the central alkyne.

Results and discussion

The starting compound for preparing **4a–c** is 1,4-diamino-2-butyne (**5**). A synthesis of this compound from the reaction of aqueous ammonia with 1,4-dichloro-2-butyne (**6**) was reported by Johnson in 1946.[10] A necessary part of this procedure is a lengthy, continuous extraction of **5** from an aqueous alkaline solution. In order to avoid this extraction, an alternative one-pot route to **5** and **4a–c** was developed; this route is shown in Scheme 2. Treatment of **6** with 2.1 equivalents of NaN_3 yields the diazide, **7**. Because the literature reports that **7** is explosive, this compound was not isolated.[11–13] Rather, the reaction solution was treated with 2.1 equivalents of PPh_3, which converted **7** to **5**. Subsequent addition of a trialkylamine base and the appropriate amino acid active ester to the reaction flask led to formation of **4a–c**. Purification of **4a–c** was achieved by an extractive workup, followed by flash chromatography.



Scheme 2 *Reagents and conditions*: (a) 2.1 equiv. NaN_3, THF–H_2O; (b) 2.1 equiv. PPh_3; (c) 2.1 equiv. Boc-Xxx-OSu, DIPEA (**a**: Xxx = Ala; **b**: Xxx = Phe; **c**: Xxx = Met).



In the course of this work it was necessary to compare the behavior of **4a–c** to their terminal alkyne counterparts **8a–c**. Compounds **8a–c** were prepared by reaction of the appropriate amino acid active ester with propargylamine, as described previously.[1]

The ^{1}H NMR spectra collected from **4a–c** provided the data necessary for deciphering the solution conformation of these compounds in $CDCl_3$. First, the ^{1}H NMR spectra for **4a–c** show that these species are symmetrically arranged in solution. This was evident from the number of resonances seen in the spectra. For example, in **4a–c** there are two NH_a protons, but these protons only show up as a single resonance in the ^{1}H NMR spectra. The same is true for every other set of protons found on both sides of the alkyne; they all appear as a single resonance. This indicates that the solution conformation of **4a–c** positions both amino acids in identical environments.

The first evidence for an ordered conformation in **4a–c** comes from the appearance of the methylene protons adjacent to the alkyne. The resonances for these two protons have significantly different chemical shifts. For example, in **4a** the two protons appear as separate resonances at 4.29 ppm and 3.71 ppm, a difference of nearly 0.6 ppm; a similar difference in chemical shift is observed in **4b** and **4c** (Table 1). This difference is in stark contrast to the same two protons in **8a–c**, which appear together as doublets located at 4.1 ppm. The different chemical shifts for the methylene in **4a–c** indicates that these two protons exist in significantly different magnetic environments, and that interconversion between these environments is slow on the NMR timescale.

The obvious source of ordering in **4a–c** would be intramolecular hydrogen bonds between the amide NH protons and the amide carbonyls. Evidence for intramolecular hydrogen bonds in **4a–c** can be gleaned in the chemical shifts of the NH protons. An NH proton involved in an intramolecular hydrogen bond would be shifted upfield relative to a similar NH not involved in an intramolecular hydrogen bond. Shown in Table 2 are the chemical shifts for the NH protons in **4a–c** as compared to the same NH protons in **8a–c**. In **4a–c** the chemical shifts of both NH_a and NH_b are shifted downfield relative to their counterparts in **8a–c**. Although there is a significant chemical shift difference of 0.8–1.0 ppm for NH_b, the difference is particularly large for NH_a, where a 1.6–1.9 ppm difference in chemical shifts is evident. There are two possible sources for these differences in chemical shift. NH_a and/or NH_b in **4a–c** could be involved in an intramolecular hydrogen bond, or NH_a and/or NH_b could be in an environment where they experience another anisotropic effect from a neighboring group, for example the alkyne. Given geometrical constraints, it is unlikely that both NH_a and NH_b are both involved in an intramolecular hydrogen bond.

Table 1 Chemical shift values for the methylene protons adjacent to the alkyne in **4a–c** in $CDCl_3$

Compound	δ_{CH_2}/ppm
4a	4.29, 3.71
4b	4.22, 3.61
4c	4.30, 3.71

Table 2 Chemical shift values for the NH protons in **4a–c** and **8a–c** in $CDCl_3$

Compound	δ_{NH_a}/ppm	δ_{NH_b}/ppm
4a	8.22	5.99
4b	8.11	6.00
4c	8.23	6.08
8a	6.61	5.03
8b	6.20	5.20
8c	6.62	5.22

To determine whether NH_a or NH_b is involved in an intramolecular hydrogen bond, a DMSO titration was performed.[14–18] Unlike $CDCl_3$, d_6-DMSO is an aggressive hydrogen bond acceptor that will quickly form interactions with amide NH protons exposed to the solvent. Replacement of the $CDCl_3$ with d_6-DMSO then leads to a sizeable shift in chemical shift of solvent exposed NH protons. In contrast, amide NH protons participating in intramolecular hydrogen bonds generally are not affected by the presence of the d_6-DMSO, and consequently their chemical shifts do not undergo a large change in chemical shift.

Shown in Fig. 2 are the data from the DMSO titration of the bis(alanine) derivative **4a**. The initial additions of d_6-DMSO cause the chemical shift of NH_a to be shifted upfield, while the chemical shift of NH_b remains unchanged. At around 15% d_6-DMSO changes in this pattern start to occur. The chemical shift of NH_a reverses direction and begins to move downfield, while the chemical shift of NH_b also begins to move downfield. The shift downfield for both NH_a and NH_b continues out to 100% d_6-DMSO. The data indicate that from 0–15% d_6-DMSO NH_b participates in an intramolecular hydrogen bond, while NH_a does not. The data also indicate that the intramolecular hydrogen bond is no longer present in the solutions having a d_6-DMSO content greater than 15%.

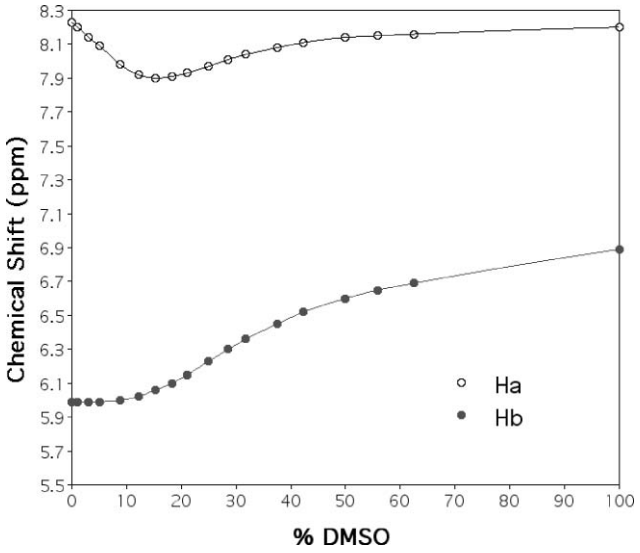


Fig. 2 A plot of the chemical shift changes for NH_a (○) and NH_b (●) in **4a** in solutions of varying compositions of $CDCl_3$ and d_6-DMSO.

To investigate whether the behavior of **4a** in the DMSO titration was typical of bis(amino acid) derivatives of **5**, **4b** and **4c** were also subjected to a DMSO titration. The results from these titrations are shown in Fig. 3. Both **4b** and **4c** show the same pattern of behavior as **4a**. Initial additions of DMSO have no effect on the chemical shift of NH_b, but do cause the chemical shift of NH_a to move upfield. Both NH_a and NH_b begin to move upfield when the amount of DMSO reaches 15–20%. That **4b** and **4c** behave the same as **4a** indicates that these and similar species all adopt the same solution coformation.

Are these chemical shift changes with addition of DMSO unusual? To answer that question, the monoalanine derivative **8a** was subjected to a DMSO titration. The chemical shift changes for NH_a and NH_b in **8a** are compared to the chemical shift changes observed for NH_a and NH_b in **4a** in Fig. 4. With **8a**, both NH_a and NH_b show the same pattern of change. As the d_6-DMSO is added their chemical shifts move downfield, sharply at first, then leveling out around at the 20–30% DMSO mark.

This pattern is typical for solvent-exposed NH protons. In contrast, the chemical shifts of NH_a and NH_b in **4a** are shifted significantly downfield relative to **8a**. As the d_6-DMSO is added to the solution, the chemical shifts of NH_a and NH_b in **4a** move

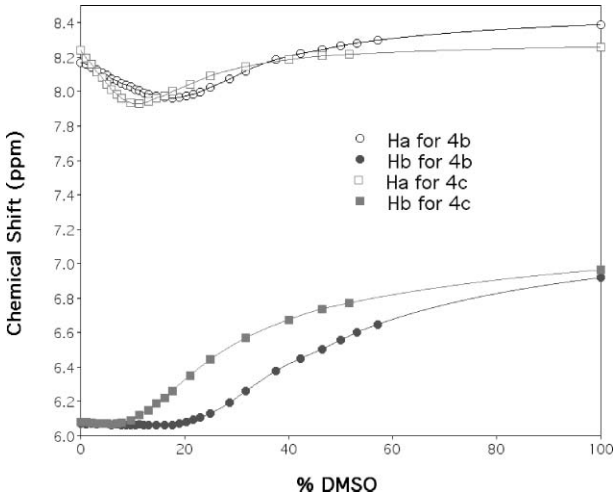


Fig. 3 A plot of the chemical shift changes for NH_a and NH_b in **4b** and **4c** in solutions of varying compositions of $CDCl_3$ and d_6-DMSO.

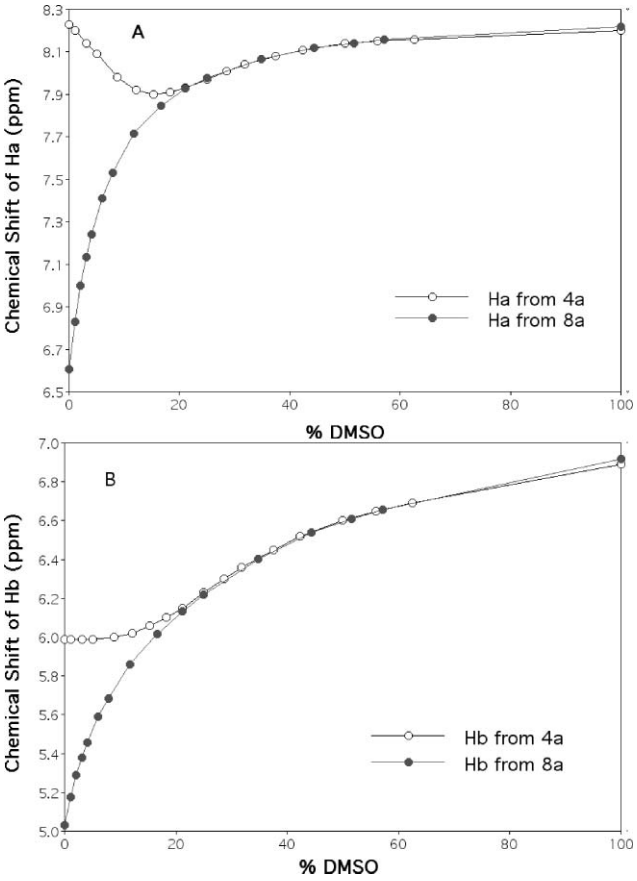


Fig. 4 (A) Comparison of the chemical shift changes that accompany proton NH_a in compounds **4a** (○) and **8a** (●) as the solvent is changed from 100% $CDCl_3$ to 100% d_6-DMSO. The two curves are indistinguishable after 20% d_6-DMSO. (B) Comparison of the chemical shift changes that accompany proton NH_b in compounds **4a** (○) and **8a** (●) as the solvent is changed from 100% $CDCl_3$ to 100% d_6-DMSO. The two curves are indistinguishable after 20% d_6-DMSO.

towards the chemical shifts of NH_a and NH_b in **8a**. The chemical shifts for NH_a and NH_b in **4a** and **8a** become indistinguishable around 20% d_6-DMSO, and continue this way out to 100% d_6-DMSO. The convergence of these chemical shifts occurs at the point when the intramolecular hydrogen bond involving NH_b in **4a** has been broken.

Although the data indicate that NH_b is involved in an intramolecular hydrogen bond, an alternative explanation for

the DMSO titration data is that **4a–c** aggregates in solution, and NH_b from one molecule of **4a–c** participates in a hydrogen bond with a second molecule of **4a–c**. To rule out this possibility, the ^1H NMR spectra of **4a–c** in $CDCl_3$ were recorded at concentrations ranging from 82 mM to 2.5 mM. Over this range of concentrations, the chemical shifts for all the resonances in **4a–c** did not change. This shows that **4a–c** do not aggregate in solution, which means that NH_b must be involved in an intramolecular hydrogen bond.

What carbonyl group forms the hydrogen bond with NH_a? Given the symmetry requirements for these molecules, the carbonyl has to be associated with the other amino acid residue bonded to the opposite end of the alkyne. Of the two possible carbonyls, the only one that is capable of lining up correctly to form an intramolecular hydrogen bond is the amide carbonyl bonded to NH_a. The structure generated by having these intramolecular hydrogen bonds is shown in Fig. 5. A conformation like the one shown in Fig. 5 has been reported for bis(amino acid) and bis(dipeptide) derivatives of 1,1′-ferrocenedicarboxylic acid.[19–25]

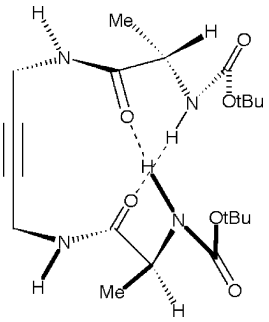


Fig. 5 Three-dimensional representation of the conformation of **4a** in $CDCl_3$.

Confirmation of this structure was obtained by analyzing the coupling constants of the two methylene hydrogens located adjacent to the alkyne. The methylene hydrogen located at 3.6 ppm appears as only a doublet, and it does not form a crosspeak with the neighboring NH when the compound is analyzed in a COSY experiment. The absence of vicinal coupling to the NH by this hydrogen indicates that the dihedral angle between the CH and NH is at or near 90°. In contrast, the methylene hydrogen located at approximately 4.2 ppm appears as a doublet of doublets, indicating geminal coupling to the other methylene hydrogen and vicinal coupling to the NH. Using the Karplus equation,[26] the vicinal NH–CH coupling constant for the resonance at 4.2 ppm of 4.4 Hz represents a dihedral angle of either 40° or 120°.

The dihedral angles calculated from the coupling constants were compared to the dihedral angles found in a Dreiding model of the hydrogen-bonded conformation of **4a** shown in Fig. 5. In the Dreiding model of **4a**, the two dihedral angles between NH_b and the methylene hydrogens are close to 90° and 40° (see Fig. 6). That the calculated dihedral angles match the dihedral angles in the model confirms that the solution conformation of **4a** is the C_2-symmetric structure shown in Fig. 5. Since **4b–c** have similar calculated dihedral angles, these compounds also adopt the same solution conformation. It would appear that this conformation will be adopted by other amino acid derivatives.

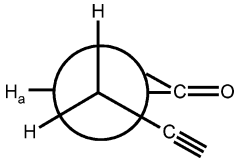


Fig. 6 Dihedral angles of NH_a and the methylene hydrogens adjacent to the alknye in **4a–c**.

Lastly, the unusual behavior of NH_a in **4a–c** deserves some comment. Its location at 8.2 ppm in 100% $CDCl_3$ strongly suggested that it was involved in an intramolecular hydrogen bond. However, the fact that the chemical shift of this resonance varies (and initially moves upfield) as DMSO is added shows that NH_a is not involved in an intramolecular hydrogen bond. The conformation of **4a** shown in Fig. 5 can explain the unusual upfield shift of NH_a during the DMSO titration. The amide carbonyl bonded to NH_a is the hydrogen bond acceptor in the $CDCl_3$ conformation of **4a–c**. Participation of this carbonyl in the hydrogen bond causes a deshielding effect on NH_a, which accounts for its location at 8.2 ppm in $CDCl_3$. As the DMSO titration proceeds and the ordered conformation of **4a–c** is lost, the deshielding effect on NH_a is lost as well.

Conclusion

Bis(amino acid) derivatives of 1,4-diamino-2-butyne (**4a–c**), which can be used to form tungsten–peptide conjugates, adopt an ordered, C_2-symmetric conformation in $CDCl_3$ (Fig. 5). This conformation features two identical hydrogen bonds between the amide carbonyl from one of the amino acids and the amide NH from the other amino acid. These findings indicate that species like **4a–c** can be used to induce a turn structure in a peptide conjugate, similar to the turn structure that can be induced by bis(amino acid) derivatives of 1,1′-ferrocenedicarboxylic acid.[19–25] Like peptide derivatives of 1,1′-ferrocenedicarboxylic acid, peptide derivatives of **5** might find use as chirality-organized receptors.[25]

Will **4a–c**, when complexed to tungsten, retain this turn structure? Experiments aimed at answering this question are in progress and will be reported in due course.

Experimental

General methods

NMR spectra were obtained on a GE Omega 300 instrument. Electrospray mass spectra were obtained on a LCQ APCI/Electrospray LC MS-MS. Samples for mass spectral analysis were dissolved in MeOH (approximately 1 mg mL^{-1}) in borosilicate glass test tubes. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). FT-ICR MS were performed by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University.

1,4-Diamino-2-butyne (5). Caution: The intermediate diazide, **7**, formed in this reaction is explosive. It should not be isolated prior to treatment with PPh_3.

To a solution of 0.468 g (3.81 mmol, 1.0 equiv.) of 1,4-dichloro-2-butyne in 20 mL THF was added 0.517 g (7.95 mmol, 2.1 equiv.) of NaN_3 dissolved in 20 mL H_2O. The resulting solution stirred at 23 °C for 16 h. To the reaction solution was then added 2.097 g (7.96 mmol, 2.1 equiv.) of PPh_3, 5 mL H_2O and 5 mL THF. After stirring at 23 °C for 24 h the solution was divided and used in the reactions described below.

(Boc-Ala-NHCH$_2$C≡)$_2$ (4a). To 25.0 mL (assuming 100% yield, 1.91 mmol, 1.0 equiv.) of the crude solution of **5** (described above) was added 1.05 g (3.78 mmol, 2.0 equiv.) of Boc-Ala-OSu and 2.52 g (19.5 mmol, 10 equiv.) of DIPEA. Next, an additional 5 mL THF and 5 mL H_2O were added. After stirring for 60 h the solvents were evaporated. The crude product was redissolved in 50 mL $CHCl_3$. The organic layer was washed 3 × 50 mL 1 M HCl, 3 × 50 mL saturated $NaHCO_3$, and 1 × 50 mL brine. The organic layer then was dried ($MgSO_4$), filtered and evaporated to yield a crude solid. Flash chromatography (2 : 1 EtOAc–hexanes) provided 1.5 g of **4a** contaminated with triphenylphosphine oxide. To remove the impurity, the crude product was dissolved in 30 mL acetone and treated with 2.0 g of Merrifield's peptide resin (1% cross-linked, 200–400 mesh, 4.38 mmol Cl g^{-1}) and 1.25 g NaI. After stirring for 16 h the

resin was filtered and washed with 3 × 10 mL THF, 1 × 10 mL H_2O, 3 × 10 mL acetone and 3 × 10 mL MeOH. The filtrate and washes were combined and evaporated. To the remaining solid was added 50 mL EtOAc, and the insoluble white solid (**4a**) was collected by vacuum filtration, to provide 0.511 g (63%) of pure **4a** : mp 63–65 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (1H, amide NH, s), 5.99 (1H, urethane NH, d, $J = 7.3$ Hz), 4.57 (1H, $C_\alpha H$, quintet, $J = 7.3$ Hz), 4.28 (1H, CH_2, dd, $J = 4.4$, 16 Hz), 3.71 (1H, CH_2, d, $J = 16$ Hz), 1.43 (9H, $(CH_3)_3$, s), 1.38 (3H, CH_3, d, $J = 7.3$ Hz); ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.20 (1H, amide NH, s), 6.89 (1H, urethane NH, d, $J = 7.8$ Hz), 3.94 (1H, $C_\alpha H$, quintet, $J = 7.3$ Hz), 3.87 (2H, CH_2, d, $J = 5.4$ Hz), 1.37 (9H, $(CH_3)_3$, s), 1.14 (3H, CH_3, d, $J = 7.3$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 156.1, 79.9, 78.9, 49.6, 29.8, 28.4, 19.2; ESMS 427 $[M + H]^+$, 449 $[M + Na]^+$, 327 $[(M + H) - C_5H_8O_2]^+$; TLC, R_f 0.61 (2 : 1 EtOAc–hexane). Anal. Calcd. for $C_{20}H_{34}N_4O_6$: C, 56.32; H, 8.04; N, 13.14. Found: C, 56.16; H, 7.94; N, 12.90.

(Boc-Phe-NHCH$_2$C≡)$_2$ (4b). To 25.0 mL (assuming 100% yield, 1.91 mmol, 1.0 equiv.) of the crude solution of **5** (described above) was added 1.37 g (3.78 mmol, 2.0 equiv.) of Boc-Phe-OSu and 2.52 g (19.5 mmol, 10 equiv.) of DIPEA. Next, an additional 5 mL THF and 5 mL H_2O were added. After stirring for 60 h the solvents were evaporated. The crude product was redissolved in 50 mL $CHCl_3$. The organic layer was washed with 3 × 50 mL 1M HCl, 3 × 50 mL saturated $NaHCO_3$, and 1 × 50 mL brine. The organic layer then was dried ($MgSO_4$), filtered and evaporated to yield a crude solid. Flash chromatography (1 : 2 EtOAc–hexanes) provided 145 mg (13%) of pure **4b** as a white solid: mp 135–137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, amide NH, s), 7.25 (5H, C_6H_5, m), 6.00 (1H, urethane NH, br s), 4.78 (1H, $C_\alpha H$, m), 4.22 (1H, CH_2N, dd, $J = 4.4$, 16 Hz), 3.61 (1H, CH_2N, d, $J = 16$ Hz), 3.06 (1H, CH_2Ph, dd, $J = 5.4$,13 Hz), 2.88 (1H, CH_2Ph, dd, $J = 8.8$, 13 Hz), 1.32 (9H, $(CH_3)_3$, s); ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.38 (1H, amide NH, s), 7.25 (5H, C_6H_5, m), 6.92 (1H, urethane NH, d, $J = 8.6$ Hz), 4.13 (1H, $C_\alpha H$, m), 3.91 (2H, CH_2N, d, $J = 4.3$ Hz), 2.92 (1H, CH_2Ph, dd, $J = 3.8$, 12.9 Hz), 2.70 (1H, CH_2Ph, dd, $J = 10.2$, 13.4 Hz), 1.28 (9H, $(CH_3)_3$, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 156.1, 137.2, 129.5, 128.2, 126.5, 79.7, 78.8, 55.4, 39.7, 29.8, 28.3; ESMS 601 $[M + Na]^+$; TLC, R_f 0.47 (1 : 2 EtOAc–hexane). FT-ICR MS Calcd. for $C_{32}H_{42}N_4O_6Na$: 601.299656; Found: 601.2977.

(Boc-Met-NHCH$_2$C≡)$_2$ (4c). To 10.0 mL (assuming 100% yield, 0.762 mmol, 1.0 equiv.) of the crude solution of **5** (described above) was added 0.538 g (1.55 mmol, 2.0 equiv.) of Boc-Met-OSu and 1.3 mL (7.5 mmol, 10 equiv.) of DIPEA. The resulting solution rapidly turned cloudy, and a white precipitate formed. After stirring for 18 h the solvents were evaporated. The crude product was redissolved in 30 mL $CHCl_3$. The organic layer was washed with 3 × 30 mL 1 M HCl, 3 × 30 mL 1 M NaOH, and 1 × 30 mL brine. The organic layer then was dried ($MgSO_4$), filtered and evaporated to yield a crude solid. Flash chromatography (1 : 1 EtOAc–hexanes) provided 99 mg (27%) of pure **4c**: mp 154–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (1H, amide NH, s), 6.08 (1H, urethane NH, br s), 4.55 (1H, $C_\alpha H$, m), 4.30 (1H, CH_2N, dd, $J = 4.4$, 16 Hz), 3.71 (1H, CH_2N, d, $J = 16$ Hz), 2.57 (2H, CH_2S, m), 2.10 (3H, CH_3S, s), 2.00 (2H, CH_2, m), 1.43 (9H, $(CH_3)_3$, s); ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.25 (1H, amide NH, s), 6.96 (1H, urethane NH, d, $J = 8.3$ Hz), 3.97 (1H, $C_\alpha H$, m), 3.87 (2H, CH_2N, d, $J = 4.9$ Hz), 2.41 (2H, CH_2S, m), 2.03 (3H, CH_3S, s), 1.75 (2H, CH_2, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 156.4, 80.2, 78.9, 53.5, 33.3, 30.4, 29.9, 28.5, 15.4; ESMS 569 $[M + Na]^+$; TLC, R_f 0.29 (1 : 1 EtOAc–hexane). Anal. Calcd. for $C_{24}H_{42}N_4O_6S_2$: C, 52.72; H, 7.74; N, 10.25. Found: C, 52.95; H, 7.76; N, 9.69.

Boc-Ala-NHCH$_2$C≡CH (8a). Prepared as previously described.[1] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (1H, amide NH, br s), 5.01 (1H, urethane NH, br s), 4.14 (1H, $C_\alpha H$, m), 4.05 (2H, CH_2N, s), 2.22 (1H, alkyne CH, t, $J = 2.4$ Hz), 1.45 (9H, $(CH_3)_3$, s), 1.37 (3H, CH_3, d, $J = 6.8$ Hz).

Boc-Phe-NHCH$_2$C≡CH (8b). Prepared as previously described.[1] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (5H, C_6H_5, m), 6.20 (1H, amide NH, br s), 5.20 (1H, urethane NH, br s), 4.42 (1H, $C_\alpha H$, m), 4.03 (2H, CH_2N, m), 3.12 (2H, CH_2Ph, m), 2.21 (1H, t, $J = 2.4$ Hz), 1.44 (9H, $(CH_3)_3$, s).

Boc-Met-NHCH$_2$C≡CH (8c). Prepared as previously described.[1] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.61 (1H, amide NH, br s), 5.22 (1H, urethane NH, d, $J = 6.55$ Hz), 4.33 (1H, $C_\alpha H$, m), 4.15, 4.06 (2H, CH_2N, 2d, $J = 16$ Hz), 2.61 (2H, CH_2, m), 2.16 (3H, CH_3S, s), 2.14, 1.99 (2H, CH_2S, 2m), 1.50 (9H, $(CH_3)_3$, s).

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