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Conformational Behavior of Symmetrical and Unsymmetrical Mono(Alkynylpeptide)-Tungsten Complexes [post-print]

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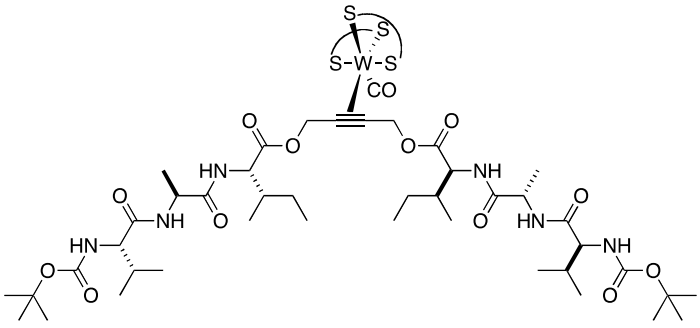
Graphical Abstract:



Two peptides linked together via ester bonds to 1,4-dihydroxy-2-butyne coordinate to a tungsten center and adopt an extended conformation.

Abstract:

A series of N-protected amino acid alkynylesters were prepared by reaction of the amino acid carboxylate group with either propargyl alcohol (to yield the asymmetric esters **2a-c**) or with 1, 4-but-2-yne diol (to yield the symmetric esters **3a-d**). The alkynyl esters were reacted with $W(CO)_3(dmtc)_2$ to yield monoalkyne complexes having the general formula $W(CO)(dmtc)_2$(alkynyl ester). The monoalkyne complexes **6a-f** were unstable in the presence of oxygen and had to be kept under an inert atmosphere. Analysis of the NMR spectra of the monoalkyne complexes showed that two diastereomers were formed in the synthesis, and that there was rapid rotation of the alkyne about the tungsten center at 23^{o}C and above with both diastereomers. At lower temperatures alkyne rotation is significantly slowed. Symmetric alkynyl esters of a dipeptide (**4**) and tripeptide (**5**) were also prepared and reacted with $W(CO)_3(dmtc)_2$ to yield monoalkyne complexes. The resulting complexes (**6g** and **6h**) also formed two diastereomers and displayed rapid rotation of the alkyne about the tungsten center at 23^{o}C and above, and slow rotation at lower temperatures. The amide NH protons in **6g** and **6h** were probed by DMSO titration to see if they were involved in intramolecular hydrogen bonds; they were not, which indicates that the peptide portions of **6g** and **6h** adopt an extended conformation in solution.

Keywords: tungsten, alkynes, peptide, conformation, bioorganometallic

1. Introduction

In recent work from our laboratory we have been exploring the possibility that tungsten-bis(alkyne) complexes [1] can be used to constrain peptides to specific three-dimensional conformations [2-4]. Specifically, we have discovered that alkynes can be readily appended to peptides at either their N-terminus, C-terminus or side chain, and that the resulting alkynylpeptides can be reacted with $W(CO)_3(dmtc)_2$ (dmtc = N,N-dimethyldithiocarbamate) [5] to yield tungsten-bis(alkynylpeptide) complexes [2]. We have also discovered that peptides bearing two alkynes, one at the N-terminus and the other at the C-terminus, will also react with $W(CO)_3(dmtc)_2$ to yield cyclic tungsten-bis(alkynylpeptide) complexes [3-4]. We have termed these cyclic complexes metallacyclicpeptides, since the metal atom is part of the macrocyclic ring structure. Analysis of a metallacyclictripeptide and a metallacyclictetrapeptide by variable temperature NMR spectroscopy indicated the presence of intramolecular hydrogen bonds in the peptide, which is consistent with the conclusion that the peptides in these complexes assume turn conformations [4].

Our initial studies with these bis-(alkynylpeptide) complexes has shown that the alkynylpeptide ligands can and do assume a variety of orientations around the tungsten, and that, at room temperature, these different conformational isomers are slow to interconvert. The relatively large number of different conformational isomers is manifested in the NMR spectra of these complexes; the result is that the ^1H NMR spectra of these species present an overlay of all the possible isomers. Thus, each proton in one of these complexes can and does appear as multiple signals; in the more crowded parts of

the spectrum, there is severe overlap of the different signals. It is this overlap of signals that makes conformational analysis of the peptide using NMR methods difficult.

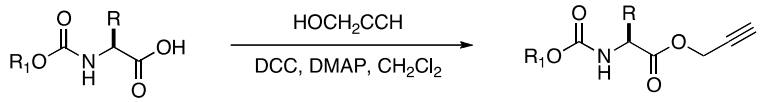
Our initial work has focused on tungsten-bis(alkynylpeptide) complexes [2-4]. An unexplored area is the behavior of tungsten-mono(alkynylpeptide) complexes. In the present work we have prepared and examined the dynamic behavior of tungsten-mono(alkynylpeptide) complexes (1). The alkynylpeptides used here are symmetric and asymmetric. In the asymmetric species one peptide is linked to one end of a terminal alkyne, while in the symmetric species two identical peptides are covalently attached to both ends of one alkyne. The advantage of examining the mono(alkynylpeptide) complexes is that these species should have fewer conformational isomers open to them than the bis(alkynylpeptide) complexes; in addition, the use of a symmetrically substituted alkynylpeptide as the ligand prevents formation of conformational isomers resulting from different alkyne orientations. It was hypothesized that the coordination of the alkyne in these species might force the two peptides on either end of the alkyne into close proximity, leading to the adoption of intramolecular hydrogen bonds between the two peptides. Peptide derivatives of 1,4-diamino-2-butyne, very similar to the peptide derivatives of 1,4-dihydroxy-2-butyne examined here, adopt a C_2-symmetric turn conformation where the two peptides on either side of the alkyne fold back to form intramolecular hydrogen bonds [6]. If the symmetrical tungsten complexes under investigation here do generate similar intramolecular hydrogen, the resulting structure would resemble a β-sheet. This paper reports the results of these studies.

2. Results and Discussion

2.1 Synthesis of Alkynylamino acids

To introduce the alkyne an amino acid with its amine group acylated with either a carbobenzyloxy (Cbz) or t-butyloxycarbonyl (Boc) protecting group was reacted with the coupling reagent dicyclohexylcarbodiimide (DCC), the acylation catalyst 4-dimethylaminopyridine (DMAP) and an alkynylalcohol. Thus, 1 equivalent of the amino acid derivative (**1a-c)** was reacted with 1 equivalent of DCC and 1 equivalent of propargyl alcohol to yield the alkynylesters **2a-c** (Scheme 1). Compounds **2a-c** were characterized by MS and ^{1}H NMR spectroscopy.

Scheme 1

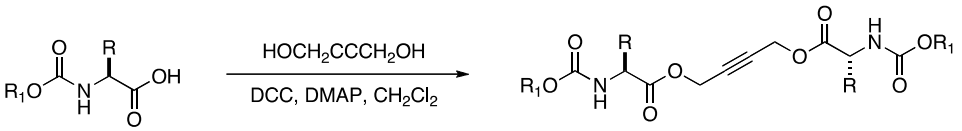


1a $R_1 = C(CH_3)_3$, $R = (CH_2)_4NHCbz$
1b $R_1 = CH_2Ph$, $R = CH_3$
1c $R_1 = CH_2Ph$, $R = CH(CH_3)CH_2CH_3$

2a $R_1 = C(CH_3)_3$, $R = (CH_2)_4NHCbz$
2b $R_1 = CH_2Ph$, $R = CH_3$
2c $R_1 = CH_2Ph$, $R = CH(CH_3)CH_2CH_3$

Similarly, the corresponding symmetrical alkynylesters **3a-d** were prepared by reacting 2 equivalents of **1a-d** with two equivalents of DCC, a catalytic amount of DMAP and one equivalent of 1,4-dihydroxy-2-butyne (Scheme 2). Compounds **3a-d** were characterized by MS and ^{1}H NMR spectroscopy.

Scheme 2



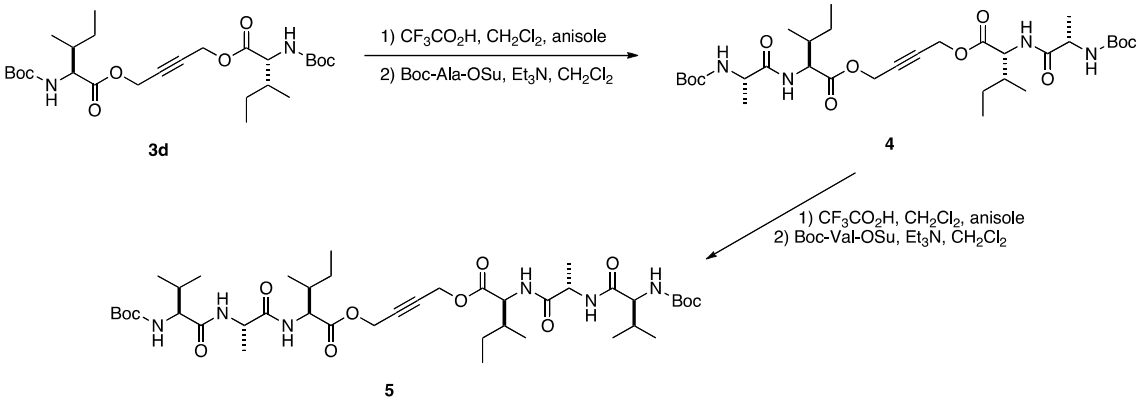
1a $R_1 = C(CH_3)_3$, R = $(CH_2)_4NHCbz$
1b $R_1 = CH_2Ph$, $R = CH_3$
1c $R_1 = CH_2Ph$, $R = CH(CH_3)CH_2CH_3$
1d $R_1 = C(CH_3)_3$, $R = CH(CH_3)CH_2CH_3$

3a $R_1 = C(CH_3)_3$, R = $(CH_2)_4NHCbz$
3b $R_1 = CH_2Ph$, $R = CH_3$
3c $R_1 = CH_2Ph$, $R = CH(CH_3)CH_2CH_3$
3d $R_1 = C(CH_3)_3$, $R = CH(CH_3)CH_2CH_3$

2.2 Synthesis of Symmetrical Alkynylpeptides

A symmetrical dipeptide (**4**) and a symmetrical tripeptide (**5**) having alkynylesters were also prepared. The synthetic route used to prepare **4** and **5** is shown in Scheme 3. Reaction of **3d** with trifluoroacetic acid in CH_2Cl_2 removed the Boc protecting group and generated the N-terminal amine as its trifluoroacetate salt. Subsequent reaction of this salt with the succinimidyl ester Boc-Ala-OSu under basic conditions yielded dipeptide **4**. Similarly, reaction of **4** under the same, two step reaction procedure yielded the tripeptide **5**. Both **4** and **5** were purified by flash chromatography and characterized by MS and ^1H NMR spectroscopy.

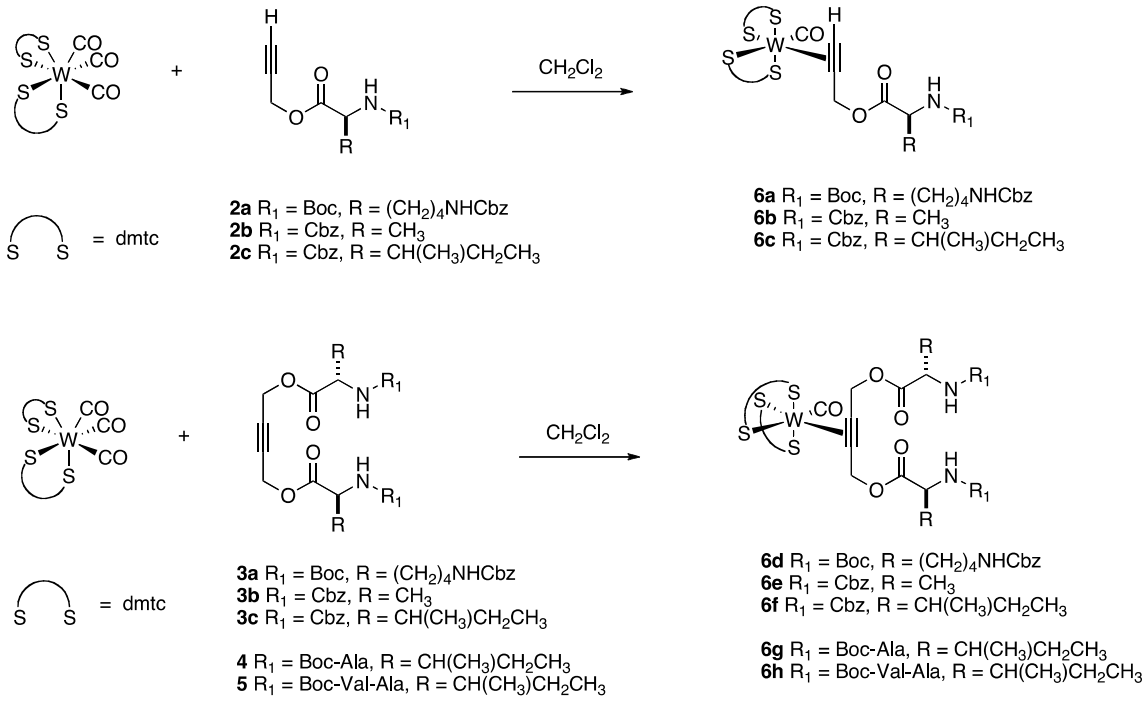
Scheme 3



2.3 Preparation of Tungsten-Mono(Alkynylamino Acid) Complexes

The tungsten-mono(alkyne) complexes **6a-h** were prepared by adaptation of literature procedures [7-8]. One equivalent of the alkynylamino acid or alkynylpeptide (**2a-c**, **3a-c**, **4**, or **5**) was reacted with one equivalent of $W(CO)_3(dmtc)_2$ in CH_2Cl_2, under N_2, for 0.5-1 h (Scheme 4). During the course of the reaction the solution went from an orange to a green color. Following evaporation of the solvents, the crude product was purified using flash chromatography. Purity was assessed using HPLC. The complexes were characterized using MS and ^1H NMR spectroscopy. The purified tungsten-mono(alkyne) complexes would decompose over time if left exposed to the air, so they were stored under N_2. All of the complexes studied here were amorphous solids; repeated attempts to induce crystal formation did not produce crystalline solid.

Scheme 4



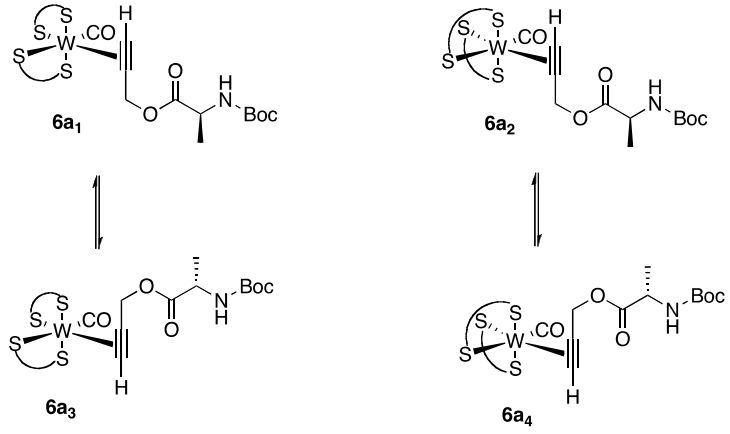
The ESMS spectra of the monoalkyne complexes **6a-h** display a unique pattern of peaks that arises primarily from the four major isotopes of tungsten. This unique isotope pattern is proof for the successful preparation of the desired complex [2-4]. In addition, these complexes also undergo fragmentation in the mass spectrometer with loss of CO. Accordingly along with the M + Na ion, one also finds the (M - CO) + Na ion.

*2.4 Dynamic Behavior of **6a-c***

Before analyzing the conformational behavior of the tungsten mono(alkynylpeptide) complexes that were made, it is important to consider the possible conformational isomers of these species. First, reaction of **2a-c** with $W(CO)_3(dmtc)_2$ will

generate **6a-c** as a mixture of two diastereomers. Consider for example the formation of **6a**. When complex **6a** is formed there are two possible ways to arrange the dmtc ligands, as shown in structures $\mathbf{6a_1}$ and $\mathbf{6a_2}$ (Figure 1). These two possible orientations for the dmtc ligands makes the tungsten a stereocenter. $\mathbf{6a_1}$ and $\mathbf{6a_2}$ also possess a second stereocenter located at C_α on the alanine residue. $\mathbf{6a_1}$ and $\mathbf{6a_2}$ superimpose at the alanine stereocenter, but do not superimpose at the tungsten stereocenter. This means that $\mathbf{6a_1}$ and $\mathbf{6a_2}$ are diastereomers, since they are not mirror images and are not superimposable. Since the steric interactions for either diastereomer are likely to be very similar, a nearly equal mixture of $\mathbf{6a_1}$ and $\mathbf{6a_2}$ should be obtained when **2a** is reacted with $W(CO)_3(dmtc)_2$.

Figure 1



Because $\mathbf{6a_1}$ and $\mathbf{6a_2}$ are diastereomers, each species will have its own unique NMR spectrum. Thus, the NMR spectrum of **6a** should be an overlay of the spectrum of $\mathbf{6a_1}$ and the spectrum of $\mathbf{6a_2}$. Given the close structural similarity between $\mathbf{6a_1}$ and $\mathbf{6a_2}$, large differences in chemical shifts between resonances in $\mathbf{6a_1}$ and $\mathbf{6a_2}$ are unlikely. So, the overlay of the spectra of $\mathbf{6a_1}$ and $\mathbf{6a_2}$ could yield complete overlap of similar resonances, or very close overlap of similar resonances.

Another source of isomerism in **6a** is rotation of the alkyne about the tungsten[8]. Thus **6a$_1$** can also adopt conformation **6a$_3$**. Similarly, **6a$_2$** can adopt the conformation **6a$_4$**.

In prior work by Ward and Templeton on the monoalkyne acetylene complex it was found that rotation of the alkyne ligand is rapid at 23^{o}C [8]. If rotation is also rapid for **6a-c**, then one would expect the NMR spectrum to consist of the overlay of the spectrum of each diastereomer. For **6a** this would mean the averaging of the spectra of **6a$_1$** and **6a$_3$**, and the averaging of the spectra of **6a$_2$** and **6a$_4$**. The result would be the appearance of two separate signals for each unique proton in **6a**. In contrast, if rotation of the alkyne ligands is not rapid at 23^{o}C, then the NMR spectrum of **6a** will consist of the overlay of the individual spectra of **6a$_1$**, **6a$_2$**, **6a$_3$** and **6a$_4$**. This overlay could produce four separate signals for each unique proton in **6a**.

The conformational behavior of monoalkyne complexes **6a-c** was examined using ^{1}H NMR spectroscopy. The complexes **6a-c** were studied first in $CDCl_3$ because this solvent is not an aggressive hydrogen bond donor or acceptor, so it should have minimal interaction with **6a-c**. The spectra of **6a-c** in $CDCl_3$ all show a resonance for the alkyne hydrogen located between 12.8 and 13.0 ppm. The alkyne hydrogen undergoes a significant chemical shift change when it is complexed to the tungsten, leading to a very large movement downfield. This chemical shift has been noted in other tungsten monoalkyne complexes [7-8] and is not remarkable. More significantly, the alkyne hydrogen resonance appears as a single peak. This behavior differs markedly from the behavior of the corresponding bis alkyne complexes, which produce multiple singlets for each alkyne hydrogen [2-4]. The appearance of a single peak for the alkyne hydrogen

indicates that the chemical shifts for this proton in each diastereromer are the same. The single peak also suggests that rotation about the alkyne-tungsten bond is relatively facile at 23^{o}C, which is in accord with previous work in this area [8].

Another resonance in the spectra of **6a-c** that is of interest is the urethane NH proton. Typically, this proton in the NMR spectrum of an amino acid will appear as a doublet. In compounds **6a-c** this proton appears as two doublets that have very similar chemical shifts. The urethane NH for **6c** can be seen in Figure 2A at 5.4 ppm. That the urethane NH shows up as two doublets indicates that there are two isomeric forms of **6a-c** in solution. This indicates that rotation of the alkyne ligand is relatively fast at 23^{o}C.

Finally, another resonance that provides a window onto the solution conformation of **6a-c** is the methylene adjacent to the alkyne carbon. Because **6a-c** possess a stereocenter in the amino acid portion of the molecule, these methylenes are prochiral. Hence, each proton in the methylene will have its own unique chemical shift in the NMR spectrum. This usually means that for each diastereomer the methylene protons would appear as two doublets; for two diastereomers, four doublets. Indeed, the NMR spectra of **6a-c** show that this methylene appears as four doublets. The resonances for the methylene in **6c** are shown in Figure 2A at 6.1 ppm. This shows that the two diastereomers of **6a-c** are present and it indicates that rotation of their alkyne ligands is relatively fast.

Figure 2

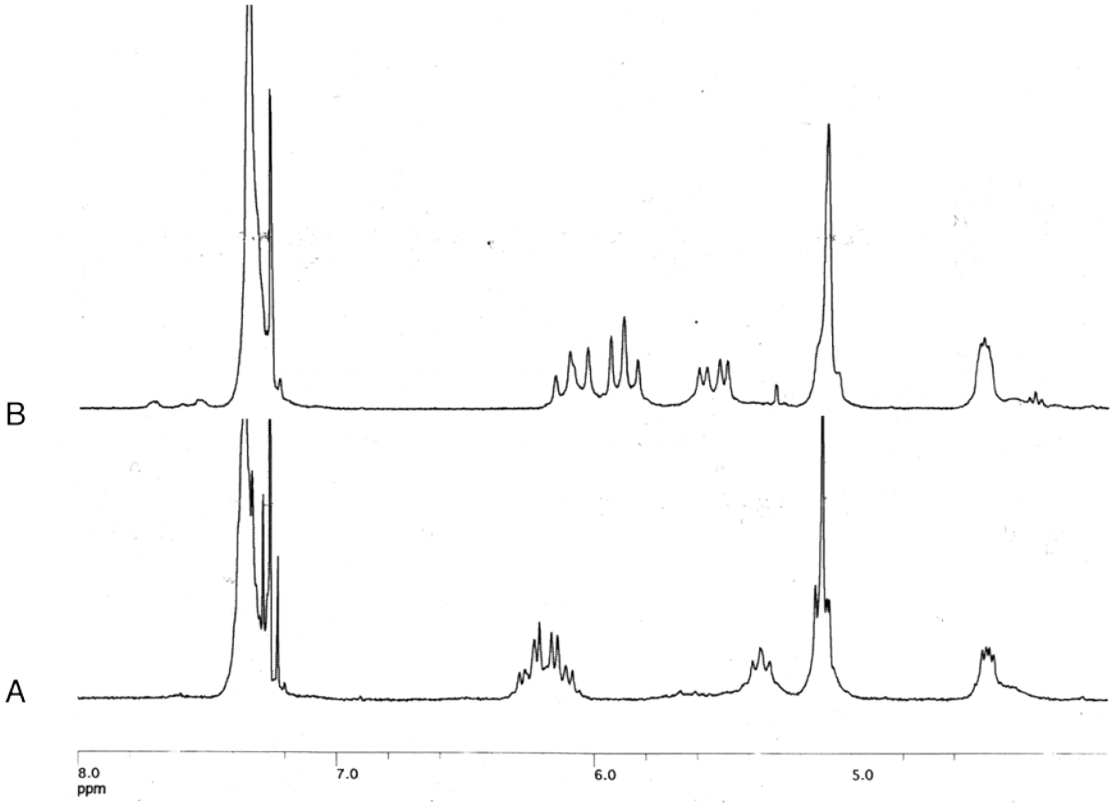


Figure 2. The NMR spectra of (A) **6c** and (B) **6f** in the region between 4-8 ppm. The resonances visible here are, from left to right, the phenyl protons (7.3 ppm), the methylene adjacent to the alkyne (6.4-6.0 ppm in A; 6.2-5.8 ppm in B), the urethane NH (5.4-5.2 ppm in A; 5.7-5.4 ppm in B), the Cbz methylene (5.1 ppm), and the $C_{\alpha}H$ proton (4.5 ppm).

Overall, the appearance of the alkyne hydrogen, the urethane NH, and the methylene adjacent to the alkyne shows that both of the expected diastereomers in **6a-c** are present. Since only two sets of peaks for each resonance were observed, interconversion between the rotational isomers is likely to be very rapid at 23^{o}C. To confirm this, samples of **6a-c** were examined by ^{1}H NMR over a range of elevated temperatures. In order to access higher temperatures d_6-DMSO was used as the solvent.

As the temperature of the sample was raised, no significant change in the appearance of the spectrum was observed. Since no change was observed, it can be concluded that at 23^{o}C and higher the rotational isomers are rapidly equilibrating, as illustrated in Figure 1 for **6a**.

To confirm this conclusion, the behavior of **6a** at lower temperatures was examined using $CDCl_3$ as the solvent. If rotation is rapid at 23^{o}C, then lowering the temperature is likely to slow down that rotation, and this should be evident in the NMR spectrum. Shown in Figure 3 are the resonances for the alkyne hydrogen (Figure 3A) and the methylene adjacent to the alkyne (Figure 3B) for **6a**. As the temperature of the sample was lowered from 23^{o}C to -27^{o}C, the signals for the alkyne hydrogen and the methylene broaden significantly. This broadening can be attributed to slowing of the rotation of the alkyne about the tungsten center. This confirms that the alkyne ligand in these complexes is rapidly rotating at 23^{o}C.

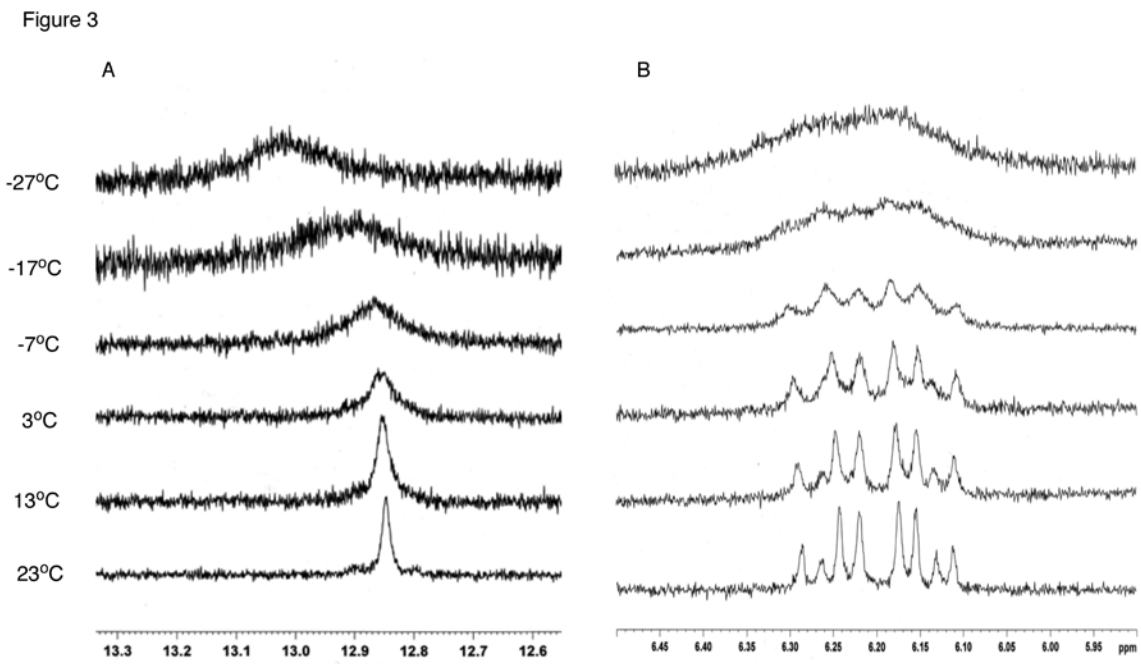


Figure 3. Portions of the ^{1}H NMR spectrum of **6a** at temperatures ranging from 23^{o}C to -27^{o}C. (A) The resonances derived from the alkyne hydrogen in **6a**. (B) The resonances derived from the methylene adjacent to the alkyne in **6a**. As the temperature is lowered these resonances broaden due to slowing of rotation of the alkyne about the tungsten center.

The alkyne hydrogen resonance in **6a** was not the only one to broaden as the temperature was lowered. The resonance for the methylene adjacent to the alkyne broadened as the temperature was lowered to -27^{o}C. Because this methylene is in close proximity to the alkyne, it is not surprising that these resonances are affected by a slowing of alkyne rotation. Protons further removed from the alkyne, for example, the resonances for the urethane NH, $C_{\alpha}H$ and the methylene adjacent to the lysine side chain nitrogen stayed sharp even down to -27^{o}C. That these resonances remained sharp is further evidence for slowing of alkyne rotation as the temperature is lowered.

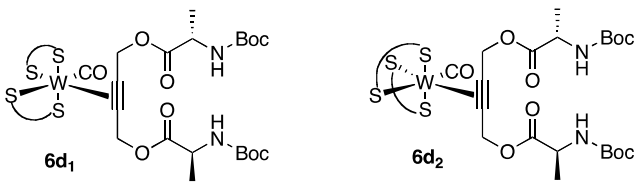
Overall, the NMR data supports the conclusion that for **6a-c** there are two diastereomers present in solution. For each diastereomer there are two rotational isomers.

At 23°C both rotational isomers are present, and interconversion between the two is rapid on the NMR time scale, indicating that the barrier to interconversion is relatively small, consistent with past findings [8]. Knowing how **6a-c** behave sets the stage for understanding the dynamic behavior of the symmetrical complexes **6d-f**.

*2.5 Dynamic Behavior of **6d-f***

Similar to **6a-c**, the syntheses of **6d-f** produces two diastereomers. The two diastereomers for **6d** are shown in Figure 4; $\mathbf{6d_1}$ and $\mathbf{6d_2}$ differ in the arrangement of the two dmtc ligands around the tungsten. Because the alkynylpeptide ligand is symmetrical there are no rotational isomers possible for $\mathbf{6d_1}$ and $\mathbf{6d_2}$. However, the two halves of the alkynylpeptides in **6d-f** are not situated in identical environments, so they are not NMR equivalent. However, due to signal averaging they would appear identical in the NMR spectrum if the alkyne ligand is rapidly rotating about the tungsten. Because **6d-f** comes as a mixture of two diastereomers, if there is rapid rotation at 23°C, then the NMR spectrum would show two resonances for each unique proton in the alkynylpeptide, one derived from each diastereomer. In contrast, if the alkyne ligand in **6d-f** is slow to rotate at 23°C, then the two halves of the alkynylpeptide would not be equivalent, and the NMR spectrum should show four resonances for each unique proton.

Figure 4



The ^{1}H NMR spectra of **6d-f** show only two resonances for each proton at 23^{o}C. For example, the NH protons in **6d-f** appear as two closely spaced doublets. Likewise, the methylenes adjacent to the alkyne appear as four doublets. This can be seen in Figure 2B, which shows the methylene adjacent to the alkyne (5.9 ppm) and the urethane NH (5.5 ppm) for **6f**. In fact, comparison of the spectra of the unsymmetrical alkyne complexes **6a-c** with their corresponding symmetrical alkyne relatives **6d-f** shows that they are very similar; the only significant difference is the presence of the alkyne hydrogen in **6a-c** and its absence in **6d-f**. The similarity of the spectra is illustrated in Figure 2, which shows the 4-8 ppm region of the ^{1}H NMR spectra of **6c** and **6f**.

Like the spectra of **6a-c**, the NMR spectra of **6d-f** show that rotation about the tungsten-alkyne bond is fast on the NMR timescale, indicating that there is a low energetic barrier to this interconversion [8]. This conclusion was supported by the behavior of **6d-f** in variable temperature NMR experiments. Heating of **6d-f** in d_6-DMSO to various elevated temperatures did not lead to any significant changes in the NMR spectra. And cooling of **6f** in $CDCl_3$ down to -53^{o}C caused broadening of the methylene adjacent to the alkyne and the urethane NH (Figure 5), indicating that the alkyne ligand is no longer rapidly rotating about the tungsten.

Figure 5

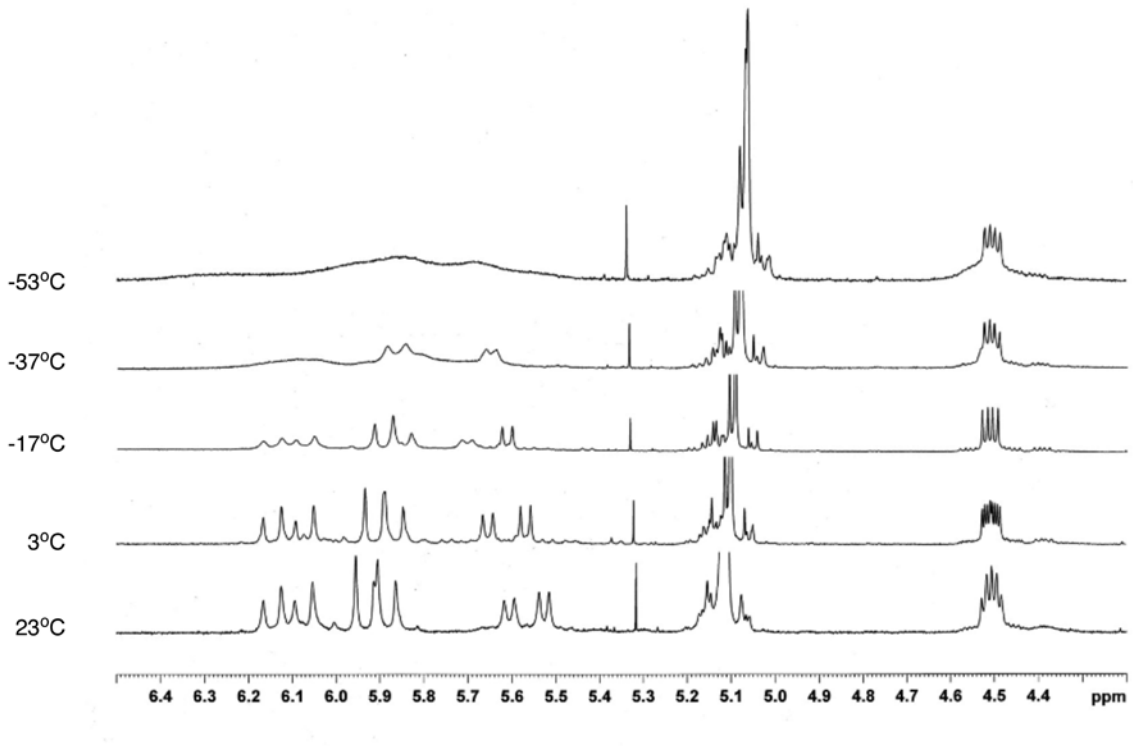


Figure 5. The NMR spectrum of **6f** at 23°C and lower between 6.5 and 4.2 ppm. This region shows resonances for the methylene adjacent to the alkyne (6.1 and 5. 9 ppm), the urethane NH proton (5.6 and 5.5 ppm), the Cbz methylene (5.1 ppm) and the $C_\alpha H$ proton (4.5 ppm). As the temperature is lowered, the methylene adjacent to the alkyne broadens, indicating that rotation of the alkyne about the tungsten is slowing.

*2.6 Dynamic Behavior of **6g-h***

Compounds **6d-f** have one amino acid residue bonded to each end of the alkyne moiety. In order to determine if intramolecular hydrogen bonds could form between the two peptide chains bonded to the alkyne, a dipeptide complexes (**6g**) and a tripeptide complex (**6h**) were prepared and studied. The NMR spectra of **6g** and **6h** were similar to the spectra of **6d-f**, except for the appearance of extra resonances arising from the additional amino acid residues. In particular, the resonances for the urethane NH protons

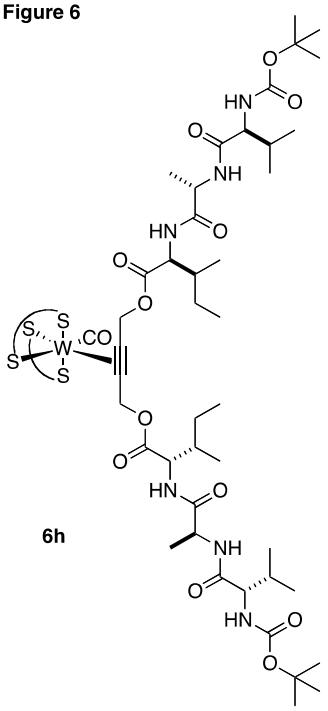
and the methylene adjacent to the alkyne had nearly identical chemical shifts. Also, the urethane NH appears as two doublets in **6g** and **6h**, and the methylene appears as four doublets. This behavior indicates that **6g** and **6h**, like **6d-f**, exist as two diastereomers in solution, and that rotation about the tungsten-alkyne bond is rapid at room temperature.

To determine if intramolecular hydrogen bonds were present in **6g-h**, these species were subjected to a DMSO titration [6, 9-13]. Unlike chloroform, DMSO is an aggressive hydrogen bond acceptor. Accordingly, the chemical shifts of the NH protons in a molecule will differ greatly between chloroform and DMSO if they interact with the solvent. If, however, an NH proton in a molecule is protected from the solvent by being involved in an intramolecular hydrogen bond, then the chemical shift of that proton will not change much in going from chloroform to DMSO. All of the NH protons in **6g** and **6h** showed large chemical shift changes as the solvent composition was changed from $CDCl_3$ to d_6-DMSO. This indicates that none of the NH protons in **6g** and **6h** are involved in intramolecular hydrogen bonds.

2.7 Conclusions

The monoalkyne complexes (**6a-h**) prepared and studied here all exhibit fast rotation of the alkyne about the tungsten center at 23^{o}C [8]. With the symmetrical complexes (**6d-h**) the data shows that they adopt an extended conformation (shown below in Figure 6) in which the two peptide chains extend away from each other; the two peptides are not held together by intramolecular hydrogen bonds.

Figure 6



6h

Figure 6. Illustration of the extended conformation adopted by complex **6h**. Only one of the two diastereomers is shown.

3. Experimental section

3.1 General procedures

Amino acid derivatives were purchased from ChemImpex International. Propargyl alcohol, 1, 4-dihydroxy-2-butyne, anisole, and DMF (dimethylformamide) were purchased from Sigma-Aldrich. TFA, DIEA, and THF were purchased from Acros Organics. $CDCl_3$, d_6-acetone and d_6-DMSO were purchased from Cambridge Isotope Labs. $W(CO)_3(dmtc)_2$ was prepared according to a literature procedure [5] starting from $W(CO)_6$, which was obtained from Strem Chemical. Silica gel for flash chromatography

was purchased from Silicycle. NMR spectra were obtained on a GE Omega 300 MHz instrument or a Bruker Avance III 400 MHz 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) in borosilicate glass test tubes. Theoretical mass spectral isotope patterns were calculated using the Sheffield Chemputer [14]. HPLC analyses were performed on an Hitachi Elite LaChrom HPLC system equipped with L-2400 detector, an L-2200 autosampler and an L-2130 pump. Each run was monitored primarily at 410 nm, which is the wavelength of maximum absorbance for the monoalkyne complexes. A Vydac C18 Peptide and Protein column was used as the stationary phase. The mobile phase involved a linear gradient program using two solvents, 0.1% trifluoroacetic acid and acetonitrile. The gradient program started at a 50:50 mixture of the two solvents and changed to 100% acetonitrile over the course of 12 minutes. The solvent was then held at 100% acetonitrile for an additional 8 minutes.

3.2 Preparation of Boc-Lys(Cbz)-OCH_2CCH, ***2a***

To a solution of 405 μL (6.81 mmol, 1.0 equiv.) of propargyl alcohol and 2.593 g (6.81 mmol, 1.0 equiv.) of Boc-Lys(Cbz)-OH (**1a**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 1.410 g (6.81 mmol, 1.0 equiv.) DCC and 0.106 g (0.68 mmol, 0.1 equiv.) DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the

insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude oil. The residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 2.68 g (94%) of pure **2a** as clear, colorless oil: TLC, R_f 0.87 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.3 ppm ($C_6\mathbf{H_5}$, 5H, m), 5.3 ppm (N**H**, 1H, m), 5.1 ppm (O-C$\mathbf{H_2}$ and N**H**, 3H, m), 4.7 ppm (C$\mathbf{H_2}$-C≡C, 2H, m), 4.3 ppm (C_α**H**, 1H, m), 3.2 ppm (C$\mathbf{H_2}$-N, 2H, m), 2.5 ppm (C≡C-**H**, 1H, s), 1.8-1.2 ppm (3 C$\mathbf{H_2}$, 6H, m), 1.4 ppm (3 C$\mathbf{H_3}$, 9H, s); ESMS: M + Na ion calculated for $C_{22}H_{30}N_2O_6Na$, 441 m/z; found, 441 m/z.

3.3 Preparation of Cbz-Ala-OCH$_2$CCH, ***2b***

To a solution of 605 μL (10.2 mmol, 1.0 equiv.) propargyl alcohol and 2.279 g (10.2 mmol, 1.0 equiv.) Cbz-Ala-OH (**1b**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 2.109 g (10.2 mmol, 1.0 equiv.) DCC and 0.154 g (1.02 mmol, 0.1 equiv.) DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. Recrystallization of this solid from hexanes produced 1.574 g (59%) of pure **2b**: TLC, R_f 0.48 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$): δ 7.3 ppm ($C_6\mathbf{H_5}$, 5H, m), 5.3 ppm (N**H**, 1H, d, J=6.4 Hz), 5.1 ppm (Ph-C$\mathbf{H_2}$, 2H, s), 4.7 ppm (C$\mathbf{H_2}$-C≡C, 2H, m), 4.4

ppm (Ala C_α**H**, 1H, m), 2.5 ppm (C≡C-**H**, 1H, m), 1.4 ppm (Ala C$\mathbf{H_3}$, 3H, d, J=7.3 Hz); ESMS: M + Na ion calculated for $C_{14}H_{15}NO_4Na$, 284 m/z; found, 284 m/z**.**

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*3.4 Preparation of Cbz-Ile-OCH_2CCH, **2c***

To a solution of 605 μL (10.2 mmol, 1.0 equiv.) of propargyl alcohol and 2.717 g (10.2 mmol, 1.0 equiv.) of Cbz-Ile-OH (**1c**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 2.119 g (10.2 mmol, 1.0 equiv.) DCC and 0.154 g (1.02 mmol, 0.1 equiv.) DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. The residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 2.256 g (73%) of pure **2c** as a clear, colorless oil: TLC, R_f 0.79 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.3 ppm ($C_6\mathbf{H_5}$, 5H, m), 5.3 ppm (N**H**, 1H, d, J=8.3 Hz), 5.1 ppm (O-C$\mathbf{H_2}$, 2H, m), 4.7 ppm (C$\mathbf{H_2}$-C≡C, 2H, m), 4.4 ppm (C_α**H**, 1H, m), 2.5 ppm (C≡C-**H**, 1H, s), 1.9 ppm (C_β**H**, 1H, m), 1.3 ppm (Ile C$\mathbf{H_2}$, 1H, m), 1.1 ppm (Ile C$\mathbf{H_2}$, 1H, m), 0.9 ppm (Ile 2 C$\mathbf{H_3}$, 6H, m); ESMS: M + Na ion calculated for $C_{17}H_{21}NO_4Na$, 326 m/z; found, 326 m/z.

*3.5 Preparation of (Boc-Lys(Cbz)-$OCH_2C)_2$, **3a***

To a solution of 0.283 g (3.29 mmol, 1.0 equiv.) of 1, 4-dihydroxy-2-butyne and 2.410 g (6.33 mmol, 2.0 equiv.) Boc-Lys(Cbz)-OH (**1a**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 1.323 g (6.41 mmol, 2.0 equiv.) DCC and 0.049 g (0.40 mmol, 0.1 equiv.) DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. The crude product was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 2.25 g (86%) of pure **3a** as a clear, colorless oil: TLC (1:1 EtOAc/hexanes): R_f 0.39; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.3 ppm (Cbz $C_6\mathbf{H_5}$, 10H, m), 5.2 ppm (Cbz $C\mathbf{H_2}$, Cbz N**H**, Lys N**H**, 8H, m), 4.7 ppm ($C\mathbf{H_2}$-CC-$C\mathbf{H_2}$, 4H, 2 d, J=15 Hz), 4.3 ppm ($C_\alpha\mathbf{H}$, 2H, m), 3.2 ppm ($C\mathbf{H_2}$-NH-Cbz, 4H, m), 1.8 ppm ($C_\beta\mathbf{H}$-$C\mathbf{H_2}$, 6H), 1.4 ppm (Boc $C\mathbf{H_3}$, C_βH-CH_2-$C\mathbf{H_2}$, 22H, m); ESMS: M + Na ion calculated for $C_{42}H_{58}N_4O_{12}Na$, 833 m/z; found, 833 m/z.

3.6 Preparation of (Cbz-Ala-$OCH_2C)_2$, ***3b***

To a solution of 0.407 g (4.728 mmol, 1.0 equiv.) 1, 4-dihydroxy-2-butyne and 2.091 g (9.367 mmol, 2.0 equiv.) Cbz-Ala-OH (**1b**) in 30 mL CH_2Cl_2 and 10 mL THF was added 1.946 g (9.431 mmol, 2.0 equiv.) DCC. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction

solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. The remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 664 mg (28 %) of pure **3b** as a white solid: TLC (1:1 EtOAc/hexanes): R_f 0.62; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.2 ppm (2 Ph, 10H, m), 5.3 ppm (N**H**, 2H, d, J=6.3 Hz), 5.1 ppm (Cbz C**H$_2$**, 4H, s), 4.7 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, s), 4.4 ppm (C_α**H**, 2H, m), 1.4 ppm (Ala C**H$_3$**, 6H, d); ESMS, M + Na ion calculated for $C_{32}H_{40}N_2O_8Na$, 519 m/z; found, 519 m/z.

3.7 Preparation of (Cbz-Ile-OCH$_2$C)$_2$, ***3c***

To a solution of 0.515 g (5.98 mmol, 1.0 equiv.) of 1, 4-dihydroxy-2-butyne and 3.058 g (11.54 mmol, 2.0 equiv.) Cbz-Ile-OH (**1c**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 2.412 g (11.69 mmol, 2.0 equiv.) DCC and 0.090 g (0.60 mmol, 0.1 equiv.) DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. The residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 3.05 g (92 %) of pure **3c** as a white solid: m.p. 84.5-86.0 ^{o}C; TLC, R_f 0.40 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ

7.3 ppm (2 Ph, 10H, m), 5.3 ppm (N**H**, 2H, d, J=6.4 Hz), 5.1 ppm (Cbz C**H$_2$**, 4H, s), 4.7 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, m), 4.4 ppm (C$_\alpha$**H**, 2H, m), 1.9 ppm (C$_\beta$**H**, 2H, m), 1.4 ppm (Ile C**H$_2$**, 2H, m), 1.2 ppm (Ile C**H$_2$**, 2H, m), 0.9 ppm (Ile 4 C**H$_3$**, 12 H, m); ESMS, M + Na ion calculated for $C_{32}H_{40}N_2O_8Na$, 603 m/z; found, 603 m/z.

3. 8 Preparation of (Boc-Ile-OCH$_2$C)$_2$, ***3d***

To a solution of 0.513 g (5.96 mmol, 1.0 equiv.) of 1, 4-dihydroxy-2-butyne and 2.704 g (11.7 mmol, 2.0 equiv.) of Boc-Ile-OH (**1d**) in 30 mL CH_2Cl_2 and 10 mL THF at 0°C was added 2.406 g (11.7 mmol, 2.0 equiv.) of DCC and 0.092 g (0.61 mmol, 0.1 equiv.) of DMAP. The resulting solution slowly warmed to 25°C over the course of 3 h. A white precipitate formed during this time. The reaction solution was chilled in an ice bath for 5 min, after which the solid was removed by filtration. The filtrate was evaporated and the residue resuspended in 20 mL EtOAc. After being chilled in an ice bath for 5 min, the insoluble white solid was removed by filtration. Again, the filtrate was evaporated to yield a crude solid. The residue was purified using flash chromatography (1:3 EtOAc/hexanes) to yield 2.152 g (73 %) of pure **3d** as a clear, colorless liquid: TLC, R_f 0.30 (1:3 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 5.1 ppm (N**H**, 2H, d, J=9.3 Hz), 4.8 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, m), 4.3 ppm (Cα**H**, 2H, q, J=4.9 Hz), 1.8 ppm (C$_\beta$**H**, 2H, m), 1.5 ppm (Boc C**H$_3$**, 18H, s), 1.2 ppm (C$_\beta$H-C**H$_2$**, 4H, m), 0.9 ppm (Ile C**H$_3$**, 12H, m); ESMS, M + Na ion calculated for $C_{26}H_{44}N_2O_8Na$, 535 m/z; found, 535 m/z.

3. 9 Preparation of (Boc-Ala-Ile-$OCH_2C)_2$, ***4***

To a solution of 1.200 g (2.34 mmol, 1.0 equiv.) of **3d** in 5 mL CH_2Cl_2 and 1.2 mL anisole at 0°C was added 5 mL TFA. After stirring for 1 h at 25°C, the solvents were evaporated; the residue that remained had a light pink color. After redissolving the residue in 10 mL CH_2Cl_2, 1.389 g (4.69 mmol, 2.0 equiv.) of Boc-Ala-OSu and 12 mL of DIEA was added. After stirring for 3-4 h, the solvents were evaporated. The crude product was redissolved in 25 mL CH_2Cl_2. The organic layer was washed: 3 x 50 mL 0.1 M HCl, 3 x 50 mL $NaHCO_3$, and 1 x 50 mL NaCl. The organic layer was then dried with $MgSO_4$, filtered, and evaporated. The residue was purified using flash chromatography (1:2 EtOAc/hexanes) to yield 852 mg (56%) of pure **4**: TLC, R_f 0.26 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 6.7 ppm (Ile N**H**, 2H, m), 4.9 ppm (Ala N**H**, 2H, d, J=5.7 Hz), 4.7 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 2 d, J=15.0 Hz), 4.6 ppm (Ile Cα**H**, 2H, m), 4.2 ppm (Ala Cα**H**, 2H, m), 1.8 ppm (Ile C_β**H**, 2H, m), 1.5 ppm (Boc C**H$_3$**, Ala C**H$_3$**, 24H, m), 1.2 ppm (C_βH-C**H$_2$**, 4H, m), 0.9 ppm (Ile C**H$_3$**, 12H, m); ESMS, M + Na ion calculated for $C_{32}H_{54}N_4O_{10}Na$, 677 m/z; found, 677 m/z.

3.10 Preparation of (Boc-Val-Ala-Ile-$OCH_2C)_2$, ***5***

To a solution of 398 mg (0.608 mmol, 1.0 equiv.) of **4** in 1 mL CH_2Cl_2 and 265 μL anisole (2.43 mmol, 4.0 equiv.) at 0°C was added 1 mL TFA. After stirring for 1 h at 25°C, the solvents were evaporated. The crude product was redissolved in 2 mL CH_2Cl_2,

then treated with 385 mg (1.22 mmol, 2.0 equiv.) of Boc-Val-OSu and 2.5 mL of DIEA. After stirring for 4 h, the solvents were evaporated. The crude product was redissolved in 5 mL CH_2Cl_2. The organic layer was washed 3 x 10 mL 0.1 M HCl, 3 x 10 mL $NaHCO_3$, and 1 x 10 mL NaCl. The organic layer was then dried with $MgSO_4$, filtered, and evaporated to yield 292 mg (56%) of pure **5** as an amorphous white solid: ^{1}H NMR (d_6-DMSO) δ 8.2 ppm (Ile N**H** or Ala N**H**, 2H, d, J=7.3 Hz), 8.0 ppm (Ile N**H** or Ala N**H**, 2H, d, J=6.8 Hz), 6.7 ppm (Val N**H**, 2H, d, J=8.8 Hz), 4.8 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, m), 4.4 ppm (Cα**H**, 2H, t, J=6.8 Hz), 4.2 ppm (Cα**H**, 2H, t, J=6.5 Hz), 3.8 ppm (Cα**H**, 2H, t, 7.5), 1.9 ppm (C_β**H**, 2H, q, J=7.0), 1.7 ppm (C_β**H**, 2H, m), 1.5 ppm (Boc C**H$_3$**, 18H, s), 1.2 ppm (Ile C_βH-C**H$_2$**, 4H, m), 0.9 ppm (Ile, Ala and Val C**H$_3$**, 30H, m); ESMS, M + Na ion calculated for $C_{42}H_{72}N_6O_{12}Na$, 875 m/z; found, 875 m/z.

3.11 Preparation of W(CO)(dmtc)$_2$(Boc-Lys(Cbz)-OCH$_2$CCH), ***6a***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) $W(dmtc)_2(CO)_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 35 mg (0.083 mmol, 1.0 equiv.) **2a**. The solution color changed from orange to green after 30 min of stirring. The solvents were evaporated. The remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 37 mg (51%) of pure **6a** as an amorphous green solid: TLC, R_f 0.39 (1:1 EtOAc/hexanes); HPLC, R_t 12.40 min; ^{1}H NMR ($CDCl_3$) δ 12.8 ppm (C≡C-**H**, 1H, s), 7.3 ppm (Ph, 5H, m), 6.2 ppm (C**H$_2$**-C≡C, 2H, 4d, J=17.8 Hz), 5.2 ppm (Ph-C**H$_2$** and N**H**, 3H, m), 4.8 ppm (N**H**, 1H, m), 4.5 ppm (Cα**H**, 1H, m), 3.2 ppm (N-C**H$_3$**, C**H$_2$**-N, 14H, m), 1.9 ppm (C_βH-C**H$_2$**, 2H, m), 1.9, 1.5 ppm (C_β**H**-C**H$_2$**-

C**H$_2$**, Boc C**H$_3$**, 14H, m); ESMS, M + Na ion pattern calculated for $WC_{29}H_{42}N_4O_7S_4Na$: 891 m/z (62.0), 892 (56.3), 893 (100), 894 (39.9), 895 (89.5), 896 (31.5), 897 (19.8), 898 (5.8); found: 891 m/z (74.8), 892 (69.7), 893 (100), 894 (34.8), 895 (56.4), 896 (39.0), 897 (21.7), 898 (10.3).

3.12 Preparation of W(CO)(dmtc)$_2$(Cbz-Ala-OCH$_2$CCH), ***6b***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) $W(dmtc)_2(CO)_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 21 mg (0.083 mmol, 1.0 equiv.) **2b**. The solution changed from orange to green after 30 min of stirring. The solvent was evaporated, and the remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 46 mg (78%) of pure **6b** as an amorphous green solid: TLC, R_f 0.41 (1:1 EtOAc/hexanes); HPLC, R_t 7.75 min (410 nm,); ^{1}H NMR ($CDCl_3$) δ 12.8 ppm (C≡C-**H**, 1H, s), 7.3 ppm (Ph, 5H, m), 6.2 ppm (C**H$_2$**-C≡C-C**H$_2$**, 2H, 4d, J=17.1 Hz), 5.3 ppm (N**H**, 1H, m), 5.1 ppm (Ph-C**H$_2$**, 2H, m), 4.5 ppm (Cα**H**, 1H, m), 3.2 ppm (N-C**H$_3$**, 12H, m), 1.5 ppm (Ala C**H$_3$**, 3H, m); ESMS, M + Na ion pattern calculated for $WC_{21}H_{27}N_3O_5S_4Na$: 734 (65.4), 735 (53.2), 736 (100), 737 (32.4), 738 (90.6), 739 (24.5), 740 (17.8); found: 734 (63.5), 735 (62.0), 736 (100), 737 (71.2), 738 (85.2), 739 (70.3), 740 (35.0).

3.13 Preparation of W(CO)(dmtc)$_2$(Cbz-Ile-OCH$_2$CCH), ***6c***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) $W(dmtc)_2(CO)_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23°C was added 35 mg (0.115 mmol, 1.4 equiv.) **2c**. The solution changed from orange to green after 30 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash chromatography (3:2 EtOAc/hexanes) to yield 16 mg (25%) of pure **6c**.

TLC, R_f 0.46 (1:1 EtOAc/hexanes); HPLC, R_t 8.97 min (410 nm); ^{1}H NMR ($CDCl_3$) δ 12.8 ppm (C≡C-**H**, 1H, s), 7.3 ppm (Ph, 5H, m), 6.0 ppm (C**H$_2$**-C≡C, 2H, 4d, J=16.4 Hz), 5.6 ppm (N**H**, H, m), 5.1 ppm (Ph-C**H$_2$**, 2H, m), 4.5 ppm (Cα**H**, 1H, m), 3.2 ppm (N-C**H$_3$**, 12H, m), 2.0 ppm (C_β**H**, 1H, m), 1.4 ppm (Ile C**H$_2$**, 1H, m), 1.2 ppm (Ile C**H$_2$**, 1H, m), 0.9 ppm (Ile 2 C**H$_3$**, 6H, m); ESMS, M + ion pattern calculated for $WC_{24}H_{33}N_3O_5S_4Na$: 776 (64.2), 777 (54.5), 778 (100), 779 (35.1), 780 (90.1), 781 (27.0), 782 (18.4); found: 776 (81.9), 777 (56.8), 778 (100), 779 (43.2), 780 (64.1), 781 (28.2), 782 (20.1).

3.14 Preparation of $W(CO)(dmtc)_2((Boc\text{-}Lys(Cbz)\text{-}OCH_2C)_2)$, ***6d***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) $W(dmtc)_2(CO)_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23°C was added 67 mg (0.083 mmol, 1.0 equiv.) **3a**. The solution changed from orange to green after 30 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 57 mg (54%) of pure **6d** as an amorphous green solid: TLC, R_f 0.35 (1:1 EtOAc/hexanes); HPLC, R_t 11.7 min (410 nm); ^{1}H NMR

($CDCl_3$): δ 7.3 ppm (2 Ph, 10H, m), 6.0 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 4d, J=16.6 Hz), 5.3 ppm (N**H**, 2H, m), 5.1 ppm (Cbz C**H$_2$** and N**H**, 6H, m), 4.5 ppm (Cα**H**, 2H, m), 3.2 ppm (N-C**H$_3$**, C**H$_2$**-N, 16H, m), 1.9 ppm (C_βH-C**H$_2$**, 2H, m), 1.7 ppm (C_βH-C**H$_2$**, 2H, m), 1.4 ppm (Boc C**H$_3$**, C_β**H**-CH_2-C**H$_2$**, 26H, m); ESMS, M + Na ion pattern calculated for $WC_{49}H_{70}N_6O_{13}S_4Na$: 1283 (53.8), 1284 (61.1), 1285 (100), 1286 (56.5), 1287 (89.1), 1288 (46.7), 1289 (26.6), 1290 (10.3); found: 1283 (49.5), 1284 (86.1), 1285 (100), 1286 (47.4), 1287 (78.7), 1288 (47.4), 1289 (25.1), 1290 (21.4).

3.15 Preparation of $W(CO)(dmtc)_2((Cbz\text{-}Ala\text{-}OCH_2C)_2)$, ***6e***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) $W(dmtc)_2(CO)_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 41 mg (0.083 mmol, 1.0 equiv.) of **3b**. The solution changed from orange to green after 30 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 65 mg (83%) of pure **6e** as an amorphous green solid: TLC, R_f 0.37 (1:1 EtOAc/hexanes); HPLC, Rt 8.1 min (410 nm); ^{1}H NMR ($CDCl_3$) δ 7.3 ppm (Ph, 10H, m), 6.0 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 4d, J=16.6 Hz), 5.5 ppm (N**H**, 2H, m), 5.1 ppm (Cbz C**H$_2$**, 4H, m), 4.5 ppm (Cα**H**, 2H, m), 3.2 ppm (N-C**H$_3$**, 12H, m), 1.5 ppm (Ala C**H$_3$**, 6H, m); ESMS, M + Na ion pattern calculated for $WC_{33}H_{40}N_4O_9S_4Na$: 969 (60.3), 970 (57.3), 971 (100), 972 (43.4), 973 (89.2), 974 (34.6), 975 (21.0); found: 969 (78.1), 970 (38.8), 971 (100), 972 (20.8), 973 (65.2), 974 (22.9), 975 (10.7).

3.16 Preparation of W(CO)(dmtc)$_2$((Cbz-Ile-OCH$_2$C)$_2$), ***6f***

To a solution of 42 mg (0.083 mmol, 1.0 equiv.) W(dmtc)$_2$(CO)$_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 48 mg (0.083 mmol, 1.0 equiv.) of **3c**. The solution changed from orange to green after 30 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 60 mg (71%) of pure **6f** as an amorphous green solid: TLC, R_f 0.51 (1:1 EtOAc/hexanes); HPLC, R_t 10.95 min (410 nm); ^{1}H NMR ($CDCl_3$) δ 7.3 ppm (2 Ph, 10H, m), 6.0 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 4d, J=16.0 Hz), 5.6 ppm (N**H**, 2H, 2d, J=9.0 Hz), 5.1 ppm (Ph-C**H$_2$**, 4H, m), 4.5 ppm (Cα**H**, 2H, m), 3.2 ppm (4 N-C**H$_3$**, 12H, m), 2.0 ppm (C$_\beta$**H**, 2H, m), 1.4 ppm (Ile C**H$_2$**, 2H, m), 1.2 ppm (Ile C**H$_2$**, 2H, m), 0.9 ppm (Ile 2 C**H$_3$**, 12H, m); ESMS, M + Na ion pattern calculated for $WC_{39}H_{52}N_4O_9S_4Na$: 1053 (58.0), 1054 (59.0), 1055 (100), 1056 (48.2), 1057 (88.8), 1058 (39.1), 1059 (22.5); found: 1053 (68.2), 1054 (42.2), 1055 (100), 1056 (45.6), 1057 (92.1), 1058 (31.1), 1059 (20.6).

3.17 Preparation of W(CO)(dmtc)$_2$((Boc-Ala-Ile-OCH$_2$C)$_2$), ***6g***

To a stirred solution of 43 mg (0.083 mmol, 1.0 equiv.) of W(dmtc)$_2$(CO)$_3$ in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 55 mg (0.083 mmol, 1.0 equiv.) of **4**. The solution changed from orange to green after 30 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash

chromatography (1:1 EtOAc/hexanes) to yield 63 mg (68%) of pure **6g** as an amorphous green solid: TLC, R_f 0.19 (1:1 EtOAc/hexanes); HPLC, R_t 9.3 min (410 nm); ^{1}H NMR ($CDCl_3$) δ 6.8 ppm (Ile N**H**, 2H, m), 6.0 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 4d, J=16.6 Hz), 5.2 ppm (Ala N**H**, 2H, m), 4.7 ppm (Ile Cα**H**, 2H, m), 4.2 ppm (Ala Cα**H**, 2H, m), 3.3 ppm (4 N-C**H$_3$**, 12H, m), 2.0 ppm (Ile C_β**H**, 2H, m), 1.5 ppm (Boc C**H$_3$**, Ala C**H$_3$**, 24H, m), 1.2 ppm (Ile C_βH-C**H$_2$**, 4H, m), 0.9 ppm (Ile 2 C**H$_3$**, 12H, m); ESMS, M + Na ion pattern calculated for $WC_{39}H_{66}N_6O_{11}S_4Na$: 1127 (57.5), 1128 (59.1), 1129 (100), 1130 (49.0), 1131 (89.0), 1132 (39.8), 1133 (23.1); found: 1127 (26.5), 1128 (54.4), 1129 (100), 1130 (54.1), 1131 (71.0), 1132 (27.8), 1133 (8.9).

3.18 Preparation of W(CO)(dmtc)$_2$((Boc-Val-Ala-Ile-OCH$_2$C)$_2$), ***6h***

To a stirred solution of 28 mg (0.055 mmol, 1.0 equiv.) of $W(dmtc)_2(CO)_3$ (REF) in 10 mL degassed CH_2Cl_2 under a N_2 atmosphere at 23^{o}C was added 47 mg (0.055 mmol, 1.0 equiv.) of **5**. The solution changed from orange to green after 60 min of stirring. The solvents were evaporated, and the remaining residue was purified using flash chromatography (1:1 EtOAc/hexanes) to yield 32 mg (44%) of pure **6h** as an amorphous green solid: TLC, R_f 0.32 (2:1 EtOAc/hexanes); HPLC, R_t 10.1 min (410 nm); ^{1}H NMR ($CDCl_3$) δ 7.1 ppm (Ile N**H** and Ala N**H**, 4H, m), 6.0 ppm (C**H$_2$**-C≡C-C**H$_2$**, 4H, 4d, J=16.6 Hz), 5.5 ppm (Val N**H**, 2H, d, J=8.3 Hz), 4.6 ppm (Ile Cα**H** and Ala Cα**H**, 4H, m), 4.0 ppm (Val Cα**H**, 2H, m), 3.5 ppm (4 N-C**H$_3$**, 12H, m), 2.1 ppm (Ile C_β**H** or Val C_β**H**, 2H, q, J=6.5 Hz), 1.9 ppm (Ile C_β**H** or Val C_β**H**, 2H, m), 1.5 ppm (Boc C**H$_3$**, 18H,

m), 1.2 ppm (Ile $C_{\beta}H$-C**H$_2$**, 4H, m), 0.9 ppm (Ile C**H$_3$**, Ala C**H$_3$**, Val C**H$_3$**, 30H, m); ESMS, M + Na ion pattern calculated for $WC_{49}H_{84}N_8O_{13}S_4Na$: 1325 (53.4), 1326 (61.3), 1327 (100), 1328 (57.1), 1329 (89.2), 1330 (47.2), 1331 (26.9), 1332 (10.5); found: 1325 (84.3), 1326 (79.5), 1327 (100), 1328 (58.6), 1329 (23.2), 1330 (40.8), 1331 (14.5), 1332 (11.1).

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