Synthesis and Conformational Behavior of Metallacyclicdipeptides Derived from Coordination of Side Chain Alkynylamino Acids to Tungsten

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Abstract:

Three dipeptides bearing alkynes on their side chains (**9** (derived from dilysine), **14** (derived from dicysteine) and **17** (derived from diglycine)) were prepared and reacted with $\text{W}(\text{CO})_3(\text{dmtc})_2$ [dmtc = dimethyldithiocarbamate] to afford, respectively, three metallacyclicpeptides, **18**, **19** and **20**. The metallacyclicpeptides were characterized by HPLC, ES-MS, and ^1H NMR. The conformational behavior of the alkynes about the tungsten center was assessed using ^1H NMR. It was found that all three metallacyclicpeptides adopt multiple conformations of the alkynes relative to the tungsten. Both **18** and **19** appear to adopt all 8 possible conformations, while **20** adopts a limited number of conformations. The ability of the alkynes to equilibrate between the syn and anti conformations was assessed by examining the alkyne hydrogen resonances using variable temperature ^1H NMR. It was found that the alkyne ligands in **18** and **19** will equilibrate between the syn and anti conformations. The alkyne hydrogen resonances in **18** coalesce to one signal around 343K, while the alkyne hydrogen resonances in **19** do not completely coalesce even by 360K. Complex **18** has a larger ring than complex **19**, and the higher temperature of coalescence for **19** is attributed to its smaller ring size. In contrast, complex **20**, which has the smallest ring size, cannot equilibrate between the syn and anti conformations, even at elevated temperatures. The results show that cyclic tungsten-bis(alkyne) complexes will form ring systems with ring sizes of approximately 10 atoms, that ring sizes of approximately 10 atoms are rigid, and that rigidity is lost as the ring size is increased.

Keywords:

Bioorganometallic, Tungsten, Alkyne, Peptide, Side Chain, Conformation

1. Introduction

Unlike other transition metals, tungsten will coordinate multiple alkyne ligands and form air stable complexes. The documented ability of $\text{W(CO)}_3(\text{dmtc})_2$ to quickly and easily react with two alkynes to form air stable bis(alkyne) complexes [1] attracted our attention, and in ongoing work from our laboratory we have been exploring the use of this chemistry to bring together [2-3], and in some cases constrain, peptides to certain secondary structures [4-6]. In this regard we developed methods for adding alkynes to the N- and C-termini of amino acid residues, and have coordinated these alkynylamino acids and alkynylpeptides to tungsten [2]. The data showed that the two coordinated

amino acids or peptides (for example **1**) do not interact via the formation of hydrogen bonds or other intramolecular interactions [2]. However, in another set of studies, peptides bearing an alkyne at both the N-terminus and the C-terminus will react with $W(CO)_3(dmtc)_2$ to yield metallacyclic species in which the two ends of the peptide are tied together via the tungsten-alkyne coordination [4-5]. These were the first reported cyclic tungsten bis-alkyne complexes. Data showed that in **2** (which has a dialkynyltetrapeptide coordinated to tungsten) the peptide part of the metallacyclicpeptide adopted a turn conformation [5].

Metallacyclicpeptides, like **2**, are constructed by linking a metal to two or more ligands on the peptide, with the metal becoming one of the atoms in the ring. Metallacyclicpeptides were reviewed in 2005 by Albrecht and Stortz [7]. All of the examples described in the review involved the use of coordination chemistry to link basic ligands on the peptide to the metal. These ligands included imidazoles, pyridines, phosphanes, catechols, hydroxyquinolines, aminodiacetates and crown ethers. The metals employed included Ti, Fe, Co, Ni, Cu, Zn, Mo, Ru, Rh, Pd, Cd and Cs. Metal-

ligand coordination in these studies yielded peptides constrained to helix, sheet and turn conformations.

Since publication of the review, newer metallacyclicpeptides have been described. Dugave and co-workers used Re complexes to constrain peptides that bind to biological targets [8-9]. Kuroda and co-workers have reported on the ability of peptide ligands to coordinate to Pd and Pt to constrain peptides to the 3_{10} -helical conformation [10]. Helical peptides were also prepared by Futaki and co-workers via coordination of dipicolyl ligands (located on amino acid side chains) to a number of transition metals [11]. Metallacyclicpeptides with defined chirality were obtained by Vazquez and co-workers via coordination of Co, Ni and Zn to bpy ligands tethered to peptides [12]. In the most recent report, Miguel and co-workers have created metallacyclicpeptides by dimerizing two peptides via metal coordination to pyridyl ligands located at the N- and C-termini [13].

In our own work we have chosen to explore metallacyclicpeptides that feature π -ligands, rather than basic ligands. Peptide helices have been generated using a ferrocene linker [14], and turn structures have been generated using coordination of tungsten to alkyne ligands located at the N- and C-termini of the peptide [4-5].

Having studied metallacyclicpeptides prepared by coordination of tungsten to dialkynylpeptides where the two alkynes are located at the N- and C-termini, we were also interested in whether metallacyclicpeptides could also be obtained from coordination of tungsten to dialkynylpeptides which have the two alkynes are located on the amino acid side chains.

This paper details our studies on the coordination of dialkynylpeptides to tungsten, where the two alkynes are located on amino acid side chains. The aim of the work was to delineate the conformational behavior of these novel species. First, the paper describes how alkynes can be located on the side chains of amino acids, and how these alkynylamino acids can be used in peptide synthesis. Second, the paper details how the dialkynylpeptides can be coordinated to tungsten via reaction with $W(CO)_3(dmtc)_2$. Finally, the conformational behavior of the resulting metallacyclicpeptides is explored. The data from this study and prior studies shed light on the factors responsible for constraining the conformations of these novel, metallacyclic molecules.

2. Results

2.1 Selection of Alkynylamino Acids

At the start of this study we sought to explore several different alkynylamino acids. The primary consideration in choosing which alkynylamino acids to use was distance of the alkyne from the peptide main chain. Given our past experience with dialkynylpeptides where the alkynes were located at the N- and C-termini [4-5], we were concerned that having the alkyne too far from the main chain would not confer enough conformational constraint to enforce a distinct conformation. On the other hand, having the alkyne too close to the main chain might prevent formation of the desired metallacycle. Thus, we studied three alkynylamino acids, **3-5**, that place the alkyne at varying distances from the amino acid C_α carbon. Acylation of the side chain amine of

lysine with propargyl chloroformate will generate alkynylamino acid **3**, which positions the alkyne 9 atoms from the C $_{\alpha}$ carbon. Alkylation of the side chain thiol of cysteine with propargyl bromide will generate alkynylamino acid **4**, which locates the alkyne 4 atoms from the C $_{\alpha}$ carbon. Finally, alkylation of glycine with propargyl bromide followed by resolution of the two enantiomers will generate alkynylamino acid **5** (propargylglycine) which places the alkyne only 2 atoms removed from the C $_{\alpha}$ carbon.

2.2 Preparation of Alkynylamino Acids and Dialkynylpeptides

Three alkynylamino acids were examined in this study. The first was the alkynyllysine, **3**. The side chain of lysine possesses a reactive primary amine that can be readily acylated. As shown in Scheme 1, the commercially available lysine derivatives **6** and **7** were coupled to yield the dipeptide **8**. The Cbz groups on **8** were then removed via catalytic hydrogenation, and the resulting diamine was acylated with propargylchloroformate to generate the dialkynyldipeptide, **9**. All of the dialkynyldipeptides (**9**, **14**, **17**) were purified by flash chromatography and its identity confirmed by ¹H NMR and ES-MS. Although racemization of the amino acid residues is

unlikely given the reaction conditions utilized here, purification would have removed any diastereomers that might have formed.

Scheme 1

The second alkynylamino acid examined was propargylcysteine. The synthesis of this amino acid from cysteine was described by Carson and Boggs [15]. Reaction of cysteine (**10**) with an excess of NaOEt and propargylbromide (Scheme 2) will yield propargylcysteine, **4**. To meet our needs we modified this literature procedure; instead of isolating the alkynylamino acid following the alkylation reaction, the crude propargylcysteine was subsequently reacted with Boc₂O to yield the known urethane **11** [16-18]. Next, **11** was acylated with anisidine using a water-soluble carbodiimide to yield **12**. Removal of the Boc group from **12** yielded the amine salt **13**. It was coupled with the acid **11** to produce the dialkynyldipeptide **14**.

Scheme 2

The final amino acid examined was propargylglycine (**5**), which can be obtained either from commercial vendors or prepared using literature procedures. We chose to purchase **5**, which is easily converted into the methyl ester hydrochloride **15** and the N-

Boc derivative **16** [17-29]. Coupling of **15** and **16** using the coupling reagent HATU produced the dialkynyl dipeptide **17** (Scheme 3).

Scheme 3

2.3 Coordination of Dialkynylpeptides to Tungsten

The dialkynyldipeptides **9**, **14**, and **17**, were reacted with $\text{W}(\text{CO})_3(\text{dmtc})_2$ [32] in refluxing methanol to produce, respectively, the metallacyclicdipeptides **18**, **19**, and **20**

(Scheme 4). We have performed these reactions two different ways, either by preparing a large stock of $W(CO)_3(dmtc)_2$ and using it when needed, or preparing a small quantity of $W(CO)_3(dmtc)_2$ and using it immediately. In our hands, $W(CO)_3(dmtc)_2$ begins decomposing shortly after it has been prepared, so reactions using a stock supply of $W(CO)_3(dmtc)_2$ have given low yields. Higher yields are obtained using freshly prepared $W(CO)_3(dmtc)_2$. All of these cyclization reactions were run at 1 mM concentrations of the dialkynyldipeptide in order to minimize the chance for oligomer formation. All of the reactions first turned from an orange color to a green color, indicating formation of the monoalkyne complex [2, 33]. The green color then turned to a lemon yellow color over the course of several hours. The appearance of the yellow color indicated formation of a bisalkyne complex [2, 34-35].

Scheme 4

To isolate the complexes **18-20**, the crude reaction mixture was evaporated, and the remaining residue was purified by flash chromatography. The unoptimized yields for **18**, **19** and **20** were 30%, 44% and 60% respectively. In all reactions some unreacted dialkyne remained behind. Analysis of the crude product by ESI-MS showed evidence for small amounts of oligomeric species, which has been seen in previous work [4]. Purity was established by HPLC, and the structures were confirmed by ^1H NMR and ESI-MS. The distinctive isotope pattern in the MS derived from the different isotopes of tungsten was used to firmly establish the identity of the metallacyclicdipeptides [2]. The pure complexes are amorphous yellow solids. They did not show any inclination to form crystalline solids.

2.4 Conformation of the Dialkynyldilysine Complex **18**

In previous work with metallacyclicpeptides derived from coordination of dialkynylpeptides where the alkynes were located at the N- and C-termini, we discovered that these cyclic species can and do adopt both the syn and anti orientations of the two alkyne ligands around the tungsten center [4-5]. Because the dialkynylpeptides examined here are not symmetrical, and because there are two ways to arrange the dmtc ligands around the tungsten, there are 8 possible isomers for these complexes. Shown in Figure 1 are the 8 possible conformations for the metallacyclicpeptide **18**. There are two sites where isomerism develops in these species. First, the dmtc ligands can adopt one of two possible unique arrangements. Isomers **18A-D** possess one of the two possible

arrangements for the dmtc ligands; isomers **18E-H** possess the other possible arrangement of the dmtc ligands. Second, the alkynes can be arranged in either a syn or anti arrangement. Isomers **18A-B** and **18E-F** have the alkyne ligands in the anti arrangement, while isomers **18C-D** and **18G-H** have the alkynes in the syn arrangement.

Figure 1

Figure 1. The 8 possible conformational isomers for complex **18**.

It is known from past work that in bis(alkyne) complexes the resonances for the alkyne hydrogens appear in the region around 11 ppm in the ^1H NMR spectrum [1]. For each of the eight possible conformations there are two different alkyne protons. This means that in the ^1H NMR spectrum of **18**, each conformation should give rise to two singlets derived from each alkyne hydrogen. If **18** adopts all 8 conformations shown in Figure 1, then the ^1H NMR spectrum should show 16 singlets in the alkyne hydrogen region, which is around 11 ppm.

Figure 2 shows the alkyne hydrogen region for complex **18** in CDCl_3 at 254 K. The spectrum shows a large number of singlets, indicating that **18** likely

Figure 2

Figure 2. The alkyne hydrogen region (11.4-10.7 ppm) of **18** in CDCl₃ at 254K.

adopts all 8 conformations shown in Figure 1. When the spectrum of **18** is taken at 274 and 294K in CDCl₃ the singlets begin to coalesce, indicating that rotation about the metal-alkyne bond at room temperature readily occurs (see Figure S1 in the Supplementary Data). When the spectrum of **18** is taken in d₆-DMSO at 294 K, two broad absorptions are observed (Figure 3). Like the NMR spectrum of **18** in CDCl₃, the spectrum of **18** in d₆-DMSO indicates a system where rotation about the metal-alkyne

bonds is taking place. Because DMSO is an aggressive hydrogen bond acceptor and CDCl_3 is not, the NMR spectra of molecules that can form hydrogen bonds (like **18**) can often show differences in chemical shifts between the two solvents. This is the likely reason why the NMR spectra of **18** in CDCl_3 and d_6 -DMSO at 294K (Figures 2 and 3) are not identical.

That the alkynes rotate about the tungsten in **18** was confirmed by the behavior of the DMSO sample when the temperature was raised (Figure 3). The two broad peaks coalesced into one peak around 343K. When the sample was returned to 294K the spectrum was identical to what it had been before heating, indicating that heating did not induce decomposition. This shows that the various conformers (unknown in number) are rapidly equilibrating at this temperature. In previous work we have found that acyclic bis(alkyne) complexes derived from peptides and amino acids also coalesce around 338K [2]. Thus, the behavior of **18** closely mirrors the behavior of these acyclic complexes, indicating that there is a great deal of conformational flexibility for the two alkynes in this metallacycle to equilibrate between conformers. This is not surprising, given that the macrocyclic ring formed by coordination of the tungsten to the two alkynes comprises 23 atoms.

Figure 3

Figure 3. The ^1H NMR spectra of the alkyne hydrogens (11.15-10.85 ppm) in complex **18** at temperatures ranging from 294K to 353K in $\text{d}_6\text{-DMSO}$.

2.5 Conformation of the Dipropargylcysteine Complex **19**

Since **18** was conformationally flexible, we next examined complex **19**, which was made by coordination of tungsten to the two alkynes in a dipeptide constructed from two propargylcysteines. The ring formed in this complex is smaller than the ring found in **18**; it is comprised of 13 atoms, including the tungsten. If **19** is as flexible as **18**, then it too should adopt all 8 conformations shown in Figure 4. This flexibility can be

Figure 4

Figure 4. The 8 possible conformational isomers for complex **19**.

detected by the presence of many singlets for the alkyne hydrogens in the ^1H NMR spectrum, and by their ability to coalesce to a single peak at elevated temperatures. This

is what the data shows. The ^1H NMR spectrum of **19** in CDCl_3 or d_6 -DMSO in the region around 11 ppm shows numerous, overlapping singlets for the alkyne hydrogens. When the d_6 -DMSO sample was heated to 360K, the alkyne hydrogen singlets begin to coalesce to a single peak, but unlike **18**, they must coalesce at a higher temperature (Figure 5). This data indicates that **19**, like **18**, is conformationally flexible and likely adopts all 8 of the possible orientations shown in Figure 4. The higher temperature for coalescence of the alkyne hydrogen peaks in **19** relative to **18** suggests that the smaller ring size in **19** makes it more difficult for the syn and anti conformers to equilibrate.

Figure 5

Figure 5. The ^1H NMR spectra of the alkyne hydrogens (11.5-10.7 ppm) in complex **19** in d_6 -DMSO at temperatures ranging from 294K to 360K.

The last complex examined here was **20**. In this molecule the tungsten is linked to the two alkynes in a dipeptide of two propargylglycine residues. This complex has the least number of atoms in the ring completed by coordination of the tungsten, 10 atoms. The ^1H NMR spectrum of **20** in CDCl_3 in the region around 11 ppm shows 8 singlets. Although it is not limited to one solution conformation, **20** is different from **18** and **19**; it only adopts 4 of the possible 8 conformations shown in Figure 6. The spectrum also indicates that there is a decided preference for one of the 4 possible conformations.

Figure 6

Figure 6. The 8 possible conformational isomers for complex **20**.

Like the other complexes, **20** was also examined by variable temperature NMR. The results are shown in Figure S2 (in the Supplementary Data). In this experiment the temperature of a d_6 -DMSO solution of **20** was varied between 293K and 343K. Unlike **18** and **19**, where the alkyne hydrogen resonances coalesce as the temperature is raised,

with **20** the alkyne hydrogens show no inclination to coalesce; rather in some cases they move apart and become more distinct as the temperature is raised. Also, the relative integrations of the singlets do not change as the temperature is raised. This indicates that the alkyne groups in **20** are unable to rotate about the tungsten center, and so cannot equilibrate between the various conformations shown in Figure 6.

3. Discussion

The results of this work show that cyclic tungsten bis(alkyne) complexes can be made by reaction of dialkynylpeptides with $\text{W(CO)}_3(\text{dmtc})_2$ where the alkynes reside on the amino acid side chains. The cyclic complexes can be made with long (**18**), medium (**19**) and short dialkynes (**20**).

The conformational mobility of **18-20** reinforces our earlier findings that most cyclic tungsten bis(alkyne) complexes adopt conformations where the alkyne ligands are both syn and anti to each other, and that often the two alkyne ligands can equilibrate between the syn and anti conformations. This is how complexes **18** and **19** behave; they likely adopt all 8 possible conformations (see Figures 1 and 4) and will equilibrate between the syn and anti conformers. In contrast, the data for the smallest cyclic structure in this study, complex **20** derived from propargylglycine, shows that this molecule exists in a limited number of the conformations shown in Figure 6, and that each conformation adopted is fixed. Complex **20** cannot equilibrate between the syn and anti conformations, even at elevated temperatures. The conformation obtained when the

second alkyne coordinates is the conformation of the complex. We found similar behavior with a dialkynylderivative of alanine, **21**, which adopts a number of different conformational isomers, but because of the rigid nature of the ring, the conformational isomers cannot interconvert [4]. Complexes **20** and **21** have similar ring sizes. There are 11 atoms in the ring (including tungsten) for **21**, while in **20** there are 10 atoms in the in the ring (including tungsten). To date, **20** is the smallest cyclic tungsten bis-alkyne complex that we have been able to make, isolate and characterize. Attempts to make a smaller cyclic tungsten bis-alkyne complex, **22** (which would have a ring size of only 8 atoms), only produced oligomeric species [4]. Thus, our data indicate that a ring of 10 atoms may be the lower limit for the possible ring sizes possible for cyclic tungsten bis-alkyne complexes.

There were two goals for this study. First, to gain a further understanding of the conformational behavior of cyclic tungsten-bis(alkyne) complexes, and second, to uncover ways to use tungsten-alkyne coordination to constrain peptide conformation.

With regards to the first goal, the data presented here and in previous work [4-5] has shown that metallacyclic species employing tungsten-alkyne coordination are

unlikely to have ring sizes less than 10 atoms. Further, rings comprising 10 or 11 atoms are rigid, the alkyne ligands are unable to rotate about the metal; unfortunately these species do not adopt one single conformation in solution. Finally, ring sizes larger than 10 or 11 atoms possess the ability to interconvert and equilibrate between syn and anti conformers.

With regards to the second goal, the ability of the tungsten alkyne bonds to interconvert between the syn and anti conformers has so far prevented the discovery of a system that constrains a peptide to one discrete conformation. The results of prior work [4-5] and this work, however, indicate that such a system must encompass a ring of around 10 atoms. Explorations in this area are ongoing.

4. Experimental

4.1 General procedures

Anisidine, L-cysteine hydrochloride and propargylchloroformate were purchased from Aldrich Chemical. Anisole, trifluoroacetic acid (TFA), propargyl bromide, tetrahydrofuran (THF), di-tert-butyl dicarbonate (Boc₂O), 1-ethyl-3-[3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), and triethylamine were purchased from Acros Organics. Diisopropylethylamine (DIEA), methanol, ethanol, hexanes, ethyl acetate, methylene chloride, toluene, ethyl ether, hydrochloric acid, sodium hydroxide, magnesium sulfate, and iodine were purchased from Fisher Scientific. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. The

propargylglycine derivatives were purchased from Bachem. Other amino acid derivatives, PyBOP, and piperidine were purchased from Chem Impex International. Silica gel for flash chromatography was purchased from Silicycle. NMR spectra were obtained on either either a Bruker Avance III 400 MHz instrument or 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) in borosilicate glass test tubes. Theoretical mass spectral isotope patterns were calculated using the online isotope pattern calculator provided on the internet by the Swiss Federal Institute of Aquatic Science and Technology (Eawag) (<http://www.envipat.eawag.ch/index.php>) [36]. 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 at 300 nm, which is the wavelength of maximum absorbance for the monoalkyne complexes. A Phenomenex Luna 250 x 4.6 mm 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 20:80 mixture of acetonitrile and trifluoroacetic acid and changed to 100% acetonitrile over the course of 12 minutes. The solvent was then held at 100% acetonitrile for an additional 2 minutes.

4.2 Preparation of Boc-Lys(Cbz)-Lys(Cbz)-OMe, **8**

To a solution of 505 mg (1.06 mmol, 1 equiv.) of Boc-Lys(Z)-OSu (**6**) and 363 mg (1.10 mmol, 1.03 equiv.) of H-Lys(Z)-OMe (**7**) in 20 mL CH₂Cl₂ was added 1.0 mL

(5.7 mmol, 5 equiv.) of DIEA. The resulting clear solution stirred at 23°C for 18 h. The solvents were evaporated and the remaining oil was redissolved in 100 mL EtOAc and washed: 3 x 25 mL 1M HCl, 3 x 25 mL saturated NaHCO₃ and 1 x 25 mL brine. The EtOAc was dried (MgSO₄), filtered, evaporated and dried under high vacuum to yield 648 mg (93%) of pure **8** as a white foam: TLC (2:1 EtOAc/hexanes), R_f 0.46; ¹H NMR (400 MHz, CDCl₃): 7.34 (10H, m, 2 x C₆H₅), 6.70 (1H, d, J=7.2 Hz, amide NH), 5.25-4.90 (7H, m, 3 x urethane NH + 2 x OCH₂), 4.55 (1H, m, C_αH), 4.09 (1H, m, C_αH), 3.69 (3H, s, OMe), 3.17 (4H, m, 2 x CH₂N), 1.97-1.27 (12H, m, 2 x CH₂CH₂CH₂), 1.43 (9H, s, OtBu) ; ESMS, M + Na ion pattern calculated for C₃₄H₄₈N₄O₉Na: 679 (100), 680 (39.2), 681 (9.3); found 679 (100), 680 (36.5), 681 (10.8).

4.3 Preparation of Boc-Lys(Poc)-Lys(Poc)-OMe, **9**

To a solution of 624 mg (0.953 mmol, 1.0 equiv.) of **8** in 50 mL EtOH was added 50 mg of 10% Pd-C. The resulting mixture was hydrogenated on a Parr apparatus for 2 h at 25°C. After 2 h the catalyst was removed by filtration through celite and the filtrate evaporated. The remaining oil was redissolved in 20 mL CH₂Cl₂, and to this solution was added 0.5 mL (2.9 mmol, 3 equiv.) of DIEA and 232 μL (2.38 mmol, 2.5 equiv.) of propargylchloroformate. After stirring at 23°C for 72 h the solvents were evaporated. The residue that remained was redissolved in 50 mL EtOAc and washed: 3 x 25 mL 1M HCl, 3 x 25 mL saturated NaHCO₃ and 1 x 25 mL brine. The EtOAc was dried (MgSO₄), filtered, evaporated and dried under high vacuum to yield 226 mg (43%) of **9** as a clear oil: TLC (4:1 EtOAc/hexanes) R_f 0.63; ¹H NMR (400 MHz, CDCl₃): 6.88 (1H,

d, J=7.7 Hz, amide NH), 5.35 (2H, m, 2 x urethane NH), 5.19 (1H, t, J=5.2 Hz, urethane NH), 4.75-4.60 (4H, m, 2 x OCH₂), 4.56 (1H, m, C_αH), 4.17 (1H, m, C_αH), 3.76 (3H, s, OMe), 3.31-3.09 (4H, m, 2 x CH₂N), 2.51 (1H, t, J=2.5 Hz, CCH), 2.49 (1H, t, J=2.4 Hz, CCH), 1.85 -1.25 (12H, m, 2 x CH₂CH₂CH₂), 1.45 (9H, s, OtBu); ESMS, M + Na ion pattern calculated for C₂₆H₄₀N₄O₉Na: 575 (100), 576 (30.4), 577 (6.3); found 575 (100), 576 (28.1), 577 (11.5).

4.4 Preparation of Boc-Cys(CH₂CCH)-OH, **11** [8-10]

To 60 mL of absolute EtOH under N₂ was added 1.030 g (44.8 mmol, 4.0 equiv.) of Na metal. Once all the Na had reacted, 1.956 g (11.1 mmol, 1.0 equiv.) of L-cysteine hydrochloride hydrate and 4.90 mL (44.8 mmol, 4.0 equiv.) of propargyl bromide were added to the solution. After stirring for 2 h, the solvent was evaporated. The remaining residue was redissolved in 15 mL THF. To this solution was added a solution of 4.865 g (22.3 mmol, 2.0 equiv.) of Boc₂O dissolved in 12.5 mL THF, followed by 5.0 mL (55 mmol, 5 equiv.) of Et₃N and 25 mL of H₂O. After stirring for 18 h the THF was evaporated, and the remaining aqueous layer was brought to pH > 11 by the addition of 1M NaOH. The alkaline aqueous layer was washed 3 x 25 mL EtOAc, then acidified to pH 2 using concentrated HCl. The resulting acidic aqueous layer was extracted 3 x 25 mL EtOAc. The combined EtOAc extracts were dried (MgSO₄), filtered and evaporated to yield 1.96 g of **11** [16-18] as a tan oil: TLC (1:1 EtOAc/MeOH), R_f 0.67; ¹H NMR (400 MHz, CDCl₃): 6.36 (1H, br s, OH), 5.39 (1H, d, J = 7.7 Hz, NH), 4.62 (1H, q, J = 5.9

Hz, C α H), 3.33 (2H, dd, J = 8.8 and 2.7 Hz, SCH₂), 3.27 (2H, dd, J = 13.3 and 4.8 Hz, β -CH₂), 2.32 (1H, s, CCH), 1.49 (9H, s, OtBu).

4.5 Preparation of Boc-Cys(CH₂CCH)-NHC₆H₄OMe, **12**

To a solution of 0.500 g (1.93 mmol, 1.0 equiv.) of **11** in 2.0 mL CH₂Cl₂ at 5°C was added 0.261 g (2.12 mmol, 1.1 equiv.) of anisidine and 0.443 g (2.31 mmol, 1.2 equiv.) of EDC. The resulting solution warmed to 23°C over the course of 4 h. The solvents were evaporated and the residue redissolved in 25 mL EtOAc and washed: 3 x 25 mL 0.1 M HCl, 3 x 25 mL saturated NaHCO₃ and 1 x 25 mL brine. The organic layer was dried (MgSO₄), filtered and evaporated. The oil that remained was purified by flash chromatography (2:1 EtOAc:hexanes) to afford 0.258 g of pure **12** (37%) as an oil: TLC (1:1 EtOAc/MeOH), R_f 0.67; ¹H NMR (400 MHz, CDCl₃): 8.17 (1H, s, ArNH), 7.43 (2H, d, J=8.9 Hz, 2 x ArH), 6.87 (2H, d, J = 9.0 Hz, 2 x ArH), 5.44 (1H, d, J = 6.6 Hz, urethane NH), 4.49 (1H, q, J = 6.8 Hz, C α H), 3.80 (3H, s, OMe), 3.34 (2H, d, J = 2.6 Hz, SCH₂), 3.15 (2H, m, β -CH₂), 2.32 (1H, s, CCH), 1.48 (9H, s, OtBu); ESMS, M + Na ion pattern calculated for C₁₈H₂₄N₂O₄Na: 387 (100), 388 (21.5), 389 (7.4); found 387 (100), 388 (19.1), 389 (6.3).

4.6 Preparation of Boc-Cys(CH₂CCH)-Cys(CH₂CCH)-NHC₆H₄OMe, **14**

To a solution of 0.100 g (0.274 mmol, 1.0 equiv.) of **12** in 1 mL CH₂Cl₂ and 50 μ L of anisole at 5°C was added 1 mL of trifluoroacetic acid. The resulting solution slowly warmed to 23°C over the course of 4 h. The solvents were evaporated and the crude amine salt (**13**) was dried under high vacuum for 14 h. Crude **13** was then redissolved in 1 mL CH₂Cl₂. To this solution was added 0.098 g (0.40 mmol, 1.5 equiv) of **16** and 0.29 mL (2.1 mmol, 7.6 equiv.) of triethylamine. After stirring at 23°C for 4 h the solvents were evaporated. The residue was redissolved in 30 mL EtOAc and washed 3 x 10 mL 0.1 M HCl, 3 x 10 mL saturated NaHCO₃ and 1 x 10 mL brine. The organic layer was dried (MgSO₄), filtered and evaporated to yield 0.042 g (30%) of **14** as an oil: TLC (3:2 EtOAc/hexanes), R_f 0.60; ¹H NMR (400 MHz, CDCl₃): 8.71 (1H, s, ArNH), 7.45 (2H, d, J = 9.1 Hz, 2 x ArH), 6.19 (2H, d, J = 9.0 Hz, 2 x ArH), 5.62 (1H, d, J = 6.4 Hz, amide NH), 5.52 (1H, d, J = 7.6 Hz, urethane NH), 4.87 (1H, m, C α H), 4.45 (1H, q, J = 5.9 Hz, C α H), 3.77 (3H, s, OMe), 3.31 (4H, m, 2 x SCH₂), 3.12 (4H, m, 2 x β -CH₂), 2.33 (2H, m, 2 x CCH), 1.44 (9H, s, OtBu); ESMS, M + Na ion pattern calculated for C₂₄H₃₁N₃O₅Na: 528 (100), 529 (29.2), 530 (9.3), 531 (2.7); found 528 (100), 529 (25.9), 530 (11.5), 531 (2.5).

4.7 Preparation of Boc-Prg-Prg-OMe, **17**

To a solution of 1.60 g (7.51 mmol, 1 equiv.) of **16** in 50 mL CH₂Cl₂ was added 1.23 g (7.50 mmol, 1 equiv.) of **15**, followed by 1.5 mL (8.6 mmol, 1.1 equiv.) of DIEA

and 1.63 g (8.49 mmol, 1.1 equiv.) of EDC. After stirring at 23°C for 18 h an additional 50 mL of CH₂Cl₂ was added, and the diluted solution was washed: 4 x 25 mL 0.1 M HCl, 3 x 25 mL saturated NaHCO₃ and 1 x 25 mL brine. The organic layer was dried (MgSO₄), filtered and evaporated to yield 1.57 g (65%) of **17** as a clear oil: TLC (Et₂O), R_f 0.71; ¹H NMR (400 MHz, CDCl₃): 7.17 (1H, s, amide NH), 5.39 (1H, s, urethane NH), 4.67 (1H, m, C_αH), 4.31 (1H, m, C_αH), 3.72 (3H, s, OMe), 2.72-2.56 (4H, m, 2 x CH₂), 2.05 (1H, s, CCH), 1.98 (1H, s, CCH), 1.40 (9H, s, OtBu); ESMS, M + Na ion pattern calculated for C₁₆H₂₂N₂O₅Na: 345 (100), 346 (17.9), 347 (2.7); found 345 (100), 346 (15.5), 347 (2.0).

4.8 Preparation of Cyclic Complex **18**

To a refluxing solution of 205 mg (0.371 mmol, 1 equiv.) of **9** in 250 mL degassed MeOH under N₂ was added dropwise over 1 h a solution of 189 mg (0.371 mmol, 1 equiv.) of W(CO)₃(dmtc)₂ [32] dissolved in 30 mL of degassed CH₂Cl₂. After 2 h the solution had taken on a lemon yellow color. Reflux was stopped the solvents evaporated. Flash chromatography (EtOAc) was used to isolate the product. Obtained 110 mg (30%) of **18** as an amorphous yellow solid: TLC (EtOAc), R_f 0.53; HPLC, R_t 7.44 min; ¹H NMR (400 MHz, CDCl₃): 11.2-10.8 (2H, ms, 2 x CCH), 7.6-6.7 (1H, m, amide NH), 6.2-4.0 (9H, m, 2 x OCH₂ + 3 x urethane NH + 2 x C_αH), 3.8-3.0 (19H, m, OMe + 2 x CH₂N + 4 x dmtc Me), 1.9-1.1 (21H, m, 2 x CH₂CH₂CH₂ + OtBu); ESMS, M + Na ion pattern calculated for WC₃₂H₅₂N₆O₉S₄Na: 997 (60.3), 998 (57.3), 999 (100),

1000 (43.1), 1001 (89.3), 1002 (34.3), 1003 (20.7), 1004 (6.4), 1005 (2.2); found 997 (62.3), 998 (63.4), 999 (100), 1000 (40.9), 1001 (91.6), 1002 (30.5), 1003 (18.9), 1004 (4.9), 1005 (1.7).

4.9 Preparation of Cyclic Complex **19**

To a refluxing solution of 0.504 g (0.992 mmol, 1 equiv.) of $W(CO)_3(dmtc)_2$ [32] in 1.1 L of degassed MeOH under N_2 was added via syringe a solution of 0.504 g (0.997 mmol, 1 equiv.) of **14** dissolved in 7 mL of degassed MeOH. The solution immediately took on a dark green color. The color turned to lemon yellow after 45 min of reflux. The methanol was evaporated and the crude product purified by flash chromatography (1:1 ethyl acetate/hexanes). A total of 0.408 g (44 %) of pure **19** was obtained as an amorphous yellow solid. TLC (1:1 EtOAc/hexanes), R_f 0.20; HPLC, R_t 8.42 min; 1H NMR (400 MHz, $CDCl_3$): 11.5-11.0 (2H, ms, 2 x CCH), 8.8-8.2 (1H, ms, ArNH), 7.7-7.4 (3H, m, amide NH + 2 x ArH), 6.9-6.7 (2H, mm 2 x ArH), 5.6-5.1 (1H, m, urethane NH), 5.0-4.0 (6H, m, 2 x $C_{\alpha}H$ + 2 x SCH_2), 3.80 (3H, ms, OMe), 3.7-2.2 (16H, m, 4 x dmtc Me + 2 x β -CH₂), 1.45 (9H, ms, OtBu); ESMS, M + Na ion pattern calculated for $WC_{30}H_{43}N_5O_5S_6Na$: 950 (57.9), 951 (54.3), 952 (100), 953 (44.6), 954 (93.0), 955 (35.2), 956 (26.5), 957 (8.5); found 950 (67.1), 951 (56.3), 952 (100), 953 (44.3), 954 (90.2), 955 (37.8), 956 (31.8), 957 (10.1).

4.10 Preparation of Cyclic Complex **20**

To a refluxing solution of 91.5 mg (0.180 mmol, 1.05 equiv.) of $\text{W}(\text{CO})_3(\text{dmtc})_2$ [32] in 200 mL of degassed MeOH under N_2 was added via syringe a solution of 55 mg (0.17 mmol, 1 equiv.) of **17** dissolved in 5 mL of degassed MeOH. The solution immediately took on a dark green color. The color turned to lemon yellow after 2 h of reflux. The methanol was evaporated and the crude product purified by flash chromatography (ethyl acetate). A total of 80 mg (63 %) of pure **20** was obtained as an amorphous yellow solid. TLC (1:1 EtOAc/hexanes), R_f 0.16; HPLC, R_t 7.02 min; ^1H NMR (400 MHz, CDCl_3): 11.5-11.2 (2H, ms, 2 x CCH), 5.5-5.0 (1H, m, amide NH), 4.88 (1H, brs, urethane NH), 4.66-4.40 (1H, m, C_αH), 4.35-3.55 (7H, m, C_αH + OMe + 1 x dmtc Me), 3.54-3.24 (7H, m, 2 x CH_2 + 1 x dmtc Me), 3.23-3.00 (6H, m, 2 x dmtc Me), 1.52-1.30 (9H, ms, OtBu); ESMS, M + Na ion pattern calculated for $\text{WC}_{22}\text{H}_{34}\text{N}_4\text{O}_5\text{S}_4\text{Na}$: 767 (64.7), 768 (53.8), 769 (100), 770 (33.5), 771 (90.4), 772 (25.5), 773 (17.8), 774 (4.3); found 767 (65.0), 768 (61.7), 769 (100), 770 (37.7), 771 (93.7), 772 (26.3), 773 (18.3), 774 (4.6).

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