ORGANIC LETTERS

2006
Vol. 8, No. 11
2381−2384

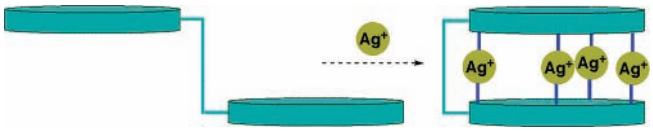
Synthesis and Ag(I) Complexation Studies of Tethered Westiellamide

Peter Wipf* and Chenbo Wang

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260
pwipf+@pitt.edu

Received March 18, 2006

ABSTRACT



A new tethered macrocyclic ring system based on the natural product westiellamide was prepared to increase the affinity and ease of complexation to Ag(I) ions. NMR and fluorescence Ag(I) titrations confirmed that the tethered macrocycles preserve the unique, high-affinity coordination mode of westiellamide.

The marine genus *Lissoclinum* has been a rich source of cyclopeptide alkaloids featuring multiple oxazolines, thiazolines, oxazoles, or thiazoles.[1] Several of these macrocyclic-heterocyclic scaffolds have been found to complex to metal ions,[2] in some cases with unique selectivities.[2o] The direct biological relevence of these metal ion binding properties, however, is still unresolved.[2k,n,p]

Previously, we reported the first example of a silver-ion-selective *Lissoclinum* cyclopeptide interaction.[2o] Westiellamide (**1**) was found to bind to Ag(I) salts in a unique $[(\mathbf{1})_2Ag_4]^{4+}$ complex (Figure 1). An association constant (K_a) of $\geq 2.8 \times 10^{13}$ M^{-5} was determined by NMR titration. Tethered cyclopeptides, or "peptide cylinders", have been

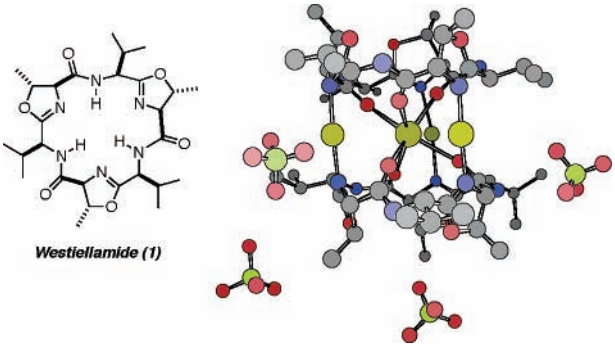


Figure 1. Westiellamide (**1**, left) and the crystal structure of the $[(\mathbf{1})_2Ag_4][ClO_4]_4$ complex.

(1) For reviews on the chemistry of *Lissoclinum* peptides, see: (a) Wipf, P. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier: Amsterdam, 1998; Vol. 12, p 187. (b) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115. (c) Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441.

(2) (a) van den Brenk, A. L.; Tyndall, J. D. A.; Cusack, R. M.; Jones, A.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R. *J. Inorg. Biochem.* **2004**, *98*, 1857. (b) Bernhardt, P. V.; Comba, P.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Lotzbeyer, L. *Chem.−Eur. J.* **2002**, *8*, 1527. (c) Morris, L. A.; Milne, B. F.; Thompson, G. S.; Jaspars, M. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1072. (d) Cusack, R. M.; Grøndahl, L.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R. *J. Chem. Soc., Perkin Trans. 2* **2002**, 556. (e) Morris, L. A.; Jaspars, M.; Kettenes-van den Bosch, J. J.; Versluis, K.; Heck, A. J. R.; Kelly, S. M.; Price, N. C. *Tetrahedron* **2001**, *57*, 3185. (f) Cusack, R. M.; Grøndahl, L.; Abbenante, G.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hambley, T. W. *J. Chem. Soc., Perkin Trans. 2* **2000**, 323. (g) Morris, L. A.; Jaspars, M. *Royal Soc. Chem.* **2000** *257*, 140. (h) Blake, A. J.; Hannam, J. S.; Jolliffee, K. A.; Pattenden, G. *Synlett* **2000**, 1515. (i) Grøndahl, L.; Sokolenko, N.; Abbenante, G.; Fairlie, D. P.; Hanson, G. R.; Gahan, L. R. *J. Chem. Soc., Dalton Trans.* **1999**, 1227. (j) Comba, P.; Cusack, R.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Kazmaier, U.; Ramlow, A. *Inorg. Chem.* **1998**, *37*, 6721. (k) Freeman, D. J.; Pattenden, G.; Drake, A. F.; Siligardi, G. *J. Chem. Soc., Perkin Trans. 2* **1998**, 129. (l) van den Brenk, A. L.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hambley, T. W. *Inorg. Chem.* **1996**, *35*, 1095. (m) van den Brenk, A. L.; Byriel, K. A.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hawkins, C. J.; Jones, A.; Kennard, C. H. L.; Moubaraki, B.; Murray, K. S. *Inorg. Chem.* **1994**, *33*, 3549. (n) van den Brenk, A. L.; Fairlie, D. P.; Hanson, G. R.; Gahan, L. R.; Hawkins, C. J.; Jones, A. *Inorg. Chem.* **1994**, *33*, 2280. (o) Wipf, P.; Venkatraman, S.; Miller, C.; Geib, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1516. (p) Michael, J. P.; Pattenden, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1. (q) van den Brenk, A. L.; Hanson, G. R.; Hawkins, C. J. *J. Inorg. Biochem.* **1989**, *36*, 165.

(3) (a) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503. (b) Pattenden, G.; Thompson, T. *Chem. Commun.* **2001**, 717. (c) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 12364.

10.1021/ol060677c CCC: $33.50
Published on Web 05/04/2006

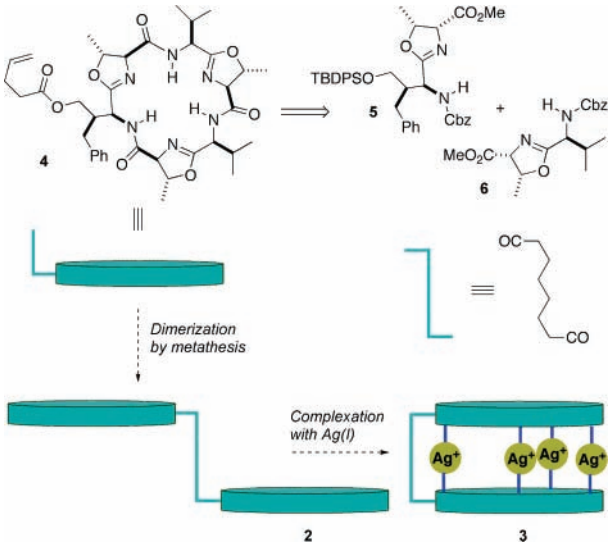


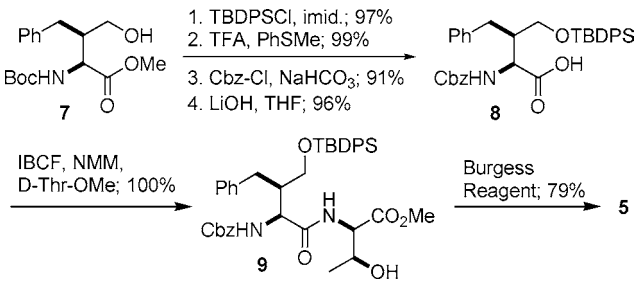
Figure 2. Ligand design and retrosynthetic analysis.

reported as macromolecular devices or scaffolds for protein mimics.[3] We were also encouraged to continue our studies of the $[(\mathbf{1})_2Ag_4][ClO_4]_4$ system because of the noteworthy antimicrobial and imaging properties of silver complexes.[4] To improve the binding affinity and simplify the stoichiometry of macrocycle−silver chelation, we designed new tethered cyclopeptide ligands (**2**, Figure 2). By tethering two westiellamide molecules, we expected the entropy loss of the binding process to be greatly reduced, thus increasing the K_a.

The westiellamide analogue **4** can be prepared from dipeptidyl oxazolines **5** and **6**.[5] The efficient synthesis of building block **5** is summarized in Scheme 1. TBDPS protection of the known compound **7**[6] was followed by a switch of the N-protective group from Boc to Cbz. Saponification of the methyl ester provided **8**, which was subsequently coupled to D-threonine methyl ester with isobutyl chloroformate (IBCF) and *N*-methylmorpholine (NMM). The dipeptidyl oxazoline **5** was formed in 79% yield by the treatment of the dipeptide **9** with Burgess reagent.[7]

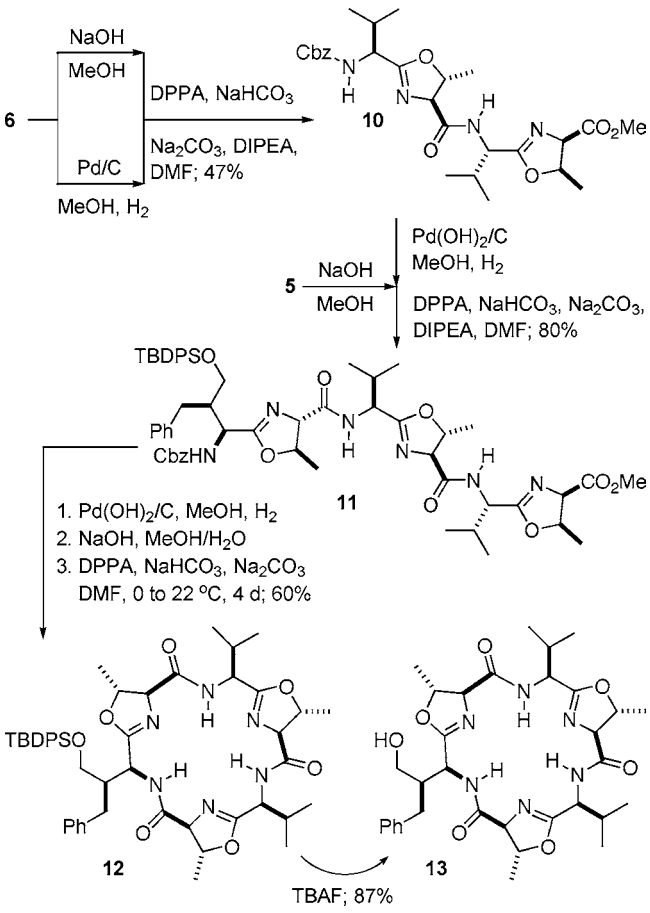
The synthesis of segment **10** was achieved by deprotection of **6**[8] followed by diphenylphosphoryl azide (DPPA)-mediated dimerization (Scheme 2). Under the conditions of methyl ester saponification, α-epimerization converts the *cis*-

Scheme 1



to the *trans*-oxazoline configuration.[7] Condensation of the carboxylic acid derived from dipeptide **5** with the primary amine derived from tetrapeptide **10** in the presence of DPPA gave the desired hexapeptide **11** in 80% yield. Upon cleavage of the Cbz group and saponification of the methyl ester, DPPA-mediated macrolactamization in a Na_2CO_3−$NaHCO_3$ buffer[8] gave the desired cyclopeptide **12** in 60% yield. The TBDPS group was removed with TBAF to provide alcohol **13**. The X-ray analysis of the *p*-bromobenzoyl ester of **13** confirmed its configuration and demonstrated an aza-crown ether macrocycle conformation analogous to that of uncomplexed westiellamide (Figure 3).[9] Acylation of **13** with pent-4-enoyl chloride gave **4**, which was dimerized by alkene

Scheme 2



(4) (a) Makarava, N.; Parfenov, A.; Baskakov, I. V. *Biophys. J.* **2005**, *89*, 572. (b) Holt, K. B.; Bard, A. J. *Biochemistry* **2005**, *44*, 13214. (c) Melaiye, A.; Simons, R. S.; Milsted, A.; Pingitore, F.; Wesdemiotis, C.; Tessier, C. A.; Youngs, W. J. *J. Med. Chem.* **2004**, *47*, 973. (d) Aymonier, C.; Schlotterbeck, U.; Antonietti, L.; Zacharias, P.; Thomann, R.; Tiller, J. C.; Mecking, S. *Chem. Commun.* **2002**, 3018. (e) Balogh, L.; Swanson, D. R.; Tomalia, D. A.; Hagnauer, G. L.; McManus, A. T. *Nano Lett.* **2001**, *1*, 18.

(5) The benzyl side chain, rather than a simple valine-like β-methyl substituent, was selected to facilitate the synthesis and improve UV visualization.

(6) Ndungu, J. M.; Gu, X.; Gross, D. E.; Cain, J. P.; Carducci, M. D.; Hruby, V. J. *Tetrahedron Lett.* **2004**, *45*, 4139.

(7) Wipf, P.; Miller, C. P. *Tetrahedron Lett.* **1992**, *33*, 907.

(8) Wipf, P.; Miller, C. P.; Grant, C. M. *Tetrahedron* **2000**, *56*, 9143.

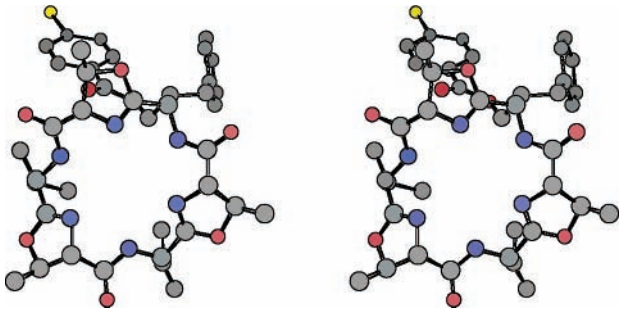
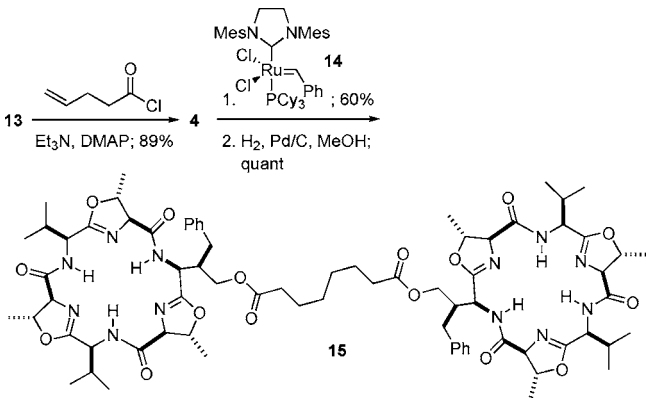


Figure 3. Stereoview of the X-ray structure of the *p*-bromobenzoyl ester of **13**.

metathesis in the presence of catalyst **14**[10] to provide the dimer as a mixture of cis/trans isomers (Scheme 3). Finally,

Scheme 3



the tethered ligand **15** was obtained in quantitative yield by hydrogenation.

The complexation of **15** with Ag(I) perchlorate was first studied by NMR titration in a fashion analogous to westiellamide.[2o] As expected, a stoichiometry of 4:1 (Ag^+/**15**) was determined, along with a $K_a \geq 1.8 \pm 1.4 \times 10^{11}$ M^{-4} in CD_3OD and D_2O (9:1, v/v). Previously, NMR titration of the natural product **1**, which binds in a 4:2 (Ag^+/**1**) stoichiometry, provided a $K_a \geq 2.8 \times 10^{13}$ M^{-5}.[2o] Obviously, the silver complexation with **1** is more sensitive to receptor concentration. However, in both cases, the binding process is highly cooperative and no intermediate states were observed during the titration. Moreover, because of the high silver affinity of both receptors and the relatively high concentrations required for analyte detection, NMR titration only provides a lower limit of the association constant.[11] Accordingly, the K_a was also determined by a competitive binding study[12] with fluorophore **16** (Figure 4).[13] In these

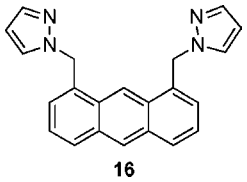


Figure 4. Fluorophore for the competitive binding study.

experiments, westiellamide and **15** exhibited considerably higher K_a's of $2.8 \pm 1.9 \times 10^{22}$ M^{-5} and $3.5 \pm 3.9 \times 10^{21}$ M^{-4}, respectively.[14] The competitive binding experiment with a fluorophore represents a more sensitive tool for the determination of high association constants. Most importantly, both analytical methods demonstrate that the tether considerably improves potential practical applications of the cyclooxazoline scaffold for silver complexation and delivery in the low micromolar range. For example, 50% complexation of a 10 μM solution of Ag(I) would require 270 μM of westiellamide vs 2 μM of **15**.

Figure 5 shows a stereoview for a model of the silver complex of **15**.[15] The overall folding of the macrocyclic azacrown ring system and all heteroatom−Ag bond lengths are close to those in the westiellamide−silver complex, and the linker chain assumes an extended zigzag conformation.

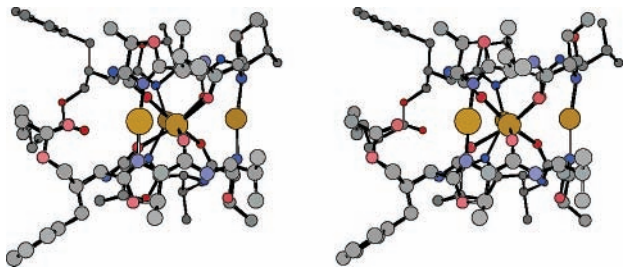


Figure 5. Computer-generated stereomodel of the $[(\mathbf{15})Ag_4]^{4+}$ complex. The model was obtained by a conformational search using CONFLEX/MM3 followed by AM1/COSMO minimization of the lowest-energy structure in CaChe 6.1.

We also briefly examined the effect of the tether length on Ag binding. Acylation of **13** with adipoyl chloride provided ligand **17**, which has two fewer methylene groups in the tether chain than **15** (Scheme 4). As expected, on the

(9) Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; van den Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341.

(10) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.

(11) See, for example: Shi, Y.; Schneider, H.-J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1797.

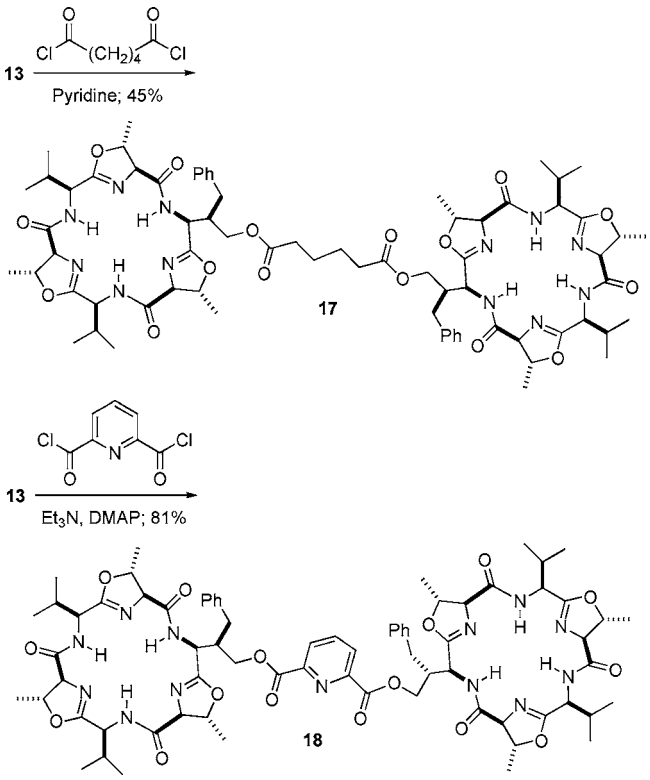
(12) Connors, K. A. *Binding Constants, The Measurement of Molecular Complex Stabiliy*; John Wiley & Sons: New York, 1987; p 175.

(13) Kang, J.; Choi, M.; Kwon, J. Y.; Lee, E. Y.; Yoon, J. *J. Org. Chem.* **2002**, *67*, 4384.

(14) The error range is relatively large due to the limited dynamic range of the fluorescence titration method (see Supporting Information).

(15) *CaChe 6.1*; Fujitsu Computer Systems, BioSciences Group: Westwood, MA. For the use of CaChe to model supramolecular clusters, see, for example: Caulder, D. L.; Brueckner, C.; Powers, R. E.; Koenig, S.; Parac, T. N.; Leary, J. A.; Raymond, K. N. *J. Am. Chem. Soc.* **2001**, *123*, 8923.

Scheme 4



basis of the reduction in entropy loss during complexation, **17** exhibited a slightly higher K_a of $3.20 \pm 2.98 \times 10^{22}$ M^{-4} (determined by competitive binding with **16**) than **15**. In a similar fashion, **13** was cross-linked with pyridine-2,6-dicarboxylate to give ligand **18**. However, NMR titration of **18** showed a complex binding pattern to Ag(I), presumably due to the shortness or rigidity of the tether, or additional silver−pyridine interactions.

In summary, we have demonstrated the feasibility of improving the unique affinity of the natural product westiellamide toward silver ions by structural modifications. The tethered ligands **15** and **17** show higher affinities toward Ag(I) than westiellamide and improve the potential for practical applications of cyclic oligooxazolines in Ag(I) detection, transport, detoxification, and targeted delivery.[16] The biological profile of these complexes is currently under investigation and will be reported in due course.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM-55433). We thank Dr. Steve Geib (University of Pittsburgh) for X-ray crystallographic analysis of the *p*-bromobenzoate of **13**.

Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL060677C

(16) Silver, S.; Phung, L. T. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 587.