ORGANIC LETTERS

2006
Vol. 8, No. 7
1259−1262

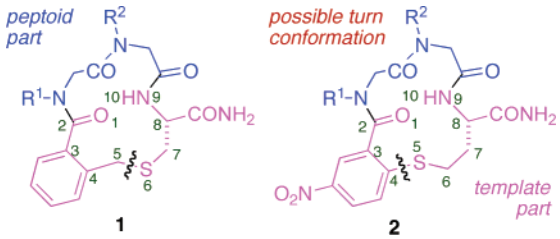
Cyclic Semipeptoids: Peptoid–Organic Hybrid Macrocycles

Ernest Nnanabu and Kevin Burgess*

Texas A&M University, Chemistry Department, P.O. Box 30012, College Station, Texas 77842
burgess@tamu.edu

Received November 26, 2005

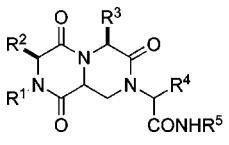
ABSTRACT



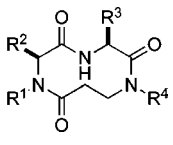
1 **2**

Cyclic semipeptoids 1 and 2 represent constrained, secondary structure mimics where the R^1 and R^2 side chains correspond to those of amino acids. Solid-phase syntheses and conformational analyses of these compounds are described.

Poly-N-alkylated glycine chains, peptoids,[1] are easily constructed via solid-phase syntheses.[2] A vast number of amines are available to use as starting materials for these compound types, so peptoids provide rapid access into diverse structures. However, they are not rigid. The next step in the evolution of this field is the production of conformationally constrained peptoid analogues, to decrease the entropy loss on binding and improve bioavailability. There have been some moves in the literature toward this goal; Golebiowski and coworkers[3] and Liskamp et al.,[4] for example, have combined a dipeptide moiety with N-alkylation to produce the β-turn analogues **A** and **B**, respectively. This Letter describes cyclic peptoid/amino acid hybrids, compounds we call "semipeptoids", **1** and **2**.



A



B

Syntheses of **1** and **2** were both based on linear precursors. Syntheses of these would be relatively straightforward for compounds that have only simple *N*-alkyl and aryl substituents. However, we are convinced that the best peptidomimetic designs for inducing diverse biological activities must allow facile incorporation of *functionalized* amino acids.[5] In the context of **1** and **2**, this means that the syntheses should accommodate protected, functionalized amines. Consequently, it was important to find conditions that were mild and generally applicable to a diverse amine set. In the event, two approaches were developed, as described below.

Solid-phase syntheses of peptoids often have been performed by heating supported bromoacyl derivatives with excess amine in DMF for approximately 80 min at 35 °C. Preliminary experiments by us using this approach gave variable results. Kodadek had previously reported that microwave heating in a domestic instrument gave shorter reaction times.[6] We were reluctant to use domestic instruments that have no temperature control and give nonuniform heating in the reaction cavity because the data tend to be hard to reproduce. Nevertheless, the prospect of dramatically reducing the reaction times was appealing.

(1) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci.* **1992**, *89*, 9367.

(2) Patch, J. A.; Kirshenbaum, K.; Seurynck, S. L.; Zuckermann, R. N.; Barron, A. E. In *Pseudo-Peptides in Drug Discovery*; Nielsen, P. E., Ed.; Wiley-VCH: Weinheim, 2004; p 1−31.

(3) Golebiowski, A.; Klopfenstein, S. R.; Shao, X.; Chen, J. J.; Colson, A.-O.; Grieb, A. L.; Russell, A. F. *Org. Lett.* **2000**, *2*, 2615.

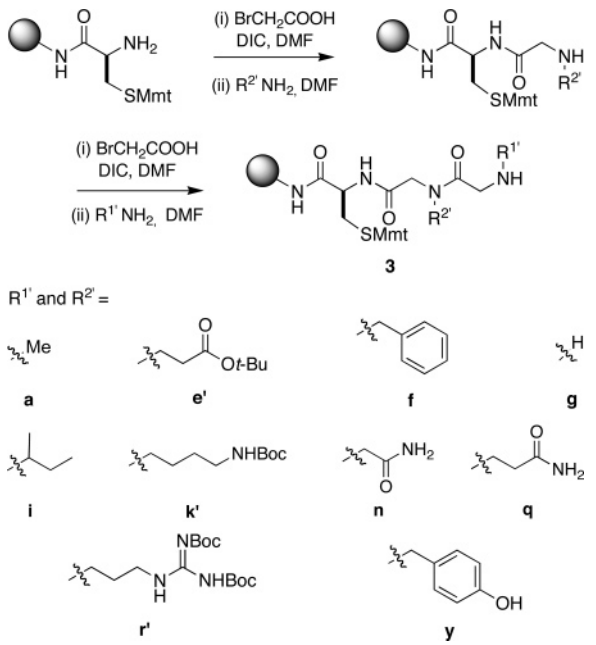
(4) Wels, B.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *Org. Lett.* **2002**, *4*, 2173.

(5) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826−35.

(6) Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057.

10.1021/ol052867d CCC: $33.50
Published on Web 03/07/2006

Scheme 1. Microwave-Accelerated Syntheses of Peptide−Peptoid Hybrids[a]

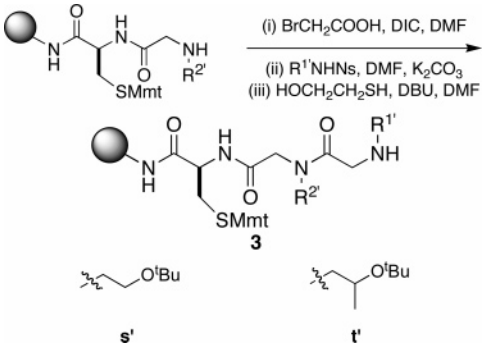


[a] The lower-case codes indicated correspond to one-letter codes for the closest amino acid side chains. For instance, a methyl side chain **a** resembles Ala or A and side chain **k** resembles Lys or K. **e′**, **k′**, and **r′** indicate masked side chains that will give **e**, **k**, and **r** side chains on deprotection. Compounds involving **a** and **g** fragments were introduced via the FMOC approach, and the rest were made as indicated in the reaction above.

On the basis of the considerations outlined above, it seemed attractive to investigate microwave-mediated acceleration of these coupling steps in a scientific microwave reactor designed to maintain constant reaction temperatures (CEM: Discover). Our initial exploratory work focused on longer reaction times and higher temperatures than were necessary. However, it was quickly demonstrated that solid-phase syntheses of peptoids on Rink-linker-functionalized[7] polystyrene proceeded rapidly and efficiently if the acylation and nucleophilic displacement steps were microwaved at 50 °C for 1 min (see Supporting Information). When this work was nearly complete, Blackwell et al. also reported solid-phase peptoid syntheses under microwave conditions.[8] They concluded that reactive, unhindered amines combine with bromoacyl groups on polystyrene beads in approximately 1 min at room temperature, even without microwave excitation. Our data are consistent with those findings because the microwave energy input is relatively mild and it is applied for a short period. However, sometimes we found that microwaves were necessary.

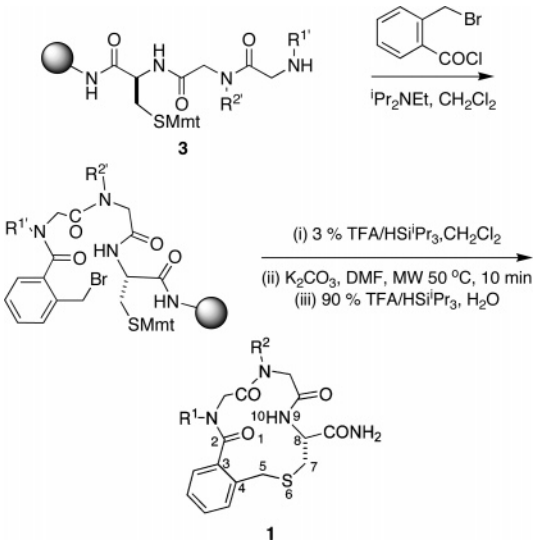
Scheme 1 shows the use of our microwave conditions to introduce a Cys and two N-alkylated Gly residues. This approach was applied using the amines indicated in Schemes 1 and 2 in various combinations (see below).

Scheme 2. Alternative Approach to Microwave-Accelerated Syntheses of Peptide−Peptoid Hybrids



In other cases, it was more convenient to prepare the amines with a nosyl protecting group[9] and to perform the alkylation step under basic conditions. This was the route used where the side-chain functionalized amines **s** and **t** were involved (Scheme 2). Finally, the "monomer approach"[10] was used to install the side chains of **a** and **g**; i.e., FMOC-sarcosine (*N*-methylglycine) and -glycine were coupled to incorporate these units directly.

Scheme 3. Syntheses of Cyclic Semipeptoids **1** via S_N2 Reactions[a]



[a] $R^{1'}$ and $R^{2'}$ indicate masked side chains.

The linear precursors **3** were converted to the cyclic semipeptoids **1** via the approach shown in Scheme 3. This featured a simple acylation of the N-terminus with 2-bromomethyl benzoyl chloride, deprotection of the 4-methoxy trityl (Mmt) protecting group, and then microwave-assisted S_N2 ring closure. Finally, the compounds were cleaved into solution, with concomitant removal of any protecting groups, by rupture of the Rink linker.

(7) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
(8) Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521.
(9) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353.
(10) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

Table 1. Purity and Yield Data for the Cyclic Semipeptoids **1**

1	R^1	R^2	purity UV[a]/ELS (%)	yield (%)
aa	Me	Me	99/98	70
ek	CO_2H	NH_2	87/89	65
gk	H	NH_2	100/98	60
ik		NH_2	85/80	45
in		$CONH_2$	90/92	60
ir		NH, NH_2	89/80	80
nk	$CONH_2$	NH_2	80/82	68
nn	$CONH_2$	$CONH_2$	80/75	35
qa	$CONH_2$	Me	91/70	55
ra	NH, NH_2	Me	95/93	50
sg	OH	H	80/80	30
sy	OH	OH	86/84	45
tg	OH	H	75/78	52

[a] 254 nm.

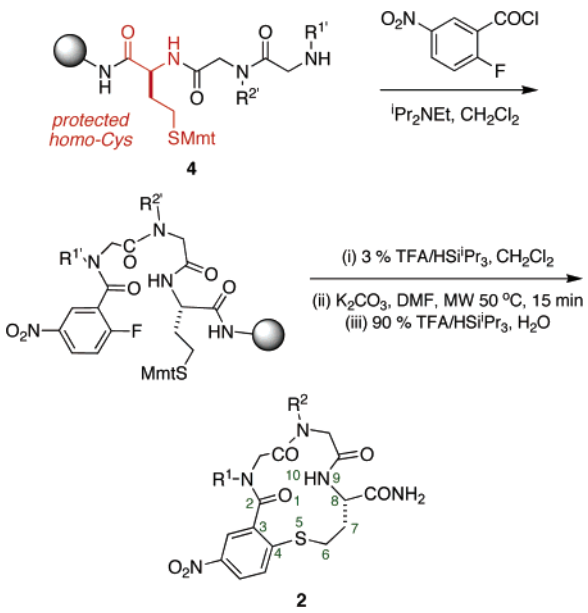
Table 1 summarizes crude purities and yields for the compounds made via the approach shown in Scheme 3. Measurements of crude purities are somewhat dependent upon the detection method (here UV and evaporative light scattering {ELS}). Many of the compounds made via this procedure would have passed an 85% purity cutoff to be entered into a high-throughput preliminary screen.

Scheme 4 shows an alternative approach to cyclizing linear peptide−peptoid hybrids. There are two key differences between this and the S_N2 strategy featured in Scheme 3. The former leads to S_NAr ring closures from homo-Cys, rather than Cys, as the amino acid foundation. Homo-Cys was used so that the final ring-closed products could support a C^{10} template arrangement in the template part of the molecule; we have hypothesized that this frequently leads to β-turn conformations in the encapsulated dipeptide or, in this case, dipeptoid fragment.[5] The Cys derivatives **1** can form this C^{10} arrangement in the template, but homologation to homo-Cys is required for the 2-fluoro-5-nitrobenzoic acid derivatives to do so.

Yield and purity data for the semipeptoids **2** formed via Scheme 4 are shown in Table 2. Overall, both parameters tend to be higher for these products than for systems **1**.

Full solution-state conformational analyses of the title cyclic semipeptoids were not possible via routine methods, for several reasons. First, these molecules lack some pivotal spectroscopic markers. For instance, the peptoid part has no N*H*/C$^{\alpha}$*H* couplings to report on dihedral angles and gives no N*H*/C$^{\alpha}$*H* or N*H*/N*H* ROESY cross-peaks. In any case, the ^{1}H NMR spectra of these compounds were broad but

Scheme 4. Syntheses of Cyclic Semipeptoids **2** via S_NAr Reactions[a]



[a] $R^{1'}$ and $R^{2'}$ indicate masked side chains.

became sharper at elevated temperatures, indicative of slow exchange on the NMR time scale. Moreover, their limited solubilities in most solvents prevented extensive variable-temperature NMR experiments. These characteristics precluded detailed analyses of the molecular conformations by 2D NMR. Further, interpretation of the CD spectra of these molecules cannot rely on the literature precedent that exists for peptides. Nevertheless, despite all these restrictions, it was possible to form some tenuous conclusions about the conformational biases of these molecules.

Compounds **1nk** and **2ff** were selected as illustrative members of the series. Their conformational states were

Table 2. Purity and Yield Data for the Cyclic Semipeptoids **2**

2	R^1	R^2	purity UV[a]/ELS (%)	yield (%)
ek	CO_2H	NH_2	91/88	62
ff			99/98	70
if			95/93	50
ik		NH_2	85/83	47
ir		NH, NH_2	89/80	40
nn	$CONH_2$	$CONH_2$	86/72	44
qa	$CONH_2$	Me	93/77	52

[a] 254 nm.

simulated in a medium of dielectric constant 45 (corresponding to DMSO) using the quenched molecular dynamics (QMD)[11,12] technique as described by us in several other publications.[13,14] Temperature coefficients were measured for the N*H* protons in DMSO-D_6; steeper slopes than −3.0 ppm are assumed to be indicative of solvent shielding and/or H-bonding.[15] Finally, CD spectra were recorded for these molecules dissolved in an aqueous methanol medium of about the same dielectric as DMSO.

Figure 1a shows the most favored conformer for **1nk** in the molecular simulation experiments. The simulated C═*O*···*H*N in the template part of this molecule was 4.7 Å, i.e., somewhat longer than in ideal β-turns. Additionally, the temperature coefficient for this proton was −4.7 ppb K^{-1}, providing no evidence for solvent shielding or H-bonding. Nevertheless, the simulated structure indicates that turnlike conformations are accessible. The CD spectrum of this compound (Figure 1b) is similar to those expected for a type 2 β-turn in a peptide, but contributions from the absorption of the aromatic groups in compound **2ff** cannot be ignored.[16−18] The distance between the two β-atoms in the peptoid side chains is very similar to that which we measured in ideal type 1 β-turns (5.3 vs 5.2 Å), so the cyclic semipeptoid template at least presents the side chains at the correct spacial separation to mimic turns.

The preferred simulated conformation of **2ff** (Figure 1c) gave a much closer C═*O*···*H*N contact (2.0 Å), and this was consistent with the temperature coefficient experimentally observed for this compound (−1.2 ppb K^{-1}). The CD spectrum of this compound was reminiscent of a type 1 β-turn. The two β-atoms in the peptoid side chains were separated by 5.36 Å in the preferred simulated conformer, about that observed in β-turns.

The research described in this paper illustrates a route[19] to combine the enormous diversity of amine-derived side chains in peptoids with amino acids in convenient solid-phase syntheses. Two routes are demonstrated to cyclize linear semipeptoids with an array of side chains that can resemble, in terms of structure and diversity, those found in the protein amino acids. These constrained compounds might be expected to have, on aggregate, superior properties with respect to loss of entropy on binding and bioavailability, relative to conformationally flexible peptides and peptoids. Further, the limited data collected on their conformational analyses indicate that molecules of this type may be designed to mimic β-turns.

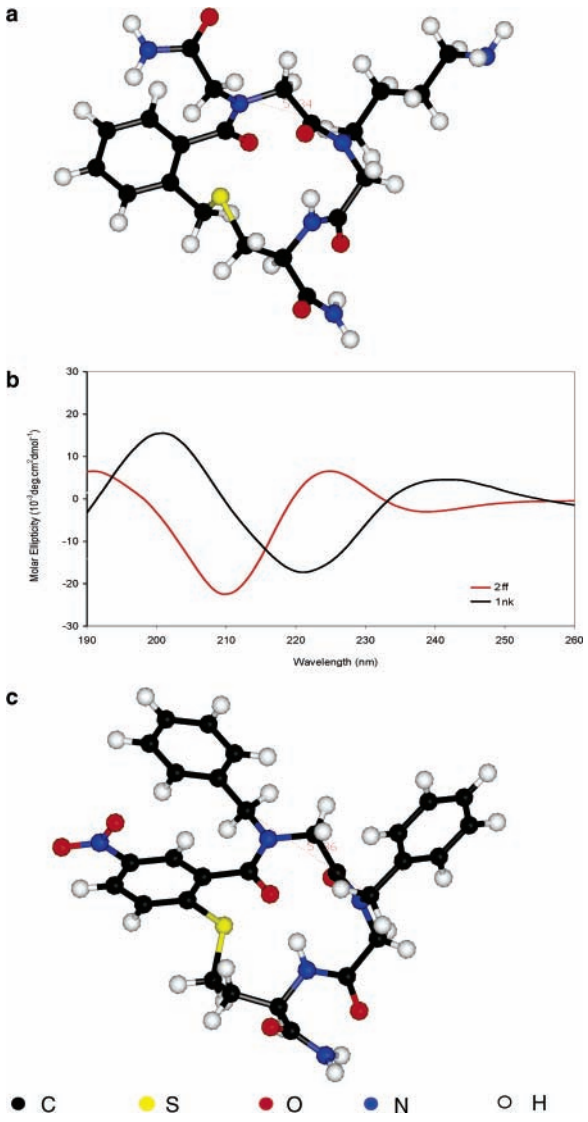


Figure 1. (a) Simulated favored conformation of **1nk**. (b) CD spectra of **1nk** and **2ff** at 0.1 mg/mL in 35% $MeOH_{(aq)}$ with 1% $NaHCO_3$. (c) Simulated favored conformation of **2ff**.

Acknowledgment. Financial support for this project was provided by the NIH (CA82642) and the Robert A. Welch Foundation. TAMU/LBMS Lab headed by Dr S. Tichy provided mass spectrometric support, and the Lab for Molecular Simulations (Dr L. Thompson) supported our simulations work. NMR instrumentation at TAMU was supported by a grant from the National Science Foundation (DBI-9970232) and the TAMU System.

Supporting Information Available: Details of optimization studies, synthetic procedures, and protocol/data for conformational analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052867D

(11) O'Connor, S. D.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870.
(12) Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophys. J. Biophys. Soc.* **1991**, *60*, 1540.
(13) Moye-Sherman, D.; Jin, S.; Ham, I.; Lim, D.; Scholtz, J. M.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 9435.
(14) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *Chem.−Eur. J.* **1999**, *5*, 3273.
(15) Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194.
(16) Manning, M. C.; Illangasekare, M.; Woody, R. W. *Biophys. Chem.* **1988**, *31*, 77.
(17) Bush, C. A.; Sarkar, S. K.; Kopple, K. D. *Biochemistry* **1978**, *17*, 4951.
(18) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York and London, 1996; p 362−364.
(19) Peptoid and peptides have been mixed in the same molecule before, in a much larger system: Shankaramma, S. C.; Moehle, K.; James, S.; Vrijbloed, J. W.; Obrecht, D.; Robinson, J. A. *Chem. Commun.* **2003**, 1842.