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Solid-Phase S_N2 Macrocyclization Reactions To Form β-Turn Mimics

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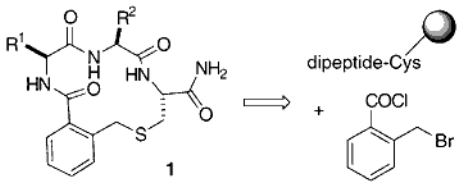
ABSTRACT



Efficient solid-phase S_N2 macrocyclization reactions were sought to facilitate preparations of focused libraries of β-turn mimetics. A very efficient, but undesired, cyclization reaction to give five-membered ring lactams 4 was identified in attempts to use O-nucleophiles. Subsequent studies focused exclusively on S-nucleophiles. These reactions gave the desired macrocyclization products 1 in high purities and good overall yields. Conformational analyses of illustrative macrocyclization products 1 via NMR, CD, and molecular simulations showed that they seem to sample both type I and type II β-turn conformations in solution. CD studies indicate a curious relationship between the preferred conformation and the amino acids encapsulated in the macrocycles.

The literature on preparations of β-turn mimics[1] contains notable solid-phase syntheses from Kahn,[2] Ellman,[3] and others.[4] Overall, however, few reports of solid-phase syntheses of β-turn mimics give efficient preparations of constrained peptidomimetics that contain two or more amino acids. Hence, most of the published work does not directly relate to turn residues in proteins. This is unfortunate, because focused libraries of β-turn analogues that are designed to mimic/disrupt specific protein−protein interactions will become increasingly valuable as The Human Genome Project matures. Consequently, developments in this area are particularly timely.

We are currently engaged in a program to devise solid-phase syntheses of β-turn peptidomimetics as pharmacological probes for protein−protein interactions. Several desirable criteria were identified at the onset of this work. The first was that the products must encapsulate amino acids that could be chosen for preparations of libraries biased toward specific protein targets. Second, the products typically should be isolated from the resin in above 80% purity. Third, those products should have detectable tendencies to adopt β-turn conformations in solution.

Our first efforts in this area involved solid-phase syntheses of β-turn mimics via S_NAr reactions.[5] This approach was chosen because the products are not entirely peptidic and, hence, could be better pharmaceutical leads and because the cyclization may be more facile than for formation of cyclic peptides in a library format.[6] The products were shown to

(1) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bos, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039−3049.

(2) (a) Kahn, M. *Synlett* **1993**, *11*, 821−826. For a β-strand mimic see: (b) Ogbu, C. O.; Qabar, M. N.; Boatman, P. D.; Urban, J.; Meara, J. P.; Ferguson, M. D.; Tulinsky, J.; Lum, C.; Babu, S.; Blaskovich, M. A.; Nakanishi, H.; Ruan, F.; Cao, B.; Minarik, R.; Little, T.; Nelson, S.; Nguyen, M.; Gall, A.; Kahn, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2321−2326.

(3) (a) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581. (b) Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996**, *37*, 6961−6964. (c) Virgilio, A. A.; Bray, A. A.; Zhang, W.; Trinh, L.; Snyder, M.; Morrissey, M. M.; Ellman, J. A. *Tetrahedron* **1997**, *53*, 6635−6644. (d) Souers, A. J.; Virgilio, A. A.; Rosenquist, A.; Fenuik, W.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 1817−1825.

(4) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **1999**, *42*, 601−608.

(5) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 10768−10769.

(6) (a) Yu, L.; Lai, Y.; Wade, J. V.; Coutts, S. M. *Tetrahedron Lett.* **1998**, *39*, 6633−6636. (b) Mayer, J. P.; Zhang, J.; Groeger, S.; Liu, C.-F.; Jarosinski, M. A. *J. Peptide Res.* **1998**, *51*, 432−436.

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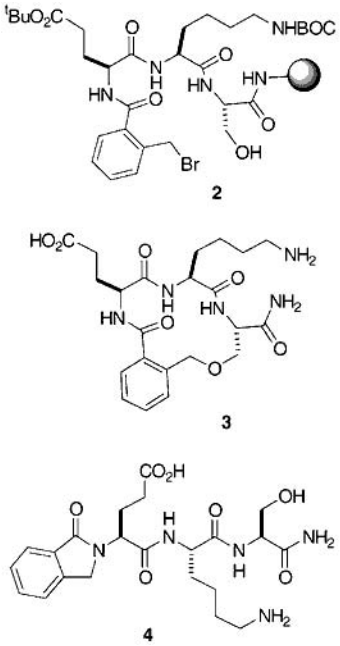
have a distinct bias toward type I β-turn conformations.[5] This letter concerns our efforts to develop efficient solid-phase syntheses of the S_N2 macrocyclization products **1**. Preliminary conformational studies are also reported.

The very first experiments performed in this study attempted to use the serine side chain as the nucleophilic component. These experiments did not give the desired products but did highlight a potential pitfall in this type of work. In an illustrative reaction, the peptidomimetic **2** (Chart 1) was prepared on Rink amide MBHA resin (NovaBiochem, San Diego, CA) via standard peptide couplings of FMOC amino acids.[7] The N-terminal benzylic bromide portion of **2** was incorporated by treating the corresponding protected peptide with 2-(bromomethyl)benzoyl chloride[8] in ethyldiisopropylamine/CH_2Cl_2. Removal of a trityl side chain protecting group used for the serine residue under mildly acidic conditions gave the cyclization precursor **2** without cleaving the peptidomimetic from the resin. Treatment of **2** with any of several bases investigated, and cleavage from the resin with concomitant removal of protecting groups, gave a product in a high state of purity, as assessed by analytical HPLC. A MALDI-MS analysis showed this product had a molecular ion at the correct *m*/*z* value for the desired macrocycle. However, 1D NMR analysis of this product indicated that one N*H* resonance was either missing or obscured. 2D NMR analyses via a combination of COSY and ROESY techniques showed that that N*H* was not obscured by coincidental overlap. Eventually, it was demonstrated via a different synthesis that the product of this reaction was not the desired macrocycle **3** but the five-membered-ring lactam **4**.

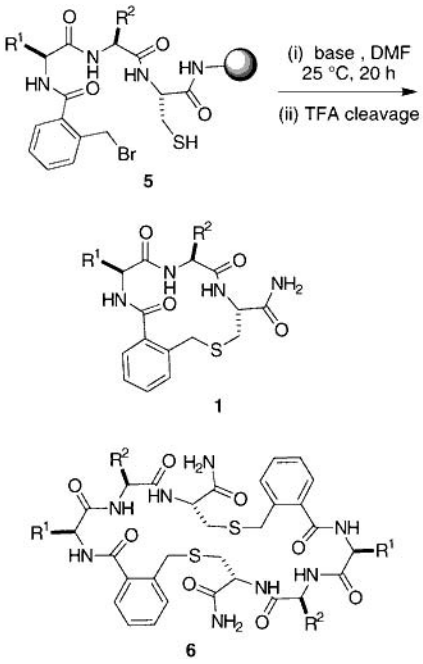
Chart 1. Substrate **2**, Desired Product **3**, and the Actual Product, Lactam **4**



The observations described above illustrate that MALDI-MS and analytical HPLC data, do not, of course, confirm that macrocyclization has occurred. Two-dimensional NMR analyses are required to characterize the library products, or at least a representative sample of the compounds produced.

In the next phase of this work, substrates **5** (Scheme 1) were prepared to test the macrocyclization via S-nucleophiles. The approach outlined above was used, except that the cysteine side chain was protected with a monomethoxytrityl (Mmt) group. That masking group was removed, without cleavage of the peptidomimetic from the resin, via a 1% TFA treatment prior to the cyclization.

Scheme 1. Successful Macrocyclization



Macrocyclization to the desired compounds **1** was the prevalent reaction when substrates **5** were allowed to cyclize under basic conditions. The only significant byproducts observed had exactly twice the desired molecular mass. We presume these are the head-to-tail dimeric structures **6**, and in one case (compound **6c**) 2D NMR data were obtained supporting this assertion. Table 1 summarizes the percentage purities of monomer and dimer obtained in these reactions and the percentage isolated yields of the desired monomeric product.

Conformational analyses of compounds **1a** and **1k** were undertaken to probe their rigidity and conformational biases. A combination of 1D and 2D spectra and N*H* temperature coefficient determinations revealed that these compounds sample rapidly equilibrating conformations on the NMR time

(7) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, U.K., 1989.

(8) (a) Pifferi, G.; Testa, E. *Tetrahedron* **1966**, *22*, 2107−2117. (b) Vitullo, V. P.; Sridharan, S.; Johnson, L. P. *J. Am. Chem. Soc.* **1979**, *101*, 2320−2322.

Table 1. Purities and Isolated Yields for Products **1**[a]

starting compd	R^1-R^2 (from parent amino acids)	products **1a**−**m** purity[b] (%)	products **1a**−**m** isolated yield[c] (%)	dimers **6a**−**m** purity[b] (%)
1a	Glu-Lys	77	25	11
1b	Ile-Lys	87	21	4
1c	Asp-Ile	63	15	30
1d	Ile-Arg	80	27	16
1e	Arg-Gly	80	44	13
1f	Lys-Tyr	88	25	9
1g	Tyr-Gly	76	27	11
1h	Lys-Thr	85	23	11
1i	Thr-Gly	76	24	21
1j	Gly-Asn	60	17	16
1k	Glu-Asn	88	35	7
1l	Asn-Asn	77	37	15
1m	Asn-Lys	77	31	15

[a] TentaGel S RAM resin (0.30 mmol/g) was used. [b] Purity was based on the peak area of HPLC traces at 215 nm. [c] Yield was ca lculated on the basis of the mass after RP-HPLC separations and the resin loading.

scale. Molecular simulations via the "quenched molecular dynamics" technique[9] supported this assertion and indicated that β-turn conformations related to both the type I and type II series were accessible. Figure 1 shows two typical simulated conformers; one resembles a type II turn, and the other is more type I like.

Circular dichroism (CD) studies gave the most informative and intriguing data in the conformational analyses. Compounds **1a**−**m** were all analyzed. The spectrum shown in Figure 2 for compound **1k** was typical; this spectral shape

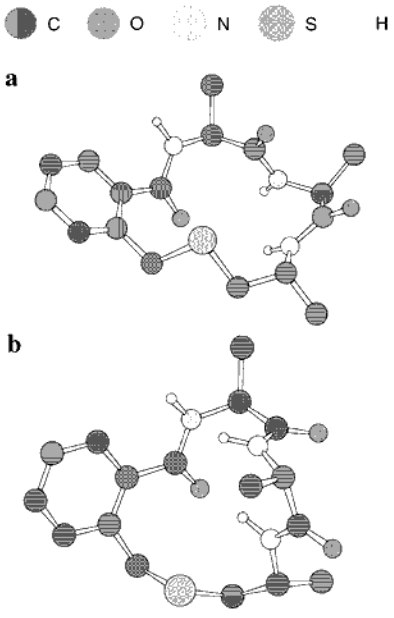


Figure 1. (a) Simulated backbone conformation for compound **1a** in a favored type II like conformation. (b) Simulated backbone conformation for **1a** in the apparently less favored type I like conformation.

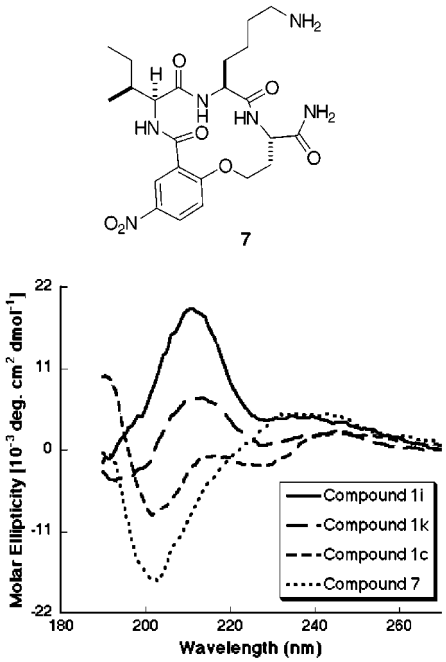


Figure 2. CD spectra for compounds **1c**,**i**,**k** and **7** (in 4:1 water/methanol at 25 °C, at 0.1 mg/mL).

is reminiscent of type II β-turns.[10] The CD spectrum for compound **1i** is even more characteristic of this conformation. For comparison, the CD spectrum of the S_NAr macrocyclization product **7**[11] is also shown in Figure 2. That compound, which has already been shown to favor a type I like turn conformation, gave a CD spectrum typical of type I turns. Moreover, the maximum absolute value of the molar ellipticity for **7** was higher than for most of the compounds **1**, supporting the assertion that the S_N2 products **1** are more conformationally flexible than similar S_NAr-based macrocycles. Another observation also implies that proposal is correct. Specifically, macrocycle **1c** and a few other compounds in the series did not give a classical type II turn CD spectrum. Instead, their CD spectra seem to feature a combination of type I and type II turn characteristics. Consequently, the conformations observed by CD are somewhat sequence-dependent, and these sequence specific factors may override the intrinsic bias of the backbone for type II turn conformation.

The rationale for selecting compounds **1** for synthesis was that they should be able to display dipeptide fragments in

(9) (a) Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophy. J. Biophys. Soc.* **1991**, *60*, 1540−1544. (b) O'Connor, S. D.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870−2881.

(10) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York and London, 1996; pp 362−364.

(11) (a) Feng, Y.; Burgess, K. *Chem. Eur. J.*, submitted for publication. (b) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *Chem. Eur. J.*, submitted for publication.

β-turn conformations via a system of two 10-membered rings created by an H-bond within the 14-atom-ring system. This supposition now has been validated. Moreover, both type I and type II turn conformations seem to be accessible to these S_N2 macrocyclization products. This contrasts with the S_NAr macrocyclization products, which have more pronounced preferences for type I turn conformations. At this stage we do not wish to speculate on the origin of the conformational differences between the S_N2 and S_NAr systems, but this is an interesting question for further investigation. In practice, both types of analogues could be useful in studies of protein−protein interactions: one to explore the efficacy of turn peptidomimetics in general, and the other as a more specific probe of type I turn mimics.

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Supporting Information Available: Experimental procedures for preparation of compounds **1a**−**m** and **4**, DQF-COSY spectra for compounds **1d**−**g**,**j**,**k** and **6c**, and a summary of the DQF-COSY, ROESY, and QMD data for **1a** and **1k**. This material is available free of charge via the Internet at http://pubs.acs.org.

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