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Diastereoselectivity in the solid-phase synthesis of peptide heterocycle hybrids

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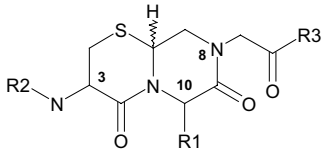
Abstract—Sixteen compounds containing the bicyclic moiety (3,8,10-trisubstituted 2,9-dioxo-5-thia-1,8-diazabicyclo[4.4.0]decane) were produced via solid-phase synthesis. Differing substitution at the 3- and 10-positions was used. These were analyzed using 2-D NMR techniques (ROESY) to determine the stereoselectivity of ring formation in the core heterocycle. Conformational analysis of the proposed transition state structure using Sybyl 6.8® was used to rationalize the stereochemical outcome of ring formation.

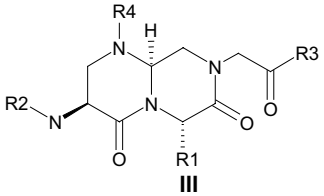
The stereocontrolled presentation of amino acid side chains is of crucial importance in molecular recognition events that govern biological interactions. Cyclization is an important method of constraint used by nature to effect the proper display of these side chains. In peptides, this is seen in bioactive compounds such as cyclosporin and marine snail conotoxins.[1]

In a previous report, the solid-phase synthesis of macrocyclic peptidic compounds containing a tripeptide-derived 6,6-fused bicyclic moiety (3,8,10-trisubstituted 2,9-dioxo-5-thia-1,8-diazabicyclo[4.4.0]decane) **I** was described.[2] This ring system was incorporated into a polypeptide chain via utilization of a novel peptoid-like, aldehyde-containing subunit. Incorporation of the bicyclic moiety involved: acylation of the peptide chain with bromoacetic acid, S_N2 displacement with aminoacetaldehyde dimethyl acetal, and coupling of amino acid corresponding to side chain R_1 (Scheme 1), which is within the ring system in the final product. This was followed by attachment of the cysteine residue, which completes the ring system, and further amino acids to the N-terminus. Alternatively, an N-terminal thiopropionic acid was incorporated with no further chain elongation (compounds **13**–**16**).



I

We believe that upon TFA mediated cleavage a transient aldehyde was formed, which then underwent condensation with the adjacent backbone amide nitrogen on the N-terminal side, forming an N-acyliminium six-membered ring intermediate.[3] Nucleophilic attack from the thiol group of the adjacent amino acid resulted in the formation of the second ring and a new stereocenter at the bridgehead carbon. Typically, we have observed only a single peak on reversed phase HPLC with the correct molecular weight, indicating that the cyclization is highly stereoselective however we had not determined the relative stereochemistry of the central ring system. We decided to undertake NMR studies of our



III

Keywords: 2-D NMR; Solid-phase synthesis; Stereoselective cyclization; Peptide-heterocycle.
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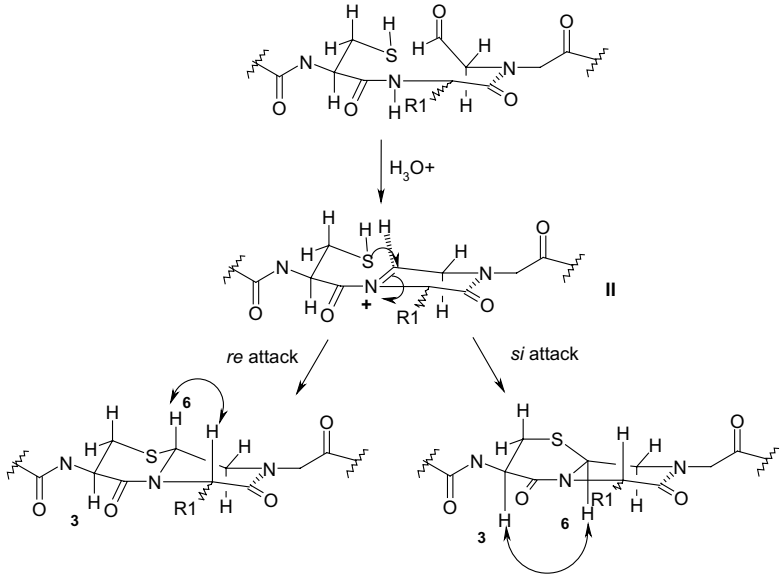


Scheme 1. Proposed mechanism for formation of central bicyclic ring system with indicative ROE's.

compounds to determine the stereoselectivity of our synthesis.[4]

A series of compounds based on the similar 1,3,6,8-substituted tetrahydro-2H-pyrazino[1,2-*a*]pyrimidine-4,7-dione system **III** had been built by Eguchi et al.[5a,b] The authors found that during the key cyclization step only one diastereomer was obtained in which the ring junction hydrogen is *cis* to R_1. We also wanted to see whether the stereoselectivity of our synthesis was analogous to that observed by the Eguchi group.

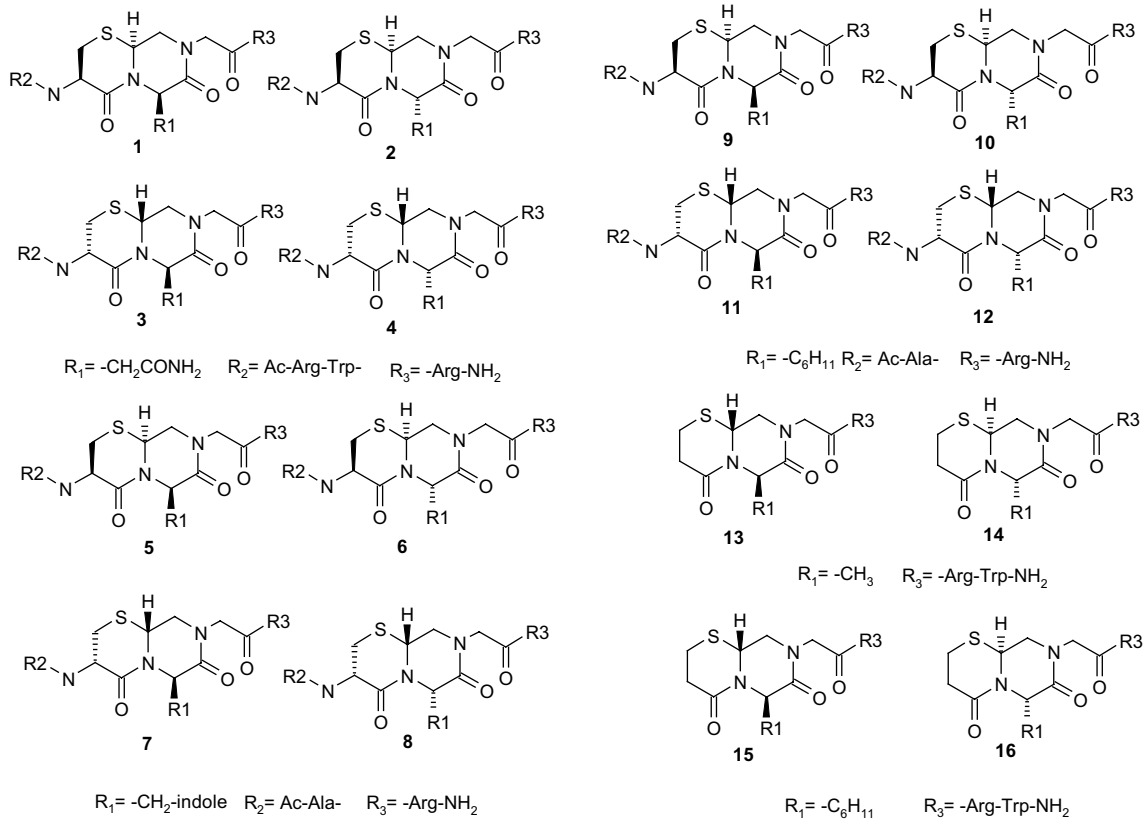
Two questions arose from the observation of a preferred stereochemistry at the ring junction: What is the stereoselectivity of nucleophilic attack by the cysteine sulfur to form the second ring? Can we influence the stereoselectivity by our choice of amino acid identity? Sixteen compounds were synthesized with varying constitution and configuration of the core residue amino acids. Disposition of the bridgehead proton (H_6) to the cysteine alpha proton (H_3) and R_1 α-proton were monitored using ROESY 2-D NMR experiments (Scheme 1). The results are summarized in Scheme 2.

We first started with a set of previously synthesized compounds (**1**–**4**) to explore the different combinations of configuration of D–L amino acids. Unexpectedly H_6 was always positioned *cis* to H_3. We reasoned that this may be a result of the bulky side chain of the amino acid on the N-terminal side of the cysteine. Compounds **5** through **8** were synthesized[6] to explore whether placing a more sterically demanding substituent at the R_1 position and a small substituent at R_2 would force the cyclization the other way but H_6 always remained *cis* to H_3. In compounds **9** through **12** an even more sterically demanding cyclohexyl group was placed at R_1. Again we observed no change in the stereoselectivity of cyclization. Attempts to synthesize analogs with a *t*-butyl substituent at the R_1 position did not generate sufficient quantity of the desired product for NMR evaluation. The *t*-butyl substituent appears to be too sterically demanding and impedes the cyclization. Indeed, it is only when we replaced cysteine with thiopropionic acid (compounds **13**–**16**) that a mixture of the two possible isomers were obtained. These were formed in a 90:10 ratio with the major constituent being that shown in Scheme 2.

Sybyl 6.8 molecular modeling software was used to generate the energy minimized conformations for the model compounds described in Figures 1 and 2. Ring 2 of the reaction intermediate **II** was first optimized and then fixed during subsequent steps of conformation generation and energy minimization for the products resulting from either *si*- or *re*-attack by sulfur (thiol of the cysteine side chain).

The pair of energy minimized conformers for *si*- and *re*-attack for compound **13** were examined and are presented in Figure 1. The two conformers are aligned with ring one of each superimposed. Taking note of the location of the sulfur atom relative to the R_1 (methyl) substituent, we see that the conformation resulting from *re*-attack (the favored product of **13** based on our NMR analysis) is more stable than that resulting from *si*-attack, in which the sulfur is much closer to the R_1 (methyl) substituent. Thus, we could conclude from this model that the steric effect from R_1 substitution determines the *cis*/*trans*-selectivity of the products (molecules-**13**–**16**).

To better systematically explain the stereoselectivity of these results, molecules **1**–**12** are grouped into two series: **1**, **5**, **9**, **4**, **8**, **12** in one series and **2**, **6**, **10**, **3**, **7**, **11** in the other. Compounds **1**, **5**, **9** are grouped together because the stereo chemical patterns for the R_1 and R_2 substitutions are the same while the actual identity of the substituents varies. Compounds **4**, **8**, and **12** are mirror images



Scheme 2. Stereochemistry of bicyclic core ring system.

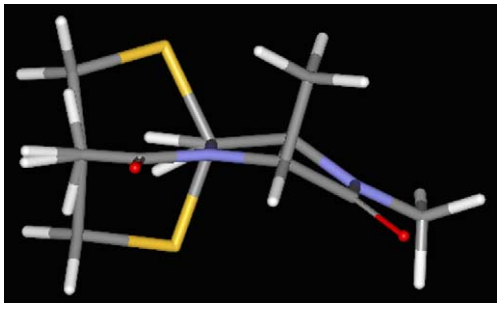


Figure 1. Comparison of the two stable conformers corresponding to *si*- and *re*-S attack relative to R_1 substitution.

of **1**, **5**, and **9** in terms of their stereo chemical patterns. Similarly, **2**, **6**, **10**, **3**, **7**, **11** are grouped together.

For group-I molecules, Figure 2a represents the product of *si*-attack, resulting in the favored product of compound **1** (and all others in group I), in which H_3 is *cis* to H_6. In this conformation, R_2 substitution is in the energetically favored equatorial position of the boat conformation. Furthermore, there may be a solvent mediated H-bond between the amide –NH and the ring C=O oxygen, which would further stabilize this conformation. By contrast, Figure 2b corresponds to S *re*-attack that would result in the product of **1** that was never experimentally observed. In fact, in this conformation, the R_2 substituents would be in the disfavored axial position of the boat conformation, and there is no potential solvent mediated H-bond between R_2 amide

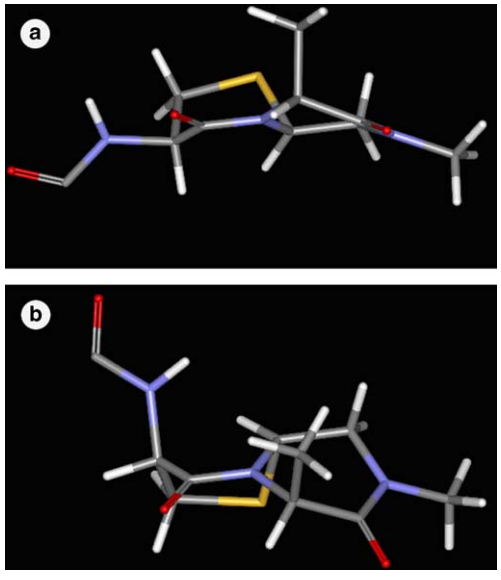


Figure 2. (a) Energy minimized conformation for S *si*-attack relative to R_1 substitution. (b) Energy minimized conformation for S *re*-attack relative to R_1 substitution.

–NH and the ring C=O oxygen. These observations are consistent with our experimental findings.

For compounds in group-II, both the R_1 and R_2 substitutions have a synergistic effect on the stereo selectivity. For example, in molecule **2**, the R_2 substitution favors

the S *si*-attack due to the above-mentioned reasons, and so does the R_1 substitution. Thus, the stereo selectivity (H_3 *cis* to H_6) can be easily explained.

In conclusion, we have shown that the stereochemistry at the ring junction upon formation of these peptide heterocycle hybrids is dependent on several factors. For substitution at the 3-position, as in cysteine, this is the dominant influence on the stereodirection of cyclization and the H_6 to H_3 relationship is always *cis*. In the absence of substitution at the 3-position, such as thiopropionic acid, R_1 exerts some influence over the stereoselectivity of cyclization. We also found that for compounds **2**, **6**, and **10**, with stereochemistry analogous to Eguchi's compounds, our results are in agreement.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.06.075.

References and notes

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4. NMR experiments were carried out on a Varian Unity Inova spectrometer at 500 MHz. All samples were observed in DMSO-d_6 at 25 °C. Assignments were made from ^{1}H, gDQCOSY, gHSQC, gHMBC, and ROESY experiments. Representative NMR data from compound **5**: ^{1}H NMR (DMSO-d_6 500 MHz, 25 °C): δ 1.23 (d, J = 7.2 Hz, 3H), 1.39–1.55 (m, 3H), 1.67–1.76 (m, 1H), 1.84 (s, 3H), 2.72 (t, J = 10.8 Hz, 1H), 3.08 (q, J = 6.1 Hz, 2H), 3.15 (dd, J = 4.5, 14.3 Hz, 1H), 3.23–3.33 (m, 3H), 3.52 (dd, J = 5.1, 13.1 Hz, 1H), 3.86 (d, J = 16.5 Hz, 1H), 4.03 (d, J = 16.5 Hz, 1H), 4.20 (q, J = 7.6 Hz, 1H), 4.35 (quintet, J = 7.2 Hz, 1H), 4.74 (quintet, J = 5.9 Hz, 1H), 4.85 (dd, J = 4.4, 8.4 Hz, 1H), 5.35 (dd, J = 4.8, 11.2 Hz, 1H), 6.53 (br s, 1H), 6.98 (t, J = 8.0 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.11 (s, 1H), 7.13 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.38 (s, 1H), 7.48 (br t, J = 5.1 Hz, 1H), 7.60 (d, J = 7.9 Hz), 8.10 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 7.3 Hz, 1H); gHSQC (multiplicity edited) (DMSO-d_6 500 MHz, 25 °C): δ 18.9 (CH_3), 23.2 (CH_3), 25.8 (CH_2), 28.2 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 41.2 (CH_2), 48.5 (CH_2), 48.8 (CH), 49.4 (CH_2), 50.3 (CH), 51.7 (CH), 52.7 (CH), 58.6 (CH), 112.1 (CH), 118.9 (CH), 119.1 (CH), 121.7 (CH), 125.1 (CH).
5. (a) Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. *J. Am. Chem. Soc.* **1999**, *121*, 12204–12205; (b) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. *Tetrahedron Lett.* **2001**, *42*, 1237–1239.
6. All synthesis carried out as stated in Ref. 2. Purification was achieved via reversed phase HPLC (vydac C_{18} 22 mm i.d.×25 cm column; linear gradient of 0.05% TFA water and 0.05% TFA/CH_3CN) followed by lyophilization.