ORGANIC LETTERS

2010
Vol. 12, No. 14
3254−3257

Cyclic Peptide Synthesis with Thioacids

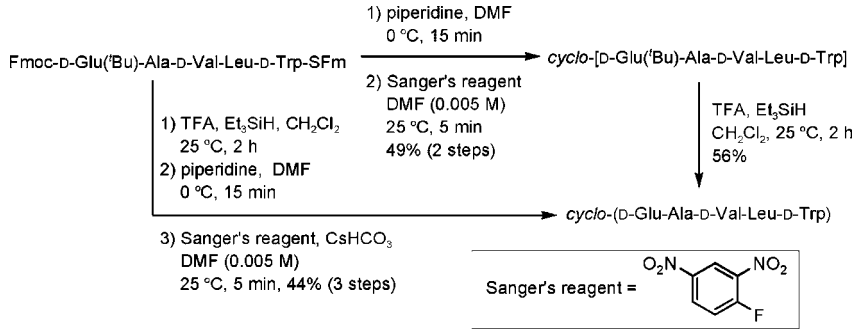
Kaname Sasaki and David Crich*

Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

dcrich@icsn.cnrs-gif.fr

Received May 25, 2010

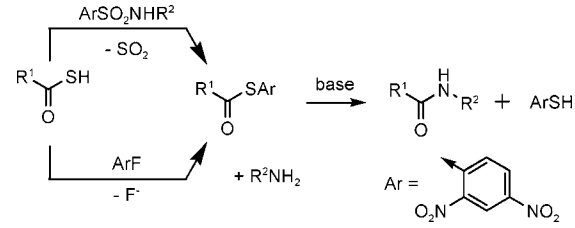
ABSTRACT



C-Terminal amino acid 9-fluorenylmethylthioesters may be carried through Boc chemistry solution phase peptide synthesis sequences. After insertion of the final residue in the form of an Fmoc carbamate, treatment with piperidine releases a *seco*-peptide as a C-terminal thioacid that on treatment with Sanger's reagent undergoes cyclization to a cyclic peptide. Cyclic penta- and hexapeptides have been synthesized in this manner, as has a cyclic glycopeptide. Functional group compatibility with alcohols and carboxylic acids is demonstrated.

By virtue of their unique reactivity profile[1] monothioacids offer numerous advantages oversimple carboxylic acids in a range of organic reactions but most notably in amide and peptide bond-forming sequences. For example, the reactions of thioacids with isonitriles[2] and with both isocyanates and isothiocyanates[3] to give amides have been revealed to operate under much milder conditions than the corresponding processes with simple carboxylic acids. Following an initial report by an industrial group,[4] our laboratory has been developing the reaction of thioacids with electron-deficient sulfonamides as well as related coupling reactions employing Sanger's reagent[5] (Scheme 1) as effective tools for peptide bond formation in both simple sequences and in block syntheses.[6]

Scheme 1. Amide Formation from Thioacids with either Sulfonamides or Amines and Sanger's Reagent



The cyclic peptides are a large and diverse class of natural products, whose backbones may contain both proteinogenic and nonproteinogenic amino acids, and whose equally diverse range of biological activities make them and their analogues important

(1) (a) Niyomura, O.; Kato, S. *Top. Curr. Chem.* **2005**, *251*, 1–12. (b) Kato, S.; Kawahara, Y.; Kageyama, H.; Yamada, R.; Murai, T.; Kanda, T. *J. Am. Chem. Soc.* **1996**, *118*, 1262–1267. (c) Hadad, C. M.; Rablen, P. R.; Wiberg, K. B. *J. Org. Chem.* **1998**, *63*, 8668–8681.

(2) Bao, Y.; Li, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 12924–12926.

(3) (a) Crich, D.; Sasaki, K. *Org. Lett.* **2009**, *11*, 3514–3517. (b) Kricheldorf, H. R.; Leppert, E. *Makromol. Chem.* **1973**, *167*, 47–68.

(4) (a) Messeri, T.; Sternbach, D. D.; Tomkinson, N. C. O. *Tetrahedron Lett.* **1998**, *39*, 1669–1672. (b) Messeri, T.; Sternbach, D. D.; Tomkinson, N. C. O. *Tetrahedron Lett.* **1998**, *39*, 1673–1676.

(5) Crich, D.; Sharma, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 2355–2358.

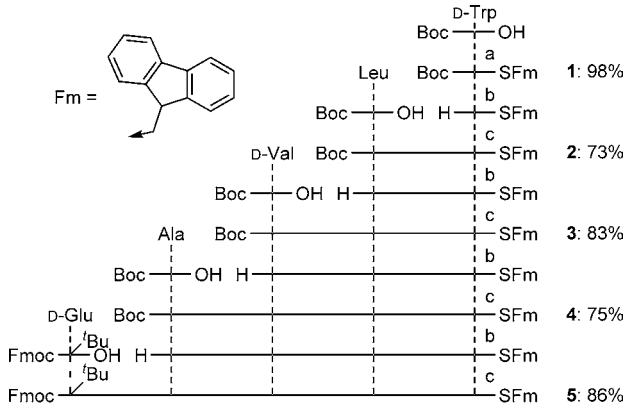
(6) (a) Crich, D.; Sana, K.; Guo, S. *Org. Lett.* **2007**, *9*, 4423–4426. (b) Crich, D.; Sana, K. *J. Org. Chem.* **2009**, *74*, 7383–7388. (c) Crich, D.; Sharma, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 7591–7594.

10.1021/ol101201w
Published on Web 06/22/2010

targets for organic synthesis.[7] Seeking to extend the chemistry of thioacids further and to probe its limits, we have investigated its application to the formation of cyclic peptides of varying size and substitution pattern and report here on our results.

As a first goal we selected the cyclic pentapeptide **7**, a known endothelin antagonist.[8] The strategy called for the release of the *seco*-thioacid from a thioester immediately prior to ring closure. Considerations of efficiency and the need to avoid potential racemization from the functionalization of C-terminal acids indicated that the thioester should be installed at the beginning of the synthesis. In view of the well-known incompatibility of thioesters to the conditions of Fmoc chemistry peptide synthesis,[9] the application of such a strategy suggested the use of Boc peptide synthesis methods for the assembly of the linear peptide. Accordingly, a tetrapeptide fluorenylmethyl thioester **4** was constructed by standard solution phase Boc chemistry peptide synthesis (Scheme 2), and after standard removal of the *N*-terminal

Scheme 2. Typical Synthesis of a *seco*-Thioester for Cyclization

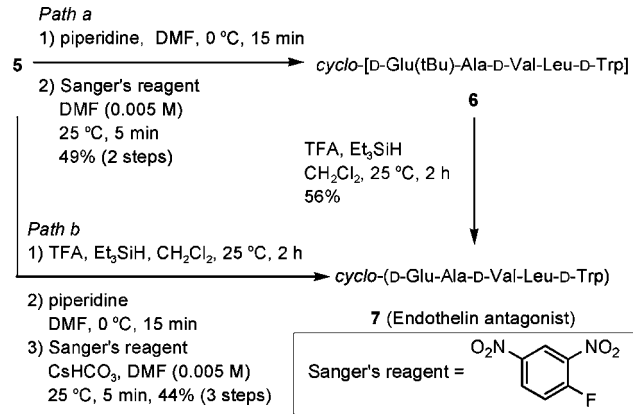


(a) FmSH, EDCI, DMAP, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) EDCI, $HOBt.H_2O$, $^iPrEt_2N$, CH_2Cl_2/DMF

Boc group with TFA in the usual manner,[10] the final amino acid was incorporated in the form of an Fmoc carbamate (Scheme 2). All other thioesters reported here were constructed analogously, and their syntheses are detailed in the Supporting Information.

Brief treatment of **5** with piperidine in DMF at 0 °C revealed both the thioacid and the nucleophilic amine, which underwent cyclization to give the desired cyclic hexapeptide **6** in 49% yield on stirring with Sanger's reagent for 5 min at 25 °C (Scheme 3, path a). Finally, exposure of **6** to

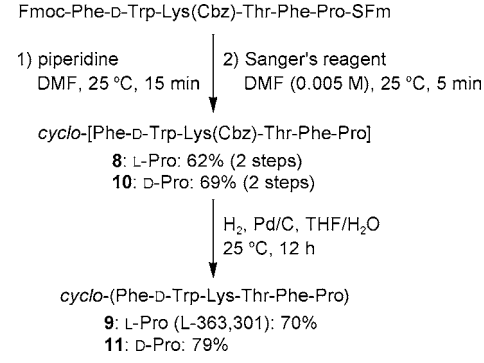
Scheme 3. Two Syntheses of a Cyclic Pentapeptide



trifluoroacetic acid removed the *tert*-butyl ester from the side chain of the glutamate residue and afforded the target molecule **7**. A higher-yielding protocol (Scheme 3, path b) involved initial cleavage of the *tert*-butyl ester before removal of the Fmoc group and deprotection of the fluorenylmethyl thioester. This second pathway also serves to reaffirm the compatibility of the thioacid coupling strategy with the presence of simple carboxylic acids, which is a function of their greater acidity and nucleophilicity.

We next targeted a cyclic hexapeptide analogue **9** of somatostatin described originally by Hirschmann and coworkers.[11] The deprotection of the N-terminal Fmoc group and the C-terminal thioester were again accomplished simultaneously with piperidine in DMF and the cyclization performed by brief exposure to Sanger's reagent in the same solvent (Scheme 4). The cyclic hexapeptide **8**

Scheme 4. Synthesis of Two Diastereomeric Hexapeptides



obtained in 62% yield in this manner was then subjected to hydrogenolysis to remove the Cbz protection from the lysine residue, when it afforded the target **9** in 70% yield.

(7) (a) Sewald, N.; Jakubke, H.-D. *Peptides: Chemistry and Biology*; Wiley-VCH: Weinheim, 2009; pp 578. (b) Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441–4482. (c) Davies, J. S. *J. Pept. Sci.* **2003**, *9*, 471–501. (d) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 471–484. (e) Gracia, S. R.; Gaus, K.; Sewald, N. *Future Med. Chem.* **2009**, *1*, 1289–1310. (f) Katsura, M.; Tselios, T.; Deraos, S.; Deraos, G.; Matsoukas, M.-T.; Lazoura, E.; Matsoukas, J.; Apostolopoulos, V. *Curr. Med. Chem.* **2006**, *13*, 2221–2232. (g) Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Mol. Diversity* **2002**, *5*, 289–304. (h) Li, P.; Roller, P. P. *Curr. Top. Med. Chem.* **2002**, *2*, 325–341. (i) Hili, R.; Rai, V.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132*, 2889–2891.

(8) Kojiri, K.; Ihara, M.; Nakajima, S.; Kawamura, K.; Funaishi, K.; Yano, M.; Suda, H. *J. Antibiot.* **1991**, *44*, 1342–1347.

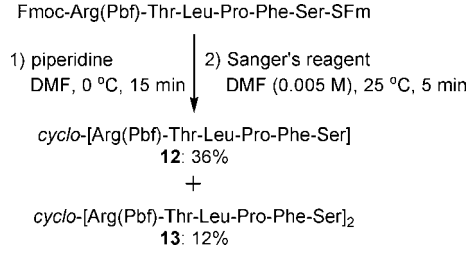
(9) MacMillan, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7668–7672.

(10) The deprotected amines were handled in the form of the TFA salts and were not isolated. In general, premature cyclization onto the thioesters to give, e.g., diketopiperazines, was not a problem.

The ^{1}H NMR data and the specific rotation of **9** so obtained differed from those reported by in the literature, for which we have no explanation at the present time; however, the CD spectrum of **9** bore a strong resemblance to that reported previously. The possibility that we might have accessed a different rotamer of **9** about the Phe−Pro bond was addressed through VT-NMR experiments. Heating of **9** in DMF-d_7 above the coalescence temperature followed by cooling to room temperature returned an ^{1}H NMR spectrum identical to the one obtained before heating, thereby ruling out the possibility that the cyclization had afforded a metastable conformer. Suspecting that the C-terminal proline residue had undergone epimerization in the course of the synthesis, an isomeric *seco*-hexapeptide thioacid, in which the original L-proline was replaced by D-proline, was prepared and cyclized. Interestingly, in this epimeric series the cyclization took place with a somewhat higher yield, thereby emphasizing the dependence of the cyclization yield on the configuration and conformation of the backbone (Scheme 4). However, after final hydrogenolysis an isomer **11** of the targeted sequence was obtained whose spectral data were clearly different from those of its L-prolinyl isomer and those reported in the literature for **9**. While this experiment does not explain the differences between the data for **9** obtained in our lab and that reported in the literature, it does serve nicely to highlight the essentially racemization-free nature of the chemistry reported here.

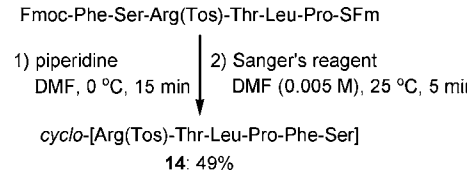
With a view to testing the scope of the method, and especially the functional group compatibility, two protected versions **12** and **14** of a further cyclic hexapeptide, of interest because of its ability to mimic structurally and functionally the human natural killer cell-1 (HNK-1) epitope,[12] were constructed by two different cyclizations as outlined in Schemes 5 and 6. In the first of these two approaches (Scheme 5) cyclization was attempted with

Scheme 5. Synthesis of Cyclic Hexapeptide **12**



formation of the peptide bond between the serine and arginine residues, with the side chain of the latter protected in the form of a pentamethyldihydrobenzofuranylsulfonamide (Pbf) group. The target compound **12** was

Scheme 6. Synthesis of Cyclc Hexapeptide **14**

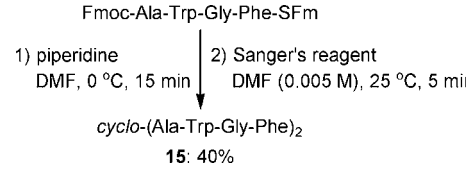


obtained in 36% yield along with 12% of the cyclic dimer **13** (Scheme 5). Interestingly, the ^{1}H NMR spectrum of **12** displayed two sets of signals in an approximate 2:1 ratio at 25 °C, and 10 amide carbonyl carbon resonances were visible in the ^{13}C NMR spectrum under the same conditions, indicating the possibility of two distinct conformers for this cyclic peptide. VT-NMR experiments confirmed this hypothesis, with coalescence of the signals into a single set at 100 °C (Supporting Information).

The second disconnection investigated, which relied on cyclization with the formation of a peptide bond between the phenylalaninyl and prolyl residues and which employed an *N*-toluenesulfonyl protected arginine residue in place of the Pbf group of the first sequence, took place with a significantly improved yield of 49% (Scheme 6).

The cyclization of a tetrapeptide was also briefly investigated. Unfortunately, we were only able to isolate the cyclic dimer **15** from this sequence (Scheme 7).

Scheme 7. Attempted Synthesis of a Cyclic Tetrapeptide



Finally, we have investigated briefly a convergent sequence for substituted cyclic peptide preparation. To this end the tetrapeptide **17** was constructed by Boc chemistry solution phase peptide synthesis (Supporting Information) starting from the bis(thioester) **16** of aspartic acid according to the standard protocol. It is especially noteworthy that the two thioester groups of the C-terminal aspartate residue were successfully carried through this sequence of reactions.

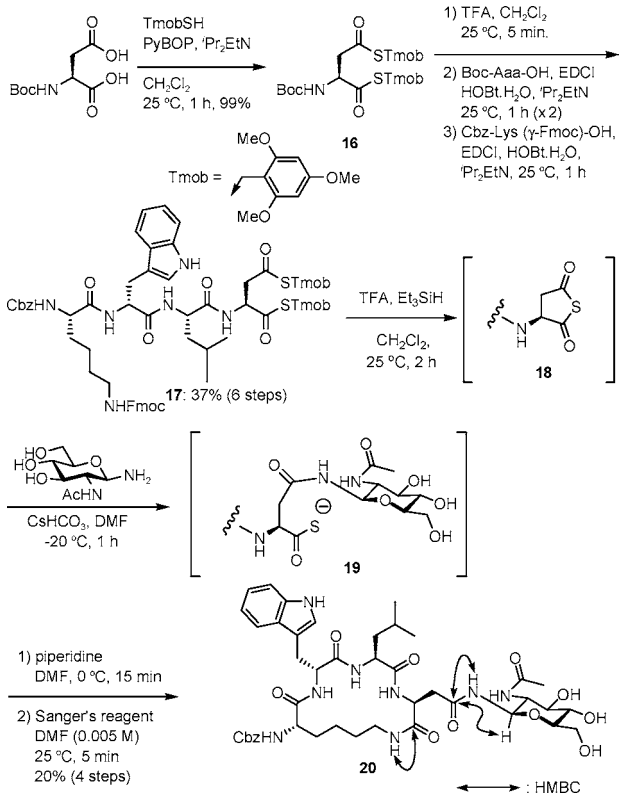
Treatment of the tetrapeptide bis(thioester) **17** with trifluoroacetic acid and triethylsilane in dichloromethane at room temperature gave, by analogy with an earlier,

(11) Veber, D. F.; Freidinger, R. M.; Perlow, D. S.; Paleveda, W. J.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C.; Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Nature* **1981**, *292*, 55–58.

(12) (a) Bhunia, A.; Vivekanandan, S.; Eckert, T.; Burg-Roderfeld, M.; Wechselberger, R.; Romanuka, J.; Bächle, D.; Kornilov, A. V.; von der Lieth, C.-W.; Jiménez-Barbero, J.; Nifantiev, N. E.; Schachner, M.; Sewald, N.; Lütteke, T.; Siebert, H.-C. *J. Am. Chem. Soc.* **2010**, *132*, 96–105. (b) Bächle, D.; Loers, G.; Guthöhrlein, E. W.; Schachner, M.; Sewald, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 6582–6585.

(13) Crich, D.; Sasaki, K.; Rahaman, M. Y.; Bowers, A. A. *J. Org. Chem.* **2009**, *74*, 3886–3894.

Scheme 8. Synthesis of a Cyclic Glycopeptide via an Intermediate Cyclic Thioanhydride



simpler example,[13] the monothioanhydride **18**. This substance was not isolated but was allowed to react directly with 2-acetamido-2-deoxy-β-D-glucosylamine in the presence of cesium hydrogen carbonate to give an expected *N*-glycosyl monothio asparagine residue **19**. Again without isolation, this compound was treated with piperidine to remove the Fmoc protecting group and the resulting amino thioacid was exposed to Sanger's reagent resulting overall in an unusual glycosylated cyclic peptide **20** in 20% overall yield for the four steps (Scheme 8). The regioselectivity of the critical step, the ring opening of the intermediate cyclic thioanhydride, was predicted on the basis of the literature precedent in simpler analogues,[13] and was confirmed in the isolated product by the observation of the indicated HMBC correlations.

Overall, in this Letter we have established that the thioacid amide bond forming sequence employing Sanger's reagent is a viable method for the formation of five- and six-residue cyclic peptides. The method has the advantages of proceeding in moderate to good yield in minutes at room temperature in DMF, is compatible with the presence of free carboxylic acids and hydroxyl groups, and enables ring closure to be effected even at hindered C-terminal groups such as proline.

Acknowledgment. We thank Indrajeet Sharma, Wayne State University, for supporting experiments and helpful discussion and Jean-François Gallard, ICSN, for help with VT-NMR experiments.

Supporting Information Available: Full experimental details and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL101201W