ORGANIC LETTERS

2009
Vol. 11, No. 20
4708−4711

Synthesis of Novel Peptide Linkers: Simultaneous Cyclization and Labeling

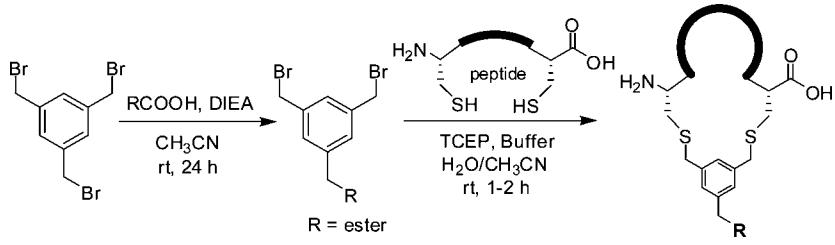
Gajanan K. Dewkar, Pedro B. Carneiro, and Matthew C. T. Hartman*

Department of Chemistry and Massey Cancer Center, Virginia Commonwealth University, 401 College Street, Richmond, Virginia 23298-0037

mchartman@vcu.edu

Received July 21, 2009

ABSTRACT



Synthesis of novel peptide linkers was accomplished by monocarboxylation of 1,3,5-tris(bomomethyl)benzene with a wide variety of carboxylic acids in the presence of diisopropylethylamine. These reagents can be used to simultaneously cyclize and label peptides containing two cysteines. Many labels are compatible with this method including lipids, fluorescent groups, and biotin.

Cyclic peptides are widely produced in nature[1] and possess a broad range of biological activities. Cyclization leads to enhanced metabolic stability[2] and may lead to increased specificity[2c,3] or affinity[4] due to the imposed conformational constraints.[5] In specific cases, cyclization can also lead to enhanced cell permeability.[2b,6] These properties make cyclic peptides promising candidates for the development of new therapeutic agents.[7]

However, cyclization also complicates the synthesis of peptides since selective, orthogonal protecting groups are typically required to unmask the reactive functional groups used in the cyclization.[8] The yields and rates of cyclization reactions can also be highly variable,[9] further complicating their synthesis. Where the construction of cyclic peptide libraries is concerned, or if the peptide needs to be both cyclized and labeled, these problems become even more acute.

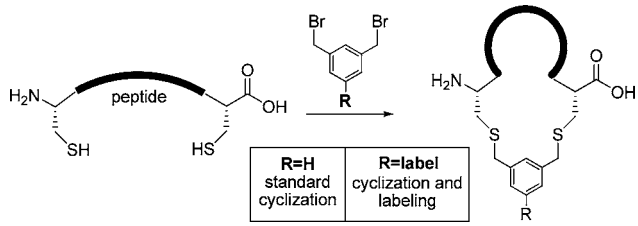
Recently, a highly chemoselective strategy for peptide cyclization has been developed which involves treating linear unprotected dicysteine-containing peptides with α,α′-dibromoxylenes, leading to a stable bis-thioether linkage (Scheme 1).[10] This reaction was found to be high yielding and relatively independent of ring size or functional groups, making it an ideal strategy for cyclization. Furthermore, by

(1) Pomilio, A. B.; Battista, M. E.; Vitale, A. A. *Curr. Org. Chem.* **2006**, *10*, 2075–2121.
(2) (a) Millward, S. W.; Fiacco, S.; Austin, R. J.; Roberts, R. W. *ACS Chem. Biol.* **2007**, *2*, 625–634. (b) Gudmundsson, O. S.; Pauletti, G. M.; Wang, W.; Shan, D.; Zhang, H.; Wang, B.; Borchardt, R. T. *Pharm. Res.* **1999**, *16*, 7–15. (c) Piserchio, A.; Salinas, G. D.; Li, T.; Marshall, J.; Spaller, M. R.; Mierke, D. F. *Chem. Biol.* **2004**, *11*, 469–473.
(3) DiMaio, J.; Nguyen, T. M.; Lemieux, C.; Schiller, P. W. *J. Med. Chem.* **1982**, *25*, 1432–1438.
(4) Khan, A. R.; Parrish, J. C.; Fraser, M. E.; Smith, W. W.; Bartlett, P. A.; James, M. N. *Biochemistry* **1998**, *37*, 16839–16845.
(5) Hruby, V. J.; al-Obeidi, F.; Kazmierski, W *Biochem. J.* **1990**, *268*, 249–262.
(6) (a) Kwon, Y. U.; Kodadek, T. *Chem. Biol.* **2007**, *14*, 671–677. (b) Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 2510–2511.
(7) (a) Katsara, M.; Tselios, T.; Deraos, S.; Deraos, G.; Matsoukas, M. T.; Lazoura, E.; Matsoukas, J.; Apostolopoulos, V. *Curr. Med. Chem.* **2006**, *13*, 2221–2232. (b) Willey, J. M.; van der Donk, W. A. *Annu. Rev. Microbiol.* **2007**, *61*, 477–501.
(8) For examples, see: (a) Liu, T.; Joo, S. H.; Voorhees, J. L.; Brooks, C. L.; Pei, D. *Bioorg. Med. Chem.* **2009**, *17*, 1026–1033. (b) Walker, M. A.; Johnson, T. *Tetrahedron Lett.* **2001**, *42*, 5801–5804. (c) Lundquist, J. T. t.; Pelletier, J. C. *Org. Lett.* **2002**, *4*, 3219–3221. (d) Lawrence, J.; Jourdan, M.; Vallee, Y.; Blandin, V. *Org. Biomol. Chem.* **2008**, *6*, 4575–4581.
(9) (a) Davies, J. S. *J. Pept. Sci.* **2003**, *9*, 471–501. (b) Fluxa, V. S.; Reymond, J. L. *Bioorg. Med. Chem.* **2009**, *17*, 1018–1025.
(10) Timmerman, P.; Beld, J.; Puijk, W. C.; Meloen, R. H. *ChemBioChem* **2005**, *6*, 821–824.

10.1021/ol901662c CCC: $40.75
Published on Web 09/08/2009

appending substituents to the benzene ring it should be possible to couple peptide cyclization with the attachment of a label (Scheme 1). In this paper, we report a novel synthesis of peptide linkers using the dibromoxylene strategy. These linkers allow peptides to be simultaneously cyclized and labeled.

Scheme 1. Strategy for Simultaneous Peptide Cyclization and Labeling

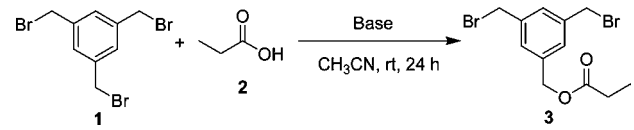


For simultaneous cyclization and labeling, we sought a flexible and efficient strategy that would lead to a small library of bis-bromomethyl compounds in a minimum number of steps. In particular, we desired an approach that did not require introduction of the benzylic bromide groups as the last step, since we did not want to limit ourselves to labels that would be inert to the bromine-introduction step. Moreover, such a strategy would require double the synthetic effort, since each member of the library would require a label attachment step followed by the bromination. However, attaching the label last presents a different challenge because the sensitive benzylic bromides must be preserved during the label addition step.

In principle, treatment of 1,3,5-tris(bromomethyl)benzene (**1**) with a nucleophile-containing label would give the desired bis-bromomethyl linkers in a single step, provided that we could control the reaction conditions to maximize monoaddition. With this in mind, we reacted several different types of nucleophiles with **1**. Thiols led to unstable monothioethers; primary amines led to insoluble products, presumably polymers; and amides and sulfonamides proved to be unreactive. However, we were able to reproduce a previously reported[11] synthesis of 3,5-bis(bromomethyl)benzyl acetate using sodium acetate and 1,3,5-tris(bromomethyl) benzene. The harsh conditions for this reaction (heating at 100 °C in DMF for 2 h) are unacceptable for many types of carboxylic acids, and the yield and selectivity for the monoadduct were low. But this initial success led us to optimize the coupling with carboxylic acids. Moreover, a wide variety of carboxylic acids are commercially available, suggesting that this reaction could be wide in scope.

A brief study on the effect of solvent and base for the reaction of propoinic acid and 1,3,5-tris(bromomethyl) benzene is shown in Table 1. We first optimized the base in the reaction. At room temperature in acetonitrile, cesium carbonate,[12] potassium carbonate, triethylamine, and pyridine (entries 1−4) were shown to be inferior to diisopropylethylamine (DIEA) (entry 5). Solvent also plays an important role in this reaction (entries 6−12) with acetonitrile proving to be most suitable. On the basis of this brief survey, MeCN

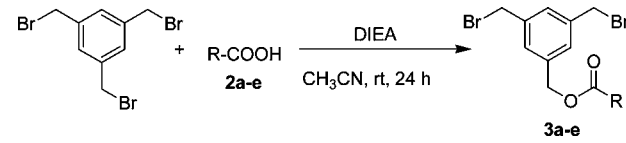
Table 1. Monoalkylation of 1,3,5-Tris(bromomethyl)benzene: Effect of Solvent and Base[a]

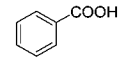
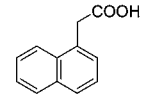
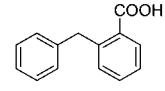


entry	solvent	base	yield (%)
1	MeCN	Cs_2CO_3	43[b]
2	MeCN	K_2CO_3	30[b]
3	MeCN	TEA	20[b]
4	MeCN	pyridine	15[b]
5	**MeCN**	**DIEA**	**75**[b]
6	DMF	DIEA	30[b]
7	THF	DIEA	<5[c]
8	toluene	DIEA	10[c]
9	acetone	DIEA	20[c]
10	chloroform	DIEA	30[c]
11	CH_2Cl_2	DIEA	50[b]
12	DMSO	DIEA	40[b]

[a] Reaction conditions: bromo **1** (1 equiv), acid **2** (1.5 equiv), base (3 equiv), rt, 24 h. [b] Isolated yield after column chromatographic purification. [c] Estimated by TLC.

Table 2. Monoalkylation of 1,3,5-Tris(bromomethyl)benzene by Aliphatic and Aromatic Carboxylic Acids[a]



entry	acid-**2**	product-**3** (yield [%])[b]
1	**2a** 	**3a** (75)
2	**2b** 	**3b** (60)
3	**2c** 	**3c** (55)
4	**2d** 	**3d** (53)
5	**2e** 	**3e** (60)

[a] Reaction conditions: bromo (1 equiv), acid (1.5 equiv), base (3 equiv), rt, 24 h. [b] Isolated yield after column chromatographic purification.

(11) Diez-Barra, E.; Garcia-Martinez, J. C.; Merino, S.; del Rey, R.; Rodriguez-Lopez, J.; Sanchez-Verdu, P.; Tejeda, J. *J. Org. Chem.* **2001**, *66*, 5664–5670.

and DIEA appeared to be the optimal solvent and base combination, and these conditions were utilized for subsequent reactions.

In addition to the desired product, these reactions gave small amounts of bis- and tris-substituted compounds. These side products could be minimized when we used 1 equiv of **1**, 1.5 equiv of carboxylic acid, and 3 equiv of DIEA. In these cases, the bis- and tris-substituted compounds were always less than 15%, and a small amount (10−15%) of unreacted starting material was also present. These byproducts could typically be easily separated from the desired monoadduct.

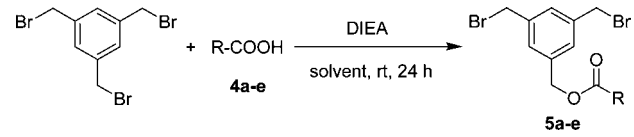
The monocarboxylation of **1** with variety of simple carboxylic acids is summarized in Table 2. Both alkyl (**2a**) and aryl (**2c** and **2d**) carboxylic acids were suitable compounds in the reaction. Hindered alkyl or aryl acids (**2b, 2e**) also led to the desired products in good yields.

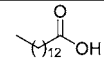
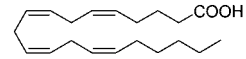
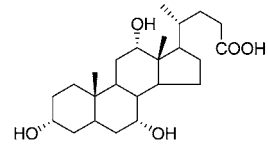
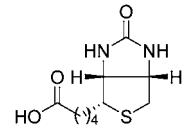
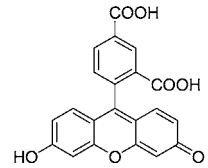
Further examples (**6a**−**g**) of simple carboxylic acids that have been successfully esterified are given in the Supporting Information.

We then proceeded to test carboxylic acids with interesting properties as well as multiple functional groups (Table 3). Long-chain fatty acids such as myristic acid (**4a**) and arachidonic acid (**4b**) were successfully esterified, although the reaction with **4b** was performed in CH_2Cl_2 due to its poor solubility in MeCN. These linkers would be useful for the synthesis of cyclic lipidated peptides. Lipopeptides are an important subset of nonribosomally prepared peptides.[13] Cholate (**4c**) was also a suitable substrate for the reaction in CH_2Cl_2; no reaction at the three hydroxyl groups was observed. Biotin (**4d**) was insoluble in both CH_2Cl_2 and acetonitrile; however, DMF proved to be a suitable solvent, leading to adduct **5d** in moderate 50% yield. We were hopeful that compound **5d** would be a suitable reagent for simultaneous biotinylation and cyclization. We also desired linkers that would allow simultaneous cyclization and fluorescent labeling of peptides or peptide libraries. 5-Carboxyfluorescein (**4e**) reacted with **1** to give the ester product in moderate yield. Since fluorescein itself (which lacks the 5-carboxy group) was unreactive when treated with **1**, we presume that the product formed is the 5-carboxy ester.

Our next step was to verify that each of these dibromomethyl reagents was active toward cyclization. We chose two commercially available disulfide containing peptides, a thyrotropin-releasing hormone derivative (TRH-SH Pro) (Cys-Lys-Arg-Gln-His-Pro-Gly-Lys-Arg-Cys) and somatostatin (Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys) for our analysis. In all cases, treatment of either of these two peptides with each these reagents led cleanly to the desired cyclic peptide products as evidenced by MALDI-TOF MS (Figure 1 and Supporting Information). Some representative MS spectra for cyclization with somatostatin are shown in Figure 1. Maximum yields of

Table 3. Monoalkylation of 1,3,5-Tris(bromomethyl)benzene by Other Carboxylic Acids[a]



entry	acid-**4**	product-**5** (yield [%])[e,f]
1	**4a** 	**5a** (52)[d,f]
2	**4b** 	**5b** (50)[b,f]
3	**4c** 	**5c** (46)[b,e]
4	**4d** 	**5d** (50)[c,e]
5	**4e** 	**5e** (45)[c,e]

[a] Reaction conditions: bromo (1 equiv), acid (1.5 equiv), base (3 equiv), [b] CH_2Cl_2. [c] DMF. [d] MeCN, rt, 24 h. [e] Isolated yield after column chromatographic purification. [f] Isolated yield after preparative TLC purification.

the cyclic peptides were observed when the concentration of the peptides was between 0.01 and 0.1 mM, along with 1.1 mM of the cyclization reagent, and 200 μM of the reducing agent tris(2-carboxyethyl)phosphine (TCEP).

A typical reaction contained 50% acetonitrile; however, this percentage could be increased to 75% to enable the solubility of hydrophobic linkers (e.g., **5a** and **5b**) or likewise decreased to 25% for more hydrophilic linkers (e.g., **5d**). It is also important to miminize the amount of TCEP since at higher concentrations it can alkylate the bisbromomethyl reagent leading to undesired phosphonium derivatives. The reaction is efficient on the largest (10 mg) scale attempted, giving yields above 90% for the cyclized peptide product.

In summary, we have developed an efficient method for monocarboxylation of 1,3,5-tris(bromomethyl)benzene using various carboxylic acids. The resulting esters are efficient peptide cyclization linkers that can be used for simultaneous cyclization and labeling of peptides. Our cyclization and labeling strategy will be particularly helpful in synthetically challenging systems like the synthesis of peptide libraries, where the single-step efficiency and chemoselectivity of the dibromoxylene-mediated cyclization will be most useful. Others have described oxidative peptide cyclization methods that lead

(12) Hennrich, G.; Lynch, V. M.; Anslyn, E. V. *Chem. Commun.* **2001**, 2436–2437.

(13) (a) Baltz, R. H.; Miao, V.; Wrigley, S. K. *Nat. Prod. Rep.* **2005**, *22*, 717–741. (b) Strieker, M.; Marahiel, M. A. *ChemBioChem* **2009**, *10*, 607–616.

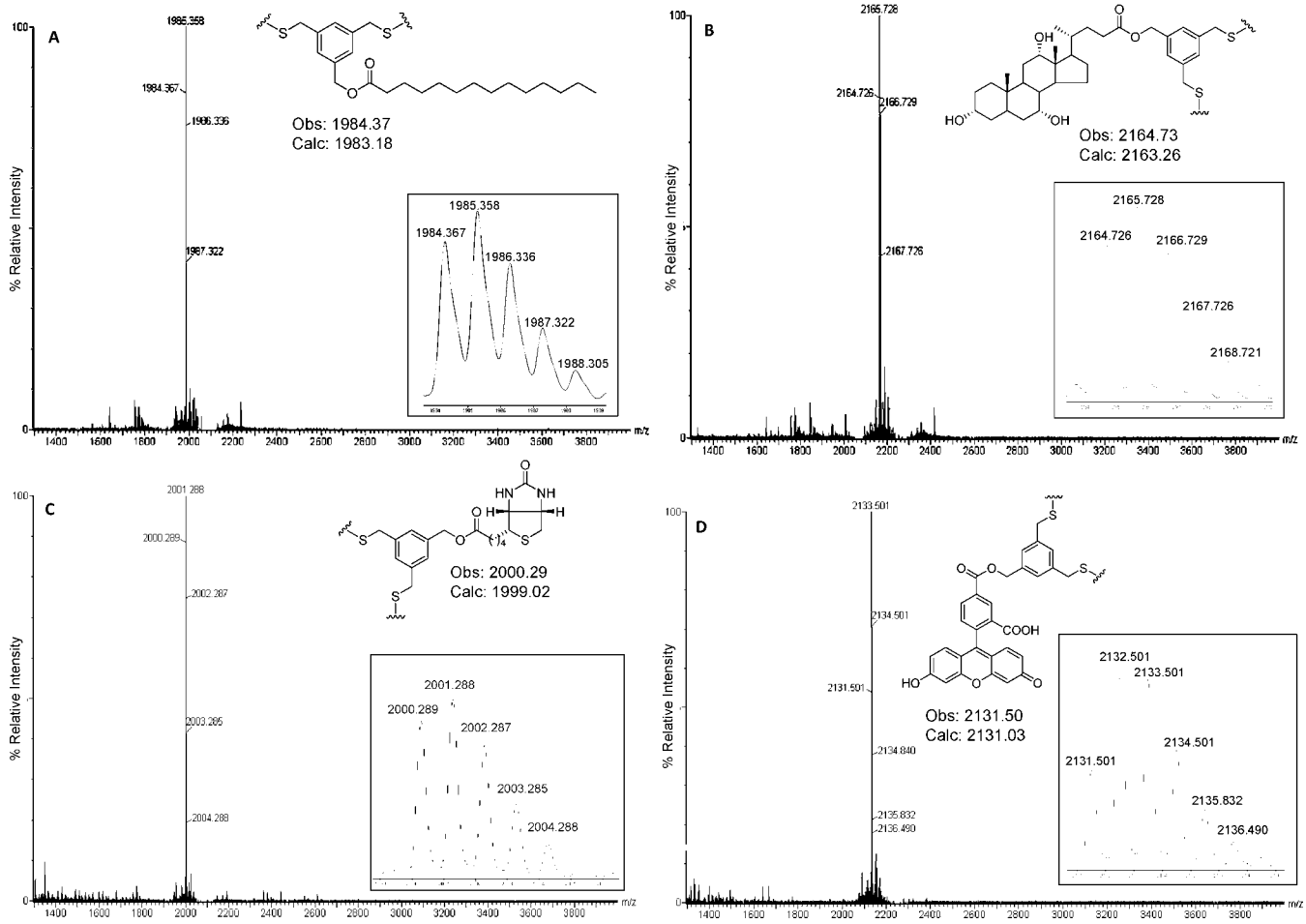


Figure 1. MALDI mass spectra of somatostatin cyclized with (A) myristic ester **5a**, (B) cholate ester **5c**, (C) biotin ester **5d**, and (D) fluorescein ester **5e**.

to fluorescent cyclic peptide products.[14] Compared to these methods, our approach offers the ability to attach many different types of labels upon cyclization and occurs under mild, nonoxidative conditions. One potential downside to our method is that the labels are linked to the peptide with an ester bond which could be labile in biological media. In principle, it should be possible to use variations on our synthetic strategy to create other types of linkages that will be more biostable. Evaluation of the stability and properties of peptide libraries cyclized and labeled using this method is currently underway.

Acknowledgment. We acknowledge the Massey Cancer Center at VCU for financial support of this project. We are also grateful to Scott Gronert (VCU) and James Coward (University of Michigan) for their helpful comments on the manuscript.

Note Added after ASAP Publication. Peak assignments in Figure 1D were incorrect in the version published ASAP September 8, 2009. The revised version was published on the Web September 11, 2009.

Supporting Information Available: Experimental procedure, characterization, and copies of ^{1}H and ^{13}C NMR spectra for all new compounds; and MALDI-TOF mass spectra of all cyclized peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901662C

(14) (a) Stachel, S. J.; Habeeb, R. L.; Van Vranken, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 1225–1226. (b) Yamagishi, Y.; Ashigai, H.; Goto, Y.; Murakami, H.; Suga, H. *ChemBioChem* **2009**, *10*, 1469–1472.