Communications

Solid-Phase Synthesis of Trisubstituted Bicyclic Guanidines via Cyclization of Reduced N-Acylated Dipeptides

John M. Ostresh, Christa C. Schoner, Vince T. Hamashin, Adel Nefzi, Jean-Philippe Meyer, and Richard A. Houghten*

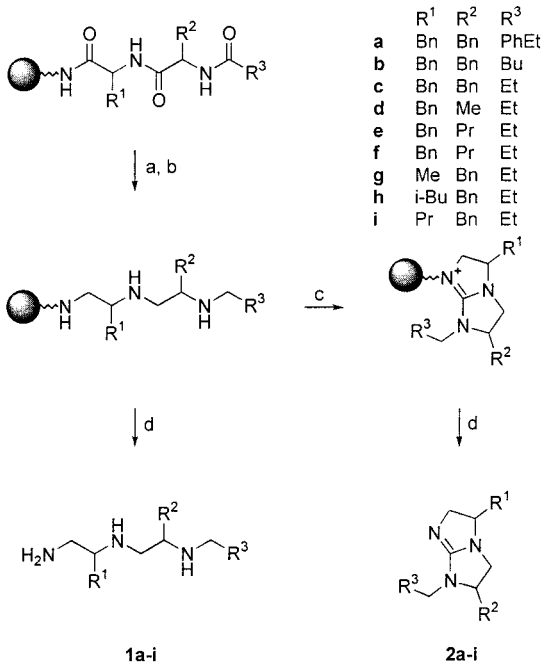
Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, California 92121

Received June 4, 1998

A novel method for the solid-phase synthesis of trisubstituted bicyclic guanidines is presented. The initial reaction step involves the exhaustive reduction of resin-bound N-acylated dipeptides using borane−THF, followed by cyclization of the resulting triamine with thiocarbonyldiimidazole to generate resin-bound trisubstituted bicyclic guanidines. Cleavage from the resin using hydrogen fluoride yields the desired trisubstituted bicyclic guanidines in excellent yield and purity. The approaches described enable efficient high-yield and purity syntheses of either polyamines or bicyclic guanidines. We have applied these methods to the synthesis of both individual compounds and combinatorial libraries.

The diversity of existing synthetic combinatorial libraries can be leveraged using the "libraries from libraries" concept. This was first demonstrated through the successsful N-permethylation[1] of a hexapeptide combinatorial library.[2−4] Later, the applicability of the concept was demonstrated again by the solution-phase transformation of the same peptide combinatorial libraries to polyamines by exhaustive reduction.[5] Furthermore, these methodologies have recently been used for the preparation of hydantoins and cyclic ureas[6] from dipeptide precursors. Polyamines are highly versatile starting materials for the generation of a wide variety of heterocyclic compounds and their respective combinatorial libraries.[7] Many naturally occurring compounds contain a guanidino moiety, and its strong cationic nature is often central to their biological activity.[8] Although many solution-phase methods for the reduction of amides have been described,[9−13] we are not aware of any general procedures for the exhaustive reduction of peptides on the solid phase. We describe here a general procedure (Scheme 1) for both the exhaustive reduction of resin-bound N-acylated dipeptides to generate triamines and their subsequent cyclization to generate trisubstituted bicyclic guanidines.[14] This synthetic approach has also been applied to generate mixture-based combinatorial libraries of both polyamines and bicyclic guanidines, each having three positions of diversity and made up of more than 100 000 compounds. In these libraries, the parent peptides were completely transformed. The residual side chain functional groups associated with the amino acids are the only indication that peptides were used in their synthesis.

Scheme 1. Solid-Phase Synthesis of Bicyclic Guanidines from N-Acylated Dipeptides[a]



	R^1	R^2	R^3
a	Bn	Bn	PhEt
b	Bn	Bn	Bu
c	Bn	Bn	Et
d	Bn	Me	Et
e	Bn	Pr	Et
f	Bn	Pr	Et
g	Me	Bn	Et
h	i-Bu	Bn	Et
i	Pr	Bn	Et

[a] Resin is methylbenzhydrylamine derivatized polystyrene resin: (a) BH_3-THF, $B(OH)_3$, $B(OCH_3)_3$, 65 °C, 72 h; (b) piperidine, 65 °C, 24 h; (c) $CSIm_2$ in CH_2Cl_2, 16 h; (d) HF, anisole, 0 °C, 9 h.

In preparation for the synthesis of a bicyclic guanidine library, more than 400 individual controls (>200 separate polyamines and >200 separate bicyclic guanidines) were synthesized. For each potential building block considered for use in the synthesis of the combinatorial library, at least one control compound was synthesized. Only those building blocks yielding individual control polyamines having crude purities greater than 90% and the corresponding control bicyclic guanidines having crude purities greater than 80% were included in the libraries. For the polyamine controls, approximately 80% of the compounds synthesized met the criteria imposed. Of the individual controls prepared, approximately 60% met the further criteria imposed for inclusion in the bicyclic guanidine library. A variety of amino acids were unacceptable in either the R^1 or R^2 positions of the bicyclic guanidine library. For amino acids such as glutamine or glutamic acid, which yield reactive functionalities following reduction, this result was expected. In addition, steric factors affected the cyclization reaction. For the R^1 position, the use of aminoisobutyric acid led to complete cyclization, while for the R^2 position the same

(1) Ostresh, J. M.; Husar, G. M.; Blondelle, S. E.; Dörner, B.; Weber, P. A.; Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11138−11142.
(2) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84−86.
(3) Pinilla, C.; Appel, J. R.; Blanc, P.; Houghten, R. A. *Biotechniques* **1992**, *13*, 901−905.
(4) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131−5135.
(5) Cuervo, J. H.; Weitl, F.; Ostresh, J. M.; Hamashin, V. T.; Hannah, A. L.; Houghten, R. A. In *Peptides 94: Proceedings of the 23rd European Peptide Symposium*; Maia, H. L. S., Ed.; ESCOM: Leiden, 1995; pp 465−466.
(6) Nefzi, A.; Ostresh, J. M.; Meyer, J.-P.; Houghten, R. A. *Tetrahedron Lett.* **1997**, *38*, 931−934.
(7) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 7, 449−472.
(8) Cotton, F. A.; Day, V. W.; Hazen, E. E., Jr.; Larsen, S. *J. Am. Chem. Soc.* **1973**, *95*, 4834.
(9) Brown, H. C.; Heim, P. *J. Org. Chem.* **1973**, *38*, 912−916.
(10) Wann, S. R.; Thorsen, P. T.; Kreevoy, M. M. *J. Org. Chem.* **1981**, *46*, 2579−2581.
(11) Raucher, S.; Klein, P. *Tetrahedron Lett.* **1980**, *21*, 4061−4064.
(12) Yoon, N. M.; Brown, H. C. *J. Am. Chem. Soc.* **1968**, *90*, 2927−2938.
(13) Lane, C. F. *Chem. Rev.* **1976**, *76*, 773−799.
(14) Corey, E. J.; Ohtani, M. *Tetrahedron Lett.* **1989**, *30*, 5227−5230.

10.1021/jo9810617 CCC: $15.00
Published on Web 11/06/1998

amino acid led to incomplete cyclization. LCMS chromatograms of incompletely cyclized products indicated the presence of cyclic thiourea intermediates in addition to the desired compounds. Benzoic acid derivatives were not used for the R^3 N-acylation step due to cleavage of the resulting benzyl functionality from the guanidine moiety during the HF cleavag

e step.

Structural characterization of compounds **1a**−**i** and **2a**−**i** is used here to demonstrate the success of the major transformations described. Following HF cleavage[15] at 0 °C for 9 h, LCMS of crude triamines **1a**−**i** were >95% pure and corresponded to completely reduced N-acylated dipeptides having an expected mass 42 Da less than the parent N-acylated dipeptides (as determined by ESI−LCMS and FAB−HRMS). Yields in all cases were greater than 70%. The use of phenylalanine in the parent N-acylated dipeptides allowed detection at 214 nm. The key to successful preparation of polyamines via borane reduction of peptides as described here is the dissociation of the resulting amine−borane complexes. By treating the resin-bound amine−borane complexes with a large excess of piperidine at 65 °C for 16 h, we were able to completely dissociate the desired triamines by disproportionation of the borane to piperidine. This procedure has been used to exhaustively reduce peptides up to 15 amino acids in length. Bicyclic guanidines **2a**−**i** demonstrate the success of the cyclization of the resulting triamines to bicyclic guanidines. In each case, a mass increase of 8 Da relative to the parent triamines was observed (as determined by ESI-LCMS and FAB−HRMS). Furthermore, the presence of the guanidino moiety was confirmed by the appearance of a peak at approximately 163−164 ppm in the ^{13}C NMR. While the bicyclic guanidines were initially cleaved under the same conditions required for the cleavage of resin-bound polyamines (HF, 0 °C, 9 h), the positively charged compounds were found to be much more labile to acidolytic cleavage than the triamines.[16] Racemization was not observed (<1%) following either the reduction or cyclization steps. Racemization was monitored as generally described earlier[1] by comparing the respective RP-HPLC absorbances at 214 nm of two diastereomeric pairs of particular bicyclic guanidines for diastereomers that do not coelute. The resin-bound mixture-based peptide combinatorial library used for preparation of the trisubstituted bicyclic guanidine combinatorial library was prepared in positional scanning format[3] using predetermined isokinetic ratios[17] of the incoming protected amino acid and carboxylic acid building blocks.[18] This library has been successfully used to identify antibacterial[19] and opioid receptor specific compounds. These results will be presented elsewhere.

In summary, an efficient and general solid-phase reduction of peptides to polyamines is presented here. This synthetic approach extends the previously described "libraries from libraries" concept. Further extension of the concept is demonstrated by the successful cyclization of triamines to yield trisubstituted bicyclic guanidines. These transformations enable both individual compounds and mixture-based combinatorial libraries to be prepared in excellent yield and purity.

Acknowledgment. We thank Eileen Weiler for her editorial assistance. This work was funded by National Science Foundation Grant No. CHE-9520142 and by Trega Biosciences, Inc.

Supporting Information Available: Experimental procedures and characterization data for compounds **1a**−**i** and **2a-i**. List of building blocks used in the synthesis of the trisubstituted bicyclic guanidine combinatorial library (6 pages).

JO9810617

(15) Houghten, R. A.; Cuervo, J. H.; Ostresh, J. M.; Bray, M. K.; Frizzell, N. D. In *Proceedings of the Tenth American Peptide Symposium*; Marshall, G. R., Ed.; Pierce Chemical Co.: Rockford, IL, 1987; pp 166−172.

(16) Polyamines can be cleaved from the methylbenzhydrylamine linker with HF at 0 °C for 9 h. This compares to the normal 1−1.5 h cleavage of peptide amides. The bicyclic guanidines were also cleaved with HF for 9 h at 0 °C to avoid further experimentation. However, cleavage of several model compounds using 100% TFA for 30 min at room temperature gave 30−50% yields. This demonstrated the positively charged resin-bound bicyclic guanidines could be cleared under much more mild conditions with further experimentation.

(17) Ostresh, J. M.; Hamashin, V. T.; Husar, G. M.; Dörner, B.; Houghten, R. A. In *Peptides 94: Proceedings of the 23rd European Peptide Symposium*; Maia, H. L. S., Ed.; ESCOM: Leiden, 1995; pp 416−417.

(18) A variation of the reported method was used in which the relative incorporation of the building blocks was quantitated by RP-HPLC.

(19) Blondelle, S.; Crooks, E.; Ostresh, J. M.; Houghten, R. A. *Antimicrob. Agents Chemother.* **1998**, in press.