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Solid-phase synthesis of bis-cyclic guanidines from tripeptides

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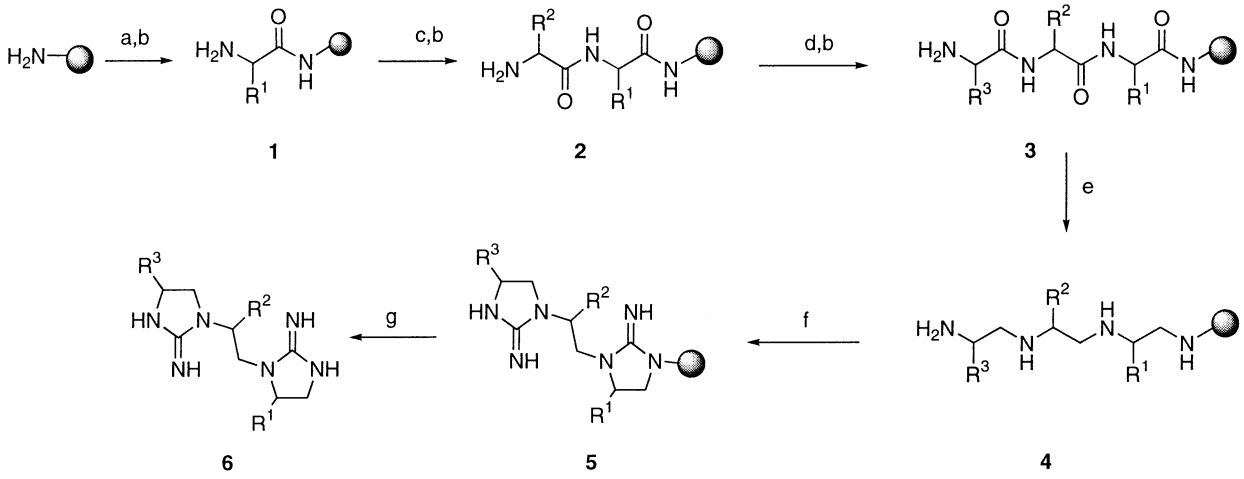
Abstract—An efficient method for the solid-phase synthesis of bis-cyclic guanidines from reduced tripeptides is described. The exhaustive reduction of the tripeptides generated tetra-amines that on treatment with cyanogen bromide, afforded bis-cyclic guanidines having three separate variable positions. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Guanidino compounds are reported to exhibit diverse biological and pharmacological activities such as neuronal Na^+ and Ca^{2+} channel blockers,[1] glutamate release inhibitors, anti-ischemic agents,[2] antiseizure agents,[3] adrenergic neuron blocking agents,[4] HIV-1 protease inhibitors,[5] potassium/ATP channel openers, antitumor agents, NO synthase inhibitors, influenza neuraminidase inhibitors,[6] cardiotonic agents,[7] histamine H_3 receptor antagonists,[8] H_2 receptor agonists/antagonists,[9] and antihistaminic, antiinflammatory, antidiabetic, antibacterial agents, and antihypertensive drugs.[10] Recent studies revealed that guanidino alkaloids exhibit antiviral activity against *Herpes simplex* virus (type 1), antifungal activity against *Candida albicans* and anti-HIV activities,[11–14] potent Na^+, K^+, Ca^{2+}-ATPase inhibitors,[15] cytotoxic against lung carcinoma A-549, colon carcinoma HT-29, melanoma MEL-28, and L1210 murine leukemia cells.[11,14,16] A known antiulcer drug, cimetidine, also contains a functionalized guanidine moiety.[17] Guanidine derivatives can also be used as strong organic bases or super bases.[18]

In a continuation of our ongoing efforts toward the solid-phase synthesis of small molecule heterocycles from amino acids and peptides, we herein, describe the straightforward



Scheme 1. (a) Boc–NHCH(R^1)CO_2H (6 equiv., 0.1 M in DMF), DIC (6 equiv.), HOBt (6 equiv.), 2 h, rt; (b) 55% TFA/45% DCM, 30 min, rt; (c) Boc–NHCH(R^2)CO_2H (6 equiv., 0.1 M in DMF), DIC (6 equiv.), HOBt (6 equiv.), 2 h, rt; (d) Boc–NHCH(R^3)CO_2H (6 equiv., 0.1 M in DMF), DIC (6 equiv.), HOBt (6 equiv.), 2 h, rt; (e) (i) BH_3–THF, 65°C, 72 h; (ii) Piperidine, 65°C, 20 h; (f) CNBr (2.2 equiv., 0.02 M in *m*-Xylene), rt, overnight; (g) HF, anisole, 0°C, 1.5 h.

Keywords: combinatorial synthesis; solid-phase synthesis; cyclic guanidine.

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Table 1. MW and RP-HPLC purity found for the bis-cyclic guanidines **6**

Product	R^1	R^2	R^3	MW (calcd)	MW (found)	Purity[a] (%)
6a	$-C_6H_{11}$	$-CH_2C_6H_5$	$-CH_3$	382.5	383.1 ($M+H^+$)	75
6b	$-CH_2C_6H_5$	$-CH_2C_6H_5$	$-CH_3$	390.5	391.0 ($M+H^+$)	69
6c	$-CH(CH_3)_2$	$-CH_2C_6H_5$	$-CH_3$	342.5	343.0 ($M+H^+$)	68
6d	$-CH_2C_{10}H_7$	$-CH_2C_6H_5$	$-CH_3$	440.6	441.0 ($M+H^+$)	59
6e	$-CH_2C_6H_4$(4-Cl)	$-CH_2C_6H_5$	$-CH_3$	425.0	425.1 ($M+H^+$)	71
6f	$-CH_2C_6H_5$	$-CH_3$	$-CH_3$	314.4	314.9 ($M+H^+$)	58
6g	$-CH_2C_6H_5$	$-CH_2C_6H_4$(4-Cl)	$-CH_3$	425.0	425.2 ($M+H^+$)	55
6h	$-CH_2C_6H_5$	$-CH_2C_6H_4$(4-OCH_3)	$-CH_3$	420.6	421.3 ($M+H^+$)	65
6i	$-CH_2C_6H_5$	$-CH_2C_6H_5$	$-CH_2CH(CH_3)_2$	432.6	433.0 ($M+H^+$)	59
6j	$-CH_2C_6H_5$	$-CH_2C_6H_5$	$-CH_2CH_2CH_3$	418.5	419.0 ($M+H^+$)	62
6k	$-CH_2C_6H_5$	$-CH_2C_6H_5$	$-C_6H_{11}$	458.6	459.4 ($M+H^+$)	53
6l	$-(CH_2)_3CH_3$	$-CH_2C_6H_5$	$-CH(CH_3)OH$	386.5	387.2 ($M+H^+$)	75
6m	$-(CH_2)_2CH_3$	$-CH_2OH$	$-CH_3$	282.4	282.9 ($M+H^+$)	57

The yields obtained were greater than 93% in all cases with respect to the initial loading of the resin (1.10 mequiv. g^{-1}).
[a] Crude purity was determined from the relative peak areas (%) of HPLC chromatograms run with a gradient of 5–95% acetonitrile in water (0.05% TFA) for 30 min at λ=214 nm.

synthesis of trisubstituted bis-cyclic guanidines from tripeptides using cyanogen bromide (CNBr) for guanidine formation.[19]

2. Results and discussion

A Boc-amino acid was coupled to 4-methylbenzhydrylamine (MBHA) resin, followed by deprotection of the Boc group generating compound **1** having a primary amine (see Scheme 1). Two subsequent couplings and deprotections yielded tripeptide **3**. Exhaustive reduction of amide bonds of the tripeptide with BH_3–THF[20] generated tetra-amine **4** having three secondary amines and a primary amine. Treatment of tetra-amine **4** with CNBr (2.2 equiv.) in a nonpolar solvent generated the resin-bound bis-cyclic guanidine **5**. It is important to maintain the reaction stochiometry (2.2 equiv. of CNBr in a non-polar solvent) to minimize the formation of undesired impurities most likely due to formation of a cyclic guanidine between the two internal secondary amines and two amides at the two other secondary amines. The selectivity of the cyclization was most likely due to the initial reaction of CNBr with the primary amine followed immediately by cyclization to form the five membered cyclic guanidine ring. The two other secondary amines then cyclized to form the second cyclic guanidine

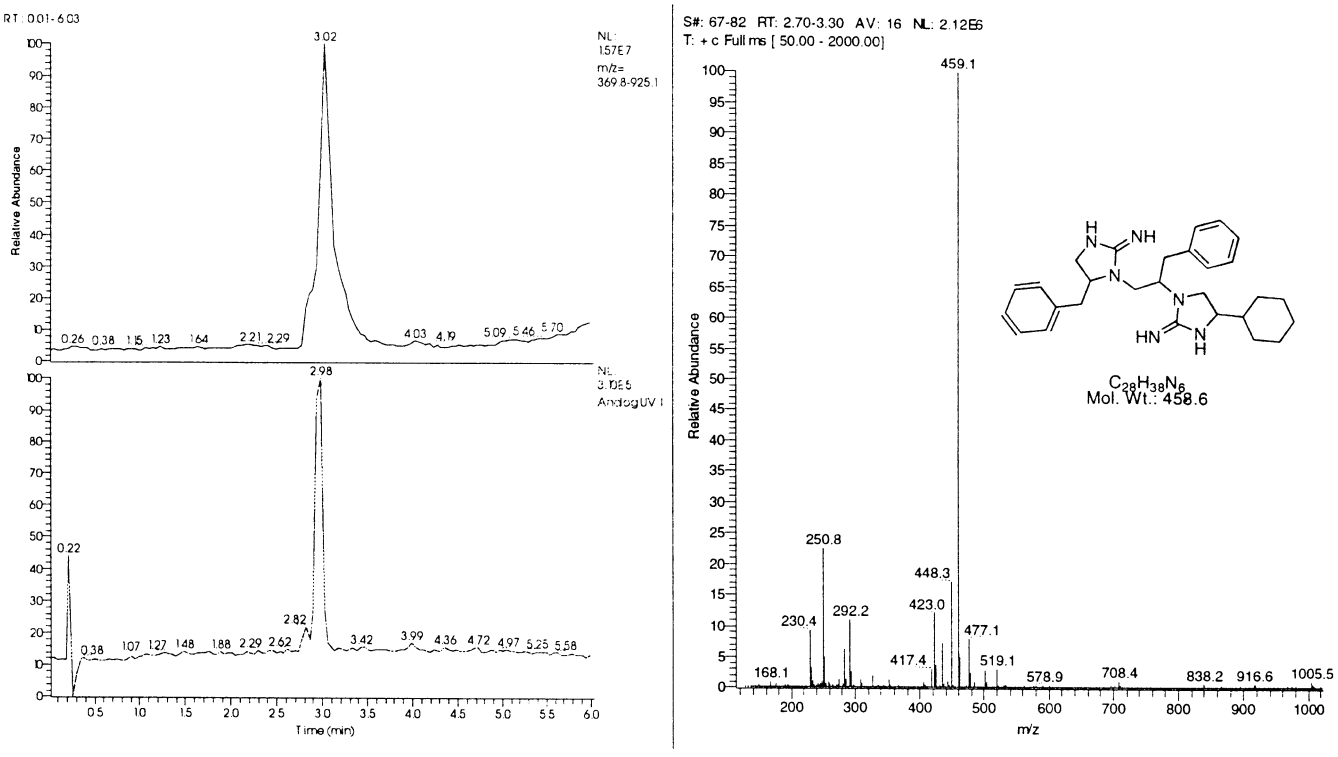

Figure 1. LC-MS of a bis-cyclic guanidine (**6k**) derived from phe–phe–cyclohexylglycine.

ring.[21] This was tested using sub-stochiometric amount of CNBr (1.1 equiv. excess) during cyclization. The product formed, as detected by LC-MS (>70% by HPLC), corresponding to the mass having one guanidine moiety. These results support this proposed reaction sequence.

The resulting compounds were cleaved from the resin using standard HF (1.5 h) conditions[22] to yield **6** in reasonable purity (purity ranged from 53–75%, see Table 1). Thirteen control compounds were prepared using seven amino acids (Phe, Val, norleucine, norvaline, 2-naphthylalanine, cyclohexylglycine, and *p*-chloro-phenylalanine) for the first (R^1) position of diversity, five amino acids (Phe, Ala, Ser, *p*-chloro-phenylalanine, and *O*-methoxy-tyrosine) for the second (R^2) position of diversity, and five amino acids (Ala, Leu, Thr, norvaline, and cyclohexylglycine) for the third (R^3) position of diversity. In all cases, complete cyclization was observed by LC-MS and reverse-phase high-pressure liquid chromatography (RP-HPLC). One exemplary LC-MS is presented in Fig. 1. Those amino acids, which have either an extra amine functionality (for example, lysine or arginine) or generate an extra amine functionality after reduction (for example, glutamine or asparagine) were not included due to formation of undesirable impurities during cyclization with CNBr. Selected samples were purified and were characterized by high-resolution mass spectra (HRMS), ^{1}H, and ^{13}C NMR spectra. It is possible to extend this approach to vary a range of amino acids (~50) for each R^1, R^2, and R^3 position of diversities.

To determine the possibility of racemization during either the BH_3–THF reduction or the final cyclization step with CNBr, two diasteriomeric analogs of intermediate **4** and final compound **6** known not to coelute, were prepared. RP-HPLC data indicated that there was negligible racemization (>99% optically pure) during exhaustive reduction of amide bonds, in conformity with our earlier observations.[20] In addition, RP-HPLC and ^{1}H NMR data indicated negligible amount of racemization (<1%) during cyclization of tetra-amine with CNBr.

Four signals appeared at δ_H~7.9–8.4 ppm in the ^{1}H NMR spectra that corresponded to the two imine protons of the two guanidine moieties and two imidazolyl protons (i.e. one each for the two guanidine rings).[20] Furthermore, two signals appearing at ~157–158 ppm in ^{13}C NMR spectra confirmed the presence of two guanidino carbons.[20,23]

3. Conclusion

A novel approach to the preparation of bis-cyclic guanidines from tripeptides is described. The solid-phase synthesis of these compounds is straightforward with high yield and moderate purity of the final products. This approach can be extended to prepare a positional scanning library using the 'libraries from libraries' concept.[24]

4. Experimental

MBHA resin (1% divinylbenzene, 100–200 mesh, 1.1 mequiv. g^{-1} substitution) and *N*,*N*′-diisopropylcarbodiimide (DIC) were purchased from Chem Impex Intl. (Wood Dale, IL). Boc-amino acid derivatives and *N*-hydroxybenzotriazole (HOBt) were purchased from Calbiochem–Novabiochem Corporation (San Diego, CA), and Bachem Bioscience (Philadelphia, PA). HF was purchased from Air Products (San Marcos, CA). All other reagents and anhydrous solvents were purchased from Aldrich Chemical (Milwaukee, WI). Analytical RP-HPLC was performed on a Beckman System Gold Instrument (Fullerton, CA). Purification of the samples was made using a Vydac 218TP54 C18 column (0.46×25 cm^2). LC-MS (ESI and APCI) were recorded on a Finnigan Mat LCQ mass spectrometer (ThermoQuest Corporation, CA) at 214 nm using Betasil C 18, 3 μm, 100 Å, 3×50 mm^2 column. High-resolution mass spectra (HRMS) were recorded at the Mass Spectrometry Facility of the University of California at Riverside. All amino acids used were of L-configurations unless otherwise indicated.

4.1. Typical procedure for the individual synthesis of bis-cyclic guanidine

Hundred milligrams of MBHA resin was sealed inside a polypropylene mesh packet.[25] Polypropylene bottles were used for all the reactions. The resin was washed with dichloromethane (DCM) followed by neutralization with 5% *N*,*N*′-diisopropylethylamine (DIEA) in DCM and washed with DCM.

4.2. Coupling of an amino acid to the resin

Boc-amino acid (6 equiv., 0.1 M in DMF) was coupled to MBHA resin using the classical coupling reagents, DIC and HOBt (6 equiv. each) for 2 h at room temperature, followed by washes with DMF (3 times) and DCM (3 times). The Boc group was deprotected using 55% TFA in DCM for 30 min followed by neutralization with 5% DIEA in DCM. Similarly, two subsequent Boc-amino acid couplings and deprotections of the Boc group were performed to yield a tripeptide. Completeness of the couplings was verified by the ninhydrin test.[26]

4.3. Exhaustive reduction of amide groups with BH_3–THF

Exhaustive reduction of the tripeptide was carried out in 50 mL glass conical tubes under nitrogen. To each tube was added the resin packet (0.110 mequiv. resin, 100 mg of starting resin) and boric acid (12 equiv.) followed by trimethyl borate (12 equiv.). Borane–THF complex (1 M, 40 equiv.) was added slowly. After cessation of hydrogen evolution, the capped tubes were heated at 65°C for 72 h followed by decantation of the reaction solution and quenching with MeOH. Following washes with DMF and MeOH (4 times) the resin was treated with piperidine at 65°C for 20 h to disproportionate the borane complexes.[20] Following decantation of the piperidine–borane solution, the resin packet was washed with DMF (4 times), DCM (4 times) and MeOH (2 times) and dried.

4.4. Cyclization to form the bis-cyclic guanidine

The resin-bound tetra-amine (Scheme 1) was treated with

CNBr (2.2 equiv., 0.02 M in *m*-xylene, overnight) under nitrogen followed by washes with *m*-xylene (2 times), DCM (2 times), IPA (2 times) and DCM (3 times). The resin-bound compound was cleaved using anhydrous HF in the presence of anisole at 0°C for 1.5 h,[22] and the cleaved product was extracted with 95% acetic acid in H_2O and lyophilized.

4.4.1. 1-[2-(2-Imino-4-methylimidazolidin-1-yl)-3-phenylpropyl]-5-isopropyl-2-imidazolidine (6c). ^{1}H NMR (500 MHz, d_6-DMSO): δ 8.42 (s, 1H), 8.34 (s, 1H), 8.27 (s, 1H), 8.10 (s, 1H), 7.24–7.36 (m, 5H), 4.48–4.49 (m, 1H), 4.09–4.12 (m, 1H), 3.85–3.96 (m, 2H), 3.72 (t, J=9.6 Hz, 1H), 3.26–3.43 (m, 4H), 2.96–3.00 (dd, J=4.9, 14.5 Hz, 1H), 2.73–2.78 (dd, J=9.85, 14.2 Hz, 1H), 2.04–2.08 (m, 1H), 1.08–1.09 (d, J=6.2 Hz, 3H), 0.84–0.85 (d, J=6.8 Hz, 3H), 0.69–0.70 (d, J=6.8 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 13.65, 17.18, 20.57, 26.89, 34.40, 40.46, 41.37, 48.07, 51.63, 60.64, 126.89, 128.49, 128.86, 136.50, 157.59, 157.77, 158.41. HRMS (DCI) *m/z* 343.2616 found ($[M+H]^+$), 343.2610 calculated for $C_{19}H_{31}N_6$ ($[M+H]^+$).

4.4.2. 5-Benzyl-1-[2-(2-imino-4-isobutylimidazolidin-1-yl)-3-phenylpropyl]-2-imidazolidine (6i). ^{1}H NMR (500 MHz, d_6-DMSO): δ 8.32 (s, 1H), 8.16 (s, 1H), 8.14 (s, 1H), 7.95 (s, 1H), 7.25–7.40 (m, 10H), 4.42–4.49 (m, 2H), 3.91–3.97 (dd, J=10.3, 15.4 Hz, 1H), 3.75–3.82 (m, 2H), 3.34–3.47 (m, 4H), 2.96–2.99 (m, 2H), 2.73–2.82 (m, 2H), 1.54–1.58 (m, 1H), 1.31–1.35 (m, 1H), 1.21–1.25 (m, 1H), 0.85 (t, J=5.4 Hz, 6H). HRMS (DCI) *m/z* 433.3061 found ($[M+H]^+$), 433.3080 calculated for $C_{26}H_{37}N_6$ ($[M+H]^+$).

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References

1. Maillard, M. C.; Perlmn, M. E.; Amitay, O.; Baxter, D.; Berlove, D.; Connaghton, S.; Fischer, J. B.; Guo, J. Q.; Hu, L. Y.; McBurney, R. N.; Nagy, P. I.; Subbarao, K.; Yost, E. A.; Zhang, L.; Durant, G. J. *J. Med. Chem.* **1998**, *41*, 3048.
2. Reddy, N. L.; Cannaughton, S.; Daly, D.; Fischer, J. B.; Goldin, S. M.; Hu, L. H.; Subbarao, K.; Durant, G. *J. Bioorg. Med. Chem. Lett.* **1995**, *5*, 2259.
3. Hu, L. Y.; Durant, G. J.; Guo, J. Q.; Maillard, M.; Wolcott, T.; Berlove, D. 214th American Chemical Society Meeting. Las Vegas, NV, p. MEDI 32, **1997**.
4. Gilman, A. G.; Goodman, L. S. *The Pharmacological Basis of Therapeutics*; 6th ed; Macmilan: New York, 1980; p. 380.
5. Jadav, P. K.; Woerner, F. J.; Lam, P. Y. S.; Nicholas Hodge, C.; Eyermann, C. J.; Man, H.; Daneker, W. F.; Bacheler, L. T.; Rayner, M. M.; Meck, J. L.; Erickson-Viitanen, S.; Jackson, D. A.; Calabrese, J. C.; Schadt, M.; Chang, C. *J. Med. Chem.* **1998**, *41*, 1446.
6. Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. *J. Med. Chem.* **1998**, *41*, 2451.
7. Kearney, P. C.; Fernandez, M.; Flygare, J. A. *Tetrahedron Lett.* **1998**, *39*, 2663.
8. Linney, I. D.; Buck, I. M.; Harper, E. A.; Kalindjian, S. B.; Pether, M. J.; Shankley, N. P.; Watt, G. F.; Wright, P. T. *J. Med. Chem.* **2000**, *43*, 2362.
9. Durant, G. J.; Ganellin, C. R.; Hills, D. W.; Miles, P. D.; Parsons, M. E.; Pepper, E. S.; White, G. R. *J. Med. Chem.* **1985**, *28*, 1414.
10. Greenhill, J. V.; Lue, L. *In Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier: New York, 1993; Vol. 30.
11. Scott Coffey, D.; McDonald, A. I.; Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **2000**, *122*, 4893.
12. Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1992**, *114*, 8472.
13. Erijman, E. A. J.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712.
14. Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925.
15. Ohizumi, Y.; Sasaki, S.; Kusumi, T.; Ohtani, I. I.; Eur *J. Pharmacol.* **1996**, *310*, 95.
16. Ohtani, I. I.; Kusumi, T.; Kakisawa, H. *Tetrahedron Lett.* **1992**, *33*, 2525.
17. Ganelin, C. R. *Chron. Drug Discovery*; Bindra, J. S., Lednicer, D., Eds.; Wiley: New York, 1982; Vol. 1, pp. 1–38.
18. (a) Isobe, T.; Fukuda, K.; Tokunaga, T.; Seki, H.; Yamaguchi, K.; Ishikawa, T. *J. Org. Chem.* **2000**, *65*, 7774. (b) Isobe, T.; Fukuda, K.; Tokunaga, T.; Seki, H.; Yamaguchi, K.; Ishikawa, T. *J. Org. Chem.* **2000**, *65*, 7770. (c) Kovacevic, B.; Maksic, Z. B. *Org. Lett.* **2001**, *3*, 1523.
19. Lundquist IV, J. T.; Orwig, K. S.; Dix, T. A. *J. Org. Chem.* **1999**, *64*, 9265.
20. Ostresh, J. M.; Schoner, C. C.; Hamashin, V. T.; Nefzi, A.; Meyer, J.-P.; Houghten, R. A. *J. Org. Chem.* **1998**, *63*, 8622.
21. Nefzi, A.; Giulianotti, M.; Ong, N. A.; Houghten, R. A. *Org. Lett.* **2000**, *2*, 3349.
22. Houghten, R. A.; Bray, M. K.; DeGraw, S. T.; Kirby, C. J. *Int. J. Pept. Protein Res.* **1986**, *27*, 673.
23. Acharya, A. N.; Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *J. Comb. Chem.* **2001**, *3*, 189.
24. Ostresh, J. M.; Husar, G. M.; Blondelle, S. E.; Dorner, B.; Weber, P. A.; Houghten, R. A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11138.
25. Houghten, R. A. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5131.
26. Kaiser, E. T.; Colescott, R. L.; Blossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.