Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2345–2350

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NEW PIPERAZINYL POLYAZACYCLOPHANE SCAFFOLDS, LIBRARIES AND BIOLOGICAL ACTIVITIES

Haoyun An, Becky D. Haly, and P. Dan Cook*

Isis Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, CA 92008, U.S.A.

Received 26 March 1998; accepted 27 May 1998

Abstract: Four novel unsymmetric piperazinyl polyazacyclophane scaffolds **1–4** were synthesized in high yields by an efficient cyclization strategy. Twenty-six libraries **12–37** (total 16000 compounds) were generated by a solution-phase combinatorial approach from **1–4** and thirty-eight functional groups. Potent antibacterial and HIV-1 tat/TAR protein–RNA disrupting activities were discovered.

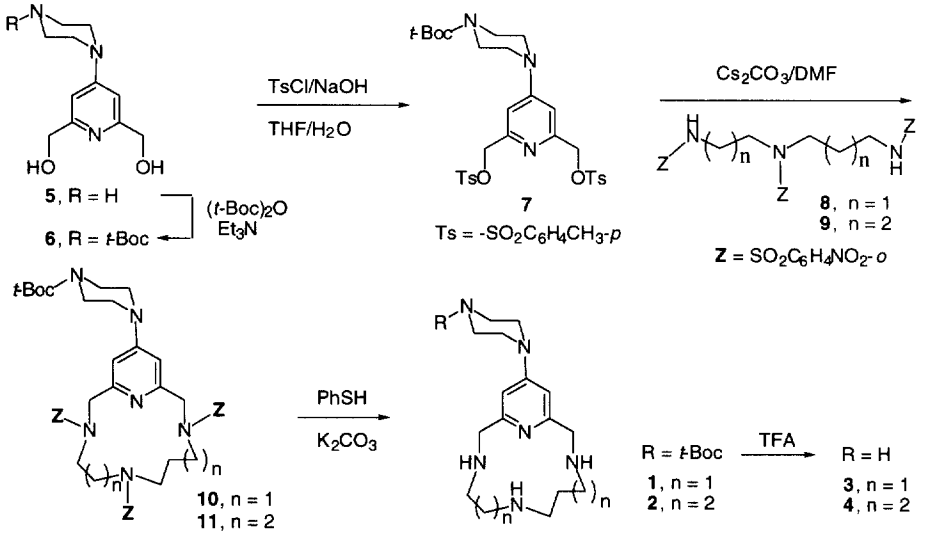
A variety of combinatorial strategies allowing creation of single-compound or mixture libraries to enhance discovery of new leads, and subsequent lead optimizations have been reported.[1] Oligomeric[2] and small-molecule[3] libraries are typically generated by solid-phase approaches. Solution-phase combinatorial approaches have recently become of interest as an alternative for the generation of parallel single-compound libraries,[4] indexed libraries,[5] and complex libraries.[6] Solid-support extraction[7] methods have been used for the purification of solution-phase libraries. We have recently reported a process to generate chemical libraries by simultaneous reacting a mixture of functionalities with several different reactive sites on a core scaffold in solution.[8,9] This solution-phase simultaneous addition of functionalities (SPSAF) combinatorial approach was designed and developed for lead discovery by incorporating diverse functionalities in large mixture libraries. In this approach, unsymmetric polyazacyclophane[8–11] and linear[12] scaffolds have been synthesized and utilized for the generation of a variety of mixture libraries. Libraries generated from the more rigid scaffolds exhibited more potent antibacterial activity than those based on the more flexible scaffolds.[10] Chemical synthesis[13] and HPLC fractionation[14] methods were used for the deconvolution of active libraries, and a series of novel antibacterial compounds were identified.

Herein we describe the synthesis of four new piperazinyl polyazacyclophane scaffolds **1–4** (Scheme 1). Twenty-six chemical libraries **12–37** (Scheme 2), having a total complexity of over 16000 compounds, were generated from these new scaffolds and a variety of functionalities (R_1–R_{38}) (Figure 1) using the SPSAF combinatorial approach. Preliminary biological activities of these libraries in antibacterial and HIV-1 tat/TAR protein–RNA disruption assays (Table 1) will be disclosed.

4′-Piperazinylpyridine-2,6-dimethanol (**5**)[15] (Scheme 1) was treated with di-*t*-butyl dicarbonate to give *t*-Boc-protected compound **6** in high yield. Compound **6** was reacted with tosyl chloride to provide the corresponding ditosylate **7**. Various cyclization methods to prepare polyazaphane related macrocyclic compounds have been reported.[16] N^{I},N^{ω}-Bis(amino) compounds protected with Cbz, COOEt, *t*-Boc, Tf, and Ts groups[16] have been used in similar cyclization steps to prepare symmetric polyazamacrocylic compounds. These methods typically provide low yields because of the requirements of strong basic conditions at elevated temperature. Removal of the protecting groups on the resulted macrocyclic compounds are generally low-yield

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00424-7

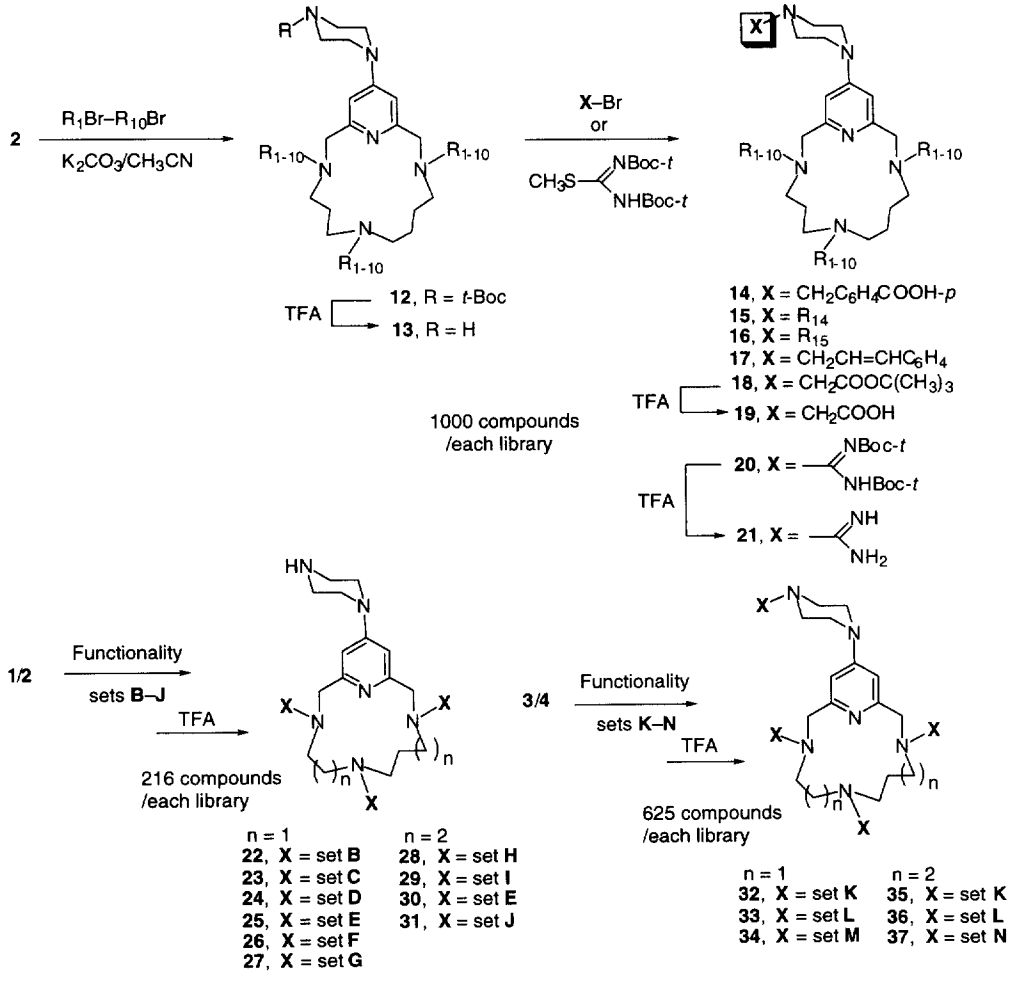
reactions because of the requirements of strong acids or bases or reducing agents. The 2-nitrobenzenesulfonyl protecting group was easily removed under mild condition; thus tris(2-nitrobenzenesulfonyl)-protected unsymmetric triamines **8** and **9**[11] were found to support efficient cyclizations. The 1:1 cyclization of ditosylate **7** with triprotected triamines **8** and **9**, using Cs_2CO_3 as a base and the Cs^+ cation serving as a template, afforded orthogonally protected piperazinyl substituted polyazacyclophanes **10** and **11** in 92% and 88% yields, respectively. The 2-nitrobenzenesulfonyl protecting groups on compounds **10** and **11** were selectively removed by thiophenol under mild conditions. The mono-protected unsymmetric piperazinyl polyaza-cyclophane scaffolds **1** and **2**, suitable for the SPSAF combinatorialization, were obtained in high yields. Scaffolds **1** and **2** were treated with trifluoroacetic acid (TFA) to provide the corresponding unprotected polyazacyclophane scaffolds **3** and **4**. Novel compounds **1–4**, **6**, **7**, **10**, and **11** were characterized by NMR and HRMS spectrometry and combustion analyses.



Scheme 1. Synthesis of new piperazinyl polyazaphane scaffolds **1–4**

Mono-*t*-Boc-protected scaffolds **1** and **2** have three 2°-amine reactive sites, while the unprotected scaffolds **3** and **4** have four 2°-amine reactive sites for the combinatorialization process. Scaffolds **1** and **3** contain the 13-membered polyazamacrocyclic ring, and scaffolds **2** and **4** share the 15-membered macrocyclic ring. Scaffolds **1–4** were utilized for the generation of twenty-six chemical libraries **12–37** (Scheme 2) using the SPSAF combinatorial approach. Thirty-eight different functionalities R_1–R_{38} (Figure 1) were grouped into fourteen functionality sets **A–N**. Their corresponding bromides or chlorides were used as starting reagents. 1,3-Propane sultone was used as the starting reagent for R_{21}. The functionality sets **A–N** have been determined to have similar reactivity.[8–12] Bromoacetonitrile and benzyl bromide derivatives Br–R_{1-10} in functionality set **A** were used for the generation of libraries **12–21** (Scheme 2). Three 2°-amine reactive sites of mono-*t*-Boc-protected scaffold **2** were combinatorialized with equimolar amounts of the ten bromides in functionality set **A** under carbonate basic condition to provide library **12** containing 1000 compounds (10^3). Treatment of library **12**

with TFA gave the intermediate library **13** containing one 2°-amine reactive site. Library **13** was reacted separately with 4-(bromomethyl)benzoic acid, 5-(bromomethyl)benzofuran, 4-(bromomethyl)-2,6-dichloropyridine, cinnamyl bromide, and *t*-butyl bromoacetate to give the corresponding libraries **14–18** possessing different functional groups at the 4′-piperazinyl fixed position. Deprotection of library **18** with TFA afforded acid library **19**. Library **13** was reacted with 2 equiv of 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-thiopseudourea to give the *t*-Boc-protected library **20**, which was then treated with TFA and HCl-saturated methanol to give guanidine type of library **21** with an amidine group at the fixed position. Libraries **12–20** were purified by flash or preparative thin-layer chromatographic techniques except the guanidine library **21**, which was obtained as its hydrochloride salt without further purification. Libraries **12–21** each contain 1000 compounds as they share the same functionality set **A**.



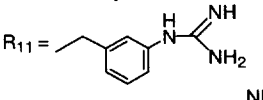
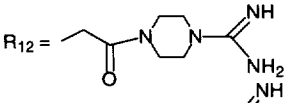
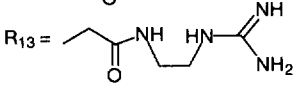
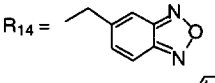
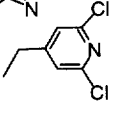
Scheme 2. Preparation of libraries **12–37**.

Mono-*t*-Boc-protected scaffold **1** was separately combinatorialized with bromides or chlorides of functionality sets **B–G**, where the guanidine and amine groups were protected by *t*-Boc group (six functionalities in each set) (Figure 1). The resulting *t*-Boc-protected intermediate libraries were purified by chromatographic techniques and then treated with TFA and HCl-saturated methanol to give the corresponding final polar libraries **22–27** as their hydrochloride salts. Libraries **28–31** were similarly obtained from the corresponding scaffold **2** and functionality sets **H**, **I**, **E**, and **J**, where the guanidine and amine groups were protected by *t*-Boc group. Each of these ten libraries **22–31** contain 216 compounds (6^3), and posses a hydrogen atom at the 4′-piperazinyl fixed position. The four 2°-amine reactive sites of the unprotected scaffolds **3** and **4** were separately combinatorialized with three sets of functionalities **K**, **L**, and **M** or **K**, **L**, and **N**, where the guanidine and amine

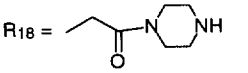
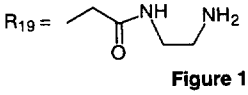
Functionality set A:

$R_1 = CH_2CN$
$R_2 = CH_2C_6H_5$
$R_3 = CH_2C_6H_4CH_3$-*m*
$R_4 = CH_2C_6H_4F$-*m*
$R_5 = CH_2C_6H_4CN$-*m*
$R_6 = CH_2C_6H_4NO_2$-*m*
$R_7 = CH_2C_6H_4Cl$-*m*
$R_8 = CH_2C_6H_4CO_2Me$-*m*
$R_9 = CH_2C_6H_4CF_3$-*m*
$R_{10} = CH_2C_6H_4Br$-*m*

Functionality set B:

$R_{11} =$ 
$R_{12} =$ 
$R_{13} =$ 
$R_{14} =$ 
$R_{15} =$ 
$R_{16} = COC_6H_5$

Functionality set C:

R_{14}, R_{15}, R_{16}
$R_{17} = CH_2C_6H_4NH_2$-*m*
$R_{18} =$ 
$R_{19} =$ 

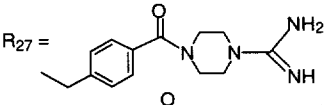
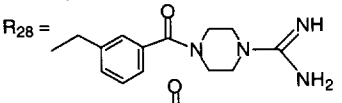
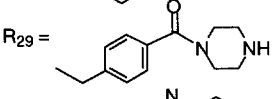
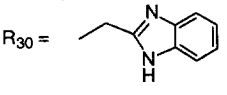
Functionality set D:

$R_{20} = CH_2COOH$
$R_{21} = CH_2CH_2CH_2SO_3H$
$R_{22} = CH_2C_6H_4COOH$-*p*
$R_{23} = CH_2C_6H_4CH_2COOH$-*p*
$R_{24} = CH_2C_6H_4CN$-*p*
$R_{25} = CH_2CONH_2$

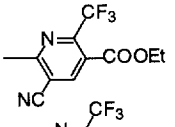
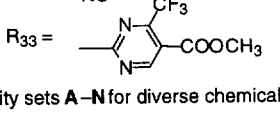
Functionalities set E:

$R_{11}, R_{12}, R_{13}, R_{17}, R_{18}, R_{19}$

Functionality set F:

$R_{23}, R_{26} = CH_2P(O)(OEt)_2$
$R_{27} =$ 
$R_{28} =$ 
$R_{29} =$ 
$R_{30} =$ 

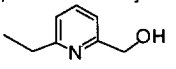
Functionality set G:

R_2, R_8, R_{16}
$R_{31} = CH_2CO_2CH_3$
$R_{32} =$ 
$R_{33} =$ 

Functionality set H:

$R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{32}$

Functionality set I:

R_{17}, R_{18}, R_{19}
$R_{34} = CH_2C_6H_4CF_3$-*o*
R_{35} = 2,6-dichlorobenzyl
$R_{36} =$ 

Functionality set J:

$R_{12}, R_{19}, R_{27}, R_{29}, R_{30}$
$R_{37} = CH_2$-4-pyridine

Functionality set K:

$R_{11}, R_{12}, R_{13}, R_{15}, R_{32}$

Functionality set L:

$R_9, R_{17}, R_{18}, R_{19}, R_{33}$

Functionality set M:

$R_{12}, R_{26}, R_{27}, R_{29}, R_{30}$

Functionality set N:

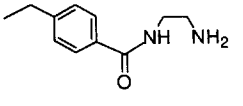
$R_{12}, R_{19}, R_{28}, R_{29}$
$R_{38} =$ 

Figure 1. Functionality sets **A–N** for diverse chemical libraries **12–37**

groups were protected by *t*-Boc group (five functionalities in each set). After purification, the *t*-Boc-protected libraries were deprotected with TFA followed by the treatment with HCl-saturated methanol to give the corresponding six final polar libraries **32–37** as their hydrochloride salts. Each of libraries **32–37** contains 625 compounds (5^4). Libraries described above were confirmed by their electrospray ionization mass spectra.

Libraries **12–37** were tested in *Streptococcus pyogenes* and *Escherichia coli imp-* antigrowth assays, and HIV-1 tat/TAR protein–RNA disrupting assay by high-throughput screening.[9,11–13] The biological activities are listed in Table 1. Libraries **13**, **19**, **21–23**, and **26–36** showed different antibacterial activity against *S. pyogenes* and *E. coli imp-*. Libraries **22**, **23**, **27**, **32**, and **33** exhibited potent antibacterial activity with MIC's as low as 2 μM. Generally, these libraries were more potent against Gram-positive bacterium *S. pyogenes* than Gram-negative bacterium *E. coli imp-* (see libraries **22**, **23**, **26**, **27**, **32**, and **34**). A hydrogen atom at the fixed position of the piperazine (library **13**) was more active than when the position was modified (libraries **14–21**). *t*-Boc-protected libraries **12**, **18**, and **20**, acid library **24**, as well as other libraries **15**, **17**, and **37** were not active in the assays. Libraries **32–34** made from the 13-membered scaffold **3** exhibited more potent activity than libraries **35–37** made from the 15-membered scaffold **4**. This results further confirmed our conclusion that more rigid scaffold provides more potent activity.[10] Guanidine libraries **22**, **25**, **26**, **32**, **34**, and amine library **23** disrupted HIV-1 tat/TAR protein–RNA interaction with **32** and **34** exhibiting activity at IC_{50}'s of 80 nM and 90 nM.

Table 1. Biological activities[a] of combinatorial libraries **12–37**

library	Complexity	*S. pyogenes*	*E. coli imp-*	tat/TAR	compd	Complexity	*S. pyogenes*	*E. coli imp-*	tat/TAR
12	1000	–	–	–	**26**	216	10–20	20–100	2.2
13	1000	4–20	4–20	–	**27**	216	2–10	<20	–
14	1000	–	–	–	**28**	216	5–20	5–20	–
15	1000	–	–	–	**29**	216	5–20	5–20	–
16	1000	–	–	–	**30**	216	20–100	20–100	–
17	1000	–	–	–	**31**	216	<100	<100	–
18	1000	–	–	–	**32**	625	2–10	10–50	0.08
19	1000	20–100	20–100	–	**33**	625	2–10	2–10	–
20	1000	–	–	–	**34**	625	2–50	10–50	0.09
21	1000	<20	<20	–	**35**	625	<100	<100	–
22	216	2–20	20–100	2.5	**36**	625	<100	<100	–
23	216	2–20	20–100	7	**37**	625	–	–	–
24	216	–	–	–					
25	216	–	–	1.5					

[a]The MIC (minimum inhibitory concentration, μM) value is given as a range of library concentration (total concentration of compounds in library). After 24 h, the complete inhibition of growth was observed at the higher concentration of the given MIC, and the growth was observed at the lower concentration. IC_{50} values (μM) were given for tat/TAR activity.

In summary, four novel piperazinyl polyazacyclophane scaffolds **1–4** were synthesized by an effective cyclization of piperazinyl pyridine ditosylate **7** with triprotected triamines **8** and **9**, followed by sequential deprotections. These novel scaffolds were combinatorialized with fifteen sets of thirty-eight diverse functionalities. Employing the SPSAF combinatorial process for the generation of twenty-six libraries, providing a total complexity of 16000 compounds, was achieved. High-throughput screening uncovered a series of antibacterial libraries with MIC's in the range of 2–10 μM. Guanidine libraries **32** and **34** were potent inhibitors (IC_{50} of 80 and 90 nM) of HIV-1 tat/TAR protein–RNA interaction.

Acknowledgments: The authors thank Richard H. Griffey, David Ecker, Rangarajan Sampath, Larry Blyn, Timothy A. Vickers, Lisa M. Risen, and Laura Wilson-Lingardo for screening combinatorial libraries, and Ramesh Bharadwaj for large scale preparation of compounds **5**, **8**, and **9**.

References

1. (a) Pirrung, M. C. *Chem. Rev.* **1997**, *97*, 473. (b) Lam, K. S.; Lebl, M.; Krchnak, V. *Chem. Rev.* **1997**, *97*, 411. (c) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489. (d) Williard, X.; Pop, I.; Bourel, L.; Horvath, D.; Baudelle, R.; Melnyk, P.; Deprez, B.; Tartar, A. *Eur. J. Med. Chem.* **1996**, *31*, 87. (e) Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123. (f) Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288.
2. (a) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82. (c) Sepetov, N. F.; Krchnak, V.; Stankova, M.; Wade, S.; Lam, K. S.; Lebl, M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5426. (d) Burger, M. T.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 7382.
3. (a) Ellman, J. A. *Acc. Chem. Res.* **1996**, *29*, 132. (b) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (c) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
4. (a) Sim, M. M.; Ganesan, A. *J. Org. Chem.* **1997**, *62*, 3230. (b) Boger, D. L.; Tarby, C. M.; Myers, P. L.; Caporale, L. H. *J. Am. Chem. Soc.* **1996**, *118*, 2109. (c) Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567. (d) Cheng, S.; Tarby, C. M.; Comer, D. D.; Williams, J. P.; Caporale, L. H.; Myers, P. L.; Boger, D. L. *Bioorg. Med. Chem.* **1996**, *4*, 727. (e) Bailey, N,; Dean, A. W.; Judd, D. B.; Middlemiss, D.; Storer, R.; Watson, S. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1409.
5. (a) Chng, B. L.; Ganesan, A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1511. (b) Neuville, L.; Zhu, J.-P. *Tetrahedron Lett.* **1997**, *38*, 4091. (c) Pirrung, M.; Chen, J. *J. Am. Chem. Soc.* **1995**, *117*, 1240. (d) Smith, P. W.; Lai, J. Y. Q.; Whittington, A. R.; Cox, B.; Houston, J. G.; Stylli, C. H.; Banks, M. N.; Tiller, P. R. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2821.
6. (a) Boger, D. L.; Chai, W.; Ozer, R. S.; Anderson, C. M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 463. (b) Boger, D. L.; Ozer, R. S.; Anderson, C. M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1903. (c) Shipps, G. W., Jr.; Spitz, U. P.; Rebek, J., Jr. *Bioorg. Med. Chem.* **1996**, *4*, 655. (d) Carell, T.; Wintner, E. A.; Sutherland, A. J.; Rebek, J., Jr.; Dunayevskiy, Y. M.; Vouros, P. *Chem. Biol.* **1995**, *2*, 171.
7. (a) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882. (b) Parlow, J. J.; Mischke, D. A.; Woodard, S. S. *J. Org. Chem.* **1997**, *62*, 5908. (c) Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874. (d) Kaldor, S. W.; Siegel, M. G.; Fritz, J. E.; Dressman, B. A.; Hahn, P. J. *Tetrahedron Lett.* **1996**, *37*, 7193. (e) Kaldor, S. W.; Fritz, J. E.; Tang, J.; McKinney, E. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3041.
8. An, H.; Cook, P. D. *Tetrahedron Lett.* **1996**, *37*, 7233.
9. An, H.; Cummins, L. L.; Griffey, R. H.; Bharadwaj, R.; Haly, B. D.; Fraser, A. S.; Wilson-Lingardo, L.; Risen, L. M.; Wyatt, J. R.; Cook, P. D. *J. Am. Chem. Soc.* **1997**, *119*, 3696.
10. An, H.; Wang, T.; Mohan, V.; Griffey, R. H.; Cook, P. D. *Tetrahedron* **1998**, *54*, 3999.
11. Wang, T. ; An, H.; Vickers, T. A.; Bharadwaj., R.; Cook, P. D. *Tetrohedron* **1998**, in press.
12. An, H.; Haly, B. D.; Fraser, A. S.; Guinosso, C. J.; Cook, P. D. *J. Org. Chem.* **1997**, *62*, 5156.
13. An, H.; Haly, B. D.; Cook, P. D. *J. Med. Chem.* **1998**, *41*, 706.
14. Griffey, R. H.; An, H.; Cummins, L. L.; Gaus, H. J.; Haly, B. D.; Herrmann, R.; Cook, P. D. *Tetrahedron* **1998**, *54*, 4067.
15. Haly, B. H.; An, H.; Griffey, R. H.; Gaus, H. J.; Herrmann, R.; Cook, P. D. unpublished results.
16. (a) Bradshaw, J. S.; Krakowiak, K. E.; Izatt, R. M. *Aza-Crown Macrocycles;* Taylor, E. C. Ed.; John Wiley and Sons: New York, 1993. (b) Krakowiak, K. E.; Bradshaw, J. S. *J. Heterocycl. Chem.* **1995**, *32*, 1639. (c) Panetta, V.; Yaouanc, J. J.; Handel, H. *Tetrahedron Lett.* **1992**, *33*, 5505.