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Solution Phase Combinatorial Chemistry
I. Synthesis of Polyazacyclophane Scaffolds and Tertiary Amine Libraries

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Abstract: Three novel unsymmetrical polyazacyclophane scaffolds **1-3** were efficiently synthesized in high yields by a new cyclization method followed by selective deprotection. Scaffold **2** was combinatorialized by solution phase simultaneous addition of functionalities to provide 16 pure tertiary amine libraries (total 1600 compounds).

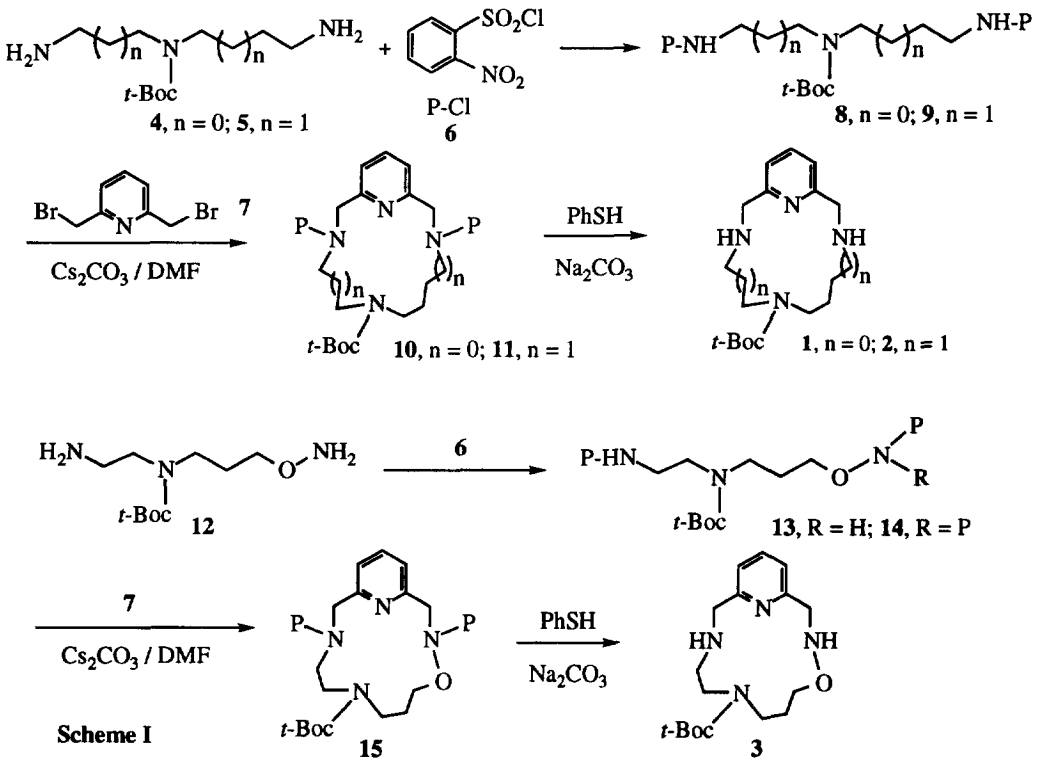
Combinatorial chemistry has recently emerged as an extremely powerful tool for generating lead compounds with desirable biological activities and for optimizing leads.[1] Early work on the synthetic chemical libraries of oligomeric peptides,[2] nucleotides[3] and saccharides,[4] and recent efforts toward chemical libraries of small organic molecules[5] generated by solid phase techniques have been well documented. Solution phase combinatorial chemistry has not been widely explored as an alternative avenue.[6-8]

In this communication we describe the synthesis of three novel conformationally constrained unsymmetrical polyazacyclophane scaffolds **1-3** (Scheme I) in high yields by a new cyclization method performed under mild conditions followed by the selective deprotection of orthogonal protecting groups. Scaffold **2** was combinatorialized by novel solution phase, simultaneous addition of functionalities strategy to provide 16 pure tertiary amine libraries (total 1600 compounds) (Scheme II). The strategy for the preparation of combinatorial libraries reported here has been validated by the generation of linear pyridino-polyamine libraries.[9]

N^1, N^ω-Bis-protected amino compounds with tosyl, Cbz, EtOCO and *t*-Boc protecting groups[10-12] have been employed in cyclization reactions to prepare symmetrical polyazamacrocyclic compounds. Most of these methods require strong basic conditions (e.g. NaH) and suffer low yields. The tosyl protecting group requires extremely strong acidic or reduction conditions,[10] e.g. 48% HBr/HOAc, 98% sulfuric acid, $LiAlH_4$ or NH_3/Na for its removal. These conditions also remove *t*-Boc group or cleave N-O bond within polyazacyclophanes. Therefore it is essential to find an efficient cyclization method which provides a suitable combination of orthogonal protecting groups such that all protecting groups can be selectively removed under mild conditions except one at the predetermined fixed position. This provides the mono-protected polyazacyclophane scaffolds suitable for our solution phase combinatorial drug discovery research. 2-Nirobenzenesulfonyl group[13] was chosen as the nitrogen protecting group to be removed first and *t*-Boc was chosen as the protecting group on the fixed position.

The *t*-Boc-protected triamines **4**[14] and **5**[15] were reacted with 2-nitrobenzenesulfonyl chloride (**6**) in CH_2Cl_2 using Et_3N as the base to give the corresponding tri-protected triamines **8** and **9** in 67% and 91%

yields, respectively (Scheme I). A mixture of tri-protected triamine **8** (18.6 mmol), anhydrous Cs_2CO_3 (4 equiv) and 2,6-bis(bromomethyl)pyridine (**7**) (1 equiv) in 500 mL of anhydrous DMF was stirred at rt for 24 h. After work-up and chromatographic purification the tri-protected polyazacyclophane **10** was obtained as a white foam in an 80% yield. The tri-protected polyazacyclophane **11** was similarly synthesized in a yield of 73%. A stirred mixture of **10** (6 mmol) and anhydrous K_2CO_3 (8 equiv) in 80 mL of anhydrous DMF was treated with thiophenol (2.4 equiv) at rt for 2 h. After aqueous work-up and chromatographic purification the desired mono-*t*-Boc-protected polyazacylophane scaffold **1** was obtained as a colorless oil in a 94% yield. Similarly, **2** was synthesized in a 72% yield.

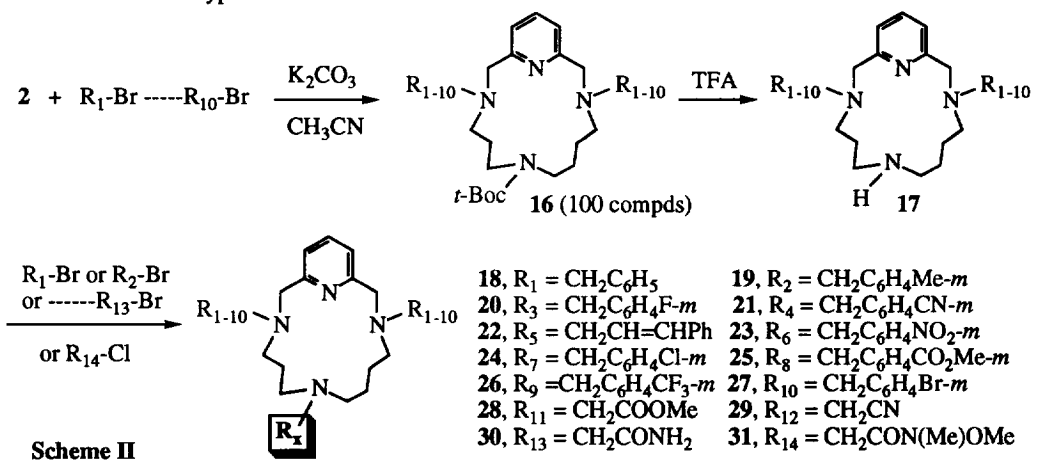


Scheme I

The same route was used for the synthesis of the N-O bond containing polyazacyclophane scaffold **3** (Scheme I). *t*-Boc-protected triamine **12**[16] (20 mmol) was reacted with 2-nitrobenzenesulfonyl chloride (**6**) (2.3 equiv) under the same conditions as for the preparation of **8** and **9**. The desired tri-protected triamine **13** was obtained as a white foam in a 33% yield; the tetra-protected triamine **14** was also isolated as a white foam in a 45% yield. The reaction conditions have not been optimized for the synthesis of the desired product **13**. The cyclization of **13** with **7** under the same conditions as described above afforded the corresponding tri-protected polyzazcyclophane **15** as a white foam in a 50% yield. The lower yield compared to those of **10** and **11** can be attributed to the different reactivities of the nitrogen nucleophiles CH_2NH- and ONH- in the triamine molecule **13**. The deprotection of **15** under the same conditions as those for **10** and **11** gave the desired N-O bond containing polyazacyclophane scaffold **3** as a colorless oil in a 97% yield. These new compounds

described above including **8-11**, **13-15** and **1-3** were characterized by ^{1}H and ^{13}C NMR, and high resolution (FAB) mass spectroscopies.

A variety of approaches for the preparation of chemical libraries have been reported including split / mixed, parallel,[17] encoded,[18] and other methods on solid supports. Up to now addition of functionalities (a set of reactive agents) in solution phase has been reported only by Rebek and coworkers[6] to make amide libraries. They used highly symmetrical scaffolds which limits the complexity, and a "reverse iterative" deconvolution procedure to find the active compounds. We chose the conformationally constrained unsymmetrical polyazacyclophane scaffold **2** and employed a novel strategy of simultaneous addition of functionalities in solution phase to generate tertiary amine libraries. Ten benzyl bromide derivatives, R_1-Br .....R_{10}-Br (see Scheme II), were selected as the first set of functionalities in our combinatorial program. The ten electorphiles have approximately the same reactivities, therefore are expected to generate libraries containing equal molar amount of compounds. Model studies of small libraries by capillary electrophoresis techniques qualitatively confirmed the above hypothesis.[14]



18, $R_1 = CH_2C_6H_5$ **19**, $R_2 = CH_2C_6H_4Me$-*m*
20, $R_3 = CH_2C_6H_4F$-*m* **21**, $R_4 = CH_2C_6H_4CN$-*m*
22, $R_5 = CH_2CH{=}CHPh$ **23**, $R_6 = CH_2C_6H_4NO_2$-*m*
24, $R_7 = CH_2C_6H_4Cl$-*m* **25**, $R_8 = CH_2C_6H_4CO_2Me$-*m*
26, $R_9 = CH_2C_6H_4CF_3$-*m* **27**, $R_{10} = CH_2C_6H_4Br$-*m*
28, $R_{11} = CH_2COOMe$ **29**, $R_{12} = CH_2CN$
30, $R_{13} = CH_2CONH_2$ **31**, $R_{14} = CH_2CON(Me)OMe$

Scheme II

The libraries (Scheme II) were synthesized as follows: a solution containing equal amount of 10 electrophiles (benzyl bromide derivatives, 1.44 mmol each for a total of 14.4 mmol, 2.4 equiv) in CH_3CN was added to a stirred mixture of **2** (6.0 mmol) and anhydrous K_2CO_3 in CH_3CN. The reaction mixture was stirred at rt overnight and the crude product was purified by flash chromatography on a silica gel column to afford the pure library **16** in a 94% yield.[19] The two reactive sites were combinatorialized with 10 functionalities resulting in 100 compounds. Deprotection of library **16** with TFA afforded library **17** in a 93% yield after chromatographic purification.

Library **17** with one reactive site was treated sequentially with each of 10 electrophiles (R_x-Br) (1.3 equiv) under similar conditions as above for the preparation of library **16**. This afforded libraries **18-27** in yields of 60-98% after preparative thin layer chromatographic purification.[20] With library **17** in hand we performed initial SAR studies by introducing different functionalities to increase the diversity of libraries. The reaction of **17** with methyl α-bromoacetate, bromoacetonitrile and α-bromoacetamide under similar conditions afforded the corresponding libraries **28**, **29** and **30**, respectively, in 95-98% yields. The commercial available 2-chloro-N-methoxy-N-methylacetamide (R_{14}-Cl) was reacted with **17** at 60-70 °C for 6 h and then at rt for 10

h affording library **31** in a 93% yield. All libraries **16-31** were confirmed by ^{1}H NMR and electrospray mass spectral data.[14,21]

In summary, we have developed a high yield cyclization and efficient synthetic route which can be used to make other types of polyazamacrocyclic compounds for combinatorial and crown ether chemistry. Sixteen pure polyazacyclophane libraries were synthesized by the novel simultaneous addition of functionalities in solution phase. The "fix last" combinatorial method allows SAR studies to be performed directly by libraries.

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19. The column loaded with crude library was first eluted with less polar eluent to remove the excess electrophiles, and further eluted with more polar eluents to wash all possible compounds in library out of the column. All fractions containing library compounds were collected. The theoretical yield was calculated according to the average molecular weight. The yield was obtained by comparing the amount of isolated library with the theoretical yield.
20. The PLC loaded with crude library was developed by selected solvents. The whole library band, which was wider than those of general single compounds, was collected to get pure library without electrophiles and other impurities.
21. The electrospray mass spectra of libraries show peaks ranged from the smallest molecule to the biggest molecule in libraries. The ^{1}H NMR spectra of libraries show the right ratio of various protons.

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