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Synthesis of Di- and Trisubstituted Guanidines on Multivalent Soluble Supports

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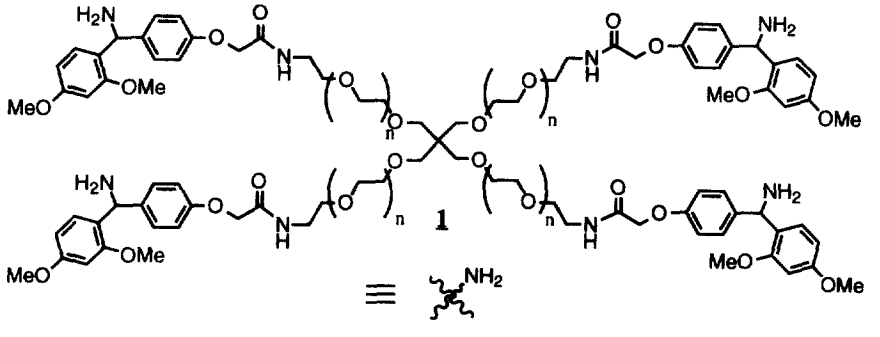
Abstract: A library of 48 di- and trisubstituted guanidines was synthesized on a soluble, tetravalent support. Support-bound intermediates and cleaved products were isolated in parallel by size exclusion chromatography using a semiautomated system. Products were generally obtained in good yield and purity.

With the increasing implementation of combinatorial and high speed synthesis techniques in drug discovery, technological advances in both solid and liquid-phase synthesis have emerged.[1,4] Recently we described a method for the preparation of combinatorial libraries on soluble supports.[2] In the process, which we now term Combinatorial Synthesis on Multivalent Oligomeric Supports (COSMOS), multiple compound copies are built on a soluble support through cycles of solution-phase synthesis, followed by size-based isolation of the support from low molecular weight reagents, solvents and detached products.[3] COSMOS therefore retains the advantages of solution phase synthesis, including homogeneous reaction conditions and routine product characterization, while incorporating a facile purification technique that, like solid-phase synthesis, exploits the intrinsic size difference between the support and small molecules for their separation.[4]

Herein we describe the application of the COSMOS methodology toward preparation of a library of 48 di- and trisubstituted guanidines. The compounds were synthesized using a commercially derived four-armed support and were isolated after each reaction step by size excusion chromatography (SEC). Most guanidines were obtained in good yield and purity, with the main side product being the corresponding thiourea or methyl isothiourea precursor. Separate experiments establish that support recovery by SEC is around 95%.



Since our initial publication, in which we used an eight-armed PAMAM dendrimer as the soluble support,[2] we have investigated the use of poly(ethylene glycol) (PEG) based oligomers due to their high solubility in a wide range of solvents, good chemical stability and favorable ^{1}H NMR properties. We have synthesized a series of supports ranging from 2 - 26 kDa and bearing 4 - 32 PEG arms, and found that the

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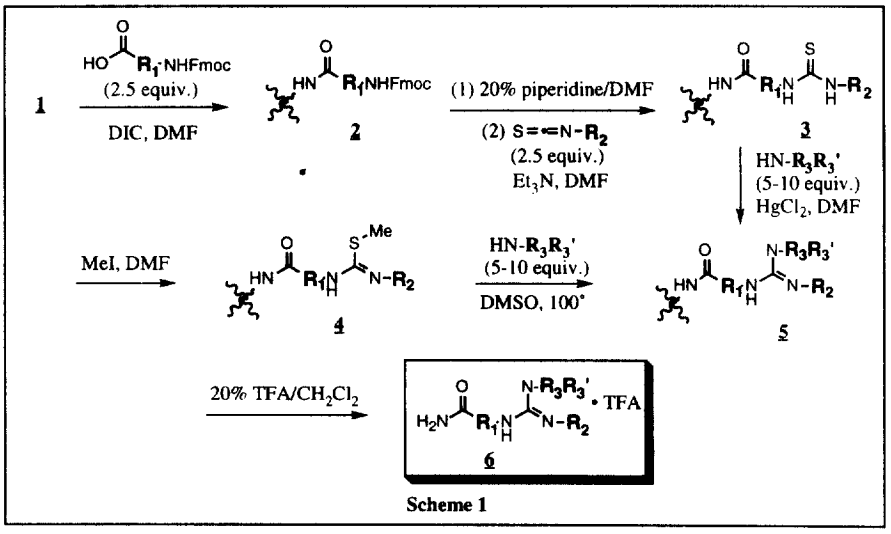
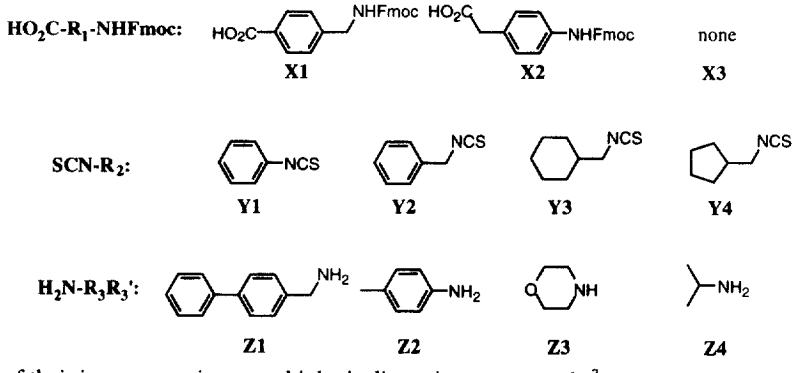
smaller species (ca. 2.5 - 5 kDa), offer advantages in characterizability, while maintaining sufficient size to be readily isolated from small molecules.[5] For this study, we employed support **1** (ca. 3.2 kDa), consisting of 4-armed PEG oligomer Sunbright PTE-2000 (Shearwater Polymers, Inc.), which was converted to the tetraamine and coupled to four Rink[6] handles. Unlike PAMAM and the aforementioned PEG supports which are discrete chemical entities, PTE-2000 is polydisperse, with an average molecular weight near 2 kDa. While this precludes full characterization of intermediates, NMR analysis is routine, and even chromatographic characterization and purification can be achieved due to the high homology of the oligomer.



Scheme 1



Because of their importance in many biologically active compounds,[7] we set out to synthesize a library of di- and trisubstituted guanidines using the COSMOS approach. Numerous solution phase methods for guanidine formation have been developed,[8] and recent examples on solid phase have been reported.[9] Our synthetic route is presented in *Scheme 1*, and consists of attaching an X-subunit Fmoc amino acid onto the support (for disubstituted guanidines, no X subunit was coupled), deprotection of the amine and reaction with a Y-subunit isothiocyanate to form the thiourea **3**, and conversion to the guanidine **5** either via the methyl isothiourea **4**, or from **3** in presence of $HgCl_2$. Cleavage from the support in 20% TFA/CH_2Cl_2 afforded the guanidine **6** as the TFA salt, which was lyophilized from 1:1 $MeCN/H_2O$.

Purifications were performed by SEC on Biorad® Biobeads S-X1 (2.5 x 10.5 cm column), eluting with 3% MeOH in CH_2Cl_2. Because the elution volume of the supports does not vary significantly with the compounds attached to the periphery, purifications resemble solid-phase extractions, and can be readily automated. SEC was performed in parallel on a four column system; crude reaction mixtures containing up to 100 mg of support were filtered as necessary and loaded manually onto a sample injection loop. Eluent was delivered via syringe pump at 4 mL/min. Support-bound intermediates were collected in the faster-eluting large molecule fraction (elution volume = 17-30 mL), while cleaved products eluted in the small molecule fraction (31-50 mL). Columns could be used more than a dozen times. Solvent was removed from isolated products under a nitrogen stream. TFA cleavage of the guanidines was accompanied by significant polymerization of the support presumably due to crosslinking of the RINK handles; any insoluble polymer was removed by filtration prior to SEC. Yields and purities of the guanidine products are listed in *Table 1*.

Table 1

Entry	subunits	% purity[c]	% recovery[d]	Entry	subunits	% purity	% recovery
1[a]	$X_1Y_1Z_1$	61	77	25[b]	$X_2Y_3Z_1$	41	63
2[a]	$X_1Y_1Z_2$	61	44	26[a]	$X_2Y_3Z_2$	95	37
3[a]	$X_1Y_1Z_3$	56	47	27[b]	$X_2Y_3Z_3$	8	74
4[a]	$X_1Y_1Z_4$	92	69	28[b]	$X_2Y_3Z_4$	90	50
5[a]	$X_1Y_2Z_1$	>95	39	29[b]	$X_2Y_4Z_1$	56	64
6[a]	$X_1Y_2Z_2$	90	37	30[a]	$X_2Y_4Z_2$	72	34
7[a]	$X_1Y_2Z_3$	>95	39	31[b]	$X_2Y_4Z_3$	10	57
8[a]	$X_1Y_2Z_4$	>95	32	32[b]	$X_2Y_4Z_4$	92	59
9[a]	$X_1Y_3Z_1$	>95	39	33[b]	Y_1Z_1	>95	54
10[a]	$X_1Y_3Z_2$	>95	40	34[a]	Y_1Z_2	85	46
11[a]	$X_1Y_3Z_3$	94	50	35[b]	Y_1Z_3	>95	29
12[a]	$X_1Y_3Z_4$	95	42	36[b]	Y_1Z_4	>95	69
13[a]	$X_1Y_4Z_1$	>95	50	37[b]	Y_2Z_1	85	19
14[a]	$X_1Y_4Z_2$	>95	56	38[a]	Y_2Z_2	>95	74
15[a]	$X_1Y_4Z_3$	94	53	39[b]	Y_2Z_3	70	11
16[a]	$X_1Y_4Z_4$	95	70	40[b]	Y_2Z_4	>95	30
17[b]	$X_2Y_1Z_1$	50	62	41[b]	Y_3Z_1	>95	14
18[a]	$X_2Y_1Z_2$	54	39	42[a]	Y_3Z_2	>95	67
19[b]	$X_2Y_1Z_3$	90	62	43[b]	Y_3Z_3	>95	14
20[b]	$X_2Y_1Z_4$	90	50	44[b]	Y_3Z_4	95	17
21[b]	$X_2Y_2Z_1$	50	73	45[b]	Y_4Z_1	>95	13
22[a]	$X_2Y_2Z_2$	>95	46	46[a]	Y_4Z_2	90	49
23[b]	$X_2Y_2Z_3$	10	46	47[b]	Y_4Z_3	0	14
24[b]	$X_2Y_2Z_4$	>95	54	48[b]	Y_4Z_4	80	26

[a] Guanidine formed from thiourea **3**. [b]Guanidine formed from methyl isothiourea **4**. [c]Crude product, determined by HPLC (230 nm) or ^{1}H NMR. [d]Crude product mass, as a fraction of the theoretical product mass based on initial Rink concentration.

Conversion of the methyl isothiourea **4** to guanidine **5** was generally clean, although extremely slow with aniline (data not shown) and secondary amine (entries 19, 23, 27 and 31) nucleophiles, affording significant amounts of methyl isothiourea sideproduct. Alternatively, Hg(II) mediated guanidine formation from the thiourea **3** (entries 1-16, 18, 22, 26 and 30) was more efficient, with product purities averaging nearly 90%. To our knowledge, mercury-mediated guanidine formation on the solid phase has not been reported, perhaps due to the inability to separate mercuric sulfide precipitate from the resin;[10] with soluble supports, however, the mercuric sulfide was simply removed by filtration prior to SEC. Secondary guanidines were also prepared in high purity (entries 33-48), although dialkyl species (entries 37, 39-41, 43-45, 47 and 48) were obtained in low yield due to inefficient cleavage from the support.

Central to the practicality of any support-based synthetic strategy is the dependable and efficient isolation of the support from the rest of the reaction medium. In this study, separation of soluble oligomeric supports by SEC was achieved in a matter of minutes, facilitating the production of guanidines in good yield and purity. Furthermore, the reproducibility of the separations enabled parallel and automated purification, raising the prospect of library construction by the COSMOS methodology on a massively parallel scale. To this end, efforts focusing on further miniaturization and parallelization of the purification process are underway.

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