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Solution-Phase Generation of Tetraurea Libraries

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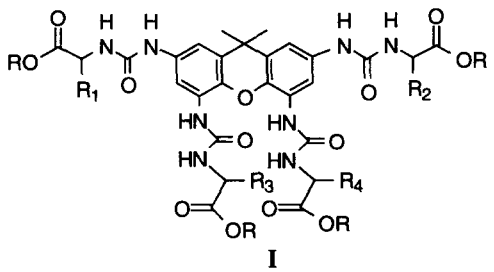
Abstract—Libraries of tetraureas tethered to a rigid core have been assembled. This simple, solution-phase methodology generates a defined, anticipated distribution of compounds. These conclusions are supported by synthesizing pure (homo) tetraurea xanthenes and by HPLC analysis of small 'microlibraries'. Copyright © 1996 Elsevier Science Ltd

Introduction

Generating diverse, yet predictable, collections of molecules on solid supports or in solution is an active area of research. While numerous reactions applicable to solid-phase methods[1] have been developed, solution phase techniques,[2] which allow for a wider range of reactions, have received less attention. In addition, combinatorial methods for drug design have begun to move away from purely amide-based systems,[3] as peptide bonds are readily cleaved in vivo. We describe here a scaffold which allows for simultaneous solution-phase functionalization of four sites. The more biologically robust urea is used as the linker. The urea functionality is used to append amino acids (and/or other amines) to the rigid xanthene core (as in the general structure **I**; R = Me or H; R_{1-4} are amino acid side chains). It may also play a role in determining activity, as the hydrogen bonding ability of the urea functional group in medicinal chemistry is well established.[4] Indeed, the libraries described here have been screened and shown to be active in a assay measuring binding to a transcription factor.[5]



I

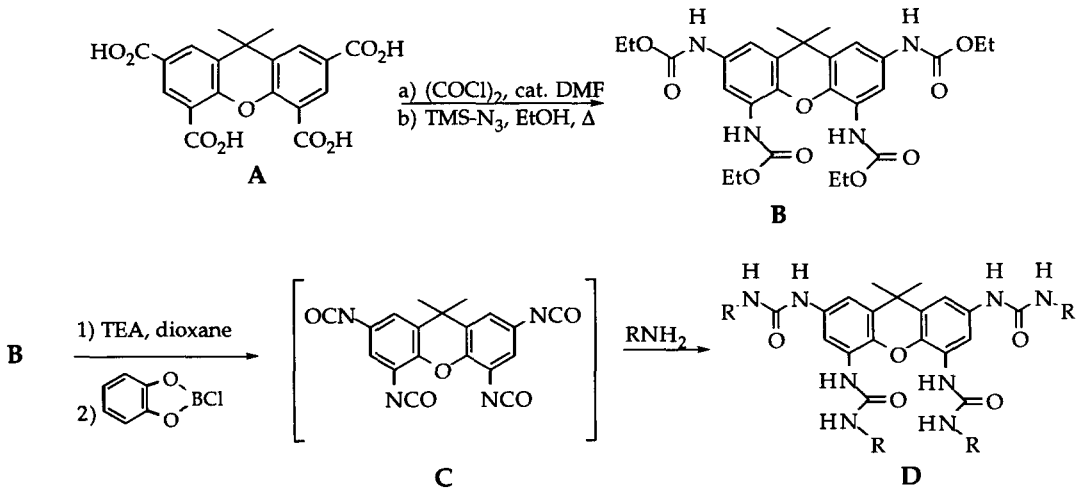
Results

In general, the approach relies on the rapid reaction of amines with isocyanates to form ureas.[6] To generate the required tetraisocyanate (**C**), we first attempted treating the tetraacid chloride[2] of **A** with TMS-azide,[7] then warming the acyl azide to effect a Curtius[8] rearrangement. However, the product isolated was too impure for direct use as a core. Nevertheless, the rearrangement did prove efficient enough to allow the tetracarbamate **B** to be isolated through silica gel chromatography. This allowed the use of the relatively new chlorocatecholborane method[9] for transforming carbamate functionalities into isocyanates. Accordingly, the reactive (tetra) isocyanate was generated in situ and reacted with amines (as the free bases) to form ureas (**D**). The carbamate to isocyanate to urea steps must occur in extremly high yield: the final products are >90% pure, a remarkable figure given that eight transformations must take place!

As it is of interest to screen libraries of tetraureas for biological activity, we chose mostly derivatives of amino acids as nucleophiles. Both methyl and *tert*-butyl esters of the amino acids were used, as the free acids were insoluble in the reaction medium. To optimize this reaction, pure (homo) tetra compounds containing amino acid methyl esters of Arg, Gly, His, Ile, Leu,[10] Lys (tBoc), Phe, Ser, Trp, Tyr, and Val were first synthesized. *t*-Butyl esters of these amino acids and *p*-methoxy benzylamine were also prepared with equally favorable results. The *t*-butyl esters were then smoothly cleaved using neat TFA[11] to give carboxylic acids. The studies revealed that a slight excess of chlorocatecholborane and amines (1.5 per site) were necessary and that the free amines could be added in methylene chloride and/or dry DMF.[12] For most (homo) tetra compounds, a simple aqueous work up with citric acid and dilute base (work up A) was sufficient to eliminate any undesired material. The yield and purity of these optimized reactions was high; in many cases, both were over 95%.

A few 'microlibraries' containing 10 compounds have been produced by reacting two and three different amines with the tetraisocyanate. However, since a slight excess of nucleophiles was necessary, it was a concern that the steric bulk of the amines would affect

Key words: solution-phase combinatorial chemistry, ureas, isocyanates, chlorocatecholborane.



the distribution of the products. A simple experimental result argues against such a size-based bias. On competing glycine methyl ester (small) with leucine *t*-butyl ester (large) in equimolar proportions, an approximately statistical distribution of products is observed (Fig. 1). All ten of the possible molecules are formed in comparable yields. When a three-fold excess of leucine *t*-butyl ester was used with glycine methyl ester, the areas changed appropriately, demonstrating that even bulky amino acid esters can be formed cleanly. A non-aqueous work up was developed for use with libraries to avoid losing hydrophilic tetraureas (work up B). This involves mere shaking of the product with basic alumina, then filtering. This quick work up eliminates nearly all the catechol-based impurities.

In summary, we have shown that ureas can be used efficiently as linkers to append biologically relevant side chains on a rigid core in solution. The libraries are not messy mixtures; rather, they are well-defined families of related, predictable compounds. Finally, the general approach should be applicable not only to other core molecules, but also to other related linkers such as carbamate and thiocarbamate—allowing ensembles of non-peptide molecules with different cores, linkages, and appendages to be rapidly produced, perhaps even by an automated process.

Experimental

Amino acid methyl esters with protected side chains and all *t*Bu ester amino acids were ordered from Novabiochem and were of the L configuration. Chlorocatecholborane was obtained from Aldrich. 1,4-Dioxane was freshly distilled from sodium/benzophenone. HPLC analysis was performed using a C18 reverse phase column (acetonitrile–H_2O mixtures) and measuring absorbances at 268 nm. The stated HPLC purities were based on peak areas, with no corrections for extinction coefficients.

General procedure for tetraureas

Tetraethylcarbamate (**B**, 1 equiv) was dissolved in dry dioxane (~0.02 M) containing triethylamine (TEA, 7

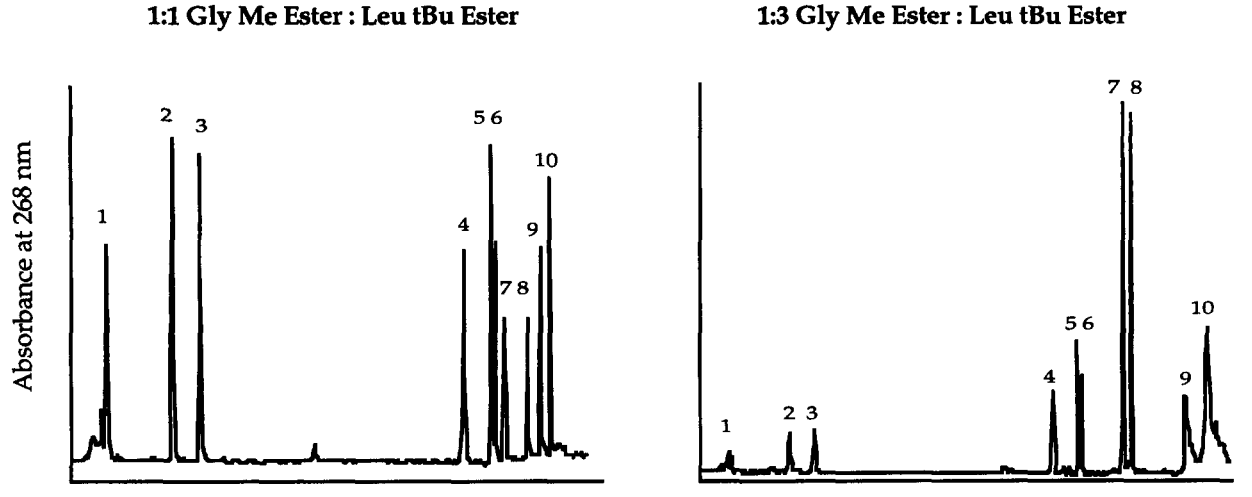


Figure 1. Reverse-phase HPLC traces of two microlibraries. Compounds **1** (tetra Gly OMe) and **10** (tetra Leu OtBu) were peak-matched to pure samples. Both samples were unpurified and merely treated with basic alumina to remove catechol-based compounds.

equiv) and the reaction heated to reflux for 10 min under Ar. While still hot, chlorocatecholborane (6 molar equiv in ~0.1 M dioxane) was added, a white ppt. formed, and the mixture refluxed for 15 min. After cooling to room temperature, the desired amino acid ester (6 equiv) was dissolved in a minimum amount of dry DMF with an equimolar amount of TEA. The reaction was stirred for 30 min at room temperature, then evaporated to dryness. The semisolid was dissolved in 5% MeOH in CH_2Cl_2 and subjected to either an aqueous work up (method A: rinsing with 1 M citric acid, then twice with 0.2 N sodium hydroxide); or (method B) a simple shaking with basic alumina, then filtration and evaporation on a high vacuum.

9,9-Dimethyl-2,4,5,7-tetraaminocarboethoxyxanthene (B). Xanthene tetraacid chloride (1.50 g, 3.4 mmol) was dissolved in dioxane (35 mL), azidotrimethylsilane (3.2 mL, 24.1 mmol) was added and the solution stirred for 10 min at 25 °C. Dry EtOH (3.2 mL, 55 mmol) was added and the solution heated to reflux under a drying tube for 12 h. After cooling, the reaction contents were poured onto 100 mL of saturated sodium bicarbonate and 100 mL EtOAc. More ethyl acetate was then added and the layers separated. The aq phase was extracted with 2 × 150 mL EtOAc; rinsed with brine and dried over Na_2SO_4. Concentration resulted in a brown foam which was dissolved and chromatographed in toluene:EtOH (15:1) on a silica gel column. Weight of the slightly yellow solid was 1.48 g (78%). Melting point: 118–120 °C (dec.); ^{1}H NMR (250 MHz, DMSO, ppm): δ 9.51 (br s, 4 H), 7.82 (d, J = 2.1 Hz, 2 H), 7.34 (d, J = 2.1Hz, 2 H), 4.19 (q, J = 7.0 Hz, 4 H), 4.10 (q, J = 7.1 Hz, 4 H), 1.49 (s, 6 H), 1.30 (t, J = 7.1Hz, 6 H), 1.23 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (62.9 MHz, DMSO, ppm): δ 153.77, 153.54, 135.53, 134.05, 129.16, 125.94, 110.96, 110.53, 60.39, 59.92, 34.08, 31.90, 14.44; HRMS (EI) calcd for $C_{27}H_{34}N_4O_9$, 558.2326; found, 558.2325.

Acknowledgments

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10. 2,4,5,7-Tetra leucine Me ester: worked up using method A, isolated yield of 88%, 94% pure by HPLC. ^{1}H NMR (250 MHz, DMSO, ppm): δ 8.54 (br s, 2 H), 8.40 (br s, 2 H), 7.97 (d, J = 2.2 Hz, 2 H), 7.29 (d, J = 2.3 Hz, 2 H), 6.89 (d, J = 7.7 Hz, 2 H), 6.33 (d, J = 7.9 Hz, 2 H), 4.26 (m, 4 H), 3.65 (s, 6 H), 3.64 (s, 6 H), 1.78–1.61 (m, 4 H), 1.57–1.51 (m, 8 H), 1.48 (s, 6 H), 0.93–0.87 (m, 14 H); ^{13}C (75.4 MHz, DMSO, ppm): 173.84 (br), 154.82, 154.36, 135.36, 133.56, 129.90, 127.39, 107.17, 107.03, 51.87, 51.81, 50.94, 50.69, 40.64, 34.54, 30.72, 24.34, 24.27, 22.69, 21.58, 21.54; HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{47}H_{71}O_{13}N_8$ (M + H), 955.5141; found, 955.5115.

11. To 50 mg of library material was added 3 mL of TFA. The yellow solution was stirred for 1 h (fine bubbles observed); then concd to a yellow, hygroscopic foam. When ether:hexane (1:1) (2 mL) was added, an off-white powder precipitated. NMR spectroscopy verified that no *t*-butyl groups remained attached to the core structures.

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