Generalized Dipeptidomimetic Template: Solution Phase Parallel Synthesis of Combinatorial Libraries

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Combinatorial synthesis, with its ability to rapidly produce large numbers of diverse compounds in a cost-effective manner in conjunction with high-throughput screening of the ever increasing number of molecular targets, has been anticipated to accelerate the drug discovery process.[1] Initially implemented with oligomeric peptide and nucleotide synthesis, more recent efforts have been directed toward conventional small-molecule synthesis. The implications of the technology are apparent both for the production of diverse lead-generation libraries and for the production of smaller targeted libraries for optimization around a promising lead candidate.

A variety of methods have been utilized for the generation of diverse chemical libraries. These include mixed, indexed, encoded, or parallel synthesis on pins,[2] beads,[3] chips,[4] and other solid supports[5] while solution phase synthesis has not been widely embraced as a viable alternative.[6] In part, this may be attributed to the evolution of combinatorial synthesis from solid phase peptide and oligonucleotide synthesis where supported phase synthesis has emerged as the medium of choice. Synthesis on a solid support offers the two important advantages of product isolation and manipulation that remain key issues in the generation of chemical libraries. It allows for the removal of reactants and nonbound byproducts by simple filtration enabling the use of excess reagents to effect high yields with no loss of product during isolation. However, the scale of solid phase synthesis is limited and generally restricted by the amount of the solid support and its loading capacity, and the production of multi-milligram quantities can be cumbersome and expensive for large libraries.[7] It requires functionalized solid supports and orthogonal chemistries suitable for substrate attachment/detachment, compatible spacer linkers, specialized protocols for monitoring the individual steps of a multistep solid phase synthesis[8] including the use of attendent orthogonal capping strategies for blocking unreacted substrate, and does not permit the purification of resin-bound intermediates. Consequently, an important complement to adapting solution phase chemistry to solid phase combinatorial synthesis is the development of protocols for solution phase combinatorial synthesis.[6] Given that solution and solid phase sample manipulation are both convenient and easily automated, the only limitation to the solution phase parallel synthesis of chemical or combinatorial libraries is isolation or purification of the reaction products. If the advantages of sample isolation attributed to solid phase synthesis may be embodied in a solution phase synthesis, its nonlimiting scale, expanded repertoire of chemical reactions, direct production of soluble intermediates and final products for assay or for purification, and the lack of required linking, attachment/detachment, or capping strategies make solution phase combinatorial synthesis a most attractive alternative. A number of potential techniques are available for such purposes, and one of the most attractive is liquid/liquid or solid/liquid extraction. Herein, we describe a high-purity solution phase parallel synthesis of a chemical library employing a dipeptidomimetic template which illustrates a simple and general isolation and purification protocol at each step.

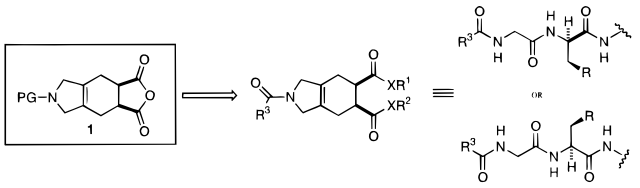

Figure 1.

Compound **1** is a designed rigid template which contains a number of important features. When fully extended, **1** contains a rigid bicyclic core with a plane of symmetry which enables it to function as a Gly-X mimic (Figure 1). When positions 1 and 3 are extended, the conformation mirrors that of an extended sheet. Extension of positions 1 and 2 introduces a turn motif. When all three positions are utilized, an interesting core peptidomimetic which explores three-dimensional space is produced. Its symmetrical structure contains three positions which can be controllably functionalized with a variety of nucleophiles and acylating agents enabling the synthesis of libraries with three variable units (Scheme 1). As an anhydride, the starting template is activated for the first functionalization, which upon reaction liberates its second functionalization site ($-CO_2H$). As such, no orthogonal protecting groups are required for the selective template functionalization and only four chemical steps are required for N^3 diversification. The same released functionality (CO_2H, NH) may be used for purification of the expected products from starting materials, reagents, and reaction byproducts by simple liquid/liquid or solid/liquid extraction. Any alcohol, amine, thiol, or nucleophile can be added to open the starting template anhydride. Following functionalization of the released acid, removal of an orthogonal protecting group on nitrogen allows an additional stage for

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(7) For a 10000-member library (three-step synthesis at 95% yield/step) to obtain 50 mg of each component (MW = 500 g/mol) on Merrifield resin with a typical loading of 1 mmol/g requires 1.166 kg of solid support = $2449/library; on Wang resin with a loading of 0.7 mmol/g, it requires 1.666 kg of solid support = $8331/library.

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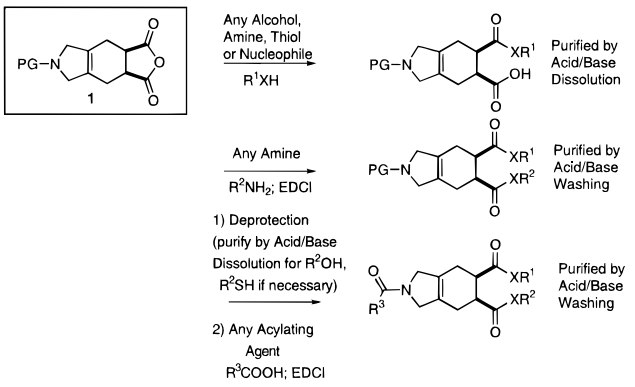
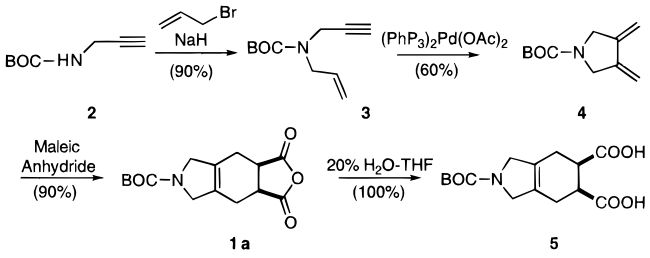
Scheme 1



Scheme 2



purification and a subsequent acylating agent to be added to complete the diversification. In addition to the use of an amine in the second diversification step detailed herein, this second step has also been modified to accommodate the use of any nucleophile (R^2OH, R^2SH, or nucleophile) by conducting an additional extraction purification on the amine liberated in the subsequent *N*-Boc deprotection. In each step of the sequence, the reactants, unreacted starting materials, reagents, and their byproducts can be removed by simple extractions, providing the intermediates and final compounds in high purities.

The template synthesis (Scheme 2) requires *N*-Boc protection of propargylamine and subsequent alkylation effected by treatment with NaH (1.1 equiv, DMF, 25 °C, 30 min) followed by allyl bromide (1.2 equiv, 0 °C, 5 h) to generate **3** (>90% yield, two steps). Treatment of **3** with catalytic $(Ph_3P)_2Pd(OAc)_2$ (0.05 equiv, 80 °C, C_6H_6, 1 h) to effect a 1,6-cycloisomerization affords diene **4** (60%).[9] The reactive diene is immediately subjected to a Diels−Alder reaction with maleic anhydride (1 equiv, C_6H_6, 40 °C, 1 h) to yield **1a**, which upon deliberate hydrolysis (20% H_2O−THF, 5 h) provides the easily purified and handled diacid **5**. The anhydride **1a** is then regenerated *in situ* upon treatment with EDCI (1 equiv) immediately prior to the addition of the first nucleophile.

To illustrate the library construction with **5**, we have detailed our initial efforts, which were conducted without prior optimization. These efforts provided a fully characterized 27-member library, which was constructed as a 3 × 3 × 3 matrix yielding 39 unique components including intermediates in individual vessels (Figure 2). Treatment of **5** with EDCI (1.1 equiv, DMF, 25 °C, 20 min) followed by addition of R^1NH_2 (1 equiv, 25 °C, 16 h) afforded the monoamides, which were purified by simple acid/base dissolution (80−99%). Importantly, only the monoamide product was generated, indicating *in situ* closure

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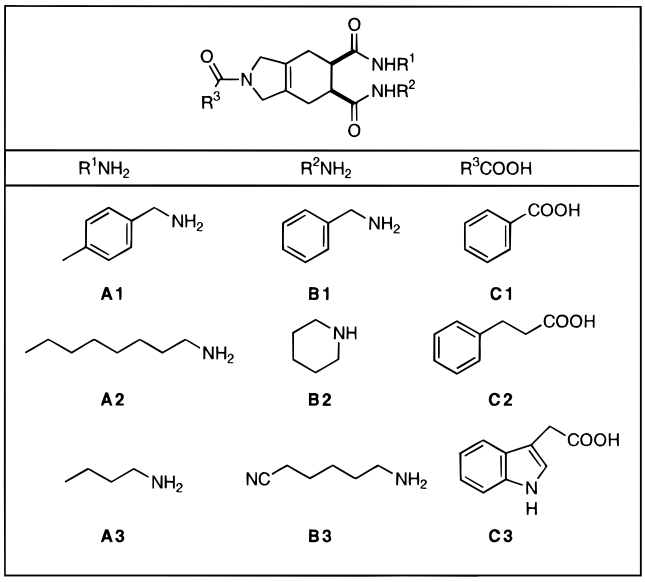

Figure 2.

of the initially generated activated carboxylate to the anhydride **1a** and its subsequent reaction with the added amine. The monoamides were split into four equal components, with one being retained for archival purposes. Each of the three remaining aliquots was treated with EDCI (3 equiv) and R^2NH_2 (3 equiv, DMF, 25 °C, 16 h), to yield 9 diamides (65−91%), which were purified by an acid/base wash removing the excess unreacted reactants, reagents, and reagent byproducts. One-quarter of the diamide was retained, and the remaining quantity was subjected to *N*-Boc deprotection (4 M HCl−EtOAc, 25 °C, 30 min). One-third of each was treated with EDCI (2 equiv) and R^3COOH (2 equiv, DMF, 25 °C) such that 27 unique products were obtained. The resulting fully functionalized peptidomimetics were purified by washing with aqueous acid and base to yield the purified final compounds (3−89%). Importantly and irrespective of individual yields, the intermediates and final compounds were ≥90−95% pure. The only contaminant observed was a small quantity of the oxidized pyrrole, which was minimized by the exclusion of oxygen during the *N*-Boc deprotection and subsequent acylation. Using a more modest criterion for purity (85% pure) than was achieved above, a three-step solid phase synthesis would require each step to proceed in ≥95% yield on each library member to provide the final released products at a ≥85% purity. Consequently, the range of reaction efficiencies acceptable for the solution phase parallel synthesis of chemical libraries as described herein is necessarily broader than would be tolerated for solid phase synthesis and avoids the need for thorough reaction optimizations. Studies employing larger targeted libraries with matrix characterization of each reaction type and their adaptation to automation will be disclosed in due time.

Supporting Information Available: Full experimental details and characterization for each library intermediate and final products (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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