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Template-induced macrocycle diversity through large ring-forming alkylations of tryptophan

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ABSTRACT

Macrocyclic peptidomimetics are valuable in research and serve as lead compounds in drug discovery efforts. New methods to prepare such structures are of considerable interest. In this pilot study, we show that an organic template harboring a latent cinnamyl cation participates in novel Friedel–Crafts macrocyclization reactions with tryptophan. Upon joining the template to Trp-Trp-Tyr, a single operation efficiently generates eight unique macrocycles. Each has been isolated and thoroughly characterized. Product distribution as a function of Brønsted and/or Lewis acidic conditions was explored, and outcomes were compared to rearrangements induced within a corresponding tyrosine-linked cyclic ether. The solution structure of a new macrocyclic pyrroloindoline was solved using a combination of two-dimensional NMR methods and molecular mechanics simulations. Template-induced structural diversification of peptide sequences harboring aromatic residues has potential to create myriad macrocycles that target surfaces involved in protein–protein interactions.

1. Introduction

As opportunities in biological research and drug discovery expand, there is a considerable need for synthetic methods that form new types of complex small molecules.[1] Conventional screening libraries often fail to produce viable lead structures when challenged with demanding targets.[2] These include receptors with relatively large, dynamic, or solvent exposed ligand binding sites and protein–protein interaction surfaces.[3] Recent drug discovery efforts aimed at such targets[4] have benefited from biophysical studies highlighting 'hot spots' within these larger binding motifs.[5] Focused libraries that incorporate structures able to interact selectively and avidly with such sites would be valuable. Molecules that recapitulate the three-dimensional display of functional groups found in native binding partners while possessing favorable pharmacological properties would be ideal.

Peptides are intrinsically relevant to this goal. They are a logical starting point to identify ligands for protein surfaces.[6] However, peptides frequently exhibit poor bioavailability and limited stability in vivo.[7] Numerous strategies have been developed to mitigate these problems, including the incorporation of D-configured[8] and non-proteinogenic amino acids,[9] pseudo-peptide bonds, and conformational constraints.[10,11] Each of these features is seen in peptide-derived macrocyclic natural products, which often possess markedly different properties relative to their acyclic precursors. With macrocycles as a central theme, we have sought to expand upon existing methods for large ring formation.

In a previous report, we outlined elements of a program aimed at systematically generating complex peptidomimetics.[12] These experiments used designed templates (Fig. 1B) to form composite structures with peptides.[13] Our current templates capitalize on reactivity of the cinnamyl cation, and transiently formed palladium

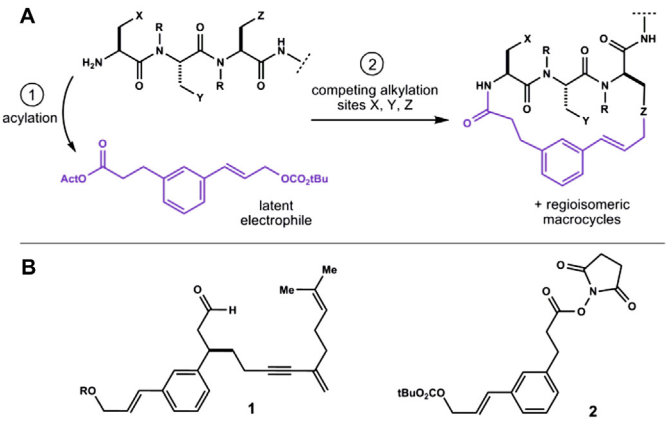

Fig. 1. (A) Current template designs (purple) utilize a cinnamyl mixed carbonate as a latent electrophile. Competing reactions with side-chain nucleophiles provide isomeric macrocycles of varying shape, (B) prototypical templates **1** and **2** (this work). R=$CO_2CMe_2CCl_3$.

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complexes thereof, to efficiently promote large ring-forming substitution reactions involving aromatic rings and heteroatom nucleophiles. While numerous methods exist to prepare peptidic macrocycles, most require tailored reacting partners.[10a,14,15] Our template chemistry exploits reactivity inherent to a subset of natural amino acid side chains.

For example, we used the allylic carbonate in template **1** (Fig. 1B) to form macrocyclic ethers by engaging the phenol of tyrosine in palladium catalyzed substitution reactions.[12,16] In the context of peptides harboring other aromatic residues, we observed that acid treatment caused these ethers to rearrange, wherein the cinnamyl unit migrated to adjacent tryptophan residues forming stable carbon–carbon bonded products. This was an exciting discovery. To probe this reactivity in greater detail, and in systems not complicated by competing rearrangements of the diene-yne appendage in **1**, we synthesized template **2**.[17] Using simple, straightforward chemistry, composites of **2** participate in remarkable reactions that alter the structure and properties of linear peptide motifs (vide infra).

Toward the goal of rapidly accessing diverse, natural product-like structures, we demonstrate use of **2** in a divergent process to prepare mixtures of macrocyclic products displaying a peptidic binding epitope.[18] In two synthetic steps, template **2** transforms a peptide harboring multiple nucleophilic side-chains (X, Y, Z Fig. 1A) into constitutionally isomeric macrocycles through competing reaction pathways. The resulting products differ in core ring size and conformation, each dictating a unique display of side chains.[19] The structural diversity derived from this process arises from the nature of the starting peptide and the reactivity of the template. Processes of this kind have potential to create composite macrocycles with inherent complementarity to protein surfaces.

2. Results and discussion

2.1. Preparation of cyclic and acyclic acidolysis precursors

Template **2** was prepared from commercial 3-(3-bromophenyl) propionic acid in six steps and 51% overall yield.[17] This material acylated the N-terminus of synthetic Trp-Trp-Tyr-NH_2 without incident, providing composite product **4** in good yield (Fig. 2). Analogous to previous work, exposure of **4** to 5 mol % $Pd(PPh_3)_4$ catalyzed efficient macrocyclization to give tyrosine O-linked cinnamyl ether **5**. Treatment of **5** with 15 equiv methanesulfonic acid in anhydrous nitromethane at room temperature provided a mixture of three isolable products (Figs. 3A and 5B). These proved to be analogous to the four macrocyclic core structures observed previously from reactions of template **1** with Trp-Trp-Tyr-NH_2.[12]

Close inspection of HPLC–MS ion chromatograms indicated the presence of five additional minor isomers (vide infra). Combined, those eight products accounted for >95% of the total peak area (HPLC–UV at 254 nm). This confirmed that template **2** was an excellent model for further study.

2.2. Purification and characterization

Acidolysis products **6**–**12** were readily separated by reverse phase preparative HPLC (Fig. 5). From macrocyclic ether **5**, we initially isolated only **6**, **7**, and **11** in sufficient quantities for NMR analyses. Upon refining the reaction conditions (see Section 2.3 and Table 1), initially trace components **8**, **9**, **10**, and **12** were also isolated. Compounds **6**–**9**, **11**, and **12** were each obtained in >95% purity. Only fraction **10** was isolated as a mixture of closely related regioisomers **10a** and **10b**, which were characterized together.

The planar structures of compounds **6**–**12** (Fig. 3A) were determined by complete assignment of their proton and carbon connectivities on the basis of homo- and heteronuclear correlations obtained from 2D and selective 1D NMR experiments (see Supplementary data). Structural elucidation involved (1) sequential assignment of the peptide backbone, (2) correlation of backbone atoms to their corresponding side chain aromatic ring, and (3) determination of the connectivity of the cinnamyl moiety to an aromatic side chain.

In this model peptide, complete resonance assignment of the peptide was required to differentiate between the two tryptophan residues. Sequential assignment of the amide backbone was made by H^N–C(O) and H^α–C(O) correlations observed by HMBC,[20] or by H^α_i–H^N_{i+1} NOE where ambiguities arose from weak correlations or overlapping carbonyl resonances.[21] Assignment of residue specific H^N–H^α–H^β spin systems from TOCSY spectra was then possible,[22] and connectivity of H^β to the aromatic portion of the side chain was established from reciprocal HMBC correlations.

The ansa bridge motif arising from electrophilic aromatic substitution causes a characteristic loss of one proton relative to the parent ^1H spin system of the aromatic amino acid side chains, which was apparent by TOCSY. The precise position of substitution was deduced from careful analysis of $^{2,3,4}J_{CH}$ correlations observed in HMBC experiments (e.g., Fig. 4A). In general, heteronuclear correlations from the cinnamyl methylene to aromatic resonances of the substituted ring, and the reciprocal thereof, allowed unambiguous structural assignment. In cases where HMBC correlations were either unclear or not observed, the connectivity of the cinnamyl unit was inferred from NOE correlations between the two proximal spin systems observed by TOCSY (Fig. 4B).

The isomeric products obtained from acidolysis of macrocyclic ether **5** comprised three of the four outcomes anticipated from previous work. We obtained compound **6**, arising from rearrangement of the O-linked macrocyclic ether to the phenolic *ortho* position, as the predominant product. The next most abundant

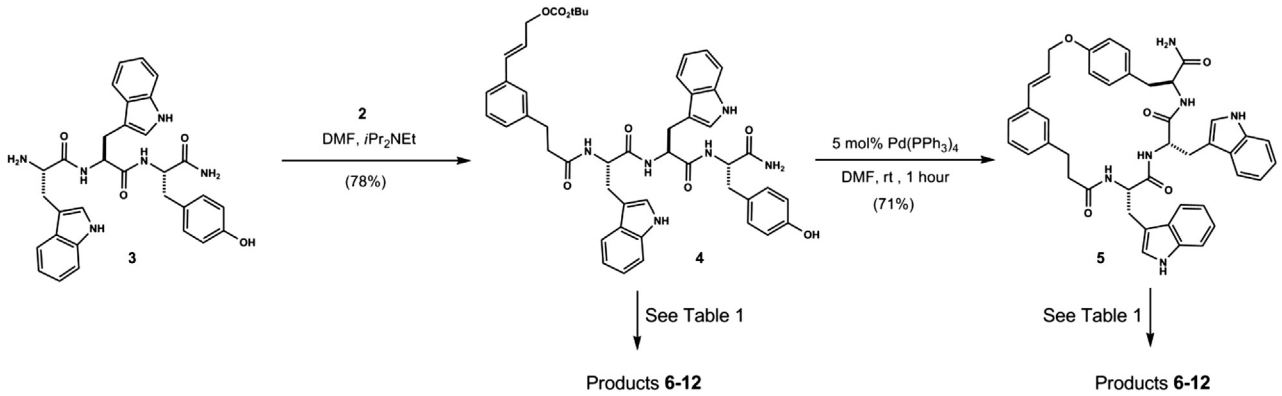

Fig. 2. Reaction conditions: (a) H-Trp-Trp-Tyr·TFA (1 equiv), template **2** (1 equiv), $^iPr_2NEt$ (4 equiv), DMF (0.1 M). (b) $Pd(PPh_3)_4$ (5 mol %), DMF (5 mM, degassed).