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Acid Promoted Cinnamyl Ion Mobility within Peptide Derived Macrocycles

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We are developing methods that restrict the conformational mobility of peptides and related heteropolymers while simultaneously altering their properties. Our experiments occur as processes wherein a conserved, lipophilic reagent (**E**, Figure 1A) is activated in stages to form composite products with unprotected polyamides in parallel. For each starting oligomer, the goal is to create not one, but rather a collection of products.[1] The intent is for those materials to retain molecular recognition elements of the biopolymer, yet display that functionality as part of stable, cyclic structures having defined shapes and enhanced membrane solubility/permeability.[2]

Here we describe compound **2** (Figure 1B) as a specific illustration of **E**.[3] The molecule harbors several electrophilic motifs, one overtly reactive and the others latent. Aldehyde **2** can be mixed, independently, with a variety of unprotected peptides to form N-terminal imines. These react in situ with, for example, aryl-substituted tosylmethylisocyanides to afford imidazole products of a net three-component condensation.[4] If the original peptide possesses a phenolic residue, the condensation product can be treated with catalytic amounts of $[allylPdCl]_2$/Xantphos[5] complex to initiate a macrocycloetherification—wherein the allylic carbonate precedes a transient metal π-allyl species that captures the pendant phenol. Synthetic G-W-Y[6] is transformed cleanly into macrocycle **4** using these two operations and the methods act similarly on a range of peptide substrates. Reagent **2** sheds its oxygenation in the process to become solely unsaturated hydrocarbon in the products.

Robust ligation and cyclization steps are a start, but the larger aim seeks more extensive alterations of the peptide brought about by extended processes (Figure 1A). The design of **2**[7] furthers this goal. Once intermediates are fashioned into rings, one can initiate promiscuous long-range rearrangements alongside varying internal cyclizations. The substance thereby permits one type of complex molecule (the peptide) to be molded into numerous others.

In the case of **4**, exposing the molecule to protic acid in anhydrous solution initiates a number of striking isomerizations.[8] Treatment with excess $MeSO_3H$ for 3 h in dry $MeNO_2$ at 0 °C affords a crude mixture from which we isolate **5**−**10** via preparative, mass-guided HPLC (Figure 2).[9] Structure assignments derive from mass spectra and combined ^1H, ^{13}C, gCOSY, gHMBC, gHMQC, and NOESY NMR analyses.

Phenol **5** is a product of noncanonical aryl Claisen rearrangement[10] of the cinnamyl ether in **4** concomitant with cyclization of its dienyne appendage to an alkynyl cyclohexene (3:1 mixture of olefin regioisomers). Structure **6** derives similarly except wherein the putative cinnamyl ion pair formed by heterolysis of the allylic ether in **4** has undergone migration to the adjacent tryptophan residue—forming a new C−C bond at the indole 5-position.

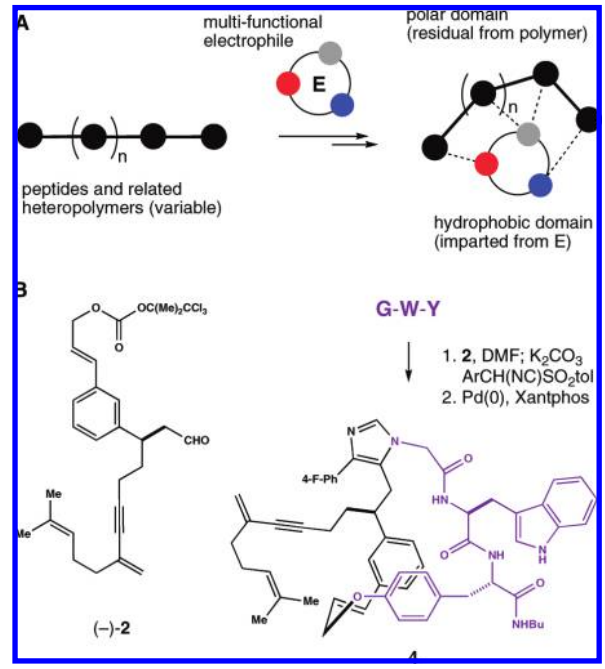

Figure 1. (A) Generic plan to mold peptides and related nucleophilic heteropolymers into complex variants having defined shapes and altered properties [black spheres = nucleophilic monomers, colored spheres = latent and/or overtly reactive electrophiles]. (B) As an example of **E**, reagent **2** can be incorporated into a range of peptides to afford cyclic composites having both polar and hydrophobic domains. For instance, mixing **2** with unprotected G-W-Y and treating the incipient imine with 4-F-PhCH(NC)SO_2tol/K_2CO_3 affords an N-terminal imidazole adduct that transforms into cyclic cinnamyl ether **4** when exposed to 2 mol % $[(C_3H_5)PdCl]_2$/Xantphos complex in DMF solution.

Congeners **7** and **8** harbor this same macrocycle and contain the dienyne of **4** intact or its formal Cope rearranged variant, respectively. Products **9** likely derive from **6** wherein a secondary cyclization installs a conjugated dihydronapthalene motif. Lastly, structures **10** result from a third mode of cinnamyl unit migration, in this case to engage the trisubstituted olefin of the dienyne appendage to generate regioisomers of the unsaturated metacyclophane shown.

Use of reagent **2** translates simple protonations into varying products and these track changes in the structure of polyamide starting material. For example, if instead of G-W-Y, our three-step process [(1) ligation with **2**, (2) cycloetherification,[11] (3) acid treatment] is repeated beginning with synthetic W-W-Y, acid induced mobility of the cinnamyl unit again transforms a 23-membered cyclic ether into products having 21, 19, and 15-membered macrocycles (**11**−**14**, Figure 2). However, in this case we also isolate materials **15** and **16**. The ansa bridge in **15** likely reflects a side-chain propargylic cation, rather than losing a proton

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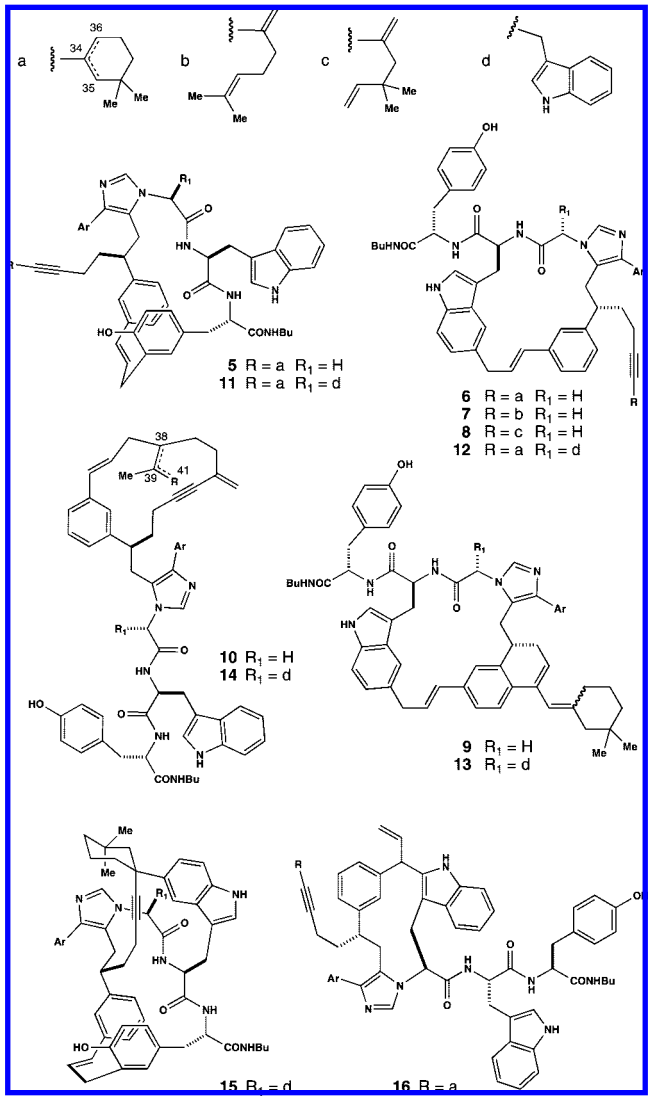

Figure 2. Products derived from processing G-W-Y and W-W-Y independently with reagent **2**, using the chemistry depicted in Figure 1B followed by treatment with MsOH in anhydrous $MeNO_2$. Ar = p-F-phenyl. Δ38,39 (R = Me) and Δ39,41 (R = CH_2) isomers of cyclophane **10** characterized separately. **14** observed in Δ38,39 form only. Where present, the alkynyl cyclohexene motif (a) is isolated as a mixture of Δ34,35 and Δ34,36 olefin isomers.

to afford **11**, engaging the proximal tryptophan residue in a transannular electrophilic aromatic substitution. Structure **16** completes a set. In **11** the cinnamyl unit has shifted from O to C at tyrosine, in **12/13** it has migrated to the adjacent tryptophan residue, and in **16** it has traversed the length of the tripeptide to engage the distal tryptophan, in this instance bonding to the indole C-2 position via a branched linkage.[12]

Structures **5**−**16** harbor six new macrocycle types. They were prepared from just two small peptides using one reagent (**2**) and three steps. The materials generally show well-dispersed ^{1}H NMR spectra and, relative to the peptides from which they derive, improved solubility in organic solvents and detergent containing buffer solutions. Precise de novo syntheses of similar molecules would be considerably more involved; and therein lies the potential. We have characterized the current examples in detail for demonstration. However, this is time-consuming. In fact, each isolate requires analyses comparable to those used in a natural product structure determination. Going forward, up-front analyses are not necessary. Rather, oligomers will be assembled systematically and processed in parallel, with product mixtures from each being fractionated and arrayed for biochemical evaluations.

As a pilot study, we processed GWY, WWY and GGY using reagent **2** and the three steps described. Product mixtures from each were divided into 10−12 fractions by preparative HPLC. These fractions were lyophilized to powders and redissolved in DMSO to provide ∼10 mM stock solutions. With this small screening set, we chose an in vivo assay where a functional response could be tracked reliably. The assay scored for hippocampal neurogenesis in the adult mouse brain. Data has established a correlation between postnatal neural stem cell proliferation in the subgranular zone of the dentate gyrus and forms of human neuropsychiatric disease characterized by cognitive deficits, such as schizophrenia.[13,14] There is interest in agents that stimulate neuronal precursor cell proliferation in the hippocampus as potential pharmacotherapy in this area.

Isolated HPLC fractions were diluted (10^3-fold) in artificial cerebrospinal fluid and infused intracerebroventricularly at a constant rate into the left lateral ventricle of adult C57BL/J6 mice by means of an implanted Alzet osmotic minipump. Mice were awake and unconstrained during the infusion and administered daily intraperitoneal injections of bromo deoxyuridine (BrdU, 50 mg/kg) as a marker of cell divisions. After infusion was complete (7 days), mice were transcardially perfused, and brain tissue was stained with antibodies against BrdU. Hippocampal neurogenesis was evaluated by light microscopy contralateral to the side of pump implantation (Figure 3A) to avoid artifacts from tissue damage. Every fifth section through the rostral-caudal extent of the hippocampus was analyzed, and the total number of BrdU+ cells was normalized against the volume of the dentate gyrus. Similarly infused recombinant fibroblast growth factor (FGF, 0.04 mg/ Kg/ day) served as a positive control (Figure 3B).

Select pools derived from each of the processed peptides displayed activity in this format. We pursued fraction five from G-G-Y because it elicited the most robust and selective[15] response (Figure 3C). This fraction contained several products (Figure 3D), and these were separated into an additional three pools and rescreened in vivo as described. The pool found active now contained four main products (Figure 3E), and these were chromatographed a third time to afford four components, one of which (Figure 3F) retained the majority of neurogenesis activity. The structure of the active constituent was subsequently assigned as meta cyclophane **17** on the basis of mass spectra and multidimensional NMR analyses. Pure **17** reproducibly elicits marked (Figure 3G) and selective neural cell proliferation in the hippocampal dentate gyrus and its activity is dose dependent in a dilution series (Figure 3H).

The mechanism by which **17** acts is not yet known. While its properties and performance are being examined further, results here show that, even from a very small array, we can track and purify an active constituent from mixtures. Iterative rescreening of increasingly homogeneous product fractions minimizes the probability of isolating pure false positives. Time invested in structure determinations is then of value.

As chemistry in this venue is refined, processes will be extended to include oxidation/oxygenations and screening sets will expand considerably. New methods will be established to amalgamate next generation reagents into diverse synthetic polyamides and variants. Unmodified, these polymers generally have poor properties for pharmacological research. Our goal is to purge those limiting characteristics while retaining the value of functionally complex, stereochemically rich raw materials that can be made systematically by machine. Doing so would provide broad potential to discover

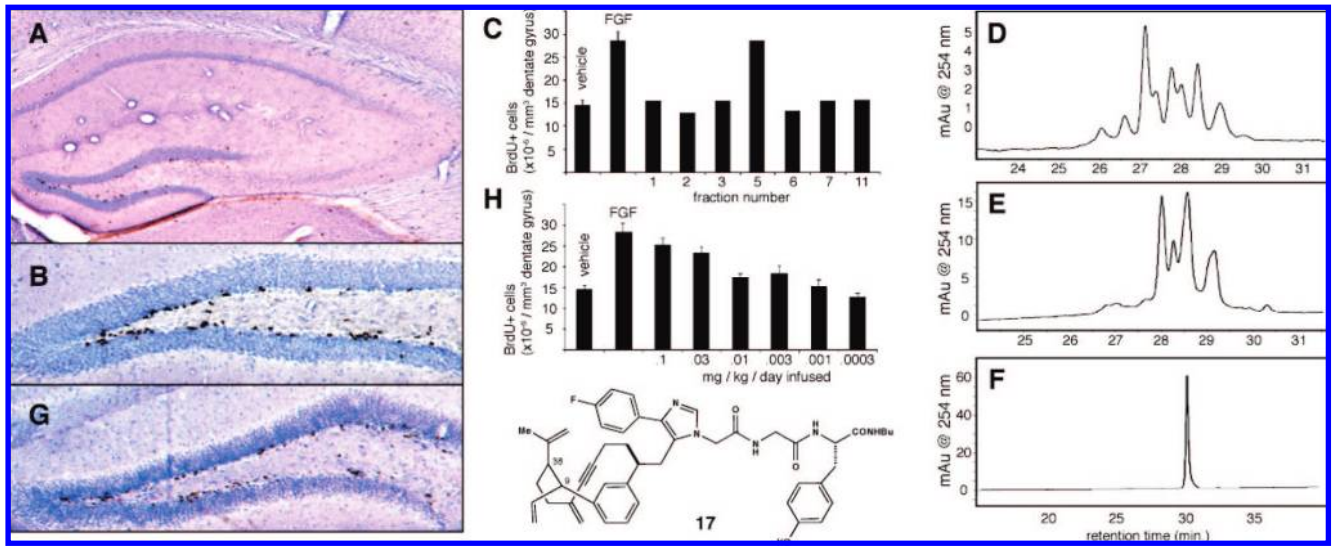

Figure 3. (A) Normally proliferating cells (anti BrdU, black dots) in the subgranular zone of the mouse hippocampal dentate gyrus (DG, lower left). (B) Magnified DG from a mouse infused with FGF (0.04 mg/Kg/d for 7 d) showing increased BrdU incorporation [see Supporting Information for detailed protocols]. (C) In vivo screening of products derived from processing G-G-Y with **2** identified a chromatography fraction (#5) that elicits BrdU incorporation comparably to FGF. HPLC analysis of this fraction (D) showed it contained several products. These were divided into three pools by HPLC and rescreened in vivo. The active fraction (E) was resolved into components, one of which (F) retained the majority of neurogenesis activity and was assigned structure **17** (∼1:1 mixture of isomers, stereochemistry at C9 and C38 unassigned). Isolated **17** elicits marked BrdU incorporation in the DG when infused at 0.1 mg/kg/d for 7d (G) and its activity is dose dependent (H).

complex peptidomimetics useful as probes in areas of biology where recombinant biologics and/or small drug-like heterocycles can be limited.[16]

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Supporting Information Available: Experimental procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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