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Triple Hybrids of Steroids, Spiroketals, and Oligopeptides as New Biomolecular Chimeras

Abhisek Banerjee,[†] Eduard Sergienko,[‡] Stefan Vasile,[‡] Vineet Gupta,[§] Kristiina Vuori,[‡] and Peter Wipf*[,†]

Department of Chemistry and Center for Chemical Methodologies & Library Development, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, Burnham Center for Chemical Genomics, Burnham Institute for Medical Research, 10901 North Torrey Pines Rd., La Jolla, California 92037, and Division of Nephrology and Hypertension, University of Miami, 1600 NW 10th Avenue, Miami, Florida 33136

pwipf@pitt.edu

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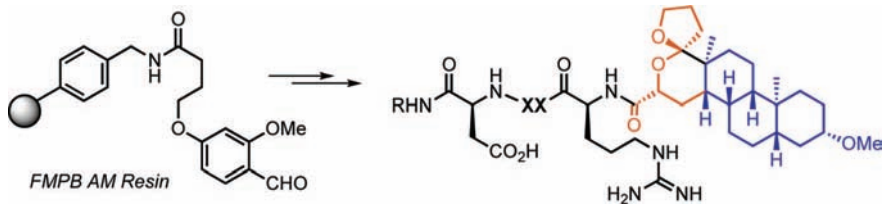
ABSTRACT



An oxidative enol ether rearrangement was the key methodology in the construction of steroid-spiroketal-RGD peptides. Biological studies demonstrated potent integrin CD11b/CD18 antagonistic effects.

Steroids are often conjugated to other building blocks that modulate their biological activity.[1] For example, carbohydrates are attached to the steroid A-ring hydroxyl groups and D-ring side chains, and the resulting hybrid molecules have improved solubility and modified physicochemical properties as well as distinct biological functions. Synthetic sugar-steroid conjugates have been shown to target phospholipid membranes.[2] Peptide-steroid conjugates have been applied as artificial proteolytic enzymes,[3] mimics of cationic antibiotics,[4] and synthetic receptors for oligopeptides (Figure 1).[5] The steroid skeleton is rearranged into a spiroketal moiety in hippurin-1,[6] cephalostatins, and ritterazine M,[7] which show potent reversal of multidrug resistance and anticancer activities. A library approach toward the synthesis of peptidomimetic spirostane hybrids took advantage of the four-component Ugi reaction,[8] and macrocyclic hybrid structures were assembled with the goal to construct chiral

[†] University of Pittsburgh.
[‡] Burnham Institute for Medical Research.
[§] University of Miami.

(1) Salunke, D. B.; Hazra, B. G.; Pore, V. S. *Curr. Med. Chem.* **2006**, *13*, 813–847.

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(6) (a) Tanaka, J.; Trianto, A.; Musman, M.; Issa, H. H.; Ohtani, I. I.; Ichiba, T.; Higa, T.; Yoshida, W. Y.; Scheuer, P. J. *Tetrahedron* **2002**, *58*, 6259–6266. See also: (b) Anjaneyulu, A. S. R.; Rao, V. L.; Sastry, V. G. *Nat. Prod. Res.* **2003**, *17*, 149–152.

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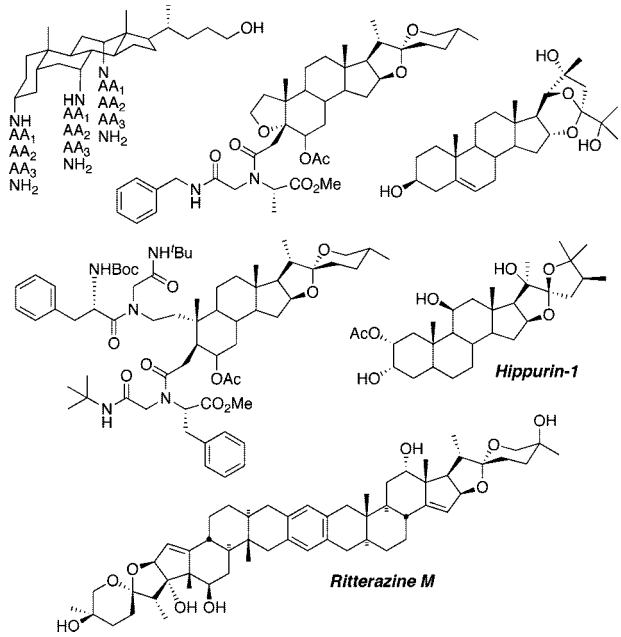
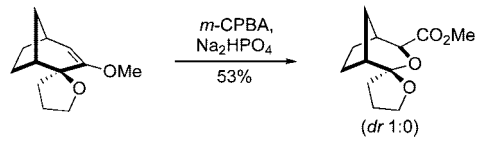


Figure 1. Selected natural and designed steroid-peptide, steroid-amino acid, and steroid-spiroketal hybrid structures.[2−7]

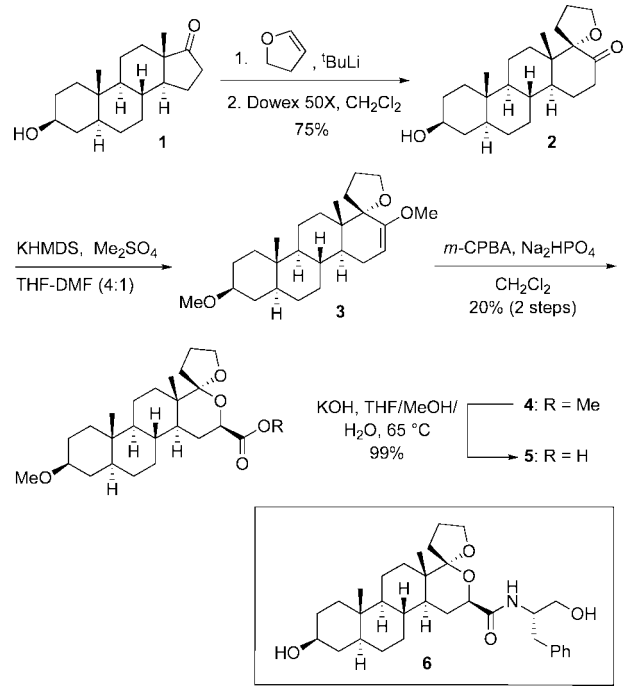
host molecules.[9] However, conjugates of spiroketal modified steroids to peptides or peptide mimetics have not yet been explored; we hypothesizd that these chimeras could demonstrate interesting membrane affinities and receptor recognition properties.

Scheme 1. Oxidative Rearrangement of Alkyl Enol Ethers



We have recently demonstrated an oxidative rearrangement of enol ethers to lactones and spiroketal esters.[10] Our methodology allows for a rapid formation of these common structural subunits (Scheme 1). We now report an extension of this methodology for the construction of a small library of steroid-spiroketal-peptide triple hybrid structures that were designed to anchor the RGD motif in the cell membrane. The tripeptide sequence Arg-Gly-Asp (RGD)[11] is widely used for cell adhesion studies, as it is recognized by many integrins, including $\alpha_V\beta_3$ ($K_d \approx 10^{-9}$ M).[12] Several integrin antagonists based on the RGD sequence have been designed for applications in the control of angiogenesis, tumor cell metastasis, osteoporosis, and other diseases.[13] However, the development of selective integrin antagonists still constitutes a major challenge in RGD mimetic design.

Scheme 2. Steroid Spiroketal α-Alkoxy Ester Synthesis



Our synthesis of the steroid spiroketal carboxylic acid **5** began with commercially available *epi*-androsterone (**1**). Treatment of **1** with 5-lithio-2,3-dihydrofuran followed by the acidic Dowex 50X initiated a pinacol-type[14] rearrangement (Scheme 2). Enolization and O-methylation of ketone **2** was performed with KHMDS and Me_2SO_4 in a 4:1 mixture of THF and DMF. Other conditions led to significant amounts of C-methylation. The reaction of the enol ether **3** with *m*-CPBA buffered with Na_2HPO_4 produced the pentacyclic spiroketal ester **4** as a single diastereomer after basic workup.[15] Saponification of **4** with KOH provided carboxylic acid **5**, and the X-ray structure of amide **6** confirmed the structural assignment of the spiroketal portion of **5**.

A microwave Fmoc-SPPS protocol[16] was selected for the preparation of the chimera. However, when Wang resin was used, cleavage of the peptide from the solid support by aminolysis with benzylamine[13] did not provide the desired

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(10) Waller, D. L.; Stephenson, C. R. J.; Wipf, P. *Org. Biomol. Chem.* **2007**, *5*, 58–60.
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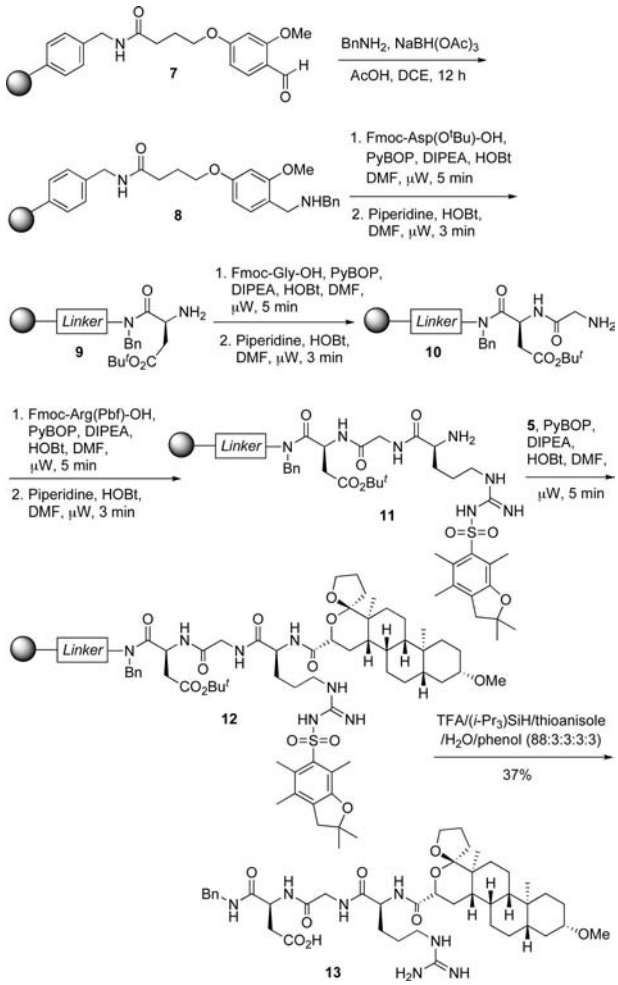
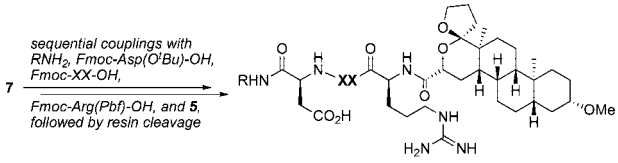
(15) Inorganic bases such as Na_2CO_3, $NaHCO_3$, Cs_2CO_3, and NaH_2PO_4/Na_2HPO_4 (1:1) gave no improvement in yield.
(16) Palasek, S. A.; Cox, Z. J.; Collins, J. M. *J. Pept. Sci.* **2007**, *13*, 143–148.

Scheme 3. Solid-Phase Synthesis of Steroid-Peptide Chimeras



product. Alternative aminolysis protocols, including benzylamine-catalytic sodium cyanide,[17] lithium aluminum benzylamide,[18] and benzylamine-dimethylaluminum complex,[19] were also unproductive. In contrast, the preparation of

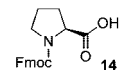
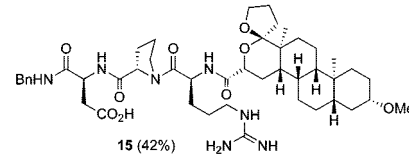
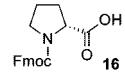
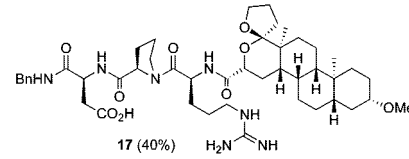
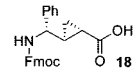
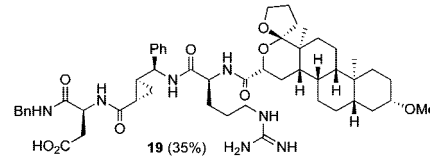
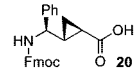
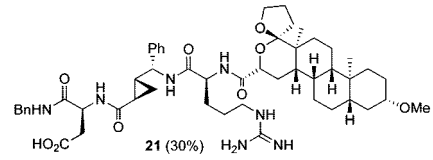
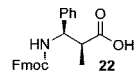
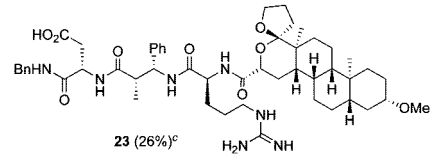
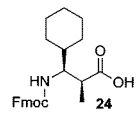
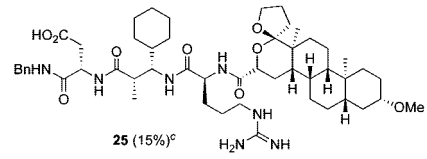
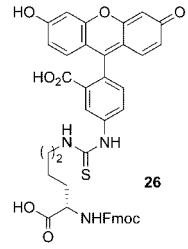
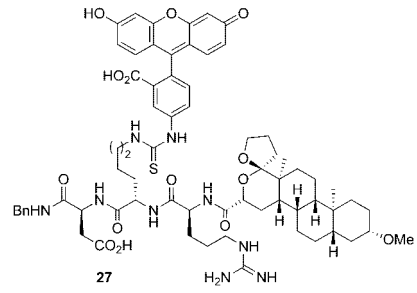
Scheme 4. Solid-Phase R-X-D Triple Hybrid Library Synthesis



chimera **13** succeded in good overall yield on FMPB-AM Rink amide resin (Scheme 3).[20,21]

Reductive amination of aldehyde **7** with benzylamine was followed by iterative coupling of **8** under microwave

Table 1. Structures of Variable Amino Acid Segments and Steroid-Spiroketal-Tripeptide (R-X-D) Products

Fmoc-XX-OH	product[a] (yield[b])
 **14**	 **15** (42%)
 **16**	 **17** (40%)
 **18**	 **19** (35%)
 **20**	 **21** (30%)
 **22**	 **23** (26%)[c]
 **24**	 **25** (15%)[c]
 **26**	 **27**

[a] All products were synthesized in a manner analogous to the procedure shown in Scheme 3. [b] Isolated yield after RP-HPLC. [c] SPPS was conducted on 0.05 mmol of resin as opposed to 0.1 mmol for all other entries.

conditions[22] to suitably *N*- and side chain protected amino acid building blocks. After conjugation of tripeptide chain **11** to acid **5**, the resin was cleaved under global deprotection conditions to provide the chimera sequence **13**. This product was purified by RP-HPLC and submitted to a cell-based

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(18) Solladie-Cavallo, A.; Bencheqroun, M. *J. Org. Chem.* **1992**, *57*, 5831–5834.

adhesion assay for high-throughput screening of regulators of the leukocyte-specific integrin CD11b/CD18, a validated therapeutic target for inflammatory diseases.[23,24]

In addition to **13**, additional RGD encoded triple hybrid chimeras were prepared for biological evaluations as well as for exploring the scope of our synthetic strategy (Scheme 4, Table 1). Coupling steps were conducted in the microwave at 40 W for 5 min at 70 °C. The coupling with Fmoc-Asp(O^{t}Bu)-OH was repeated with 2.5 equiv of acid to ensure complete loading. For other acids, a single acylation with 3.5 equiv of Fmoc-amino acid was used, and 2.0 equiv of **5** were employed in the final amide bond formation. The cleavage of the Fmoc groups with 20% piperidine in DMF also took advantage of microwave heating (50 W, 3 min, 50 °C). Products were released from the resin by treatment with a TFA cleavage cocktail at room temperature for 2 h.

In the design of our chimera, we hypothesized that the steroid scaffold would anchor the peptide in the membrane, the spiroketal linker would rigidly project the peptide strand and provide selectivity, and the replacement of the glycine residue in the RGD sequence would induce features such as conformational preorganization, rigidity, β-turn stabilization, and resistance toward proteolytic degradation. Thus, we synthesized both L- and D-proline containing scaffolds **15** and **17**. Similarly, the Fmoc-protected enantiomeric γ-amino-α,β-cyclopropyl acids **18** and **20** were used to introduce turn structures.[25] β-Amino acids also have a profound effect on secondary sturctures and are finding increasing applications in peptide mimicry.[26] The β-amino acids **22** and **24**[27] were readily inserted into the triple hybrid scaffold. Finally, for cellular localization studies, we prepared the fluorescein labeled derivative **27** by inserting the modified Fmoc-lysine residue **26**[28] into the R-X-D tripeptide sequence.

The biological evaluation[24] of all triple hybrid RGD mimics revealed no agonist activities. However, two compounds, **13** and the corresponding C-terminal NH_2amide, displayed potent antagonistic effects and IC_{50}'s of 10.9 and 6.5 μM, respectively. These assay data validated our hypothesis and provide the basis for planned expansions of the chimera motif for integrin antagonist design.

In conclusion, we have successfully extended our oxidative enol ether rearrangement methodology toward the construction of steroidal RGD mimics. These chimeras contain structural elements from three major classes of natural products, i.e., steroids, spiroketals, and peptides. The biological studies provided evidence for potent integrin CD11b/CD18 antagonistic effects for the glycine-containing **13** and its corresponding N-debenzylated primary amide analog. Further work will mainly focus on determining selectivity and binding sites for these and related antagonists.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds, including copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) See, for example Gentilucci, L.; Cardillo, G.; Squassabia, F.; Tolomelli, A.; Spampinato, S.; Sparta, A.; Baiula, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2329–2333.

(24) The assay was an adaptation of Park, J. Y.; Arnaout, M. A.; Gupta, V. *J. Biomol. Screen.* **2007**, *12*, 406–417.

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(28) Generated in situ from fluorescein isothiocyanate and Fmoc-lysine.