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Single-Step Formation of Structurally Defined Bicyclic Peptides via S_NAr Cyclization

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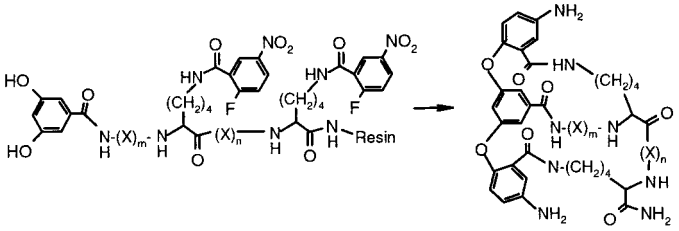
ABSTRACT



A solid-phase methodology for macrocyclization via an S_NAr reaction has been developed for the unambiguous formation of bicyclic peptidic compounds in a single cyclization step. The cyclization strategy involves the reaction of a 3,5-dihydroxybenzoyl group with two nitrofluorobenzoyl moieties. The symmetry of the dihydroxy aromatic ring results in a single product, and the remaining nitro groups are subsequently reduced to anilines and acylated.

Peptidic molecules continue to be popular as initial leads for drug discovery, particularly in the increasingly important area of protein−protein interactions.[1] Despite generally unfavorable pharmacological characteristics, peptides often provide attractive starting points for drug design and often are developed as drug candidates.[2] Linear peptides are generally unstructured in solution but bind to receptors in a discrete conformation. Macrocyclization constrains the conformation, which can increase binding affinity, reduce enzymatic degradation rate, and make peptidomimetic design easier. Indeed, a number of cyclic naturally occuring and synthetic peptides have been identified as potent leads or drug candidates.[1d,2a,3]

A number of naturally occurring cyclic peptides with potent biological activities, such as the conotoxins[4] and other peptide toxins,[5] have more elaborate polycyclic structures. These molecules display high potency and selectivity and have afforded structure-based design of new compounds based on their rigid structures and well-defined structure−activity relationships.[6] Other polycyclic peptidic molecules that have been identified include the mammalian defensin

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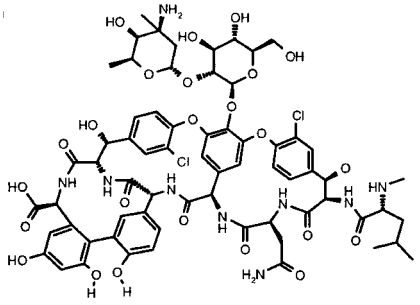
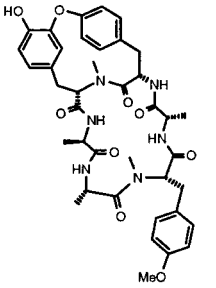
antimicrobial peptides[7] and a variety of plant-derived peptides with antimicrobial and other interesting activities.[8] For example, a 21-residue tricyclic peptide from a *Streptomyces* strain has shown inhibition of HIV replication with low cytotoxicity,[9] and more recently, the unique, plant-derived bicyclic peptide moroidin was found to possess antimitotic activity.[10]

Biaryl-ether containing compounds, such as the potent antibiotic vancomycin and bicyclic hexapeptide antitumor antibiotic deoxybouvardin, represent another interesting class of cyclic peptidic molecules.[11] There has been significant



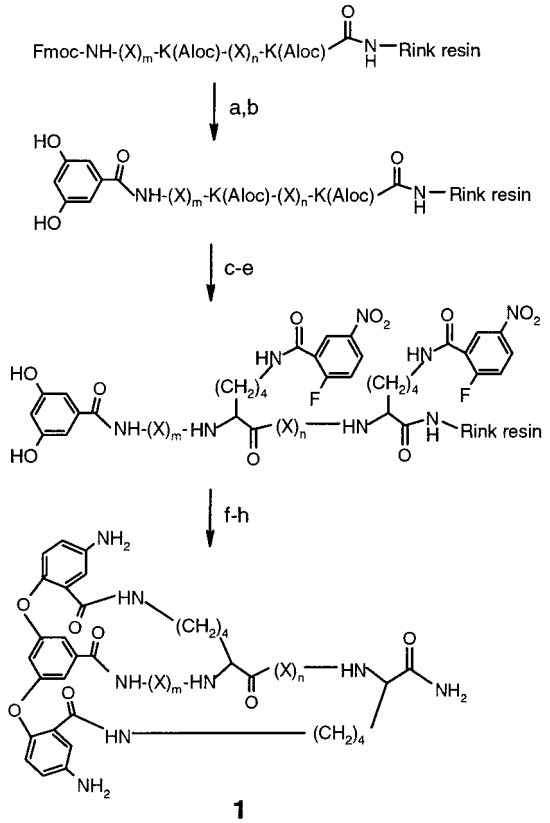
Vancomycin



Deoxybouvardin

interest in the synthesis of such molecules recently.[11b,12] Of particular utility has been the S_NAr reaction in which cyclization occurs through an electrophilic, nitro-substituted aromatic ring.[12a,13] A bicyclic vancomycin-derived compound was obtained via a "one-pot" double S_NAr reaction in solution.[14] As part of our program to design new polycyclic, conformationally constrained platforms for drug discovery, we have developed a versatile, one-step, solid-phase approach to formation of bicyclic structures via the S_NAr reaction. The key reagent is a symmetrical 3,5-dihydroxybenzoyl moiety, which reacts with two fluoronitrobenzoyl rings elsewhere in a linear precursor resulting in a single bicyclic product. Here the chemistry is described, and the general versatility of the synthetic methodology is illustrated.

Scheme 1. Solid-Phase Synthesis of Bicyclic Biaryl-Containing Compounds via N-Terminal Dihydroxybenzoyl Group[a]



[a] Reagents and conditions: (a) 20% piperidine/DMF; (b) 3,5-dihydroxybenzoic acid, DIC/DMF; (c) 3 equiv of $(Ph_3P)_4Pd$, $CHCl_3$/piperidine/HOAc (92:5:3); (d) $(C_2H_5)_2NCS_2Na$, DIEA/DMF; (e) 2-fluoro-5-nitrobenzoic acid, DIC/DMF; (f) 5% DBU/DMF; (g) 2 M $SnCl_2 \cdot 2H_2O$/DMF; (h) TFA/anisole/H_2O/TiPS (91:3:3:3).

The overall synthetic process is shown in Scheme 1. The linear peptide precursor was assembled via standard Fmoc peptide synthesis protocols.[15] After removal of the N-terminal Fmoc group, the free amine was acylated with 3,5-dihydroxybenzoic acid, which was preactivated with DIC in a 2:1 ratio to form the symmetric anhydride. Complete acylation was achieved in under 1 h with a 10-fold excess of symmetric anhydride. Deprotection of the Lys(Aloc) side chains by $(Ph_3P)_4Pd$ was followed by treatment with sodium diethyldithiocarbamate to remove the remaining Pd reagent. The resulting free amino groups were acylated with 2-fluoro-5-nitrobenzoic acid, which was preactivated with DIC in a 2:1 ratio to form the symmetric anhydride. The coupling reaction was generally complete in under 1 h with a 10-fold excess of symmetric anhydride. Cyclization was initiated by addition of 5% DBU/DMF and was completed after 4−5 h at room temperature. The color of the resin became deep orange over time. Other authors have used K_2CO_3 to catalyze S_NAr reactions with phenolic nucleophiles[13c,d,e] while Kise-

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lyov et al.[13b] reported problems with K_2CO_3 and excellent results with DBU. Cyclization of **1a** with 0.02 M K_2CO_3 in DMF for 4 h gave the desired bicyclic product with a purity and yield similar to that obtained using DBU/DMF.

Treatment of solid-supported cyclic peptides and other molecules with a 2 M solution of $SnCl_2 \cdot 2H_2O$ in DMF has been found to efficiently reduce the nitro groups,[13a,16] allowing further elaboration via acylation of the resultant aniline.[13a] Similarly, a 2 M $SnCl_2 \cdot 2H_2O$ solution reduced the two nitro groups on these bicyclic compounds cleanly within 4−5 h at room temperature. Acetylation with a 25:5:70 mixture of Ac_2O:DIEA:DCM was complete after 1 h. Acylation of the aniline nitrogen with a variety of Fmoc-protected amino acids was carried out overnight with a 10-fold excess of amino acid activated with PyAOP. Cleavage under standard TFA conditions (91:3:3:3 TFA:anisole:TIPS:H_2O) generated the cyclic products in generally good purity for a number of sequences (Table 1). Overall yields, based on the initial substitution level of the Fmoc-amide resin, were in the range 40−50% (Table 1), comparable to previously reported S_NAr-derived macrocyclic peptides.[13a−c]

Table 1. Representative Biaryl Ether Containing Bicyclic Peptides

compd	$(X)_m$	$(X)_n$	purity[a]	yield (%)
1a	Q-L-K	dF-R-W	77	47
1b	Y-R-L	dF-R-W	62	41
1c	K-Q-L	dF-R-W	80	48
1d	Q-L-K	P-R-W	81	40
1e	Q-L-K	dR-W-Q	70	43
1f	Q-L-K	R-W-Q	74	53
1g	Q-L	K-P-R-W	81	47
1h	Q-L-K-dF	R-W	72	41
1i	Q-dL-K-F	R-W	76	48
2a	Q-L-K	P-R-W	82	41
2b	Y-R-L	dF-R-W	70	47

[a] Purity (in %) based on reversed-phase HPLC at 220 nm.

Comparison of the sequences **1a** to **1c**, in which the N-terminal loop was varied and the C-terminal loop residues dPhe-Arg-Trp were constant, suggested the methodology had general utility. Often, a D-amino acid or Pro residue serves to induce a turn. Substitution of the dPhe residue present in **1a**−**c** with Pro (in **1d**) or dArg (in **1e**) resulted in equally efficient cyclization. Other sequence changes were also made in the C-terminal loop of **1e** with no adverse effect on cyclization efficiency. The importance of including a turn-inducing residue was probed with analogue **1f** (Arg at position 5), for which both the yield and purity were similar to that of **1e** (dArg at position 5), suggesting that such a turn-inducer is not necessary. Analogues **1g**,**h**,**i** have different loop sizes than **1a**−**f** (either two residues in the first loop and four in the second or vice versa). These molecules also cyclized efficiently, further demonstrating the versatility of this macrocyclization approach. In summary, the purities, as judged by the reversed-phase and size-exclusion HPLC results, were generally over 80%, similar to those reported for other S_NAr macrocyclization studies in which monocyclic products were generated.[13a,b,f]

Representative reversed-phase HPLC results for **1a** are given in Figure 1. The bicyclic product with nitro groups

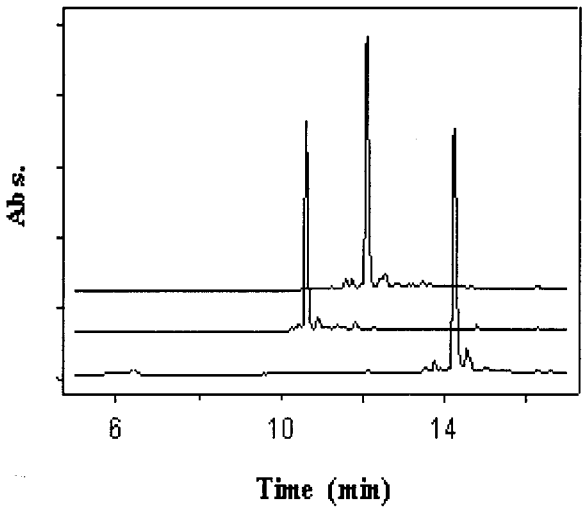

Figure 1. Reversed-phase HPLC results for **1a**. Chromatograms of peptide with nitro groups (lower trace), with nitro groups reduced to amines (middle trace), and with the amines acetylated (top trace). The mobile phase is an AB linear gradient of 10−100% B over 15 min at 1 mL/min where A = 0.05% TFA/H_2O and B = 0.05% TFA in 60:40 CH_3CN:H_2O. Column used is a Zorbax SB-C18 4.6 mm × 15 cm with 5 μm particle size.

remaining has a retention time of 14.2, which is reduced by almost 4 min upon reduction of the nitro groups to amines without any effect on purity. The acetylated peptide again displayed similar purity and a retention time increase of about 1.5 min. Size-exclusion HPLC confirmed the purity and the identity of the cyclic product as a monomeric form. Mass spectrometry (LC-MS) confirmed the identity of all products and showed that some of the latter-eluting impurities were due to oligomerization.

An additional strategy for formation of bicyclic peptides via S_NAr macrocyclization was pursued: in this case, the 3,5-dihydroxybenzoyl group was attached to a Lys side chain at the C-terminus of the peptide (Scheme 2). Acylation of the side chain amine of Fmoc-Lys with the dihydroxybenzoyl group was performed in solution prior to attachment of the amino acid to the solid phase. Chain elongation via the standard Fmoc protocol was then carried out, followed by $(Ph_3P)_4Pd$ removal of the Aloc group from the internal Lys residue and deblocking of the N-terminal amine. Subsequent acylation with 2-fluoro-5-nitrobenzoic acid and cyclization, as in Scheme 1, generated the cyclic product **2**. The analogues **2a** and **2b** were synthesized; they contain the same intervening tripeptide loops as **1d** and **1b**, respectively. Both yielded clean, cyclic products.

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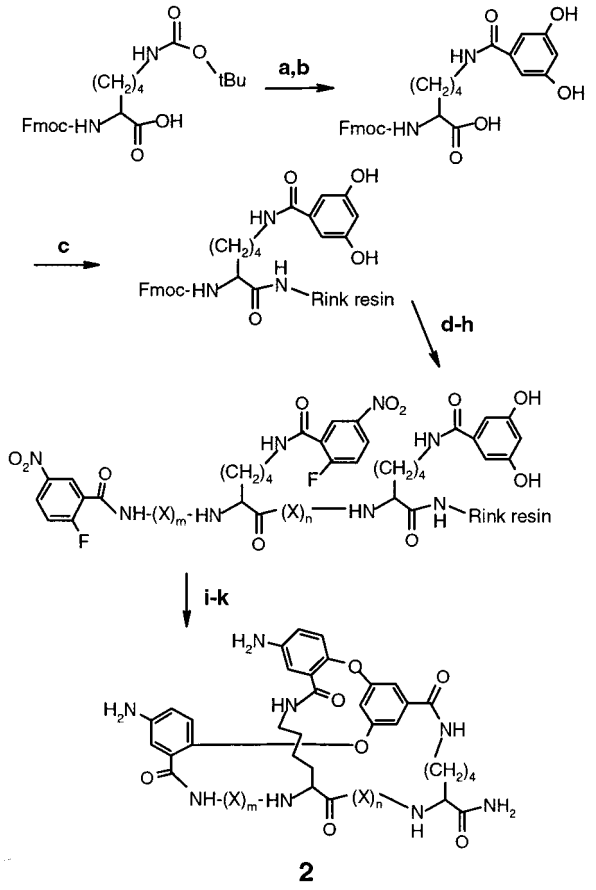
Scheme 2. Solid-Phase Synthesis of Bicyclic Biaryl-Containing Compounds via Lys Side Chain Attached Dihydroxybenzoyl Group[a]



[a] Reagents and conditions: (a) TFA/DCM/anisole; (b) 3,5-dihydroxybenzoic acid, DIC/CH_3CN:dioxane:H_2O; (c) DIC/DMF, NH_2-Rink resin; (d) solid-phase peptide synthesis (see ref 15); (e) 3 equiv of $(Ph_3P)_4Pd$, $CHCl_3$/piperidine/HOAc (92:5:3); (f) $(C_2H_5)_2NCS_2Na$, DIEA/DMF; (g) 20% piperidine/DMF; (h) 2-fluoro-5-nitrobenzoic acid, DIC/DMF; (i) 5% DBU/DMF; (j) 2 M $SnCl_2 \cdot 2H_2O$/DMF; (k) TFA/anisole/H_2O/TiPS (91:3:3:3).

Computer-generated, energy-minimized structures of **1d** and **2a** (Figure 2) suggest that the general shape of the compounds is similar. Comparison of the backbone conformations indicated a substantial degree of alignment for the α carbons of the amino acids in the tripeptide loops. The biaryl ether portion of the molecules differs more, particularly the orientation of the aniline group, which is highlighted. A structural difference is also suggested by the observation of a significantly higher retention time for **2a** and **2b** than for the corresponding analogues **1d** and **1b**, respectively, on reversed-phase HPLC. The different orientations of the aniline groups in compounds **1** and **2** offer further opportunity

(17) Kohn, W. K. Unpublished results.

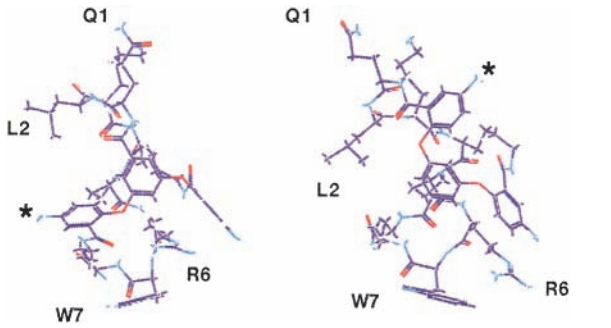


Figure 2. Structures of **1d** (left) and **2a** (right) generated using the program Cerius[2] version 4.0 from Molecular Simulations Inc. Energy minimization was carried out in vacuo using the cff1.01 force field. Marked with an asterisk (*) are the aniline groups of the aromatic rings attached to either the side chain amine of Lys8 (in **1d**) or the N-terminal amine (in **2a**). Also, for comparison, the positions of side chains of Gln, Leu, Arg, and Trp at positions 1, 2, 6, and 7, respectively, are indicated.

for molecular design in which constituents could be displayed from different faces of the molecule, while the core structure remains similar. However, NMR structures would be necessary to more accurately compare compounds **1** and **2**.

An alternative electrophilic ring moiety, 4-F-3-NO_2-benzoic acid, is commercially available and has been used successfully in S_NAr macrocyclization.[13a,17] However, attempts to cyclize **1a**−**c** using the 4-F-3-NO_2-benzoyl group in place of 2-F-5-NO_2-benzoyl proved unsuccessful in all three cases. The products displayed primarily oligomeric species, apparently due to intermolecular S_NAr reaction, which was evident from size-exclusion HPLC analysis.

In summary, we have developed a novel solid-phase macrocyclization methodology for single-step formation of unambiguous bicyclic structures in intermediate-sized peptidic molecules. The synthesis utilizes readily available building blocks and mild conditions. This and the general applicability of the reaction, as shown here for several different peptide sequences and intervening loop sizes, suggest it could be applied to parallel synthesis in order to generate large numbers of molecules for screening in biological assays or to structure-based design of modulators of protein−protein interactions.

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Supporting Information Available: Detailed synthetic and analytical procedures and mass spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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