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Tetrahedron Letters 45 (2004) 4153–4156
Tetrahedron Letters

Synthesis of a novel 14-membered highly constrained cyclic peptidic scaffold

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Received 26 February 2004; revised 15 March 2004; accepted 22 March 2004

Abstract—The synthesis and NMR analysis of a novel highly constrained scaffold is described. The 14-membered macrocyclic ring structure was inspired by many medicinally relevant natural products that also contain the bi-aryl ether moiety. The synthesis required only commercially available starting materials and involved a base mediated S_NAr cyclization. A conformational search was performed, which indicated a strong preference for a single conformation, which was consistent with observed ROE signals by NMR.

Cyclic peptides[1] are important compounds for medicinal purposes and represent an important class of natural products. Compared to their acyclic counterparts, cyclic peptides have more constrained conformations and are more resistant to degradation in vivo. The constriction of a peptide into a certain conformation can greatly enhance its binding to receptors due to reduced entropy loss upon binding. Many efforts have been made to develop methods of cyclization and a variety of reagents and catalysts have been developed for this purpose. Macrolactamization,[2] disulfide formation,[3] ring closing olefin metathesis,[4] and various cycloetherification techniques such as S_NAr cyclizations[5] are important methods for a synthetic chemist in the design of a desired cyclic peptide. By the same token, these tools are essential for the synthesis of mimics or derivatives of biologically active compounds aiming at improved activity or function. We here present a short synthesis of a highly constrained cyclic peptide scaffold that includes several sites that allow the introduction of structural variations.

The bi-aryl ether moiety is a common element in many natural products and also in synthetically constrained peptides. Numerous examples include the vancomycin class of antibiotics,[6] cycloisodityrosine derivatives,[7] piperazinomycin,[8] deoxybouvardin,[9] K-13,[10] and OF4949[11] (Fig. 1). The medicinal functions of these products range from antitumor agents, enzyme inhibitors (including HIV integrase, and angiotensin I converting enzyme), and antibiotics.

A feature that differentiates the structure of these medicinally relevant molecules is the size of the

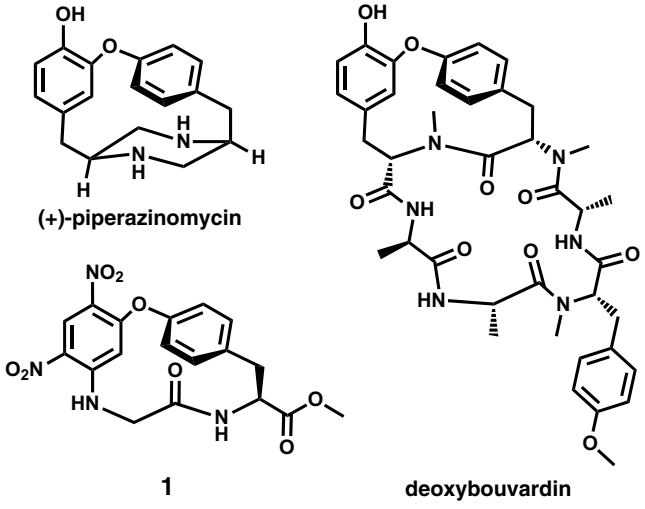

Figure 1. Structures of (+)-piperazinomycin and deoxybouvardin compared with **1**. These 14-membered macrocyclic examples contain a biphenyl ether moiety.

Keywords: S_NAR reaction; Cyclic peptide; Bi-aryl ether.
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doi:10.1016/j.tetlet.2004.03.131

macrocyclic ring, which ranges from 14 to 17. Without considering the differences in functionality elsewhere in the molecule, a general scaffold that harbors such a highly constrained macrocyclic ring, and that allows functionalization could be a useful starting point to find novel biologically active entities. Previously in our laboratory small mimics for vancomycin were studied.[12,13] An S_NAr cyclization was employed to form biphenyl ether macrocyclic compounds (16-membered rings) and was successful for solution and solid-phase applications. Described herein is the solution phase synthesis of **1**, a mimic for piperazinomycin and deoxybouvardin. Similar to these natural products, the novel highly constrained scaffold **1** also contains a 14-membered ring, and includes a biphenyl ether bridge. Compound **1** was synthesized using commercially available starting materials in five steps, which included 1,5-difluoro-2,4-dinitrobenzene, an inexpensive reagent used for protein crosslinking[14] but also used in organic synthesis.[15]

The solution phase synthesis began with tyrosine, which was protected with a methyl ester on the C-terminus and a benzyl group on the phenolic OH (Scheme 1). This compound was coupled to Boc-protected glycine with BOP and *i*-Pr_2NEt in CH_2Cl_2. Removal of the protecting groups was achieved by sequential hydrogenation on Pd/C and TFA/CH_2Cl_2 1:1 treatment. The N-terminus of the dipeptide was first linked to 1,5-difluoro-2,4-dinitrobenzene using NEt_3 as a base to give **3**,[16] which was isolated in 58% overall yield after column chromatography. 1,5-Difluoro-2,4-dinitrobenzene is highly reactive in nucleophilic aromatic substitutions because of the nitro functionalities *ortho* and *para* to the carbons bearing a fluorine. Upon substitution with the first nucleophile, the second point of substitution was observed to be slightly less electrophilic since compound **3** was a stable yellow solid, and could be stored at room temperature for months without degradation. Cyclization was achieved by treatment with 4 equiv of K_2CO_3 in DMF, which gave the highly constrained **1**[17] in an isolated yield of 38%. Mass spectrometry confirmed the cyclization to **1**, which proved to be very stable in air at room temperature.

Scaffold **1** is highly functionalizable due to its five potential points of derivatization (Fig. 2). The C-terminus can be easily functionalized, the secondary aromatic amine is a possible second point of variation, and upon reduction of the nitro functionalities, a third and fourth handle for functionalization are possible although no differentiation is easily envisioned. A fifth potential point of derivatization represents the amino acid side chain by using other amino acids instead of glycine.

The structural identity of **1** was confirmed by NMR. To this end TOCSY, ROESY, and HSQC experiments measured at 500 MHz were performed. The cyclization reaction was clearly observed by the disappearance of the fluorine–hydrogen coupling observed in **3**. The aromatic proton flanked by both nitro groups was seen at 8.87 ppm ($J_{H-F} = 8$ Hz), and the other aromatic hydrogen on the nitro-aromatic was seen at 6.68 ppm ($J_{H-F} = 14.6$ Hz). Upon cyclization, these signals became singlets and were observed at 8.82 and 4.02 ppm! The large pronounced shift in the latter can be explained by

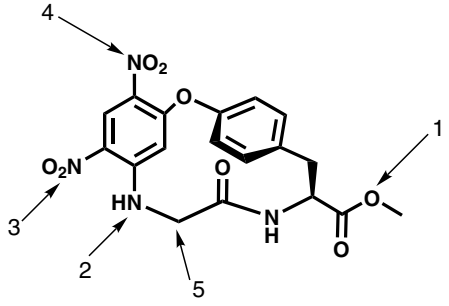
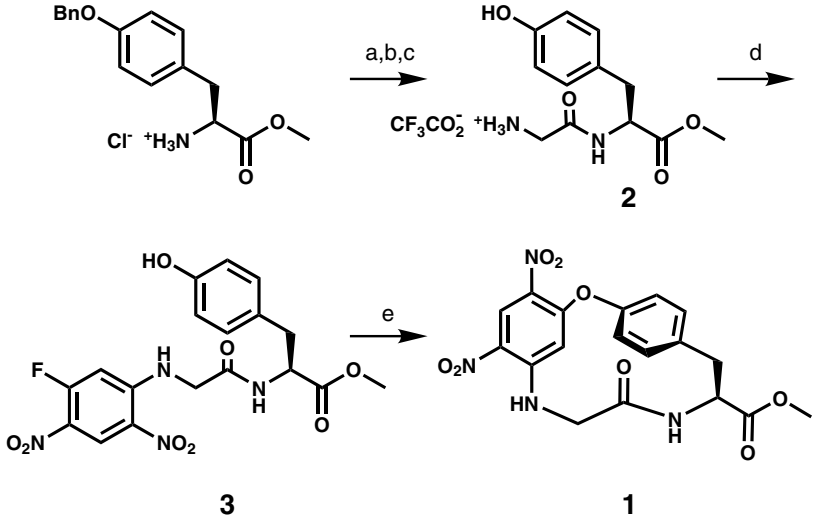


Figure 2. Potential points of variation in **1**.



Scheme 1. Reagents and conditions: (a) Boc–Gly–OH, BOP, *i*-Pr_2NEt, CH_2Cl_2, 3 h. (b) H_2 Pd/C, EtOAc/MeOH, 3 h. (c) TFA/CH_2Cl_2 1:1, 1 h. (d) 1,5-Difluoro-2,4-dinitrobenzene 1 equiv, NEt_3, EtOAc, 30 min., steps a–d 58%. (e) K_2CO_3, DMF, 3 d. rt 38%.

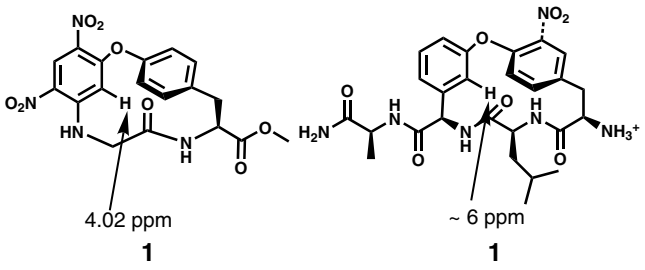


Figure 3. Chemical shifts of **1** in DMSO-d_6 and vancomycin mimic **4**.

its close proximity to the flanking aromatic ring. This effect was also previously seen in our 16-membered vancomycin mimics,[12,13] although less pronounced as the relevant resonance was observed at ~6 ppm (Fig. 3). The assignment of the high-field aromatic proton was confirmed by the observation of a signal at 4.02 (^{1}H) and 106 (^{13}C) ppm in the ^{1}H–^{13}C HSQC spectrum of **1**. The ^{13}C chemical shift confirmed the aromatic nature of the carbon. Another interesting observation was the differentiation of all the tyrosine aromatic protons due to their distinct chemical environments, resulting in four unique chemical shifts: 7.52, 7.36, 7.28, 6.94 ppm (in CD_3OD).

In order to obtain an idea about the preferred conformation of the rigid structure of **1**, a conformational search[18] was undertaken using MacroModel v. 7.0.[19] A group of highly similar conformations was found to have the lowest energy (Fig. 4). This conformation was consistent with ROE signals observed by NMR. The ROESY experiment indicated a short interproton distance between the amide NH and one of the glycine CH signals (3.28 ppm) and a significant longer one to the other glycine CH signal (4.07 ppm). Furthermore only a weak ROE signal was observed between the amide NH and the aromatic-H at 4.02 ppm. This profile is consistent with the structure shown and not with higher energy alternative structures with either the glycine CH_2 group or the amide bond rotated away.

In summary, the synthesis of a novel bio-inspired scaffold was achieved in five synthetic steps in an overall yield of 22%. Its identity and conformation were elucidated by NMR and molecular modeling.

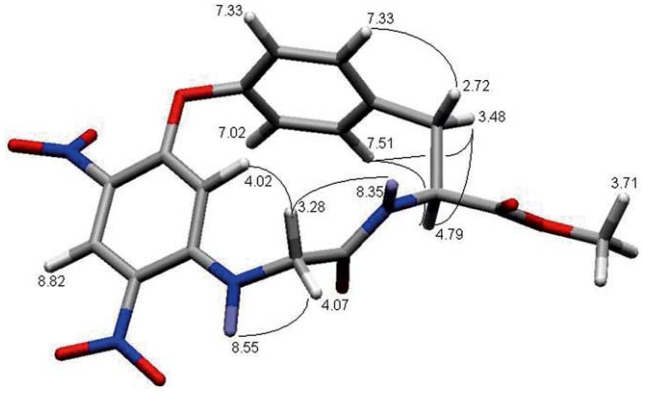


Figure 4. Lowest energy structure of **1** found by molecular modeling. Chemical shift assignment in DMSO-d_6 are indicated and also the observed ROE signals labeled as strong.

Acknowledgements

We would like to thank Hans Hilbers for help with modeling and Dr. Johan Kemmink for help with NMR. We also would like to thank the Royal Netherlands Academy of Arts and Sciences (KNAW) for a fellowship to R.J.P. and acknowledge support from Prof. Dr. R. M. J. Liskamp.

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16. Compound **3**: $R_f = 0.63$ (EtOAc). ^{1}H NMR (DMSO-d_6): $\delta = 2.78$ (dd, $J = 9.3$ Hz, $J = 13.7$ Hz, 1H), 2.93 (dd, $J = 5.2$ Hz, $J = 14$ Hz, 1H), 3.60 (s, 3H), 4.13 (d, $J = 5.5$ Hz, 2H), 4.40–4.49 (m, 1H), 6.60 (d, $J = 8.2$ Hz, 2H), 6.68 (d, $J = 14.6$ Hz, 1H), 6.95 (d, $J = 8.2$ Hz, 2H), 8.63 (d, $J = 7.7$ Hz, 1H), 8.87 (d, $J = 8$ Hz, 1H), 9.05 (br s, 1H), 9.22 (s, 1H).
17. Compound **1**: $R_f = 0.47$ (EtOAc). ^{1}H NMR (DMSO-d_6): $\delta = 2.72$ (t, $J = 12.6$ Hz, 1H), 3.28 (d, $J = 17.0$ Hz, 1H), 3.48 (dd, $J = 4.6$ Hz, $J = 12.6$ Hz, 1H), 3.71 (s, 3 H), 4.02 (s, 1H), 4.07 (d, $J = 16.5$ Hz, 1H), 4.79 (m, 1H), 7.02 (dd, $J = 2.2$ Hz, $J = 8.5$ Hz, 1H), 7.33 (m, 2 H), 7.51 (dd, $J = 1.7$ Hz, $J = 7.2$ Hz, 1H), 8.35 (d, $J = 10.1$ Hz, 1H), 8.55 (br s, 1H), 8.82 (s, 1H). MS: $m/z = 417.2$ $[M + H]^+$.
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