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ON-RESIN MACROCYCLIZATION OF PEPTIDES VIA INTRAMOLECULAR SNAr REACTIONS

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Abstract: On-resin macrocyclization via an SNAr reaction is employed in the synthesis of tocinoic acid analogs. Specifically, an N-terminal nitrofluorobenzene is attacked by a nucleophilic C-terminal sidechain. The remaining nitro group can be reduced and acylated. NMR is used to compare the conformation of the new macrocyclic peptides to tocinoic acid.

Introduction

Macrocyclic peptides in which the cystine bridge has been replaced have been the subject of considerable study.[3] These novel macrocycles often have improved pharmacokinetic[4] and conformational properties relative to their cognate peptides. Peptide macrocyclization through aromatic sidechains is found in strategies toward the synthesis of natural products such as vancomycin,[5] and teicoplanin,[6] and in the total synthesis of RA-VII,[7] deoxybouvardin[8] and K-13.[9] In the course of their total syntheses of RA-VII and deoxybouvardin, Boger et al. first[7ab] closed the macrocycles via substitution on the aromatic sidechain using the Ullman reaction and subsequent improvements enlisted the SNAr reaction.[7cd] Zhu's approach to K-13 is to use the SNAr reaction, the electrophilic ring being activated by a nitro group, which is later replaced.[9] Of particular interest has been Burgess' use of SNAr macrocyclizations to impose β-turn conformations on the peptide residues of the macrocycle.[9] As part of our search for efficient, on-resin routes to new macrocycles, we have studied the SNAr reaction as a general method for preparing conformationally constrained cyclic peptides on the solid phase. To illustrate the synthetic method and assess the conformational impact of incorporating the nitrobenzene into the macrocycle, we prepared a number of analogs of tocinoic acid (Cys-Tyr-Ile-Gln-Asn-Cys, cyclic disulfide) which has been studied extensively by NMR.

Results and Discussion

Tocinoic acid derivative **2** was prepared using the standard Fmoc protocol for peptide synthesis[10] on polystyrene Wang resin[11] with trityl as the sidechain protecting group on the C-terminal Cys. After removal of the Fmoc from the Tyr residue, the free amine was acylated with 2-fluoro-5-nitrobenzoic acid using diisopropylcarbodiimide and 1-hydroxy-7-azabenzotriazole (DIC and HOAT).[12] The presence of aromatic

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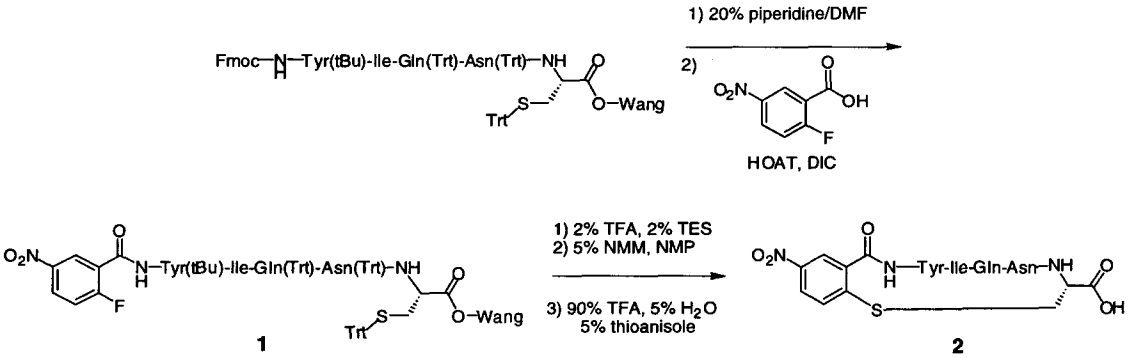
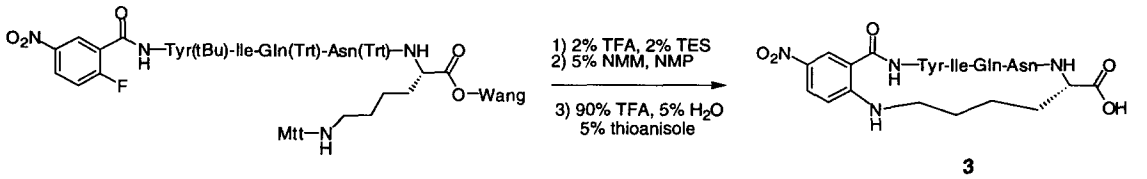
fluoride can be confirmed by gel-phase ^{19}F NMR.[13] The resulting solid-supported benzamide **1** was treated with 2% TFA and 2% Et_3SiH (TES) in CH_2Cl_2 four times (total time = 60 min) to expose the cysteine sulfhydryl. With longer reaction times or higher acid concentrations the yields of the product decreased due to the premature cleavage of the peptide from the resin.[14] Cyclization was initiated with 5% *N*-methylmorpholine (NMM) in *N*-methylpyrrolidinone (NMP) for 2–12 h. During this time, the disappearance of free thiol was monitored by Ellman's reagent.[15] Once liberated from the resin using a standard cleavage cocktail (90% TFA, 5% H_2O, 5% thioanisole), the crude material (71 mg, 37% yield, based on the loading of FmocCys(Trt)-Wang) showed only cyclized product **2** present in >90% purity by RP-HPLC (C_{18}).

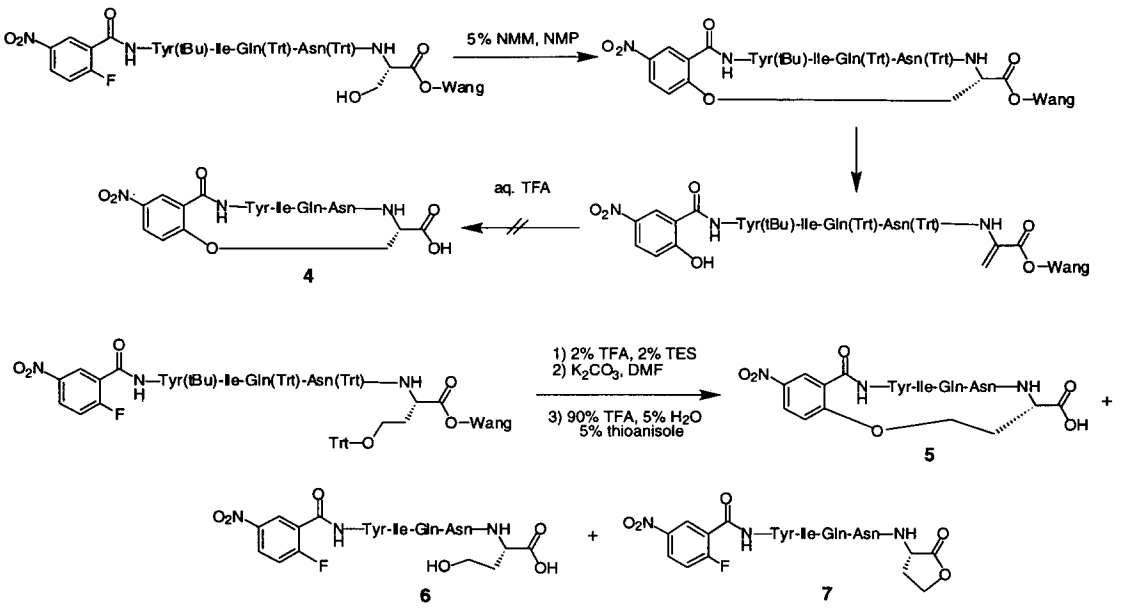


Scheme 1

In addition to Cys, closure of the macrocycle was successful with Lys as the internal nucleophile (**3** in Scheme 2). Closure via Ser did not yield the desired macrocycle **4**. The Ser β-OH is the poorest nucleophile of the series, but even if occurs, the resulting phenyl ether may undergo facile elimination to produce an N-terminal phenol and C-terminal dehydroalanine (Scheme 3). When the γ-OH of homoserine serves as the nucleophile, macrocyclization did not proceed at ambient temperature in 5% NMM/NMP; K_2CO_3 in DMF at 80°C was required to produce **5**. However, the uncyclized product **6** and the lactone **7** were also present in the sample after cleavage from the resin.[9]

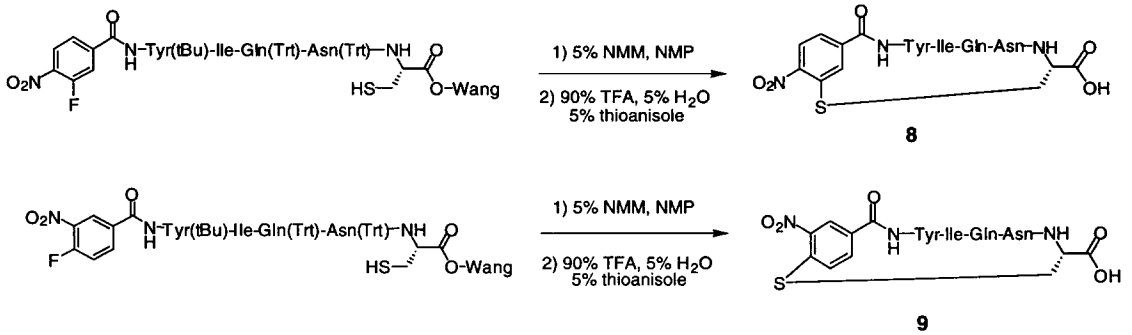


Scheme 2



Scheme 3

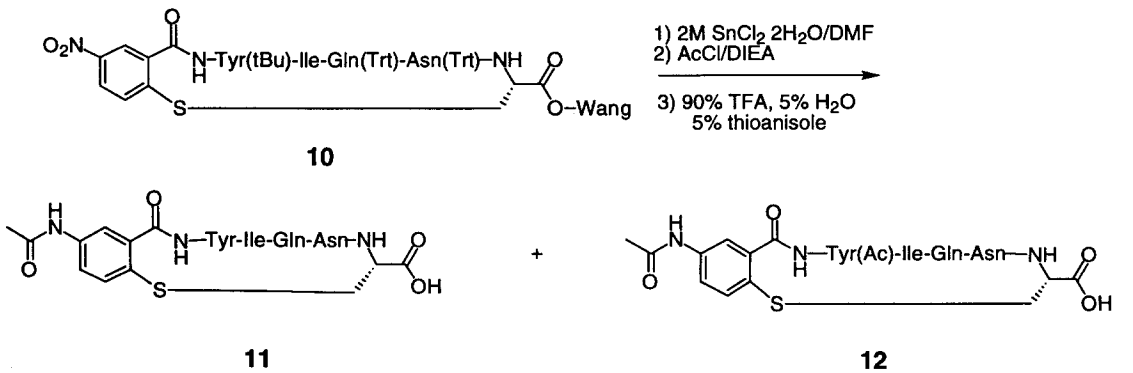
With the Cys sulfhydryl as nucleophile the macrocyclization was relatively insensitive to the regiochemistry on the nitrobenzoate: in addition to *ortho* (see Scheme 1), the *meta* and *para* fluorides were readily displaced, yielding peptides **8** and **9**, respectively (Scheme 4).



Scheme 4

The principal function of the nitro group is to activate the ring toward addition of the nucleophile. In Zhu's synthesis of K-13[8] the nitro is converted to the requisite hydroxyl in three steps by palladium-catalyzed hydrogenation, diazotization, and hydrolysis of the diazonium salt. We chose to reduce the nitro to the amine to provide a handle for attachment of further functionality. Chemoselective reductions of aromatic nitro groups are frequently heterogeneous and, therefore, unsuited to solid-supported substrates. Pavia et al.[16] circumvented

this problem by using homogeneous $SnCl_2 \bullet 2H_2O$ in DMF, a modification of a method reported by Bellamy and Ou. [17] Thus, exposure of solid-supported, cyclized peptide **10** to a 2 M solution of $SnCl_2 \bullet 2H_2O$ in DMF efficiently reduced the nitro group. Subsequent treatment with acetyl chloride followed by cleavage from the resin afforded peptide **11** (7 mg, 5% yield, based on the loading of FmocCys(Trt)-Wang; see Scheme 5). The *bis*-acylated product **12** was also isolated (6 mg, 4%), indicating that the partial deprotection of the *tert*-butyl ether on Tyr probably occurred during treatment with TFA.



Scheme 5

NMR Analysis

^{1}H NMR spectroscopy was used to compare the peptide conformation of **2** and tocinoic acid in DMSO. As expected, the largest chemical shift differences between corresponding proton resonances in **2** and tocinoic acid were observed for the protons closest to the Aryl-1 group, in particular for the Cys-6 α-proton ($\Delta\delta$ = 0.46 ppm). Interestingly, the Ile-3 NH resonance in tocinoic acid is significantly broadened, while for **2** no such line broadening was observed. Analysis of $^3J_{HH}$ coupling constant and quantitative NOE data indicated that both **2** and tocinoic acid adopt highly populated conformations with β-turns centered on Gln-4 and Asn-5.[18] For both peptides this conformation is supported by the presence of a non-sequential Gln-4 $_{\alpha CH}$-Cys-6$_{NH}$ NOE, a strong Asn-5$_{NH}$-Cys-6$_{NH}$ NOE cross-peak, and a small Gln-4 $^3J_{N\alpha}$ coupling constant (4.0 Hz and 4.2 Hz in **2** and tocinoic acid, respectively).

The lowest energy conformations of tocinoic acid and **2** generated from the NMR data using XPLOR[19] are shown in Figure 1. The structures of the two peptides reveal that the greatest conformational perturbation caused by replacing the Cys-1 with the nitrobenzene ring occurs for the ϕ and χ_1 dihedral angles of Cys-6. In tocinoic acid the Cys-6 ϕ and χ_1 dihedral angles are approximately +50° and -80°, respectively. In contrast, for **2**, the Cys-6 ϕ is approximately -85° and the Cys-6 χ_1 is approximately +75°.

The NMR data therefore suggest that the primary influence of substitution of the nitrobenzene ring for Cys-1 in tocinoic acid is on the local conformation of the residues immediately adjacent to the point of substitution (i.e. Cys-6). Also, the predominant conformational feature of the β-turn found in tocinoic acid is retained in the nitrothioether **2**.

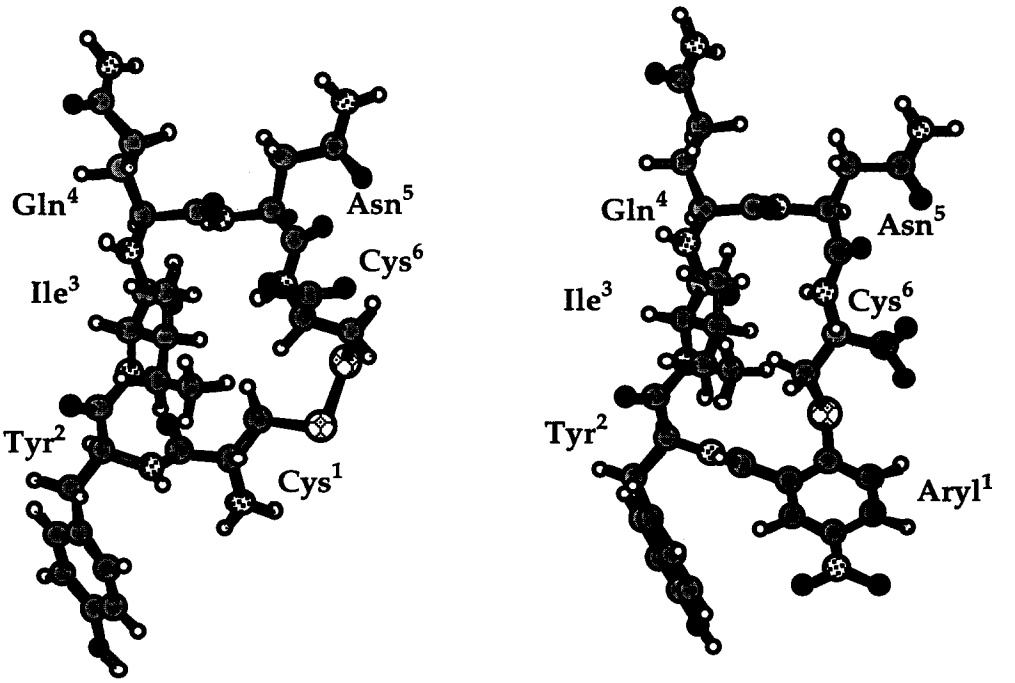


Figure 1. Left, tocinoic acid; right, compound **2**

Conclusions

On-resin macrocyclization of peptides via intramolecular SNAr reaction yields novel peptide analogs using a variety of sidechain nucleophiles and all three regioisomeric fluorobenzoyl groups. In the case of tocinoic acid, the replacement of the N-terminal Cys with *ortho*-nitrobenzoate yielded a new analog with conformational properties similar to those of the parent peptide. The activating nitro group can be reduced and acylated, allowing further elaboration of the macrocycle.

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References and Notes

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