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3,7-FUNCTIONALIZED-10-METHYL PHENOTHIAZINE: A POTENTIAL TURN SCAFFOLD IN PEPTIDOMIMETICS

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Abstract. Molecular modeling studies suggest that the phenothiazine nucleus, embedded in a peptide via attachments at the 3- and 7-positions, may be a possible surrogate for the α-carbon backbone of five residue turns in a variety of proteins. The synthesis of the orthogonally-protected Fmoc 3-aminoethyl-7-carboxyethyl-10-methylphenothiazine (**1**) is described. © 1997 Elsevier Science Ltd.

Scaffolding of the backbone is an established way to probe for the active conformation of a protein or peptide.[1] A suitable scaffold should mimic the active backbone motif (e.g., α helix, turn, or β sheet) and should permit suitable functionalization to append the required sidechain groups. The phenothiazine nucleus was selected as a potential scaffold for the middle three residues for a five residue turn. Figure 1 shows how a 3,7-functionalized phenothiazine orients X and Y, the peptide chains at, respectively, the amino and carboxy termini of the scaffold, with respect to the *i* and *i+4* residues flanking the turn. A ring-substituted phenothiazine might also be designed to add substituents congruent to the $R_{(i+1)}$, $R_{(i+2)}$, and $R_{(i+3)}$ sidechains, although α carbon chirality would be lost.

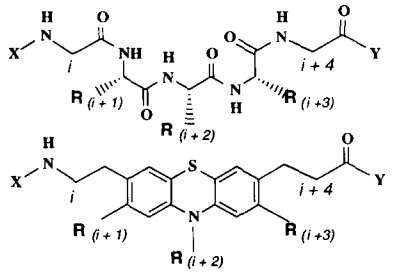

Figure 1: Sequence of a peptide replaced by phenothiazine scaffold. The portions X and Y of the peptide (above) are outside the turn; residues with sidechains $R_{(i)}$-$R_{(i+4)}$ describe the turn. These functional groups can be substituted onto the phenothiazine (below).

A search of secondary structures gave over 75 proteins containing this turn/loop motif in which the backbone atoms of the *i*+1, *i*+2, and *i*+3 residues overlap well with the phenothiazine template. In these proteins the distances between Cα(*i*) and Cα(*i*+4) are within 9.2-9.6 Å, and the distances between Cα(*i*+1) and Cα(*i*+3) are between 6.9-7.1Å.[2] The corresponding distances in the phenothiazine compound are 9.6-12.3 Å and 7.3 Å. In Figure 2, the phenothiazine is shown superimposed on one example of this turn motif that spans Asp133-Asn137 in the calmodulin structure determined by Babu et al.[3] In this example, the congruent region of the protein is one of the calcium-binding sites.

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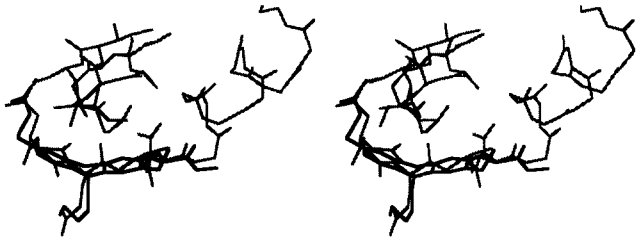
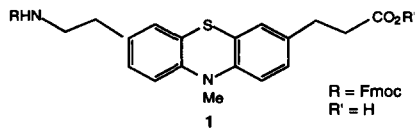


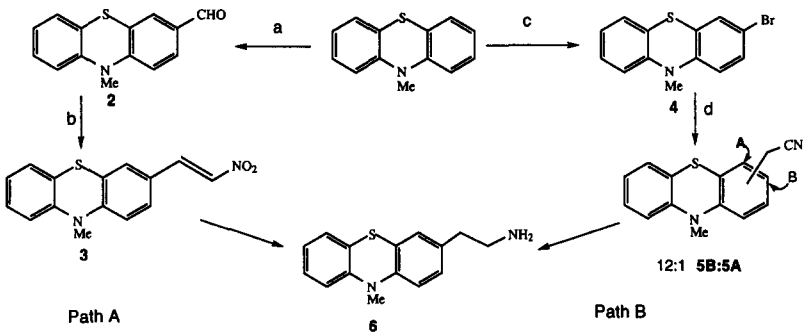
Figure 2: Stereo view of superimposed structures of calmodulin Asp133-Asn137 and 2-ethyl, 3-(2-N-methylamido)ethyl, 7-(2-aceto)ethyl, 10-pentylphenothiazine. The view shows the region of the protein backbone corresponding to the template.

In our target, phenothiazine **1**, the residues that compose the turn are replaced by the tricyclic ring system. Thus successful use of this surrogate requires that the sacrifice of the sidechains of *i+1*, *i+2*, and *i+3* be compensated by the proper orientation of the *i* and *i+4* sidechains. Positions 3 and 7, with orthogonal protection on the N- and C-termini, are the points of attachment to the peptide chains. It was expected that **1** would be amenable to both solution- and solid-phase chemistry.



1

Our initial attempts for the synthesis of **1** followed literature reports of phenothiazine formylation[1,4] and cyanomethylation.[5] In our hands, however, these reactions (Scheme I) gave disappointing yields and tedious separations. The generation of 2- cyanomethyl compound **5A** along with the desired regioisomer **5B** is likely to arise from an aryne intermediate.[6]

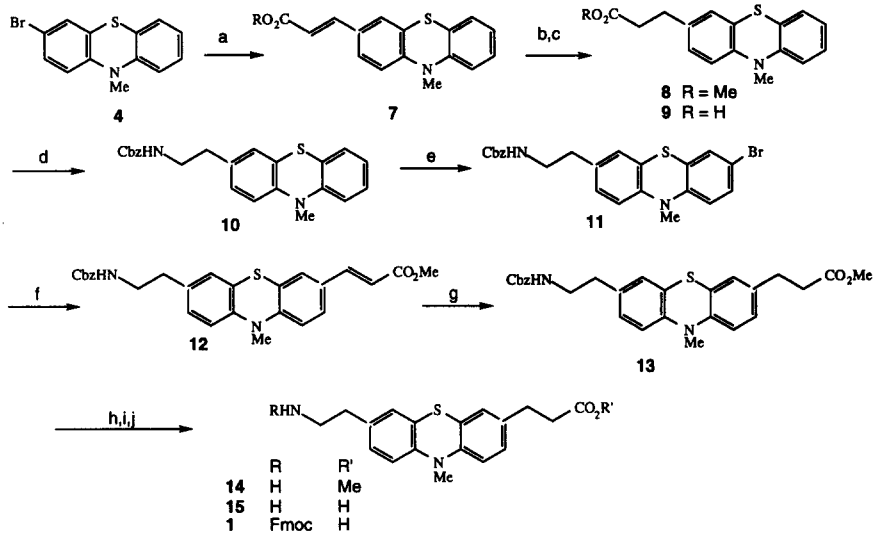


a. $POCl_3$, $HCONPh(Me)_2$ DMF b. CH_3NO_2, NH_4OAc c. CH_2Cl_2 - 10 °C
d. LDA, CH_3CN, THF - 40 °C

Scheme I

A more efficient strategy uses essentially the same chemistry to append both the aminoethyl and carboxyethyl sidechains.[7] As shown in Scheme II, arylbromide **4** was readily alkylated by the Heck reaction[8] to the α,β–unsaturated ester **7**.[9] Reduction,[10] saponification, and Curtius rearrangement gave **10**, protected as a benzyl carbamate. Partial repetition of the sequence (regioselective bromination, Heck reaction, reduction) gave the

methyl ester **12**, an intermediate (unlike **7**) unstable to silica chromatography. The Cu/$NaBH_4$ system was highly sensitive to the impurities in crude **12**, whereas Ni/$NaBH_4$ was not; the latter gave good yields of **13** that could then be purified. The Cbz protecting group at the N-terminus was exchanged for a Fmoc group as shown, and the ester saponified.



a. CO_2Me, $Pd(OAc)_2$, (*o*-tol)$_3$P, NEt_3, 155 °C, 74 %

b. $NaBH_4$ - Cu_2Cl_2, MeOH-THF (7:3) 0 °C, 87% c. LiOH, THF-H_2O, 95%

d. (i) EtOCOCl/NMM, (ii) NaN_3, (iii) BzOH/ Δ toluene, 55% e.  CH_2Cl_2, -10 °C, 72% f. reaction as in a

g.. $NaBH_4$ - $NiCl_2$, MeOH-THF (7:3) 0 °C, 31% from **11** h. HBr/ AcOH i. 2.1 equiv. NaOH j. Fmoc-OSu, THF-dioxane-10% Na_2CO_3, 73% from **13**

Scheme II

The phenothiazine nucleus occurs in biologically-active compounds that span a wide spectrum of therapeutic effects; atoms 3, 7, and 10 are frequently (although not exclusively) the substituted positions.[11] By modeling studies, we have superimposed peptide-substituted 3-aminoethyl-7-carboxyethyl phenothiazines on a commonly-observed turn in diverse proteins. We then synthesized a scaffold having a methylated N-10 and orthogonally protected amino and carboxy groups at, respectively, the 3- and 7-sidechains in order to test the hypothesis that active peptidomimetics could be generated from this ring system. Results of incorporating scaffold **1** in a series of novel compounds will be presented in a subsequent report.

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2. A search was carried on SYBYL's binary protein database using the protein database search option: SYBYL Molecular Modeling System, Version 6.1, TRIPOS Assoc., St. Louis, MO, U.S.A. Segments from various proteins in which the inter-Cα distance between residue *i* and *i+4* was between 9.2 to 9.6 Å and where the inter-C_α distance between residue *i+1* and *i+3* was between 6.9 Å and 7.1 Å were identified. When these segments (75 segments obtained from various proteins) were overlaid with Cα atoms of the calmodulin segment (3cln) Asp 133-Asn 137, they were found to have RMS deviation of ≤0.5Å.

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6. The regio isomers were separated by flash chromatography on silica gel (5% ethyl acetate/hexane). The assignments were based on distinct ^{1}H NMR signals for the CH_2 protons: δ 3.83 for **5A**, the earlier-eluting fraction, and δ 3.64 for **5B**. This is consistent with literature reports (ref 4) for this reaction and ^{1}H NMR assignments for a compound analogous to **5B**.

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9. On a large (25 g, 86 mmol) scale, a substantial amount of dealkylation to the α,β-unsaturated acid occurred, giving a ratio of 1.3:1 acid to ester. Although the mechanism was not rigorosuly investigated, we attribute this to incompletely neutralized HBr. Following realkylation to **7** with $KHCO_3$ and CH_3I, Scheme II was resumed and proceeded as shown.

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