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THE SYNTHESIS OF AMINOBENZAZEPINONES AS *ANTI*-PHENYLALANINE DIPEPTIDE MIMICS AND THEIR USE IN NEP INHIBITION

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Abstract: A general and stereoselective synthesis of 4-aminobenzazepinones is presented. This peptidomimetic structure was used in the preparation of MDL 100,407, a potent inhibitor of NEP.

In peptide/protein interactions, the interplay of amino acid side chains with their biological target is believed to be fundamental in conferring the ultimate biological activity. The side chain orientations with respect to a particular peptide backbone are defined by the χ dihedral angles. An analysis of structures in the Brookhaven Protein Data Bank revealed that the *anti* (χ_1=180°), *gauche*(−) (χ_1=−60°), and gauche(+) (χ_1=+60°) staggered conformations of an amino acid occur predominantly (Figure 1).[1] Compounds containing side chain constrained amino acids are particularly valuable in probing the active site of biological targets such as neutral endopeptidase (NEP; EC 3.4.24.11) where crystal structure data is not available. NEP is a zinc dependent protease which cleaves a number of biologically important peptides at the amino side of a hydrophobic residue (e.g., phenylalanine).[2] This report details a general and stereoselective approach to 4-aminobenzazepinones as *anti*-phenylalanine dipeptide mimics, and the incorporation of an *anti*-PheLeu mimic into MDL 100,407, a potential inhibitor of NEP.

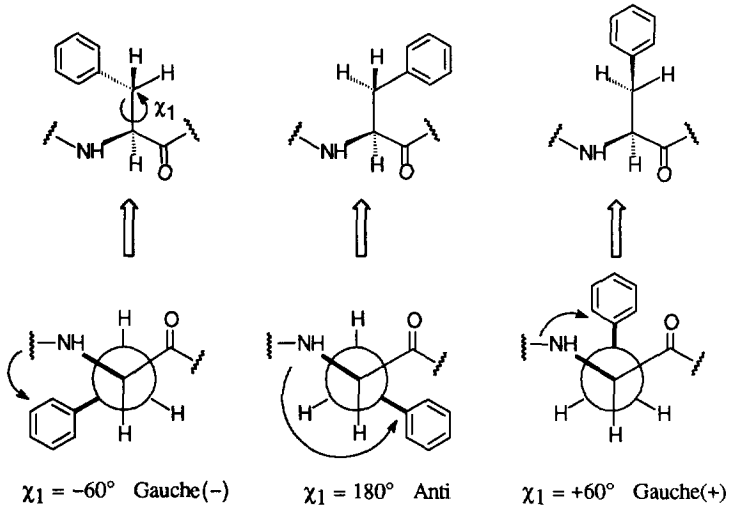


Figure 1. Amino acid side chain conformations: χ_1 preferences.

Two retrosynthetic strategies were devised for the preparation of the target molecule (Figure 2). Path A utilized an N-acyliminium ion cyclization to form the 7-membered lactam. This methodology has been used by our group for the efficient synthesis of *anti*-PheGly dipeptide mimics (R=H).[3] Unfortunately, only racemic product was formed in this case. At the start of the current study, we attempted to extend this methodology to *anti*-PheAla dipeptide mimics ($R=CH_3$) to examine the effects of an additional chiral center on stereoselectivity. Under various Lewis acid conditions, the desired lactam was isolated in good yields; however, at best a 4:1 mixture of diastereomers was observed.[4] An alternative path (B) using a reductive amination-lactam formation sequence was developed to overcome the lack of diastereoselection found in the iminium ion approach. A description of the successful implementation of this route follows.

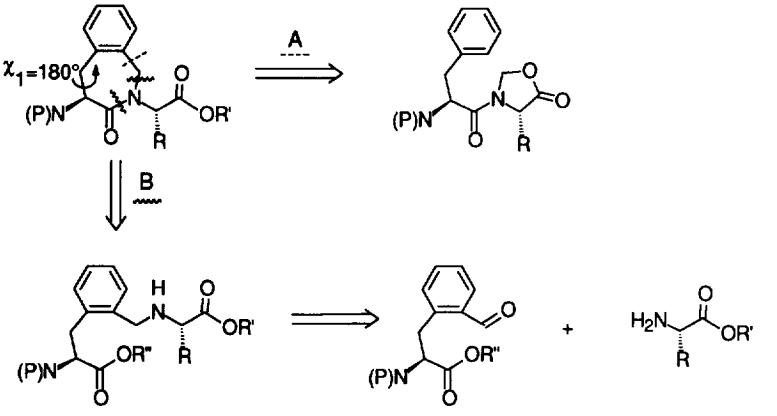
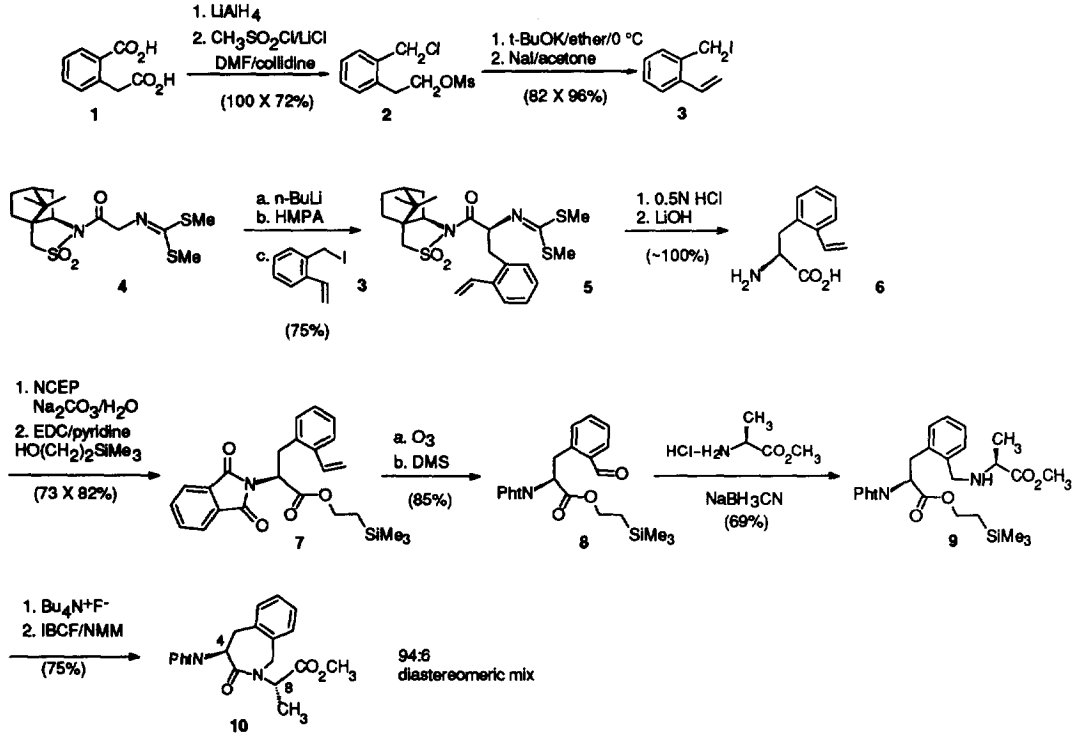


Figure 2. Retrosynthesis.

The success of Path B required the efficient synthesis of unnatural amino acid L-*ortho*-vinyl-phenylalanine (**6**) (Scheme 1). The Oppolzer sultam methodology proved effective in this regard.[5] The requisite electrophile (**3**) was prepared in 4 steps and 57% overall yield from commercially available homophthalic acid (**1**).[6] This diacid was reduced with $LiAlH_4$ in THF to the corresponding diol which was converted to the chloro mesylate **2** using mesyl chloride/LiCl/collidine in DMF.[7] Elimination of the mesyl group using KO*t*-Bu in ether followed by chloride displacement with NaI in acetone gave benzylic iodide **3**. Alkylation of sultam **4** yielded **5** in 75% yield, a single diastereomer as determined by ^1H NMR spectroscopy. Deprotection by the standard protocol afforded *ortho*-vinyl-phenylalanine (**6**) quantitatively. Protection of amino acid **6** using N-carbethoxyphthalimide followed by EDC/2-trimethylsilylethanol gave phthalimido ester **7** in 60% overall yield. Ozonolysis provided key aldehyde **8** in 85% yield. This aldehyde serves as a general intermediate for the preparation of *anti*-phenylalanine dipeptide mimics. Reductive amination of aldehyde **8** with L-alanine methyl ester and sodium cyanoborohydride afforded amino diester **9** in 69% yield. Fluoride induced silyl ester cleavage

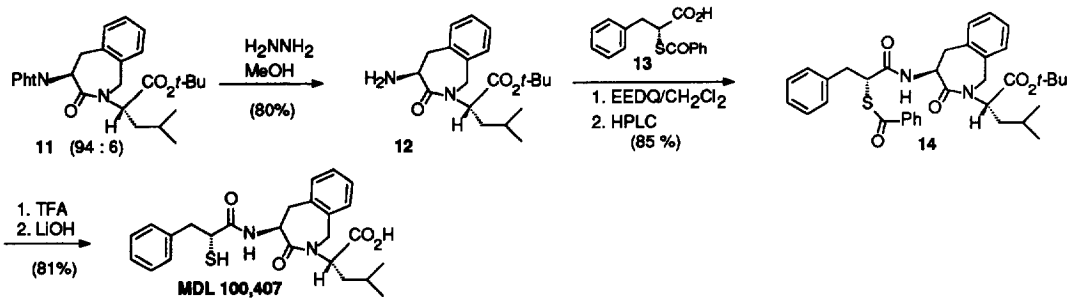
followed by isobutyl chloroformate-mediated cyclization led smoothly to the desired lactam (**10**) as a 94:6 mixture of diastereomers in 75% yield.

The stereochemical nature of these diastereomers was determined by substituting D-alanine methyl ester for the L-isomer in this sequence. Chiral HPLC analysis revealed that the chiral center bearing the phthalimide group had epimerized (i.e., lactam **10** was 94(4*S*,8*S*):6(4*R*,8*S*)). Although the point(s) at which epimerization occurs in this synthesis remains undetermined, this route represents a significant improvement over the N-acyliminium ion-based approach to *anti*-phenylalanine dipeptide mimics.



Scheme 1. Lactam synthesis.

The potential NEP inhibitor, MDL 100,407, was synthesized using L-leucine *tert*-butyl ester in the reductive amination-lactam formation sequence (Scheme 2). Again, the bicyclic lactam (**11**) was formed as a 94:6 mixture of diastereomers. The phthalimide group was removed using hydrazine in 80% yield. The resulting amine (**12**) was coupled with (*R*)-2-benzoylthio-3-phenylpropionic acid (**13**)[8] using EEDQ to give isomerically pure amide **14** in 85% yield after HPLC purification. Sequential ester cleavages with acid then base afforded MDL 100,407 in 81% yield.



Scheme 2. Preparation of NEP inhibitor MDL 100,407.

MDL 100,407 proved to be an excellent inhibitor of NEP (K_i = 0.4 nM) (Figure 3). Compared to the previously reported *gauche*(−) oxazepinone analog MDL 100,192[9], MDL 100,407 was greater than 800-fold more potent. Therefore, the S1' binding site of NEP prefers the *anti*-phenylalanine conformation over the *gauche*(−).[10] Interestingly, only a six-fold loss in potency was observed with MDL 100,407 compared to the highly constrained tricyclic inhibitor MDL 101,628.[11,12]

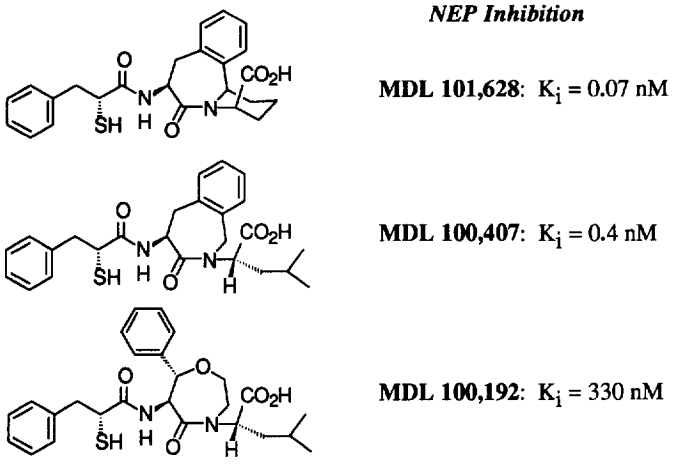


Figure 3. PheLeu constraint variation: NEP inhibition.[13]

Molecular modeling studies revealed differences in the space accessible to the bicyclic and tricyclic *anti*-phenylalanine containing compounds (Figure 4). Low energy conformations were generated with SYBYL using a systematic search. The conformations were minimized with MAXIMIN followed by a minimization with the AM1 Hamiltonian in MOPAC. Two low energy conformations for the bicyclic lactam were found. In one conformation, the carboxyl group does not overlap with the carboxyl of the tricyclic lactam. In both

conformations, the leucine side chain fails to overlap the methylenes of the tricyclic 6-membered ring. Either one or a combination of these observations may account for the differences in activities.

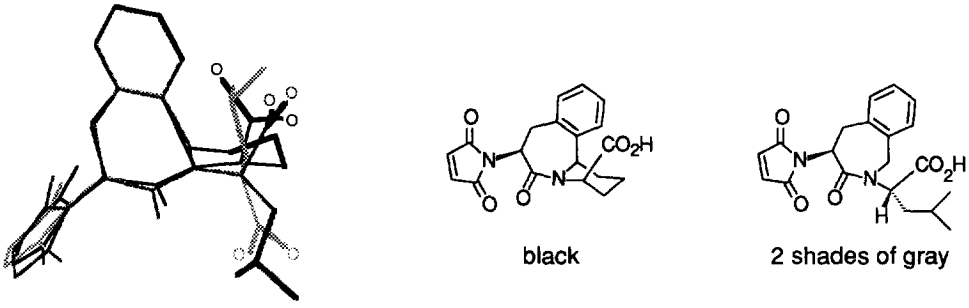


Figure 4. Overlay of models for MDL 101,628 and the two conformers of MDL 100,407. Carboxylic acid and butyl groups are highlighted by greater line widths.

In summary, a flexible and stereoselective approach to *anti*-phenylalanine dipeptide mimics has been developed and used in the preparation of MDL 100,407, a potent *anti*-PheLeu-based inhibitor of NEP. This inhibitor has served as a useful tool to probe the active site of NEP for which crystal stucture data is unavailable. Further SAR studies related to conformationally constrained NEP inhibitors are forthcoming.

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