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STEREOCHEMISTRY OF THE BENZODIAZEPINE BASED RAS FARNESYLTRANSFERASE INHIBITORS

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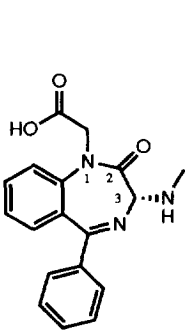
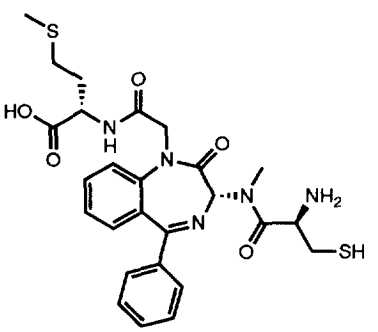
Abstract: Chiral benzodiazepine I is the preferred dipeptide turn mimic enantiomer employed in a series of Ras farnesyltransferase inhibitors. It was resolved as the camphorsulfonic acid salt of its methyl ester via a directed crystallization process. Crystallographic analysis of a derivative established R stereochemistry at C-3. The stereochemistry of the additional two chiral centers in derived inhibitor **II** is addressed.



I

(R)-N-Me-BZA



II

BZA-2B

Fig. 1. Dipeptide turn mimic (R)-N-Me-BZA and it's incorporation into Ras farnesyltransferase inhibitor BZA-2B, Cys(N-Me-BZA)Met-COOH. Inverting the stereocenter at position 3 drops activity over 400 fold.

Oncogenic Ras proteins have been implicated in many human cancers and are functionally dependent on the farnesylation of a cysteine residue near the C-terminus for their cell transforming ability. Compounds which inhibit Ras farnesylation may therefore have chemotheraputic value.[1] The enzyme that catalyzes the attachment of the farnesyl group, Ras farnesyltransferase (Ras FTase), is a logical target for intervention. Compound **I**, (R)-3-methylamino-1-carboxymethyl-2-3-dihydro-5-phenyl-1*H* -1,4-benzodiazepin-2-one), abbreviated (R)-N-Me-BZA, is a very effective replacement for the two central amino acids in a series of tetrapeptide Ras FTase inhibitors of the general formula CAAX, where C is cysteine, A is usually an aliphatic amino acid, and X is a carboxyl terminal methionine or serine.[2] Compound **II** illustrates the incorporation of (R)-N-Me-BZA into one such inhibitor, BZA-2B, which is over 400 times as active in the Ras FTase inhibition assay as the diastereomer

with inverted stereochemistry at position 3 (IC_{50}=0.85 nM vs. IC_{50}=370 nM).[3] Modeling studies suggest that the benzodiazepine scaffold functions as a natural dipeptide turn mimic, defining a favorable presentation of the cysteine and C-terminal methionine residues.[2]

Racemic N-Me-BZA (**III**) was used in the original synthesis of these inhibitors resulting in a mixture of diastereomers. Once separated, the individual isomers were reported as BZA-A or BZA-B according to their relative retention times on reversed phase HPLC.[4] The later eluting (B) diastereomer has always exhibited much greater activity. Following an elegant lead by Reider *et al.*[5], we have developed a directed resolution process (shown in **Fig.2**) for isolation of the preferred (R)-N-Me-BZA enantiomer as the (R)-camphorsulfonic acid salt of its methyl ester (**IV**).[6] The process was enabled by the facile racemization of the protonated benzodiazepine in warm solution. Racemization, very slow in solution at room temperature, is complete in less than two hours at 75 °C. By choosing a solvent and temperature combination where crystallization and racemization proceed concurrently, crystallization was directed to the desired enantiomer in ~80% yield.[7] Methanol is required in the crystallization solvent. Incorporated into the crystal lattice at 0.5 M relative levels,[8] it prevents formation of an almost unfilterable gel. The benzodiazepine amine must be protonated, increasing the acidity of the hydrogen at C-3, for racemization to occur. Saponification of the methyl ester and N-Boc protection allowed incorporation of the resolved (R)-N-Me-BZA into normal solid phase peptide synthesis [9] and was thereby shown to correspond to the active diastereomer BZA-2B (**II**). Enantiomeric purity of >95% for compound **IV** was measured by a chiral solid support Pirkle HPLC method.[10]

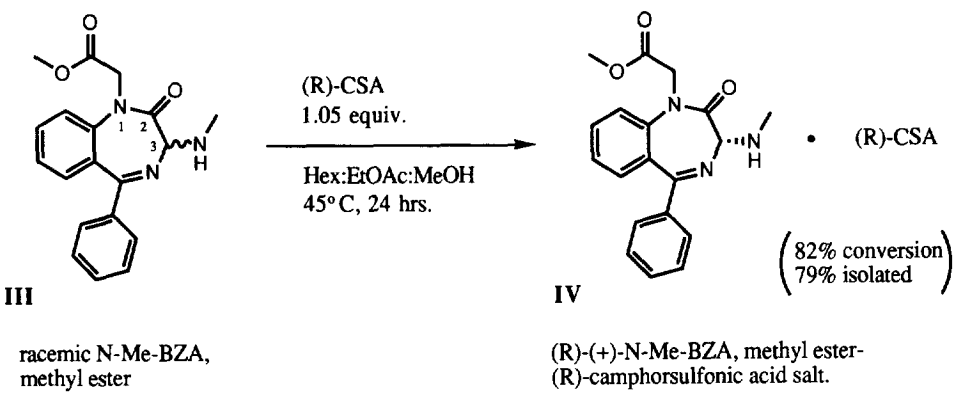
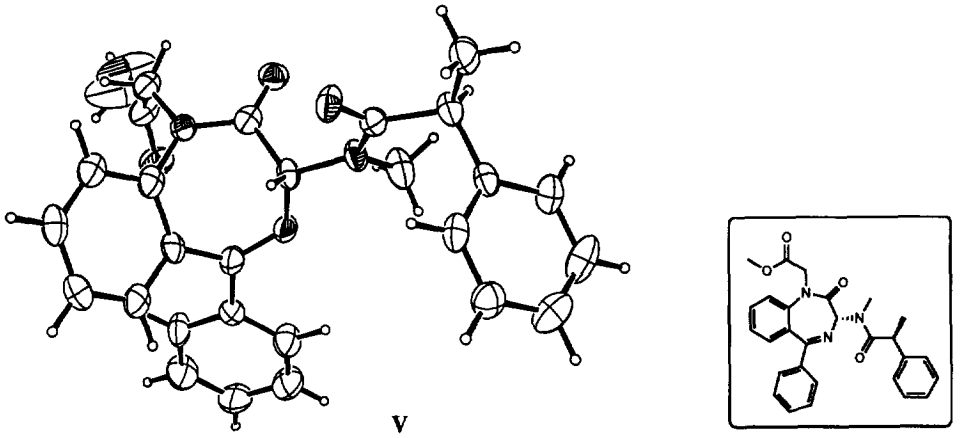


Fig.2. Directed resolution of N-Me-BZA. Under conditions where crystallization and racemization proceed concurrently, crystallization can be directed to the desired enantiomer in ~80% yield.[7] Resolved benzodiazepine **IV** was incorporated into inhibitor **II**. A crystal structure was obtained on a derivative (see **Fig.3.**).

The absolute stereochemistry of the resolved methyl ester (**IV**) was established by single crystal X-ray analysis on the derived (S)-2-phenylpropionamide (**V**), (**Fig.3**).[11] Data collection was done on colorless tabular crystals (grown from methylene chloride/hexane) at -115 °C on an Enraf-Nonius CAD-4 diffractometer. The structure was solved by direct methods (SHELXS) and refined via standard least-squares and Fourier techniques. The space group enantiomorph was chosen to correctly give the known (S) optical center.



V

Fig.3. ORTEP drawing from X-ray coordinates of crystalline (R)-N-Me-BZA-(S)-2-phenylpropionamide. Ellipsoids are scaled to the 50% probability surface while hydrogen atoms are represented by arbitrary small spheres for clarity.

The N to C terminal stereochemistry of inhibitor **II** is (R)-(S)-(S). By convention, the stereochemical designation at position 3 is reversed once the amine is acylated. The effect of stereochemical variation at the remaining two chiral centers in inhibitor **II** was assessed by solid phase synthesis of D-cysteine and D-methionine containing analogs following procedures previously described for inhibitor **II**.[2] The original CAAX tetrapeptide inhibitors did not tolerate sequential D amino acid substitution. Analogous substitution of D for L cysteine in inhibitor **II** dropped activity almost 100 fold (IC_{50}=0.85 nM to IC_{50}=80 nM). D-Methionine substitution was fairly well tolerated dropping activity only 2.5 fold (IC_{50}=0.85 nM to IC_{50}=2.1 nM). Inversion at the 3 position in the benzodiazepine, as previously mentioned, drops activity over 400 fold (IC_{50}=0.85 nM to IC_{50}=370 nM). Clearly, the three stereocenters in inhibitor **II** are selectively sensitive to change, and with the design of simpler Ras FTase inhibitors in mind, this finding may indicate where structural modifications are more likely to be accepted. In addition, model scaffolds may now be further refined.

Acknowledgment: We thank Dr. Frederick J. Hollander, Dept. of Chemistry, University of California, Berkeley, for the crystallographic analysis.

References and Notes:

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3. The assay for inhibition of Ras farnesyltransferase has been described previously (a, b and c) and was conducted at the Brown and Goldstein laboratories, Dept. of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75235. Briefly, the amount of [^{3}H] farnesyl transferred from all-trans-[^{3}H] farnesylpyrophosphate to recombinant p21H-ras was measured using a filter binding assay. IC_{50} values generated in this competition assay are reported as the concentration of inhibitor required to reduce farnesylation of Ras by 50%. (a) Reiss, Y.; Stradley, S. J.; Gierasch, L.M.; Brown, M.S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 732. (b) Goldstein, J. L.; Brown, M. S.; Stradley, S. J.; Reiss, Y.; Gierasch, L. M. *J. Biol. Chem.* **1991**, *266*, 15575. (c) Brown, M. S.; Goldstein, J. L.; Paris, K. J.; Burnier, J. P.; Marsters, J. C., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8313.
4. In a typical separation, 580 mg of crude racemic **II** was loaded on a 2.5 cm x 26 cm Vydac C18, 300 Å column and eluted with a gradient of 10% to 50% acetonitrile/water (0.1%TFA) over 80 min at 18 ml/min with 280/214 nm detection. Isomer (A) rt=23-30 min, isomer (B) rt=37-47 min.
5. Reider, P.J.; Davis, P.; Hughes, D.L.; Grabowski, E.J.J. *J. Org. Chem.* **1987**, *52*, 955.
6. In a typical experiment, 13.71 g (40.7 mmol) of racemic free amine **III** was dissolved in 550 ml of 50:45:5 Hex:EtOAc:MeOH and warmed to 50 °C. Anhydrous (R)-(-)-Camphorsulfonic acid (9.91 g, 42.7 mmol) was added and the mixture brought briefly to reflux (60 °C) then cooled to 45 °C and held there with stirring for 24 hr while crystallization proceeded. Pirkle analysis[10] of the crystallization solution indicated equilibrium had been reached.[7] The slurry was cooled to 25 °C and stirred an additional 24 hr. Very little racemization takes place at room temperature as indicated by failure to re-equilibrate. The slurry was cooled to 5 °C, filtered and washed with 1:1 hex:EtOAc. Yield: 18.22 g (79%) of colorless crystals. >95% *ee* by Pirkle HPLC. mp=115-118 °C. A second 2.58 g batch was obtained by recycling the mother liquors through the above process bringing the overall isolated yield to 90%.
7. The resolution stalls when equilibrium is reached, indicated by equivalent concentration of isomers in solution. Obviously there is a trade off between the temperature required to get an appreciable rate of racemization and the solubility of the compound at that temperature. Conditions were not optimized.
8. Measured by ^{1}H-NMR, Varian VXR300S, 300MHz, d_6-DMSO.
9. The free amine (as prepared in note 11) was N-Boc protected with di-t-butyl dicarbonate in $MeCl_2$ / triethylamine and the methyl ester carefully hydrolyzed (0 °C, 4 hr)with aqueous methanolic LiOH (1.5 eq). Peptides were synthesized via standard solid phase methodologies.[2] See also: Barany and Merrifield, *The Peptides*; E. Gross and J.Meienhofer, Ed.; Academic Press, New York: 1980;Vol. 2; pp 1-284.
10. A covalently bonded D-phenylglycine column (4.6 mm x 25 cm) from Regis Technologies, Morton Grove, IL. was used. Acetylation of the free benzodiazepine amines (acetic anhydride and triethylamine) was required prior to analysis. Mobile phase: 75:23:2 (Hex:$MeCl_2$:IpOH), 3 ml/min, 254 nm, rt = 23 min (R), 25.5 min (S). Baseline separation was not achieved but detection limits of about 5% are realistic.
11. Compound **V** was prepared from 1.5 g (2.64 mmol) of **IV** which was partitioned between ethyl acetate and 10% potassium carbonate and the organic phase concentrated to give the glassy free amine $[\alpha]^{20}_D$=+107°, c=4.7, methanol. Treatment with 3.16 mmol of (S)-2-phenylpropionylchloride [from (S)-2-phenylpropionic acid and oxalyl chloride] in $MeCl_2$/TEA gave the desired covalent diastereomer which after standard aqueous/organic work-up was crystallized from ethyl acetate/hexane. mp=130-132 °C, $[\alpha]^{20}_D$=+221°, c=2.6, methanol. X-ray quality crystals were grown from $MeCl_2$/hexane.

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