would be very interesting if it were important for transportation of taste compounds to a certain receptor and for exertion of taste. For applying pattern-recognition techniques to SAR studies, it is important to consider the data structure in parameter space of objective samples with a visible display method.

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Probes of the Active Site of Norepinephrine *N*-Methyltransferase: Effect of Hydrophobic and Hydrophilic Interactions on Side-Chain Binding of Amphetamine and α-Methylbenzylamine[1a]

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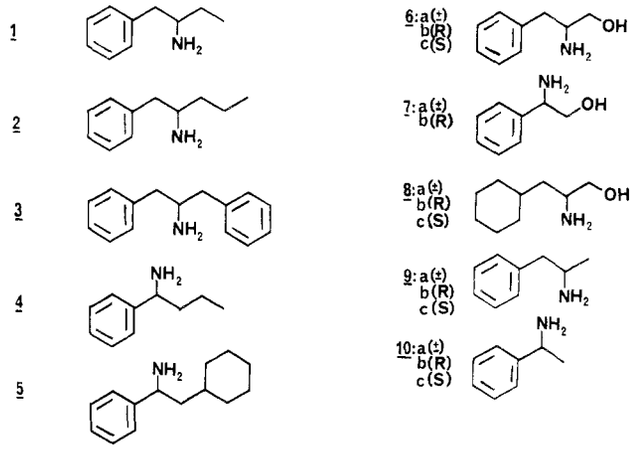
A series of ω-substituted analogues of amphetamine and α-methylbenzylamine were prepared and evaluated as inhibitors of norepinephrine *N*-methyltransferase (NMT). These included several alkyl side chain extended analogues (**1–5**), as well as the terminally hydroxylated derivatives phenylalanol (**6a**) and phenylglycinol (**7a**). None of the alkyl-substituted derivatives displayed appreciable activity as inhibitors; however, the hydroxylated analogues were up to twofold more potent than the parent compounds. The positive contribution of the side-chain hydroxy suggests that the terminal methyl group of the lead compounds is situated close to a hydrophilic area or hydrogen bonding functional group within the active site.

The enzyme norepinephrine *N*-methyltransferase (NMT, EC 2.1.1.28; also known as phenylethanolamine *N*-methyltransferase, PNMT) catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amino group of norepinephrine, yielding epinephrine. In pursuit of specific inhibitors of NMT which could prove to be useful pharmacological tools, we have been attempting to characterize select features of the active site through the use of analogues of norepinephrine and of the competitive NMT inhibitors amphetamine and α-methylbenzylamine. Our efforts to date have focused on the properties of the active-site region that accommodates the aromatic ring of bound substrates[2,3] and inhibitors,[4] and also on the side-chain conformation of bound phenylethylamines.[5] In this report, we describe initial results of an investigation that is aimed at characterizing a different region of the active site, the area which lies in the vicinity of the terminal methyl group of bound amphetamine (**9a**) and α-methylbenzylamine (**10a**).

The ability of phenylethylamines, such as amphetamine, to inhibit NMT was first noted by Axelrod[6] and later explored in depth by Fuller et al.[7] The potent inhibitory activity of NMT by benzylamines was first reported by



Fuller et al.[8] The α-methyl derivatives of phenylethylamine and benzylamine were of interest with respect to the development of some metabolically stable inhibitors suitable for in vivo studies, since the added methyl group renders these compounds resistant to oxidation by the amine metabolizing enzyme monoamine oxidase (MAO). To our knowledge, no further investigations have been conducted regarding the nature and tolerances of the region of the active site of NMT occupied by this additional methyl group. Since an understanding of the active site area that binds substrates and inhibitors is important for the rational design of feasible inhibitors to bind to this site, we have prepared some simple side chain extended analogues of **9a** and **10a** that were designed to probe the characteristics of the region of the enzyme in the vicinity of the methyl group of the ligands. Basically, the compounds were selected in order to detect either hydrophobic or hydrophilic character within this region by adding additional hydrocarbon bulk to the methyl group or by adding an hydroxy group to the side-chain terminus. The

(1) (a) Taken from the Ph.D. Dissertation submitted to the Graduate School of the University of Kansas by M.F.R., 1982. (b) University of Kansas undergraduate research participant (Grant KU-3944). (c) Supported by NIH Predoctoral Training Grant GM 07775.
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Table I. Inhibition Constants (K_i) for Side Chain Extended Analogues of Amphetamine and α-Methylbenzylamine

compd	K_i ± SEM, μM	formula	mp (lit.), °C	anal.
1	>1000	$C_{10}H_{15}N \cdot HCl$	143-145	C, H, N
2	>1000	$C_{11}H_{17}N \cdot HCl$	130-132	C, H, N
3	>1000	$C_{15}H_{17}N \cdot HCl$	202-203 (200-202)[a]	C, H, N
4	>1000	$C_{10}H_{15}N \cdot HCl$	282 (282)[b]	C, H, N
5	>1000	$C_{14}H_{21}N \cdot HCl$	299 (313-314)[c]	C, H, N
6a (±)	385 ± 47			
6b (*R*)	379 ± 39			
6c (*S*)	664 ± 70			
7a (±)	1223 ± 212			
7b (*R*)	88 ± 19			
8a (±)	47 ± 4.5		148-150	C, H, N
8b (*R*)	33 ± 7	$C_9H_{19}NO \cdot HCl$	152-154	C, H, N
8c (*S*)	125 ± 10		151-154	C, H, N
9a (±)	740 ± 68			
9b (*R*)	1381 ± 147			
9c (*S*)	442 ± 43			
10a (±)	460 ± 52			
10b (*R*)	1679 ± 209			
10c (*S*)	149 ± 13			

[a] Reference 15. [b] Reference 16. [c] Reference 17.

hydroxylated compounds, phenylalanol (**6a**) and phenylglycinol (**7a**), are readily available or conveniently prepared in optically active form from the corresponding amino acids. The optically active ligands were of interest, since our recent examination of stereospecificity of binding showed that modification of the ring portion of substrates and inhibitors can affect the orientation of the side chain within the active site.[4] By comparing the stereochemical behavior of the enzyme toward these hydroxylated derivatives with that found for the unsubstituted **9a** and **10a**, we hope to detect any changes in binding orientation and also establish a relationship that would allow us to confidently attribute the conclusions of this investigation to the region of the active site under study.

Results and Discussion

The hydrophobic side chain extended analogues **1–5** were all prepared by Leuckardt reductive amination of the necessary ketones. Phenylalanol isomer **6b** was prepared by $NaBH_4$ reduction of D-phenylalanine ethyl ester. The cyclohexyl derivatives **8a–c** were prepared by catalytic reduction of compounds **6a–c**. Interest in these saturated analogues stems from our earlier findings that fully saturated analogues of amphetamine are significantly more potent as inhibitors of NMT.[4] All of the compounds were assayed against bovine adrenal NMT by a procedure that has been previously described,[4,5] and compounds with significant inhibitory activity were all found to be competitive with respect to the amine substrate.

Table I presents the K_i values that were determined for these compounds. It is apparent from the data that any additional hydrophobic bulk to the side-chain terminus of either amphetamine or α-methylbenzylamine had a detrimental effect on binding, as indicated by the relative inactivity of compounds **1–5**. The conclusion is that either steric interferences in the active site preclude binding or that the additional hydrophobic character of **1–5** is incompatible with the region of the active site that it encounters. By contrast, the hydroxylated derivatives (**6a–c** and **7a,b**) were all more potent than the unsubstituted compounds **9** and **10**, the difference in activities being up to twofold. This positive contribution by the added hydroxy group toward binding suggests that the area of the active site in the vicinity of the side-chain methyl group of **9a** and **10a** is predominantly hydrophilic in character or contains a hydrogen-bonding functionality. Compound **7a** (phenylglycinol) had previously been examined by Fuller et al.[9] for NMT substrate activity and was found to be inactive; however, the ability of **7a** to competitively inhibit NMT had not been previously determined.

We have found as hydrophobicity increases in nonaromatic analogues of NMT substrates and competitive inhibitors, they are bound in a slightly different orientation within the active site. This is reflected in the differing stereoselectivity displayed by NMT toward aromatic compounds and the nonaromatic analogues.[4] If the added hydroxy group of **6a** and **7a** were causing similar changes in binding orientation of the side chain, then it is possible that the area with which the hydroxy group is binding might be somewhat removed from the region that accommodates the side-chain methyl group. In order to probe for such a change in binding mode, we determined the absolute and relative stereochemical requirements of **6a** for NMT inhibition. The *R* isomer (**6b**) proved to be roughly twice as potent an inhibitor as the *S* isomer (**6c**). The *R* isomer **6b** corresponds to (*S*)-(+)-amphetamine, which is the more potent isomer for NMT inhibition by approximately threefold.[4,7] Thus, the stereochemical influences of the active site appear to be the same for both **6a** and **9a**, which suggests that the side chains occupy the same region of the active site.

Recently, we reported that saturation of the phenyl ring of **9a–c** resulted in a substantial (7- to 10-fold) increase in NMT inhibition.[2,4] The positive effect of the added hydroxy group encouraged us to evaluate the saturated analogues of **6a–c** in order to establish that the two modifications were compatible and would result in a further increase in inhibitory activity. A K_i of 109 μM was found for the saturated analogue of **9a**; compound **8a** was found to be twice as potent, with a K_i value of 47 μM, suggesting that the contributions of ring saturation and the terminal hydroxy group are indeed additive.

In summary, we have evaluated some simple side chain extended analogues of amphetamine and α-methylbenzylamine as NMT inhibitors in an effort to determine the nature of the active-site region near the terminal side-chain methyl group. Compounds that bore additional hydrocarbon bulk on the side chain were found to be ineffective at inhibiting NMT; however, derivatives in which an hydroxy substituent had been added to the terminal methyl group were more active than the unsubstituted parent compounds. Stereochemical behavior of

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NMT toward the hydroxylated derivatives was found to be essentially identical with that displayed by NMT toward the parent compounds, thus confirming that the enhanced activity of the hydroxylated analogues resulted from favorable interactions in the region that we sought to investigate. The preliminary conclusion based on these results is that the active site in the vicinity of the side-chain terminus is hydrophilic in character, and added hydrophilic character in this region would supplement the binding of inhibitors to the active site. Efforts to carry out a more detailed characterization of this region and to assess its potential utility for inhibitor design are continuing.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus that had been calibrated with known compounds. Combustion analyses were performed on a Hewlett-Packard 185B CHN Analyzer at the University of Kansas. IR spectra were recorded on a Beckmann IR-33 spectrophotometer and NMR spectra on a Varian T-60 spectrometer with Me_4Si as an internal standard. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter at 589 nm. The following compounds were purchased from Sigma Chemical Co. and used directly: **6a,c** and **7a,b**. Compound **6b** was prepared from phenylalanine by the method of Seki et al.[10] Butyrophenone, 1-phenyl-2-butanone, and 1,3-diphenylacetone required for the preparation of **4**, **1**, and **3**, respectively, were purchased from Aldrich Chemical Co. α-Cyclohexylacetophenone was prepared as described by Dodds et al.[11] Unless specified as absolute, EtOH and ethanol refer to 95% ethanol.

1-Phenyl-2-pentanone. 1-Phenyl-2-pentanol (1.27 g, 7.83 mmol) in 50 mL of hexane was treated with 15.0 g of pyridinium chlorochromate on alumina[12] at room temperature for 1 h. Filtration and evaporation left 1.09 g (87%) of the desired ketone, which was suitable for use in the next step: IR (film) 1718 cm^{-1}; NMR ($CDCl_3$) δ 7.23 (s, 5, arom), 3.60 (s, 2, benzylic), 2.36 (t, J = 7 Hz, 2, $COCH_2$), 1.50 (m, 2, CH_2CH_2), 0.80 (t, J = 7 Hz, 3, CH_3).

Leuckardt Reaction. Synthesis of 1-Phenyl-2-aminopentane (2). The procedure of Ingersol et al.[13] was employed. Ammonium formate (9.0 g, 0.143 mol) was heated in a flask equipped with a 10-cm Vigreux column and distillation head to 185 °C and maintained at that temperature for 2 h. The clear distillate that was collected during this period was returned to the pot (after cooling), and 0.86 g (5.3 mmol) of 1-phenyl-2-pentanone was added. The heterogeneous mixture was heated with stirring to a final temperature of 165 °C, which was maintained for 3 h. Over this period the contents of the pot became homogeneous. After the mixture was cooled, 20 mL of 6 N HCl was added, and the mixture was heated at reflux for 16 h. The cooled solution was diluted with 25 mL of H_2O and made basic by the careful addition of KOH pellets. Extraction with ether (3 × 30 mL), followed by drying over K_2CO_3 and evaporation, left 0.81 g of a yellow oil, which was distilled (bp 72 °C, 0.3 mm) to yield 0.77 g (88%) of free amine **2**: IR (film) 3370, 3310 (NH_2) cm^{-1}; NMR ($CDCl_3$) δ 7.23 (br s, 5, arom), 2.61 (t, J = 4.5 Hz, 2, benzylic), 2.35 (d, J = 4.5 Hz, 1, $CHNH_2$), 1.36 (br m, 4, CH_2CH_2), 0.98 (s, 2, D_2O exchangeable, NH_2), 0.88 ppm (t, 3, CH_3).

The same procedure was used for compounds **1** and **3–5**: yield (distilled product): **1**, 62%; **3**, 20%; **4**, 27%; **5**, 57.7%.

Catalytic Hydrogenation of 6b,c. The method of Leithe was followed.[14] Compound **6b** [308 mg; $[\alpha]^{22}_D$ 20.8° (c 1.77, EtOH), lit.[10] $[\alpha]^{23}_D$ 24.1° (EtOH)] was dissolved in 30 mL of 95% EtOH, to which 0.5 mL of concentrated HCl had been added, in a Parr shaker flask. PtO_2 (40 mg) was added and hydrogenation commenced at 50 psi for 18 h at room temperature. Filtration of the catalyst and evaporation of the solvent left 380 mg of a white residue, which contained no aromatic protons by NMR analysis (D_2O). The residue was recrystallized from ethanol/ethyl acetate to yield **8b**·HCl, mp 152–154 °C (phase transition to clear gel at 135 °C): $[\alpha]^{20}_D$ −2.24° (c 2.99, H_2O). Anal. ($C_9H_{19}NO$·HCl) C, H, N. Compound **6c** was reduced as above to yield **8c**·HCl: mp 151–154 °C (phase transition at 135 °C), $[\alpha]^{21}_D$ +2.05° (c 4.87, H_2O).

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Importance of the Aromatic Ring in Adrenergic Amines. 8. 2-(Aminomethyl)-*trans*-2-decalols as Inhibitors of Norepinephrine *N*-Methyltransferase[1a]

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In an effort to determine the surface appearance of the hydrophobic ring binding region of the norepinephrine *N*-methyltransferase active site, we employed some *trans*-decalin analogues of 1-(aminomethyl)cycloundecanol (1), a potent NMT inhibitor. These analogues [axial and equatorial 2-(aminomethyl)-*trans*-2-decalol, **2** and **3**] closely resemble a low energy "crown" conformation of **1**. Both compounds were as potent as **1** at inhibiting NMT (K_i = 3.6, 5.6, and 3.8 μM for **1**, **2**, and **3**, respectively), indicating that this conformation is most likely adopted within the active site in order to optimize contact with a flat hydrophobic area. None of the compounds showed significant substrate activity for NMT, a fact that is consistent with our proposed active site binding model.

Interest in developing more potent and specific inhibitors of norepinephrine *N*-methyltransferase (NMT,[2] EC 2.1.1.28) as potential pharmacological tools for the selective modulation of brain epinephrine prompted us to begin an investigation of the active site of this enzyme, using se-

(1) (a) Taken from the Ph.D. Dissertation presented to the Graduate School of the University of Kansas by M.F.R., 1982. (b) NIH Predoctoral Trainee (Grant GM 07775).

(2) This enzyme has also been referred to as phenylethanolamine *N*-methyltransferase (PNMT).

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