at this temperature for 20 h. (The reaction progress was followed by taking from the mixture a sample which was treated with 2 M sodium hydroxide and extracted with diethyl ether. The extract was subjected to silica TLC with methylene chloride and methanol (10:1).) The reaction mixture was allowed to cool to 120 °C and 2 M sodium hydroxide (650 mL) was added. The temperature was lowered to room temperature and the mixture stirred about 0.5–1 h and extracted with ether. After evaporation, the residue was distilled at 225 °C/1 Torr. Compound 2b (5 g, 37%) was obtained by recrystallization from ligroin (100–140 °C), mp 186–187 °C (lit.[29] mp 186 °C).

9-[(2-Methoxyphenyl)amino]-1,2,3,4-tetrahydroacridine (2c). Phosphorus pentoxide (1.8 mol, 256 g) was mixed with triethylamine hydrochloride (1.8 mol, 448 g) in a flask fitted with a mechanical stirrer and a reflux condenser with a drying tube (calcium chloride) at room temperature. 2-Methoxyaniline (1.8 mol, 222 g) was added dropwise while the mixture was heated in an oil bath to 60 °C (oil-bath temperature). The mixture was further heated to 160 °C until a homogeneous mixture was achieved (0.5 h). The oil-bath temperature was then lowered to 130 °C. Methyl anthranilate (0.3 mol, 45.4 g) and cyclohexanone (0.54 mol, 53.0 g) were added dropwise. The temperature was again increased to 160 °C and maintained at that temperature for 15 min. The reaction mixture was allowed to cool to 110 °C and 2 M sodium hydroxide (4000 mL) was added. The aqueous suspension was extracted with 2 × 1500 mL of diethyl ether. The solvent was stripped off under reduced pressure. The residue was further evaporated at 0.1 mmHg to remove 2-methoxyaniline. A black solid formed and was recrystallized twice from ligroin (80–100 °C) to yield **2c** (32.4 g, 36%) as white-yellow crystals: mp 131–132 °C; ^{1}H NMR ($CDCl_3$/TMS) δ/ppm 1.91 (m, 4 H), 2.79 (m, 2 H), 3.19 (m, 2 H), 4.02 (s, 3 H), 6.27 (s, NH), 6.10–8.20 (m, 8 H); ^{13}C NMR ($CDCl_3$/TMS) δ/ppm 25.09 (C-1), 22.75 (C-2), 22.49 (C-3), 33.98 (C-4), 159.77 (C-4a), 128.60 (C-5) 128.41 (C-6), 124.84 (C-7), 123.08 (C-8), 123.67 (C-8a), 142.96 (C-9), 124.45 (C-9a), 147.24 (C-10a); MS *m/e* 304 (100), 289 (13), 273 (11), 197 (11), 182 (11). Anal. ($C_{20}H_{20}N_2O$) C, H, N.

9-[(4-Nitro-2-methoxyphenyl)amino]-1,2,3,4-tetrahydroacridine (3). Compound **2c** (20 mmol, 6.1 g) was dissolved in acetic acid (99%, 80 mL). After addition of nitric acid (68%) at 70 °C and stirring for 4 h, it was poured into ice (500 mL). When the ice had melted, 2 M sodium hydroxide (0.5 L) was added. A yellow precipitate 6.5 g (ortho and paraisomer) was washed with water and dried in vacuum. Recrystallization twice from ethyl acetate yielded **3** (2.4 g, 34%), mp 221–223 °C. Anal. ($C_{20}H_{19}N_3O_3$) C, H, N.

9-[(4-Amino-2-methoxyphenyl)amino]-1,2,3,4-tetrahydroacridine (4). A mixture of **3** (13 mmol, 4.6 g), tin (83 mmol, 9.9 g), and 6 M hydrochloride acid (100 mL) was heated with stirring to 120 °C (oil-bath temperature) by which time the tetrahydroacridine had dissolved. The mixture was refluxed for 2 h and cooled. The precipitate was dissolved in water and, after addition of 2 M sodium hydroxide (50 mL), extracted into chloroform. The chloroform extract was evaporated and the residue was recrystallized from 50% ethanol to yield **4** (2.5 g, 56%), mp 155–156 °C.

***N*-[4-[(1,2,3,4-Tetrahydro-9-acridinyl)amino]-3-methoxyphenyl]methanesulfonamide (2d).** Compound **4** (7.5 mmol, 2.4 g) was dissolved in 25 mL of dry pyridine. Mesyl chloride (15 mmol, 1.16 mL) was added slowly at −5 °C and the mixture was stirred for 1 h. The mixture was evaporated under reduced pressure and dissolved in water (200 mL). Hydrochloric acid (4 M, 20 mL) was added to precipitate the hydrochloride, which was taken up in 200 mL of water. 10 mL of sodium hydrogen carbonate was added and the precipitate was crystallized from ethanol (96%) to yield **2d** (1.5 g, 50%), mp 248–249 °C (lit.[30] mp 243–245 °C.

NMR Experiments. All DNA samples for NMR were made 5 mM in DNA phosphate by diluting with distilled water. This gave $[Na^+]/[P]$ ratios of 1.2. The NMR intercalator titrations were performed by adding successive aliquots (corresponding to $r \sim 0.005$) of a drug stock solution directly to the DNA solution in the NMR tube. For titrations up to $r \sim 0.05$, the volume increases by 10%. A control experiment, in which water alone was added up to 20% volume increase, showed that T_1, within measuring uncertainties, was unchanged by such dilution. pH was measured to be 7 both before and after the addition of intercalators.

^{23}Na NMR spectra were recorded at 5.9 T on a Bruker AC 250 and obtained without lock. The inversion-recovery (180°–τ–90°–acq) pulse sequence was used for the T_1 measurements with 15 different values of τ for each experiment. The T_1 values were obtained by a three-parameter linear least square fitting procedure. Each T_1 value is the average of at least two measurements. The temperature for the NMR measurements was 27 °C.

^{13}C and ^{1}H spectral data given were also obtained at 5.9 T on a Bruker AC 250 NMR instrument.

Registry No. 1, 321-64-2; **2a**, 14807-16-0; **2b**, 110245-49-3; **2c**, 123333-18-6; **2d**, 111232-55-4; **3**, 123333-19-7; **4**, 123333-20-0; *o*-$NH_2C_6H_4CO_2Me$, 134-20-3; 4-methylcyclohexanone, 589-92-4; cyclohexanone, 108-94-1; aniline, 62-53-3; 2-methoxyaniline, 90-04-0.

(29) Endicott, M.; Alden, B. W.; Sherrill, M. L. *J. Am. Chem. Soc.* **1946**, *68*, 1303.

(30) Yamato, M.; Takeuchi, Y.; Ikeda, Y. *Heterocycles* **1987**, *26*, 191.

Cyclization-Activated Prodrugs. Basic Carbamates of 4-Hydroxyanisole

Walfred S. Saari,* John E. Schwering, Paulette A. Lyle, Steven J. Smith, and Edward L. Engelhardt

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. *Received January 18, 1989*

A series of basic carbamates of 4-hydroxyanisole was prepared and evaluated as progenitors of this melanocytotoxic phenol. All of the carbamates were relatively stable at low pH but released 4-hydroxyanisole cleanly at pH 7.4 at rates that were structure dependent. A detailed study of the *N*-methyl-*N*-[2-(methylamino)ethyl]carbamate showed that generation of the parent phenol followed first-order kinetics with $t_{1/2}$ = 36.3 min at pH 7.4, 37 °C, and was accompanied by formation of *N,N'*-dimethylimidazolidinone. These basic carbamates are examples of cyclization-activated prodrugs in which generation of the active drug is not linked to enzymatic cleavage but rather depends solely upon a predictable, intramolecular cyclization–elimination reaction.

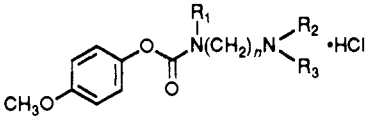
Esterification of therapeutically active agents to provide prodrugs with improved properties has become a familiar strategy for the circumvention of adverse physicochemical limitations. Ester prodrugs of alcohols and phenols are frequently explored to improve solubility, absorption, and bioavailability and to extend the duration of action of the parent drug.[1,2] It is of course essential for the success of this strategy that the ester progenitor be capable of delivering the parent drug at a practical rate in vivo. Generally, ester prodrugs have depended upon chemical or

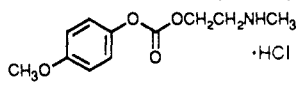
(1) Stella, V. J.; Charman, W. N. A.; Naringrekar, V. H. *Drugs.* **1985**, *29*, 455.

(2) Leinweber, F.-J. *Drug. Metab. Rev.* **1987**, *18*, 379.

0022-2623/90/1833-0097$02.50/0

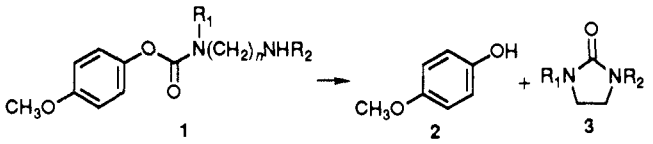
Table I. Carbamates of 4-Hydroxyanisole. Chemical Properties and Half-Lives for Conversion to 4-Hydroxyanisole



compd[a]	n	R_1	R_2	R_3	mp, °C	$t_{1/2}$, min, at 37 °C pH 7.4	pH 6.8	$t_{1/2}$ at 37 °C pH 5.2	pH 4.2
1a[b]	2	CH_3	CH_3	H	148.0–19.0 (softens at 125)	36.3 ± 1.3 56.0[c] ± 1.4	140 ± 5	57.8 ± 1.9 h	>15 days
1b	2	CH_3	CH_3	CH_3	153.0–154.5	39.6 ± 0.7			
1c	2	C_2H_5	C_2H_5	H	149.0–150.0	118 ± 2			
1d[d]	2	CH_3	H	H	152.5–154.5	304 ± 1			
1e	2	H	CH_3	H	159.0–160.5	335 ± 1			
1f[e]	2	H	H	H	185.0–192.0	724 ± 2			
1g[f]	3	H	H	H	162.0–165.0 (softens at 110)	910 ± 10			
1h	3	CH_3	CH_3	H	143.0–145.0	942 ± 1			
8					53.0–55.0	<0.3			

[a] Anal. C, H, N. [b] pK_a = 8.88. [c] Murine plasma. [d] pK_a = 8.90. [e] pK_a = 8.65. [f] pK_a = 9.60.

Scheme I



enzymatic hydrolysis of the ester bond for useful rates of conversion of prodrug to drug. However, this requirement is not always attainable or may be subject to much variability between species or even between individual members of a particular species.

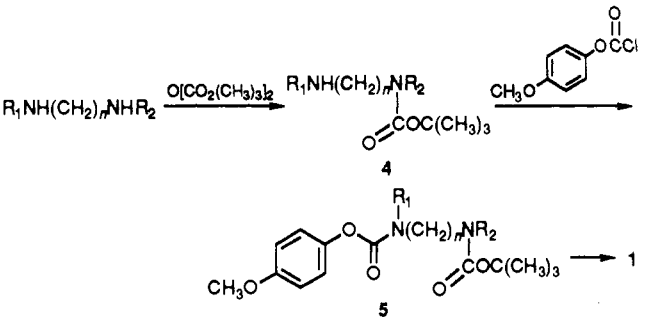
An alternate approach to this problem is release of the parent drug from the prodrug through mechanisms not involving hydrolysis of the ester bond, but rather through an intramolecular cyclization–elimination reaction such as that depicted in Scheme I. In this way, ideally, generation of active drug is not dependent upon the host environment but rather solely upon the rate of the cyclization reaction.

In this work we describe the synthesis of basic carbamates 1 of the clinically effective melanocytotoxic agent 4-hydroxyanisole (2)[3,4] and their evaluation as progenitors of this phenol by an intramolecular cyclization reaction.

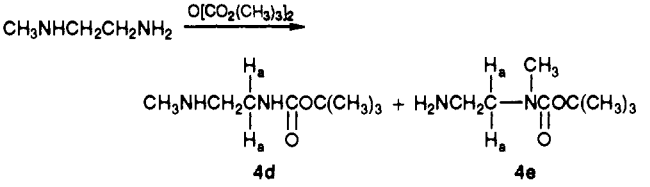
Chemistry

Synthesis of the basic carbamates 1 required monoprotected diamine intermediates which could ultimately be deblocked in the last step without destruction of the carbamate function. The *tert*-butoxycarbonyl (BOC) group appeared to be compatible with this approach. Although several different syntheses of mono-alkoxycarbonyl-protected diamines have been reported,[5-10] we found that direct acylation of excess diamine with di-*tert*-butyl dicarbonate ($^1/_3$ molar equiv) in THF (Scheme

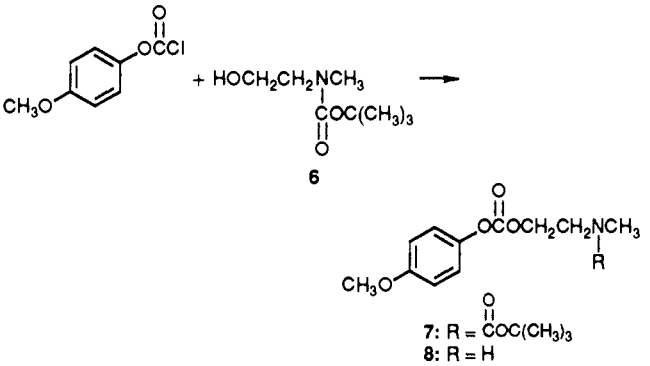
Scheme II



Scheme III



Scheme IV

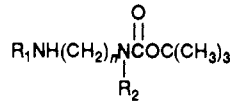


II) proved to be a convenient source of the protected diamines 4 (Table II). The products from symmetrical diamines were generally of satisfactory purity for use in the next step without requiring extensive purification.

In the case of the unsymmetrical diamine *N*-methylethylenediamine, acylation occurred at each nitrogen to provide 4d and -e in 12 and 38% yields, respectively, after separation by flash chromatography (Scheme III). Structural assignments could be made from the ^{1}H NMR

(3) Morgan, B. D. G.; O'Neill, T.; Dewey, D. L.; Galpine, A. R.; Riley, P. A. *Clin. Oncol.* **1981**, *7*, 227.
(4) Kanclerz, A.; Chapman, J. D. *Br. J. Cancer* **1986**, *54*, 693.
(5) Hansen, J. B.; Nielsen, M. C.; Erhbar, U.; Buchardt, O. *Synthesis* **1982**, 404.
(6) Fuchs, S.; Klingler, W.; Voelter, W. *Liebigs Ann. Chem.* **1977**, 602.
(7) Geiger, R. *Justus Liebigs Ann. Chem.* **1971**, *750*, 165.
(8) Herrin, T. R.; Pauvlik, J. M.; Schuber, E. V.; Geiszler, A. O. *J. Med. Chem.* **1975**, *18*, 1216.
(9) Houssin, R.; Bernier, J.-L.; Henichart, J.-P. *Synthesis* **1988**, 259.
(10) Atwell, G. J.; Denny, W. A. *Synthesis* **1984**, 1032.

Table II. Mono-BOC-Protected Diamines



compd	n	R_1	R_2	yield, %	method	^{1}H NMR, δ (H)	hydrogen fumarate mp, °C dec	hydrogen fumarate ^{1}H NMR, δ (H)
4a	2	CH_3	CH_3	81	B	1.46 (s, 9), 2.48 (s, 3), 2.75 (br t, 2), 2.88 (s, 3), 3.35 (br t, 2)[a]		
4c	2	C_2H_5	C_2H_5	83	B	1.10 (t, t, 6), 1.45 (s, 9), 1.72 (br s, 1), 2.72 (t, q, 4), 3.28 (m, 4)[a]		
4d	2	CH_3	H	12	B		118.0–120.0[b]	1.38 (s, 9), 2.45 (s, 3), 2.80 (t, 2), 3.16 (q, 2), 6.43 (s, 2), 7.17 (m, 1)[c]
4e	2	H	CH_3	38	B		146.0–148.0[b]	1.40 (s, 9), 2.80 (s 3), 2.88 (t, 2), 3.36 (t, 2), 6.44 (s, 2)[c]
4f	2	H	H	94	A	1.38 (s, 9), 2.53 (t, 2), 2.91 (q, 2), 6.72 (br, s, 1)[c]	172.5–173.5[b]	1.38 (s, 9), 2.80 (t, 2), 3.16 (q, 2), 6.44 (s, 2), 7.14 (t, 1)[c]
4g	3	H	H	85	A		147.0–148.0[b]	1.38 (s, 9), 1.65 (m, 2), 2.75 (t, 2), 2.98 (q, 2), 6.43 (s, 2), 6.98 (br t, 1)[c]
4h	3	CH_3	CH_3	72	B	1.48 (s, 9), 1.74 (m, 3), 2.44 (s, 3), 2.58 (t, 2), 2.83 (s, 3), 3.29 (br s, 2)[a]		

[a] $CDCl_3$. [b] Anal. C, H, N. [c] DMSO-d_6.

of the corresponding hydrogen fumarate salts. The ^{1}H NMR spectrum of **4d** hydrogen fumarate showed H_a protons as a quartet (coupling with the BOC NH) while those of **4e** were found to be a triplet. In addition, the hydrogen fumarate salt of **4e** did not exhibit a BOC NH peak while that of **4d** did.

The basic carbamates of Table I were synthesized by reaction of BOC-protected diamines **4** with 4-methoxyphenyl chloroformate[11] followed by removal of the BOC group with anhydrous HCl (Scheme II). Deprotection under acidic conditions allowed direct isolation of the basic carbamates as the stable HCl salts in high yields. The trimethyl derivative **1b** (Table I) was obtained directly from reaction of *N,N,N'*-trimethylethylenediamine with 4-methoxyphenyl chloroformate.

Basic carbonate **8** was prepared for comparison with carbamate **1a** by a similar sequence using (*N*-BOC-*N*-methylamino)ethanol (**6**) (Scheme IV).

Results and Discussion

Stability of the carbamates of Table I at 37 °C in aqueous solution at pH 7.4 was determined conveniently by HPLC analysis. In every case, 4-hydroxyanisole was generated smoothly and cleanly but at different rates depending upon the structure of the particular carbamate. Detailed kinetic analysis of the *N,N'*-dimethylcarbamate **1a** showed that the phenol was formed following first-order kinetics.

Confirmation that 4-hydroxyanisole is formed through the cyclization mechanism of Scheme I was obtained by the isolation of *N,N'*-dimethylimidazolidinone (**3**, $R_1 = R_2 = CH_3$) in 49.5% yield after heating **1a** at 37 °C and pH 7.4. In addition, formation of 4-hydroxyanisole was slower at pH 6.8 than at pH 7.4, and the carbamates are more stable at even lower pH (Table I). This suggests that a nonprotonated amine function is critical for reaction to occur and further substantiates that phenol release is the result of a cyclization reaction.

The *N*-methyl substituent of **1a** ($R_1 = CH_3$) precludes formation of 4-hydroxyanisole through an elimination mechanism involving an isocyanate intermediate. In addition, carbamates with N–H substituents normally react faster than *N*-alkyl analogues by this route.[14,15] However, in this series, the N–H derivative **1e** formed 4-hydroxyanisole at a much slower rate than the corresponding *N*-methylcarbamate **1a**.

Although carbamate esters have been shown to be stable under mild hydrolytic conditions and unreactive toward intermolecular amine addition, reaction with intramolecular nucleophiles may occur with great facility. For example, cyclization of phenyl *N*-(2-aminophenyl)-*N*-methylcarbamate to 1-methyl-2-benzimidazolone proved to be very rapid under conditions where bimolecular reaction of the carbamate with amines could not be detected.[15]

Inspection of Table I shows that the trimethyl analogue **1b** generates 4-hydroxyanisole as well as the dimethyl derivative **1a**. In this case, addition of the dimethylamino group to the carbamate carbonyl followed by elimination of 4-hydroxyanisole is still possible.[17] All other changes in the carbamate portion of **1a** that were examined led to a slower release of the phenol. These included conversion of the N-substituent to ethyl (**1c**), deletion of *N*-alkyl groups (**1d–f**), or extension of the carbon chain to three methylenes (**1g** and **-h**). The carbonate **8** corresponding to carbamate **1e** was found to be extremely reactive at pH 7.4 and 37 °C and released the phenol with $t_{1/2}$ <20 s. This carbonate, like the carbamates, was also relatively stable under more acidic conditions.

The *N,N'*-dimethylcarbamate **1a** was also found to be stable toward murine plasma esterases. Formation of 4-hydroxyanisole from **1a** was not faster in murine plasma at pH 7.4 and 37 °C ($t_{1/2}$ = 56.0 min) when compared to

(11) Zabik, M. J.; Schuetz, R. D. *J. Org. Chem.* **1967**, *32*, 300.
(12) Adams, P.; Baron, F. A. *Chem. Rev.* **1965**, *65*, 567.
(13) Christenson, I. *Acta Chem. Scand.* **1964**, *18*, 904.
(14) Hegarty, A. F.; Frost, L. N.; Coy, J. H. *J. Org. Chem.* **1974**, *39*, 1089.
(15) Fife, T. H.; Hutchins, J. E. C.; Wang, M. S. *J. Am. Chem. Soc.* **1975**, *97*, 5878.
(16) We thank the manuscript reviewer for refs 12–15.
(17) For example, reaction of tertiary amines with the carbonyl group of chloroformates is the first step in the well-known dealkylation procedure using these reagents.[18,19]
(18) Hobson, J. S.; McCluskey, J. G. *J. Chem. Soc. C* **1967**, 2015.
(19) Olofson, R. A.; Schnur, R. C.; Bunes, L.; Pepe, J. B. *Tetrahedron Lett.* **1977**, 1567.

nonenzymatic conditions, but even slightly slower. This apparent stabilizing effect of the murine plasma could be due to binding of the carbamate to plasma proteins, resulting in partial inhibition of the cyclization mechanism. Resistance of carbamates to enzymatic hydrolysis has been noted previously.[20-23]

This series of basic carbamates of 4-hydroxyanisole appears to satisfy the requirements for cyclization-activated prodrugs. That is, formation of the active drug is not linked to enzymatic cleavage but rather depends solely upon a predictable, intramolecular cyclization–elimination reaction. The pH dependence of the critical cyclization step can also be advantageous in the design of practical prodrugs. For example, relatively stable, weakly acidic solutions of the basic carbamate can be prepared prior to administration. Also, the release of a relatively lipophilic and neutral or weakly acidic drug from a basic, hydrophilic prodrug offers interesting possibilities which might be exploited for drug distribution applications.

Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are uncorrected. Analytical results are indicated by atom symbols and are within ±0.4% of theoretical values. ^{1}H NMR spectra were recorded for all intermediates and final products on either a Varian XL-300 or a GE NT-360 instrument using tetramethylsilane as an internal standard and are consistent with assigned structures. E. Merck silica gel, 230–400 mesh, was used for the flash chromatographies.

Preparation of Mono-BOC Diamines. Method A. *tert*-Butyl *N*-(2-Aminoethyl)carbamate (4f). A solution of di-*tert*-butyl dicarbonate (7.27 g, 33.3 mmol) in THF (30 mL) was added over 30 min to a stirred, cooled solution of ethylenediamine (6.7 mL, 100 mmol) in THF (30 mL) at 0 °C. After addition was complete, the reaction mixture was stirred in an ice bath for 30 min and then at room temperature for 18 h. Solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and brine. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated to 5.0 g (94%) of clear oil.

Method B. *tert*-Butyl *N*-[2-(Methylamino)ethyl]carbamate (4d) and *tert*-Butyl *N*-Methyl-*N*-(2-aminoethyl)carbamate (4e). A solution of di-*tert*-butyl dicarbonate (3.27 g, 15 mmol) in THF (30 mL) was added over 35 min to a stirred, ice bath cooled solution of *N*-methylethylenediamine (4.4 mL, 50 mmol) in THF (100 mL). After stirring at room temperature overnight, the reaction was processed by the same procedure as in method A. Flash chromatography over silica gel and elution with 20% MeOH–80% $CHCl_3$ gave first 1.0 g (38%) of **4e** as the faster moving material and then 0.30 g (12%) of **4d** as the slower moving product.

Preparation of 4-Hydroxyanisole Carbamates. General Procedure. *N*-Methyl-*N*-[2-(methylamino)ethyl]carbamic Acid 4-Methoxyphenyl Ester Hydrochloride (1a). A solution of *tert*-butyl *N*-methyl-*N*-[2-(methylamino)ethyl]carbamate (3.0 g, 16.1 mmol) and *N*,*N*-diisopropylethylamine (2.8 mL, 16.1 mmol) in THF (50 mL) was added over 30 min to a stirred, cooled solution of 4-methoxyphenyl chloroformate[11] (3.0 g, 16.1 mmol) in THF (50 mL). After addition was complete, the reaction mixture was stirred in an ice bath for 30 min and then at room temperature for 20 h. Solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and brine. The EtOAc extract was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 15% EtOAc–85% *n*-butyl chloride gave pure BOC-protected carbamate (4.9 g, 90%) as a clear oil.

A solution of the BOC-protected carbamate (4.9 g, 14.5 mmol) was dissolved in EtOAc (100 mL) and cooled in an ice bath. After saturating with anhydrous HCl, the reaction mixture was allowed to warm to room temperature over 3 h. Solvents were removed under reduced pressure, and the residue was recrystallized from MeOH–EtOAc–hexane to give *N*-methyl-*N*-[2-(methylamino)-ethyl]carbamic acid 4-methoxyphenyl ester hydrochloride (3.17 g, 79.6%), mp 148.0–149.0 °C with softening at 125 °C.

***N*-Methyl-*N*-[2-(dimethylamino)ethyl]carbamic Acid 4-Methoxyphenyl Ester Hydrochloride (1b).** A solution of *N*,*N*,*N*′-trimethylethylenediamine (1.3 mL, 10 mmol) and *N*,*N*-diisopropylethylamine (1.75 mL, 10 mmol) in THF (20 mL) was added over 0.5 h to a stirred, cooled solution of 4-methoxyphenyl chloroformate[11] (1.86 g, 10 mmol) in THF (30 mL). After stirring at ice bath temperature for 1 h and then at room temperature for 2 h, solvent was removed under reduced pressure and the residue partitioned between EtOAc and H_2O. The organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave 1.0 g (35%) of product as an oil. The HCl salt, mp 153.0–154.5 °C, was prepared with anhydrous HCl in EtOH followed by recrystallization from MeOH–EtOAc.

Isolation of *N*,*N*′-Dimethylimidazolidinone from Hydrolysis of 1a. A solution of *N*-methyl-*N*-[2-(methylamino)-ethyl]carbamic acid 4-methoxyphenyl ester hydrochloride (1a) (500 mg, 1.82 mmol) in pH 7.4 phosphate buffer (25 mL) was heated at 37 °C for 6.5 h and then cooled to room temperature overnight. The pH of the solution was maintained at 7.3–7.5 by the dropwise addition of 1 N NaOH. After lyophilization, the residue was extracted with CH_2Cl_2, which was then filtered, dried (Na_2SO_4), filtered, and concentrated to 272 mg of liquid. ^{1}H NMR analysis showed that *N*,*N*′-dimethylimidazolidinone was present (49.5% yield). To further substantiate the presence of the imidazolidinone, this product was dissolved in $CHCl_3$ extracted with 10% NaOH then H_2O to remove 4-methoxyphenol, dried (Na_2SO_4), filtered, and concentrated. The residue was distilled (bath temperature 120 °C, 14 mmHg) to give 30 mg (14.4%) of *N*,*N*′-dimethylimidazolidinone identical with an authentic sample by ^{1}H NMR and MS.

***tert*-Butyl *N*-(2-Hydroxyethyl)-*N*-methylcarbamate (6).** A solution of di-*tert*-butyl dicarbonate (43.6 g, 0.20 mol) in DMF (100 mL) was added over 45 min to a stirred solution of *N*-methylethanolamine (15.0, 0.20 mol) in DMF (250 mL) at 10–15 °C. After stirring at room temperature overnight, DMF was mol) at 50 °C and 0.1 mmHg, and the residue was dissolved in EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. Distillation gave the BOC-protected alcohol (25.7 g, 73.5%): bp 83–84 °C (0.05 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 2.92 (s, 3 H), 3.41 (m, 2 H), 3.77 (m, 2 H).

4-Methoxyphenyl 2-(Methylamino)ethyl Carbonate Hydrochloride (8). 4-Methoxyphenyl chloroformate[11] (0.94 g, 5.4 mmol) was added to a solution of *tert*-butyl *N*-(2-hydroxyethyl)-*N*-methylcarbamate (0.88 g, 4.7 mmol) and the mixture stirred at room temperature overnight. After concentrating under reduced pressure at 45 °C, the residue was partitioned between EtOAc and H_2O containing a little NaOH. The EtOAc was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with $CHCl_3$ gave 1.9 g of the protected carbonate as a clear, colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9 H), 2.95 (s, 3 H), 3.55 (m, 2 H), 3.80 (s, 3 H), 4.32 (m, 2 H), 6.89 (d, 2 H), 7.10 (d, 2 H).

A solution of this BOC-protected carbonate (1.9 g) in dry EtOAc (30 mL) was cooled in an ice bath and saturated with anhydrous HCl. After stirring at ice bath temperature for 20 min and then at room temperature for 30 min, solvents were removed under reduced pressure and the residue was recrystallized from MeOH–EtOAc–hexane to give the deprotected carbonate 8 (0.75 g, 61%).

Determination of Carbamate Half-Lives in Buffer. Buffer solution (2.0 mL), preheated to 37 °C, was added quickly to approximately 0.5 mg of the carbamate hydrochloride salt to give a final concentration of approximately 1 mM. The resulting solution was heated at 37 °C while 20-μL samples were removed at intervals and injected directly into the HPLC injection port.

(20) Verbiscar, A. J.; Abood, L. G. *J. Med. Chem.* **1970**, *13*, 1176.
(21) Firestone, R. A.; Pisano, J. M.; Falck, J. R.; McPhaul, M. M.; Kreiger, M. J. *J. Med. Chem.* **1984**, *27*, 1037.
(22) Alexander, J.; Cargill, R.; Michelson, S. R.; Schwam, H. *J. Med. Chem.* **1988**, *31*, 318.
(23) Svensson, L.-A.; Tunek, A. *Drug Metab. Rev.* **1988**, *19*, 165.

In some cases, 0.10-mL aliquots were quenched in 1 N HCl (1.8 mL) prior to injection. Unreacted carbamate and 4-methoxyphenol concentrations were determined by HPLC analysis with a C-18 reverse-phase column using either a gradient mobile phase of 95% dilute H_3PO_4 (1.0 mL of 85% H_3PO_4 in 1.0 L of H_2O)–5% CH_3CN to 5% dilute H_3PO_4–95% CH_3CN over 30 min or isocratic elution with a mobile phase of 87.5% dilute H_3PO_4–12.5% CH_3CN, flow = 1.0 or 3.0 mL/min. The carbamates are stable at the pH of the mobile phase, and therefore injection into the HPLC effectively stops the reaction. The detector was set at 220 nm. The half-life is the time required for 50% conversion of carbamate to 4-methoxyphenol and was calculated by using first-order kinetics. Results in Table I are the average of at least two separate determinations.

Stability of *N*-Methyl-*N*-[2-(methylamino)ethyl]carbamic Acid 4-Methoxyphenyl Ester Hydrochloride (1a) in Murine Plasma. A solution of the carbamate hydrochloride **1a** (0.27 mg) in 0.10 mL of dilute H_3PO_4 (1 mL of 85% H_3PO_4 in 1 L of H_2O) was added to a magnetically stirred mixture of fresh murine plasma (1.6 mL) and pH 7.4 phosphate buffer (0.40 mL) preheated to 37 °C to give a carbamate concentration of 5×10^{-4} M. This solution was maintained at 37 °C and pH 7.4 with a Radiometer Copenhagen pH stat. At various intervals, aliquots (25 μL) were removed, quenched in 7% $HClO_4$ (0.20 mL) to stop the reaction, and shaken. After centrifugation (14000 *g*, 8 min) clear supernatant was pipetted from the insoluble pellet and analyzed by the same HPLC method used in the buffer reactions.

Acknowledgment. We are indebted to J. Moreau for elemental analyses, Y. Lee for the pK_a determinations, S. Pitzenberger, J. Murphy, and S. Varga for NMR spectra, and D. Frankenfield for supplying the murine plasma. We also thank W. Randall for helpful advice on determining half-lives of the carbamates and V. Finley for preparation of the manuscript.

Registry No. **1a**, 122734-28-5; **1a** (base), 122734-36-5; **1b**, 122734-30-9; **1b** (base), 122734-37-6; **1c**, 122754-64-7; **1d**, 122734-29-6; **1e**, 123183-68-6; **1f**, 123183-69-7; **1g**, 122734-33-2; **1h**, 123183-70-0; **2**, 150-76-5; **4a**, 112257-19-9; **4c**, 122734-34-3; **4d**, 122734-32-1; **4d**·fumarate, 123183-73-3; **4e**, 121492-06-6; **4e**·fumarate, 123183-74-4; **4f**, 57260-73-8; **4f**·fumarate, 123183-75-5; **4g**, 75178-96-0; **4g**·fumarate, 123183-76-6; **4h**, 123183-72-2; **5a**, 122734-31-0; **5c**, 122734-35-4; **5d**, 123183-77-7; **5e**, 123183-78-8; **5f**, 123183-79-9; **5g**, 123183-80-2; **5h**, 123183-81-3; **6**, 57561-39-4; **7**, 123183-82-4; **8**, 123183-71-1; di-*tert*-butyl dicarbonate, 24424-99-5; ethylenediamine, 107-15-3; *N*-methylethylenediamine, 109-81-9; 4-methoxyphenyl chloroformate, 7693-41-6; *N,N'*-dimethylimidazolidinone, 80-73-9; *N,N'*-dimethylethylenediamine, 110-70-3; *N*-methylethanolamine, 109-83-1; *N,N'*-diethylethylenediamine, 111-74-0; 1,3-propanediamine, 78-90-0; *N,N'*-dimethyl-1,3-propanediamine, 111-33-1.

Synthesis and Biochemical Studies of 7-Substituted 4,6-Androstadiene-3,17-diones as Aromatase Inhibitors[1]

Pui-Kai Li and Robert W. Brueggemeier*

College of Pharmacy and OSU Comprehensive Cancer Center, 500 West 12th Avenue, The Ohio State University, Columbus, Ohio 43210. *Received March 6, 1989*

Inhibitors of aromatase, the cytochrome P-450 enzyme complex responsible for the biosynthesis of estrogens, may be useful as therapeutic agents for the treatment of estrogen-dependent disease states such as breast and endometrial cancer. Several 7α-thio-substituted androstenediones have proven to be potent inhibitors of aromatase in vitro and in vivo. Recent research efforts have focused on designing aromatase inhibitors with both substitution at C-7 and extended linear conjugation in rings A and B of the steroid nucleus. The targeted compounds, 7-substituted 4,6-androstadiene-3,17-diones **4–10**, were prepared by the addition of either Grignard or lithium reagents to 3,3:17,17-bis(ethylenedioxy)-5-androsten-7-one (**3**). Inhibitory activities of the compounds were evaluated in vitro by enzyme kinetic studies employing the microsomal fraction isolated from human term placenta. 7-Benzyl- and 7-phenethyl-4,6-androstadiene-3,17-dione analogues are effective inhibitors with apparent K_i's of 60.9–174 nM, while the 7-phenyl analogue exhibited an apparent K_i of 1.424 μM. Thus, several 7-substituted 4,6-androstadiene-3,17-diones were prepared and exhibited good competitive inhibition of aromatase in vitro in human placental microsomes.

Aromatase is the cytochrome P-450 enzyme complex responsible for the conversion of androgens to estrogens. Estrogens are involved in reproductive processes and are also implicated in estrogen-dependent disease states such as breast and endometrial cancers. Thus, inhibitors of aromatase may be useful in controlling these physiological processes and disease states. The aromatase inhibitors 4-hydroxyandrostenedione and aminoglutethimide have demonstrated therapeutic effectiveness in the treatment of hormone-dependent breast tumors in both animals[2-4] and humans.[5-7]

Previous work from our laboratory has illustrated that several 7α-thio-substituted derivatives of androstenedione were effective inhibitors of aromatase.[8-12] Among the compounds synthesized, 7α-[(4'-aminophenyl)thio]-4-androstene-3,17-dione (7α-APTA) was found to be one of the most potent inhibitors with an apparent K_i of 18 nM. These aromatase inhibitors have also demonstrated activity in inhibiting aromatase activity in MCF-7 cells[13] and in reducing tumor volumes in the DMBA-induced rat

(1) A portion of this work was presented: 70th Annual Meeting of the Endocrine Society, New Orleans, LA, June 8–11, 1988; Abstract 616.
(2) Brodie, A. M. H.; Schwarzel, W. C.; Shaikh, A. A.; Brodie, H. J. *Endocrinology* **1977**, *100*, 1684.
(3) Brodie, A. M. H.; Marsh, D. A.; Brodie, H. J. *J. Steroid Biochem.* **1979**, *10*, 423.
(4) Brodie, A. M. H.; Brodie, H. J.; Garrett, W. M.; Hendrickson, J. R.; Marsh, D. A.; Tsai-Morris, C.-H. *Biochem. Pharmacol.* **1982**, *31*, 2017.
(5) Santen, R. J.; Santner, S.; Davis, B.; Veldhuis, J.; Samojlik, E.; Ruby, E. *Clin. Endocrinol. Metab.* **1978**, *47*, 1257.
(6) Coombes, R. C.; Goss, P.; Dowsett, M.; Gazet, J.-C.; Brodie, A. M. H. *Lancet* **1984**, *2*, 1237.
(7) Sjoerdsma, A. *Clin. Pharmacol. Ther.* **1981**, *30*, 3.
(8) Brueggemeier, R. W.; Floyd, E. E.; Counsell, R. E. *J. Med. Chem.* **1978**, *21*, 1008.
(9) Brueggemeier, R. W.; Snider, C. E.; Counsell, R. E. *Cancer Res. (Suppl.)* **1982**, *42*, 3334s.
(10) Darby, M. V.; Lovett, J. A.; Brueggemeier, R. W.; Groziak, M. P.; Counsell, R. E. *J. Med. Chem.* **1985**, *28*, 803.
(11) Snider, C. E.; Brueggemeier, R. W. *J. Steroid Biochem.* **1985**, *22*, 325.
(12) Snider, C. E.; Brueggemeier, R. W. *J. Biol. Chem.* **1987**, *262*, 8685.
(13) Brueggemeier, R. W.; Katlic, N. E. *Cancer Res.* **1987**, *47*, 4548.

0022-2623/90/1833-0101$02.50/0