(5.00 mL, 49.6 mmol) over a period of 5 min. The mixture was stirred in an ice bath for an additional 5 min and the crude product collected, washed with H_2O, and dried in vacuo. A solution of the solid (4.78 g) in cyclohexane (100 mL) was filtered and refrigerated to give crystalline **14** which was collected and washed with cold cyclohexane: yield 3.28 g (25%); mp 45 °C (lit.[8] mp 42–46 °C). Anal. ($C_9H_8BrNO_4$) C, H, N.

***N*-[(3-Bromopropionyl)oxy]succinimide (18).** *N*-Hydroxysuccinimide (4.97 g, 43.1 mmol), dried at 56 °C in vacuo, 2-bromopropionic acid (6.60 g, 43.1 mmol), and DCC (8.90 g, 43.1 mmol) were added successively under N_2 with stirring to anhydrous ethyl acetate (1.2 L). After 20 h, the solution was filtered under N_2 and evaporated at 25 °C in vacuo to a syrup. A solution of the syrup in EtOAc (20 mL) was filtered and evaporated under high vacuum to a crystalline solid which was triturated with EtOH (50 mL), collected, washed with EtOH, and dried: yield 7.72 g (72%); mp 86 °C. Anal. ($C_7H_8BrNO_4$) C, H, N.

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Registry No. **1**, 25152-20-9; **2**, 50700-63-5; **3**, 101314-73-2; **4**, 72164-50-2; **5**, 101314-74-3; **6**, 101314-75-4; **7**, 4356-92-7; **8**, 101314-76-5; **9**, 75191-50-3; **10**, 101314-77-6; **11**, 101314-78-7; **12**, 101314-79-8; **13**, 101314-80-1; **14**, 101314-81-2; **15**, 101314-82-3; **16**, 101314-83-4; **17**, 56985-87-6; **18**, 101314-84-5; CH_3NH_2, 74-89-5; 3-bromopropionyl chloride, 15486-96-1; thymidine, 50-89-5; bromoacetyl bromide, 598-21-0; silver methanesulfonate, 2386-52-9; 5′-*O*-tosylthymidine, 7253-19-2; 4-nitrophenyl bromoacetate, 19199-82-7; bromomethanesulfonyl chloride, 10099-08-8; diethyl oxalate, 95-92-1; 4-(fluorosulfonyl)benzoyl chloride, 402-55-1; 3-(fluorosulfonyl)benzoyl chloride, 454-93-3; phenyl chloroformate, 1885-14-9; sodium *p*-nitrophenoxide, 824-78-2; 2-bromopropionyl chloride, 7148-74-5; *N*-hydroxysuccinimide, 6066-82-6; 2-bromopropionic acid, 598-72-1; chloroacetyl chloride, 79-04-9.

Synthesis and Biological Activity of Resolved C-10 Diastereomers of 10-Methyl- and 10-Ethyl-10-deazaminopterin

J. I. DeGraw,* P. H. Christie,† H. Tagawa,† R. L. Kisliuk,‡ Y. Gaumont,‡ F. A. Schmid,§ and F. M. Sirotnak§

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025, Department of Biochemistry, Tufts University Medical School, Boston, Massachusetts 02111, and Laboratory for Molecular Therapeutics, Memorial Sloan Kettering Cancer Center, New York, New York 10021. *Received September 30, 1985*

Synthesis and evaluation of the antitumor drugs 10-methyl- and 10-ethyl-10-deazaminopterin (**15a,b**) were previously reported for the diastereomeric mixtures, lacking resolution at the C-10 position. In order to assess biological properties of the individual diastereomers, the C-10 isomers of 4-amino-4-deoxy-10-methyl- and 10-ethyl-10-deazapteroic acids (**13a,b**) were prepared by total synthesis. Coupling with L-glutamate afforded the appropriate diastereomers of the title compounds. Biochemical, transport, and cell growth inhibitory properties in L1210 cells and folate-dependent bacteria were measured. Differences were generally less than 2-fold between diastereomeric pairs, but a factor of 3 was noted for *d*,L-**15b** vs. *l*,L-**15b** in inhibition of DHFR from L1210 cells and in cytotoxicity toward L1210 cells. An in vivo comparison of the isomers of **15b** with racemic compound against L1210 in mice did not show a significant efficacy difference (ILS) among the compounds. However, *d*,L-**15b** showed an acute toxicity about 2.5 times that of *l*,L-**15b**.

In a previous paper[1] we reported the synthesis, in vitro observations on bacterial and L1210 cells, and antileukemic activity in L1210 bearing mice for 10-methyl- and 10-ethyl-10-deazaminopterin (**15a,b**). The latter drug has been found to be considerably more efficacious than methotrexate or 10-deazaminopterin in a number of experimental murine tumor models.[2-4] More recently it was shown to cause regressions in human mammary, lung, and colon tumor xenografts in nude mice.[5] Clinical trials have been initiated[6] for this agent, whose primary advantage appears to lie in its enhanced differential penetration and polyglutamylation in tumor vs. normal tissue.[7] The enhanced transport takes place via an active-transport protein in the cell wall and represents one of the few examples whereby an antitumor drug takes advantage of a fundamental difference in the nature of this transport system between tumor and normal cells. The advantage of an enhanced polyglutamation of the compound once it enters the cell may result from diminished efflux of the polyglutamate species from the cell and also its increased inhibitory potency for folate-dependent thymidine and purine synthetic enzymes.

The 10-alkyl-10-deazaminopterin molecules have two chiral centers, namely, at the 10-position and the α-carbon of the glutamate moiety. The latter is conveniently fixed as the L isomer by incorporation of L-glutamate into the synthetic scheme. However, our synthetic route previously reported[1] afforded compounds that were completely ra-

† SRI International.
‡ Tufts University of Medical School.
§ Memorial Sloan Kettering Cancer Center.

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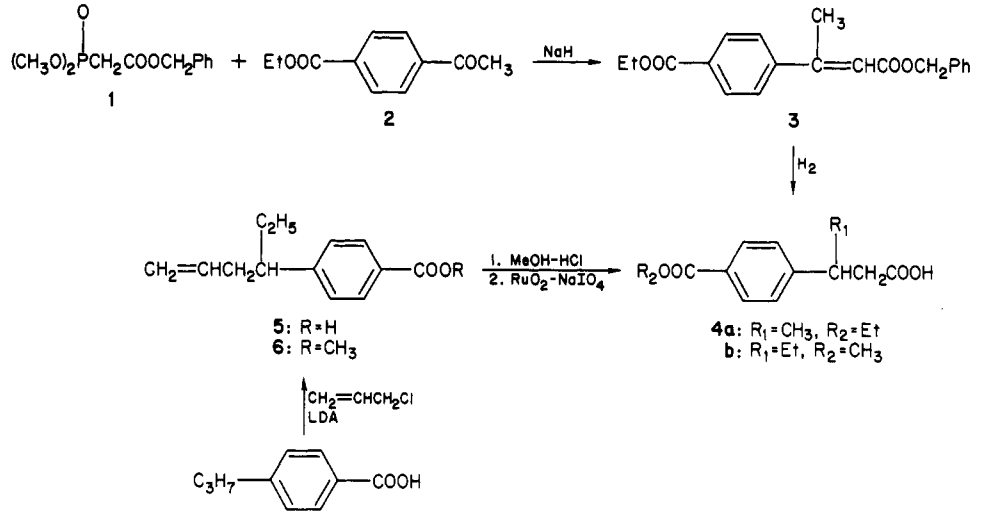
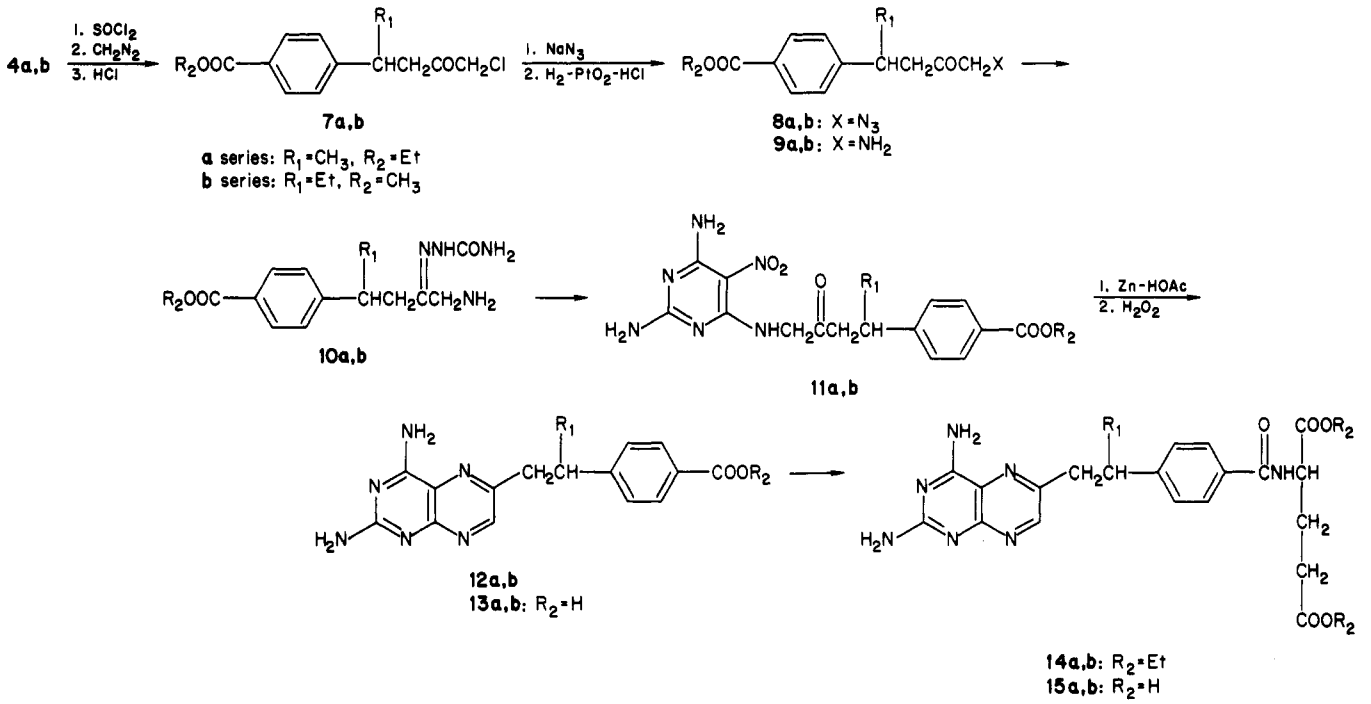
0022-2623/86/1829-1056$01.50/0

Scheme I



Scheme II



cemic about the 10-position. In this paper we describe the synthesis of the individual diastereoisomers for **15a** and **15b** at C-10 and report observations on their respective biological parameters.

Chemistry

The synthesis of the chiral forms of 10-methyl- (**15a**) and 10-ethyl-10-deazaminopterin (**15b**) is outlined in Schemes I and II. Key intermediates are the 4-amino-4-deoxy-10-alkylpteroic acids (**13a** and **13b**), which were coupled with diethyl L-glutamate to eventually yield the individual target diastereomers. Resolution of racemic precursors was accomplished at the intermediates 3-(*p*-carbethoxyphenyl)butyric acid (**4a**) in the 10-methyl series and 3-(*p*-carbomethoxyphenyl)valeric acid (**4b**) for the 10-ethyl series.

Condensation of benzyl (dimethoxyphosphono)acetate (**1**) with ethyl *p*-acetylbenzoate (**2**) gave benzyl 3-(*p*-carbethoxyphenyl)crotonate (**3**) in 60% yield. Hydrogenation of the unsaturated diester over 10% Pd/C smoothly afforded saturation of the olefin with concomitant hydrogenolytic cleavage of the benzyl ester to yield crystalline 3-(*p*-carbethoxyphenyl)butyric acid (**4a**). Recrystallization of the quinine salt of **4a** from EtOH followed by regeneration with 1 N HCl gave the *d* form of **4a**, $[\alpha]$ +46°. The partially racemic acid obtained from first formation of the quinine salt was converted to the *l*-α-methylbenzylamine salt and recrystallized to eventually yield the *l* form of **4a**, $[\alpha]$ −39°.

We initially attempted to use a similar synthetic strategy for the ethyl homologue **4b**, but condensation of **1** with ethyl *p*-propionylbenzoate led to complex mixtures. Alternatively, alkylation of the dianion of *p*-propylbenzoic acid with allyl bromide followed by esterification of the acid **5** yielded 4-(*p*-carbomethoxyphenyl)-1-hexene (**6**). Oxidation of the olefinic group with $NaIO_4$ in aqueous acetone as catalyzed by RuO_2 resulted in degradation of the terminal carbon to afford 3-(*p*-carbomethoxyphenyl)valeric acid (**4b**). Recrystallization of the salt with *d*-α-methylbenzylamine led to the *d* form of **4b**, $[\alpha]$ +22.7°, while similar treatment of the *l*-α-methylbenzylamine salt gave the *l* form of **4b**, $[\alpha]$ −24.1°.

The racemic acids **4a,b** and their resolved forms were then carried through the sequence shown in Scheme II to

Table I. Biochemical and Growth Inhibition Data Derived with L1210 Cells[a]

compd	DHFR inhibn (K_i),[b] pM	growth inhibn (MIC_{50}),[c] nM	transport[b] influx: K_i, μM	transport[b] efflux: k, min^{-1}
l,L-**15a**	3.17	1.18	1.90	0.267
d,L-**15a**	4.24	0.68	1.91	0.258
l,L-**15b**	12.40	1.52	1.62	0.249
d,L-**15b**	3.93	0.43	1.58	0.253
MTX	4.42	2.48	5.61	0.213

[a] Average of three to six runs; SE less than 15%. [b] See ref 1 for methods. [c] See Sirotnak, F.; et al. *Biochem. Pharmacol.* **1979**, *28*, 2993.

yield the target diastereomers, **15a,b**. This process, which is similar to that originally used for synthesis of 10-deazaminopterin,[8] began with conversion of the acid chlorides of **4a,b** to the chloromethyl ketones **7a,b** via the diazomethyl ketone intermediates. Displacement of the chlorides with sodium azide in 80% MeOH afforded the azidomethyl ketones **8a,b**. Hydrogenation of the azides over Pd black in the presence of HCl gave the amino ketones, which were isolated and characterized as the crystalline picrate salts. The ketone picrates **9a,b** were directly converted to the semicarbazone derivatives **10a,b** by treatment with semicarbazide hydrochloride in 70% EtOH. Exchange of picrate salt for hydrochloride was easily accomplished in quantitative yield by stirring with Dowex 2 (Cl^-) resin in aqueous EtOH.

Alkylation of the free bases of **10a,b** with 2,4-diamino-6-chloro-5-nitropyrimidine in the presence of 1 equiv of collidine, followed by hydrolysis of the intermediate semicarbazones in 90% CF_3COOH, afforded the (2,4-diamino-6-pyrimidinyl)amino ketones **11a,b**. Reduction of the nitro group with zinc dust in acetic acid caused ring closure to the dihydro pteridines, which were oxidized in situ to the diamino pteridine esters **12a,b** by treatment with dilute H_2O_2. The benzoate esters were saponified by brief warming with aqueous NaOH in 2-methoxyethanol. The 4-amino-4-deoxy-10-alkyl-10-deazapteroic acids (**13a,b**) so obtained were identical with those reported in our previous publication.[1] Coupling with diethyl L-glutamate followed by saponification of the diethyl esters **14a,b** gave the target diastereomers of 10-methyl-10-deazaaminopterin (*d*,L-**15a** and *l*,L-**15a**) and the 10-ethyl analogue (*d*,L-**15b** and *l*,L-**15b**). Chromatographic and spectral properties for the final products were likewise identical with those previously reported.[1]

It is interesting to note that the NMR signal for the pterin 7-proton was shifted incrementally with variation of the 10-substituent. The 10-H compound showed a chemical shift of 8.59 ppm (in Me_2SO-d_6), 10-Me (8.45), 10-Et (8.35), 10-Pr (8.33) drawing to a constant value of 8.32 for the 10-amyl analogue. This association of the 10-substituent with the pterin ring may also be reflected in the spectrum of biological activities. The apparent partial association of the pterin and benzoate rings could affect rates of hydroxylation at C-7 on the pteridine ring.

Biological Results

In Table I data are presented that show some biochemical and transport properties in L1210 cells for the resolved forms of **15a,b** in comparison with MTX. All of the diastereomers showed similar affinities for the transport protein of L1210. The K_i values against MTX were about 1.9×10^{-6} M for the 10-methyl diastereomers and 1.6×10^{-6} M for the 10-ethyl homologues. All had stronger affinities than MTX at 5.6×10^{-6} M and similar ratios as previously observed[1] for the racemates. Likewise the efflux rate constants (k) were very similar for all compounds including MTX. For inhibition of dihydrofolate reductase derived from L1210, the diastereomeric forms of **15a** were not significantly different from one another or MTX with K_i values in the $3–4 \times 10^{-12}$ range. However, there was a 3-fold difference between *l*,L-**15b** and *d*,L-**15b** isomers, with the latter also approximating MTX. This factor of 3 difference was also reflected in the inhibition of growth for L1210 cells with *l*,L-**15b** being only one-third as potent as the *d*,L form. There was not a significant difference observed between the 10-methyl isomers in the growth inhibition.

Table II. Bacterial Growth and Enzyme Inhibition[a]

compd	growth inhibn: IC_{50},[b,c] ng/mL *S. faecium* ATCC8043	growth inhibn: IC_{50},[b,c] ng/mL *L. casei* ATCC7469	DHFR inhibn: IC_{50}, nm *L. casei*	DHFR inhibn: IC_{50}, nm chicken liver
l,L-**15b**	2.2	0.23	38	22
d,L-**15b**	3.9	0.29	14	19
rac-**15b**	2.7	0.26	22	20
7,8-H_2-*l*,L-**15b**[d]	1.1	0.165	120	50
7,8-H_2-*d*,L-**15b**	1.1	0.027	25	26
7,8-H_2-rac-**15b**	1.1	0.055	32	34
5,6,7,8-H_4-*l*,L-**15b**[e]	3.1	0.30	120	76
5,6,7,8-H_4-*d*,L-**15b**	2.5	0.08	35	20
5,6,7,8-H_4-rac-**15b**	2.6	0.14	50	34
MTX	0.4	0.013	10	19

[a] See ref 8 for conditions. [b] Folate concentration = 1 ng/mL. [c] All compounds were weak inhibitors or inactive against MTX-resistant strains of these bacteria. [d] UV, pH 7.4, 292 nm, 324; reduced with $Na_2S_2O_4$. [e] UV, pH 7.4, 296 nm; reduced with H_2–PtO_2.

In Table II we present comparisons of the diastereomers and racemates of **15b** with regard to their abilities to inhibit growth of folate dependent bacteria *Streptococcus faecium* and *Lactobacillus casei* and we have also included observations on the 7,8-dihydro and 5,6,7,8-tetrahydro forms of these compounds. The table further contains data on the inhibitory potencies (IC_{50}) against the dihydrofolate reductases derived from *L. casei* and chicken liver. Against *S. faecium* *l*,L-**15b** was slightly more potent than the *d*,L isomer. Reduction to the dihydro form caused an increase in potency with no difference observed among the compounds. Further reduction to tetrahydro derivatives caused the inhibitory potencies to return to the range seen for the fully aromatic substrates but with a reversal in the *l*,L to *d*,L relationship. In *L. casei* the compounds were more active by 1 order of magnitude. Again little difference was noted among the unreduced inhibitors; however, dihydro *d*,L-**15b** was about 11 times more active than the precursor and 6 times more active than the corresponding dihydro *l*,L-**15b**. Further reduction to tetrahydro caused activity to decrease, but the superiority of the *d*,L isomer was maintained.

Inhibition of the dihydrofolate reductases derived from *L. casei* and chicken liver was fairly similar among the isomers and the MTX control. A notable difference was the ca. 3-fold greater potency of the *d*,L-**15b** over *l*,L-**15b** against the *L. casei* enzyme. Reduction decreased potencies but only slightly in the *d*,L series. In all cases involving the enzymes or whole cell inhibitions, the racemic **15b** was intermediate between the values for the resolved diastereomers.

The isomers of **15b** and the unresolved drug were also evaluated in vivo against L1210 leukemia in mice. As

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Table III. Antitumor Activity against L1210 Leukemia in Mice

compd[a]	dose, mg/kg	MST, days	ILS, %	30-day survivors, obsd/total	toxic deaths, no./total
control		6.6	0	0/5	0/5
d,L-**15b**	9	18.3 ± 1.7	+177	0/6	0/6
	12	19.8 ± 2.3	+199	0/9	3/9
	18	17.5 ± 1.5	>+165	1/9	5/9
	24	10.0 ± 0	+55	0/3	3/3
l,L-**15b**	12	15.7 ± 0.5	+138	0/3	0/3
	18	17.3 ± 0.7	+163	0/9	0/9
	24	19.0 ± 1.0	+187	0/9	0/9
	32	18.8 ± 2.2	+181	0/6	1/6
rac-**15b**	9	15.5 ± 0.6	+135	0/10	0/10
	12	16.7 ± 0.7	+153	0/10	0/10
	18	19.3 ± 0.8	+193	0/10	0/10
	24	20.3 ± 1.9	>+207	2/10	2/10
	32	16.0 ± 1.1	+67	0/10	10/10
MTX	9	15.7 ± 0.4	+137	0/10	0/10
	12	16.3 ± 0.7	+147	0/10	1/10
	18	14.5 ± 1.6	+119	0/10	6/10
	24	9.6 ± 1.1	+46	0/10	10/10

[a] Drug given sc (Q2D×5) 24 h after 10^6 cells implanted ip.

shown in Table III there was not a significant difference in the percent increase in life span among the test compounds. However, the results indicate that *d*,L-**15b** is ca. 2.5 times as toxic as *l*,L-**15b**. The higher toxicity of *d*,L-**15b** correlates with its greater inhibition of dihydrofolate reductase.

In general the observations in the in vitro and in vivo systems shown in Tables I–III do not indicate major differences among stereoisomeric forms in the fully aromatic state. The only outstanding difference noted in the study was the 6-fold greater potency of the dihydro *d*,L-**15b** vs. the dihydro *l*,L-**15b** for growth inhibition of *L. casei*. The *d*,L isomers were generally more inhibitory than the *l*,L isomers in the various in vitro assays. Insofar as the results from a single animal model, L1210, can be predictive, they indicate that continued use of the equal diastereomeric mixture of **15b** is acceptable for clinical application. The formidable task of preparation of pure diastereomers is unwarranted at present; however, future research may indicate the desirability of a chiral synthesis.

Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. The ^{1}H NMR spectra were taken on a Varian EM 360A or a JEOL FX90Q spectrometer. Mass spectra were run on a LKB 9000 GC–MS spectrometer. Ultraviolet spectra were taken on a Perkin-Elmer 552 spectrophotometer. TLC was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Melting points were determined on a Thomas-Hoover Uni-melt apparatus. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter.

Benzyl 3-(*p*-Carbethoxyphenyl)crotonate (3). To an ice-cooled stirred mixture of 4.83 g (0.1 mol) of 50% NaH in oil and 244 mL of DMF was added dropwise a solution of 24.4 g (0.094 mol) of benzyl (dimethoxyphosphono)acetate (1).[9] The mixture was stirred for 1 h at room temperature and a solution of ethyl *p*-acetylbenzoate (2) (18.2 g, 0.094 mol) in 70 mL of DMF was added. After 20 h the solvent was evaporated in vacuo and the residue partitioned between 165 mL of 10% HOAc and 400 mL of Et_2O. After two additional extractions with 100-mL portions of Et_2O, the Et_2O extract was dried ($MgSO_4$) and evaporated to leave 32.0 g of a brown oil. The crude product was chromatographed on 400 g of Baker Flash Chromatography silica gel with elution by hexane–Et_2O (85:15) to afford 18.3 g (60%) of the product as a colorless oil: NMR ($CDCl_3$) δ 1.37 (3 H, t, CH_2CH_3), 2.58 (3 H, s, CH_3), 4.38 (2 H, q, CH_2O), 5.22 (2 H, s, $-OCH_2Ph$), 6.23 (1 H, s, olefin), 7.38 (5 H, s, C_6H_5), 7.78 (4 H, q, benzoate).

3-(*p*-Carbethoxyphenyl)butyric Acid (4a). A mixture of 3.95 g of the benzyl ester **3**, 0.4 g of 10% Pd/C, and 100 mL of EtOH was stirred under an atmosphere of hydrogen until 2 equiv were taken up (2 h). The catalyst was removed by filtration and the filtrate evaporated. The residue was crystallized from Et_2O–cyclohexane to afford white crystals, 2.55 g (89%), mp 95–97 °C. Anal. $C_{13}H_{16}O_4$ (C, H).

Three recrystallizations of the quinine salt of **4a** from EtOH afforded white crystals, mp 183–184 °C. The salt was treated with 1 N HCl and the liberated product extracted into $CHCl_3$. Evaporation of solvent gave *d*-3-(*p*-carbethoxyphenyl)butyric acid, $[\alpha]_D$ +46.0° ($CHCl_3$).

The mother liquor from the first crystallization of the quinine salt was evaporated and the product acid extracted into $CHCl_3$ after liberation by 1 N HCl. The $CHCl_3$ extract was evaporated and the residue was treated with 1 equiv of *l*-α-methylbenzylamine. The salt was recrystallized six times from *i*-PrOH tc give white crystals, mp 147–148 °C. Liberation of the free acid as above gave *l*-3-(*p*-carbethoxyphenyl)butyric acid, $[\alpha]_D$ −39° ($CHCl_3$).

4-(*p*-Carboxyphenyl)-1-hexene (5) and Methyl Ester (6). To an ice-cold solution of diisopropylamine (8.5 mL, 0.061 mol) in 100 mL of dry THF was added, dropwise, 45.6 mL (0.073 mol) of 1.6 M BuLi in hexane. The solution was chilled for 1 h when a solution of *p*-propylbenzoic acid (5.0 g, 0.03 mol) in 30 mL of THF was added dropwise followed by 5.8 mL of HMPA. The mixture was kept at ambient temperature for 63 h and treated dropwise with 3.6 g (0.03 mol)e of allyl bromide in 30 mL of THF to quench the color of the dark red dianion. The solvent was evaporated in vacuo and the residue partitioned between water (200 mL) and Et_2O (50 mL). After two additional Et_2O washes the aqueous solution was acidified with 2 N HCl to precipitate the product. The mixture was extracted with three 50-mL portions of $CHCl_3$, which were dried ($MgSO_4$) and evaporated to leave 10 g of crude product (contained HMPA).

The material was immediately esterified by stirring with 100 mL of 4.5% HCl in MeOH at room temperature for 42 h. The solvent was removed in vacuo and the residue taken up in 100 mL of $CHCl_3$ and washed with 25 mL of saturated $NaHCO_3$. The $CHCl_3$ was evaporated and the residue dissolved in 100 mL of pentane. The solution was washed with water (25 mL), dried over $MgSO_4$, and evaporated to leave 4.1 g (63%) of the methyl ester **6** as a liquid; TLC ($CHCl_3$–MeOH, 9:1; silica gel) R_f 0.90; NMR ($CDCl_3$) δ 0.80 (3 H, t, CH_3), 1.70 (2 H, m, CH_2CH_3), 2.50 (3 H, m, allylic, benzylic H's), 3.90 (3 H, s, OCH_3), 5.0 (3 H, m, olefins), 7.25 (2 H, d, 3′,5′-Ar), 8.00 (2 H, d, 2′,6′-Ar).

A portion of ester **6** was saponified (10% NaOH–MeOH, 1:7) and the liberated acid was purified by preparative TLC (slica gel; $CHCl_3$–MeOH, 9:1) to afford an analytical sample of **5** as a gum. Anal. $C_{13}H_{16}O_2 \cdot {}^1/_4H_2O$ (C, H).

3-(*p*-Carbomethoxyphenyl)valeric Acid (4b). A solution of the hexenyl benzoate **6** (21.0 g, 0.096 mol) in 990 mL of acetone was cooled in ice, and a cold solution of $NaIO_4$ (103 g, 0.48 mol) in 660 mL of water containing 1.25 g of RuO_2 hydrate was added to cause formation of a copious light green precipitate. The mixture was stirred for 10 min in the cold and another 20 min at room temperature. The mixture was filtered through a Celite pad, followed by a wash with 60% acetone. The filtrate was

treated with 10 mL of 2-PrOH, kept at room temperature for 10 min, and saturated with NaCl. The mixture was extracted with three 500-mL portions of $CHCl_3$, which were washed with 200 mL of brine and extracted with 500 mL of 1.5 N NH_4OH. The NH_4OH extract was acidified with concentrated HCl and thrice extracted with 100-mL portions of $CHCl_3$. After drying ($MgSO_4$), the $CHCl_3$ was evaporated to leave 17.5 g (77%) of the product as a brown partially crystalline syrup; NMR ($CDCl_3$) δ 0.82 (3 H, t, CH_3), 1.73 (2 H, m, CH_2CH_3), 2.60 (2 H, m, CH_2COOH), 3.90 (3 H, s, OCH_3), 7.33 (2 H, d, 3',5'-Ar), 8.10 (2 H, d, 2',6'-Ar). The acid was characterized as the salt with *d*-α-methylbenzylamine as described below.

Four crystallizations of the salt of **4b** with *d*-α-methylbenzylamine from 2-propanol gave white crystals, mp 165–166 °C. Anal. $C_{21}H_{27}NO_4$ (C, H, N). Liberation of the free acid by partition between 1 N HCl and $CHCl_3$ afforded a gum, $[\alpha]_D$ +22.7° ($CHCl_3$).

The mother liquors from the first crystallization were evaporated, and the free acid was liberated as above. The salt prepared by mixture with 1 equiv of *l*-α-methylbenzylamine was crystallized twice from 2-propanol to give white crystals, mp 164–165 °C. The free acid showed a rotation of $[\alpha]_D$ −24.1° ($CHCl_3$).

1-Chloro-4-(*p*-carbomethoxyphenyl)-2-hexanone (7b). A solution of the racemic acid **4b** (5.9 g, 0.025 mol) in 30 mL of benzene containing 38 mL of $SOCl_2$ was heated under reflux for 1 h. The solvent was removed in vacuo and the residual acid chloride dissolved in 20 mL of Et_2O. The solution was added dropwise at 0–5 °C to an ethereal solution of diazomethane (from 22.6 g of nitrosomethylurea). The yellow solution of the incipient diazo ketone was treated with gaseous HCl for 30 min and poured into 135 mL of ice H_2O. The Et_2O phase was separated and washed with cold 0.5 N Na_2CO_3 (25 mL) and H_2O (25 mL) and dried over $MgSO_4$. Evaporation of solvent afforded 5.9 g (88%) of a yellow oil; TLC (silica gel; $CHCl_3$–MeOH, 40:1) R_f 0.75 single spot; NMR ($CDCl_3$) δ 0.80 (3 H, t, CH_3), 1.73 (2 H, m, CH_2CH_3), 2.97 (2 H, m, $CH_2C{=}O$), 3.20 (1 H, m, Ar CH), 3.90 (3 H, s, OCH_3), 3.95 (2 H, s, CH_2Cl), 7.30 (2 H, d, 3',5'-Ar H), 8.10 (2 H, d, 2',6'-Ar H).

The chloromethyl ketone **7a** was obtained also in 88% yield by a similar procedure from the acid **4a**. The optically resolved forms of **4b** and **4a** were carried through the same processes described for the chloromethyl ketone preparation and all subsequent steps as described below. Physical and chromatographic properties were equal to those of their racemic counterparts except as noted.

1-Azido-4-(*p*-carbomethoxyphenyl)-2-hexanone (8b). A mixture of the chloromethyl ketone **7b** (5.9 g, 0.022 mol), 10.8 g (0.16 mol) of NaN_3, and 180 mL of 80% MeOH was stirred at room temperature for 20 h. After evaporation of MeOH the aqueous residue was diluted to 100 mL with H_2O and extracted with three 50-mL portions of $CHCl_3$. The $CHCl_3$ was dried ($MgSO_4$) and evaporated to leave 5.5 g (91%) of a yellow oil; TLC (silica gel; $CHCl_3$–C_6H_6, 1:1) R_f 0.30 single spot; IR cm^{-1} 2105 (N_3); NMR ($CDCl_3$) δ 0.80 (3 H, t, CH_3), 1.70 (2 H, m, CH_2CH_3), 2.80 (2 H, m, $CH_2C{=}O$), 3.15 (1 H, m, Ar CH), 3.77 (2 H, s, CH_2N_3), 3.90 (3 H, s, OCH_3), 7.30 (2 H, d, 3',5'-Ar H), 8.05 (2 H, d, 2',6'-Ar H).

The corresponding azido ketone in the methyl series **8a** was similarly obtained as an oil in 90% yield.

1-Amino-4-(*p*-carbomethoxyphenyl)-2-hexanone Picrate (9b). A mixture of the azide **8b** (5.50 g, 0.02 mol), 3.05 mL (0.037 mol) of 12 N HCl, 1.88 g of palladium black, and 100 mL of EtOH was stirred under an atmosphere of H_2 for 6 h. The catalyst was removed by filtration and the filtrate was evaporated in vacuo to leave a yellow foam (5.6 g). This residue was treated with 50 mL of H_2O and stirred until homogeneous. The aqueous supernatant was decanted from some insoluble gum and added to a warm solution of picric acid (5.0 g) in 275 mL of H_2O with stirring. The yellow crystalline precipitate was collected, washed with H_2O, and dried to leave 6.70 g (70%), mp 144–145 °C. An analytical sample, mp 162–163 °C, was obtained by recrystallization from EtOH. Anal. $C_{20}H_{22}N_4O_{10}$ (C, H, N). **9b**: *d* isomer, mp 129–131 °C; *l* isomer, mp 125–130 °C.

The amino ketone picrate derived from hydrogenation of **8a** was obtained in 85% yield, mp 112–113 °C after recrystallization from 95% EtOH. Anal. $C_{20}H_{22}N_4O_{10}\cdot{}^1/_2H_2O$ (C, H, N). An NMR spectrum was obtained for the hydrochloride salt in D_2O; δ 1.16 (3 H, d, CH_3), 1.18 (3 H, t, CH_3 of OEt), 2.82 (2 H, m, $CH_2C{=}O$), 3.30 (1 H, m, Ar CH), 3.91 (2 H, s, CH_2N), 4.55 (2 H, q, OCH_2), 7.25 (2 H, d, 3',5'-Ar H), 7.75 (2 H, d, 2',6'-Ar H).

1-Amino-4-(*p*-carbomethoxyphenyl)-2-hexanone Semicarbazone Picrate (10b). A mixture of the amino ketone picrate **9b** (6.4 g, 0.133 mol), semicarbazide hydrochloride (2.6 g, 0.023 mol), and 190 mL of 70% EtOH was stirred at room temperature for 90 h. The solid was collected and washed with 70% EtOH and the filtrate was evaporated and similarly retreated with 1.27 g of semicarbazide hydrochloride in 75 mL of 70% EtOH to afford 0.76 g of yellow crystals, mp 200–201 °C. The original solid above was treated with 225 mL of warm EtOH and the insoluble portion collected to give 2.67 g, mp 196–197 °C. The combined mother liquors were evaporated and retreated with 0.84 g of semicarbazide hydrochloride in 61 mL of 70% EtOH to afford another 1.66 g, mp 200–201 °C, for a total yield of 5.09 g (71%). Anal. $C_{21}H_{25}N_7O_{10}$ (C, H, N). **10b**: *d* isomer, mp 196–197 °C; *l* isomer, mp 196–196.5 °C.

The semicarbazone picrate in the methyl series was similarly obtained in 83% yield, mp 177–180 °C. Anal. $C_{21}H_{25}N_7O_{10}\cdot H_2O$ (C, H, N). **10a**: *d* isomer, mp 172–173 °C; *l* isomer, mp 172–173 °C.

When the picrate salts of **10b** or **10a** were stirred with a 10-fold excess of Dowex 2 (×8) chloride resin in 75% EtOH, followed by filtration and evaporation of solvent, the respective hydrochloride salts were obtained in quantitative yield as white crystals; **10b**·HCl, mp 187–191 °C; **10a**·HCl, mp 188–190 °C.

1-[(2,4-Diamino-5-nitropyrimidin-6-yl)amino]-4-(*p*-carbomethoxyphenyl)-2-hexanone Trifluoroacetate (11b). To a solution of sodium ethoxide (from 219 mg, 0.0095 g-atom of Na) in EtOH (69 mL) was added 3.3 g (0.0095 mol) of the semicarbazone hydrochloride **10b**. The mixture was stirred at room temperature for 30 min and evaporated to dryness in vacuo. The residue was taken up in 170 mL of dry DMF and treated with 1.89 g (0.01 mol) of 2,4-diamino-5-nitro-6-chloropyrimidine[10] and 1.26 mL (0.0095 mol) of *s*-collidine. The mixture was stirred at 90–100 °C for 30 min and evaporated in vacuo. The residue was stirred with 225 mL of ice water and the yellow solid semicarbazone intermediate collected and dried to leave 2.86 g.

The material was stirred with 48 mL of 90% trifluoroacetic acid for 18 h and the solvent removed in vacuo. The residue was treated with 50 mL of water and the pH adjusted to 8.9 with 15% K_2CO_3. The mixture was stirred 15 min and the supernatant decanted from the gummy solid, which was washed with 50 mL of water. The solid was taken up in 100 mL of hot 2-methoxyethanol and some insoluble material removed by filtration, followed by evaporation of the filtrate to afford 2.90 g. This material was redissolved in 5 mL of 2-methoxyethanol and applied to a column of 90 g of Baker Flash Chromatography silica gel. The column was preeluted by $CHCl_3$ and the product removed with $CHCl_3$–MeOH, 93:7, to yield 1.11 g (44%) of a foamy solid. A portion was crystallized from 50% EtOH–Et_2O for analysis, mp 73 °C; NMR ($CDCl_3$) δ 0.80 (3 H, t, CH_3), 1.75 (2 H, m, CH_2CH_3), 2.85 (2 H, m, $CH_2C{=}O$), 3.25 (1 H, m, Ar CH), 3.90 (3 H, s, OCH_3), 4.20 (2 H, d, $NHCH_2C{=}O$), 6.50 (2 H, m, NH_2), 7.35 (2 H, m, 3',5'-Ar H), 8.05 (2 H, d, 2',6'-Ar H), 9.00 (2 H, m, NH_2), 9.80 (1 H, t, $NHCH_2$). Anal. $C_{18}H_{22}N_6O_5\cdot{}^3/_4CF_3COOH\cdot{}^3/_4H_2O$ (C, H, N, F).

The corresponding product in the methyl series **11a** was a solid that could not be purified to a standard acceptable for elemental analysis (mp 126–130 °C). It was used directly in the next step.

Methyl 4-Amino-4-deoxy-10-ethyl-10-deazapteroate (12b). A solution of 1.52 g (0.0038 mol) of the nitro ketone **11b** in 33 mL of HOAc was stirred at 90–100 °C while Zn dust (1.5 g) was added portionwise over 30 min. The mixture was stirred another 15 min at 90–100 °C, cooled to room temperature, and filtered. The filter cake was washed with 17 mL of 50% HOAc, and the combined filtrate and wash were treated with 1.3 mL of 30% H_2O_2. After 1 h the solvent was removed under vacuum and the residue treated with 65 mL of H_2O. The pH was adjusted to 8 with concentrated NH_4OH and the mixture was stirred overnight. The precipitate was collected, washed thoroughly with H_2O, and dried to afford 1.11 g (83%) of pale yellow crystals; TLC ($CHCl_3$–MeOH, 9:1) single UV spot, R_f 0.40; NMR (Me_2SO-d_6) δ 0.78 (3 H, t, CH_3), 1.70 (2 H, m, CH_2CH_3), 3.20 (3 H, m, C-9, 10 H's), 3.85 (3 H, s,

OCH_3), 6.70 (2 H, s, NH_2), 7.33 (2 H, d, 3',5'-Ar H), 7.60 (2 H, m, NH_2), 7.83 (2 H, d, 2',6'-Ar H), 8.37 (1 H, s, C-7 H). Anal. $C_{18}H_{20}N_6O_2 \cdot {}^1/_2H_2O$ (C, H, N).

The 10-methyl compound **12a** was similarly obtained from **11a** in 87% yield. Anal. $C_{18}H_{20}N_6O_2 \cdot {}^3/_4H_2O$ (C, H, N).

Amino-4-deoxy-10-ethyl-10-deazapteroic Acid (13b). A solution of 1.17 g of the diamino ester **12b** in 36 mL of 2-methoxyethanol was warmed to 100 °C and 2.71 mL of 10% NaOH was added. Heating was continued for 15 min and the solvent was evaporated in vacuo. The residue was dissolved in 35 mL of H_2O and adjusted to pH 5–6 with concentrated HCl. The precipitate was collected, washed with H_2O and EtOH, and dried to leave 0.90 g (80%) of pale yellow crystals; HPLC (C_{18} Bondapak reverse phase, MeOH–0.1 M KH_2PO_4 (pH 6.7), 1:3) 98% pure; UV max at pH 13 235 nm, 255, 370; NMR (Me_2SO-d_6) δ 0.74 (3 H, t, CH_3), 1.70 (2 H, m, CH_2CH_3), 3.10 (3 H, m, C-9,10 H's), 6.56 (2 H, s, NH_2), 7.31 (2 H, d, 3',5'-Ar H), 7.50 (2 H, br s, NH_2), 7.80 (2 H, d, 2',6'-Ar H), 8.34 (1 H, s, C-7 H). The HPLC, UV, and NMR spectra were identical with those measured for **13b** previously reported.[1]

The 10-methyl analogue **13a** was similarly prepared from the ester **12a** in 84% yield. The HPLC, UV, and NMR spectra were also equal to the previously reported material.

Coupling of the acids **13a,b** with diethyl L-glutamate and saponification of the intermediate esters **14a,b** to yield the resolved diastereomeric acids **15a,b** was carried out via procedures reported in ref 1.

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Synthesis and Dopaminergic Activity of *trans*-6-Methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline and *trans*-1,2,3,4,4a,5,6,10b-Octahydro-4,7-phenanthroline Derivatives

Francesco Claudi,*† Gloria Cristalli,† Sante Martelli,† Vincenzo Perlini,† Maurizio Massi,‡ and Fabrizio Venturi‡

Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino 1, 62032 Camerino (MC), Italy, and Istituto di Farmacologia, Farmacognosia e Tecnica Farmaceutica, Università di Camerino, Via Scalzino 5, 62032 Camerino (MC), Italy.
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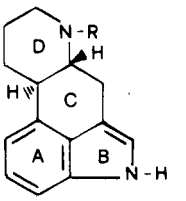
The synthesis and dopamine agonist activity of some derivatives of *trans*-6-methyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (**6a–c**) are reported. These compounds can be regarded as analogues of ergoline derivatives with the indole nucleus replaced by indolizine. These congeners have been evaluated as inhibitors of prolactin release in vivo. *trans*-6-Methyl-8-ethyl-7a,8,9,10,11,11a-hexahydro-7*H*-pyrrolo[3,2,1-*gh*]-4,7-phenanthroline (**6b**) proved to produce a dose-dependent inhibition of serum prolactin that was almost complete at the highest dose employed. Although effective, this compound was far less potent than bromocriptine. The 8-propyl derivative **6c** was weakly active only at very high doses, and the 8-methyl derivative **6a** proved to be completely ineffective. *trans*-4-Propyl-1,2,3,4,4a,5,6,10b-octahydro-4,7-phenanthroline (**7**), a molecular simplification of hexahydropyrrolo-4,7-phenanthroline, proved to be the most potent among the newly synthesized compounds. These results, taken together with those of previous studies, suggest that the presence of the nitrogen of the indolizine nucleus and the N-7 in the octahydro-4,7-phenanthroline **7** are significant for the interaction with the dopamine receptor involved in the control of prolactin release.

A large variety of ergoline (**1**) derivatives, such as bromocriptine,[1] lisuride,[2] and pergolide,[3] have been extensively investigated over the last 10 years, and some of them are now used in the treatment of Parkinson's disease and prolactin-dependent diseases. The antiimplantative, antilactation, and tumor-regression effects reported for the various ergoline derivatives can be attributed to the inhibition of prolactin release by virtue of their dopamine (DA) agonist properties. Recently, some 9-oxaergolines have been found to possess potent dopaminergic activity in vivo.[4,5] The structure–activity relationships for central dopaminergic activity of ergoline derivatives and related compounds indicate that the presence of an aromatic ring system replacing either the indole or the pyrrole ring is required.[6] Also the presence of a tertiary and basic nitrogen atom, separated from the aromatic nucleus by two carbon atoms in a given conformation, is essential. The junction of the rings C and D must be trans, and the *N*-*n*-propyl group frequently enhances dopamine agonist effects.[7]

Kornfeld et al.[6] have suggested and confirmed that the dopaminergic activity of ergoline derivatives is attributable to the moiety of the rigid pyrroloethylamine included in the ergoline structure as shown in **1**.



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In previous papers[8] we reported the biological activities of some indolizinylalkylamines and hydrazides of indol-

† Dipartimento di Scienze Chimiche.
‡ Istituto di Farmacologia.

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