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Medicinal Chemical Studies on Antiplasmin Drugs. 4. Chemical Modification of *trans*-4-Aminomethylcyclohexanecarboxylic Acid and Its Effect on Antiplasmin Activity†

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A series of N-substituted derivatives, amides, and esters of *trans*-4-aminomethylcyclohexanecarboxylic acid (*trans*-AMCHA) were synthesized and evaluated for their antiplasmin activity. Among those, Ph ester derivatives were found to be superior to *trans*-AMCHA. In particular, a high order of the activity was achieved with para-substituted Ph esters. This paper reports the synthetic method, the antiplasmin activity, and the structure-activity relationship.

Several synthetic inhibitors of plasmin have been reported, including ε-aminocaproic acid (EACA), p-aminomethylbenzoic acid (PAMBA), *trans*-4-aminomethylcyclohexanecarboxylic acid (*trans*-AMCHA), and 4-aminomethylbicyclo[2,2,2]octanecarboxylic acid. Some of them have been subjected to chemical modifications in a search for a new inhibitor. Nagamatsu, *et al.*[1], reported the inhibitory effects of various N-substituted compounds of L-lysine and esters of EACA on plasmin activity, and Muramatsu, *et al.*[2-6] described the extensive inhibitory effect of various esters on plasmin and trypsin activities and the relationship between their chemical structure and the inhibitory effect. Among the various saturated aliphatic esters of EACA, the *n*-hexyl ester showed the most extensive inhibitory effect, while branching of the alkyl chain resulted in a decrease of this effect. Markwardt[7-9] and his coworkers synthesized various PAMBA derivatives and studied the relationship between chemical structure and antiproteolytic activity of these compounds, and they demonstrated that the benzyl esters were most potent. Modification of *trans*-AMCHA had been limited to hexyl[6,10] and *p*-nitrophenyl[11] esters. The preceding paper[12] from our laboratories indicated that introduction of Me into the cyclohexane ring or the side chain of *trans*-AMCHA resulted in a decrease of the antifibrinolytic activity.

Recently, however, Muramatsu and Fujii[13] observed the excellent inhibitory effects of Ph ester and *p*-carboxyethylphenyl ester of *trans*-AMCHA on plasmin, trypsin, plasma kallikrein, and thrombin. The present paper deals with the relationship between the antiplasmin activity and the chemical structure of ester derivatives of *trans*-AMCHA including these Ph esters. Other chemical modifications of *trans*-AMCHA, N-substitution and amidation, are also described here.

Chemistry. *trans*-AMCHA derivatives used in this study were synthesized mainly according to the methods A-J described in the Experimental Section, and are shown in Tables I and II. Most of these methods were used widely to obtain N-substituted amide and ester derivatives of the amino acid. Carbobenzoxy (Cbz) *trans*-AMCHA and its acid chloride were found very useful for the preparation of *trans*-AMCHA derivatives. Physical properties of Cbz intermediates are tabulated in Table III.

Structure-Activity Relationships. The substances listed in Tables I and II were examined for their antiplasmin activity in the caseinolytic and fibrinolytic reactions using *trans*-AMCHA, its benzyl ester (**63**), or its phenyl ester (**75**) as reference standards.

From the data in Table I, it was apparent that introduction of substituent groups into the aminomethyl moiety or amidation of *trans*-AMCHA caused a drastic decrease in the antiplasmin activity with only one exception (**14**).

As shown in Table II, the antiplasmin activity of a series of alkyl esters (**35-46**) was somewhat superior to that of *trans*-AMCHA in caseinolysis, and the relationship between the activity and the length of the ester moiety was in good agreement with the result of the EACA ester investigated earlier,[1-6] that is, the *n*-hexyl ester was found to be the most active agent in this series and the activity of the unbranched ester (**37**,**39**) was greater than that of the branched chain compd with the same number of C atoms (**38**,**40**,**41**). Furthermore, it was very interesting to find that the unsaturated alkyl esters (**54**,**55**) having a double or triple bond at the β position of the alkoxy group were more potent than the corresponding saturated alkyl ester (**37**).

The potency of the benzyl ester (**63**) relative to *trans*-

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Table I. N-Substituted or Amide Derivatives of *trans*-AMCHA

No.	R	R'	HX	Mp, °C	Method	Yield, %	Formula	Analyses	Relative act.[a] fibrinolysis
				RCH_2-(H)···COR'HX					
1	CH_3SO_2NH	OH		153–155	A	30	$C_9H_{17}NO_4S$	C, H, N	<0.01
2	CH_3-(C$_6$H$_4$)-SO_2NH	OH		193–195	A	80	$C_{15}H_{21}NO_4S$	C, H, N	<0.01
3	Ph-CONH	OH		177–178	A	89	$C_{15}H_{19}NO_3$	C, H, N	
4	CH_3CONH	OH		154–155	A	70	$C_{10}H_{17}NO_3$	C, H, N	0.01
5	CH_3NH	OH	*b*	230–232 dec	B[c]	86	$C_9H_{17}NO_2$	C,[d] H, N	0.23
6	C_2H_5NH	OH		219–220 dec	B[e]	84	$C_{10}H_{19}NO_2$	C, H, N	0.03
7	$(CH_3)_2N$	OH	*f*	149–152	C	70	$C_{10}H_{19}NO_2$	C, H, N	0.03
8	$(C_2H_5)_2N$	OH	HCl	196–198	C	40	$C_{12}H_{23}NO_2 \cdot HCl$	C, H, N	0.03
9	$((CH_3)_2CHCH_2)_2N$	OH	HCl[g]	205–207	C	45	$C_{16}H_{31}NO_2 \cdot HCl$	C, H, N	0.03
10	$H_2NC(NH)NH$	OH	*h*	349 dec	*i*	50	$C_9H_{17}N_3O_2$	C, H, N	<0.04
11	H_2NCONH	OH		195–197	*i*	27	$C_9H_{16}N_2O_3$	C, H, N[j]	<0.01
12	$HOOCCH_2NH$	OH	HCl	217–220 dec	*i*	35	$C_{10}H_{17}NO_4 \cdot HCl$	C, H, N	<0.01
13	C_2H_5OCONH	OH		130–132	*i*	70	$C_{11}H_{19}NO_4$	C, H, N[k]	<0.01
14	HO_3SCH_2NH	OH		181–183 dec	*i*	81	$C_9H_{17}NO_5S$	C, H, N	1.05
15	H_2NNH	OH	*p*-TsOH	228–230 dec	*i*		$C_8H_{16}N_2O_2 \cdot$ *p*-TsOH	C, H, N	0.13
16	$H_2NCONHNH$	OH	HCl	198–200 dec	*i*		$C_9H_{17}N_3O_3 \cdot HCl$	C, H, N[l]	<0.01
17	$(CH_3)_3N^+$	OH	I^-	191–193	*i*	60	$C_{11}H_{22}INO_2$	C, H, N	<0.02
18	NH_2	NH_2	HCl	251–252 dec	E	70	$C_8H_{16}N_2O \cdot HCl$	C, H, N	0.02
19	NH_2	$NHCH_3$	HCl	239–241 dec	E	80	$C_9H_{18}N_2O \cdot HCl$	C, H, N[m]	<0.02
20	NH_2	$NH(CH_2)_3CH_3$	HCl	248–250 dec	D	92	$C_{12}H_{24}N_2O \cdot HCl$	C, H, N	<0.02
21	NH_2	$NH(CH_2)_5CH_3$	HCl	233–237 dec	E	90	$C_{14}H_{28}N_2O \cdot HCl$	C, H, N	0.02
22	NH_2	$NH(CH_2)_6CH_3$	HCl	232–235 dec	E	79	$C_{15}H_{30}N_2O \cdot HCl$	C, H, N[n]	<0.02
23	NH_2	$NH(CH_2)_2N(CH_3)_2$	2HCl	193–195 dec	E	54	$C_{12}H_{25}N_3O \cdot 2HCl$	C, H, N	<0.02
24	NH_2	NHPh	HCl	260–262 dec	*i*	89	$C_{14}H_{20}N_2O \cdot HCl$	C, H, N	<0.02
25	NH_2	NH-(H)	HCl	305 dec	E	91	$C_{14}H_{26}N_2O \cdot HCl$	C,[o] H, N	<0.02
26	NH_2	$N(C_2H_5)_2$	HCl	190–192	E	81	$C_{12}H_{24}N_2O \cdot HCl$	C, H, N	0.02
27	NH_2	N((H))$_2$	HCl	239–240 dec	D	42	$C_{20}H_{36}N_2O \cdot HCl$	C, H,[p] N	0.02
28	NH_2	Piperidino	HCl	246	E	91	$C_{13}H_{24}N_2O \cdot HCl$	C, H, N	<0.02
29	NH_2	Morpholino	HCl	268–270 dec	F	77	$C_{12}H_{22}N_2O_2 \cdot HCl$	C,[q] H, N	<0.02
30	NH_2	$NH(C_6H_4)OCH_3(p)$	HCl	281–282 dec	F	70	$C_{15}H_{22}N_2O_2 \cdot HCl$	C, H, N	<0.02
31	NH_2	$NH(C_6H_4)OC_2H_5(p)$	HCl	287–289 dec	F	68	$C_{16}H_{24}N_2O_2 \cdot HCl$	C, H, N	<0.02
32	NH_2	$NH(C_6H_4)CH_3(o)$	HCl	299–300 dec	F	65	$C_{15}H_{22}N_2O \cdot HCl$	C, H, N	<0.02
33	NH_2	$NH(C_6H_4)CH_3(m)$	HCl	248–249 dec	F	47	$C_{15}H_{22}N_2O \cdot HCl$	C, H, N	<0.02
34	NH_2	$NH(C_6H_4)Cl(p)$	HCl	285	F	67	$C_{14}H_{19}ClNO_2 \cdot HCl$	C, H, N	<0.02

[a] Figures indicate the relative activity to *trans*-AMCHA (=1.0). [b] HCl, mp 254–257° dec. [c] Intermediate, *N*-methyl-*N*-tosyl-*trans*-AMCHA, mp 173–176°, prepd from I, yield 78%. [d] C: calcd, 63.12; found, 62.62. [e] Intermediate, *N*-ethyl-*N*-tosyl-*trans*-AMCHA, mp 133–135°, prepd from I, yield 58%. [f] HCl, mp 230–235°. [g] Free base, mp 93–95°. [h] HCl, mp 231°. [i] See in Experimental Section. [j] N: calcd, 13.99; found, 14.49. [k] N: calcd, 6.11; found, 6.59. [l] N: calcd, 16.69; found, 16.18. [m] N: calcd, 13.54; found, 13.11. [n] N: calcd, 9.63; found, 10.23. [o] H: calcd, 9.90; found, 9.40. [p] H: calcd, 10.45; found, 10.93. [q] C: calcd, 54.84; found, 54.19.

AMCHA was 41.8 and 1.6 in caseinolysis and fibrinolysis, respectively. And as we would expect from the above evidence (**54**,**55**,**63**) the introduction of $CH{=}CH_2$ at the α-CH_2 portion of **63** caused an enhanced activity. Substitution in the benzene ring of the benzyl esters, however, gave no clear relationship (see **64–70**).

Furthermore, the conversion of the benzyl moiety into phenyl resulted in an outstanding enhancement of the antiplasmin activity. For example, the activity of the Ph ester (**75**) relative to *trans*-AMCHA was increased about 32 times in fibrinolysis. The following characteristics between the substituent groups and the activity were observed. (1) Generally, the presence of the substituent groups at the para position, such as halogen, nitro, carboxy, aldehyde, sulfamoyl, or alkyl, enhanced the activity (**78**, **80**, **83**, **94**, **95**, **96**, **97**, **99**, **101**, **102**, **105**, **106**, **109**, **110**, **114**, **118**, **119**), with only one exception (**104**). (2) The activity of the meta-substituted Ph esters (**77**, **82**. **100**) was inferior to that of the corresponding para-substituted compounds (**78**, **83**, **99**). (3) The activity of the ortho, para-disubstituted esters (**85**, **92**, **98**, **111**, **112**, **113**) was the same as or a little lower than that of the para-substituted esters. (4) On the other hand, the introduction of the substituent groups into the ortho, ortho positions of the Ph moiety (**91**, **93**) resulted in lowering of the activity. These findings suggest that the antiplasmin activity of these Ph esters was affected by the steric as well as electronic effects of the substituents.

On the basis of the solubility and the stability in H_2O in addition to the excellent antiplasmin activity, it was assumed that **99** (our abbreviation was DV-1006) was the most promising substance as a novel antiplasmin drug.

Experimental Section

Melting points were detd on a Melting Point Apparatus (Yamato Scientific Co., Ltd.) and are uncor. Ir spectra were obtd with a Hitachi infrared spectrophotometer type EPI-G2. Tlc was carried out on silica gel (Silica Rider, Daiichi Pure Chemicals Co., Ltd.), the upper layer consisting of *n*-BuOH–AcOH–H_2O (4:1:5) (solvent A) and ppc on Toyo Roshi No. 50 filter paper with solvents A and B (*n*-PrOH–H_2O; 65:35). The ascending technique was used in both chromatographies and *trans*-AMCHA derivatives were detected by spray-

ing with ninhydrine in ppc, and the same reagent and I_2 were used in tlc. Hydrogenations were carried out at room temp and atm pressure unless otherwise stated. Where analyses are indicated only by the symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Materials. Amines, benzyl alcohols, and most phenols used in this work were obtd from commercial sources. Phenols having benzyloxycarbonyl groups, not commercially available, were prepd by benzylation of the corresponding carboxylic acid derivs as follows. To a soln of 4-hydroxyphenylacetic acid (3.0 g, 0.02 mole) in 4% aq NaOH (20 ml, 0.02 mole) and EtOH (30 ml), $C_6H_5CH_2Cl$ (3.0 g, 0.024 mole) was added and the resulting mixt was refluxed for 1.5 hr. After completion of the reaction, EtOH was removed to give a syrup which was solidified on cooling. The resulting solid was treated with Et_2O (20 ml). The sepd H_2O layer was removed and the Et_2O layer was washed with 5% aq Na_2CO_3 soln and dried. After removal of the solvent, the white residue was recrystd from petr ether to give benzyl 4-hydroxyphenylacetate (2.3 g, 46.5%) as white prisms, mp 88–92°. *Anal.* ($C_{15}H_{14}O_3$) C, H.

In the same manner as described above, the hydroxyaryl derivatives were synthesized (Table IV).

Assay Methods of the Antiplasmin Activity. Anticaseinolytic activity of the compds was detd by the method of Shimizu, *et al.*[14] Euglobulin soln (0.5 ml) prepd from human blood was preincubated with 1 ml of 2% casein soln in phosphate buffer–saline (pH 7.4) and 0.4 ml of the phosphate buffer–saline contg various amts of an inhibitor to be tested at 37° for 3 min. Then, 0.1 ml of streptokinase soln (200 units) was added and the mixt was incubated at 37° for 20 min. After incubation, 2 ml of 17% $HClO_4$ was added, allowed to stand at room temp for about 1 hr, and centrifuged. The extinction of the clear supernatant was measured at 280 mμ against an enzyme blank to which the streptokinase soln was added after the addn of $HClO_4$. The incubation rates were calcd by comparison with the control run which contd no inhibitor.

Antifibrinolytic activity was detd according to the method of Okamoto.[15] Human euglobulin soln (0.1 ml) was mixed with 0.5 ml of the phosphate buffer–saline contg various amts of an inhibitor to be tested, 0.1 ml of thrombin soln (5 units) and 0.1 ml of streptokinase soln (100 units). Then 0.2 ml of 0.5% bovine fibrinogen in phosphate buffer–saline was added to the above mixt. The lysis time of the fibrin clot formed was measured at 25° after the addn of fibrinogen. Inhibitory actions of the compds are represented as the concns of the compds for doubling the clot lysis time of the control run which contd no inhibitor.

The relative antiplasmin activity of the ester derivs varied depending on the assay system employed and was variable, to some extent, even in the same assay system, when *trans*-AMCHA was used as a reference standard, because the mechanism of action was entirely different between *trans*-AMCHA and its ester derivative.[14,16,17]

Method A. *trans*-4-*p*-Toluenesulfonylaminomethylcyclohexanecarboxylic Acid (2). *trans*-AMCHA (I) (52.5 g, 0.334 mole) and *p*-TsCl (69 g, 0.363 mole) were added to 1 *N* NaOH (800 ml, 0.8 mole) and the mixt was vigorously stirred at room temp for 4 hr. Undissolved *p*-TsCl was filtered off, and the filtrate was acidified with concd HCl. The ppt was collected and recryst from AcOEt to give 2 (83.0 g) as colorless prisms.

Method B. *trans*-4-Methylaminomethylcyclohexanecarboxylic Acid (5). To a soln of 2 (2.2 g, 0.007 mole) in 2 *N* NaOH (11 ml, 0.002 mole), MeI (2.0 g, 0.014 mole) was added and the soln was stirred at 65° for 1 hr and gradually cryst materials separated out. H_2O (20 ml) was added to the soln and neutralized with aq HCl. Pptd crystals were recryst from MeOH–H_2O to give *trans*-4-(*N*-methyl-*N*-*p*-toluenesulfonyl)aminomethylcyclohexanecarboxylic acid (1.8 g, 78%), as prisms, mp 173–176°. *Anal.* ($C_{16}H_{23}NO_4S$) C, H, N.

The *N*-Me derivative (1.0 g, 0.003 mole) described above was dissolved in dry liquid NH_3 (80 ml) at −55~−60°. To this soln Na (0.3 g, 0.013 g-atom) was added portionwise and for decolorization the dry anionic ion-exchange resin DIAION SK #1 (NH_4 form; 2.2 g) was added. After removal of NH_3, the residue was passed through a column of the same resin (H form; 20 ml). After washing of the column with H_2O, it was eluted with 5% NH_4OH and the effluent was concd *in vacuo* and the residue was recrystd from MeOH–Me_2CO to give 0.45 g of 5, as prisms: tlc, solvent A, R_f, 0.34.

Method C. *trans*-4-Diisobutylaminomethylcyclohexanecarboxylic Acid (9). To a soln of I (1.6 g, 0.01 mole) in 25% aq MeOH (40 ml), isobutylaldehyde (2.9 g, 0.04 mole) and 10% Pd/C (1.6 g) were added. This suspension was catalytically hydrogenated at 50° for 3 hr. After absorption of the theoretical amt of H_2 (450 ml), the catalyst was filtered off, and the filtrate was concd *in vacuo*. The residue was recrystd twice from H_2O to furnish 9, as prisms: ppc, solvent A, R_f, 0.84.

trans-4-Carbamoylaminomethylcyclohexanecarboxylic Acid (11). To a soln of I (10 g, 0.064 mole) in H_2O (70 ml), a soln of KCNO (6.25 g, 0.077 mole) in H_2O (10 ml) was added with chilling and stirring. The reaction mixt was allowed to stand overnight at room temp, then acidified by adding of aq HCl and the sepd crystals were collected. Recrystn from *n*-PrOH gave 11 (3.5 g).

trans-4-Carboxymethylaminomethylcyclohexanecarboxylic Acid (12). To a soln of $ClCH_2COOH$ (7.55 g, 0.08 mole) in H_2O (11 ml), 8% aq NaOH (80 ml, 0.16 mole) was added with cooling and stirring. To this soln, a soln of I (12.6 g, 0.08 mole) in 8% aq NaOH (40 ml, 0.08 mole) was added with cooling and stirring and the mixt was allowed to stand for 5 hr at room temp. The soln was passed through a column of the ion-exchange resin DIAION SK #1 (H form) and was eluted with H_2O (500 ml). The eluate was concd *in vacuo* under 50° to give raw 12 (8.5 g), mp 205–210° dec. Recrystn from Me_2CO–H_2O gave pure 12, as prisms.

trans-4-Ethoxycarbonylaminomethylcyclohexanecarboxylic Acid (13). I (12.6 g, 0.08 mole) was dissolved in 8% aq NaOH (40 ml, 0.08 mole). To this soln, $ClCOOC_2H_5$ (9.6 g, 0.088 mole) was added with chilling and stirring. Na_2CO_3 (4.2 g, 0.044 mole) was gradually added and the reaction mixt allowed to stand at room temp for 5 hr. HCl (1 *N*, 82 ml, 0.08 mole) was added to the soln and the pptd crystals, mp 70–110°, were collected and recrystd from Me_2CO to give pure 13.

trans-4-Sulfomethylaminomethylcyclohexanecarboxylic Acid (14). I (15.7 g, 0.1 mole), $HOCH_2SO_3Na$ (15.2 g, 0.11 mole), and $NaHCO_3$ (8.4 g, 0.1 mole) were dissolved in H_2O (79 ml), and the soln was heated on a boiling water bath for 4 hr. After cooling with an ice bath, 12 *N* HCl (16.7 ml) was added to the soln to acidity (pH 3) with stirring. White crystals were gradually pptd from the soln and the mixt was kept in a refrigerator overnight. The crystals were collected, washed (H_2O, EtOH, Et_2O), and dried to give 14 (20.3 g).

trans-4-Hydrazinomethylcyclohexanecarboxylic Acid *p*-Toluenesulfonate (15). A soln of $NaHSO_3$ (40 g, 0.38 mole) in H_2O (280 ml) was cooled to 15° and methyl 4-oxocyclohexanecarboxylate[18] (47 g, 0.3 mole) was gradually added over 10 min under stirring. After stirring the soln for 30 min at 15°, NaCN (37 g, 0.75 mole) was added and the mixt was stirred for 20 min at 10–15°. The sepd upper oily layer was extd with trichloroethylene and the ext was washed (H_2O), dried (Na_2SO_4), and concd to a syrup *in vacuo*. The yellow oil (52.5 g) was dissolved in α-picoline (123 g, 1.32 mole) and to this soln $POCl_3$ (50 g, 0.32 mole) was added for 30 min at 0–5° under stirring. After stirring for 1 hr at 0°, an ice bath was removed. Heat was evolved and the color changed to reddish brown at the end. After standing at room temp overnight, the soln was poured onto ice water and the sepd oil solidified. It was extd with trichloroethylene and the soln was washed (H_2O) and dried (Na_2SO_4). After removal of Na_2SO_4 and the solvent, the residue was distd; a colorless transparent oil, bp 125–128° (6 mm), was obtd in 34.5 g yield (70% from methyl 4-oxocyclohexanecarboxylate). It solidified on ice cooling, mp 33–35°.

To a soln of methyl 4-cyano-3-cyclohexenecarboxylate (11.0 g, 0.067 mole), described above in pyridine (150 ml), a soln of $NaH_2PO_2 \cdot H_2O$ (20 g, 0.19 mole) in H_2O (20 ml) was added at room temp with stirring, and then a suspension of Raney Ni catalyst (20 ml) in AcOH (75 ml). The mixt was warmed at 40–45° for 2 hr under stirring. The catalyst was filtered off and washed with enough EtOH, and the washings were combined with the filtrate. It was concd to a syrup under reduced pressure, and the syrup was dissolved in H_2O (50 ml) and extd with *i*-Pr_2O. The *i*-Pr_2O layer was washed with aq NaCl soln and dried. After removal of the solvent, the residual syrup was distd. Methyl 4-formyl-3-cyclohexenecarboxylate (6.7 g, 60%) was obtd as colorless oil, bp 113–117° (5 mm). The 2,4-dinitrophenylhydrazone, mp 207–210° dec, was obtd as orange needles in the usual way. *Anal.* ($C_{15}H_{16}N_4O_6$) C, H, N.‡

Methyl 4-formyl-3-cyclohexenecarboxylate (5.0 g, 0.03 mole) and $CH_3CONHNH_2$ (2.3 g, 0.03 mole) were dissolved in EtOH (20 ml) and the soln was refluxed for 4 hr and then cooled. The pptd white crystals, mp 155–157°, of the hydrazone were collected by filtration, yield, 6.2 g (92%). Recrystn from EtOH gave white prisms, mp 156–158°. *Anal.* ($C_{11}H_{16}N_2O_3$) C, H, N.

A soln of the above hydrazone (2.7 g, 0.012 mole) in AcOH (24 ml) was catalytically hydrogenated over PtO_2 (0.25 g). After removal of the catalyst and the solvent, the residual syrup was refluxed

‡N: calcd, 16.87; found, 16.26.

Table II. Ester Derivatives of *trans*-AMCHA

No.	R	HX	Mp, °C	Method	Yield, %	Formula	Analyses	Relative act. Caseinolysis A[a]	Caseinolysis B[b]	Caseinolysis C[c]	Fibrinolysis
			H_2NCH_2–⟨H⟩···COOR HX								
35	CH_3	HCl	168–170	G J	91 70	$C_9H_{17}NO_2 \cdot HCl$	C, H, N	2.4			0.17
36	C_2H_5	HCl	185–188	G	92	$C_{10}H_{19}NO_2 \cdot HCl$	C,[d] H, N	1.5			0.04
37	$C_3H_7(n)$	HCl	160–164	G	79	$C_{11}H_{21}NO_2 \cdot HCl$	C,[e] H. N	2.5			0.05
38	$C_3H_7(i)$	HCl	180–181 dec	I	86	$C_{11}H_{21}NO_2 \cdot HCl$	C, H, N	1.8			
39	$C_4H_9(n)$	HCl	135–138	G	81	$C_{12}H_{23}NO_2 \cdot HCl$	C, H, N	3.7			
40	C_4H_9(i)	HCl	154–156 dec	I	80	$C_{12}H_{23}NO_2 \cdot HCl$	C, H, N	3.0			
41	$C_4H_9(tert)$	HCl	197–203 dec	I	48	$C_{12}H_{23}NO_2 \cdot HCl$	C. H. N	0.8			
42	$C_5H_{11}(n)$	HCl	120–124	G	71	$C_{13}H_{25}NO_2 \cdot HCl$	C, H, N	4.6			
43	$C_6H_{13}(n)$	HCl	121–123	G	76	$C_{14}H_{27}NO_2 \cdot HCl$	C, H, N	8.0			0.4
44	$C_7H_{15}(n)$	HCl	118–122	G	75	$C_{15}H_{29}NO_2 \cdot HCl$	C, H, N	7.0			
45	$C_8H_{17}(n)$	HCl	125–127	G	72	$C_{16}H_{31}NO_2 \cdot HCl$	C, H, N	5.4			
46	$CH_2CH(C_2H_5)(CH_2)_3CH_3$	$0.5(COOH)_2$	190–200	G	45	$C_{16}H_{31}NO_2 \cdot 0.5(COOH)_2$	C, H, N	2.4			
47	Cyclohexyl	HCl	200–203	G	83	$C_{14}H_{25}NO_2 \cdot HCl$	C, H, N	5.0			
48	$CH_2CH_2OC_2H_5$	HCl	91–93	G	67	$C_{12}H_{23}NO_3 \cdot HCl$	C,[f] H, N	6.5			
49	$CH_2CH_2OC_3H_7(n)$	*p*-TsOH	120–130	I	52	$C_{13}H_{25}NO_3$[g] $\cdot$ *p*-TsOH	C, H, N	10.0			
50	$CH_2(CH_2)_3CH_2OH$	*p*-TsOH	132–135	H	87	$C_{13}H_{25}NO_3 \cdot$ *p*-TsOH	C, H, N	7.3			
51	$CH_2(CH_2)_3CH_2$	2HCl	213–215	H	78	$C_{21}H_{38}N_2O_4 \cdot 2HCl$	C, H, N		0.6		
52	$CH_2(CH_2)_4CH_2OH$	*p*-TsOH	84–85	H	83	$C_{14}H_{27}NO_3$[g] $\cdot$ *p*-TsOH	C, H, N	8.3			
53	$CH_2(CH_2)_4CH_2$	2HCl[h]	261–263	I	79	$C_{22}H_{40}N_2O_4 \cdot 2HCl$	C, H, N		0.4		
54	$CH_2CH{=}CH_2$	HCl	139–142	I	85	$C_{11}H_{19}NO_2 \cdot HCl$	C, H, N	12.0	0.4		
55	$CH_2C{\equiv}CH$	HCl	188–190	I	89	$C_{11}H_{17}NO_2 \cdot HCl$	C, H, N		1.2		3.7
56	CH_2COOH		210–214 dec	*i*	85	$C_{10}H_{17}NO_4$[g]	C, H, N	1.8			0.1
57	CH_2CONH_2	HBr	188–190	*i*	76	$C_{10}H_{18}N_2O_3 \cdot HBr$	C,[j] H, N		0.8		1.8
58	$CH(C_6H_5)CONHC_6H_4CH_3(p)$	HCl	247–248 dec	*i*	89	$C_{23}H_{28}N_2O_3 \cdot HCl$	C, H, N		8.4		13.3
59	$CH_2COC_6H_5$	HBr	168–170	*i*	50	$C_{16}H_{21}NO_3 \cdot HBr$	C,[k] H, N		0.1		
60	$CH_2CH_2C_6H_5$	HCl	161–163	I	81	$C_{16}H_{23}NO_2 \cdot HCl$	C, H, N		0.3		
61	$CH_2CH{=}CHC_6H_5$	HCl	139–142	I	79	$C_{17}H_{23}NO_2 \cdot HCl$	C, H, N		1.5		2.2
62	CH_2–⟨H, O⟩	*p*-TsOH	140–143 dec	I	35	$C_{13}H_{23}NO_3$[g] $\cdot$ *p*-TsOH	C, H, N		0.3		
63	$CH_2C_6H_5$	HCl[l]	151–153	1	90	$C_{15}H_{21}NO_2 \cdot HCl$	C, H, N	41.8	1.0		1.6
64	$CH_2C_6H_4OCH_3(p)$	HCl	148–150	I	73	$C_{16}H_{23}NO_3 \cdot HCl$	C,[m] H, N		1.3		
65	$CH_2C_6H_3(OCH_3)_2(m,p)$	HCl	157–158	I	65	$C_{17}H_{25}NO_4 \cdot HCl$[g]	C, H, N		0.9		2.1
66	$CH_2C_6H_4Cl(o)$	HCl	158–160	I	80	$C_{15}H_{20}ClNO_2 \cdot HCl$	C, H, N		1.1		2.4
67	$CH_2C_6H_4Cl(p)$	HCl	173–177	I	75	$C_{15}H_{20}ClNO_2 \cdot HCl$	C, H, N		0.9		
68	$CH_2C_6H_4CH_3(p)$	HCl	173–176	I	91	$C_{16}H_{23}NO_2 \cdot HCl$	C, H, N[n]		1.6		
69	$CH_2C_6H_4NO_2(m)$	HCl	140	I	70	$C_{15}H_{20}N_2O_4 \cdot HCl\ 2/3\ H_2O$	C, H, N		1.2		1.5
70	$CH_2C_6H_4CH_2OH(p)$	HBr	157–160 dec	H	14	$C_{16}H_{23}NO_3 \cdot HBr$	C,[o] H, N		2.2		
71	$CH(C_6H_5)C{\equiv}CH$	HCl	140–142	H	77	$C_{17}H_{21}NO_2 \cdot HCl$[g]	C, H, N[p]		4.2		15.8
72	$CH(C_6H_5)CH_3$	*p*-TsOH	135–137	I	81	$C_{16}H_{23}NO_2 \cdot p$-TsOH	C,[q] H, N		*r*		
73	CH_2–⟨O⟩	*p*-TsOH	153–154	1	41	$C_{13}H_{20}NO_3 \cdot p$-TsOH	C, H, N		1.7		

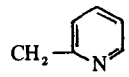
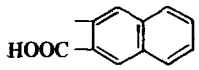
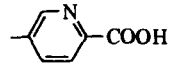
74		2HBr	202 dec	H	50	$C_{14}H_{20}N_2O_2 \cdot 2HBr$	C, H, N		0.4		
75	C_6H_5	HCl	213–215 dec	I	75	$C_{16}H_{19}NO_2 \cdot HCl$	C, H, N	903		1.0	32.1
76	$C_6H_4CH_3(o)$	HCl	181–183	I	81	$C_{15}H_{21}NO_2 \cdot HCl$	C, H, N			0.4	10.4
77	$C_6H_4CH_3(m)$	HCl	183–185	I	79	$C_{15}H_{21}NO_2 \cdot HCl$	C, H, N			1.0	34.7
78	$C_6H_4CH_3(p)$	HCl	240–242	I	88	$C_{15}H_{21}NO_2 \cdot HCl$	C, H, N[s]			1.7	53.0
79	$C_6H_4OCH_3(o)$	HCl	212–213	I	77	$C_{15}H_{21}NO_3 \cdot HCl$	C, H, N			0.3	17.3
80	$C_6H_4OCH_3(p)$	HCl	215–217 dec	I	83	$C_{15}H_{21}NO_3 \cdot HCl$	C, H, Cl			1.2	47.2
81	$C_6H_4Cl(o)$	HCl	175–177	I	85	$C_{14}H_{18}ClNO_2 \cdot HCl$	C, H, Cl			1.2	52.0
82	$C_6H_4Cl(m)$	HCl	191–194	I	80	$C_{14}H_{18}ClNO_2 \cdot HCl$	C, H, Cl			1.4	27.4
83	$C_6H_4Cl(p)$	HCl	224–225 dec	I	80	$C_{14}H_{18}ClNO_2 \cdot HCl$	C,[t] H, Cl			2.5	50.5
84	$C_6H_4Br(o)$	HCl	169–171	I	83	$C_{14}H_{18}BrNO_2 \cdot HCl$	C,[u] H, N			1.0	55.3
85	$C_6H_3(CH_3)_2(o,p)$	HCl	215 dec	I	85	$C_{16}H_{23}NO_2 \cdot HCl$	C, H, N			0.2	7.8
86	Biphenyl	HCl	243 dec	I	65	$C_{20}H_{23}NO_2 \cdot HCl$	C, H, N[v]			r	
87	α-Naphthyl	HCl	200–203	I	75	$C_{18}H_{21}NO_2 \cdot HCl$[g]	C, H, N			0.7	
88	β-Naphthyl	HCl	239 dec	I	62	$C_{18}H_{21}NO_2 \cdot HCl$	C,[w] H, N			0.9	
89	Thiophenyl	HCl	217 dec	I	56	$C_{14}H_{19}NOS \cdot HCl$	C, H,[x] N			0.6	24.8
90	$C_6H_3(CH_3)_2(m,p)$	HCl	241 dec	H	78	$C_{16}H_{23}NO_2 \cdot HCl$	C, H, N			0.9	40.9
91	$C_6H_3(CH_3)_2(o,o)$	HCl	221–223 dec	H	68	$C_{16}H_{23}NO_2 \cdot HCl$	C, H, N			<0.01	<1.0
92	$C_6H_3Cl_2(o,p)$	HCl	201 dec	H	88	$C_{14}H_{17}Cl_2NO_2 \cdot HCl$	C, H, N			1.4	64.3
93	$C_6H_2Cl_3(o,o,p)$	HCl	210–213 dec	H	78	$C_{14}H_{16}Cl_3NO_2 \cdot HCl$	C, H, N			0.05	<1.0
94	$C_6H_4C(CH_3)_3(p)$	HCl	256 dec	H	86	$C_{18}H_{27}NO_2 \cdot HCl$	C, H, N[y]			1.5	54.9
95	$C_6H_4OH(p)$	HCl	212–214 dec	H	42	$C_{14}H_{19}NO_3 \cdot HCl$	C, H,[z] N			0.7	31.0
96	$C_6H_4CH_2OH(p)$	HCl	241–242 dec	H	52	$C_{15}H_{21}NO_3 \cdot HCl$	C, H, N			1.7	54.2
97	$C_6H_4COOH(p)$	HCl	255 dec	H	65	$C_{15}H_{19}NO_4 \cdot HCl$	C, H, N			1.2	59.4
98	$C_6H_3(COOH)_2(o,p)$	HCl	181–183 dec	H	40	$C_{16}H_{19}NO_6 \cdot HCl$	C, H, N			0.1	3.3
99	$C_6H_4CH_2CH_2COOH(p)$	HCl[aa]	238–240 dec	H ji	88	$C_{17}H_{23}NO_4 \cdot HCl$	C, H, N			1.1	47.3
100	$C_6H_4CH_2CH_2COOH(m)$	HCl	197–199	H	81	$C_{17}H_{23}NO_4 \cdot HCl$	C, H, N			0.4	15.1
101	$C_6H_4CO(CH_2)_4COOH(p)$	HCl	173–175	H	62	$C_{20}H_{27}NO_5 \cdot HCl$	C, H, N			2.5	74.3
102	$C_6H_4CH(OH)(CH_2)_4COOH(p)$	HCl	150–154	H	53	$C_{20}H_{29}NO_5 \cdot HCl$	C, H, N			2.1	62.5
103	$C_6H_4CHBr(CH_2)_4COOH(p)$	HBr	139[bb] dec	cc	39	$C_{20}H_{28}NO_4Br \cdot HBr$	C, H, N				
104	$C_6H_4NHCH_2COOH(p)$	HCl	213–214 dec	H	76	$C_{16}H_{22}N_2O_4 \cdot HCl$	C, H, N			0.02	0.5
105	$C_6H_4CH_2CH(NH_2)COOH(p)$	2HCl[dd]	251 dec	H	85	$C_{17}H_{24}N_2O_4 \cdot 2HCl$	C, H, N			0.7	30.9
106	$C_6H_4OCH_2CH_2COOH(p)$	HCl	213 dec	H	84	$C_{17}H_{23}NO_5 \cdot HCl$	C, H, N			1.0	38.8
107		HCl	208–210 dec	H	20	$C_{19}H_{21}NO_4 \cdot HCl$	C,[ee] H, N			0.1	4.8
108		HCl	216–218 dec	H	40	$C_{14}H_{18}N_2O_4 \cdot HCl$	C,[ff] H, N			v	
109	$C_6H_4NO_2(p)$	HBr	190–192 dec	i	85	$C_{14}H_{18}N_2O_4 \cdot HBr$	C, H, N			1.5	78.9
110	$C_6H_4NH_2(p)$	2HCl	262 dec	gg	75	$C_{14}H_{20}N_2O_2 \cdot 2HCl$	C, H, N			0.7	31.0
111	$C_6H_3(OCH_3)(CHO)(o,p)$	HBr	242–245 dec	H	75	$C_{16}H_{21}NO_4 \cdot HBr$	C, H, N			2.3	83.4
112	$C_6H_3(OCH_3)(CH_3)(o,p)$	HCl	188–191 dec	hh	40	$C_{16}H_{23}NO_3 \cdot HCl$	C,[ii] H, N			1.0	31.0
113	$C_6H_3(NO_2)(COOCH_3)(o,p)$	HBr	183–186	H	56	$C_{16}H_{20}N_2O_6 \cdot HBr$	C, H, N				103.6
114	$C_6H_4SO_2NH_2(p)$	HBr	261 dec	H	86	$C_{14}H_{20}N_2O_4 \cdot HBr$	C, H, N			4.3	136
115		2HBr	193–195 dec	H	52	$C_{13}H_{18}N_2O_2 \cdot 2HBr$	C, H, N			1.6	64.5

Table II (Continued)

No.	R	HX	Mp, °C	Method	Yield, %	Formula	Analyses	Relative act. Caseinolysis A^a	B^b	C^c	Fibrinolysis
116		HBr	278 dec	H	58	$C_{13}H_{18}N_2O_3 \cdot HBr$	C, H, N				47.5
117	$C_6H_4CH_2COOH(p)$	HBr	210 dec	H	60	$C_{16}H_{21}NO_4 \cdot HBr$	C, H, N			1.0	22.8
118	$C_6H_4CH{=}CHCOOH(p)$	HBr	252–254 dec	H	62	$C_{17}H_{21}NO_4 \cdot HBr$	C, H,*jj* N			1.9	72.9
119	$C_6H_4(CH_2)_5COOH(p)$	HBr	195 dec	H	65	$C_{20}H_{29}NO_4 \cdot HBr$	C,*kk* H, N			2.0	81.4

[a] Figures indicate the relative activity to *trans*-AMCHA(=1.0). [b] Figures indicate the relative activity to benzyl *trans*-AMCHA(=1.0). [c] Figures indicate the relative activity to phenyl *trans*-AMCHA(=1.0). [d] C: calcd, 54.17; found, 54.58. [e] C: calcd, 56.04; found 56.47. [f] C: calcd, 54.23; found, 53.68. [g] Hemihydrate. [h] *p*-Toluenesulfonate, mp 239–242°. [i] See in Experiment Section. [j] C: calcd, 40.69; found, 40.17. [k] C: calcd, 53.94; found, 54.43. [l] *p*-Toluenesulfonate, mp 159–161°. [m] C: calcd, 61.23; found, 60.77; [n] N: calcd, 4.70; found, 5.18. [o] C: calcd, 53.63; found, 54.39. [p] N: calcd, 5.05; found, 4.42. [q] C: calcd, 63.71; found, 63.06. [r] Insoluble in H_2O. [s] N: calcd, 4.94; found, 5.35. [t] C: calcd, 55.27; found, 56.21. [u] C: calcd, 48.22; found, 47.81. [v] N: calcd, 4.05; found, 4.53. [w] C: calcd, 67.59; found, 66.67. [x] H: calcd, 7.05; found, 7.60. [y] N: calcd, 4.30; found, 4.86. [z] H: calcd, 7.05; found, 7.59. [aa] Methanesulfonate, mp 213–215°. [bb] Decompd in a few days. [cc] Treatment of 4-(1-hydroxy-5-benzyloxycarbonyl-*n*-pentyl)phenyl *trans*-4-*N*-carbobenzoxyaminomethylcyclohexanecarboxylate, intermediate of 102, with 35% HBr–AcOH gave 103. [dd] Monohydrochloride, mp 233–235° dec, dimethanesulfonate, mp 204–206° dec, dihydrobromide, mp 205–207° dec. [ee] C: calcd, 62.72; found, 62.28. [ff] C: calcd, 53.42; found, 52.59. [gg] Catalytic hydrogenation of 4-nitrophenyl *trans*-4-*N*-carbobenzoxyaminomethylcyclohexanecarboxylate, intermediate of 109, over Pd/C gave 110. [hh] Catalytic hydrogenation of (4-formyl-1-methoxy)phenyl *trans*-4-carbobenzoxyaminomethylcyclohexanecarboxylate, intermediate of 111, over Pd/C gave 112. [ii] C: calcd, 61.23; found, 60.44. [jj] H: calcd, 5.77; found, 6.29. [kk] C: calcd, 56.07; found, 55.60.

for 3 hr with 1 *N* HCl (30 ml) under N_2. The amino acids fractions obtd after treatment with an ion-exchange resin were concd *in vacuo*, and the residue was crystd with EtOH–Et_2O to give a white powder (1.4 g, 67.5%), mp 105–130° dec, of crude 4-hydrazinomethylcyclohexanecarboxylic acid.

To a soln of this crude acid (1.4 g) in H_2O (15 ml), a soln of *p*-TsOH (2.3 g) in H_2O (5 ml) was added with stirring, and gradually prisms, mp 218–227° dec, sepd from the reaction mixt, yield, 0.93 g. Recrystn from H_2O yielded colorless prisms, mp 228–230° dec, of 15.

The stereochemical configuration of this compound was confirmed as follows. A soln of 15 in H_2O or 95% EtOH was refluxed with Raney Ni catalyst and the crystals obtd from this reaction mixt were identical with I by comparison of ir and ppc.

trans-4-(2-Carbamoylhydrazino)methylcyclohexanecarboxylic Acid (16). To a soln of methyl *trans*-4-formylcyclohexanecarboxylate (1.7 g, 0.01 mole), prepd from methyl *trans*-4-cyanocyclohexanecarboxylate in a similar manner as above in EtOH (24 ml) and H_2O (18 ml), $H_2NCONHNH_2 \cdot HCl$ (1.2 g, 0.011 mole) and AcONa (0.9 g, 0.011 mole) were added and vigorously stirred to make the soln homogeneous. After the soln was warmed on a water bath for 15 min, it was concd *in vacuo*, and the residue was treated with Et_2O to give the semicarbazone (0.94 g, 41%), mp 162–164°. A soln of this semicarbazone (0.94 g) in AcOH (18 ml) was catalytically hydrogenated over PtO_2 (0.1 g) and a theoretical amt of H_2 was absorbed. After removal of the catalyst and the solvent, the resulting syrup was dissolved in 4 *N* HCl and the soln was heated for 1 hr on a boiling water bath and was concd *in vacuo*. The sepd crystals were collected by filtration and washed with EtOH. Recrystn from EtOH–Et_2O gave colorless prisms (0.68 g, 66%) of 16.

N-(*trans*-4-Carboxycyclohexylmethyl)trimethylammonium Iodide (17). A soln of 7 (8.0 g, 0.043 mole) in hot Me_2CO (500 ml) was cooled to room temp and MeI (12.4 g, 0.087 mole) was added to the soln and the mixt was allowed to stand at room temp overnight. The soln was concd to one-third of its original vol. The sepd crystals were recrystd from Me_2CO to give 17 (8.5 g) as prisms.

Method D. *trans*-4-Aminomethylcyclohexanecarboxamide Hydrochloride (18). To a soln of I (6.3 g, 0.04 mole) in 10% aq NaOH (16 ml, 0.04 mole), CbzCl (8.2 g, 0.048 mole) and 10% aq NaOH (20 ml, 0.05 mole) were added for 15 min with cooling and vigorous stirring and the reaction mixt was stirred for another hr. The soln was acidified with aq HCl under cooling. The white ppt was collected, washed with H_2O, dried, and recrystd from C_6H_6–petr ether to give needles of II (10.7 g, 92%), mp 115–117°. *Anal.* ($C_{16}H_{21}NO_4$) C, H, N.

II (5.0 g, 0.017 mole) was mixed with $SOCl_2$ (5 ml) and warmed to 40° for 30 min. A vigorous reaction took place and a homogeneous soln was formed. After cooling, dry petr ether (50 ml) was added to the soln to ppt white crystals, which were collected, washed with dry petr ether, and dried *in vacuo* to give 4.4 g (82%) of acid chloride of II, as hygroscopic white crystals, mp 77–82°. *Anal.* ($C_{16}H_{20}ClNO_3$) C, H, N.

Dry NH_3 was introduced to the soln of II-acid chloride (4.4 g, 0.014 mole) in dry C_6H_6 (30 ml) under cooling. The reaction mixt was concd to dryness *in vacuo* and the residue was recrystd from Me_2CO–*n*-C_6H_{14} to give an amide, 3.5 g (83%). This amide (3.2 g, 0.011 mole) was dissolved in MeOH (20 ml), concd HCl (3 ml) was added, and the soln was catalytically hydrogenated over 10% Pd/C (0.5 g). After removal of the catalyst, the filtrate was concd to give 2.4 g of 18, mp 242–247° dec. Recrystallization from MeOH–Me_2Co gave the pure sample.

Method E. *N*-*n*-Hexyl-*trans*-4-aminomethylcyclohexanecarboxamide Hydrochloride (21). II (5.8 g, 0.02 mole) and NEt_3 (2.1 g, 0.02 mole) were dissolved in $CHCl_3$ (70 ml) and the soln was cooled to 0°. $ClCOOC_2H_5$ (2.2 g, 0.02 mole) was dropwise added for 15 min at 0–5° and the mixt kept at this temp for 30 min under stirring. *n*-Hexylamine (2.1 g, 0.02 mole) was added to the soln at the same temp for 30 min, and after being kept at room temp for 2.5 hr the mixture was washed with H_2O and dried. After removal of the solvent, the residue was recrystd from Me_2CO to give 5.9 g of *trans*-4-*N*-Cbz-aminomethylcyclohexanecarbox-*n*-hexylamide as needles.

This intermediate was catalytically hydrogenated in methanolic HCl over 10% Pd/C. After removal of the catalyst and the solvent, the residue was dissolved in H_2O and the soln was passed through a column of Amberlite IR-45 (OH form), and the eluate was concd to dryness *in vacuo*. Recrystn from MeOH–Me_2CO yielded 21, as leaflets.

trans-4-Aminomethylcyclohexanecarboxanilide Hydrochloride (24). To a soln of II (2.9 g, 0.01 mole) and $C_6H_5NH_2$ (0.93 g, 0.01 mole) in CH_2Cl_2 (20 ml), DCC (2.3 g, 0.011 mole) was added and the

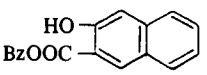
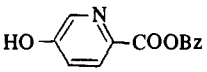
reaction mixt was allowed to stand at room temp overnight. After addn of several drops of AcOH, the sepd dicyclohexylurea was filtered off. The filtrate was concd *in vacuo* and the residual white mass was recrystd from EtOH to give a white cryst powder, mp 188–190°; yield, 2.93 g (78%). *Anal.* ($C_{22}H_{26}N_2O_3$) C, H, N. These crystals (1.83 g, 0.005 mole) were dissolved in 2% MeOH–HCl (50 ml)

Table III. Intermediate, Cbz Derivatives

No.	R	Mp, °C	Method	Yield, %	Formula	Analyses
		CbzHNCH$_2$–C$_6$H$_4$–···COR				
18	NH_2	185–188	E	91	$C_{16}H_{22}N_2O_3$	C, H, N
19	$NHCH_3$	198–200	E	86		*a*
20	$NH(CH_2)_3CH_3$	160–162	D	78		*a*
21	$NH(CH_2)_5CH_3$	162–163	E	85		*a*
22	$NH(CH_2)_6CH_3$	160–162	E	85		*a*
23	$NHCH_2CH_2N(CH_3)_2$	139–140	E	89	$C_{20}H_{31}N_3O_3$	C, H, N
25	NH–C$_6$H$_4$	211–212	E	92	$C_{22}H_{32}N_2O_3$	C, H, N
26	$N(C_2H_5)_2$	117–119	E	71	$C_{20}H_{30}N_2O_3$	C, H,[b] N
27	N(C$_6$H$_4$)$_2$	183	D	18	$C_{28}H_{42}N_2O_3$	C, H, N
28	Piperidino	127–129	E	28	$C_{21}H_{30}N_2O_3$	C, H, N
50	$O(CH_2)_5OH$	*c*	H			
51	$OCH_2(CH_2)_3CH_2O$	*c*	H			
52	$OCH_2(CH_2)_5OH$	*c*	H			
53	$OCH_2(CH_2)_4CH_2O$	*c*	H			
70	$OCH_2C_6H_4CH_2OH(p)$	*c*	H			
71	$OCH(C_6H_5)C{\equiv}CH$	*c*	H			
74	OCH$_2$–C$_5$H$_4$N	*c*	H			
90	$OC_6H_3(CH_3)_2(m,p)$	97–98	H	81	$C_{24}H_{29}NO_4$	C, H, N
91	$OC_6H_3(CH_3)_2(o,o)$	110–112	H	28	$C_{24}H_{29}NO_4$	C, H, N
92	$OC_6H_3Cl_2(o,p)$	122–123	H	87	$C_{22}H_{23}Cl_2NO_4$	C, H, N
93	$OC_6H_2Cl_3(o,o,p)$	107–109	H	73	$C_{22}H_{22}Cl_3NO_4$	C, H,[d] N
94	$OC_6H_4C(CH_3)_3(p)$	88–94	H	87	$C_{26}H_{33}NO_4$	C, H, N
95	$OC_6H_4OBz^e(p)$	106–108	H	68	$C_{29}H_{31}NO_5$	C, H, N
96	$OC_6H_4CH_2OH(p)$	112–113	H	64	$C_{23}H_{27}NO_5$	C, H, N
97	$OC_6H_4COOBz(p)$	98–100	H	88	$C_{30}H_{31}NO_6$	C,[f] H, N
98	$OC_6H_3(COOBz)_2(o,p)$	118–121	H	67	$C_{38}H_{37}NO_8$	C, H
99	$OC_6H_4CH_2CH_2COOBz(p)$	83	H	82	$C_{32}H_{35}NO_6$	C,[g] H, N
100	$OC_6H_4CH_2CH_2COOBz(m)$	Syrup	H			
101	$OC_6H_4CO(CH_2)_4COOBz(p)$	72–75	H	87	$C_{35}H_{39}NO_7$	C,[h] H, N
102	$OC_6H_4CH(OH)(CH_2)_4COOBz(p)$	62–65	H	79	$C_{35}H_{41}NO_7$	C, H, N
104	$OC_6H_4NHCH_2COOBz(p)$	169–170	H	50	$C_{31}H_{34}N_2O_6$	C, H, N
105	$OC_6H_4CH_2CH(NHCbz)COOBz(p)$	137–139	H	70	$C_{40}H_{42}N_2O_8$	C, H, N
106	$OC_6H_4O(CH_2)_2COOBz(p)$	108–110	H	86	$C_{32}H_{35}NO_7$	C, H, N
107	BzOOC–C$_8$H$_5$O	117–119	H	84	$C_{34}H_{33}NO_6$	C, H
108	O–C$_5$H$_3$N–COOBz	94–96	H	63	$C_{29}H_{30}N_2O_6$	C,[i] H, N
109	$OC_6H_4NO_2(p)$	132–134	*j*	85	$C_{22}H_{24}N_2O_6$	C, H, N
111	$OC_6H_3(OCH_3)(CHO)(o,p)$	94–97	H	78	$C_{24}H_{27}NO_6$	C, H, N
113	$OC_6H_3(NO_2)(COOCH_3)(o,p)$	126–128	H	81	$C_{24}H_{26}N_2O_8$	C, H, N
114	$OC_6H_4SO_2NH_2(p)$	174–176	H	59	$C_{22}H_{26}N_2O_6S$	C, H, N
115	O–C$_5$H$_4$N	88–90	H	85	$C_{21}H_{24}N_2O_4$	C, H, N
116	O–C$_5$H$_4$NO	100–103	H	27	$C_{21}H_{24}N_2O_5$	C, H, N
117	$OC_6H_4CH_2COOBz(p)$	106–108	H	77	$C_{31}H_{33}NO_6$	C, H, N
118	$OC_6H_4CH{=}CHCOOBz(p)$	121–123	H	75	$C_{32}H_{33}NO_6$	C,[k] H
119	$OC_6H_4(CH_2)_5COOBz(p)$	76.5–77.5	H	81	$C_{35}H_{41}NO_6$	C, H, N

[a] Used to the next procedure without further purification. [b] N: calcd, 8.09; found, 8.71. [c] Not isolated. [d] H: calcd, 4.67; found, 4.23. [e] Benzyl. [f] C: calcd, 71.84; found, 72.32. [g] C: calcd, 72.56; found, 71.97. [h] C: calcd, 71.77; found, 71.19. [i] C: calcd, 69.31; found, 69.95. [j] See in Experimental Section. [k] C: calcd, 72.82; found, 72.23.

Table IV. Hydroxyaryl Derivatives

No.	Hydroxyaryl derivatives	Mp (or bp (mm), °C)	Yield, %	Formula	Analyses
1	$HOC_6H_4CH{=}CHCOOBz^a(p)$	89–91	37	$C_{16}H_{14}O_3$	C, H
2	$HOC_6H_4CH_2CH_2COOBz(p)$	199 (1 mm)	35	$C_{16}H_{16}O_3$	C, H
3	$HOC_6H_4CO(CH_2)_4COOBz(p)$	90–96	32	$C_{19}H_{20}O_4$	C, H
4	$HOC_6H_4(CH_2)_5COOBz(p)$	40–41; 213.5 (1 mm)	37	$C_{19}H_{22}O_3$	C, H
5	$HOC_6H_4NHCH_2COOBz(p)\cdot p$-TsOH	186 dec	81	$C_{15}H_{15}NO_3$ p-TsOH	C,[b] H, N
6	$HOC_6H_4CH_2CH(NHCbz^c)COOBz(p)$	116–118	30	$C_{24}H_{23}NO_5$	C, H,[d] N
7	$HOC_6H_4O(CH_2)_2COOBz(p)$	74–78; 200–201 (1 mm)	26	$C_{16}H_{16}O_4$	C, H
8	$HOC_6H_4CH_2CH_2COOBz(m)$	194–196 (1 mm)	29	$C_{16}H_{16}O_3$	C, H
9	$HOC_6H_3(COOBz)_2(o,p)$	81–83	29	$C_{22}H_{18}O_5$	C, H
10		86–87	37	$C_{18}H_{14}O_3$	C,[e] H
11		142–145 dec	16	$C_{13}H_{11}NO_3\cdot HCl$	C, H, N, Cl

[a]Benzyl. [b]C: calcd, 61.52; found, 62.58. [c]Carbobenzoxy. [d]H: calcd, 6.23; found, 5.72. [e]C: calcd, 76.67; found, 76.13.

and catalytically hydrogenated over 10% Pd/C (0.5 g). After filtration of the catalyst, the filtrate was concd *in vacuo* and the residue was recrystd from EtOH–Me_2CO to render white prisms of **24**.

Method F. *trans*-4-Aminomethylcyclohexanecarboxyl-*o*-toluidide Hydrochloride (32). I (5 g, 0.032 mole) was dissolved in $SOCl_2$ (20 ml). Gradually white crystals began to ppt. After 30 min, Et_2O was added to the reaction mixt and the crystals were collected by filtration and dried in a desiccator to give the acid chloride hydrochloride of I (5.4 g), as highly hygroscopic needles, mp 126–128° dec. *Anal.* ($C_8H_{14}ClNO$ HCl) C, H, N.

To a soln of the acid chloride hydrochloride of I (5.0 g, 0.024 mole) in PhMe (50 ml), *o*-toluidine (5.0 g, 0.047 mole) was added with chilling and stirring. Stirring was contd for 30 min. The ppt was collected by filtration and recrystd from MeOH–Me_2CO to give 32 as white crystals.

Method G. Ethyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrochloride (36). I (0.5 g, 0.003 mole) was dissolved in EtOH (20 ml) and the soln was refluxed for 1 hr while bubbling through dry HCl. The soln was concd under diminished pressure and the residual white powder was recrystd from EtOH–Et_2O to give white needles of 36.

Carboxymethyl *trans*-4-Aminomethylcyclohexanecarboxylate (56). To a soln of II (5.82 g, 0.02 mole) and NEt_3 (2.2 g, 0.02 mole) in AcOEt (40 ml), a soln of $ClCH_2COOCH_2C_6H_5$ (3.7 g, 0.02 mole) in AcOEt (20 ml) was added. The reaction mixt was refluxed for 8 hr and then cooled. H_2O was added and the soln was extd with C_6H_6; the C_6H_6 layer was washed with H_2O repeatedly and dried. After removal of the solvent *in vacuo*, the residue was recrystd from AcOEt to give 3.3 g (40%) of benzyloxycarbonylmethyl ester of II, mp 108–110°, as needles. *Anal.* ($C_{25}H_{29}NO_6$) C,§ H, N.# This ester (2.2 g, 0.005 mole) was dissolved in AcOH (20 ml) and THF (10 ml) and the soln was catalytically hydrogenated over 10% Pd/C (1 g). After completion of the hydrogenation, the catalyst was removed, Et_2O and petr ether were added to the filtrate to ppt crystals. Recrystallization from H_2O–EtOH–Me_2CO gave 56.

Carbamoylmethyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrobromide (57). A soln of II (29.1 g, 0.1 mole), NEt_3 (15.2 g, 0.15 mole), and $ClCH_2CN$ (11.4 g, 0.15 mole) in AcOEt (150 ml) was refluxed for 4 hr. The soln was washed with H_2O, 5% aq $NaHCO_3$, 1 *N* HCl, and H_2O and dried. After removal of the solvent, the residue was recrystd from AcOEt–petr ether to afford 26.1 g (79%) of cyanomethyl ester of II. *Anal.* ($C_{18}H_{22}N_2O_4$) C, H, N.** The cyanomethyl ester (16.5 g, 0.05 mole) was dissolved in AcOH (17 ml), 44% HBr–AcOH (50 ml) was added, and the mixt was allowed to stand for 1 hr at room temp. Et_2O (700 ml) was added to the soln, the sepd syrup was dissolved in *tert*-BuOH (80 ml) and the soln was warmed at 80° for 5 min. The solidified material was collected and recrystd from EtOH–Et_2O to give 57.

α-(4-Tolylcarbamoyl)benzyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrochloride (58). To a soln of II (12 g, 0.04 mole) in PhCHO (29.1 g, 0.27 mole), 4-tolylisonitrile (3.7 g, 0.032 mole) was added and the mixt was allowed to stand in a refrigerator for 3 days. Petr ether was added, and the sepd oily residue was washed with petr ether repeatedly; Et_2O (100 ml) was added to the residue to solidify it. The cryst mass (7.1 g), mp 145–149°, was dissolved in EtOH–MeOH and the soln was decolorized with activated C. After removal of the solvent, the residue was triturated with Et_2O to give 5.0 g of 4-*N*-Cbz-58, mp 149–151°. *Anal.* ($C_{31}H_{35}N_2O_5$) C, H, N.

4-*N*-Cbz-58 (515 mg, 0.001 mole) was dissolved in warm MeOH (20 ml) and 34% HCl–MeOH (0.5 ml). The soln was catalytically hydrogenated over 5% Pd/C (500 mg). After completion of the hydrogenation (5 min), the catalyst was removed and the filtrate was concd *in vacuo* below 40° (bath temp). Et_2O was added to the residue to induce crystn. The crystals were collected and washed with Et_2O to give 370 mg of 58. A pure sample was obtd by recrystn from MeOH–Et_2O, as prisms: ppc, solvent A, R_f, 0.86.

Phenacyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrobromide (59). To a soln of II (2.9 g, 0.01 mole) in AcOEt (20 ml), phenacyl bromide (2.0 g, 0.01 mole) and NEt_3 (1.4 ml, 0.01 mole) were added and the mixt was allowed to stand at room temp overnight. After removal of $NEt_3\cdot HBr$, the filtrate was washed enough with H_2O and dried. The soln was concd to a syrup which solidified on cooling. Recrystn from MeOH gave white needles, mp 92–94°, in yield of 2.6 g (65%). *Anal.* ($C_{24}H_{27}NO_5$) C, H, N.

These crystals (2.6 g) were dissolved in AcOH (6 ml), 30% HBr–AcOH (6 ml) was added to the soln, and the mixt was allowed to stand at room temp for 30 min when dry Et_2O was added to the soln. The sepd crystals were recryst from EtOH–Et_2O to yield white needles of 59.

Method I. Phenyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrochloride (75). A soln of phenol (2.3 g, 0.024 mole) in dry dioxane (20 ml) was added to the acid chloride hydrochloride of I (4.2 g, 0.02 mole) and the mixt was warmed for 30 min and then concd. The sepd crystals were collected and recrystd from EtOH–Et_2O to give 75 (4.3 g).

Method H. 4-(2-Carboxyethyl)phenyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrochloride (99). To a soln of benzyl 4-hydroxyphenylpropionate (60.4 g, 0.236 mole) and NEt_3 (28.6 g, 0.283 mole) in dry C_6H_6 (250 ml), a soln of the acid chloride of II (73.0 g, 0.236 mole) in dry C_6H_6 (450 ml) was dropwise added for 30 min under stirring. The reaction mixt was allowed to stand at room temp for 2 hr and then warmed at 50–60° for 30 min. After cooling, the sepd $NEt_3\cdot HCl$ was filtered off and the filtrate was washed several times with H_2O and dried (Na_2SO_4). After removal of the solvent, Et_2O was added to the residual syrup which crystd. The crystals were collected, washed with Et_2O, and dried. This Cbz-ester (30 g, 0.057 mole) was dissolved in AcOH (150 ml) and the soln was hydrogenated over 10% Pd/C (5 g). After removal of the catalyst, Et_2O (200 ml) and petr ether (400 ml) were added and the mixt was allowed to stand in a refrigerator overnight. The sepd crystals, mp 280°, were collected and washed with Et_2O, yield 16.1 g.

To a soln of free 99 (16.1 g, 0.053 mole) in AcOH (100 ml), 9% HCl–AcOH (25.6 g, 0.063 mole) was added under chilling and stirring and then *i*-Pr_2O (300 ml) was added and the mixt cooled with an ice bath for 1 hr. The sepd crystals of 99 were collected, washed with *i*-Pr_2O, and dried; yield, 17.1 g.

Method J. 4-(2-Carboxyethyl)phenyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrochloride (99). *trans*-4-Cyanocyclohex-

§C: calcd, 68.32; found, 67.76.
#N: calcd, 3.19; found, 4.07.
**N: calcd, 8.48; found, 7.81.

anecarboxylic acid[19] (1.53 g, 0.01 mole) was dissolved in $SOCl_2$ (5 ml) and the soln was gently refluxed for 1 hr and then concd to a syrup *in vacuo* at low temp (bath temp was below 80°). A soln of this syrup in dry C_6H_6 (20 ml) was added to a soln of benzyl 4-hydroxyphenylpropionate (2.6 g, 0.01 mole) and NEt_3 (2.0 g, 0.02 mole) in dry C_6H_6 (20 ml) with stirring. The reaction mixt was warmed on a water bath for 30 min with stirring. The sepd $NEt_3 \cdot HCl$ was filtered off, and the filtrate was concd to dryness *in vacuo*. The white residue (3.5 g, 89.5%), mp 58–62°, was recrystd from MeOH to give a pure sample of the cyano ester, mp 61–65°; yield, 3.1 g (80%). *Anal.* ($C_{24}H_{25}NO_4$) C, H, N.

A soln of this ester (1.5 g, 0.004 mole) in a mixt of AcOEt–EtOH–H_2O (15:40:20) (75 ml), and 28% NH_4OH (0.25 ml, *ca.* 0.002 mole) was placed in an autoclave and Raney Ni catalyst (W-5) (1.5 ml) was added. Hydrogenation was achieved at an initial pressure of 140 kg/cm² at 70°. After completion of the hydrogenation, the catalyst was filtered off and washed with MeOH and H_2O and the washings were combined with the filtrate and the combined soln was concd *in vacuo* at low temp (bath temp was 40–50°). The sepd crystals were collected by filtration and washed with hot MeOH; yield, 0.8 g. The crystals, mp 200–280° dec, were dissolved in a small amt of AcOH, an equimolar HCl–AcOH was added, and then *i*-Pr_2O was added to the soln. The ppt was collected, washed with enough *i*-Pr_2O, and recrystd from MeOH–Et_2O to give 0.7 g of 99.

4-Nitrophenyl *trans*-4-Aminomethylcyclohexanecarboxylate Hydrobromide (109). A soln of II (14.6 g, 0.05 mole) and *p*-nitrophenol (8.4 g, 0.06 mole) in AcOEt (90 ml), DCC (12.4 g, 0.06 mole) was added at room temp. Gradually cryst materials sepd from the reaction mixt which was allowed to stand at room temp overnight. White crystals were collected and washed with cold AcOEt. Recrystn from EtOH gave pale yellow crystals, mp 130–132°, yield, 17.5 g (85%). These crystals (2.1 g, 0.005 mole) were suspended in 15% HBr–AcOH (10 ml) and warmed at 50° for 10 min and then the resulting homogeneous soln was cooled. Dry Et_2O was added to the soln and the ppt was recrystd from EtOH to give pale yellow needles of 109.

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Potential Antileukemic and Immunosuppressive Drugs. 3. Effects of Homocyclic Ring Substitution on the *in Vitro* Drug Activity of 4-Nitrobenzo-2,1,3-oxadiazoles (4-Nitrobenzofurazans) and Their *N*-Oxides (4-Nitrobenzofuroxans)[1]

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4-Nitrobenzofuroxans and benzofurazans bearing electron-withdrawing substituents in the 5 and 6 positions (relative to NO_2) have been examined for their ability to inhibit nucleic acid synthesis in rabbit thymocytes *in vitro*. None of the compds tested were more potent in this screen than the unsubstituted nitrobenzofurazan or nitrobenzofuroxan, suggesting that the formation and stability of Meisenheimer complexes with cellular thiols and amino groups is diminished by the presence of substituents in the 5 as well as 6 position. The 5-halogeno (F, Cl, Br) benzofuroxans nitrated in the 4 position, in contrast to 5-CF_3, 5-CN, 5-CONHR, and 5-COOR benzofuroxans which direct NO_2 to the 7 position. Some unique chemical and biological properties of 5-F (*vs.* 5-Cl or 5-Br) benzofuroxan are discussed.

Benzofuroxans and benzofurazans bearing NO_2 groups in the 4 and 5 positions have been shown to be potent *in vitro* inhibitors of nucleic acid synthesis in lymphocytes.[2] It was suggested[2] that a possible mode of action of these compounds at the cellular level was by forming Meisenheimer complexes with essential cellular SH and/or amino groups.