Highly Selective Inhibitors of Thromboxane Synthetase. 2. Pyridine Derivatives

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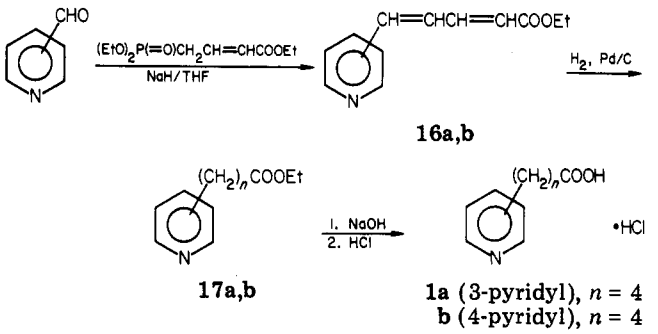
The enzyme thromboxane (TX) synthetase is inhibited by pyridine. The β-substituted pyridine derivatives showed higher inhibitory potency than the γ-substituted ones having the same side chain. Among the β-substituted derivatives containing the ω-carboxyalkyl group, the compounds with 6–8 carbon atoms in the side chain were especially effective. The derivatives holding the phenylene group in the side chain exhibited much higher inhibitory activity than those of the alkylene type. Among them, (*E*)-3-[4-(3-pyridylmethyl)phenyl]-2-methylacrylic acid hydrochloride (**5a**) had the highest potency ($IC_{50} = 3 \times 10^{-9}$ M). The β-substituted pyridine derivatives and 1-substituted imidazole derivatives which had the same side chain showed almost the same potency. The β-substituted pyridine derivatives do not inhibit arachidonic acid cyclooxygenase or prostaglandin I_2 synthetase, two other enzymes of the arachidonic cascade.

Prostaglandin H_2 (PGH_2) is transformed to thromboxane A_2 (TXA_2) by TX synthetase in human platelets. The product is a potent aggregatory agent.[1] On the basis of the previous findings that imidazole and its derivatives blocked the action of TX synthetase,[2-5] we synthesized various imidazole derivatives as described in the preceding paper[6] and found that *p*-(1-imidazolylmethyl)-α-methylcinnamic acid hydrochloride (**5c**) was about 12 000 times more potent than imidazole. We also tested the inhibitory effects of various heterocyclic compounds on TX synthetase and observed that pyridine had almost the same inhibitory potency as imidazole. From this point of view, a number of pyridine derivatives were synthesized and their inhibitory potency on TX synthetase was examined.

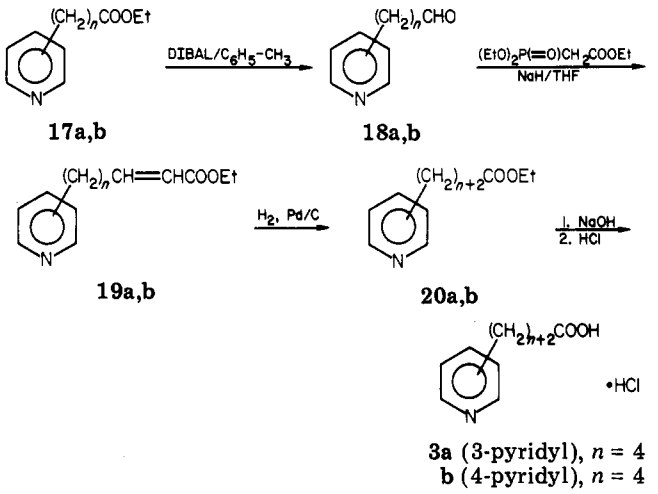
Chemistry. The ω-carboxalkyl derivatives were synthesized as shown in Schemes I–III (methods A–C). The β- or γ-substituted compounds having the same side chain were prepared by the same procedure. The preparation of the 5-pyridylpentanoic acid hydrochlorides (**1a** and **1b**, method A) was effected by the following sequence: (1) Wadsworth–Emmons reaction of 3- or 4-formylpyridine, (2) catalytic hydrogenation on Pd/C, and (3) hydrolysis of the ester function with 2 N NaOH. The synthesis of the 7-pyridylheptanoic acid hydrochlorides (**3a** and **3b**, method B) was performed as follows: (1) reduction of **17** with $(i\text{-Bu})_2AlH$ (DIBAL), (2) Wadsworth–Emmons reaction of **18**, (3) catalytic reduction on Pd/C, and (4) saponification of the ester **20**. A one-carbon extension (method C) was achieved by the following route: (1) reduction of **17** with DIBAL, (2) chlorination of **21** with $SOCl_2$, (3) treatment with NaCN, and (4) hydrolysis with concentrated HCl.

The derivatives having phenylene or thiophene in the side chains were obtained by the procedures shown in Schemes IV–VIII (methods D–H). The unsaturated derivatives (**5a**, **6**, **12**, and **13**) were derived from nicotinyl chloride hydrochloride by the following reactions (method D): (1) reaction with the Grignard reagent, (2) Wadsworth–Emmons reaction of the aldehyde **24**, and (3) hydrolysis of the esters **25**. The saturated compounds (**7** and **8**) were prepared from the unsaturated esters **25** by catalytic hydrogenation and saponification. The synthesis of **9** was accomplished by using the ketone **28** instead of the aldehyde **24**. The Wadsworth–Emmons reaction of **28** was very slow because of the steric hindrance of the methyl group. The thiophene derivatives (**14** and **15**) were synthesized from nicotinaldehyde as shown in Scheme VI (method F). The hydroxyl group of **30** was removed by treatment with Zn in AcOH after acetylation. Removal

Scheme I. Synthetic Method A



Scheme II. Synthetic Method B



(1) M. Hamberg, J. Svensson, and B. Samulesson *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2994 (1975).
(2) P. Needleman, A. Raz, J. A. Ferrendelli, and M. Minkes, *Proc. Natl. Acad. Sci. U.S.A*, **74**, 1716 (1977).
(3) S. Moncada, S. Bunting, K. Mullane, P. Thorogood, J. R. Vane, A. Raz, and P. Needleman *Prostaglandins*, **13**, 611 (1977).
(4) T. Yoshimoto, S. Yamamoto, and O. Hayaishi, *Prostaglandins*, **16**, 529 (1978).
(5) H.-H. Tai, and B. Yuan, *Biochem. Biophys. Res. Commun.*, **80**, 236 (1978).
(6) K. Iizuka, K. Akahane, D. Momose, T. Tanouchi, M. Kawamura, I. Ohyama, I. Kajiwara, Y. Iguchi, T. Okada, T. Miyamoto, K. Taniguchi, and M. Hayashi, *J. Med. Chem.*, preceding paper in this issue.

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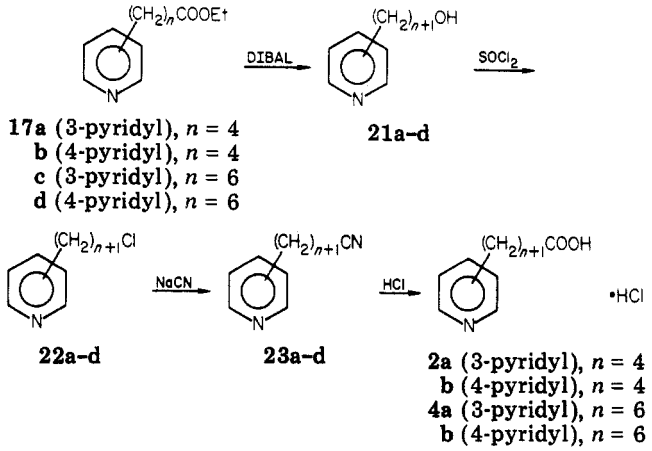
Scheme III. Synthetic Method C



17a (3-pyridyl), $n = 4$
b (4-pyridyl), $n = 4$
c (3-pyridyl), $n = 6$
d (4-pyridyl), $n = 6$

21a-d

22a-d **23a-d**

2a (3-pyridyl), $n = 4$
b (4-pyridyl), $n = 4$
4a (3-pyridyl), $n = 6$
b (4-pyridyl), $n = 6$

Table I. Inhibitory Potencies of Heterocyclic Compounds on TX Synthetase

compd	% inhibn at 100 μM
pyrrole	−0.8
pyrazole	8.9
1,2,4-triazole	4.1
thiophene	−1.6
thiazole	17.3
imidazole	67.8
piperidine	15.8
pyridine	71.2
pyridazine	16.8
pyrimidine	12.0
pyrazine	12.0
s-triazine	11.0

Table II. Inhibitory Potencies of Picolines on TX Synthetase

compd	% inhibn at 100 μM
α-picoline	14.7
β-picoline	66.4
γ-picoline	67.8

of the hydroxyl group in **34** was achieved successfully by dehydration with $SOCl_2$ and catalytic hydrogenation on Pd/C. In method H, the hydroxyl group of compound **42** was removed after Wittig reaction by (1) chlorination with $SOCl_2$ and (2) reduction with Zn in AcOH.

Enzyme Assay. PGH_2 was prepared with sheep vesicular gland microsomes as described previously.[7] TX isomerase was prepared from rabbit platelets by a previous method.[7] Enzyme reactions were carried out by the procedure of Yoshimoto et al.[4,6]

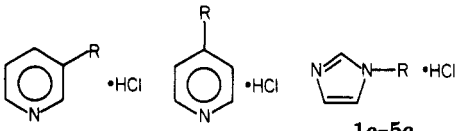
Results and Discussion

We tested the inhibitory activity of various five- or six-membered heterocyclic compounds for TX synthetase. As previously reported,[8] pyridine was as active as imidazole (Table I). The tested compounds, other than pyridine and imidazole, showed no significant inhibitory potency at 100 μM.

The inhibitory activity of picolines was examined as a simple system to determine the relationship of the position of the side chain on the pyridine ring (Table II) to activity. Both β- and γ-picolines were as active as pyridine, but

(7) T. Yoshimoto, S. Yamamoto, M. Okuma, and O. Hayaishi, *J. Biol. Chem.*, **252**, 5871 (1977).
(8) T. Miyamoto, K. Taniguchi, T. Tanouchi, and M. Hirata, *Adv. Prostaglandin Thromboxane Res.*, **6**, 443 (1980).

Table III. Inhibitory Potencies on TX Synthetase of β-Substituted Pyridines, γ-Substituted Pyridines, and 1-Substituted Imidazoles



1a-5a **1b-5b** **1c-5c**

no.	R	IC_{50}, nM	synth method
1a		500	A
1b	$(CH_2)_4COOH$	>1000	A
1c		>1000	*a*
2a		86	C
2b	$(CH_2)_5COOH$	>1000	C
2c		>1000	*a*
3a		68	B
3b	$(CH_2)_6COOH$	>1000	B[b]
3c		39	*a*
4a		76	C
4b	$(CH_2)_7COOH$	>1000	C[b]
4c		32	*a*
5a		3	D
5b	$CH_2C_6H_4CH{=}CH(CH_3)COOH$	>1000	D
5c		4	*a*

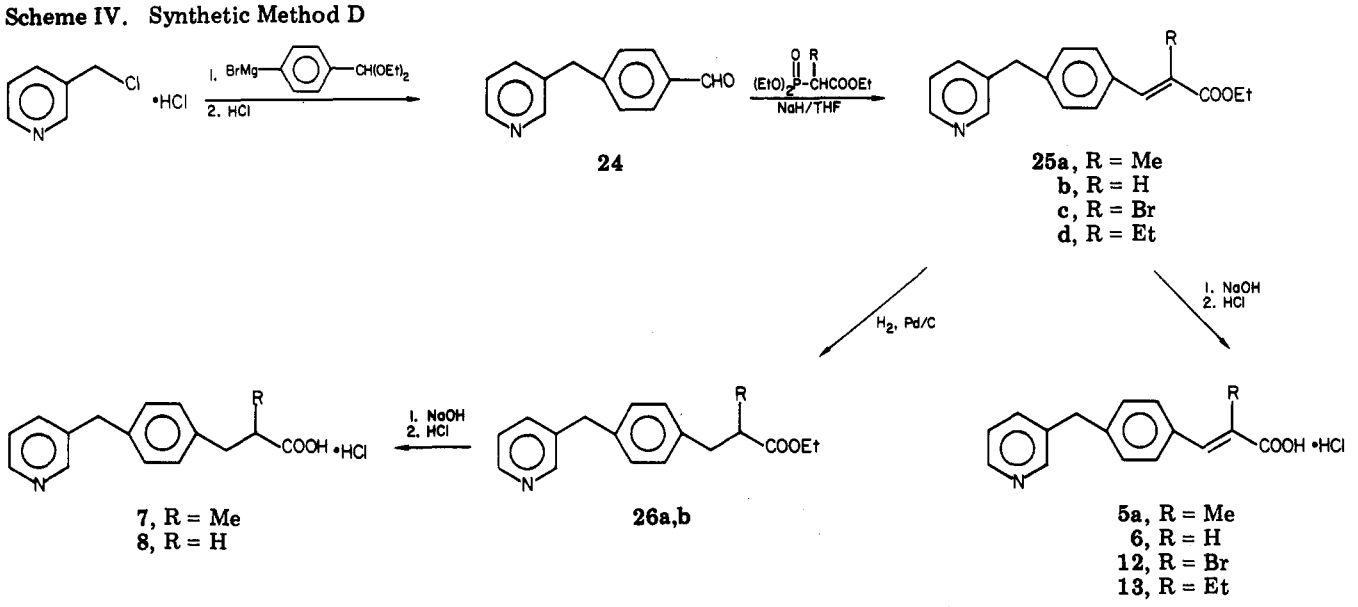
[a] See ref 6. [b] HCl free compounds were reported: J. A. Gautier, I. Marszak, M. Olomucki, and M. Miocque, *Bull. Soc. Chim. Fr.*, 2569 (1965).

α-picoline was less active than pyridine. Consequently, the same side chain was introduced into the β or γ positions of the pyridine ring. As shown in Table III, the inhibitory potency of β-substituted pyridine derivatives (**1a–4a**) was related to the length of the side chain, and the derivatives having the ω-carboxyalkyl group with six to eight carbon atoms showed high inhibitory activity. On the other hand, the γ-substituted derivatives (**1b–4b**) showed no significant effect on the enzyme at 1 μM. This was an unexpected result in sharp contrast to the results of β- and γ-picolines described above. Therefore, it is obvious that both the length of the molecule and the position of the side chain on the pyridine ring are important factors to reveal activity.

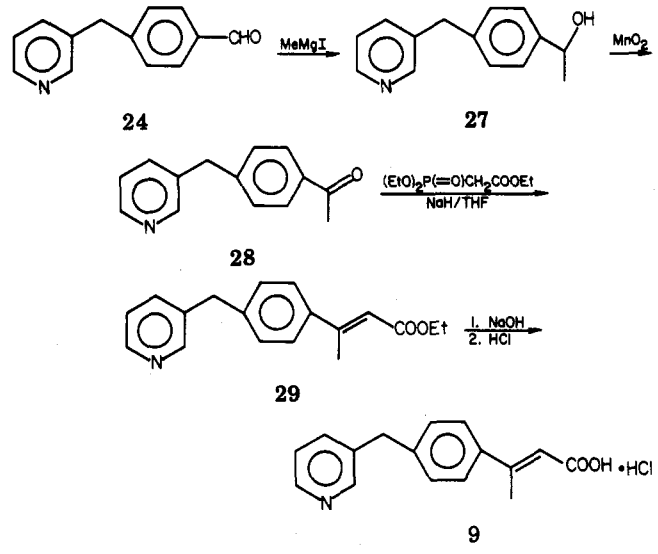
Several kinds of side chains were introduced into imidazole and pyridine (Table III). The pyridine derivative **1a** inhibited TX synthetase at 0.5 μM, while the imidazole derivative **1c** did not block it at 1 μM. Among the derivatives **2a** and **2c** posessing longer side chains than those of **1a** and **1c**, **2a** manifested much higher activity than **2c**. Of the pyridine derivatives, **2a**, **3a**, and **4a** had almost the same activity, although the length of the side chain was different. On the other hand, imidazole derivatives with longer side chains showed higher inhibitory potency (**2c**, **3c**, and **4c**).[4,6] These results suggest the different properties of pyridine and imidazole as TX synthetase inhibitors.

The pyridine derivative **5a** with the same side chain as that of **5c**[6] was the most active inhibitor of TX synthetase. Therefore, various modified compounds of **5a** were synthesized (Table IV). Saturation of the double bond (**7**) or removal of the methyl group (**6**) decreased the original potency to some degree. Compound **8** lost its inhibitory potency by saturation of the double bond and demethylation. The transfer of the methyl group in **5a** to the β position of the carboxyl group (**9**) considerably decreased the potency. The introduction of one more methyl group into **5a** (**10** and **11**) lowered the inhibitory potency. It seems that the methyl group of these compounds (**10** and **11**) hinders the binding of the compounds to the enzyme.

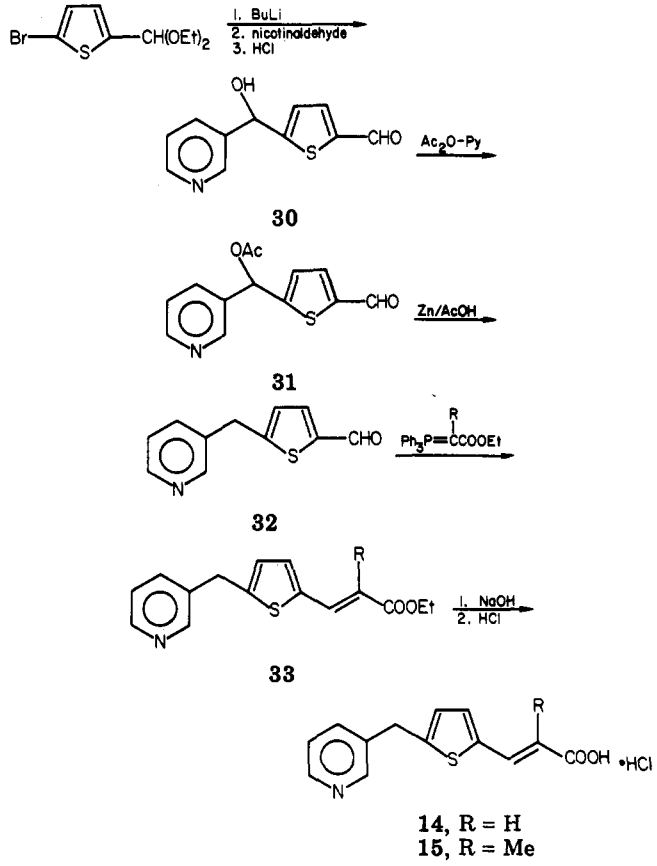
Scheme IV. Synthetic Method D



Scheme V. Synthetic Method E



Scheme VI. Synthetic Method F



The replacement of the methyl group in **5a** by other functions, such as the bromo (**12**) or the ethyl group (**13**), led to approximately the same potency as that of **5a**. It is clear that the potency does not depend upon the electronic effect of the substituent at the α position of the terminal carboxyl group, since the bromo derivative **12** is as active as the alkyl derivatives **5a** and **13**. The thiophene derivative **14** and **15** showed fairly high inhibitory potency, but they were less potent than **5a**.

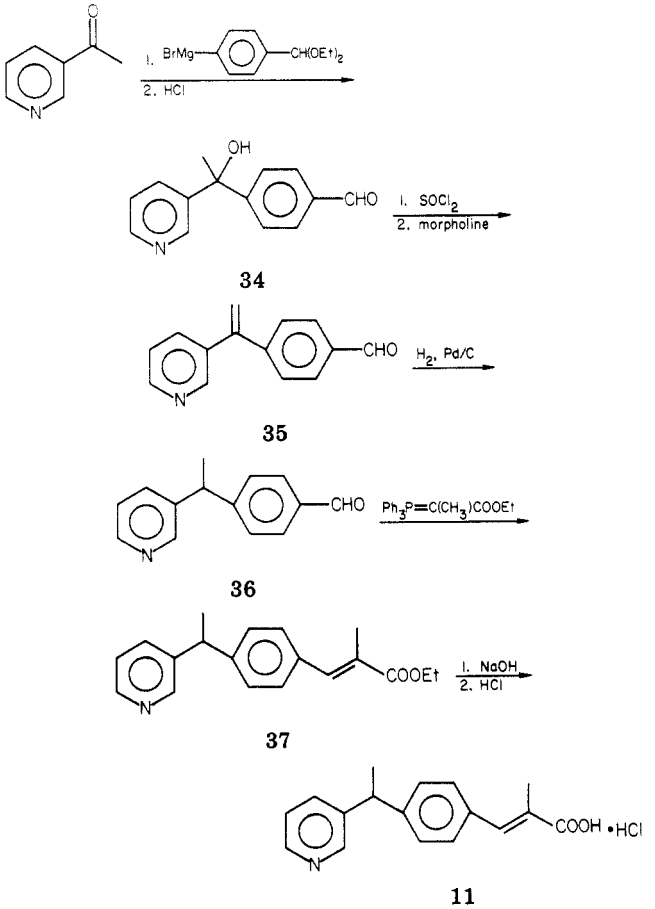
Thus, the inhibitory potency on TX synthetase was influenced by the position of the substituent on pyridine and the kind of side chain. As with the inhibitors reported in the previous paper,[8] the most active compound (**5a**) of the tested pyridine derivatives does not have an effect at 10^{-4} M on other enzymes in the PG biosynthetic enzyme system, such as cyclo-oxygenase and PGI_2 synthetase, and thus appears to inhibit TX synthetase specifically. It also supresses arachidonic acid (AA) induced platelet aggregation (IC_{50} = 0.1 μM) and strikingly prevents rabbits form the AA-induced sudden death.

Experimental Section

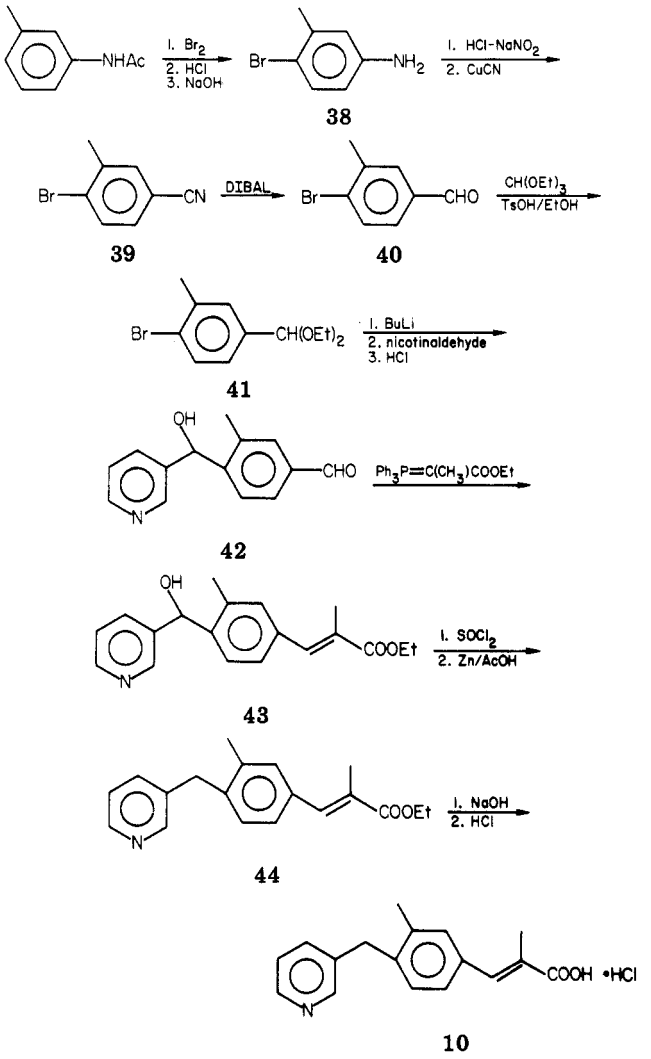
Chemistry. IR spectra were taken with a Hitachi GPI-G2 spectrophotometer. NMR spectra were recorded on a PMX-60 (JEOL) or XL-100 (Varian) spectrometer. Mass spectra (MS) were obtained on a JMS-OISG double-focusing mass spectrometer. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analysis were performed with Yanaco CHN Corder Model MT-2.

Method A. 5-(3-Pyridyl)pentanoic Acid Hydrochloride (1a). To a dry THF (100 mL) solution of lithium diisopropylamide (LDA; 33 mmol) was added triethyl phosphonocrotonate (8.6 g, 34.5 mmol) at −70 °C with stirring under a nitrogen atmosphere. After 15 min, nicotinaldehyde (3.21 g, 30 mmol) was added to the reaction mixture at −70 °C, and the mixture was warmed to 0 °C in 1.5 h. The reaction was quenched by the addition of AcOH, and the solvent was evaporated. An aqueous $NaHCO_3$ solution was added to the residue, and the mixture was extracted with EtOAc. Purification of the concentrated extracts by column chromatography on silica gel (40% cyclohexane in EtOAc) af-

Scheme VII. Synthetic Method G



Scheme VIII. Synthetic Method H



forded **16a** as a colorless oil (5.62 g, 92%).

To an EtOH (130 mL) solution of **16a** (5.4) was added 5% Pd/C (1.5 g) at room temperature, and the mixture was stirred for 1 h under a hydrogen stream. The catalyst was filtered off, and removal of the solvent in vacuo gave an oil, which was chromatographed on a silica gel column (40% cyclohexane in EtOAc) to give ethyl 5-(3-pyridyl)pentanoate (**17a**; 5.0 g, 91%).

Saponification of **17a** (207 mg, 1 mmol) with 2 N NaOH (1 mL) at room temperature for 1 h, followed by acidification with 6 N HCl and concentration in vacuo, afforded white crystals. Recrystallization of the residue from EtOH–Et_2O gave the title compound **1a** (71 mg, 33%): mp 142–145 °C; IR (KBr) 3300–2150, 1720, 1635, 1618, 1560, 1475, 1405, 1375, 1195 cm^{-1}; NMR (D_2O) δ 1.40–2.10 (m, 3 H), 2.48 (t, 2 H), 2.95 (t, 2 H), 7.90–8.20 (m, 1 H), 8.30–8.90 (m, 3 H); MS, *m/e* 179 (M^+). Anal. ($C_{10}H_{14}ClNO_2$) C, H, N.

5-(4-Pyridyl)pentanoic Acid Hydrochloride (1b): mp 193–196 °C (EtOH–Et_2O); IR (KBr) 3350–2200, 1730, 1645, 1610, 1510, 1400, 1240, 1180 cm^{-1}; NMR (D_2O) δ 1.40–2.10 (m, 4 H), 2.48 (t, 2 H), 3.04 (t, 3 H), 8.00 (d, 2 H), 8.72 (d, 2 H); MS, *m/e* 179 (M^+). Anal. ($C_{10}H_{14}ClNO_2$) C, H, N.

Method B. 7-(3-Pyridyl)heptanoic Acid Hydrochloride (3a). To a dry toluene (90 mL) solution of **17a** (1.82 g, 8.8 mmol) was added dropwise DIBAL (25% in toluene, 6.5 mL, 11.4 mmol) at −70 °C under a nitrogen atmosphere, and the solution was stirred for 30 min at the same temperature. The reaction was quenched by the addition of MeOH–H_2O, and the solution was stirred for 30 min after the cooling bath was removed. The white solid was filtered off, and concentration of the dried ($MgSO_4$) filtrate in vacuo gave a colorless oil, which was purified by column chromatography on silica gel (35% EtOAc in cyclohexane) to yield **18a** (500 mg, 35%).

To a suspension of NaH (63.5% in mineral oil, 348 mg, 9.6 mmol) in dry THF (40 mL) was added triethyl phosphonoacetate (2.24 g, 10 mmol) at room temperature, and the solution was stirred for 10 min. A solution of **18a** (1.3 g, 8 mmol) in THF (10 mL) was added dropwise into the reaction mixture, and the resulting mixture was stirred for 1 h. The same workup as method A (synthesis of **16a**) gave **19a** (1.568 g, 84%) as a colorless oil after column chromatography on silica gel (40% cyclohexane in EtOAc).

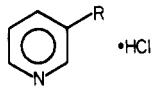
Catalytic hydrogenation of **19a** to **20a** was achieved by the same method as in method A (**16a** to **17a**) in 81% yield. The ester **20a** was hydrolyzed to the title compound **3a** in 92% yield under the same conditions as in method A: mp 151–153 °C (EtOH–Et_2O); IR (KBr) 3300–2200, 1720, 1638, 1550, 1440, 1230, 1190 cm^{-1}; NMR (D_2O) δ 2.20–2.70 (m, 2 H), 2.70–3.20 (m, 2 H), 7.80–8.40 (m, 1 H), 8.40–9.10 (m, 3 H); MS, *m/e* 207 (M^+). Anal. ($C_{12}H_{18}ClNO_2$) C, H, N.

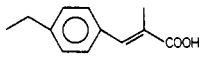
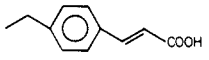
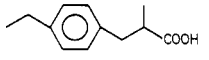
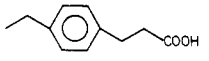
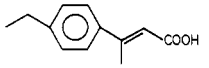
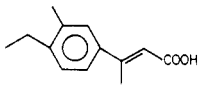
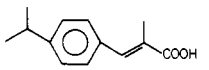
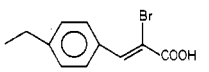
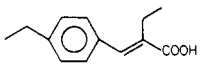
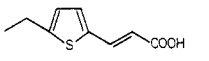
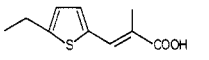
7-(4-Pyridyl)heptanoic Acid Hydrochloride (3b): mp 180–182 °C (EtOH–Et_2O); IR (KBr) 3400–2350, 1720, 1635, 1605, 1530, 1500, 1460, 1420, 1400, 1265, 1230, 1220, 1170, 1105, 1080 cm^{-1}; NMR (D_2O) δ 2.40 (t, 2 H), 3.00 (t, 2 H), 7.97 (d, 2 H), 8.69 (d, 2 H); MS, *m/e* 207 (M^+). Anal. ($C_{12}H_{18}ClNO_2$) C, H, N.

Method C. 6-(4-Pyridyl)hexanoic Acid Hydrochloride (2b). To a solution of ethyl 5-(4-pyridyl)pentanoate (**17b**; 2.1 g, 10.14 mmol) in dry toluene (50 mL) was added DIBAL (25% in toluene, 20 mL, 33 mmol) dropwise at −40 °C, and the solution was stirred for 30 min. The reaction was quenched by the addition of MeOH, and the mixture was warmed to 0 °C. Water (10 mL) was added to the reaction mixture, and the mixture was stirred for 30 min at room temperature. The formed white solid was filtered off. The filtrate was concentrated and the residue was chromatographed on silica gel (2% MeOH in $CHCl_3$) to yield the hydroxy compound **21b** as a colorless oil (1.3 g, 78%).

A mixture of **21b** (1.3 g, 7.88 mmol) and $SOCl_2$ (0.6 mL) was stirred at room temperature for 30 min. A saturated aqueous $NaHCO_3$ solution was added to the reaction mixture, and the mixture was extracted with $CHCl_3$. The extracts were washed, in turn, with a saturated aqueous $NaHCO_3$ solution, H_2O, and brine. The organic layer was dried ($MgSO_4$) and concentrated

Table IV. Modification of 5a



no.	R	IC_{50},[a] nM	synth method
5a		3	D
6		10	D
7		20	D
8		210	D
9		56	E
10		18	H
11		25	G
12		5	D
13		7	D
14		47	F
15		12	F

[a] Inhibitory potency on TX synthetase.

in vacuo. The residue was chromatographed on silica gel (2% MeOH in $CHCl_3$) to give the chloride **22b** (1.3 g, 90%) as a colorless oil.

To a mixture of NaCN (417 mg, 8.51 mmol) and Me_2SO (3.5 mL) was added a solution of the chloride **22b** (1.3 g, 7.1 mmol) in Me_2SO (3.5 mL) at 60 °C, and the solution was stirred for 3 h. The reaction mixture was poured into cold H_2O (40 mL) and extracted with $CHCl_3$. The extracts were washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2% MeOH in $CHCl_3$) to afford the cyanide **23b** as a colorless oil (1.1 g, 89%).

A solution of the cyanide **23b** (1.1 g, 6.3 mmol) in concentrated HCl (3 mL) was stirred at 60–70 °C for 3 days. The reaction mixture was washed with $CHCl_3$, and the aqueous layer was basified with 2 N NaOH. The solution was washed with $CHCl_3$ and neutralized with 2 N HCl. The white solid was filtered and recrystallized (EtOH–Et_2O) to give the title compound **2b** (1.14 g, 82%): mp 195–198 °C; IR (KBr) 3090, 3000, 2950, 1730, 1640, 1610, 1520, 1500, 1400, 1240, 1180 cm^{-1}; NMR (D_2O) δ 1.20–2.00 (m, 6 H), 2.42 (t, 2 H), 3.02 (t, 2 H), 7.80–8.10 (m, 2 H), 8.50–8.90 (m, 2 H); MS, *m/e* 193 (M^+). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

6-(3-Pyridyl)hexanoic Acid Hydrochloride (2a): mp 146–148 °C (EtOH–Et_2O); IR (KBr) 3400–1720, 1550, 1465, 1405, 1190 cm^{-1}; NMR (D_2O) δ 1.00–2.00 (m, 6 H), 2.42 (t, 2 H), 2.94 (t, 2 H), 8.08 (dd, 1 H), 8.40–8.90 (m, 3 H); MS, *m/e* 193 (M^+). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

8-(3-Pyridyl)octanoic Acid Hydrochloride (4a): mp 158–160 °C (EtOH–Et_2O); IR (KBr) 3400–2300, 1720, 1555, 1465, 1405, 1265, 1220, 1185 cm^{-1}; NMR (D_2O) δ 1.10–2.00 (m, 10 H), 2.40 (t, 2 H), 2.92 (t, 2 H), 8.05 (dd, 1 H), 8.54 (dt, 1 H), 8.64–8.80 (m, 2 H); MS, *m/e* 221 (M^+). Anal. ($C_{13}H_{20}ClNO_2$) C, H, N.

8-(4-Pyridyl)octanoic Acid Hydrochloride (4b): mp 190–193 °C (EtOH–Et_2O); IR (KBr) 3400, 3090, 2950, 2860, 1730, 1640, 1610, 1510, 1410 cm^{-1}; NMR (D_2O) δ 1.00–2.00 (m, 10 H), 2.40 (t, 2 H), 3.00 (t, 2 H), 7.80–8.20 (m, 2 H), 8.50–8.80 (m, 2 H); MS, *m/e* 221 (M^+). Anal. ($C_{13}H_{20}ClNO_2$) C, H, N.

Method D. (*E*)-3-[4-(3-Pyridylmethyl)phenyl]-2-methylacrylic Acid Hydrochloride (5a). To a dry THF (20 mL) suspension of 3-picolyl chloride hydrochloride (3.26 g, 20 mmol) was added the Grignard reagent (prepared from 1.07 g of Mg and 11.4 g of *p*-bromobenzaldehyde diethyl acetal) at 0 °C under a nitrogen atmosphere, and the mixture was stirred for 2 h at room temperature. The reaction mixture was poured into a saturated aqueous NH_4Cl solution and extracted with Et_2O. The dried ($MgSO_4$) extracts were concentrated in vacuo, and the residual oil was chromatographed on silica gel (1.5% EtOH in $CHCl_3$) to give 4-(3-pyridylmethyl)benzaldehyde diethyl acetal (2.40 g, 44%).

A mixture of the acetal (2.4 g, 8.9 mmol) and 1 N HCl (15 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with $CHCl_3$ and neutralized with $NaHCO_3$. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (1% EtOH in $CHCl_3$) to give the aldehyde **24** as a colorless oil. To a dry THF (5 mL) suspension of NaH (4% in mineral oil, 41.3 mg, 1.1 mmol) was added triethyl 2-methylphosphonoacetate (274 mg, 1.15 mmol) at room temperature under a nitrogen atmosphere, and the solution was stirred for 10 min. A solution of **24** (197 mg, 1 mmol) in THF (2 mL) was added to the reaction mixture, and the solution was stirred for 1 h at room temperature. The same workup of the reaction mixture as in method A (synthesis of **16a**) gave the ester **25a** as a colorless oil. The ester **25a** (225 mg, 0.8 mmol) was hydrolyzed with 2 N NaOH (1 mL) in EtOH (1 mL) at room temperature for 1 h. The solution was acidified with 6 N HCl and concentrated in vacuo. The residue was recrystallized from EtOH–Et_2O to give the title compound **5a** as white crystals (181 mg, 78%): mp 178–180 °C; IR (KBr) 1690, 1525, 1450, 1240, 1216, 1123, 851, 800 cm^{-1}; NMR (D_2O) δ 2.10 (d, 3 H), 4.39 (s, 2 H), 7.54 (s, 4 H), 7.72 (br s, 1 H), 8.17 (dd, 1 H), 8.68 (d, 1 H), 8.95 (m, 2 H); MS, *m/e* 253 (M^+). Anal. ($C_{16}H_{16}ClNO_2$) C, H, N.

(*E*)-3-[4-(4-Pyridylmethyl)phenyl]-2-methylacrylic Acid Hydrochloride (5b): mp 211–213 °C (EtOH–Et_2O); IR (KBr) 3700–2600, 1700, 1640, 1610, 1500, 1385, 1200, 1190, 1120, 825 cm^{-1}; NMR (Me_2SO-d_6) δ 2.06 (s, 3 H), 4.40 (s, 2 H), 7.20–7.80 (m, 5 H), 7.80–8.20 (m, 2 H), 8.60–8.90 (m, 2 H); MS, *m/e* 253 (M^+). Anal. ($C_{16}H_{16}ClNO_2$) C, H, N.

(*E*)-3-[4-(3-Pyridylmethyl)phenyl]-2-bromoacrylic Acid Hydrochloride (12): mp 192–196 °C (EtOH–Et_2O); IR (KBr) 1700, 1607, 1531, 1380, 1214, 808, 681 cm^{-1}; NMR (D_2O) δ 4.44 (s, 1 H), 7.49 (d, 2 H), 7.88 (d, 2 H), 8.17 (s, 1 H), 8.27 (dd, 1 H), 8.65 (d, 1 H), 9.00 (m, 2 H); MS, *m/e* 319 (M^+), 317 (M^+). Anal. ($C_{15}H_{13}BrClNO_2$) C, H, N.

(*E*)-3-[4-(3-Pyridylmethyl)phenyl]-2-ethylacrylic Acid Hydrochloride (13): mp 153–163 °C (EtOH–Et_2O); IR (KBr) 3040, 2960, 2810, 1700, 1632, 1614, 1553, 1390, 1181, 1128, 780, 683, 610 cm^{-1}; NMR (D_2O) δ 1.10 (t, 3 H), 2.50 (m, 2 H), 4.31 (s, 2 H), 7.47 (s, 4 H), 7.59 (s, 1 H), 8.09 (dd, 1 H), 8.57 (br d, 1 H), 8.59 (br d, 1 H), 9.01 (br s, 1 H); MS, *m/e* 267 (M^+). Anal. ($C_{17}H_{18}ClNO_2$) C, H, N.

(*E*)-3-[4-(3-Pyridylmethyl)phenyl]acrylic Acid Hydrochloride (6): mp 219–222 °C (EtOH–Et_2O); IR (KBr) 1702, 1634, 1323, 1226, 1175, 685 cm^{-1}; NMR (D_2O) δ 4.39 (s, 2 H), 6.54 (d, 1 H), 7.40–7.90 (m, 5 H), 8.18 (dd, 1 H), 8.65 (br d, 1 H), 8.88 (m, 2 H); MS, *m/e* 239 (M^+). Anal. ($C_{15}H_{14}ClNO_2$) C, H, N.

2-Methyl-3-[4-(3-pyridylmethyl)phenyl]propionic Acid Hydrochloride (7). A mixture of **25a** (338 mg, 1.2 mmol), 5% Pd/C (150 mg), and EtOH (8 mL) was stirred at room temperature for 10 h under a hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give a colorless oil, which was chromatographed on silica gel (35% EtOAc in cyclohexane) to afford the ester **26a** (245 mg, 72%). The ester **26a** (104 mg, 0.368 mmol) was hydrolyzed to the title compound **7** by the same procedure as in method A (79 mg, 83%): mp 126–131 °C (EtOH–Et_2O); IR (KBr) 1720, 1630, 1553, 1466, 1180, 684 cm^{-1}; NMR (D_2O) δ 1.20 (m, 3 H), 2.86 (m, 3 H), 4.29 (s, 2 H), 7.32 (s, 4 H), 8.10 (dd, 1 H), 8.55 (d, 1 H), 8.90 (m, 2 H); MS, *m/e* 240 (M – Me). Anal. ($C_{16}H_{18}ClNO_2$) C, H, N.

3-[4-(3-Pyridylmethyl)phenyl]propionic Acid Hydrochloride (8): mp 114–117 °C (EtOH–Et_2O); IR (KBr) 3160, 3120, 1720, 1534, 1450, 1405, 1177, 1008, 822, 791, 680 cm^{-1}; NMR (D_2O) δ 2.60–3.10 (m, 4 H), 4.26 (s, 2 H), 7.31 (s, 4 H), 8.07 (dd, 1 H),

8.53 (br d, 1 H), 8.76 (m, 2 H); MS, *m/e* 241 (M^+). Anal. ($C_{15}H_{16}ClNO_2$) C, H, N.

Method E. (*E*)-3-[4-(3-Pyridylmethyl)phenyl]-2-butenoic Acid Hydrochloride (9). To a dry THF (20 mL) solution of the aldehyde **24** (830 mg, 4.2 mmol) was added a solution of MeMgI in Et_2O (3 M, 1.5 mL, 4.5 mmol) at −70 °C under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature over a period of 1 h with stirring. The mixture was poured into a saturated aqueous NH_4Cl solution and extracted with Et_2O. The extracts were washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (50% cyclohexane in EtOAc) to give the alcohol **27** as a colorless oil (547 mg, 61%). A mixture of the alcohol **27** (547 mg, 2.6 mmol), MnO_2 (1.5 g, 17 mmol), and CH_2Cl_2 (10 mL) was stirred for 3 h and filtered. The filtrate was concentrated in vacuo to yield the ketone **28** as a colorless oil (407 mg, 75%). To a suspension of NaH (63% in mineral oil, 152 mg, 4 mmol) in dry THF (10 mL) was added triethyl phosphonoacetate (936 mg, 4.18 mmol) under a nitrogen atmosphere, and the mixture was stirred for 10 min at room temperature. The ketone **28** (400 mg, 1.90 mmol) was added, and the mixture was stirred at 60 °C for 48 h. The mixture was poured into a saturated aqueous NH_4Cl solution and extracted with Et_2O. The extracts were washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (35% cyclohexane in EtOAc) to afford the ester **29** as a colorless oil (423 mg, 79%). Hydrolysis of the ester **29** (181 mg, 0.64 mmol) with 2 N NaOH (0.65 mL, 1.3 mmol) in EtOH (1.6 mL) at 60 °C for 4 h and the same workup as in method A (**17a** to **1a**) gave the title compound **9** as white crystals (119 mg, 64%): mp 162–167 °C (EtOH–Et_2O); IR (KBr) 3660–2640, 1705, 1175 cm^{-1}; NMR (D_2O) δ 2.47 (s, 3 H), 4.22 (s, 2 H), 6.12 (s, 1 H), 7.20–7.70 (m, 4 H), 7.80–8.06 (m, 1 H), 8.30–8.50 (m, 1 H), 8.70–8.90 (m, 2 H); MS, *m/e* 253 (M^+). Anal. ($C_{16}H_{16}ClNO_2$) C, H, N.

Method F. (*E*)-5-(3-Pyridylmethyl)-2-(2-carboxyvinyl)thiophene Hydrochloride (14). A mixture of 5-bromo-2-thiophenecarboxaldehyde (17.2 g, 90.5 mmol), $CH(OEt)_3$ (25 mL, 150 mmol), EtOH (20 mL), and *p*-TsOH (0.20 g) was stirred under reflux for 30 min and cooled to room temperature. The reaction was quenched by the addition of triethylamine (0.5 mL). The mixture was subjected to distillation, and 5-bromo-2-thiophenecarboxaldehyde diethyl acetal (21.3 g, 89%) was collected, bp 95–97 °C (3 mmHg). To a solution of the acetal (3.76 g, 14.2 mmol) in anhydrous Et_2O (45 mL) was added dropwise *n*-BuLi (1.4 M in hexane, 10 mL, 14 mmol) at −70 °C, and the solution was stirred for 1 h. A solution of nicotinaldehyde (1.5 g, 14.1 mmol) in Et_2O (15 mL) was dropped into the reaction mixture at the same temperature, and the solution was warmed to room temperature gradually and concentrated in vacuo. Hydrochloric acid (2 N, 30 mL) was added to the residual oil. The mixture was stirred for 30 min at room temperature, neutralized with $NaHCO_3$, and extracted with CH_2Cl_2. The extracts were dried (Na_2SO_4), concentrated in vacuo, and chromatographed on a silica gel column (4% EtOH in $CHCl_3$) to give **30** (2.718 g, 87%): mp 96–99 °C (acetone–*i*-Pr_2O). A mixture of the alcohol **30** (2.718 g, 12.4 mmol), pyridine (13 mL), and $(AcO)_2O$ (1.91 g, 18.6 mmol) was stirred at room temperature for 20 h and concentrated in vacuo. To the residue was added a saturated aqueous $NaHCO_3$ solution, and the solution was extracted with Et_2O. The extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (1% EtOH in $CHCl_3$) to give the acetate **31** (3.08 g, 95%). To a solution of the acetate **31** (3.08 g, 11.8 mmol) in AcOH (15 mL) was added, all at once, Zn dust (0.9 g); after 5 min, the same amount of Zn dust (total 27.7 mg-atoms) was added to the suspension. After the mixture was stirred for 5 min, it was filtered and the filtrate was concentrated in vacuo. A saturated aqueous $NaHCO_3$ solution was added to the residue, and the mixture was extracted with Et_2O. The extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (40% cyclohexane in EtOAc) to yield **32** as yellow crystals (592 mg, 25%): mp 60–61 °C (Et_2O–pentane). A mixture of the aldehyde **32** (106 mg, 0.785 mmol), (ethoxycarbonyl)methylenetriphenylphosphorane (273 mg, 0.785 mmol), and $CHCl_3$ (3 mL) was stirred for 15 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (35% EtOAc in cyclohexane) to give the ester **33** (126 mg, 88%) as a colorless oil. The ethyl ester **33** (117 mg, 0.428 mmol) was hydrolyzed to the title compound **14** (108 mg, 90%) as white crystals under the same conditions as in method A (**17a** to **1a**): mp 169–173 °C (EtOH–Et_2O); IR (KBr) 3050, 3000, 1690, 1618, 1529, 1227, 1196, 1177, 957, 800, 690 cm^{-1}; NMR (Me_2SO-d_6) δ 4.48 (s, 2 H), 6.10 (d, 1 H), 7.07 (d, 1 H), 7.39 (d, 1 H), 7.68 (d, 1 H), 8.06 (dd, 1 H), 8.54 (dt, 1 H), 8.85 (br d, 1 H), 8.93 (br s, 1 H); MS, *m/e* 245 (M^+). Anal. ($C_{13}H_{12}ClNO_2S$) C, H, N.

(*E*)-5-(3-Pyridylmethyl)-2-(2-carboxy-2-propenyl)thiophene Hydrochloride (15): mp 186–191 °C (EtOH–Et_2O); IR (KBr) 2710, 1680, 1612, 1365, 1248, 1197, 1110, 808, 685 cm^{-1}; NMR (Me_2SO-d_6) δ 2.06 (d, 3 H), 4.52 (s, 2 H), 7.14 (d, 1 H), 7.95 (br s, 1 H), 8.06 (dd, 1 H), 8.55 (dt, 1 H), 8.88 (br d, 1 H), 8.99 (d, 1 H); MS, *m/e* 259 (M^+). Anal. ($C_{14}H_{14}ClNO_2S$) C, H, N.

Method G. (*E*)-2-Methyl-3-[4-[1-(3-pyridyl)ethyl]phenyl]acrylic Acid Hydrochloride (11). To a dry THF (50 mL) solution of 3-acetylpyridine (4.5 mL, 41.3 mmol) was added dropwise the Grignard reagent (prepared from 2 g of Mg and 23.36 g of *p*-bromobenzaldehyde diethyl acetal in THF) at 0 °C under a nitrogen atmosphere, and the mixture was stirred for 1 h at room temperature. The reaction was quenched with a saturated aqueous NH_4Cl solution, and the solution was concentrated in vacuo. To the residue was added a saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with $CHCl_3$. The extracts were washed with H_2O and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30% cyclohexane in EtOAc) to yield 4-[1-(3-pyridyl)-1-hydroxyethyl]benzaldehyde diethyl acetal (7.05 g, 56%). To the acetal was added 1 N HCl (40 mL). The mixture was stirred for 30 min and washed with Et_2O. The aqueous layer was basified with $NaHCO_3$ and extracted with $CHCl_3$. The extracts were dried (Na_2SO_4) and concentrated in vacuo, and the residue was chromatographed on a silica gel column (50% EtOAc in cyclohexane) to yield **34** as white crystals (4.67 g, 88%): mp 107–110 °C (EtOAc–*n*-hexane). A mixture of the aldehyde **34** (2.462 g, 10.85 mmol), $SOCl_2$ (1.56 mL, 21.69 mmol), and benzene (10 mL) was stirred at 70–80 °C for 1.5 h and concentrated in vacuo. To the residue was added morpholine (3.4 mL), and the mixture was stirred at 70–80 °C for 3 h. After cooling, the reaction mixture was poured into cold 6 N HCl, basified with $NaHCO_3$, and extracted with $CHCl_3$. The extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (50% EtOAc in cyclohexane) to give **35** (1.225 g, 54%). The aldehyde **35** (583 mg, 2.8 mmol) was hydrogenated with 5% Pd/C (200 mg) in EtOH (6 mL), and the product was purified by column chromatography on silica gel (35% cyclohexane in EtOAc) to afford the aldehyde **36** (337 mg, 57%). The aldehyde **36** (337 mg, 1.6 mmol) was treated with (ethoxycarbonyl)methylene triphenylphosphorane (1.16 g, 3.2 mmol) in $CHCl_3$ (3 mL) at room temperature for 1 h, and the mixture was chromatographed on a silica gel column (35% cyclohexane in EtOAc) to give the ester **37** (480 mg, 100%) as a colorless oil. The ester **37** (150 mg, 0.51 mmol) was hydrolyzed to the title compound **11** (126 mg, 82%) as white crystals under the same conditions as in method A: mp 165–167 °C (EtOH–Et_2O); IR (KBr) 3200–2700, 1700, 1535, 1455, 1380, 1250, 1130, 830, 690 cm^{-1}; NMR (D_2O) δ 1.81 (d, 3 H), 1.88 (s, 3 H), 4.20–4.60 (m, 1 H), 6.90–7.50 (m, 5 H), 7.60–8.50 (m, 2 H), 8.50–8.80 (m, 2 H); MS, *m/e* 267 (M^+). Anal. ($C_{17}H_{18}ClNO_2$) C, H, N.

Method H. (*E*)-3-[4-(3-Pyridylmethyl)-3-methylphenyl]-2-methylacrylic Acid Hydrochloride (10). To a solution of *m*-acetotoluide (75 g, 0.50 mol) in AcOH (400 mL) was added dropwise Br_2 (25.5 mL, 0.508 mol) at 50–55 °C, and the solution was stirred for 30 min. After cooling, the reaction mixture was poured into cold H_2O (2.5 L) containing $NaHCO_3$ (6.25 g). The formed precipitate was filtered and washed with H_2O. The product was added to EtOH (125 mL). To the mixture was added concentrated HCl (125 mL) under reflux, and the mixture was stirred for 3 h. The white precipitate was filtered and washed with EtOH. To a suspension of the precipitate in H_2O (200 mL) was added aqueous NaOH (28 g in 140 mL). The white precipitate was filtered and dried to give the bromide **38** (71 g, 76%). To a suspension of CuCl (17 g, 0.17 mol) in H_2O (100 mL) was added NaCN (21 g, 0.42 mol), followed by toluene (50 mL). To a mixture

of **38** (25 g, 0.134 mol), H_2O (210 mL), and concentrated HCl (27 mL) was added $NaNO_2$ (9.8 g, 0.142 mol) in H_2O (27 mL) at 0–5 °C. After completion of dropping, the reaction mixture was neutralized with Na_2CO_3. The mixture was added to the suspension of CuCN obtained above at 0–5 °C. The resulting mixture was stirred at room temperature for 2 h and warmed to 50 °C. The organic layer was separated after cooling, dried (Na_2SO_4), and sistilled to yield the cyanide **39** (6.51 g, 23%): bp 78–82 °C (0.3 mmHg). To a mixture of **39** (6.4 g, 33 mmol), toluene (60 mL), and CH_2Cl_2 (10 mL) was added DIBAL (25% in toluene, 28 mL, 50 mmol) at −50 to −60 °C, and the solution was stirred for 30 min at the same temperature. The reaction mixture was warmed to room temperature and stirred for 2 h. To the mixture was added MeOH (10 mL) at 0–10 °C, and the solution was stirred for 30 min. The white precipitate was filtered off. The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (50% $CHCl_3$ in cyclohexane) to give the aldehyde **40** (5.34 g, 82%). A mixture of the aldehyde **40** (5.3 g, 26.6 mmol), $CH(OEt)_3$ (7.1 g, 48 mmol), EtOH (7 mL), and *p*-TsOH (0.2 g) was refluxed for 1 h. After the mixture was cooled, Et_3N (1 mL) was added to the mixture, which was concentrated in vacuo and distilled to give the acetal **41** (5.7 g, 80%): bp 102–104 °C (0.3 mmHg). To a solution of **41** (1.88 g, 6.9 mmol) in Et_2O (20 mL) was added *n*-BuLi (1.4 M in hexane, 5 mL, 6.9 mmol) at −70 °C. The mixture was warmed to 0 °C gradually and stirred for 1 h at the same temperature. Nicotinaldehyde (0.89 g, 7.5 mmol) was added to the mixture at −30 °C, and the resulting mixture was warmed to room temperature within 1 h. The solution was concentrated in vacuo. The residual oil was treated with 2 N HCl (10 mL) in THF (10 mL) at room temperature for 1 h. The mixture was basified with $NaHCO_3$ and extracted with Et_2O. The extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed on a silica gel column (35% EtOAc in cyclohexane) to yield **42** (1.20 g, 77%) as white crystals: mp 97.5–99 °C (EtOAc–*i*-Pr_2O). A mixture of the aldehyde **42** (405 mg, 1.78 mmol), (ethoxycarbonyl)methylenetriphenylphosphorane (0.91 g, 2.5 mmol) and $CHCl_3$ (9 mL) was stirred at room temperature for 18 h. The mixture was purified by column chromatography on silica gel (3% EtOH in $CHCl_3$) to afford **43** (520 mg, 94%) as a colorless oil. A mixture of **43** (520 mg, 1.67 mmol) and $SOCl_2$ (2 mL, 28 mmol) was stirred at 60 °C for 1 h and concentrated in vacuo. The residue was basified with $NaHCO_3$ and extracted with Et_2O. The extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. To a solution of the residue in AcOH (4 mL) was added Zn dust (220 mg, 3.34 mg-atoms) at room temperature. The mixture was stirred for 2 h and concentrated in vacuo. The residue was purified by column chromatography on silica gel (35% cyclohexane in EtOAc) to give **44** (404 mg, 82%) as a pale yellow oil. The ester **44** (165 mg, 0.56 mmol) was treated with a mixture of 2 N NaOH (0.5 mL) and EtOH (2 mL) to give the title compound **10** (150 mg, 88%) as white crystals: mp 153–157 °C (EtOH–Et_2O; IR (KBr) 3420, 3050, 2760, 2710, 1690, 1630, 1550, 1394, 1370, 1210, 1120, 833, 690 cm^{-1}; NMR (D_2O) δ 2.02 (d, 3 H), 2.37 (s, 3 H), 4.43 (s, 2 H), 7.10–7.60 (m, 4 H), 8.23 (dd, 1 H), 8.55 (br d, 1 H), 8.89 (br s, 1 H), 8.99 (br d, 1 H); MS, *m/e* 267 (M^+). Anal. ($C_{17}H_{18}ClNO_2$) C, H, N.

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Inhibition of Inosinic Acid Dehydrogenase by 8-Substituted Purine Nucleotides[1]

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A series of 8-substituted derivatives of adenosine monophosphate (AMP) and inosine monophosphate (IMP) were synthesized and examined for their ability to inhibit *Escherichia coli* IMP dehydrogenase. All compounds studied were competitive inhibitors in IMP-dependent competition studies and lacked substrate activity. In oxidized nicotinamide adenine dinucleotide dependent studies, 8-(*p*-NO_2PhCH_2S)-IMP was noncompetitive and 8-(*p*-NO_2PhCH_2S)-AMP showed mixed inhibition. Multiple regression analysis showed that for the series of 8-(para-substituted-benzylthio)-AMPs and -IMPs, the electron-withdrawing ability of the para substituent on the benzylthio moiety correlated best with log K_i of the analogues.

The first of two reactions in the biochemical conversion of IMP to GMP is catalyzed by IMP dehydrogenase (EC 1.2.1.14, IMP-DH), which converts IMP to XMP. This enzyme is of vital importance to rapidly growing cells; the levels of IMP-DH in a series of rat hepatomas have been found to be markedly elevated over normal rat liver levels.[2] Furthermore, many inhibitors of IMP-DH have been shown to have anticancer activity; these include mycophenolic acid,[3] the ribonucleotides of 6-chloropurine and 6-mercaptopurine,[4–6] and a nucleotide derived from 2-amino-1,3,4-thiadiazole.[7] Studies carried out with IMP-DH from various sources indicate that removal of an OH from the phosphate is detrimental to binding,[8] the 5′-oxygen can be replaced by S or NH,[9] and a number of analogues of IMP with modifications in the heterocyclic ring bound somewhat less tightly than IMP itself.[10] Other nucleotide analogues found to bind better than IMP have been 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate[11] and 3-deaza-GMP.[12] We report here the effect of substitution of arylthio, aralkylthio, and alkylthio

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