Studies on Nilvadipine. III. Syntheses of Metabolites of Nilvadipine and Their Related Compounds

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Nilvadipine (I), isopropyl 2-cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate, has a unique 1,4-dihydropyridine structure in that the substituents at all five positions of the nucleus differ from one another. It is an exellent calcium antagonist drug in terms of its potency, its duration of action and its selectivity in the blood vascular system.

During the development of I, some metabolites were isolated from the urine and bile of both rats and dogs after oral administration. With data obtained from the metabolism of known 1,4-dihydropyridines at hand, we proposed the synthesis of a series of compounds (1—11) for comparison with the metabolites isolated from I as a method for structure determination. Indeed, of the compounds synthesized five of them (3—7) were found to coincide with the metabolites from both rat and dog urine and bile isolates.

Keywords nilvadipine; 1,4-dihydropyridine; calcium antagonist; metabolite

Introduction

In our previous publications,[1,2] we reported the syntheses of novel 1,4-dihydropyridine derivatives containing a new substituent at the 2-position of the nucleus, and we selected isopropyl 2-cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate, nilvadipine (I), as a candidate compound for further biological evaluation. From the results of the evaluations,[3] I was found to be an excellent calcium antagonist in terms of its potency, its duration of action and its selectivity for blood vessels in comparison to nifedipine (II), diltiazem (III) and verapamil (IV), which are typical of the original calcium antagonist.

The metabolism of known 1,4-dihydropyridine calcium antagonists, II, nicardipine (V), nimodipine (VI) and nitrendipine (VII) had been published[4-7] and they were metabolized *via* the oxidation of the 1,4-dihydropyridine nucleus to the corresponding pyridine ring, the hydrolysis of the ester group to the corresponding carboxylic acid and the hydroxylation of the methyl group neighbouring the carboxylic acid moiety to the hydroxymethyl group.

Nilvadipine (I) has a unique 1,4-dihydropyridine structure which contains a cyano group at the 2-position, and the substituents at all five positions of the nucleus differ from one another. Since the metabolic pathways of I and the structures, activities and toxicity of metabolites isolated from the urine and bile of both rats and dogs had not yet been determined, it was necessary to identify such pathways and structures *via* synthetic methods.

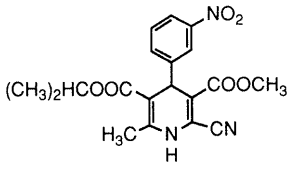
First of all, in order to determine the structures of the metabolites of I, while refering to the data on the metabolism of the known 1,4-dihydropyridines described above, we proposed the synthesis of eleven compounds (**1**—**11**) as shown in Chart 1 for comparison studies.

The five synthesized compounds were found to coincide with the metabolites isolated from the urine and bile of both rats and dogs. Thus, one was able to assume possible metabolic pathways for I and to evaluate the metabolites' biological activities.

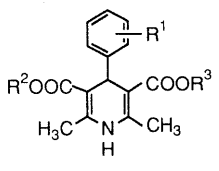
In the present paper we describe the syntheses of metabolites of I and their related compounds which allowed us to identify the metabolites isolated from the urine and bile of both rats and dogs after oral administration.

Results and Discussion

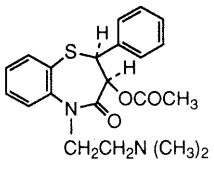
Compound **1**, which is a pyridine derivative oxidized from the corresponding 1,4-dihydropyridine nucleus of I, was obtained easily by treatment of I with diluted aqueous nitric acid and glacial acetic acid at ambient



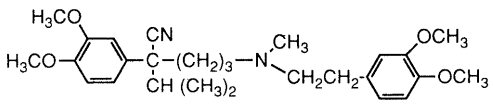
nilvadipine (I)



nifedipine (II) : R^1=2-NO_2, R^2=CH_3, R^3=CH_3
nicardipine (V) : R^1=3-NO_2, R^2=CH_3, R^3=$CH_2CH_2N(CH_3)CH_2C_6H_5$
nimodipine (VI) : R^1=3-NO_2, R^2=$CH(CH_3)_2$, R^3=$CH_2CH_2OCH_3$
nitrendipine (VII) : R^1=3-NO_2, R^2=CH_2CH_3, R^3=CH_3



diltiazem (III)



verapamil (IV)

Fig. 1

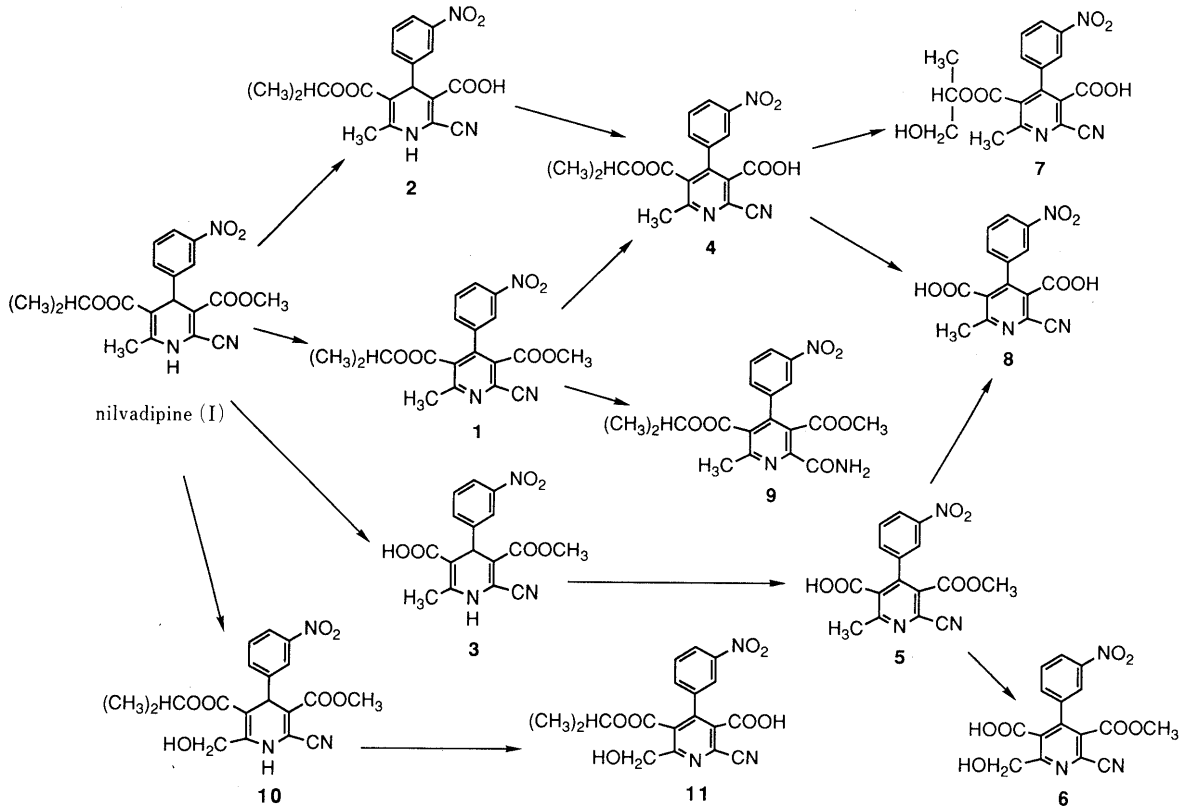


Chart 1. Estimated Metabolites of Nilvadipine (I)

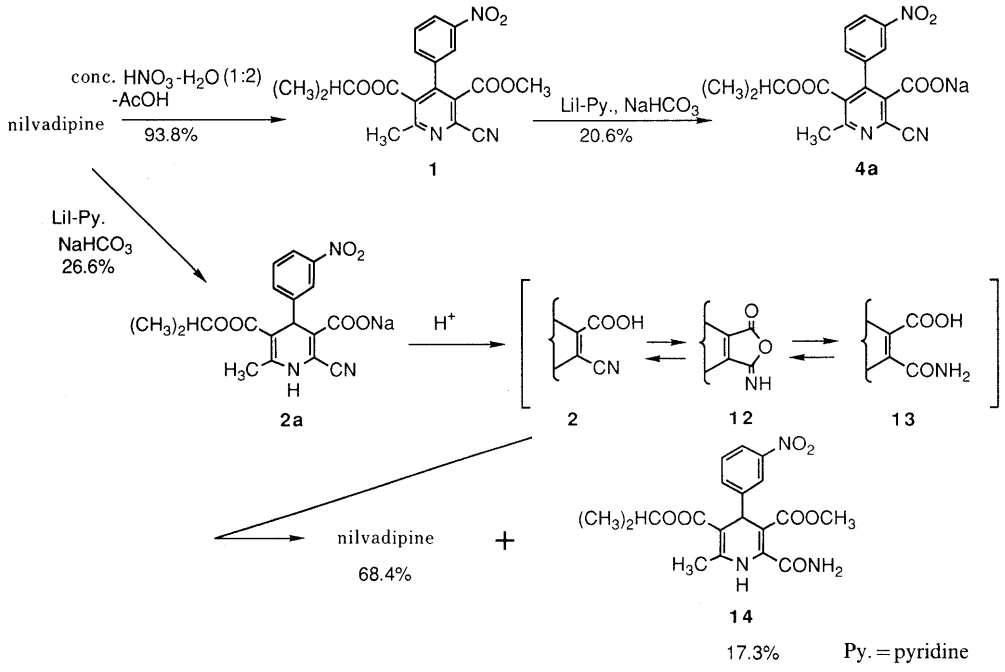


Chart 2

temperature in 93.8% yield.

Compound **2**, which is a hydrolized-form of the methyl ester at the 3-position next to the cyano group of I, was impossible to isolate as the free acid form owing to its instability in acidic media. Nevertheless, the sodium salt (**2a**) could be isolated by cleavage of the methyl ester of I with lithium iodide in pyridine, followed by the formation of sodium salt with sodium bicarbonate as shown in Chart 2. When the isolated sodium salt (**2a**) was treated with diazomethane immediately after acidification with dilute hydrochloric acid, I and **14** were obtained in 68.4% and 17.3% yields respectively. It is considered that **14** was obtained *via* the iminolactone derivative (**12**), followed by ring-opening to form the 2-carbamide carboxylic acid (**13**) and subsequent esterification with diazomethane, as shown in Chart 2. All compounds whose methyl ester

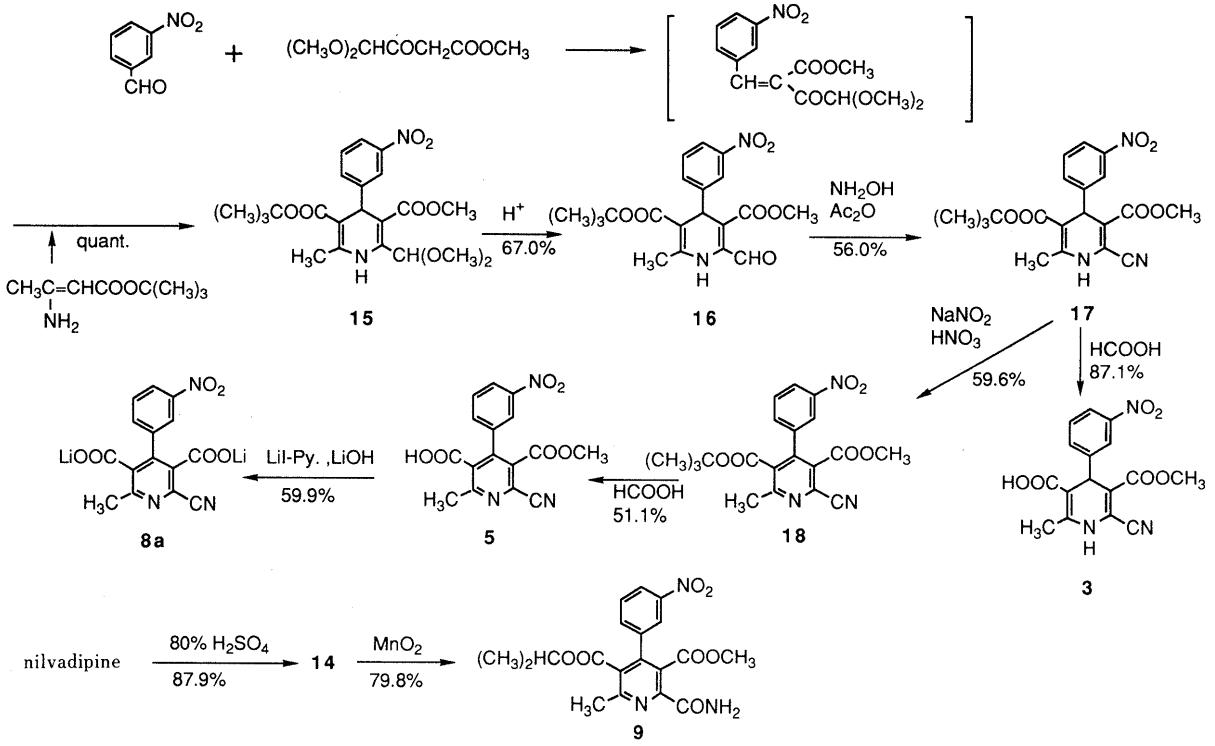


Chart 3

the 3-position neighbouring the 2-cyano group were hydrolized were isolated as metal salts in the following manner.

Compound **4**, an oxidized-form of **2** or a hydrolized-form at the methyl ester of **1**, was prepared by treatment of **1** with lithium iodide in pyridine, followed by sodium bicarbonate in a similar manner to that of **2a** in 20.6% yield as a sodium salt (**4a**).

Compound **3**, which is a hydrolized-form of the isopropyl ester group at the 5-position of I, was prepared by treatment of the *tert*-butyl ester analogue of I (**17**), obtained *via* **15** and **16** according to similar methods described in previous papers,[1,2] with formic acid in 87.1% yield. In addition, **15** was prepared by the modified Hantzsch reaction using *m*-nitrobenzaldehyde, methyl 4,4-dimethoxyacetoacetate and *tert*-butyl 3-aminocrotonate in quantitative yield.

Compound **5**, an oxidized-form of **3**, was obtained *via* **18** in a similar manner to that of **3** in 51.5% yield. Compound **18** was prepared in 59.6% yield from **17** by oxidation with nitric acid and sodium nitrite as shown in Chart 3.

Compound **8** was obtained as a dilithium salt (**8a**) by treating **5** with lithium iodide in pyridine, followed by lithium hydroxide in 59.9% yield.

Compound **9**, which was assumed to be one of the metabolites possessing both an oxidized pyridine ring and a carbamido group hydrolized of a cyano group, was prepared by hydrolysis of the cyano group of I with 80% aqueous sulfuric acid to **14** in 87.9% yield, followed by oxidation with manganese dioxide in 79.8% yield (Chart 3).

In order to prepare some of the assumed metabolitic structures *i.e.* (**6**, **10**, and **11**) which possess a hydroxylated methyl group at the 6-position of the nucleus, we introduced an acetoxymethyl group at the 6-position to afford **19** according to similar methods described above on **17** in Chart 3 or reported in previous papers.[1,2] Namely, the 1,4-dihydropyridine nucleus containing the acetoxymethyl substituent at the 6-position was constructed by the modified Hantzsch method[8] by using *m*-nitrobenzaldehyde, isopropyl 4-acetoxyacetoacetate, prepared from acetoxyacetyl chloride and malonic acid accetonide followed by treatment with isopropyl alcohol, and methyl 3-amino-4,4-dimethoxycrotonate. The dimethoxymethyl group at the 2-position was deprotected with formic acid to give a 2-formyl derivative, which was converted to a 2-cyano derivative (**19**) by reacting with hydroxylamine, followed by treatment with acetic anhydride.

Compound **10**, which is a hydroxylated form at the methyl group of I, was easily prepared by treatment of **19** with 28% aqueous ammonia at 0—5 °C in a methanol/chloroform mixed solvent system in 95.4% yield. Compound **10** was found to be easily cyclized to form lactone (**20**) in 91.7% yield by refluxing with *p*-toluenesulfonic acid in methanol. It was also found that **21**, which is the methyl ester of the presumed metabolite **11**, could be formed by the oxidation of **10** with nitric acid in 39.3% yield, accompanied by a lactone (**22**) in 38.8% yield. The oxidized lactone (**22**) was obtained from the isolated **21** by treatment with a catalytic amount of *p*-toluenesulfonic acid in 81.9% yield (Chart 4).

One of the presumed metabolites **6** was obtained from **22** as a sodium salt by hydrolysis with sodium bicarbonate in methanol in 74.6% yield.

On the other hand, **19** could be oxidized quantitatively with manganese dioxide to form **24**, which was also prepared by a rearangement reaction of pyridine-*N*-oxide (**23**) with acetic anhydride. Compound **23** was obtained from **1** by treatment with hydrogen peroxide in glacial

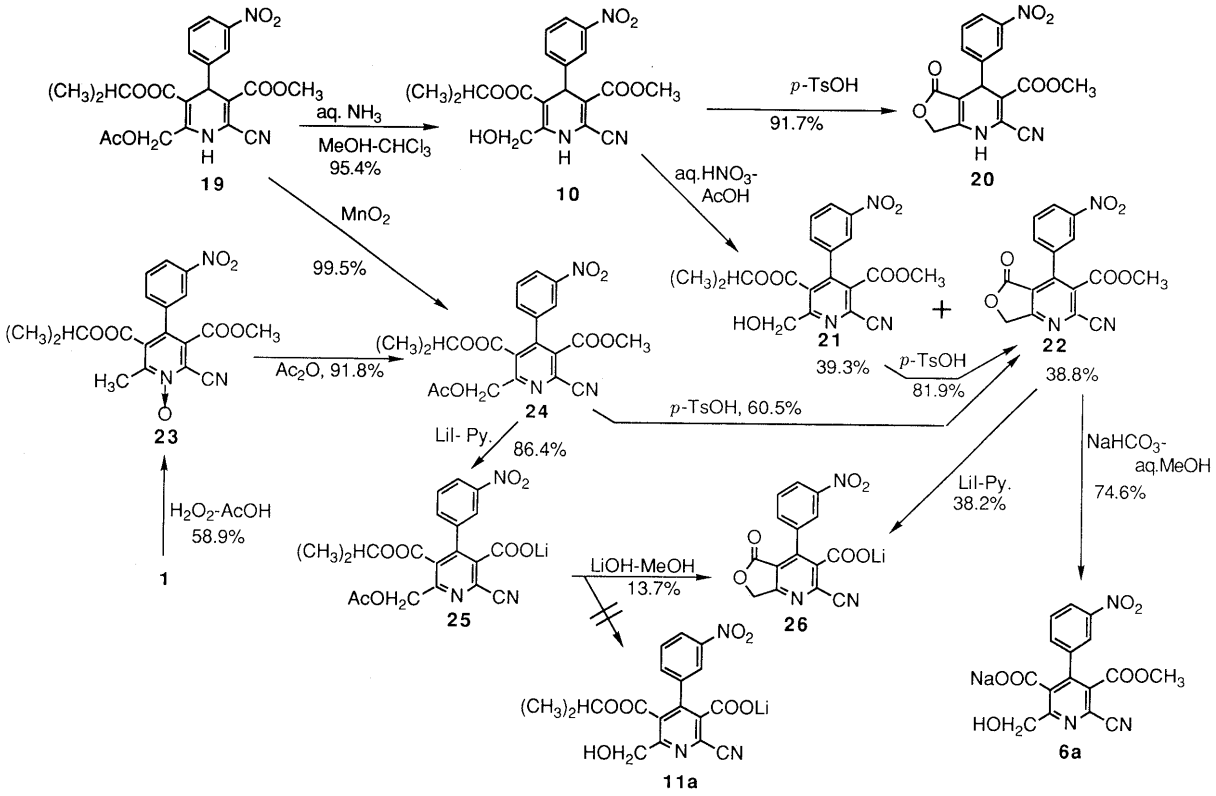


Chart 4

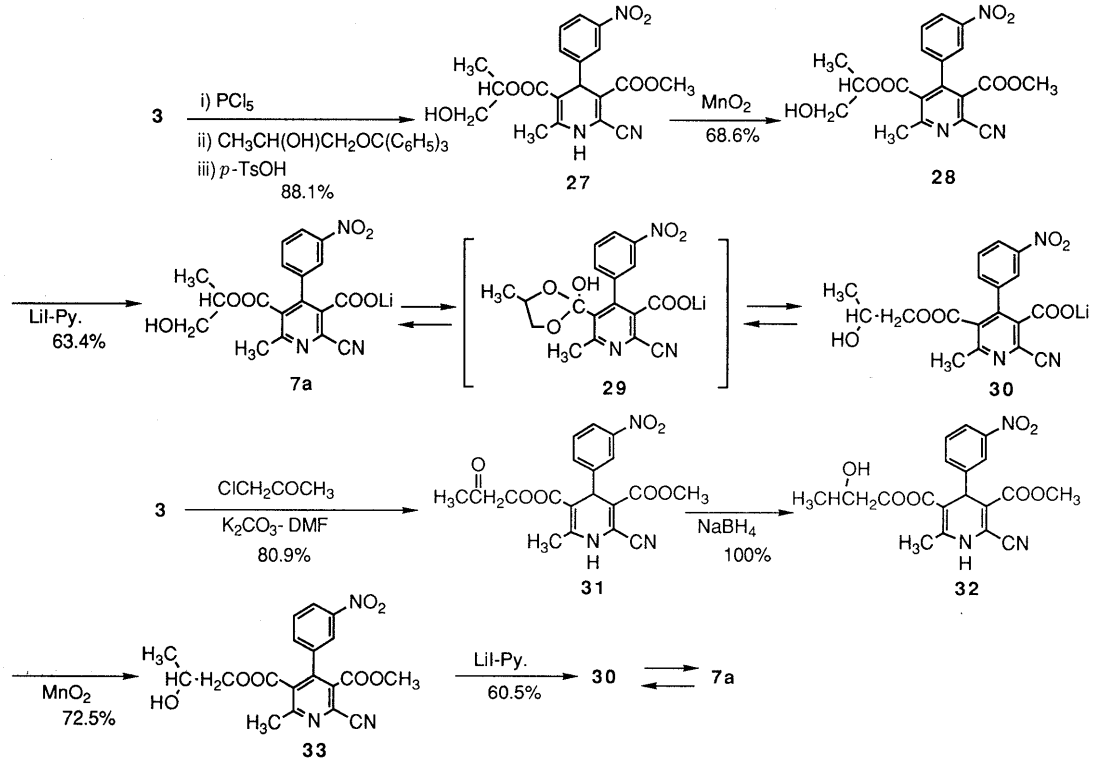


Chart 5

acetic acid in 58.9% yield.

In order to obtain one of the presumed metabolites **11** as a lithium salt (**11a**), **25** was prepared from **24** by treatment with lithium iodide in pyridine in 86.4% yield. Hydrolysis of **25** under mild conditions with lithium hydroxide resulted in failure. The only isolated product was found to be a lithium salt of lactonized-3-carboxylic acid (**26**), which was also obtained from **22** by treatment with lithium iodide in pyridine in 38.2% yield.

Compound **7**, in which one of the methyls of the isopropyl ester function is replaced by a hydroxymethyl and in which the methyl ester at the 3-position of the

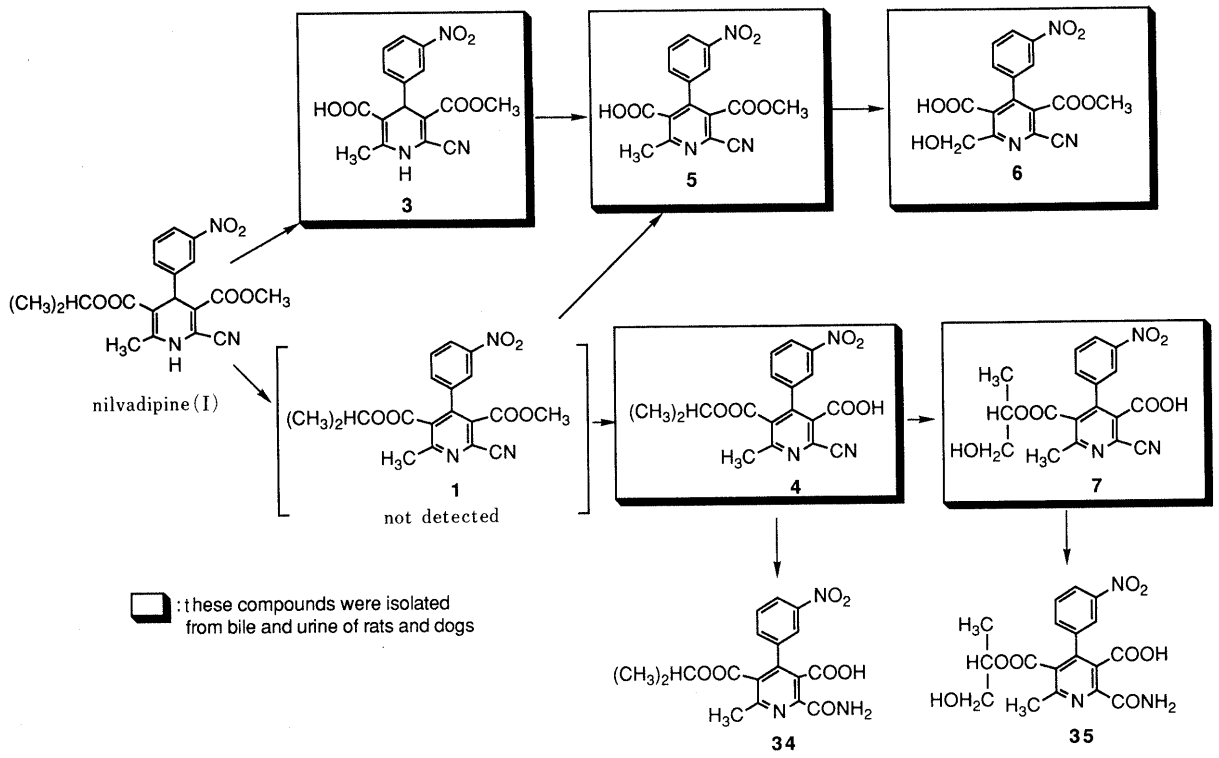


Chart 6. Possible Metabolic Pathways of Nilvadipine in Rats and Dogs

nucleus of I is hydrolized, was hoped to be obtained from **3**, which was prepared from **17** as shown in Chart 3. Compound **3** was activated with phosphorus pentachloride followed by esterification with 1-triphenylmethyloxy-2-propanol. The triphenylmethyl protective group was removed with *p*-toluenesulfonic acid in methanol to afford **27** in 88.1% yield. Compound **27** was oxidized with manganese dioxide to afford **28**. The methyl ester of **28** was cleaved with lithium iodide in pyridine to give the object **7** as a lithium salt (**7a**), as shown in Chart 6. However, it was found by high performance liquid chromatography (HPLC) measurement that **7a** was a mixture of two components. The ratio of the two components was found to be 8:2 immediately after dissolution, but this ratio was found to change after a lapse of several hours. From this finding it could be considered that **7a** was converted to isomer **30** by an ester-exchange reaction *via* intermediate **29** since **7a** is a monoester of propyleneglycol as shown in Chart 5. Isomer **30** was prepared in another process, *i.e.*; (i) esterification of **3** with chloroacetone to afford **31** in 80.9% yield, (ii) reduction with sodium borohydride to give **32** in quantitative yield, (iii) oxidation with manganese dioxide to **33** in 72.5% yield and (iv) cleavage of methyl ester with lithium iodide in pyridine to provide **30** in 60.5% yield as shown in Chart 6. The synthesized **30** was also found to be a mixture of two components by HPLC measurement. The ratio of **30** and **7a** was about 9:1 immediately after dissolution. Thus, pure **7a** or **30** could not be isolated, even though the intermediates for **7a** or **30** were found to contain no contamination from their isomer forms by nuclear magnetic resonance (NMR) spectroscopy.

Conclusion

Of all the compounds prepared in this paper, five compounds were found to coincide with the metabolites isolated from bile and urine of both rats and dogs. Namely, **3** and **5** in Chart 3 and the free acid forms of **4a** in Chart 2, **6a** in Chart 4 and **7a** in Chart 5 were proposed to be intermediates in the possible metabolic pathway of I on the bases of metabolites isolated by Noguchi[9] as shown in Chart 6. The compounds **34** and **35**, proposed in Chart 6, were thought to be artifacts from **4** and **7** respectively in the process of handling, as described above in Chart 2.

Compounds **3**, **5**, **4a** **6a** and **7a** were evaluated for their biological activities but they were found to possess little activity with regard to hypotensive effect in normotensive rats after oral administration.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a JNN-PMX NMR spectrometer using tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on either a Hitachi 260-10 spectrophotometer or a Shimadzu IR-420 spectrophotometer. Mass spectra (MS) were recorded on a Hitachi M-80 mass spectrometer. Column chromatography was performed on silica gel (Merck Kieselgel 60, 230—400 mesh).

Isopropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-5-carboxylate (1) To a suspension of I (1.49 g) in a mixture of conc. HNO_3 and H_2O (1:2, 10 ml) was added AcOH (10 ml) under stirring at ambient temperature. After 3 h stirring at room temperature, H_2O was added to the reaction mixture and extracted with AcOEt. The organic extract was washed with an aqueous solution of $NaHCO_3$ and H_2O in turn and dried over $MgSO_4$. Removal of the solvent *in vacuo* afforded the title compound as yellow crystals (1.39 g, 93.8%). An analytical sample was obtained by recrystallization from a mixture of (iso-Pro)$_2$O and Et_2O, mp 91—93 °C. *Anal.* Calcd for $C_{19}H_{17}N_3O_6$: C, 59.52; H, 4.47; N, 10.96. Found: C, 59.53; H, 4.43; N, 11.01. IR (Nujol) cm^{-1}: 2230 (CN). NMR ($CDCl_3$) δ: 1.05 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 1.12 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 2.68 (3H, s, C_6-CH_3), 3.74 (3H, s, $COOCH_3$), 4.99 (1H, septet, J=6.5 Hz, $C\underline{H}(CH_3)_2$), 7.5—8.5 (4H, m, aromatic protons).

Sodium 2-Cyano-5-isopropoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (2a) A mixture of I (5.77 g, 15 mmol) and LiI (6.03 g, 45 mmol) in dry pyridine (30 ml) was refluxed under a nitrogen atmosphere for 6 h. Pyridine was removed *in vacuo* and to the residue were added an aqueous solution of $NaHCO_3$ and Et_2O with vigorous stirring. The separated aqueous layer was washed with Et_2O and extracted with *n*-BuOH. After washing with H_2O, *n*-BuOH was removed *in vacuo*. The residue was triturated in Et_2O and the obtained precipitates were collected by filtration and dried. The obtained powder was dissolved in CH_2Cl_2 and to the solution was added Et_2O. The resultant precipitate was collected by filtration, recrystallized from a mixture of CH_2Cl_2 and Et_2O and dried to afford the title compound, (1.57 g, 26.6%). mp 159—161 °C (dec.). *Anal.* Calcd for $C_{18}H_{16}N_3NaO_6 \cdot 2/3H_2O$: C, 53.33; H, 4.31; N, 10.36. Found: C, 53.47; H, 4.41; N, 9.73. IR (Nujol) cm^{-1}: 2240 (CN). NMR (DMSO-d_6) δ: 1.01 (3H, d, $J=6$ Hz, $(CH_3)_2CH$), 1.18 (3H, d, $J=6$ Hz, $(CH_3)_2CH$), 2.31 (3H, s, C_6-CH_3), 4.79 (1H, septet, $J=6$ Hz, $(CH_3)_2CH$), 5.20 (1H, s, C_4-H), 7.3—8.2 (4H, m, aromatic protons), 9.30 (1H, m, NH).

Isopropyl 2-Carbamoyl-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (14) To a suspension of **2a** (0.197 g, 0.5 mmol) in AcOEt (5 ml) was added 5% aqueous HCl (5 ml). The mixture was shaken and the separated organic layer was washed with H_2O. To the AcOEt solution was added dropwise an ethereal solution of CH_2N_2. The resultant solution was evaporated *in vacuo* and the residue was subjected to gradient column chromatography on silica gel with $CHCl_3$, $CHCl_3$: MeOH = 100 : 1 and $CHCl_3$: MeOH = 50 : 1 as eluents. After the fractions containing I (132 mg, 68.4%) were eluted, the fractions containing the titled compound were combined and evaporated *in vacuo* to afford yellow crystals (35 mg, 17.3%). Recrystallization from aqueous MeOH afforded an analytically pure sample of the title compound, mp 182—183.5 °C. *Anal.* Calcd for $C_{19}H_{21}N_3O_7$: C, 56.57; H, 5.25; N, 10.42. Found: C, 56.96; H, 5.20; N, 10.48. MS *m/z*: 402 (M^+). IR (Nujol) cm^{-1}: 3350, 3280, 3230 (sh), 3100, 1690 (sh), 1680 (sh), 1670 (sh), 1660, 1625. NMR ($CDCl_3$) δ: 1.26 (6H, d, $J=6$ Hz, $(CH_3)_2CH$), 2.43 (3H, s, C_6-CH_3), 3.78 (3H, s, $COOCH_3$), 5.01 (1H, septet, $J=6$ Hz, $CH(CH_3)_2$), 5.31 (1H, s, C_4-H), 6.41 (1H, br s, NH), 7.2—8.25 (4H, m, aromatic protons), 10.1 (2H, br s, NH_2).

Compound **14** was also prepared from I as follows: I (11.55 g, 30 mmol) was gradually added to an 80% aqueous solution of H_2SO_4 (120 ml) over a period of 5 min with stirring and ice-bath cooling. After addition was complete, the ice-bath was removed and the mixture was stirred for an additional 10 min. The reaction mixture was poured into crushed ice and extracted with AcOEt. The extract was washed with H_2O and dried over $MgSO_4$. Removal of the solvent afforded a crystalline mass, which was washed with AcOEt and collected by filtration to give semicrude **14** (10.63 g, 87.9%). Recrystallization from aqueous MeOH gave pure **14**, mp 182—183.5 °C, which was analytically identical with the sample obtained by the previously described method.

Sodium 2-Cyano-5-isopropoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-3-carboxylate (4a) A mixture of **1** (1.12 g, 2.9 mmol) and LiI (1.28 g, 9.6 mmol) in dried pyridine (10 ml) was refluxed under a nitrogen atmosphere for 5 h. The work-up procedure was similar to that of **2a** described before and afforded the title compound **4a** as yellow crystals (0.26 g, 20.6%). mp 136 °C (recrystallization from a mixture of CH_3COCH_3 and AcOEt). *Anal.* Calcd for $C_{18}H_{14}N_3NaO_6 \cdot 2.5H_2O$: C, 49.55; H, 4.39; N, 9.63. Found: C, 49.38; H, 4.42; N, 9.71. IR (Nujol): 3500, 2245 (CN), 1720 (COOR), 1705 (sh, COO^-). NMR (DMSO-d_6) δ: 0.91 (6H, d, $J=6$ Hz, $CH(CH_3)_2$), 2.48 (3H, s, C_6-CH_3), 4.87 (1H, septet, $J=6$ Hz, $CH(CH_3)_2$), 7.6—8.4 (4H, m, aromatic protons).

***tert*-Butyl 2-Dimethoxymethyl-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (15)** A mixture of methyl 4,4-dimethoxy-2-(3-nitrobenzylidene)-acetoacetate (9.40 g), prepared from *m*-nitrobenzaldehyde and methyl 4,4-dimethoxyacetoacetate in the presence of a catalytic amount of morpholine and AcOH by the Knoevenagel reaction, and *tert*-butyl 3-aminocrotonate (5.26 g), synthesized from *tert*-butyl acetoacetate and ammonia, was heated at 70 °C for 0.5 h and then at 120 °C for 2.5 h. The reaction mixture was allowed to cool, and then dissolved in AcOEt. The solution was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give the title compound **15** (14.68 g, quant. yield) as an oil, which was used in the following reaction without further purification. IR (Nujol) cm^{-1}: 3345 (NH), 1690 (COOR × 2), 1645 (C=C–N), 1605. NMR ($CDCl_3$) δ: 1.42 (9H, s, $C(CH_3)_3$), 2.36 (3H, s, C_6-CH_3), 3.46, 3.50, 3.69 (each 3H, each s, $COOCH_3$, OCH_3 × 2), 5.13 (1H, s, C_4-H), 6.03 (1H, s, $CH(OR)_2$), 6.79 (1H, br s, NH), 7.35—8.21 (4H, m, aromatic protons).

***tert*-Butyl 2-Formyl-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (16)** To a solution of **15** (12.93 g) in acetone (130 ml) was added 6 N HCl (12.9 ml) and the resultant mixture was stirred at ambient temperature for 6 h. Acetone was removed *in vacuo* and the residue was extracted with AcOEt. The extract was washed with H_2O, an aqueous solution of $NaHCO_3$ and brine successively and dried. The solvent was removed *in vacuo* to give **16** (7.77 g, 67.0%), which was recrystallized from MeOH, mp 132.5—134 °C. *Anal.* Calcd for $C_{20}H_{22}N_2O_7$: C, 59.70; H, 5.51; N, 6.96. Found: C, 59.58; H, 5.62; N, 7.02. IR (Nujol) cm^{-1}: 3350 (NH), 1690, 1680 (COOR, CHO), 1635 (C=C–N). 1600. NMR ($CDCl_3$) δ: 1.42 (9H, s, $C(CH_3)_3$), 2.43 (3H, s, C_6-CH_3), 3.79 (3H, s, $COOCH_3$), 5.22 (1H, s, C_4-H), 6.98 (1H, br s, NH), 7.35—8.2 (4H, m, aromatic protons), 10.47 (1H, s, CHO).

***tert*-Butyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (17)** To a suspension of **16** (120.8 g) in AcOH (470 ml) were added $NH_2OH \cdot HCl$ (25 g) and NaOAc (36.9 g) under stirring at ambient temperature. The mixture was stirred at the same temperature for 1 h and to the reaction mixture was added Ac_2O (99 ml). The mixture was stirred at ambient temperature for 1 h and at 97—105 °C for an additional 4.5 h. After cooling to 80 °C, H_2O (700 ml) was added dropwise to the mixture and then allowed to cool to room temperature. The resulting precipitates were collected by filtration, washed with H_2O, $(iso\text{-}Pro)_2O$ and cold MeOH successively. Recrystallization from MeOH afforded the pure title compound **17** (67.3 g, 56.0%), mp 168—169 °C. *Anal.* Calcd for $C_{20}H_{21}N_3O_6$: C, 60.14; H, 5.30; N, 10.52. Found: C, 60.13; H, 5.44; N, 10.65. IR (Nujol) cm^{-1}: 3305 (NH), 2250 (CN), 1700 (COOR), 1640 (C=C), 1625. NMR ($CDCl_3$) δ: 1.43 (9H, s, $C(CH_3)_3$), 2.40 (3H, s, C_6-CH_3), 3.77 (3H, s, $COOCH_3$), 5.15 (1H, s, C_4-H), 6.73 (1H, br s, NH), 7.3—8.3 (4H, m, aromatic protons).

2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic Acid (3) A mixture of **17** (7.24 g, 18.1 mmol) in 98% HCOOH (30 ml) was stirred at ambient temperature for 5 h. The resultant precipitates were collected by filtration, washed with CH_2Cl_2 and dried to give **3** (5.42 g, 87.1%), as a yellow powder. An analytical sample was obtained by recrystallization from MeOH, mp 233 °C (dec.). *Anal.* Calcd for $C_{16}H_{13}N_3O_6$: C, 55.98; H, 3.82; N, 12.24. Found: C, 55.70; H, 3.78; N, 12.16. IR (Nujol) cm^{-1}: 3310 (NH), 2255 (CN), 1730 ($COOCH_3$), 1708 (COOH). NMR (DMSO-d_6) δ: 2.35 (3H, s, C_6-CH_3), 3.71 (3H, s, $COOCH_3$), 5.13 (1H, s, C_4-H), 7.55—8.2 (4H, m, aromatic protons), 10.26 (1H, br s, COOH).

***tert*-Butyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-5-carboxylate (18)** To a solution of **17** (12.36 g, 30.9 mmol) in AcOH (25 ml) were added $NaNO_2$ (2.1 g, 30.9 mmol) and conc. HNO_3 (2.5 g) under cooling in an ice-bath. The mixture was stirred for 2 h at ambient temperature. To the reaction mixture was added AcOEt and the solution was washed with H_2O, an aqueous solution of $NaHCO_3$ and brine successively. After drying over $MgSO_4$, the solvent was removed *in vacuo* to give a residue, which was subjected to column chromatography on silica gel with a mixture of C_6H_6 and AcOEt (9 : 1) as eluent to afford pure **18** (7.33 g, 59.6%) as an oil. IR (neat) cm^{-1}: 2220 (CN), 1720 (COOR). NMR (CCl_4) δ: 1.26 (9H, s, $C(CH_3)_3$), 2.66 (3H, s, C_6-CH_3), 3.77 (3H, s, $COOCH_3$), 7.5—8.5 (4H, m, aromatic protons).

2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-5-carboxylic Acid (5) A mixture of **18** (7.00 g, 17.6 mmol) and 98% HCOOH (20 ml) was warmed at 70 °C for 2 h. The reaction mixture was concentrated *in vacuo* and to the residue was added H_2O. The mixture was then made alkaline with an aqueous solution of $NaHCO_3$. The mixture was extracted with AcOEt and washed with H_2O twice. After drying over $MgSO_4$, the solvent was removed *in vacuo* to give semicrude crystals, which were recrystallized from AcOEt to give pure **5** (3.07 g, 51.1%), mp 229.5—231.5 °C (dec.). *Anal.* Calcd for $C_{16}H_{11}N_3O_6$: C, 56.31; N, 3.25; N, 12.31. Found: C, 56.46; H, 3.34; N, 12.42. MS *m/z*: 341 (M^+). IR (Nujol) cm^{-1}: 2250 (CN), 1735 ($COOCH_3$), 1715 (COOH). NMR (DMSO-d_6) δ: 2.65 (3H, s, C_6-CH_3), 3.62 (3H, s, $COOCH_3$), 7.7—8.55 (4H, m, aromatic protons).

Dilithium 2-Cyano-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (8a) A mixture of **5** (2.37 g, 6.94 mmol) and LiI (3.30 g, 24.6 mmol) in dry pyridine (70 ml) was heated at 90—100 °C for 10 h under a nitrogen atmosphere with stirring. After cooling to room temperature, the resultant precipitate was collected by filtration, washed with cold pyridine and dried *in vacuo*. The obtained mass was dissolved in MeOH, and to the solution was added $LiOH \cdot H_2O$ (0.29 g) with stirring and ice-bath cooling. The mixture was filtered off and the filtrate was evaporated *in vacuo* to dryness, which was subjected to column chromatography on

silica gel with a gradient elution using a mixture of $CHCl_3$ and MeOH (5 : 1 to 1 : 1) as eluent. The obtained dilithium salt was recrystallized from MeOH to afford pure **8a** (1.41 g, 59.9%), mp >340 °C. *Anal.* Calcd for $C_{15}H_7N_3O_6 \cdot 2Li$: C, 53.12; H, 2.08; N, 12.39. Found: C, 52.93; H, 2.31; N, 12.26. IR (Nujol) cm^{-1}: 2240 (CN), 1645, 1600. NMR (CD_3OD) δ: 2.59 (3H, s, C_6-CH_3), 7.53 (1H, t, J=8 Hz, Ar-5-H), 7.84 (1H, dd, J=8, 2 Hz, Ar-6-H), 8.18 (1H, dd, J=8, 2 Hz, Ar-4-H), 8.36 (1H, t, J=2 Hz, Ar-2-H).

Isopropyl 2-Carbamoyl-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (9) A mixture of **14** (4.03 g, 10 mmol) and MnO_2 (4.0 g) in $CHCl_3$ (100 ml) was stirred at ambient temperature for 5 h. The reaction mixture was filtered and washed with $CHCl_3$. The filtrate and the washings were combined and evaporated *in vacuo* to give an oil, which was subjected to column chromatography on silica gel with $CHCl_3$ and a mixture of $CHCl_3$: MeOH (100 : 1) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give an oil, which crystallized on standing. Recrystallization from a mixture of CH_2Cl_2 and Et_2O gave pure **9** (3.20 g, 79.8%) as light yellow crystals, mp 156—157.5 °C. *Anal.* Calcd for $C_{19}H_{19}N_3O_7$: C, 56.86; H, 4.77; N, 10.47. Found: C, 57.22; H, 4.84; N, 10.63. MS *m/z*: 401 (M^+). IR (Nujol) cm^{-1}: 3450, 3250 (sh), 3180, 3080 (sh) (NH_2), 1720, 1710 (COOR), 1685 ($CONH_2$), 1520, 1250, 1110. NMR ($CDCl_3$) δ: 1.05 (6H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$, 2.70 (3H, s, C_6-CH_3), 3.69 (3H, s, $COOCH_3$), 4.99 (1H, septet, J=6.5 Hz, $C\underline{H}(CH_3)_2$), 6.32 (1H, br s, NH), 7.4—8.45 (5H, m, aromatic 4 protons and NH).

Isopropyl 6-Acetoxymethyl-2-cyano-3-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (19) To a solution of isopropyl 4-acetoxyacetoacetate (16.80 g, 83.1 mmol), prepared from acetoxyacetyl chloride and malonic acid acetonide and followed by treatment with iso-PrOH, and *m*-nitrobenzaldehyde (12.56 g, 83.1 mmol) in C_6H_6 (100 ml) were added morpholine (0.4 g) and AcOH (0.4 g) successively. The mixture was refluxed under stirring for 1 h. Removal of the solvent *in vacuo* afforded an oil, to which was added methyl 4,4-dimethoxy-3-aminocrotonate (17.42 g, 99.7 mmol). After the mixture was heated at 80 °C for 0.5 h, C_6H_6 (10 ml) and AcOH (1 ml) were added. The mixture was refluxed for 2 h. The resultant reaction mixture was washed successively with H_2O, dil. HCl and dil. $NaHCO_3$. Removal of the solvent gave an oil, which was subjected to column chromatography on silica gel with toluene and toluene–AcOEt (20 : 1 and 10 : 1) successively as eluents to afford pure 2-dimethoxymethyl-6-acetoxymethyl-1,4-dihydropyridine intermediate (32.04 g, 78.3%) as an oil. NMR (CD_3OD) δ: 1.14, 1.27 (each 3H, each d, J=6 Hz, $CH(C\underline{H}_3)_2$), 2.19 (3H, s, CH_3CO), 3.48 (6H, s, $OCH_3 \times 2$), 3.68 (3H, s, $COOCH_3$), 4.99 (1H, septet, J=6 Hz, $C\underline{H}(CH_3)_2$), 5.16 (1H, s, C_4-H), 5.35 (2H, d, J=6.4 Hz, C_6-CH_2O), 6.00 (1H, s, C_2-$CH(OR)_2$), 7.2—8.25 (5H, m, aromatic protons and NH).

A mixture of the oil (32.01 g) obtained above and formic acid (50 ml) was stirred for 5 h at room temperature. The mixture was evaporated *in vacuo* to dryness and subjected to column chromatography on silica gel with a mixture of toluene and AcOEt (20 : 1 to 10 : 1) as eluents. The fractions containing the desired compound were combined and evaporated *in vacuo* to afford the 2-formyl intermediate (26.13 g, 90.0%) as an oil, which crystallized on standing with a small amount of Et_2O and was collected by filtration after washing with *n*-hexane. An analytical sample was obtained by recrystallization from Et_2O, mp 85—86 °C. *Anal.* Calcd for $C_{21}H_{22}N_2O_9$: C, 56.50; H, 4.97; N, 6.28. Found: C, 56.39; H, 5.06; N, 6.23. IR (Nujol) cm^{-1}: 3390 (NH), 1750 (AcO), 1700, 1680 (each COOR, CHO), 1640 (C=C), 1595, 1520. NMR ($CDCl_3$) δ: 1.12, 1.28 (each 3H, each d, J=6 Hz, $CH(C\underline{H}_3)_2$), 2.24 (3H, s, CH_3CO-O), 3.80 (3H, s, $COOCH_3$), 4.99 (1H, septet, J=6 Hz, $C\underline{H}(CH_3)_2$), 5.25 (1H, s, C_4-H), 5.40 (2H, s, C_6-CH_2O), 7.14—8.20 (5H, m, aromatic protons and NH), 10.47 (1H, s, CHO).

To a solution of the 2-formyl derivative (21.11 g, 47.3 mmol), obtained above, in AcOH (80 ml) were added successively $NH_2OH \cdot HCl$ (3.95 g, 56.8 mmol) and AcONa (5.82 g, 71.0 mmol) under stirring at ambient temperature. The stirring was continued for a further 45 min and then Ac_2O (20 ml, 0.312 mol) was added to the reaction mixture. After stirring at the same temperature for 1 h, the resultant mixture was heated on an oil bath at 100—105 °C for 3 h and at 115—120 °C for 4.5 h. The mixture was evaporated *in vacuo* to afford a residue, which was dissolved in $CHCl_3$ and successively washed with H_2O, aqueous $NaHCO_3$ and H_2O. After drying over $MgSO_4$, the solvent was removed *in vacuo* to give a residue, which was subjected to column chromatography on silica gel with a mixture of AcOEt and $CHCl_3$ (1 : 10) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give an oil, which crystallized on standing at ambient temperature (15.39 g, 73.4%). An analytical sample was obtained by recrystallization from AcOEt, mp 165—166.5 °C. *Anal.* Calcd for $C_{21}H_{21}N_3O_8$: C, 56.88; H, 4.77; N, 9.48. Found: C, 56.68; H, 4.82; N, 9.43. MS *m/z*: 443 (M^+). IR (Nujol) cm^{-1}: 3300 (NH), 2220 (CN), 1740 (AcO), 1680 (COOR), 1620 (C=C), 1595, 1510. NMR ($CDCl_3$) δ: 1.11, 1.28 (each 3H, each d, J=6 Hz, $CH(C\underline{H}_3)_2$), 2.25 (3H, s, CH_3CO), 3.80 (3H, s, $COOCH_3$), 4.98 (1H, septet, J=6 Hz, $C\underline{H}(CH_3)_2$), 5.22 (1H, s, C_4-H), 5.35 (2H, s, C_6-CH_2O), 7.23—8.20 (5H, m, aromatic protons, NH).

Isopropyl 2-Cyano-6-hydroxymethyl-3-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (10) A mixture of **19** (2.12 g), conc. aqueous NH_3 (1 ml), MeOH (40 ml) and $CHCl_3$ (20 ml) was stirred under cooling in an ice-bath for 3 h. After removal of the solvent *in vacuo*, the residue was dissolved in AcOEt and washed with brine and H_2O. The extract was dried over $MgSO_4$ and evaporated to dryness. The obtained semicrude crystals were recrystallized from Et_2O to afford pure **10** (1.83 g, 95.4%) as yellow granules, mp 154—155 °C. *Anal.* Calcd for $C_{19}H_{19}N_3O_7$: C, 56.86; H, 4.77; N, 10.47. Found: C, 56.79; H, 4.75; N, 10.44. MS *m/z*: 401 (M^+). IR (Nujol) cm^{-1}: 3480 (OH), 3300 (NH), 2245 (CN), 1685 (COOR), 1640 (C=C), 1605, 1625. NMR ($CDCl_3$) δ: 1.09, 1.29 (each 3H, each d, J=6 Hz, $CH(C\underline{H}_3)_2$), 3.12 (1H, t, J=5.5 Hz, $CH_2O\underline{H}$), 3.80 (3H, s, $COOCH_3$), 4.92 (2H, d, J=5.5 Hz, $C\underline{H}_2OH$), 4.65—5.03 (1H, m, $C\underline{H}(CH_3)_2$), 5.22 (1H, s, C_4-H), 7.27—8.20 (5H, m, aromatic protons, NH).

Methyl 2-Cyano-4-(3-nitrophenyl)-5-oxo-1,4,5,7-tetrahydrofuro[3,4-*b*]-pyridine-3-carboxylate (20) To a solution of **10** (0.50 g, 1.25 mmol) in MeOH (15 ml) was added *p*-TsOH (0.10 g) under stirring at ambient temperature. The mixture was refluxed for 4.5 h. Removal of the solvent afforded a crystalline mass, which was washed with AcOEt and collected by filtration to give semicrude **20** (0.39 g, 91.7%). An analytical sample was obtained by recrystallization from MeOH as fine yellow needles, mp 250—253 °C (dec.). *Anal.* Calcd for $C_{16}H_{11}N_3O_6$: C, 56.31; H, 3.25; N, 12.31. Found: C, 56.44; H, 3.28; N, 12.12. MS *m/z*: 341 (M^+). IR (Nujol) cm^{-1}: 3302 (NH), 3180 (sh), 3100, 2230 (CN), 1746 (lactone), 1695, 1680 (COOR), 1600. NMR (DMSO-d_6) δ: 3.34 (1H, br s, NH), 3.60 (3H, s, $COOCH_3$), 4.92 (2H, dd, J=16, 22 Hz, CH_2 in lactone), 5.04 (1H, s, C_4-H), 7.6—8.15 (4H, m, aromatic protons).

Isopropyl 2-Cyano-6-hydroxymethyl-3-methoxycarbonyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (21) and Methyl 2-Cyano-4-(3-nitrophenyl)-5-oxo-5,7-dihydrofuro[3,4-*b*]pyridine-3-carboxylate (22) To a suspension of **10** (0.64 g, 1.57 mmol) in a mixture of conc. HNO_3 and H_2O (1 : 2, 7 ml) was added AcOH (7 ml) under stirring at ambient temperature. The mixture was warmed to 40 °C to obtain a clear solution for about 5 min. The solution was stirred at ambient temperature for 1.5 h. The reaction mixture was diluted with H_2O, its pH was adjusted to 8.0, and the resultant mixture was extracted with AcOEt. The extract was washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo* to give a yellow oil, which was subjected to column chromatography on silica gel with $CHCl_3$ as eluent. The first eluted compound crystallized on standing and was recrystallized from a mixture of C_6H_6 and MeOH (10 : 3) to give pure **22** (0.21 g, 38.8%), mp 193—195 °C. The fractions containing the second eluted compound were combined and evaporated *in vacuo* to give pure **21** (0.25 g, 39.3%) as an oil.

Compound **21**: MS *m/z*: 399 (M^+). IR (neat) cm^{-1}: 3450 (OH), 3080, 2230 (CN), 1745 (COOR), 1720 (COOR'), 1615. NMR ($CDCl_3$) δ: 1.02 (6H, d, J=6.4 Hz, $CH(C\underline{H}_3)_2$), 3.79 (3H, s, $COOCH_3$), 4.91 (2H, s, C_6-CH_2O), 4.99 (1H, septet, J=6.4 Hz, $C\underline{H}(CH_3)_2$), 7.55—8.4 (4H, m, aromatic protons).

Compound **22**: *Anal.* Calcd for $C_{16}H_9N_3O_6$: C, 56.65; H, 2.67; N, 12.39. Found: C, 56.73; H, 2.46; N, 12.11. MS *m/z*: 339 (M^+). IR (Nujol) cm^{-1}: 2250 (CN), 1750 (lactone), 1730 (COOR). NMR (DMSO-d_6) δ: 3.69 (3H, s, $COOCH_3$), 5.56 (2H, s, C_6-CH_2O), 7.75—8.55 (4H, m, aromatic protons).

Compound **22** was also obtained from isolated **21** as follows: A mixture of **21** (0.25 g, 0.62 mmol) and a catalytic amount of *p*-TsOH in MeOH (10 ml) was refluxed for 2.5 h. The reaction mixture was allowed to cool to ambient temperature and the resultant white crystals were collected by filtration. Recrystallization from a mixture of C_6H_6 and MeOH afforded pure **22** (174 mg, 81.9%), which was identical spectroscopically with the previously prepared sample.

Compound **22** was also prepared from compound **24** as follows: To a solution of **24** (74.7 g, 169.2 mmol) in MeOH (400 ml) was added *p*-TsOH (3.12 g), and the mixture was refluxed under stirring for 15.5 h. After the reaction mixture was cooled, the resultant precipitates were collected by filtration, washed with cold MeOH and dried to give semicrude **22**, which

was subjected to column chromatography on silica gel with a mixture of AcOEt and CH_2Cl_2 (1 : 20) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to afford **22** as crystals. Recrystallization from a mixture of C_6H_6 and MeOH gave an analytically pure sample of **22** (34.74 g, 60.5%), which was identical with the sample obtained above.

2-Cyano-5-isopropoxycarbonyl-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-*N*-oxide (23) To a solution of **1** (183.4 g, 0.478 mol) in AcOH (1500 ml) was added 30% aqueous H_2O_2 (200 ml, *ca.* 3.7 eq mol), and the mixture was stirred with warming at 70 °C for 5 d. 30% aqueous H_2O_2 (200 ml) addition was repeated twice with additional stirring periods at 70 °C of 5 and 3 d respectively. The reaction mixture was neutralized with Na_2CO_3 under cooling in an ice-bath and the mixture was extracted with C_6H_6 twice. The extracts were combined and washed with H_2O and dried. Removal of the solvent afforded an oily residue, which was subjected to column chromatography on silica gel with a mixture of AcOEt and C_6H_6 (5 : 1) as eluent to give pure **23** (112.5 g, 58.9%) as yellow crystals. An analytical sample was obtained by recrystallization from Et_2O, mp 119—121.5 °C. *Anal.* Calcd for $C_{19}H_{17}N_3O_7$: C, 57.14; H, 4.29; N, 10.52. Found: C, 57.08; H, 4.21; N, 10.41. IR (Nujol) cm^{-1}: 2230 (CN), 1730 (COOR). NMR ($CDCl_3$) δ: 1.04 (6H, d, J=6.3 Hz, $CH(C\underline{H}_3)_2$), 2.54 (3H, s, C_6-CH_3), 3.75 (3H, s, $COOCH_3$), 4.97 (1H, septet, J=6.3 Hz, $C\underline{H}(CH_3)_2$), 7.5—8.5 (4H, m, aromatic protons).

Isopropyl 6-Acetoxymethyl-2-cyano-3-methoxycarbonyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (24) To a solution of **19** (3.17 g, 7.15 mmol) in CH_2Cl_2 (40 ml) was added activated MnO_2 (0.70 g). The mixture was stirred for 8 h at ambient temperature. Removal of the insoluble mass by filtration and concentration of the filtrate *in vacuo* gave a residue, which was subjected to column chromatography on silica gel with CH_2Cl_2 and a mixture of CH_2Cl_2 and AcOEt (10 : 1) as eluents. The fractions containing the desired compound were combined and evaporated *in vacuo* to give **24** (3.14 g, 99.5%) as a crystalline mass. An analytical sample was obtained by recrystallization from Et_2O as yellow crystals, mp 87.5—88.5 °C. *Anal.* Calcd for $C_{21}H_{19}N_3O_8$: C, 57.14; H, 4.34; N, 9.52. Found: C, 56.96; H, 4.18; N, 9.48. MS *m/z*: 442 ($M^+ + 1$). IR (Nujol) cm^{-1}: 3080, 2980, 2870, 2245 (CN), 1720 (COOR, $COCH_3$), 1605, 1520. NMR ($CDCl_3$) δ: 1.03 (6H, d, J=7 Hz, $CH(C\underline{H}_3)_2$), 2.13 (3H, s, $COCH_3$), 3.76 (3H, s, $COOCH_3$), 4.97 (1H, septet, J=7 Hz, $C\underline{H}(CH_3)_2$), 5.35 (2H, s, C_6-CH_2O), 7.55—8.45 (4H, m, aromatic protons).

Compound **24** was also obtained from **23** as follows: To a solution of **23** (0.70 g, 1.75 mmol) in Ac_2O (2.5 ml) was added a catalytic amount of *p*-TsOH. The mixture was refluxed for 1 h with stirring. After cooling, Ac_2O was removed *in vacuo* to dryness, and to the residue was added C_6H_6. The mixture was washed with H_2O, an aqueous solution of $NaHCO_3$ and brine successively and dried over $MgSO_4$. Filtration and removal of the solvent afforded an oil, which was subjected to column chromatography on silica gel with a mixture of C_6H_6 and AcOEt (9 : 1) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give pure **24** (0.71 g, 91.8%), which was recrystallized from Et_2O to afford pure crystals, mp 88—88.5 °C. This was found to be identical with the sample obtained above.

Lithium 6-Acetoxymethyl-2-cyano-5-isopropoxycarbonyl-4-(3-nitrophenyl)pyridine-3-carboxylate (25) A mixture of **24** (2.30 g, 5.2 mmol) and LiI (2.4 g, 17.9 mmol) in dry pyridine (40 ml) was stirred under a nitrogen atmosphere at 100 °C for 3 h. The solvent was removed *in vacuo* to dryness to give a residue, which was dissolved in AcOEt and filtered. The filtrate was evaporated *in vacuo* and the obtained residue was subjected to column chromatography on silica gel with a mixture of $CHCl_3$ and MeOH (10 : 1) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to dryness to afford pure **25** (1.95 g, 86.4%) as a powder, which was used in the following reaction without further purification due to its hygroscopicity. MS *m/z*: 427 ($M^+ - Li$). IR (Nujol) cm^{-1}: 2250 (CN). NMR (DMSO-d_6) δ: 0.95 (6H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 2.06 (3H, s, CH_3CO), 4.84 (1H, septet, J=6 Hz, $C\underline{H}(CH_3)_2$), 5.22 (2H, s, C_6-CH_2O), 7.59—8.50 (4H, m, aromatic protons).

Lithium 2-Cyano-4-(3-nitrophenyl)-5-oxo-5,7-dihydrofuro[3,4-*b*]pyridine-3-carboxylate (26) A mixture of **22** (5.35 g, 15.8 mmol) and LiI (5.27 g, 39.4 mmol) in dry pyridine (80 ml) was stirred for 2.5 h at 100 °C under a nitrogen atmosphere. The reaction mixture was evaporated *in vacuo* to give a residue, which was dissolved in AcOEt. An insoluble mass was removed by filtration. The filtrate and the washings were combined and evaporated *in vacuo* to dryness. The residue was subjected to column chromatography on silica gel with a mixture of $CHCl_3$ and MeOH (5 : 2) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give a residue, which was dissolved in acetone and evaporated to dryness. This residue was dissolved in AcOEt and to the solution was added gradually C_6H_6 to give a powder, which was collected by filtration and dried in a desiccator over P_2O_5. As this compound was highly hygroscopic, an elemental analysis could be obtained. IR (Nujol) cm^{-1}: 2245 (CN). NMR (DMSO-d_6) δ: 5.43 (2H, s, C_6-CH_2O), 7.7—8.5 (4H, m, aromatic protons).

Compound **26** was also obtained from **25** as follows; To a solution of **25** (0.77 g) was added a methanolic solution (1 ml) of $LiOH \cdot H_2O$ (35 mg/ml). The mixture was stirred for 3 h under reflux. The work-up procedure was similar to that described above. Yield: 0.1 g, 13.7%.

Sodium 2-Cyano-6-hydroxymethyl-3-methoxycarbonyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (6a) A mixture of **22** (18.29 g, 53.9 mmol), $NaHCO_3$ (4.53 g, 53.9 mmol) in MeOH (180 ml) and H_2O (900 ml) was refluxed at 85—90 °C with stirring for 11 h. After cooling and treatment with active carbon, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in H_2O and washed with CH_2Cl_2. The aqueous layer was saturated with NaCl, made acidic with AcOH and cooled in an ice-bath. The resultant precipitate was collected by filtration, washed with AcOEt and dried to give **6a** (15.24 g, 74.6%). An analytical sample was obtained by recrystallization from MeOH, mp >350 °C. *Anal.* Calcd for $C_{16}H_{10}N_3NaO_7$: C, 50.69; H, 2.92; N, 11.08. Found: C, 50.77; H, 2.72; N, 11.18. MS *m/z*: 357 ($M^+ - Na$). IR (Nujol) cm^{-1}: 3100 (OH), 1745, 1720 (COOR, COONa), 1595, 1530, 1350. (NMR (DMSO-d_6) δ: 3.62 (3H, s, $COOCH_3$), 4.63 (2H, d, J=6 Hz, $C\underline{H}_2OH$), 5.67 (1H, br t, J=6 Hz, OH), 7.84—8.10 (4H, m, aromatic protons).

***β*-Hydroxyisopropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (27)** To a solution of **3** (5.42 g, 15.8 mmol) in $CHCl_3$ (50 ml) was added PCl_5 (4.40 g, 21.0 mmol, 1.33 eq mol), with stirring and cooling in an ice-bath. The mixture was stirred for 1.5 h under the same conditions. To the reaction mixture obtained above was added dropwise a solution of 1,2-propyleneglycol-1-triphenylmethylether (5.89 g, 17.6 mmol) and pyridine (3 ml, 37.2 mmol) in $CHCl_3$ (30 ml) under ice cooling. The mixture was stirred for a further 2.5 h under the same conditions. To the resultant reaction mixture was added a 10% aqueous solution of $NaHCO_3$. The separated $CHCl_3$ layer was washed with an aqueous solution of $NaHCO_3$, dil. HCl and brine successively and dried over $MgSO_4$. Removal of the solvent *in vacuo* afforded a residue, which was subjected to column chromatography on silica gel with a mixture of AcOEt and $CHCl_3$ (1 : 2) as eluent. The fractions containing the desired compound were combined and evaporated to give a pure intermediate protected with a triphenylmethyl ether at the *β*-hydroxyisopropyl group (10.1 g, 99.3%) as a yellow viscous oil. IR (neat) cm^{-1}: 2230 (CN). NMR ($CDCl_3$) δ: 1.06, 1.25 (3H, each d, J=7 Hz, $CHCl_3$), 2.37 (3H, s, C_6-CH_3), 2.95—3.25 (2H, br m, $CHC\underline{H}_2O$), 3.68, 3.74 (3H, each s, $COOCH_3$), 4.87—5.36 (2H, m, C_4-H and COOCH), 7.0—8.15 (20H, m, aromatic 19 protons and NH).

To the solution of the oil (10.0 g) obtained above in MeOH (45 ml) was added *p*-TsOH·H_2O (0.33 g). The mixture was stirred for 4 h at ambient temperature. After neutralization with an aqueous solution of NH_3, the mixture was concentrated *in vacuo* to give a residue, to which was added H_2O and extracted with $CHCl_3$. The extract was washed with brine twice and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was subjected to column chromatography on silica gel by gradient eluting with AcOEt and $CHCl_3$ (1 : 10) to AcOEt as eluent. The fractions containing the desired compound were combined and evaporated to give an oil (5.30 g, 88.5%), which was used in the following reaction without further purification. MS *m/z*: 401 (M^+). IR (neat) cm^{-1}: 2250 (CN). NMR ($CDCl_3$) δ: 1.07, 1.26 (3H, each d, J=7 Hz, $COOCHC\underline{H}_3$), 2.41 (3H, s, C_6-CH_3), 3.54, 3.69 (2H, each br d, J=5 Hz, $COOCHC\underline{H}_2O$), 3.77 (3H, s, $COOCH_3$), 4.77—5.23 (1H, m, COOCH), 5.20 (1H, s, C_4-H), 7.03—8.24 (5H, m, aromatic protons, NH).

***β*-Hydroxyisopropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-5-carboxylate (28)** To a stirred solution of **27** (5.71 g) in CH_2Cl_2 (100 ml) was added MnO_2 (20 g), and stirring was continued for 8 h at ambient temperature. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue, which was subjected to column chromatography on silica gel with a mixture of AcOEt and $CHCl_3$ (1 : 20 to 2 : 5) as eluent. The fractions containing the desired compound were combined and evaporated to afford pure **28** (3.90 g, 68.6%) as a yellow oil. MS *m/z*: 400 (M^+), 368 ($M^+ - OCH_3$). IR (neat) cm^{-1}: 2240 (CN). NMR ($CDCl_3$) δ: 1.00 (3H, d, J=7 Hz, $COOCHC\underline{H}_3$), 1.43—1.60 (2H, m, OH, H_2O), 2.72 (3H, s, C_6-CH_3), 3.43—3.63 (2H, m, $COOCHC\underline{H}_2O$), 3.76 (3H, s, $COOCH_3$), 4.83—5.21 (1H, m, COOCH),

7.60—8.47 (4H, m, aromatic protons).

Lithium 2-Cyano-5-(β-hydroxyisopropoxy)carbonyl-6-methyl-4-(3-nitrophenyl)pyridine-3-carboxylate (7a) A mixture of **28** (3.54 g, 8.86 mmol) and LiI (2.97 g, 2.5 eq mol) in dry pyridine (45 ml) was stirred at 90 to 100 °C for 4 h under a nitrogen atmosphere. The reaction mixture was evaporated to dryness *in vacuo* to give a residue, which was dissolved in AcOEt. The insoluble mass formed was filtered off, and to the filtrate was added $(iso\text{-}Pro)_2O$ to afford a second precipitate, which was washed with $(iso\text{-}Pro)_2O$ by decantation three times and dissolved in H_2O. The aqueous solution was washed with CCl_4 and extracted with *n*-BuOH. The extract was concentrated *in vacuo* and dissolved in acetone. The solution was filtered through silica gel by suction and evaporated *in vacuo* to dryness to give a residue, which precipitated out from a mixture of AcOEt and $(iso\text{-}Pro)_2O$. Filtration, washing with $(iso\text{-}Pro)_2O$ and drying under reduced pressure gave a mixture of **7a** and **30** (ratio; 80.6 : 19.4 by HPLC; anal. column: NOVAPAK™C_{18}, 8 mm i.d. × 10 cm, ϕ: 5 μ; guard column: LS-410, 4 mm i.d. × 10 mm; eluent: 0.02 M Pi buffer (pH 6.8) (75%)–MeOH (25%); eluting speed: 1 ml/min; detection: 254 nm (Varian 5000 Liquid Chromatograph). MS *m/z*: 385 ($M^+ - Li$), IR (Nujol) cm^{-1}: 2240 (CN), NMR (CD_3OD) δ: 0.87 (3H, d, J=6 Hz, $COOCHC\underline{H}_3$), 2.61 (3H, s, C_6-CH_3), 3.33 (2H, d, J=6 Hz, $COOCHC\underline{H}_2O$), 4.60—5.10 (1H, m, COOCH), 7.43—8.38 (4H, m, aromatic protons).

2-Oxopropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (31) To a solution of **3** (1.30 g, 3.79 mmol) in dimethylformamide (DMF) (5 ml) was added monochloroacetone (0.39 g, 4.22 mmol, 1.11 eq) and K_2CO_3 (0.49 g, 3.53 mmol, 1.86 eq) successively. The mixture was stirred for 1.5 h at ambient temperature. To the reaction mixture was added H_2O, and extraction with AcOEt was carried out. The extract was washed with H_2O and brine and dried over $MgSO_4$. Removal of the solvent *in vacuo* afforded a crystalline mass, which was washed with $CHCl_3$ and collected by filtration to give **31** (1.04 g, 80.9%). An analytical sample was obtained by recrystallization from AcOEt, mp 189—190 °C. *Anal.* Calcd for $C_{19}H_{17}N_3O_7$: C, 57.14; H, 4.29; N, 10.52. Found: C, 57.37; H, 4.23; N, 10.54. IR (Nujol) cm^{-1}: 2245 (CN). NMR (DMSO-d_6) δ: 2.03 (3H, s, CH_3CO), 2.39 (3H, s, C_6-CH_3), 3.74 (3H, s, $COOCH_3$), 4.77 (2H, s, $COOCH_2$), 5.17 (1H, s, C_4-H), 7.4—8.23 (4H, m, aromatic protons), 10.44 (1H, s, NH).

2-Hydroxypropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (32) To a solution of **31** (1.00 g, 2.5 mmol) in tetrahydrofuran (THF) (10 ml) was gradually added $NaBH_4$ (0.14 g, 3.7 mmol) under ice-cooling and stirring, which was continued for 1.5 h under the same conditions. To the reaction mixture was added H_2O and extracted with AcOEt twice. The combined extract was washed with brine and dried over $MgSO_4$. Removal of the solvent *in vacuo* afforded a residue, which was subjected to column chromatography on silica gel with a mixture of $CHCl_3$ and AcOEt (10 : 1) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give pure **32** (1.00 g, quant. yield). An analytical sample was obtained by recrystallization from a mixture of AcOEt and Et_2O as yellow crystals, mp 135—140 °C (dec.). *Anal.* Calcd for $C_{19}H_{19}N_3O_7$: C, 56.86; H, 4.77; N, 10.47. Found: C, 56.76; H, 4.84; N, 10.11. MS *m/z*: 401 (M^+). IR (Nujol) cm^{-1}: 2220 (CN). NMR ($CDCl_3$) δ: 1.14, 1.20 (3H, each d, J=each 6 Hz, $C\underline{H}_3CHOH$), 1.72—2.15 (1H, m, OH), 2.39 (3H, s, C_6-CH_3), 3.75 (3H, s, $COCH_3$), 3.75—4.30 (3H, m, $COOC\underline{H}_2$, $C\underline{H}OH$), 5.17 (1H, s, C_4-H), 6.9—8.2 (5H, m, aromatic protons, NH).

2-Hydroxypropyl 2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (33) To a solution of **32** (1.0 g, 2.49 mmol) in CH_2Cl_2 (8 ml) was added MnO_2 (7.62 g) under stirring at ambient temperature. The mixture was stirred under the same conditions for 2 h. The mixture was filtered off and the filtrate was concentrated *in vacuo* to give a residue, which was subjected to column chromatography on silica gel with a mixture of $CHCl_3$ and AcOEt (10 : 1 to 5 : 2) as eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to afford pure **33** (0.75 g, 72.5%) as an oil. MS *m/z*: 399 (M^+). IR (neat): 2250 (CN). NMR ($CDCl_3$) δ: 1.07 (3H, d, J=6 Hz, $C\underline{H}_3CHOH$), 1.52—1.77 (1H, br m, OH), 2.71 (3H, s, C_6-CH_3), 3.75 (3H, s, $COOCH_3$), 3.75—4.17 (3H, m, $COOC\underline{H}_2$, $C\underline{H}OH$), 7.46—8.45 (4H, m, aromatic protons).

Lithium 2-Cyano-5-(2-hydroxy)propoxycarbonyl-6-methyl-4-(3-nitrophenyl)pyridine-3-carboxylate (30) A mixture of **33** (3.14 g, 7.86 mmol) and LiI (3.35 g, 25.0 mmol) in dry pyridine (40 ml) was heated at 85—90 °C for 2.5 h under stirring and a nitrogen atmosphere. The reaction mixture was evaporated *in vacuo* to dryness to afford a residue, which was dissolved in AcOEt. The precipitate which formed was filtered off and the filtrate and the washings were evaporated *in vacuo*. To the residue was added H_2O (10 ml) and the solution was washed with $(iso\text{-}Pro)_2O$. From the aqueous layer the reaction product was extracted with *n*-BuOH twice. Removal of the solvent *in vacuo* afforded a residue, which crystallized on standing. Washing and decantation with Et_2O once, a mixture of Et_2O and AcOEt (5 : 1) three times and collection by filtration gave a mixture of **30** and **7a** (1.86 g, 60.5%) as yellow crystals (90.4 : 9.6 by HPLC; anal. column: NOVAPAK™C_{18}, 8 mm i.d. × 10 cm, ϕ: 5 μ; guard column: LS-410, 4 mm i.d. × 10 mm; eluent: 0.02 M Pi buffer pH 6.8 (75%)–MeOH (25%); eluting speed: 1 ml/min; detection: 254 nm (Varian 5000 Liquid Chromatograph). MS *m/z*: 385 ($M^+ - Li$). IR (Nujol) cm^{-1}: 2245 (CN). NMR (CD_3OD) δ: 0.99, 1.32 (3H, each d, J=5, 6 Hz, $C\underline{H}_3CH$), 2.64 (3H, s, C_6-CH_3), 3.61—4.00 (3H, m, $COOC\underline{H}_2$, $C\underline{H}OH$), 7.57—8.38 (4H, m, aromatic protons).

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