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

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Nilvadipine (I), isopropyl 2-cyano-3-methoxy carbonyl-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 excellent 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, 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-methoxy carbonyl-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, 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 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 referring 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 I 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
Compounds I, 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
temperature in 93.8% yield.

Compound 2, which is a hydrolyzed-form of the methyl ester at the 3-position next to the cyano group of 1, 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 1 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, 1 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-carbamido carboxylic acid (13) and subsequent esterification with diazomethane, as shown in Chart 2. All compounds whose methyl ester
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 hydrodized-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 hydrodized-form of the isopropyl ester group at the 5-position of 1, was prepared by treatment of the tert-butyl ester analogue of 1 (17), obtained via 15 and 16 according to similar methods described in previous papers, with formic acid in 87.1% yield. In addition, 15 was prepared by the modified Hantzsch reaction using m-nitrobenzaldehyde, isopropyl 4-acetoxyacetocetate, prepared from acetoxy-acetyl chloride and malonic acid acetone followed by treatment with isopropyl alcohol, and methyl 3-amino-4,4-dimethoxyacetonate. The dimethoxyethyl 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 of 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-toluene sulfonic 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-toluene sulfonic 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 rearrangement reaction of pyridine-N-oxide (23) with acetic anhydride. Compound 23 was obtained from 1 by treatment with hydrogen peroxide in glacial
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
nucleus of I is hydrolyzed, 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-triphenylmethylxoy-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 propylene glycol 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.

Experimental
Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a JNM-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-methoxy-carbonyl-6-methyl-4-(3-nitrophenyl)pyridine-5-carboxylate (I) To a suspension of I (1.49 g) in a mixture of conc. HNO₃ and H₂O (1:2, 10 ml) was added AcOH (10 ml) under stirring at ambient temperature. After 3 h stirring at room temperature, H₂O was added to the reaction mixture and extracted with AcOEt. The organic extract was washed with an aqueous solution of NaHCO₃ and H₂O in turn and dried over MgSO₄. 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)₂O and Et₂O, mp 91—93 °C. Anal. Calcd for C₁₅H₁₂N₂O₈: 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 (CDCl₃) δ: 1.05 (3H, d, J = 6.5 Hz, CH₂(CH₃)), 1.12 (3H, d, J = 6.5 Hz, CH₂(CH₃)), 2.68 (3H, s, C₆H₃CH₃), 3.74 (3H, s, COOCH₃), 4.99 (1H, sept, J = 6.5 Hz, CH(CH₃)₂), 7.5—8.5 (4H, m, aromatic protons).
Sodium 2-Cyano-5-isopropoxycarbonyl-6-methyl-1-(3-nitrophenyl)-1,4-dihydroxyridine-3-carboxylate (2a) A mixture of 1 (5.77 g, 15.0 mmol) and LiI (5.90 g, 30 mmol) was refluxed under a nitrogen atmosphere for 6 h. Pyridine was removed in vacuo and to the residue was added an aqueous solution of NaHCO₃ and Et₂O with vigorous stirring. The separated aqueous layer was washed with Et₂O and extracted with n-ButOH. After washing with H₂O, n-ButOH was removed in vacuo. The residue was triturated in Et₂O and the obtained precipitates were collected by filtration and dried. The obtained powder was dissolved in CH₂Cl₂ and to the solution was added Et₂O. The resultant precipitate was collected by filtration, recrystallized from a mixture of CH₂Cl₂ and Et₂O and dried to afford the title compound, (1.57 g, 26.6%), mp 159—161°C (dec. Anal. Calc. for C₁₉H₂₄N₂O₄: 2H₂O: C, 53.33; H, 4.31; N, 10.36. Found: C, 53.47; H, 4.41; N, 9.73. IR (Nujol) cm⁻¹: 2290 (CN); NMR (DMF-d₇): δ 1.01 (3H, d, J=6 Hz, (CH₃)₂); 1.18 (3H, d, J=6 Hz, (CH₃)₂); 1.27 (3H, s, CH₃); 2.74 (1H, s, CH₃); 3.65 (1H, s, COOC₂H₅); 5.12 (1H, d, J=6 Hz, 3H, CH₃); 7.04—8.12 (4H, m, aromatic protons). 9.30 (1H, m, NH).

Isopropyl 2-Carboxamido-3-methoxy carbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydroxyridine-5-carboxylate (14) To a suspension of 2 (0.197 g, 0.59 mmol) in AcOEt (5 ml) was added 5% aqueous HCl (5 ml). The mixture was shaken and the separated organic layer was washed with H₂O. To the AcOEt solution was added dropwise an ethereal solution of CH₂Cl₂. The resultant solution was evaporated in vacuo and the residue was subjected to gradient column chromatography on silica gel with CHCl₃: MeOH=100:1 and CHCl₃:MeOH=50:1 as eluents. After the fractions containing I (2250 mg) were eluted, the fractions containing the title 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. Calc. for C₂₃H₂₅N₂O₄·C₆H₅OH: C, 56.57; H, 5.45; N, 10.42. Found: C, 56.81; H, 5.20; N, 10.48. MS m/z: 402 (M⁺). IR (Nujol) cm⁻¹: 3350, 3280, 3290 (sh), 1600 (sh), 1620, 1650, NMR (CDCl₃): δ 1.36 (6H, d, J=6 Hz, (CH₃)₂), 2.43 (3H, s, CH₃), 3.78 (3H, s, COOCH₃), 5.01 (1H, s, CH₃), 6.17 (1H, s, COOCH₃), 7.51 (1H, s, C₆H₅), 6.41 (1H, br, NH), 7.2—8.25 (4H, m, aromatic protons), 10.1 (2H, brs, NH).

Compound 14 was also prepared from 1 as follows: 1 (1.155 g, 30 mmol) was gradually added to an 80% aqueous solution of H₂SO₄ (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 H₂O and dried over MgSO₄. Evaporation of the solvent afforded a crystalline mass, which was washed with AcOEt and collected by filtration to give semicrude 14 (1.063 g, 97.9%). 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-1-(3-nitrophenyl)pyridine-3-carboxylate (4a) A mixture of 1 (1.12 g, 2.2 g) and LiI (1.28 g, 9.6 mmol) in dry 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 (0.206, 20.6%), mp 136°C (recrystallization from a mixture of CH₂COCH₃ and AcOEt). Anal. Calc. for C₁₉H₂₄N₂O₄·2H₂O: C, 55.55; H, 4.39; N, 9.63. Found: C, 54.89; H, 4.42; N, 9.71. IR (Nujol): 3500, 2254 (CN), 1720 (COOR), 1705 (sh, COO⁻). NMR (DMSO-d₆): δ 0.91 (6H, d, J=6 Hz, CH₂(CH₃)₂), 2.48 (3H, s, CH₃), 4.87 (1H, s, J=6 Hz, CH₃(CH₃)₂), 7.6—8.43 (4H, m, aromatic protons).

terr-Butyl 2-Dimethoxymethyl-3-methoxy carbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydroxyridine-5-carboxylate (15) A mixture of methyl 4,4-dimethyl-3-oxobutanoyl (9.40 g) prepared from -nitrobenzaldehyde and methyl 4,4-dimethoxycetocetate in the presence of a catalytic amount of morpholino and AcOH by the Knoevenagel reaction, and terr-butyl 3-aminoxocetionate (5.26g), synthesized from terr-butyl acetacetoacetate and amonia, was heated at 70°C for 0.5 h and then at 120°C for 2.5 h. The reaction mixture was cooled to room temperature, added with Li₂CO₃ (132 mg, 0.24 mmol) and MgSO₄ and evaporated in vacuo to give the title compound 15 (14.68 g, 98.36%, quant. yield) as an oil, which was used in the following reaction without further purification. IR (Nujol) cm⁻¹: 3345 (NH), 1690 (COOR x 2), 1645 (C=C-N), 1605 (NMR (CDCl₃): δ 1.42 (4H, s, (CH₂CH₃)), 2.36 (3H, s, CH₃), 3.46, 3.59, 2.59 (each 3H, each s, CH₂COCH₃, OCH₃ x 2), 5.12 (1H, s, CH₂COCH₃), 6.25 (1H, s, (COOR) x 2), 6.79 (1H, br. s, OH), 7.35—8.21 (4H, m, aromatic protons).
vacuo to give an oil, which crystallized on standing at ambient temperature (15.9%, 73.4%). An analytical sample was obtained by recrystallization from AcOEt, mp 165–166.5°C. Anal. Caled for C22H32NO4: C, 56.88; H, 4.77; N, 9.48. Found: C, 56.86; H, 4.82; N, 9.43. MS m/z: 447 (M+), 429 (NH2), 220 (CN), 1740 (AcO), 1620 (COOR), 1595, 1510. NMR (CDCl3) δ: 1.11, 1.28 (each 3H, d, J = 6.7 Hz, CH3(CH3)), 2.25 (3H, s, CH3CO), 3.80 (3H, s, COOCH3), 4.98 (1H, septet, J = 6.7 Hz, CH3(CH3)), 5.25 (1H, s, C-O-C), 5.22 (1H, d, J = 4.6 Hz, C-CH(2)OH), 6.00 (1H, s, C-CH2OR), 7.8–8.25 (5H, m, aromatic protons, NH).

**Isopropyli 2-Cyano-6-hydroxymethyl-3-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (21) and Methyl 2-Cyano-4-(3-nitrophenyl)-5-oxo-4,5,6,7-tetrahydrofurao[3,4-b]-pyridine-3-carboxylate (22)** 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 2 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. Caled for C22H32NO4: 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: 3340 (NH), 3180 (sh), 3100, 2230 (CN), 1746 (lactone), 1695, 1680 (COOR). 1600. NMR (DMSO-d₆): δ: 3.34 (1H, s, NH), 3.60 (3H, s, COOCH3), 4.92 (2H, dd, J = 16, 22 Hz, CH2 in lactone), 5.04 (1H, s, C-H), 7.6–8.15 (4H, m, aromatic protons).

**Isopropyli 2-Hydroxyethyl-3-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (21) and Methyl 2-Cyano-4-(3-nitrophenyl)-5-oxo-4,5,6,7-tetrahydrofurao[3,4-b]-pyridine-3-carboxylate (22)** To a suspension of (0.64 g, 1.57 mmol) in a mixture of conc. HNO3 and H2O (1:2, 7 ml) was added AcOH (7 ml) under stirring at ambient temperature. The mixture was warm to 40°C to obtain a clear solution for about 5 min. The solution was stirred at ambient temperature for 1.5h. The reaction mixture was diluted with H2O, its pH was adjusted to 8.0, and the excess reagent was extracted with AcOEt. The extract was washed with H2O, dried over MgSO4 and evaporated in vacuo to give a yellow oil, which was subjected to column chromatography on silica gel with CH3Cl as eluent. The first eluted compound crystallized on standing and was recrystallized from a mixture of C6H6 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.
was subjected to column chromatography on silica gel with a mixture of AcOEt and CHCl₃ (1:20) as eluent. The fractions containing the desired compound were combined and evaporated in vacuo to afford 22 as colorless needles. The residual (CH₂Cl₂ and MeOH) was collected by filtration and dried in a desiccator over P₂O₅. As this compound was highly hygroscopic, an elemental analysis could be obtained. IR (Nujol) cm⁻¹: 2245 (CN), NMR (DMSO-d₆): δ: 5.43 (2H, s, C₂H₂O), 7.7-8.5 (4H, m, aromatic protons).

Compound 26 was also obtained from 25 as follows: To a solution of 25 (0.77g) in anhydrous methanol (10mL), a methanolic solution (1mL) of LiOH·H₂O (35mg/mL) was added and the mixture was stirred for 3hr under reflux. The work-up procedure was similar to that described above. Yield: 0.1g, 13.7%.

Sodium 2-Cyano-6-hydroxy-3-methoxycarbonyl-4-(3-nitrophenyl)-pyridine-5-carboxylate (6a): A mixture of 22 (18.29g, 53.99mmol), NaHCO₃ (4.35g, 53.99mmol) in MeOH (180mL) and H₂O (900mL) was refluxed at 85-90°C with stirring for 11hr. After cooling and treatment with activated carbon, the reaction mixture was concentrated in vacuo. The residue was dissolved in H₂O and washed with CH₂Cl₂. 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 (15.24g, 74.6%). An analytical sample was obtained by recrystallization from MeOH, mp > 130°C. Anal. Calc. For C₂H₂N₂O₃Na₂CO₃: 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, 1350, 1300. (NMR (DMSO-d₆): δ: 3.62 (3H, s, COOHCH₂), 4.63 (2H, d, J = 6Hz, COOH), 5.76 (1H, br, J, 10Hz, OH), 7.84-8.10 (4H, m, aromatic protons).

β-Hydroxyisopropyl-2-Cyano-3-methoxycarbonyl-6-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (27) To a solution of 3 (5.42g, 15.8mmol) in CHCl₃ (50mL) was added PCl₅ (4.40g, 21.0mmol, 1.33eq), with stirring and cooling in an ice-bath. The mixture was stirred for 1.5hr under the same conditions. To the reaction mixture obtained above was added dropwise a solution of 1,2-propanediol (1.25g, 19.8mmol) and triphenylmethylether (5.89g, 17.66mmol) and pyridine (3ml, 21.7mmol) in CHCl₃ (30mL) under ice cooling. The mixture was stirred for further 2.5hr under the same conditions. To the resultant reaction mixture was added a 10% aqueous solution of Na₂CO₃. The separated CHCl₃ layer was washed with an aqueous solution of NaHCO₃, dill HCl and brine successively and dried over MgSO₄. Removal of the solvent in vacuo afforded a residue, which was subjected to column chromatography on silica gel with a mixture of AcOEt and CHCl₃ (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 (10.1g, 99.3%) as a yellow viscous oil.

Lithium 6-Acetoxyethyl-2-cyano-5-isopropoxycarbonyl-4-(3-nitrophenyl)pyridine-3-carboxylate (25) A mixture of 24 (2.3g, 5.2mmol) and Lii (2.4g, 17.9mmol) in dry pyridine (40ml) was stirred under a nitrogen atmosphere at 100°C for 3hr. The solution was concentrated in vacuo 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 CHCl₃ and MeOH (10:1) as eluent. The fractions containing the desired compound were combined and evaporated in vacuo to afford pure 25 (1.95g, 66.4%) 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). NR (DMSO-d₆): δ: 0.95 (6H, d, J = 6Hz, CH₂CH₂), 2.06 (3H, s, CH₃CO), 4.84 (1H, sept, J = 6Hz, CH₂CH₂), 5.22 (2H, s, CH₂CO), 7.59-8.0 (4H, m, aromatic protons).

Lithium 5-Cyano-4-(3-nitrophenyl)-5-oxo-3,7-dihydropururolo[3,4-d]pyridine-3-carboxylate (26) A mixture of 22 (2.53g, 15.8mmol) and LiI (5.27g, 39.4mmol) in dry pyridine (80ml) was stirred for 2.5hr at 100°C under a nitrogen atmosphere. The reaction mixture was concentrated 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 CHCl₃ 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 CH₂Cl₂ (35mL) was added and the mixture was collected by filtration and dried in a desiccator over P₂O₅. As this compound was highly hygroscopic, an elemental analysis could be obtained. IR (Nujol) cm⁻¹: 2245 (CN), NMR (DMSO-d₆): δ: 5.43 (2H, s, C₂H₂O), 7.7-8.5 (4H, m, aromatic protons).

β-Hydroxyisopropyl-2-Cyano-3-methoxycarbonyl-6-(3-nitrophenyl)pyridine-5-carboxylate (28) To a solution of 27 (5.71g) in CH₂Cl₂ (100mL) was added MnO₂ (20g), and stirring continued for 8hr 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 CHCl₃ (1:20 to 2:5) as eluent. The fractions containing the desired compound were combined and evaporated to afford pure 28 (3.96g, 66.3%) as a yellowish oil. MS m/z: 400 (M⁺), 368 (M⁺ - OCH₃), IR (neat) cm⁻¹: 2240 (CN), NR (DMSO-d₆): δ: 1.00 (3H, d, J = 7Hz, COOCH₂), 1.45-1.60 (CH₃, s, CH₂CO), 2.72 (3H, s, CH₃), 3.45-3.65 (2H, m, CH₂COOCH₃), 3.76 (3H, s, COOH), 4.83-5.21 (1H, s, COOCH₃).
7.60—8.47 (4H, m, aromatic protons).

Lithium 2-Cyano-5-(β-hydroxypropoxycarbonyl)-6-methyl-4-(3-nitrophenyl)pyridine-3-carboxylate (7A) To a solution of 2B (3.54g, 8.86mmol) and LiI (2.97g, 2.5eq mol) in dry pyridine (45ml) 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 the filtrate was added (iso-Pro)O to afford a second precipitate, which was washed with (iso-Pro)O by deanization three times and dissolved in H2O. The aqueous solution was washed with CCl4 and extracted with n-BuOH. The extract was concentrated in vacuo and dissolved in aceton. 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-Pro)O. Filtration, washing with (iso-Pro)O and drying under reduced pressure gave a mixture of 7A and 30 (ratio: 80.6: 19.4 by HPLC; anal. column: NovaPak CI8, 5 mm i.d. x 10 cm, φ: 5 µ; guard column: LS-410, 4 mm i.d. x 10 mm; eluent: 0.02 M Pi buffer (pH 6.8) (75%)-MeOH (25%); eluting speed: 1ml/min; detection: 254nm (Varian 5000 Liquid Chromatograph). MS m/z: 385 (M+−Li), IR (Nujol) cm−1: 2240 (CN), NMR (CD3OD) δ: 0.87 (3H, d, J=6Hz, COOC6H4CH3), 2.61 (3H, s, C6H4-CH3), 3.33 (2H, d, J=6Hz, COOC6H4CH2O), 4.60−5.10 (1H, m, COOCH3), 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 (1.30g, 3.79mmol) in dimethylformamide (DMF) (5ml) was added monochloroacetone (0.39g, 4.22mmol, 1.11 eq) and K2CO3 (0.49g, 3.53mmol, 1.86 eq) successively. The mixture was stirred for 1.5h at ambient temperature. To the reaction mixture was added H2O and the reaction with AcOEt was carried out. The extract was washed with H2O and brine and dried over MgSO4. Removal of the solvent in vacuo afforded a crystalline mass, which was washed with CHCl3 and collected by filtration to give 31 (1.04g, 80.9%). An analytical sample was obtained by recrystallization from AcOEt, mp 189−190°C. Anal. Calc'd for C18H16N2O2·C5: 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-d6) δ: 2.03 (3H, s, CH3CO), 2.39 (3H, s, C6H4-CH3), 3.74 (3H, s, COOCH3), 4.77 (2H, s, COOCH2), 5.17 (1H, s, C6H4), 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 (1.09g, 2.5mmol) in tetrahydrofuran (THF) (10ml) was gradually added NaBH4 (0.14g, 3.7mmol) under ice-cooling and stirring, which was continued for 1.5h under the same conditions. The reaction mixture was added H2O and extracted with AcOEt twice. The combined extract was washed with brine and dried over MgSO4. Removal of the solvent in vacuo afforded a residue, which was subjected to column chromatography on silica gel with a mixture of CHCl3 and AcOEt (10:1) as eluent. The fractions containing the desired compound were combined and evaporated in vacuo to give pure 32 (1.00g, quant. yield). An analytical sample was obtained by recrystallization from a mixture of AcOEt and Et2O as yellow crystals, mp 135−140°C (dec.). Anal. Calc'd for C24H21N2O2·C5: 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 (CDCl3) δ: 1.14, 1.20 (3H, each d, J=6Hz, C6H4-CHOH), 1.72−2.15 (1H, m, OH), 2.39 (3H, s, C6H4-CH3), 3.75 (3H, s, COOCH3), 3.75−4.30 (3H, m, COOCH2, C6H4-CHOH), 5.17 (1H, s, C6H4), 6.9−8.2 (5H, m, aromatic protons, NH).

References