Synthesis and Biological Activity of the Metabolites of Diethyl 4-[(4-Bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (NO-1886)

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Five metabolites of diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (NO-1886) (1) were synthesized to confirm their proposed structures. The metabolites (2—6) were found to be identical with the synthesized compounds. These metabolites were orally administrated to Triton WR-1339-induced hypertriglyceridemic rats, and the plasma levels of triglycerides were measured to estimate lipoprotein lipase activity. All the metabolites showed lower potency than NO-1886.

Key words diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate; metabolite; lipoprotein lipase activity; Triton WR-1339; synthesis

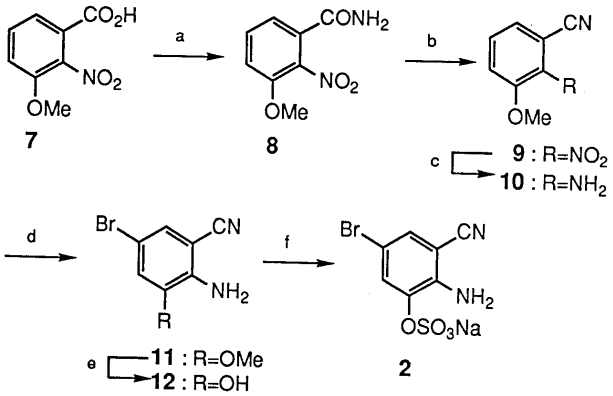
We have previously reported the synthesis of diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (NO-1886) (**1**),[1] which increases lipoprotein lipase (LPL) activity with resulting reduction of plasma triglyceride levels and elevation of high-density-lipoprotein (HDL) cholesterol. Its long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis.[2] Furthermore, Tsutsumi *et al.* recently reported that **1** is potentially beneficial for the treatment of hypertriglyceridemia with low HDL cholesterol in diabetes.[3] Compound **1** is under clinical study as a candidate antilipidemic agent. In metabolic studies of **1**, two metabolites (**2**, **3**) were isolated from dog urine and three (**4**—**6**) from monkey plasma (Fig. 1). Their structures were proposed to be a sulfate derivative (**2**), two glucuronide derivatives (**3**, **5**), a hydroxylated derivative (**4**) and a hydrolyzed derivative (**6**), mainly based on spectroscopic analysis.[4] The present study was undertaken to confirm their structures and to clarify their biological activities.

Synthesis

The metabolite **2** was prepared by the method shown in Chart 1. Commercial 2-nitro-3-methoxybenzoic acid (**7**) was treated with thionyl chloride ($SOCl_2$) and then with 28% ammonium hydroxide (NH_4OH) to give the amide **8**, which was dehydrated with $SOCl_2$ to provide the nitrile **9**. Catalytic hydrogenation with 10% palladium on charcoal (10% Pd/C) in ethyl acetate (AcOEt) gave the amine **10**, which was treated with *N*-bromosuccinimide (NBS) to give the brominated product **11** and then demethylation of **11** with boron tribromide (BBr_3) provided the phenol **12**. Finally, **12** was converted into **2** with sulfur trioxide trimethylamine complex ($SO_3 \cdot NMe_3$).[5]

The metabolite **2** was identical with this synthetic compound in HPLC,[6] ^{1}H-NMR and electron impact–mass spectrometry (EI-MS) comparisons.[7]

We next attempted to obtain the metabolite **3** *via* the



a) i) $SOCl_2$, THF, ii) NH_4OH. b) $SOCl_2$, THF. c) Pd/C, H_2, AcOEt. d) NBS, DMF. e) BBr_3, CH_2Cl_2. f) SO_3•NMe_3, NaOH-$NaHCO_3$.

Chart 1

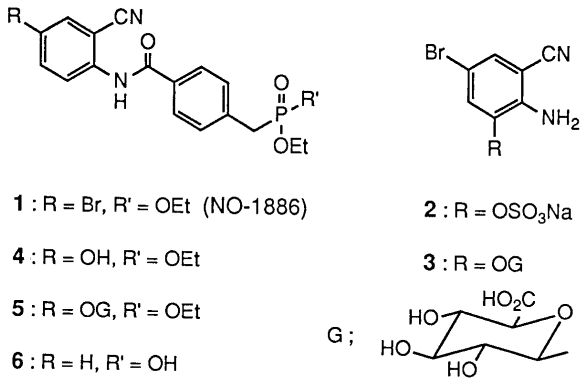
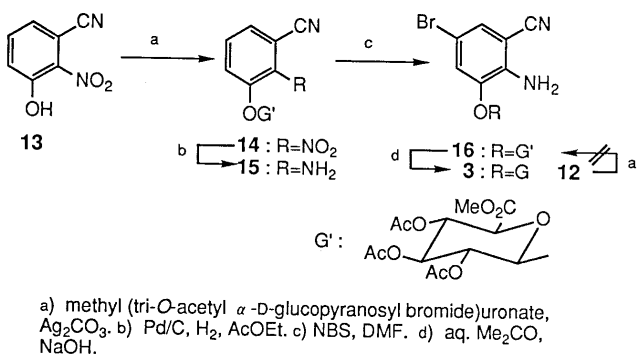


Fig. 1



a) methyl (tri-*O*-acetyl α-D-glucopyranosyl bromide)uronate, Ag_2CO_3. b) Pd/C, H_2, AcOEt. c) NBS, DMF. d) aq. Me_2CO, NaOH.

Chart 2

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conversion of **12** into the precursor **16** for **3**. However, since the approach to **16** from **12** did not give an acceptable result, **3** was synthesized according to the method shown in Chart 2. Thus, glucosidation of 3-hydroxy-2-nitrobenzonitrile (**13**),[8] which was prepared in 28% yield from commercial 3-cyanophenol, with methyl (tri-*O*-acetyl α-D-glucopyranosyl bromide)uronate,[9] followed by hydrogenation of the resulting compound **14** with 10% Pd/C gave the amine **15**. Subsequently, conversion of **15** into the desired compound **3** was executed in 50% overall yield by bromination and deprotection of the product **16**.

The metabolite **3** was identical with this synthetic compound on the basis of HPLC,[6] ^{1}H-NMR and secondary ion mass spectrometry (SI-MS) comparisons.

The synthetic route to the metabolite **4** from commercial 5-hydroxy-2-nitrobenzaldehyde (**17**) is shown in Chart 3. The reaction of **17** with hydroxylammonium chloride ($NH_2OH \cdot HCl$) in pyridine afforded the oxime intermediate, followed by dehydration with acetic anhydride and sodium acetate to produce the nitrile **18**, which was then hydrogenated to provide the amine **19** in 38% overall yield. The treatment of 4-[(diethoxyphosphoryl)methyl]-benzoic acid[1] with $SOCl_2$ gave the corresponding benzoyl chloride, which was transformed into the amide **20** on treatment with **19** in pyridine. Finally, hydrolysis of **20** with aqueous 2 N NaOH at room temperature afforded **4** in 81% yield.

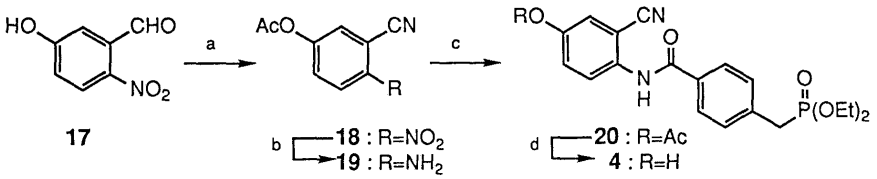
The metabolite **4** was identical with this synthetic compound on the basis of HPLC,[6] ^{1}H-NMR and EI-MS comparisons.

Chart 4 shows the synthesis of the metabolite **5**, which is the *O*-glucuronide of **4**. We initially examined glycosidation of **4**. Unexpectedly, however, when **4** was treated with methyl (tri-*O*-acetyl α-D-glucopyranosyl bromide)uronate in the presence of silver carbonate, the desired glucuronide derivative **25** was not obtained. Consequently, a stepwise route was pursued. Thus, we chose 5-hydroxy-2-nitorobenzonitrile (**22**),[10] prepared by the hydrolysis of **18**, as a starting material instead of **17**, since the attempt to convert **17** into an appropriate glucuronide derivative **21** failed, and transformation of **22** into **5** was achieved uneventfully by a synthetic sequence parallel to that shown in Charts 2 and 3 (glucosidation, hydrogenation, acylation and deprotection).

The metabolite **5** was identical with this synthetic compound on the basis of HPLC,[6] ^{1}H-NMR and SI-MS comparisons.

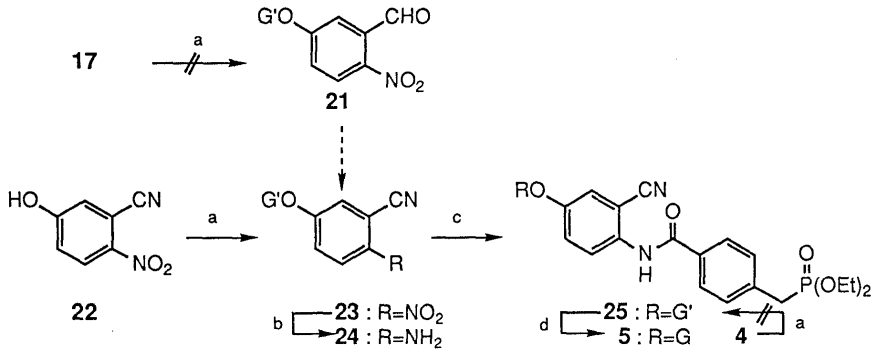
The metabolite **6** was prepared by the method shown in Chart 5. In accordance with the known method,[11] selective monodealkylation of compound **26**[1] by lithium bromide (LiBr) in acetonitrile (CH_3CN) produced **6**.

The metabolite **6** was identical with this synthetic compound on the basis of HPLC,[6] ^{1}H-NMR and EI-MS comparisons.



a) i) NH_2OH•HCl, pyridine. ii) Ac_2O, AcONa. b) Pd/C, H_2, AcOEt. c) 4-[(diethoxyphosphoryl)methyl)]benzoyl chloride, pyridine. d) NaOH.

Chart 3



a) methyl (tri-*O*-acetyl α-D-glucopyranosyl bromide)uronate, Ag_2CO_3. b) Pd/C, H_2, AcOEt. c) 4-[(diethoxyphosphoryl)methyl)]benzoyl chloride, pyridine. d) NaOH.

Chart 4

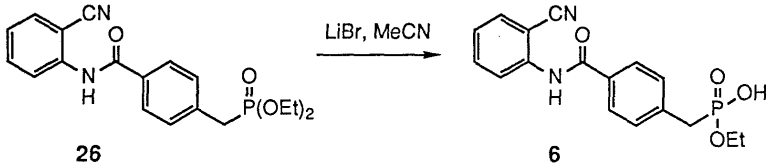


Chart 5

Table 1. Plasma Triglyceride Levels after Administration of NO-1886 and Its Metabolites in Triton WR-1339-Induced Hypertriglyceridemic Rats

Compound	Plasma triglycerides (mg/dl)
Control	611±172
2	435±248
3	462±324
4	498±295
5	314±222
6	383±120
NO-1886 (**1**)	70±22*

The significance of differences between the groups was calculated by the use of Dunnett's test. Data are expressed as means±S.D. ($n=6$). Significantly different from the value in the respective control rats: * $p<0.01$.

Biological Activity

Hypertriglyceridemia due to low LPL activity can be induced by Triton WR-1339.[12] We used this model and measured plasma triglyceride levels to evaluate the LPL activities of metabolites. The data are shown in Table 1. NO-1886 caused a significant decrease in plasma triglycerides in hypertriglyceridemic rats. However, none of the metabolites decreased plasma triglycerides. Based on the pharmacological data, it is suggested that NO-1886 itself contributes predominantly to increasing the LPL activity, and that the metabolites do not play a decisive role in the pharmacological effect of NO-1886.

Experimental

Melting points (mp) were measured on a Yamato MP-21 melting point apparatus and are uncorrected. IR spectra were obtained on a Hitachi 270-30 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL GX-270 (270 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) relative to tetramethylsilane (TMS). MS were obtained with a Hitachi M-80A mass spectrometer. Elemental analysis was carried out with a Yanagimoto MT-3 CHN Corder. All optical rotations were measured at 24 °C in chloroform ($CHCl_3$) or dimethyl sulfoxide (DMSO) solution on a JASCO DIP-370 digital polarimeter. Analytical thin-layer chromatography (TLC) was performed on E. Merck Silica gel GOF-254 (0.25 mm thickness). Column chromatography was carried out with E. Merck Silica gel 60 (70—230 mesh).

3-Methoxy-2-nitrobenzamide (8) A solution of 3-methoxy-2-nitrobenzoic acid **7** (20 g, 0.1 mol) and $SOCl_2$ (27 ml) in tetrahydrofuran (THF) (130 ml) was refluxed for 2 h and evaporated *in vacuo* to afford the benzoyl chloride. NH_4OH (80 ml) was added to a stirred solution of the benzoyl chloride in THF (80 ml) at 0 °C. After having been stirred at room temperature for 30 min, the mixture was concentrated to a small volume, yielding crude crystalline **8**. The crude product was washed with H_2O and EtOH to provide **8** (14.2 g, 70.8%) as yellow crystals, mp 209—211 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 7.32 (1H, d, $J=7.0$ Hz, arom.), 7.47 (1H, d, $J=8.2$ Hz, arom.), 7.61 (1H, dd, $J=8.2$, 7.0 Hz, arom. 5-H), 7.70 (1H, br s, $CONH_2$), 8.18 (1H, br s, $CONH_2$). IR (KBr) cm^{-1}: 3384, 3184, 1658, 1580, 1534. MS *m/z*: 196 (M^+).

3-Methoxy-2-nitrobenzonitrile (9) A solution of 3-methoxy-2-nitrobenzamide **8** (12.4 g, 62.4 mmol) and $SOCl_2$ (71 ml) in THF (100 ml) was stirred at room temperature overnight. The mixture was concentrated to a small volume and ice was added to afford crude crystalline **9**. The crude product was washed with diethyl ether (Et_2O) to provide **9** (8.4 g, 74.6%) as yellow crystals, mp 119—121 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.98 (3H, s, OCH_3), 7.35 (2H, d, $J=8.1$ Hz, arom.), 7.62 (1H, t, $J=8.1$ Hz, arom. 5-H). IR (KBr) cm^{-1}: 3092, 2236, 1608, 1574, 1526. MS *m/z*: 178 (M^+).

2-Amino-3-methoxybenzonitrile (10) A solution of **9** (8.0 g, 44.9 mmol) in AcOEt (80 ml) was hydrogenated under atmospheric pressure with 10% Pd/C (800 mg) for 2 h. The catalyst was removed by filtration and the filtrate was concentrated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30 : 1) to provide **10** (5.9 g, 87.5%) as a colorless powder, mp 64—67 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (3H, s, OCH_3), 4.59 (2H, br s, NH_2), 6.59 (1H, dd, $J=8.0$, 8.0 Hz, arom. 5-H), 6.88 (1H, d-like, $J=8.0$ Hz, arom.), 6.98 (1H, dd, $J=8.0$, 1.3 Hz, arom.). IR (KBr) cm^{-1}: 3452, 3320, 2216, 1630, 1490. MS *m/z*: 148 (M^+).

2-Amino-5-bromo-3-methoxybenzonitrile (11) A solution of NBS (6.4 g, 36.0 mmol) in *N*,*N*-dimethylformamide (DMF) (20 ml) was added to a stirred solution of **10** (4.8 g, 32.7 mmol) in DMF (30 ml) at 0 °C. The mixture was stirred at room temperature for 1 h, then diluted with water and extracted with AcOEt. The extracts were washed with saturated brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel ($CHCl_3$) to afford **11** (5.9 g, 78.6%) as a colorless powder, mp 126—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (3H, s, OCH_3), 4.62 (2H, br s, NH_2), 6.96 (1H, d, $J=2.0$ Hz, arom), 7.10 (1H, d, $J=2.0$ Hz, arom.). IR (KBr) cm^{-1}: 3480, 3376, 2220, 1642, 1498. MS *m/z*: 226, 228 (M^+).

2-Amino-5-bromo-3-hydroxybenzonitrile (12) A solution of BBr_3 (1.0 M) in dichloromethane (CH_2Cl_2) (39.5 ml, 39.5 mmol) was added to a stirred solution of **11** (3.0 g, 13.2 mmol) in CH_2Cl_2 (50 ml) at −78 °C, and the mixture was stirred at room temperature overnight, then diluted with water and extracted with CH_2Cl_2. The extracts were washed with saturated brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30 : 1) to provide **12** (2.1 g, 72.6%) as a colorless powder, mp 207—210 °C. ^{1}H-NMR ($CDCl_3$–DMSO-d_6) δ: 5.03 (2H, br s, NH_2), 6.93 (1H, d, $J=2.0$ Hz, arom.), 7.00 (1H, d, $J=2.0$ Hz, arom.), 10.11 (1H, br s, OH). IR (KBr) cm^{-1}: 3508, 3408, 3296, 2216, 1644, 1572, 1510. MS *m/z*: 212, 214 (M^+). *Anal.* Calcd for $C_7H_5BrN_2O$: C, 39.47; H, 2.37; N, 13.15. Found: C, 39.45; H, 2.37; N, 13.06.

Sodium 2-Amino-5-bromo-3-cyanophenyl Sulfate (2) Sodium bicarbonate ($NaHCO_3$) (2.42 g, 28.8 mmol) and $SO_3 \cdot NMe_3$ (2.71 g, 19.5 mmol) were added to a solution of **12** (1.58 g, 7.4 mmol) and NaOH (940 mg, 23.5 mmol) in H_2O (12 ml), and the mixture was stirred at room temperature for 3 d. After addition of EtOH to the solution, the resulting solid was filtered off and the filtrate was concentrated to give crude crystalline **2**. Recrystallization of the crude product from MeOH and EtOH provided **2** (1.1 g, 49.7%) as yellow crystals, mp 173—176 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.79 (2H, br s, NH_2), 7.49—7.53 (2H, m, arom.). IR (KBr) cm^{-1}: 3624, 3484, 3380, 2220, 1634, 1484. MS, *m/z*: 212, 214 ($M-SO_3Na+H)^+$. *Anal.* Calcd for $C_7H_4BrNaN_2O_4 \cdot H_2O$: C, 25.24; H, 1.82; N, 8.41. Found: C, 25.01; H, 2.11; N, 8.17.

Methyl (3-Cyano-2-nitrophenyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (14) 3-Hydroxy-2-nitrobenzonitrile **13** (300 mg, 1.8 mmol) was dissolved in quinoline (7.5 ml) and then silver carbonate (262 mg, 0.95 mmol) was added. The reaction mixture was stirred at room temperature for 20 min in the dark. Methyl (tri-*O*-acetyl α-D-glucopyranosyl bromide)uronate (523 mg, 1.3 mmol) was added to the reaction mixture and stirring was continued for an additional 15 h. Then, benzene (7.5 ml) was added to dissolve the crystals and the reaction mixture was filtered through Celite. The filtrate was sequentially washed with aqueous 3 N HCl (7.5 ml × 3), aqueous 1 N KOH (7.5 ml × 2) and finally saturated brine. The washed filtrate was dried with $MgSO_4$, and evaporated. The residue was chromatographed on silica gel ($CHCl_3$–AcOEt, 5 : 1) to give **14** (510 mg, 80.7%) as a colorless powder, mp 172—174 °C. $[\alpha]_D$ +5.6° ($c=0.16$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.05 (6H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 3.74 (3H, s, CO_2CH_3), 4.26 (1H, d, $J=8.2$ Hz, $CHCO_2CH_3$), 5.17—5.41 (4H, m), 7.52 (1H, dd, $J=7.3$, 1.8 Hz, arom.), 7.62 (1H, dd, $J=8.5$, 7.3 Hz, arom. 5-H), 7.67 (1H, dd, $J=8.5$, 1.8 Hz, arom.). IR (KBr) cm^{-1}: 3092, 2964, 2240, 1768, 1612, 1578, 1552. MS *m/z*: 421 $(M-CO_2Me)^+$. *Anal.* Calcd for $C_{20}H_{20}N_2O_{12}$: C, 50.01; H, 4.20; N, 5.83. Found: C, 50.07; H, 4.21; N, 5.63.

Methyl (2-Amino-3-cyanophenyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (15) By means of the procedure described for the preparation of **10**, **14** was converted into **15** (73.2%), which was obtained after chromatographic purification ($CHCl_3$–AcOEt, 15 : 1) as a colorless powder, mp 159—161 °C. $[\alpha]_D$ −51.7° ($c=0.24$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 3.76 (3H, s, CO_2CH_3), 4.19 (1H, d, $J=9.3$ Hz, $CHCO_2CH_3$), 4.61 (2H, s, NH_2), 5.03 (1H, d, $J=6.9$ Hz, O–CH–O), 5.23—5.38 (3H, m), 6.66 (1H, dd, $J=8.0$, 7.8 Hz, arom. 5-H), 7.06 (1H, d, $J=7.8$ Hz, arom.), 7.13 (1H, d, $J=8.0$ Hz, arom.). IR (KBr) cm^{-1}: 3508, 3380, 2956, 2220, 1756, 1628, 1574. SIMS *m/z*: 451 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{22}N_2O_{10}$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.56; H, 5.01; N, 6.04.

Methyl (2-Amino-5-bromo-3-cyanophenyl 2,3,4-Tri-*O*-acetyl-β-D-

glucopyranosid)uronate (16) By means of the procedure described for the preparation of **11**, **15** was converted into **16** (90.2%), which was obtained after chromatographic purification ($CHCl_3$–AcOEt, 20 : 1) as a colorless powder, mp 170—172 °C. $[\alpha]_D$ −34.0° (c=0.25, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.06 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 3.77 (3H, s, CO_2CH_3), 4.23 (1H, d, J=9.1 Hz, C<u>H</u>CO_2CH_3), 4.66 (2H, s, NH_2), 5.06 (1H, d, J=7.3 Hz, O–C<u>H</u>–O), 5.23—5.41 (3H, m), 7.15 (1H, d, J=2.0 Hz, arom.), 7.24 (1H, d, J=2.0 Hz, arom.). IR (KBr) cm^{-1}: 3488, 3388, 2220, 1756. SIMS m/z: 531 $(M+1+H)^+$, 529 $(M-1+H)^+$. *Anal.* Calcd for $C_{20}H_{21}BrN_2O_{10}$: C, 45.38; H, 4.00; N, 5.29. Found: C, 45.48; H, 3.99; N, 5.13.

2-Amino-5-bromo-3-cyanophenyl β-D-Glucopyranosiduronic Acid (3) An aqueous solution (23.4 ml) of 1 N NaOH was added dropwise to a solution of **16** (1.9 g, 3.6 mmol) in acetone (60 ml) and the mixture was stirred at room temperature for 15 min. Dowex 50-X4 (H^+ form) was added to remove sodium ion and the filtrate obtained was concentrated *in vacuo* to a small volume. The residue was taken up in EtOH and the mixture was kept standing at room temperature to afford crude crystalline **3**. Recrystallization of the crude product **3** from 80% EtOH afforded **3** (770 mg, 55.3%) as a colorless powder, mp 231—232 °C. $[\alpha]_D$ −79.6° (c=0.27, DMSO). ^{1}H-NMR (DMSO-d_6–D_2O) δ: 3.37—3.58 (3H, m), 3.94 (1H, d, J=9.4 Hz, C<u>H</u>CO_2CH_3), 4.88—4.96 (1H, m), 7.29 (1H, d, J=2.1 Hz, arom.), 7.32 (1H, d, J=2.1 Hz, arom.). IR (KBr) cm^{-1}: 3527, 3344, 2232, 1690, 1624, 1572. SIMS m/z: 391 $(M+1+H)^+$, 389 $(M-1+H)^+$. *Anal.* Calcd for $C_{13}H_{13}BrN_2O_7$: C, 40.12; H, 3.36; N, 7.19. Found: C, 40.30; H, 3.40; N, 6.92.

5-Acetoxy-2-nitrobenzonitrile (18) A solution of 5-hydroxy-2-nitrobenzaldehyde **17** (15 g, 90 mmol) in pyridine (7.5 ml) was treated with $NH_2OH \cdot HCl$ (6.8 g, 98 mmol) and the mixture was stirred at 100 °C for 2 h. After cooling, the mixture was diluted with 5% aqueous HCl and extracted with AcOEt. The extracts were concentrated and the residue was mixed with acetic anhydride (60 ml) and sodium acetate (2.0 g, 24 mmol). This mixture was stirred at 130 °C for 1.5 h. After evaporation of the solvent, the residue was diluted with water, and extracted with CH_2Cl_2. The organic solution was washed with 10% aqueous sodium carbonate and saturated brine, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30 : 1) to afford **18** (10.1 g, 54.6%) as a colorless powder, mp 96—98 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s, $COCH_3$), 7.59 (1H, dd, J=9.2, 2.6 Hz, arom. 4-H), 7.71 (1H, d, J=2.6 Hz, arom. 5-H), 8.37 (1H, d, J=9.2 Hz, arom. 3-H). IR (KBr) cm^{-1}: 3120, 3076, 2236, 1770, 1612, 1586, 1540. MS m/z: 206 (M^+). *Anal.* Calcd for $C_9H_6N_2O_4$: C, 52.44; H, 2.93; N, 13.59. Found: C, 52.62; H, 2.86; N, 13.39.

5-Acetoxy-2-aminobenzonitrile (19) By means of the procedure described for the preparation of **10**, **18** was converted into **19** (70.3%), which was obtained after chromatographic purification (AcOEt–hexane, 3 : 1) as a colorless powder, mp 96—98 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s, $COCH_3$), 4.34 (2H, br s, NH_2), 6.73 (1H, d, J=8.9 Hz, arom. 3-H), 7.08 (1H, dd, J=8.9, 2.6 Hz, arom. 4-H), 7.13 (1H, d, J=2.6 Hz, arom. 5-H). IR (KBr) cm^{-1}: 3488, 3388, 2216, 1758, 1640, 1500. MS m/z: 176 (M^+). *Anal.* Calcd for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.50; H, 4.52; N, 15.66.

Diethyl 4-[(4-Acetoxy-2-cyanophenyl)carbamoyl]benzylphosphonate (20) A solution of 4-[(diethoxyphosphoryl)methyl)]benzoic acid (1.6 g, 5.7 mmol), DMF (0.3 g) and $SOCl_2$ (0.5 ml) in CH_2Cl_2 (10 ml) was heated under reflux for 3 h and evaporated *in vacuo* to afford 4-[(diethoxyphosphoryl)methyl]benzoyl chloride. A solution of 4-[(diethoxyphosphoryl)methyl]benzoyl chloride in pyridine (4 ml) was added slowly to a stirred solution of **19** (0.5 g, 2.8 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 45 min. It was then diluted with water, and extracted with CH_2Cl_2. The organic solution was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried ($MgSO_4$) and concentrated to give crude crystalline **20**. Recrystallization of the crude product from CH_2Cl_2–Et_2O provided **20** (0.75 g, 62.3%) as a colorless powder, mp 186—188 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (6H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 2.33 (3H, s, $COCH_3$), 3.24 (2H, d, J=22.1 Hz, CH_2P=O), 4.02—4.10 (4H, m), 7.38—7.51 (4H, m), 7.89 (2H, d, J=7.6 Hz, arom. 3-H), 8.43 (1H, s, NHCO), 8.58 (1H, d, J=8.3 Hz, arom. (6)-H). IR (KBr) cm^{-1}: 3284, 2228, 1774, 1678, 1612, 1512. MS m/z: 430 (M^+). *Anal.* Calcd for $C_{21}H_{23}N_2O_6P$: C, 58.61; H, 5.39; N, 6.51. Found: C, 58.38; H, 5.36; N, 6.26.

Diethyl 4-[(2-Cyano-4-hydroxyphenyl)carbamoyl]benzylphosphonate (4) Aqueous NaOH (2 N, 7.6 ml) was added to a solution of **20** (2.2 g, 5.1 mmol) in CH_3CN (50 ml). After having been stirred at room temperature for 1 h, the mixture was acidified with 10% HCl. Evaporation of the solvent followed by recrystallization from CH_2Cl_2 gave **4** (1.6 g, 80.5%) as a colorless powder, mp 200—203 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (6H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 3.24 (2H, d, J=22.1 Hz, CH_2P=O), 4.08—4.20 (4H, m), 6.78 (1H, d, J=2.8 Hz, arom. (3)-H), 6.88 (1H, dd, J=8.9, 2.8 Hz, arom. (5)-H), 7.33 (2H, dd, J=8.2, 2.3 Hz, arom. 2-H), 7.73 (2H, d, J=8.2 Hz, arom. 3-H), 7.81 (1H, d, J=8.9 Hz, arom. (6)-H), 8.35 (1H, s), 8.98 (1H, s). IR (KBr) cm^{-1}: 3148, 2224, 1678, 1538, 1512. MS m/z: 388 (M^+). *Anal.* Calcd for $C_{19}H_{21}N_2O_5P$: C, 58.76; H, 5.45; N, 7.21. Found: C, 58.85; H, 5.44; N, 7.05.

Methyl (3-Cyano-4-nitrophenyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (23) By means of the procedure described for the preparation of **14**, 5-hydroxy-2-nitrobenzonitrile **22** was converted into **23** (46.8%), which was obtained after chromatographic purification (AcOEt–hexane, 1 : 2) as a colorless powder, mp 157—159 °C. $[\alpha]_D$ −57.7° (c=0.26, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s, $COCH_3$), 2.09 (6H, s, $COCH_3$), 3.73 (3H, s, CO_2CH_3), 4.31 (1H, d, J=8.2 Hz, C<u>H</u>CO_2CH_3), 5.29—5.41 (4H, m), 7.35 (1H, dd, J=9.2, 2.6 Hz, arom. 4-H), 7.47 (1H, d, J=2.6 Hz, arom. 6-H), 8.33 (1H, d, J=9.2 Hz, arom. 3-H). IR (KBr) cm^{-1}: 3480, 3116, 2956, 2236, 1756, 1588, 1534. MS m/z: 421 $(M-CO_2Me)^+$. *Anal.* Calcd for $C_{20}H_{20}N_2O_{12}$: C, 50.01; H, 4.20; N, 5.83. Found: C, 50.07; H, 4.13; N, 5.65.

Methyl (4-Amino-3-cyanophenyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (24) By means of the procedure described for the prepatation of **10**, **23** was converted into **24** (83.0%), which was obtained after chromatographic purification ($CHCl_3$–MeOH, 30 : 1) as a colorless powder, mp 153—155 °C. $[\alpha]_D$ −37.0° (c=0.27, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.04 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 3.77 (3H, s, CO_2CH_3), 4.12 (1H, d, J=9.3 Hz, C<u>H</u>CO_2CH_3), 4.27 (2H, s, NH_2), 4.95 (1H, d, J=7.3 Hz, O–C<u>H</u>–O), 5.18—5.33 (3H, m), 6.68 (1H, d, J=8.0 Hz, arom. 3-H), 7.04—7.08 (2H, m). IR (KBr) cm^{-1}: 3402, 2960, 2212, 1748, 1628, 1506. MS m/z: 391 $(M-CO_2Me)^+$. *Anal.* Calcd for $C_{20}H_{22}N_2O_{10}$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.21; H, 4.91; N, 6.02.

Methyl {3-Cyano-4-[4-(diethoxyphosphorylmethyl)benzoylamino]-phenyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid}uronate (25) By means of the procedure described for the preparation of **20**, **24** was converted into **25** (68.0%), which was obtained after chromatographic purification ($CHCl_3$–AcOEt, 25 : 1) as a colorless powder, mp 80—83 °C. $[\alpha]_D$ −27.9° (c=0.40, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (6H, t, J=7.3 Hz, $OCH_2C\underline{H}_3$), 2.06 (6H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 3.23 (2H, d, J=22.1 Hz, CH_2P=O), 5.14 (1H, d, J=6.9 Hz, O–C<u>H</u>–O), 5.24—5.40 (3H, m), 7.24—7.34 (2H, m), 7.46 (2H, dd, J=8.3, 2.3 Hz, arom. 2-H), 7.88 (2H, d, J=8.3 Hz, arom. 3-H), 8.30 (1H, s), 8.47 (1H, d, J=9.2 Hz, arom. (6)-H). IR (KBr) cm^{-1}: 2232, 1758, 1670. SIMS m/z: 705 (M^+). *Anal.* Calcd for $C_{32}H_{37}N_2O_{14}P \cdot H_2O$: C, 53.18; H, 5.44; N, 3.88. Found: C, 53.68; H, 5.31; N, 3.79.

3-Cyano-4-[4-(diethoxyphosphorylmethyl)benzoylamino]phenyl β-D-Glucopyranosiduronic Acid (5) By means of the procedure described for the preparation of **3**, **25** was converted into **5** (67.0%), which was recrystallized from EtOH–$CHCl_3$–AcOEt to afford a colorless powder, mp 82—85 °C. $[\alpha]_D$ −45.0° (c=0.41, DMSO). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (6H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 3.48—3.68 (3H, m), 3.99—4.15 (5H, m), 5.07 (1H, d, J=7.6 Hz, O–C<u>H</u>–O), 7.40—7.53 (4H, m), 7.58 (1H, d, J=7.6 Hz, arom. (6)-H), 7.95 (2H, d, J=8.0 Hz, arom. 3-H). IR (KBr) cm^{-1}: 3392, 2232, 1732, 1658, 1506. SIMS m/z: 565 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{29}N_2O_{11}P \cdot H_2O$: C, 51.55; H, 5.36; N, 4.81. Found: C, 51.99; H, 5.38; N, 4.61.

Ethyl 4-[(2-Cyanophenyl)carbamoyl]benzylphosphonate (6) Diethyl 4-[(2-cyanophenyl)carbamoyl]benzylphosphonate **26** (10.0 g, 26.9 mmol) was dissolved in CH_3CN (150 ml), then LiBr (11.7 g, 136 mmol) was added, and the reaction mixture was heated under reflux overnight. The lithium salt, which was isolated by filtration, was dissolved in H_2O, and 3 N HCl was added to give a crude crystalline **6**. Recrystallization of the crude product from EtOH afforded (3.4 g, 36.9%) as a colorless powder, mp 227—229 °C. ^{1}H-NMR (CD_3OD) δ: 1.27 (3H, t, J=7.3 Hz, $OCH_2C\underline{H}_3$), 3.28 (2H, d, J=22.1 Hz, CH_2P=O), 3.97—4.08 (2H, m), 7.40—7.45 (1H, m), 7.49 (2H, dd, J=8.2, 2.3 Hz, arom. 2-H), 7.69—7.72 (2H, m), 7.77 (1H, d, J=6.9 Hz, arom.), 7.95 (2H, d, J=8.2 Hz, arom. 3-H). IR (KBr) cm^{-1}: 2216, 1682, 1608, 1584, 1534, 1512. MS m/z: 344 (M^+). *Anal.* Calcd for $C_{17}H_{17}N_2O_4P \cdot 1/2H_2O$: C, 57.79; H, 5.13; N, 7.93. Found: C, 58.03; H, 4.96; N, 7.83.

Biological Methods Male Sprague–Dawley rats were obtained from Charles River Japan Inc., and used at 7 weeks, when they weighed

200—220 g. Triton WR-1339 was purchased from Ruger Chemical Co. (New York, U.S.A.). The rats were injected *via* the tail vein with 200 mg of Triton WR-1339 in 0.9% sodium chloride, and then a test drug, which was suspended in 5% gum arabic, was administered to the rats *via* a gastric tube at a dose of 10 mg/kg body weight. Blood samples were collected 24 h later, and plasma lipids were measured. Plasma triglycerides were determined by a conventional enzymatic method.

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6) HPLC analysis of each metabolite and the corresponding synthetic compound showed an identical retention time (**2**: $t_R = 13.5$ min, **3**: $t_R = 11.7$ min, **4**: $t_R = 29.9$ min, **5**: $t_R = 12.7$ min, **6**: $t_R = 10.1$ min).
Chromatographic conditions: column, TSK gel ODS-120A (4.6 i.d. × 250 mm, Tosoh); elution, gradient of 50 mM phosphate buffer (pH 2.20) to CH_3CN: solvent flow rate, 1.2 ml/min; detection at 260 nm.
7) Shortly afterward, the structure of **2** was confirmed by an X-ray crystallographic analysis (Fig. 2).

X-ray study of **2**: crystal size = 0.2 × 0.20 × 0.25 mm, Rigaku AFC diffractometer (45 kV, 200 mA), CuK_α radiation ($\lambda = 1.54178$ Å), $a = 20.499$ (4), $b = 4.976$ (1), $c = 12.495$ (2) Å, $\alpha = 101.280$ (2)°, $V = 1249.9$ (5) Å^3, space group $P2_1/a$, $Z = 4$, Dcalc = 1.59 g/cm^3, ω scan mode, scan speed of 3° min^{-1}, measured reflections = 2589, reflections used for refinement = 2039 [$I_O > 3\sigma(I_O)$]. The final R value was 0.042 ($R_w = 0.80$).

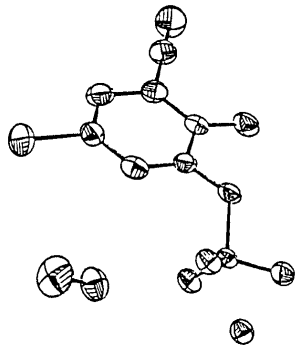

Fig. 2. ORTEP Drawing of **2**

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