Synthesis of Methyl 1-*O*-(4-Hydroxymethamphetaminyl)-α-D-glucopyranouronate

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For the purpose of the direct characterization of the intact conjugated form in the urine of a methamphetamine (MA) abuser, 4-hydroxymethamphetamine (4-OHMA) glucuronate, corresponding to one of the metabolites of MA, was synthesized from the commercially available methyl 4-hydroxyphenylacetate.

Key words 4-hydroxymethamphetamine; glucuronate; synthesis

Drug abuse has become a serious problem and is increasingly widespread in the world. In Japan, methamphetamine (MA, **1**) is the most frequently abused drug. Nowadays, arrests for violation of the Stimulant Control Law are on the verge of 20000. A MA addict is identified by the detection of unchanged MA (**1**) and its metabolite amphetamine (AP, **2**), then MA (**1**) analysis in urine samples is carried out routinely by a standard screening test and thin layer chromatography (TLC) method, followed by gas chromatography/mass spectrometry (GC/MS). Recently, the drug abuse situation has been internationalized. It is possible that AP (**2**) and/or a variety of analogues are imported from overseas. MA (**1**) is metabolized by way of two pathways, either by hydroxylation of the aromatic ring or demethylation of the side chain. It has been reported that the metabolites of MA (**1**) in urine were composed of the unchanged drug (18—27%), the free **3** and the conjugated forms (14—16%) of 4-hydroxymethamphetamine (4-OHMA, **3**) and AP (**2**) (2—3%).[1] It has been reported that analysis of a conjugated form, such as glucuronide **4** of 4-OHMA (**3**), in the urine samples of MA (**1**) abusers were carried out by comparison of the ratios of between free 4-OHMA (**3**) obtained by β-glucuronidase or HCl treatment, and the total 4-OHMA (**3**).[2,3] Direct characterization of the intact conjugated form of **3** has not been reported so far.

In the present paper, we describe the synthesis of 4-OHMA glucuronide **4** for the direct characterization of an intact conjugated form in the metabolism of MA (**1**) abusers without hydrolysis. Moreover, 4-OHMA (**3**) as a metabolite of MA (**1**) is on the market, but it is an expensive product. Buzas *et al.* have synthesized 4-OHMA (**3**) from a controlled substance by Stimulants Raw Material in Japan,[4] therefore, we now report the synthesis of glucuronate (**5**) of 4-OHMA (**3**) corresponding the above-mentioned glucuronide (**4**) by unregulated materials.

The synthesis of glucuronide congener (**5**) is shown in Charts 2—4.

Synthesis of 4-Hydroxy-*N*-benzylmethamphetamine (±)-13 Silylation of the commercially available methyl 4-hydroxyphenylacetate (**6**) gave the corresponding *tert*-butyldimethylsilyl (TBDMS)-ether **7** in 95% yield, which was reduced with $LiAlH_4$ to afford the primary alcohol **8** in 99% yield. Pyridinium chlorochromate (PCC) oxidation of **8** yielded an aldehyde **9**, which was used for the next reaction without further purification. The aldehyde **9** was treated with methyllithium (MeLi) gave the secondary alcohol (±)-**10** in 42% from **8**. Swern's oxidation of (±)-**10** afforded a ketone **11** in 83% yield. The reaction of **11** and *N*-benzyl *N*-methylamine in the presence of 1.8 M HCl/MeOH, followed by reduction with cyanoborohydride ($NaBH_3CN$), gave the tertiary amine (±)-**12** in 51% yield. Deprotection of the silyl group of (±)-**12** with 1 M H_2SO_4 provided the desired 4-hydroxy-*N*-benzylmethamphetamine (±)-**13** in 83% yield.

Synthesis of Glucuronide Imidate 18 Treatment of the commercially available D-(+)-glucuronolactone (**14**) with MeOH in the presence of triethylamine (Et_3N), followed by subjection to acetylation, gave both tetraacetyl-α-glucuronate **15** (25%) and β-glucuronate **16** (38%). By applying the reported procedure,[5] α-glucuronate **15** and β-glucuronate **16** were independently treated with tributyltin methoxide (Bu_3SnOMe) to provide the triacetyl-α-glucuronate **17** (60%), and (**17**) (99%), respectively. By applying the reported procedure,[6] the reaction of **17** with K_2CO_3 in the presence of molecular sieves (MS, 3 Å), followed by

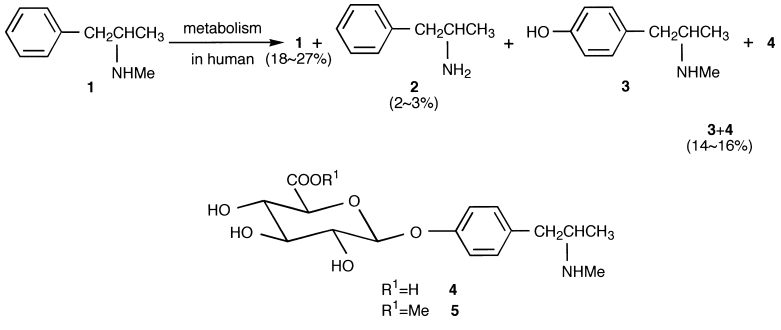

Chart 1

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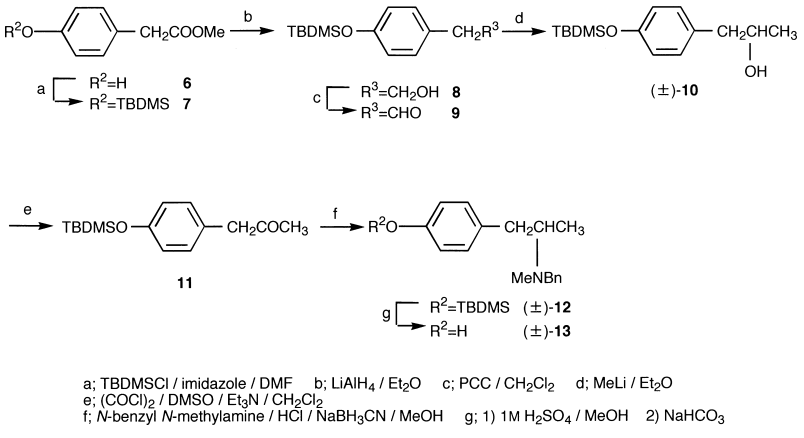

Chart 2

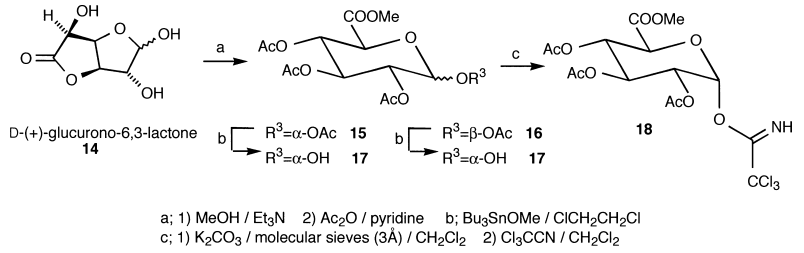

Chart 3

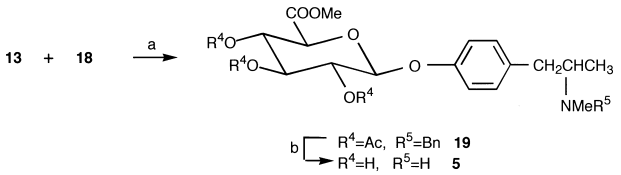

Chart 4

treatment with trichloroacetonitrile (CCl_3CN), provided the desired α-imidate **18** in 75%.

Coupling Reaction between 4-Hydroxy-*N*-benzylmethamphetamine (±)-13 and α-Imidate 18 The reaction of phenol **13** and α-imidate **18** in the presence of $BF_3 \cdot Et_2O$ afforded the coupled product **19** as a diastereomeric mixture in 97% yield. Deprotection of the acetyl group of **19** with 1 M NaOH gave the deacetylated compound along with the partial hydrolysis of a methyl ester group, which was used for the next reaction without further purification. This mixture was subjected hydrogenolysis in the presence of 10% $Pd(OH)_2$–C to provide a debenzylated mixture which was treated with MeOH in the presence of 1.8 M HCl/MeOH for the purpose of the esterification of the partially generated carboxylic acid to give the desired compound **5** in 57% yield.

In conclusion, for the purpose of the direct characterization of the intact conjugated form (glucuronide congener) in the urine samples of a methamphetamine (MA) abuser, the diastereomeric mixture of glucuronate (**5**) of 4-hydroxymethamphetamine (**3**) corresponding to one of the metabolites (**4**) of MA, was synthesized from the commercially available methyl 4-hydroxyphenylacetate (**6**). Treatment of the diastereomeric mixture of glucuronates (**5**) under an alkaline condition should afford the corresponding glucuronide (**4**), which should be identical with the intact conjugated form. Methyl 4-hydroxyphenylacetate (**6**) was converted to 4-hydroxy-*N*-benzylmethamphetamine ((±)-**13**) in 7 steps. Coupling reaction of (±)-**13** and methyl 2,3,4-triacetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucopyranouronate (**18**) derived from D-(+)-glucurono-6,3-lactone (**14**) in the presence of $BF_3 \cdot Et_2O$ afforded the glucuronide congener **19**, which was subjected to deprotection to give the methamphetamine glucuronate (**5**).

Experimental

^{1}H-NMR spectra were recorded by a JEOL AL 400 spectrometer (Tokyo, Japan). Spectra were taken with 5—10% (w/v) solution in $CDCl_3$ with Me_4Si as an internal reference. The fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS-600H (matrix; dithiothreitol : α-thioglycerol=1 : 1 mixture) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrophotometer. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

Methyl 4-*tert*-Butyldimethylsiloxyphenylacetone (7) A mixture of methyl 4-hydroxyphenylacetate (**6**; 10.00 g, 60 mmol), imidazole (12.25 g, 180 mmol) and *tert*-butyldimethylsilylchloride (TBDMSCl; 13.57 g, 90 mmol) in *N*,*N*-dimethylformamide (DMF; 60 ml) was stirred for 30 min at 0 °C. The reaction mixture was diluted with H_2O and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was chromatographed on silica gel (400 g, hexane/AcOEt=20 : 1) to afford **7** (15.99 g, 95%) as a colorless oil.

IR (neat) cm^{-1}: 2950, 1741, 1511. ^{1}H-NMR δ: 0.17 (6H, s), 0.96 (9H, s), 3.53 (2H, s), 3.67 (3H, s), 6.76 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz). *Anal.* Calcd for $C_{15}H_{24}SiO_3$: C, 64.24; H, 8.63. Found: C, 63.96; H, 8.58. FAB-MS (*m*/*z*): M^+ 280.

2-(4-*tert*-Butyldimethylsiloxyphenyl) Ethanol (8) To a stirred suspension of lithium aluminium hydride ($LiAlH_4$; 1.40 g, 37 mmol) in Et_2O (60 ml) at 0 °C was added dropwise a solution of **7** (10.38 g, 37 mmol) in

Et_2O (100 ml), and the reaction mixture was stirred for 1.75 h at room temperature. The reaction mixture was treated with acetone (6 ml) and H_2O, and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (195 g, hexane/AcOEt=5 : 1) to give **8** (9.26 g, 99%) as a colorless oil.

IR (neat) cm^{-1}: 3339, 2938, 1509. ^{1}H-NMR δ: 0.18 (6H, s), 0.97 (9H, s), 1.54 (1H, s), 2.78 (2H, t, J=6.0 Hz), 3.79 (2H, t, J=6.0 Hz), 6.77 (2H, d, J=8.0 Hz), 7.06 (2H, d, J=8.0 Hz). *Anal.* Calcd for $C_{14}H_{24}SiO_2$: C, 66.61; H, 9.58. Found: C, 66.80; H, 9.69. FAB-MS (m/z): M^+ 252.

2-(4-*tert*-Butyldimethylsiloxyphenyl) Acetaldehyde (9) A solution of **8** (4.50 g, 18 mmol) in CH_2Cl_2 (10 ml) was added to a mixture of pyridinium chlorochromate (PCC; 7.76 g, 36 mmol) in CH_2Cl_2 (100 ml) and celite (18 g) at 0 °C, and the reaction mixture was stirred for 5 h at the same temperature. The reaction mixture was filtered with the aid of celite and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (100 g, hexane/AcOEt=10 : 1) to give **9** (3.59 g) as a colorless oil.

^{1}H-NMR δ: 0.18 (6H, s), 0.97 (9H, s), 3.59 (2H, d, J=2.0 Hz), 6.82 (2H, d, J=8.0 Hz), 7.05 (2H, d, J=8.0 Hz), 9.70 (1H, t, J=2.0 Hz).

2-(4-*tert*-Butyldimethylsiloxyphenyl) Propanol ((±)-10) To a stirred solution of **9** (3.59 g, 14 mmol) in Et_2O (100 ml) was added dropwise 1 M methyllithium (MeLi)–Et_2O solution (14 ml, 14 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 1 h at the same temperature and quenched by the addition of aqueous ammonium chloride (NH_4Cl). The mixture was extracted with Et_2O and the organic layer was washed with brine (6 ml), and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (75 g, hexane/AcOEt=20 : 1) to provide **10** (1.99 g, 42%, from **8**) as a colorless oil.

IR (neat) cm^{-1}: 3361, 2934, 1509. ^{1}H-NMR δ: 0.18 (6H, s), 0.97 (9H, s), 1.20 (3H, d, J=6.0 Hz), 1.64 (1H, br s), 2.60 (1H, dd, J=8.0, 14.0 Hz), 2.69 (1H, dd, J=4.0, 14.0 Hz), 3.94 (1H, qdd, J=4.0, 6.0, 8.0 Hz), 6.77 (2H, d, J=8.0 Hz), 7.04 (2H, d, J=8.0 Hz). *Anal.* Calcd for $C_{15}H_{26}SiO_2$: C, 67.62; H, 9.84. Found: C, 67.60; H, 9.98. FAB-MS (m/z): M^+ 266.

2-(4-*tert*-Butyldimethylsiloxyphenyl) Propanone (11) Dimethyl sulfoxide (DMSO; 1.25 g, 16 mmol) was added to a stirred solution of oxalyl chloride ($(COCl)_2$; 1.02 g, 8 mmol) in CH_2Cl_2 (40 ml) at −78 °C under argon. After 10 min, a solution of **10** (1.01 g, 4 mmol) in CH_2Cl_2 (8 ml) was added to the above mentioned reaction mixture and the mixture was kept at the same temperature for 1 h. Triethylamine (Et_3N; 3.24 g, 32 mmol) was added to the reaction mixture and the whole mixture was warmed to −20 °C for 1 h. The whole mixture was diluted with brine (40 ml) and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (25 g, hexane/AcOEt=20 : 1) to give **11** (0.88 g, 83%) as a colorless oil.

IR (neat) cm^{-1}: 2938, 1715, 1509. ^{1}H-NMR δ: 0.17 (6H, s), 0.96 (9H, s), 2.10 (3H, s), 3.59 (2H, s), 6.78 (2H, d, J=8.0 Hz), 7.03 (2H, d, J=8.0 Hz). *Anal.* Calcd for $C_{15}H_{24}SiO_2$: C, 68.13; H, 9.15. Found: C, 68.00; H, 9.26. FAB-MS (m/z): $[M+H]^+$ 265.

1-(4-*tert*-Butyldimethylsiloxyphenyl)-2-*N*-benzyl-*N*-methylaminopropane ((±)-12) To a solution of *N*-methylbenzylamine (2.04 g, 16.8 mmol) and 1.8 M HCl/MeOH solution (3.1 ml, 5.6 mmol) in MeOH (5 ml) was added a solution of **11** (0.73 g, 2.8 mmol) in MeOH (5 ml) at 0 °C. Cyanoborohydride ($NaBH_3CN$; 0.35 g, 5.6 mmol) was added to the above reaction mixture, and the whole mixture was stirred for 24 h at room temperature. The reaction mixture was treated with $NaHCO_3$ (0.47 g) and concentrated to give a residue. It was diluted with H_2O and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude residue, which was chromatographed on silica gel (60 g, benzene/AcOEt=10 : 1) to give **12** (0.15 g, 51%) as a colorless oil.

IR (neat) cm^{-1}: 2934, 1506, 1258. ^{1}H-NMR δ: 0.19 (6H, s), 0.97 (3H, d, J=5.0 Hz), 0.98 (9H, s), 2.22 (3H, s), 2.42 (1H, dd, J=16.0, 12.0 Hz), 2.91 (1H, dd, J=16.0, 6.0 Hz), 2.94 (1H, qdd, J=12.0, 6.0, 6.0 Hz), 3.56 (1H, d, J=14.0 Hz), 3.60 (1H, d, J=14.0 Hz), 6.74 (2H, d, J=8.0 Hz), 6.99 (2H, d, J=8.0 Hz), 7.18—7.31 (5H, m). *Anal.* Calcd for $C_{23}H_{35}SiNO$: C, 74.74; H, 9.54; N, 3.79. Found: C, 74.55; H, 9.68; N, 3.51. FAB-MS (m/z): $[M+H]^+$ 370.

1-(4-Hydroxyphenyl)-2-*N*-benzyl-*N*-methylaminopropane ((±)-13) A solution of **12** (0.60 g, 1.6 mmol) and 1 M H_2SO_4 solution (4 ml) in MeOH (8 ml) was stirred for 3.5 h at room temperature, then the reaction mixture was treated with $NaHCO_3$ (0.21 g). The reaction mixture was diluted with H_2O and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to provide a residue, which was chromatographed on silica gel (30 g, benzene/AcOEt=20 : 1) to give **13** (0.34 g, 83%) as a colorless oil.

IR (KBr) cm^{-1}: 3404, 1608, 1509, 1243. ^{1}H-NMR δ: 0.98 (3H, s), 2.23 (3H, s), 2.40 (1H, dd, J=15.0, 10.0 Hz), 2.91 (1H, qdd, J=6.0, 10.0, 6.0 Hz), 2.94 (1H, dd, J=15.0, 6.0 Hz), 3.59 (1H, d, J=14.0 Hz), 3.62 (1H, d, J=14.0 Hz), 6.71 (2H, d, J=8.0 Hz), 6.98 (2H, d, J=8.0 Hz), 7.18—7.31 (5H, m). *Anal.* Calcd for $C_{17}H_{21}NO$: C, 79.96; H, 8.29; N, 5.49. Found: C, 79.56; H, 8.38; N, 5.46. FAB-MS (m/z): $[M+H]^+$ 256.

Methyl 1,2,3,4-Tetraacetyl-α- and β-D-glucopyranuronates (15, 16) A solution of glucuronolactone (**14**; 8.0 g, 45 mmol) and Et_3N (0.8 ml) in MeOH (60 ml) was stirred for 2 h at room temperature, and then MeOH was removed *in vacuo*. The resulting syrup was dissolved in pyridine (20 ml) and acetic anhydride (30 ml). The mixture was allowed to stand in the refrigerator for 5 d, then concentrated *in vacuo*. Et_2O was added to the resulting residue, and the less soluble product was filtered to give a solid (β-isomer **16**). The mother liquor was evaporated to give a crude oil (13.82 g), which was chromatographed on silica gel (50 g, hexane/AcOEt=3 : 1) to yield **16** (6.43 g, 38%). The NMR data of **16** were identical with those of the reported **16**.[7] The crude oil from the mother liquor was again chromatographed on silica gel (160 g, hexane/AcOEt=4 : 1) to provide **15** (4.30 g, 25% as α-isomer).

15: ^{1}H-NMR δ: 1.97 (3H, s), 2.00 (3H, s), 2.00 (3H, s), 2.14 (3H, s), 3.70 (3H, s), 4.37 (1H, d, J=10.0 Hz), 5.07 (1H, dd, J=10.0, 4.0 Hz), 5.18 (1H, dd, J=10.0, 10.0 Hz), 5.47 (1H, dd, J=10.0, 10.0 Hz), 6.35 (1H, d, J=4.0 Hz).

Methyl 2,3,4-Triacetyl-α-D-glucopyranuronate (17) (1) A mixture of **15** (2.0 g, 5.3 mmol) and tributyltin methoxide (Bu_3SnOMe; 1.57 ml, 5.3 mmol) in $ClCH_2CH_2Cl$ (30 ml) was stirred for 5 h at 90 °C, and the whole mixture was evaporated to give a crude residue. It was chromatographed on silica gel (70 g, hexane/AcOEt=3 : 1) to give **17** (1.06 g, 60%) as a colorless oil. The NMR data of **17** were identical with those of the reported **17**.[8]

(2) A mixture of **16** (1.0 g, 2.7 mmol) and Bu_3SnOMe (0.8 ml, 2.7 mmol) in $ClCH_2CH_2Cl$ (15 ml) was stirred for 2 h at 90 °C. The reaction mixture was worked up in the same way as for **15** to give **17** (0.90 g, 99%). The NMR data of **17** were identical with those of the reported **17**.[8]

Methyl 2,3,4-Triacetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucopyranouronate (18) A mixture of **17** (1.50 g, 4 mmol), K_2CO_3 (0.94 g, 6.8 mmol) and molecular sieves (MS 3 Å, 0.5 g) in CH_2Cl_2 (5 ml) under an argon atmosphere was stirred for 25 min at 0 °C. A solution of trichloroacetonitrile (Cl_3CCN; 1.73 g, 12 mmol) in CH_2Cl_2 (5 ml) was added to the above mentioned reaction mixture, and the whole mixture was stirred for 2 h at the same temperature. The reaction mixture, including a solid, was filtered and the filtrate was treated with 7% aqueous $NaHCO_3$, then concentrated *in vacuo*. The resulting residue was chromatographed on silica gel (50 g, hexane/AcOEt=5 : 1) to give **18** (1.44 g, 75%) as a solid. The NMR data of **18** were identical with those of the reported **18**.[6]

Coupling Reaction between (±)-13 and 18 A mixture of **13** (0.1214 g, 0.48 mmol), **18** (0.45 g, 0.94 mmol) and MS 3 Å (0.2 g) was dried under reduced pressure, and CH_2Cl_2 (10 ml) was added to the above mentioned mixture. The whole mixture was stirred for 10 min at room temperature under an argon atmosphere. Boron trifluoride ether complex ($BF_3 \cdot Et_2O$; 0.06 ml, 0.42 mmol) was added to the above mentioned whole mixture at 0 °C and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was filtered and the filtrate was washed with 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (20 g, benzene/AcOEt=5 : 1) to provide **19** (0.225 g, 97%) as a colorless oil.

IR (KBr) cm^{-1}: 1755, 1375. ^{1}H-NMR δ: 0.96 (3H, d, J=6.0 Hz), 2.22 (3H, s), 2.43 (1H, dd, J=16.0, 12.0 Hz), 2.92 (1H, dd, J=16.0, 6.0 Hz), 2.94 (1H, qdd, J=12.0, 6.0, 6.0 Hz), 3.56 (1H, d, J=14.0 Hz), 3.60 (1H, d, J=14.0 Hz), 3.71 (3H, s), 4.15 (1H, d, J=10.0 Hz), 5.09 (1H, d, J=8.0 Hz), 5.22—5.28 (1H, m), 5.29—5.35 (2H, m), 6.88 (2H, d, J=8.0 Hz), 7.05 (2H, d, J=8.0 Hz), 7.17—7.30 (5H, m). *Anal.* Calcd for $C_{30}H_{37}NO_{10}$: C, 63.04; H, 6.52; N, 2.45. Found: C, 63.26; H, 6.71; N, 2.19. FAB-MS (m/z): $[M+H]^+$ 572.

Methyl 1-*O*-(4-Hydroxymethamphetaminyl)-α-D-glucopyranouronate (5) A solution of **19** (0.1875 g, 0.3 mmol) and 1 M NaOH solution (2.4 ml) in MeOH (3 ml) was stirred for 1.5 h at 0 °C, and the reaction mixture was acidified with 1 M HCl (3 ml). The whole mixture was stirred for 20 min at 0 °C and evaporated *in vacuo*. The resulting residue was dissolved in MeOH and the precipitate was filtered. The filtrate was concentrated *in vacuo* to provide a residue which was dissolved in MeOH (2 ml). The MeOH solution was subjected to hydrogenolysis in the presence of 10% palladium hydroxide on carbon ($Pd(OH)_2$–C; 0.1 g) for 12 h at ambient temperature. The reac-

tion mixture was filtered and the filtrate was evaporated to provide a residue. The residue was dissolved in MeOH (1 ml) in the presence of MS 3 Å (0.15 g). 1.8 M HCl (gas)/MeOH solution (2 ml) and the whole mixture was stirred for 1 h at room temperature. The reaction mixture was filtered and the filtrate was treated with 7% aqueous $NaHCO_3$ (1 ml) and evaporated *in vacuo*. The resulting residue was chromatographed on silica gel (10 g, $CHCl_3$/MeOH=5 : 1) to give **5** (0.061 g, 57%) as a colorless oil.

IR (KBr) cm^{-1}: 3395, 1735, 1629, 1516, 1233. ^{1}H-NMR (CD_3OD, 60 °C, 400 MHz) δ: 1.24 (3H, d, J=7.0 Hz), 2.70 (3H, s), 2.75 (1H, dd, J=9.0, 14.0 Hz), 3.09 (1H, dd, J=6.0, 14.0 Hz), 3.42 (1H, qdd, J=7.0, 9.0, 6.0 Hz), 3.47—3.52 (2H, m), 3.64 (1H, dd, J=10.0, 10.0 Hz), 3.75 (3H, s), 4.00 (1H, d, J=10.0 Hz), 4.96 (1H, d, J=8.0 Hz), 7.05 (2H, d, J=9.0 Hz), 7.20 (2H, d, J=9.0 Hz). ^{13}C-NMR (CD_3OD, 45 °C, 125 MHz) δ: 15.85 (q), 31.05 (q), 39.53 (t), 52.93 (q), 57.99 (d), 73.01 (d), 74.68 (d), 76.83 (d), 77.31 (d), 102.56 (d), 118.50 (d), 131.29 (s), 131.57 (d), 158.15 (s), 170.95 (s). FAB-HR-MS (m/z) $C_{17}H_{26}NO_7$: Calcd 356.1709. Found. 356.1706.

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