# Synthesis and Antidiuretic Activities of Novel Glycoconjugates of Arginine-Vasopressin[1)]

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**Arginine-vasopressin (AVP) was acylated with various acyl azides (2a—j) in pH 9.1 buffer to give AVP derivatives (11a—j) modified at the tyrosine side chain with a carbohydrate *via* a spacer arm. Glycoconjugates of AVP modified at the N-terminal amide (12a—e) were also synthesized from AVP and carboxylic acids (3a—e) using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as coupling agent. Analogues (11a—j) exhibited greater *in vivo* antidiuretic activity than AVP. AVP and glycoconjugates (12a—e) were stable in rat plasma. On the other hand, glycoconjugates (11a—i) were found to readily convert to AVP according to first order kinetics. Hence, 11a—j are considered to be prodrugs of AVP.**

**Key words** arginine-vasopressin; glycoconjugate; antidiuretic activity; Brattleboro rat

The development of medicinal peptides or proteins holds great promise for future therapeutics, but has been severely limited by problems of low oral bioavailability and short plasma half-lives.[4)] Carbohydrate moieties in glycoproteins have been shown to play an important role,[5)] leading to dramatic changes in activity, stability, and metabolism of various glycosylated peptide drugs.[6)] A number of glycoconjugates of protein,[7)] poly(L-glutamic acid)[8)] and poly(L-lysine),[9)] containing an alkylene spacer arm between the polymer and the carbohydrate, have been also reported.

Arginine-vasopressin (AVP, Chart 1) is an antidiuretic hormone.[10)] The half life of AVP *in vivo* is very short.[11)] Many studies have been described on the effects of structural change of AVP.[12)] In our previous paper,[13)] we reported AVP derivatives modified at the glutamine side chain amide and at the C-terminal amide with carbohydrate *via* an alkylene spacer arm. In this work, to investigate the influence of the position of glycosylation on antidiuretic activities of AVP derivatives, we synthesized glycosylated derivatives modified at the phenolic hydroxy group of the tyrosine side chain and the N-terminal amine. Antidiuretic activities and stabilities in rat plasma were determined.

## Results and Discussion

**Synthesis of Glycoconjugates of AVP** Glycopeptides are usually synthesized stepwise using glycosylated amino acids or by linking suitably protected peptides with carbohydrate derivatives.[14)] We used non-protected AVP for coupling to carbohydrate derivatives *via* an acyl bond. Hydrazides (**1a—j**) as precursors of acyl azides (**2a—j**) and carboxylic acids (**3a—e**) were used for modification of AVP, and are shown in Chart 2.

Hydrazides **1a**, **b**[15)] and **1d—f**[9)] are known compounds. The preparation of hydrazides **1c** and **1g—j** was as follows. In the first step, methyl 4-[2-[2-(2-hydroxyethoxy)ethoxy]-ethoxy]butanoate (**5**) was prepared (Chart 3). Swern oxidation of 2-[2-[2-(*tert*-butyldimethylsilyloxy)ethoxy]ethoxy]-ethanol,[16)] followed by Horner–Emmons reaction gave **6**. Hydrogenation and desilylation of **6** gave **5**. Condensation of 1,2,3,4,6-penta-*O*-acetyl-$\alpha$-D-glucopyranose with 8-(methoxycarbonyl)octanol (**7**)[17)] and **5** promoted by boron trifluoride etherate[18)] gave $\beta$-glycosides **8c** and **8i**, respectively. Reac-


AVP

Chart 1

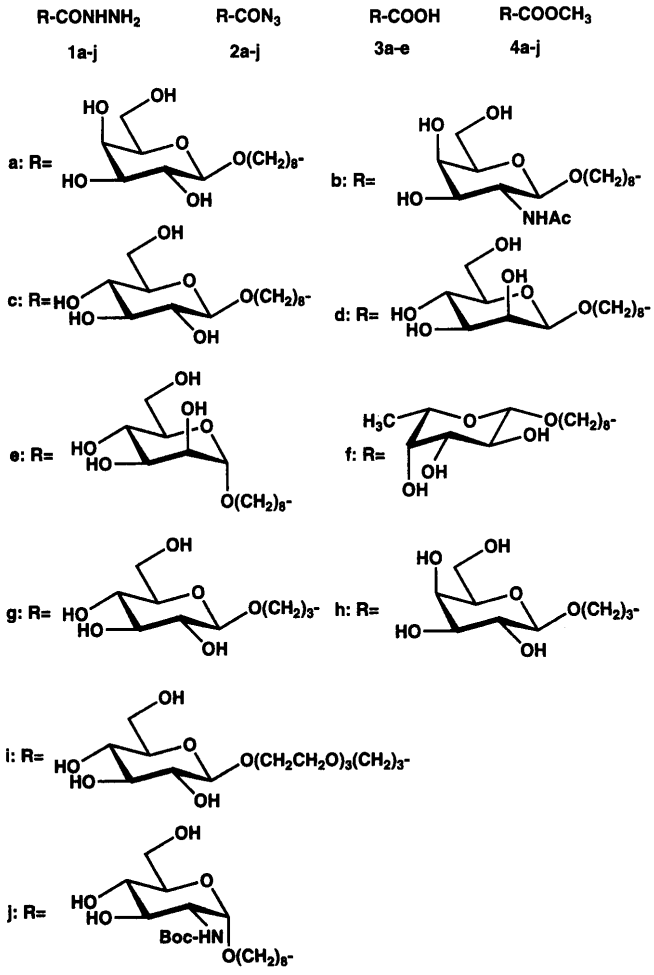


Chart 2

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tion of 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-glucopyranosyl bromide and 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-galactopyranosyl bromide with 3-(benzyloxycarbonyl)propanol[19] in the presence of silver carbonate gave $\beta$-glycosides **8g** and **8h**, respectively (Chart 4). $\alpha$-Selective glycosidation of glucosamine derivative **9**[20] with **7**, promoted by zinc triflate and trimethylsilyl bromide[21] gave **10**. The Troc group of **10** was replaced with a Boc group by zinc treatment in acetic acid followed by reaction with di-*tert*-butyl dicarbonate to give **8j** (Chart 5). Treatment of **8c** and **8g—j** with sodium methoxide in methanol gave the *O*-deacetylated products **4c** and **4g—j**, respectively. $^1$H-NMR spectra of **4g** and **4h** revealed that they were methyl esters formed by ester exchange reaction. Conversion of the methyl ester group of **4c** and **4g—j** by treatment with hydrazine monohydrate gave hydrazides **1c** and **1g—j**, respectively.

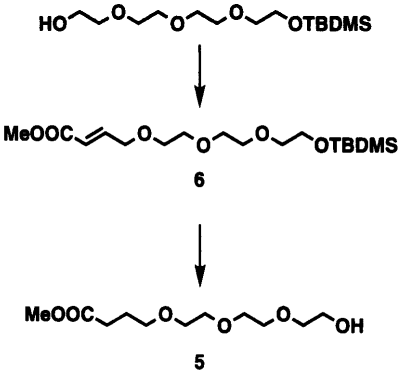


Chart 3

Carboxylic acids **3a—e** were prepared by alkaline hydrolysis of methyl esters **4a**, **b**[15], **4c** and **4d**, **e**,[19] respectively.

A solution of AVP in borate buffer (pH 9.1) reacted with the azide **2a**, prepared from hydrazide **1a**, to give glycopeptide **11a**. On the other hand, AVP was acylated with carboxylic acid **3a** using DCC and HOBt in DMF to give a different product **12a** having a different retention time from that of **11a** on an octadecyl silica (ODS) column. Compound **12a** was not detectable in the reaction mixture of **2a** and AVP by HPLC analysis. In the FAB-MS spectra, **11a** and **12a** both showed an ion peak at *m/z* 1403 ($MH^+$). This result suggested that both compounds were monoacylated AVP derivatives. The signals of the aromatic protons and carbons of Tyr$^2$ of **11a** in the $^1$H-NMR and $^{13}$C-NMR spectra were similar to those of Boc-Tyr(OAc)-OH, but different from those of AVP and **12a** (Table 1). The UV spectrum of **12a** and AVP in pH 8.5 phosphate buffer showed a peak near 270 nm due to the $\pi$–$\pi^*$ transition of Tyr$^2$. However, the UV spectrum of **11a** showed no peak near 270 nm. These results indicated that **11a** was modified at the phenolic hydroxy group of Tyr$^2$. Futhermore, reaction of **11a** with hydrazine hydrate gave AVP as a deacylated product within 1 h, but **12a** was not changed by hydrazine hydrate treatment. As a result, it was determined that **11a** was [*O*-acyl-Tyr$^2$]AVP and **12a** was $N^{\alpha}$-acyl AVP (Chart 6).

By using essentially the same method as mentioned above, AVP was acylated in pH 9.1 buffer solution with acyl azides **2b—i**, prepared from hydrazides **1b—i**, to give Tyr$^2$-*O*-acylated products **11b—i**. AVP was acylated with acyl azide **2j** prepared from **1j** and then treated with trifluoroacetic acid

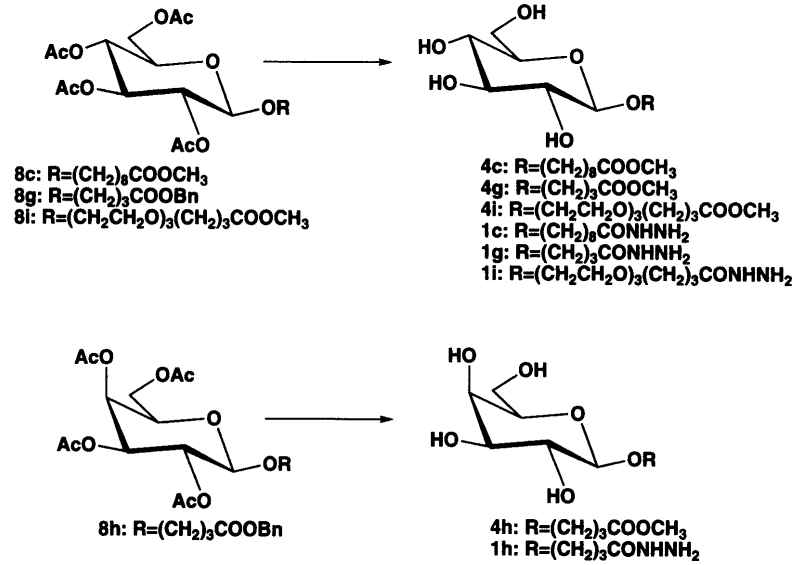


Chart 4

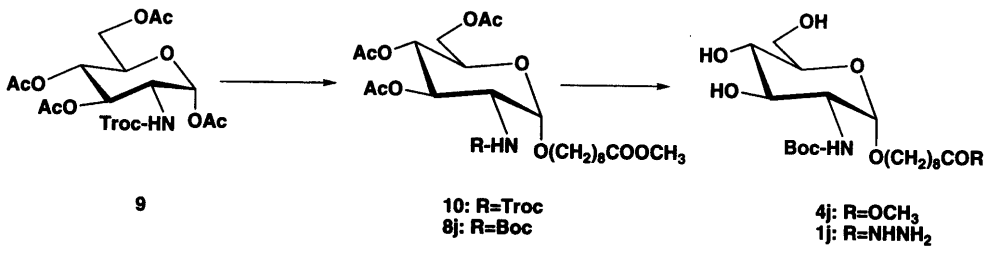


Chart 5

for removal of the Boc group to give **11j**. Also, reaction of AVP with acids **3b—e** using DCC and HOBt in DMF gave *N*-acylated AVP analogs **12b—e**.

**Biological Activity** Initially, *in vivo* antidiuretic activity of AVP and glycosylated AVP derivatives was determined by i.v. injection into Brattleboro rats[22] with hereditary hypothalamic diabetes insipidus. Urine volume during the 2 h before injection ($U_B$), and urine volume during the 2 h after injection ($U_A$) are shown in Table 2. Two parameters which indicate the magnitude of the antidiuretic response, namely, duration and depression ratio, are also shown in Table 2. These results suggest that glycoconjugates modified at the N-terminal amine (**12a—e**) showed almost no antidiuretic activity, even at a dose of 4 nmol/kg. On the other hand, glycoconjugates modified at the tyrosine hydroxyl group (**11a—j**) expressed antidiuretic activity which was stronger than that of AVP. In the previous paper,[13] we reported AVP derivatives modified at the glutamine side chain amide and at the C-terminal amide with carbohydrate. These glycoconjugates expressed antidiuretic activity which was somewhat weaker than that of AVP. Also, glycoconjugates of 1-(3-mercaptopropanoic acid)-2-D-tyrosine-4-serine-8-D-arginine vasopressin bound directly to galactose at the $Ser^2$ residue have been reported.[23] As far as we know, these are the only reports on glycosylated vasopressin analogs except from us, though there are many reports on various approaches to the modification of AVP.[10,12] However, the glycopeptides had almost no antidiuretic activity.

From the standpoint of structure-activity relationships, differences in the antidiuretic activities among analogs modified at the tyrosine side chain were not significant. However, α-mannoside derivative (**11e**) showed lower activity than β-mannoside derivative (**11d**) and other β-glycoside derivatives.

The functions of vasopressins are mediated by either of two receptor subtypes, termed $V_1$ and $V_2$. For the most part, the $V_2$ receptor mediates antidiuretic activities, while the $V_1$ receptor mediates vasoconstriction.[24] In LLC-PK1 cells, vasopressins stimulate adenylate cyclase activities through $V_2$ receptors.[25] Thus, *in vitro* antidiuretic activities of the glycosylated AVP analogs were determined using cAMP accumulation in LLC-PK1 cells with AVP as reference. The results are shown in Table 2. All compounds caused very low cAMP

Table 1. Selected $^1$H-NMR[a] and $^{13}$C-NMR[b] Chemical Shifts of Tyr of **11a**, **12a**, AVP, Boc-Tyr-OH, and Boc-Tyr(OAc)-OH

| | AVP | **11a** | **12a** | Boc-Tyr-OH | Boc-Tyr(OAc)-OH |
|---|---|---|---|---|---|
| H-2′ and 6′ | 7.04 | 7.25 | 7.00 | 7.21 | 7.38 |
| H-3′ and 5 | 6.81 | 7.11 | 6.77 | 6.90 | 7.15 |
| C-1′ | 127.2 | 134.7 | 127.6 | 128.0 | 135.4 |
| C-3′ and 5′ | 115.0 | 121.4 | 114.8 | 115.0 | 121.3 |
| C-4′ | 155.9 | 149.1 | 155.7 | 155.9 | 149.0 |

*a*) Spectra were obtained in $D_2O$ on a Varian VXR-500 spectrometer (500 MHz) at 25 °C. The HOD peak is assigned at 4.80 ppm. *b*) Spectra were obtained in dimethylsulfoxide-$d_6$ on a Varian Gemini 300 spectrometer (75 MHz) at 25 °C. The dimethylsulfoxide peak is assigned at 39.5 ppm.

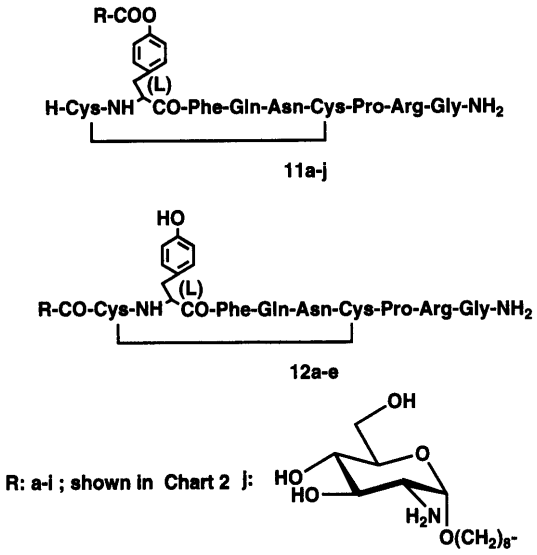


Chart 6

Table 2. Biological Activity of Glycosylated AVP[a]

| | $U_B$[b] | $U_A$[c] | Depression ratio[d] (%) | Duration[e] (h) | cAMP accumulation[f] | $t_{1/2}$[g] (min) |
|---|---|---|---|---|---|---|
| AVP | 15.52±3.26 | 6.92±2.74[h] | 55.1±17.2 | 1.20±0.84 | 100 | Stable[i] |
| **11a** | 16.82±4.03 | 2.78±1.42[h] | 84.1±7.1 | 2.00±0.00 | 1.12 | 14.9 |
| **11b** | 13.65±2.96 | 1.73±1.31[h] | 87.3±10.3 | 2.17±0.41 | 1.35 | 11.1 |
| **11c** | 14.70±2.16 | 2.10±0.89[h] | 86.0±4.7 | 2.33±0.52 | 2.24 | 11.3 |
| **11d** | 17.03±2.55 | 1.88±1.24[h] | 89.3±6.5 | 2.67±0.82 | 1.48 | 10.9 |
| **11e** | 16.78±3.45 | 6.05±3.15[h] | 64.2±16.3 | 1.67±0.52 | 2.34 | 6.0 |
| **11f** | 13.43±1.51 | 2.59±0.78[h] | 80.7±5.5 | 2.67±0.52 | 5.25 | 9.9 |
| **11g** | 12.07±2.87 | 0.73±0.84[h] | 92.9±8.6 | 2.00±0.00 | 2.14 | 242 |
| **11h** | 11.90±2.31 | 0.53±0.54[h] | 95.7±4.6 | 2.33±0.52 | 0.56 | 205 |
| **11i** | 14.00±1.15 | 1.43±0.84[h] | 89.9±5.8 | 2.83±0.75 | 5.13 | 131 |
| **11j** | 16.36±7.05 | 2.54±1.85[h] | 84.4±12.6 | 2.60±0.55 | 1.66 | N.T.[j] |
| **12a** | 11.98±2.38 | 10.40±3.04 | 12.5±25.4 | 0.50±0.55 | 0.02 | Stable |
| **12b** | 14.62±4.45 | 17.18±1.57 | −24.8±30.7 | 0.17±0.41 | 0.07 | Stable |
| **12c** | 11.68±3.22 | 10.38±1.51 | 7.1±22.0 | 0.80±0.45 | 0.02 | Stable |
| **12d** | 13.06±3.63 | 14.32±3.94 | −11.2±22.5 | 0.00±0.00 | 0.01 | Stable |
| **12e** | 16.28±5.15 | 21.18±3.39[h] | −35.8±26.3 | 0.00±0.00 | 0.01 | Stable |

*a*) Each value of *in vivo* antidiuretic activity, namely $U_B$, $U_A$, depression ratio and duration, represents mean±S.D. for five or six animals. AVP and **11a—j** were administered at a dose of 400 pmol/kg and **12a—e** were administered at a dose of 4 nmol/kg. *b*) Urine volume during 2 h before treatment. *c*) Urine volume during the 2 h after treatment. *d*) Depression ratio (%)=$(1-(U_A/U_B))\times 100$. *e*) The number of hours during which urine flow remained at less than 50% of the control volume for each rat. *f*) cAMP accumulation in LLC-PK1 cells (AVP=100). *g*) Half-lives were calculted from the time course studies of degradation in plasma of Brattleboro rat. *h*) Significant differences between $U_B$ and $U_A$ were determined by *t* test, $p<0.05$. *i*) Less than 10% degradation occurred upon incubation for 1 h. *j*) Not tested.

accumulation. The low potencies and sometimes antagonistic properties of AVP analogs with an alkylated tyrosine hydroxyl group have been reported.[12] The $V_2$ receptor in its agonist state cannot accommodate bulky aromatic residues. Thus, compounds (**11a—j**) had low stimulating activities for adenylate cyclase.

The stability of these compounds in the plasma of Brattleboro rats was also investigated, and results are shown in Table 2. AVP and glycoconjugates modified at the N-terminal amine (**12a—e**) were degraded to below 10% by incubation in plasma for 1 h. On the other hand, glycoconjugates modified at the tyrosine hydroxyl group (**11a—i**) were found to convert to AVP according to first order kinetics. The half-lives ($t_{1/2}$) of the compounds are shown in Table 2. Aliphatic carboxylic acid esters of the tyrosine phenolic group in 1-(3-mercaptopropanoic acid)-8-D-arginine vasopressin (desmopressin) were converted to desmopressin by enzymatic hydrolysis in plasma.[26] These results showed that **11a—j** were prodrugs of AVP and were the reason that compounds (**12a—e**) had no antidiuretic activities *in vivo*.

Derivatives with a trimethylene spacer arm (**11g—h**) were much more stable in plasma than the derivatives with an octamethylene spacer arm (**11a—f**). However, the antidiuretic activities of the derivatives with a trimethylene spacer arm were not long-acting *in vivo*, judging from the duration in Table 2. The precise reason is not clear, but these derivatives may be eliminated from the blood before conversion to AVP.

In conclusion, our methodology is useful for synthesis of glycopeptides modified at the hydroxy group of Tyr and the N-terminal group with carbohydrate derivatives containing a spacer arm from non-protected peptides in one step. We obtained a number of glycosylated AVP analogs (**11a—j**), prodrugs of AVP, which exhibited *in vivo* antidiuretic activity that was higher than that of AVP. Other biological properties, such as biodistribution, will be reported in forthcoming papers.[27]

## Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. $^1$H-NMR spectra were obtained on a Varian VXR-500 spectrometer at 25 °C. Tetramethylsilane was used as an internal standard, except for spectra taken in $D_2O$. In this case, no internal standard was used; the HOD peak is assigned at 4.80 ppm. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. IR spectra were obtained on a Hitachi 270-30 infrared spectrophotometer. FAB-MS spectra were obtained on a JEOL JMS-HX110 mass spectrometer. Column chromatography was performed with Merck Silica gel 60 (230—430 mesh) or Silica gel 60 from Nacalai Tesque (230—430 mesh). Preparative HPLC was performed with a YMC SH 345-5 S5 120A ODS column (20 i.d.×300 mm) and eluted with a mixture of 0.05% TFA-containing acetonitrile–water at a flow rate of 10 ml/min. Amino acid analyses were performed on a JEOL JLC-300 amino acid analyzer after hydrolysis of the glycopeptides with 6 N HCl at 110 °C for 22 h at the Peptide Institute, Inc. (Minoh, Japan). Borate buffer (pH 9.1) was prepared with 0.08 M sodium tetraborate and 0.35 M potassium hydrogen carbonate.

**Methyl 4-[2-[2-[2-(*tert*-Butyldimethylsilyloxy)ethoxy]ethoxy]ethoxy]-2-butenoate (6)** A solution of dimethylsulfoxide (2.0 ml, 28 mmol) in dichloromethane (5 ml) was added to a solution of oxalyl chloride (1.2 ml, 13.8 mmol) in dichloromethane (35 ml) at −78 °C. The mixture was stirred at this temperature for 0.5 h, and then a solution of 2-[2-[2-[2-(*tert*-butyldimethylsilyloxy)ethoxy]ethoxy]ethoxy]ethanol[16] (3.08 g, 10.0 mmol) in dichloromethane was added dropwise. After stirring for 20 min at −78 °C, triethylamine (8.4 ml, 60 mmol) was added dropwise and stirring was continued at this temperature for 10 min. The mixture was allowed to warm to 0 °C and poured into water. The organic layer was washed with 2% citric acid solution and 1 : 1 water-saturated NaCl solution, dried over $MgSO_4$, and concentrated to give 3.11 g of crude aldehyde. This product was immediately used for the next step without purification. A solution of trimethyl phosphonoacetate (2.43 g, 13.2 mmol) was added to an ice-cooled solution of potassium *tert*-butoxide (1.68 g, 15 mmol) in THF (40 ml). After stirring for 2 h at room temperature, the mixture was cooled to −78 °C. To the stirred solution, a solution of the above crude aldehyde in THF (12 ml) was added. The mixture was stirred for 1 h at this temperature and for 14 h at room temperature and then concentrated under reduced pressure. A solution of the residue in ethyl acetate was washed with saturated NaCl solution, dried over $MgSO_4$, concentrated and chromatographed on silica gel with hexane–ethyl acetate (9 : 1) to give **6** (2.12 g, 59%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 0.06 (6H, s), 0.89 (9H, s), 3.56 (2H, t, $J$=6.5 Hz), 3.62—3.70 (8H, s), 3.77 (2H, t, $J$=6.5 Hz), 4.20 (1H, dd, $J$=2.0, 4.5 Hz), 6.01 (1H, dt, $J$=16.0, 2.0 Hz), 6.96 (1H, dt, $J$=16.0, 4.5 Hz). IR (neat): 1720 cm$^{-1}$. FAB-MS *m/z*: 363 ($MH^+$).

**Methyl 4-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]butanoate (5)** A mixture of 10% Pd–C (51 mg) and **6** (1.35 g, 3.7 mmol) in THF (20 ml) was stirred under a hydrogen atmosphere overnight. After filtration, 1 M solution of tetrabutylammonium fluoride in THF (7.6 ml) was added to the filtrate. The mixture was stirred at room temperature for 5 h and then concentrated, chromatographed by silica gel with hexane-ethyl acetate (3 : 1) and ethyl acetate to give **5** (867 mg, 88%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 1.91 (2H, quintet, $J$=7.0 Hz), 2.42 (2H, t, $J$=7.0 Hz), 3.50 (2H, t, $J$=6.0 Hz), 3.67 (3H, s), 3.57—3.75 (12H, m). IR (neat): 3450, 1735 cm$^{-1}$. FAB-MS *m/z*: 251 ($MH^+$).

**8-(Methoxycarbonyl)octyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (8c)** Boron trifluoride etherate (4.0 ml, 32 mmol) was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (2.5 g, 8.0 mmol) and **7**[17] (3.0 g, 16.0 mmol) in dichloromethane (30 ml). The mixture was stirred at room temperature for 5 h and then washed with water. The organic phase was dried over $MgSO_4$ and evaporated. Chromatography of the residue on a column of silica gel with toluene–acetone (9 : 1) gave **8c** (2.45 g, 61%) as an oil. $[\alpha]_D$ +3.73° ($c$=1.42, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.56 (8H, s), 1.57—1.63 (4H, m), 2.01 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.30 (2H, t, $J$=7.6 Hz), 3.46 (1H, dt, $J$=9.5, 6.8 Hz), 3.67 (3H, s), 3.69 (1H, ddd, $J$=2.4, 4.6, 9.8 Hz), 3.86 (1H, dt, $J$=9.5, 6.4 Hz), 4.15 (1H, dd, $J$=12.4, 2.4 Hz), 4.28 (1H, dd, $J$=12.4, 4.6 Hz), 4.49 (1H, d, $J$=8.1 Hz), 4.98 (1H, dd, $J$=9.5, 8.1 Hz), 5.09 (1H, dd, $J$=9.8, 9.5 Hz), 5.20 (1H, dd, $J$=9.5, 9.5 Hz). IR (KBr): 2944, 1756, 1628, 1232, 1042 cm$^{-1}$. FAB-MS *m/z*: 519 ($MH^+$).

**3-(Benzyloxycarbonyl)propyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (8g)** To a mixture of 3-(benzyloxycarbonyl)propanol[19] (1.75 g, 9.0 mmol), silver carbonate (3.0 g), and calcium sulfate (3.0 g) in dichloromethane (15 ml), 2,3,4,6-penta-*O*-acetyl-α-D-glucopyranosyl bromide (4.11 g, 10.0 mmol) was added. The mixture was stirred for 16 h at room temperature. The mixture was filtered and the filtrate was evaporated. Chromatography of the residue on a silica gel column with toluene–acetone (9 : 1) gave **8g** as a colorless oil (2.10 g, 40%). $[\alpha]_D$ −8.5° ($c$=1.45, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.89—1.95 (2H, m), 2.00 (3H, s), 2.01 (3H, s), 2.02 (3H, s), 2.08 (3H, s), 2.43 (2H, t, $J$=7.3 Hz), 3.53 (1H, dt, $J$=9.8, 6.2 Hz), 3.66 (1H, ddd, $J$=2.4, 4.9, 7.8 Hz), 3.90 (1H, dt, $J$=9.8, 5.9 Hz), 4.13 (1H, dd, $J$=12.2, 2.4 Hz), 4.28 (1H, dd, $J$=12.2, 4.9 Hz), 4.45 (1H, d, $J$=8.7 Hz), 4.98 (1H, dd, $J$=9.5, 8.7 Hz), 5.07 (1H, dd, $J$=7.8, 9.5 Hz), 5.10 (1H, d, $J$=12.5 Hz), 5.13 (1H, d, $J$=12.5 Hz), 5.18 (1H, dd, $J$=9.5, 9.5 Hz), 7.31—7.39 (5H, m). IR (KBr): 1756, 1230, 1038 cm$^{-1}$. FAB-MS *m/z*: 525 ($MH^+$).

**3-(Benzyloxycarbonyl)propyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (8h)** Condensation of 2,3,4,6-penta-*O*-acetyl-α-D-galactopyranosyl bromide (6.32 g, 15.4 mmol) with 3-(benzyloxycarbonyl)propanol (2.6 g, 13.4 mmol) as described for the synthesis of **8g**, gave **8h** (2.45 g, 35%) as a colorless oil. $[\alpha]_D$ −6.7° ($c$=1.10, methanol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.90—1.95 (2H, m), 1.98 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.14 (3H, s), 2.43 (2H, t, $J$=7.3 Hz), 3.53 (1H, dt, $J$=9.8, 7.1 Hz), 3.86 (1H, dd, $J$=6.8, 6.8 Hz), 3.92 (1H, dt, $J$=9.8, 7.1 Hz), 4.11 (1H, dd, $J$=11.2, 6.8 Hz), 4.16 (1H, dd, $J$=11.2, 6.8 Hz), 4.40 (1H, d, $J$=8.1 Hz), 4.99 (1H, dd, $J$=3.4, 10.5 Hz), 5.10 (1H, d, $J$=12.5 Hz), 5.13 (1H, d, $J$=12.5 Hz), 5.18 (1H, dd, $J$=8.1, 10.5 Hz), 5.38 (1H, d, $J$=3.4 Hz), 7.15—7.18 (2H, m), 7.24—7.27 (1H, m), 7.32—7.38 (2H, m). IR (KBr): 1752, 1372, 1226, 1056 cm$^{-1}$. FAB-MS *m/z*: 547 ($M+Na^+$), 525 ($MH^+$).

**2-[2-[2-[3-(Methoxycarbonyl)propyloxy]ethoxy]ethoxy]ethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (8i)** Condensation of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (1.28 g, 3.3 mmol) with **5** (860 mg, 3.3 mmol), as described for the synthesis of **8c**, gave **8i** (924 mg, 41%) as a colorless oil. $[\alpha]_D$ −10.9° ($c$=1.71, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.90 (2H, quintet, $J$=6.5 Hz), 2.01 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 2.09 (3H, s),

2.41 (2H, t, $J$=8.0 Hz), 3.49 (2H, t, $J$=6.0 Hz), 3.56—3.68 (10H, m), 3.67 (3H, s), 3.67—3.77 (1H, m), 3.95 (1H, dt, $J$=11.5, 4.0 Hz), 4.14 (1H, dd, $J$=2.0, 12.5 Hz), 4.25 (1H, dd, $J$=4.5, 12.5 Hz), 4.61 (1H, d, $J$=8.0 Hz), 4.99 (1H, dd, $J$=8.0, 9.5 Hz), 5.09 (1H, dd, $J$=9.5, 9.5 Hz), 5.20 (1H, dd, $J$=9.5, 9.5 Hz). IR (neat): 1755, 1440, 1370 cm$^{-1}$. FAB-MS *m/z*: 603 ($M+Na^+$).

**8-(Methoxycarbonyl)octyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (10)** Trimethylsilyl bromide (642 mg, 4.2 mmol) was added to a mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranoside **9**[20] (1.50 g, 2.9 mmol), **7** (1.22 g, 6.5 mmol) and zinc triflate (1.56 g, 4.3 mmol) in dichloromethane (150 ml) under an argon atmosphere, and the mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with ethyl acetate, washed with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel with toluene–ethyl acetate (9 : 1) to give **10** (1.48 g, 79%) as a colorless oil. $[\alpha]_D$ +69.7° ($c$=1.90, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.28—1.33 (8H, m), 1.58—1.66 (4H, m), 2.01 (3H, s), 2.04 (3H, s), 2.10 (3H, s), 2.31 (2H, t, $J$=7.5 Hz), 3.45 (1H, dt, $J$=9.8, 6.6 Hz), 3.67 (3H, s), 3.69 (1H, m), 3.96 (1H, m), 4.06 (1H, dt, $J$=10.0, 3.5 Hz), 4.09 (1H, dd, $J$=2.5, 12.5 Hz), 4.26 (1H, dd, $J$=4.5, 12.5 Hz), 4.65 (1H, d, $J$=12.0 Hz), 4.80 (1H, d, $J$=12.0 Hz), 4.87 (1H, d, $J$=3.5 Hz), 5.10 (1H, t, $J$=10.0 Hz), 5.22 (1H, d, $J$=10.0 Hz), 5.23 (1H, t, $J$=10.0 Hz). IR (KBr): 3340, 1750, 1530 cm$^{-1}$. FAB-MS *m/z*: 650 ($MH^+$).

**8-(Methoxycarbonyl)octyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*tert*-butoxycarbonylamino)-α-D-glucopyranose (8j)** Zinc dust (915 mg, 14.0 mmol) was added to a solution of **10** (590 mg, 0.9 mmol) in acetic acid (12 ml) and the mixture stirred at room temperature for 21 h. The insoluble matter was filtered off and the filtrate was diluted with ethyl acetate and washed with $NaHCO_3$ solution, water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue (409 mg) was used in the next step without further purification. To the residue, dioxane (20 ml), di-*tert*-butyl dicarbonate (255 mg, 1.2 mmol), and triethylamine (0.35 ml, 2.5 mmol) were added. After stirring for 14 h, the solvent was evaporated. The residue was dissolved in ethyl acetate and washed with $NaHCO_3$ solution, water and saturated NaCl solution, then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel with toluene-ethyl acetate (9 : 1) to give **8j** (425 mg, 82%, 2 steps) as a colorless oil. $[\alpha]_D$ +73.2° ($c$=1.33, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.28—1.38 (8H, m), 1.32 (9H, s), 1.42 (12H, brs), 1.56—1.66 (4H, m), 2.02 (3H, s), 2.03 (3H, brs), 2.90 (6H, s), 2.31 (1H, t, $J$=7.5 Hz), 3.43 (1H, dt, $J$=9.8, 7.0 Hz), 3.67 (3H, s), 3.90—4.20 (2H, m), 4.09 (1H, dd, $J$=1.5, 12.0 Hz), 4.23 (1H, dd, $J$=5.0, 12.0 Hz), 4.72 (1H, d, $J$=10.0 Hz), 4.82 (1H, d, $J$=3.5 Hz), 5.07 (1H, t, $J$=10.0 Hz), 5.18 (1H, d, $J$=10.0 Hz). IR (neat): 3460, 3370, 1750, 1500 cm$^{-1}$. FAB-MS *m/z*: 576 ($MH^+$), 520 ($MH^+$-$C_4H_8$).

**8-(Methoxycarbonyl)octyl β-D-Glucopyranoside (4c)** A mixture of **8c** (1123 mg, 3.32 mmol) in methanol (4 ml) and 28% sodium methoxide in methanol (4 ml) was stirred for 4 h. After addition of acetic acid (0.1 ml), the mixture was evaporated to dryness. Chromatography of the residue on a column of silica gel with chloroform–methanol (9 : 1) gave **4c** (587 mg, 59%) as a colorless solid. Mp 69—70 °C, $[\alpha]_D$ −12.9° ($c$=1.16, $CH_3OH$). *Anal.* Calcd for $C_{16}H_{30}O_8$: C, 54.84; H, 8.63. Found: C, 54.88; H, 8.57. $^1$H-NMR ($D_2O$) $\delta$: 1.32 (8H, m), 1.59—1.66 (4H, m), 2.40 (2H, t, $J$=7.6 Hz), 3.26 (1H, dd, $J$=7.8, 9.0 Hz), 3.39 (1H, dd, $J$=9.3, 9.5 Hz), 3.44—3.46 (1H, m), 3.49 (1H, dd, $J$=9.0, 9.3 Hz), 3.66—3.70 (1H, m), 3.70 (3H, s), 3.73 (1H, dd, $J$=5.9, 12.2 Hz), 3.90—3.95 (2H, m), 4.46 (1H, d, $J$=8.1 Hz). IR (KBr): 3396, 2932, 1734, 1032 cm$^{-1}$. FAB-MS *m/z*: 351 ($MH^+$).

The following compounds **4g—j** were synthesized similarly from **8g—j** respectively.

**3-(Methoxycarbonyl)propyl β-D-Glucopyranoside (4g)** mp 69—71 °C, $[\alpha]_D$ −18.4° ($c$=1.29, $CH_3OH$). *Anal.* Calcd for $C_{11}H_{20}O_8 \cdot 1/2H_2O$: C, 45.67; H, 7.32. Found: C, 45.94; H, 7.33. $^1$H-NMR ($CD_3OD$) $\delta$: 1.89—1.92 (2H, m), 2.45—2.48 (2H, m), 3.17 (1H, dd, $J$=7.8, 7.8 Hz), 3.20—3.37 (3H, m), 3.58—3.66 (2H, m), 3.66 (3H, s), 3.90—3.93 (2H, m), 4.24 (1H, d, $J$=7.8 Hz). IR (KBr): 3412, 1732, 1166, 1076 cm$^{-1}$. FAB-MS *m/z*: 281 ($MH^+$).

**3-(Methoxycarbonyl)propyl β-D-Galactopyranoside (4h)** mp 70—72 °C, $[\alpha]_D$ −1.39° ($c$=1.05, $CH_3OH$). *Anal.* Calcd for $C_{11}H_{20}O_8$: C, 47.14; H, 7.19. Found: C, 47.16; H, 7.13. $^1$H-NMR ($D_2O$) $\delta$: 1.90—2.05 (2H, m), 2.56 (2H, t, $J$=7.1 Hz), 3.47—3.88 (6H, m), 3.76 (3H, s), 3.97—4.02 (2H, m), 4.43 (1H, d, $J$=8.3 Hz). IR (KBr): 3420, 1732, 1068 cm$^{-1}$. FAB-MS *m/z*: 303 ($M+Na^+$), 281 ($MH^+$).

**2-[2-[2-[3-(Methoxycarbonyl)propyloxy]ethoxy]ethoxy]ethyl β-D-Glucopyranoside (4i)** Colorless oil, $[\alpha]_D$ −12.8° ($c$=1.63, $CHCl_3$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.86 (2H, qq, $J$=6.0, 7.0 Hz), 2.41 (2H, t, $J$=7.0 Hz), 3.20 (1H, dd, $J$=8.0, 9.0 Hz), 3.25—3.30 (2H, m), 3.36 (1H, t, $J$=9.0 Hz), 3.50 (2H, t, $J$=6.0 Hz), 3.57—3.76 (12H, m), 3.65 (3H, s), 3.86 (1H, dd, $J$=2.0, 12.0 Hz), 3.98—4.04 (1H, m), 4.30 (1H, d, $J$=8.0 Hz). IR (neat): 3400, 1735 cm$^{-1}$. FAB-MS *m/z*: 435 ($M+Na^+$).

**8-(Methoxycarbonyl)octyl 2-Deoxy-2-(*tert*-butoxycarbonylamino)-α-D-glucopyranoside (4j)** mp 63—66 °C, $[\alpha]_D$ +94.6° ($c$=1.12, $CH_3OH$). *Anal.* Calcd for $C_{21}H_{39}NO_9$: C, 56.11; H, 8.74; N, 3.11. Found: C, 56.05; H, 8.82; N, 2.83. $^1$H-NMR ($CD_3OD$) $\delta$: 1.30—1.36 (8H, m), 1.45 (9H, s), 1.56—1.65 (4H, m), 2.32 (2H, t, $J$=7.5 Hz), 3.34 (1H, d, $J$=9.5 Hz), 3.40 (1H, dt, $J$=10.0, 6.5 Hz), 3.50—3.60 (3H, m), 3.65 (3H, s), 3.65—3.75 (3H, m), 3.80 (1H, dt, $J$=2.0, 12.0 Hz), 4.78 (1H, d, $J$=3.5 Hz). IR (KBr): 3450, 3350, 1740, 1690, 1530 cm$^{-1}$. FAB-MS *m/z*: 472 ($M+Na^+$), 450 ($MH^+$).

**8-(Hydrazinocarbonyl)octyl β-D-Glucopyranoside (1c)** A mixture of **9c** (587 mg, 1.17 mmol) and hydrazine hydrate (1.8 ml) in ethanol (9 ml) was stirred at room temperature for 65 h. The mixture was then concentrated and water was added to the residue, followed by evaporation (repeat 3×). The residue (**1c**) was used for the next step without further purification. mp 142—144 °C, $[\alpha]_D$ −13.1 ($c$=1.20, $CH_3OH$). *Anal.* Calcd for $C_{15}H_{30}N_2O_7 \cdot 1/4H_2O$: C, 50.76; H, 8.66; N, 7.89. Found: C, 50.71; H, 8.46; N, 7.78. $^1$H-NMR ($D_2O$) $\delta$: 1.31 (8H, s), 1.55—1.64 (4H, m), 2.21 (2H, t, $J$=7.3 Hz), 3.25 (1H, dd, $J$=7.8, 9.0 Hz), 3.39 (1H, dd, $J$=9.3, 9.5 Hz), 3.43—3.46 (1H, m), 3.48 (1H, dd, $J$=9.0, 9.3 Hz), 3.64—3.70 (1H, m), 3.72 (1H, dd, $J$=6.1, 12.5 Hz), 3.89—3.94 (2H, m), 4.46 (1H, d, $J$=7.8 Hz). IR (KBr): 3300, 2928, 1650, 1622, 1088, 1034 cm$^{-1}$. FAB-MS *m/z*: 351 ($MH^+$).

Compounds **1g—j** were synthesized similarly from **4g—j**, respectively.

**3-(Hydrazinocarbonyl)propyl β-D-Glucopyranoside (1g)** mp 85—87 °C, $[\alpha]_D$ −19.3° ($c$=1.05, $CH_3OH$). *Anal.* Calcd for $C_{10}H_{20}N_2O_7$: C, 42.85; H, 7.19; N, 9.99. Found: C, 41.69; H, 7.40; N, 10.14. $^1$H-NMR ($D_2O$) $\delta$: 1.90—1.95 (2H, m), 2.34 (2H, t, $J$=7.3 Hz), 3.28 (1H, dd, $J$=8.1, 9.2 Hz), 3.39 (1H, dd, $J$=9.8, 9.5 Hz), 3.45—3.48 (1H, m), 3.50 (1H, dd, $J$=9.2, 9.5 Hz), 3.67—3.75 (2H, m), 3.91—3.96 (2H, m), 4.46 (1H, d, $J$=8.1 Hz). IR (KBr): 3292, 1650, 1626, 1026 cm$^{-1}$. FAB-MS *m/z*: 281 ($MH^+$).

**3-(Hydrazinocarbonyl)propyl β-D-Galactopyranoside (1h)** Mp 172—174 °C, $[\alpha]_D$ −4.9° ($c$=1.06, $H_2O$). *Anal.* Calcd for $C_{10}H_{20}N_2O_7$: C, 42.85; H, 7.19; N, 9.99. Found: C, 43.12; H, 7.07; N, 9.86. $^1$H-NMR ($D_2O$) $\delta$:1.93—1.96 (2H, m), 2.35 (2H, t, $J$=7.1 Hz), 3.54 (1H, dd, $J$=4.2, 7.9 Hz), 3.66—3.72 (3H, m), 3.78—3.82 (2H, m), 3.93—3.96 (2H, m), 4.41 (1H, d, $J$=7.6 Hz). IR (KBr): 3296, 1650, 1538, 1266, 1194 cm$^{-1}$. FAB-MS *m/z*: 281 ($MH^+$).

**2-[2-[2-[3-(Hydrazinocarbonyl)propyloxy]ethoxy]ethoxy]ethyl β-D-Glucopyranoside (1i)** Colorless viscous oil, $[\alpha]_D$ −12.4° ($c$=0.51, $CH_3OH$). $^1$H-NMR ($CDCl_3$) $\delta$: 1.86 (2H, quintet, $J$=7.0 Hz), 2.25 (2H, t, $J$=7.0 Hz), 3.20 (1H, dd, $J$=8.0, 9.0 Hz), 3.25—3.30 (2H, m), 3.36 (1H, t, $J$=9.0 Hz), 3.49 (1H, t, $J$=7.0 Hz), 3.56—3.76 (12H, m), 3.86 (1H, dd, $J$=2.0, 11.5 Hz), 4.00—4.05 (1H, m), 4.30 (1H, d, $J$=8.0 Hz). IR (neat): 3400, 1650, 1540, 1460 cm$^{-1}$. FAB-MS *m/z*: 413 ($MH^+$).

**8-(Hydrazinocarbonyl)octyl 2-Deoxy-2-(*tert*-butoxycarbonylamino)-α-D-glucopyranose (1j)** mp 136—138.5 °C, $[\alpha]_D$ +84.1° ($c$=1.35, $CH_3OH$). *Anal.* Calcd for $C_{21}H_{39}N_3O_8$: C, 54.65; H, 8.52; N, 9.10. Found: C, 54.65; H, 9.04; N, 9.35. $^1$H-NMR ($CD_3OD$) $\delta$: 1.34 (8H, m), 1.44 (9H, s), 1.60 (4H, m), 2.14 (2H, t, $J$=7.0 Hz), 3.30 (1H, m), 3.35 (1H, m), 3.40 (1H, dt, $J$=10.0, 6.5 Hz), 3.50—3.60 (3H, m), 3.65—3.75 (2H, m), 3.80 (1H, dd, $J$=2.0, 12.0 Hz), 4.78 (1H, d, $J$=3.5 Hz). IR (KBr): 3540, 1670, 1540 cm$^{-1}$. FAB-MS *m/z*: 472 ($M+Na^+$), 450 ($MH^+$).

**9-(β-D-Galactopyranosyloxy)nonanoic Acid (3a)** To a solution of 8-(methoxycarbonyl)octyl β-D-galactopyranoside[15] (350 mg, 1 mmol) in methanol (5 ml) and THF (4 ml), 1 N sodium hydroxide (1.2 ml) was added. The mixture was stirred at room temperature for 18 h. The pH of the reaction mixture was brought to 3 by the addition of Amberlyst IR-120B ($H^+$ form). The resin was filtered off, and the filtrate evaporated to give **3a** (327 mg, 90%) as a colorless solid. mp 107.5—109 °C, $[\alpha]_D$ −10.3° ($c$=1.0, $CH_3OH$). *Anal.* Calcd for $C_{15}H_{28}O_8 \cdot 1/3H_2O$: C, 51.74; H, 8.11 Found: C, 51.76; H, 8.25. $^1$H-NMR ($CD_3OD$) $\delta$: 1.30—1.44 (8H, m), 1.55—1.66 (4H, m), 2.26 (2H, t, $J$=7.0 Hz), 3.45 (1H, dd, $J$=3.5, 9.5 Hz), 3.46—3.52 (2H, m), 3.53 (1H, dt, $J$=8.5, 7.0 Hz), 3.69—3.78 (2H, m), 3.83 (1H, d, $J$=3.5 Hz), 3.89 (1H, dt, $J$=8.5, 7.0 Hz), 4.20 (1H, d, $J$=8.0 Hz). IR (KBr): 3420, 1730, 1704, 1635 cm$^{-1}$. FAB-MS *m/z*: 359 ($M+Na^+$).

Compounds **3b—e** were synthesized similarly from **4b—e**, respectively.

**9-(2-Acetamido-2-deoxy-β-D-galactopyranosyloxy)nonanoic Acid (3b)** mp 161—163 °C, $[\alpha]_D$ +8.8° ($c$=0.6, $H_2O$). *Anal.* Calcd for $C_{17}H_{31}NO_8$: C, 54.10; H, 8.28; N, 3.71. Found: C, 54.27; H, 8.40; N, 3.75. $^1$H-NMR ($CD_3OD$) $\delta$: 1.30—1.40 (8H, m), 1.50—1.64 (4H, m), 1.97 (3H, s), 2.27 (2H, t, $J$=7.5 Hz), 3.45—3.49 (2H, m), 3.59 (1H, dd, $J$=3.0, 11.0 Hz),

3.70—3.80 (2H, m), 3.83 (1H, d, $J$=3.0 Hz), 3.85—3.90 (1H, m), 3.89 (1H, dd, $J$=8.5, 11.0 Hz), 4.36 (1H, d, $J$=8.5 Hz). IR (KBr): 3340, 1740, 1640, 1550 $cm^{-1}$. FAB-MS $m/z$: 378 ($MH^+$).

**9-(β-D-Glucopyranosyloxy)nonanoic Acid (3c)** mp 75—77 °C, $[\alpha]_D$ −20.7° ($c$=1.1, $CH_3OH$). *Anal.* Calcd for $C_{15}H_{28}O_8 \cdot 1/2H_2O$: C, 52.16; H, 8.46. Found: C, 52.29; H, 8.49. $^1$H-NMR ($D_2O$) $\delta$: 1.30—1.45 (8H, m), 1.55—1.64 (4H, m), 2.21 (2H, t, $J$=7.3 Hz), 3.27 (1H, dd, $J$=8.1, 9.3 Hz), 3.40 (1H, dd, $J$=9.0, 9.8 Hz), 3.47 (1H, ddd, $J$=2.2, 5.9, 9.8 Hz), 3.50 (1H, dd, $J$=9.0, 9.3 Hz), 3.69 (1H, dt, $J$=10.0, 6.8 Hz), 3.73 (1H, dd, $J$=5.9, 12.2 Hz), 3.93 (1H, dd, $J$=7.1, 10.0 Hz), 3.27 (1H, dd, $J$=8.1, 9.3 Hz), 4.47 (1H, d, $J$=8.1 Hz). IR (KBr): 3424, 1710 $cm^{-1}$. FAB-MS $m/z$: 359 ($M+Na^+$).

**9-(β-D-Mannopyranosyloxy)nonanoic Acid (3d)** mp 146—149 °C, $[\alpha]_D$ −39.0° ($c$=1.1, $CH_3OH$). *Anal.* Calcd for $C_{15}H_{28}O_8 \cdot 1/2H_2O$: C, 52.16; H, 8.46 Found: C, 52.43; H, 8.29. $^1$H-NMR ($D_2O$) $\delta$: 1.33—1.41 (8H, m), 1.62—1.66 (4H, m), 2.41 (2H, t, $J$=7.3 Hz), 3.41 (1H, ddd, $J$=2.2, 7.1, 12.0 Hz), 3.62 (1H, dd, $J$=9.5, 9.8 Hz), 3.67—3.72 (2H, m), 3.78 (1H, dd, J=6.4, 12.2 Hz), 3.92 (1H, dt, $J$=10.0, 6.8 Hz), 3.97 (1H, dd, $J$=2.2, 12.2 Hz), 4.02 (1H, d, $J$=2.4 Hz), 4.71 (1H, d, $J$=1.2 Hz). IR (KBr): 3406, 2932, 1725 $cm^{-1}$. FAB-MS $m/z$: 359 ($M+Na^+$), 337 ($MH^+$).

**9-(α-D-Mannopyranosyloxy)nonanoic Acid (3e)** mp 60—62 °C, $[\alpha]_D$ +50.9° ($c$=1.1, $CH_3OH$). *Anal.* Calcd for $C_{15}H_{28}O_8 \cdot 1/2H_2O$: C, 52.16; H, 8.46 Found: C, 52.19; H, 8.35. $^1$H-NMR ($D_2O$) $\delta$: 1.30—1.45 (8H, m), 1.60—1.70 (4H, m), 2.41 (2H, t, $J$=7.3 Hz), 3.59 (1H, dd, $J$=7.3, 9.8 Hz), 3.68—3.83 (5H, m), 3.92 (1H, d, $J$=12.2 Hz), 3.97 (1H, dd, $J$=1.7, 3.4 Hz), 4.90 (1H, d, $J$=1.7 Hz). IR (KBr): 3420, 1738, 1714 $cm^{-1}$. FAB-MS $m/z$: 359 ($M+Na^+$), 337 ($MH^+$).

**Synthesis of 11a** To a solution of **1a** (67 mg, 0.18 mmol) in DMF (3 ml) was added 4 N HCl-dioxane (0.19 ml) and a solution of *tert*-butyl nitrite (0.031 ml, 0.26 mmol) at −20 °C After stirring at this temperature for 1 h, sulfamic acid (18 mg, 0.19 mmol) was added and the mixture was stirred for 45 min. This mixture containing acyl azide **2a** was then added to a solution of AVP (49 mg, 0.046 mmol) in pH 9.1 borate buffer (24 ml). The mixture was stirred at room temperature for 2 h. The pH of the reaction mixture was brought to 0.85 by the addtion of 1 N HCl and insoluble material was filtered off. The filtrate was purified by preparative HPLC to give **11a** (15 mg, 23%) and starting AVP (12 mg, 24%). $[\alpha]_D$ −22.0° ($c$=0.2, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.29—1.51 (8H, m), 1.64—2.02 (9H, m), 2.03—2.43 (7H, m), 2.66 (2H, t, $J$=7.0 Hz), 2.88—3.13 (7H, m), 3.18—3.38 (5H, m), 3.47—3.57 (2H, m), 3.65—4.03 (12H, m), 4.16 (1H, dd, $J$=6.0, 9.0 Hz), 4.33—4.37 (2H, m), 4.02 (2H, d, $J$=8.0 Hz), 4.49 (1H, dd, $J$=6.0, 9.0 Hz), 4.54 (1H, dd, $J$=6.0, 9.0 Hz), 4.96 (1H, dd, $J$=6.0, 9.0 Hz), 7.11 (2H, d, $J$=8.5 Hz), 7.25 (2H, d, $J$=8.5 Hz), 7.26—7.48 (5H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.99, Pro 0.98, Cys 1.75, Asp 1.00, Glu 1.00, Phe 1.00, Tyr 0.91, ammonia 3.05.

Compounds (**11b—i**) were synthesized similarly from hydrazides (**1b—i**), respectively.

**11b**: $[\alpha]_D$ −15.8° ($c$=0.33, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.28—1.48 (8H, m), 1.54—2.00 (8H, m), 2.05 (3H, s), 2.00—2.40 (8H, m), 2.67 (2H, t, $J$=7.0 Hz), 2.85—3.10 (6H, m), 3.18—3.50 (6H, m), 3.58—4.00 (12H, m), 4.14 (1H, dd, $J$=5.5, 8.5 Hz), 4.26—4.31 (1H, m), 4.33 (1H, dd, $J$=5.5, 8.5 Hz), 4.45—4.50 (1H, m), 4.50—4.55 (1H, m), 4.94 (1H, dd, $J$=3.5, 10.0 Hz), 7.10 (2H, d, $J$=8.5 Hz), 7.24 (2H, d, $J$=8.5 Hz), 7.25—7.46 (5H, m). FAB-MS $m/z$: 1444 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.99, Pro 0.98, Cys 1.74, Asp 1.00, Glu 0.94, Phe 1.39, Tyr 1.01, ammonia 3.68.

**11c**: $[\alpha]_D$ −23.0° ($c$=0.20, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.28—1.48 (8H, m), 1.60—1.96 (8H, m), 2.02—2.15 (3H, m), 2.25—2.33 (3H, m), 2.62—2.66 (2H, m), 2.84—3.06 (5H, m), 3.14—3.43 (7H, m), 3.50—3.56 (2H, m), 3.64—3.96 (8H, m), 4.08—4.12 (1H, m), 4.28—4.32 (1H, m), 4.42—4.46 (1H, m), 4.48—4.55 (1H, m), 7.06 (2H, d, $J$=7.8 Hz), 7.12 (2H, d, $J$=7.8 Hz), 7.22—7.26 (2H, m), 7.33—7.37 (1H, m), 7.38—7.43 (2H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.99, Pro 0.98, Cys 1.79, Asp 1.00, Glu 0.99, Phe 1.00, Tyr 0.91, ammonia 2.99.

**11d**: $[\alpha]_D$ −24.0° ($c$=0.34, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.28—1.48 (8H, s), 1.60—1.98 (8H, m), 2.02—2.16 (3H, m), 2.28—2.36 (3H, m), 2.64—2.69 (2H, m), 2.84—3.08 (5H, m), 3.17—3.36 (7H, m), 3.28—3.34 (1H, m), 3.38—3.46 (1H, m), 3.52—3.58 (1H, m), 3.62—3.70 (2H, m), 3.72—3.81 (3H, m), 3.82—3.98 (1H, m), 4.10—4.14 (1H, m), 4.17—4.24 (1H, m), 4.30—4.34 (1H, m), 4.45—4.48 (1H, m), 4.50—4.55 (1H, m), 7.08 (2H, d, J =7.8 Hz), 7.23 (2H, d, $J$=7.8 Hz), 7.23—7.28 (2H, m), 7.34—7.39 (1H, m), 7.40—7.44 (2H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.98, Arg 0.98, Pro 0.97, Cys 1.77, Asp 0.99, Glu 0.98, Phe 1.00, Tyr 0.90, ammonia 3.01.

**11e**: $[\alpha]_D$ −9.4° ($c$=0.31, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.25—1.41 (8H, m), 1.60—2.00 (8H, m), 2.05—2.20 (3H, m), 2.28—2.32 (3H, m), 2.63—2.67 (2H, m), 2.87—3.08 (6H, m), 3.17—3.36 (6H, m), 3.43—3.44 (1H, m), 3.56—3.75 (6H, m), 3.83—3.96 (6H, m), 4.09—4.12 (1H, m), 4.25—4.32 (2H, m), 4.43—4.51 (2H, m), 7.06 (2H, d, $J$=8.0 Hz), 7.20—7.26 (4H, m), 7.35—7.42 (3H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.98, Arg 0.99, Pro 0.98, Cys 1.77, Asp 1.00, Glu 1.00, Phe 1.00, Tyr 0.92, ammonia 3.07.

**11f**: $[\alpha]_D$ −7.75° ($c$=0.40, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.25 (3H, d, $J$= 6.5 Hz), 1.30—1.46 (6H, m), 1.60—1.85 (7H, m), 1.85—1.95 (2H, m), 2.00—2.17 (4H, m), 2.27—2.34 (3H, m), 2.65 (2H, t, $J$=7.0 Hz), 2.86—3.06 (7H, m), 3.15—3.35 (4H, m), 3.38—3.48 (2H, m), 3.60—3.68 (2H, m), 3.70—3.78 (2H, m), 3.80—3.90 (2H, m), 3.90—3.96 (2H, m), 4.09—4.14 (1H, m), 4.30—4.35 (3H, m), 4.49—4.55 (1H, m), 7.07 (2H, d, $J$=8.5 Hz), 7.20—7.27 (4H, m), 7.34—7.45 (3H, m). FAB-MS $m/z$: 1386 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.98, Arg 0.99, Pro 0.98, Cys 1.75, Asp 0.99, Glu 0.99, Phe 1.00, Tyr 0.91, ammonia 3.07.

**11g**: $[\alpha]_D$ −27.1° ($c$=0.34, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.64—2.00 (6H, m), 2.06—2.15 (3H, m), 2.31—2.35 (1H, m), 2.81 (1H, t, $J$=7.3 Hz), 2.87—3.10 (5H, m), 3.20—3.27 (3H, m), 3.32 (1H, dd, $J$=9.1, 8.3 Hz), 3.32—3.36 (1H, m), 3.40—3.51 (2H, m), 3.53 (1H, dd, $J$=9.3, 9.0 Hz), 3.73—3.95 (1H, m), 3.93 (1H, d, $J$=17.1 Hz), 3.98 (1H, d, $J$=17.1 Hz), 4.05 (1H, dt, $J$=10.0, 6.4 Hz), 4.16 (1H, dd, $J$=8.5, 5.6 Hz), 4.35 (1H, dd, $J$=8.8, 5.4 Hz), 4.49 (1H, dd, $J$=8.3, 5.9 Hz), 4.50 (1H, d, $J$=8.1 Hz), 4.55—4.57 (1H, m), 4.95—4.96 (1H, m), 7.12 (2H, d, $J$=8.5 Hz), 7.25 (2H, d, $J$=8.5 Hz), 7.28—7.31 (2H, m), 7.39—7.41 (1H, m), 7.43—7.47 (2H, m). FAB-MS $m/z$: 1333 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 1.00, Arg 0.99, Pro 0.96, Cys 1.84, Asp 1.01, Glu 1.01, Phe 1.00, Tyr 0.91, ammonia 3.17.

**11h**: $[\alpha]_D$ −18.9° ($c$=0.22, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.64—1.76 (2H, m), 1.78—1.87 (1H, m), 1.88—2.00 (2H, m), 2.04—2.18 (3H, m), 2.08 (1H, t, $J$=6.4 Hz), 2.26—2.36 (3H, m), 2.80 (1H, t, $J$=7.1 Hz), 2.88—2.98 (3H, m), 3.00—3.10 (2H, m), 3.16—3.26 (3H, m), 3.24 (1H, dd, $J$=6.6, 7.1 Hz), 3.30—3.38 (2H, m), 3.55 (1H, dd, $J$=8.1, 8.1 Hz), 3.65—3.90 (8H, m), 3.92 (1H, d, $J$=17.1 Hz), 3.97 (1H, d, $J$=17.1 Hz), 4.02—4.06 (1H, m), 4.13—4.15 (1H, m), 4.32—4.35 (1H, m), 4.43 (1H, d, $J$=7.8 Hz), 4.47 (1H, dd, $J$=6.4, 6.6 Hz), 4.56—4.62 (1H, m), 4.91—4.94 (1H, m), 7.11 (2H, d, $J$=8.6 Hz), 7.24 (2H, d, $J$=8.3 Hz), 7.27—7.29 (2H, m), 7.36—7.40 (1H, m), 7.43—7.47 (2H, m). FAB-MS $m/z$: 1333 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.98, Pro 0.99, Cys 1.86, Asp 1.00, Glu 1.00, Phe 1.00, Tyr 0.87, ammonia 3.16.

**11i**: $[\alpha]_D$ −17.2° ($c$=0.32, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.64—1.78 (2H, m), 1.79—1.89 (1H, m), 1.89—2.03 (2H, m), 2.77 (3H, t, $J$=7.0 Hz), 2.89—3.11 (6H, m), 3.20—3.55 (8H, m), 3.67—3.81 (9H, m), 3.83—4.01 (5H, m), 4.06—4.21 (1H, m), 4.16 (1H, dd, $J$=5.5, 8.5 Hz), 4.35 (1H, dd, $J$=5.5, 8.5 Hz), 4.47—4.51 (2H, m), 4.53—4.58 (1H, m), 4.95 (1H, dd, $J$=3.5, 10.5 Hz), 7.12 (2H, d, $J$=8.5 Hz), 7.26 (2H, d, $J$=8.5 Hz), 7.27—7.31 (2H, m), 7.39—7.53 (3H, m). FAB-MS $m/z$: 1464 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.98, Pro 1.00, Cys 1.79, Asp 1.00, Glu 0.99, Phe 1.01, Tyr 0.78, ammonia 3.04.

**Synthesis of 11j** To a solution of hydrazide **1j** (66 mg, 0.18 mmol) in dry DMF (3 ml) was added 4 N HCl–dioxane (0.19 ml) and a solution of *tert*-butyl nitrite (0.087 ml, 0.73 mmol) at −20 °C After stirring at this temperature for 60 min, sulfamic acid (15 mg, 0.15 mmol) was added and the mixture was stirred for 45 min. This whole mixture, containing acyl azide **2k** was added to a solution of AVP (48 mg, 0.046 mmol) in pH 9.1 borate buffer (18 ml). The mixture was stirred at room temperature for 1.5 h. The pH of the reaction mixture was brought to 3.1 by the addtion of 1 N HCl and the solvent was evaporated to dryness. Trifluoroacetic acid was added to the residue and stirred at room temperature for 1 h. After the solvent was evaporated, the mixture was purified by preparative HPLC to give **11j** (18 mg, 29%) and starting AVP (25 mg, 52%). $[\alpha]_D$ +7.3° ($c$=0.4, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.36—1.50 (8H, m)), 1.66—1.82 (6H, m), 1.82—2.02 (3H, m), 2.06—2.22 (4H, m), 2.30—2.40 (3H, m), 2.70 (2H, t, $J$=7.0 Hz), 2.85—3.12 (6H, m), 3.22—3.40 (6H, m), 3.45—3.65 (4H, m), 3.75—4.02 (9H, m), 4.16 (1H, dd, $J$=6.0, 8.5 Hz), 4.26—4.32 (1H, m), 4.35 (1H, dd, $J$=6.0, 9.0 Hz), 4.50 (1H, dd, $J$=5.5, 8.5 Hz), 4.55 (1H, dd, $J$=6.5, 9.0 Hz), 4.95—4.98 (1H, m), 7.11 (2H, d, $J$=8.5 Hz), 7.26 (2H, d, $J$=8.5 Hz), 7.28—7.50 (5H, m). FAB-MS $m/z$: 1402 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.96, Arg 0.99, Pro 0.98, Cys 1.80, Asp 1.07, Glu 0.97, Phe 1.00, Tyr 1.39, ammonia 3.57.

**Synthesis of 12a** Triethylamine (0.049 ml, 0.35 mmol) was added to a methanol solution of AVP (80 mg, 0.07 mmol), and the mixture was evaporated to dryness. To the resulting residue, DMF (3 ml), **3a** (50 mg,

0.15 mmol), HOBt (20 mg, 0.15 mmol) and DCC (31 mg, 0.15 mmol) were added and the mixture was stirred overnight. Solvent was removed by evaporation and water added to the residue. Insoluble material was filtered off and the filtrate was purified by preparative HPLC to give **12a** (60 mg, 61%). $[\alpha]_D$ −79.1° ($c$=0.44, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.15—1.37 (8H, m)), 1.49—1.96 (9H, m), 2.01—2.35 (9H, m), 2.67 (1H, dd, $J$=10.0, 14.0 Hz), 2.82—2.85 (2H, m), 2.91—3.07 (4H, m), 3.11—3.24 (4H, m), 3.30—3.37 (1H, m), 3.46—3.52 (1H, m), 3.58—3.73 (9H, m), 3.87—3.96 (4H, m), 4.11—4.16 (2H, m), 4.30—4.33 (1H, m), 4.34 (2H, d, $J$=8.0 Hz), 4.42—4.46 (1H, m), 4.55—4.69 (3H, m), 4.87—4.92 (1H, m), 6.77 (2H, d, $J$=8.5 Hz), 7.00 (2H, d, $J$=8.5 Hz), 7.25—7.41 (5H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.97, Arg 0.98, Pro 0.97, Cys 1.76, Asp 0.98, Glu 0.97, Phe 1.00, Tyr 0.93, ammonia 2.88.

Compounds (**12b—e**) were synthesized similarly from AVP and carboxylic acids (**3b—e**), respectively.

**12b**: $[\alpha]_D$ −70.0° ($c$=0.12, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.23—1.39 (8H, m)), 1.51—2.00 (9H, m), 2.05 (3H, s), 2.03—2.25 (6H, m), 2.39—2.45 (3H, m), 2.67—2.75 (1H, m), 2.85—2.89 (2H, m), 2.95—3.09 (4H, m), 3.15—3.29 (4H, m), 3.35—3.41 (2H, m), 3.53—3.99 (15H, m), 4.15—4.20 (1H, m), 4.31—4.39 (1H, m), 4.33—4.37 (2H, m), 4.39—4.53 (2H, m), 6.83 (2H, d, $J$=7.5 Hz), 7.04 (2H, d, $J$=7.5 Hz), 7.27—7.47 (5H, m). FAB-MS $m/z$: 1444 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 0.99, Pro 0.97, Cys 1.80, Asp 1.00, Glu 0.99, Phe 1.33, Tyr 1.06, ammonia 3.48.

**12c**: $[\alpha]_D$ −82.2° ($c$=0.23, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.27—1.39 (8H, m), 1.54—1.76 (5H, m), 1.80—1.88 (1H, m), 1.92—2.01 (2H, m), 2.08—2.26 (5H, m), 2.30—2.38 (3H, m), 2.75—2.78 (1H, m), 2.86—2.90 (2H, m), 2.98—3.10 (4H, m), 3.17—3.32 (4H, m), 3.36—3.54 (4H, m), 3.64—3.86 (4H, m), 3.90—3.99 (3H, m), 4.16—4.22 (1H, m), 4.34—4.40 (1H, m), 4.44—4.52 (2H, m), 6.82 (2H, d, $J$=8.3 Hz), 6.94 (2H, d, $J$=8.3 Hz), 7.28—7.32 (2H, m), 7.38—7.48 (3H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 1.00, Pro 0.96, Cys 1.81, Asp 1.00, Glu 0.99, Phe 1.00, Tyr 0.92, ammonia 2.95.

**12d**: $[\alpha]_D$ −77.8° ($c$=0.23, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.27—1.39 (8H, m), 1.56—1.73 (5H, m), 1.83—1.86 (1H, m), 1.92—2.00 (2H, m), 2.08—2.25 (3H, m), 2.31—2.38 (2H, m), 2.42—2.45 (1H, m), 2.70—2.75 (1H, m), 2.88 (2H, d, $J$=6.6 Hz), 2.98—3.10 (3H, m), 3.18—3.28 (3H, m), 3.36—3.42 (2H, m), 3.59—4.00 (8H, m), 3.81—4.02 (5H, m), 4.17—4.21 (1H, m), 4.36—4.39 (1H, m), 4.48—4.51 (1H, m), 4.93—4.96 (1H, m), 6.83 (2H, d, $J$=8.5 Hz), 7.06 (2H, d, $J$=8.5 Hz), 7.30 (2H, d, $J$=7.6 Hz), 7.40—7.47 (3H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.99, Arg 1.00, Pro 0.96, Cys 1.81, Asp 1.00, Glu 0.97, Phe 1.01, Tyr 0.91, ammonia 3.25.

**12e**: $[\alpha]_D$ −62.9° ($c$=0.28, $H_2O$). $^1$H-NMR ($D_2O$) $\delta$: 1.27—1.40 (8H, m), 1.57—1.80 (5H, m), 1.80—1.90 (1H, m), 1.92—2.02 (2H, m), 2.05—2.24 (5H, m), 2.32—2.40 (3H, m), 2.70—2.75 (1H, m), 2.88 (2H, d, $J$=6.6 Hz), 2.98—3.10 (3H, m), 3.18—3.28 (3H, m), 3.36—3.40 (1H, m), 3.53—3.58 (2H, m), 3.64—3.85 (7H, m), 3.90—3.97 (4H, m), 4.17—4.20 (4H, m), 4.36—4.38 (1H, m), 4.47—4.50 (1H, m), 6.82 (2H, d, $J$=8.6 Hz), 7.06 (2H, d, $J$=8.6 Hz), 7.30 (2H, d, $J$=7.1 Hz), 7.44—7.46 (3H, m). FAB-MS $m/z$: 1403 ($MH^+$). Amino acid ratios of the 6 N HCl hydrolysate; Gly 0.98, Arg 0.99, Pro 0.99, Cys 1.83, Asp 1.00, Glu 0.97, Phe 1.02, Tyr 0.92, ammonia 3.21.

**Antidiuretic Activity *in vivo*** Male adult Brattleboro rats[22] weighing 250 to 300 g were purchased from Harlan CPB, the Netherlands. The animals were maintained under standard conditions with food and water *ad libitum*. The animals were acclimatized in metabolic cages for at least 2 d and baseline levels of urine flow established. Rats receiving saline including 0.1% rat serum albumin (RSA) were used as controls. The solutions of AVP and glycosylated AVP were prepared to a concentration of 200 pmol/ml (**11a—j**) or 2 nmol/ml (**12a—e**) in saline with 0.1% RSA. The solutions were injected intravenously (400 pmol/kg (**11a—j**) or 4 nmol/kg (**12a—e**)) and urine was collected hourly thereafter. The mean of the two hourly urine volume just before injections was considered as the predosage urine volume for each rat. Two methods were used to express the magnitude of the antidiuretic response. 1) Duration was defined as the number of hours during which urine flow remained at less than 50% of the control for each rat. 2) Depression ratio was expressed by the following equation

$$\text{Depression ratio } (\%) = (1 - (U_A/U_B)) \times 100$$

$U_A$=urine volume during 2 h after treatment.

$U_B$=urine volume during 2 h before treatment.

**cAMP Accumulation in LLC-PK1 Cells as an Indicator of Antidiuretic Activities *in vitro*** LLC-PK1 cells (American Type Culture Collection, Rockville, U.S.A.) were incubated in Dulbecco's Modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and penicillin (100 U/ml)/streptomycin (100 $\mu$m/ml) under 5% $CO_2$ atmosphere at 37 °C. Subculturing was performed every 3—5 d. For the cAMP accumulation assay, $4\times10^5$ cells were seeded onto 12 well culture plate and cultured for 3 d. After washing the cells twice with 0.5 ml of experimental medium (Hanks containing 0.5 mM 3-isobutyl-1-methylxanthine, Aldrich, Milwaukee, U.S.A.), 0.9 ml of the medium was added to the wells followed by preincubation at 37 °C for 10 min. Experimental incubations (10 min) were performed after 100 $\mu$l of solutions of various concentration of the samples were added. Incubation were concluded by adding 100 $\mu$l of 100% TCA (w/v), and the cells were transferred to glass tubes. TCA in the solutions was excluded by extracting 5 times with diethyl ether (5 ml). cAMP in the aqueous phases were determined by an EIA Kit (Cayman Chemical Company, Michigan, U.S.A.). The concentration of for 50% cAMP accumulation of the maximum accumulation caused by AVP ($ED_{50}$) was calculated by log-logit regression analysis. The relative value of each $ED_{50}$ is compared with that of AVP as shown in Table 2.

**Stability in the Presence of Brattleboro's Plasma** Mixed solutions of 40 $\mu$l Tris–HCl buffer (pH 7.4), containing 0.1% RSA, 50 $\mu$l of plasma obtained from Brattleboro rats and 10 $\mu$l of a 20 $\mu$M solution of glycosylated AVP (**11a—i** and **12a—e**) were incubated at appropriate intervals at 37 °C, The reactions were terminated by adding 900 $\mu$l of 0.2 M glycine–HCl buffer (pH 3.0). The glycosylated AVP and AVP formed by hydrolysis were extracted with Sep-Pak C18 Light (Waters, Milford, U.S.A.) using 60% acetonitrile containing 0.05% TFA as eluent. The extract was subjected to HPLC using a Beckman System Gold (Beckman, Fullerton, U.S.A.) equipped with a column (4.6 i.d.×150 mm) of Inertsil ODS-2 ( GL science, Tokyo, Japan). The mobile phase was 0.5% TFA containing 12—60% acetonitrile and the flow rate was 0.5 ml/min. The detection wave length was 210 nm and peak areas were used to calculate concentrations of the samples. Under these conditions, compounds were found to convert to AVP according to first order kinetics. The half-lives ($t_{1/2}$) of the compounds were calculated from time course studies of degradation, using the formula for single exponential decay.

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**References and Notes**

1) *a*) Presented in part at the 113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, March 1993 and at the 10th International Conference on Organic Synthesis, Bangalore India, December 1994; *b*) The amino acids used here are of the L-configuration. Standard abbreviations for amino acids are used [*Eur. J. Biochem.*, **138**, 9—37 (1984)]. The following abbreviations are also used: Ac=acetyl, Boc=*tert*-butyloxycarbonyl, Bn=benzyl, DCC=dicyclohexylcarbodiimide, DMF=*N*,*N*-dimethylformamide, HOBt=1-hydroxybenzotriazole, TBDMS=*tert*-butyldimethylsilyl, TCA=trichloroacetic acid, TFA=trifluoroacetic acid, THF=tetrahydrofuran, Troc=2,2,2-trichloroethoxycarbonyl.

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4) Haneishi T., Nakajima M., Katayama M., Torikata A., Kawahara Y., Kurihara K., Arai M., Aoyagi T., *Antimicrob. Agents Chemother.*, **32**, 110 (1988).

5) *a*) Kunz H., *Angew. Chem. Int. Ed. Engl.*, **26**, 294 (1987), and references cited therein; *b*) Montreuil J., *Adv. Carbohydr. Chem. Biochem.*, **37**, 157 (1980), and references cited therein.

6) *a*) Rodriguez R. E., Rodriguez F. D., Sacristan M. P., Torres J. L., Va-

lencia G., Garcia-Anton J. M., *Neurosci. Lett.*, **101**, 89 (1989); *b*) Fisher J. F.,. Harrison A. W, Bundy G. L., Wilkinson K. F., Rush B. D., Ruwart M. J., *J. Med. Chem.*, **34**, 3140 (1991); *c*) Bundy G. L., Pals D. T., Lawson J. A., Couch S. J., Lipton M. F., Mauragis M. A., *J. Med. Chem.*, **33**, 2276 (1990); *d*) V.-Defterdarovic L., Horvat S., Chung N. N., Schiller P.W., *Int. J. Peptide Protein Res.*, **39**, 12 (1992).

7) *a*) Lemieux R. U., Bundle D. R., Baker D. A., *J. Am. Chem. Soc.*, **97**, 4076 (1975); *b*) Lemieux R. U., Baker D. A., Bundle D. R., *Can. J. Biochem.*, **55**, 507 (1977); *c*) Kojima S., Ishido M., Kubota K., Kubodera A., Hellmann T., Kohnke-Godt B., Wosgien B., Gabius H-J., *Biol. Chem. Hoppe-Seyler*, **371**, 331—338 (1990).

8) Sugawara T., Susaki H., Nogusa H., Gonsho A., Iwasawa H., Irie K., Ito Y., Shibukawa M., *Carbohydr. Res.*, **238**, 163—184 (1993).

9) Gonsho A., Irie K., Susaki H., Iwasawa H., Okuno S., Sugawara T., *Biol. Pharm. Bull.*, **17**, 275 (1994).

10) *a*) Cowley A. W., Jr., Liard J.-F., Ausiello D. A. (eds.), "Vasopressin: Cellular and Integrative Functions," Raven Press, New York, 1988, and references cited therein; *b*) Schrier R. W. (ed.), "Vasopressin," Raven Press, New York, 1985, and references cited therein.

11) *a*) Janáky T., László F. A., Sirokmán F., Morgat J.-L., *J. Endocr.*, **93**, 295—303 (1982); *b*) Janáky T., Laczi F., László F. A., *Ann. N. Y. Acad. Sci.*, **394**, 116—127 (1982).

12) *a*) Hruby V. J., Chow M-S., Smith D. D., *Annu. Rev. Pharmacol. Toxicol.*, **30**, 501—534 (1990), and references cited therein; *b*) Hruby V. J., Smith D. D., "Comprehensive Medicinal Chemistry," Vol. 3, ed. by Emmet J. C., Pergamon Press, Oxford, 1990, pp. 881—899, and references cited therein.

13) Susaki H., Suzuki K., Ikeda M., Yamada H., Watanabe H. K., *Chem. Pharm. Bull.*, **42**, 2090—2096 (1994).

14) *a*) Paulsen H., *Angew. Chem., Int. Ed. Engl.*, **29**, 823—838 (1990), and references cited therein; *b*) Kunz H., *Pure Appl. Chem.*, **65**, 1223—1232 (1993), and references cited therein; *c*) Norberg T., Lünig B., Tejbrant J., *Methods Enzymol.*, **247**, 87—106 (1994), and references cited therein.

15) Lemieux R. U., Bundle D. R., Baker D. A., U.S. Patent 4238473 (1980) [*Chem. Abstr.*, **94**, 154863*m* (1981)].

16) Bertozzi C. R., Bednarski M. D., *J. Org. Chem.*, **56**, 4326—4329 (1991).

17) Kann N., Rein T., Åkermark B., Helquist P., *J. Org. Chem.*, **55**, 5312—5323 (1990).

18) Magnuson G., Noori G., Dahmén J., Frejd T., Lave T., *Acta Chem. Scand., Ser. B*, **35**, 213—216 (1981).

19) Sugawara T., Irie K., Iwasawa H., Yoshikawa T., Okuno S., Watanabe H. K., Kato T., Shibukawa M., Ito Y., *Carbohydr. Res.*, **230**, 117—149 (1992).

20) Imoto M., Yoshimura H., Shimamoto T., Sakaguchi N., Kusumoto S., Shiba T., *Bull. Chem. Soc. Jpn.*, **60**, 2205—2214 (1987).

21) Higashi K., Susaki H., *Chem. Pharm. Bull.*, **40**, 2019—2022 (1992).

22) Valtin H., *Am. J. Med.*, **42**, 814—827 (1967).

23) *a*) Kihlberg J., Åhman J., Walse B., Drakenberg T., Nilsson A., Söderberg-Ahlm C., Bengtsson B., Olsson H., *J. Med. Chem.*, **38**, 161—169 (1995); *b*) Kihlberg J., Åhman J., Walse B., Nilsson A., Olsson H., Söderberg-Ahlm C., *Glycoconjugate J.*, **10**, 263—264 (1993).

24) Margolis B., Angle J., Kremer S., Skorecki K., "Vasopressin: Cellular and Integrative Functions," ed. by Cowley A. W., Jr., Liard J.-F., Ausiello D. A. , Raven Press, New York, 1988, pp. 97—106.

25) Roy C., Hall D., Karish M., Ausiello D.A., *J. Biol. Chem.*, **256**, 3423—3427 (1981).

26) Kahns A. H., Buur A., Bundagaard H., *Pharm. Res.*, **10**, 68—74 (1993).

27) We presented part of the biodistribution of the glycoconjugates of AVP at the 12th Annual Meeting of the Japanese Society for the Study of Xenobiotics, Nagoya, November 1997, for abstract see: Suzuki K., Susaki H., Okuno S., Yamada H., Watanabe H. K., Sugiyama Y., *Xenobio. Metabol. Dispos.*, **9**, S292—S293 (1997); Suzuki K., Susaki H., Okuno S., Yamada H., Watanabe H. K., Sugiyama Y., *J. Pharmacol. Exp. Ther.*, accepted.