Chem. Pharm. Bull. **42**(10) 2090—2096 (1994) Vol. 42, No. 10

Synthesis of Artificial Glycoconjugates of Arginine-Vasopressin and Their Antidiuretic Activities[1)]

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Received April 8, 1994; accepted June 14, 1994

Arginine-vasopressin (AVP) derivatives modified at the glutamine side chain amide with carbohydrate *via* an alkylene spacer (1a—d) were synthesized from new glycosylated glutamine derivatives (3a—d) by solid-phase synthesis. Glycoconjugates of AVP modified at the C-terminal amide (2a—d) were also synthesized from vasopressionic acid. All of them exhibited antidiuretic activity.

Keywords arginine-vasopressin; glycoconjugate; antidiuretic activity; solid-phase synthesis; glucose; galactose

The development of medicinal peptides or proteins holds great promise for future therapeutics but has been limited by the problems of low oral bioavailability[3)] and short plasma half-lives.[4)] The carbohydrate moieties in glycoproteins play an important role,[5)] dramatic changes in the activity, stability, and metabolism of glycosylated peptide drugs have been reported.[6)] Various glycoconjugates of peptides have been synthesized.[7,8)] In most of them, the carbohydrate moieties were bound directly to the side chains of Ser, Thr and Asn of the peptides. Glycoconjugates of protein,[9)] poly(L-glutamic acid)[10)] and poly(L-lysine),[11)] containing an alkylene spacer arm between the peptide and the carbohydrate, have also been reported. Octylene (eight carbon atoms)[9-11)] and ethylene (two carbon atoms)[12)] spacer arms have frequently been used as alkylene spacer arms. We reported that the octylene spacer arm was the most suitable one for recognition of lectins in the case of glycoconjugates of human serum albumin.[13)] In this paper, we wish to report a method for synthesis of glycopeptide analogs possessing an alkylene (octylene or ethylene) spacer arm. Arginine-vasopressin (AVP, Chart 1) is an antidiuretic hormone,[14)] and many studies have been done on the effect of the structural change of AVP.[14,15)] The lipophilic character of the side chain on the amino acid in the 4-position (Gln^4) plays a key role in enhancing antidiuretic specificity.[16)] The

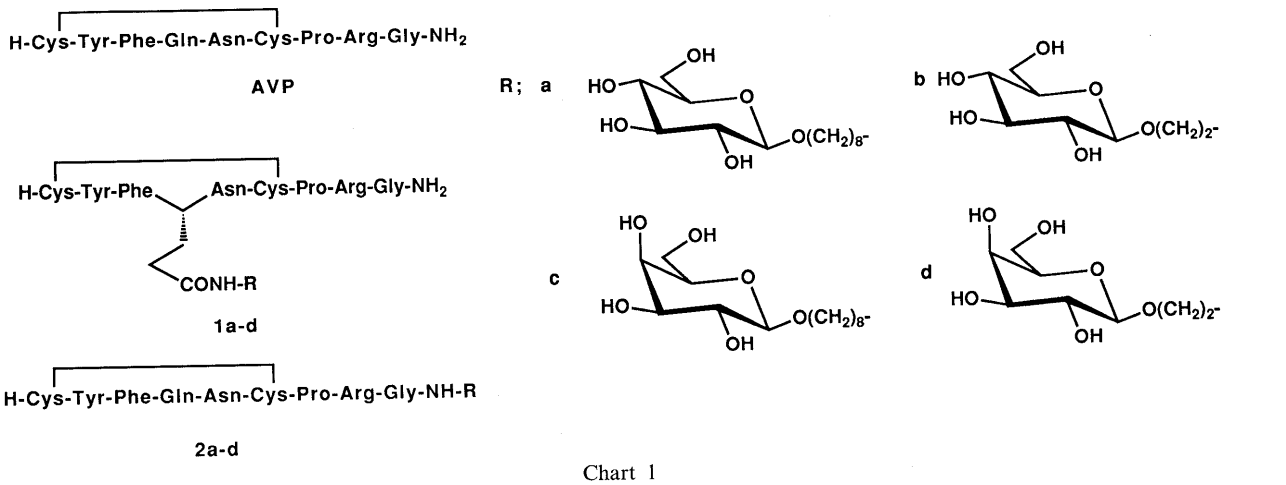

Chart 1

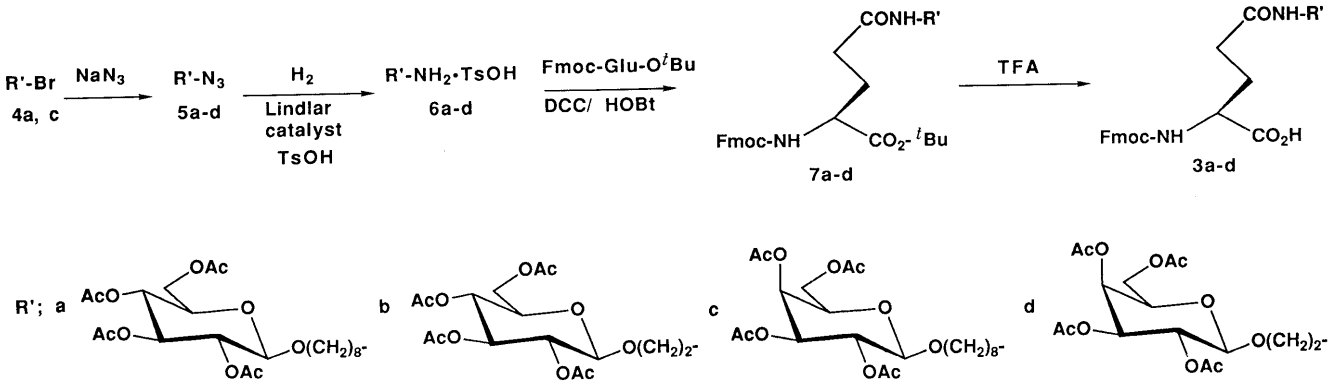

Chart 2

C-terminal position of the bioactive peptides is also important for bioactivity.[17] Thus, in this work, we synthesized glycoconjugates of AVP linked at Gln^4 and Gly^9, including an alkylene spacer arm, and determined their antidiuretic activities.

Results and Discussion

Synthesis of Glycoconjugates of AVP Glycopeptides were usually synthesized stepwise using glycosylated amino acid derivatives[7] or by linking suitably protected peptides with carbohydrate derivatives.[8] We used glutamine derivatives (**3a—d**) for the solid-phase synthesis of AVP derivatives modified at the glutamine side chain amide (**1a—d**). *O*-Acetyl groups of **3a—d** were employed for protection of the carbohydrate moiety due to their easy removal with hydrazine.

The syntheses of **3a—d** were conducted as follows (Chart 2). Condensation of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose and 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose with 8-bromooctanol promoted by boron trifluoride etherate[18] gave the β-glycoside **4a** and **4c** in 19% and 16% yields, respectively. When trimethylsilyl triflate[19] was used instead of borontrifluoride etherate as a promoter, the yield of **4a** was decreased to 10%. Treatment of **4a** and **4c** with sodium azide in DMF gave the azides **5a** and **5c** in 99% and 81% yields, respectively. The azides **5b** and **5d**[20] were known. Reduction of the azide group of **5a—d** by catalytic hydrogenolysis over a Lindlar catalyst in ethanol containing TsOH (1.0 eq) gave the amines **6a—d** as the tosylate. Condensation of **6a—d** with Fmoc–Glu–O^{t}Bu using DCC and HOBt in DMF gave **7a—d** in 61%, 78%, 82% and 90% yields, respectively, followed by TFA treatment for removal of the *tert*-butyl group to give the building blocks **3a—d**.

The Fmoc-based peptide methodology is suited to glycopeptide synthesis because the deprotection step with piperidine is compatible with the acetyl group as a hydroxyl protecting group. The synthetic scheme is depicted in Chart 3. The synthesis of the peptide fragment **9** from resin **8** was carried out on a peptide synthesizer. The acid lability of the 2-(2′,4′-dimethoxyphenyl)(9-fluorenylmethoxycarbonyl)aminomethyl)phenoxy group as a linker[21] is adequate considering the sensitivity of the glycosidic bond to strong acids. Because **3a—d** are much more sterically encumbered than the amino acids normally used in solid phase peptide synthesis, coupling of the carbohydrate-bearing residue **3a—d** with **9** was much slower than usual, so the reaction proceeded to only 70% completion even after 2 d. Fmoc derivatives of Phe, Tyr(O^{t}Bu) and Cys(Trt) were successively introduced by using DCC and HOSu in NMP in more than 99% yield.

After completion of the chain elongation, removal of acetate groups from the carbohydrate portion was performed at this stage with hydrazine hydrate in methanol.[22] Deprotection of other protective groups and cleavage from the polymer support was carried out with 95% trifluoroacetic acid (TFA) containing phenol, 1,2-ethanedithiol and thioanisole. Then the disulfide bond was formed with potassium ferricyanide(III) in dilute aqueous solution to give the desired products **1a—d** in 2.9%, 3.7%, 2.6% and 3.7% overall yields from **8**, respectively. De-*O*-acetylation after cleavage or after disulfide bond formation gave a complex degraded mixture. Thus, de-*O*-acetylation had to be performed on the resin. The glutamine derivatives **3a—d** are useful intermediates for the synthesis of artificial glycopeptides modified at the glutamine side chain amide with carbohydrate through an alkylene spacer.

Next, we synthesized AVP derivatives (**2a—d**) modified at the C-terminal amide with carbohydrate *via* an alkylene spacer (Chart 4). Treatment of **4a—d** with sodium methoxide in methanol gave the *O*-deacetylated products **10a—d** in 87%, 95%, 90% and 99% yields, respectively. Conversion of the azide group of **10a—d** by reduction

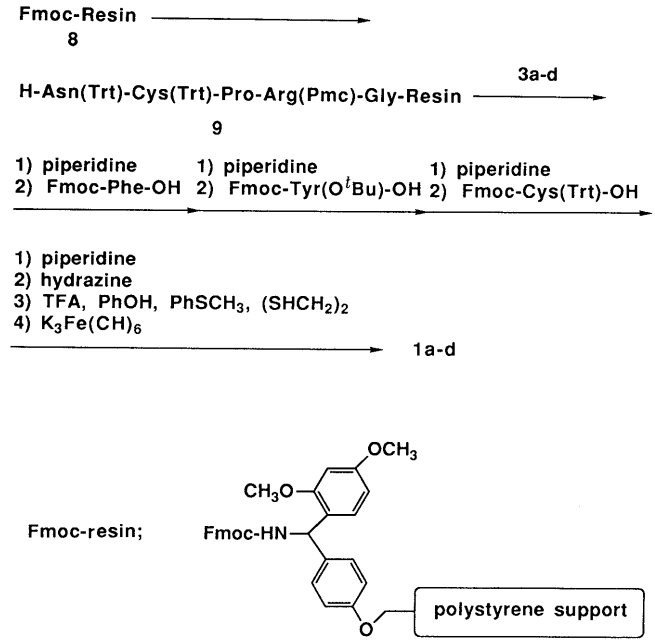

Chart 3

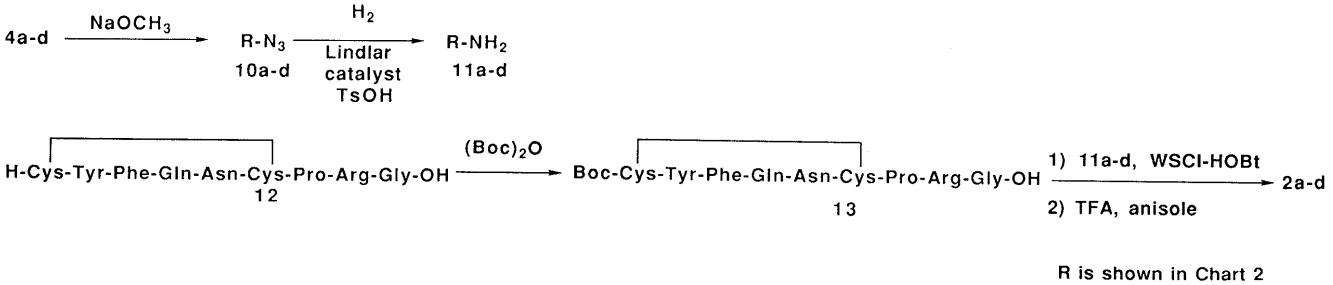

Chart 4

TABLE I. The Antidiuretic Activity of Glycosylated AVP[a]

	Dose = 400 pmol/kg				Dose = 4 nmol/kg			
	U_B[b] (ml)	U_A[c]	The depression ratio (%)	The duration (h)	U_B[b] (ml)	U_A[c] (ml)	The depression ratio (%)	The duration (h)
AVP	15.52±3.26	6.92±2.74[d]	55.1±17.2	1.20±0.84	[e]			
1a	14.23±3.31	9.63±4.12	31.4±28.1	0.83±0.41	17.68±4.88	2.22±1.18[d]	85.9±11.0	2.17±0.41
1b	13.10±2.81	6.72±3.06[d]	49.8±19.5	1.00±0.00	16.25±2.03	0.92±0.69[d]	94.5± 4.3	2.00±0.00
1c	16.63±3.06	11.83±5.20	27.8±32.9	1.00±0.63	18.78±2.70	4.67±1.63[d]	74.0±12.2	1.50±0.55
1d	16.93±2.78	12.37±4.79[d]	28.2±18.2	1.00±0.00	13.70±3.36	2.28±1.66[d]	84.4± 9.0	1.83±0.41
2a	16.03±3.47	12.90±2.34[d]	18.4±10.3	0.33±0.52	16.67±2.21	10.73±4.06[d]	36.0±22.6	1.00±0.00
2b	21.00±2.22	18.32±4.03	12.8±17.4	0.83±0.41	17.47±3.37	4.62±1.96[d]	72.5±10.7	1.83±0.41
2c	19.63±5.20	11.39±4.01[d]	43.2±12.4	0.80±0.45	15.05±4.43	12.98±4.10	12.4±18.4	1.00±0.00
2d	18.53±3.32	8.19±1.46[d]	54.9± 9.0	1.17±0.41	16.88±4.60	7.15±4.77[d]	58.9±20.0	1.33±0.52

a) Each value represents mean±S.D. for five or six animals. *b*) U_B = urine volume during 2 h before treatment. *c*) U_A = urine volume during 2 h after treatment. *d*) Significant difference: differences between U_B and U_A were evaluated by means of t test, with $p<0.05$ as the criterion of significance. *e*) This dose is a lethal one.

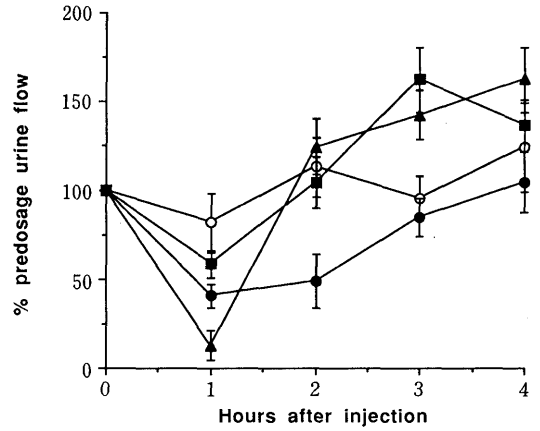

Fig. 1. Antidiuretic Responses by Brattleboro Rats to AVP, **1a** and **2a**

Each sample was given *i.v.* at the dose of 400 pmol/kg. Vertical lines indicate S.E.'s. ○, control (saline) ($n=6$); ●, AVP ($n=5$); ▲, **1a** ($n=6$); ■, **2a** ($n=6$).

gave the amines **11a—d**. The N-terminal amine of vasopressinoic acid **12**[23] was protected with a Boc group by treatment of **12** with di-*tert*-butyl dicarbonate to give **13**. Compound **13** was reacted with one of the amines **11a—d**, WSCI and HOBt in DMF, then treated with TFA and anisole for removal of the Boc group to give **3a—d** in 20%, 45%, 33% and 29% yields based on **12**, respectively.

Antidiuretic Activity The antidiuretic activity of AVP and glycosylated AVP were determined by i.v. injection into Brattleboro rats[24] with hereditary hypothalamic diabetes insipidus. The Gln[4] is an important amino acid for enhancing the antidiuretic action of AVP. Sawyer *et al.*[16] reported that substituting amino acids with more lipophilic side chains for the Gln[4] in 1-deamino-AVP further enhanced the persistence of its antidiuretic action. We substituted the amino acid with glycosylated glutamine having an ethylene or octylene spacer arm. Antidiuretic responses by Brattleboro rats to AVP, **1a** and **2a** are shown in Figure 1. Urine flow decreased on treatment by AVP, **1a** and **2a**. Urine volume during 2 h before injection (U_B) and urine volume during 2 h after injection (U_A) are shown in Table I. Also, two parameters to indicate the magnitude of the antidiuretic response, namely, the duration and the depression ratio, are shown in Table I. All glycoconjugates expressed antidiuretic activity which was somewhat weaker than that of AVP. From the standpoint of the structure–activity relationship, the antidiuretic activities of the derivatives containing an ethylene spacer arm were higher than those of the compounds containing an octylene spacer arm. Among analogs modified at the glutamine residue, glucose derivatives had higher activity than galactose derivatives. Among analogs modified at the C-terminal, galactose derivatives had higher activity than glucose derivatives at a dose of 400 pmol/kg. Recently, glycoconjugates of 1-(3-mercaptopropanoic acid)-2-D-tyrosine-4-serine-8-D-arginine vasopressin bound directly to galactose at the Ser[2] residue were reported.[25] As far as we know, this is the only previous report on glycosylated vasopressin analogs, though there are many reports on various approaches to the modification of AVP.[14,15] However, the glycopeptides had no antidiuretic activity.

In conclusion, our methodology is useful for synthesis of artificial glycopeptides modified at the amide group of Gln and at the C-terminal with carbohydrate derivatives containing an alkylene spacer arm. Also, we obtained the first glycosylated AVP analog to retain antidiuretic activity.

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). Preparative HPLC was performed with a column of YMC SH 345-5 S5 120A ODS (i.d. 20 mm × 300 mm) and developed 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).

1-(8-Bromooctyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (4a) Boron trifluoride etherate (5.5 ml, 44 mmol) was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (4.0 g, 11.0 mmol), 8-bromo-

octanol (4.4 ml, 22.0 mmol) and molecular sieves 4A (4.0 g) in 1,2-dichloroethane (50 ml). The mixture was stirred at room temperature for 8 h and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The organic phase was dried over $MgSO_4$ and evaporated. Chromatography of the residue on a column of silica gel with hexane–ethyl acetate (3 : 1) gave **4a** (1.1 g, 19%) as an oil. $[\alpha]_D$ −17.2° (c=1.25, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.90—1.30 (12H, m, $(CH_2)_6$), 2.01 (3H, s, Ac), 2.02 (3H, s, Ac), 2.04 (3H, s, Ac), 2.09 (3H, s, Ac), 3.26 (2H, t, J=7.0 Hz, CH_2Br), 3.47 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 3.69 (1H, ddd, J=2.5, 4.5, 10.0 Hz, H-5), 3.87 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 4.14 (1H, dd, J=2.5, 12.5 Hz, H-6), 4.26 (1H, dd, J=4.5, 12.5 Hz, H-6), 4.49 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.09 (1H, dd, J=9.5, 9.5 Hz, H-4), 5.20 (1H, t, J=9.5, 9.5 Hz, H-3). IR ($CHCl_3$): 1755, 1367, 1251, 1039 cm^{-1}. FAB-MS m/z: 539 (MH^+), 537 (MH^+).

1-(8-Bromooctyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (4c) Condensation of 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose (5.0 g, 14.0 mmol) with 8-bromooctanol (5.5 g, 28.0 mmol) as described for the synthesis of **4a** gave **4c** (1.1 g, 16%) as a colorless oil. $[\alpha]_D$ −10.6° (c=1.10, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.90 (12H, m, $(CH_2)_6$), 1.99 (3H, s, Ac), 2.05 (3H, s, Ac), 2.05 (3H, s, Ac), 2.15 (3H, s, Ac), 3.41 (1H, t, J=7.0 Hz, CH_2Br), 3.47 (1H, dt, J=6.5, 9.5 Hz, CH_2O), 3.87—3.91 (2H, m, CH_2O, H-5), 4.13 (1H, dd, J=6.5, 11.0 Hz, H-6), 4.19 (1H, dd, J=6.5, 11.0 Hz, H-6), 4.45 (1H, d, J=8.0 Hz, H-1), 5.02 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.20 (1H, dd, J=10.5, 8.0 Hz, H-2), 5.38—5.39 (1H, m, H-4). IR ($CHCl_3$): 1749, 1369, 1250, 1059 cm^{-1}. FAB-MS m/z: 539 (MH^+), 537 (MH^+).

1-(8-Azideoctyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (5a) A mixture of the bromide **4a** (1.69 g, 3.13 mmol) and sodium azide (603 mg, 9.39 mmol) in DMF (15 ml) was stirred at 50 °C for 38 h. After addition of ethyl acetate, the mixture was washed with water. The organic phase was dried over $MgSO_4$ and evaporated. Chromatography of the residue on a column of silica gel with hexane–ethyl acetate (1 : 1) gave **5a** (1.56 g, 99%) as an oil. $[\alpha]_D$ −15.0° (c=1.13, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.90—1.30 (12H, m, $(CH_2)_6$), 2.01 (3H, s, Ac), 2.02 (3H, s, Ac), 2.04 (3H, s, Ac), 2.09 (3H, s, Ac), 3.26 (2H, t, J=7.0 Hz, CH_2N), 3.47 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 3.69 (1H, ddd, J=2.5, 4.5, 10.0 Hz, H-5), 3.87 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 4.14 (1H, dd, J=2.5, 12.5 Hz, H-6), 4.26 (1H, dd, J=4.5, 12.5 Hz, H-6), 4.49 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.09 (1H, dd, J=9.5, 9.5 Hz, H-4), 5.20 (1H, dd, J=9.5, 9.5 Hz, H-3). IR ($CHCl_3$): 2401, 2100, 1755, 1205 cm^{-1}.

1-(8-Azideoctyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (5c) This compound was synthesized by a procedure similar to the above, from **4c**, in 81% yield. $[\alpha]_D$ −15.0° (c=1.13, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.90—1.30 (12H, m, $(CH_2)_6$), 1.99 (3H, s, Ac), 2.05 (3H, s, Ac), 2.05 (3H, s, Ac), 2.15 (3H, s, Ac), 3.26 (2H, t, J=7.0 Hz, CH_2N), 3.47 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 3.86—3.92 (2H, m, CH_2O, H-5), 4.13 (1H, dd, J=6.5, 11.0 Hz, H-6), 4.19 (1H, dd, J=6.0, 11.0 Hz, H-6), 4.45 (1H, d, J=8.0 Hz, H-1), 5.02 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.20 (1H, dd, J=10.5, 8.0 Hz, H-2), 5.39 (1H, d, J=3.5 Hz, H-4). IR ($CHCl_3$): 2100, 1754, 1265 cm^{-1}. FAB-MS m/z: 524 ($M+Na^+$), 502 (MH^+).

1-(8-(α-*tert*-Butyl-*N*-(9-fluorenylmethoxycarbonyl)-L-glutamide)octyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (7a) A mixture of HOSu (110 mg, 0.93 mmol), Fmoc–Glu–O^{t}Bu (400 mg, 0.93 mmol) and DCC (190 mg, 0.93 mmol) in dichloromethane (8 ml) was stirred for 1 h at room temperature. This solution was used directly as an HOSu ester solution. TsOH (130 mg, 1.3 mmol) and **5a** (500 mg, 1.2 mmol) were dissolved in ethanol (10 ml). Then a Lindlar catalyst (400 mg) was added, and the mixture was subjected to catalytic hydrogenation for 1 h at 3.2 atmospheres pressure of H_2. After addition of the Lindlar catalyst (300 mg), the mixture was hydrogenated for 1 h at 3.2 atmospheres pressure of H_2. After removal of the catalyst by filtration, the solvent was removed by evaporation. The residue (600 mg) was dissolved in dichloromethane (4 ml). To this solution, triethylamine (0.13 ml, 0.93 mmol) and the HOSu ester solution, prepared as above, were added at 0 °C. The mixture was stirred overnight at room temperature. After filtration, the reaction mixture was washed with water and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure and chromatographed on silica gel with chloroform–methanol (99 : 1) to give **7a** (500 mg, 61%). mp 52—56 °C. $[\alpha]_D$ −7.29° (c=1.03, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{62}N_2O_{15}\cdot 1/2H_2O$: C, 61.94; H, 7.12; N, 3.14. Found: C, 62.06; H, 7.13; N, 3.16. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (12H, s, $(CH_2)_6$), 1.47 (9H, s, ^{t}Bu), 1.85—1.95 (1H, m, Glu β-CH_2), 2.00 (3H, s, Ac), 2.02 (3H, s, Ac), 2.03 (3H, s, Ac), 2.08 (3H, s, Ac), 2.21—2.23 (3H, m, Glu γ-CH_2, Glu β-CH_2), 3.20—3.26 (2H, m, CH_2N), 3.45 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 3.68 (1H, ddd, J=2.5, 4.5, 10 Hz, H-5), 3.85 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 4.13 (1H, dd, J=2.5, 12.0 Hz, H-6), 4.21—4.28 (3H, m, Fmoc CH, Glu α-CH), 4.36—4.43 (2H, m, Fmoc CH_2), 4.47 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.08 (1H, dd, J=9.5, 9.5 Hz, H-4), 5.20 (1H, dd, J=9.5, 9.5 Hz, H-3), 5.61 (1H, d, J=8.0 Hz, Glu α-NH), 5.95 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.61 (2H, t, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR (KBr): 1747, 1666, 1514, 1371, 1251 cm^{-1}. FAB-MS m/z: 883 (MH^+).

The following compounds **7b—d** were synthesized similarly from **5b—d** in 78%, 82% and 90% yields, respectively.

1-(2-(α-*tert*-Butyl-*N*-(9-fluorenylmethoxycarbonyl)-L-glutamide)ethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (7b) mp 62—66 °C. $[\alpha]_D$ −2.70° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{50}N_2O_{15}\cdot 2H_2O$: C, 57.54; H, 6.52; N, 3.36. Found: C, 57.84; H, 6.26; N, 3.45. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, ^{t}Bu), 1.92—1.98 (1H, m, Glu β-CH_2), 2.00 (3H, s, Ac), 2.02 (3H, s, Ac), 2.03 (3H, s, Ac), 2.06 (3H, s, Ac), 2.15—2.30 (3H, m, Glu β-CH_2, Glu γ-CH_2), 3.43—3.47 (2H, m, CH_2N), 3.65—3.72 (2H, m, CH_2O, H-5), 3.79—3.84 (1H, m, CH_2O), 4.25—4.12 (4H, m, Fmoc CH, Glu α-CH, H-6), 4.36 (1H, dd, J=6.5, 11.0 Hz, Fmoc CH_2), 4.41 (1H, dd, J=6.5, 11.0 Hz, Fmoc CH_2), 4.49 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.06 (1H, dd, J=9.5, 9.5 Hz, H-4), 5.20 (1H, dd, J=9.5, 9.5 Hz, H-3), 5.65 (1H, d, J=8.0 Hz, Glu α-NH), 6.12 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.62 (2H, dd, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR (KBr): 1755, 1668, 1512, 1369, 1251 cm^{-1}. FAB-MS m/z: 799 (MH^+).

1-(8-(α-*tert*-Butyl-*N*-(9-fluorenylmethoxycarbonyl)-L-glutamide)octyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (7c) mp 58—62 °C. $[\alpha]_D$ −6.26° (c=1.03, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{62}N_2O_{15}\cdot 1/2H_2O$: C, 61.94; H, 7.12; N, 3.14. Found: C, 62.22; H, 7.13; N, 3.24. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (12H, s, $(CH_2)_6$), 1.47 (9H, s, ^{t}Bu), 1.85—1.96 (1H, m, Glu β-CH_2), 1.98 (3H, s, Ac), 2.04 (3H, s, Ac), 2.05 (3H, s, Ac) 2.14 (3H, s, Ac), 2.20—2.24 (3H, m, Glu β-CH_2, Glu γ-CH_2), 3.19—3.27 (2H, m, CH_2N), 3.45 (1H, dt, J=9.5, 6.5 Hz, CH_2O), 3.84—3.90 (2H, m, CH_2O, H-5), 4.13 (1H, dd, J=2.5, 12.0 Hz, H-6), 4.21—4.28 (3H, m, Fmoc CH, Glu α-CH, H-6), 4.36—4.43 (2H, m, Fmoc CH_2), 4.43 (1H, d, J=8.0 Hz, H-1), 5.01 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.19 (1H, dd, J=8.0, 10.5 Hz, H-2), 5.39 (1H, d, J=3.5 Hz, H-4), 5.61 (1H, d, J=8.0 Hz, Glu α-NH), 5.93—5.97 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.61 (2H, t, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR (KBr): 1747, 1666, 1514, 1371, 1251 cm^{-1}. FAB-MS m/z: 883 (MH^+).

1-(2-(α-*tert*-Butyl-*N*-(9-fluorenylmethoxycarbonyl)-L-glutamide)ethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (7d) mp 59—64 °C. $[\alpha]_D$ −2.60° (c=1.03, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{50}N_2O_{15}\cdot 5/2H_2O$: C, 56.93; H, 6.57; N, 3.31. Found: C, 57.23; H, 6.29; N, 3.25. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, ^{t}Bu), 1.85—1.95 (1H, m, Glu β-CH_2), 1.98 (3H, s, Ac), 2.04 (3H, s, Ac), 2.05 (3H, s, Ac), 2.18—2.26 (3H, m, Glu β-CH_2, Glu γ-CH_2), 3.44—3.48 (2H, m, CH_2N), 3.67 (1H, ddd, J=4.0, 6.0, 10.0 Hz, CH_2O), 3.84—3.90 (2H, m, CH_2O, H-5), 4.10 (1H, dd, J=6.5, 11.0 Hz, H-6), 4.16 (1H, dd, J=6.5, 11.0 Hz, H-6), 4.23 (2H, m, Fmoc CH, Glu α-CH), 4.37 (1H, dd, J=11.0, 7.0 Hz, Fmoc CH_2), 4.42 (1H, dd, J=11.0, 7.0 Hz, Fmoc CH_2), 4.46 (1H, d, J=8.0 Hz, H-1), 5.01 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.19 (1H, dd, J=8.0, 10.5 Hz, H-2), 5.38 (1H, d, J=3.5 Hz, H-4), 5.65 (1H, d, J=8.0 Hz, Glu α-NH), 6.08 (1H, d, J=8.0 Hz, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.61 (2H, t, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR (KBr): 1755, 1668, 1512, 1369, 1251 cm^{-1}. FAB-MS m/z: 799 (MH^+).

1-(8-(*N*-(9-Fluorenylmethoxycarbonyl)-L-glutamide)octyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (3a) A solution of **7a** (576 mg, 0.75 mmol) in TFA (1 ml) was stirred for 1 h at room temperature and the solvent was removed by evaporation. The residue was washed with hexane and ether, dried *in vacuo* and used as **3a** for the next step without further purification. mp 106—111 °C. $[\alpha]_D$ −1.80° (c=0.91, $CHCl_3$). *Anal.* Calcd for $C_{42}H_{54}N_2O_{15}$: C, 61.00; H, 6.58; N, 3.39. Found: C, 61.17; H, 6.84; N, 3.35. ^{1}H-NMR ($CDCl_3$) δ: 1.27—1.60 (12H, m, $(CH_2)_6$), 2.00 (3H, s, Ac), 2.02 (3H, s, Ac), 2.03 (3H, s, Ac), 2.08 (3H, s, Ac), 2.09—2.12 (1H, m, Glu β-CH_2), 2.14—2.22 (1H, m, Glu β-CH_2), 2.40—2.50 (1H, m, Glu γ-CH_2), 2.50—2.60 (1H, m, Glu γ-CH_2), 3.24—3.30 (2H, m, $NHCH_2$), 3.45 (1H, dt, J=6.5, 9.5 Hz, OCH_2), 3.68 (1H, ddd, J=2.5, 4.5, 10.0 Hz, H-5), 3.85 (1H, dt, J=6.5, 9.5 Hz, OCH_2), 4.13 (1H, dd, J=2.5, 12.0 Hz, H-6), 4.20—4.29 (2H, m, Fmoc CH, H-6), 4.32—4.42

(3H, m, Fmoc CH_2, Glu α-CH), 4.47 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.08 (1H, t, J=9.5 Hz, H-4), 5.20 (1H, t, J=9.5 Hz, H-3), 6.04 (1H, d, J=8.0 Hz, Glu α-NH), 6.15 (1H, br, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.59 (2H, t, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR ($CHCl_3$): 1755, 1230, 1043 cm^{-1}. FAB-MS m/z: 827 (MH^+).

The following compounds **3b—d** were synthesized similarly from **7b—d**, respectively.

1-(2-(*N*-(9-Fluorenylmethoxycarbonyl)-L-glutamide)ethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (3b) mp 93—96 °C. $[\alpha]_D$ = +6.90° (c=0.95, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{42}N_2O_{15}$: C, 58.21; H, 5.70; N, 3.77. Found: C, 58.38; H, 6.00; N, 3.73. ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s, Ac), 2.02 (3H, s, Ac), 2.03 (3H, s, Ac), 2.06 (3H, s, Ac), 2.09—2.12 (1H, m, Glu β-CH_2), 2.12—2.21 (1H, m, Glu β-CH_2), 2.41—2.50 (1H, m, Glu γ-CH_2), 2.52—2.61 (1H, m, Glu γ-CH_2), 3.48 (2H, ddd, J=2.0, 5.8, 9.5 Hz, CH_2N), 3.69—3.76 (2H, m, CH_2O, H-5), 3.80—3.85 (1H, m, CH_2O), 4.15—4.25 (3H, m, Fmoc CH, H-6), 4.34—4.43 (3H, m, Fmoc CH_2, Glu α-CH_2), 4.52 (1H, d, J=8.0 Hz, H-1), 4.98 (1H, dd, J=8.0, 9.5 Hz, H-2), 5.07 (1H, dd, J=9.5, 9.5 Hz, H-4), 5.22 (1H, dd, J=9.5, 9.5 Hz, H-3), 6.05 (1H, d, J=8.0 Hz, Glu α-NH), 6.35 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.60 (2H, dd, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR ($CHCl_3$): 1757, 1222, 1039 cm^{-1}. FAB-MS m/z: 743 (MH^+).

1-(2-(*N*-(9-Fluorenylmethoxycarbonyl)-L-glutamide)octyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (3c) mp 67—72 °C. $[\alpha]_D$ = +0.71° (c=0.99, $CHCl_3$). *Anal.* Calcd for $C_{42}H_{54}N_2O_{15}\cdot H_2O$: C, 59.71; H, 6.68; N, 3.32. Found: C, 59.93; H, 6.60; N, 3.21. ^{1}H-NMR ($CDCl_3$) δ: 1.27—1.60 (12H, m, $(CH_2)_6$), 1.98 (3H, s, Ac), 2.04 (3H, s, Ac), 2.05 (3H, s, Ac), 2.10—2.13 (1H, m, Glu β-CH_2), 2.14 (3H, s, Ac), 2.16—2.26 (1H, m, Glu β-CH_2), 2.39—2.46 (1H, m, Glu γ-CH_2), 2.48—2.56 (1H, m, Glu γ-CH_2), 3.25—3.31 (2H, m, CH_2N), 3.43—3.51 (1H, m, CH_2O), 3.85—3.92 (2H, m, CH_2O, H-5), 4.08—4.24 (3H, m, Fmoc CH, H-6), 4.32—4.40 (3H, m, Fmoc CH_2, Glu α-CH), 4.49 (1H, d, J=8.0 Hz, H-1), 5.04 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.19 (1H, dd, J=8.0, 10.5 Hz, H-2), 5.39 (1H, dd, J=3.5 Hz, H-4), 6.01 (1H, d, J=8.0 Hz, Glu α-NH), 6.38—6.42 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.60 (2H, dd, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR ($CHCl_3$): 1753, 1230, 1078, 1055 cm^{-1}. FAB-MS m/z: 827 (MH^+).

1-(2-(*N*-(9-Fluorenylmethoxycarbonyl)-L-glutamide)ethyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (3d) mp 84—88 °C. $[\alpha]_D$ = +6.40° (c=1.21, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{42}N_2O_{15}\cdot 1/2H_2O$: C, 57.52; H, 5.77; N, 3.73. Found: C, 57.42; H, 5.87; N, 3.53. ^{1}H-NMR ($CDCl_3$) δ: 1.98 (3H, s, Ac), 2.04 (3H, s, Ac), 2.05 (3H, s, Ac), 2.10—2.13 (1H, m, Glu β-CH_2), 2.14 (3H, s, Ac), 2.16—2.26 (1H, m, Glu β-CH_2), 2.39—2.46 (1H, m, Glu γ-CH_2), 2.48—2.56 (1H, m, Glu γ-CH_2), 3.45—3.52 (2H, m, CH_2N), 3.69—3.74 (1H, m, H-5), 3.84—3.89 (1H, m, CH_2O), 3.90—3.94 (1H, m, CH_2O), 4.08—4.24 (3H, m, Fmoc CH, H-6), 4.34—4.43 (3H, m, Fmoc CH_2, Glu α-CH), 4.49 (1H, d, J=8.0 Hz, H-1), 5.04 (1H, dd, J=3.5, 10.5 Hz, H-3), 5.19 (1H, dd, J=8.0, 10.5 Hz, H-2), 5.39 (1H, dd, J=3.5 Hz, H-4), 6.01 (1H, d, J=8.0 Hz, Glu α-NH), 6.38—6.42 (1H, m, Glu γ-CONH), 7.32 (2H, t, J=7.5 Hz, ArH), 7.40 (2H, t, J=7.5 Hz, ArH), 7.60 (2H, dd, J=7.5, 4.0 Hz, ArH), 7.77 (2H, d, J=7.5 Hz, ArH). IR ($CHCl_3$): 1751, 1230, 1076 cm^{-1}. FAB-MS m/z: 743 (MH^+).

H-Asn(Trt)-Cys(Trt)-Pro-Arg(Pmc)-Gly-Resin (9) Solid-phase synthesis of this resin from 2-(2′,4′-dimethoxyphenyl)(9-fluorenylmethoxycarbonyl)aminomethyl)-phenoxy resin **8** (Calbiochem-Novabiochem Japan, Tokyo, Japan) was carried out on a peptide synthesizer 430A (Applied Biosystems Inc., Foster City, U.S.A.) using the 0.25 mmol scale protocol of the system software version 1.40 with NMP/HObT Fmoc cycles.

Synthesis of 1a The resin **9** (0.25 mmol) was placed in the reaction vessel of a Kokkusan peptide synthesizer (Kokkusan Chemical Works, Ltd., Tokyo, Japan). To the reaction vessel, NMP (6 ml), **3a** (620 mg, 0.75 mmol), DCC (155 mg, 0.75 mmol) and HOSu (86 mg, 0.75 mmol) were added. After 2 days' vortex mixing, a ninhydrin test[26] showed that the reaction had proceeded to the extent of only about 70%. The removal of the protective group of the α-amine and elongation of the peptides were carried out using Fmoc derivatives of Phe, Tyr(O^{t}Bu) and Cys(Trt). The schedule of reaction was as follows: (1) washing with NMP (6 ml for 1 min, 4 times); (2) deprotection of Fmoc with 20% piperidine in NMP (6 ml for 3 min, 3 times and additional 6 ml for 20 min, once); (3) washing with NMP (6 ml for 1 min, 7 times); (4) coupling, 1.00 mmol of Fmoc amino acid, DCC (206 mg, 1.0 mmol) and HOSu (115 mg, 1.0 mmol) in NMP (6 ml) for 2 h; (5) the ninhydrin test showed that the yield had reached 99%.

Deprotection of Fmoc of the N-terminal was carried out according to schedule (1)—(3) described above. To the reaction vessel, 12.5% hydrazine in methanol (8 ml) was added. After overnight vortex mixing, the reaction mixture was washed with NMP (6 ml for 1 min, 7 times). To the resin in the flask, TFA–water (95 : 5, 10 ml), phenol (750 mg), 1,2-ethandithiol (0.25 ml) and thioanisole (0.5 ml) were added with ice-cooling. The mixture was stirred for 2 h at room temperature for the cleavage and removal of protecting groups from the amino acid side chain. After addition of cold ether (100 ml), the reaction mixture was filtered. The precipitate was dissolved in TFA and filtered. The filtrate was added to ether (200 ml) and filtered. The precipitate was dissolved in methanol. The solution was concentrated to about 1 ml and filtered after addition of ether. The precipitate was dissolved in methanol and evaporated. The residue was dissolved in water (1000 ml) and saturated aqueous $NaHCO_3$ was added to adjust the pH to 7.0. To this mixture, potassium ferricyanide(III) (90 mg) in water (100 ml) was added, and the whole was stirred for 2 h. Ion exchange resin AG1-X2 (Bio Rad, Richmond, U.S.A.) was added and the reaction mixture was filtered. The filtrate was concentrated, lyophilized and purified by preparative HPLC to give glycosylated AVP **1a** (10 mg, 2.9% based on **8**). $[\alpha]_D$ −2.0° (c=0.40, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.20—1.30 (12H, m, $(CH_2)_6$), 1.33—1.42 (2H, m), 1.43—1.66 (5H, m), 1.70—2.10 (6H, m), 2.10—2.20 (2H, m), 2.45—2.48 (1H, m), 2.53—2.56 (1H, m), 2.60—2.66 (2H, m), 2.80—2.88 (2H, m), 2.90—2.96 (4H, m), 3.00—3.08 (2H, m), 3.08—3.14 (2H, m), 3.14—3.20 (2H, m), 3.57—3.70 (2H, m), 3.72—3.77 (1H, m), 3.90—3.94 (1H, m), 4.04—4.07 (1H, m), 4.09 (1H, d, J=8.0 Hz), 4.15—4.36 (4H, m), 4.36—4.61 (3H, m), 4.76—4.80 (2H, m), 4.86—4.95 (3H, m), 6.64 (2H, d, J=8.0 Hz, Tyr^2 ArH), 6.94—6.95 (3H, m, Tyr^2 ArH), 7.11 (2H, s), 7.18—7.27 (4H, m, Phe^3 ArH), 7.27—7.32 (2H, m, Phe^3 ArH), 7.44—7.47 (1H, m), 7.79—7.83 (1H, m), 7.90—7.95 (1H, m), 7.95—8.00 (1H, m), 8.20—8.35 (10H, m), 8.46—8.50 (1H, m), 8.70—8.80 (1H, m), 9.24 (1H, s). Amino acid ratios; Asp 0.98, Glu 0.74, Gly 0.99, Cys 1.60, Tyr 0.73, Phe 0.83, Arg 1.00, Pro 1.00, ammonia 2.01.

Compounds **1b—d** were synthesized similarly from the resin **9** and carboxylic acids **3b—d** in 3.7%, 2.6% and 3.7% yields (based on **8**), respectively.

1b: $[\alpha]_D$ −8.3° (c=0.40, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.48—1.58 (2H, m), 1.70—2.20 (5H, m) , 2.45—2.48 (2H, m), 2.58—2.62 (2H, m), 2.80—3.20 (13H, m), 3.60—3.70 (2H, m), 3.72—3.77 (1H, m), 3.90—3.94 (2H, m), 4.04—4.07 (2H, m), 4.09—4.10 (2H, m), 4.12—4.36 (6H, m), 4.36—4.61 (4H, m), 4.72—4.80 (2H, m), 4.86—4.95 (4H, m), 6.64 (2H, d, J=8.0 Hz, Tyr^2 ArH), 6.94—6.95 (3H, m, Tyr^2 ArH), 7.11 (2H, s), 7.18—7.27 (4H, m, Phe^3 ArH), 7.27—7.32 (2H, m, Phe^3 ArH), 7.44—7.47 (1H, m), 7.84—7.86 (1H, m), 7.90—7.94 (1H, m), 7.95—7.98 (1H, m), 8.20—8.40 (11H, m), 8.60—8.64 (1H, m), 9.24 (1H, s). Amino acid ratios; Asp 1.03, Glu 1.00, Gly 1.02, Cys 1.88, Tyr 0.92, Phe 1.0, Arg 1.05, Pro 1.03, ammonia 2.84.

1c: $[\alpha]_D$ +2.0° (c=0.10, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.20—1.30 (12H, m, $(CH_2)_6$), 1.33—1.42 (2H, m), 1.43—1.66 (5H, m), 1.70—2.00 (6H, m), 2.00—2.20 (2H, m), 2.45—2.48 (1H, m), 2.53—2.56 (1H, m), 2.60—2.66 (2H, m), 2.80—2.88 (2H, m), 2.90—2.96 (4H, m), 3.00—3.08 (2H, m), 3.08—3.14 (2H, m), 3.14—3.20 (2H, m), 3.52—3.75 (2H, m), 3.84—3.88 (1H, m), 3.90—3.94 (1H, m), 4.04—4.14 (3H, m), 4.15—4.22 (4H, m), 4.28—4.36 (4H, m), 4.40—4.90 (4H, m), 6.52 (1H, s), 6.60—6.68 (2H, m, Tyr^2 ArH), 6.94—6.95 (2H, m, Tyr^2 ArH), 7.09—7.12 (1H, m), 7.18—7.32 (4H, m, Phe^3 ArH), 7.34—7.38 (1H, m, Phe^3 ArH), 7.44—7.47 (2H, m), 7.79—7.83 (1H, m), 7.90—7.95 (1H, m), 7.95—8.00 (1H, m), 8.10—8.35 (10H, m), 8.40—8.44 (1H, m), 8.56—8.62 (1H, m), 9.24 (1H, s). Amino acid ratios; Asp 0.98, Glu 0.51, Gly 1.00, Cys 1.44, Tyr 0.65, Phe 0.83, Arg 1.00, Pro 1.02, ammonia 2.01.

1d: $[\alpha]_D$ +8.2° (c=0.32, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.43—1.66 (3H, m), 1.70—2.10 (3H, m), 2.10—2.20 (2H, m), 2.45—2.48 (2H, m), 2.53—2.56 (2H, m), 2.60—2.66 (5H, m), 2.80—2.88 (2H, m), 2.90—2.96 (6H, m), 3.08—3.14 (2H, m), 3.14—3.20 (2H, m), 3.57—3.70 (2H, m), 3.72—3.77 (1H, m), 3.90—3.94 (2H, m), 4.04—4.14 (3H, m), 4.15—4.30 (6H, m), 4.36—4.44 (2H, m), 4.50—4.54 (2H, m), 4.76—4.81 (2H, m), 6.64 (2H, d, J=8.0 Hz, Tyr^2 ArH), 6.94—6.95 (3H, m, Tyr^2 ArH), 7.11 (1H, s), 7.18—7.27 (4H, m, Phe^3 ArH), 7.27—7.32 (2H, m, Phe^3 ArH), 7.44—7.47 (2H, m), 7.79—7.83 (1H, m), 7.90—7.95 (1H, m), 7.95—8.00 (1H, m), 8.20—8.35 (10H, m), 8.46—8.50 (1H, m), 8.70—8.80 (1H, m), 9.24 (1H, s). Amino acid ratios; Asp 1.03, Glu 0.98, Gly 1.02, Cys 1.87,

Tyr 0.92, Phe 1.0, Arg 1.06, Pro 1.05, ammonia 2.75.

1-(8-Azideoctyl)-β-D-glucopyranose (10a) A mixture of **4a** (1.55 g, 3.1 mmol) in methanol (20 ml) and 28% sodium methoxide in methanol (0.38 ml) was stirred for 2.5 h. After addition of acetic acid (0.11 ml), the mixture was evaporated to dryness. Chromatography of the residue on a column of silica gel with chloroform–methanol (9 : 1) gave **10a** (897 mg, 87%) as a colorless viscous oil. $[\alpha]_D$ −19.9° (c=1.07, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.36—1.40 (8H, m, $(CH_2)_4$), 1.59—1.64 (4H, m, C$\underline{H}_2$CH$_2$O, C$\underline{H}_2$CH$_2$N), 3.17 (1H, dd, J=7.8, 9.8 Hz, H-2), 3.24—3.30 (4H, m, CH$_2$N, H-4, 5), 3.34 (1H, dd, J=8.8, 9.0 Hz, H-3), 3.53 (1H, dt, J=9.5, 6.8 Hz, CH$_2$O), 3.66 (1H, dd, J=5.4, 12.0 Hz, H-6), 3.85 (1H, dd, J=1.5, 12.0 Hz, H-6), 3.90 (1H, dt, J=9.5, 6.8 Hz, CH$_2$O), 4.24 (1H, d, J=7.8 Hz, H-1). IR (KBr): 3408, 2100 cm^{-1}. FAB-MS m/z: 356 (M+Na$^+$), 334 (MH$^+$).

The following compounds **10b—d** were synthesized similarly from **4b—d** in 95%, 90% and 99% yields, respectively.

1-(2-Azideethyl)-β-D-glucopyranose (10b) A pale yellow oil. $[\alpha]_D$ −14.6° (c=1.44, MeOH). ^{1}H-NMR (D_2O) δ: 3.28 (1H, dd, J=9.2, 9.8 Hz, H-2), 3.38 (1H, dd, J=9.0, 10.7 Hz, H-4), 3.44—3.47 (1H, m, H-5), 3.48 (1H, dt, J=9.0, 10.7 Hz, H-3), 3.55 (2H, dd, J=4.2, 4.9 Hz, CH$_2$N), 3.71 (1H, dd, J=6.1, 12.5 Hz, H-6), 3.83 (1H, dt, J=12.2, 4.9 Hz, CH$_2$O), 3.91 (1H, d, J=12.5 Hz, H-6), 4.04 (1H, dt, J=10.5, 4.2 Hz, CH$_2$O), 4.50 (1H, d, J=7.8 Hz, H-1). IR (KBr): 3400, 2112 cm^{-1}. FAB-MS m/z: 250 (MH$^+$).

1-(8-Azideoctyl)-β-D-galactopyranose (10c) mp 80—83 °C. $[\alpha]_D$ −14.8° (c=1.03, MeOH). *Anal.* Calcd for $C_{14}H_{27}N_3O_6 \cdot 1/4H_2O$: C, 49.76; H, 8.20; N, 12.43. Found: C, 49.79; H, 8.06; N, 12.04. ^{1}H-NMR (D_2O) δ: 1.30—1.40 (8H, m, $(CH_2)_4$), 1.59—1.65 (4H, m, C$\underline{H}_2$CH$_2$O, C$\underline{H}_2$CH$_2$N), 3.22 (2H, t, J=7.1 Hz, CH$_2$N), 3.55 (1H, dd, J=8.1, 9.8 Hz, H-2), 3.63—3.70 (3H, m, CH$_2$O, H-3, 5), 3.76—3.98 (2H, m, H-6), 3.92—3.94 (2H, m, CH$_2$O, H-4), 4.39 (1H, d, J=8.1 Hz, H-1). IR (KBr): 3456, 2104 cm^{-1}. FAB-MS m/z: 356 (M+Na$^+$), 334 (MH$^+$).

1-(2-Azideethyl)-β-D-galactopyranose (10d) mp 67—67 °C. $[\alpha]_D$ −6.3° (c=1.14, MeOH). *Anal.* Calcd for $C_8H_{15}N_3O_6$: C, 38.55; H, 6.07; N, 16.86. Found: C, 38.64; H, 6.12; N, 16.57. ^{1}H-NMR ($CDCl_3$) δ: 3.51—3.57 (3H, m, CH$_2$N, H-2), 3.65 (1H, dd, J=3.4, 10.0 Hz, H-3), 3.68—3.80 (3H, m, H-5, 6), 3.84 (1H, dt, J=11.2, 5.4 Hz, CH$_2$O), 3.93 (1H, d, J=3.4 Hz, H-4), 4.06 (1H, dt, J=11.7, 4.6 Hz, CH$_2$O), 4.44 (1H, d, J=7.8 Hz, H-2). IR (KBr): 3392, 2104 cm^{-1}. FAB-MS m/z: 250 (MH$^+$).

1-(8-Aminooctyl)-β-D-glucopyranose (11a) A solution of **10a** (167 mg, 0.5 mmol) in methanol (10 ml) containing 1 N HCl (0.5 ml) was hydrogenated over a Lindlar catalyst (170 mg) for 1 h at 3.2 atmospheres pressure of H_2. After addition of the Lindlar catalyst (80 mg), the mixture was hydrogenated for 1 h at 3.2 atmospheres pressure of H_2. After removal of the catalyst by filtration, the solvent was removed by evaporation. The residue (340 mg, 99%) was used for the next step as 11a hydrochloride without further purification. $[\alpha]_D$ −22.9° (c=1.03, DMF). ^{1}H-NMR (D_2O) δ: 1.34—1.44 (8H, m, $(CH_2)_4$), 1.62—1.68 (4H, m, C$\underline{H}_2$CH$_2$O, C$\underline{H}_2$CH$_2$N), 2.96 (2H, t, J=8.1 Hz, CH$_2$N), 3.27 (1H, dd, J=7.8, 8.3 Hz, H-2), 3.41 (1H, dd, J=6.6, 9.3 Hz, H-4), 3.45—3.52 (2H, m, CH$_2$O, H-3), 3.68—3.96 (2H, m, CH$_2$O, H-5), 3.92—3.96 (2H, m, H-6), 4.47 (1H, d, J=7.8 Hz, H-1). IR (KBr): 3412, 1580 cm^{-1}. FAB-MS m/z: 308 (MH$^+$). The hydrochroride was dissolved in water, absorbed on a column of Amberlite IRC-50 (NH_4^+ form) resin, and eluted with 7.5% ammonia, giving the free base as a colorless amorphous material used as an analytical sample. $[\alpha]_D$ −24.1° (c=1.06, MeOH). *Anal.* Calcd for $C_8H_{11}NO_6 \cdot 1/3H_2O$: C, 42.19; H, 7.75; N, 6.15. Found: C, 42.00; H, 7.69; N, 5.96. ^{1}H-NMR (D_2O) δ: 1.35—1.42 (8H, m, $(CH_2)_4$), 1.56—1.60 (2H, m, C$\underline{H}_2$CH$_2$N), 1.65—1.70 (2H, m, C$\underline{H}_2$CH$_2$O), 2.82 (2H, t, J=6.8 Hz, CH$_2$N), 3.31 (1H, dd, J=8.3, 9.0 Hz, H-2), 3.43 (1H, dd, J=9.3, 9.8 Hz, H-4), 3.48—3.55 (2H, m, H-3, 5), 3.70—3.79 (2H, m, CH$_2$O, H-6), 3.95—4.00 (2H, m, CH$_2$O, H-6), 4.50 (1H, d, J=8.1 Hz, H-1). IR (KBr): 3384, 1576 cm^{-1}. FAB-MS m/z: 308 (MH$^+$).

The following compounds **11b—d** were synthesized similarly from **10b—d** respectively.

1-(2-Aminoethyl)-β-D-glucopyranose (11b) A colorless amorphous solid. $[\alpha]_D$ −17.2° (c=1.23, MeOH). *Anal.* Calcd for $C_{14}H_{29}NO_6 \cdot 1/4H_2O$: C, 42.19; H, 7.75; N, 6.15. Found: C, 42.00; H, 7.69; N, 5.96. ^{1}H-NMR (D_2O) δ: 3.01—3.06 (2H, m, CH$_2$N), 3.34 (1H, dd, J=8.1, 9.3 Hz, H-2), 3.43 (1H, dd, J=9.3, 9.5 Hz, H-4), 3.49—3.55 (1H, m, H-5), 3.54 (1H, dd, J=9.3, 9.3 Hz, H-3), 3.55 (2H, dd, J=4.2, 4.9 Hz, H-6), 3.76 (1H, dd, J=4.6, 12.2 Hz, H-6), 3.82—3.84 (1H, m, CH$_2$O), 3.95 (1H, d, J=12.2 Hz, H-6), 4.03—4.05 (1H, m, CH$_2$O), 4.52 (1H, d, J=8.1 Hz, H-1). IR (KBr): 3364, 1590 cm^{-1}. FAB-MS m/z: 224 (MH$^+$).

1-(8-Aminooctyl)-β-D-galactopyranose (11c) The spectral data for the reduction product were identical with the reported[10] values.

1-(2-Aminoethyl)-β-D-galactopyranose (11d) A colorless amorphous solid. $[\alpha]_D$ −11.3° (c=0.23, MeOH). *Anal.* Calcd for $C_8H_{11}NO_6 \cdot 1/4H_2O$: C, 42.19; H, 7.75; N, 6.15. Found: C, 42.40; H, 7.74; N, 6.13. ^{1}H-NMR (D_2O) δ: 2.80—2.90 (2H, m, CH$_2$N), 3.55 (1H, dd, J=7.8, 9.8 Hz, H-2), 3.66—3.82 (5H, m, CH$_2$O, H-3, 5, 6), 3.94—4.00 (2H, m, CH$_2$O, H-4), 4.43 (1H, d, J=7.8 Hz, H-1). IR (KBr): 3396, 1606 cm^{-1}. FAB-MS m/z: 224 (MH$^+$).

Synthesis of 2a Triethylamine (0.028 ml, 0.2 mmol) was added to a methanol (2 ml) solution of **12**[23] (40 mg, 0.04 mmol). The mixture was evaporated to dryness. To the residue, DMF (2 ml) and *tert*-butyldicarbonate (40 mg, 0.12 mmol) were added, and the mixture was stirred for 5 h. The solvent was removed and DMF (2 ml), **11a** hydrochloride (41 mg, 0.12 mmol), HOBt (20 mg, 0.15 mmol) and WSCI (31 mg, 0.12 mmol) were added to the residue. The mixture was stirred for 18 h, then the solvent was removed. Anisole (0.5 ml) and TFA (4.5 ml) were added to the residue, and the mixture was stirred for 1.5 h. The solvent was removed, then ether and water were added to the residue. The water layer was purified by preparative HPLC to give **2a** (11 mg, 20% based on **12**). $[\alpha]_D$ −13.3° (c=0.21, H_2O). ^{1}H-NMR (D_2O) δ: 1.28—1.35 (8H, m, $(CH_2)_4$), 1.49—1.50 (2H, m, C$\underline{H}_2$CH$_2$N), 1.61—1.64 (3H, m), 1.80—2.00 (3H, m), 2.04—2.09 (3H, m), 2.28—2.31 (3H, m), 2.88—2.95 (5H, m), 3.17—3.28 (6H, m), 3.38—3.49 (3H, m), 3.67—3.74 (2H, m), 3.84—3.93 (4H, m), 4.10—4.18 (1H, m), 4.25—4.26 (1H, m), 4.44—4.45 (2H, m), 4.62 (1H, dd, J=7.3, 7.6 Hz), 6.84 (2H, d, J=8.3 Hz, Tyr^2 ArH), 7.09 (2H, d, J=8.3 Hz, Tyr^2 ArH), 7.20—7.22 (2H, m, Phe^3 ArH), 7.36—7.43 (3H, m, Phe^3 ArH). FAB-MS m/z: 1375 (MH$^+$). Amino acid ratios; Gly 0.98, Arg, 0.98, Pro 0.91, Cys 1.75, Asp 0.98, Glu 0.97, Phe 1.00, Tyr 0.90, ammonia 2.10.

The following compounds **2b—d** were synthesized similarly from **12** and **11b—d** in 45%, 33% and 29% yields, respectively.

2b: $[\alpha]_D$ −20.5° (c=0.22, H_2O). ^{1}H-NMR (D_2O) δ: 1.75—1.95 (5H, m), 1.95—2.15 (4H, m), 2.20—2.36 (3H, m), 3.00—3.13 (5H, m), 3.15—3.33 (6H, m), 3.37—3.52 (5H, m), 3.71—3.98 (6H, m), 4.11—4.14 (2H, m), 4.31—4.35 (1H, m), 4.43—4.47 (2H, m), 4.90—4.92 (1H, m), 6.82 (2H, d, J=7.6 Hz, Tyr^2 ArH), 7.07 (2H, d, J=7.6 Hz, Tyr^2 ArH), 7.20—7.24 (2H, m, Phe^3 ArH), 7.35—7.43 (3H, m, Phe^3 ArH). FAB-MS m/z: 1291 (MH$^+$). Amino acid ratios; Gly 0.98, Arg, 0.99, Pro 0.96, Cys 1.79, Asp 0.98, Glu 0.98, Phe 1.00, Tyr 0.92, ammonia 2.78.

2c: $[\alpha]_D$ −10.0° (c=0.33, H_2O). ^{1}H-NMR (D_2O) δ: 1.29—1.54 (8H, m, $(CH_2)_4$), 1.61—1.72 (2H, m, C$\underline{H}_2$CH$_2$N), 1.83—1.96 (3H, m), 2.04—2.14 (3H, m), 2.30—2.35 (3H, m), 2.80—3.06 (5H, m), 3.15—3.34 (6H, m), 3.53 (1H, dd, J=7.8, 9.8 Hz, Gal H-2), 3.64—3.95 (8H, m), 4.12—4.15 (1H, d, J=7.8 Hz, Gal H-1), 4.27—4.30 (1H, m), 4.39—4.41 (1H, m), 4.46—4.53 (2H, m), 4.64—4.68 (1H, m), 4.90—4.94 (1H, m), 6.86 (2H, d, J=8.3 Hz, Tyr^2 ArH), 7.09 (2H, d, J=8.3 Hz, Tyr^2 ArH), 7.23—7.27 (2H, m, Phe^3 ArH), 7.38—7.45 (3H, m, Phe^3 ArH). FAB-MS m/z: 1375 (MH$^+$). Amino acid ratios; Gly 0.99, Arg, 1.00, Pro 0.98, Cys 1.75, Asp 1.00, Glu 0.98, Phe 1.00, Tyr 0.71, ammonia 2.11.

2d: $[\alpha]_D$ −13.9° (c=0.28, H_2O). ^{1}H-NMR (D_2O) δ: 1.65—2.00 (5H, m), 2.00—2.15 (4H, m), 2.30—2.34 (3H, m), 2.86—3.04 (5H, m), 3.20—3.30 (6H, m), 3.42—3.52 (5H, m), 3.67—3.88 (4H, m), 3.94—4.01 (2H, m), 4.12—4.15 (1H, m), 4.20—4.30 (1H, m), 4.34—4.37 (1H, m), 4.42—4.50 (1H, m), 4.92—4.95 (1H, m), 6.86 (2H, d, J=8.5 Hz, Tyr^2 ArH), 7.08 (2H, d, J=8.5 Hz, Tyr^2 ArH), 7.12—7.25 (2H, m, Phe^3 ArH), 7.38—7.45 (3H, m, Phe^3 ArH). FAB-MS m/z: 1291 (MH$^+$). Amino acid ratios; Gly 1.00, Arg, 0.99, Pro 0.99, Cys 1.85, Asp 1.00, Glu 0.99, Phe 1.00, Tyr 0.83, ammonia 3.02.

Antidiuretic Activity Male adult Brattleboro rats[24] 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 acclimated to metabolic cages for at least 2 days 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 (**1a—d**, **2a—d**) were prepared to a concentration of 200 pmol/ml or 2 nmol/ml in saline with 0.1% RSA. The solutions were injected intravenously (400 pmol/kg or 4 nmol/kg) and urine was collected hourly thereafter. The mean of the two hourly urine flow rates just before injections was considered as the predosage urine flow rate for each rat. Two methods were used to express the magnitude of the antidiuretic response. (1) The duration was defined

as the number of hours during which urine flow remained at less than 50% of the control rate for each rat. (2) The 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.

Acknowledgment We are grateful to Mrs. S. Fukushima, Mrs. J. Hosoda, Miss A. Imamura, Miss Y. Katori, Mrs. K. Kikuchi, Miss J. Komatsuzaki, Miss K. Nakamura, Miss H. Tsukamoto, and Mrs. M. Yoshii for their technical assistance.

References and Notes

1) *a*) Presented in part at the 114th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, March 1994; *b*) The amino acids used here are of the L-configuration. Standard abbreviations for amino acids are used [*Eur. J. Biochem.*, **138**, 9 (1984)]. The following abbreviations are also used: Ac = acetyl, Boc = *tert*-butoxycarbonyl, DCC = dicyclohexylcarbodiimide, DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethoxycarbonyl, HOBt = 1-hydroxybenzotriazole, HOSu = *N*-hydroxysuccinimide, NMP = *N*-methylpyrrolidinone, Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, TFA = trifluoroacetic acid, Trt = triphenylmethyl, TsOH = *p*-toluenesulfonic acid, WSCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.
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