Structural Requirements for Alkylglycoside-Type Renal Targeting Vector

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Purpose. We have previously shown Glc-S-C7-Me (octyl β-D-thioglucoside) exhibits renal targeting potential in vivo in addition to its specific binding to the renal membrane fraction in vitro. Thus, "alkylglycoside" is considered to be a novel targeting vector for the kidney (1,2). The present study is designed to clarify the structural requirements for alkylglycoside as a renal targeting vector.

Methods. Inhibitory effects of various sugars and glycosides on ^{3}H-Glc-S-C7-Me binding to the kidney membrane fraction were evaluated by a centrifugation method.

Results. As far as the sugar moiety is concerned, no other sugars except D-aldohexose and D-aldohexose derivatives (containing F, S, and N) showed greater inhibition than D-glucose. Therefore, octylthio derivatives of various D-aldohexose were prepared and their inhibitory effects were investigated. The following findings were obtained: Equatorial OH at 4 position is essential; OH at 2 position can have either orientation or be deleted. As far as the alkyl moiety is concerned, the length, branching and electrical environment in the region of the glycoside bond are important; aromatic structures can substitute for the alkyl portion; the preferred glycoside bonding atom is as follows: S > NH > O.

Conclusions. The structural requirements for the renal targeting vector have been identified to be as follows: a hydrophobic group (alkyl chain or aromatic ring) should be introduced to a sugar (D-glucose, D-mannose, or 2-deoxy-D-glucose) via a β-glycoside binding atom (S > NH > O).

KEY WORDS: DDS; kidney; targeting; sugar; glycoside.

INTRODUCTION

It is very important to develop tissue-specific targeting systems in order to make drugs safer and more effective. To this end, a number of different methods have been developed and are currently under investigation (3). While DDS (Drug Targeting System) for the liver has been thoroughly investigated and a number of successful developments have resulted, DDS targeting other tissues has received much less attention and further study is needed. In carrying out such studies, tissue-specific targeting vectors could play an important role (4). So far, some of the vectors investigated include polypeptides (5), antibodies (6,7), viruses (8,9), fatty acids (10), sugars (11,12), and low-molecular-weight peptide-(13). In our laboratory, we have focused on sugars to develop a new targeting DDS. During our recent investigations we found the low-molecular-weight model peptide, Arg-vasopressin (AVP), when modified by linking it to some sugars via an octamethylene group, exhibits renal-selective and efficient uptake (1). The renal uptake of these glycosylated peptides depends on the structure of the sugar and the uptake itself takes place by a saturable process from blood and involves specific binding to the renal microsomal fraction. Following this uptake, these peptides are then distributed in the proximal tubules (1). In addition, S-glycoside has greater renal targeting potential than O-glycoside, and Glc-S-C7-Me (Octyl β-D-thioglucoside) itself is recognized by the kidney. Therefore, we believe Glc-S-C7-Me may be a useful vector for renal targeting (2). So far, the structural requirements for this alkylglycoside-type renal targeting vector have not been defined and so we focused on this in the present report in order to obtain fundamental information that can be used to achieve the practical renal targeting of therapeutic agents.

Chemistry

The target thioglycosides were prepared according to Scheme 1. Treatment of commercially available peracetylated sugars with n- or t-octylmercaptan in dry dichloromethane in the presence of $BF_3 \cdot Et_2O$ gave the thioglycosides as an anomeric mixture, which was separated by silica gel chromatography, and then O-deacetylation with catalytic sodium methoxide in methanol gave target compounds; **6–12**, respectively (Standard Procedure A). While 1-thio-β-D-glucopyranose sodium salt was treated with alkyl tosylates, prepared from corresponding alcohols, or n-octanoyl chloride in DMF-water gave compounds **13–15**, respectively (Standard Procedure B).

The synthesis of 4-deoxyglucose **5** was carried out via introduction of (phenoxy) thiocarbonyl group (14), reductive dethioacylation with tributyltin hydride in the presence of α,α′-azobisisobutyronitrile (AIBN), replacement of O-benzyl group by O-acetyl group, selective transformation of 2-(trimethylsilyl)ethyl group into O-acetyl group (15,16), and finally deprotection, starting from 2-(trimethylsilyl)ethyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (15).

N-Glycoside compound **16** was also prepared according to the known method (17,18).

MATERIALS AND METHODS

Materials

^{3}H-Glc-S-C7-Me was synthesized by Amersham. All other reagents were commercially available.

Procedures

TLC plates coated with silica gel 60 F_{254} (Merck) were used, and flush chromatography was performed on silica gel (Nacalai Tesque, 230–400 mesh). Melting points were determined with Yanaco Micro Melting Point Apparatus and were uncorrected. Optical rotations were determined with a JASCO

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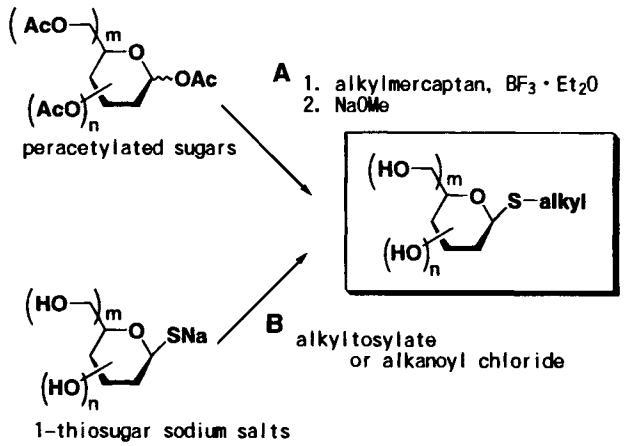


Scheme 1. Preparation of thioglycosides.

Digital Polarimater DIP-370. ^{1}H-NMR spectra were recorded with a JEOL-GSX400FT spectrometer. Mass spectra were recorded with JEOL JMS-700 (FAB), HITACHI M-80B (FD, SIMS), and HEWLETT PACKARD 5989A (TSP) spectrometers, respectively. Concentrations were conducted in vacuo.

General Procedure for Obtaining Sugars and Glycosides

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-deoxy-*β-D-*glucopyranoside* (**2**)

Phenyl chlorothionoformate (0.5 ml, 3.64 mmol) was added to a solution of 2-(trimethylsilyl)ethyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (1, 1.00 g, 1.82 mmol) in 1:1 dichloromethane-pyridine (18 ml), and the mixture was stirred overnight at room temperature then heated for 4 h at 45°C. After completion of the reaction, methanol (3ml) was added to the solution and it was concentrated, then extracted with chloroform. The extract was successively washed with 2M-HCl and water, dried (Na_2SO_4), concentrated. Silica gel column chromatography (200 ml, 1:10 AcOEt-Hexane) of the residue gave the (phenoxy-)thiocarbonyl derivative. To a solution of (phenoxy)thiocarbonyl derivative in toluene (50 ml) was added tributyltin hydride (4.0 ml) and α,α′-azobisisobutyronitrile (AIBN, 123 mg), and the mixture was stirred for 2 h at 100°C then concentrated. Silica gel chromatography (1:10 AcOEt-Hexane) of the residue gave **2** (0.54g, 56%) as a syrup; ^{1}H-NMR($CDCl_3$):δ 1.01 (m, 2H), 1.36 (m, 1H), 2.10 (m, 1H), 3.29 (t, 1H, J 8.2 Hz), 3.46–3.61 (m, 5H), 4.00 (m, 1H), 4.33 (d, 1H, J 7.8 Hz, H-1), 4.51–4.93 (m, 6H), 7.20–7.37 (m, 15H).

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-deoxy-*β-D-*glucopyranoside* (**3**)

A solution of **2** (0.54 g 1.01 mmol) in ethanol (36 ml) and acetic acid (6 ml) was hydrogenolyzed in the presence of 10% Pd-C (0.5 g) for 3 h at 50°C, then filtered and concentrated. The residue was acetylated with Ac_2O (5 ml) in pyridine (10 ml) for 16 h at room temperature. The product was purified by chromatography on a column of silica gel (250 ml) with 1:3 AcOEt-Hexane, affording triacetate **3** (0.44 g, 87%) as a syrup; ^{1}H-NMR($CDCl_3$):δ 0.92 (m, 2H), 1.59 (dt, 1H, J 11.7, 12.5 Hz, H-4ax), 2.02–2.07 (3s, 9H), 2.12 (ddd, 1H, J 2.0, 5.3, 12.7 Hz, H-4eq), 3.54 (m, 1H), 3.74 (m, 1H, H-5), 3.95 (m, 1H), 4.09 (dd, 1H, J 4.4, 11.7 Hz, H-6), 4.19 (dd, 1H, J 5.8, 11.7 Hz, H-6′), 4.41 (d, 1H, J 8.0 Hz, H-1), 4.87 (dd, 1H, J 7.8, 9.7 Hz, H-2), 4.98 (ddd, 1H, J 5.3, 9.7, 11.4 Hz, H-3).

*1,2,3,6-Tetra-*O*-acetyl-4-deoxy-*α,β-D-*glucopyranose* (**4**)

To a solution of **3** (21 mg, 53.7 μmol) in toluene (0.5 ml) and Ac_2O (76 μl, 0.81 mmol), cooled to 0°C, was added $BF_3 \cdot Et_2O$ (6 μl, 48.3 μmol). The mixture was stirred for 2 h at room temperature, chloroform (30 ml) was added, and the solution was successively washed with saturated $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Silica gel chromatography (20 ml, 1:3 AcOEt-Hexane) of the residue gave **4** (17 mg, 92%); ^{1}H-NMR($CDCl_3$):δ 2.03–2.15 (8s, 12H), 5.65 (d, 1H, J 7.8 Hz, H-1 for β-anomer), 6.34 (d, 1H, J 3.6 Hz, H-1 for α-anomer).

*4-deoxy-*D*-glucopyranose (4-deoxy glucose)* (**5**)

O-Deacetylation of **4** (65 mg, 0.20 mmol) with catalytic sodium methoxide in methanol gave **5** (quantitative) as an amorphous mass; $[\alpha]^{24}_D$ +44.3° (c 1.52, $CHCl_3$- CH_3OH 1:5); ^{1}H-NMR(CD_3OD):δ 1.36 (m, 1H, H-4ax), 1.91 (m, 1H, H-4eq), 4.42 (d, 1H, J 7.8 Hz, H-1 for β-anomer), 5.15 (d, 1H, J 3.6 Hz, H-1 for α-anomer); MS (FAB) m/z 187 $(M + Na)^+$.

*n-Octyl 1-thio-*β*-D-allopyranoside* (**9**) (Standard Procedure A)

To a solution of 1,2,3,4,6-penta-O-acetyl-β-*D*-allopyranoside (220 mg, 0.56 mmol) in dry dichloromethane (2 ml), cooled to 0°C, was added $BF_3 \cdot ET_2O$ (103 μl, 0.84 mmol), the mixture was stirred for 5 h at 0°C, and the stirring was continued overnight at room temperature. Chloroform (30 ml) was added, and the solution was successively washed with saturated $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Silica gel chromatography (50 ml, 1:3 AcOEt-Hexane) of the residue gave the β-glycoside (141 mg, 53%) and the corresponding α-glycoside (37 mg, 14%), respectively. O-Deacetylation of the β-glycoside (141 mg, 0.29 mmol) as described for **5** gave **9** (84 mg, 94%) as an amorphous mass; $[\alpha]^{24}$D-31.0° (c 0.84, $CHCl_3$); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.30 (m, 10H), 1.63 (m, 2H), 2.71 (m, 2H), 3.36 (dd, 1H, J 2.9, 9.9 Hz), 3.48 (dd, 1H, J 2.9, 9.6 Hz), 3.63 (m, 2H, H-6), 3.83 (m, 1H, H-5), 4.07 (t, 1H, J 3.0 Hz, H-3), 4.69 (d, 1H, J 10.0 Hz, H-1); MS(FD) m/z 308 $(M)^+$.

Compounds **6**, **7**, **8**, **10**, **11** and **12** were prepared in the same way (Standard Procedure A) from corresponding peracetylated sugars, respectively.

*n-Octyl 1-thio-*β*-D-mannopyranoside* (**6**). mp 118–119°C; $[\alpha]^{24}_D$ −87.6° (c 0.60, $CHCl_3$-CH_3OH 9:1); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.30 (m, 10H), 1.62 (m, 2H), 2.71 (m, 2H), 3.32 (ddd, 1H, J 2.4, 5.4, 9.6 Hz, H-5), 3.47 (dd, 1H, J 3.6, 9.5 Hz, H-3), 3.60 (t, 1H, J 9.5 Hz, H-4), 3.72 (dd, 1H, J 5.4, 11.8 Hz, H-6), 3.85 (dd, 1H, J 2.4, 11.9 Hz, H-6′), 3.89 (dd, 1H, J 0.9, 3.5 Hz, H-2), 4.69 (d, 1H, J 1.0 Hz, H-1); MS(SIMS) m/z 309 $(M + H)^+$.

n-Octyl 2-deoxy-1-thio-β-D-glucopyranoside (**7**). mp 103–104°C; $[\alpha]^{27}D$ −39.5° (c 0.94, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.30 (m, 10H), 1.58 (dt, 1H, J 11.5, 12.8 Hz, H-2ax), 1.61 (m, 2H), 2.14 (ddd, 1H, J 1.9, 5.3, 12.8 Hz, H-2eq), 2.71 (m, 2H), 3.21 (m, 2H), 3.58 (m, 1H, H-5), 3.68 (dd, 1H, J 5.3, 11.9 Hz, H-6), 3.86 (dd, 1H, J 1.9, 11.9 Hz, H-6′), 4.64 (dd, 1H, J 1.8, 11.8 Hz, H-1); MS(TSP) m/z 315 $(M + Na)^+$.

n-Octyl 4-deoxy-1-thio-β-D-glucopyranoside (**8**). $[\alpha]^{24}D$ −32.2° (c 0.93, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):δ 0.88 (t, 3H), 1.28 (m, 10H), 1.61 (m, 3H), 1.94 (ddd, 1H, J 1.8, 5.1, 12.7 Hz, H-4eq), 2.68 (m, 2H), 3.08 (t, 1H, J 9.1 Hz, H-2), 3.49 (m, 4H), 4.27 (d, 1H, J 9.5 Hz, H-1); LC-MS(TSP) m/z 315 $(M + Na)^+$.

n-Octyl 1-thio-β-D-talopyranoside (**10**). $[\alpha]^{24}D$ −14.8° (c 0.38, $CHCl_3$); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.30 (m, 10H), 1.64 (m, 2H), 2.73 (m, 2H), 3.47 (dt, 1H, J 1.0, 5.6 Hz, H-5), 3.58 (t, 1H, J 3.3 Hz, H-3), 3.73 (dd, 1H, J 5.6, 11.3 Hz, H-6), 3.76 (dd, 1H, J 4.9, 11.5 Hz, H-6′), 3.81 (dd, 1H, J 1.3, 3.1 Hz), 3.85 (dd, 1H, J 1.3, 3.3 Hz), 4.64 (d, 1H, J 1.0 Hz, H-1); MS(FD) m/z 309 $(M + H)^+$.

n-Octyl 1-thio-β-D-xylopyranoside (**11**). $[\alpha]^{24}D$ −43.7° (c 0.78, CH_3OH); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.31 (m, 10H), 1.62 (m, 2H), 2.66 (m, 2H), 3.19 (m, 2H), 3.30 (t, 1H, J 8.6 Hz), 3.49 (m, 1H, H-4), 3.91 (dd, 1H, J 5.3, 11.4 Hz, H-5eq), 4.29 (d, 1H, J 9.4 Hz, H-1); MS(FD) m/z 278 $(M)^+$.

t-Octyl 1-thio-β-D-glucopyranoside (**12**). mp 88–91°C; $[\alpha]^{24}D$ −44.8° (c 0.90, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):δ 1.05 (s, 9H), 1.49 (d, 6H), 1.75 (s, 2H), 3.13 (t, 1H, J 9.2 Hz), 3.28 (m, 1H, H-5), 3.33 (t, 1H, J 8.9 Hz), 3.39 (t, 1H, J 8.5 Hz), 3.65 (dd, 1H, J 5.3, 11.9 Hz, H-6), 3.82 (dd, 1H, J 2.2, 11.9 Hz, H-6′), 4.50 (d, 1H, J 10.0 Hz, H-1); MS(TSP) m/z 331 $(M + Na)^+$.

t-Butyl 1-thio-β-D-glucopyranoside (**13**) (Standard Procedure B)

To a solution of t-butanol (0.1 g, 1.35 mmol) in 1:1 dichloromethane-pyridine (6 ml) was added p-toluenesulfonyl chloride (0.51 g, 2.70 mmol) at 0°C, and the mixture was stirred 5 h at room temperature. After completion of the reaction, methanol (3 ml) was added to the solution and it was concentrated, then extracted with chloroform. The extract was successively washed with 2M-HCl and water, dried (Na_2SO_4), concentrated. The residue in DMF (1ml) was added a solution of 1-thio-β-D-glucopyranose sodium salt (147 mg, 0.67 mmol) in 1:1 H_2O-DMF (2 ml), and the mixture was stirred overnight at room temperature, then concentrated. The product was purified by chromatography on a column of silica gel (30 ml) with 10:1 $CHCl_3$-CH_3OH, affording **13** (80 mg, 47%) as a syrup; $[\alpha]^{24}D$ −22.7° (c 0.80, CH_3OH); ^{1}H-NMR(CD_3OD):δ 2.17 (s, 9H), 3.20–3.37 (m, 4H), 3.65 (dd, 1H, J 5.4, 12.1 Hz, H-6), 3.84 (dd, 1H, J 2.1, 11.8 Hz, H-6′), 4.25 (d, 1H, J 9.7 Hz, H-1).

Compounds **14** and **15** were prepared in the similar manner (Standard Procedure B) from 1-thio-β-D-glucopyranose sodium salt and tosylate of 5-nonaol or n-octanoyl chloride, respectively.

5-Nonyl 1-thio-β-D-glucopyranoside (**14**). $[\alpha]^{24}D$ −52.2° (c 1.42, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):° 0.90 (t, 6H), 1.34 (m, 8H), 1.61 (m, 4H), 2.87 (m, 1H), 3.36 (m, 2H), 3.58 (t, 1H, J 8.9 Hz), 3.65 (t, 1H, J 9.2 Hz), 3.84 (br d, 2H, H-6), 4.42 (d, 1H, J 10.0 Hz, H-1); MS(FD) m/z 322 $(M)^+$.

n-Octanoyl 1-thio-β-D-glucopyranose (**15**). $[\alpha]^{24}D$ −14.8° (c 0.80, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.31 (m, 8H), 1.66 (m, 2H), 2.62 (dd, 2H), 3.36 (m, 4H), 3.67 (dd, 1H, J 4.6, 12.1 Hz, H-6), 3.82 (dd, 1H, J 1.9, 12.2 Hz, H-6′), 5.06 (d, 1H, J 10.3 Hz, H-1); MS(TSP) m/z 345 $(M + Na)^+$.

n-Octyl β-D-glucopyranosyl amine **16** was prepared from D-glucose and n-octylamine according to the known method (17,18) as follows. To a solution of D-glucose (3.00 g, 16.70 mmol) in H_2O was added a solution of n-octylamine (4.13 ml, 25.05 mmol) in ethanol (15 ml), and the mixture was stirred 3 days at room temperature, then concentrated. The solid residue was recrystallized from AcOEt, affording **16** (4.56 g, 94%); mp 98–100°C; $[\alpha]^{22}D$ −15.5° (c 0.54, $CHCl_3$-CH_3OH 1:1); ^{1}H-NMR(CD_3OD):δ 0.90 (t, 3H), 1.32 (m, 10H), 1.50 (m, 2H), 2.62 (m, 1H), 2.90 (m, 1H), 3.06 (t, 1H, J 8.7 Hz), 3.22 (ddd, 1H, J 2.1, 5.3, 9.3 Hz, H-5), 3.27 (t, 1H, J 9.0 Hz), 3.35 (t, 1H, J 8.7 Hz), 3.65 (dd, 1H, J 5.3, 11.9 Hz, H-6), 3.82 (d, 1H, J 8.5 Hz, H-1), 3.84 (dd, 1H, J 2.3, 11.8 Hz, H-6′); MS(FAB) m/z 292 $(M + H)^+$.

Inhibition of the Binding of ^{3}H-Glc-S-C7-Me to Rat Kidney Membrane Fraction

Rat kidney microsomes were prepared by centrifugation (19). ^{3}H-Glc-S-C7-Me (20 pmol/ml), inhibitors and kidney microsomes (1 mg/ml as protein concentration) were incubated in phosphate-buffered saline (pH 7.4) on ice for 1 h. The other conditions were the same as described in the previous report (2). After ultracentrifugation (50,000 × g for 5 min at 4°C), the supernatant was aspirated and the precipitate was dissolved in 1% Tween20 to determine the radioactivity using a liquid scintillation counter. To correct for any supernatant remaining in the precipitate, ^{14}C-sucrose was added to the incubation buffer. All samples were examined in triplicate and IC_{50}s were evaluated by log-logit analysis of the mean values from 3 to 4 points involving a ligand concentration around 50% inhibition.

RESULTS

Inhibitory Effects of Various Sugars

The % inhibition by D-glucose of the specific binding of ^{3}H-Glc-S-C7-Me to the kidney membrane fraction was 17.4% at 20 mM and 36.3% at 100 mM (Table 1). Various sugars (D-aldohexose, L-aldohexose, D-aldopentose, L-aldopentose, ketohexose and oligosugar) were tested and all the sugars, except D-aldohexose and D-aldohexose derivatives (containing F, S and N), failed to show greater inhibition than D-glucose (Table 1).

Inhibitory Effects of Various Octylthioglycosides

Structures and abbreviations of the various octylthioglycosides tested in this report are shown in Fig. 1. These compounds are all β-thioglycoside, and the only difference is in the sugar

Table 1. Inhibitory Effects of Various Sugars on ^{3}H-Glc-S-C7-Me Binding to Kidney Membrane

Sugar		% inhibition (Mean ± SD)	
		20 mM	100 mM
D-aldohexose	D-allose (All)	22.2 ± 2.6	52.0 ± 2.4
	D-altrose	9.4 ± 1.6	18.5 ± 2.0
	D-glucose (Glc)	17.4 ± 0.4	36.3 ± 5.1
	D-mannose (Man)	18.7 ± 3.6	51.0 ± 1.5
	D-gulose	18.9 ± 2.1	47.8 ± 3.4
	D-idose	35.1 ± 8.8	62.0 ± 0.8
	D-galactose (Gal)	2.5 ± 1.7	18.9 ± 0.6
	D-talose	31.0 ± 0.3	61.8 ± 2.2
deoxy D-aldohexose	2-deoxy-D-glucose (2d-Glc)	29.2 ± 0.5	58.3 ± 0.5
	3-deoxy-D-glucose (3d-Glc)	6.7 ± 0.7	18.9 ± 1.8
	4-deoxy-D-glucose (4d-Glc)	33.3 ± 1.0	69.6 ± 3.3
	6-deoxy-D-glucose (6d-Glc)	9.9 ± 0.7	23.4 ± 1.0
D-aldohexose derivatives containing F	1-Fluoro-1-deoxy-α-D-glucose	99.6 ± 2.5	105.7 ± 1.8
	1-Fluoro-1-deoxy-β-D-glucose	42.3 ± 5.2	98.7 ± 4.9
	2-Fluoro-2-deoxy-D-glucose	37.8 ± 1.1	67.9 ± 1.8
	3-Fluoro-3-deoxy-D-glucose	9.7 ± 0.9	18.7 ± 2.7
	4-Fluoro-4-deoxy-D-glucose	70.2 ± 1.1	83.9 ± 1.5
	6-Fluoro-6-deoxy-D-glucose	17.8 ± 5.6	32.5 ± 0.7
D-aldohexose derivatives containing S	1-thio-β-D-glucose	66.0 ± 1.5	78.2 ± 0.6
	1-thio-β-D-galactose	64.8 ± 2.4	60.6 ± 0.8
	5-thio-D-glucose	11.2 ± 0.5	30.7 ± 0.9
D-aldohexose derivatives containing N	deoxynojirimycin	98.9 ± 2.7	99.6 ± 0.6
	deoxynojirimycin 1-sulfonic acid	52.1 ± 1.0	85.4 ± 2.5
	1-amino-1-deoxy-β-D-glucose	39.7 ± 0.8	82.7 ± 2.4
L-aldohexose	L-glucose	6.7 ± 0.4	19.1 ± 2.3
	L-galactose	3.1 ± 0.6	11.3 ± 0.8
	L-(−)-mannose	5.5 ± 1.3	18.4 ± 7.8
	L-(−)-idose	12.1 ± 2.2	35.1 ± 1.3
Ketohexose	D-xylulose	4.1 ± 4.0	0.4 ± 10.4
	D-fructose	12.8 ± 2.6	23.5 ± 1.4
	D-psicose	8.2 ± 8.2	25.8 ± 1.3
	D-tagatose	8.1 ± 1.0	11.1 ± 1.6
	D-(+)-sorbose	10.4 ± 3.1	20.4 ± 0.8
	D-ribulose	2.9 ± 3.6	7.0 ± 3.9
	L-sorbose	−4.0 ± 1.0	2.9 ± 2.7
	L-xylulose	6.9 ± 2.8	22.1 ± 2.4
D-aldopentose	D-ribose	3.5 ± 2.3	20.9 ± 1.1
	D-arabinose	4.8 ± 0.5	13.8 ± 1.1
	D-xylose	4.2 ± 1.7	15.7 ± 1.6
	D-lyxose	4.0 ± 0.8	12.9 ± 0.1
L-aldopentose	L-arabinose	2.8 ± 1.7	8.5 ± 1.2
	L-xylose	7.0 ± 1.3	13.3 ± 1.8
Oligosugar	α,α-trehalose	−0.2 ± 0.8	−6.1 ± 3.2
	lactose	−1.3 ± 1.5	−2.3 ± 2.3
	maltose	15.9 ± 3.3	15.7 ± 1.3
	sucrose	−1.7 ± 2.4	−9.6 ± 8.2
	cellobiose	−1.6 ± 3.1	15.6 ± 1.3
	melibiose	−0.7 ± 1.7	−2.8 ± 1.4
	raffinose	−1.2 ± 3.5	−4.6 ± 4.3
Amino sugar	D-glucosamine	−2.3 ± 2.5	−6.4 ± 8.3
	D-galactosamine	−3.4 ± 2.7	−8.0 ± 4.0
	N-acetyl-D-glucosamine	0.9 ± 2.1	0.7 ± 1.2
	N-acetyl-D-galactosamine	3.3 ± 0.7	−2.3 ± 1.0
Miscellaneous	3-0-methyl-D-glucopyranose	9.8 ± 4.1	22.8 ± 2.4
	myo-inositol	7.2 ± 2.9	15.8 ± 2.6
	L-rhamnose	7.8 ± 2.3	23.7 ± 3.7
	L-fucose	10.1 ± 2.7	23.6 ± 1.4
	glucronic acid	3.9 ± 2.8	2.8 ± 2.4
	D-galacturonic acid	5.3 ± 1.7	4.2 ± 3.7
	n-acetylneuraminic acid	5.7 ± 1.8	0.4 ± 0.5
	galactonic acid	−9.3 ± 0.7	ND
	D-glucose-6-phosphate	13.7 ± 3.9	25.4 ± 3.6
	2-deoxy-D-ribose	3.6 ± 1.8	6.4 ± 3.0

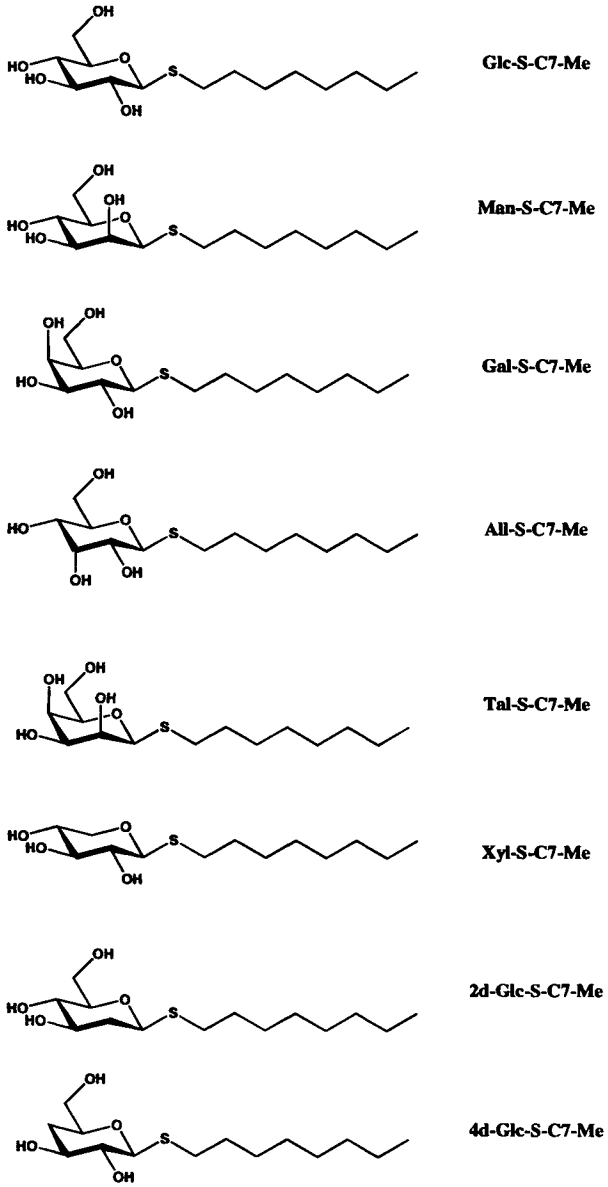


Fig. 1. Structures of the various n-octyl 1-thioglycosides tested in this report.

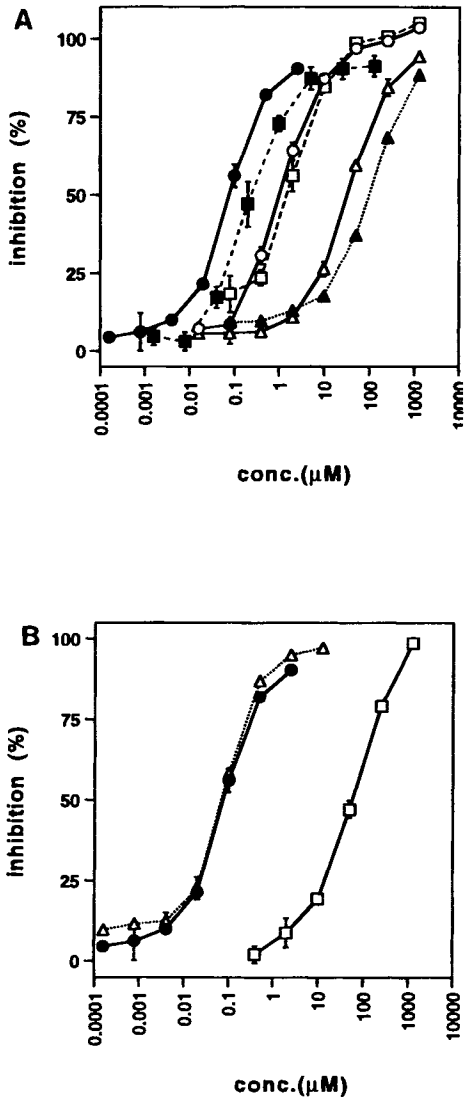


Fig. 2. Inhibititory effects of the glycoside derivatives on the specific binding of ^{3}H-Glc-S-C7-Me to rat kidney membrane fraction. (A): The binding of ^{3}H-Glc-S-C7-Me (20 pmol/ml) to membrane (1mg/ml) was measured in the presence of the inhibitors, i.e., Glc-S-C7-Me (●), Gal-S-C7-Me (▲), Man-S-C7-Me (■), All-S-C7-Me (○), Tal-S-C7-Me (△) and Xyl-S-C7-Me (□). (B): The binding of ^{3}H-Glc-S-C7-Me (20pmol/ml) to membrane (1mg/ml) was measured in the presence of the inhibitors, i.e., Glc-S-C7-Me (●), 2d-Glc-S-C7-Me (△) and 4d-Glc-S-C7-Me (□). Values represent means ± SD of triplicate assay.

moiety. The inhibitory effects of these octylthioglycosides are shown in Fig.2A and the IC_{50} values are shown in Table 2. Comparing the IC_{50} of non-labeled Glc-S-C7-Me (0.0775 μM), those of Man-S-C7-Me, Gal-S-C7-Me, All-S-C7-Me, Tal-S-C7-Me and Xyl-S-C7-Me were 0.296, 87.4, 1.09, 31.5 and 1.52 μM, respectively. The inhibitory effect of Man-S-C7-Me was reduced slightly, that of All or Xyl derivatives was reduced less than to 1/10, and that of Gal or Tal derivatives was reduced less than to 1/400.

The inhibitory effects of 2d-Glc-S-C7-Me and 4d-Glc-S-C7-Me, i.e., Glc-S-C7-Me derivatives substituted with H at the C-2 or C-4 OH in the pyranose ring, respectively, are shown in Fig. 2B. These IC_{50} values were 0.071 and 54.5 μM, respectively. Deletion of the C-2 OH had no clear effect while deletion of the C-4 OH reduced markedly reduced inhibition.

Fig. 3A shows the effects of the derivatives of which alkyl portion differ. In comparison with the effect of Glc-S-C7-Me (n-octyl as alkyl portion), the inhibitory effects of Glc-S-t-Octyl and Glc-S-CH(-$CH_2CH_2CH_2CH_3$)$_2$ (carbon number are nearly the same but a branch present) were reduced from 1/10 to 1/20. In addition, the inhibitory effect of Glc-S-t-Bu (carbon number of the alkyl portion is half of octyl group) was markedly reduced to less than 1/12000 that of Glc-S-C7-Me.

Inhibitory effects of the derivatives in which n-octyl portion is substituted by moieties other than alkyl chains are shown in Fig. 3B. While the p-nitrophenyl derivative produced almost

Table 2. Inhibitory Effects of Various Glycosides on ^{3}H-Glc-S-C7-Me Binding to Kidney Membrane

Glycosides		IC_{50} (μM)	Relative Activity	
S-glucoside				
n-octyl 1-thio-β-D-glucopyranoside	Glc-S-C7-Me	0.0775	1	
n-octyl 1-thio-β-D-galactopyranoside	Gal-S-C7-Me	87.4	0.000887	1/1100
n-octyl 1-thio-β-D-mannopyranoside [6]	Man-S-C7-Me	0.296	0.262	1/3.8
n-octyl 1-thio-β-D-allopyranoside [9]	All-S-C7-Me	1.09	0.0713	1/14.0
n-octyl 1-thio-β-D-talopyranoside [10]	Tal-S-C7-Me	31.5	0.00246	1/410
n-octyl 1-thio-β-D-xylopyranoside [11]	Xyl-S-C7-Me	1.52	0.0511	1/20.0
n-octyl 1-thio-2-deoxy-β-D-glucopyranoside [7]	2d-Glc-S-C7-Me	0.071	1.09	1/0.92
n-octyl 1-thio-4-deoxy-β-D-glucopyranoside [8]	4d-Glc-S-C7-Me	54.5	0.00142	1/700
n-octyl 1-thio-α-D-glucopyranoside	Glc(α)-S-C7-Me	1.33	0.0583	1/17.0
n-octyl 1-thio-α-D-mannopyranoside	Man(α)-S-C7-Me	1.27	0.0610	1/16.0
n-octyl 1-thio-α-D-allopyranoside	All(α)-S-C7-Me	10.4	0.00744	1/134
n-octyl 1-thio-α-D-talopyranoside	Tal(α)-S-C7-Me	14.9	0.00520	1/190
n-octyl 1-thio-α-D-xylopyranoside	Xyl(α)-S-C7-Me	21.4	0.00362	1/276
n-octyl 1-thio-2-deoxy-α-D-glucopyranoside	2d-Glc(α)-S-C7-Me	2.77	0.0279	1/38
n-octyl 1-thio-4-deoxy-α-D-glucopyranoside	4d-Glc(α)-S-C7-Me	55.2	0.00140	1/710
p-nitrophenyl 1-thio-β-D-glucopyranoside	Glc-S-pNP	0.226	0.343	1/2.9
p-nitrophenyl 1-thio-β-D-galactopyranoside	Gal-S-pNP	>1000	<0.0000775	<1/13000
p-nitrophenyl 1-thio-β-D-mannopyranoside	Man-S-pNP	2.37	0.0327	1/31.0
p-nitrobenzyl 1-thio-β-D-glucopyranoside	Glc-S-pNBn	5.33	0.0146	1/68.7
t-butyl 1-thio-β-D-glucopyranoside [13]	Glc-S-tBu	910	0.000085	1/12000
t-octyl 1-thio-β-D-glucopyranoside [12]	Glc-S-$C(CH_3)_2CH_2C(CH_3)_3$	1.00	0.0773	1/13.0
5-nonyl 1-thio-β-D-glucopyranoside [14]	Glc-S-$CH(C_4H_9)_2$	1.49	0.0521	1/19.0
n-octanoyl 1-thio-β-D-glucopyranose [15]	Glc-S-CO-C6-Me	701	0.000111	1/9000
O-glucoside				
n-octyl β-D-glucopyranoside	Glc-O-C7-Me	4.61	0.0168	1/60
n-hexyl β-D-glucopyranoside	Glc-O-C5-Me	15.6	0.00498	1/201
n-decyl β-D-glucopyranoside	Glc-O-C9-Me	6.39	0.0121	1/82.5
phenyl β-D-glucopyranoside	Glc-O-Ph	667	0.000116	1/8600
p-nitrophenyl β-D-glucopyranoside	Glc-O-pNP	2.86	0.0271	1/37
p-nitrophenyl α-D-glucopyranoside	Glc(α)-O-pNP	1.73	0.0448	1/22.0
o-nitrophenyl β-D-glucopyranoside	Glc-O-oNP	267	0.000290	1/3400
4-methylumbelliferyl β-D-glucopyranoside	Glc-O-4MU	6.21	0.0125	1/80
N-Glucoside				
n-octyl β-D-glucopyranosyl amine [16]	Glc-NH-C7-Me	0.378	0.205	1/4.9

Note: [ ]: compound No. in Materials.

the same inhibition as Glc-S-C7-Me, the p-nitrobenzyl derivative reduced the effects to about 1/70. In addition, the inhibitory effects of Glc-S-CO-C6-Me, which has a carbonyl introduced next to the glycosidic binding atom, reduced the inhibitory effect to 1/9000.

Inhibitory effects of various glycosides are summarized in Table 2. The compounds without any anomer description in the table are β-glycosides. As far as Glc, Man and 2d-Glc derivatives are concerned, α-glycosides showed 1/4 to 1/40 the inhibition of the corresponding β-glycosides, and so β-glycoside is suitable for the renal recognition.

Similarly, in the case of O-glucoside derivatives, Glc-O-pNP showed a relatively strong inhibitory effect but the effects of Glc-O-oNP and Glc-O-Ph were about 1/1000 that of Glc-O-pNP (Table 2).

DISCUSSION

The previously reported results described below suggest the alkylglycoside-type renal targeting vector is taken up by the kidney via binding to the renal membrane fraction. (I) In the Arg-vasopressin (AVP, selected as a model peptide) derivatives, those modified by linkage to a variety of sugars via an octamethylene group, glucose, mannose, and 2-deoxyglucose derivatives exhibited renal targeting potential in rats as well as specific binding to the kidney membrane fraction (1). On the other hand, the galactose derivative exhibited neither of these effects (1). (II) From a pharmacokinetic analysis of the O-glucoside derivative of AVP, renal uptake in vivo is a specific mechanism which is saturable. Also, since the apparent Km estimated from the in vivo uptake is almost the same as Kd estimated from the in vitro binding study, binding to the renal membrane is considered to involve renal uptake in vivo (1). (III) Glc-S-C7-Me itself binds to the renal membrane fraction as well as exhibiting renal targeting potential in vivo (2). In addition, Glc-S-C8- derivatives of AVP, tryptamine and NBD exhibit renal targeting ability in vivo and inhibitory effects on the binding of ^{3}H-Glc-S-C7-Me to the renal membrane fraction (2).

Considering these results, we believe the structural requirements for renal recognition can be identified from the in vitro study. In fact, the inhibitory effects of some sugars (glucose,

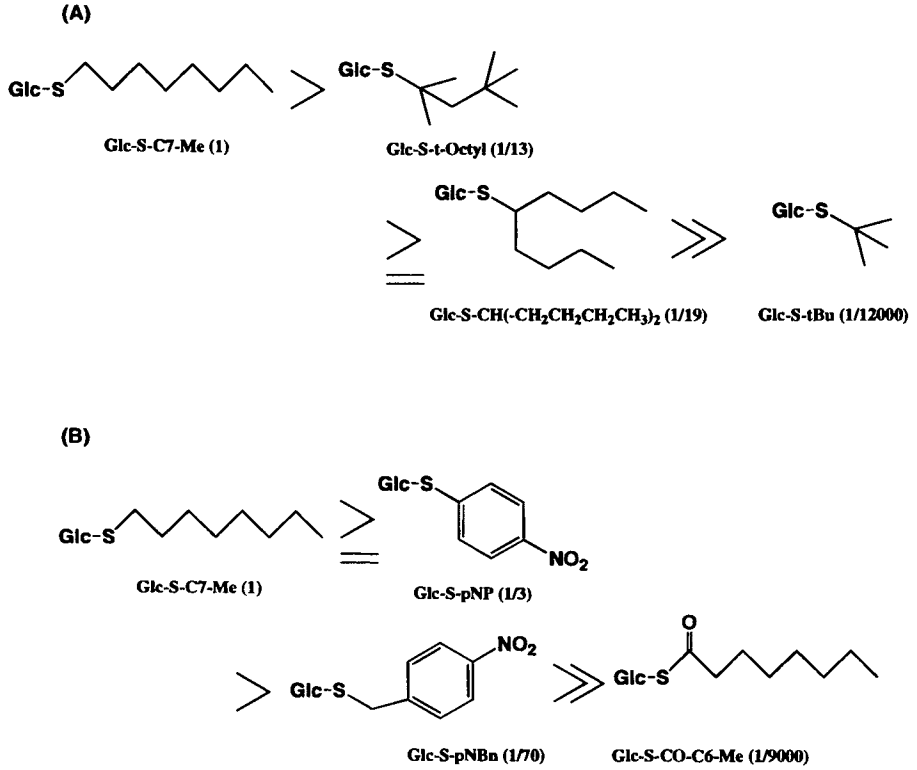
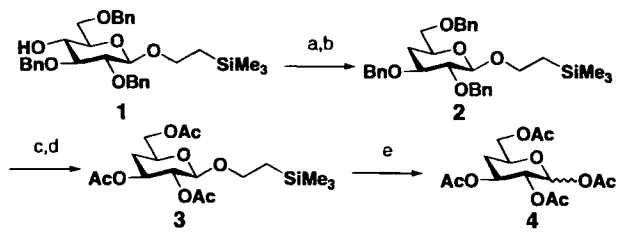


Fig. 3. Inhibitory effects and structures of various alkylthioglucosides (A) and glucosides containing phenyl or carbonyl (B). Relative effects of the compounds compared to that of Glc-S-C7-Me were showed in the parenthesis.

mannose, and galactose) on the specific binding of ^{3}H-Glc-S-C7-Me to the kidney membrane were determined in preliminary experiments in which glucose and mannose exhibited more potent inhibition than galactose (Table 1). Also, from the preliminary in vitro study using octylthio derivatives, while Glc-S-C7-Me and Man-S-C7-Me exhibited potent effects, Gal-S-C7-Me was about 1000-fold less effective than Glc-S-C7-Me (Table 2). These results confirm the structural requirements of an alkylglycoside as a renal targeting vector can be analyzed by investigating the inhibitory effects of various sugars and glycosides on ^{3}H-Glc-S-C7-Me binding to the kidney membrane fraction.

First, various sugars (D-aldohexose, L-aldohexose, D-aldopentose, L-aldopentose, ketohexose, and oligosugar) were tested and all the sugars, except D-aldohexose and D-aldohexose derivatives containing F, S, and N, failed to show greater inhibition than D-glucose (Table 1). This results suggest the D-aldohexose structure is essential for the sugar portion of the vector. Since the basic structure of the sugar portion necessary for renal recognition is considered to be glucose, various deoxy-, F, and N-glucose derivatives were evaluated and 2 and 4-deoxy-D-glucose and 1-F-, 2-F-, 4-F-, 5-N-(nojirimycin) and 1-N-glucose exhibited strong inhibition (Table 1). Because sugars adopt various configurations in solution, these results cannot be applied directly. Therefore, octylthio derivatives of various D-aldohexose compounds were prepared and their inhibitory effects were investigated. These derivatives have a pyranose ring structure, so the α- and β-glycosides could be separately evaluated. Consequently, the preferred sugar moiety follows the order: glucose, mannose, 2-deoxyglucose > allose, xylose >> talose, galactose, 4-deoxyglucose (Table 2, Fig. 2A, and B). Thus, some key properties have been identified, e.g. an equatorial OH at 4 position in the pyranose ring is essential; OH at the 2 position can have either orientation or be deleted. Compared with the relative inhibitory effects of the sugar and corresponding glycoside, although talose, for example, exhibited greater inhibition than glucose (Table 1), Tal-S-C7-Me was about 400-fold weaker than Glc-S-C7-Me. The reason for this is unclear, and it might be the sugar could be positioned in a different way from the sugar moiety of the glycoside described above. Also, the β-glycoside exhibited greater inhibition than the corresponding α-glycoside so β-glycosides might be more favorable as the vector. This corresponds to the finding showing that the β-mannosilated derivative of AVP exhibited high renal



a: PhOC(=S)Cl b: Bu_3SnH, AIBN c: H_2, Pd-C d: Ac_2O, Pyr.
e: Ac_2O, $BF_3 \cdot Et_2O$

Scheme 2. Preparation of 4-deoxyglucose delivative.

targeting ability, unlike its α-mannosilated counterpart (1). As far as the recognition specificity of these sugars is concerned, this system is similar to the mannose/glucose lectin molecule (20,21) and glucose transporters, which have been the subject of detailed studies as sugar-binding molecules. Hydrogen and hydrophobic bonds are both important for specific sugar binding (20,21). The mammalian kidney lectin which recognize glucose/mannose has not been identified. Sugar transporters can be classified as facilitated sugar transporters and Na^+/glucose cotransporters (22). As the former transporters, five human transporters has been cloned (GLUT1 - GLUT5) (23). They transport sugars passively and are characterized by moderate-affinity D-glucose binding (Km in the mM range) (23). These characteristics are distinct from the alkylglycoside recognition mechanism. The latter transporter cannot be involved in the renal uptake of alkylglycoside, as shown by in vivo uptake studies (1).

As far as alkyl portion is concerned, its length and degree of branching affected the degree of inhibition. In particular, Glc-S-tBu exhibited 1/12000 the inhibition of Glc-S-C7-Me so that the alkyl portion must be a certain length of (Table 2, Fig. 3A). In addition, the preferred glycosidic bonding atom is as follows; S > NH > O. The S-glucoside has 60-fold greater affinity than O-glucoside, and Glc-S-CO-C6-Me, which has a carbonyl group situated next to the glycoside atom, showed 9000-fold less inhibition than Glc-S-C7-Me. Therefore this shows the electrical environment near the glycoside bond is important. Aromatic structures could be substituted for the alkyl portion (Table 2, Fig. 3B), but there was a difference between p-nitrophenyl derivatives and p-nitrobenzyl derivative and further studies are needed before these aromatic structures can be used. At present, Glc-S-C7-Me exhibited the greatest inhibition among the various glycosides tested in this report. This molecule is commonly used as a detergent (24) and studies using it as a transmucosal absorption promoter have been performed (25). Since some model drugs modified with this structure (Glc-S-C8-) exhibited renal targeting ability (2), Glc-S-C8- is considered to have the highest potential as a renal targeting vector. In addition, as far as the design of renally targeted drugs is concerned, the physicochemical properties and renal targeting potential of all the modified drugs should be considered. Some of the findings described in this report will be help in designing novel renally targeted drugs.

Some methods have already been developed to target the kidney; e.g., peptides reabsorption after glomerular filtration through the brush-border membrane (26) and prodrug activation by kidney-specific enzymes (27–29). Compared with these methods, the kidney targeting using the alkylglycoside vector described in this report is a novel, new method for directly delivering drugs using drug-targeting devices from the blood side, and this applies not only to pharmacologically active modified drugs but also to prodrugs.

In conclusion, the structural requirements for the renal targeting vector have been identified as follows: a hydrophobic group (alkyl chain or aromatic ring) should be introduced to a sugar (D-glucose, D-mannose or 2-deoxy-D-glucose) via a β-glycoside binding atom (S > NH > 0).

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