Chinese Journal of Chemistry, **2004**, *22*, 482—486 **Article**

Synthesis of Glutamic Acid-based Cluster Galactosides and Their Binding Affinities with Liver Cells

ZHANG, Xiao-Ru(张晓茹) LI, Ying-Xia*(李英霞) CHU, Shi-Dong (褚世栋)
DING, Ning(丁宁) LI, Chun-Xia(李春霞) GUAN, Hua-Shi(管华诗)

Institute of Marine Drugs and Food, Ocean University of China, Qingdao, Shandong 266003, China

Structurally well defined di-, tri- and tetra-valent cluster galactosides were synthesized in a convenient way. Oligo-glutamic acids were assembled as scaffolds. The presence of amine groups in these three ligands is expected to couple with drugs or genes for delivery. The binding affinities of these cluster galactoses to liver cells were determined by *in vitro* binding studies. Among them, the tetravalent cluster galactose (**19**) showed the highest affinity to liver cell. It is therefore a promising targeting device for the specific delivery of drugs or genes to parenchymal liver cells.

Keywords glycocluster, cluster galactoside, multivalent effect, glutamic acid

Introduction

β-D-Galacto- or 2-acetamido-2-deoxy-*β-D*-galactopyranosyl-terminated glycoproteins are recognized by the asialoglycoprotein receptor (ASGPR) on hepatocytes specifically, which makes them ideal targeting devices for the delivery of drugs or genes to liver cells.[1,2]

It has been generally admitted that the interactions between proteins and monovalent carbohydrate determinants are often weak, and yet the strength and specificity required for recognition in physiological settings are high. To achieve the desired affinity, nature employs simultaneous formation of multiple protein-carbohydrate interactions.[3] In this respect, the "glycocluster", a ligand with organized multivalent carbohydrate branches, is introduced as mimic of multivalent ligand in nature.[4]

Based on the point of view mentioned above, many people engaged in synthesizing cluster galactosides for liver targeting. Lee *et al*.[5] have developed a very potent trivalent *N*-acetylgalactosaminide, YEE $(GalNAcAH)_3$, which is the best known synthetic ligand for human hepatic ASGPR. This cluster is now being evaluated as a carrier to direct nucleosides to liver.[6] Kichler and Schuber have synthesized trivalent *β-D*-galacopyranosides which are almost as potent as YEE $(GalNAcAH)_3$ towards Gal/GalNAc receptors of human hepatic cell.[7] Encouraged by these results, we tried to find a convenient method to synthesize cluster galactosides, and determine the interactions between the cluster galactosides and liver cells by *in vitro* binding studies.

Results and discussion

Because the binding mode available to a multivalent ligand results from its architecture, altering structural feature of a multivalent ligand, such as its valency density of binding epitopes, can change its activity.[8] Further design of such molecules can shed some lights on the geometric factors affecting multivalent carbohydrate-protein interactions. We describe herein a convenient approach to the synthesis of di-, tri-, tetra-valent cluster galactosides.

Glutamic acid was assembled into di-, tri-, or tetra-branched scaffold, respectively (Scheme 1). Benzyloxycarbonyl (Cbz) protected *L*-glutamic acid (**2)** was directly used as the divalent scaffold. Trivalent scaffold **4** was synthesized by hydrolysis of **3** which was obtained by condensation of *γ*-methyl *N*-benzyloxycarbonyl-*L*-glutamate (**1)** with *L*-glutamic acid diethyl hydrochloride. Under similar conditions, the reaction of benzyloxycarbonyl protected *L*-glutamic acid (**2**) with *L*-glutamic acid diethyl ester hydrochloride afforded tetravalent scaffold **6**.[9]

An appropriate spacer arm is usually required in constructing glycoclusters. One end of the spacer arm is condensed with saccharide residue via glycosidation, to the other end is attached an active group for linking scaffolds effectively. Here, 2-(2′-chloroethoxy) ethanol was chosen as the arm for this purpose. Also the flexibility of this spacer arm makes it easy for the cluster galactosides to bind to the ASGPR receptor. Thus 2-(2′-chloroethoxy) ethanol first reacted with peracetated galactose in the presence of BF_3•Et_2O[10] to give rise to 2-(2′-chloroethoxy) ethyl 2,3,4,6-tetra-*O*-acetyl-

* E-mail: liyx417@ouc.edu.cn
Received by July 1, 2003; revised October 13, 2003; accepted February 2, 2004.
Project supported by the Ministry of Science and Technology of China (No. 2001CCA01600).

β-D-galactopyranoside (**8)** in 73.1% yield. The *β* configuration of galactoside is necessary for the ASGPR recognization. After azidation of **8** with NaN_3, providing **9** in 96.3% yield, the terminal azide was then reduced into active amine group by catalytic hydrogenation (H_2, 10% Pd/C) to give the key intermediate **10**, which was directly used in the next step without purification (Scheme 2)

The solution of **10** in CH_2Cl_2 was added to the activated di-, tri-, or tetra- scaffold with DCC/NHS in CH_2Cl_2/DMF. Corresponding precursors of the clusters (**11**, **14**, and **17**) were obtained in 82.4%, 65.3%, and 61.1% yield, respectively. Final removal of all protection groups furnished the target di-, tri- or tetra-valent cluster galactosides **13**, **16** and **19** in satisfactory yields (Scheme 3).

All the structures of cluster galactosides were determined by ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra analysis.

The binding affinities of cluster galactoses **13**, **16** and **19** to liver cells asialoglycoprotein receptor (ASGPR) were determined by *in vitro* binding studies.[11] The galactosyl-neoglycoalbumin [NGA refers to human serum albumin (HSA) modified by galactoside] has known to bind ASGPR specifically.[12] Compounds **13**, **16** and **19** competed with ^{125}I-NGA to bind with the liver cell. The IC_{50} values of each compound are shown in Table 1. Galactose was used as the monovalent standard. Among these cluster galactosides, an increase in valency results in a steady increase in inhibitory potential. The IC_{50} values of **13**, **16** and **19** are 1.24, 0.130, and 0.0138 mmol/L, respectively, while IC_{50} of galactose is 38.0 mmol/L. After valent correction, the binding affinity of the synthesized clusters is 1, 15, 97, 688 times stronger than that of galactose. Tetravalent cluster galactose **19** shows the highest affinity to liver cell. The increased binding between carbohydrates and proteins can be attributed to an increase in multivalency as reflected by the well-known "cluster effect".

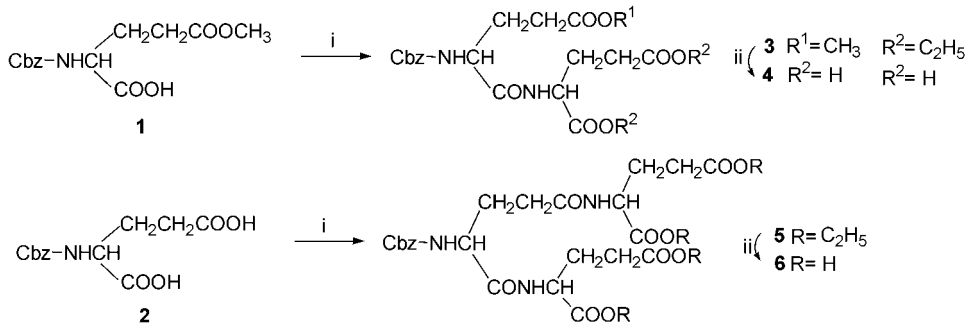
For the well known compound $(GalNAcAH)_3$, the binding affinity of trivalent cluster YEE is 10,000 times stronger than that of GalNAc mono-saccharide,[13] which performs better than that of our tetravalent cluster **19**. Unlike 2-acetamidogalactopyranose, the galactose is cheap and easily available, while the binding affinity of **19** still increases a lot than that of galactose, making it a potential drug carrier for further study.

Table 1 Inhibition of ^{125}I-NGA binding to ASGPR by synthesized ligands

Compound	IC_{50}/(mmol•L^{-1})	Relative potency[a]
galactose	38.0	1
13	1.24	30 (15)
16	0.130	292 (97)
19	0.0138	2753 (688)

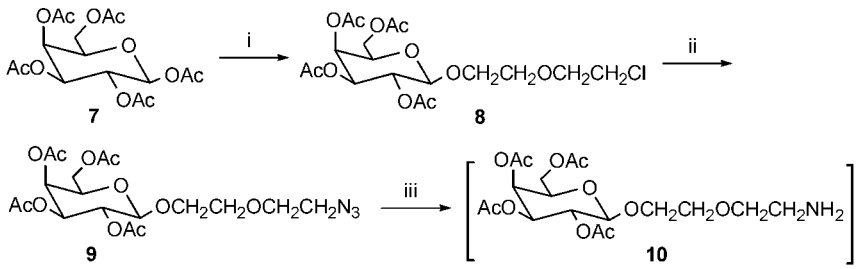
[a] Values in parentheses are based on the potency of a per galacoside residue.

Scheme 1



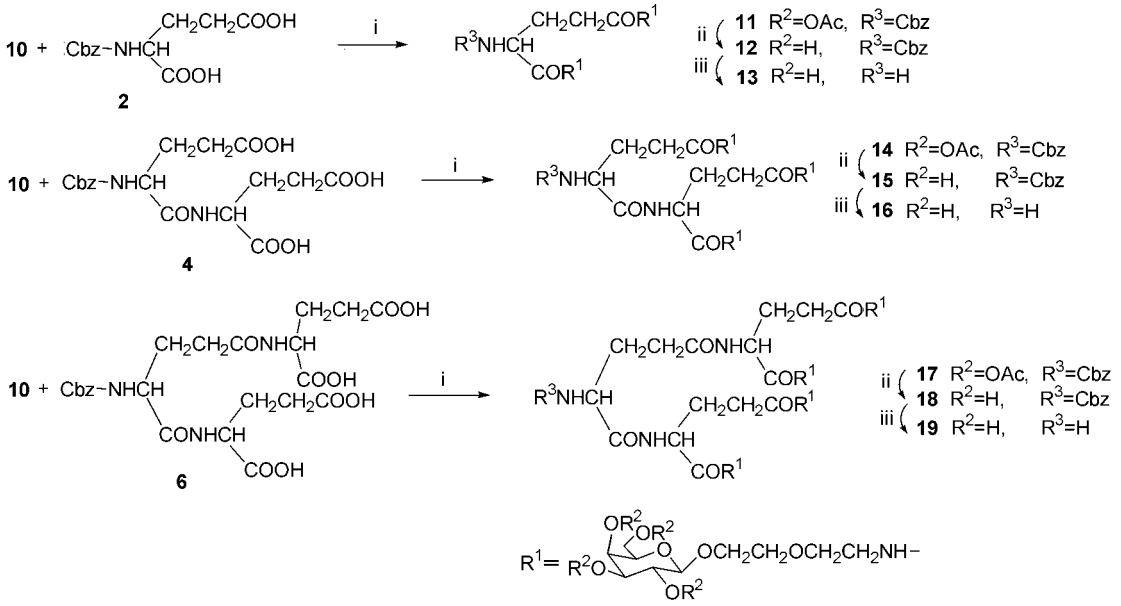
Reagents and conditions: i) *L*-glutamic acid diethyl ester hydrochloride, Et_3N, DCC, NHS, DMF/CH_2Cl_2, 80.0% for **3**, 81.2% for **5**. ii) NaOMe/MeOH, MeOH, 90.0% for **4**, 87.0% for **6**.

Scheme 2



Reagents and conditions: i) $HOCH_2CH_2OCH_2CH_2Cl$, BF_3•Et_2O, CH_2Cl_2, 73.1%. ii) NaN_3, DMF, 60 ℃, 16 h, 96.3%. iii) H_2, 10% Pd/C, CH_2Cl_2/MeOH.

Scheme 3



Reagents and conditions: i) DCC, NHS, CH_2Cl_2, DMF, 82.4% for **11**, 65.3% for **14**, 61.1% for **17**. ii) NaOMe/MeOH, MeOH, 90.0% for 12, 92.0% for **15**, 91.5% for **18**. iii) H_2, Pd/C, MeOH, 95.1% for **13**, 93.0% for **16**, 91.7% for **19**.

Conclusion

An efficient approach to the synthesis of di-, tri- and tetra-valent cluster galactosides was described in this paper, by which target di-, tri-, and tetra-valent cluster galactosides **13**, **16** and **19** were obtained in good yields. Additionally, this approach would allow us to obtain higher valent cluster galactosides. The presence of amine groups in these three ligands is expected to couple with drugs or genes for delivery.

The binding affinities of cluster galactoses to liver cells were determined by *in vitro* binding studies. The cluster galactoses **13**, **16** and **19** all proved to be good ligands for ASGPR. Among them, tetravalent cluster galactose **19** showed the highest affinity to liver cell. Such a glycocluster would be of great value not only in providing compounds valuable on liver targeting, but also in understanding multivalent interactions.

Experimental

Reagents and instruments

Cbz-Glu(OMe)-OH was purchased from Advanced ChemTech， Cbz-Glu-OH was from GL Biochem (Shanghai, China), and *L*-Glutamic acid diethyl ester hydrochloride was from Aldrich. All other chemicals were of reagent grade and used without further purification. NGA was prepared by Jiangsu Institute of Nuclear Medicine. Thin layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck) and column chromatography on silica Gel (100—200 mesh, Qingdao, China). Sephadex G-10 and G-15 were purchased from Amersham Pharmacia Biotech AB. The ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL JNM-ECP 600 spectrometer, and assignments were based on COSY, DEPT and HMQC experiments. IR spectra were performed on a Nicolet Nexus 470 spectrometer. Mass spectra were recorded on a Q-Tof micro spectrometer and a Bruker Daltonics. Inc. APEX II spectrometer.

2-(2'-Chloroethoxy)ethyl 2,3,4,6-tetra-*O*-acetyl-*β*-*D*-galactopyranoside (8)

2-(2′-Chloroethoxy) ethanol (162.3 μL, 1.538 mmol) and 1,2,3,4,6-penta-*O*-acetyl-*α*-*D*-galactopyranoside (0.500 g, 1.282 mmol) were dissolved in dry CH_2Cl_2 (25 mL) and the glycosylation reaction was started by the addition of BF_3•Et_2O (241.5 μL, 1.923 mmol). After stirring of the mixture for 24 h at room temperature, triethylamine (0.2 mL) was added. The reaction mixture was concentrated under high vacuum and the residue was purified by chromatography to yield compound **8** (0.426 g, 73.1%). ^{1}H NMR（$CDCl_3$, 600 MHz）δ: 5.40 (dd, J=1.1, 3.3 Hz, 1H, H-4), 5.22 (dd, J=10.3, 8.0 Hz, 1H, H-2), 5.02 (dd, J=10.6, 3.3 Hz, 1H, H-3), 4.59 (d, J=8.0 Hz, 1H, H-1), 4.10—4.20 (m, 2H, H-6a, H-6b), 3.95—3.99 (m, 1H, OC**H**HCH$_2$OCH$_2$CH$_2$Cl), 3.92 (dt, J=6.6, 1.1 Hz, 1H, H-5), 3.75—3.78 (m, 1H, OCH**H**-CH$_2$OCH$_2$CH$_2$Cl), 3.73—3.74 (m, 2H, OCH$_2$C**H**$_2$OC-H$_2$CH$_2$Cl), 3.67—3.69 (m, 2H, OCH$_2$CH$_2$OC**H**$_2$CH$_2$Cl), 3.61—3.63 (m, 2H, OCH$_2$CH$_2$OCH$_2$C**H**$_2$Cl), 2.15, 2.07, 2.05, 1.99 (4s, 12H, 4×COCH$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 170.4, 170.2, 170.1, 169.5 (4×**C**OCH$_3$), 101.3 (C-1), 71.4 (OCH$_2$**C**H$_2$OCH$_2$CH$_2$Cl), 70.9 (C-3), 70.6 (C-5), 70.3 (OCH$_2$CH$_2$O**C**H$_2$CH$_2$Cl), 69.0 (O**C**H$_2$-CH$_2$OCH$_2$CH$_2$Cl), 68.7 (C-2), 67.0 (C-4), 61.2 (C-6), 42.8 (OCH$_2$CH$_2$OCH$_2$**C**H$_2$Cl), 20.8, 20.7, 20.7, 20.6 (4×CO**C**H$_3$); IR (KBr) v: 2940, 2880 (CH$_3$, CH$_2$), 1750 (C=O) cm^{-1}.

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-*β*-*D*-galactopyranoside (9)

Compound **8** (1.000 g, 2.200 mmol) and sodium azide (1.140 g, 17.600 mmol) were dissolved in dry DMF (40 mL), and the reaction mixture was stirred at 60 ℃ for 16 h. Then the solvent was removed under vacuum, the residue was dissolved in ethyl acetate, washed with water and dried over anhydrous Na_2SO_4. The solution was evaporated under reduced pressure and purified by chromatography to yield compound **9** (0.977 g, 96.3%). ^{1}H NMR（$CDCl_3$, 600 MHz） δ: 5.39 (dd, J=3.3, 0.7 Hz, 1H, H-4), 5.22 (dd, J=10.3, 7.7 Hz, 1H, H-2), 5.03 (dd, J=10.6, 3.7 Hz, 1H, H-3), 4.59 (d, J=8.0 Hz, 1H, H-1), 4.10—4.19 (m, 2H, H-6a, H-6b), 3.96—3.99 (m, 1H, OC**H**HCH$_2$OCH$_2$CH$_2$N$_3$), 3.91 (dt, J=6.5, 0.7 Hz, 1H, H-5), 3.75—3.78 (m, 1H, OCH**H**CH$_2$OCH$_2$CH$_2$N$_3$), 3.64—3.68 (m, 4H, OCH$_2$C**H**$_2$OC**H**$_2$CH$_2$N$_3$), 3.36—3.39 (m, 2H, OCH$_2$CH$_2$OCH$_2$C**H**$_2$N$_3$), 2.15, 2.07, 2.05, 1.99 (4s, 12H, 4×COCH$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 170.4, 170.2, 170.1, 169.5 (4×**C**OCH$_3$), 101.3 (C-1), 70.9 (C-3), 70.6 (C-5), 70.3, 70.2 (OCH$_2$**C**H$_2$O**C**H$_2$CH$_2$N$_3$), 69.1 (O**C**H$_2$CH$_2$OCH$_2$CH$_2$N$_3$), 68.2 (C-2), 67.0 (C-4), 61.2 (C-6), 50.8 (OCH$_2$CH$_2$OCH$_2$**C**H$_2$N$_3$), 20.8, 20.7, 20.7, 20.6 (4×CO**C**H$_3$); IR (KBr) v: 2937, 2875 (CH$_3$, CH$_2$), 2110 (N$_3$), 1750 (C=O) cm^{-1}.

Divalent cluster galactoside (13)

Compound **2** (0.14 g, 0.5 mmol) was dissolved in CH_2Cl_2∶DMF (6 mL, 2∶1, V∶V), NHS (120 mg, 1.0 mmol) and DCC (210 mg, 1.0 mmol) were added under Ar. The reaction mixture was kept for later use. At the same time, compound **9** (0.693 g, 1.5 mmol) was dissolved in the solvent of CH_2Cl_2∶MeOH (30 mL, 1∶1, V∶V). 10% Pd/C (0.25 g) was added, then the reaction mixture stirred at room temperature for 40 min under hydrogen. The mixture was filtered over a pad of Celite quickly, and concentrated to a syrup. Then the intermediate was removed directly to the reaction mixture of the active scaffold with CH_2Cl_2. After being stirred at r.t. for 48 h under Ar atmosphere, the mixture was filtered through Celite. The filtrate was washed with water, dried over Na_2SO_4, concentrated and purified by chromatography on silica gel ($CHCl_3$∶MeOH, 60∶1 to 40∶1, V∶V) to give the desired divalent cluster **11** (0.460 g, 82.4%). To a solution of divalent cluster **11** (0.46 g, 0.412 mmol) in dry methanol (3 mL) was added sodium methoxide and the pH of the solution was kept at 9. The solution was stirred at room temperature for 1 h, then neutralized with Dowex-50x-8(H^+), filtered and concentrated. The residue was purified by Sephadex G-10, lyophilized to give compound **12** (0.289 g, 90.0%). The product **12** (0.147 g, 0.192 mmol) in MeOH (3 mL) was hydrogenolyzed in the presence of 10% Pd/C (50 mg) for 1.5 h. The mixture was filtered through Celite, concentrated and purified by Sephadex G-10. The solution was concentrated and lyophilized to give the target divalent cluster galactoside **13** (0.116 g, 95.1%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.55—8.57 (m, 1H, NH), 8.22—8.31 (m, 2H, NH$_2$), 8.05—8.06 (m, 1H, NH), 4.89—4.90 (m, 2H, 2×OH-2), 4.75—4.76 (m, 2H, 2×OH-3), 4.60—4.61 (m, 2H, 2×OH-6), 4.42—4.43 (m, 2H, 2×OH-4), 4.10 (t, J=6.2 Hz, 2H, 2×H-1), 3.83—3.87 (m, 2H, 2×GalOC**H**H), 3.78 (t, J=6.3 Hz, 1H, NHC**H**), 3.63 (bs, 2H, 2×H-4), 3.41—3.58 (m, 14H, 2×H-6a, 2×H-6b, 2×GalOCH**HC**H$_2$OC**H**$_2$), 3.25—3.31 (m, 6H, 2×H-5, 2×H-2, 2×H-3), 3.19—3.21 (m, 4H, 2×GalOCH$_2$CH$_2$OCH$_2$C**H**$_2$), 2.20 (t, J=7.9 Hz, 2H, NHCHCH$_2$C**H**$_2$), 1.86—1.96 (m, 2H, NHCHC**H**$_2$CH$_2$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 168.3, 171.1 (2×C=O), 103.5 (2×C-1), 75.1 (2×C-5), 73.4 (2×C-3), 70.5 (2×C-2), 69.5 (2×GalOCH$_2$**C**H$_2$), 68.9 (2×GalOCH$_2$CH$_2$O**C**H$_2$), 68.1 (2×C-4), 67.7 (2×GalCH$_2$), 60.4 (2×C-6), 51.9 (NH**C**H), 38.6 (2×GalOCH$_2$CH$_2$OCH$_2$**C**H$_2$), 30.6 (NHCHCH$_2$**C**H$_2$), 27.1 (NHCH**C**H$_2$CH$_2$); IR (KBr) v: 3339 (OH), 1678, 1650, 1559 (CONH) cm^{-1}; HRMS [M+H]$^+$ m/z: calcd for $C_{25}H_{48}N_3O_{16}$ 646.3029, found 646.3031.

Trivalent cluster galactoside (16)

Adopting the same procedure as described for **11** but using **9** (0.893 g, 1.936 mmol) and **4** (0.192 g, 0.484 mmol), after chromatography on silica gel ($CHCl_3$∶MeOH, 50∶1 to 30∶1, V∶V) intermediate **14** was obtained (0.520 g, 65.3%). The protected trivalent cluster **14 (**0.280 g, 0.242 mmol) was treated in the same way as **12** and purified on Sephadex G-15 to give **15** (0.179 g, yield 92.0%). Then Cbz group of compound **15** (0.139 g, 0.1199 mmol) was removed as described above. After be purified on Sephadex G-15 target trivalent cluster galactose **16** (0.114 g, 93.0%) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.04—8.06 (m, 3H, CHN**H**CO, 2×CH$_2$N**H**CO), 7.86—7.89 (m, 3H, CH$_2$N**H**CO, N**H**$_2$CH), 4.90 (bs, 3H, 3×OH-2), 4.72 (bs, 3H, 3×OH-3), 4.58 (bs, 3H, 3×OH-6), 4.38 (bs, 3H, 3×OH-4), 4.23 (bs, 1H, CONHC**H**), 4.09—4.11 (m, 3H, 3×H-1), 3.82—3.86 (m, 3H, 3×GalOC**H**H), 3.62 (bs, 3H, 3×H-4), 3.53—3.57 (m, 9H, 3×GalOCH**H**, 3×GalOCH$_2$C**H**$_2$O), 3.50—3.53 (m, 3H, 3×H-6a), 3.45—3.48 (m, 3H, 3×H-6b), 3.38—3.42 (m, 6H, 3×GalOCH$_2$CH$_2$OC**H**$_2$CH$_2$), 3.31—3.33 (m, 3H, 3×H-5), 3.27—3.28 (m, 3H, 3×H-2), 3.25—3.27 (m, 3H, 3×H-3), 3.21—3.23 (m, 1H, NH$_2$C**H**), 3.15—3.23 (m, 6H, 3×GalOCH$_2$CH$_2$OCH$_2$C**H**$_2$), 2.13—2.16 (m, 2H, NH$_2$CHCH$_2$C**H**$_2$CO), 2.06—2.09 (m, 2H, NHCHCH$_2$C**H**$_2$), 1.80—1.87 (m, 2H, NHCHC**H**H, NH$_2$CHC**H**H), 1.70—1.75 (m, 1H, NHCHCH**H**), 1.55—1.58 (m, 1H, NH$_2$CHCH**H**); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 172.0, 171.5, 171.2 (4×C=O), 103.4 (3×C-1), 75.0 (3×C-5), 73.3 (3×C-3), 70.3 (3×C-2), 69.4 (3×GalOCH$_2$**C**H$_2$), 68.9 (3×GalOCH$_2$CH$_2$O**C**H$_2$), 68.0 (3×C-4), 67.5 (3×GalO**C**H$_2$CH$_2$), 60.3 (3×C-6), 54.0 (NH$_2$**C**HCO), 51.6 (NH**C**HCO), 38.4 (3×GalOCH$_2$CH$_2$OCH$_2$**C**H$_2$), 31.8, 31.4 (2×CHCH$_2$**C**H$_2$), 28.5 (2×CH**C**H$_2$CH$_2$); IR (KBr) v: 3386 (OH), 1650, 1549 (CONH) cm^{-1}; HRMS [M+H]$^+$ m/z: calcd for $C_{40}H_{74}O_{25}N_5$ 1024.4667, found 1024.4672.

Tetravalent cluster galactoside (19)

Compounds **9** (0.471 g, 1.022 mmol) and **6** (100 mg, 0.186 mmol) were treated as the preparation of compound **14** to afford the protected tetravalent compound **17** (0.251 g, 61.1%) after chromatography on silica gel ($CHCl_3$ ∶ MeOH, 50 ∶ 1 to 30 ∶ 1, *V* ∶ *V*). Compound **18** (0.134 g, 91.5%) was obtained after deacetylation of compound **17** (0.211 g, 0.096 mmol) and purified on Sephadex G-15. The Cbz group of compound **18** (93.6 mg, 0.061 mmol) was removed according to the procedure described above. After purified on Sephadex G-15 target tetravalent compound **19** (80.4 mg, 91.7%) was obtained. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.93 (d, J= 8.0 Hz, 1H, CON**H**CH), 8.40 (d, J=7.7 Hz, 1H, CON**H**CH), 8.33—8.34 (m, 1H, Gal$OCH_2CH_2OCH_2CH_2$-N**H**), 8.22—8.24 (m, 3H, 3×Gal$OCH_2CH_2OCH_2CH_2$N**H**), 7.90—7.91 (m, 2H, NH_2), 4.88—4.91 (m, 4H, 4×OH-2), 4.73—4.75 (m, 4H, 4×OH-3), 4.58—4.60 (m, 4H, 4×OH-6), 4.08—4.41 (m, 4H, 4×OH-4), 4.34—4.38 (m, 1H, CONHC**H**), 4.19—4.23 (m, 1H, CONHC**H**), 4.10 (d, J=5.5 Hz, 4H, 4×H-1), 3.83—3.86 (m, 4H, 4×GalOC**H**H), 3.63 (bs, 4H, 4×H-4), 3.53—3.57 (m, 12H, 4×GalOCH**H**C**H**$_2$), 3.50—3.52 (m, 4H, 4×H-6a), 3.44—3.48 (m, 4H, 4×H-6b), 3.40—3.43 (m, 8H, 4×GalOCH_2CH_2OC**H**$_2CH_2$), 3.31—3.34 (m, 4H, 4×H-5), 3.25—3.28 (m, 8H, 4×H-2, 4×H-3), 3.23—3.25 (m, 1H, NH_2C**H**), 3.18—3.21 (m, 8H, 4×Gal$OCH_2CH_2OCH_2$C**H**$_2$), 2.11—2.21 (m, 6H, 3×$CHCH_2$C**H**$_2$CONH), 1.80—1.92 (m, 3H, 3×CHC**H**HCH_2CONH), 1.71—1.77 (m, 3H, 3×CHCH**H**CH_2CONH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 172.3, 171.4, 171.3, 171.2 (6×CO), 103.4 (4×C-1), 75.1 (4×C-5), 73.3 (4×C-3), 70.0 (4×C-2), 69.4, 69.3 (4×GalOCH_2**C**H_2), 68.9, 68.6 (4×GalOCH_2CH_2O**C**H_2), 67.9 (4×C-4), 67.5 (4×Gal**O**CH_2CH_2O), 60.3 (4×C-6), 52.4, 52.1 (2×NH**C**HCO), 39.1 (4×Gal$OCH_2CH_2OCH_2$**C**H_2), 38.9 (NH_2CH), 31.6, 31.3, 30.5 (3×$CHCH_2$**C**H_2CONH), 28.1, 27.8 (3×CH**C**H_2CH_2CONH); IR (KBr) ν: 3380 (OH), 1651, 1548 (CONH) cm^{-1}; HRMS $[M+H]^+$ *m/z*: calcd for $C_{55}H_{100}O_{34}N_7$ 1402.6292, found 1402.6296.

Inhibition of liver uptakes of ^{125}I-labeled compounds by unlabeled compounds *in vivo*

Iodination of NGA The NGA was labeled with $Na^{125}I$ using Iodogen reagent according to usual method[14] and purified on Sephadex G-25 column. The radioactivities were measured by using γ-counter.

Isolation of parenchymal liver cells Patenchymal liver cells were isolated according to usual method.[15]

***In vitro* binding studies** The synthesized ligands of different concentration were added to a series of tubes, then 100 μL of ^{125}I-NGA and 400 μL of newly prepared parenchymal liver cells were added to the tubes above. Following incubation for 30 min at 37 ℃ under gentle agitation, the medium was removed by aspiration and the cells were washed with 1000 μL of TBBSC buffer, then centrifuged under 4000 r/min. The medium was removed again by aspiration. Subsequently, cells were counted for radioactivity. Non-specific binding was measured in the presence of 100 times of NGA.

Acknowledgment

We are thankful to Mr. Zhang Rongjun (Jiangsu Institute of Nuclear Medicine, National Laboratory of Nuclear Medicine) for his kindly providing the bioactivity test.

References

1 Roy, R. *Curr. Opin. Struct. Biol*. **1996**, *6*, 692.
2 Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215.
3 Bertozzi, C. R.; Kiessling L. L. *Science* **2001**, *291*, 2357.
4 Lundquist, J. J.; Toone, E. *J. Chem. Rev*. **2002**. *102*, 555.
5 Ozaki, K.; Lee, R. T.; Lee, Y. C.; Kawasaki, T. *Glycoconjugate J.* **1995**, *12*, 268.
6 Hangeland, J. J.; Levis, J. T.; Lee, Y. C.; Tso, P. O. *Bioconjugate Chem*. **1995**, *6*, 695.
7 Kichler, A.; Schuber, F. *Glycoconjugate J*. **1995**, *12*, 275.
8 Gestwick, J. E.; Cairo, C. W.; Strong, L. E. *J. Am. Chem. Soc*. **2002**, *124*, 14922.
9 Ranganathan, D.; Kurer, S.; Madhususanan, K. P.; Roy, R.; Karle, I. *J. Pept. Res*. **1998**, *51*, 297.
10 Sjölin, P.; Kihlberg, J. *Tetrahedron Lett*. **2000**, *41*, 4435.
11 Valentijin, A. R. P. M.; Van der Marel, G. A.; Sliedregt, L. A. J. M.; van Berkel, T. J. C.; Biessen E. A. L.; van Boom, J. H. *Tetrahedron* **1997**, *53*, 759.
12 Li, W. X.; Zhang, R. J.; Tan, C.; Tao, Y. H.; Ji, J. *Prog. Biochem. Biophys*. **2000**, *27*, 394.
13 Lee, Y. C.; Townsend, R. R.; Hardy, M. R.; Longren, J.; Arnarp, J.; Haraldsson, M.; Lonn, H. *J. Biol. Chem*. **1983**, *258*, 199.
14 Greenwood, F. C.; Hunter, W. H.; Glover, J. S. *Biochem. J*. **1963**, *89*, 114.
15 Casey, C. A.; Kragskow, S. L.; Sorrel. M. F. *J. Biol. Chem*. **1987**, *262*, 2704.

(E0307019 SONG, J. P.)