Bioconjugate Chemistry

Article

pubs.acs.org/bc

Synthesis and Evaluation of a Photoactive Probe with a Multivalent Carbohydrate for Capturing Carbohydrate–Lectin Interactions

Tsung-Che Chang,[†] Chian-Hui Lai,[†] Chih-Wei Chien,[†,§] Chien-Fu Liang,[†] Avijit Kumar Adak,[†] Yung-Jen Chuang,[‡] Yu-Ju Chen,[§] and Chun-Cheng Lin*[,†]

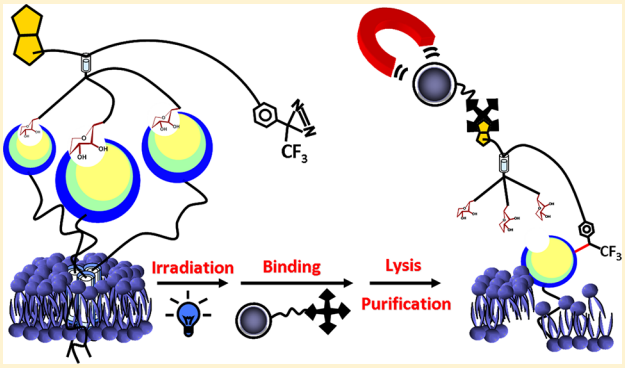
[†]Department of Chemistry and

[‡]Institute of Bioinformatics and Structural Biology, National Tsing Hua University, 101 Sec. 2, Kuang Fu Rd., Hsinchu 30013, Taiwan

[§]Institute of Chemistry, Academia Sinica, 128 Sec. 2, Academia Rd, Nankang Taipei 115, Taiwan

Supporting Information

ABSTRACT: Lectins are ubiquitous carbohydrate-binding proteins of nonimmune origin that are characterized by their specific recognition of defined monosaccharide or oligosaccharide structures. However, the use of carbohydrates to study lectin has been restricted by the weak binding affinity and noncovalent character of the interaction between carbohydrates and lectin. In this report, we designed and synthesized a multifunctional photoaffinity reagent composed of a trialkyne chain, a masked latent amine group, and a photoreactive 3-trifluoromethyl-3-phenyl-diazirine group in high overall yield. Two well-defined chemistries, Huisgen-Sharpless click chemistry and amide bond coupling, were the key steps for installing the multivalent character and tag in our designed photoaffinity probe. The photolabeling results demonstrated that the designed probe selectively labeled the target lectin, RCA_{120} (*Ricinus communis* Agglutinin), in an *E. coli* lysate and an asialoglycoprotein receptor (ASGP-R) on intact HepG2 cell membranes. Moreover, the probe also enabled the detection of weak protein–protein interactions between RCA_{120} and ovalbumin (OVA).



INTRODUCTION

Carbohydrate–lectin interactions are involved in various biological recognitions and mediate many processes including cellular recognition,[1] adhesion,[2] signal transduction,[3] glycoprotein clearance,[4] immunomodulation,[5,6] inflammation,[7] and host–pathogen recognition.[8] To fully understand the biological implications of carbohydrate–lectin interactions in living organisms, it is imperative to investigate and profile their functions under physiological conditions. Moreover, a quantitative assessment of the variations in lectins expressed in different cell states and cell types is essential for elucidating their biological roles in disease development. However, probing carbohydrate interacting proteins is challenging due to the properties of the carbohydrate–lectin interaction: it is noncovalent, reversible, and weak (K_d in the mM range).[9]

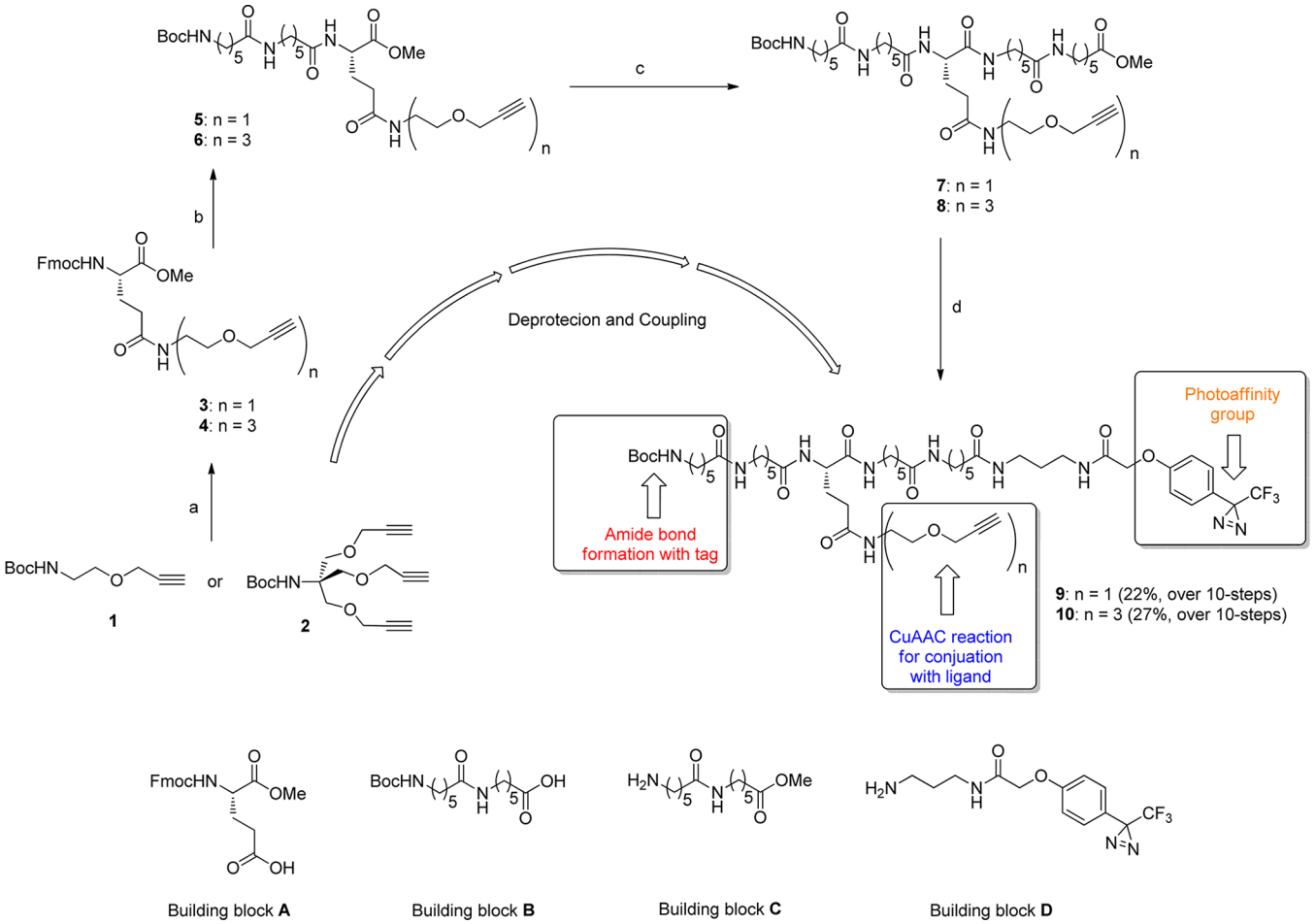
Photoaffinity labeling was introduced in the 1960s[10] and has proven to be a powerful tool to probe proteins by attaching a photoaffinity reagent to the corresponding small molecule ligands.[11−18] Although these photoaffinity probes worked very well by forming covalent bonds with target proteins upon UV irradiation, the success of labeling interacting proteins is highly reliant on high-affinity (K_d in the nM range) interactions between the probe and the protein. Thus, for the low affinity interaction between a carbohydrate ligand and a lectin, a monovalent carbohydrate ligand conjugated with a photoaffinity probe may not be suitable for lectin labeling. Multivalent carbohydrate structures, which greatly enhance the weak affinity of individual monoligands to their binding lectins, is a solution to overcome weak affinity.[19−21] For example, the asialoglycoprotein receptor (ASGP-R) in rat liver membranes was photoaffinity-labeled by a multivalent YEE-$(ahGalNAc)_3$-glycoprobe containing three terminal GalNAc residues.[22,23] In addition, Shin and co-workers developed photoaffinity probes possessing a trivalent fucose or mannose structure and used them to specifically photolabel spiked exogenous ConA from bacterial cell lysates of *A. aurantia*.[24]

Several photoaffinity functionalities are known and these functionalities are stable in the absence of ultraviolet (UV) radiation.[25,26] The most notable feature is that these functional groups can be appropriately incorporated and localized within the proteins upon UV irradiation. However, native carbohydrate–lectin binding interactions may be impeded by the steric bulk of the proximal photoaffinity groups,[27] resulting in a low efficiency of photolabeling and, consequently, hampering

Received: July 2, 2013
Revised: October 11, 2013
Published: October 23, 2013

   dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

Scheme 1. Convergent Synthesis of the Multifunctional Skeleton Precursor of the Multivalent Photoaffinity Probes[a]



[a]Reagents and conditions: (a) (i) TFA, DCM, r.t., 2 h, and then neutralization; (ii) block **A**, HBTU, DMF, Et_3N, r.t., overnight; yield for two steps, n = 1, 86%; n = 3, 78%; (b) (i) DBU, DCM, r.t., 1 h; (ii) block **B**, HBTU, DMF, Et_3N, r.t., 12 h, yield for two steps, n = 1, 80%; n = 3, 85%; (c) (i) LiOH, MeOH, H_2O, r.t., 1 h, and then neutralization; (ii) block **C**, HBTU, DMF, Et_3N, r.t., overnight, yield for two steps, n = 1, 72%; n = 3, 89%; (d) (i) LiOH, MeOH, H_2O, r.t., 1 h, and then neutralization; (ii) block **D**, HBTU, DMF, Et_3N, r.t., overnight, yield for two steps, n = 1, 73%; n = 3, 81%. HBTU: *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate; DBU: 1,8-diazabicycloundec-7-ene.

purification and/or identification. As shown in Scheme 1 (see the Supporting Information for details), we designed a multifunctional photoaffinity reagent (**10**) derived from a glutamic acid core and carrying a powerful photocross-linker, 3-trifluoromethyl-3-phenyl-diazirine,[25,26] and two well-defined synthetic handles to enable subsequent conjugations. The presence of a trialkyne in the probe could be simply and smoothly used to assemble carbohydrate ligands by Cu(I)-assisted click chemistry[28] for efficient photolabeling. The important multivalent ligand character of the probe could strengthen some weak ligand/receptor interactions and consequently increase the efficiency of covalent bond formation between the probe and the interacting protein. Subsequently, the amine of the probe could be ligated[29] with a variety of tags for visualization (e.g., fluorescence) or enrichment (e.g., biotin) of the labeled proteins. Furthermore, in **10**, the photoreactive group is not close to the multivalent ligand, minimizing negative effects on the ligand−receptor interaction.

EXPERIMENTAL PROCEDURES

Materials and Methods. Dimethyl formaldehyde (DMF, Merck), 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetra-methyluronium hexafluorophosphate (HBTU, Merck), triethylamine (NEt_3, Merck), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris, Acros), 6-aminohexanoic acid (Acros), Fmoc-L-glutamic acid 5-*tert*-butyl ester (Fmoc-Glu(OtBu)-OH, AnaSpec), suberic acid bis-*N*-hydroxysuccinimide ester (DSS, Fluka), and ethanolamine (Acros) were used as received. Bovine serum albumin (BSA, A9418), *Ricinus communis* agglutinin 120 (RCA_{120}, L7886), concanavalin A (ConA, C7642), wheat germ agglutinin (WGA, L9640), and ovalbumin (OVA, A5378) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The BCA protein assay kit was obtained from Pierce (Rockford, IL, USA). The P-2 gel was purchased from Bio-Rad. Monoclonal antibiotin antibody conjugated with HRP (A0185) was purchased from Sigma-Aldrich. Polyclonal anti-ASGP1 (asialoglycoprotein receptor 1) antibody (ARP33805_T100) and anti-ASGP2 (asialoglycoprotein receptor 2) antibody (ARP33820_P050) were purchased from AVIVA systems biology. Donkey antirabbit HRP-conjugated antibody (RPN510) was obtained from GE Healthcare. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AV-400 or an AV-600 MHz NMR instrument. Chemical shifts are expressed in ppm using residual $CDCl_3$ (7.24 ppm), CD_3OD (3.30 ppm), or D_2O (4.67 ppm at 298 K) as an internal standard. Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet,

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

q = quartet, m = multiplet, br = broad. J = coupling constant values are expressed in Hertz. Low-resolution and high-resolution mass spectra were recorded under ESI-TOF mass spectroscopy conditions. Analytical thin-layer chromatography (TLC) was performed on precoated plates (silica gel 60). Silica gel 60 (E. Merck) was employed for all flash chromatography. All reactions were performed in oven-dried glassware (120 °C) under an atmosphere of argon unless indicated otherwise. All solvents were dried and distilled by standard techniques.

Building Block A. The Fmoc-Glu-OMe was prepared by following the procedures described in ref 30.

Methyl 6-[(6-{[*tert*-Butoxycarbonyl]amino}hexanoyl)-amino]hexanoate. Commercially available 6-[(*tert*-butoxycarbonyl)amino]hexanoic acid (1.08 g, 4.67 mmol), *N*-hydroxysuccinimide (537 mg, 4.67 mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.07 g, 5.61 mmol) were dissolved in dry dichloromethane (23 mL), and the resulting mixture was stirred at room temperature under a nitrogen atmosphere for 3 h, until TLC indicated complete disappearance of starting material. The mixture was partitioned between dichloromethane and H_2O. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the desired activated ester. To a stirred solution of the activated ester in dry dichloromethane (25 mL) was added 6-aminohexanoic acid (613 mg, 4.67 mmol) and DIPEA (1.63 mL, 9.34 mmol). After being stirred at room temperature for 7 h, the mixture was concentrated to dryness *in vacuo*. The residue was dissolved in dry DMF (10 mL), and then iodomethane (2.33 mL, 37.36 mmol) and cesium carbonate (2.28 g, 7.00 mmol) were added at room temperature. After being stirred at room temperature for 1.5 h, the mixture was concentrated to dryness *in vacuo*. The above residue was dissolved in ethyl acetate and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to give the title compound (1.22 g, 73% for three steps) as a colorless oil. R_f 0.3 (EtOAc/Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24−1.33 (m, 4H), 1.39 (s, 9H), 1.42−1.50 (m, 4H), 1.55−1.63 (m, 4H) 2.11 (t, J = 7.6 Hz, 2H), 2.27 (t, J = 7.4 Hz, 2H), 3.05 (q, J = 6.6 Hz, 2H), 3.19 (q, J = 7.0 Hz, 2H), 3.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.0, 24.9, 25.8, 25.9, 27.9, 28.7, 29.2, 33.3, 35.8, 38.6, 39.8, 50.9, 78.1, 155.6, 172.7, 173.5. HRMS (ESI) m/z Calcd for $C_{18}H_{35}N_2O_5$ $[M+H]^+$ 359.2546; found: 359.2556.

Building Block B. To a solution of methyl 6-[(6-{[*tert*-butoxycarbonyl]amino}hexanoyl)amine]-hexanate (4.50 g, 12.52 mmol) in MeOH (12.5 mL)/H_2O (12.5 mL) was added LiOH (301.5 mg, 12.57 mmol) at room temperature. After being stirred at room temperature for 0.5 h, the mixture was neutralized with Amberlite IR-120 (H^+) resin and then filtered and concentrated *in vacuo* to give building block **B** as a colorless oil (4.29 g, 99%), which was used in the next step without further purification. R_f 0.65 (MeOH/DCM = 1/9); ^{1}H NMR (400 MHz, CD_3OD): δ 1.29−1.39 (m, 4H), 1.42 (s, 9H), 1.44−1.54 (m, 4H), 1.56−1.65 (m, 4H), 2.16 (t, J = 7.6 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.98−3.04 (m, 2H), 3.13−3.18 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 25.8, 26.6, 27.3, 27.4, 28.8, 30.0, 30.5, 35.4, 36.9, 40.1, 41.1, 79.7, 158.3, 175.8, 178.2. HRMS (ESI) m/z Calcd for $C_{17}H_{33}N_2O_5$ $[M+H]^+$ 345.2389; found: 345.2390.

Building Block C. To a solution of methyl 6-[(6-{[*tert*-butoxycarbonyl]amino}hexanoyl)amino]-hexaneate (1.08 g, 3.02 mmol) in dry DCM (6 mL) was added TFA (1.20 mL) dropwise at 4 °C. After being stirred for 2.5 h at room temperature, the mixture was then concentrated to dryness *in vacuo* by coevaporation with toluene. The residue in MeOH (10 mL) was neutralized with Dowex resin 550 (OH^-) at 4 °C followed by filtration and then concentration *in vacuo* to give building block **C** as a colorless oil (770 mg, 99%). R_f 0.15 (MeOH/DCM = 1/9); ^{1}H NMR (400 MHz, CD_3OD): δ 1.29−1.42 (m, 4H), 1.46−1.53 (m, 2H), 1.57−1.69 (m, 6H), 2.19 (t, J = 7.4 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.90 (t, J = 7.6 Hz, 2H), 3.15 (t, J = 7.0 Hz, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 25.0, 25.9, 26.3, 29.0, 33.7 (x2), 36.0, 39.1 (x2), 51.4, 173.2, 174.1. HRMS (ESI) m/z Calcd for $C_{13}H_{27}N_2O_3$ $[M+H]^+$ 259.2021; found: 259.2022.

***N*-*tert*-Butoxycarbonyl-1-[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]carbonyl-3-propane-diamine.** [4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic acid[31,32] (160 mg, 0.615 mmol), commercially available *N*-Boc-1,3-propanediamine (161 mg, 0.923 mmol), and HBTU (280 mg, 0.738 mmol) were dissolved in dry DMF (6 mL) and treated with triethylamine (129 μL, 0.923 mmol) at room temperature. After being stirred at room temperature under a nitrogen atmosphere for 8 h, the mixture was concentrated to dryness *in vacuo*, and ethyl acetate was added. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the crude product. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to give the desired product as a yellow oil (210 mg, 80%). R_f 0.26 (EtOAc/Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 9H), 1.57−1.63 (m, 2H), 3.11 (q, J = 6.1 Hz, 2H), 3.34 (q, J = 6.3 Hz, 2H), 4.45 (s, 2H), 4.88 (br, 1H), 6,93 (d, J = 8.7 Hz, 2H), 7.13(d, J = 8.7 Hz, 2H), 7.35 (br, 1H); ^{13}C (100 MHz, $CDCl_3$): δ 28.1 (q, $^2J_{CF}$ = 32 Hz), 28.3, 30.1, 35.3, 36.9, 67.2, 79.4, 115.1, 122.1 (q, $^1J_{CF}$ = 272 Hz), 122.3, 128.3, 156.6, 158.3, 167.8. HRMS (ESI) m/z Calcd for $C_{18}H_{23}F_3N_4O_4Na$ $[M+Na]^+$ 439.1569; found: 439.1565.

Building Block D. To a solution of the above coupling product (210 mg, 0.505 mmol) in dry DCM (4 mL) was added TFA (1 mL) dropwise at 4 °C. After being stirred at room temperature for 45 min, the mixture was concentrated to dryness *in vacuo* by coevaporation with toluene. The residue in MeOH (5 mL) was neutralized with Dowex resin 550 (OH^-) at 4 °C and then filtered and concentrated *in vacuo* to give building block **D** as a yellow oil (153 mg, 96%). R_f 0.15 (EtOAc/Hexane = 1/1 containing 10% MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.83−1.90 (m, 2H), 2.92 (t, J = 7.3 Hz, 2H), 3.37 (t, J = 6.6 Hz, 2H), 4.58 (s, 2H), 7.08 (d, J = 8.8 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 29.2 (q, $^2J_{CF}$ = 40.2 Hz), 29.6, 37.3, 38.0, 68.1, 116.5, 122.8, 123.6 (q, $^1J_{CF}$ = 273.0 Hz), 129.4, 160.4, 170.7. HRMS (ESI) m/z Calcd for $C_{13}H_{16}F_3N_4O_2$ $[M+H]^+$ 317.1225; found: 317.1234.

1-Azido-3,6-dioxaoct-8-yl 2,3,4,6-*tetra*-*O*-acetyl-β-D-galactopyranoside (β-D-Galactoside 15). The β-D-Galactoside **15** was prepared by following the procedure described in ref 33.

***N*-(*tert*-Butyloxycarbonyl)-2-(propargyloxy)-aminoethane (1).** To a solution of ethanolamine (3.05 g, 50 mmol) in dioxane (50 mL) was added $(Boc)_2O$ (16.42 g, 75 mmol), and the resulting mixture was stirred at room temperature overnight. After the complete absence of starting material (as judged by TLC), the volatiles were removed under reduced pressure, and the residue was redissolved in 30 mL of

CH_2Cl_2. The solution was washed successively with 1% HCl (60 mL), brine (2 × 30 mL), and H_2O (30 mL), then dried over $MgSO_4$, filtered, and concentrated to yield *N*-(*tert*-butoxycarbonyl)ethanolamine, which was used directly in the subsequent steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H), 2.50 (br, 1H), 3.26 (q, J = 5.2 Hz, 2H), 3.67 (q, J = 4.5 Hz, 2H), 4.97 (br, 1H). HRMS (FAB) m/z Calcd for $C_7H_{16}NO_3$ $[M+H]^+$ 162.1130; found: 162.1129. A solution of *N*-(*tert*-butoxycarbonyl)ethanolamine (1.60 g, 9.938 mmol) and propargyl bromide (80 wt % in toluene, 2.13 mL, 19.87 mmol) in dry DMF (10.7 mL) was stirred at 0 °C. Portions of finely ground KOH (1.11 g, 19.87 mmol) were added over a period of 15 min. The mixture was then heated to 35 °C and stirred for 16 h under a nitrogen atmosphere. The mixture was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/10) to give compound **1** (1.23 g, 63%) as a yellow oil. R_f 0.50 (EtOAc/Hexane = 1/2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H), 2.42 (t, J = 2.4 Hz, 1H), 3.31 (q, J = 5.0 Hz, 2H), 3.56 (t, J = 5.0 Hz, 2H), 4.13 (d, J = 2.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.7, 41.0, 58.8, 69.6, 75.9, 80.0, 80.5, 158.3. HRMS (FAB) m/z Calcd for $C_{10}H_{18}NO_3$ $[M+H]^+$ 200.1287; found: 200.1291.

***N*-(*tert*-Butyloxycarbonyl)tris[(propargyloxy)methyl]-aminomethane (2).** The compound **2** was prepared according to the procedure described in ref 34.

Compound 3. To a solution of compound **1** (1.20 g, 6.03 mmol) in dry DCM (10 mL) was added TFA dropwise (2.5 mL) at 4 °C. After being stirred at room temperature for 2 h, the mixture was concentrated to dryness *in vacuo* by coevaporation with toluene. The residue was dissolved in MeOH (12 mL) and neutralized with Dowex resin 550 (OH^-) at 4 °C, followed by filtration and concentration *in vacuo* to give a yellow oil. To a solution of the above compound (598 mg), building block **A** (2.30 g, 6.03 mmol), and HBTU (3.43 g, 9.05 mmol) in dry DMF (6 mL) was added triethylamine (1.68 mL, 12.06 mmol) at room temperature under a nitrogen atmosphere. After being stirred at room temperature overnight, the mixture was concentrated to dryness *in vacuo,* and ethyl acetate was added. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and then concentrated *in vacuo* to give the crude product. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/2) to give compound **3** (2.40 g, 86%) as a white syrup. R_f 0.42 (EtOAc/Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.92−2.05 (m, 1H), 2.17−2.26 (m, 3H), 2.41 (t, J = 2.4 Hz, 1H), 3.39−3.50 (m, 2H), 3.56 (t, J = 5.0 Hz, 2H), 3.72 (s, 3H), 4.11 (d, J = 2.4 Hz, 2H), 4.19 (t, J = 7.0 Hz, 1H), 4.32−4.40 (m, 3H), 5.79 (d, J = 8.0 Hz, 1H), 6.16 (br, 1H), 7.29 (t, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.58 (dd, J = 5.0, 7.0 Hz, 2H), 7.74(d, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2, 32.3, 39.2, 47.1, 52.5, 53.4, 58.2, 66.9, 68.5, 74.8, 79.3, 120.0, 125.0, 127.0, 127.6, 141.2, 143.6, 143.8, 156.2, 171.9, 172.4. HRMS (ESI) m/z Calcd for $C_{26}H_{28}N_2NaO_6$ $[M+Na]^+$ 487.1845; found: 487.1836.

Compound 4. The synthesis of compound **4** is similar to the synthesis of compound **3**. Compound **4**: White syrup (yield: 78%). R_f 0.40 (EtOAc/Hexane = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90−1.97 (m, 1H), 2.16−2.24 (m, 3H), 2.39 (t, J = 2.4 Hz, 3H), 3.73 (s, 3H), 3.79−3.86 (m, 6H), 4.11 (d, J = 2.4 Hz, 6H), 4.21 (t, J = 6.8 Hz, 1H), 4.34−4.43 (m, 3H), 5.69 (d, J = 8.0 Hz, 1H), 5.99 (br, 1H), 7.30 (t, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.59 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3, 32.8, 47.0, 52.3, 53.2, 58.5, 59.2, 66.8, 68.2, 74.6, 79.4, 119.8, 125.0, 126.9, 127.5, 141.1, 143.5, 143.7, 156.1, 171.8, 172.4. HRMS (ESI) m/z Calcd for $C_{34}H_{36}N_2NaO_8$ $[M+Na]^+$ 623.2369; found: 623.2368.

Compound 5. To a solution of compound **3** (2.79 g, 6.03 mmol) in dry DCM (20 mL) was added 8-diazabicycloundec-7-ene (1.8 mL, 12.06 mmol) at room temperature. After being stirred at room temperature for 1 h, the mixture was concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1 + 20% MeOH) to afford the desired product. The above compound, building block **B** (2.07 g, 6.03 mmol), and HBTU (2.74 g, 7.24 mmol) were dissolved in dry DMF (30 mL), and triethylamine (1.26 mL, 9.05 mmol) was added at room temperature. After being stirred under a nitrogen atmosphere at room temperature for 12 h, the mixture was concentrated to dryness *in vacuo* and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/1 + 10% MeOH) to give compound **5** (2.74 g, 80%) as a white syrup. R_f 0.50 (EtOAc/Hexane = 1/1 + 15% MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25−1.36 (m, 4H), 1.39 (s, 9H), 1.43−1.51 (m, 4H), 1.56−1.65 (m, 4H), 2.11−2.30(m, 7H), 2.43 (t, J = 2.4 Hz, 1H), 3.06 (q, J = 6.4 Hz, 2H), 3.21 (q, J = 6.4 Hz, 2H), 3.36−3.51 (m, 2H), 3.57 (t, J = 5.2 Hz, 2H), 3.70 (s, 3H), 4.12 (d, J = 2.4 Hz, 2H), 4.47−4.52 (m, 1H), 4.66 (br, 1H), 5.87 (br, 1H), 6.49 (br, 1H), 6.88 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.7, 25.1, 25.9, 26.1, 27.1, 28.1, 28.8, 29.3, 29.5, 32.0, 35.6, 36.1, 38.8, 38.9, 40.1, 51.8, 52.1, 57.9, 68.1, 74.7, 78.6, 79.1, 155.9, 172.2, 172.3, 173.0, 173.2. HRMS (ESI) m/z Calcd for $C_{28}H_{48}N_4O_8Na$ $[M+Na]^+$ 591.3363; found 591.3370.

Compound 6. The synthesis of compound **6** is similar as described in the synthesis of compound **5**. The crude compound was purified by column chromatography in silica gel (Hexane/Acetone = 1/1) to give compound **6** (yield: 85%) as white syrup R_f 0.3 (EtOAc/Hexane = 1/1 + 10% MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26−1.37 (m, 4H), 1.40 (s, 9H), 1.44−1.52 (m, 4H), 1.89−1.98 (m, 1H), 2.07−2.31 (m, 7H), 2.42 (t, J = 2.4 Hz, 3H), 3.07 (q, J = 6.6 Hz, 2H), 3.22 (q, J = 6.8 Hz, 2H), 3.71 (s, 3H), 3.81 (dd, J = 9.2, 14.0 Hz, 6H), 4.12 (d, J = 2.4 Hz, 6H), 4.50−4.55 (m, 1H), 4.62 (br, 1H), 5.77 (br, 1H), 6.70 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8, 25.3, 26.1, 26.3, 27.6, 28.3, 29.0, 29.7, 33.0, 35.9, 36.4, 39.0, 40.3, 51.8, 52.4, 58.6, 59.4, 68.3, 74.7, 79.4, 156.0, 172.3, 172.6, 172.9, 173.2. HRMS (ESI) m/z Calcd for $C_{36}H_{56}N_4NaO_{10}$ $[M+Na]^+$ 727.3894; found: 727.3900.

Compound 7. To a solution of compound **5** (900 mg, 1.583 mmol) in MeOH (10 mL)/H_2O (10 mL) was added LiOH (37.9 mg, 1.583 mmol) at room temperature. After being stirred at room temperature for 1 h, the mixture was neutralized with Amberlite IR-120 (H^+) resin, then filtered and concentrated *in vacuo* to afford the acid as a white solid. The above acid, building block **C** (766 mg, 2.058 mmol), and HBTU (900 mg, 2.374 mmol) were dissolved in dry DMF (7.9 mL) and treated with triethylamine (440 μL, 3.166 mmol) at room temperature under a nitrogen atmosphere. After being stirred overnight at room temperature, the reaction was concentrated to dryness *in vacuo*, and ethyl acetate was added. The organic layer was washed with brine, dried over

$MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1/1 + 15% MeOH) to give compound **7** (873 mg, 72%) as a white syrup. R_f 0.25 (EtOAc/Hexane = 1/1 + 20% MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 1.28−1.36 (m, 8H), 1.41 (s, 9H), 1.44−1.53 (m, 8H), 1.57−1.65 (m, 8H), 1.84−1.91 (m, 1H), 2.00−2.07 (m, 1H), 2.85 (t, J = 1.5 Hz, 1H), 3.01 (t, J = 4.7 Hz, 1H), 3.12−3.18 (m, 6H), 3.32−3.43 (m, 2H), 3.54−3.58 (m, 2H), 3.64 (s, 3H), 4.15 (d, J = 1.5 Hz, 2H), 4.24−4.30 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 25.6, 26.4, 26.6 (x2), 26.7 27.4, 27.5, 28.8, 29.1, 30.0 (x2), 30.1, 30.6, 33.2, 34.6, 36.6, 36.9, 37.0, 40.1 (x2), 40.2, 41.2, 51.9, 54.3, 58.8, 58.9, 69.2, 76.0, 79.7, 80.5, 158.5, 173.8,174.8,175.7, 175.9 (x2), 176.0. HRMS (ESI) m/z Calcd for $C_{40}H_{70}N_6NaO_{10}$ $[M+Na]^+$ 817.5051; found: 817.5049.

Compound 8. Compound **8** was prepared similarly as described in the synthesis of compound **7**. The residue was purified by column chromatography in silica gel (EtOAc/Hexane = 1/1 + 17% MeOH) to give compound **8** (yield: 89%) as white syrup. R_f 0.55 (EtOAc/Hexane = 1/1 + 20% MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.27−1.37 (m, 8H), 1.42 (s, 9H), 1.45−1.54 (m, 9H), 1.58−1.65 (m, 8H), 1.83−1.91 (m, 1H), 1.96−2.04 (m, 1H), 2.16 (t, J = 7.5 Hz, 4H), 2.24 (t, J = 7.3 Hz, 4H), 2.32 (t, J = 7.3 Hz, 4H), 2.83 (t, J = 2.3 Hz, 3H), 2.99−3.04 (m, 2H), 3.12−3.19 (m, 6H), 3.64 (s, 3H), 3.75−3.80 (m, 6H), 4.14 (d, J = 2.3 Hz, 6H), 4.21−4.26 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 25.5, 26.3, 26.6 (x2), 27.3, 27.5, 28.8, 29.1, 29.9, 30.0 (x2), 30.6, 33.6, 34.6, 36.6, 36.9, 40.0, 40.1, 40.2, 41.1, 52.0, 54.2, 59.4, 60.9 (x2), 69.0, 76.0, 79.7, 80.5, 158.3, 173.7, 174.8, 175.6, 175.8 (x3). HMRS (ESI) m/z Calcd for $C_{48}H_{78}N_6NaO_{12}$ $[M+Na]^+$ 953.5575; found: 953.5572.

Compound 9. Compound **9** was prepared by following the procedures as described in the synthesis of compound **7**. The residue was purified by column chromatography in silica gel (MeOH/DCM = 1/12) to give compound **9** (yield: 73%) as lightly yellow syrup. R_f 0.50 (MeOH/DCM = 1/9); ^{1}H NMR (400 MHz, CD_3OD): δ 1.27−136 (m, 8H), 1.41 (s, 9H), 1.44−1.51 (m, 8H), 1.55−1.69 (m, 10H), 1.83−1.92 (m, 1H), 1.98−2.08 (m, 1H), 2.14−2.20 (m, 6H), 2.22−2.28 (m, 4H), 2.85 (t, J = 2.3 Hz, 1H), 2.98−3.03 (m, 2H), 3.12−3.18 (m, 8H), 3.26−3.40 (m 4H), 3.56 (t, J = 5.5 Hz, 2H), 4.14 (d, J = 2.3 Hz, 2H), 4.24−4.31 (m, 1H), 4.54 (s, 2H), 7.08 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H); ^{13}C NMR (150 MHz, CD_3OD): δ 26.3, 26.5, 26.6, 26.7, 27.3, 27.4, 27.5, 28.8, 29.1, 29.2 (q, $^2J_{CF}$ = 40 Hz), 29.9, 30.0, 30.2, 30.5, 33.0, 33.1, 36.6, 36.9, 37.3, 37.4, 40.1, 40.2, 41.1, 49.8, 54.1, 54.2, 58.8, 68.1, 69.0 69.1, 76.0, 76.1, 79.6, 80.5, 116.5, 122.6, 123.5 (q, $^1J_{CF}$ = 272 Hz), 129.3, 158.3, 160.3, 170.3, 173.7, 174.7, 175.8 (x3), 176.0. HMRS (ESI) m/z Calcd for $C_{52}H_{81}F_3N_{10}NaO_{11}$ $[M+Na]^+$ 1101.5936; found: 1101.5945.

Compound 10. Compound **10** was prepared by following the procedures as described in the synthesis of compound **7**. The residue was purified by column chromatography in silica gel (EtOAc/Hexane = 1/1 + 15−30% MeOH) to give compound **10** (yield: 81%) as light yellow syrup. R_f 0.50 (EtOAc/Hexane = 1/1 + 20% MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.27−1.36 (m, 8H), 1.41 (s, 9H), 1.45−1.53 (m, 8H), 1.56−1.71 (m, 10H), 1.81−1.91 (m, 1H), 1.95−2.03 (m, 1H), 2.14−2.20 (m, 6H), 2.22−2.27 (m, 4H), 2.83 (t, J = 2.3 Hz, 3H), 3.01 (t, J = 7.0 Hz, 2H), 3.13 (m, 8H), 3.27−3.29 (m, 2H), 3.75−3.80 (m, 6H), 4.13 (d, J = 2.3 Hz, 6H), 4.23−4.26 (m, 1H), 4.54 (s, 2H), 7.08 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.9, 25.0, 25.1, 25.2, 26.1, 26.2, 27.9 (q, $^2J_{CF}$ = 40 Hz), 28.1, 28.2, 28.7, 28.9, 29.4, 29.5, 33.0, 35.6, 35.7, 35.8, 36.0, 36.1, 38.9, 39.0, 40.2, 52.6, 58.5, 59.3, 67.0, 68.2, 74.8, 78.8, 79.3, 115.0, 121.9 (q, $^1J_{CF}$ = 273 Hz), 122.0, 128.1, 156.0, 158.1, 167.9, 171.6, 172.6, 173.1, 173.2, 173.4, 173.7. HMRS (ESI) m/z Calcd for $C_{60}H_{89}F_3N_{10}NaO_{13}$ $[M+Na]^+$ 1237.6460; found: 1237.6461.

Compound 11. To a solution of compound **9** (182 mg, 0.169 mmol) in EtOH/H_2O/*t*-BuOH (5.6 mL; 3:2:5 = v/v/v) was added 1-azido-3,6-dioxaoct-8-yl 2,3,4,6-*tetra*-*O*-acetyl-β-D-galacto-pyranoside (94 mg, 0.186 mmol) and DIPEA (27 μL, 0.169 mmol) at room temperature. The mixture was stirred for 5 min, and then copper iodide (3 mg, 0.0169 mmol), a saturated solution of copper(II) sulfate (49 μL), and sodium ascorbate (6.7 mg, 0.0338 mmol) were added. The mixture was stirred at 35 °C for another 6 h. The solvent was concentrated to dryness *in vacuo*, and the resulting residue was purified by column chromatography on silica gel (MeOH/DCM = 1/10) to give compound **11** (193 mg, 72%) as a yellow syrup. R_f 0.45 (MeOH/DCM = 1/9); ^{1}H NMR (400 MHz, CD_3OD): δ 1.26−1.34 (m, 8H), 1.41 (s, 9H), 1.44−1.53 (m, 8H), 1.56−1.71 (m, 10H), 1.84−1.90 (m, 1H), 1.93 (s, 3H), 1.98−2.05 (m, 1H), 2.01 (s, 3H), 2.02 (s, 3H), 2.11 (s, 3H), 2.12−2.20 (m, 6H), 2.22−2.27 (m, 4H), 3.01 (q, J = 6.6 Hz, 2H), 3.12−3.18 (m, 8H), 3.21−3.28 (m, 2H), 3.35−3.44 (m, 2H), 3.51−3.58 (m, 8H), 3.67−3.75 (m, 1H), 3.87−3.93 (m, 3H), 4.08−4.15 (m, 3H), 4.22−4.31 (m, 1H), 4.54 (s, 2H), 4.57−4.61 (m, 4H), 4.68 (d, J = 7.5 Hz, 1H), 5.04−5.14 (m, 2H), 5.37 (d, J = 3.2 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.94 (s, 1H), 8.04 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 20.5, 20.6, 20.8, 26.4, 26.6, 26.7, 27.4, 27.5, 28.8, 29.0 (q, $^2J_{CF}$ = 39 Hz), 29.1, 29.2, 30.0, 30.1, 30.3, 30.6, 33.1, 33.2, 36.7, 37.0, 37.4, 37.5, 40.1, 40.3, 41.2, 43.8, 51.4, 54.2, 54.3, 55.8, 62.5, 64.7, 68.2, 68.8, 69.7, 69.8, 70.3, 70.4, 71.3, 71.4, 71.5, 72.3, 79.7, 102.3, 116.6, 122.8, 123.6 (q, $^1J_{CF}$ = 272 Hz), 125.9, 129.4, 145.6, 158.5, 160.4, 170.5, 171.3, 171.4, 171.9, 172.0, 173.8, 174.7, 175.9 (x3), 176.2. HMRS (ESI) m/z Calcd for $C_{72}H_{112}F_3N_{13}NaO_{23}$ $[M+Na]^+$ 1606.7844; found: 1606.7844.

Compound 12. Compound **12** was prepared by following the procedures as described in the synthesis of compound **11**. The residue was purified by column chromatography in silica gel (MeOH/DCM = 1/8) to give compound **12** (157 mg, 70%) as yellow syrup. R_f 0.43 (MeOH/DCM = 1/9); ^{1}H NMR (400 MHz, CD_3OD): δ 1.28−1.36 (m, 8H), 1.41 (s, 9H), 1.44−1.51 (m, 8H), 1.56−1.69 (m, 10H), 1.77−1.85 (m, 1H), 1.86−1.97 (m, 1H), 1.93 (s, 6H), 1.94 (s, 3H), 2.01 (s, 18H), 2.11−2.24 (m, 19H), 2.99−3.05 (m, 4H), 3.12−3.18 (m, 8H), 3.50−3.71 (m, 26H), 3.75−3.80 (m, 2H), 3.85−4.00 (m, 9H), 4.10−4.21 (m, 9H), 4.24−4.47 (m, 6H), 4.53−4.60 (m, 8H), 4.67−4.70 (m, 3H), 5.04−5.15 (m, 6H), 5.37−5.39 (m, 3H), 7.04 (dd, J = 8.8, 14.2 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 8.8 Hz, 1H), 7.41 (dd, J = 8.8, 14.2 Hz, 1H), 7.94−8.00 (m, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 20.5, 20.6, 20.7, 20.8, 26.4, 26.6, 26.7, 27.4, 27.5, 28.8, 29.0 (q, $^2J_{CF}$ = 38 Hz), 29.2, 30.0, 30.1, 30.3, 30.6, 30.7, 33.6, 36.7, 37.0, 37.4, 37.5, 40.2, 41.2, 50.5, 51.4, 51.5, 54.4, 62.5, 62.6, 64.1, 64.3, 65.2, 68.1, 68.2, 68.8, 70.2, 70.3, 70.4, 71.1, 71.2, 71.4, 71.5, 71.8, 72.3, 79.8, 102.2, 115.7, 115.9, 116.6, 122.8, 123.6 (q, $^1J_{CF}$ = 273 Hz), 125.2, 129.4, 130.2, 147.6, 158.5, 160.4, 170.5, 171.3 (x3), 171.5 (x3), 171.9 (x3), 172.0 (x3), 173.7, 174.7, 175.9 (x3), 176.2. HMRS (ESI) m/z Calcd for $C_{120}H_{182}F_3N_{19}NaO_{49}$ $[M+Na]^+$ 2753.2184; found: 2753.2190.

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

Compound 13. To a solution of compound **11** (133 mg, 0.084 mmol) in dry DCM (2 mL) was added TFA (0.6 mL) dropwise at 4 °C. After being stirred at room temperature for 1.5 h, the mixture was concentrated to dryness *in vacuo* by coevaporation with toluene. The residue in MeOH (12 mL) was neutralized with Dowex resin 550 (OH^-) at 4 °C, filtered, and concentrated *in vacuo* to give a crude product, which was used in the next reaction without purification. The above material and biotin-OSu[35] (42.9 mg, 0.126 mmol) were dissolved in dry DMF (2 mL), and Et_3N (17.5 μL, 0.126 mmol) was added. The reaction was stirred at room temperature for 12 h under a nitrogen atmosphere. After the reaction was complete (as judged by TLC), the mixture was concentrated to dryness *in vacuo* by coevaporation with toluene. The resulting residue was dissolved in MeOH (4 mL) and stirred with NaOMe (13.6 mg, 0.252 mmol) at room temperature. After 1 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated *in vacuo* to give the crude product. The residue was purified by Bio-Gel P2 size exclusion chromatography to give compound **13** as a colorless syrup (60 mg, 46% overall yield for three steps). R_f 0.22 (MeOH/DCM = 1/2); ^{1}H NMR (600 MHz, CD_3OD): δ 1.29−1.36 (m, 8H), 1.39−1.45 (m, 2H), 1.47−1.52 (m, 10H), 1.57−1.75 (m, 12H), 1.84−1.90 (m, 1H), 1.99−2.05 (m, 1H), 2.14−2.19 (m, 8H), 2.22−2.27 (m, 4H), 2.69 (d, *J* = 12.7 Hz, 1H), 2.91 (dd, *J* = 5.0 Hz, 12.7 Hz, 1H), 3.13−3.21 (m, 12H), 3.28 (dd, *J* = 9.4 Hz, 16.2 Hz, 1H), 3.37(t, *J* = 5.4 Hz, 2H), 3.45−3.53 (m, 3H), 3.55−3.58 (m, 2H), 3.60 (s, 4H), 3.62−3.64 (m, 3H), 3.67−3.76 (m, 3H), 3.82 (d, *J* = 3.3 Hz, 1H), 3.89 (t, *J* = 5.0 Hz, 2H), 3.96−3.99 (m, 1H), 4.24−4.30 (m, 3H), 4.48 (dd, *J* = 5.0, 8.0 Hz, 1H), 4.54 (s, 2H), 4.58 (t, *J* = 5.0 Hz, 2H), 4.61 (s, 2H), 7.01−7.06 (m, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 7.38−7.43 (m, 1H), 8.04 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 26.4, 26.6, 26.7, 26.9, 27.5, 27.6, 29.0 (q, $^2J_{CF}$ = 39 Hz), 29.2, 29.5, 29.7, 30.0, 30.1, 30.3, 30.4, 30.7, 33.0, 33.2, 36.7, 36.8, 37.0, 37.4, 37.5, 40.2, 40.3, 40.4, 41.0, 51.4, 54.4, 57.0, 61.6, 62.5, 63.4, 64.7, 68.2, 69.6, 69.8, 70.3, 71.3, 71.4, 72.5, 74.9, 76.7, 105.1, 115.9, 116.6, 122.8, 123.7 (q, $^1J_{CF}$ = 273 Hz), 125.9, 129.4, 130.8, 145.6, 160.5, 166.1, 170.5, 173.8, 174.9, 175.9 (x3), 176.0, 176.2. HMRS (ESI) *m*/*z* Calcd for $C_{69}H_{110}F_3N_{15}NaO_{19}S$ $[M+Na]^+$ 1564.7673; found: 1564.7659.

Compound 14. Compound **14** was synthesized by the same methods as described in the synthesis of compound **13**. The residue was purified by Bio-Gel P2 size exclusion chromatography to give compound **14** as colorless syrup (43% for three steps). R_f 0.1 (MeOH/DCM = 1/1); ^{1}H NMR (600 MHz, CD_3OD): δ 1.29−1.36 (m, 8H), 1.41−1.44 (m, 2H), 1.47−1.53 (m, 10H), 1.56−1.74 (m, 12H), 1.78−1.87 (m, 1H), 1.91−2.01 (m, 1H), 2.16−2.19 (m, 8H), 2.23−2.26 (m, 4H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.91 (dd, *J* = 4.0, 12.7 Hz, 1H), 2.98−3.05 (m, 1H), 3.12−3.20 (m, 13H), 3.27−3.28 (m, 1H), 3.33−3.35 (m, 1H), 3.47−3.77 (m, 52H), 3.82−3.84 (m, 4H), 3.88−3.91 (m, 5H), 3.93−3.98 (m, 6H), 4.20−4.31 (m, 8H), 4.46−4.58 (m, 16H), 7.01−7.06 (m, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.38−7.43 (m, 1H), 8.00 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 26.4, 26.6, 26.7, 26.9, 27.5, 29.0 (q, $^2J_{CF}$ = 39 Hz), 29.5, 29.7, 30.0, 30.1, 30.3, 36.7, 36.8, 37.0, 37.4, 37.5, 40.2, 41.0, 51.4, 56.9, 61.6, 62.5, 63.3, 65.2, 68.2, 69.6, 70.3, 71.3, 71.4, 72.5, 74.9, 76.7, 105.1, 115.9, 116.6, 122.8, 123.6 (q, $^1J_{CF}$ = 273 Hz), 125.9, 126.0, 129.4, 130.8, 145.6, 160.4, 166.0, 170.5, 173.8, 174.9, 176.0 (x4), 176.2. HMRS (MALDI-TOF, Matrix: CHCA (10 mg/mL) 0.1% TFA and 50% acetonitrile) *m*/*z* Calcd for $C_{101}H_{164}F_3N_{21}NaO_{37}S$ $[M+Na]^+$ 2375.11; found: 2375.12.

Labeling of RCA_{120} by the Trivalent (14) Galactose Photoaffinity Probe. RCA_{120} (0.1 μM, 500 μL in PBS buffer, pH 7.4) was incubated with various concentrations of compound **14** (25, 50, 75, and 100 μM). The mixture was briefly agitated at 4 °C for 30 min to ensure proper mixing and then irradiated with a UV lamp (365 nm, 2.76 mW/cm^2) (Panchum, photochemical reactor, PR-2000) at 4 °C for 30 min. Excess trigalactose photoaffinity probe was removed from the reaction via spin concentration (Microcon centrifugal filter 10,000 MWCO, Millipore, MA). An aliquot of this purified mixture was treated with SDS-PAGE loading buffer (0.3 M Tris-Cl, 10% SDS, 30% glycerol, 9.3% DTT, pH 6.8) and heated at 95 °C for 10 min followed by electrophoresis. Samples were visualized by Coomassie blue staining and verified by Western blotting. Negative control experiments were performed as described above without UV irradiation.

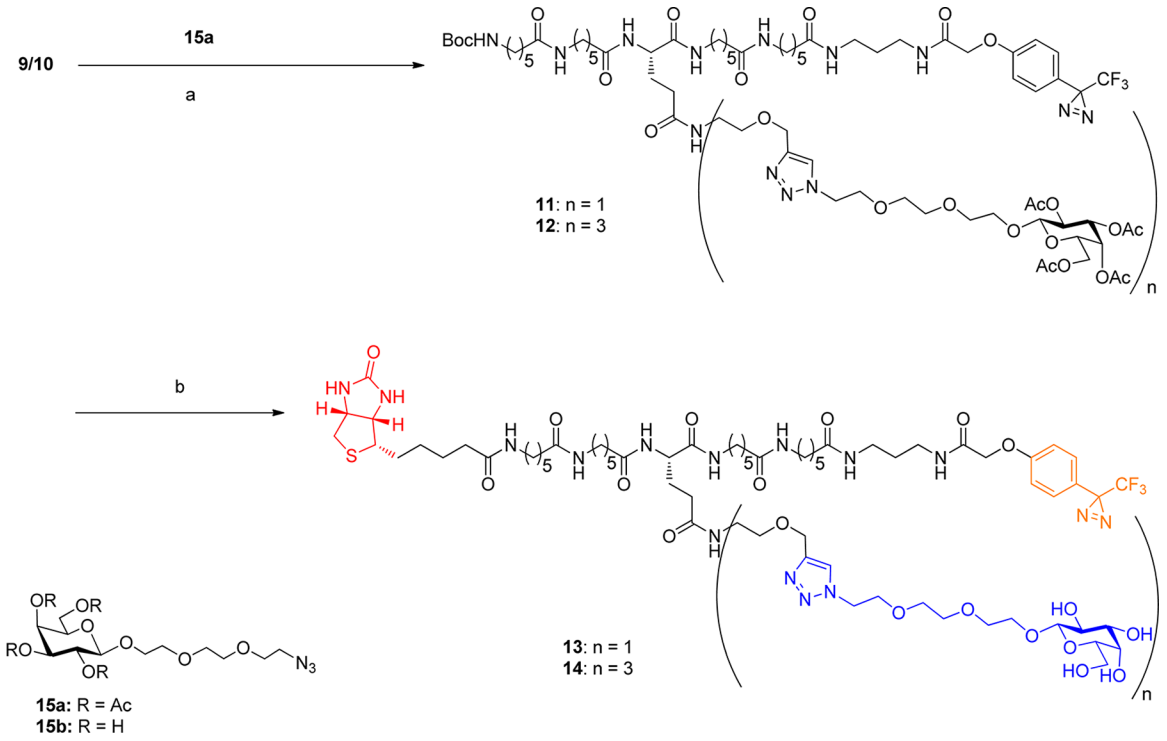
Labeling of RCA_{120} by Monovalent (13) Galactose Photoaffinity Probe. RCA_{120} (0.1 μM, 500 μL in PBS buffer at pH 7.4) was incubated with various concentrations of compound **13** (12.5, 25, 50, 100, and 200 μM). The subsequent procedure was the same as that described above.

Selective Labeling of RCA_{120} by Trivalent Galactose Photoaffinity Probe (14) in a Protein Mixture. A 1.7-mL Eppendorf tube was charged with a solution of protein mixture I (OVA, BSA, ConA, RCA_{120}, and WGA) or II (BSA, ConA, RCA_{120}, and WGA) (0.1 μM for each protein, 500 μL in PBS buffer at pH 7.4) and a solution of compound **14** (final 50 μM). The subsequent procedure was the same as previously described. A negative control experiment was performed by omitting the irradiation step.

Synthesis of Magnetic Nanoparticles. The core Fe_3O_4 particles (MNPs) and the amine-functionalized MNPs (NH_2-MNPs) were prepared following reported procedures.[36]

Synthesis of Streptavidin-MNP (Streptavidin-Conjugated MNP). NH_2-MNPs (10 mg) and DSS (50 mg) were mixed in DMSO (500 μL), and the resulting mixture was gently rotated at 37 °C for 6 h. After magnetic separation, the supernatant was discarded, and the MNPs were washed three times with DMSO, ddH_2O, and PBS (pH 7.4) to give (OSu)-activated MNPs. To conjugate streptavidin with MNP, the (OSu)-activated MNPs were dispersed in PBS, and a streptavidin solution (10 mg in 1.0 mL PBS) was added. The resulting mixture was stirred at 4 °C overnight. The resulting MNPs were separated from the supernatant by applying a magnet and were washed three times with ddH_2O. The protein amount on the MNPs was determined with a BCA Protein Assay Kit (PIERCE, Rockford, IL). An Agilent spectrometer 8453 was used to measure the absorbance of the dye solutions. The calibration curve for the quantitation was obtained by measuring the absorbance at 562 nm of a 1 mL dye solution containing bovine serum albumin (BSA; 0 μg, 2 μg, 4 μg, 6 μg, 8 μg, and 10 μg). The BCA working reagent was prepared by mixing 50 parts BCA working reagent A and 1 part BCA working reagent B (reagent A:reagent B = 50:1 (v/v)). Each standard protein or unknown sample was pipetted into 1.7 mL Eppendorf centrifuge tubes. One milliliter of BCA working reagent was added to each tube and then vortexed to mix well. After incubation at 60 °C for 30 min, the samples were separated by applying a magnet. The supernatants were interrogated with an UV/vis spectrometer at 562 nm. This

Scheme 2. Syntheses of Mono- and Tri-Galactoses of Photoaffinity Probes 13 and 14[a]



[a]Reagents and conditions: (a) β-D-Galactoside **15a**, CuI (catalytic), $CuSO_4$ (catalytic), sodium ascorbate, DIPEA, $EtOH:H_2O:CH_3CN$ = 3:2:5, r.t. 6 h, *n* = 1, 80%; *n* = 3, 82%. (b) (i) TFA, DCM, r.t., 1.5 h, and then neutralization; (ii) D-biotin, HBTU, Et_3N, DMF, r.t.,12 h; (iii) NaOMe (catalytic), MeOH, r.t., 1 h, *n* = 1, 46%; *n* = 3, 43% (over three steps). DIPEA: *N,N*-diisopropylethylamine.

measurement should be completed within 10 min. The absorbance intensity of the blank standard was subtracted from the absorbance intensity of the measured sample. The concentration of the tested sample was determined from the calibration curve. The amount of streptavidin was calculated to be 106 μg/mg MNP.

Labeling and purification of RCA_{120} from *E. coli* Cell LysateA. A 1.7-mL Eppendorf tube was charged with a solution of *E. coli* BL21 (DE3) cell lysate (delivered as 300 μL of solution in 20 mM Tris-HCl buffer at pH 8.0), RCA_{120} (delivered as 24 μL of a 56.4 μM stock solution in PBS buffer at pH 7.4), PBS buffer (delivered as 1.0 mL at pH 7.4), and a solution of compound **14** (delivered as 50 μL of a 2 mM solution in PBS buffer at pH 7.4). The mixture was briefly agitated to ensure proper mixing and then incubated with agitation at 4 °C for 30 min, followed by subsequent irradiation with a UV lamp (365 nm, 2.76 mW/cm^2) at 4 °C for 30 min. Excess compound **14** was removed from the reaction via spin concentration (Microcon centrifugal filter 10,000 MWCO, Millipore, MA). An aliquot of this purified mixture (50 μL) was diluted with PBS buffer at pH 7.4 (950 μL) and then incubated with Streptavidin-MNP (1.5 mg/protein) at 4 °C for 30 min with vortexing. The RCA_{120}-Streptavidin-MNP complex was isolated by applying a magnet, and the resulting MNPs were washed three times with 500 μL washing buffer (sodium dodecyl sulfate:PBS = 0.3:9.7% (v/v)). The samples were then treated with SDS-PAGE loading buffer (0.3 M Tris-Cl, 10% SDS, 30% glycerol, 9.3% DTT, pH 6.8), heated at 95 °C for 10 min, and then analyzed by electrophoresis, visualized by Coomassie blue staining, and verified by Western blotting.

Cell Culture. HepG2 cells were cultured in T-75 flasks with Dulbecco's Modified Eagle's Medium (DMEM, Gibco), 10% heat-inactivated fetal bovine serum (FBS, Gibco), 100 U mL^{-1} penicillin (Gibco), 100 μg mL^{-1} streptomycin (Gibco), and 0.25 μg mL^{-1} Fungizone (BioSOURCE). Cells were incubated at 37 °C in an ambient air/5% CO_2 atmosphere and subcultured every 3 days.

Labeling the Membrane Protein ASGP-R (Asialoglycoprotein receptor) on HepG2 cells with the Trivalent Galactose Photoaffinity Probe (14) and the Purification of Labeled Proteins by Streptavidin-MNP. HepG2 cells (1×10^7) were seeded onto sterile glass coverslips, placed in a 100 mm dish, and cultured in 10.0 mL DMEM medium with 10% FBS overnight. The cells were washed three times with serum-free DMEM and then serum-starved for 30 min on ice in the same medium. After 30 min, the cells were removed from their Petri dish for passage by gently pipetting up and down several times with 1.0 mL of serum-free DMEM on ice. To the above solution was added compound **14** (2 mM, in ddH_2O (5 μL)), followed by incubation on ice for 1.5 h. After the binding step, the cells were irradiated for 30 min with a UV lamp (365 nm, 2.76 mW/cm^2) at 4 °C. Then, Streptavidin-MNPs (1.0 mg in PBS buffer (20 μL) at pH 7.4) were added, and the resulting mixture was vortexed for 30 min at 4 °C, followed by centrifugation at 200 *g* for 2 min to collect the cells. The cells were then lysed with 1.0 mL lysis buffer (1xRIPA buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM -glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin], 1 mM PMSF, and protease inhibitor cocktail (Roche, 04693116001)) for 30 min at 4 °C. The MNPs in the above mixture were magnetically isolated, and the resulting particles were washed three times with 500 μL washing buffer (sodium dodecyl sulfate: PBS = 0.3: 99.7%, v/

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

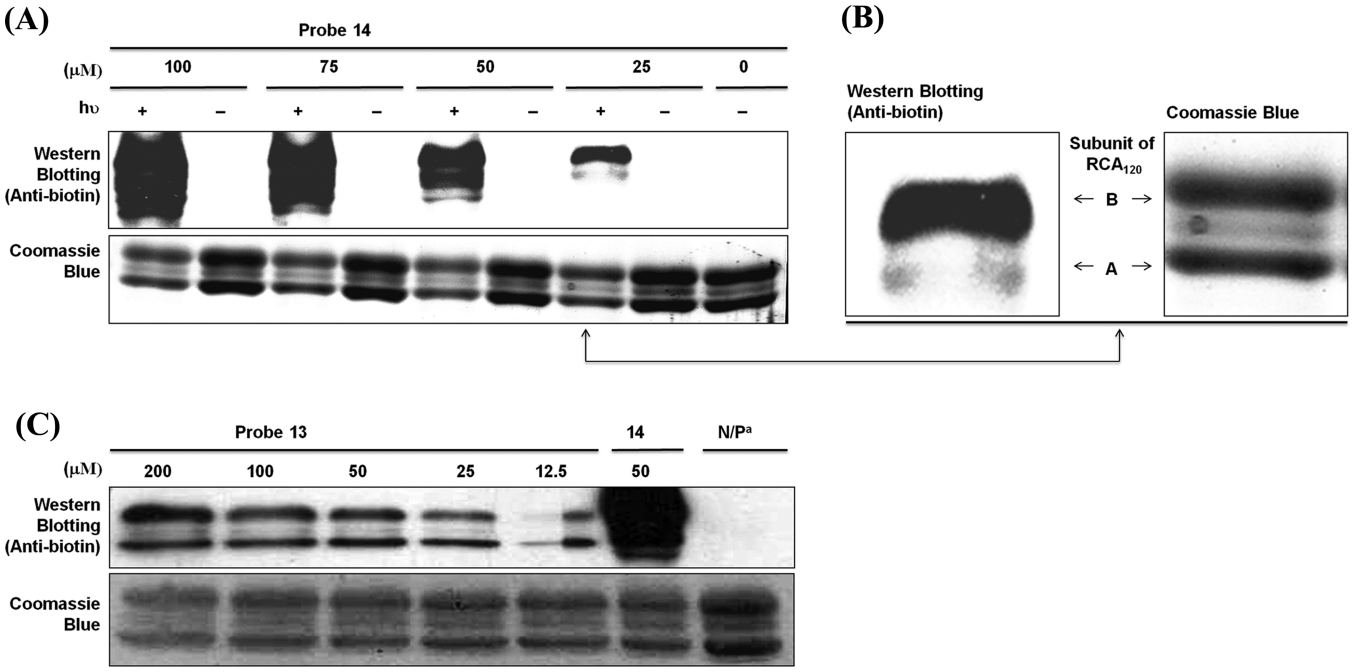


Figure 1. (A) Concentration-dependent labeling of RCA_{120} (0.1 μM) by probe **14** with and without UV irradiation. (B) A partial enlargement of the Western blot (*left*), and Coomassie stained (*right*) SDS-PAGE in part A (marked with an arrow), indicative of cross-linking at A chain of RCA_{120}. (C) Comparison of RCA_{120} (0.1 μM) labeling efficiencies with various concentrations of probe **13** and probe **14** (50 μM). N/P lane: only RCA_{120} (0.2 μM) as a negative control.

v). The captured proteins were divided into four aliquots and analyzed by SDS-PAGE with Coomassie blue visualization and Western blotting with antibodies against biotin and the ASGP-R subunits (H1 and H2).

Western Blotting. Proteins were transferred onto a PVDF membrane with a semidry transfer blot apparatus (BioRad). Samples were resolved on SDS-polyacrylamide gels. The gels were then rinsed with transfer buffer (50 mM Tris-HCl, pH 8.3, 20% methanol, 40 mM glycine in distilled water). The PVDF membrane (Millipore) was activated in a bath of methanol and then equilibrated in transfer buffer. The membranes were blocked with 5% milk/TBST at room temperature for 1 h followed by incubation with the corresponding primary antibody in 5% milk/TBST at 4 °C for 16 h. The membranes were washed with TBST (4 × 10 min) and then incubated with an appropriate secondary antibody at room temperature for 1 h. Finally, the membranes were washed again with TBST before visualization by chemiluminescence (PerkinElmer, NEL102001EA).

■ RESULTS AND DISCUSSION

Synthesis of Trigalactose Photoaffinity Probe. To highlight the effect of the multivalent character of the designed probe on the photolabeling of target proteins, the monovalent probe **9** (Scheme 1) was also synthesized for comparison. To readily access scaffolds with flexible spatial arrangements, we used commercially available 6-aminohexanoic acid, which is commonly employed as a heterobifunctional linker in glycocluster synthesis.[37] The multifunctional skeletons **9** and **10** were efficiently constructed using monoalkyne **1** and trialkyne **2**, respectively, via a series of deprotection and amide bond formations with building blocks **A**, **B**, **C**, and **D** (see Supporting Information for detailed syntheses). For example, the Boc group of **2** was removed with 20% TFA/DCM followed by coupling with building block **A** to give **4**. The linker length was then elongated by (i) cleavage of Fmoc with 1,8-diazabicycloundec-7-ene, (ii) coupling with building block **B** using HBTU, (iii) hydrolysis of methyl esters under basic conditions, and (iv) coupling with building block **C** to furnish compound **8**. Hydrolysis of the methyl ester of **8** was followed by amide bond formation with photoreactive group **D** to yield photoaffinity probe **10** in ten steps with 27% overall yield from **2**. Following these same procedures, compound **9** (22% overall yield from **1**) was synthesized.

To demonstrate the photolabeling efficiency of this multivalent ligand, *Ricinus communis* Agglutinin 120 (RCA_{120}) was chosen as the target for a proof-of-concept study. RCA_{120} was isolated from the seed of the castor bean and consists of a dimeric chain [BAAB] in which the two chains, A–B, are covalently connected by a disulfide bridage.[38,39] RCA_{120} recognizes nonreducing terminal β-D-galactose (β-Gal) and shows low avidity for monosaccharide (K_a 7.7 × 10^3 M^{-1} for methyl-β-D-galactoside),[40] while that for poly-Gal (more than 37 gal) was enhanced to K_a 1.8 × 10^8 M^{-1}.[41] Thus, the β-D-galactopyranoside **15** was assembled on skeletons **9** and **10** by Cu(I)-catalyzed click chemistry to give the mono- and trigalactosyl precursors **11** and **12**, respectively (Scheme 2). Removal of the Boc group on precursor **11** (or **12**) was followed by coupling with biotin, which was used as a tag to purify the labeled protein. Finally, global hydrolysis of the acetyl groups on the galactose was performed with sodium methoxide to furnish the desired photoaffinity probes **13** and **14** (see the SI).

Evaluation and Characterization of Photolabeling Ability of Probe (14). To assess the photolabeling efficiency of probes on RCA_{120}, RCA_{120} (0.1 μM) was incubated with probe **14** for 30 min at 4 °C and then irradiated with or without UV (365 nm) at 4 °C for 30 min. The excess labeling reagent in the resulting solution was removed by ultrafiltration (10 kDa molecular weight cutoff), and the resulting mixture was

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895–1906

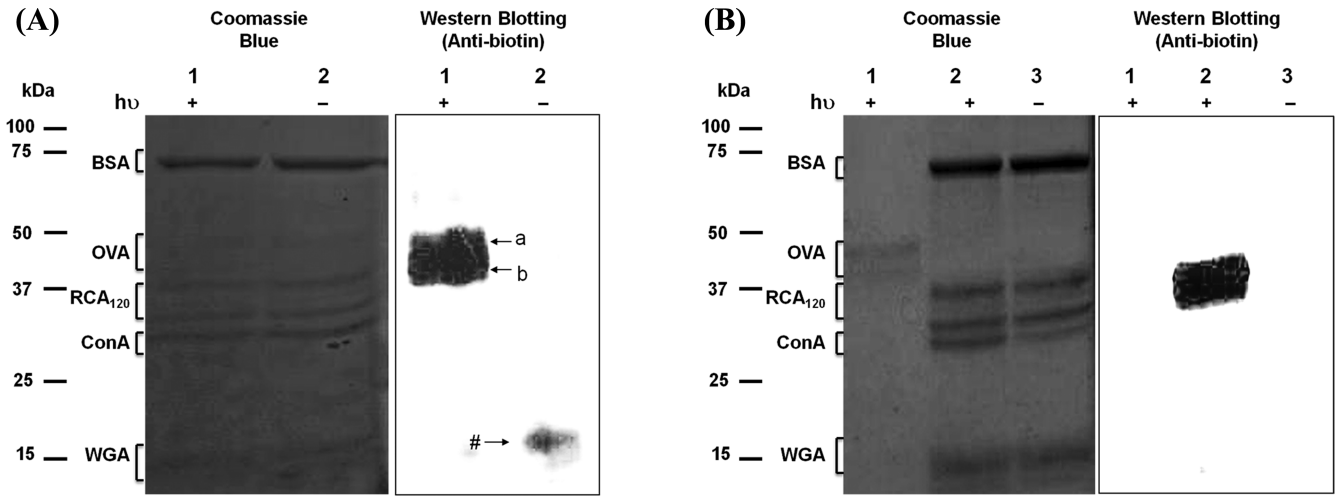


Figure 2. (A) OVA and RCA_{120} were labeled simultaneously by probe **14** (50 μM) from a protein pool containing BSA, ConA, RCA_{120}, OVA, and WGA (lane 1: presence of UV; lane 2: absence of UV). Indicated bands: "a" OVA (lane 1 of Western blot); "b" B chain of RCA_{120} (lane 1 of Western blot); "#" excess probe **14**. (B) OVA was incubated with probe **14** and then irradiated with UV (lane 1); RCA_{120} was specifically labeled by probe **14** (50 μM) from a protein pool containing BSA, ConA, RCA_{120}, and WGA (lane 2: presence of UV; lane 3: absence of UV). The concentration of each protein was 0.1 μM. BSA: bovine serum albumin; OVA: ovalbumin; ConA: concanavalin A; WGA: wheat germ agglutinin.

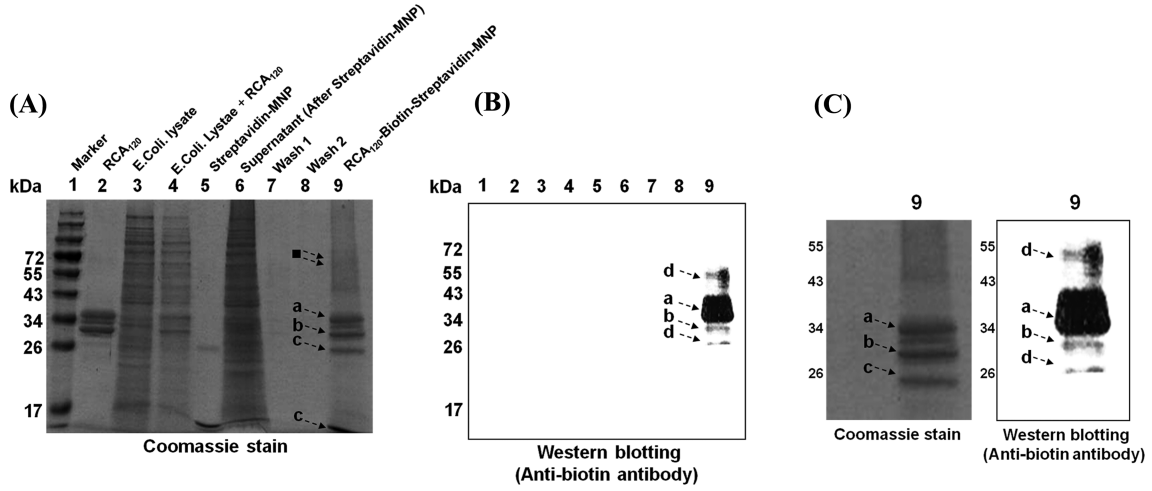


Figure 3. RCA_{120} in *E. coli* lysate was photolabeled with **14** and purified by streptavidin-MNP. Wash 1 was performed with 0.3% SDS in PBS to remove nonspecifically adsorbed proteins (lane 7 in Figure 3A); Wash 2 was performed with PBS to remove excess detergent (SDS) (lane 8 in Figure 3A); Indicated bands: ■, nonspecific adsorption of proteins on streptavidin-MNP (lane 9 in Figure 3A); **a**, B chain of RCA_{120} (lane 9 in Figure 3A, B); **b**, A chain of RCA_{120} (lane 9 in Figure 3A, B); **c**, fragment of streptavidin protein on MNPs (lane 9 in Figure 3A); **d**, unknown photolabeled proteins that associated with the galactose of **14** in the *E. coli* lysate. (C) Comparison of expansion (20−55 kDa) of gel electrophoresis images (lane 9) of A and B.

analyzed by Western blotting with an antibiotin antibody and Coomassie blue staining on the SDS-PAGE. As shown in Figure 1A, probe **14** successfully labeled RCA_{120} in a dose-dependent manner after UV irradiation. The effective concentration of probe **14** for efficient labeling of the B chain of RCA_{120} was found to be 25 μM (Figure 1A). It is not surprising that the major labeling occurred on the B chain because the B chain (37 kDa) is a lectin moiety that binds β-D-galactosides, while the A chain (29.5 kDa) is a toxin bearing rRNA *N*-glycosidase activity. However, because the diazirine group in probe **14** is flexible and the carbohydrate binding site of the B chain is linked with the A chain in RCA_{120}, the A chain was also labeled (Figure 1B). Increasing the concentration of **14** resulted in increased labeling of the A chain due to the proximity effect.[42] To avoid undesired labeling, 50 μM **14** was used in the subsequent experiments. The efficiency of RCA_{120} labeling by the mono- and trigalactose photoaffinity probes **13** and **14** was also evaluated. Therefore, various concentrations of monovalent probe **13** (200−12.5 μM) were incubated with RCA_{120} following the same photolabeling process described in Figure 1A. As evident from Figure 1C, **13**-treated RCA_{120} displays a considerably decreased band intensity compared to **14**-treated ones, directly suggesting the superior photolabeling efficiency of **14**. Conducting a competitive binding experiment using different concentrations (1−200 μM) of galactoside (**15b**) also revealed suppression of cross-linking as the concentration **15b** increased; however, a strong band is still visible even in the presence of large excess (200 μM) of **15b** (Figure S5). The results clearly demonstrated that photoaffinity probe **14** containing a multivalent ligand provided much better labeling efficiency due to the higher affinity of the multivalent interaction through statistical rebinding mode,[19] where a

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

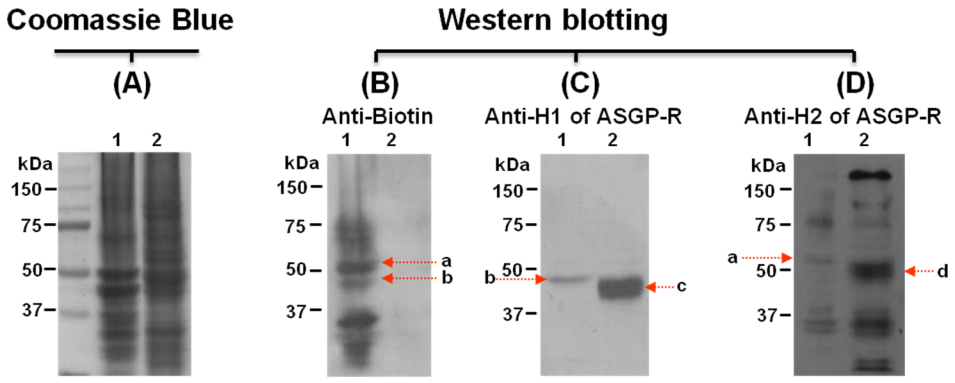


Figure 4. Photolabeling of the asialoglycoprotein receptor (ASGP-R) with trigalactose photoaffinity probe **14**. Lane 1, HepG2 cells (1×10^7, total protein was 45 mg) were incubated with probe **14** (10 μM) followed by UV irradiation, incubation with streptavidin-coated magnetic nanoparticles, cell lysis, purification with a magnet and subjected to SDS-PAGE followed by (A) Coomassie staining; Western blot with an antibody against (B) biotin, (C) ASGP-R subunit H1, and (D) ASGP-R subunit H2. Lane 2, HepG2 cells (1×10^7, total protein was 45 mg) were lysed, and a portion was subjected to SDS-PAGE as described for lane 1 as a negative control. Indicated bands: "a" H2 subunit of ASGP-R associated with probe **14**; "b" H1 subunit of ASGP-R associated with probe **14**; "c" H1 subunit of ASGP-R; "d" H2 subunit of ASGP-R.

nearby ligand quickly replaces the bound ligand due to its proximity, leading to reduce off-rate.

Evaluation of Photolabeling Selectivity of Probe (14) in a Protein Pool. To examine the labeling specificity of probe **14**, a mixture of five proteins (BSA, OVA, RCA_{120}, ConA, and WGA at 0.1 μM) was incubated with probe **14** (50 μM) followed by UV irradiation (Figures S3 and S4). Unexpectedly, OVA and the B chain of RCA_{120} were concurrently labeled instead of specifically labeling of RCA_{120} (Figure 2A, lane 1 in the Western blot). OVA is a glycosylated protein with nonreducing LacNAc on the protein surface.[43] Thus, OVA may have been labeled due to the interaction of the carbohydrate moiety of ovalbumin with RCA_{120} and cross labeling by probe **14**. However, a nonspecific hydrophobic interaction between ovalbumin and probe **14** could not be excluded. To further investigate this undesired labeling, ovalbumin (0.1 μM) was incubated with probe **14** (50 μM) and then irradiated. However, no labeled band was observed in the Western blot (Figure 2B, lane 1), indicating the absence of nonspecific interactions between probe **14** and OVA. Furthermore, incubation of probe **14** with the above protein mixture without OVA revealed specific labeling of the B chain of RCA_{120} (Figure 2B, lane 2). The labeling of OVA by probe **14** could thus be clearly attributed to the presence of RCA_{120}. RCA_{120} has four carbohydrate binding sites, two of which exhibit high affinity for β-D-galactopyranoside and are separated by 11 nm.[41] The protein microarray based assay was used to further confirm the interaction between RCA_{120} and OVA, as shown in Figure S6. Thus, probe **14** and OVA may bind the galactose binding sites of RCA_{120}, with a proximal orientation of **14** and OVA in the resulting complex. Upon UV irradiation, the generated carbene can reach and label OVA. Despite the short lifetime of the carbene derived from **14**, the photo-cross-linking yield of **14** to RCA_{120} was estimated to be ~36 ± 5% (Figure S7). Thus, probe **14** could serve as an efficient probe for lectin labeling and protein−protein interactions.

To demonstrate the potential applications of **14** in specific labeling, we employed probe **14** to detect an exogenous lectin in *E. coli* lysates. Accordingly, an *E. coli* lysate (300 μg protein) spiked with RCA_{120} (24 μg) was incubated with **14** (100 μM) and then irradiated, ultrafiltered (cutoff of 10 kDa), and separated using streptavidin-coated magnetic nanoparticles (streptavidin-MNPs). The captured proteins were washed with detergent (0.3% SDS in PBS buffer), and the resulting captured complex was divided into two aliquots, analyzed by SDS-PAGE, and visualized by Coomassie blue staining (Figure 3A) and Western blotting (Figure 3B). The results demonstrated that probe **14** selectively labeled the B chain of RCA_{120} (band **a** in Figure 3A and B) in a complicated cell lysate, although trace amounts of unknown photolabeled proteins were observed (band **d** in Figure 3B). The visualized bands **a** and **b** in Figure 3A were further subjected to in-gel digestion followed by LC-MS/MS analysis. RCA_{120} was indeed identified by multiple peptides (sequence coverage 37%) with high confidence ($p < 0.05$) (see Figure S8 for MS/MS spectra of all identified peptide sequences).

Photolabeling ASGP-R by Probe 14 on the Membrane of HepG2 Cells. Encouraged by the above results, we attempted to evaluate the potential of probe **14** for labeling cell surface carbohydrate binding proteins. The ASGP-R is located on hepatocytes and is a Ca^{2+}-dependent carbohydrate-binding protein.[44] ASGP-R is expressed on the plasma membrane of mammalian liver cells (highly expressed in liver cancer cells) and maintains serum glycoprotein homeostasis through endocytosis of asialoglycoproteins (ASGPs), desialylated glycoproteins with terminal galactose or GalNAc residues. ASGP-R consists of two homologous subunits, designated H1 (46 kDa) and H2 (50 kDa), in human hepatocytes; these subunits form a noncovalent hetero-oligomeric complex with an estimated ratio of 2−5:1.[45] Previously, we demonstrated that preassembled trigalactose multivalent ligands provided better endocytosis efficiency than monogalactose multivalent ligands by targeting ASGP-R on the HepG2 cell surface.[46] Thus, we anticipate that the trigalactose probe **14** should exhibit high affinity and specificity for ASGP-R.

HepG2 cells derived from a human hepatocellular carcinoma expressing ASGP-R on the cell surface were incubated with probe **14** (10 μM) for 1.5 h on ice to permit binding of the probe with the cell surface receptor while preventing endocytosis. The unbound probe **14** was removed from the plasma membrane by washing the cells with PBS buffer (pH 7.4) followed by UV irradiation for 30 min at 4 °C to furnish photocrossing with ASGP-R. Due to interference from endogenous biotinylated proteins in HepG2 cells during purification,[47] the streptavidin−biotin interaction was performed before cell lysis. Streptavidin-MNPs were applied to

dx.doi.org/10.1021/bc400306g | *Bioconjugate Chem.* 2013, 24, 1895−1906

capture biotinylated ASGP-R on the plasma membrane. The cells were lysed, and the labeled proteins were separated by applying a magnet and then washed with detergent (0.3% SDS in PBS buffer). The resulting captured proteins were divided into four aliquots and analyzed by SDS-PAGE with Coomassie blue visualization (Figures 4A and S9) and Western blotting with antibodies against biotin and the ASGP-R subunits. Two bands with molecular weights of approximately 46 and 50 kDa were observed in the Western blot with an antibiotin antibody (Figure 4B, lane 1, "a" and "b" markers), indicating successful photolabeling of H1 and H2 of ASGP-R by **14**. To confirm the two observed bands, Western blotting with anti-H1 and H2 of ASGP-R was performed and the results were consistent with the results of Western Blotting with the antibiotin antibody (Figure 4C and D, lane 1). The strong cross-linking at these two subunits of ASGP-R is in good agreement with the results obtained from a previous glycoprobe-based photoaffinity labeling method using terminal GalNAc (*N*-acetylgalactosamine) residues.[23] Notably, the bands at 25−37 kDa and 55−65 kDa in the Western blot with antibiotin antibody (Figure 4B, lane 1) may be the result of an undesired streptavidin−biotin interaction with endogenous biotinylated proteins. However, unidentified galactose binding proteins may also contribute to these bands. Overall, probe **14** was demonstrated to be an efficient photoaffinity probe for labeling the membrane protein ASGP-R on HepG2 cells.

CONCLUSIONS

In conclusion, a convergent synthesis of a versatile photoaffinity scaffold was developed. The desired ligand and tag of the probe were conveniently conjugated via orthogonal reactions: Cu(I)-catalyzed click reaction and amide bond formation. The advantage of the developed synthetic route was that the use of click chemistry permitted the efficient assembly of various ligands on the probe to form a multivalent ligand scaffold that provided greater affinity and thus improved labeling efficiency. The trigalactose photoaffinity probe was designed to demonstrate the importance of multivalent ligand binding in lectin labeling. The results demonstrated selective labeling of RCA_{120} in a protein mixture and *E. coli* lysates, as well as labeling of ASGP-R on the surface of HepG2 cells. Furthermore, transient and weak protein−protein interactions between the LacNAc moiety of OVA and the carbohydrate binding chain of RCA_{120} were detected by the use of probe **14**. It is anticipated that the designed photoaffinity scaffold could be used to quickly assemble diverse affinity ligands and facilitate the interrogation of unknown protein interactions.

ASSOCIATED CONTENT

Supporting Information

Spectra, photolabeling yields, and additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: cclin66@mx.nthu.edu.tw. Tel: (+) 886-3-5753147.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the National Tsing Hua University, Academia Sinica, and the National Science Council, Taiwan.

REFERENCES

(1) Bertozzi, C. R., and Kiessling, L. L. (2001) Chemical glycobiology. *Science 291*, 2357−2364.
(2) Drickamer, K., and Taylor, M. E. (1993) Biology of animal lectin. *Annu. Rev. Cell Biol. 9*, 237−264.
(3) Kiessling, L. L., Gestwichi, J. E., and Strong, L. E. (2006) Synthetic multivalent ligands as probes of signal transduction. *Angew. Chem., Int. Ed. 45*, 2348−2368.
(4) Lee, S. J., Evers, S., Roeder, D., Parlow, A. F., Risteli, J., Risteli, L., Lee, Y. C., Feizi, T., Langen, H., and Nussenzweig, M. C. (2002) Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science 295*, 1898−1901.
(5) Sperandio, M., Gleissner, C. A., and Ley, K. (2009) Glycosylation in immune cell trafficking. *Immunol. Rev. 230*, 97−113.
(6) Rudd, P. M., Elliott, T., Cresswell, P., Wilson, I. A., and Dwek, R. A. (2001) Glycosylation and the immune system. *Science 291*, 2370−2376.
(7) Dube, D. H., and Bertozzi, C. R. (2005) Glycans in cancer and inflammation-potential for therapeutics and diagnostics. *Nat. Rev. Drug Discovery 4*, 477−488.
(8) Lee, Y. C. (2010) Warfare between pathogens and hosts: the trickery of sugars. *Trends Glycosci. Glycotechnol. 22*, 95−106.
(9) Sharon, N., and Lis, H. (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev. 98*, 637−674.
(10) Singh, A., Thorton, E. R., and Westheimer, F. H. (1962) The photolysis of diazoacetylchymotrypsin. *J. Biol. Chem. 237*, 3006−3008.
(11) Han, S., Collins, B. E., Bengtson, P., and Paulson, J. C. (2005) Homomultimeric complexes of CD22 in B cells revealed by protein-glycan cross-linking. *Nat. Chem. Biol. 1*, 93−97.
(12) Ballell, L., Alink, K. J., Slijper, M., Versluis, C., Liskamp, R. M. J., and Pieters, R. J. (2005) A new chemical probe for proteomics of carbohydrate-binding proteins. *ChemBioChem 6*, 291−295.
(13) Walter, M., Wiegand, M., and Lindhorst, T. K. (2006) Synthesis of cluster mannosides carrying a photolabile diazirine group. *Eur. J. Org. Chem.*, 719−728.
(14) Wiegand, M., and Lindhorst, T. K. (2006) Synthesis of photoactive α-mannosides and mannosyl peptides and their evaluation for lectin labeling. *Eur. J. Org. Chem.*, 4841−4851.
(15) Vodovozova, E. L. (2007) Photoaffinity labeling and its application in structural biology. *Biochemistry (Mosc.) 72*, 1−20.
(16) Tanaka, Y., and Kohler, J. J. (2008) Photoactivatable crosslinking sugars for capturing glycoprotein interactions. *J. Am. Chem. Soc. 130*, 3278−3279.
(17) Nakamura, Y., Miyatake, R., and Ueda, M. (2008) Enantiodifferential approach for the detection of the target membrane protein of the jasmonate glycoside that controls the leafmovement of albizzia saman. *Angew. Chem., Int. Ed. 47*, 7289−7292.
(18) Tamura, T., Tsukiji, S., and Hamachi, I. (2012) Native FKBP12 engineering by ligand-directed tosyl chemistry: labeling properties and application to photo-cross-linking of protein complexes in vitro and in living Cells. *J. Am. Chem. Soc. 134*, 2216−2226.
(19) Gestwichi, J. E., Cairo, C. W., Strong, L. E., Oetjen, K. A., and Kiessling, L. L. (2002) Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. *J. Am. Chem. Soc. 124*, 14922−14933.
(20) Jayaraman, N. (2009) Multivalent ligand presentation as a central concept to study intricate carbohydrate-protein interactions. *Chem. Soc. Rev. 38*, 3463−3483.
(21) Lahmann, M. (2009) Architectures of multivalent glycomimetics for probing carbohydrate-lectin interactions. *Top. Curr. Chem. 288*, 17−65.
(22) Lee, R. T., and Lee, Y. C. (1987) Affinity labeling of the galactose/N-acetylgalactosamine-specific receptor of rat hepatocytes:

preferential labeling of one of the subunits. *Biochemistry 26*, 6320−6329.
(23) Lauc, G., Lee, R. T., Dumić, J., and Lee, Y. C. (2000) Photoaffinity glycoprobes-a new tool for the identification of lectins. *Glycobiology 10*, 357−364.
(24) Lee, M.-R., Jung, D.-W., Williams, D., and Shin, I. (2005) Efficient solid-phase synthesis of trifunctional probes and their application to the detection of carbohydrate-binding proteins. *Org. Lett. 7*, 5477−5480.
(25) Moss, R. A. (2006) Diazirines: carbene precursors par excellence. *Acc. Chem. Res. 39*, 267−272.
(26) Das, J. (2011) Aliphatic diazirines as photoaffinity probes for proteins: recent developments. *Chem. Rev. 111*, 4405−4417.
(27) Ingenhorst, G., Bindseil, K. U., Boddien, C., Dröse, S., Gaßel, M., Altendorf, K., and Zeeck, A. (2001) Synthesis of a doubly labelled concanamycin derivative for ATPase binding studies. *Eur. J. Org. Chem. 2001*, 4525−4532.
(28) Becer, C. R., Hoogenboom, R., and Schubert, U. S. (2009) Click chemistry beyond metal-catalyzed cycloaddition. *Angew. Chem., Int. Ed. 48*, 4900−4908.
(29) Montalbetti, C. A. G. N., and Falque, V. (2005) Amide bond formation and peptide coupling. *Tetrahedron 61*, 10827−10852.
(30) Dumez, E., Snaith, J. S., and Jackson, R. F. W. (2009) Synthesis of macrocyclic potential protease inhibitors using a generic scaffold. *J. Org. Chem. 67*, 4882−4892.
(31) Hatanaka, Y., Hashimoto, M., Nakayama, H., and Kanaoka, Y. (1994) An entry to chromogenic carbene precursors for photoaffinity labeling. *Chem. Pharm. Bull. 42*, 826−831.
(32) Lu, X., Cseh, S., Byun, H.-S., Tigyi, G., and Bittman, R. (2003) Total synthesis of two photoactivatable analogues of the growth-factor-like mediator sphingosine 1-phosphate: differential interaction with protein targets. *J. Org. Chem. 68*, 7046−7050.
(33) Cecioni, S., Oerthel, V., Iehl, J., Holler, M., Goyard, D., Praly, J.-P., Imberty, A., Nierengarten, J.-F., and Vidal, S. (2001) Synthesis of dodecavalent fullerene-based glycoclusters and evaluation of their binding properties towards a bacterial lectin. *Chem.—Eur. J. 17*, 3252−3261.
(34) Chabre, Y. M., Contino-Pépin, C., Placide, V., Shiao, T. C., and Roy, R. (2008) Expeditive synthesis of glycodendrimer scaffolds based on versatile TRIS and mannoside derivatives. *J. Org. Chem. 73*, 5602−5605.
(35) He, B., Velaparthi, S., Pieffet, G., Pennington, C., Mahesh, A., Holzle, D. L., Brunsteiner, M., Breemen, R. V., Blond, S. Y., and Petukhov, P. A. (2009) Binding ensemble profiling with photoaffinity labeling (BEProFL) approach: mapping the binding poses of HDAC8 inhibitors. *J. Med. Chem. 52*, 7003−7013.
(36) Wu, H.-T., Hsu, C.-C., Tasi, C.-F., Lin, P.-C., Lin, C.-C., and Chen, Y.-J. (2011) Nanoprobe-based immobilized metal affinity chromatography for sensitive and complementary enrichment of multiply phosphorylated peptides. *Proteomics 11*, 2639−2653.
(37) Tian, X., Pai, J., Baek, K.-H., Ko, S.-K., and Shin, I. (2011) Fluorophore-labeled, peptide-based glycoclusters: synthesis, binding properties for lectins, and detection of carbohydrate-binding proteins in cells. *Chem. Asian J. 6*, 2107−2113.
(38) Nicolson, G. L., Blaustein, J., and Etzler, M. E. (1974) Characterization of two plant lectins from ricinus communis and their quantitative interaction with a murine lymphomat. *Biochemistry 13*, 196−204.
(39) Sweeney, E. C., Tonevitsky, A. G., Temiakov, D. E., Agapov, I. I., Saward, S., and Palmer, R. A. (1997) Preliminary crystallographic characterization of ricin agglutinin. *Proteins: Struct., Funct., Genet. 28*, 586−589.
(40) Sharma, S., Bharadwaj, S., Surolia, A., and Podder, S. K. (1998) Evaluation of the stoichiometry and energetics of carbohydrate binding to Ricinus communis agglutinin: a calorimetric study. *Biochem. J. 333*, 539−542.
(41) Spain, S. G., and Cameron, N. R. (2011) The binding of polyvalent galactosides to the lectin Ricinus communis agglutinin 120 (RCA120): an ITC and SPR study. *Polym. Chem. 2*, 1552−1560.
(42) Vila-Perelló′, M., Pratt, M. R., Tulin, F., and Muir, T. W. (2007) Covalent capture of phospho-dependent protein oligomerization by site-specific incorporation of a diazirine photo-cross-linker. *J. Am. Chem. Soc. 129*, 8068−8069.
(43) Yamashita, K., Tachibana, Y., and Kobata, A. (1978) The structures of the galactose-containing sugar chains of ovalbumin. *J. Biol. Chem. 253*, 3862−3869.
(44) Ashwell, G., and Harford, J. (1982) Carbohydrate-specific receptors of the liver. *Annu. Rev. Biochem. 51*, 531−554.
(45) Weigel, P. H., and Yik, J. H. N. (2002) Glycans as endocytosis signals: the cases of the asialoglycoprotein and hyaluronan/chondroitin sulfate receptors. *Biochim. Biophys. Acta 1572*, 341−363.
(46) Lai, C.-H., Lin, C.-Y., Wu, H.-T., Chan, H.-S., Chuang, Y.-J., Chen, C.-T., and Lin, C.-C. (2010) Galactose encapsulated multifunctional nanoparticle for HepG2 cell internalization. *Adv. Funct. Mater. 20*, 3948−3958.
(47) The detail of endogenous biotinylated proteins in HepG2 cells was viewed in Figure S7.