Synthesis of Neu5Ac-\(\alpha\)-C-galactopyranosyl-functionalized magnetic nanoparticles via click chemistry

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**A B S T R A C T**

A new type of glyco-nanoparticle has been accomplished through Cu(I)-catalyzed 1,3-dipolar cycloaddition of 6-azidohexyl Neu5Ac-\(\alpha\)-C-galactoside and N-propargyl derivative, which was attached on a silica-wrapping magnetic nanoparticle. Neu5Ac-\(\alpha\)-C-galactoside was stereo-selectively synthesized in 11 steps from isopropyl thiogalactopyranoside and per-acetylated Neu5Ac phenylsulfone using samarium-mediated reductive desulfonation as the key step. Magnetic nanoparticles were prepared with one-step hydrothermal method and a subsequent coating with (\(NN\)-di-propargyl-3-aminopropyl) triethoxysilane derivative.

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1. Introduction

The interaction between cell surface carbohydrates and their protein receptors is implicated in many important biological events.\(^1\) Due to the poor availability of carbohydrate probes in glycobiology, synthetic carbohydrate derivatives are potentially useful tools to study cellular interactions, the biosynthesis of glycoproteins, the catabolism of glycoconjugates, and the mechanism of their enzymatic processing.\(^4\) One major research orientation in glycobiology is the sialic acids and the related neuraminidases. Sialic acids, especially the most common N-acetylneuraminic acid (Neu5Ac), appear in a wide range of biologically important glycoconjugates, and involve in cell recognition and interactions, neuronal transmission, reproduction, differentiation, epitope masking, and also pathological processes.\(^5\) Neuraminidases are hydrolases, obtained from diverse species and tissues that bind to sialic acid present on the outer space of cell membranes and evoke catabolism.\(^7\) This de-sialation has dramatic consequence for infection, adhesion, and recognition events. Inhibitors of the neuraminidases may represent potent antiviral,\(^8\)–\(^10\) antibacterial,\(^11\) and anticancer agents.\(^12,13\) In most cases, natural Neu5Ac are terminally \(\alpha-(2\rightarrow3)\) or \(\alpha-(2\rightarrow6)\)-O-linked to a galactose of the oligosaccharide cell epitope, or polymerized in the form of \(\alpha-(2\rightarrow8)\) or \(\alpha-(2\rightarrow9)\) linkages.\(^5\) The replacement of the interglycosidic oxygen atom with Neu5Ac-containing compounds are thus of particular interest for their potential pharmaceutical applications with respect to improved enzymatic hydrolytic stability and exoanomeric conformational similarity to the corresponding O-glycosides.\(^15\)

Recently, glyco-nanotechnology is growing fast enabling the development of nanoparticles (NPs) with specific functional properties that address the shortcomings of traditional clinic diagnoses and therapeutic agents.\(^16\) Magnetic nanoparticles (MNPs) have attracted more interests due to their potential applications in nanobiotechnology, such as magnetic resonance imaging (MRI), bio-separation, immunoassay, antitumor therapy, and so on.\(^17\)–\(^19\) The application of MNPs as drug delivery carriers has also been demonstrated to be a new solution for in vivo site-specific magnetic targeting to the liver, spleen, lymph nodes, and bone marrow,\(^20\)–\(^24\) allowing them to function either as an MRI contrast agent or as a drug carrier. However, to the best of our knowledge, seldom research has been reported regarding the synthesis and application of Neu5Ac-containing MNPs.\(^25\) Herein, we report for the first time the synthesis of silica-coating MNPs functionalized with Neu5Ac-\(\alpha\)-(2\(\rightarrow6\))-C-Gal residue through Huisgen reaction (Fig. 1).

2. Results and discussion

Except for several elegant methods for direct C–C bond formation at the anomeric center of carbohydrates, few advances have been reported in the synthesis of Neu5Ac C-glycosides.\(^26\) The major problem confounding such preparation is the C–C bond formation generating a quaternary carbon atom. Following our co-authored Neu5Ac-C-glycosylation procedure under Barbier conditions,\(^27\) we
explored a way toward the designed compound through SmI2-mediated coupling of the 6-aldehyde galactose derivative with N-acetylneuraminic acid phenylsulfone derivative.

Modification of galactose aldehyde started from per-acetylated isopropyl galactoside (1) as shown in Scheme 1. Glycosylation of 1 and 6-benzyloxy-hexan-1-ol in the presence of NIS and TMSOTf in CH2Cl2 obtained 6-benzyloxy-hexyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2), which was fully deacetylated with NaOMe in MeOH to afford 6-benzyloxy-hexyl β-D-galactopyranoside (3). Treatment of 3 with tert-butyldimethylsilyl chloride (TBSCI) in CH2Cl2 using catalytic amount of triethylamine (TEA) blocked the primary hydroxyl group (→4), and the subsequent ketalation with 2,2-dimethoxypropane in the presence of toluene sulfonic acid, provided 5 in 50% yield over four steps. Acetylation of 5 with acetic anhydride in pyridine (→6), followed by chemoselective removal of TBS using tetrabutylammonium fluoride (TBAF) gave 7 in excellent yield. Oxidation of 7 with 2-iodoxybenzoic acid (IBX) in DMSO afforded the desired galactosyl aldehyde (8) in 81% yield, which could be used for the next step without further purification.28 Barbier coupling of 8 with the known Neu5Ac sulfone 9 in the presence of freshly prepared SmI2 in dry THF furnished the corresponding C-disaccharides 10 (S) and 10 (R) (Scheme 2) in approximately 1:1 ratio and in 80% total yield.29 Spectra of 1H, 13C NMR, and 1H−1H COESY for these two stereo-isomers were obtained, respectively, and the absolute configurations were identified based on 1H NMR and NOESY analyses. In 10 (S), the chemical shift of H-4 for Neu5Ac residue appeared at 4.78 ppm, the coupling constant of J7,8 was 9.6 Hz, and the shift differentiation of H-9a and H-9b was less than 0.3 ppm. These characteristic data confirmed the α-configuration of Neu5Ac in 10 (S). A strong NOE between H-6 of galactose and H-3ax of Neu5Ac observed in 10 (R) also supported the assignment for 10 (R). Besides, the bridge hydroxyl group points to

![Scheme 1. Synthesis of galactose aldehyde 8.](image)

![Scheme 2. Synthesis of Neu5Ac-α-C-galactopyranoside 13.](image)
H-3eq in 10 (R) resulting in downfield chemical shift of H-3eq, while in 10 (S), the bridge hydroxy group resulting in downfield chemical shift of H-3ax.²⁹ Debenzylation of the diastereoisomeric mixture 10 (S) and 10 (R) afforded the corresponding disaccharides 11 (S) and 11 (R) in 95% yield. Selective transformation of the chain-end hydroxy to azide was carried out smoothly under the Mitsunobu conditions to give a mixture of 12 (S) and 12 (R) in 85% yield.³⁰ Global saponification of 12 with NaOMe in MeOH, and subsequent H⁺—Na⁺ exchanging with acidic resin, afforded Neu5Ac-α-C-galactopyranosides 13 (S) and 13 (R) in an isolated overall yield of 90%.

The synthesis of alkyn modified magnetic nanoparticles was depicted in Scheme 3. The process started from the synthesis of super-paramagnetic Fe₃O₄ nanoparticles through a high-temperature hydrolysis and reduction of FeCl₃ in diethylene glycol (DEG) and ethylene glycol (EG) under the presence of sodium acrylate.³¹ TEM images of the prepared Fe₃O₄ nanoparticles (Fig. 2a) indicated that the average size of the nanoparticles was approximately 70 nm and every single particle was composed of tiny primary nanocrystals. A modified Stöber reaction associated with the hydrolysis of tetraethyloxysilicate (TEOS) was employed to cover the Fe₃O₄ nanoparticles with an amorphous silica layer.³² TEM images of silica-coated MNPs indicated the well-defined core-shell nanostructure (Fig. 2b). The shell thickness could be controlled by variation of the TEOS concentration and the reaction time. The resulting silica-coated MNPs were then modified by dipropargyl derivative 14, which was prepared from the reaction of (3-aminopropyl) triethoxysilane and propargyl bromide in the presence of TEA in THF, to graft silica surface with alkynyl group (alkyne—MNPs). The coupling reaction did not generate significant change to the size and appearance of nanoparticles (Fig. 2c).

To confirm the presence and demonstrate the activity of propargyl groups on nanoparticles, we synthesized a fluorescent molecule 16 from rhodamine B in two steps (Scheme 4). The fluorescent molecule, having azido tag, was bound covalently to the MNPs by click chemistry in adequate reaction conditions.³³ As a result, the solution color of the alkyn-coated particles in methanol switched from deep-yellow to brownish-red (Fig. 3b). The confocal fluorescence micrograph (CFM) image (Fig. 4a) showed the nanoparticles emitting green fluorescence (green spots on the black background). The fluorescence spectrum of rhodamine—MNPs (0.1 mg/mL in MeOH) was also examined with the corresponding alkyne—MNPs (0.1 mg/mL in MeOH) as a control (Fig. 4b), showing a significant fluorescence signal at λ_em=574/530 nm in emission spectrum, which is similar to that of rhodamine B chromophore.³⁴ These results indicated that terminal alkynyl groups have been successfully incorporated onto the MNPs surface and shown good activity under click chemistry environment.

Next, Neu5Ac-α-C-glycosides 13 was covalently bound to the MNPs surface under the same click chemistry conditions as described in the preparation of rhodamine—MNPs, to give Neu5Ac—MNPs (Scheme 4). The methanol solution of the resulting nanoparticles (0.1 mg/mL) showed deep-yellow color, and also has good response to external magnetic field (Fig. 3c). FTIR analyses of alkyn—MNPs, Neu5Ac-α-C-glycosides 13, and the synthetic Neu5Ac—MNPs (Fig. 5) were in good accordance with the corresponding structures. The FTIR spectrum of 13 (Fig. 5a) presented characteristic asymmetric stretching of the azido at around 2103 cm⁻¹, and the absorptions at 1632 and 1600 cm⁻¹ ascribing to the carboxyl and amide groups in the molecule. In Fig. 5c, the bands appearing at 580 cm⁻¹ were related to the Fe—O vibration, and 1558, 1406 cm⁻¹ to the asymmetric and symmetric C—O vibration of —COOH. In addition, the peaks at 1091, 954 cm⁻¹ correspond to
the symmetric and asymmetric stretching vibrations of Si-O bonds. The FTIR spectrum of the Neu5Ac-MNPs (Fig. 5b), comparing with the one for alkyne-MNPs, possessed not only the similar characteristic signals at 580, 954, 1091, 1406, 1558 cm⁻¹, but also two new distinct signals at 1631 and 631 cm⁻¹ corresponding to the bands of C-glycoside. From thermogravimetric analysis (TGA), the weight losses exhibited 83.8% for alkyne-MNPs and 76.7% for Neu5Ac-MNPs (Fig. 6). Based on this calculation, the quantity for immobilized C-glycosides on MNPs was approximately 0.07 mg/mg (carbohydrates/solid). These observations confirmed that Neu5Ac-C-glycoside 13 has been incorporated successfully onto the MNPs surface.

3. Conclusions

We have developed a strategy for the synthesis of magnetic Neu5Ac-α-C-galactosyl nanoparticles, where the magnetic cores of the nanoparticles were wrapped with a silica-layer coating with C-glycoside residues at the outer space. The presence of alkyne groups on MNPs was confirmed by the fluorescent-labeled approach, the confocal fluorescence micrograph, and fluorescence spectra. The designed Neu5Ac-α-C-galactosyl MNPs were characterized by FTIR and TGA, and shown good magnetic property to the external field, demonstrating the successful grafting of Neu5Ac C-glycosides to magnetic nanoparticles. Our results should be applicable to the detection of carbohydrate-related diseases via magnetic resonance imaging (MRI), or as inhibitors for the related remediation.35

4. Experimental section

4.1. General methods

Anhydrous solvents were distilled prior to use: THF from Na and benzenophene; CH₂Cl₂ from CaH₂; MeOH from Mg. Commercial reagents were used without purification. Thin-layer chromatography (TLC) was performed using glass-packed silica gel plates (0.15–0.2 mm thickness). Column chromatography was carried out by using silica gel (100–200 mesh). Optical rotations were recorded on a digital polarimeter at ambient temperature. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Bruker (400 MHz) spectrometer using CDCl₃, CD₃OD or D₂O as solvent, and chemical shifts were reported as δ values in parts per million based on internal (CH₃)₄Si (0.00 ppm, ¹H) or solvent peak. FTIR transmission spectra were recorded using a Spectrum GX FTIR spectrometer at a resolution of 4 cm⁻¹ using the KBr pellet technique. Transmission electron microscopy (TEM) images were recorded on a Hitachi H-7500 field-emission transmission electron microscope. Confocal fluorescence microscopy images were captured using a Leica TCS SP5 confocal laser scanning microscope. HRMS spectra were taken in ESI (TOF) mode.

4.1.1. 6-Benzoxylhexyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2). A mixture of 1 (15 g, 37 mmol), 6-(benzoxyl)hexan-1-ol
Fig. 4. (a) Confocal fluorescence micrograph (CFM) image of rhodamine–MNPs; (b) Fluorescence spectra of rhodamine–MNPs.

Fig. 5. FTIR spectra of (a) Neu5Ac C-glycosides 13; (b) Neu5Ac–MNPs; (c) Alkyne–MNPs.

Fig. 6. TGA curve of (a) Alkyne–MNPs; (b) Neu5Ac–MNPs.

(9.22 g, 44.3 mmol), and 4 Å molecular sieves (1 g) in anhydrous CH₂Cl₂ (50 mL) was stirred for 10 min under nitrogen at 0 °C, then NIS (12.46 g, 55.4 mmol) was added, followed immediately by dropwise addition of TMSOTf (1 mL, 0.54 mmol). The reaction completed in 1 h, then neutralized with TEA, and filtered. The filtrate was evaporated under vacuo, and the syrup was purified by silica gel column chromatography to give 2 (13.9 g, 70%), as colorless oil. TLC Rf = 0.24 (Petroleum ether/EtOAc = 2:1); [α]D20 = −69 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃); δ = 7.32–7.24 (m, 5H, Ph), 5.36 (d, J = 2.8 Hz, 1H, H-4); 5.18 (dd, J = 10.4, 8.0 Hz, 1H, H-2); 4.99 (dd, J = 10.4, 3.6 Hz, 1H, H-3); 4.47 (s, 2H, CH₂Ph), 4.43 (d, J = 8.0 Hz, 1H, H-1); 4.18–4.08 (m, 2H, H-6a,b), 3.89–3.84 (m, 2H, H-5, CH₂O), 3.46–3.42 (m, 3H, –CH₂–OAc), 2.12 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.61–1.54 (m, 4H, CH₂), 1.38–1.30 (m, 4H, CH₂); ¹³C NMR (100 MHz, CDCl₃); δ = 170.7, 170.6, 170.5, 169.6, 138.9, 128.6, 127.9, 127.8, 101.6, 77.7, 77.4, 77.0, 73.2, 71.3, 70.9, 70.6, 70.4, 69.2, 67.4, 61.6, 30.0, 29.6, 26.2, 26.0, 21.0, 20.9, 20.8; HRMS (ESI) calcd m/z for C₂₇H₃₈O₁₁Na [(M+Na)⁺] 561.2312, found m/z 561.2319.

4.1.2. 6-Benzoyloxyhexyl β-D-galactopyranoside (3). To a solution of 2 (12 g, 22.3 mmol) in anhydrous methanol (25 mL) under nitrogen was added 1 N NaOMe to keep pH 9.5. The reaction mixture was stirred at rt for 3 h, and the end of which time TLC indicated a complete consumption of 2, neutralized with Amberlite IR-120 (H⁺), filtered, and evaporated in vacuo to give 3 (8.25 g, 100%) as a colorless solid. TLC Rf = 0.23 (EtOAc/MeOH = 6:1); [α]D20 = 53 (c 1, CHCl₃); ¹H NMR (400 MHz, CD₂OD); δ = 7.34–7.29 (m, 5H, Ph), 4.52 (s, 2H, CH₂Ph), 4.24 (d, J = 7.2 Hz, 1H, H-1), 3.96–3.90 (m, 1H, –CH₂O), 3.86 (dd, J = 3.0, 0.8 Hz, 1H, H-4), 3.78–3.76 (m, 2H, H-6a,b), 3.60–3.47 (m, 6H, H-2, H-3, H-5, –CH₂O), 1.68–1.62 (m, 4H, CH₂), 1.46–1.44 (m, 4H, CH₂); ¹³C NMR (100 MHz, CDCl₃); δ = 138.3, 127.9, 127.4, 127.1, 103.5, 75.1, 73.5, 72.3, 71.1, 69.9, 69.2, 68.8, 61.0, 29.3, 29.2, 25.6, 25.4; HRMS (ESI) calcd m/z for C₉₉H₉₀O₁₉Na [(M+Na)⁺] 393.1889, found m/z 393.1880.

4.1.3. 6-Benzoyloxyhexyl 6-O-tert-butyldimethylsilyl-β-D-galactopyranoside (4). To a solution of 3 (8 g, 21.6 mmol) in anhydrous CH₂Cl₂ (20 mL) were added triethylamine (9 mL, 64.8 mmol) and TBSCI (9.76 g, 64.8 mmol). The resulting mixture was stirred at rt for 3 h, then quenched with saturated aqueous NH₄Cl, and extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel column chromatography to afford 4 (9.95 g, 95%) as a colorless oil. TLC Rf = 0.12 (Petroleum ether/EtOAc = 1:1); [α]D20 = −55 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃); δ = 7.33–7.26 (m, 5H, Ph), 4.48 (s, 2H, CH₂Ph), 4.19 (d, J = 7.6 Hz, 1H, H-1), 3.95 (d, J = 3.2 Hz, 1H, H-4), 3.89–3.78 (m, 3H, H-5, 6a, –CH₂O), 3.63 (dd, J = 9.2, 7.6 Hz, 1H, H-2), 3.35–3.43 (m, 5H, H-3, H-6b, –CH₂O), 1.66–1.57 (m, 4H, CH₂), 1.37–1.36 (m, 4H, CH₂), 0.89 (s, 9H, Si-tert-buty), 0.10 (s, 6H, Si–CH₃); ¹³C NMR (100 MHz, CDCl₃); δ = 138.9, 128.7, 128.0, 127.8, 103.4, 75.0, 73.9, 73.2, 71.8, 70.7, 70.3, 69.0, 62.7, 30.0, 29.7, 26.3, 26.2, 18.6, −5.0, −5.1; HRMS (ESI) calcd m/z for C₉₅H₉₄O₁₉SiNa [(M+Na)⁺] 507.2754, found m/z 507.2764.

4.1.4. 6-Benzoyloxyhexyl 3,4-O-isopropylidene-6-O-tert-butyldimethylsilyl-β-D-galactopyranoside (5). To a solution of 4 (9 g, 18.6 mmol) in anhydrous CH₂Cl₂ (20 mL) were added 2,2-dimethoxypropane (4.57 mL, 37.2 mmol) and toluene sulfonic
acid (0.35 g, 1.86 mmol). The reaction mixture was stirred at rt for 4 h, quenched by triethylamine (1 mL), and evaporated under diminished pressure. The syrup was purified by silica gel column chromatography to give 5 (7.4 g, 76%) as colorless oil. TLC Rf = 0.70 (Petroleum ether/ EtOAc=1:1); [α]βD +13 (+ c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ = 7.33–7.26 (m, 5H, Ph), 4.49 (s, 2H, CH2Ph), 4.18 (dd, J=5.6, 2.0 Hz, 1H, H-4), 4.13 (d, J=8.0 Hz, 1H, H-1), 4.04 (dd, J=7.2, 5.6 Hz, 1H, H-3) 3.93–3.78 (m, 4H, H-5, H-6a,b, –CH2O), 3.54–3.43 (m, 4H, –CH2O), 1.65–1.58 (m, 4H, 18C, 1.51 (s, 3H, isopropylidene CH3), 1.40–1.33 (m, 7H, isopropylidene CH2), 0.89 (s, 9H, Si-tet-butyldimethylsilane), and the residue was purified, and the solvents were evaporated. The combined organic layers were dried over anhydrous Na2SO4, and filtered, and the solvents were evaporated. The reaction mixture was stirred at rt for 3 h, and then evaporated under vacuo, and the residue was purified by silica gel column chromatography to give 6 (6.4 g, 11.3 mmol) in hydrous tetrahydrofuran (10 mL) was added TBAF (8.35 g, 22.6 mmol). The reaction mixture was stirred at rt for 1.5 h, then evaporated under vacuo, and the residue was purified by silica gel column chromatography to give 7 (6.4 g, 9.6%) as colorless oil. TLC Rf = 0.36 (Petroleum ether/EtOAc=1:1); [α]βD +11 (+ c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ = 7.34–7.24 (m, 5H, Ph), 4.94 (dd, J=8.0, 6.8 Hz, 1H, H-2), 4.48 (s, 2H, CH2Ph), 4.30 (d, J=8.4 Hz, 1H, H-1), 4.18–4.13 (m, 2H, H-4, H-3), 3.98–3.92 (m, 1H, H-5), 3.88–3.79 (m, 3H, H-6a,b, –CH2O), 3.46–3.39 (m, 3H, –CH2O), 2.06 (s, 3H, OAc), 1.61–1.54 (m, 7H, isopropylidene CH2), 1.37–1.32 (m, 7H, isopropylidene CH2), and the residue was purified, and the solvents were evaporated. The reaction mixture was stirred at rt for 4 h, and then ether (20 mL) and saturated NaHCO3 (10 mL) were added. The aqueous phase was extracted with ether (20–3). The combined organic layers were washed with H2O, dried over anhydrous Na2SO4, filter, and the salts were evaporated under vacuo to give 8 (3.2 g, 81%) without further purification. TLC Rf = 0.65 (Petroleum ether/ EtOAc=1:1); [α]βD +28 (+ c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ = 5.973 (s, 1H, H-1), 7.37–7.30 (m, 5H, Ph), 5.06 (t, J=7.6 Hz, 1H, H-2), 4.53 (s, 2H, CH2Ph), 4.50–4.44 (m, 2H, H-1, H-4), 4.27 (t, J=6.4 Hz, 1H, H-3), 4.19 (s, 1H, H-5), 3.99–3.94 (m, 1H, CH2O), 3.54–3.48 (m, 3H, –CH2O), 2.13 (s, 3H, OAc), 1.65–1.59 (m, 7H, isopropylidene CH2), 1.41–1.36 (m, 7H, isopropylidene CH2), 1.37–1.32 (m, 7H, isopropylidene CH2), and the residue was purified, and the solvents were evaporated. The reaction mixture was stirred at rt for 4 h, quenched by triethylamine (1 mL), and evaporated under diminished pressure. The syrup was purified by silica gel column chromatography to give 5 (7.4 g, 76%) as colorless oil. TLC Rf = 0.70 (Petroleum ether/ EtOAc=1:1); [α]βD +13 (+ c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ = 7.33–7.26 (m, 5H, Ph), 4.49 (s, 2H, CH2Ph), 4.18 (dd, J=5.6, 2.0 Hz, 1H, H-4), 4.13 (d, J=8.0 Hz, 1H, H-1), 4.04 (dd, J=7.2, 5.6 Hz, 1H, H-3) 3.93–3.78 (m, 4H, H-5, H-6a,b, –CH2O), 3.54–3.43 (m, 4H, –CH2O), 1.65–1.58 (m, 4H, 18C, 1.51 (s, 3H, isopropylidene CH3), 1.40–1.33 (m, 7H, isopropylidene CH2), 0.89 (s, 9H, Si-tet-butyldimethylsilane), and the residue was purified, and the solvents were evaporated. The combined organic layers were washed with H2O, dried over anhydrous Na2SO4, filter, and the salts were evaporated under vacuo to give 8 (3.2 g, 81%) without further purification. TLC Rf = 0.65 (Petroleum ether/ EtOAc=1:1); [α]βD +28 (+ c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ = 5.973 (s, 1H, H-1), 7.37–7.30 (m, 5H, Ph), 5.06 (t, J=7.6 Hz, 1H, H-2), 4.53 (s, 2H, CH2Ph), 4.50–4.44 (m, 2H, H-1, H-4), 4.27 (t, J=6.4 Hz, 1H, H-3), 4.19 (s, 1H, H-5), 3.99–3.94 (m, 1H, CH2O), 3.54–3.48 (m, 3H, –CH2O), 2.13 (s, 3H, OAc), 1.65–1.59 (m, 7H, isopropylidene CH2), 1.41–1.36 (m, 7H, isopropylidene CH2), 1.37–1.32 (m, 7H, isopropylidene CH2), and the residue was purified, and the solvents were evaporated.
1H, C′G′-OH), 2.91 (dd, J = 13.2, 4.4 Hz, 1H, H-3eq), 2.16 (s, 3H, OA′c), 2.13 (s, 3H, OA′c), 2.09 (s, 3H, OA′c), 2.05 (s, 3H, OA′c), 2.03 (s, 3H, OA′c), 1.87 (s, 3H, OA′c), 1.80 (t, J = 13.2 Hz 1H, H-3ax), 1.56–1.51 (m, 7H, isopropylidene CH2), CH3); 13C NMR (100 MHz, CDC13) δ = 171.1, 170.7, 170.2, 170.1, 170.0, 169.7, 169.8, 100.1, 79.4, 77.4, 72.5, 72.3, 70.3, 69.7, 67.5, 67.0, 62.6, 62.5, 53.2, 49.6, 36.7, 32.5, 29.4, 27.7, 26.5, 25.6, 25.4, 23.1, 20.9, 20.8, 20.7, 20.6; HRMS (ESI) calc m/z for C27H30N2O11Na [(M+Na)+] = 883.3436, found m/z 883.3430.

4.1.11. 5-Acetamido-2,6-anhydro-3,5-dideoxy-2-C(S,R)-hydroxy-[6-(6-azido-hexyl 2-O-acetyl-3,4-O-isooxy)]-methyl-o-erythro-I-manno-noronoate (13). To a solution of 12 (366 mg, 0.43 mmol) in MeOH (10 mL) was added 1 N NaOMe until Ph=9.5. The reaction mixture was stirred at rt for 10 h, then Amberlite IR-120 (H+) (30 mg) was added. The reaction mixture was stirred vigorously for another 24 h followed by filtering. The filtrate was evaporated and the dried residue was dissolved in 0.1 N NaOH and stirred for overnight. The resulting mixture was neutralized with Amberlite IR-120 (H+) and filtered and solvent was evaporated to dryness. The residue was purified with Sephadex G-15 and desired fractions were lyophilized to give 13 (217 mg, 90%) as white powder. TLC Rf = 0.27 (CH3Cl/MeOH/H2O/TEA=2:1:0.2:0.04); δH NMR (400 MHz, D2O): δ = 4.21 (d, J = 8.0 Hz, 1H, 1H), 4.06 (d, J = 8.0 Hz, 1H), 3.95 (d, J = 3.2 Hz, 1H), 3.88–3.79 (m, 4H), 3.74–3.31 (m, 23H), 3.19 (t, J = 6.8 Hz, 2H), 2.60 (dd, J = 13.2, 4.4 Hz, 1H), 2.38 (dd, J = 13.2, 4.4 Hz, 1H), 1.98–1.84 (m, 7H), 1.61 (t, J = 12.0 Hz 1H, 1.51–1.47 (m, 8H), 1.28–1.27 (m, 8H); 13C NMR (100 MHz, CDC13) δ = 176.5, 175.6, 174.9, 162.7, 102.3, 83.7, 73.5, 73.6, 73.1, 73.0, 72.8, 72.7, 72.6, 72.1, 71.9, 71.8, 71.6, 71.0, 70.5, 70.4, 70.2, 69.0, 68.9, 68.5, 68.2, 67.2, 67.5, 52.1, 52.0, 51.1, 37.2, 36.2, 28.5, 28.4, 27.9, 25.7, 25.6, 24.7, 24.6, 22.0; HRMS (ESI) calc m/z for C23H30N2O11Na [(M+Na)+] = 619.2439, found m/z 619.2447.

4.1.12. Synthesis of Fe3O4 magnetic nanoparticles (MNP). FeCl3 · 6H2O (0.54 g) and sodium acrylate (1.5 g) were dissolved in ethylene glycol (5 mL) and diethylene glycol (15 mL) under magnetic stirring. The obtained homogeneous yellow solution was transferred to a Teflon-lined stainless-steel autoclave and sealed to heat 200 °C. After reaction for 10 h, the autoclave was cooled to rt. The obtained magnetic particles were washed with MeOH and water several times and dried in a vacuum for 12 h.

4.1.13. Synthesis of silica-coated magnetic nanoparticles (silica-coated MNPs). The prepared MNPs (25 mg) was mixed with water (3 mL) and EtOH (20 mL). The mixture was homogenized by ultrasonication for 30 min prior to the addition of an ammonium solution (1 mL). After that, TEOS (0.2 mL) was injected into the solution. After 2 h, the products were collected with the help of a magnet and washed with EtOH and water several times. Finally, the product was dried in vacuum for 12 h to obtain the silica-coated MNPs.

4.1.14. N-(Prop-2-yn-1-yl)-N-(3-((triethoxysilyl)prop)prop-2-yn-1-amine (14). To a solution of 3-aminopropyltriethoxysilane (2.3 mL, 10 mol) in CH2Cl2 (20 mL) were added propargyl bromide (2.2 mL, 25 mmol) and TEA (4.2 mL, 30 mmol). The reaction mixture was stirred at rt for 3 h and evaporated under vacuo to give a yellow oil. The oil was purified by flash chromatography to give 14 (2.77 g, 93%) as colorless oil. TLC Rf = 0.25 (Petroleum ether/EtOAc=10:1); δH NMR (400 MHz, CDC13) δ = 3.77 (q, J = 6.8 Hz, 6H), 3.39 (d, J = 2.4 Hz, 4H), 2.48 (t, J = 7.6 Hz, 2H), 2.18 (t, J = 2.4 Hz, 2H), 1.50–1.51 (m, 2H), 1.18 (t, J = 7.2 Hz, 9H), 0.62–0.57 (m, 2H); δ13C NMR (100 MHz, CDC13) δ = 78.7, 72.6, 58.2, 55.7, 41.8, 20.7, 18.2, 7.7; HRMS (ESI) calc m/z for C15H35N2O2SiNa [(M+Na)+] = 320.1658, found m/z = 320.1650.

4.1.15. Synthesis of magnetic nanoparticles functionalized with alkyne (alkyne–MNPs). The prepared silica-coated MNPs (25 mg) was mixed with water (3 mL) and EtOH (20 mL). The mixture was homogenized by ultrasonication for 30 min prior to the addition of an ammonium solution (1 mL). After that, 14 (100 mg) was injected into the solution. After 3 h, the products were collected with the help of a magnet and washed with EtOH and water several times. Finally,
the product was dried in vacuum for 12 h to obtain the alkyne–MNP.

4.1.16. N-(9-(2-(3-Azidopropoxy)carboxyl)phenyl)amino)-6-(dithyramino)-3H-xanthen-3-ylidene-N-ethyl ethanaminium (16). To a solution of rhodamine B (4.79 g, 10 mmol) in CH2Cl2 (30 mL) with a trace of DMF (0.5 mL) was added oxalyl chloride (0.86 mL, 20 mmol), and the resulting mixture was stirred at rt for 3 h. The reaction mixture was concentrated in vacuo and further dried at 80 °C under vacuum for 12 h to obtain Neu5Ac–MNPs.

4.1.17. Synthesis of magnetic nanoparticles functionalized with rhodamine (rhodamine–MNP). Alkyne–MNP (5 mg) dispersed in dimethylsulfoxide (5 mL) were treated with ultrasound for 15 min. CuSO4 (5 mg) and sodium ascorbate (10 mg) were added to the solution. Compound 16 (20 mg) was added and the dispersion was sonicated at 50 °C for 40 min. The products were collected with the help of a magnet and washed with DMSO and water several times until the washing solution showed no sign of fluorescence, and then were separated magnetically. The particles were dried in vacuum for 12 h to obtain the rhodamine–MNP.

4.1.18. Synthesis of magnetic nanoparticles functionalized with Neu5Ac–MGNPs. Alkyne–MNP (20 mg) dispersed in PBS (5 mL) were treated with ultrasound for 15 min. CuSO4 (5 mg) and sodium ascorbate (10 mg) were added to the solution. Compound 13 (5 mg) (30 mg) was added and the dispersion was sonicated at 50 °C for 40 min. The products were collected with the help of a magnet and washed with water and MeOH several times and then were separated magnetically. The particles were dried in vacuum for 12 h to obtain the Neu5Ac–MGNPs.

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References and notes