Tetrahedron Letters 50 (2009) 4488–4491

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of biotinylated sialoside to probe CD22–ligand interactions

Hajjaj H. M. Abdu-Allah [a,*], Kozo Watanabe [b,c], Koji Hayashizaki [b], Yuki Iwayama [a], Hiromu Takematsu [d], Yasunori Kozutsumi [d], Takeshi Tsubata [b,c], Hideharu Ishida [a], Makoto Kiso [a,e,*]

[a] Department of Applied Bio-organic Chemistry, The United Graduate School of Agricultural Sciences, Gifu University, Gifu 501-1193, Japan
[b] Laboratory of Immunology, Graduate School of Biomedical Sciences, Tokyo Medical and Dental University, Tokyo, Japan
[c] CREST, Science and Technology Agency (JST), Saitama, Japan
[d] Laboratory of Membrane Biochemistry and Biophysics, Graduate School of Biostudies, Kyoto University, Kyoto, Japan
[e] Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan

ARTICLE INFO

Article history:
Received 2 May 2009
Accepted 18 May 2009
Available online 22 May 2009

Keywords:
Biotinylated CD22 ligand
Hanessian reaction
Click chemistry

ABSTRACT

Biotin conjugate of the most potent ligand for mouse CD22 was designed and synthesized. The key synthetic step involved the dual capability of Hanessian reaction for debenzylation and simultaneous conversion of the anomeric *p*-methoxyphenyl group into the corresponding phenylthioglycoside in one step. Competition enzyme-linked immunosorbent assay (ELISA) for testing the binding affinity of synthetic sialosides was developed based on this biotinylated ligand.

CD22 (Siglec-2) is an α2,6 linked sialic acid-recognizing receptor that is specifically expressed on B lymphocytes (B cells) and B cell lymphoma cells, and is involved in regulation of B cell signalling. It has been established as a target for immunotherapy of B cell lymphomas and autoimmune diseases.[1,2] Recently, we have reported the design and synthesis of a series of sialic acid-based compounds that competitively block binding of CD22 to its natural ligands. Compound **1** (Fig. 1) is the most potent compound for mouse CD22 (mCD22) that we have obtained.[3]

Synthetic ligands tagged with biotin have frequently been utilized to detect, localize, characterize and recover the target protein.[4] Moreover, in order to pursue CD22 as a drug target, precise and reproducible binding assays need to be developed. To address this goal we synthesized a biotinylated derivative of **1** (compound **2**) which could bind to streptavidin coated materials such as plastic plates, sepharose/agarose beads and sensor chips. Introduction of an inert hydrophilic oligoethylene glycol spacer between the biotin and the carbohydrate moiety is expected to give a well defined interface, minimize non specific binding, enable the desired orientational flexibility and enhance the access of binding partners.[5] Accordingly, compound **2** would be a versatile probe for studying the specificity and sensitivity of CD22 binding. In the design of a biotinylated ligand, it is critical to identify a suitable position for biotinylation of the ligand so that the biotin tag will not compromise the binding of the ligand to its target protein. Based upon our predicted binding model for compound **1**,[3] the aglycone part does not have a specific interaction with CD22. In comparison, the 9-(4′-hydroxy-4-biphenyl)acetamido substituent buries into a hydrophobic pocket adjacent to C-9 of sialic acid moiety, so a biotin tag at this ring system would clash with the binding site and decrease ligand binding to CD22.

Therefore, the biotinylated compound **2** (Fig. 1) was designed in which the *p*-methoxyphenyl group was replaced with propargyl group. The azidolinked biotin tag **7** is then coupled to alkyne via the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction. The synthesis of compound **2** is detailed in Scheme 1. We designed two synthetic strategies to synthesize the key intermediate **5**. In the first route, **1** was postulated as a precursor to introduce the anomeric propargyl group. In the second strategy, **3** was envisioned as a precursor to **5**. Our initial strategy was to start with **1** and was supposed to involve the following sequence of reactions; preparation of methyl ester, benzoylation, deprotection of the anomeric *p*-methoxyphenyl, imidate formation and finally glycosylation with propargyl alcohol. However, methods for the esterification that we could use were severely limited. In addition to the known sensitivity of glycosidic bond for acidic medium, we could use neither diazomethane nor MeI/Na_2CO_3 due to the presence of phenolic hydroxyl group. Esterification of **1** by dowex 50W-X8 in methanol, as recently reported for closely related sialoside,[6] gave 30–40% yield. This disappointing result lets us to focus on our second strategy; that is to start with **3** since we have sufficient amount from our previous study.[3] Cleavage of benzyl ether protecting group with $PhSSiMe_3$ in the presence of ZnI_2 and Bu_4NI was first reported by Hanessian.[7] The same condition has also been used for the direct

* Corresponding authors. Tel./fax: +81 (0) 58 293 2918/2840 (M.K.).
E-mail addresses: moazhajjaj@yahoo.com (H.H.M. Abdu-Allah), Kiso@gifu-u.ac.jp (M. Kiso).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.05.044

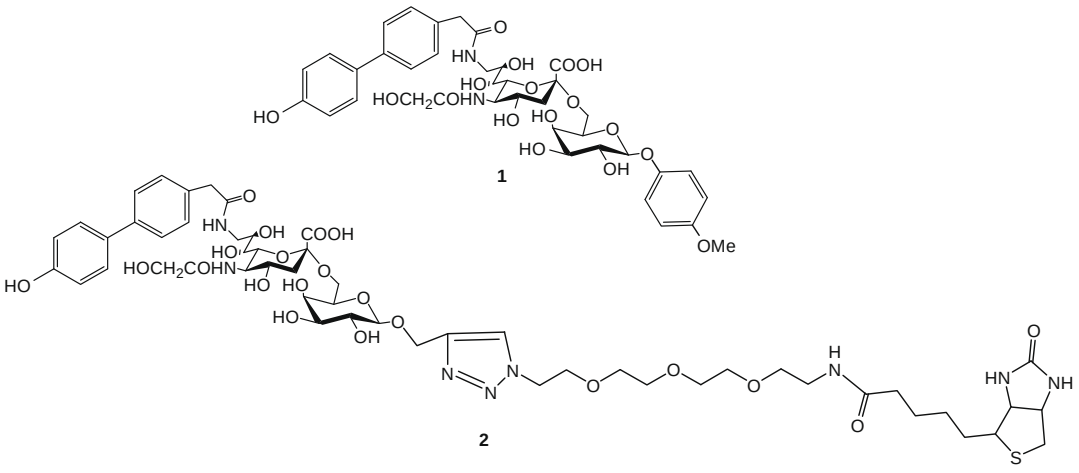
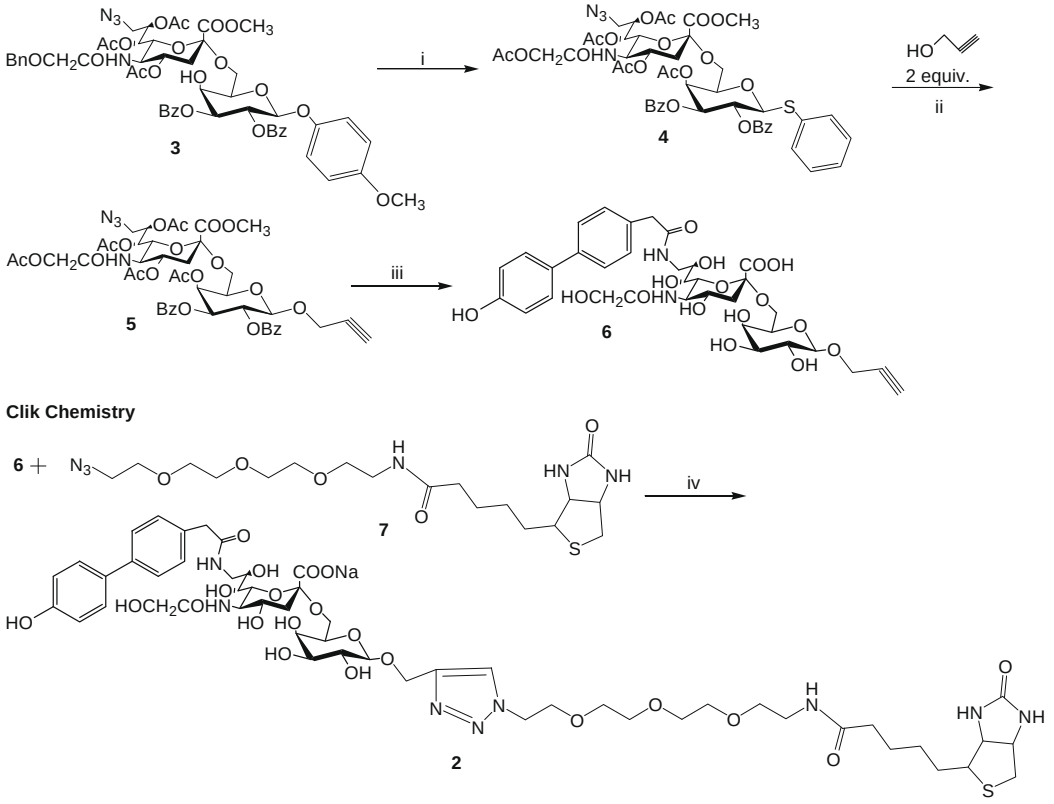


Figure 1. CD22 ligand and its biotinylated form.

conversion of alkyl glycoside to their corresponding thioglycosides.[8] On the other hand, the anomeric *p*-methoxyphenyl was converted into the corresponding phenylthioglycoside with $PhSH/BF_3$[9] or $PhSSiMe_3/BF_3$.[10]

Based on these data, Hanessian reaction method was employed for selective debenzylation of **3**. Fortunately, simultaneous conversion of *p*-methoxyphenyl to the corresponding phenylthioglycoside has occurred in one step. Thioglycoside formation probably occurred before debenzylation since the former reaction normally proceeds relatively fast.[10] Direct acetylation of the product in the presence of DMAP afforded compound **4** in 80% yield (two steps).[11]

For glycosylation of phenylthioglycoside donor **4** with the propargyl alcohol acceptor we tried the promoter system; 1-benzenesulfinyl piperidine/triflic anhydride (BSP/Tf_2O) as a potent thiophilic activator system was developed by Crich and coworkers.[12] This method has been demonstrated to afford high glycosylation yield and excellent stereoselectivity through the formation of glycosyl triflate intermediates. Fortunately, clean reaction was observed using the reported protocol; thioglycoside **4** was treated with BSP, tri-*tert*-butylpyrimidine and molecular sieves in dichloromethane at −60 °C, followed by the addition of the Tf_2O and a solution of propargyl alcohol in dichloromethane. The propargyl



Scheme 1. Reagents and conditions: (i) (a) 10 equiv $PhSSiMe_3$, 5 equiv ZnI_2, 1.5 equiv Bu4NI, DCE, 60 °C, 36 h; (b) Ac_2O, DMAP/pyridine, two steps 87%; (ii) BSP, TTBP, Tf_2O, CH_2Cl_2, MS 4 , −60 °C to 0 °C, 8 h,70%; (iii) (a) LiOH EtOH/H_2O; (b) Me_3P, MeOH/H_2O, rt, 24 h; (c) NHS ester, $NaHCO_3$, MeCN/H_2O, three steps 50%; (iv) $CuSO_4$, sodium ascorbate, H_2O/*t*-BuOH = 1/1, 60%.

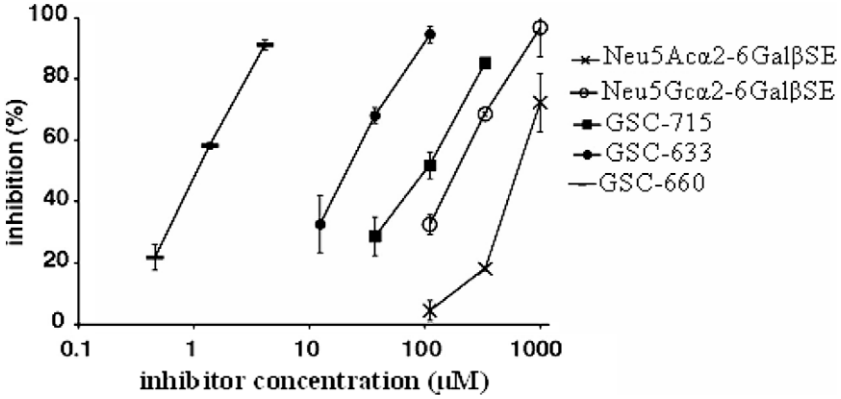
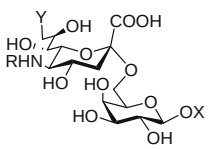


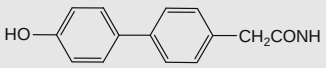
Figure 2. Results of competition ELISA. Inhibition of binding of CD22Fc to compound **2** by model compounds are shown (mean ± SD of triplicates).

glycoside **5** was obtained in good yield (70%) and excellent stereoselectivity, only trace amount of the α-glycoside was detected in ^{1}H NMR spectrum of the crude product, and none could be isolated. The stereochemistry of the new glycoside was confirmed to be β from ^{1}H NMR spectrum, δ 5.03 (d, $J_{1,2}$ = 8.24 Hz, H-1^{Gal}).[13] Complete deprotection of **5** by hydrolysis with LiOH followed by chemoselective reduction of azide using trimethylphosphine in aqueous methanol afforded the corresponding free amine, which acylated directly with the *N*-hydroxysuccinimide ester of 4′-hydroxylbiphenyl-4-acetic acid[3] to provide **6** in 50% yield.[14] The azidolinked biotin **7** was prepared as reported.[15] Cu(I)-catalyzed 1, 3-dipolar cycloaddition reaction of the alkyne derivative **6** with the azido derivative **7** was carried out at ambient temperature in the presence of $CuSO_4$ and sodium ascorbate in a mixture of 1:1 *t*-BuOH–H_2O to furnish **2** in 60% yield.[16] This method of click bioconjugation is attractive because it is simple, completely atom economical, tolerant of virtually all other chemical functionality and compatible with aqueous reaction conditions.[17]

Previously, we used the cell-based assay for measuring the binding affinity of synthetic sialosides for CD22.[3] The reproducibility of the results was not so high and extensive experimentation was required to get accurate results. To overcome this problem we developed a competition enzyme-linked immunosorbent assay (ELISA) based on compound **2**.[18] The binding affinity of various synthetic sialosides was accurately and reliably determined as shown in Figure 2 for model compounds (Table 1).

Table 1
Structures of the model compounds



Compd	Y	R	X
Neu5Acα2-6GalβSE	OH	Ac	SE
Neu5Gcα2-6GalβSE	OH	Gc	SE
GSC-715	OH	Gc	MP
GSC-633	NH_2	Gc	MP
GSC-660		Gc	MP

MP, *p*-methoxyphenyl; SE, 2-(trimethylsilyl)ethyl.

In conclusion, we have described the design and synthesis of biotinylated CD22–ligand **2**. The dual capability of Hanessian reaction method for selective debenzylation and simultaneous conversion of the anomeric *p*-methoxyphenyl group into the corresponding phenylthioglycoside is reported for the first time. Compound **2** is being used as a biological tool to study its application for the analysis of CD22 interactions and generation of B cell-binding materials.

Acknowledgements

H.H.M.A. is grateful to the Egyptian Government for Ph.D. funding scholarship. This work was partly supported by the ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (Grant-in-Aid for Scientific Research to M. Kiso, No. 17101007) and CREST of JST (Japan Science and Technology Corporation).

References and notes

1. Steinfeld, S. D.; Tant, L.; Burmester, G. R.; Teoh, N. K.; Wegener, W. A.; Goldenberg, D. M.; Pradier, O. *Arthritis Res. Ther.* **2006**, *8*, R129.
2. Fiorina, P.; Vergani, A.; Dada, S.; Jurewicz, M.; Wong, M.; Law, K.; Wu, E.; Tian, Z.; Abdi, R.; Guleria, I.; Rodig, S.; Dunussi-Joannopoulos, K.; Bluestone, J.; Sayegh, M. H. *Diabetes* **2008**, *57*, 3013–3024.
3. Abdu-Allah, H. H. M.; Tamanaka, T.; Yu, J.; Zhuoyuan, L.; Sadagopan, M.; Adachi, T.; Tsubata, T.; Kelm, S.; Ishida, H.; Kiso, M. *J. Med. Chem.* **2008**, *51*, 6665–6681.
4. For instance: (a) Honda, T.; Janosik, T.; Honda, Y.; Han, J.; Liby, K. T.; Williams, C. R.; Couch, R. D.; Anderson, A. C.; Sporn, M. B.; Gribble, G. W. *J. Med. Chem.* **2004**, *47*, 4923–4932; (b) Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Wang, S. *Tetrahedron Lett.* **2005**, *46*, 7015–7018; (c) Zhao, X. Z.; Semenova, E. A.; Liao, C.; Nicklaus, M.; Pommier, Y.; Burke, T. R., Jr. *Bioorg. Med. Chem.* **2006**, *14*, 7816–7825; (d) Linman, M. J.; Taylor, J. D.; Yu, H.; Chen, X.; Cheng, Q. *Anal. Chem.* **2008**, *80*, 4007–4013.
5. Kale, R. R.; Mukundan, H.; Price, D. N.; Harris, J. F.; Lewallen, D. M.; Swanson, B. I.; Schmidt, J. G.; Lyer, S. S. *J. Am. Chem. Soc.* **2008**, *130*, 8169–8171.
6. Okamoto, R.; Souma, S.; Kajihara, Y. *J. Org. Chem.* **2008**, *73*, 3460–3466.
7. (a) Hanessian, S.; Guindon, Y. *Tetrahedron Lett.* **1980**, *21*, 2305–2308; (b) Nicolaou, K. C.; Pavia, M. R.; Seitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 2027–2029.
8. Hanessian, S.; Guindon, Y. *Carbohydr. Res.* **1980**, *86*, C3–C6.
9. Zhang, Z.; Magnusson, G. *Carbohydr. Res.* **1996**, *295*, 41–55.
10. Lichtenthaler, F. W.; Oberthur, M.; Peters, S. *Eur. J. Org. Chem.* **2001**, 3849–3869.
11. *Selected chemical data of compound* **4**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.97 (d, J = 7.5 Hz, 2H, Ar-H), 7.85 (d, J = 7.5 Hz, 2H, Ar-H), 7.57–7.31 (m, 11H, Ar-H), 5.88 (d, J = 13.1 Hz, 1H, NH), 5.72–5.69 (m, 2H, H_{2a}, H_{4a}), 5.46 (br d, 10.3 Hz, 1H, H_{3a}), 5.35 (m, 1H, H_{8b}), 5.26 (d, J = 7.5 Hz, 1H, H_{7b}), 5.07 (d, J = 10.3 Hz, 1H, H_{1a}), 4.93 (m, 1H, H_{4b}), 4.61 (d, J = 15.4 Hz, 1H, PhCH_2), 4.29 (d, J = 15.4 Hz, 1H, PhCH_2), 4.17–4.09 (m, 2H, $H_{6a'}$, H_{5b}, H_{6b}), 3.94–3.91 (m, 1H, H_{5a}), 3.82 (s, 3H, COOCH_3), 3.74 (br d, J = 13.7 Hz, 1H, $H_{6a''}$), 3.50 (br q, 1H, H9$_{b'}$), 3.33 (br q, 1H, $H_{9b''}$), 2.60 (br d, J = 12.2 Hz, 1H, H_{3beq}), 2.24–2.02 (m, 15H, 5OAc), 1.97 (t, J = 12.4 Hz, 1H, H_{3bax}); ^{13}C NMR ($CDCl_3$): δ 171.0, 170.5, 169.9, 169.6, 167.8, 167.6, 165.3, 133.3, 133.0, 132.3, 129.8, 129.6, 129.5, 129.3, 128.9, 128.8, 128.4, 128.3, 127.9, 99.0, 86.5, 73.0, 72.9, 69.9, 68.0, 67.9, 67.8, 67.7, 62.7, 53.0, 51.0, 49.3, 38.0, 29.6, 21.0, 20.7, 20.6, 20.5. MALDI-TOF MS calcd for $C_{46}H_{50}N_4O_{19}NaS$ $(M+Na)^+$, 1017.26; found 1017.13.
12. (a) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020; (b) Crich, D.; Karatholuvhu, S. M. *J. Org. Chem.* **2008**, *73*, 5173–5176.

13. *Selected chemical data of compound* **5**: IR spectrum (KBr) showed characteristic peaks at 2150 and 2350 cm^{-1} for azide and C–C triple bond, respectively. The stereochemistry of the new glycoside was confirmed to be β from ^1H NMR (600 MHz, $CDCl_3$); δ 5.05 (d, $J_{1,2}$ = 8.6 Hz, H_{1a}). The propargyl moiety is evidenced by 4.50–4.38 (m, 2H, propargylic CH_2) and 2.40 (t, J = 2.4 Hz, alkynyl H). Two ^{13}C alkynyl signals at 78.4 and 75.2 ppm.
14. *Compound* **6**: ^1H NMR (600 MHz, CD_3OD): δ 7.49–7.38 (m, 4H, Ar-H), 7.30 (d, J = 8.0 Hz, 2H, Ar-H), 7.03 (d, J = 8.0 Hz, 2H, Ar-H), 4.38–4.29 (m, 3H, propargylic CH_2, H_{1a}), 4.03 (s, 2H, glycolyl CH_2CO), 3.95–3.85 (m, 3H, H_{4a}, $H_{6a'}$, H_{8b}), 3.85–3.63 (m, 6H, H_{2a}, H_{5a}, $H_{6a''}$, H_{4b}, H_{5b}, H_{6b},), 3.56 (s, 2H, acetamido CH_2), 3.50–3.44 (m, 3H, H_{3a}, H_{7b}, $H_{9b'}$), 3.20 (dd, J = 8.0, 12.0 Hz, 1H, $H_{9b''}$) 2.83 (m, 2H, H_{3beq}, Alkynyl H), 1.61 (t, J = 12.0 Hz, 1H, H_{3bax}).
15. Fusz, S.; Srivatsan, S. G.; Ackermann, D.; Famulok, M. *J. Org. Chem.* **2008**, *73*, 5069–5077.
16. *Selected chemical data of compound* **2**: it was purified by a silica reversed-phase column. The compound was eluted with a gradient of methanol–water (0:1–1:1 methanol/water) to afford compound **2** as a white fluffy solid after a final lyophilization from water. ^1H NMR (600 MHz, D_2O + CD_3OD): δ 8.26 (br s, 1H, a triazole H), 7.54–7.51 (m, 4H, Ar-H), 7.35 (d, J = 6.8 Hz, 2H, Ar-H), 7.03 (d, J = 6.8 Hz, 2H, Ar-H), 4.74–4.46 (m, 5H), 4.30 (br s, 1H, SCH_2CHN from biotin), 4.11 (br s, 2H, glycolyl CH_2CO), 3.95–3.36 (m, 28H), 3.21 (m, 2H, –SCH biotin), 2.9 (m, 1H, –SCH*H* biotin), 2.73 (m, 2H, H_{3beq}, –SCH*H* biotin), 2.19 (m, 2H, $COCH_2$), 1.81–1.33 (m, 7H, H_{3bax}, biotin spacer). HRMS calcd for $C_{52}H_{73}N_8O_{21}S$ $(M-H)^-$, 1177.461; found 1177.466.
17. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064; (c) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2005**, *5*, 1–68; (d) Baskin, J. M.; Bertozzi, C. R. *QSAR Comb. Sci.* **2007**, *26*, 1211–1219; (e) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249–1262; (f) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664–667.
18. Binding of synthetic sialosides to CD22 was analyzed by competition ELISA. 96-well plates (Greiner) were coated overnight with 50 μL of streptavidin (40 μg/mL) dissolved in 50 mM of $NaHCO_3$ (*p*H 8.5). Plates were then incubated with 50 μL of 4 μg/mL of compound **2** in PBS for 1 h. After blocking the wells with 0.5% BSA in PBS for 3 h, various concentrations of synthetic sialosides were incubated for 1 h together with 0.5 μg of the CD22Fc fusion protein. After washing with PBS containing 0.05% Tween 20, CD22Fc bound to the plates was detected using alkaline phosphatase-conjugated anti-human IgG Fc antibody (Southernbiotech).