Synthesis of Solid-Supported Mirror-Image Sugars: A Novel Method for Selecting Receptors for Cellular-Surface Carbohydrates

Igor A. Kozlov, Shenlan Mao, Yue Xu, Xuefei Huang, Lac Lee, Pamela S. Sears, Changshou Gao, Avery R. Coyle, Kim D. Janda,* and Chi-Huey Wong[a]*

We introduced a novel method, through mirror-image phage display, for the identification of high-affinity D-peptides to target specific cell-surface carbohydrates. Both 3-deoxy-α-L-manno-2-octulosonic acid (L-KDO) and L-sialic acid and an L-sialo-disaccharide have been synthesized and attached to a solid support for selection of high-affinity peptide binders displayed on phages. Our initial studies in this effort produce single-chain Fab sequences and dodecapeptides that bind to sialic acid and KDO with nanomolar and high micromolar affinity.

KEYWORDS:

carbohydrates · drug research · L-carbohydrates · phage display · surface plasmon resonance spectroscopy

Introduction

Carbohydrates on cell surfaces often form unique complex structures that act as key elements in various molecular recognition processes.[1–6] Compounds that are capable of interfering with recognition of cell-surface carbohydrates thus hold great potential in biomedical applications. Such molecules, including inhibitors of carbohydrate-processing and -synthesizing enzymes,[7, 8] cell-surface carbohydrate mimetics,[8–10] as well as carbohydrate-binding antibodies,[11] may be used as new drug candidates for the treatment of inflammation, cancer metastasis, bacterial or viral infection, and as biosensors for detecting carbohydrates.

We introduce a novel method in which specific carbohydrate receptors that are enantiomers of natural peptides or nucleic acids can be identified through in vitro phage selection of peptides[12] or "evolution" of nucleic acids.[13, 14] To enable this strategy it requires the synthesis of the mirror image (the L-form) of the naturally occurring D-configured carbohydrates. This L-sugar is then used to screen phages expressing a peptide library on the coat proteins to identify specific clones that bind to the L-sugar. The mirror image of the L-peptide identified (i.e. the corresponding D-peptide) is then chemically synthesized and the D-peptide should bind to the natural form of the target (i.e. naturally occurring D-sugar). Similarly, unnatural L-DNA or L-RNA can be created to target specific cell-surface carbohydrates. This approach provides a new technology platform to study sugar–protein and sugar–nucleic acid interaction. It also serves as an effective way to design high-affinity and hydrolase-resistant molecules as artificial receptors that are capable of binding to the natural carbohydrates (Figure 1). The advantage of this method is that large libraries of peptides or DNA can be easily generated and there are good methods available for selection of high-affinity binders that can be further improved through iterative processes. This mirror-image approach has been used in the identification of D-peptides[15] and L-RNA[16] to target L-peptides and D-adenosine, respectively. By using this approach

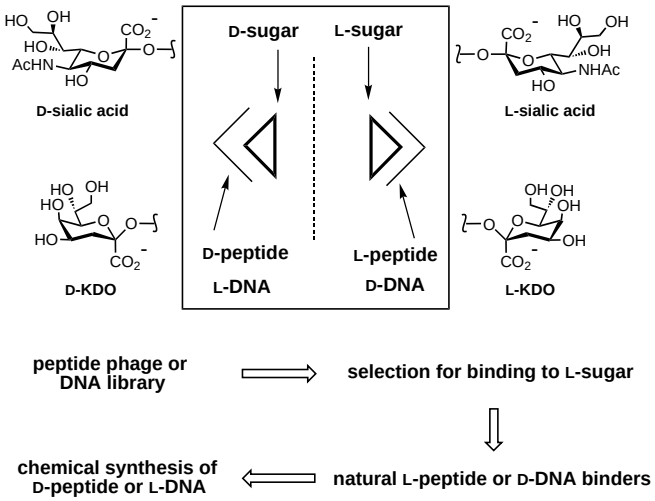


Figure 1. *Design of D-peptides and L-nucleic acids that target cell-surface sugars. The enantiomer of a cell-surface sugar (L-sugar) is used as an affinity ligand for the identification of L-peptides (from a phage display library) or D-DNA (through in vitro evolution) that bind the ligand. Through an iterative process, more tight-binding peptides or nucleic acids are selected and the corresponding enantiomers (i.e. D-peptides or L-DNA, respectively) are synthesized chemically to target the natural surface sugar. KDO = 3-deoxy-α-manno-2-octulosonic acid.*

[a] *Prof. K. D. Janda, Prof. C.-H. Wong, Dr. I. A. Kozlov, Dr. S. Mao, Dr. Y. Xu, Dr. X. Huang, Dr. L. Lee, Dr. P. S. Sears, Dr. C. Gao, A. R. Coyle*
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2409
E-mail: kdjanda@scripps.edu
E-mail: wong@scripps.edu

to target cell-surface carbohydrates, the unnatural enantiomers of carbohydrates should be readily available. Herein, we report our synthesis of the enantiomers of commonly found cell-surface carbohydrates, including *N*-acetyl-L-neuraminic acid (L-NeuNAc, sialic acid; **1**), the disaccharide L-NeuNAc-α-(2→3)-L-Gal (**2**), 3-deoxy-α-L-manno-2-octulosonic acid (L-KDO, **3**), and 3-deoxy-L-glycerogalacto-2-octulosonic acid (L-KDN, **4**) and describe a general strategy for immobilization of these compounds on various solid supports for in vitro selection studies. To demonstrate the feasibility of this approach, we also report the selection of single-chain (sc) antibodies that bind to D-NeuNAc with nanomolar affinity by using the phage display method.

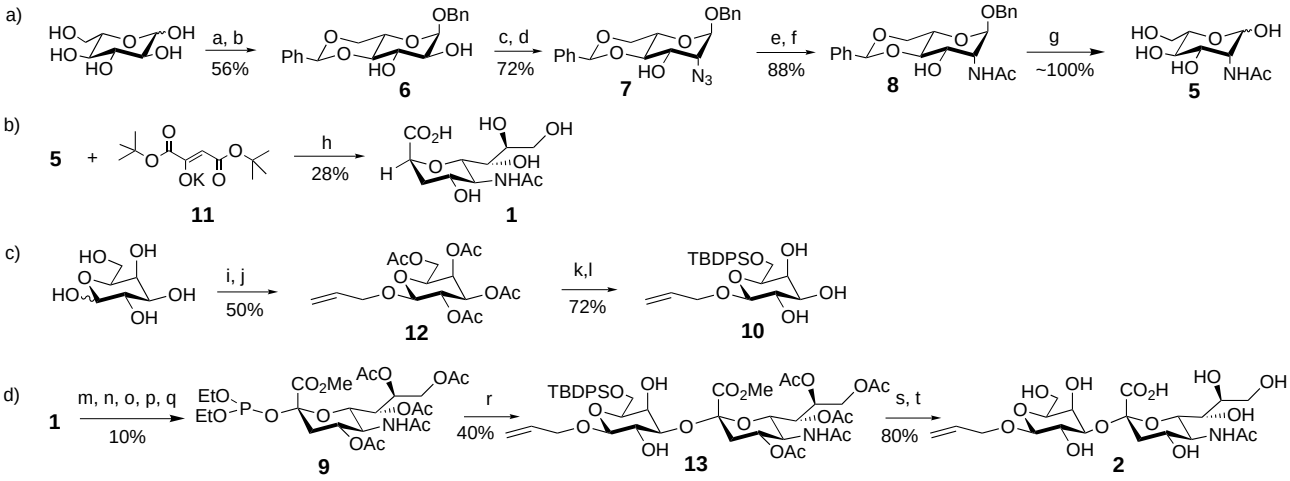
Results and Discussion

L-NeuNAc (**1**) can be synthesized by either the neuraminic acid aldolase catalyzed addition of pyruvate to *N*-acetyl-L-mannosamine (L-ManNAc, **5**)[17, 18] or by chemical condensation of L-ManNAc with di-*tert*-butyl oxaloacetate.[19] L-ManNAc (**5**) was prepared from L-glucose as shown in Scheme 1a. Anomeric benzylation of L-glucose followed by benzylidenation gave the diol **6** as white crystals.[20] Treatment of **6** with triflic anhydride[21, 22] followed by S_N2 substitution with sodium azide[23, 24] provided the azide **7** in 72% yield with complete inversion at C-2. A subsequent one-step catalytic reduction of the azido, benzyl, and benzylidene groups was attempted, but the reaction did not proceed to completion. Compound **7** was therefore reduced in a stepwise fashion. Reduction of the azido group by triphenylphosphine followed by acetylation provided the mannosamine derivative **8** in 88% yield after crystallization. Catalytic hydrogenation over palladium(II) hydroxide then gave the desired L-ManNAc (**5**) in gram quantities, with 33% overall yield for the seven steps starting from L-glucose. Enzymatic synthesis of L-NeuNAc (**1**) by the neuraminic acid aldolase catalyzed condensation of mannosamine (**5**) with pyruvic acid[18] was too slow to be useful for preparative synthesis. L-NeuNAc (**1**) was synthesized in 28% yield by chemical condensation of L-ManNAc (**5**) and the potassium salt of di-*tert*-butyl oxalacetate (**11**) (Scheme 1b).[19]

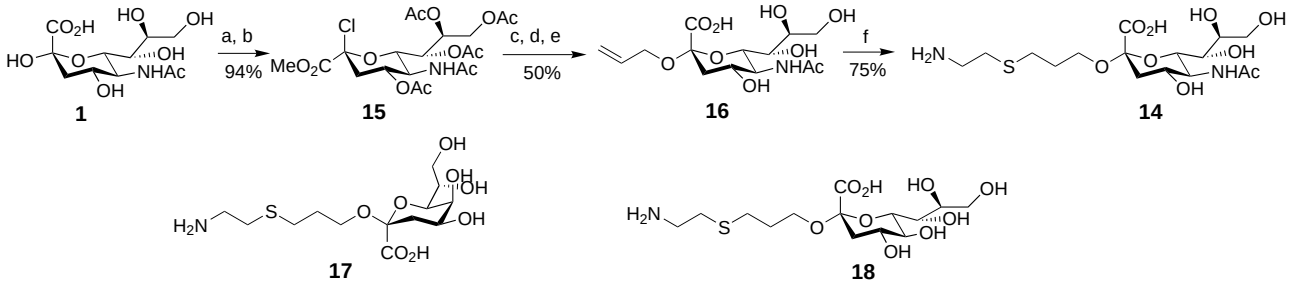
Compound **1** was further linked to L-galactose to provide **2**. D-NeuNAc-α-(2→3)-D-Gal exists as a common moiety in typical tumor-associated glycosphingolipid antigens and the binding site of influenza virus hemagglutinin.[3, 25] The enantiomer **2** was synthesized by coupling of the L-sialyl phosphite **9** with 6-TBDPS-1-*O*-allyl-L-galactose (**10**), which was prepared from L-galactose via 1-*O*-allyl-2,3,4,6-tetraacetate-L-galactose (**12**) as shown in Scheme 1c. The L-sialyl phosphite **9** was synthesized from L-sialic acid (**1**) by methylation of the carboxylic acid function, acetylation of all the free hydroxy groups, and exchange of the anomeric acetate to phosphite. Coupling of **9** and **10** was accomplished by using TMSOTf at $-78\,^{\circ}C$ to give the protected disaccharide **13** in 40% yield (Scheme 1d).[26] Deprotection with TBAF followed by NaOH gave **2** in 80% yield. The anomeric configuration of the sialic acid moiety in disaccharide **2** was determined to be α exclusively based on EXCIDE[27, 28] ($^3J_{C(1)\text{-}H(3)ax} = 5.95$ Hz).

D-KDO and D-KDN are key components of the core region of bacterial lipopolysaccharides.[29] The enantiomers L-KDO (**3**) and L-KDN (**4**) were prepared on gram scales by neuraminic acid aldolase catalyzed addition of pyruvate to L-arabinose or L-mannose, respectively, according to modified literature procedures.[17, 18] We found that an increased amount of the enzyme was necessary to achieve high yields.

To immobilize the L-saccharides to a solid support, the 3-(2-aminoethylthio)propyl moiety was chosen as the linker. It can be readily synthesized by irradiating allyl glycosides in the presence of cysteamine. Aminoethylthiopropyl-L-sialic acid (**14**) was prepared in five steps from L-NeuNAc (**1**) (Scheme 2).[30–32] Methylation and acetyl chloride treatment gave the fully protected sialyl chloride **15** in 94% yield. Allylation of **15**



Scheme 1. *Reagents and conditions: a) BnOH, TsOH; b) $PhCH(OMe)_2$, camphor sulfonic acid, 56%; c) Tf_2O, pyridine, CH_2Cl_2; d) NaN_3, DMF, 72%; e) H_2, $Pd(OH)_2$/C, MeOH, Ac_2O; f) PPh_3, THF followed by Ac_2O, MeOH, 88%; g) H_2, $Pd(OH)_2$/C, MeOH, AcOH, 100%; h) MeOH, $NiCl_2/H_2O$, 70%, 2 h, 28%; i) Ac_2O, NaOAc, reflux, 3 h, 48%; j) $BF_3 \cdot Et_2O$, allyl alcohol, CH_2Cl_2, 0 °C, 2 h, 50%; k) NaOMe, MeOH, RT, 1 h; l) TBDPSCl, DMF, imidazole, RT, 2 h, 72%; m) MeOH, amberlite IR-120, 2 h, RT; n) pyridine, Ac_2O, RT, 12 h; o) $BF_3 \cdot Et_2O$, p-thiocresol, CH_2Cl_2, RT, 2 h; p) NBS, acetone, water, RT 2 h; q) diethyl chlorophosphite, CH_2Cl_2, iPr_2EtN, 1 h, RT, 10%; r) **10**, TMSOTf, CH_2Cl_2, CH_3CN, $-78\,^{\circ}C$, 1 h, 40%; s) TBAF, THF, RT, 12 h; t) NaOH, THF, RT, 1 h, 80%. – Bn = benzyl, NBS = N-bromosuccinimide, TBAF = tetrabutylammonium fluoride, TBDPS = tert-butyldiphenylsilyl, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl, Ts = toluene-4-sulfonyl.*



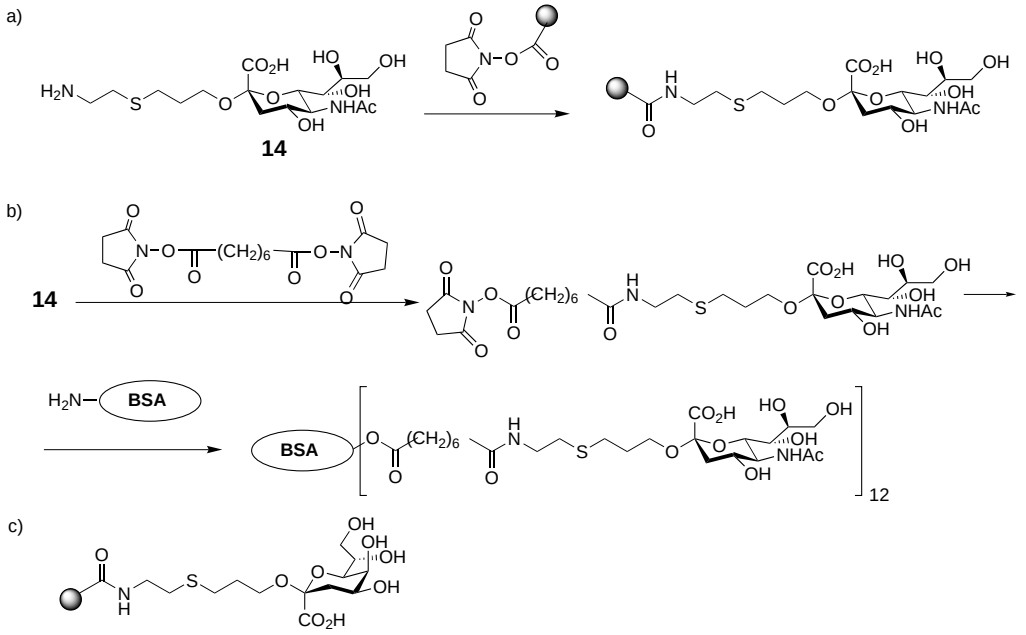
Scheme 2. *Reagents and conditions: a) MeOH, HCl, RT, 12 h; b) AcCl, AcOH, MeOH, RT, 48 h, 94%; c) allyl alcohol, silver(I) salicylate, 3 Å molecular sieves, 25 °C, 18 h; d) MeOH, NaOMe, RT, 1 h; e) NaOH, H_2O, RT, 1 h, 50%; f) $HSCH_2CH_2NH_2$, H_2O, UV light (254 nm), 18 h, 75%.*

followed by basic hydrolysis provided the allyl sialoside **16** (50% yield), which was converted into **14** in 75% yield. It was necessary to keep the pH neutral to avoid decomposition of the compounds. Aminoethylthiopropyl-L-KDO (**17**) and -L-KDN (**18**) were obtained in a similar manner.

Aminoethylthiopropyl-L-NeuNAc **14** and -L-KDO **17** were immobilized through an amide bond on a number of solid supports such as Affi-Gel 15, Affi-Prep 10, Dynabeads M-270, and BIACORE sensor chip CM5 (Scheme 3a). In addition, we immobilized compounds **14** and **17** on Nunc immunoplates with two approaches. In the first approach, we conjugated the compounds to bovine serum albumin (BSA, Scheme 3b) and then absorbed the obtained conjugate on hydrophobic Nunc plates. In the second approach, we directly immobilized compounds **14** and **17** on Nunc plates containing amino groups by using the dicarboxylate linker or cyanuric chloride.

To evaluate these novel carbohydrate conjugates the phage display technology was used.[33] We have previously shown that a human single-chain antibody phage library can be used to select for high-affinity human single-chain antibodies against tumor-associated carbohydrate antigens such as sialyl Lewis^x and Lewis^x.[34] We believed that the applicability of our immobilized carbohydrate entities for in vitro selection could be tested by utilizing this antibody phage display library.

The library was subjected to four rounds of panning by using Maxisorb Nunc plates coated with either D-KDO – BSA or D-NeuNAc – BSA (Figure 2). Phages were pooled after each successive round of panning and tested for their ability to bind either BSA conjugate. The enrichment clone number using either conjugate went from 2×10^5 to 8×10^8. Thus, after the fourth round of panning, a total of 40 phage clones (20 to D-KDO – BSA and 20 to D-NeuNAc – BSA) were randomly selected that survived the rigorous individual selection process. It was found that 14 and 16 clones exhibited substantial binding activity to D-KDO – BSA and D-NeuNAc – BSA, respectively, as determined by an enzyme-linked immunosorbent assay (ELISA). As a control, 20 random clones were collected from the unpanned library, and none of these clones showed any affinity (as determined by ELISA) to either D-KDO – BSA or D-NeuNAc – BSA.



Scheme 3. *a) The immobilization of aminoethylthiopropyl-L-sialic acid on different solid supports. b) The conjugation of aminoethylthiopropyl-L-sialic acid to BSA. c) The immobilization of aminoethylthiopropyl-L-KDO on different solid supports.*

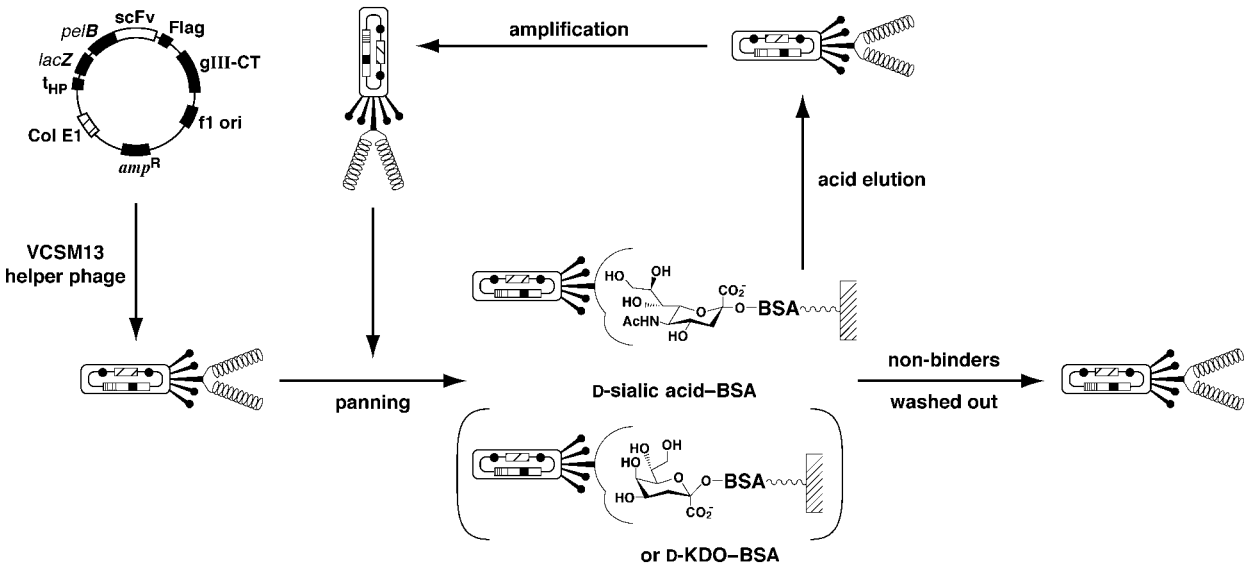


Figure 2. *Schematic representation of the phage display method to select for antibodies against sugar – BSA conjugates.*

Clones with the greatest affinity as judged by ELISA were subjected to sequencing, and the nucleotide sequences encoding the V_H and V_L regions were determined. Of the 8 D-NeuNAc-binding clones picked, two sequences were found that differed in the V_H and V_L regions, while of the 7 D-KDO-binding clones chosen a single consensus sequence for both the V_H and V_L chains was uncovered (Table 1). To analyze their binding specificity and affinity, the genes of the three novel single-chain antibodies (K18, S11, and S18, see Table 1) were subcloned into the pETFlag expression vector and purified from *E. coli*.

Table 1. *Amino acid sequences for selected single-chain human antibody variable fragments (scFv).*

Clone[a]	Heavy chain	Light chain
FR1		
K18	QVQLVESGAEVKKPGASVKVSCRASGY	QAVLTQPSSLSASPGASASLTC
S11	QVQLVHSGAEVKKPGASVKVSCKASGY	QAVLTQPSFPSLHLPGASVSLTC
S18	QVQLVESGAEVKRPGASVKVSCRASGY	QAVLTQPSSLSASPGASVSLTC
CDR1		
K18	TFTSYYMH	TLRSDINVRSYRIY
S11	TFTGYYMH	TLRSGINVGAYRIY
S18	TFTNNYIH	TLRSGINVGAYRIY
FR2		
K18	WVRQAPGQGLEWMG	WYQQKPGSPPQFLLR
S11	WVRQAPGQGLEWMG	WYQQKPGSPPQFLLR
S18	WLRQAPGQGLEWVG	WYQQKPGSPPQFLLR
CDR2		
K18	WINPHSGGTNSAQKFQG	YKSDSDKQQGS
S11	WINPNSGGTNYAQKFQG	YKSDSDKQQGS
S18	WMNPNSGNTAYAQKFQG	YKSDSDKRKGP
FR3		
K18	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	GVPSRFSGSRDASANAGILLISGLRSEDEADYYC
S11	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	GVPSRFSGSKDASANAGILLISGLRSEDEADYYC
S18	RVTMTRDTSISTAYLELSGLRPDDTAVYYCAR	GVPSRFSGSKDASANAGILLISGLRSEDEADYYC
CDR3		
K18	YGSKGGFDP	AIWHSSAWV
S11	VGRDRTGDIRAFDI	AIWHSSAWV
S18	GGNYYSLDS	AIWHSSAWV
FR4		
K18	WGQGTLVTVSS	FGEGTKLTVLG
S11	WGQGTLVTVSS	FGEGTKLTVLG
S18	WGQGTTVTVSS	FGGGTKLTVLG

[a] FR = framework region, CDR = complementarity-determining region. Clone K18 was selected from D-KDO, clones S11 and S18 were selected from D-NeuNAc.

Table 2 illustrates the specificity and affinity of the single-chain antibodies for the carbohydrate conjugates as determined by SPR spectroscopy. Since the antibodies were selected against D-sugars, they showed greater affinity for their respective D-sugar conjugates versus the L-sugar conjugates. The difference in dissociation constants is very significant between D-NeuNAc – BSA and L-NeuNAc – BSA. As expected, K18 displayed the greatest specificity for D-KDO – BSA as compared with L-KDO – BSA, since it was selected against D-KDO during the panning process. The antibodies selected against D-NeuNAc, S11 and S18, displayed the greatest specificity for D-NeuNAc – BSA as opposed to L-NeuNAc – BSA, but showed no specificity between the D- and L-KDO – BSA conjugates. As a control experiment, no interaction

Table 2. *Dissociation constants [nM] for single-chain human antibody variable fragments (scFv) K18, S11, and S18.*[a]

Clone	D-NeuNAc – BSA	L-NeuNAc – BSA	D-KDO – BSA	L-KDO – BSA
K18	18 ± 3	530 ± 40	96 ± 4	130 ± 20
S11	24 ± 2	120 ± 20	54 ± 5	45 ± 8
S18	13 ± 3	310 ± 90	97 ± 5	94 ± 16

[a] The dissociation constants were measured by the method in which the single-chain antibodies were immobilized on the BIACORE CM5 chip surface and the carbohydrate – BSA conjugate was injected into the flow cell at different concentrations. K18 was selected from D-KDO, S11 and S18 were selected from D-NeuNAc.

between the antibodies and BSA that contained only conjugated linker but no sugar was observed.

The mechanism of binding of the carbohydrate conjugates to the immobilized antibodies appears to be by polyvalent interactions (Figure 3). Unconjugated D-KDO (up to 50 mM) showed no interactions with any of the antibodies, and

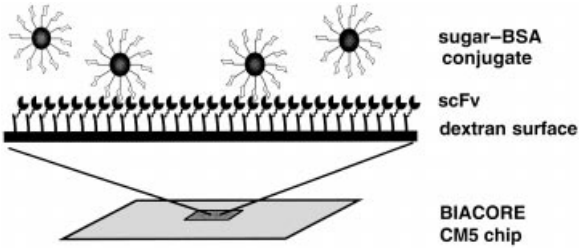


Figure 3. *Polyvalent interactions between carbohydrate conjugates and surface-immobilized single-chain antibodies. The interaction was detected by surface plasmon resonance on the CM5 chip surface through binding experiments with a BIACORE 2000 system.*

unconjugated D-NeuNAc showed binding at concentrations of 12.5 mM and above as judged by SPR spectroscopy (data not shown). When the carbohydrates were immobilized on the CM5 chip and the single-chain antibodies were passed over the chip surface, no significant interactions were observed in the micromolar range. Because each carbohydrate conjugate consists of 12 sugars linked to BSA (see Experimental Section), the observed nanomolar dissociation constants are probably a result of polyvalent interactions between the two interacting species.

This research report has presented our work toward the selection of biologically stable molecules capable of binding sugars and saccharides. A method for selecting the unnatural enantiomers of biopolymers that are capable of binding to the natural sugars and saccharides is outlined. Synthetic techniques for preparing the unnatural enantiomers of the sugars have been worked out. Preliminary studies using phage display libraries to select single-chain antibodies against natural sugars have demonstrated the feasibility of this approach. Identification of short peptides capable of binding sugars and saccharides would enhance our understanding of sugar – protein interactions and provide a new approach to developing antibiotics and drug delivery systems.[36]

Experimental Section

Immobilization of L-saccharides onto polymer supports: The aminoethylthiopropyl-L-sialic acid (**14**) and L-KDO (**3**) were immobilized on a number of solid supports. Affi-Gel 15 (an agarose-based resin) and Affi-Prep 10 (a methacrylate resin) were purchased from Bio-Rad in their *N*-hydroxysuccinimide-activated forms. The resin (1 mL) was shaken with a solution of aminoethylthiopropyl-sugar (20 mM) and triethylamine (5 μL) in anhydrous DMSO (500 μL) at 25 °C for 4 h. The excess carboxylate groups on the resin was blocked by incubating the resin with 500 μL of 1 M aqueous ethanolamine solution at pH 8.5 for 1 h. This afforded an immobilization efficiency of about 90%. Dynabeads M-270 carboxylic acid magnetic beads (Dynal) were preactivated by treatment with 0.1 M *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS) solution in DMSO for 2 h at 25 °C. The resin was shaken with a solution of aminoethylthiopropyl-sugar (20 mM) and triethylamine (30 mM) in anhydrous DMSO at 25 °C for 4 h. BIACORE sensor chip CM5 was supplied by the manufacturer in the free-carboxylate form. It was activated by flushing with a solution of NHS and EDC for 7 min at 10 μL min^{-1}. Flushing with aminoethylthiopropyl-sugar in a HEPES buffer (pH 8.0) containing 1 M sodium chloride (5 μL min^{-1} for 14 min) then provided the labeled chip. Ethanolamine solution (1 M, pH 8.5, 10 μL min^{-1} for 7 min) was then applied to quench the excess activated acid on the chip.

A 50 mM aminoethylthiopropyl-sugar and 60 mM 3-(trimethylsilyl) 1-propanesulfonic acid (DSS) solution in anhydrous DMSO (0.1 mL) was incubated at 25 °C for 1 h. BSA (5 mg) solution in 0.1 M carbonate/bicarbonate buffer (0.9 mL, pH 9) was added and incubated at 25 °C for 3 h. The BSA – sugar conjugate was purified by using YM-10 Micron centrifuge filter devices (Millipore). We obtained about 3 mg of BSA conjugate with each sugar. A MALDI-TOF MS analysis of the conjugates showed that on average 12 molecules of sugar are attached to each modified BSA molecule.

Determination of dissociation constants of single-chain antibodies on a BIACORE 2000 instrument: According to standard procedures, 4 μM of the single-chain antibodies were immobilized on the CM5 chip through the ammino groups on the protein. Injections of the carbohydrate conjugates were preprogrammed into the BIACORE Control Software (version 1.3) and the concentrations ranged from 1.35 nM to 1350 nM to encompass the K_D value. At equilibrium conditions, 4 min after each injection (10 μL min^{-1}), the response unit was recorded. The values were fitted to the equation $[AB]_{eq} = AB_{max}(1/(1 + K_D/[A]))$ to determine K_D.[35]

Physical and NMR spectroscopic data

2: $[\alpha]_D^{25} = +1.2$ ($c = 0.1$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta =$ 5.92 – 6.01 (m, 1H), 5.32 (dd, $J = 1.8$, 17.2 Hz, 1H), 5.32 (dd, $J = 1.3$, 10.2 Hz, 1H), 4.34 – 4.40 (m, 1H), 4.30 (d, $J = 8.0$ Hz, 1H), 4.11 – 4.17 (m, 1H), 4.01 (dd, $J = 3.8$, 9.5 Hz, 1H), 5.32 (dd, $J = 0.7$, 3.1 Hz, 1H), 3.79 – 3.87 (m, 2H), 3.53 – 3.76 (m, 8H), 3.49 (dd, $J = 1.7$, 9.0 Hz, 1H), 3.43 – 3.48 (m, 1H), 2.85 (dd, $J = 3.9$, 12.4 Hz, 1H), 1.68 – 1.75 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 175.4$, 174.9, 135.8, 117.4, 103.8, 101.0, 76.6, 74.9, 72.9, 70.9, 70.7, 70.0, 69.4, 68.9, 64.4, 62.8, 53.9, 42.2, 22.6; $^3J_{C(1)\text{-}H(3)ax} = 5.95$ Hz; HR-MS: *m/z*: calcd for $C_{20}H_{33}NO_{14}Na$: 534.1793, found: 534.1773.

14: ^{1}H NMR (500 MHz, D_2O): $\delta = 3.83 - 3.96$ (m, 3H), 3.59 – 3.75 (m, 6H), 3.27 (t, $J = 6.4$ Hz, 2H), 2.91 (t, $J = 6.6$ Hz, 2H), 2.78 (dd, $J = 4.4$, 12.9 Hz, 1H), 2.71 (dt, $J = 1.9$, 7.3 Hz, 2H), 2.08 (s, 3H), 1.87 – 1.93 (m, 2H), 1.7 (t, $J = 12.1$ Hz, 1H); ^{13}C NMR (125 MHz, D_2O): $\delta = 175.5$, 174.0, 101.0, 73.0, 72.3, 68.8, 68.7, 63.0, 61.9, 52.5, 38.5, 28.5, 27.8, 24.1, 22.4; HR-MS: *m/z*: calcd for $C_{15}H_{31}N_2O_9S$: 427.17, found: 427.1745.

17: ^{1}H NMR (500 MHz, D_2O): $\delta = 3.92 - 4.02$ (m, 3H), 3.77 – 3.87 (m, 3H), 3.65 – 3.56 (m, 2H), 3.27 (t, $J = 6.6$ Hz, 2H), 2.91 (t, $J = 6.6$ Hz, 2H), 2.70 (t, $J = 6.9$ Hz, 2H), 2.46 (dd, $J = 4.8$, 12.1 Hz, 1H), 1.86 – 1.92 (m, 1H), 1.83 (t, $J = 12.4$ Hz, 1H); ^{13}C NMR (125 MHz, D_2O): $\delta = 174.0$, 102.0, 73.8, 69.2, 67.7, 65.6, 64.5, 63.3, 39.0, 35.1, 29.3, 28.5, 27.6; HR-MS: *m/z*: calcd for $C_{13}H_{26}NO_8S$: 356.14, found: 356.1379.

The ^{1}H NMR and ^{13}C NMR spectra of compounds **14** and **17** matched those of their enantiomers.

This research was supported by the NIH.

[1] P. Sears, C.-H. Wong, *Cell. Mol. Life Sci.* **1998**, *54*, 223 – 52.
[2] H. Lis, N. Sharon, *Eur. J. Biochem.* **1993**, *218*, 1 – 27.
[3] S. Hakomori, Y. Zhang, *Chem. Biol.* **1997**, *4*, 97 – 104.
[4] Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321 – 327.
[5] T. Kolter, K. Sandhoff, *Angew. Chem.* **1999**, *111*, 1632 – 1670; *Angew. Chem. Int. Ed.* **1999**, *38*, 1532 – 1568.
[6] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683.
[7] J. D. McCarter, S. G. Withers, *Curr. Opin. Struct. Biol.* **1994**, *4*, 885 – 892.
[8] P. Sears, C.-H. Wong, *Angew. Chem.* **1999**, *111*, 2446 – 2471; *Angew. Chem. Int. Ed.* **1999**, *38*, 2300 – 2324.
[9] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2935; *Angew. Chem. Int. Ed.* **1998**, 2754 – 2794.
[10] E. J. Gordon, W. J. Sanders, L. L. Kiessling, *Nature* **1998**, *392*, 30 – 31.
[11] S. J. Danishefsky, J. R. Allen, *Angew. Chem.* **2000**, *112*, 882 – 912; *Angew. Chem. Int. Ed.* **2000**, *39*, 836 – 863.
[12] K. Johnsson, L. Ge in *Combinatorial Chemistry in Biology, Vol. 243* (Eds.: M. Famulok, E.-L. Winnacker, C.-H. Wong), Springer, Berlin, **1999**, pp. 87 – 106.
[13] M. Famulok, *Curr. Opin. Struct. Biol.* **1999**, *9*, 324 – 329.
[14] T. Hermann, D. J. Patel, *Science* **2000**, *287*, 820 – 825.
[15] T. N. M. Schumacher, L. M. Mayr, D. L. Minor, M. A. Milhollen, M. W. Burgess, P. S. Kim, *Science* **1996**, *271*, 1854 – 1857.
[16] S. Klussmann, A. Nolte, R. Bald, V. A. Erdmann, J. P. Furste, *Nat. Biotechnol.* **1996**, *14*, 1112 – 1115.
[17] C. Gautheronlenarvor, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1991**, *113*, 7816 – 7818.
[18] C. H. Lin, T. Sugai, R. L. Halcomb, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 10138 – 10145.
[19] R. Kuhn, G. Baschang, *Justus Liebigs Ann. Chem.* **1962**, *659*, 156 – 161.
[20] K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, Y. Tsuda, *Chem. Pharm. Bull.* **1979**, *27*, 2661 – 2674.
[21] S. Knapp, P. J. Kukkola, S. Sharma, M. T. G. Dhar, A. B. J. Naughton, *J. Org. Chem.* **1990**, *55*, 5700 – 5710.
[22] S. Knapp, A. B. J. Naughton, P. J. Kukkola, W. C. Shieh, *J. Carbohydr. Chem.* **1991**, *10*, 981 – 993.
[23] S. Knapp, A. B. J. Naughton, C. Jaramillo, B. Pipik, *J. Org. Chem.* **1992**, *57*, 7328 – 7334.
[24] M. A. Probert, M. J. Milton, R. Harris, S. Schenkman, J. M. Brown, S. W. Homans, R. A. Field, *Tetrahedron Lett.* **1997**, *38*, 5861 – 5864.
[25] Y. Ito, J. J. Gaudino, J. C. Paulson, *Pure Appl. Chem.* **1993**, *65*, 753 – 762.
[26] T. J. Martin, R. R. Schmidt, *Tetrahedron Lett.* **1992**, *33*, 6123 – 6126.
[27] V. V. Krishnamurthy, *J. Magn. Reson. Ser. A* **1996**, *121*, 33 – 41.
[28] T. Ercegovic, G. Magnusson, *J. Chem. Soc. Chem. Commun.* **1994**, 831 – 832.
[29] J. Hansson, S. Oscarson, *Curr. Org. Chem.* **2000**, *4*, 535 – 564.
[30] R. Roy, C. A. Laferrière, *Carbohydr. Res.* **1988**, *177*, c1 – c4.
[31] R. Roy, C. A. Laferrière, *Can. J. Chem.* **1990**, *68*, 2045 – 2054.
[32] C. A. Laferrière, F. O. Andersson, R. Roy, *Methods Enzymol.* **1994**, *242*, 271 – 280.
[33] J. K. Scott, G. P. Smith, *Science* **1990**, *249*, 386 – 390.
[34] S. Mao, C. Gao, C.-H. L. Lo, P. Wirsching, C.-H. Wong, K. D. Janda, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6953 – 6958.
[35] D. G. Myszka, *Methods Enzymol.* **2000**, *323*, 325 – 340.
[36] Note added in proof: Our initial effort toward this goal has identified a dodecapeptide (SQATGPKRSNPA) on phage that binds to D- and L-NeuNAc with $K_d = 0.46$ and 1.04 mM, respectively, in a monovalent fashion. Work is in progress to further improve the selectivity and affinity by directed evolution.

Received: March 26, 2001
Revised version: May 4, 2001 [F 222]