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Supporting Information

Galectin–Glycan Interactions: Guidelines for Monitoring by ^{77}Se NMR Spectroscopy, and Solvent (H_2O/D_2O) Impact on Binding

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Contents

NMR pulse programs

The following NMR pulse programs for 2D $^{1}H,^{77}Se$ long-range correlation were written and tested under TopSpin 3.2.7 on a BRUKER AVANCE III 600 MHz spectrometer. Auxiliary files (e.g., for shaped pulses and CPD sequences) can be obtained from tdiercks@cicbiogune.es upon request.

2D $^{1}H,^{77}Se$ HSQMBC-CPMG

```
;tdHSQMBCcpmg.gs                    ;17/05/2019

;#### adapted for TopSpin 3 and AVANCE III ###

;2D H,X HSQMBC (long-range correlation) using CPMG-INEPT
;- CPMG-INEPT to enforce inphase (TOCSY) evolution of competing nJHH coupling
;  using XY16 phase cycling (i.e. MOCCA mixing on H)
;  NOTE: ### XY16-CPMG bandwidth BW_XY16 depends on 2 criteria: ###
;       ### a) BW_XY16 <= Brf/4 = 1/(16*p10) ###
;       ### b) BW_XY16 <= Bcpmg = cnst15     ###
;- with coherence selection by echo/antiecho gradients
;- further on-resonant water suppression by presaturation (plw9) and flip-back
;- with pre-scan X depolarisation to reduce t1 noise
; -Dd4opt: optimise evolution delay d4 in steps of 5ms
;  Analysis: optimal d4 = 5ms * row_max

;------------- Processing info --------------
;Dimension:  FnMode    WDW   SSB  REVERSE  FCOR  PHC0  PHC1
;F1(X)       E/A      QSINE  2    FALSE    0.5   0     0

;------------ Parameter settings -----------------
;## DIGMOD = baseopt ###
;ns = ## 8*n (4*n: minimal F1 artefacts) ##
;zgoptns = -Dd4opt
;cnst15 = computed Bcpmg [Hz] = BANDWIDTH
;l1 = X decoupling in F2: 0(OFF),1(ON)
;--- 1H (f1) ---
;sfo1 = ### H2O on-resonant ###
;p1 = 90deg on 1H (@ pl1)
;pl1 = for p1=90deg on 1H
;p2 <= 200us*600/BF1 (power check on sp2!)
;spnam2 = ### BIP_720_(50 or 75).. ###
;pl9 < 20uW (optional presaturation, 0 = OFF)
;--- X (f2) ---
;p3  = 90deg on X (@pl2)
;pl2 = for p3=90deg on X
;cnst62 = bandwidth[ppm] of X dec. in F2 (UNFOLDED WINDOW)
;cpdprg2 = ### GARP4.p62 ###
;---- Delays ---
;d1 = relaxation delay (~ 1.25 T1(H))
;d4 < 0.5/nJHX
;l4 = sets desired echo delay d40 & bandwidth = cnst15
;d16 > 100 us (eddy current recovery)
;d1 = relaxation delay (~ 1.25 T1(H))
;---------- GRADIENTS ----------
;p16 >= 400us (gradient pulse)
;gpnam1 = SINE.50 or SMSQ10.50
;gpz1 > 70 (strong z-spoil)
```

```
;gpnam4 = SINE.100 or SMSQ10.50
;gpz4 > 50 (strong z-spoil)

;---------- calculated parameters ----------
#include <Grad.incl>
#include <Avance.incl>
;--- 1H pulses ---
#define H f1
"plw10=plw1*pow(p1/p10,2)"
"spoff2=0"                              ;for 1H BIP on ALL 1H
"spoal2=0.5"
"spw2 = plw1*pow(8*p1/p2,2)"            ;for p2 = BIP_720_...
;--- X pulses ---
#define X f2
"p62=1000000/(bf2*cnst62)"              ;for GARP4 decoupling
if(l1)
{;--- with X decoupling in F2 ---
"plw12 = plw2*pow(p3/p62,2)"            ;for p62 = 90deg square pulse
;--- 15N pulses ---
}
else
{
"plw12=0"
}
"plw20=plw2*pow(p3/p10,2)"
;--- delays ---
define delay d0c
define delay d1c
define delay d12c
define delay d40c
define delay comp
define delay prompt
"cnst16=sfo2/sfo1"
"d11=10m"
"d12=5u"
"in0=inf1/2"
"d0=4u"
"d0c=d0*2+p2"
#ifdef d4opt
"l4=1"
"d4=5m"
#endif
"l14=(l4*16)-2"
"d40=d4/(4+2*l14)-p10"
"d40c=d40-p10*2/PI"
"comp=p10*4/PI+d12+2*(p16+d16+p3)+d0c"
"cnst15=250000/(p10+d40)"               ;Bcpmg field strength (Hz)
"d1c=d1-(aq+d11+3m+d12*2)"
"prompt=1u+cnst15*1u+l1*1u"
"acqt0 = -d12"

1 ze
  prompt
2 d11 do:X
3 d12 pl9:H pl2:X
  d1c cw:H
  d12 do:H
```

```
  (p3 ph0):X                                ;depolarise X prior to scan to reduce t1 noise
  d12 UNBLKGRAD
  2mp:gp4                                   ;z-spoil
  0.7m pl10:H pl20:X
  0.3m rpp20
;---- CPMG-INEPT using XY16 ------
  (p10 ph1):H
  d40c
  (p10*2 ph20):H (p10*2 ph20^):X
  d40
14 d40
   (p10*2 ph20):H (p10*2 ph20^):X
   d40
   lo to 14 times l14
  d40
  (p10*2 ph20):H (p10*2 ph20^):X
  d40c
  (p10 ph12):H
;---- z-spoil and H2Ox dephasing ----
  d12
  (p2:sp2 ph0):H                            ;compensating BIP
  1.3mp:gp4
  0.5m pl1:H pl2:X
  0.2m rpp20
;---- t1(X) ----
  (p3 ph5):X
  d0
  (p2:sp2 ph1):H                            ;invert H2O to -x
  d0
  p16:gp1*EA
  d16
  (p3*2 ph4):X
  p16:gp1*EA*-1
  d16
  d0c
  (p3 ph15):X
;---- z-spoil and H2O-x rephasing ----
  1.3mp:gp4*-1
  0.7m pl10:H pl20:X
  comp
;---- CPMG-INEPT using XY16 ------
  (p10 ph1):H                               ;flips H2O-x to +z
  d40c
  (p10*2 ph20):H (p10*2 ph20^):X
  d40
24 d40
   (p10*2 ph20):H (p10*2 ph20^):X
   d40
   lo to 24 times l14
  d40
  (p10*2 ph20):H (p10*2 ph20^):X
  d40
;--- decoding gradient echo ---
  p16:gp1*cnst16*-0.93
  d16 pl1:H
  (p1*2 ph0):H
  p16:gp1*cnst16*1.07
```

```
 d16 pl12:X
 d12 BLKGRAD
 go=2 ph31 cpd2:X
 10u do:X
 20u cpds2:X                          ;reset decoupler pointer after complete complex point
 10u do:X
  d11 mc #0 to 2

#ifdef d4opt
 F1QF(calclc(l14,+16))
#endif

#ifndef d4opt
  F1EA(calgrad(EA), caldel(d0,+in0) & calph(ph5,+180) & calph(ph31,+180))
#endif
exit

ph0= 0
ph1= 1
ph2= 2
ph3= 3
ph4= 1
ph5= 0 2                              ;(X) excitation pulse with axial peak suppression in t1
ph12={{2}*4}^2
ph15= 0 0 2 2                         ;(X) read-out pulse after t1 (CRITICAL)
ph20= 0 1 0 1 1 0 1 0 2 3 2 3 3 2 3 2  ;XY16 cycle
ph31={0 2 2 0}^2
```

2D ^{1}H,^{77}Se HeHaHa (heteronuclear Hartmann-Hahn transfer)

Depending on the DIPSI2 field strength, this experiment achieves broadband or narrow-band (selective) HeHaHa transfer. For this, parameter “cnst15” must be set as described below.

```
;tdHXhehaha.gs                        ;16/05/2019
;#### adapted for TopSpin 3 and AVANCE III ###

;2D H,X hetero-TOCSY (Hetero Hartmann-Hahn transfer)
;- with DIPSI2 heHaHa to simultaneously enforce pure inphase evolution of
;  competing nJHH coupling (for pure phase long-range H,X transfer)
;- with coherence selection by echo/antiecho gradients
;- with universal flip-back
;- with optional on-resonant presaturation (during d1, power plw9)
;- with pre-scan X depolarisation to reduce t1 noise
;- zgoption: -Dl15opt to optimise l15 in steps of 'dDIPSI2'
;######### Important Set-up NOTES ##########
;- Set-up for DIPSI2 mixing:
;1. Define bandwidth for HeHaHa:
;   cnst15[Hz] = 2*(most distant signal H or X from offset H or X)
;2. Define mixing time d15
;   Set l15 such that resulting d15 is ca. 0.5/J(CX)
;   Possibly increase cnst15 (-> decreases p10) to fine-adjust d15!
;-  Set-up for l15 (mixing time) optimisation:
;   set zgoptn = -Dl15opt to measure l15 dependent transfer efficiency
;   set FnMode = QF, TD1 = SI1 = 8-16, possibly increase cnst15
;   process by 'xf2' and select row_max with maximal transfer
;   -> Corresponding mixing time: d15 = dDIPSI2*row_max
```

```
;------------- Processing info --------------
;Dimension:  FnMode    WDW   SSB  REVERSE  FCOR  PHC0  PHC1
;F1(X)       E/A      QSINE   2    FALSE    0.5   0     0

;------------ Parameter settings -----------------
;## DIGMOD = baseopt ###
;ns = ## 8*n (4*n: minimal F1 artefacts) ##
;zgoptns = -Dl15opt
;l1 = X decoupling in F2:0(OFF),1(ON)
;--- 1H (f1) ---
;sfo1 = ## center of H for HeHaHa mixing ##
;cnst1 = H2O offset for presaturation (o1 from zgpr)
;p1 = 90deg on 1H (@ pl1)
;pl1 = for p1=90deg on 1H
;p2 <= 200us*600/BF1 (power check on sp2!)
;spnam2 = ### BIP_720_(50 or 75).. ###
;p10 ~ 1/(4*BW) [BW = desired bandwidth in Hz]
;--- X (f2) ---
;sfo2 = ## center of X for HeHaHa mixing ##
;p3  = 90deg on X (@pl2)
;pl2 = for p3=90deg on X
;cnst62 = bandwidth[ppm] of X dec. in F2 (UNFOLDED WINDOW)
;cpdprg2 = ### GARP4.p62 ###
;p62 = [600/bf1]*75u(Call),*120u(Caliph),*85u(Carom)
;---- Delays ---
;d1 = relaxation delay (~ 1.25 T1(H))
;cnst15 = minimal BW[Hz] for mixing
;l15 = DIPSI2 loops for desired d15 = mixing time
;d15 = computed mixing time (=< 0.5/nJHX)
;d16 > 100 us (eddy current recovery)
;---------- GRADIENTS ----------
;p16 >= 400us (gradient pulse length)
;gpnam1 = SINE.50
;gpz1 = 73 (coherence selection gradient)
;gpnam4 = SINE.100
;gpz4 = 47.31 (z-spoil gradient)

;--------------- calculated parameters --------------
#include <Grad.incl>
#include <Avance.incl>
;--- 1H pulses ---
#define H f1
;"cnst10=cnst1-o1"
"p10=1s/(4*cnst15)"
"plw10=plw1*pow(p1/p10,2)"
"spoff2=0"                                  ;for 1H BIP on ALL 1H
"spoal2=0.5"
"spw2 = plw1*pow(8*p1/p2,2)"                ;for p2 = BIP_720_...
;--- X pulses ---
#define X f2
"p62=1000000/(bf2*cnst62)"                  ;for GARP4 decoupling
if(l1)
{;--- with X decoupling ---
"plw12 = plw2*pow(p3/p62,2)"                ;for p62 = 90deg square pulse
;--- 15N pulses ---
}
else
```

```
{;--- without X decoupling ---
"plw12 = 0"
}
"plw20=plw2*pow(p3/p10,2)"
;--- delays ---
define delay d0c
define delay d1c
define delay dDIPSI2
define delay prompt
"cnst16=sfo2/sfo1"
"dDIPSI2=28.78*4*p10"
"d11=10m"
"d12=5u"
"in0=inf1/2"
"d0=4u"
"d0c=d0*2+p2"
"d1c=d1-(aq+d11)"
#ifdef l15opt
"l15=1"
#endif
"d15=l15*dDIPSI2"
#define DIPSI2a p10*3.556 ph20 p10*4.556 ph22 p10*3.222 ph20 p10*3.167 ph22 p10*0.333 ph20 p10*2.722
ph22 p10*4.167 ph20 p10*2.944 ph22 p10*4.111 ph20
#define DIPSI2b p10*3.556 ph22 p10*4.556 ph20 p10*3.222 ph22 p10*3.167 ph20 p10*0.333 ph22 p10*2.722
ph20 p10*4.167 ph22 p10*2.944 ph20 p10*4.111 ph22

"prompt = d12+l1*1u"
"acqt0 = 0"

1 ze
  prompt
2 d11 do:X
3 d12 pl9:H pl2:X
  1m fq=cnst1 (bf):H                ;jump to H2O for optional presaturation
  d1c cw:H
  d12 do:H
  1m fq=0:H                         ;jump to H center of HeHaHa mixing
  (p3 ph0):X                        ;depolarise X prior to scan to reduce t1 noise
  d12 UNBLKGRAD
  2mp:gp4                           ;z-spoil
  1m pl10:H pl20:X
;---- HeHaHa ------
  (p10 ph1):H                       ;create Hx for mixing
15 (DIPSI2a):H (DIPSI2a):X          ;DIPSI2 along x
   (DIPSI2b):H (DIPSI2b):X          ;DIPSI2 along -x
   (DIPSI2b):H (DIPSI2b):X          ;DIPSI2 along -x
   (DIPSI2a):H (DIPSI2a):X          ;DIPSI2 along x
  lo to 15 times l15
  (p10 ph1):H (p10 ph3):X           ;flip back Hx->-Hz and Xx->Xz
;---- z-spoil, unused Hu in -z ----
  1.7mp:gp4                         ;suppress radiation damping on H2O-z
  1.3m pl1:H pl2:X
;---- t1(X) ----
  (p3 ph5):X
  d0
  (p2:sp2 ph1):H                    ;flip Hu to +z
  d0
```

```
 p16:gp1*EA
 d16
 (p3*2 ph4):X
 p16:gp1*EA*-1
 d16
 d0c
 (p3 ph15):X
;---- z-spoil ----
 (p2:sp2 ph1):H                ;flip Hu forward to -z
 1.3mp:gp4
 1m pl10:H pl20:X
;---- HeHaHa to Hx ------
 (p10 ph25):X                  ;create Xx for mixing
16 (DIPSI2a):H (DIPSI2a):X     ;DIPSI2 along x
  (DIPSI2b):H (DIPSI2b):X      ;DIPSI2 along -x
  (DIPSI2b):H (DIPSI2b):X      ;DIPSI2 along -x
  (DIPSI2a):H (DIPSI2a):X      ;DIPSI2 along x
 lo to 16 times l15
 p16:gp1*cnst16*-0.93
 d16 pl1:H pl12:X
 d12
 (p1*2 ph0):H                  ;universal flip-back
 p16:gp1*cnst16*1.07
 d16
 d12 BLKGRAD
 go=2 ph31 cpd2:X
 10u do:X
 20u cpds2:X                   ;reset decoupler pointer after complete complex points
 10u do:X
  d11 mc #0 to 2
#ifdef l15opt
  F1QF(calclc(l15,+1))
#endif
#ifndef l15opt
  F1EA(calgrad(EA), caldel(d0,+in0) & calph(ph5,+180) & calph(ph31,+180))
#endif
exit

ph0= 0
ph1= 1
ph2= 2
ph3= 3
ph4= {{1}*4}^1          ;(X) rephasing pulse in t1
ph5= 0 2                ;(X) excitation pulse with axial peak suppression in t1
ph15= {{0}*8}^2          ;(X) read-out pulse after t1 (CRITICAL)
ph25= {{1}*2}^2          ;(X) reTOCSY excitation pulse
ph20= 0                 ;dipsi phase 1
ph22= 2                 ;dipsi phase 2
ph31={{0 2 2 0}^2}^2
```

1D ^{1}H spectra of SeDG with/out ^{77}Se decoupling

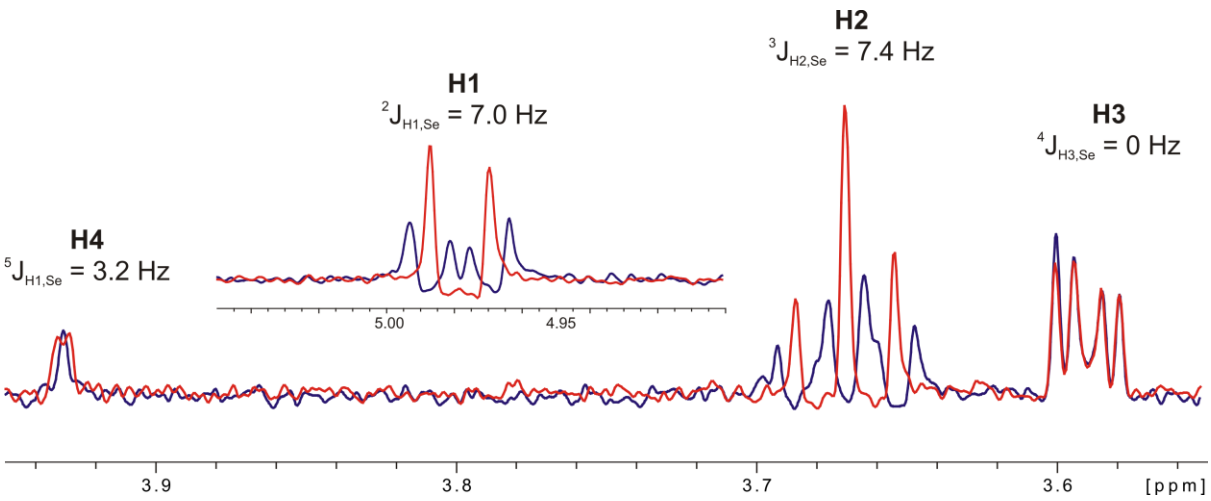


Figure S1: 1D ^{1}H spectrum of SeDG with (red) and without (blue) ^{77}Se decoupling, evincing the indicated direct n-bond $^{n}J_{H,Se}$ coupling constants.

^{1}H→^{77}Se correlation: transfer intensity versus transfer delay (SeDG)

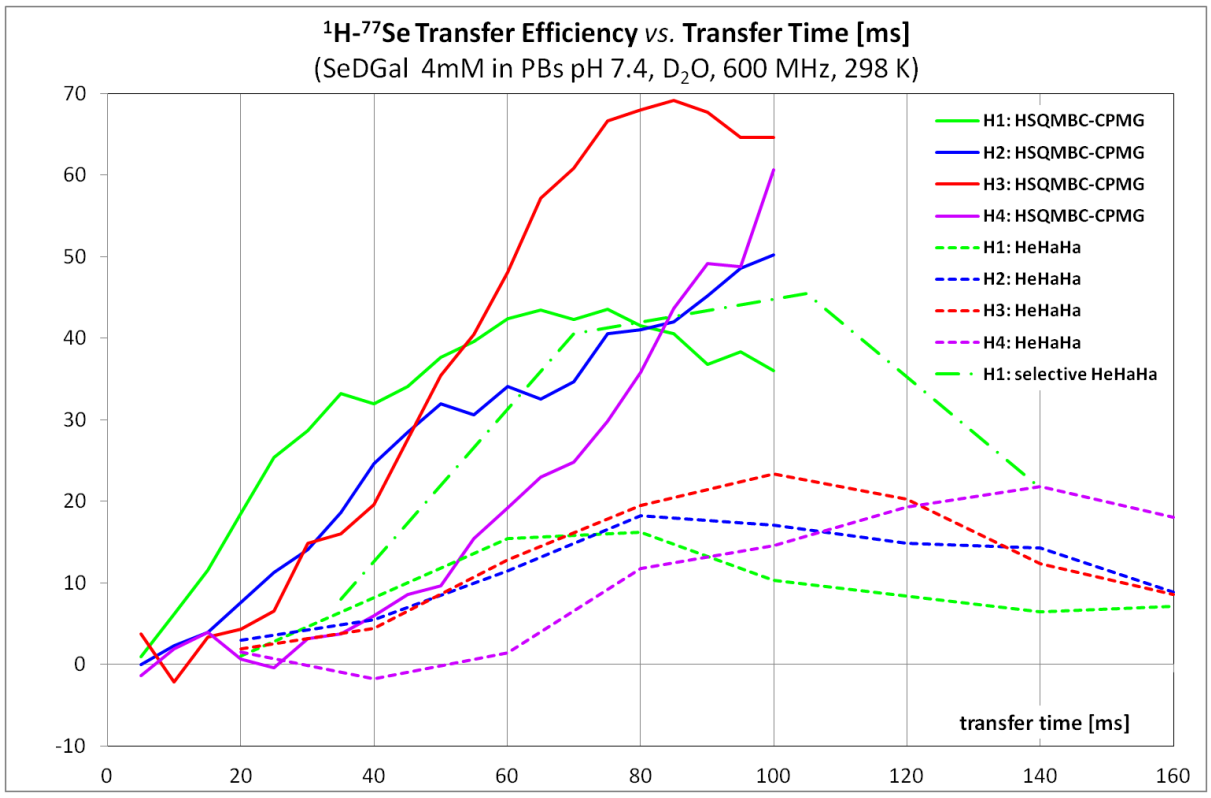


Figure S2. Transfer time dependence of SeDGal H1 to H4 signal intensities for different heteronuclear ^{1}H,^{77}Se correlation schemes: (*i*) CPMG-INEPT (straight lines) with B_{CPMG} = 1371 Hz, δ_{CPMG} = 157.3 µs, (*ii*) broadband heteronuclear Hartmann-Hahn (HeHaHa) transfer by high-power DIPSI2 mixing with B_{DIPSI2} = 1450 Hz (dotted lines), and (*iii*) H1 selective heteronuclear Hartmann-Hahn (sel. HeHaHa) transfer by low-power DIPSI2 mixing with B_{DIPSI2} = 285 Hz centered on H1 (dashed-dotted lines).

2D $^{1}H,^{77}Se$ NMR correlation spectra

All experiments were acquired at 298 K on a BRUKER AVIII 600 MHz spectrometer equipped with z-TBI probehead using the pulse programs printed above and identical total interscan recovery time (d1 = 1.5 s), scan number (ns = 8), FID resolution (1.2 Hz for ^{1}H, 2 Hz for ^{77}Se), resulting in 0.5 h total measurement time. All spectra are processed and plotted identically.

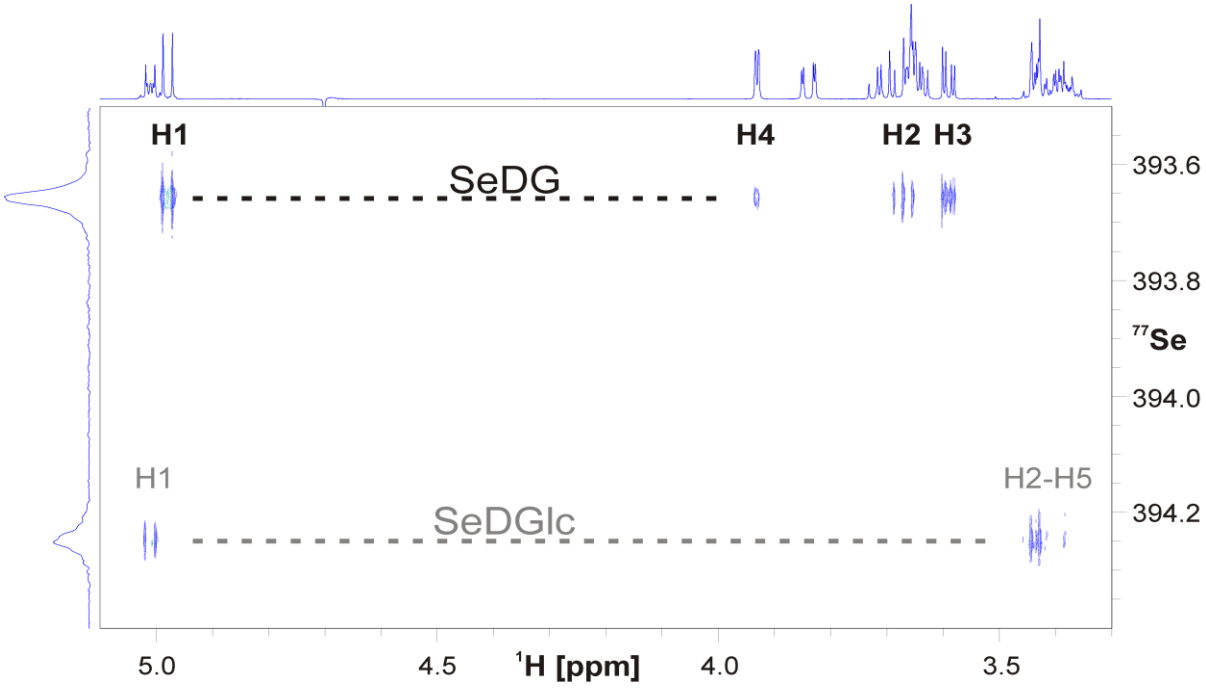


Figure S3a. 2D $^{1}H,^{77}Se$ HSQMBC-CPMG with B_{CPMG} (cnst15) = 1143 Hz at B_{RF} = 10.000 Hz (p10 = 25 µs), Δ (d4) = 70ms.

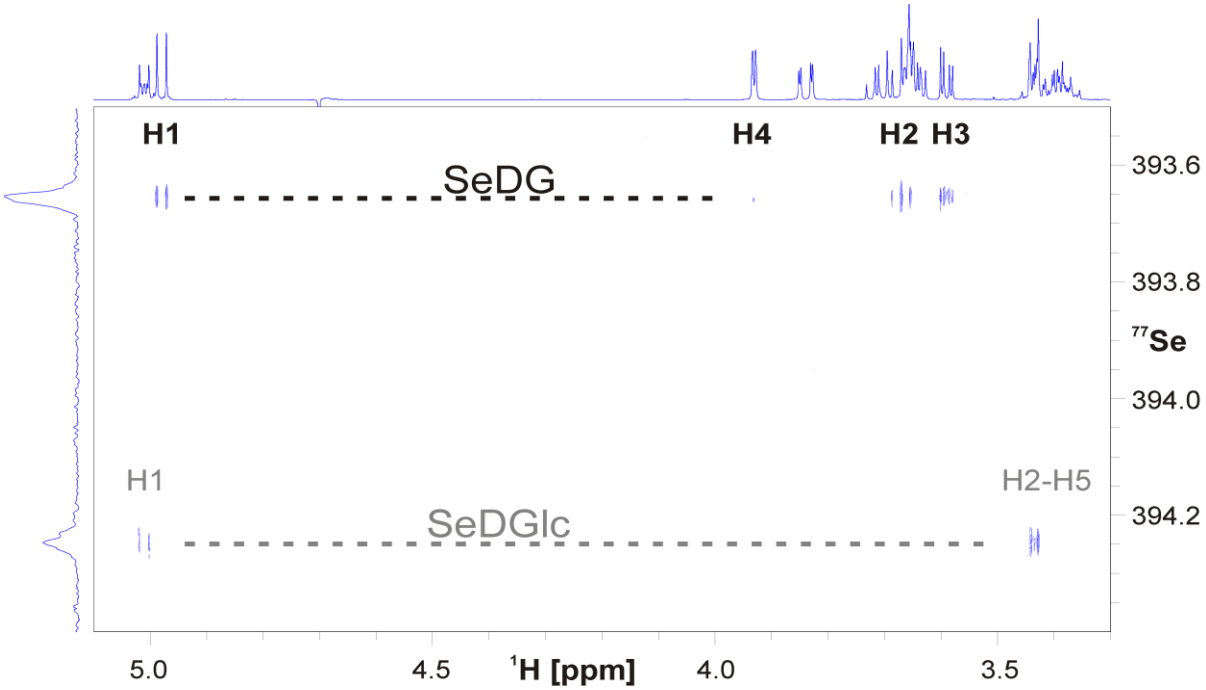


Figure S3b. 2D $^{1}H,^{77}Se$ HeHaHa with B_{DIPSI2} (cnst15) = 1450 Hz (p10 = 172.4 µs), Δ (d8) = 80ms.

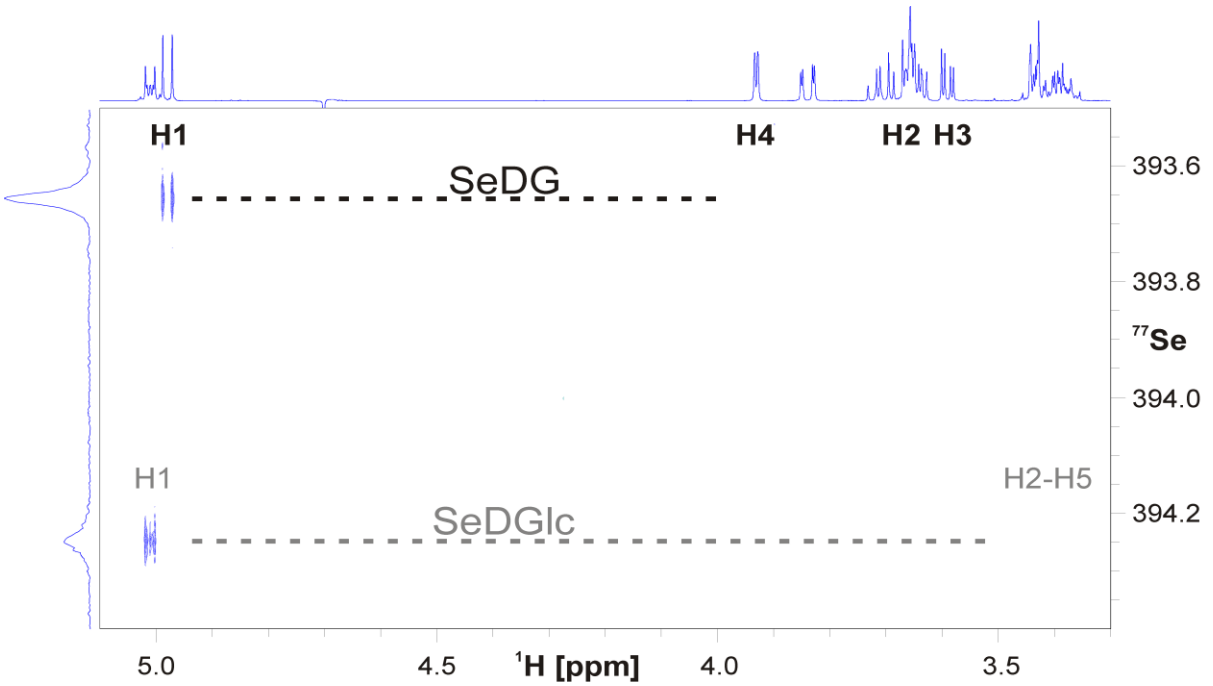


Figure S3c. 2D $^{1}H,^{77}Se$ H1-selective HeHaHa with B_{DIPSI2} (cnst15) = 285 Hz (p10 = 877.2 µs) centered at 4.98 ppm (center of H1 for SeDG and SeDGal), Δ (d8) = 100ms.

1D $^{1}H[^{77}Se]$ traces for SeDGlc

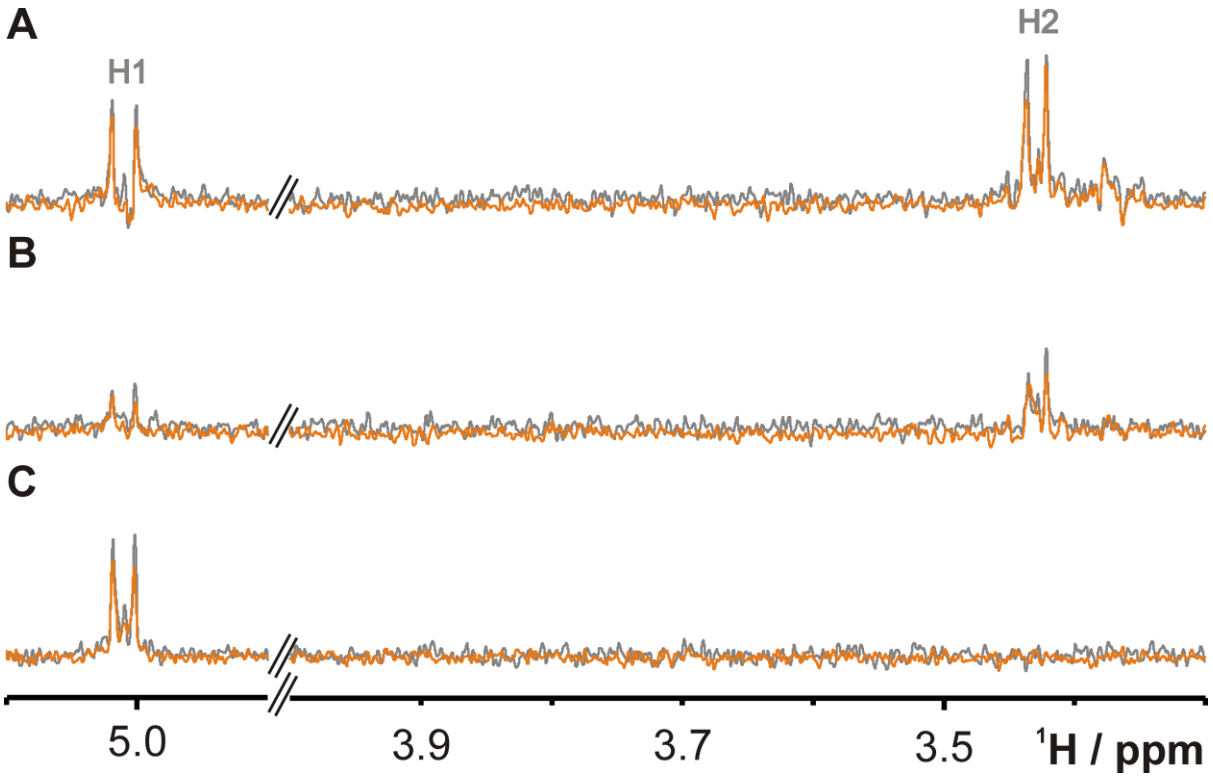


Figure S4. 1D ^{1}H traces from 2D $^{1}H,^{77}Se$ correlation spectra taken at the ^{77}Se chemical shift of SeDGal (394.25 ppm) in the absence (grey) or presence (orange) of hGal-3 (0.125 mM). (**A**) CPMG-HSQMBC (Δ_{opt}(H1) = 70 ms, B_{CPMG} = 1143 Hz). The indirect H3 correlation signal ($^{4}J_{H3,Se}$ = 0) derives from HoHaHa (TOCSY) transfer. (**B**) HeHaHa (B_{DIPSI2} = 1450 Hz, Δ_{opt}(H1) = 80 ms). (**C**) H1 selective HeHaHa (B_{DIPSI2} = 285 Hz, Δ_{opt}(H1) = 100 ms). The small intensity reduction (ca. -10%) in the presence of hGal-3 is likely due to a dilution effect.

Fluorescence anisotropy

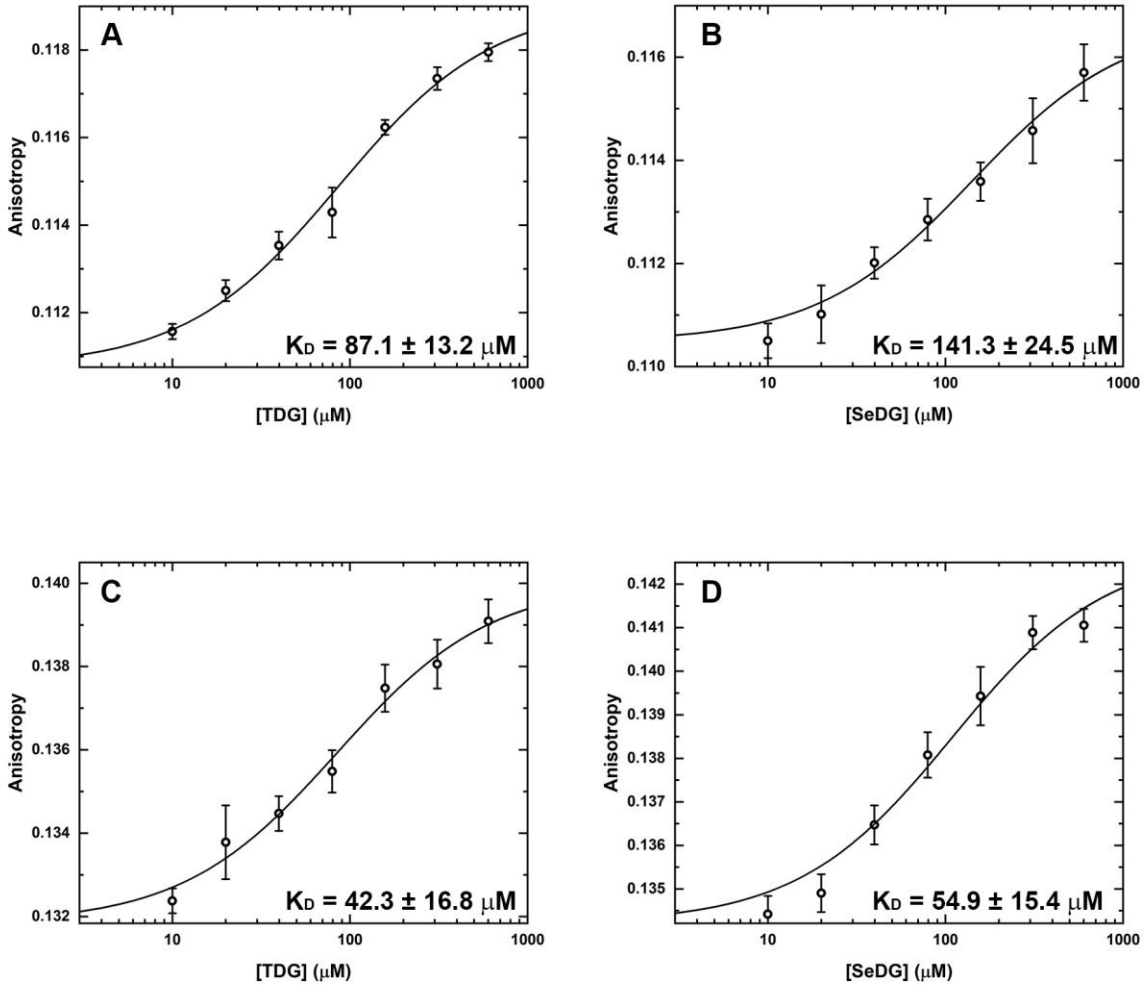


Figure S5. Determination of hGal-3 CRD and hGal3-hGal3 interactions with increasing concentrations of TDG and SeDG by intrinsic Trp fluorescence anisotropy. Binding isotherms of TDG (A) and SeDG (B) to Gal-3 CRD, and of TDG (C) and SeDG (D) to Gal3-Gal3 at 25 °C. Equilibrium KD values were determined as described in methods. Error bars represent standard deviations from five repeated anisotropy measurements. Errors of KD represent error estimates from the fitting algorithm.

CD spectroscopy

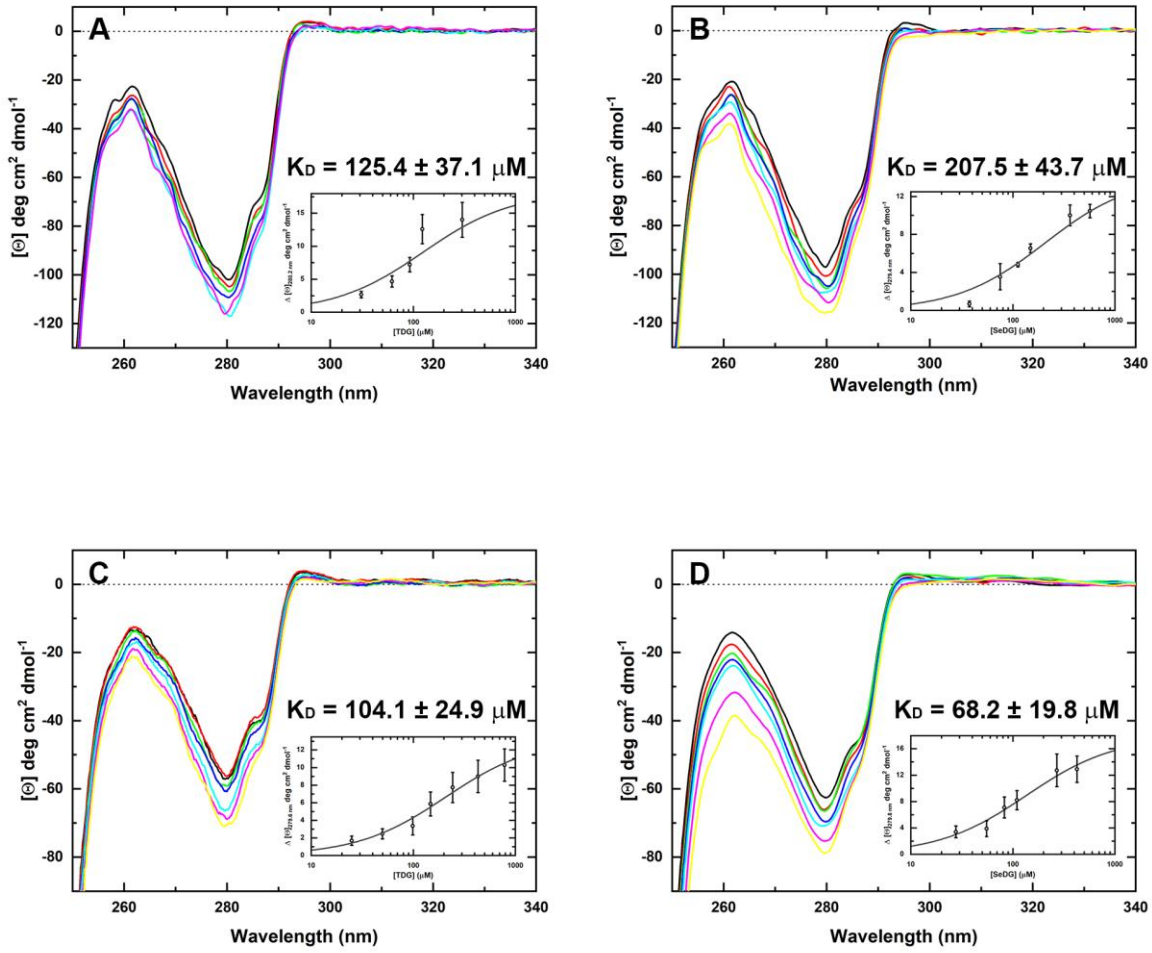


Figure S6. Interaction of Gal-3 CRD and Gal3-Gal3 with the ligands TDG and SeDG, observed by circular dichroism. (**A**) Near-UV CD spectra of Gal-3 CRD in the absence (black) and in the presence of increasing amounts of TDG (red, 31.0 μM; green, 61.8 μM; blue, 92.5 μM; cyan, 122.9 μM and magenta, 301.7 μM). Inset: binding isotherms of TDG to Gal-3 CRD at 25 °C. (**B**) Near-UV CD spectra of Gal-3 CRD in the absence (black) and in the presence of increasing amounts of SeDG (red, 37.6 μM; green, 75.0 μM; blue, 112.1 μM; cyan, 148.9 μM; magenta, 364.0 μM and yellow, 570.0 μM). Inset: binding isotherm of SeDG to Gal-3 CRD at 25 °C. (**C**) Near-UV CD spectra of Gal3-Gal3 in the absence (black) and in the presence of increasing amounts of TDG (red, 24.9 μM; green, 49.8 μM; blue, 99.0 μM; cyan, 147.8 μM; magenta, 243.9 μM and yellow, 430.6 μM). Inset: binding isotherm of TDG to Gal3-Gal3 at 25 °C. (**D**) Near-UV CD spectra of Gal3-Gal3 in the absence (black) and in the presence of increasing amounts of SeDG (red, 27.7 μM; green, 55.2 μM; blue, 82.6 μM; cyan, 109.9 μM; magenta, 270.3 μM and yellow, 425.5 μM). Inset: binding isotherm of SeDG to Gal3-Gal3 at 25 °C. Equilibrium K_D values were determined as described in methods. Error bars represent an estimation of the noise of the CD spectra. Errors of K_D represent error estimates from the fitting algorithm.

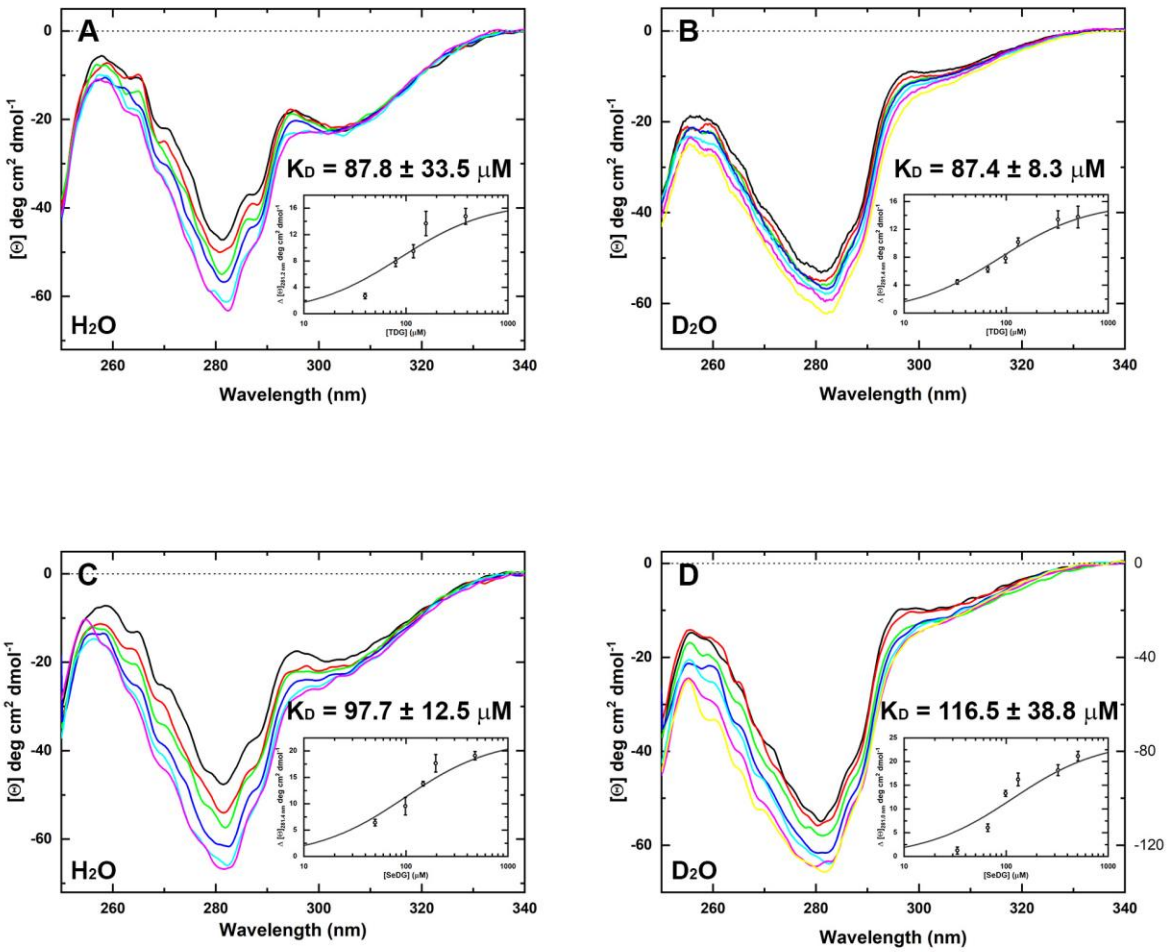


Figure S7. Effect of H_2O vs. D_2O on the interactions of Gal-1 with the ligands TDG and SeDG, observed by circular dichroism. (**A**) Near-UV CD spectra of Gal-1 in H_2O in the absence (black) and in the presence of increasing amounts of TDG (red, 39.8 μM; green, 79.4 μM; blue, 118.6 μM; cyan, 157.5 μM and magenta, 385.5 μM). Inset: binding isotherms of TDG to Gal-1 at 25 °C. (**B**) Near-UV CD spectra of Gal-1 in D_2O in the absence (black) and in the presence of increasing amounts of TDG (red, 33.2 μM; green, 66.2 μM; blue, 99.0 μM; cyan, 131.6 μM; magenta, 322.6 μM and yellow, 506.3 μM). Inset: binding isotherms of SeDG to Gal-1 at 25 °C. (**C**) Near-UV CD spectra of Gal-1 in H_2O in the absence (black) and in the presence of increasing amounts of SeDG (red, 49.8 μM; green, 99.0 μM; blue, 147.8 μM; cyan, 196.1 μM and magenta, 476.2 μM). Inset: binding isotherms of SeDG to Gal-1 at 25 °C. (**D**) Near-UV CD spectra of Gal-1 in D_2O in the absence (black) and in the presence of increasing amounts of SeDG (red, 33.2 μM; green, 66.2 μM; blue, 99.0 μM; cyan, 131.6 μM; magenta, 322.6 μM and yellow, 506.3 μM). Inset: binding isotherms of SeDG to Gal-1 at 25 °C. Equilibrium K_D values were determined as described in methods. Error bars represent an estimation of the noise of the CD spectra. Errors of K_D represent error estimates from the fitting algorithm.

ITC Titration Profiles

ITC measurements were done on samples dissolved in phosphate buffer (pH 7.2) containing 20 mM phosphate, 10 mM NaCl, 10 mM BME, in either H_2O or D_2O. Ligands were injected every 150 s at 298 K. Data fitting to a one-site binding model used MicroCal PEAQ-ITC analysis software; the resulting values for stoichiometry (n), binding affinity (K_a), dissociation constant (K_d), enthalpy (ΔH), and change in entropy with respect to temperature ($T\Delta S$) are summarized in Table 3 of the article.

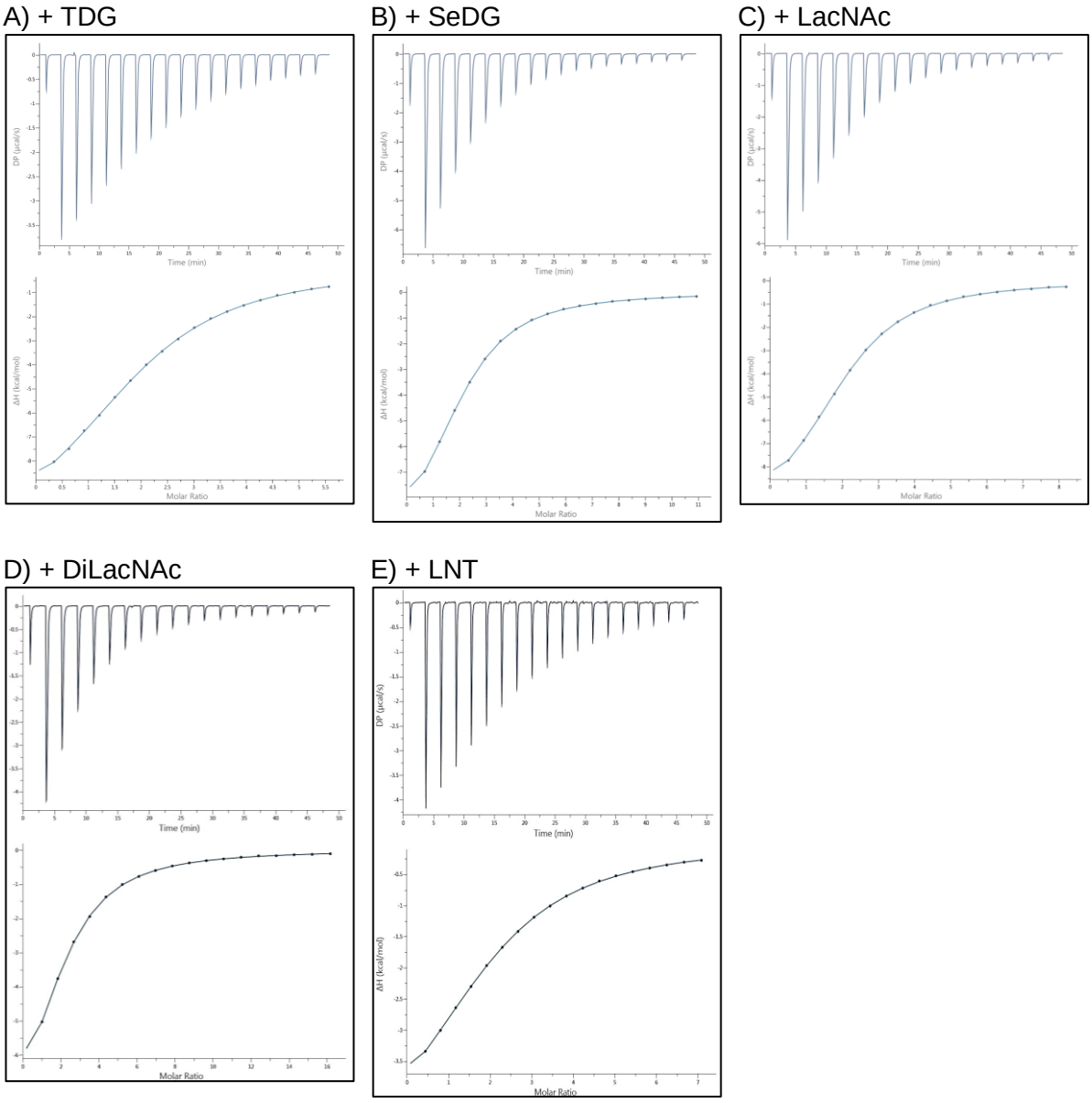


Figure S8a. ITC titration profiles for Gal-1 in H_2O. (A) Gal-1 (110 µM) with TDG (3.2 mM), (B) Gal-1 (140 µM) with SeDG (8.0 mM), (C) Gal-1 (140 µM) with LacNAc (6.0 mM), (D) Gal-1 (171 µM) with DiLacNAc (6.0 mM), (E) Gal-1 (162 µM) with LNT (6.0 mM). Top panels: experimental ITC data. Bottom panels: data fitting to a one-site binding model.

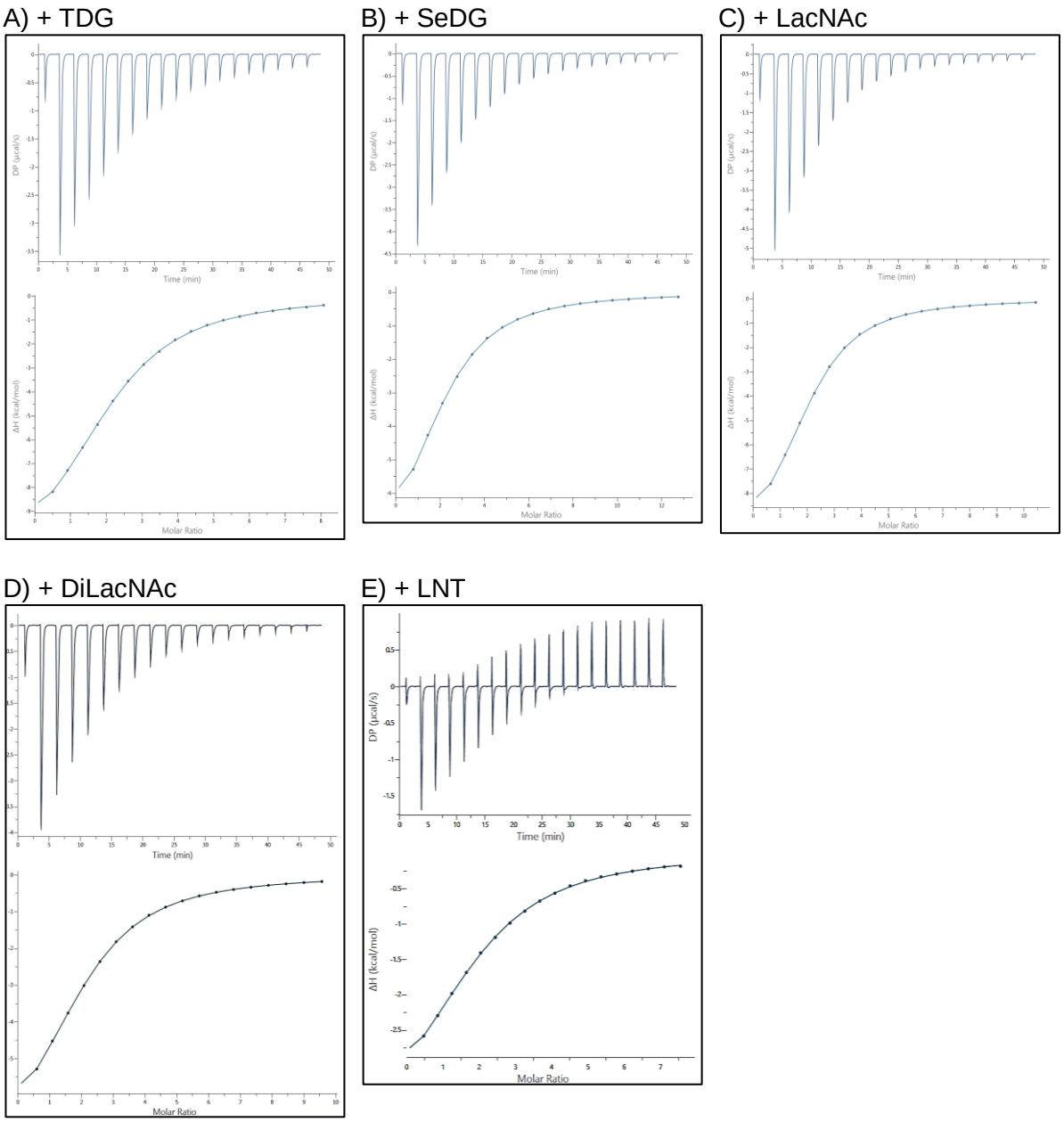


Figure S8b. ITC titration profiles for Gal-1 in D_2O. (A) Gal-1 (90 µM) with TDG (3.8 mM), (B) Gal-1 (120 µM) with SeDG (8.0 mM), (C) Gal-1 (110 µM) with LacNAc (6.0 mM), (D) Gal-1 (120 µM) with DiLacNAc (6.0 mM), (E) Gal-1 (152 µM) with LNT (6.0 mM). Top panels: experimental ITC data. Bottom panels: data fitting to a one-site binding model.

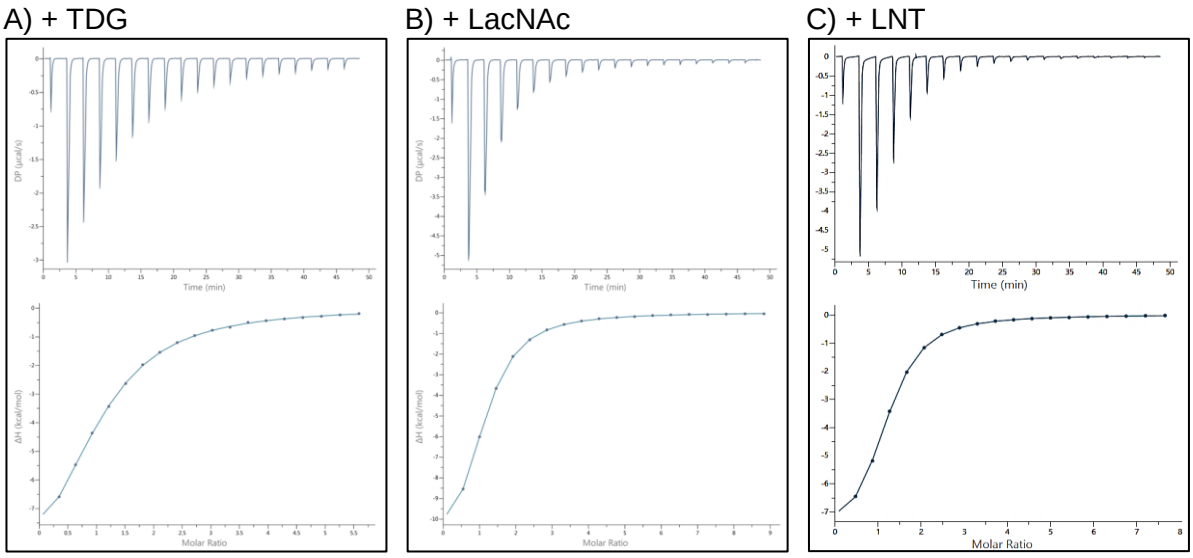


Figure S9a. ITC titration profiles for Gal-3 in H_2O. (A) Gal-3 (130 μM) with TDG (3.8 mM), (C) Gal-3 (130 μM) with LacNAc (6.0 mM), (D) Gal-3 (150 μM) with LNT (6.0 mM). Top panels: experimental ITC data. Bottom panels: data fitting to a one-site binding model.

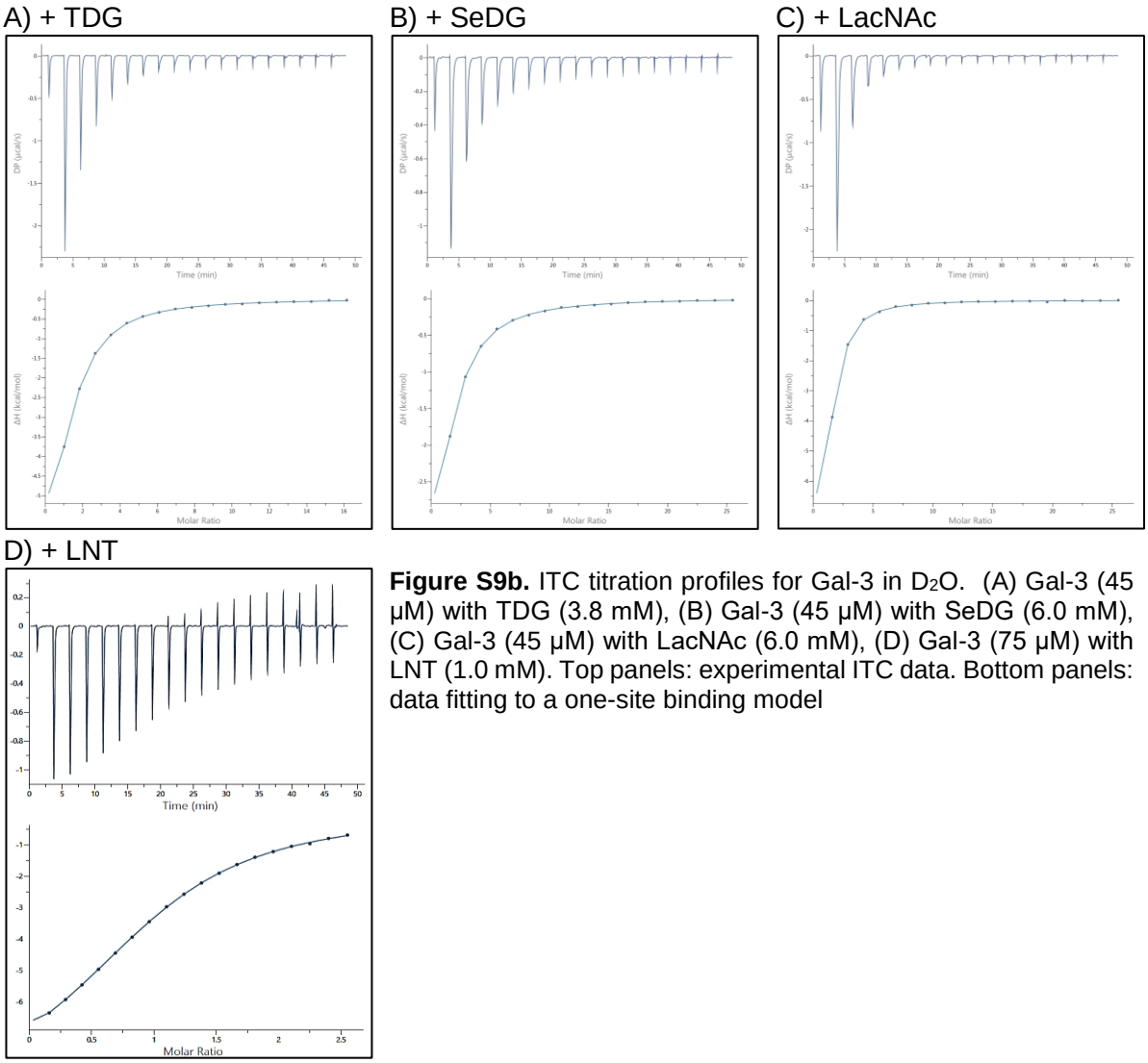
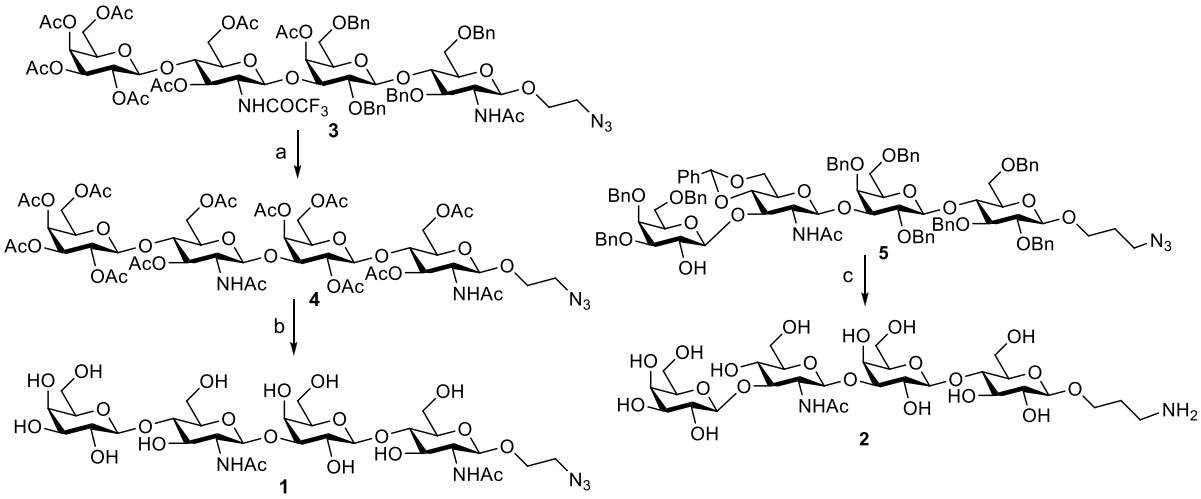


Figure S9b. ITC titration profiles for Gal-3 in D_2O. (A) Gal-3 (45 μM) with TDG (3.8 mM), (B) Gal-3 (45 μM) with SeDG (6.0 mM), (C) Gal-3 (45 μM) with LacNAc (6.0 mM), (D) Gal-3 (75 μM) with LNT (1.0 mM). Top panels: experimental ITC data. Bottom panels: data fitting to a one-site binding model

Synthesis of DiLacNAc and LNT tetrasaccharides

The DiLacNAc tetrasaccharide **1** and the LNT tetrasaccharide **2** were synthesized through deprotection of tetrasaccharides **3**[1] and **5**[3], respectively (Scheme). The benzyl groups in DiLacNAc precursor **3**, efficiently obtained using a trifluoroacetamide protected lactosamine thioglycoside donor and a benzylated LacNAc acceptor, were removed under oxidative conditions which do not reduce the azide.[2] Following base treatment and peracetylation afforded compound **4**, which was deacetylated under Zemplen conditions to give target DiLacNAc compound **1**. The benzyl groups in the LNT precursor **5**, synthesized as an intermediate for continued Lewis b and H antigen synthesis,[3] were removed using catalytic hydrogenolysis, which also reduced the azide to an amino group, to afford target LNT compound **2**.



Scheme S1 Synthesis of DiLacNac compound **1** and LNT compound **2**. Reagents: (a) i. $NaBrO_3$, $Na_2S_2O_4$, H_2O, AcOEt, ii. NaOMe, MeOH, iii. Ac_2O, pyridine; (b) NaOMe, DMSO, MeOH; (c) H2, Pd/C, $Pd(OH)_2$/C, HCl (aq), H_2O, EtOH, EtOAc.

General Methods

Reactions were monitored by thin-layer chromatography (TLC) on Merck DC-Alufolien plates precoated with silica gel 60 F_{254}. Visualisation was performed with UV-light (254 nm) and/or staining with 8% H_2SO_4/EtOH solution. All chemicals were purchased from commercial suppliers (Acros, Carbosynth Ltd., Fisher Scientific Ltd., Glycom A/S, Merck, Sigma-Aldrich, VWR) and were used without purification. Dry solvents were obtained from a PureSolv-EN™ solvent purification system (Innovative Technology Inc.) or were used as purchased from Sigma-Aldrich in AcroSeal® bottles. NMR spectra were recorded on Varian Inova spectrometers at 25 °C. High-resolution mass spectrometry (HRMS) data were recorded on a Waters Micromass LCT LC-TOF instrument using electrospray ionisation (ESI) in positive mode. Specific rotations were recorded on a Perkin-Elmer polarimeter (Model 343) at the sodium D-line (589 nm) at 20 °C in a 1 dm cell. Deprotected sugars were lyophilised using an Alpha 1-2 LDplus (Christ Ltd.) freeze-dryer: pressure: 0.035 mbar; ice-condenser temperature: -55 °C.

References:

1. C. Romanò, S. Oscarson, *Org. Biomol. Chem*., **2019**, 17, 2265-2278.
2. M. Niemietz, L. Perkams, J. Hoffman, S. Eller, C. Unverzagt, Y. Ito, M. Niemietz, M. Pischl and C. Raps, *Chem. Commun*., **2011**, 47, 10485.
3. M. Lahmann, L. Bülow, P. Teodorovic, H. Gybäck, and S. Oscarson, *Glycoconj. J*., **2004**, 21, 251-256.

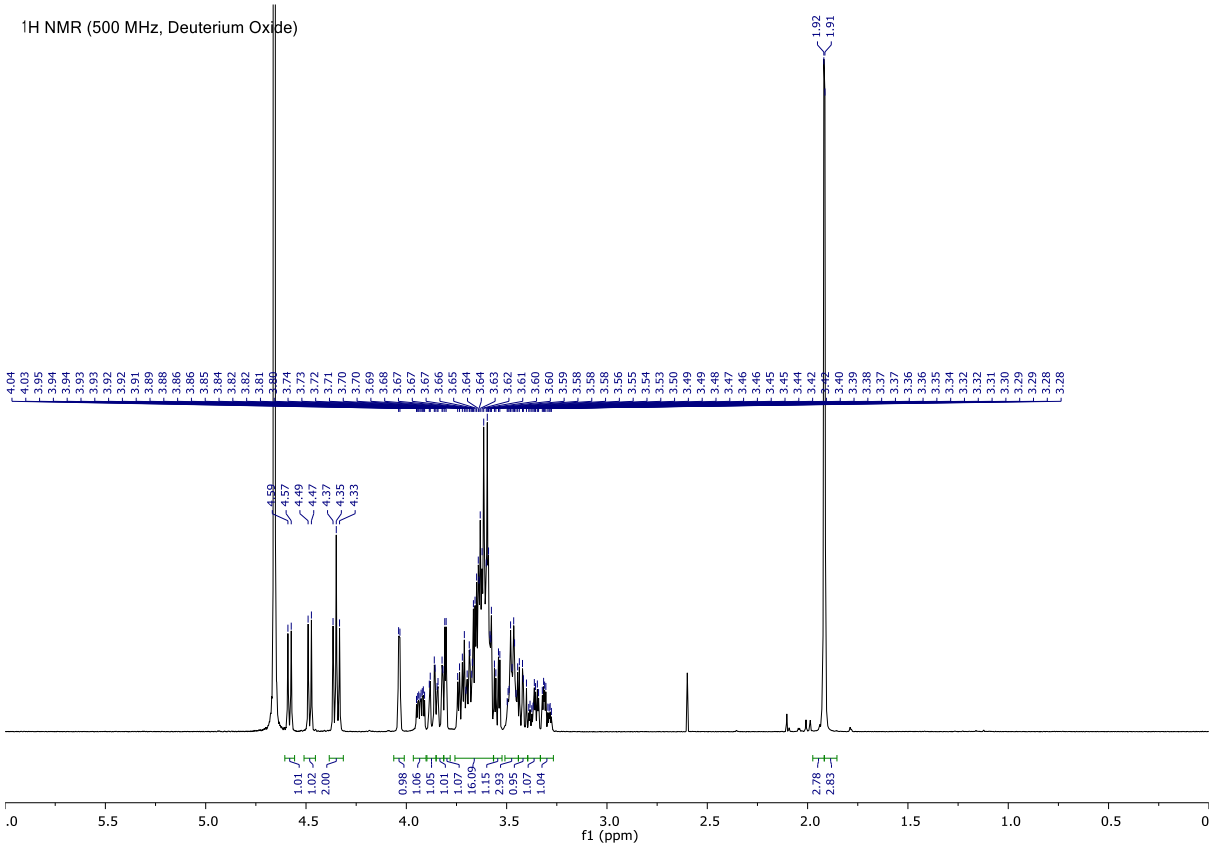


Figure S10A. ^{1}H-NMR of compound **1** (298 K, in D_2O, 500 MHz)

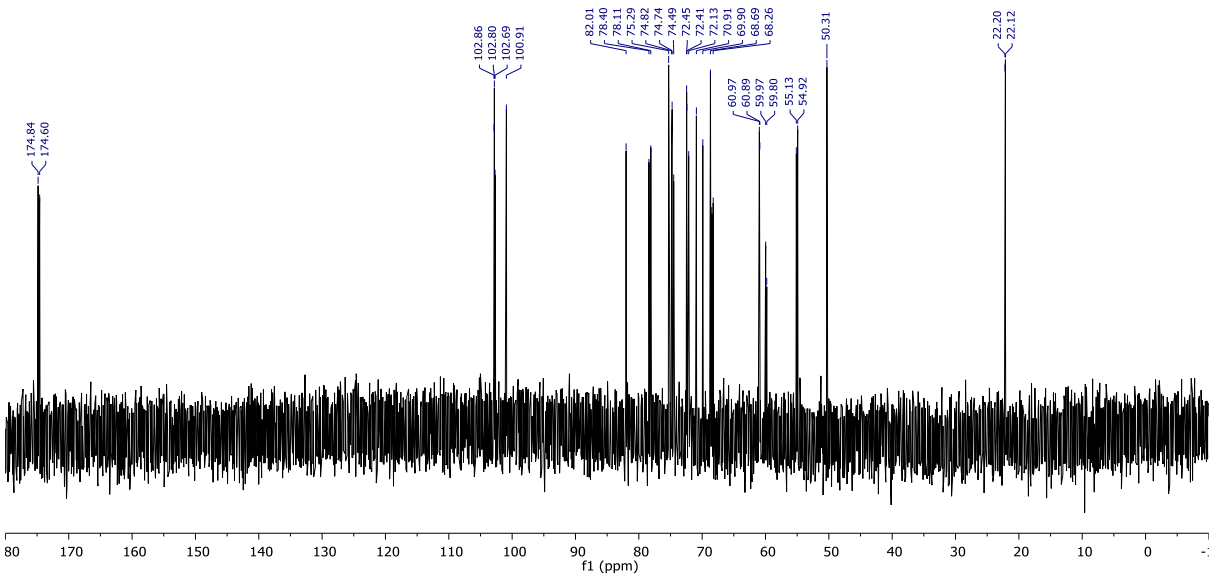


Figure S10B. ^{13}C-NMR of compound **1** (298 K, in D_2O, 500 MHz)

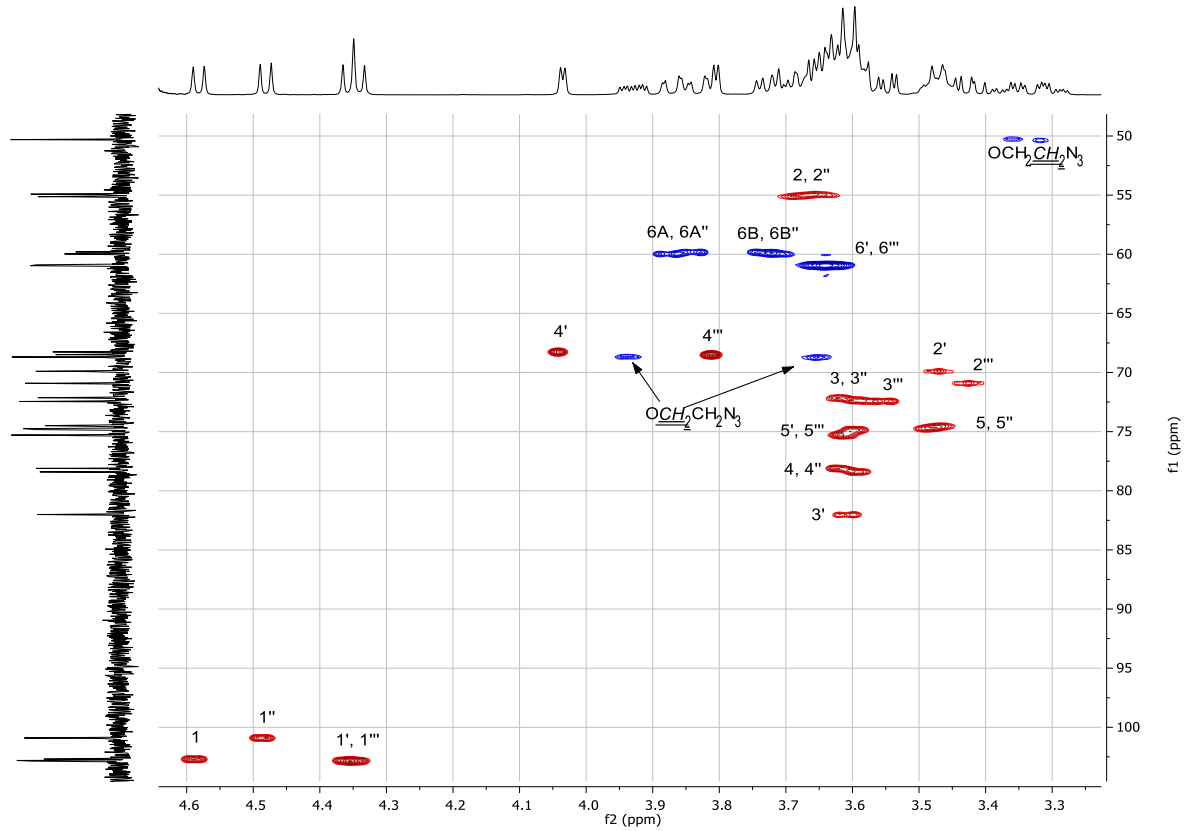


Figure S10C. CH_n multiplicity edited 2D $^{1}H,^{13}C$ HSQC of compound **1**.

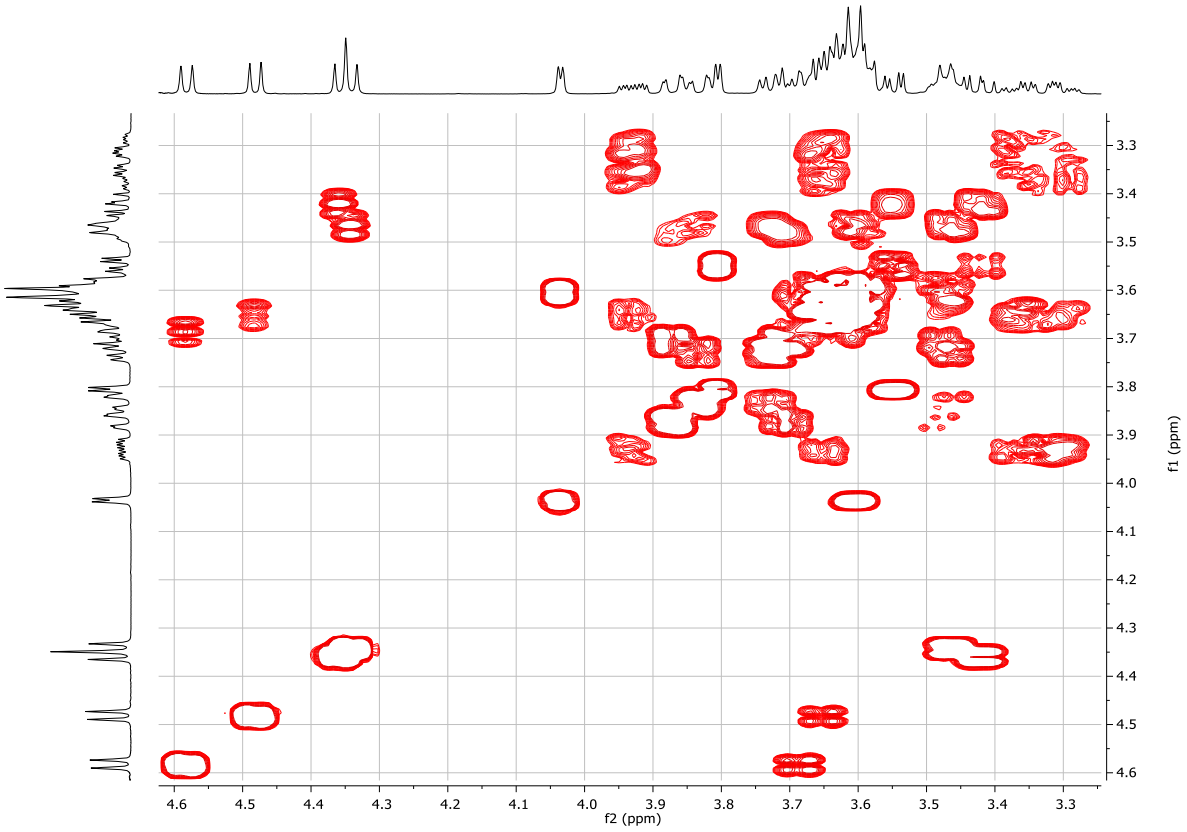


Figure S10D. 2D $^{1}H,^{1}H$ COSY spectrum (magnitude mode) of compound **1**

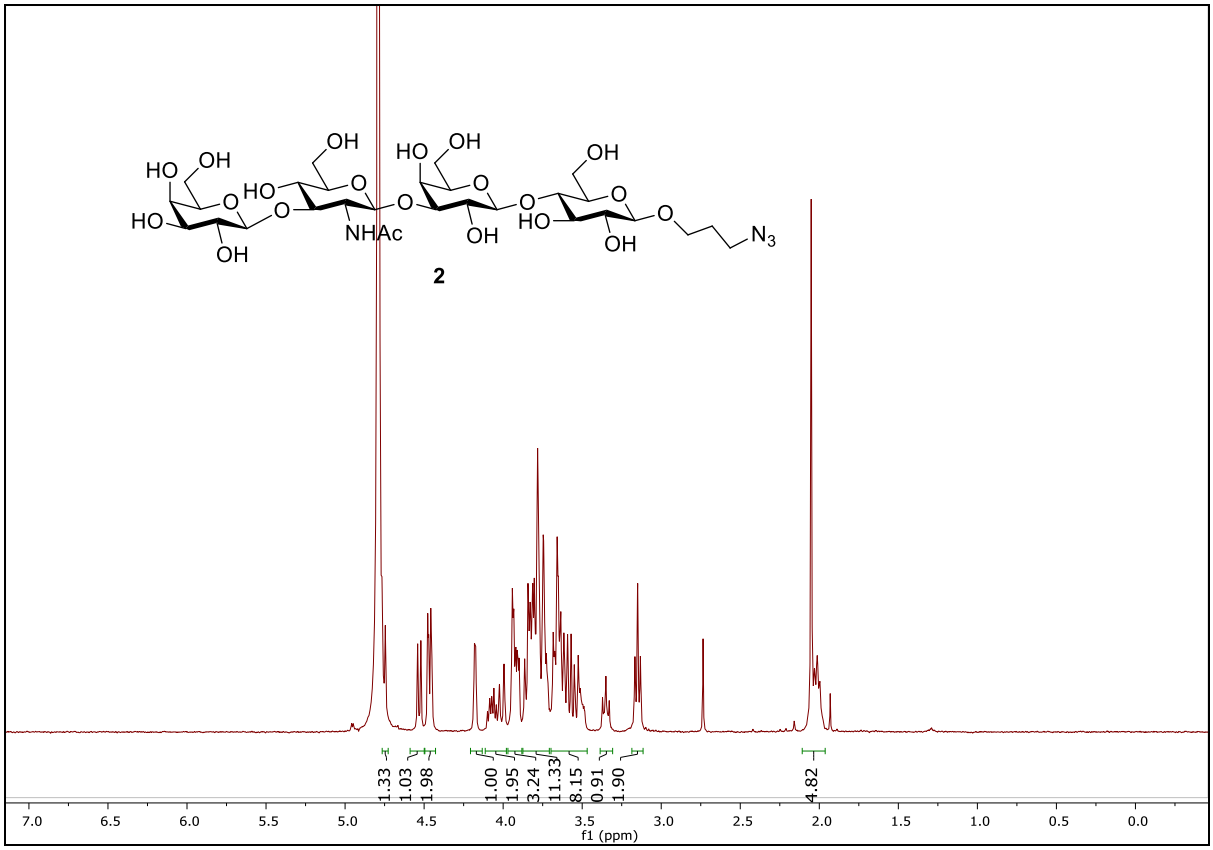


Figure S11A. 1D ^{1}H NMR spectrum of compound **2**

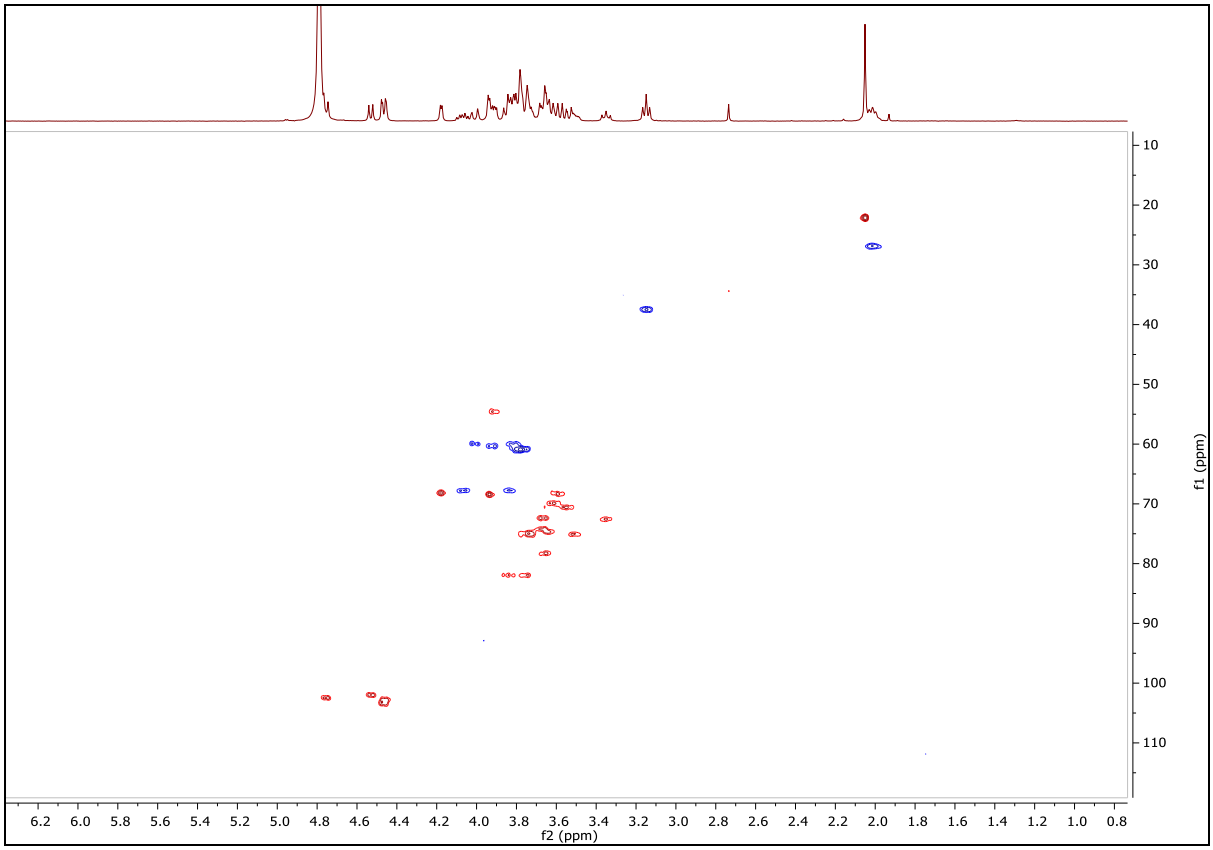


Figure S11B. CH_n multiplicity edited 2D $^{1}H,^{13}C$ HSQC spectrum of compound **2**