Chemistry–A European Journal

Supporting Information

Midkine Interaction with Chondroitin Sulfate Model Synthetic Tetrasaccharides and Their Mimetics: The Role of Aromatic Interactions

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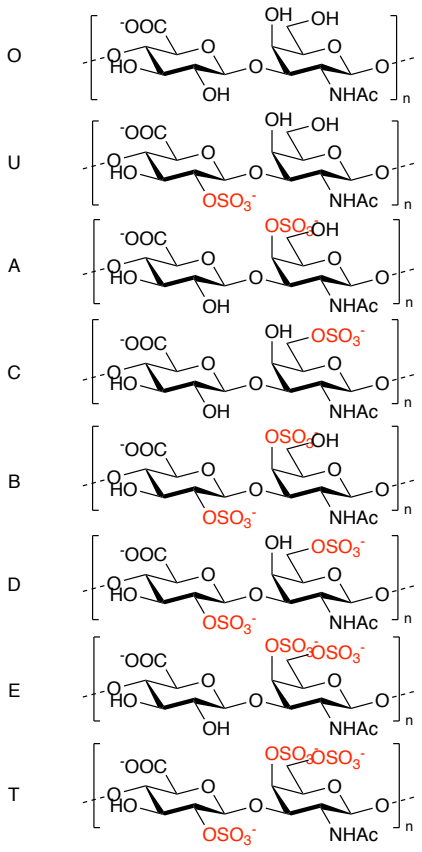
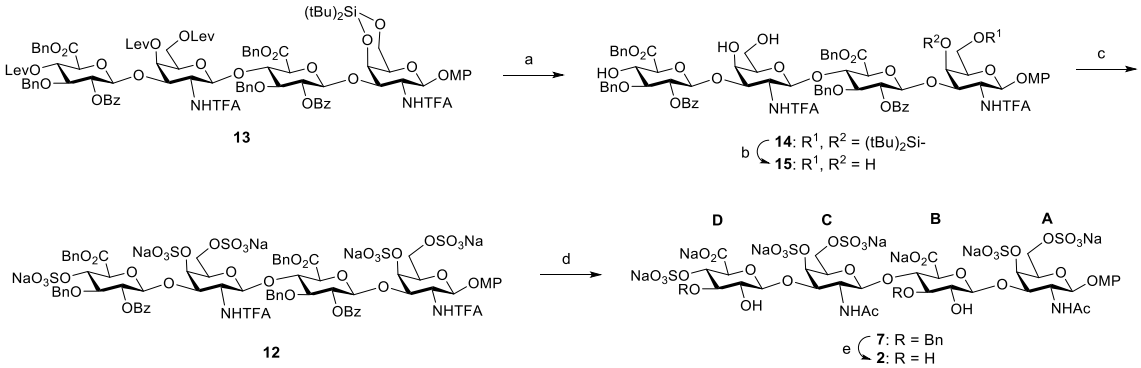


Figure S1. Types of CS according to their sulfation patterns.

Synthesis of new tetrasaccharides 2, 7 and 12.



Scheme S1. Reagents and conditions: a) $NH_2NH_2 \cdot H_2O$, Py/AcOH, CH_2Cl_2, 92%; b) $(HF)_n \cdot Py$, THF, 0 °C, 95%; c) $SO_3 \cdot Me_3N$, DMF, 100 °C, MW, 30 min, 75%; d) H_2O_2, LiOH, THF; NaOH, MeOH; Ac_2O, MeOH/H_2O, Et_3N, 89%; e) H_2, $Pd(OH)_2/C$, H_2O/MeOH, 78%. Lev = levulinoyl.

General synthetic procedures: Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck). The compounds were detected by UV visualization (λ = 254 nm) and by staining with cerium (IV) sulfate (1 g)/ ammonium molybdate tetrahydrate (21 g)/sulfuric acid (30 mL) solution in water (0.47 L), or with anisaldehyde solution [anisaldehyde (25 mL), sulfuric acid (25 mL) and acetic acid (1 mL) in ethanol (450 mL)], followed by heating at over 200ºC. Column chromatography was performed on silica gel 60 from Merck (15-40 μm or 63-200 μm). Size exclusion chromatography was performed using Sephadex LH-20 and G10 gels from GE Healthcare. Ion-exchange chromatography was carried out using Dowex 50WX2 Na^+ resin from Sigma-Aldrich. Microwave-based sulfation reaction was performed using a Biotage Initiator Eight synthesizer in sealed reaction vessels. Optical rotations were measured with a Perkin Elmer 341 polarimeter, using a sodium lamp (λ = 589 nm) at 25 ºC in 1 dm tubes. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III-400 spectrometer. Bidimensional COSY and HSQC NMR experiments were carried out to assist in signal assignment. Unit A refers to the reducing end monosaccharide in the NMR data. For electrospray mass spectra (ESI MS), we employed a Bruker Esquire 6000 equipment. High resolution mass spectra (HR MS) were performed at CITIUS, University of Seville.

Tetrasaccharide 14: Compound **13**[1] (272 mg, 0.137 mmol) was dissolved in CH_2Cl_2 (3 mL) and hydrazine monohydrate (1.6 mL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 1 h, the reaction mixture was quenched with acetone (0.6 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-EtOAc 2:1) to afford **14** (214 mg, 92%) as

a white foam. TLC (toluene-EtOAc 2:1) Rf 0.28; $[\alpha]^{20}_{D}$ +7º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.99-7.15 (m, 30H, Ar), 6.94-6.81 (m, 5H, Ar, NH), 6.57 (br d, 1H, $J_{2,NH}$ = 7.4 Hz, NH), 5.32-5.12 (m, 8H, 4 x CH_2(Bn), H-2B, H-2D, H-1A, H-1B or D), 4.86-4.67 (m, 4H, 3 x CH_2(Bn), H-1B or D), 4.51 (m, 3H, CH_2(Bn), H-1C, H-4A), 4.33 (br d, 1H, H-3A), 4.17-4.10 (m, 3H, H-4B, H-4D, H-6A), 4.04-3.91 (m, 6H, H-2A, H-2C, H-6A, H-5B, H-5D, H-4C), 3.78 (s, 3H, Me (OMP)), 3.74 (m, 2H, H-3C, H-3B or D), 3.66 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.2 Hz, H-3B or D), 3.55 (dd, 1H, $J_{5,6}$ = 7.3 Hz, $J_{6,6}$ = 11.7 Hz, H-6C), 3.37 (dd, 1H, $J_{5,6}$ = 4.3 Hz, H-6C), 3.28 (br s, 1H, H-5A), 3.14 (m, 1H, H-5C), 1.06-0.99 (2s, 18H, $C(CH_3)_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.3, 168.2, 165.3, 165.1 (4 x CO), 157.5 (2q, *C*OCF_3), 155.9-114.0 (Ar), 116.9 (2q, CO*C*F_3), 101.3 (C-1 B or D), 100.2, 99.4 (C-1A, C-1B or D), 99.1 (C-1C), 80.6, 80.3, 79.2 (C-3B, C-3C, C-3D), 77.1 (C-4B), 75.4, 75.3, 74.6, (C-3A, 2 x CH_2(Bn)), 74.4 (C-5C), 74.2, 74.0 (C-5B, C-5D), 72.90, 72.83, 72.5 (C-2B, C-2D, C-4A), 71.8 (C-4D), 71.2 (C-5A), 68.2, 68.0 (CH_2(Bn)), 67.5 (C-4C), 66.9 (C-6A), 61.8 (C-6C), 55.6 (Me (OMP)), 53.6 (C-2A), 52.9 (C-2C), 27.5 (C(*C*$H_3)_3$), 23.2-20.7 (*C*$(CH_3)_3$); HR MS: *m/z*: calcd for $C_{85}H_{92}F_6N_2O_{26}NaSi$: 1721.5504; found: 1721.5470 [*M*+Na]$^+$.

Tetrasaccharide 15: An excess of $(HF)_n$·Py (314 µL, 12.1 mmol) was added at 0°C under an argon atmosphere to a solution of **14** (103 mg, 0.061 mmol) in dry THF (4.0 mL). After 24 h at 0ºC the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo to give **15** (90 mg, 95%) as a white amorphous solid. TLC (CH_2Cl_2-MeOH 20:1) Rf 0.37; ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 5:2): δ 7.98-7.06 (m, 30H, Ar), 6.90-6.73 (m, 4H, Ar), 5.27-5.13 (m, 5H, 3 x CH_2(Bn), H-2B, H-2D), 5.06 (d, 1H, *J* = 12.0 Hz, CH_2(Bn)), 4.90-4.81 (m, 5H, 2 x CH_2(Bn), H-1A, H-1B, H-1D), 4.66 (d, 1H, CH_2(Bn)), 4.57 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1C), 4.50 (d, 1H, CH_2(Bn)), 4.21 (t, 1H, $J_{3,4} = J_{4,5}$ = 8.2 Hz, H-4B or D), 4.12 (m, 1H, H-2A), 4.08-3.94 (m, 7H, H-3A, H-4B or D, H-5B, H-5D, H-4A, H-4C, H-2C), 3.86-3.73 (m, 8H, H-3C, H-3B, H-3D, Me (OMP), H-6A, H-6C), 3.56 (dd, 1H, $J_{5,6}$ = 4.3 Hz, $J_{6,6}$ = 11.6 Hz, H-6A or C), 3.49 (m, 1H, H-5A or C), 3.42 (dd, 1H, H-6A or C), 3.33 (m, 1H, H-5A or C); ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD 5:2): δ 168.9, 167.9, 165.8, 165.6 (4 x CO), 155.4-114.4 (Ar), 101.3 (C-1 B, C-1D), 100.3 (C-1A), 99.6 (C-1C), 81.4, 80.0 (C-3B, C-3D), 78.9, 78.4, 75.8, 75.7, 75.0, 74.92, 74.85, 74.3, 72.9, 72.3, 71.7 (C-3A, C-3C, 2 x CH_2(Bn), C-2B, C-2D, C-4B, C-4D, C-5A, C-5B, C-5C, C-5D), 67.9, 67.6, 67.5 (C-4A, C-4C, 2 x CH_2(Bn)), 61.9, 61.6 (C-6A, C-6C), 55.4 (Me (OMP)), 52.4 (C-2C), 52.1 (C-2A); HR MS: *m/z*: calcd for $C_{77}H_{76}F_6N_2O_{26}Na$: 1581.4483; found: 1581.4423 [*M*+Na]$^+$.

Tetrasaccharide 12: Compound **15** (15 mg, 9.6 µmol) and sulfur trioxide–trimethylamine complex (67 mg, 0.48 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100ºC for 30 min using microwave radiation (20 W average power). The reaction vessel was cooled and Et_3N (300 µL),

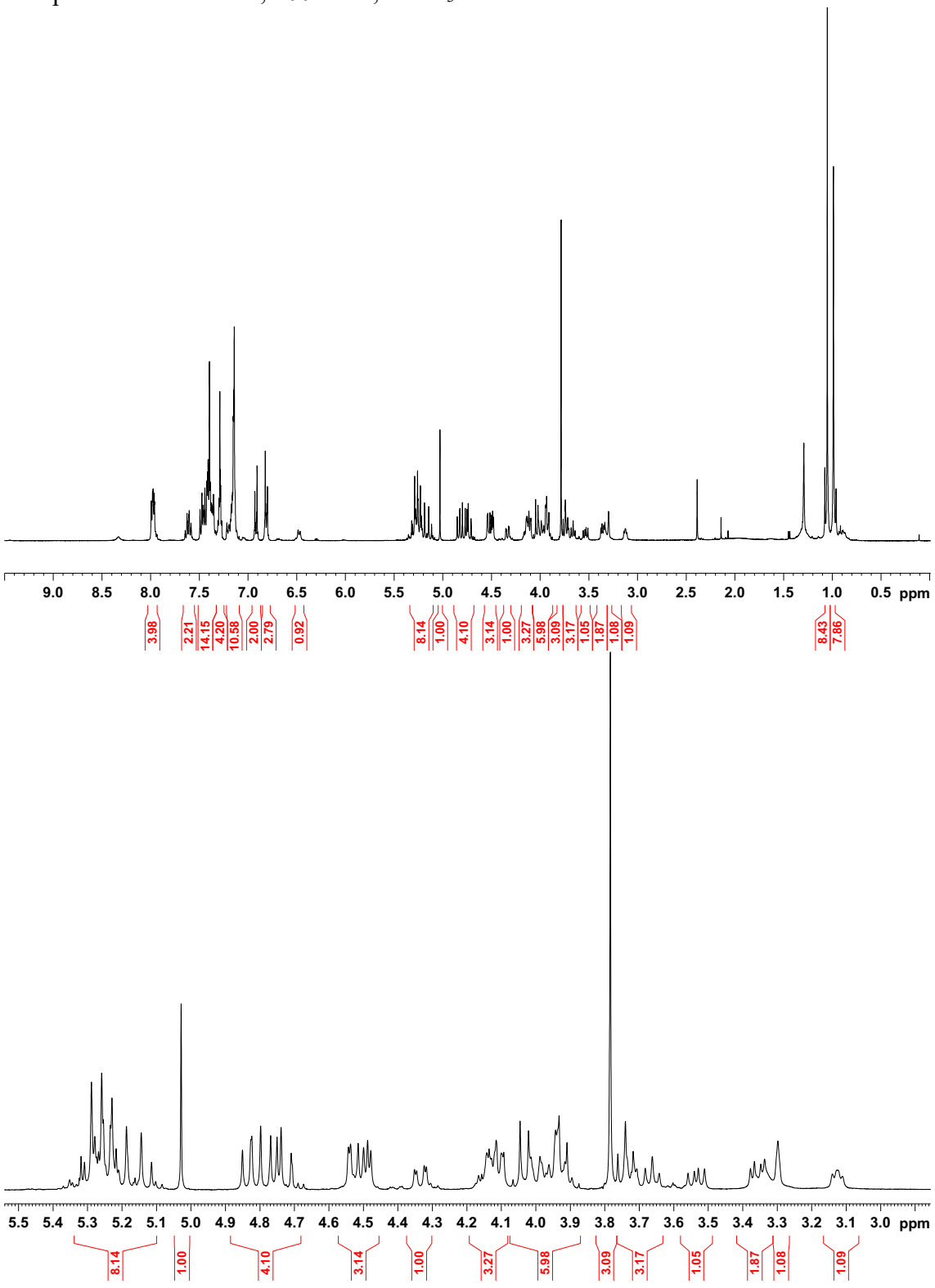
MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was first purified by Sephadex LH 20 chromatography (CH_2Cl_2-MeOH 1:1). The residue was then purified by silica gel column chromatography (EtOAc-MeOH-H_2O 32:5:3 → 28:5:3) and finally eluted from a Dowex 50WX2-Na^+ column (MeOH-H_2O 9:1) to obtain **12** as sodium salt (15 mg, 75%). TLC (EtOAc/pyridine/H_2O/AcOH 12:5:3:1) Rf 0.13; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.96-7.00 (m, 30H, Ar), 6.97-6.81 (m, 4H, Ar), 5.39-5.35 (m, 3H, 2 x CH_2(Bn), H-2D), 5.30 (t, 1H, $J_{1,2} = J_{2,3}$ = 7.7 Hz, H-2B), 5.20 (m, 2H, CH_2(Bn)), 5.02 (d, 1H, J = 11.2 Hz, CH_2(Bn)), 4.96-4.93 (m, 2H, H-1D, H-4A or C), 4.87 (m, 3H, H-4A or C, CH_2(Bn), H-1B), 4.83 (t, 1H, H-4D), 4.76 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1A), 4.60 (m, 2H, CH_2(Bn), H-1C), 4.53 (d, 1H, CH_2(Bn)), 4.43-4.27 (m, 6H, H-4B, H-5D, H-2A, 3 x H-6A/C), 4.17-4.05 (m, 3H, H-6A or C, H-5B, H-2C), 3.96-3.92 (m, 4H, H-5A or C, H-3A, H-3C, H-3D), 3.87-3.81 (m, 2H, H-3B, H-5A or C), 3.74 (s, 3H, Me (OMP)); ^{13}C-NMR (100 MHz, CD_3OD, selected data from HSQC experiment): δ 101.2 (C-1 B), 100.8 (C-1D), 100.5 (C-1A), 99.7 (C-1C), 79.6 (C-3D), 79.1 (C-3B), 76.1 (C-4B), 75.9 (C-4D), 75.7-73.2 (C-5A or C, C-3A, C-3C), 75.2, 75.0 (C-4A, C-4C), 74.7 (C-5B), 74.4-74.0 (2 x CH_2(Bn)), 73.9 (C-5D), 72.8 (C-2D), 72.7 (C-5A or C), 72.6 (C-2B), 67.5 (2 x CH_2(Bn)), 67.5-66.8 (C-6A, C-6C), 54.3 (Me (OMP)), 52.3 (C-2C), 51.5 (C-2A); ESI MS: *m/z*: calcd for $C_{77}H_{72}F_6N_2O_{41}S_5NaK$: 1008.1; found: 1008.0 $[M\text{-}4Na+K+H]^{2-}$.

Tetrasaccharide 7: H_2O_2 (30%, 0.27 mL) and an aqueous solution of LiOH (0.7 M, 167 µL) were added at 0ºC to a solution of **12** (14.2 mg, 6.9 µmol) in THF (0.74 mL). After stirring for 20 h at room temperature, MeOH (1.4 mL), H_2O (0.43 mL) and an aqueous solution of NaOH (4 M, 343 µL) were added. After stirring for 72 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give the desired diamine intermediate. Triethylamine (25 µL, 0.18 mmol) and acetic anhydride (26 µL, 0.27 mmol) were added to a cooled (0ºC) solution of this diamine derivative in MeOH-H_2O 4:1 (2.0 mL). After stirring for 2.5 h at room temperature, Et_3N (300 µL) was added and the mixture was concentrated to dryness. The residue was purified by Sephadex G 10 chromatography column which was eluted with H_2O-MeOH (9:1) to obtain **7**. This compound was then dissolved in H_2O (2 mL) and Amberlite IR-120 H^+ resin was added (pH = 3.0). The mixture was immediately filtered, treated with 0.04 M NaOH (pH = 7.1) and lyophilized. The white solid was finally eluted from a column of Dowex 50WX2-Na^+ (H_2O-MeOH 9:1) to obtain **7** as sodium salt (9.9 mg, 89%) after lyophilization. TLC (EtOAc/MeOH/H_2O 10:5:3) Rf 0.42; ^{1}H-NMR (400 MHz, D_2O): δ 7.50-7.28 (m, 10H, Ar), 7.02 (m, 2H, Ar), 6.90 (m, 2H, Ar), 4.95 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.89 (m, 2H, CH_2(Bn)), 4.79 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4C), 4.76 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4A), 4.74-4.66 (m, 2H, CH_2(Bn)), 4.58 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1C), 4.47 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1B), 4.46 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1D), 4.40 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4D), 4.29-4.20 (m, 2H, H-6aA or C, H-2A), 4.18-4.14 (m, 3H, H-6bA or C, H-6aA or C, H-5A or C), 4.10 (dd,

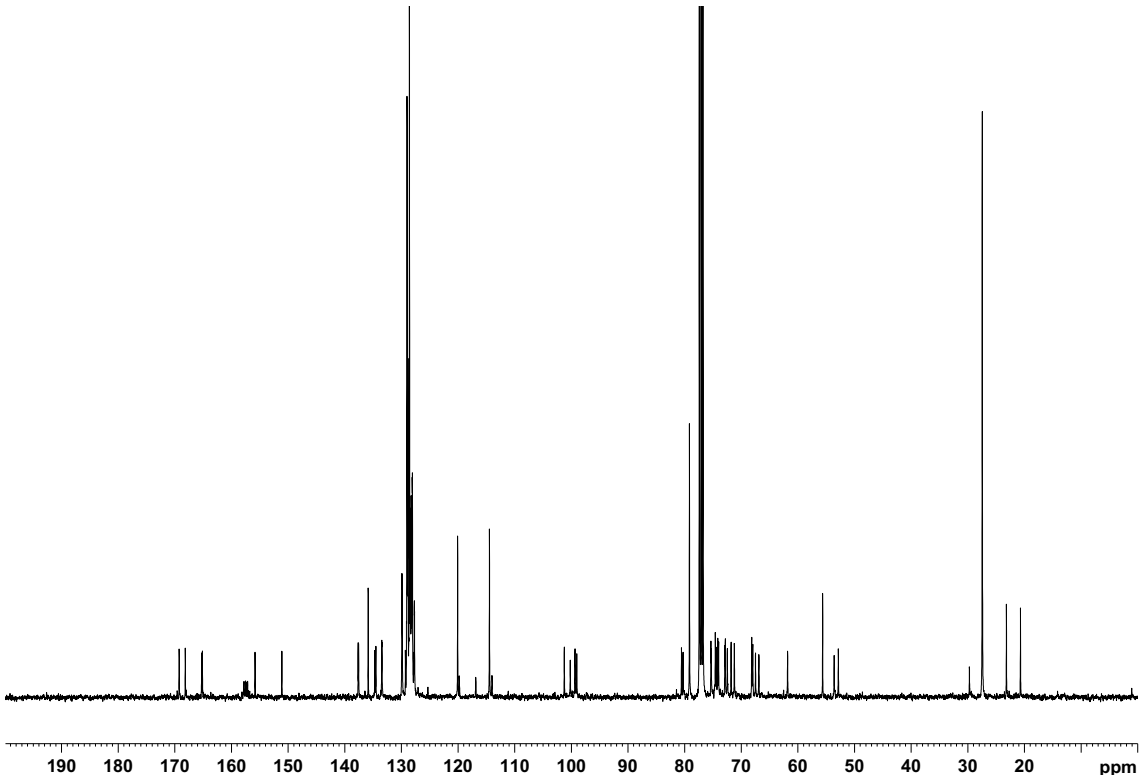
1H, $J_{2,3}$ = 11.0 Hz, H-3A), 4.07-3.93 (m, 4H, H-2C, H-6bA or C, H-4B, H-3C), 3.88 (m, 1H, H-5A or C), 3.77 (d, 1H, H-5D), 3.74 (s, 3H, Me (OMP)), 3.66-3.61 (m, 2H, H-3D, H-5B), 3.58-3.50 (m, 2H, H-3B, H-2B), 3.47 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2D), 1.99, 1.97 (2s, 6H, NHAc); ^{13}C-NMR (100 MHz, D_2O, selected data from HSQC experiment): δ 128.9-114.6 (Ar), 103.2 (C-1B, C-1D), 100.5 (C-1A), 100.0 (C-1C), 81.9 (C-3D), 81.1 (C-3B), 77.4 (C-4D), 76.9 (C-4B, C-5B), 76.2 (C-4A, C-5D), 75.5 (C-3C, C-4C), 74.4 (C-3A), 74.0, 73.1(2 CH_2(Bn)), 72.2 (C-5A or C), 71.9 (C-5A or C, C-2D), 71.1 (C-2B), 68.0, 67.0 (C-6A, C-6C), 55.6 (Me (OMP)), 51.4 (C-2A, C-2C), 22.4 (NHAc); ESI MS: *m/z*: calcd for $C_{49}H_{56}N_2O_{39}Na_4S_5$: 774.0; found: 773.8 [*M*-3Na+H]$^{2-}$.

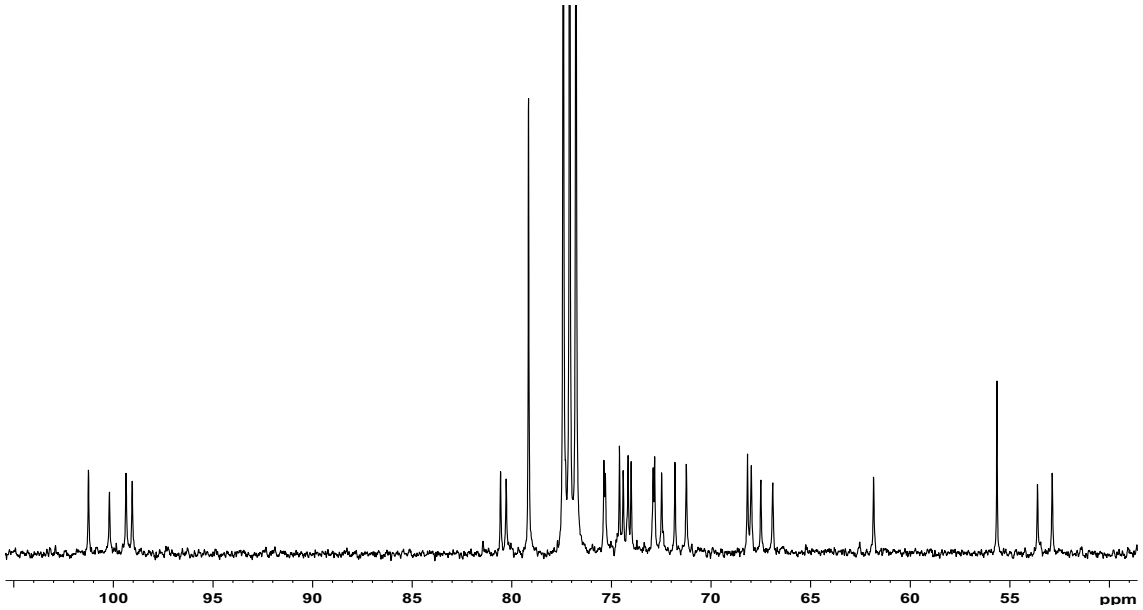
Tetrasaccharide 2: A solution of **7** (5.3 mg, 3.3 µmol, sodium salt) in H_2O/MeOH (4.5 mL/0.5 mL) was hydrogenated in the presence of 20% $Pd(OH)_2$/C (12 mg). After 24 h, the suspension was filtered over celite and concentrated. The residue was purified by Sephadex LH 20 chromatography column which was eluted with H_2O-MeOH (9:1) to obtain **2** as a white amorphous solid (3.7 mg, sodium salt, 78%) after lyophilization. ^{1}H-NMR (400 MHz, D_2O): δ 7.02 (m, 2H, Ar), 6.90 (m, 2H, Ar), 4.95 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1A), 4.78 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4A), 4.76 (br d, 1H, H-4C), 4.55 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1C), 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1B), 4.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1D), 4.29-4.13 (m, 7H, H-2A, H-4D, H-5C, H-6aA, H-6bA, H-6aC, H-6bC), 4.11-3.95 (m, 4H, H-3A, H-5A, H-2C, H-3C), 3.74-3.69 (m, 5H, Me (OMP), H-5D, H-4B), 3.66-3.61 (m, 2H, H-3D, H-5B), 3.54 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, H-3B), 3.36 (m, 2H, H-2B, H-2D), 1.96-1.95 (2s, 6H, NHAc); ^{13}C-NMR (100 MHz, D_2O, selected data from HSQC experiment): δ 118.4, 115.0 (Ar), 103.4 (C-1B, C-1D), 101.5 (C-1C), 100.5 (C-1A), 82.0 (C-4B), 78.5 (C-4D), 76.8 (C-5B), 75.8 (C-4A, C-4C), 75.6 (C-5D), 74.7 (C-3A, C-3C), 73.9 (C-3D), 73.8 (C-3B), 72.3 (C-5C), 72.1 (C-5A), 71.9 (C-2B, C-2D), 67.8, 67.4 (C-6A, C-6C), 55.4 (Me (OMP)), 51.2 (C-2A, C-2C), 22.1 (NHAc); ESI MS: *m/z*: calcd for $C_{35}H_{45}N_2O_{39}Na_3S_5$: 673.0; found: 672.7 [*M*-4Na+2H]$^{2-}$.

Compound **14**. ^{1}H-NMR, 400 MHz, $CDCl_3$

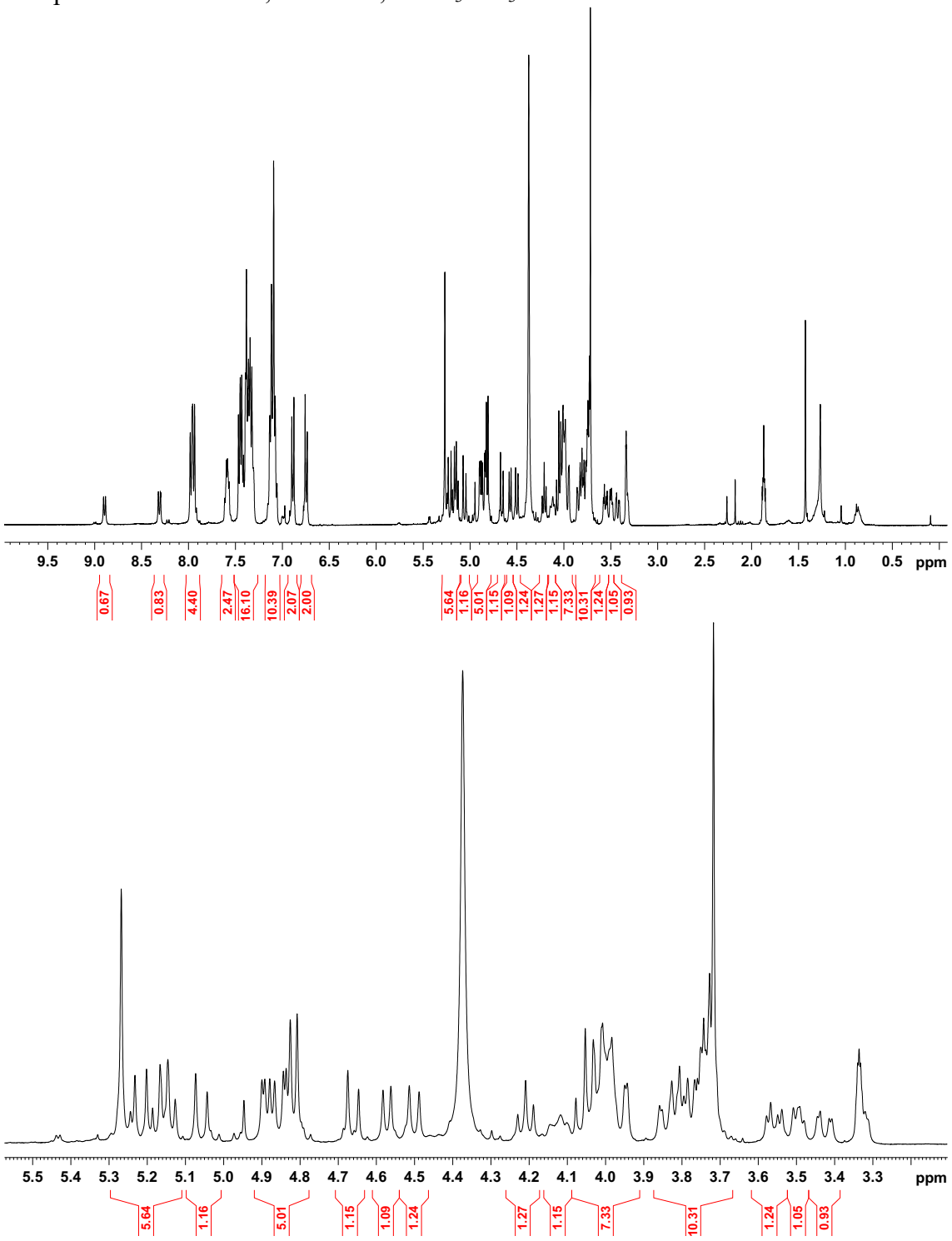


Compound **14**. ^{13}C-NMR, 400 MHz, $CDCl_3$

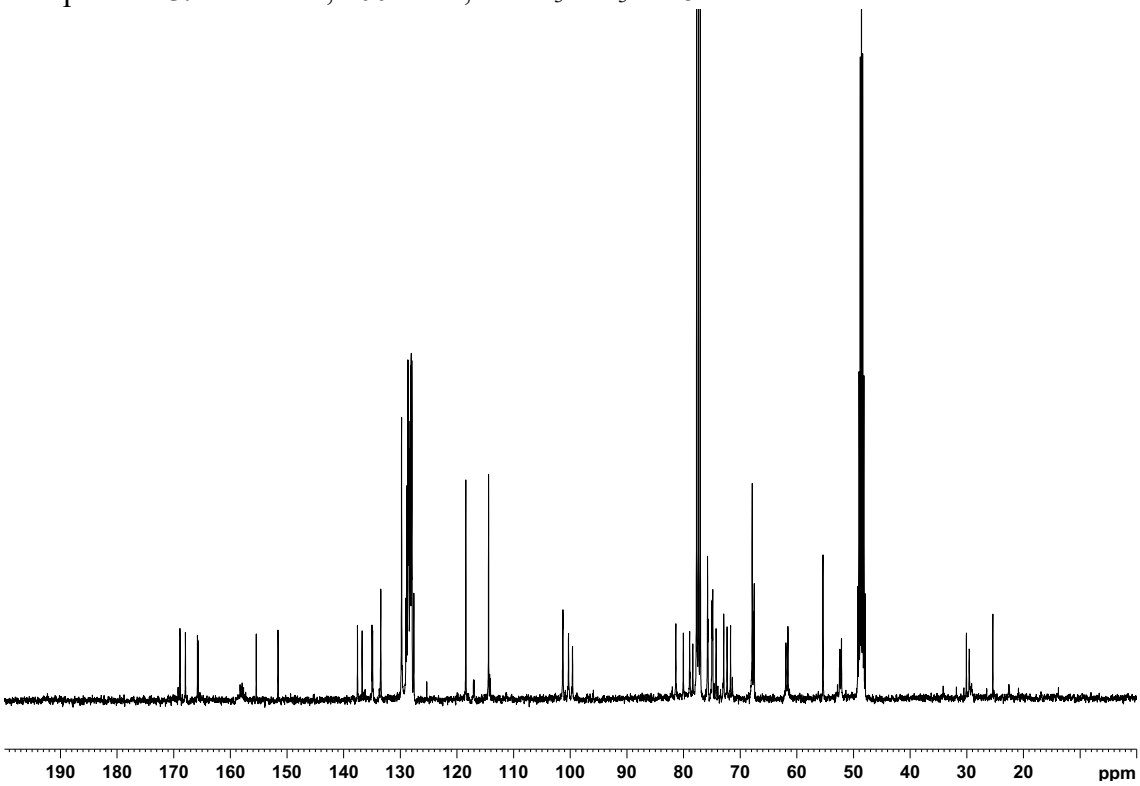


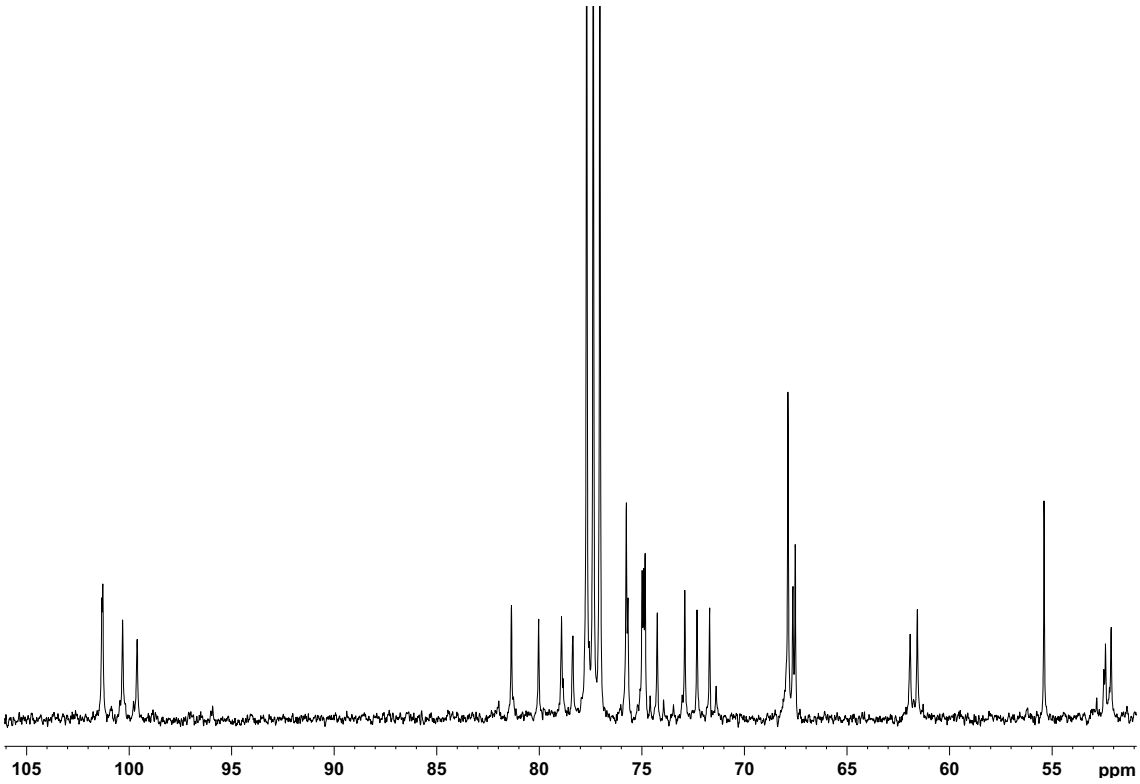


Compound **15.** ^{1}H-NMR, 400 MHz, $CDCl_3/CD_3OD$ 5:2

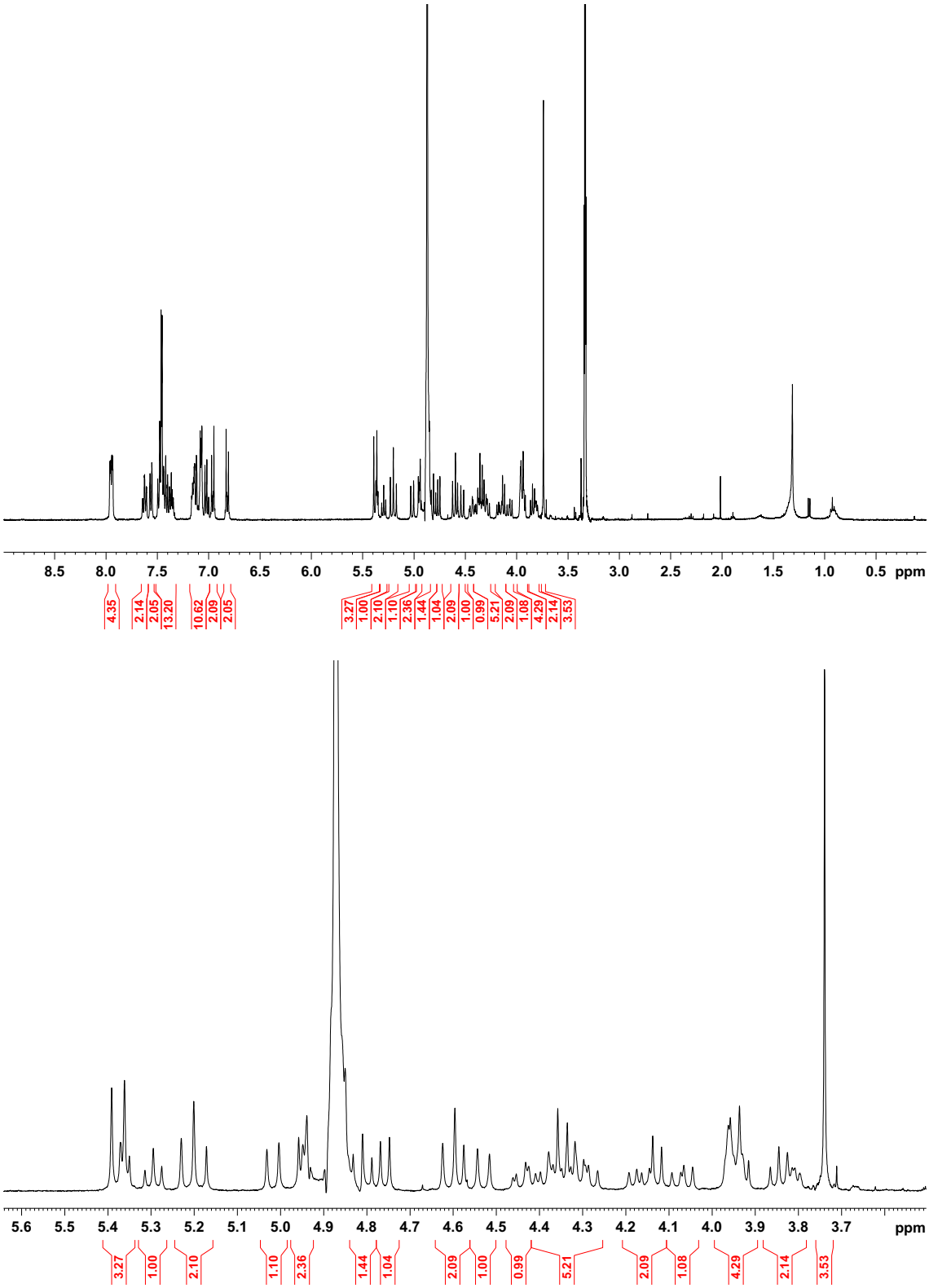


Compound **15.** ^{13}C-NMR, 400 MHz, $CDCl_3/CD_3OD$ 5:2

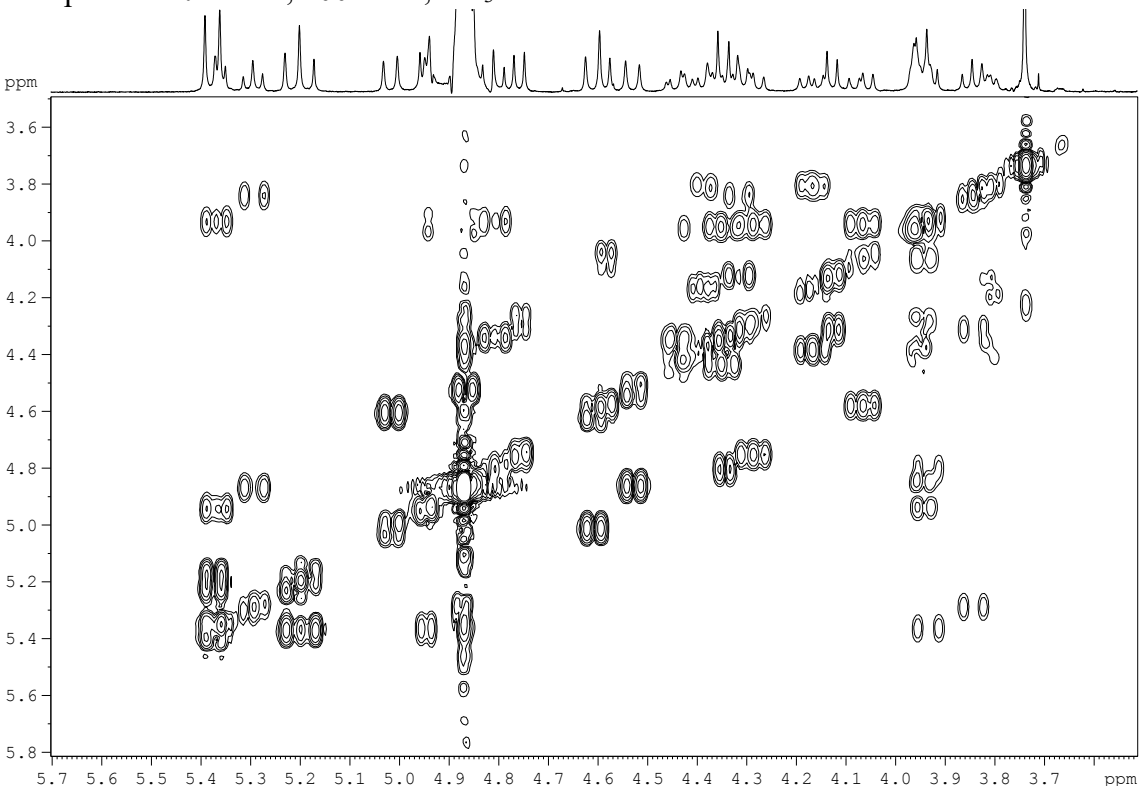




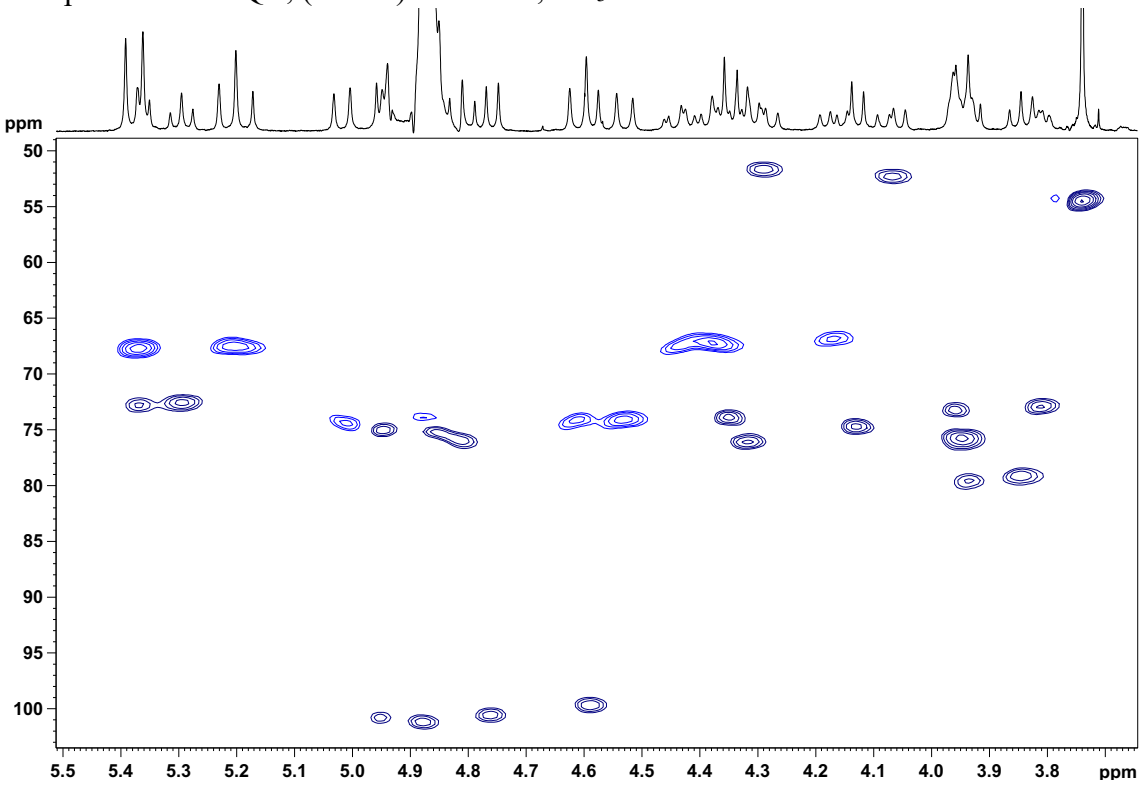
Compound **12.** ^{1}H-NMR, 400 MHz, CD_3OD



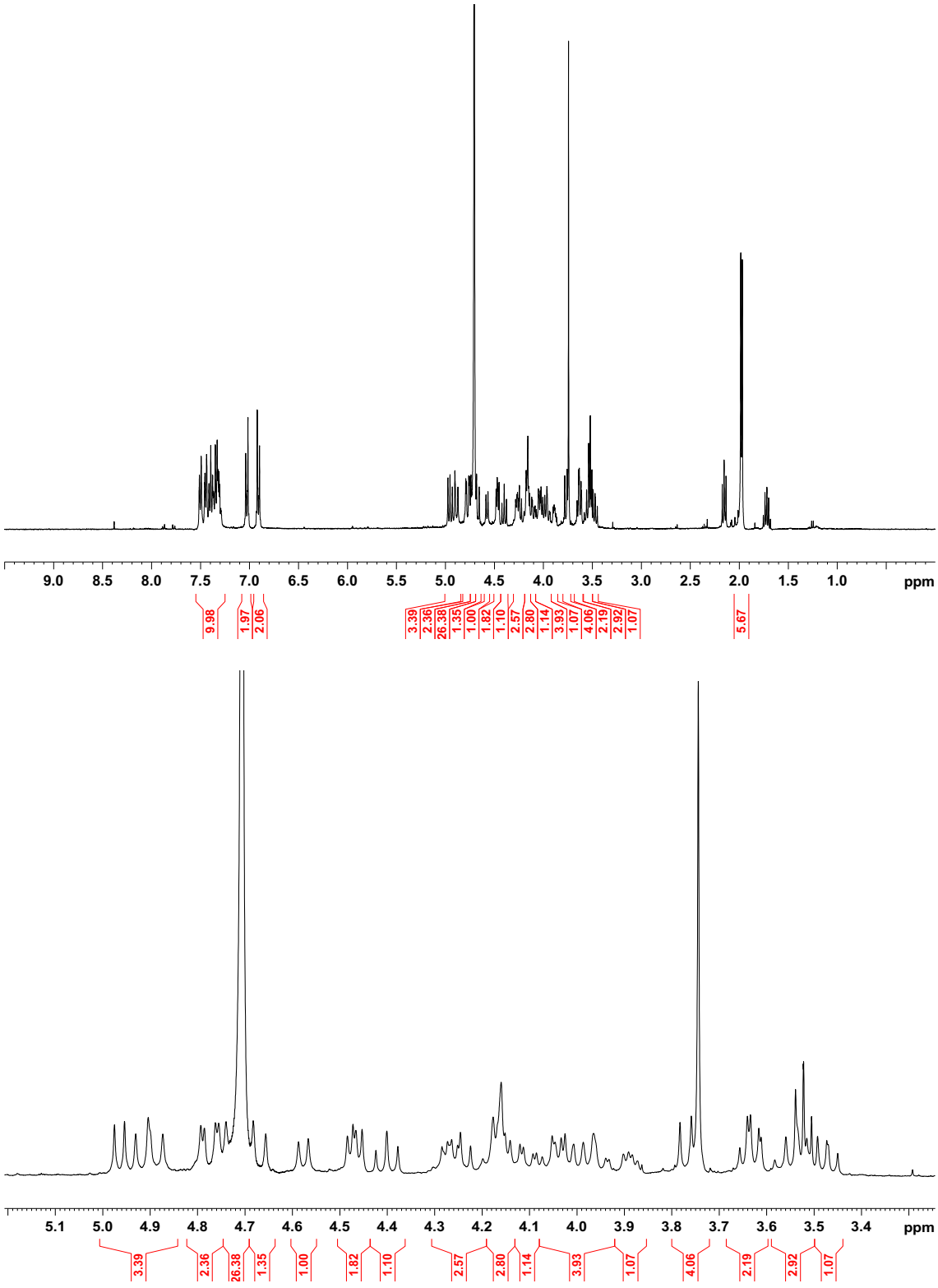
Compound **12.** COSY, 400 MHz, CD_3OD



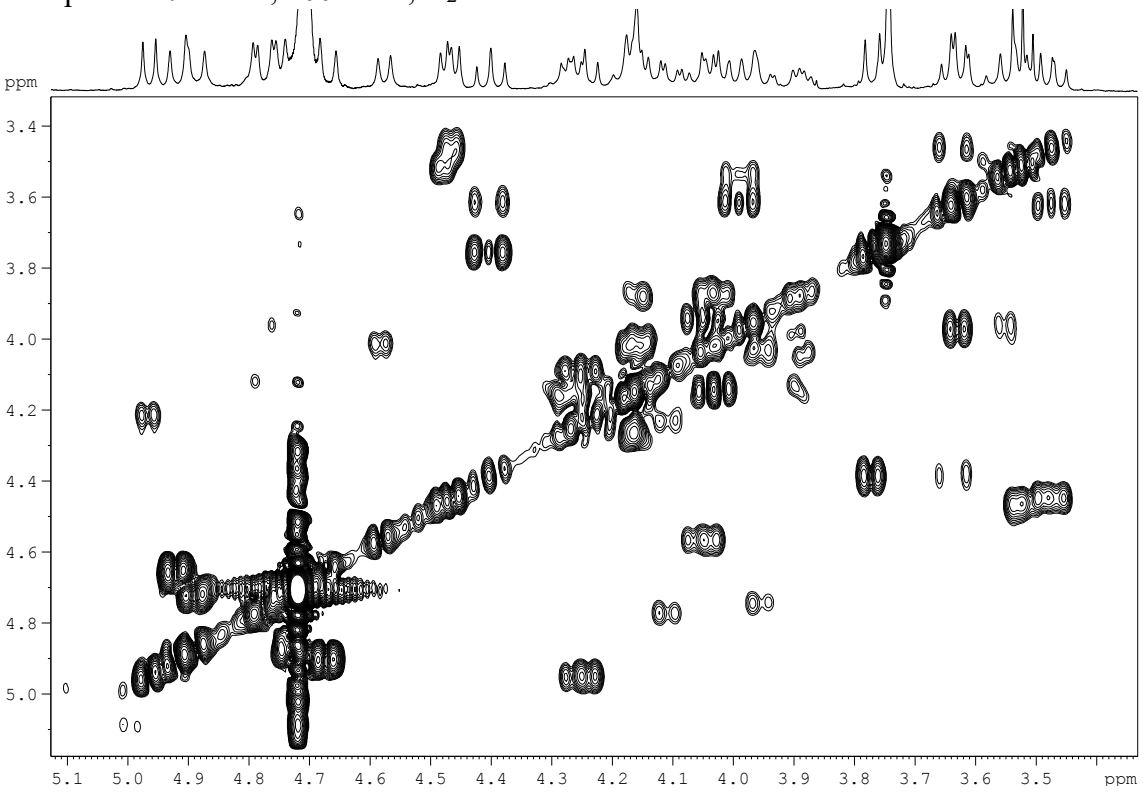
Compound **12.** HSQC, (Edited) 400 MHz, CD_3OD



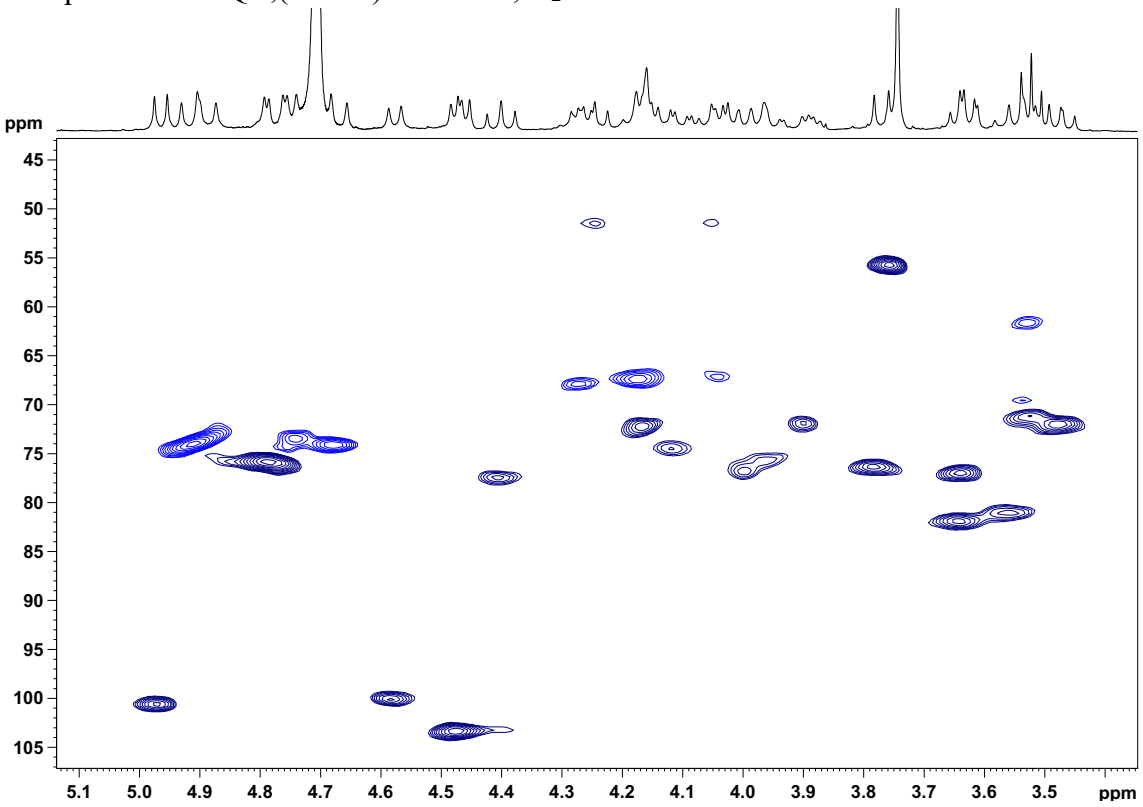
Compound **7.** ^{1}H-NMR, 400 MHz, D_2O



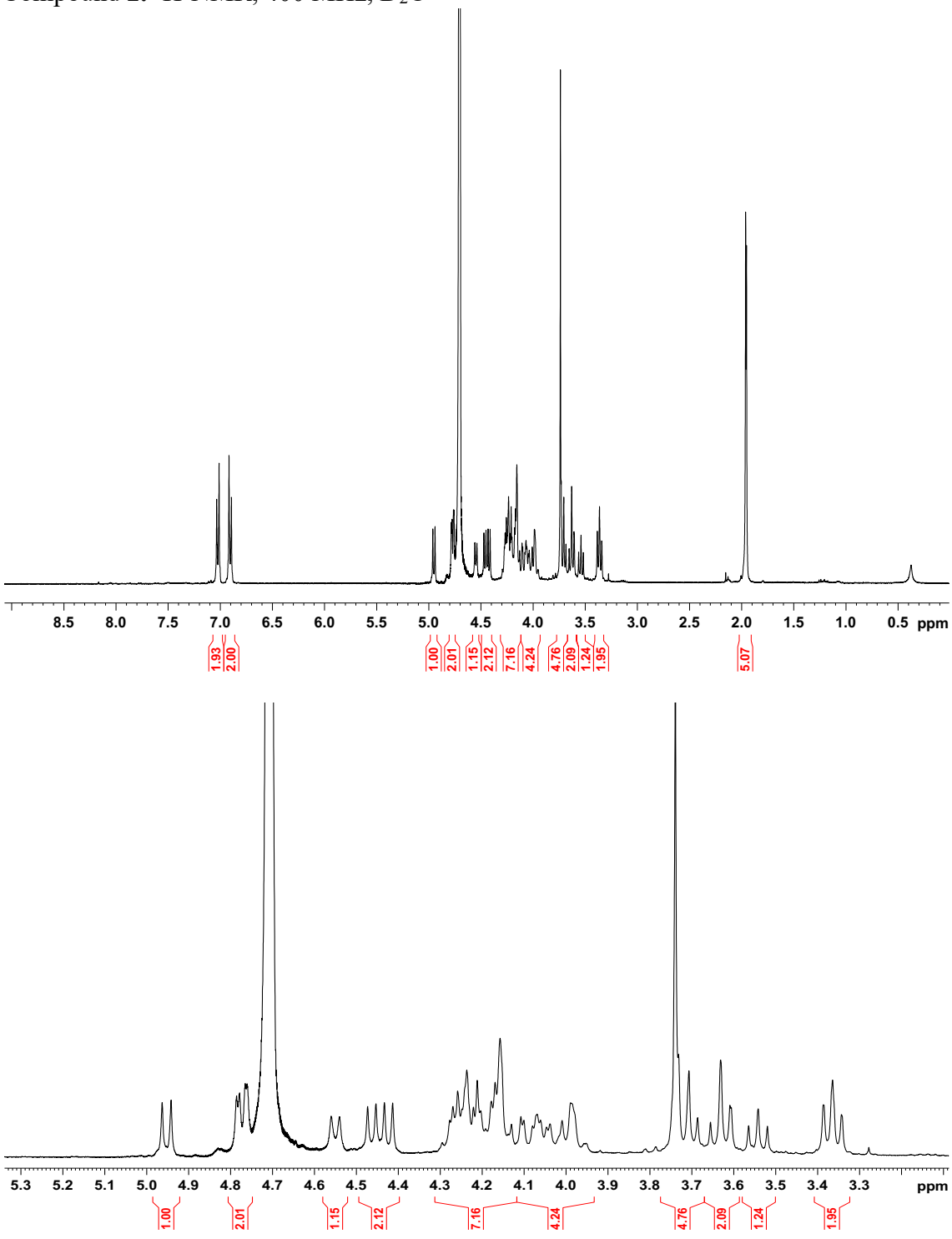
Compound **7.** COSY, 400 MHz, D_2O



Compound **7.** HSQC,(Edited) 400 MHz, D_2O



Compound **2.** ^{1}H-NMR, 400 MHz, D_2O



Fluorescence polarization competition experiments

In order to calculate the binding affinities of tetrasaccharides **2**, **7** and **12** for midkine, expressed as IC_{50} values, fluorescence polarization competition experiments were run as previously reported.[2-5] Thus, we recorded the fluorescence polarization from wells containing 20 µL of a 125 nM midkine solution and 10 µL of a 40 nM probe solution (a fluorescein labelled heparin-like hexasaccharide) in the presence of 10 µL of tetrasaccharide solutions with different concentrations. The measurements were performed in 384-well microplates (from Corning), using a TRIAD multimode microplate reader (from Dynex) with excitation and emission wavelengths of 485 and 535 nm, respectively. The mean polarization values of three replicates were plotted against the logarithm of tetrasaccharide concentration and the resulting curve was fitted to the equation for a one-site competition: $y = A_2 + (A_1-A_2)/[1+10^{(x-\log IC_{50})}]$ where A_1 and A_2 are the maximal and minimal values of fluorescence polarization, and IC_{50} is the tetrasaccharide concentration required for 50% inhibition. At least two independent experiments were carried out for each IC_{50} calculation.

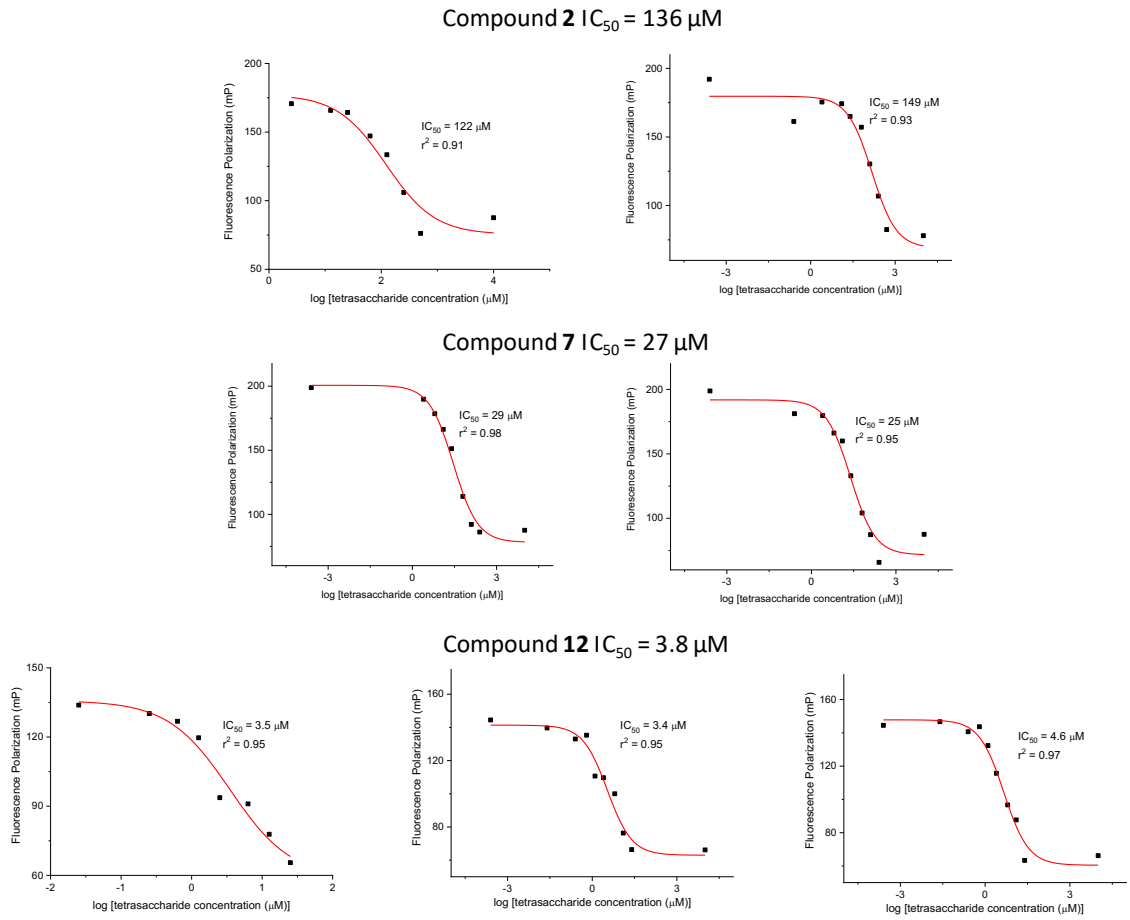


Figure S2. Inhibition curves showing the ability of compounds **2**, **7** and **12** to inhibit the interaction between midkine and the fluorescent probe. All the fluorescence polarization values are the average of three replicate wells. The reported IC_{50} values (Table 1, main text) represent the mean of these two (or three) independent experiments.

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NMR / MD:

	$^3J_{HH}$ (exptal) (Hz)							$^3J_{HH}$ (MD) (Hz)						$^3J_{HH}$ (tar-MD) (Hz)						
	1	**2**	**6**	**7**	**9**	**11**	**12**	**1**	**2**	**6**	**7**	**9**	**12**	**1**	**2**	**6**	**7**	**9**	**11**	**12**
H1A / H2A	7.94	8.60	6.70	8.60	8.50	8.00		7.18	8.20	5.70	8.20	8.20	8.20	7.50	8.10	7.60	8.20	8.2		8.20
H2A / H3A		9.70	9.00	10.80		7.80	10.25	8.43	10.50	6.70	10.40	10.4	10.40	8.31	10.40	9.10	10.50	10.4		10.30
H3A / H4A		3.10	8.40	3.10	3.50	7.90		8.61	2.50	6.50	2.60	2.60	3.00	8.41	2.50	8.50	2.60	2.8		3.00
H4A / H5A			9.40	-				9.91	1.00	7.80	1.00	1	1.00	10.00	1.00	9.80	1.00	0.9		1.00
H1B / H2B	7.59	8.00	8.30	7.40		8.40	8.20	8.21	7.30	8.30	4.60	6.5	7.80	8.22	7.40	8.30	8.30	7.5		7.80
H2B / H3B		9.00	10.40	9.20	8.30	11.10	8.20	10.49	8.50	10.40	5.60	9.30	8.60	10.50	8.60	10.40	10.00	8.9		8.90
H3B / H4B		9.00	2.70	9.20	8.70		8.30	2.54	8.60	2.80	5.80	7.90	8.50	2.52	8.70	2.80	9.10	8.6		8.90
H4B / H5B		9.90	2.70	9.60	10.40		8.64	1.06	9.90	1.10	6.00	8.70	10.00	1.05	10.00	1.10	1.10	9.9		10.10
H1C / H2C	7.84	8.50	7.70	8.80		7.60	8.20	7.24	8.20	7.30	8.22	8.20	8.30	7.69	8.20	7.50	8.30	8.3		8.30
H2C / H3C	8.66	10.90	8.50	10.90	7.80	6.25		8.50	10.50	8.70	10.30	10.50	10.50	8.67	10.50	8.90	10.40	10.5		10.40
H3C / H4C	8.99	2.10	8.80	2.10	2.20	6.80		8.66	2.50	8.40	3.00	2.60	2.40	8.58	2.40	8.60	3.10	2.5		3.40
H4C / H5C	9.73	-	9.90	-	1.20			9.91	1.07	9.70	1.30	0.90	1.00	9.99	1.00	9.90	1.10	1.5		1.50
H1D / H2D	7.21	8.00	8.40	7.80	5.60	8.44	8.20	8.25	7.30	8.30	3.80	1.80	3.20	8.27	7.40	8.30	7.40	5.69		7.50
H2D / H3D		9.70	10.80	9.20	9.90	10.00	8.20	10.38	8.40	10.60	3.80	1.30	4.20	10.41	8.50	10.50	8.30	9.59		9.00
H3D / H4D	2.71	9.70	3.10	9.20	6.00	3.02	9.10	2.74	8.50	3.00	4.20	2.80	4.60	2.74	8.50	2.90	8.40	5.31		8.70
H4D / H5D		10.30	3.30	9.60		6.20	9.80	1.12	9.90	1.30	2.00		3.70	1.14	9.90	1.20	9.90			9.90

Table S1. $^3J_{HH}$ **1, 2, 6, 7, 9, 11, 12**: experimental, averaged along MD simulations without restriction or with time-averaged restrains.

	1[a]			**2**			**3**[a]			**4**[a]			**5**[a]			**6**			**7**			**9**			**11**		**12**[b]	
	Exp	MD	tar-MD	Exp	MD	tar-MD	Exp	MD	tar-MD	Exp	MD	tar-MD	Exp	MD	tar-MD	expt	MD	tar-MD	Exp	MD	tar-MD	Exp	MD	tar-MD	Exp.	tar-MD	Exp	tar-MD
H3A / H1A	2.8	2.8	2.7	2.6	2.7	2.7	2.6	2.6	2.6	2.5	2.8	2.7	2.6	3.2	2.5	2.6	3.2	2.5	2.6	2.7	2.7	2.5	2.7	2.7	3.0	2.0	2.6	3.0
H5A / H1A	2.2	2.5	2.3	2.7	2.6	2.7	2.6	2.6	2.6	2.5	2.5	2.3	2.8	2.8	2.7	2.8	2.8	2.7	2.5	2.6	2.5	2.3	2.6	2.6	3.0	2.4	2.7	2.7
H3A / H5A																										2.4		2.8
HxA / H1B		2.5		2.7	2.5	2.4	2.5	3.0	3.0	2.4	2.5		3.0	2.6	2.5	3.0	2.6	2.5	2.4	2.5	2.4	2.6	2.6	2.8	2.6	2.3	2.6	2.7
H3B / H1B	2.4	2.6	2.6	2.6	2.8	2.8	2.5	2.8	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.4	2.7	2.3	3.0	2.7	2.4	2.2		2.5
H5B / H1B	2.4	2.6	2.6	2.9	2.6	2.5	2.2	2.6	2.4	2.3	2.6	2.6		2.6	2.6		2.6	2.6	2.7	3.1	2.5	2.4	2.6	2.5	2.4	2.4	2.9	3.0
H3B / H5B																										2.3		3.1
HxB / H1C	2.3	2.5	2.4	2.9	2.4	2.3	2.2	2.3	2.3		2.4	2.4	2.8	2.6	2.6	2.8	2.6	2.6	2.9	2.9	3.6				2.5	2.4	3.4	2.5
H3C / H1C	2.5	2.7	2.6	2.6	2.6	2.6					2.7	2.6	2.6	2.8	2.7	2.6	2.8	2.7		2.7	2.6				4.4		2.3	2.5
H5C / H1C	2.2	2.5	2.3	2.7	2.6	2.6					2.5	2.4	3.0	2.6	2.5	3.0	2.6	2.5	2.6	2.6	2.5				2.5	3.4	3.7	2.7
H3C / H5C																									4.1			3.1
HxC / H1D	2.3		2.3	2.7	2.5	2.5	2.4	2.4	2.3	2.2		2.3	2.7	2.5	2.5	2.7	2.5	2.5	2.6	2.5						2.3	3.1	2.7
H3D / H1D	2.6	2.6	2.6	2.6	2.8	2.8	2.5	2.4	2.3	2.2	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	3.8	2.8	2.8	4.0		4.2	2.7	2.7	2.7
H5D / H1D	2.5	2.6	2.6	2.9	2.5	2.5	2.5	2.9	2.6		2.6	2.6	2.9	2.5	2.5	2.9	2.5	2.5	3.3	2.5	2.4	4.3	2.4		2.4		2.6	3.50*
H3D / H5D																										2.6		2.7
H2D/ H5D										2.7	2.4	2.4	nd	nd	nd							2.8	nd	2.9				

Table S2. Distances for compounds **1-12**, experimental at 600MHz, from molecular dynamics in explicit solvent (PME) and with time averaged restrains (tar-MD).[a] Partially reported in reference 20.[b] 900MHz

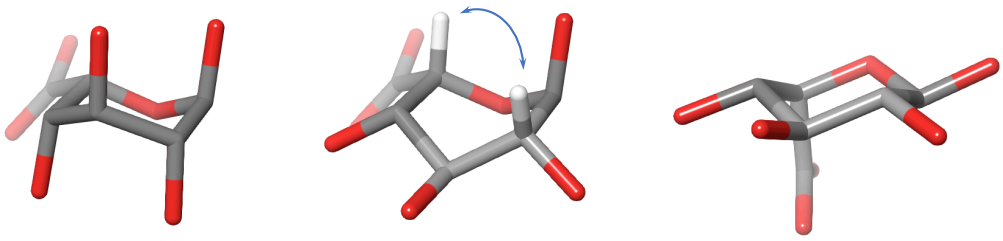

Figure S3. Iduronate main conformations $^{1}C_{4}$, $^{2}S_{O,}$ and $^{4}C_{1}$. The $^{2}S_{O}$ exclusive NOE between protons 2 and 5 is shown

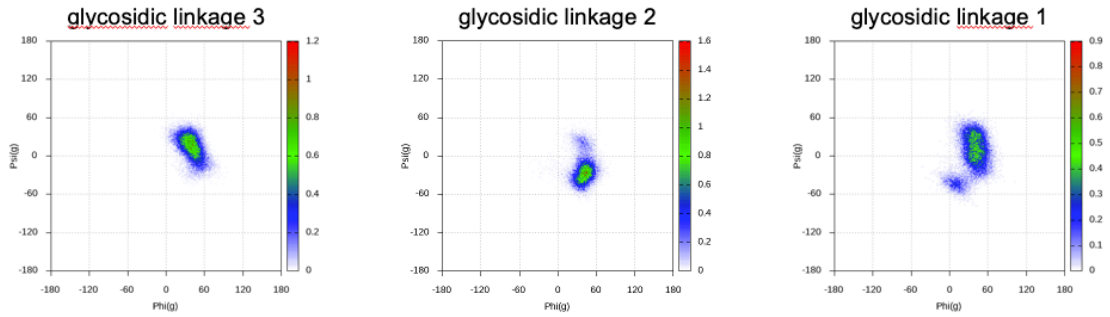

Figure S4. **11** Φ/Ψ Glycosidic maps

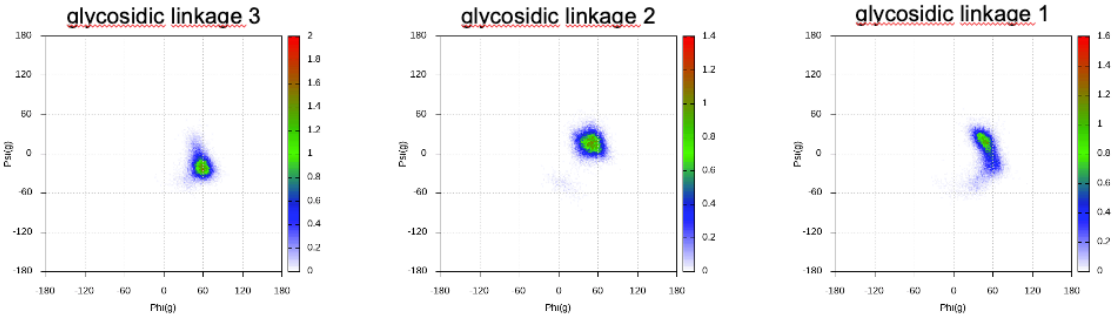

Figure S5. **12** Φ/Ψ Glycosidic maps.

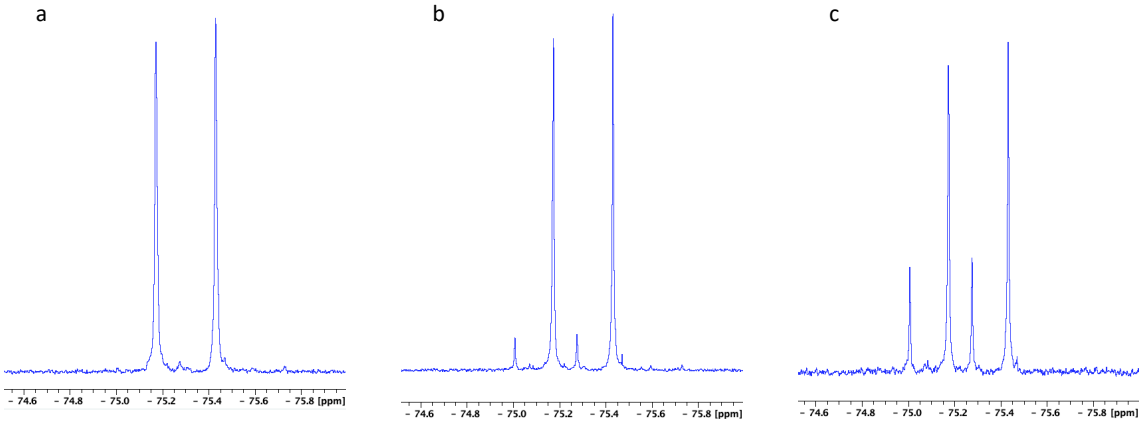

Figure S6. Hydrolysis of **12** benzylic ester at position 6 of GlcA residues, by ^{19}F NMR a) sample recently prepared, b) after two days, and c) after one month.

Note on stability of compound 11.

Compound **11** hydrolyzes faster than **12**, and we could not perform an accurate analysis of the NOESY in the presence of midkine. The signals from the hydrolysis product appear in the spectrum close to the non-hydrolyzed ones, interfering in the spectra. Though, the STD experiments only showed the signals corresponding to **11**, without evidence of the partially hydrolyzed tetrasaccharide. This can be explained if the hydrolyzed tetrasaccharide were a worse binder than the fully protected one. We confirmed that the signals in the difference spectra correspond to **11**, and no evidence for the partially hydrolyzed tetrasaccharide was appreciated in the difference experiment. Therefore, the STD can be assimilated to the binding of **11** to the midkine without the participation of the partially hydrolyzed tetrasaccharide.

ANALYSIS OF BOUND COMPOUNDS

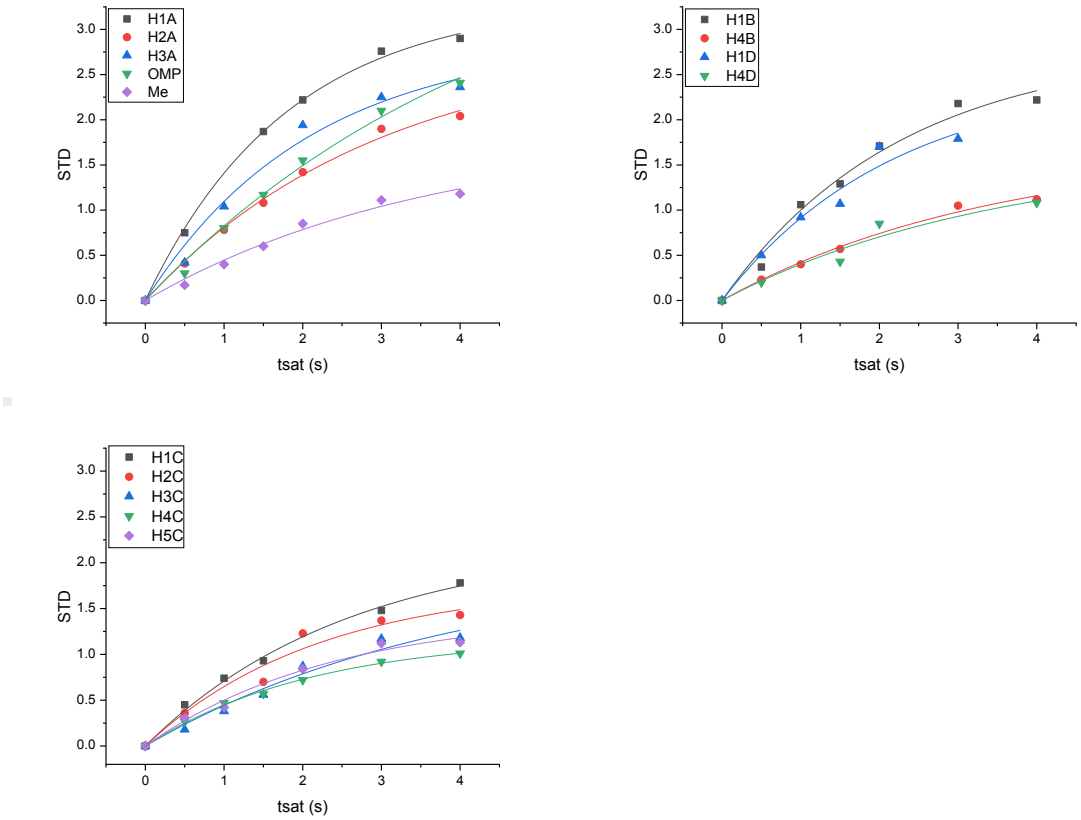

Figure S7. 1 STD growing curves at 600MHz and 298.2 K.

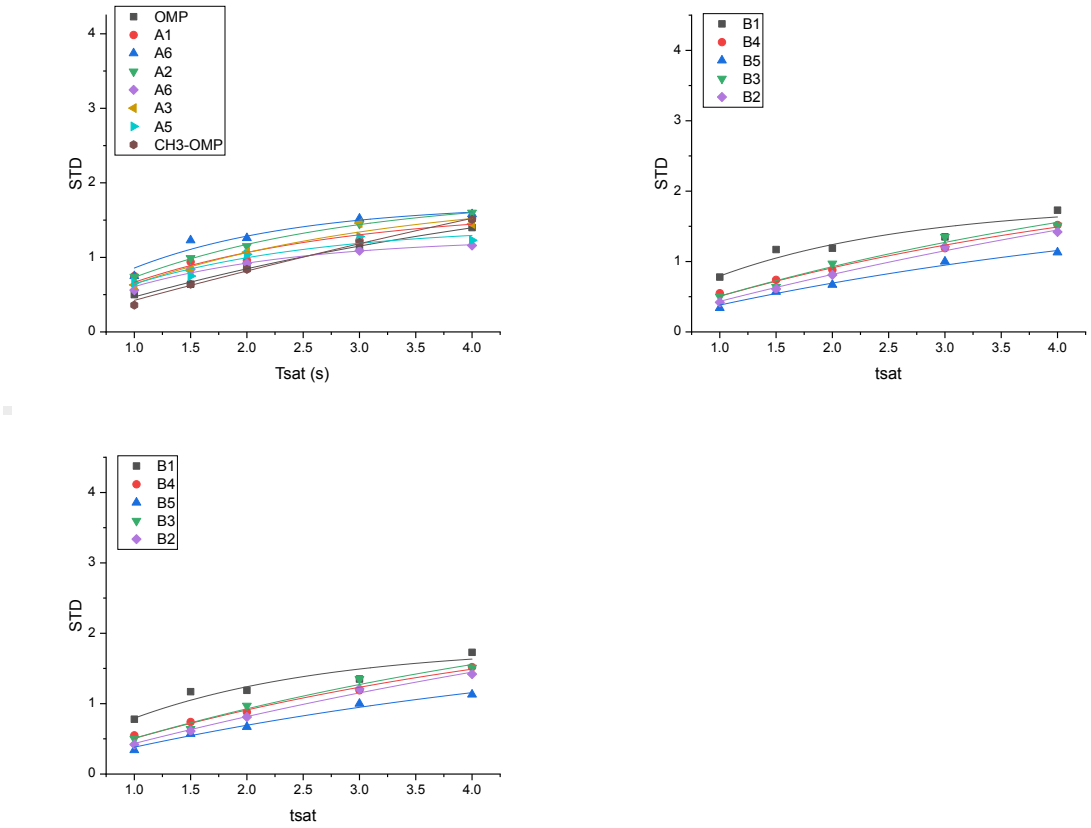

Figure S8. **2** STD growing curves at 600MHz and 298.2 K.

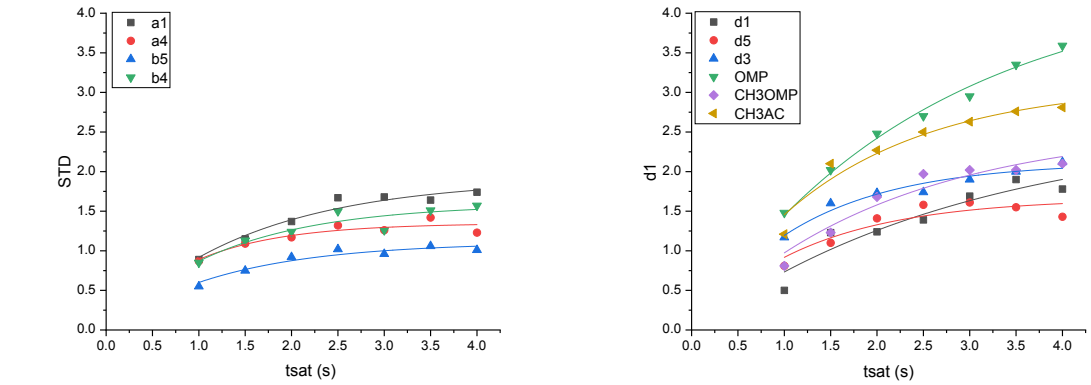

Figure S9. **3** STD growing curves at 600MHz and 298.2 K.

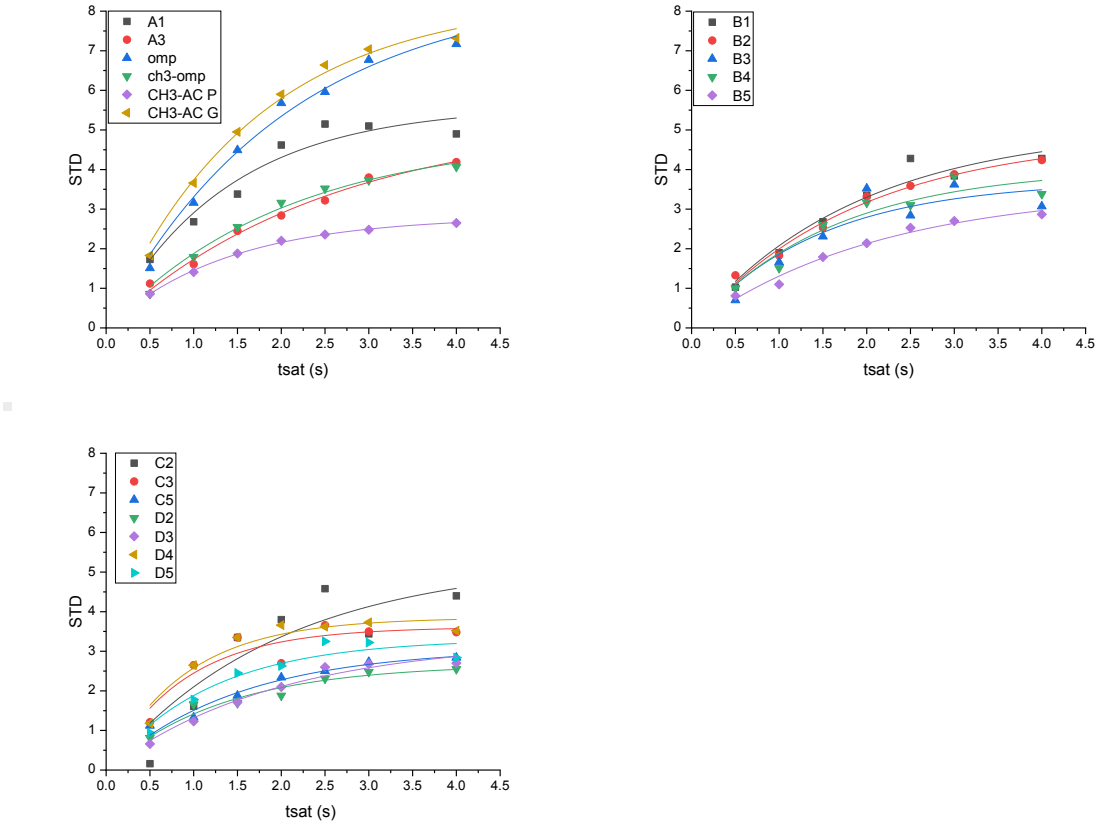

Figure S10. **4** STD growing curves at 600MHz and 298.2 K.

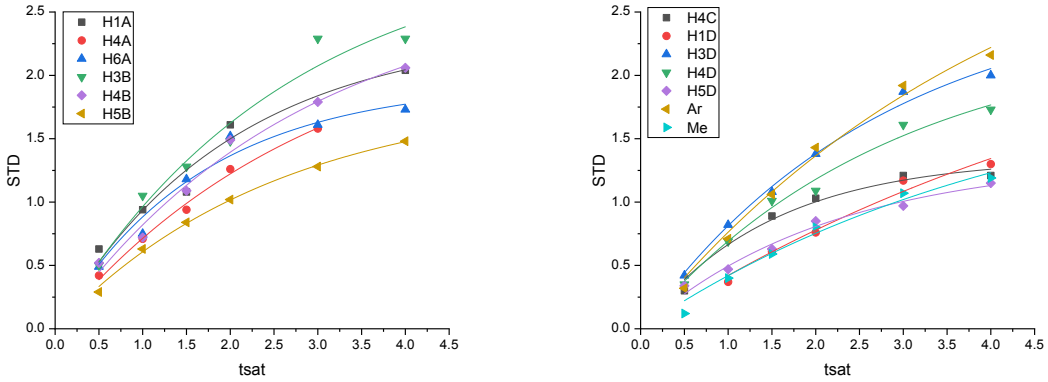

Figure S11. **5** STD growing curves at 600MHz and 298.2 K.

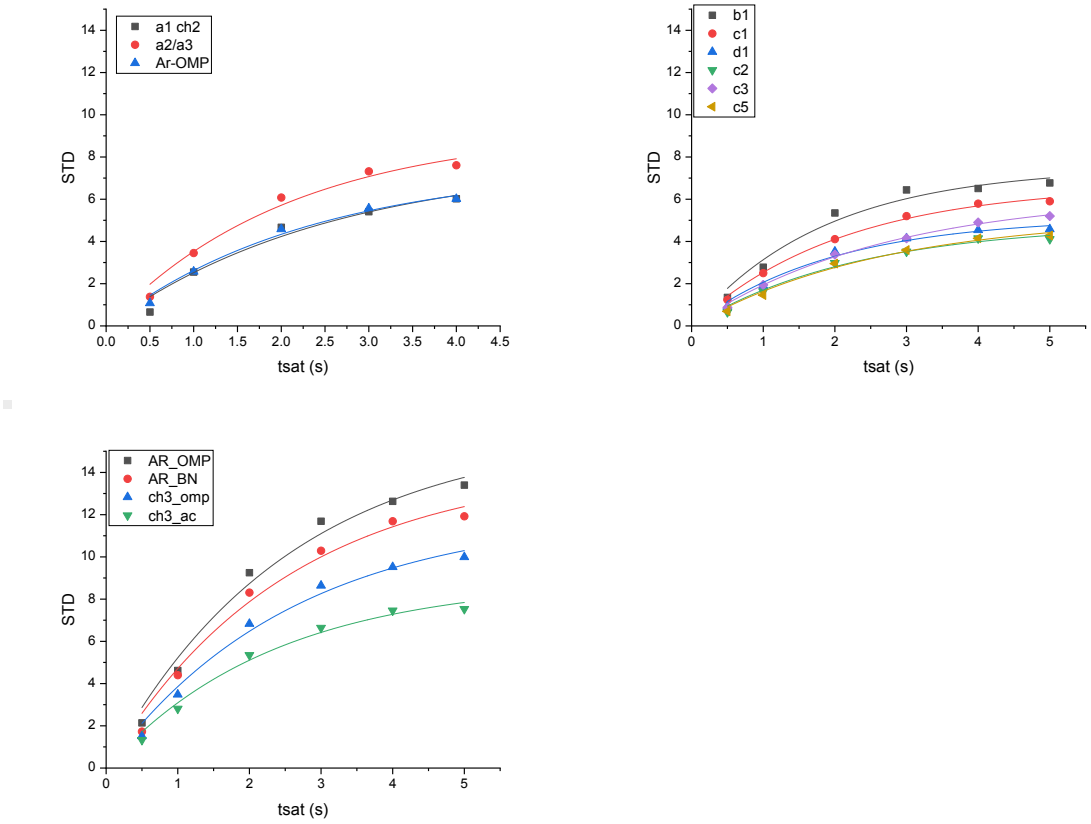

Figure S12. **6** STD growing curves at 600MHz and 298.2 K.

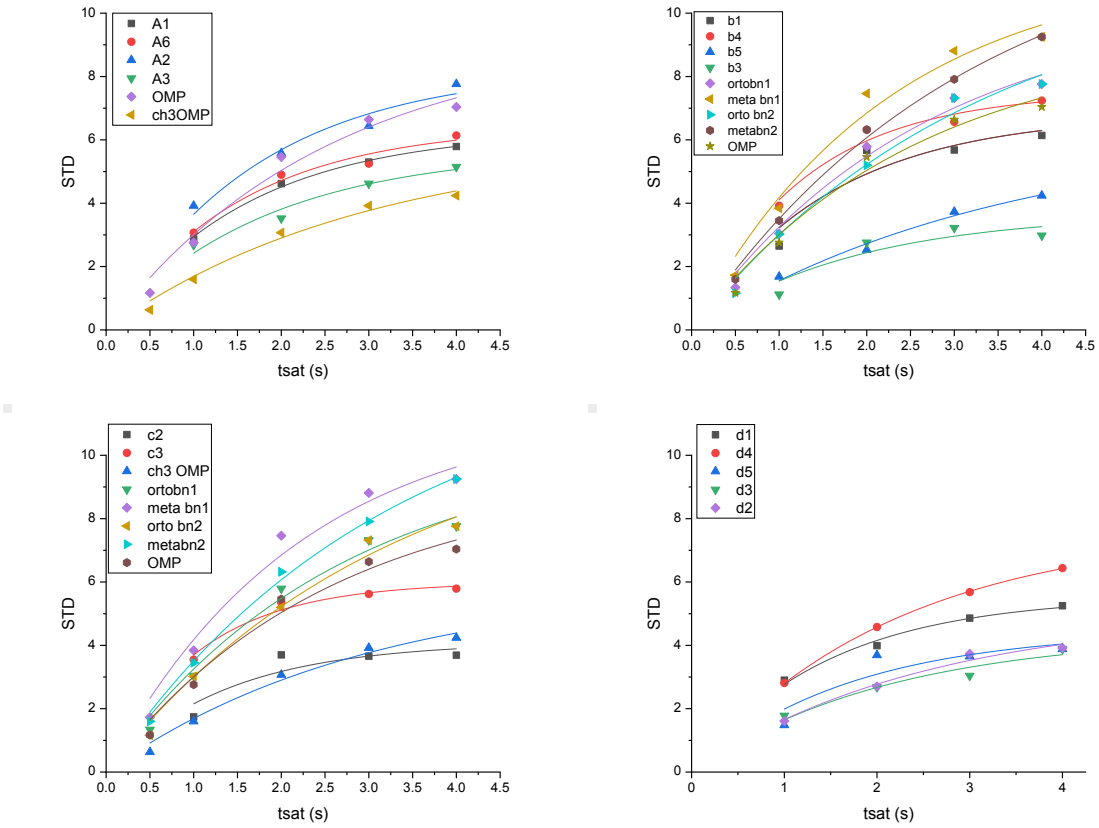

Figure S13. **7** STD growing curves at 600MHz and 298.2 K.

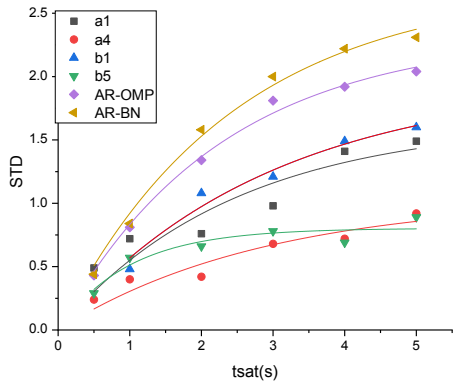

Figure S14. **9** STD growing curves at 600MHz and 298.2 K.

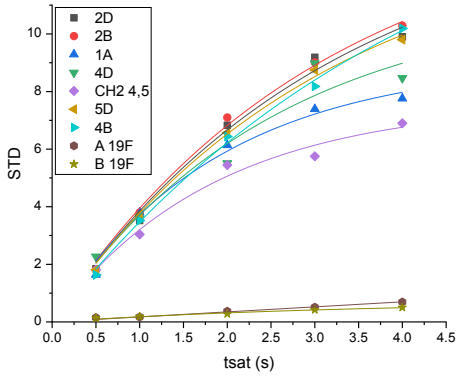

Figure S15. **12** STD growing curves at 600MHz and 298.2 K.

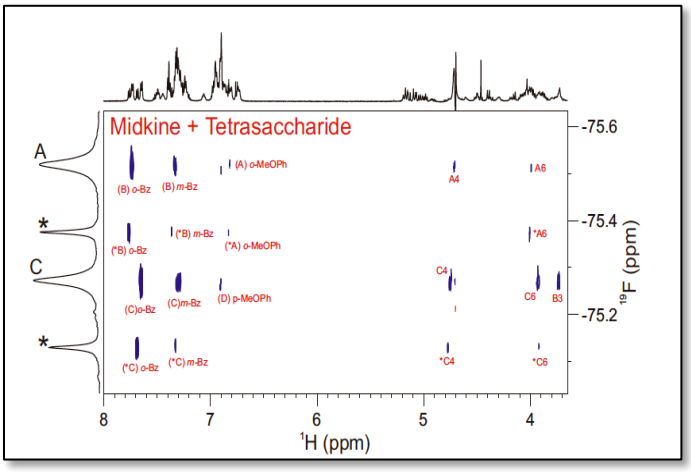

Figure S16. Expansion of transfer ^{19}F HOESY experiment used for the assignment of the ^{19}F signals.

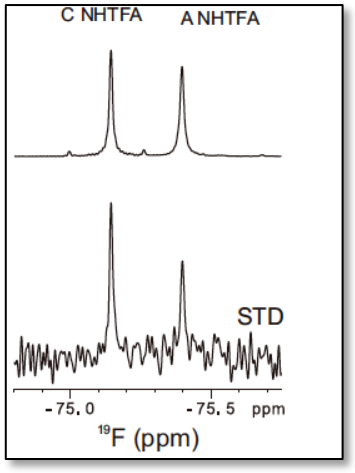

Figure S17. **12** ^{19}F-STD experiment at 600MHz and 298.2 K

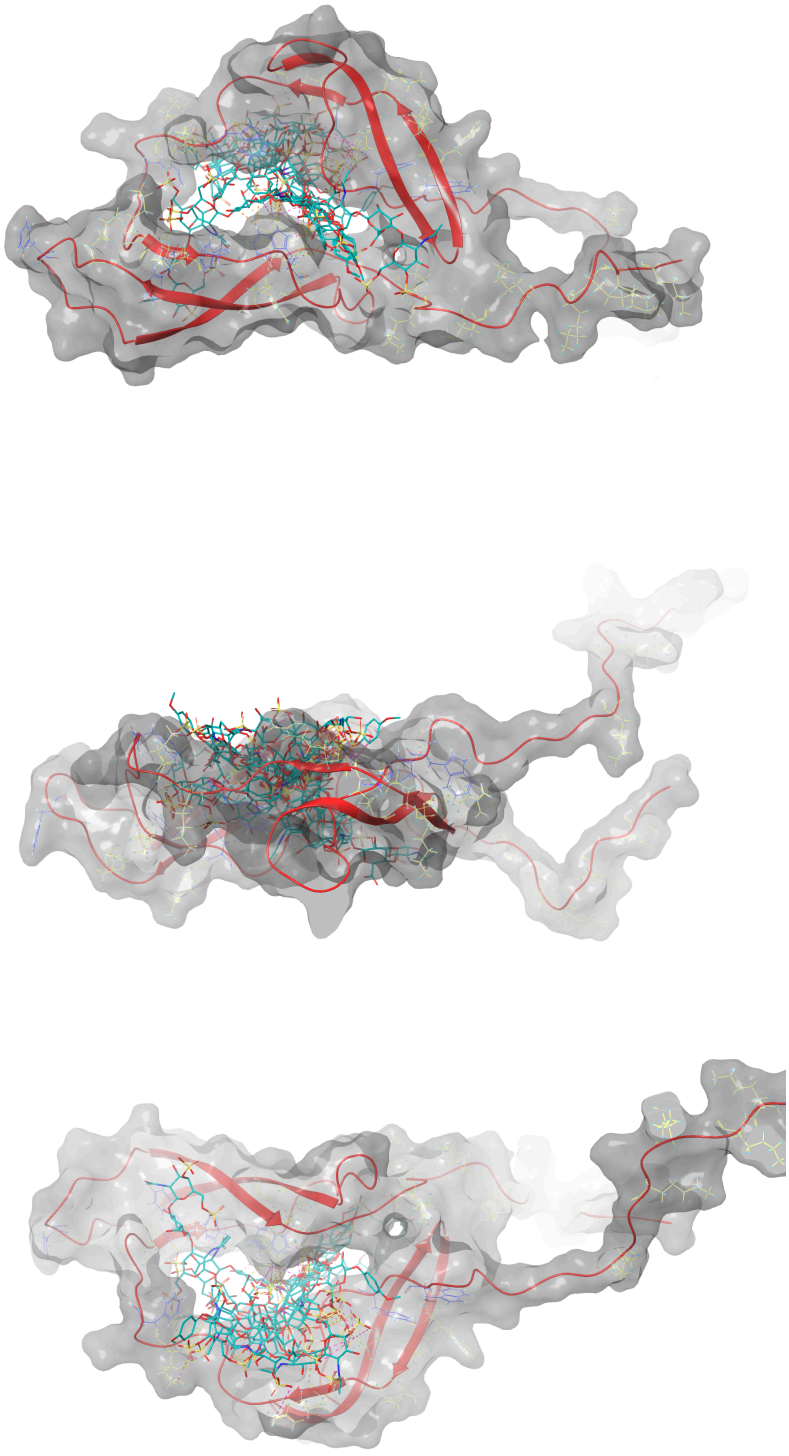

Figure S18. **1** docking structures obtained using Autodock Vina. (orthogonal view)

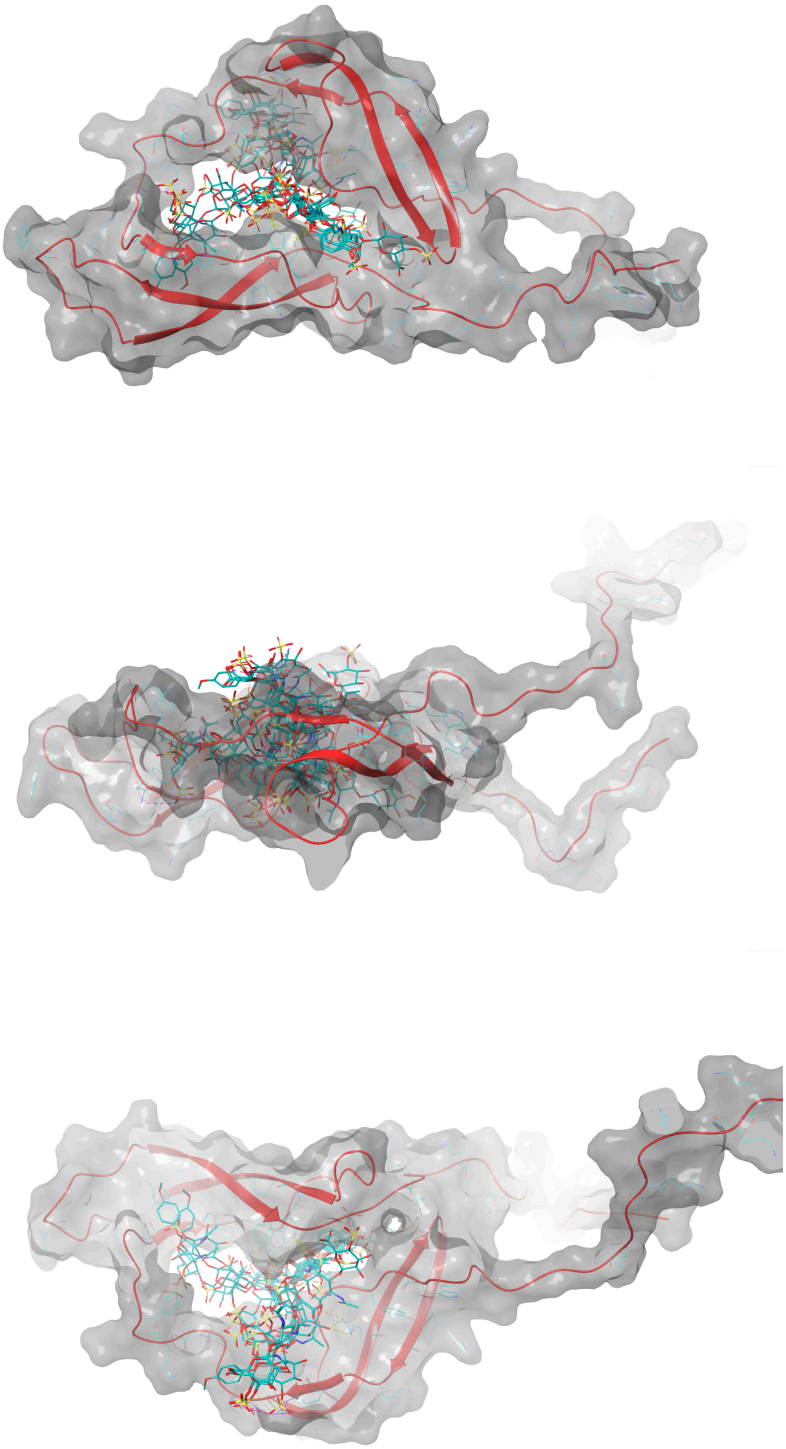

Figure S19. **2** docking structures obtained using Autodock Vina. (orthogonal view)

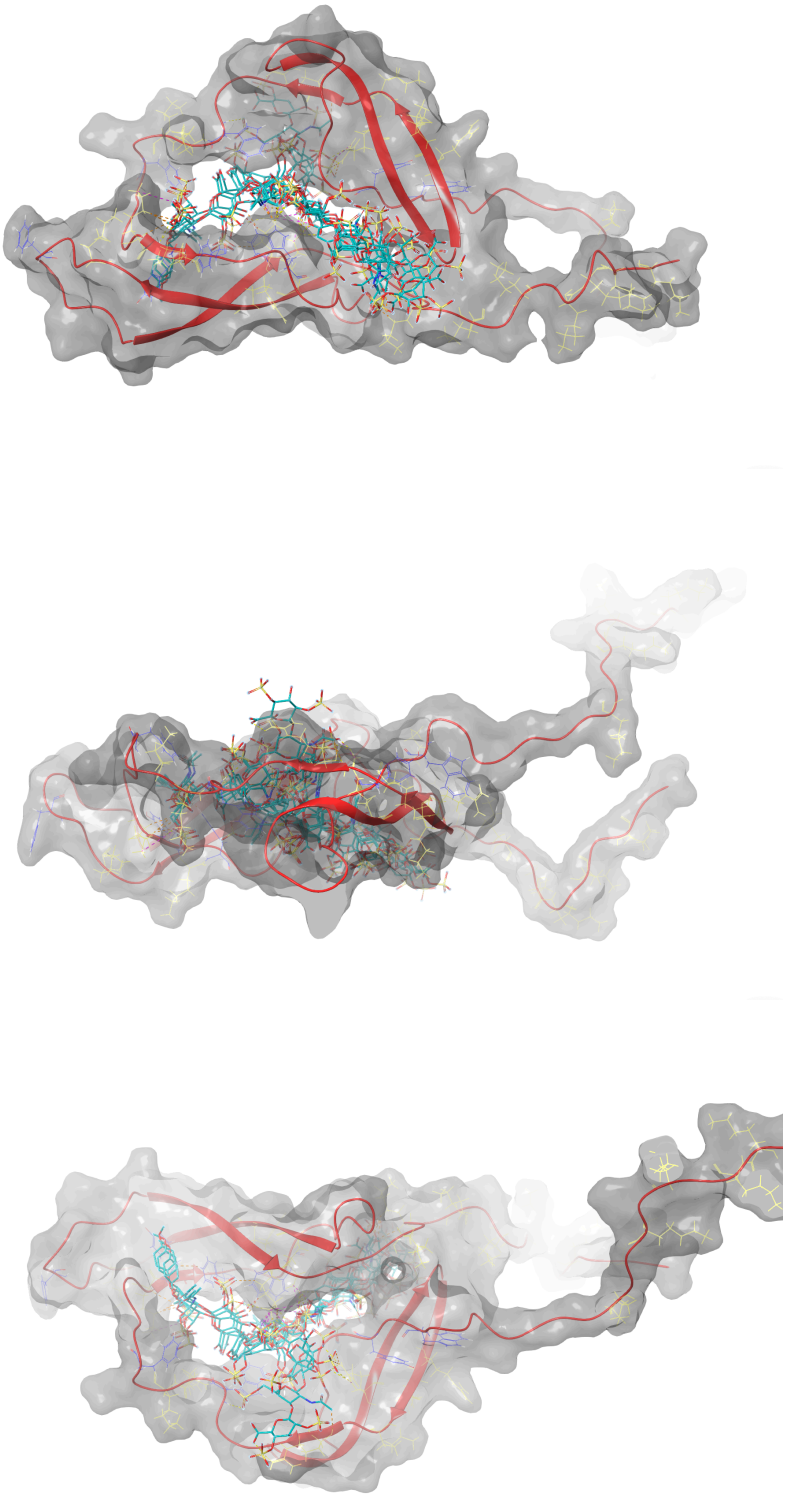

Figure S20. **3** docking structures obtained using Autodock Vina. (orthogonal view)

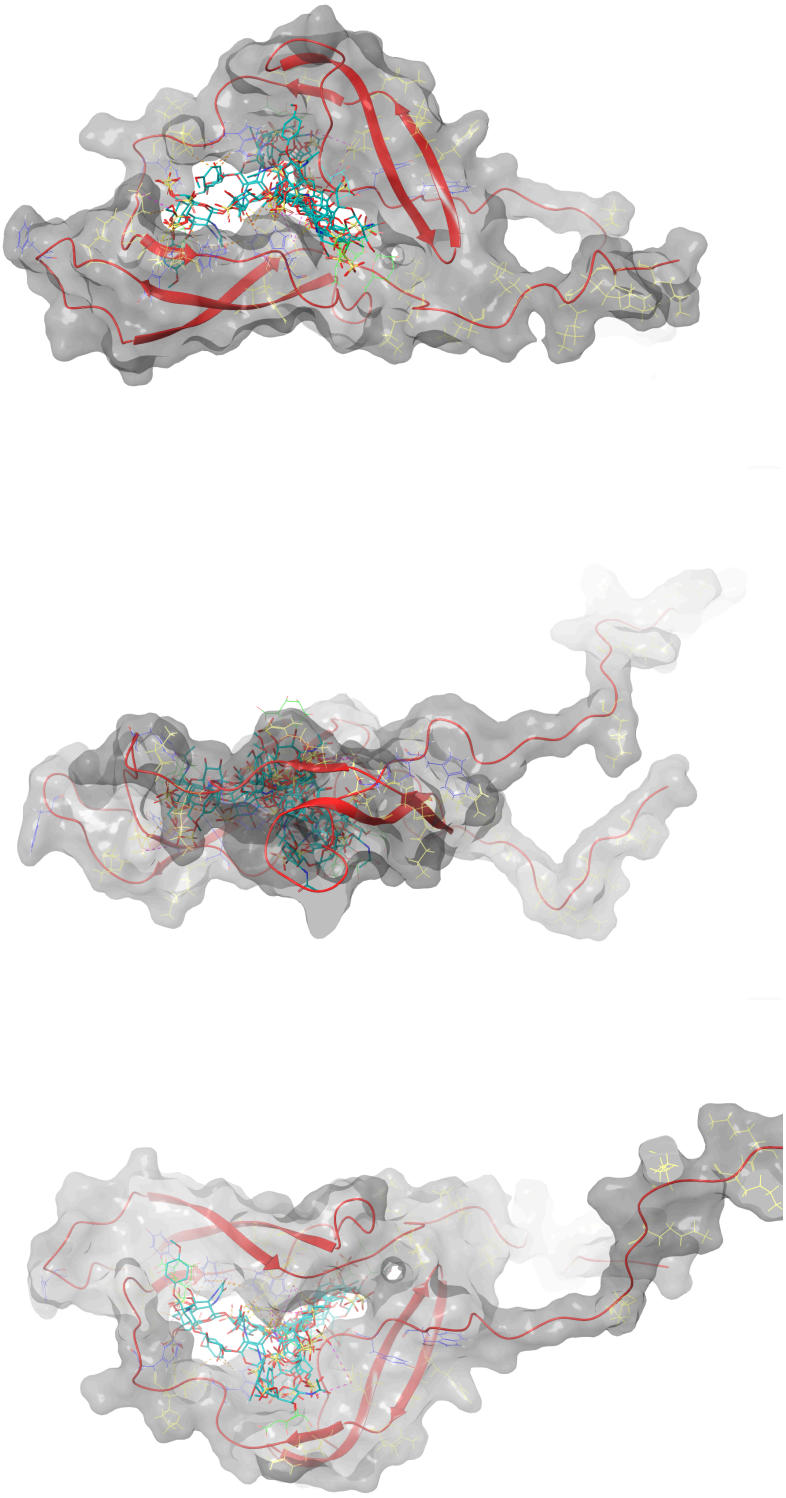

Figure S21. **4** docking structures obtained using Autodock Vina. (orthogonal view)

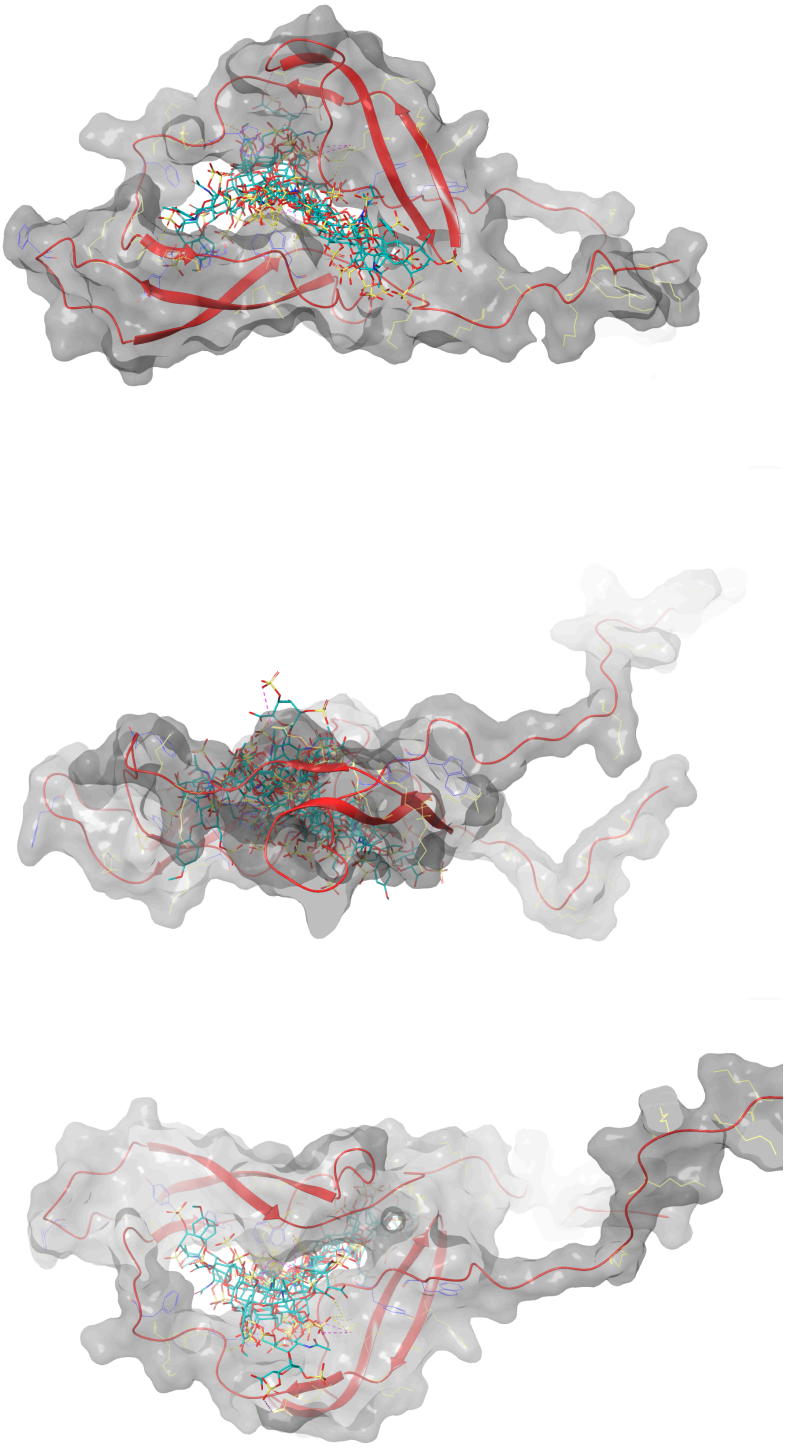

Figure S22. **5** docking structures obtained using Autodock Vina. (orthogonal view)

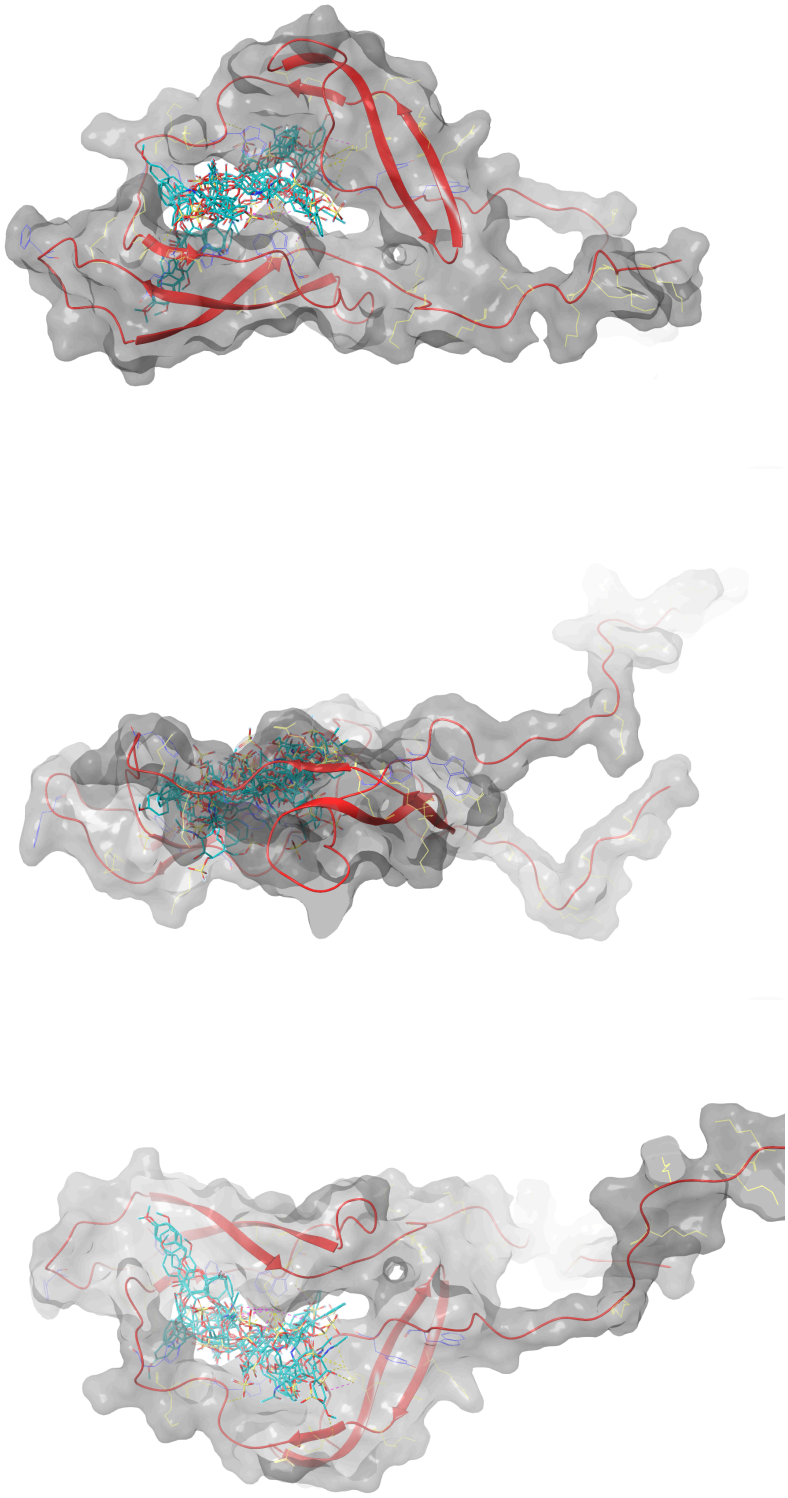

Figure S23. **6** docking structures obtained using Autodock Vina. (orthogonal view)

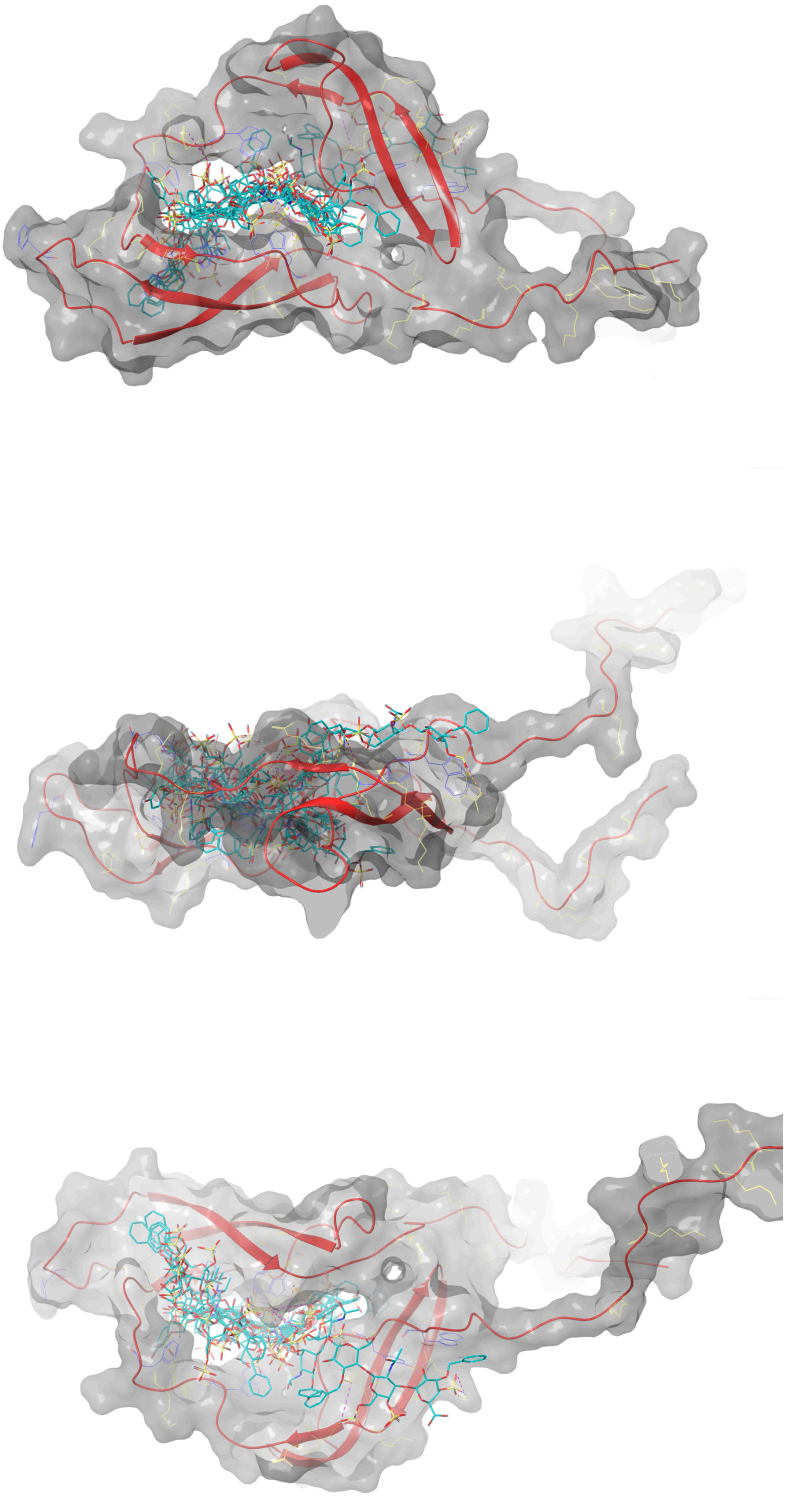

Figure S24. 7 docking structures obtained using Autodock Vina. (orthogonal view)

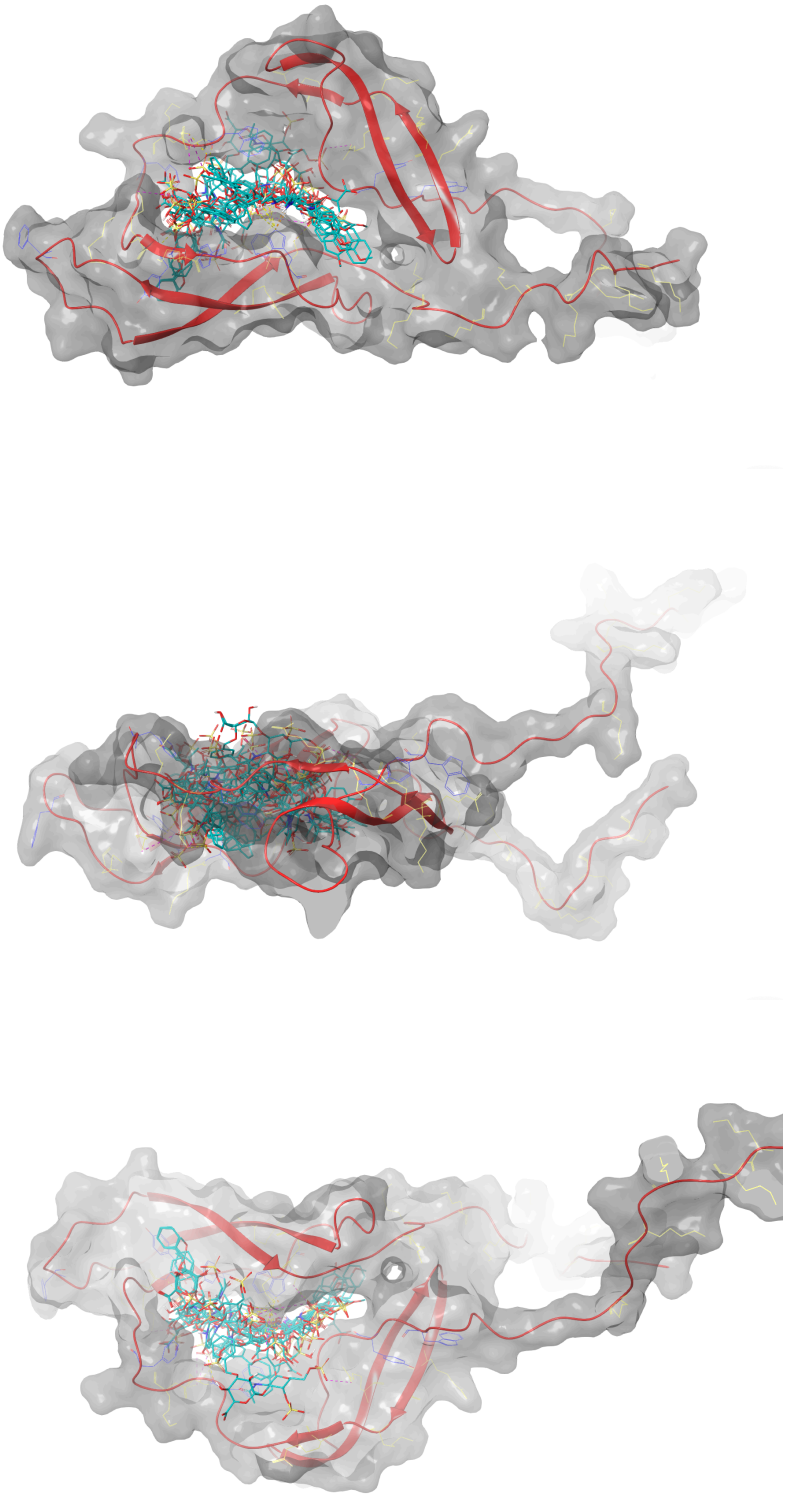

Figure S25. **9** docking structures obtained using Autodock Vina. (orthogonal view)

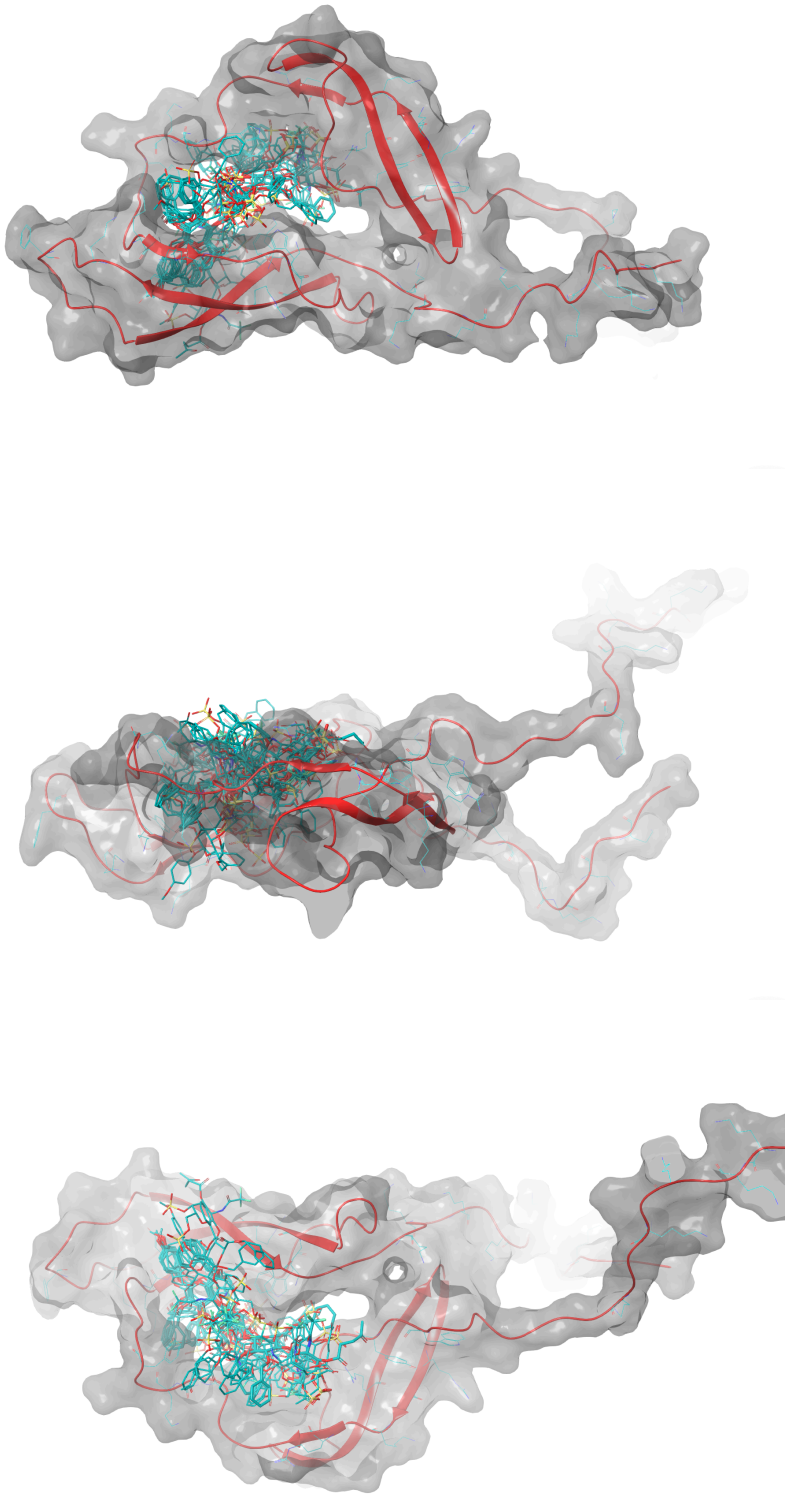

Figure S26. **11** docking structures obtained using Autodock Vina. (orthogonal view)

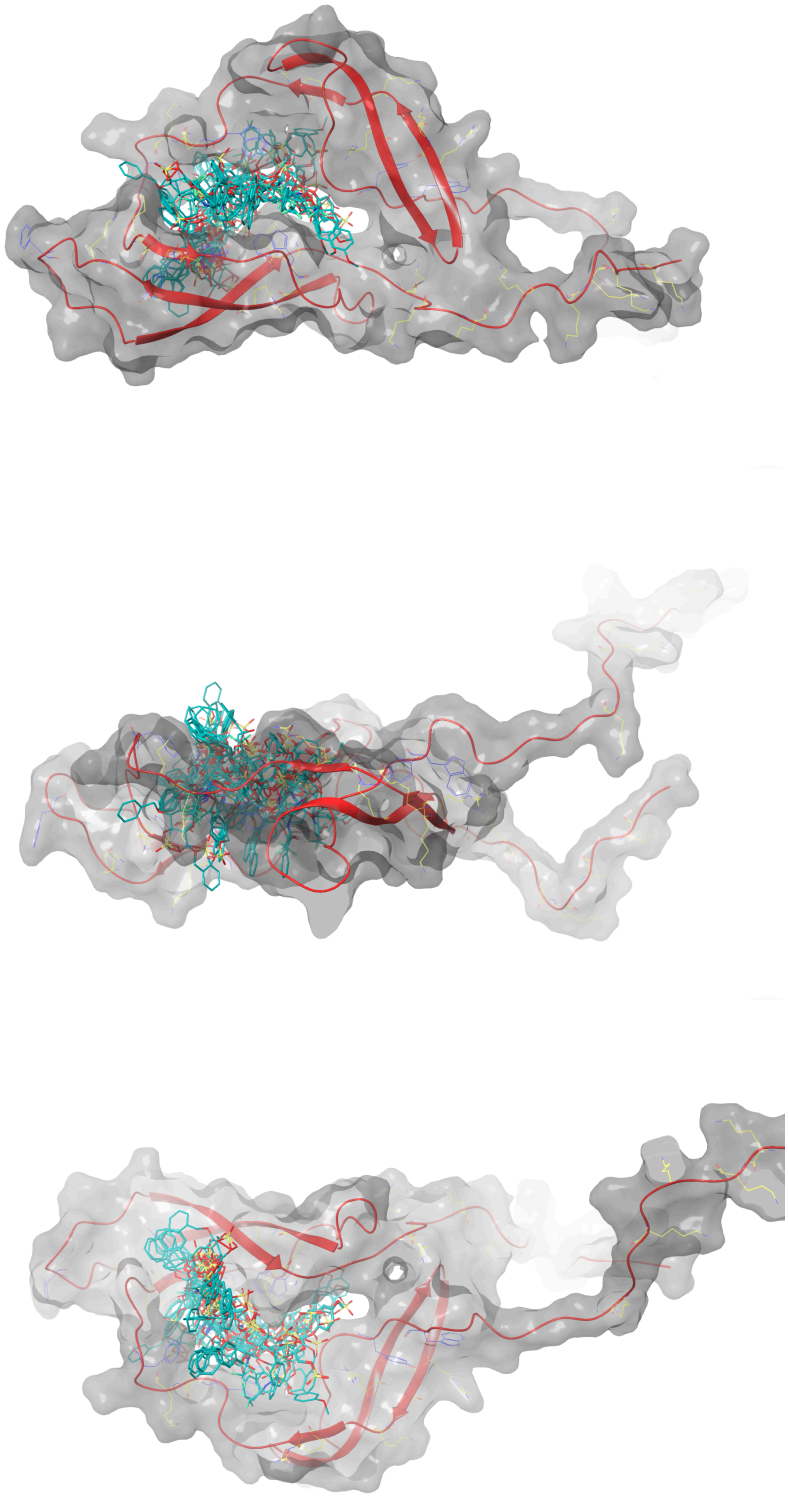

Figure S27. **12** docking structures obtained using Autodock Vina. (orthogonal view)

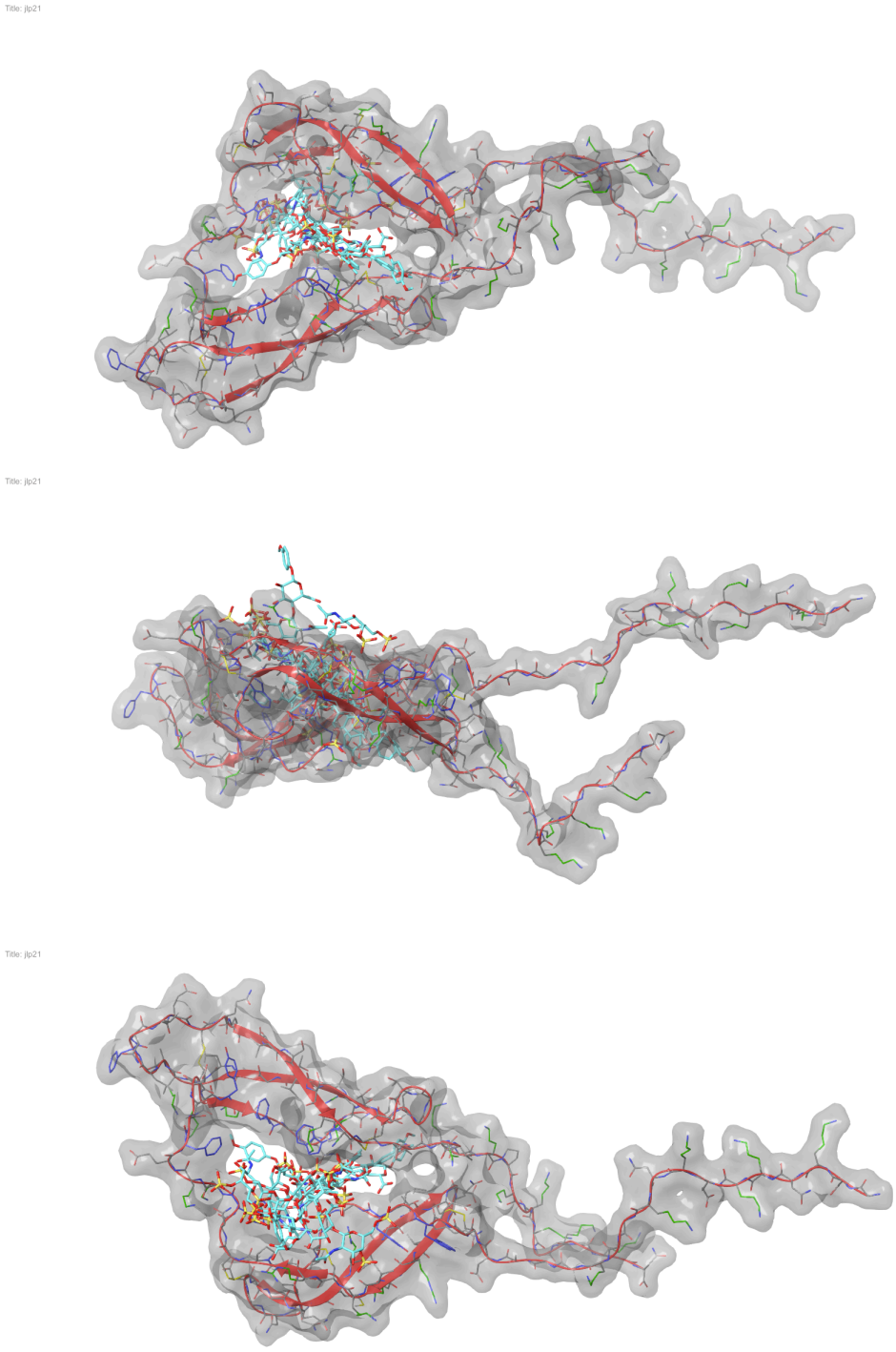

Figure S28. **1** docking structures obtained using Grid. (orthogonal view)

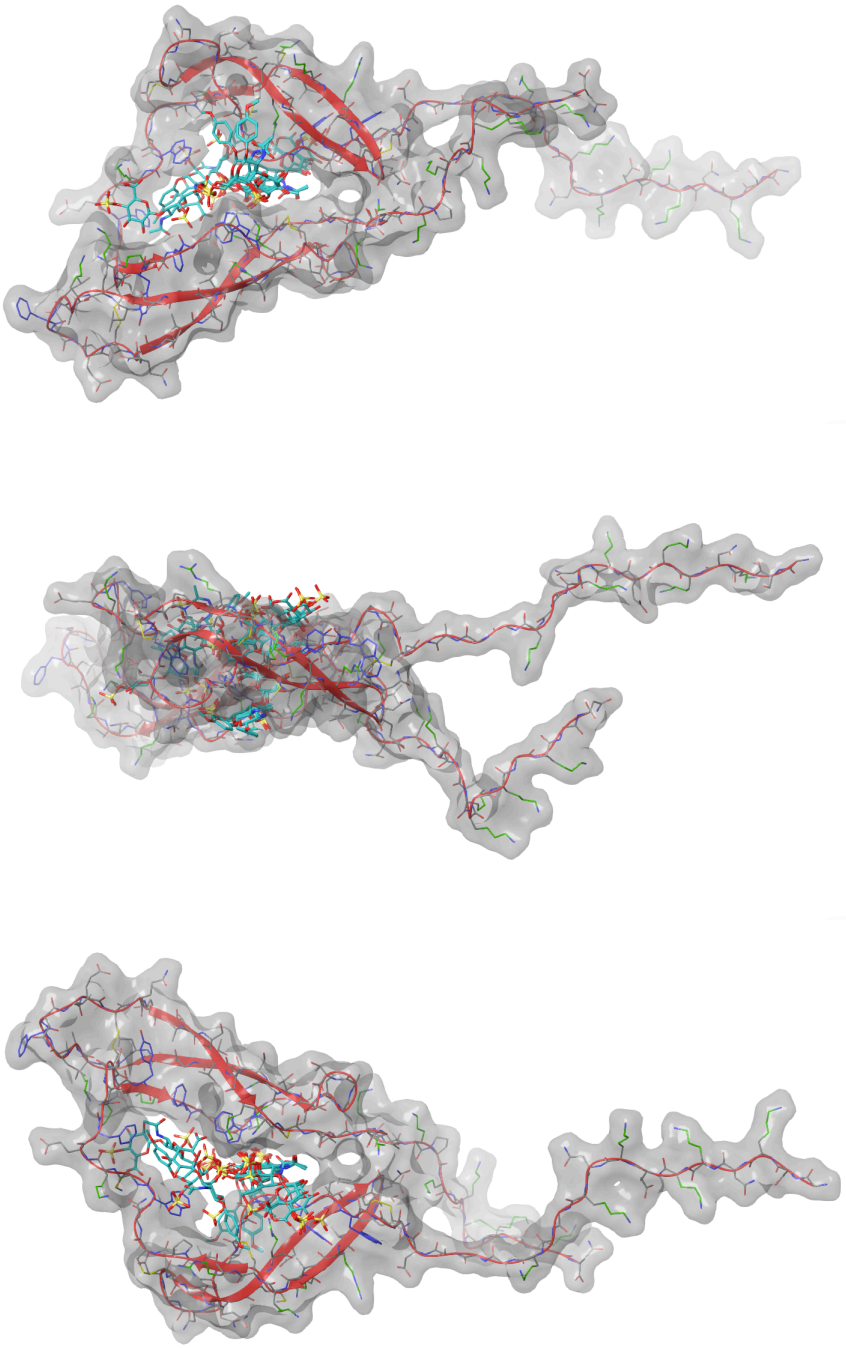

Figure S29. **2** docking structures obtained using Grid. (orthogonal view)

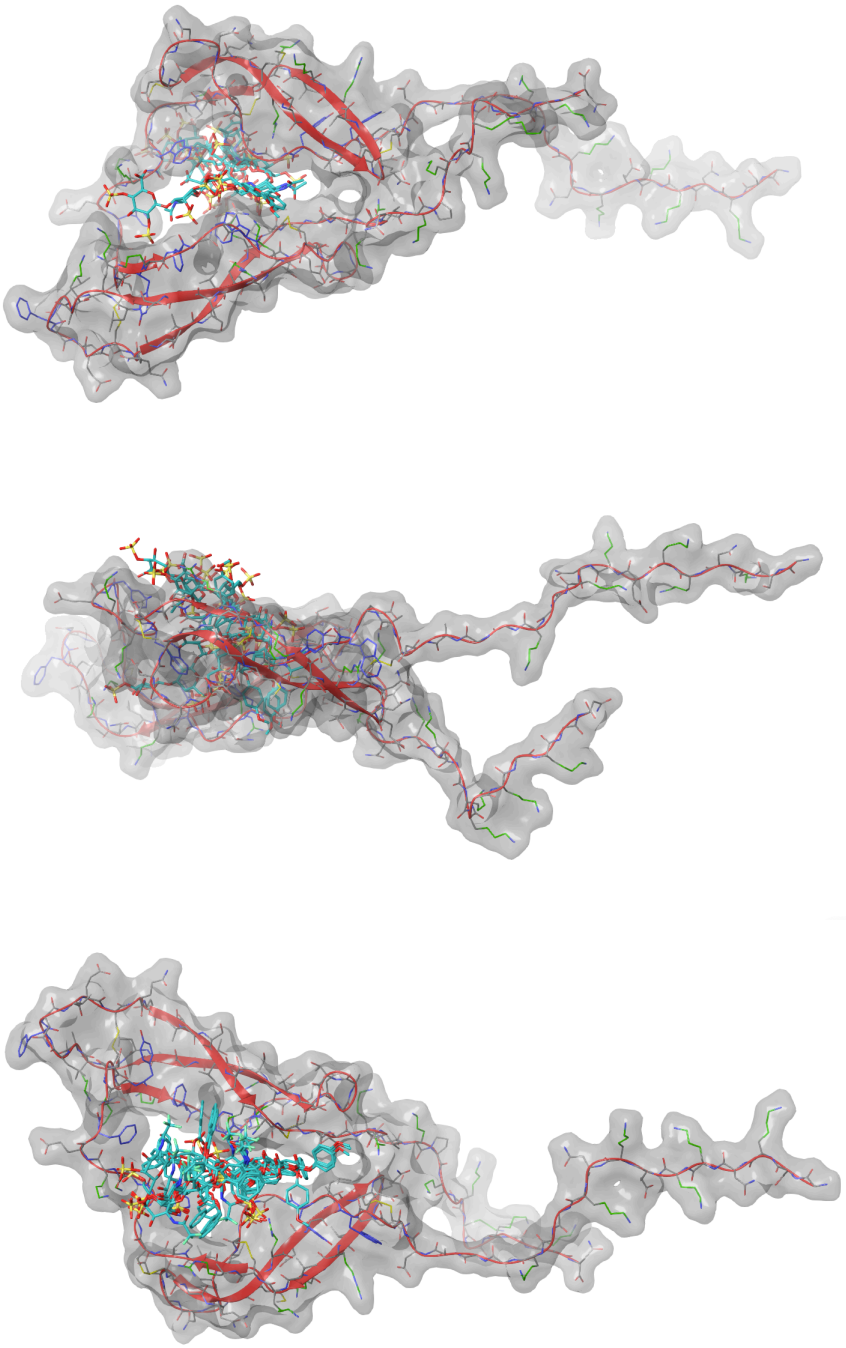

Figure S30. **3** docking structures obtained using Grid. (orthogonal view)

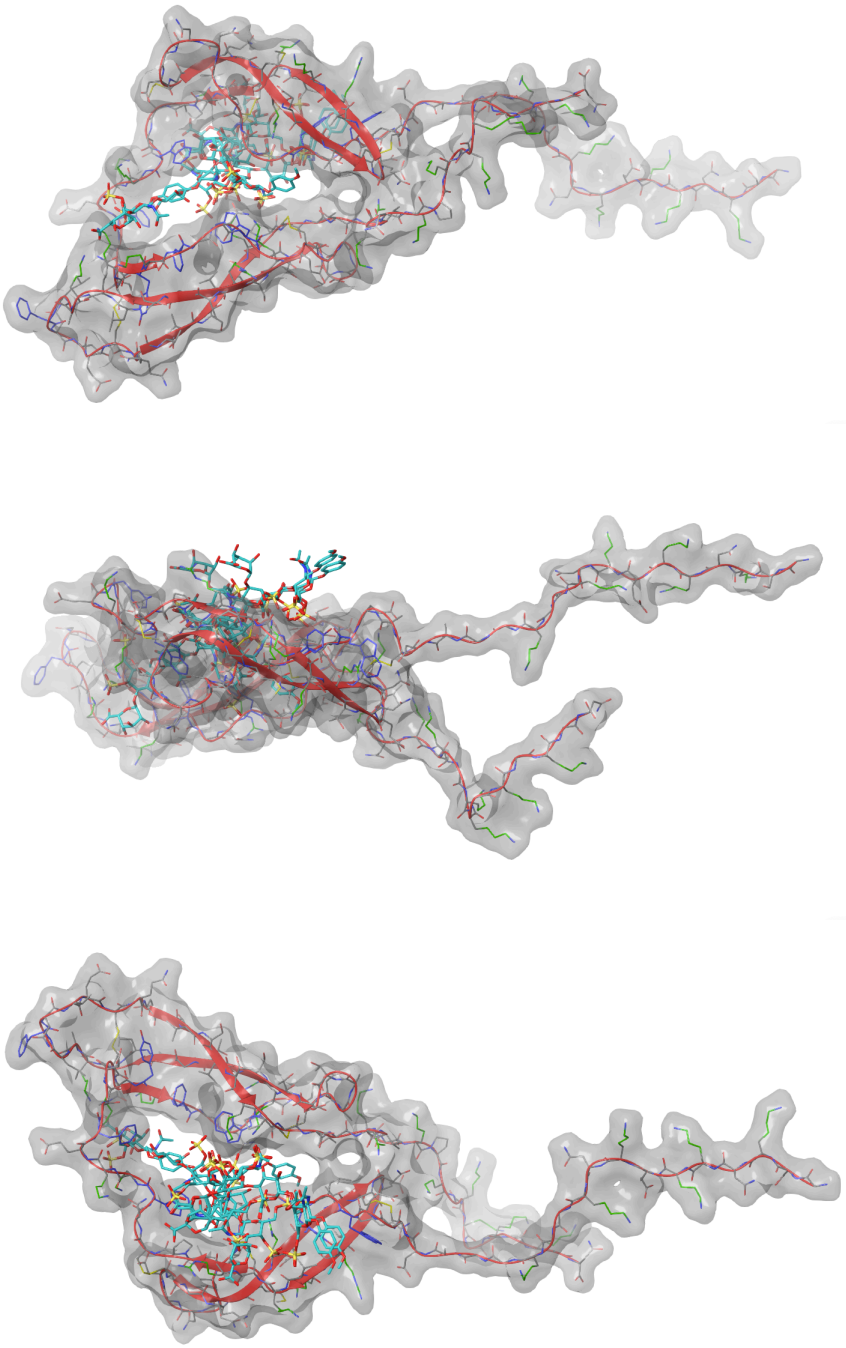

Figure S31. **4** docking structures obtained using Grid. (orthogonal view)

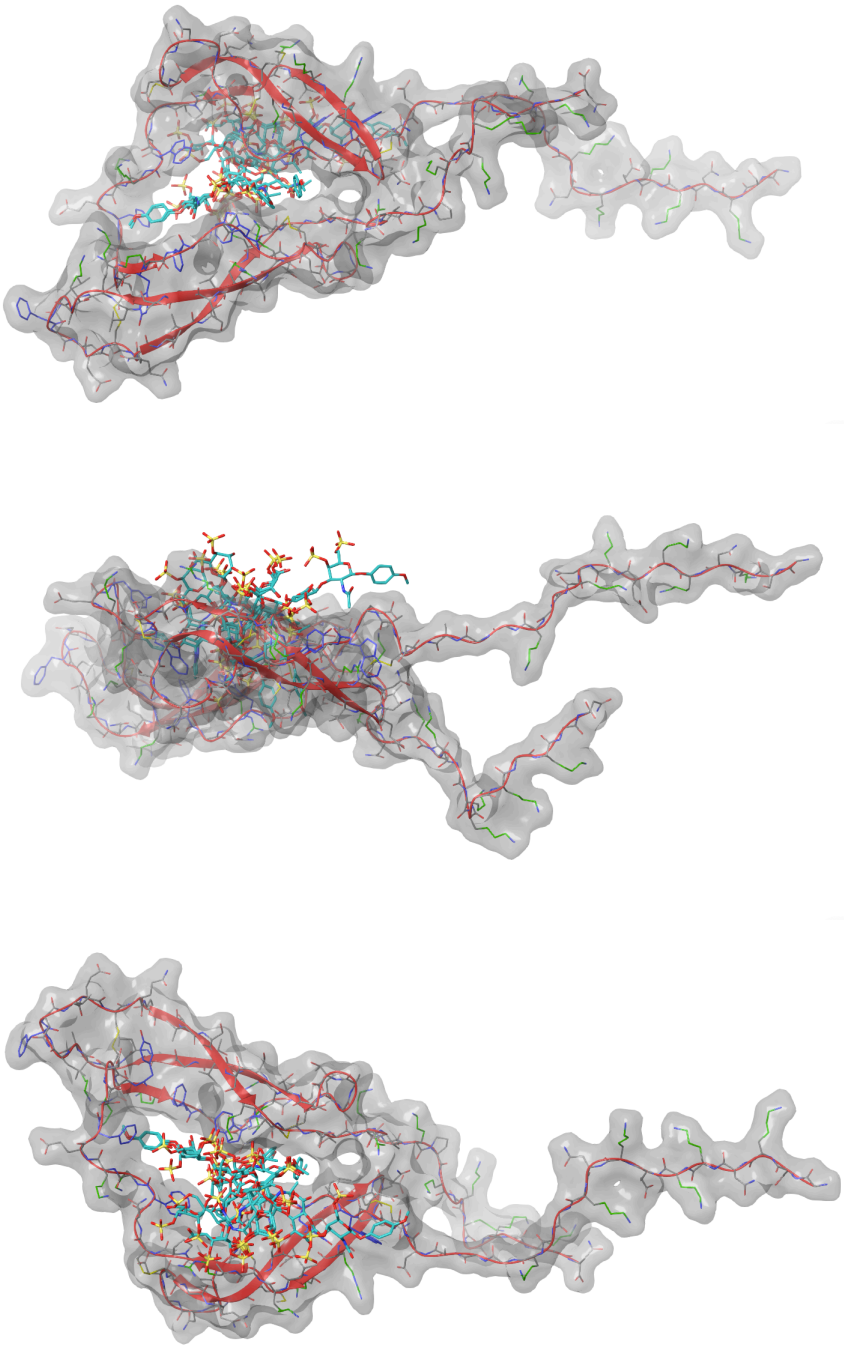

Figure S32. **5** docking structures obtained using Grid. (orthogonal view)

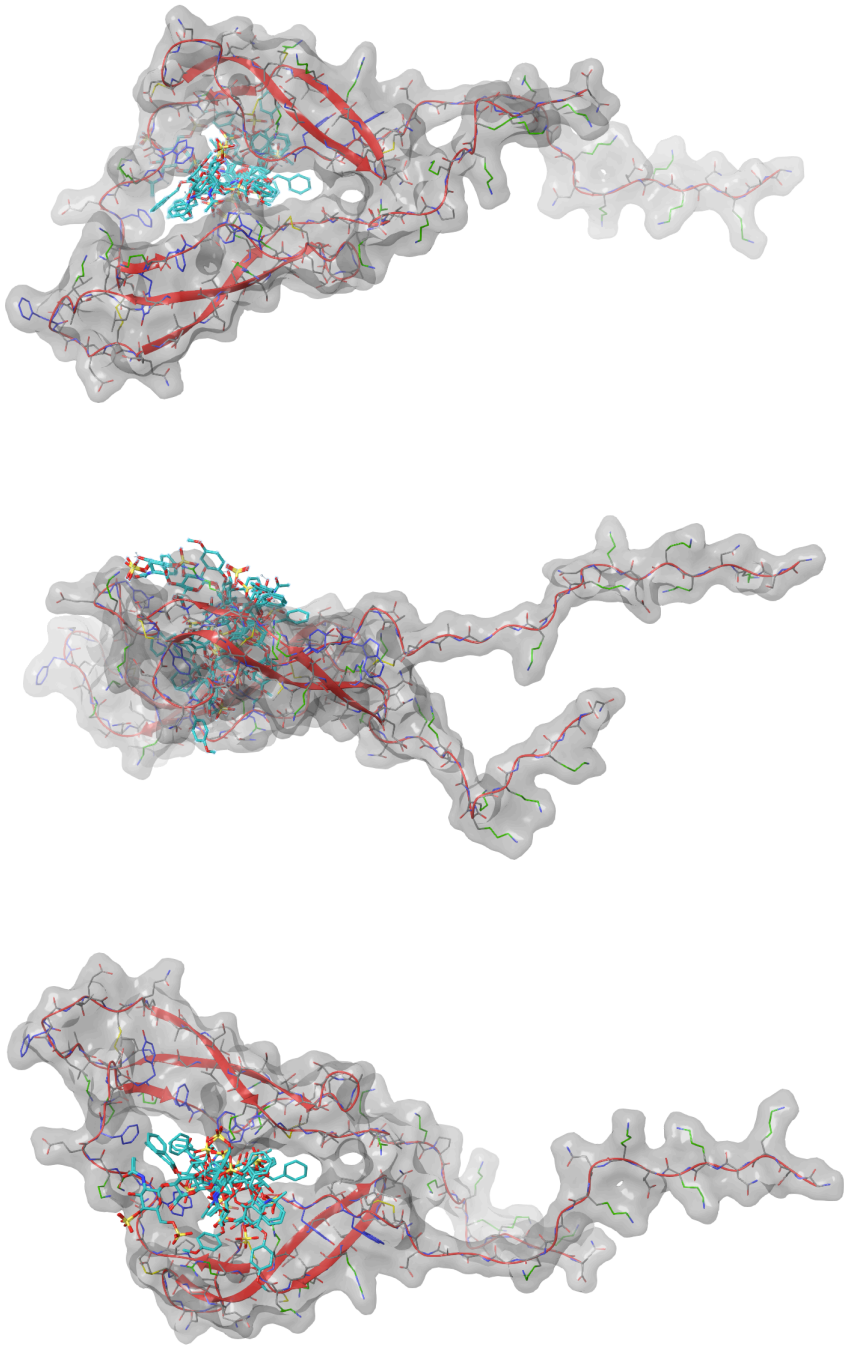

Figure S33. **6** docking structures obtained using Grid. (orthogonal view)

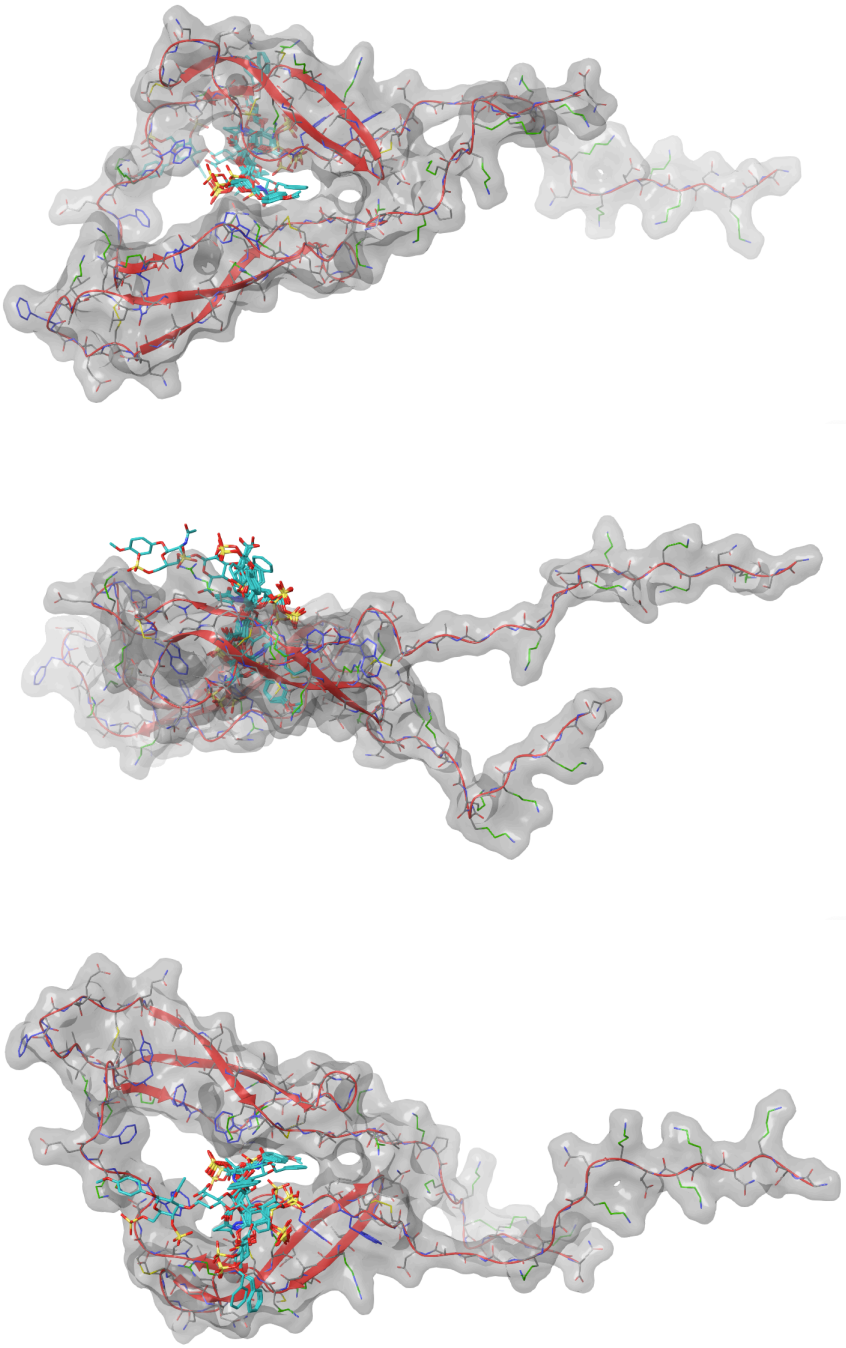

Figure S34. **7** docking structures obtained using Grid. (orthogonal view)

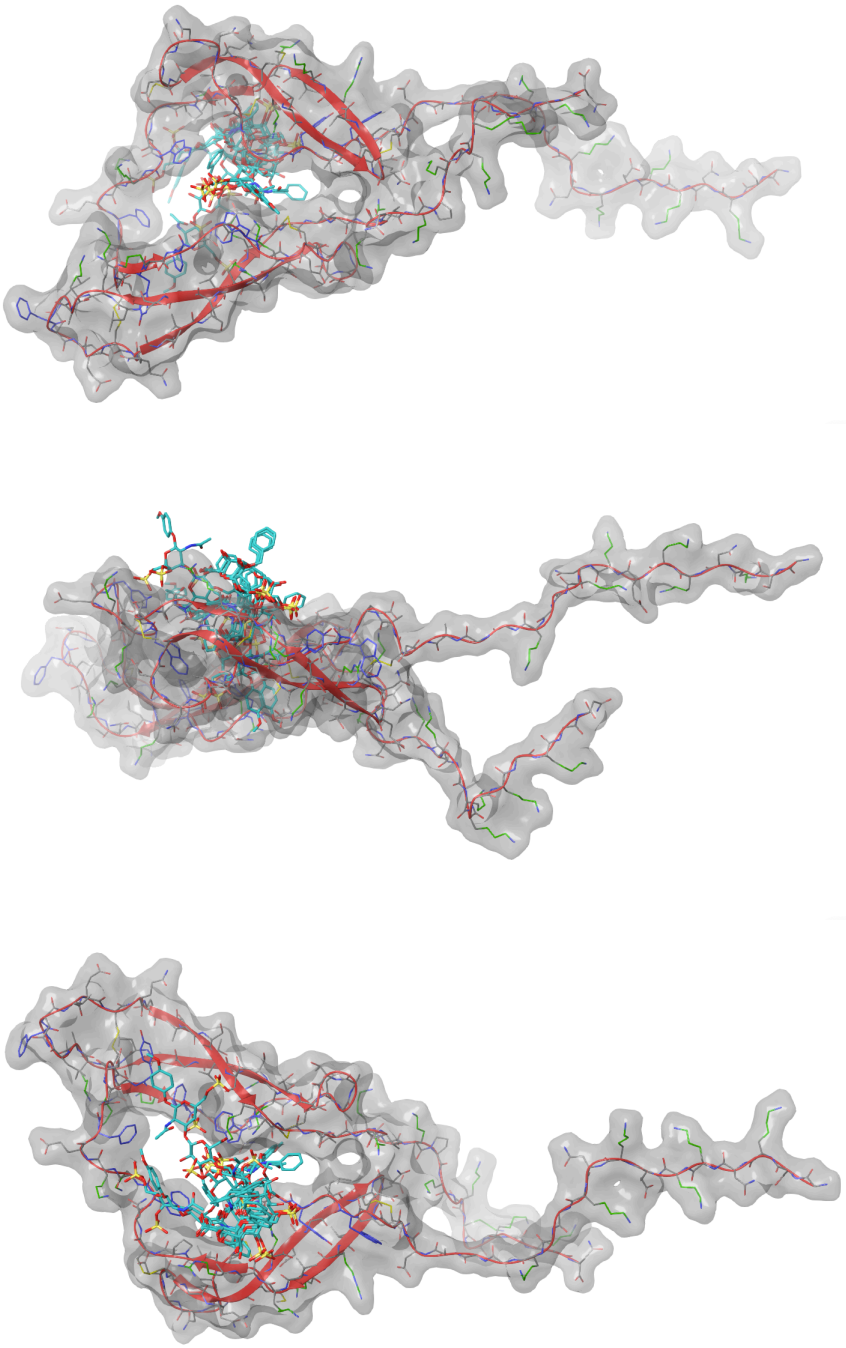

Figure S35. **9** docking structures obtained using Grid. (orthogonal view)

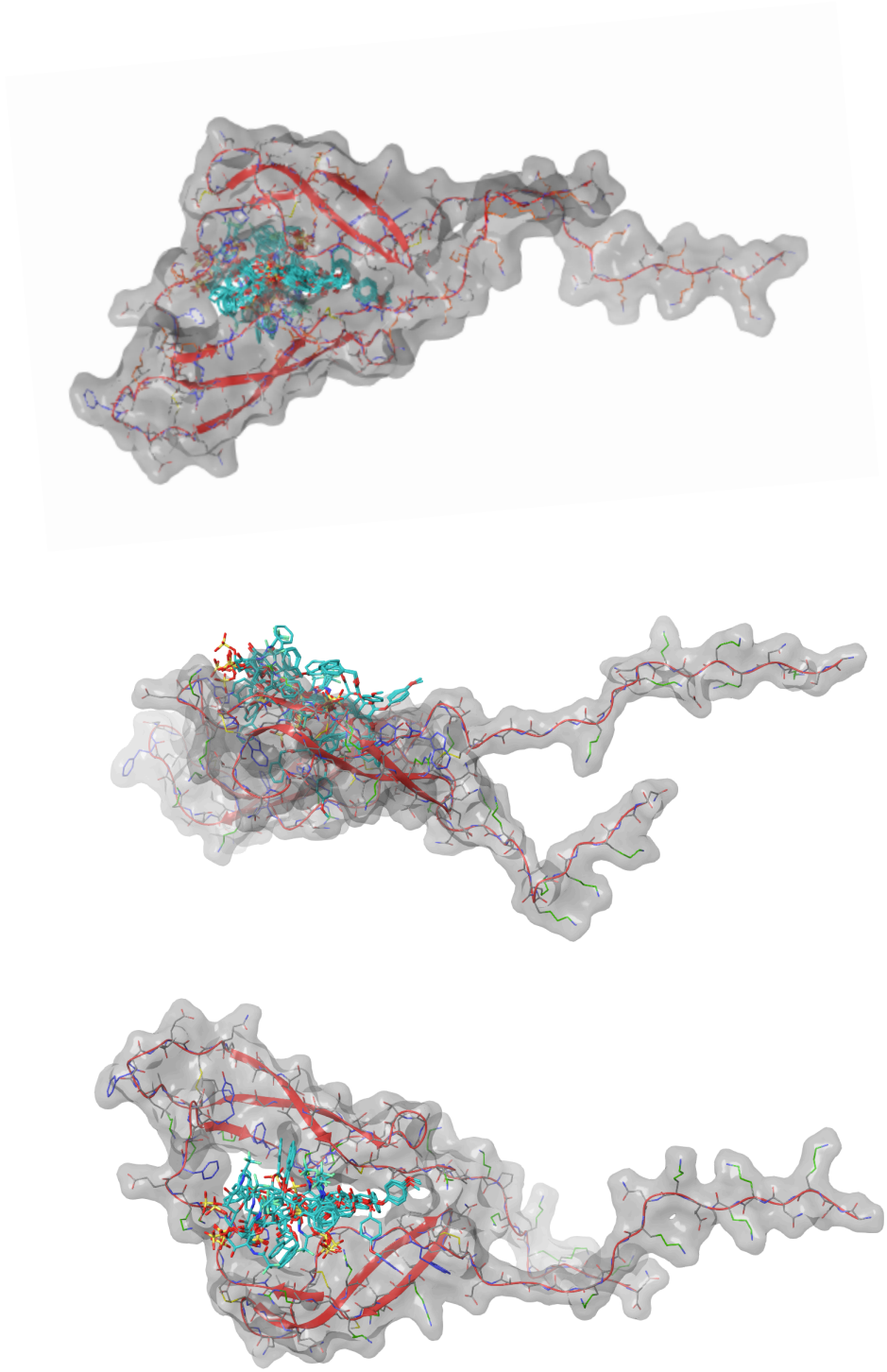

Figure S36. **11** docking structures obtained using Grid. (orthogonal view)

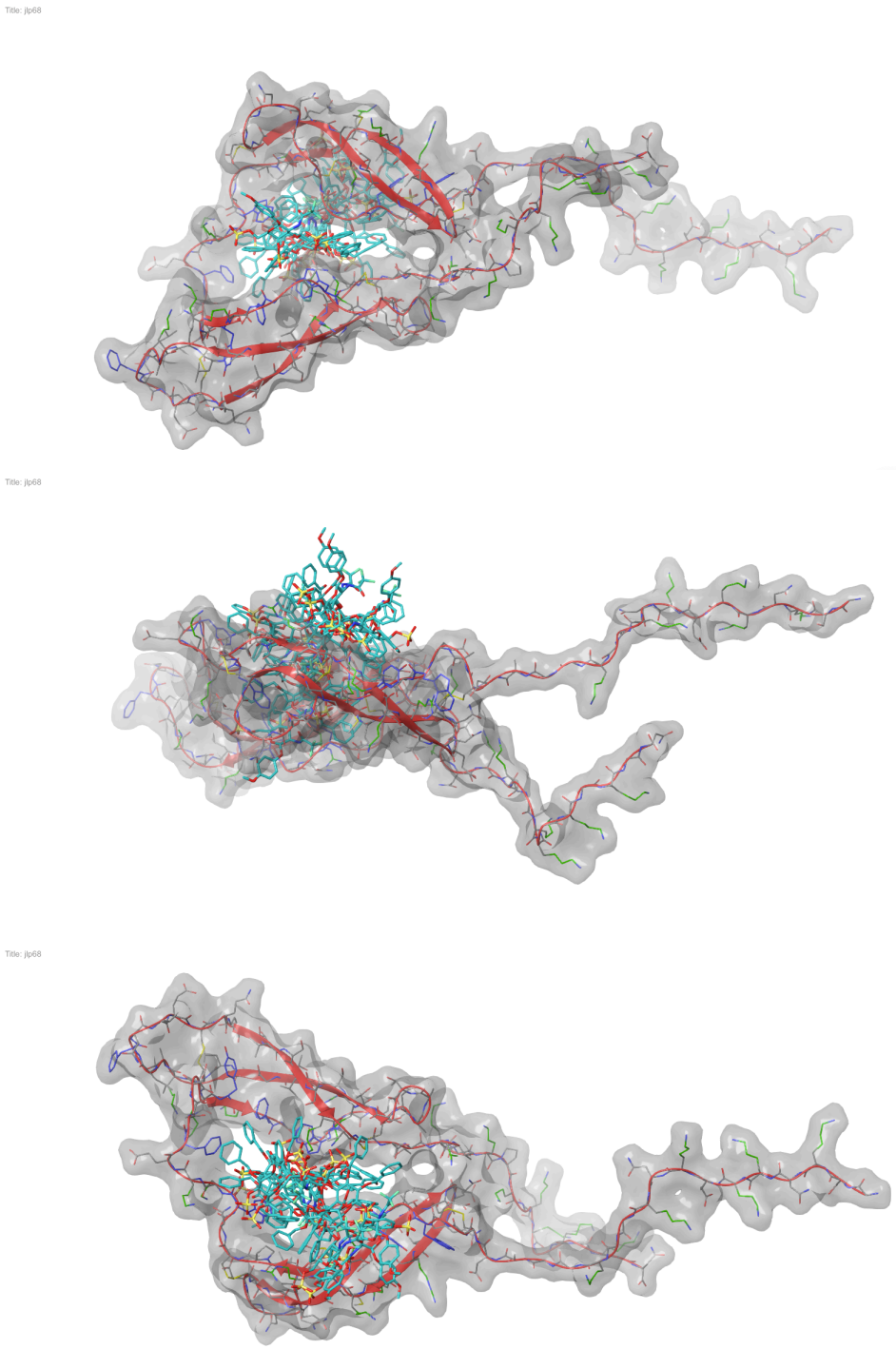

Figure S37. 12 docking structures obtained using Grid. (orthogonal view)