Supporting Information for:

Alginate Trisaccharide Binding Sites on the Surface of β-Lactoglobulin Identified by NMR Spectroscopy - Implications for Molecular Network Formation

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Primary structure of BLGA.

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   1         10         20         30         40         50         60
EAEAYVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRVYVEELKPTPEGDLEILLQKWEN

      70         80         90         100        110        120
DECAQKKIIAEKTKIPAVFKIDALNENKVLVLDTDYKKYLLFCMENSAEPEQSLVCQCLVRTPEVD

130        140        150        160
DEALEKFDKALKALPMHIRLSFNPTQLEEQCHI
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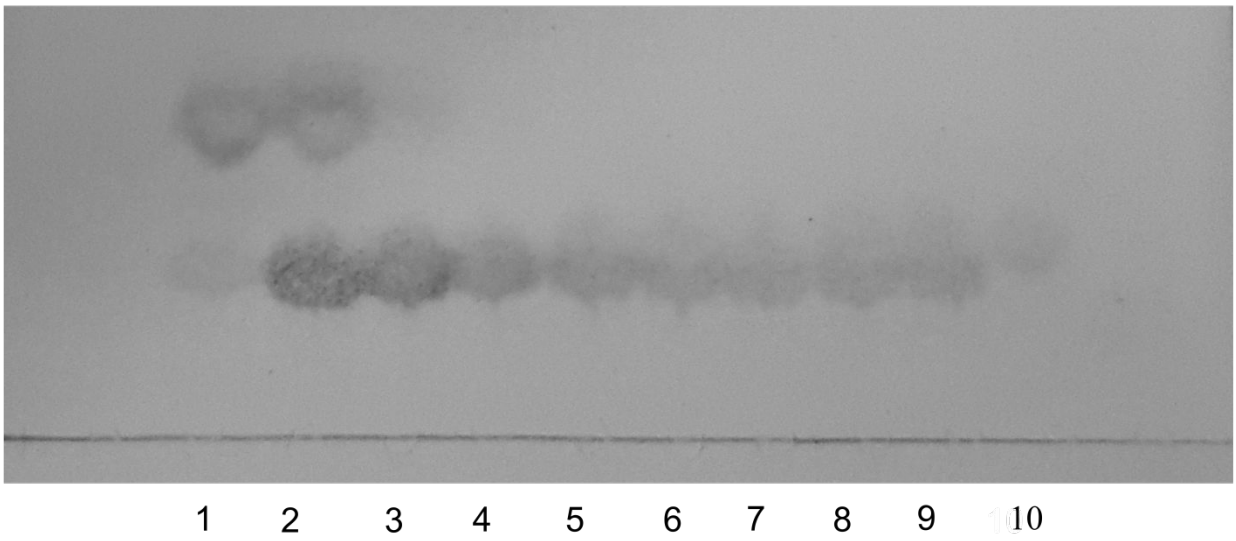


Supporting Figure S1. Top: Primary structure of the BLGA used in the current work. Three cloning residues (EAE) and two mutations (LI->AY) of residues 1 and 2 are included, just at V105 appear as F105 to match the NMR assignments of Uhrínová et al. (ref 7). The numbering this adds +3 to the sequences, such that F105 becomes F108 when the 3 N-terminal residues are considered. Bottom: TLC of AOSs purification (see Methods). Numbers represent individual fractions from the HPLC chromatogram. The upper spot represent the disaccharide and the lower the trisaccharides

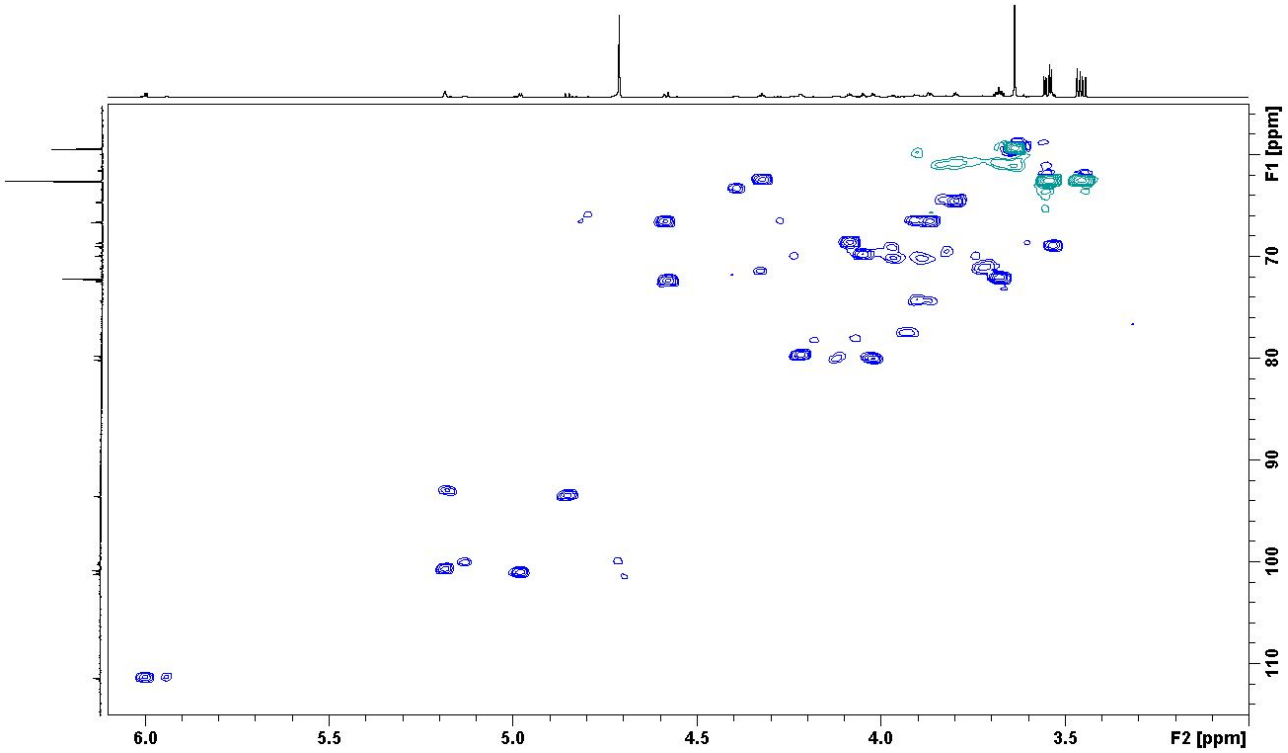
products.

Structure determination of alginate oligosaccharides (AOSs)

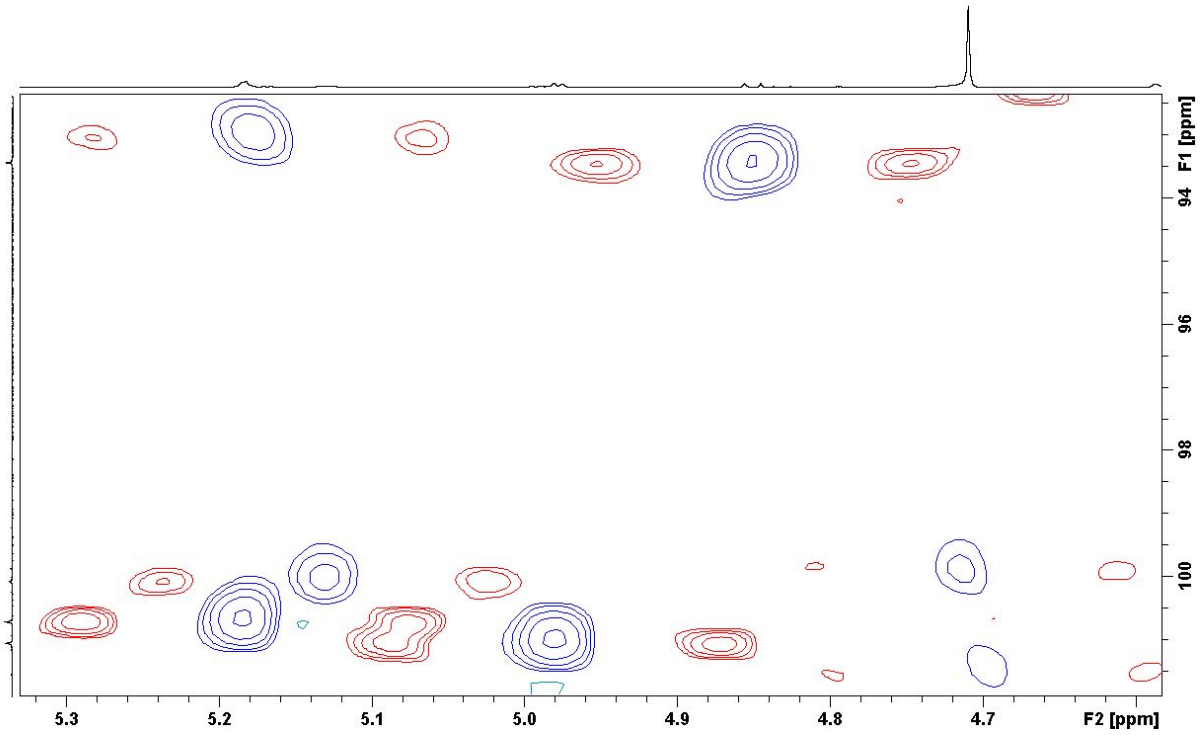
AOS structures were determined by NMR spectroscopy (Bruker Avance III (799.90 MHz for ^{1}H and 201.14 MHz for ^{13}C) equipped with a 5 mm TCI ^{1}H/(^{13}C, ^{15}N) cryoprobe using 4,4-dimethyl-4-silapentane-1-sulfonic acid as reference (–0.093 ppm for ^{1}H and –2.76 ppm for ^{13}C). Chemical shifts were assigned using 1D ^{1}H with presaturation, 1D ^{13}C, 2D double quantum filter correlated spectroscopy (DQF-COSY), 2D Rotating frame nuclear Overhauser effect spectroscopy (ROESY), 2D ^{13}C heteronuclear single quantum coherence (HSQC) with multiplicity editing, 2D ^{13}C HSQC-[^{1}H,^{1}H] total correlation spectroscopy (HSQC-TOSCY) with 60 ms mixing time, 2D ^{13}C heteronuclear multi-bond correlation (HMBC) optimized for 10 Hz long range coupling constants and CLIP-HSQC. All spectra were recorded at 298 K using TopSpin 3.5 and processed and analysed (TopSpin 3.5 software; Bruker).

The ^{1}H, ^{13}C HSQC NMR spectrum of the AOSs revealed seven distinct peaks corresponding to anomeric positions of monosaccharide units labelled **A**-**G** in order of descending anomeric ^{13}C chemical shift (Table S1), which were assigned to the individual monosaccharide units using DQF-COSY, ROESY, HSQC-TOCSY and HMBC. The anomeric configuration was determined using $^{1}J_{H1,C1}$ coupling constants[35] obtained using CLIP-HSQC.[36] **F** and **G** being from a reducing end and not of mannose configuration. Instead they were of gulose configuration assigned from the 8.4 Hz $^{3}J_{H,H}$ coupling constant between the anomeric proton and the C2-proton. Anomeric signals **A** and **E** were of lower intensity than the other five and corresponded to a central mannuronic acid linked to a reducing end guluronic acid in α/β configuration. Signal **B** was from the central guluronic acid; CLIP-HSQC showing the anomeric proton to be equatorial, and as the $^{3}J_{H3,H4}$ coupling constant was 3.6 Hz, this was in α-configuration. Signal **C** was from the non-reducing end contained a double

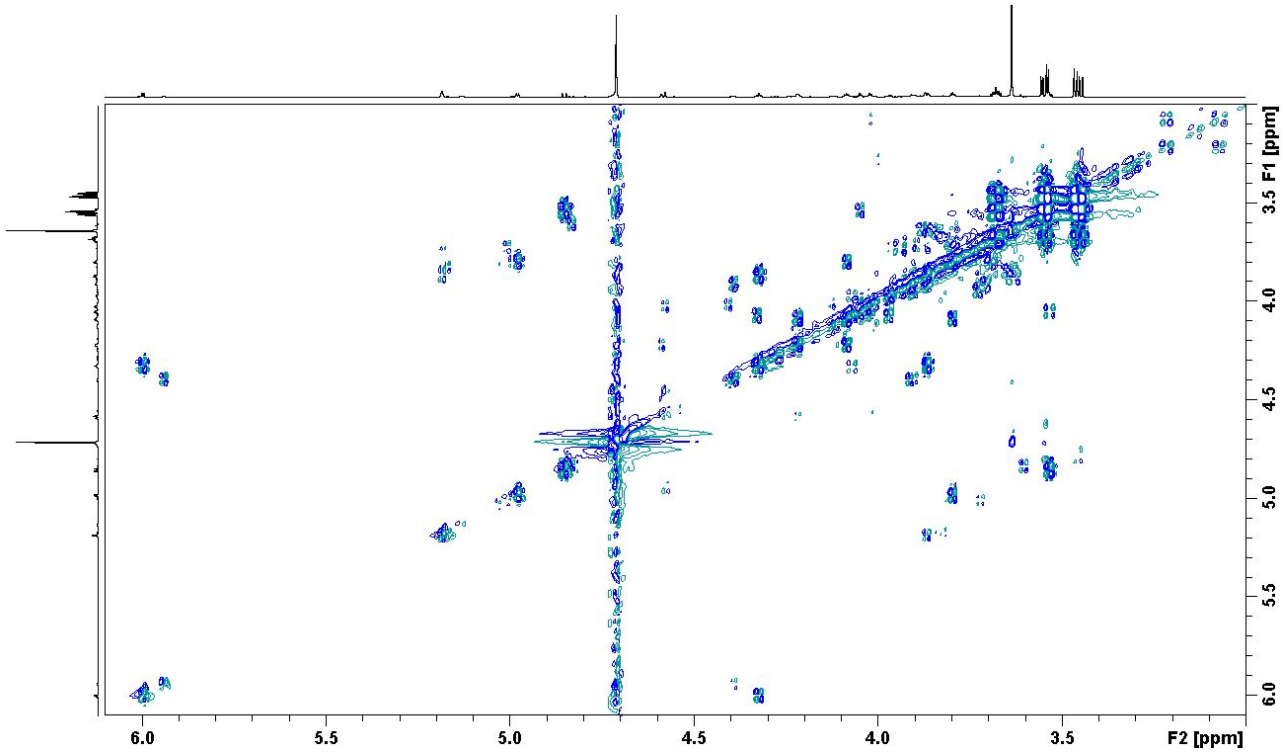
bond between C4 and C5 and signal **D** was from an almost identical unit as **C**, hence at a non-reducing end containing a double bond. The differences between **C** and **D** stems from whether they were attached to a guluronic acid or a mannuronic acid residue.[37] **E** and **F** were from the remaining anomeric positions of α- and β-guluronic acid reducing ends. Using HMBC and ROESY experiments all connections between monosaccharides were determined to be 1,4-linkages. In conclusion, the purified AOSs of DP 3 were the trisaccharides, (4-deoxy-α-L-erythro-hex-4-enopyranosyluronate)-(1,4)-(α-L-gulopyranosyluronate)-(1,4)-(L-gulopyranosyluronate) (**1**, 75% major component) and (4-deoxy-α-L-erythro-hex-4-enopyranosyluronate)-(1,4)-(β-D-mannopyranosyluronate)-(1,4)-(L-gulopyranosyluronate) (**2**, 25%, minor product), depicted in Figure 2.



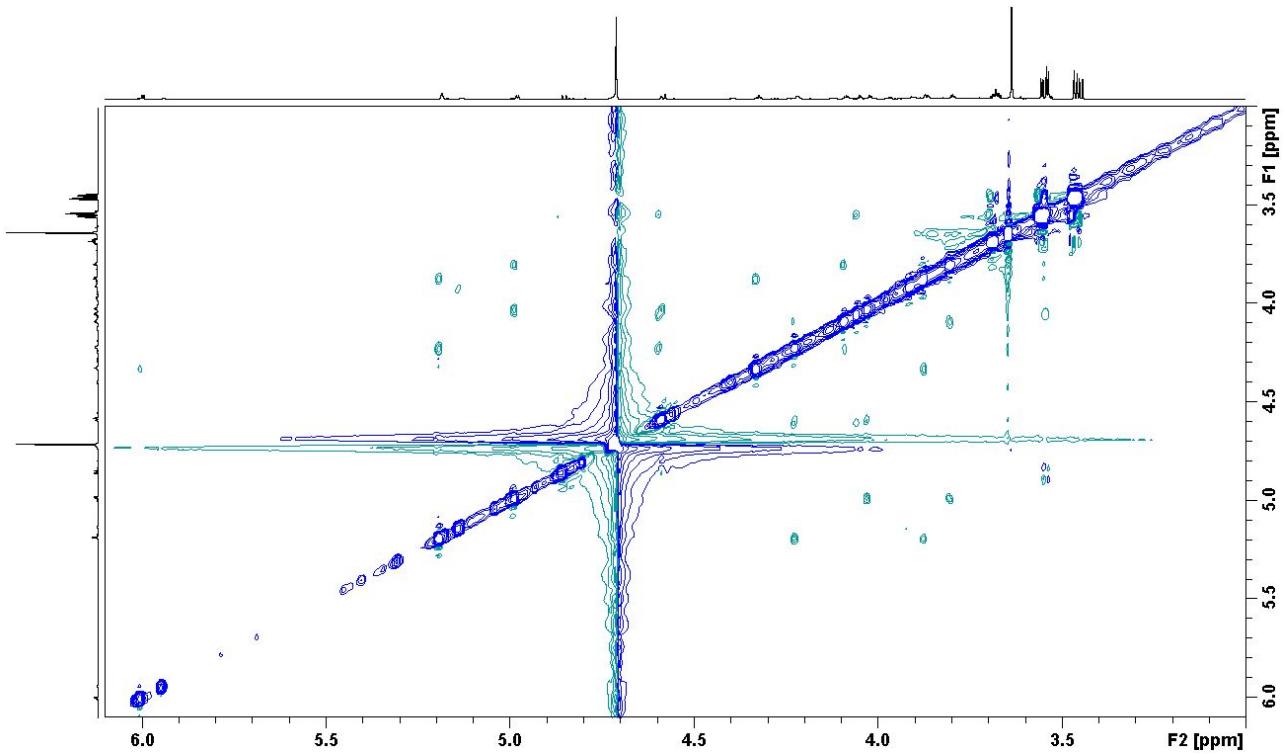
Supporting Figure S2. HSQC spectrum of the AOS sample. The projections are from separate experiments.



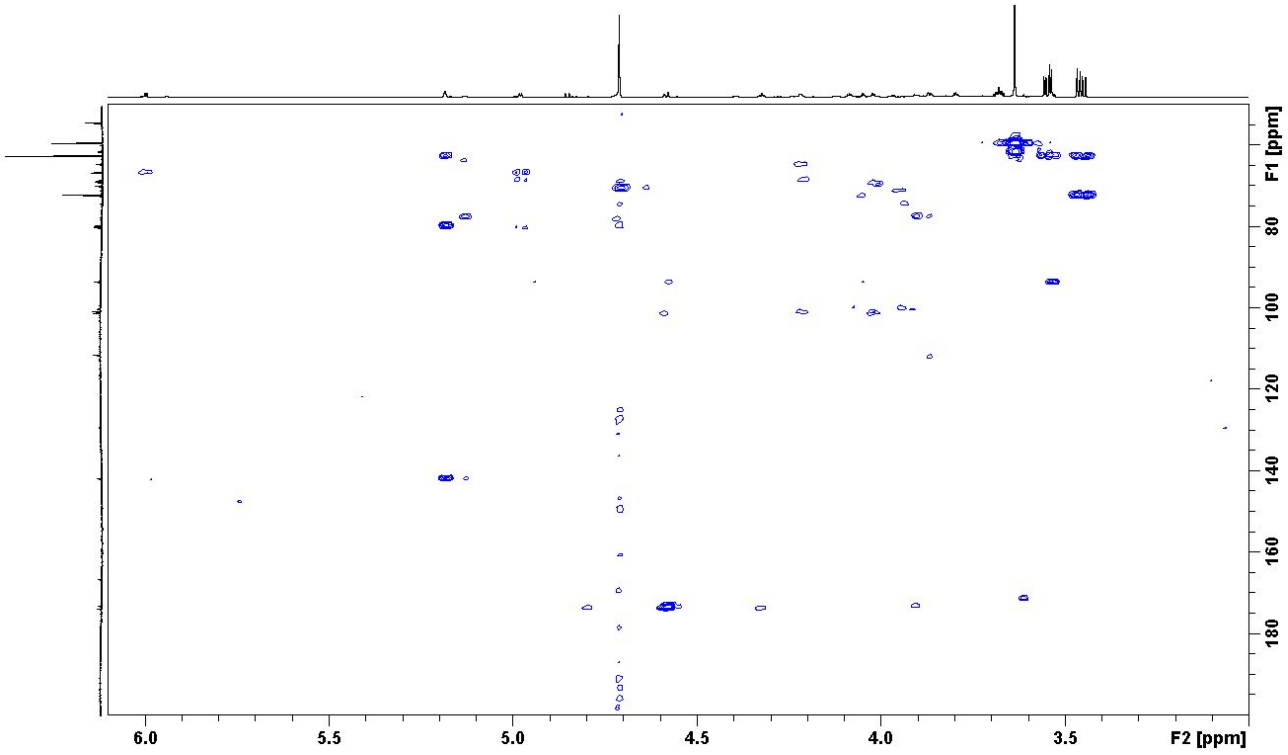
Supporting Figure S3. HSQC spectrum (blue) with the CLIP-HSQC spectrum (red) of the AOS sample overlayed, zoomed to the anomeric position. The projections are from separate experiments.



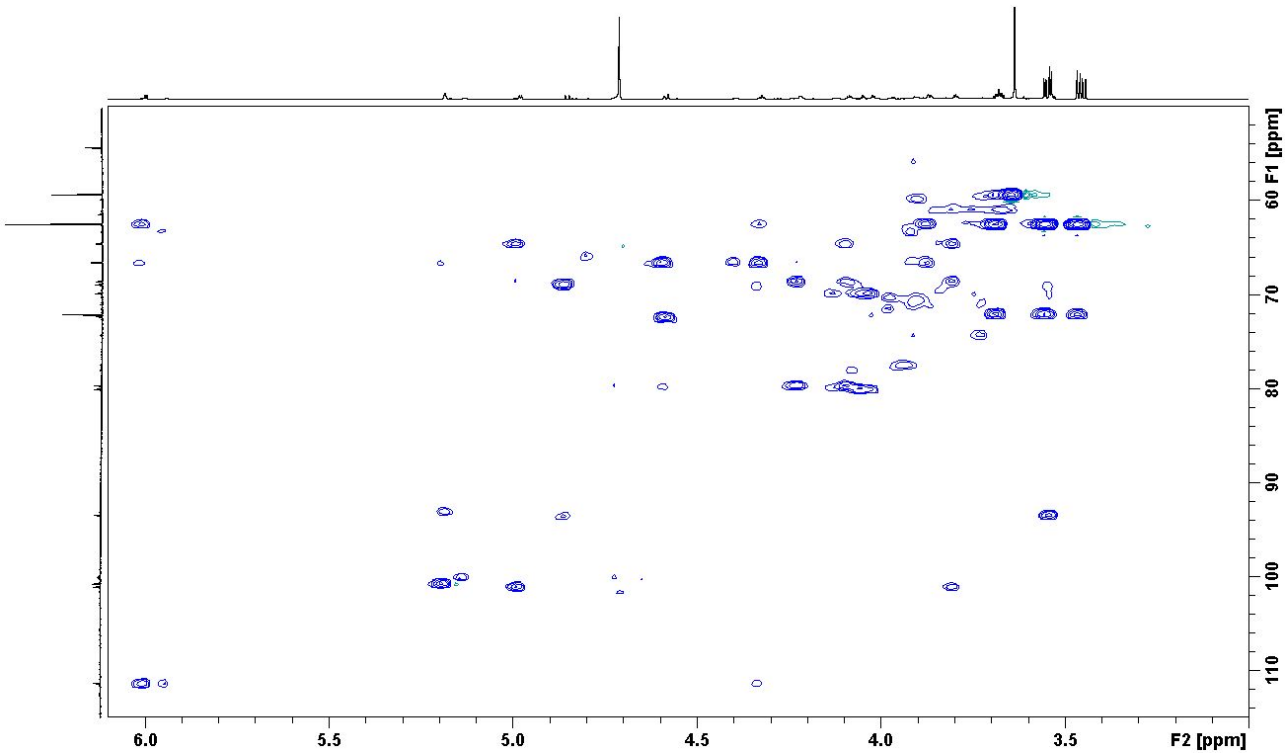
Supporting Figure S4. DQC-COSY spectrum of the AOS sample. The projections are from separate experiments.



Supporting Figure S5. ROESY spectrum of the AOS sample. The projections are from separate experiments.



Supporting Figure S6. HMBC spectrum of the AOS sample. The projections are from separate experiments.

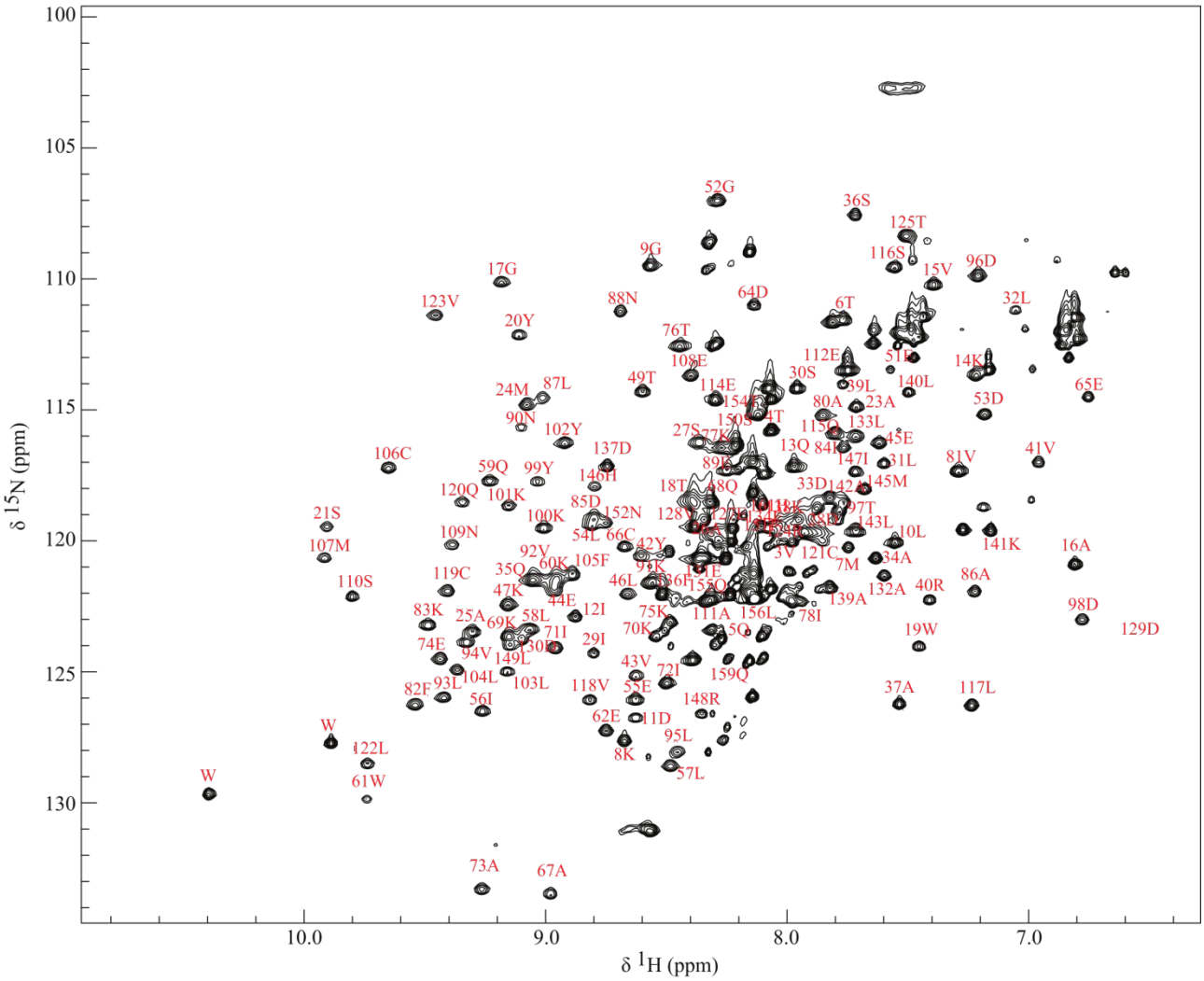


Supporting Figure S7. HSQC-TOCSY spectrum of the AOS sample. The projections are from separate experiments.

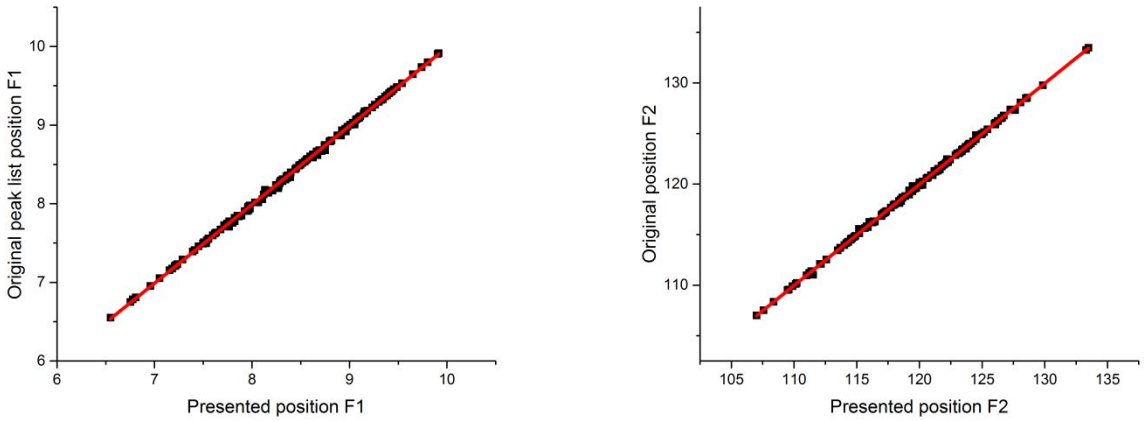
Supporting Table S1. NMR assignment of the two trisaccharides. NA means not applicable and ND means non detectable.

1β	$^{1}J_{H1,C1}$	1	2	3	4	5	6
Term	171 hz	5.185	3.867	4.322	5.995	NA	NA
C		100.73	66.43	62.35	111.29	141.73	ND
GulA mid	171 hz	4.978	3.798	4.086	4.217	4.586	NA
B		101.01	64.45	68.55	79.55	66.46	ND
b-GulA red	164 hz	4.856	3.534	4.049	4.02	4.577	NA
F		93.57	68.87	69.78	79.99	72.28	ND
1α	$^{1}J_{H1,C1}$	1	2	3	4	5	6
Term	171 hz	5.185	3.867	4.322	5.995	NA	NA 0
C		100.73	66.43	62.35	111.29	141.73	
GulA mid	171 hz	4.978	3.798	4.086	4.217	4.586	NA
B		101.01	64.45	68.55	79.55	66.46	ND
a-GulA red	172 hz	5.18	3.823	0	4.123	4.794	NA
G		93.03	69.39	0	79.97	65.86	ND
2β	$^{1}J_{H1,C1}$	1	2	3	4	5	6
Term	171 hz	5.13	3.907	4.394	5.94	NA	NA
D		99.95	66.42	63.27	111.32	141.73	ND
ManA mid	159 hz	4.717	3.968	3.726	3.935	3.904	NA
E		99.85	70.17	71.02	77.47	74.29	ND
b-GulA red	164 hz	4.856	3.534	4.049	4.02	4.577	NA

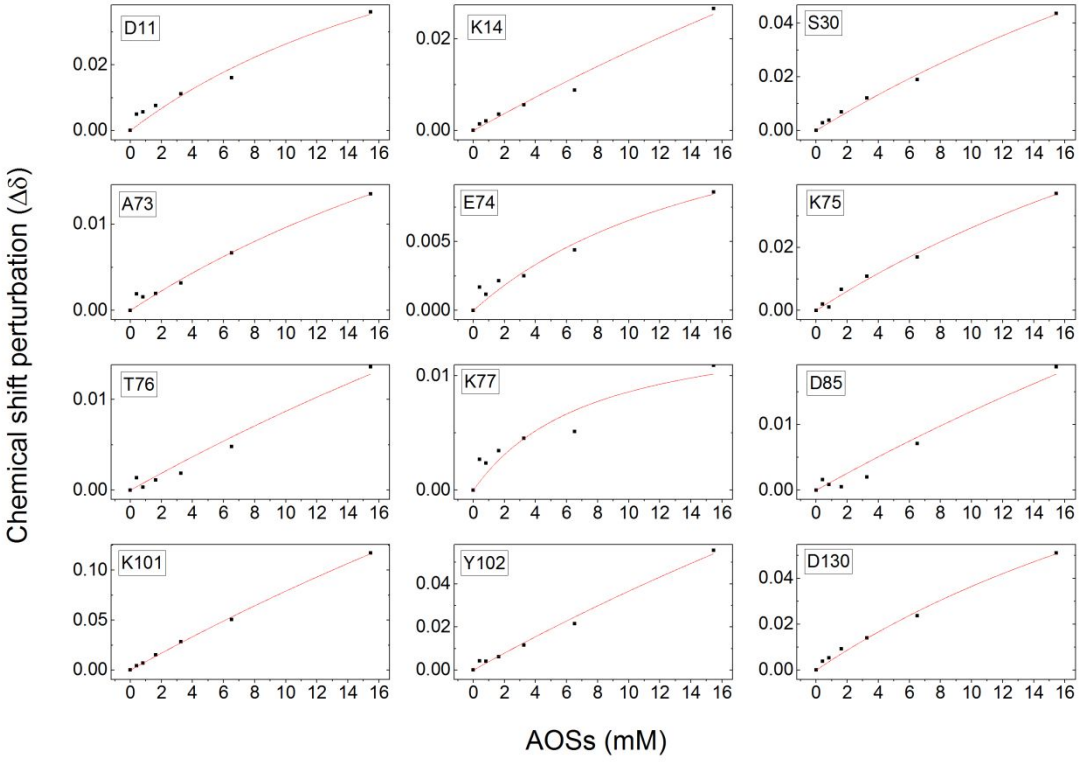
F		93.57	68.87	69.78	79.99	72.28	ND
2α	$^{1}J_{H1,C1}$	1	2	3	4	5	6
Term	171 hz	5.13	3.907	4.394	5.94	NA	NA
D		99.95	66.42	63.27	111.32	141.73	ND
ManA mid	163 hz	4.696	3.972	3.726	3.935	3.87	NA
A		101.54	69.05	71.02	77.47	74.36	ND
a-GulA red	172 hz	5.18	3.823	0	4.123	4.794	NA
G		93.03	69.39	0	79.97	65.86	ND



Supporting Figure S8. ^{1}H ^{15}N HSQC spectrum and chemical shift assignments of BLGA at pH 2.65

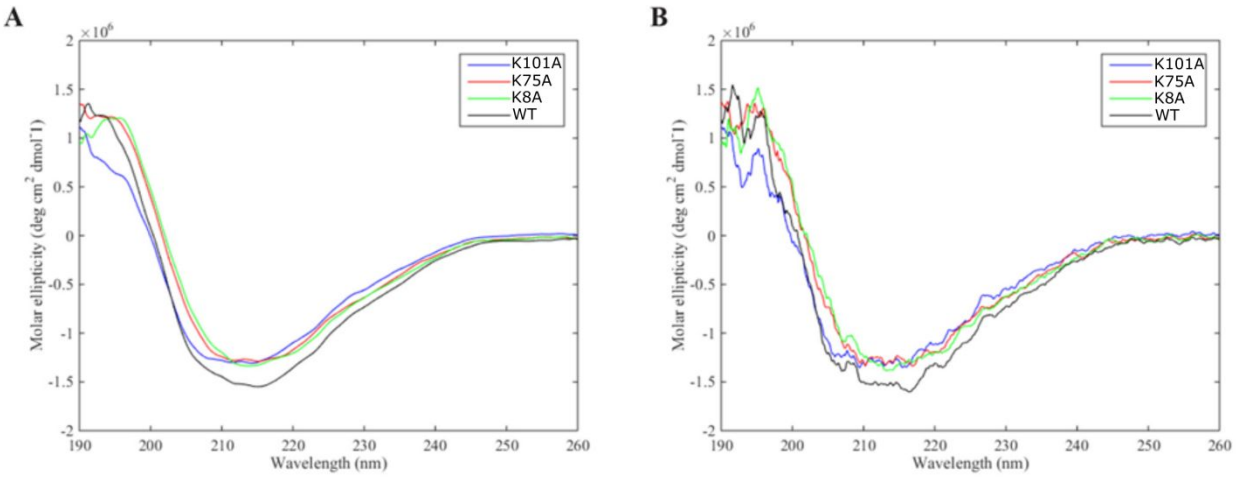


Supporting Figure S9. Assignment of BLGA chemical shifts at pH 2.65. The assigned chemical shifts in this work correlate well (R^2=0.9995) with previously published assignments listed in Table 1 of (ref 7).

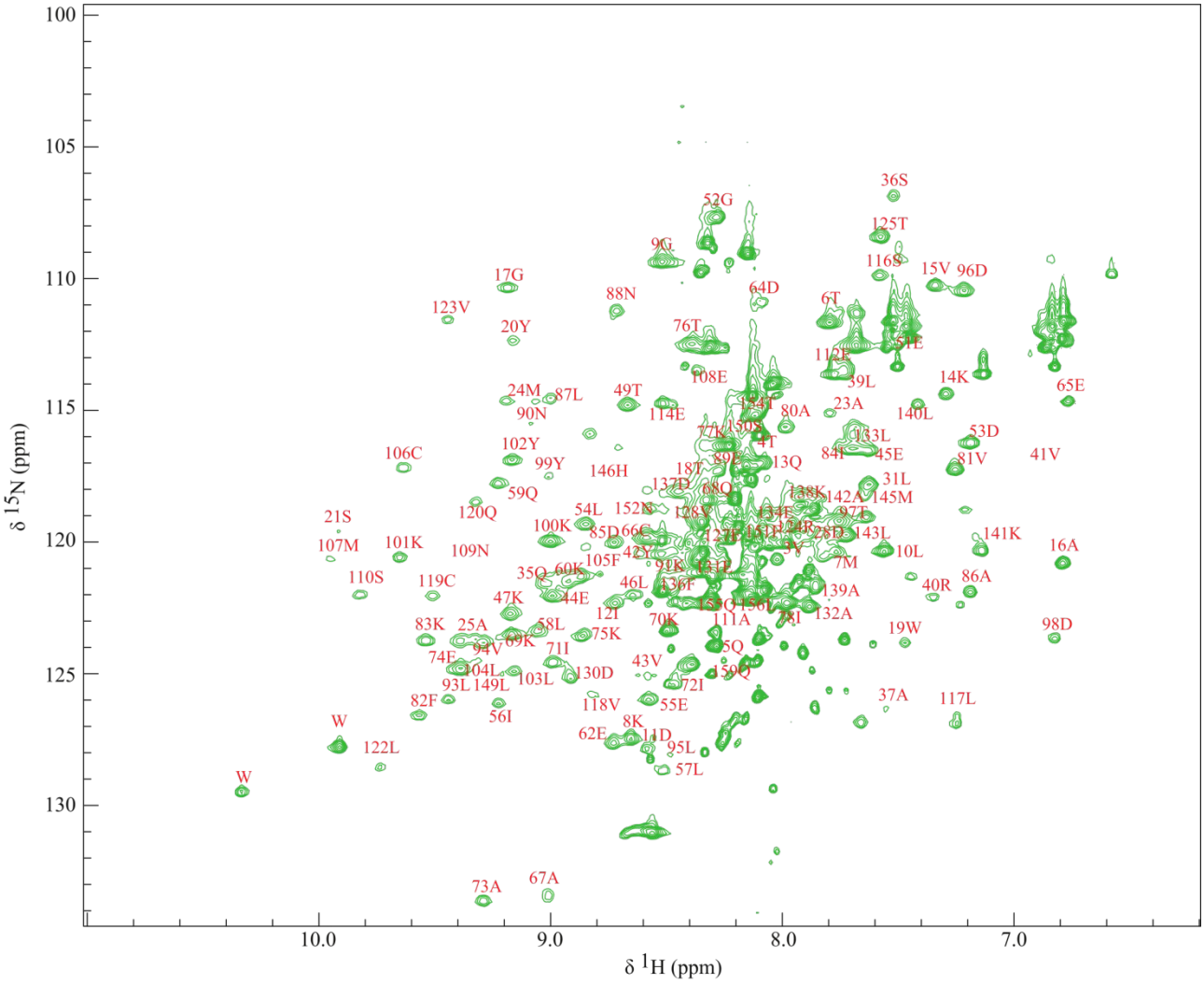


Supporting Figure S10. Fit of classical binding model to chemical shift perturbations as a function

of AOSs concentration. Decent fit is observed for significant chemical shift perturbations e.g. K101, Y102 and K75, while poor fit is observe for chemical shift perturbations below the average plus 1 standard deviation e.g. for K77 (Figure 4).



Supporting Figure S11. Far-UV CD spectra of BLGA WT (black), K75A (red), K8A (green) and K101A (blue). A) Fourier transformed spectra B) untreated spectra. For details on the sample see Methods.



Supporting Figure S12. ^{1}H ^{15}N HSQC spectrum and chemical shift assignments of BLGA at pH 4.0

Supporting Table S2. Assignment of BLGA WT at pH 2.65 and 4.0. For details on sample conditions, see methods (BMRB entry 27668).

pH 2.65 assignment				pH 4.0 assignment			
Position F1	Position F2	Assign F1	Assign F2	Position F1	Position F2	Assign F1	Assign F2
8.714	127.01	-1AlaH	-1AlaN	8.714	127.01	-1AlaH	-1AlaN
8.15643	124.5946	1AlaH	1AlaN	8.15249	124.60326	None	1AlaN
7.894	116.98	2TyrH	2TyrN			2TyrH	2TyrN

7.97976	120.0125	3ValH	3ValN	7.98107	120.03856	3ValH	3ValN
8.06521	115.7838	4ThrH	4ThrN	8.09869	115.95741	4ThrH	4ThrN
8.27049	123.7363	5GlnH	5GlnN	8.28391	123.95411	5GlnH	5GlnN
7.76571	111.5255	6ThrH	6ThrN	7.67919	111.31498	6ThrH	6ThrN
7.74564	120.2697	7MetH	7MetN	7.76694	120.3676	7MetH	7MetN
8.67298	127.6449	8LysH	8LysN	8.6534	127.46774	8LysH	8LysN
8.56641	109.4976	9GlyH	9GlyN	8.5181	109.36035	9GlyH	9GlyN
7.55358	120.1035	10LeuH	10LeuN	7.56091	120.36403	10LeuH	10LeuN
8.62551	126.7537	11AspH	11AspN	8.58382	127.84335	11AspH	11AspN
8.87636	122.891	12IleH	12IleN	8.72169	122.32619	12IleH	12IleN
7.96968	117.1562	13GlnH	13GlnN	8.09266	117.00496	13GlnH	13GlnN
7.21671	113.686	14LysH	14LysN	7.29469	114.37819	14LysH	14LysN
7.39204	110.2285	15ValH	15ValN	7.34103	110.25788	15ValH	15ValN
6.80901	120.9193	16AlaH	16AlaN	6.78999	120.80588	16AlaH	16AlaN
9.18302	110.1356	17GlyH	17GlyN	9.18423	110.34642	17GlyH	17GlyN
8.39327	118.4161	18ThrH	18ThrN	8.335	118.06956	18ThrH	18ThrN
7.4529	124.024	19TrpH	19TrpN	7.47022	123.8326	19TrpH	19TrpN
9.11053	112.1596	20TyrH	20TyrN	9.16544	112.35702	20TyrH	20TyrN
9.90568	119.4652	21SerH	21SerN	9.91494	119.60535	21SerH	21SerN
7.7138	114.8999	23AlaH	23AlaN	7.79469	115.09779	23AlaH	23AlaN
9.07795	114.8068	24MetH	24MetN	9.06504	114.67118	24MetH	24MetN
9.30156	123.4667	25AlaH	25AlaN	9.323	123.69839	25AlaH	25AlaN
8.31501	119.7417	26AlaH	26AlaN			26AlaH	26AlaN
8.36407	116.245	27SerH	27SerN			27SerH	27SerN
7.86704	119.7309	28AspH	28AspN	7.87439	119.58716	28AspH	28AspN
8.80004	124.2856	29IleH	29IleN			29IleH	29IleN
7.95915	114.1799	30SerH	30SerN			30SerH	30SerN
7.59857	117.0407	31LeuH	31LeuN	7.6256	117.82315	31LeuH	31LeuN
7.05274	111.2054	32LeuH	32LeuN			32LeuH	32LeuN
7.82273	118.3684	33AspH	33AspN			33AspH	33AspN
7.6322	120.6785	34AlaH	34AlaN			34AlaH	34AlaN
9.05349	121.4988	35GlnH	35GlnN	9.02323	121.55195	35GlnH	35GlnN
7.71685	107.569	36SerH	36SerN	7.52043	106.86304	36SerH	36SerN
7.53554	126.2455	37AlaH	37AlaN	7.55159	126.31237	37AlaH	37AlaN
7.76613	114.0162	39LeuH	39LeuN	7.71813	114.00216	39LeuH	39LeuN
7.41082	122.2755	40ArgH	40ArgN	7.33993	122.0839	40ArgH	40ArgN
6.95828	117	41ValH	41ValN	6.93893	117.0467	41ValH	41ValN
8.60354	120.5741	42TyrH	42TyrN	8.61541	120.42029	42TyrH	42TyrN
8.62631	125.1627	43ValH	43ValN	8.62408	125.05276	43ValH	43ValN
8.96265	121.7778	44GluH	44GluN	8.99272	122.04012	44GluH	44GluN
7.6186	116.2857	45GluH	45GluN	7.63043	116.53426	45GluH	45GluN
8.66171	122.0424	46LeuH	46LeuN	8.64507	122.0205	46LeuH	46LeuN
9.15635	122.4511	47LysH	47LysN	9.17446	122.7257	47LysH	47LysN
8.59743	114.3018	49ThrH	49ThrN	8.66702	114.80277	49ThrH	49ThrN
7.52999	112.0727	51GluH	51GluN	7.51061	112.13326	51GluH	51GluN

8.28883	107.0215	52GlyH	52GlyN	8.2869	107.66962	52GlyH	52GlyN
7.18097	115.1938	53AspH	53AspN	7.18685	116.24506	53AspH	53AspN
8.80694	119.4394	54LeuH	54LeuN	8.84793	119.32414	54LeuH	54LeuN
8.62626	126.0635	55GluH	55GluN	8.57733	125.9837	55GluH	55GluN
9.26173	126.5153	56IleH	56IleN	9.22524	126.13213	56IleH	56IleN
8.48296	128.6041	57LeuH	57LeuN	8.5193	128.69465	57LeuH	57LeuN
9.06596	123.3919	58LeuH	58LeuN	9.05892	123.36032	58LeuH	58LeuN
9.23144	117.7148	59GlnH	59GlnN	9.22422	117.78442	59GlnH	59GlnN
8.95487	121.5045	60LysH	60LysN	8.92294	121.5005	60LysH	60LysN
9.74021	129.856	61TrpH	61TrpN			61TrpH	61TrpN
8.74944	127.2512	62GluH	62GluN	8.72787	127.63268	62GluH	62GluN
8.91417	121.6213	63AsnH	63AsnN			63AsnH	63AsnN
8.13732	110.9992	64AspH	64AspN	8.11994	110.97099	64AspH	64AspN
6.75262	114.5227	65GluH	65GluN	6.76569	114.6642	65GluH	65GluN
8.67315	120.2401	66CysH	66CysN	8.58824	119.84009	66CysH	66CysN
8.97902	133.4899	67AlaH	67AlaN	9.00822	133.41585	67AlaH	67AlaN
8.38211	118.635	68GlnH	68GlnN	8.37675	118.73442	68GlnH	68GlnN
9.15043	123.6905	69LysH	69LysN	9.16777	123.52064	69LysH	69LysN
8.54061	123.6847	70LysH	70LysN	8.49975	123.38297	70LysH	70LysN
8.95827	124.1015	71IleH	71IleN	8.98812	124.57064	71IleH	71IleN
8.49789	125.4454	72IleH	72IleN	8.4856	125.42674	72IleH	72IleN
9.26399	133.2975	73AlaH	73AlaN	9.29059	133.63675	73AlaH	73AlaN
9.43616	124.5123	74GluH	74GluN	9.40332	124.79317	74GluH	74GluN
8.4843	123.1493	75LysH	75LysN	8.86128	123.56929	75LysH	75LysN
8.44455	112.5842	76ThrH	76ThrN	8.38966	112.48585	76ThrH	76ThrN
8.27246	116.4517	77LysH	77LysN	8.27008	116.36791	77LysH	77LysN
7.97976	122.2947	78IleH	78IleN	7.97952	122.31686	78IleH	78IleN
7.85116	115.217	80AlaH	80AlaN	7.98791	115.6413	80AlaH	80AlaN
7.28916	117.3683	81ValH	81ValN	7.2541	117.25123	81ValH	81ValN
9.53946	126.2751	82PheH	82PheN	9.56952	126.57629	82PheH	82PheN
9.48594	123.2371	83LysH	83LysN	9.53814	123.74872	83LysH	83LysN
7.7646	116.4437	84IleH	84IleN	7.75112	116.38482	84IleH	84IleN
8.79959	119.195	85AspH	85AspN	8.72482	120.01021	85AspH	85AspN
7.22122	121.9317	86AlaH	86AlaN	7.18809	121.90603	86AlaH	86AlaN
9.01196	114.5393	87LeuH	87LeuN	9.00004	114.55674	87LeuH	87LeuN
8.68998	111.244	88AsnH	88AsnN	8.71409	111.23332	88AsnH	88AsnN
8.24907	117.3076	89GluH	89GluN	8.27401	117.27395	89GluH	89GluN
9.10042	115.6569	90AsnH	90AsnN	9.08647	115.52173	90AsnH	90AsnN
8.55857	121.6032	91LysH	91LysN	8.5147	121.59359	91LysH	91LysN
9.05227	121.2037	92ValH	92ValN			92ValH	92ValN
9.42034	125.9924	93LeuH	93LeuN	9.44071	125.98654	93LeuH	93LeuN
9.32618	123.8768	94ValH	94ValN	9.28846	123.81946	94ValH	94ValN
8.45414	128.07	95LeuH	95LeuN	8.48537	128.06782	95LeuH	95LeuN
7.20926	109.8899	96AspH	96AspN	7.21482	110.46173	96AspH	96AspN
7.78325	118.8771	97ThrH	97ThrN	7.75403	119.1947	97ThrH	97ThrN

6.77772	123.0158	98AspH	98AspN	6.82303	123.66726	98AspH	98AspN
9.0333	117.7347	99TyrH	99TyrN	9.00943	117.5086	99TyrH	99TyrN
9.00755	119.5293	100LysH	100LysN	9.00086	119.97522	100LysH	100LysN
9.15464	118.6572	101LysH	101LysN	9.6492	120.5814	101LysH	101LysN
8.92234	116.313	102TyrH	102TyrN	9.1665	116.88936	102TyrH	102TyrN
9.15925	125.0007	103LeuH	103LeuN	9.15791	124.91022	103LeuH	103LeuN
9.36536	124.9315	104LeuH	104LeuN	9.38556	124.80012	104LeuH	104LeuN
8.8884	121.2799	105PheH	105PheN	8.86883	121.31019	105PheH	105PheN
9.65116	117.241	106CysH	106CysN	9.6324	117.19327	106CysH	106CysN
9.91503	120.6674	107MetH	107MetN	9.94944	120.6503	107MetH	107MetN
8.39827	113.7012	108GluH	108GluN	8.36756	113.5401	108GluH	108GluN
9.38562	120.1509	109AsnH	109AsnN	9.31585	120.13682	109AsnH	109AsnN
9.80254	122.1423	110SerH	110SerN	9.82524	122.02011	110SerH	110SerN
8.31275	123.4047	111AlaH	111AlaN	8.28885	123.42175	111AlaH	111AlaN
7.74809	113.4934	112GluH	112GluN	7.77428	113.62941	112GluH	112GluN
8.29799	114.6368	114GluH	114GluN	8.51444	114.7389	114GluH	114GluN
7.80129	115.9036	115GlnH	115GlnN			115GlnH	115GlnN
7.55463	109.5774	116SerH	116SerN	7.57996	109.8805	116SerH	116SerN
7.23519	126.2904	117LeuH	117LeuN	7.24807	126.90872	117LeuH	117LeuN
8.81571	126.0732	118ValH	118ValN	8.8264	125.78253	118ValH	118ValN
9.40553	121.9189	119CysH	119CysN	9.5114	122.05082	119CysH	119CysN
9.34365	118.5203	120GlnH	120GlnN	9.32355	118.48955	120GlnH	120GlnN
7.85199	119.9971	121CysH	121CysN			121CysH	121CysN
9.73782	128.505	122LeuH	122LeuN	9.73866	128.56131	122LeuH	122LeuN
9.45469	111.3953	123ValH	123ValN	9.4416	111.54708	123ValH	123ValN
7.92677	119.6724	124ArgH	124ArgN	7.93195	119.76318	124ArgH	124ArgN
7.50602	108.3734	125ThrH	125ThrN	7.57508	108.4092	125ThrH	125ThrN
8.28294	119.9774	127GluH	127GluN	8.34175	120.41194	127GluH	127GluN
8.34333	119.1833	128ValH	128ValN	8.34697	119.25082	128ValH	128ValN
6.54905	123.9292	129AspH	129AspN			129AspH	129AspN
9.099	123.7389	130AspH	130AspN	8.91334	125.16318	130AspH	130AspN
8.36184	121.0666	131GluH	131GluN	8.32864	121.14207	131GluH	131GluN
7.59799	121.3508	132AlaH	132AlaN	7.8827	122.45506	132AlaH	132AlaN
7.71521	116.0186	133LeuH	133LeuN	7.69738	116.45833	133LeuH	133LeuN
8.03054	119.4525	134GluH	134GluN	8.05092	119.54641	134GluH	134GluN
7.96565	119.7573	135LysH	135LysN			135LysH	135LysN
8.51648	121.6446	136PheH	136PheN	8.51634	121.87362	136PheH	136PheN
8.74399	117.1587	137AspH	137AspN	8.57401	118.74543	137AspH	137AspN
7.95303	119.1884	138LysH	138LysN	7.95684	119.32023	138LysH	138LysN
7.82219	121.8021	139AlaH	139AlaN	7.85513	121.68615	139AlaH	139AlaN
7.49697	114.3457	140LeuH	140LeuN	7.49057	114.77317	140LeuH	140LeuN
7.15578	119.6065	141LysH	141LysN	7.16841	119.78887	141LysH	141LysN
7.76552	118.5511	142AlaH	142AlaN	7.755	118.53939	142AlaH	142AlaN
7.71446	119.6259	143LeuH	143LeuN	7.72371	119.72597	143LeuH	143LeuN
7.67849	118.0502	145MetH	145MetN	7.63753	118.12747	145MetH	145MetN

8.79564	117.935	146HisH	146HisN	8.81493	117.8165	146HisH	146HisN
7.71735	117.3738	147IleH	147IleN			147IleH	147IleN
8.35305	126.5955	148ArgH	148ArgN			148ArgH	148ArgN
9.14659	123.9575	149LeuH	149LeuN	9.20937	125.01044	149LeuH	149LeuN
8.21158	116.3462	150SerH	150SerN	8.22909	116.33363	150SerH	150SerN
8.10716	119.4649	151PheH	151PheN	8.14169	119.74648	151PheH	151PheN
8.75075	119.3184	152AsnH	152AsnN	8.7258	119.25046	152AsnH	152AsnN
8.12013	115.1838	154ThrH	154ThrN	8.11869	115.19077	154ThrH	154ThrN
8.32129	122.2797	155GlnH	155GlnN	8.30967	122.34969	155GlnH	155GlnN
8.13395	122.2049	156LeuH	156LeuN	8.12967	122.20482	156LeuH	156LeuN
8.24512	124.5139	159GlnH	159GlnN	8.30288	125.02645	159GlnH	159GlnN
8.27406	121.4658	160CysH	160CysN			160CysH	160CysN
8.16857	120.2484	161HisH	161HisN			161HisH	161HisN
				8.15198	109.00202	{153}H[306]	{153}N[305]
				7.99379	121.15781	{159}H[318]	{159}N[317]
				8.38869	124.6487	{167}[340]	{167}N[338]
				8.10204	117.50465	{168}H[341]	{168}N[342]
				8.13905	121.24688	{169}H[343]	{169}N[344]

Supporting table S3) Difference between the assignment at pH 2.65 and the assignment by Uhrínová. S et al.

Presented BLGA assingment				Original peak list by Uhrínová. S et al.				Difference in chemical shift		
Position F1	Position F2	Assign F1	Assign F2	Position F1	Position F2	Assign F1	Assign F2	delta 1H	delta 15N	Delta-delta
8.714	127.01	-1AlaH	-1AlaN	8.66671	18.84152	-1AlaH	-1AlaN	0.04729	108.1685	21.63375
8.15643	124.5946	1AlaH	1AlaN	8.15671	124.796	1AlaH	1AlaN	-0.00028	-0.20139	0.040279
7.894	116.98	2TyrH	2TyrN	7.84671	116.836	2TyrH	2TyrN	0.04729	0.14404	0.055374
7.97976	120.0125	3ValH	3ValN	7.93671	120.166	3ValH	3ValN	0.04305	-0.15351	0.052876
8.06521	115.7838	4ThrH	4ThrN	8.01671	115.816	4ThrH	4ThrN	0.0485	-0.03218	0.048925
8.27049	123.7363	5GlnH	5GlnN	8.19671	123.486	5GlnH	5GlnN	0.07378	0.25033	0.089163
7.76571	111.525	6ThrH	6ThrN	7.70671	111.026	6ThrH	6ThrN	0.059	0.4995	0.11603

	5								5	
7.74564	120.2697	7MetH	7MetN	7.74771	120.264	7MetH	7MetN	-0.00207	0.00578	0.002371
8.67298	127.6449	8LysH	8LysN	8.61671	127.316	8LysH	8LysN	0.05627	0.32896	0.086573
8.56641	109.4976	9GlyH	9GlyN	8.56871	109.51	9GlyH	9GlyN	-0.0023	-0.01233	0.003372
7.55358	120.1035	10LeuH	10LeuN	7.55271	120.04	10LeuH	10LeuN	0.00087	0.06356	0.012742
8.62551	126.7537	11AspH	11AspN	8.58671	126.786	11AspH	11AspN	0.0388	-0.03224	0.039332
8.87636	122.891	12IleH	12IleN	8.86971	122.854	12IleH	12IleN	0.00665	0.03701	0.00995
7.96968	117.1562	13GlnH	13GlnN	7.96671	117.145	13GlnH	13GlnN	0.00297	0.01122	0.003722
7.21671	113.686	14LysH	14LysN	7.21671	113.686	14LysH	14LysN	0	0	0
7.39204	110.2285	15ValH	15ValN	7.39071	110.21	15ValH	15ValN	0.00133	0.01851	0.003934
6.80901	120.9193	16AlaH	16AlaN	6.80871	120.877	16AlaH	16AlaN	0.0003	0.04237	0.008479
9.18302	110.1356	17GlyH	17GlyN	9.18271	110.115	17GlyH	17GlyN	0.00031	0.02062	0.004136
8.39327	118.4161	18ThrH	18ThrN	8.33671	118.116	18ThrH	18ThrN	0.05656	0.30016	0.08248
7.4529	124.024	19TrpH	19TrpN	7.45771	123.997	19TrpH	19TrpN	-0.00481	0.02701	0.007233
9.11053	112.1596	20TyrH	20TyrN	9.11071	112.1	20TyrH	20TyrN	-0.00018	0.05959	0.011919
9.90568	119.4652	21SerH	21SerN	9.90271	119.456	21SerH	21SerN	0.00297	0.00922	0.003496
7.7138	114.8999	23AlaH	23AlaN	7.70871	114.873	23AlaH	23AlaN	0.00509	0.02689	0.007405

9.07795	114.8068	24MetH	24MetN	9.07771	114.776	24MetH	24MetN	0.00024	0.03081	0.006167
9.30156	123.4667	25AlaH	25AlaN	9.29571	123.439	25AlaH	25AlaN	0.00585	0.02778	0.008068
8.31501	119.7417	26AlaH	26AlaN	8.30971	119.687	26AlaH	26AlaN	0.0053	0.05475	0.012165
8.36407	116.245	27SerH	27SerN	8.36071	116.204	27SerH	27SerN	0.00336	0.04101	0.008864
7.86704	119.7309	28AspH	28AspN	7.83671	119.656	28AspH	28AspN	0.03033	0.0749	0.033828
8.80004	124.2856	29IleH	29IleN	8.79271	124.22	29IleH	29IleN	0.00733	0.06567	0.015041
7.95915	114.1799	30SerH	30SerN	7.95671	114.158	30SerH	30SerN	0.00244	0.02194	0.005021
7.59857	117.0407	31LeuH	31LeuN	7.59471	117.005	31LeuH	31LeuN	0.00386	0.03576	0.008127
7.05274	111.2054	32LeuH	32LeuN	7.05271	111.156	32LeuH	32LeuN	3E-05	0.04947	0.009894
7.82273	118.3684	33AspH	33AspN	7.77671	118.306	33AspH	33AspN	0.04602	0.06242	0.047683
7.6322	120.6785	34AlaH	34AlaN	7.63271	120.642	34AlaH	34AlaN	-0.00051	0.03657	0.007332
9.05349	121.4988	35GlnH	35GlnN	9.01671	121.376	35GlnH	35GlnN	0.03678	0.12283	0.04423
7.71685	107.569	36SerH	36SerN	7.70971	107.522	36SerH	36SerN	0.00714	0.04704	0.011811
7.53554	126.2455	37AlaH	37AlaN	7.53471	126.215	37AlaH	37AlaN	0.00083	0.03056	0.006168
7.76613	114.0162	39LeuH	39LeuN	7.75571	113.949	39LeuH	39LeuN	0.01042	0.06724	0.017012
7.41082	122.2755	40ArgH	40ArgN	7.40971	122.227	40ArgH	40ArgN	0.00111	0.04855	0.009773
6.95828	117			6.95271	116.975			0.005	0.0250	0.00749

		41Val H	41Val N			41Val H	41Val N	57	8	6
8.60354	120.5741	42Tyr H	42Tyr N	8.59971	120.572	42Tyr H	42Tyr N	0.00383	0.00218	0.003855
8.62631	125.1627	43Val H	43Val N	8.62271	125.123	43Val H	43Val N	0.0036	0.03976	0.008729
8.96265	121.7778	44Glu H	44Glu N	8.91671	121.806	44Glu H	44Glu N	-0.04594	-0.02815	0.046284
7.6186	116.2857	45Glu H	45Glu N	7.62071	116.223	45Glu H	45Glu N	-0.00211	0.06272	0.01272
8.66171	122.0424	46Leu H	46Leu N	8.66171	122.004	46Leu H	46Leu N	0	0.03846	0.007692
9.15635	122.4511	47Lys H	47Lys N	9.15771	122.424	47Lys H	47Lys N	-0.00136	0.02713	0.005594
8.59743	114.3018	49Thr H	49Thr N	8.60071	114.288	49Thr H	49Thr N	-0.00328	0.01386	0.004294
7.52999	112.0727	51Glu H	51Glu N	7.49671	112.086	51Glu H	51Glu N	0.03328	-0.0133	0.033386
8.28883	107.0215	52Gly H	52Gly N	8.28071	106.994	52Gly H	52Gly N	0.00812	0.02751	0.009808
7.18097	115.1938	53Asp H	53Asp N	7.17571	115.13	53Asp H	53Asp N	0.00526	0.06381	0.013803
8.80694	119.4394	54Leu H	54Leu N	8.79671	119.287	54Leu H	54Leu N	0.01023	0.15239	0.032149
8.62626	126.0635	55Glu H	55Glu N	8.62671	125.876	55Glu H	55Glu N	-0.00045	0.18757	0.037517
9.26173	126.5153	56IleH	56IleN	9.25971	126.498	56IleH	56IleN	0.00202	0.01737	0.004019
8.48296	128.6041	57Leu H	57Leu N	8.48571	128.546	57Leu H	57Leu N	-0.00275	0.05813	0.011947
9.06596	123.3919	58Leu H	58Leu N	9.06671	123.367	58Leu H	58Leu N	-0.00075	0.02498	0.005052
9.23144	117.7148	59Gln	59Gln	9.22371	117.655	59Gln	59Gln	0.00773	0.05983	0.014246

		H	N			H	N			
8.95487	121.5045	60LysH	60LysN	8.91671	121.496	60LysH	60LysN	0.03816	0.0085	0.038198
9.74021	129.856	61TrpH	61TrpN	9.73471	129.762	61TrpH	61TrpN	0.0055	0.09404	0.019596
8.74944	127.2512	62GluH	62GluN	8.67671	127.376	62GluH	62GluN	0.07273	-0.12476	0.076891
8.91417	121.6213	63AsnH	63AsnN	8.86671	121.476	63AsnH	63AsnN	0.04746	0.14538	0.055658
8.13732	110.9992	64AspH	64AspN	8.13871	110.966	64AspH	64AspN	-0.00139	0.03326	0.006796
6.75262	114.5227	65GluH	65GluN	6.75071	114.489	65GluH	65GluN	0.00191	0.03375	0.007015
8.67315	120.2401	66CysH	66CysN	8.66571	120.173	66CysH	66CysN	0.00744	0.06715	0.015353
8.97902	133.4899	67AlaH	67AlaN	8.97871	133.464	67AlaH	67AlaN	0.00031	0.0259	0.005189
8.38211	118.635	68GlnH	68GlnN	8.34671	118.636	68GlnH	68GlnN	0.0354	-0.00096	0.035401
9.15043	123.6905	69LysH	69LysN	9.14771	123.65	69LysH	69LysN	0.00272	0.04051	0.008546
8.54061	123.6847	70LysH	70LysN	8.53471	123.623	70LysH	70LysN	0.0059	0.06175	0.013687
8.95827	124.1015	71IleH	71IleN	8.95371	124.008	71IleH	71IleN	0.00456	0.09353	0.019254
8.49789	125.4454	72IleH	72IleN	8.49371	125.402	72IleH	72IleN	0.00418	0.04339	0.009632
9.26399	133.2975	73AlaH	73AlaN	9.25771	133.246	73AlaH	73AlaN	0.00628	0.05153	0.012069
9.43616	124.5123	74GluH	74GluN	9.42871	124.432	74GluH	74GluN	0.00745	0.08031	0.017706
8.4843	123.1493	75LysH	75LysN	8.48471	123.032	75LysH	75LysN	-0.00041	0.11733	0.02347

8.44455	112.5842	76ThrH	76ThrN	8.43871	112.528	76ThrH	76ThrN	0.00584	0.05627	0.012679
8.27246	116.4517	77LysH	77LysN	8.21671	116.306	77LysH	77LysN	0.05575	0.1457	0.062906
7.97976	122.2947	78IleH	78IleN	7.97671	122.156	78IleH	78IleN	0.00305	0.1387	0.027907
7.85116	115.217	80AlaH	80AlaN	7.83971	115.155	80AlaH	80AlaN	0.01145	0.06201	0.016879
7.28916	117.3683	81ValH	81ValN	7.29071	117.327	81ValH	81ValN	-0.00155	0.04136	0.008416
9.53946	126.2751	82PheH	82PheN	9.53171	126.222	82PheH	82PheN	0.00775	0.05314	0.013154
9.48594	123.2371	83LysH	83LysN	9.48371	123.137	83LysH	83LysN	0.00223	0.10012	0.020148
7.7646	116.4437	84IleH	84IleN	7.72671	116.283	84IleH	84IleN	0.03789	0.16071	0.049687
8.79959	119.195	85AspH	85AspN	8.79471	119.212	85AspH	85AspN	0.00488	-0.01692	0.005939
7.22122	121.9317	86AlaH	86AlaN	7.21771	121.909	86AlaH	86AlaN	0.00351	0.02274	0.005745
9.01196	114.5393	87LeuH	87LeuN	9.00271	114.586	87LeuH	87LeuN	0.00925	-0.04667	0.013141
8.68998	111.244	88AsnH	88AsnN	8.68071	111.222	88AsnH	88AsnN	0.00927	0.02205	0.010266
8.24907	117.3076	89GluH	89GluN	8.22671	117.226	89GluH	89GluN	0.02236	0.08164	0.027687
9.10042	115.6569	90AsnH	90AsnN	9.09471	115.64	90AsnH	90AsnN	0.00571	0.01696	0.006642
8.55857	121.6032	91LysH	91LysN	8.55671	121.486	91LysH	91LysN	0.00186	0.11721	0.023516
9.05227	121.2037	92ValH	92ValN	9.00671	121.296	92ValH	92ValN	0.04556	-0.09229	0.049157
9.42034	125.9924	93Leu	93Leu	9.42171	125.949	93Leu	93Leu	-0.001	0.04346	0.008799

		H	N			H	N	37		
9.32618	123.8768	94ValH	94ValN	9.32071	123.826	94ValH	94ValN	0.00547	0.05085	0.011548
8.45414	128.07	95LeuH	95LeuN	8.45471	128.055	95LeuH	95LeuN	-0.00057	0.01507	0.003067
7.20926	109.8899	96AspH	96AspN	7.20571	109.887	96AspH	96AspN	0.00355	0.00298	0.0036
7.78325	118.8771	97ThrH	97ThrN	7.75671	118.816	97ThrH	97ThrN	0.02654	0.06118	0.029225
6.77772	123.0158	98AspH	98AspN	6.78371	123.026	98AspH	98AspN	-0.00599	-0.01015	0.006325
9.0333	117.7347	99TyrH	99TyrN	9.02871	117.67	99TyrH	99TyrN	0.00459	0.06475	0.013739
9.00755	119.5293	100LysH	100LysN	9.00471	119.499	100LysH	100LysN	0.00284	0.03034	0.0067
9.15464	118.6572	101LysH	101LysN	9.17471	118.672	101LysH	101LysN	-0.02007	-0.01474	0.020285
8.92234	116.313	102TyrH	102TyrN	8.93171	116.264	102TyrH	102TyrN	-0.00937	0.04904	0.013564
9.15925	125.0007	103LeuH	103LeuN	9.15671	124.966	103LeuH	103LeuN	0.00254	0.03475	0.0074
9.36536	124.9315	104LeuH	104LeuN	9.36271	124.899	104LeuH	104LeuN	0.00265	0.0325	0.007019
8.8884	121.2799	105PheH	105PheN	8.86671	121.216	105PheH	105PheN	0.02169	0.06391	0.025176
9.65116	117.241	106CysH	106CysN	9.64471	117.185	106CysH	106CysN	0.00645	0.056	0.012924
9.91503	120.6674	107MetH	107MetN	9.91471	120.639	107MetH	107MetN	0.00032	0.02846	0.005701
8.39827	113.7012	108GluH	108GluN	8.39871	113.701	108GluH	108GluN	-0.00044	0.00023	0.000442
9.38562	120.1509	109As	109As	9.37971	120.102	109As	109As	0.00591	0.04891	0.011429

		nH	nN			nH	nN			
9.80254	122.1423	110SerH	110SerN	9.79871	122.111	110SerH	110SerN	0.00383	0.03133	0.007344
8.31275	123.4047	111AlaH	111AlaN	8.28671	123.226	111AlaH	111AlaN	0.02604	0.17874	0.044227
7.74809	113.4934	112GluH	112GluN	7.74071	113.434	112GluH	112GluN	0.00738	0.05941	0.013987
8.29799	114.6368	114GluH	114GluN	8.29471	114.629	114GluH	114GluN	0.00328	0.00783	0.003635
7.80129	115.9036	115GlnH	115GlnN	7.75671	115.736	115GlnH	115GlnN	0.04458	0.16766	0.055783
7.55463	109.5774	116SerH	116SerN	7.55771	109.554	116SerH	116SerN	-0.00308	0.02342	0.005606
7.23519	126.2904	117LeuH	117LeuN	7.23071	126.251	117LeuH	117LeuN	0.00448	0.03939	0.009063
8.81571	126.0732	118ValH	118ValN	8.80571	126.07	118ValH	118ValN	0.01	0.00327	0.010021
9.40553	121.9189	119CysH	119CysN	9.40371	121.903	119CysH	119CysN	0.00182	0.01591	0.003666
9.34365	118.5203	120GlnH	120GlnN	9.32671	118.446	120GlnH	120GlnN	0.01694	0.07436	0.022542
7.85199	119.9971	121CysH	121CysN	7.84671	120.076	121CysH	121CysN	-0.00528	-0.07889	0.016638
9.73782	128.505	122LeuH	122LeuN	9.73771	128.487	122LeuH	122LeuN	0.00011	0.01802	0.003606
9.45469	111.3953	123ValH	123ValN	9.45371	111.378	123ValH	123ValN	0.00098	0.01733	0.003602
7.92677	119.6724	124ArgH	124ArgN	7.90671	119.556	124ArgH	124ArgN	0.02006	0.11644	0.030737
7.50602	108.3734	125ThrH	125ThrN	7.50771	108.354	125ThrH	125ThrN	-0.00169	0.01948	0.004247
8.28294	119.9774	127Glu	127Glu	8.25671	119.856	127Glu	127Glu	0.02623	0.12142	0.035745

		H	N			H	N			
8.34333	119.1833	128ValH	128ValN	8.30671	118.956	128ValH	128ValN	0.03662	0.22731	0.058377
6.54905	123.9292	129AspH	129AspN	6.54971	123.869	129AspH	129AspN	-0.00066	0.06024	0.012066
9.099	123.7389	130AspH	130AspN	9.08971	123.691	130AspH	130AspN	0.00929	0.04794	0.01335
8.36184	121.0666	131GluH	131GluN	8.32671	120.876	131GluH	131GluN	0.03513	0.19064	0.051845
7.59799	121.3508	132AlaH	132AlaN	7.59871	121.317	132AlaH	132AlaN	-0.00072	0.03383	0.006804
7.71521	116.0186	133LeuH	133LeuN	7.72671	116.256	133LeuH	133LeuN	-0.0115	-0.23733	0.048839
8.03054	119.4525	134GluH	134GluN	8.01671	119.626	134GluH	134GluN	0.01383	-0.17346	0.037347
7.96565	119.7573	135LysH	135LysN	7.91671	119.616	135LysH	135LysN	0.04894	0.14129	0.056512
8.51648	121.6446	136PheH	136PheN	8.51671	121.446	136PheH	136PheN	-0.00023	0.19859	0.039719
8.74399	117.1587	137AspH	137AspN	8.74171	117.114	137AspH	137AspN	0.00228	0.04477	0.00924
7.95303	119.1884	138LysH	138LysN	7.90671	119.366	138LysH	138LysN	0.04632	-0.17753	0.058363
7.82219	121.8021	139AlaH	139AlaN	7.81871	121.75	139AlaH	139AlaN	0.00348	0.05216	0.010997
7.49697	114.3457	140LeuH	140LeuN	7.49171	114.282	140LeuH	140LeuN	0.00526	0.0637	0.013783
7.15578	119.6065	141LysH	141LysN	7.15371	119.582	141LysH	141LysN	0.00207	0.02458	0.005334
7.76552	118.5511	142AlaH	142AlaN	7.77671	118.306	142AlaH	142AlaN	-0.01119	0.24512	0.050285
7.71446	119.6259	143Le	143Le	7.71171	119.606	143Le	143Le	0.00275	0.01989	0.004836

		uH	uN			uH	uN			
7.67849	118.0502	145MetH	145MetN	7.67171	118.024	145MetH	145MetN	0.00678	0.02625	0.008575
8.79564	117.935	146HisH	146HisN	8.78971	117.917	146HisH	146HisN	0.00593	0.01806	0.006943
7.71735	117.3738	147IleH	147IleN	7.71471	117.335	147IleH	147IleN	0.00264	0.03883	0.008202
8.35305	126.5955	148ArgH	148ArgN	8.34471	126.557	148ArgH	148ArgN	0.00834	0.03853	0.011355
9.14659	123.9575	149LeuH	149LeuN	9.14471	123.935	149LeuH	149LeuN	0.00188	0.02257	0.00489
8.21158	116.3462	150SerH	150SerN	8.16671	116.306	150SerH	150SerN	0.04487	0.04019	0.045584
8.10716	119.4649	151PheH	151PheN	8.05671	119.806	151PheH	151PheN	-0.05045	-0.34104	0.084838
8.75075	119.3184	152AsnH	152AsnN	8.74671	119.306	152AsnH	152AsnN	0.00404	0.01245	0.004746
8.12013	115.1838	154ThrH	154ThrN	8.11671	115.566	154ThrH	154ThrN	-0.00342	-0.38218	0.076512
8.32129	122.2797	155GlnH	155GlnN	8.31171	122.264	155GlnH	155GlnN	0.00958	0.01578	0.010086
8.13395	122.2049	156LeuH	156LeuN	8.17671	122.466	156LeuH	156LeuN	-0.04276	-0.26103	0.067482
8.24512	124.5139	159GlnH	159GlnN	8.23671	124.826	159GlnH	159GlnN	0.00841	-0.31209	0.062982
8.27406	121.4658	160CysH	160CysN	8.22671	121.306	160CysH	160CysN	0.04735	0.15979	0.057126
8.16857	120.2484	161HisH	161HisN	8.13671	119.896	161HisH	161HisN	0.03186	0.35247	0.077359

Supporting Table S4. Unassigned residues and residues with peaks disappearing during pH titration to pH 4.0.

Unassigned residues		Residues with disappearing NMR signal during pH titration	Titration step for disappearing NMR signal
Y2	38Pro	A26	3.20 → 4.00
L22	48Pro	S27	3.20 → 4.00
N63	50Pro	I29	3.20 → 4.00
D129	79Pro	S30	2.65 → 3.20
K135	113Pro	L32	2.65 → 3.20
E157	126Pro	D33	3.20 → 4.00
E158	144Pro	A34	3.20 → 4.00
C160	153Pro	W61	3.20 → 4.00
I162		V92	3.20 → 4.00
		Q115	3.20 → 4.00
		C121	3.20 → 4.00
		D129	2.65 → 3.20
		I147	3.20 → 4.00
		R148	3.20 → 4.00
		H161	3.20 → 4.00