



Full wwPDB X-ray Structure Validation Report ⓘ

Aug 7, 2020 – 06:02 PM BST

PDB ID : 2P1S
Title : Crystal structure of the C-terminal lobe of bovine lactoferrin complexed with O-alpha-D-Glucopyranosyl-(1 3)-alpha-D-fructofuranosyl- (2 1)- alpha-D-glucopyranoside at 1.93 Å resolution
Authors : Mir, R.; Singh, N.; Sinha, M.; Sharma, S.; Kaur, P.; Singh, T.P.
Deposited on : 2007-03-06
Resolution : 1.93 Å(reported)

This is a Full wwPDB X-ray Structure Validation Report for a publicly released PDB entry.

We welcome your comments at validation@mail.wwpdb.org

A user guide is available at

<https://www.wwpdb.org/validation/2017/XrayValidationReportHelp>

with specific help available everywhere you see the ⓘ symbol.

The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

MolProbity : 4.02b-467
Mogul : 1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix) : **NOT EXECUTED**
EDS : **NOT EXECUTED**
Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.13.1

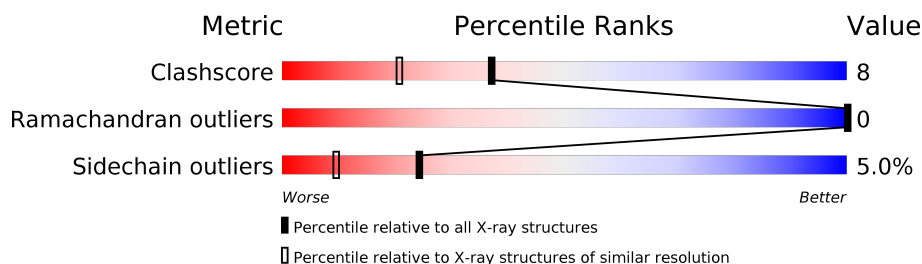
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.93 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
Clashscore	141614	1023 (1.94-1.94)
Ramachandran outliers	138981	1007 (1.94-1.94)
Sidechain outliers	138945	1007 (1.94-1.94)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for ≥ 3 , 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions $\leq 5\%$.

Note EDS was not executed.

Mol	Chain	Length	Quality of chain
1	A	344	81% 16% ..
2	B	3	33% 67%
3	C	4	100%
4	D	3	33% 67%

2 Entry composition [i](#)

There are 10 unique types of molecules in this entry. The entry contains 3067 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

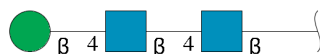
- Molecule 1 is a protein called Lactotransferrin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	340	Total	C	N	O	S	0	0	0
			2594	1613	453	507	21			

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	565	LYS	ASN	SEE REMARK 999	UNP P24627
A	608	GLU	LYS	SEE REMARK 999	UNP P24627

- Molecule 2 is an oligosaccharide called beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose.



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace
2	B	3	Total	C	N	O	0	0	0
			39	22	2	15			

- Molecule 3 is an oligosaccharide called alpha-D-mannopyranose-(1-4)-beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose.



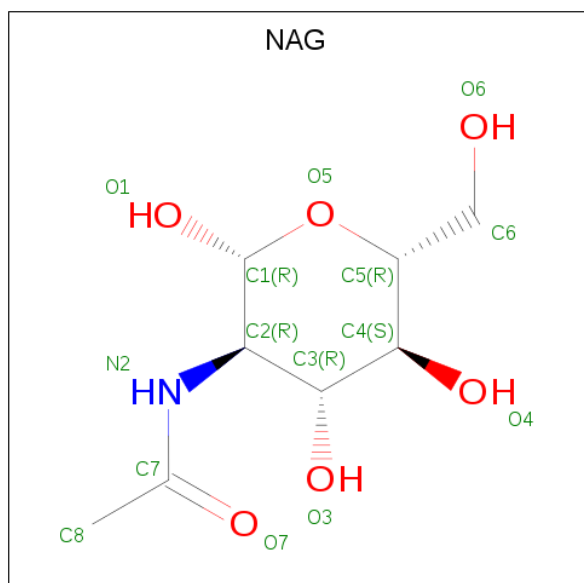
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace
3	C	4	Total	C	N	O	0	0	0
			50	28	2	20			

- Molecule 4 is an oligosaccharide called alpha-D-galactopyranose-(1-2)-alpha-D-fructofuranose-(1-3)-alpha-D-glucopyranose.



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace
4	D	3	Total	C	O	0	0	0
			34	18	16			

- Molecule 5 is 2-acetamido-2-deoxy-beta-D-glucopyranose (three-letter code: NAG) (formula: $C_8H_{15}NO_6$).

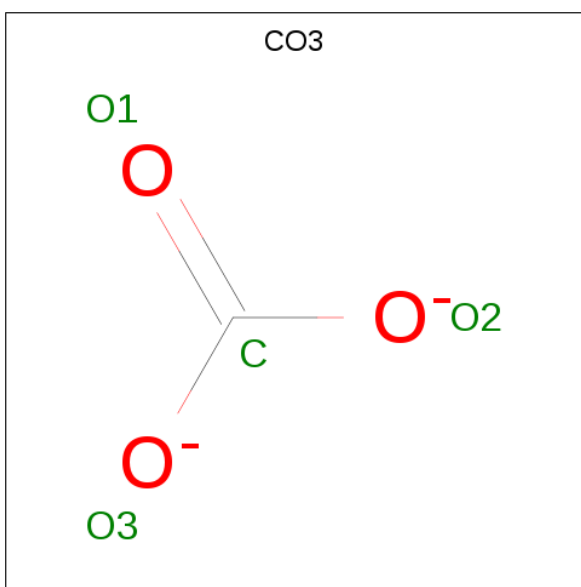


Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
5	A	1	Total	C	N	O	0	0
			14	8	1	5		

- Molecule 6 is FE (III) ION (three-letter code: FE) (formula: Fe).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	1	Total	Fe	0	0
			1	1		

- Molecule 7 is CARBONATE ION (three-letter code: CO3) (formula: CO_3).

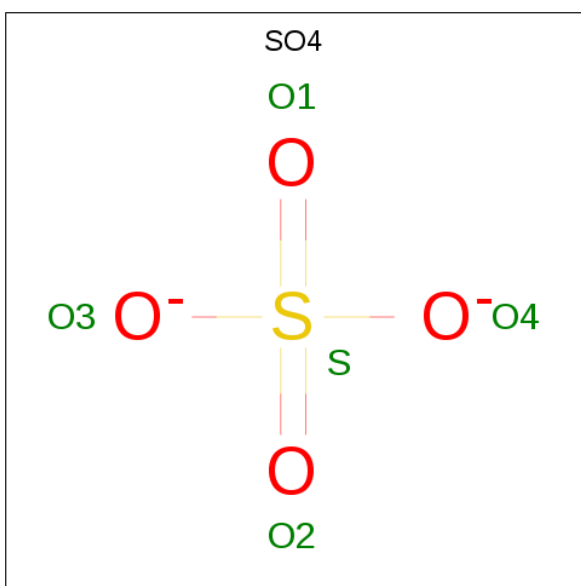


Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
7	A	1	Total	C	O	0	0
			4	1	3		

- Molecule 8 is ZINC ION (three-letter code: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
8	A	2	Total	Zn	0	0
			2	2		

- Molecule 9 is SULFATE ION (three-letter code: SO4) (formula: O₄S).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
9	A	1	Total	O	S	0	0
			5	4	1		

- Molecule 10 is water.

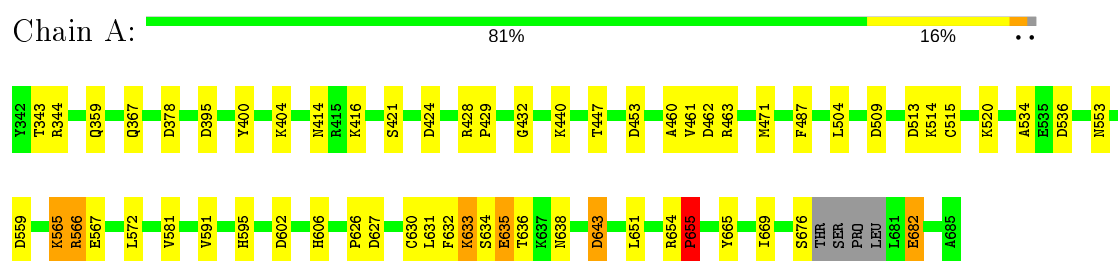
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
10	A	324	Total	O	0	0
			324	324		

3 Residue-property plots [i](#)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

Note EDS was not executed.

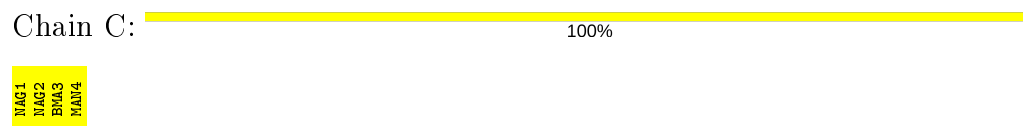
- Molecule 1: Lactotransferrin



- Molecule 2: beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose



- Molecule 3: alpha-D-mannopyranose-(1-4)-beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose



- Molecule 4: alpha-D-galactopyranose-(1-2)-alpha-D-fructofuranose-(1-3)-alpha-D-glucopyranose



4 Data and refinement statistics

Xtriage (Phenix) and EDS were not executed - this section is therefore incomplete.

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	63.16 Å 50.42 Å 65.88 Å 90.00° 107.70° 90.00°	Depositor
Resolution (Å)	63.25 – 1.93	Depositor
% Data completeness (in resolution range)	94.1 (63.25-1.93)	Depositor
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
Refinement program	REFMAC 5.0	Depositor
R, R_{free}	0.170 , 0.217	Depositor
Estimated twinning fraction	No twinning to report.	Xtriage
Total number of atoms	3067	wwPDB-VP
Average B, all atoms (Å ²)	32.0	wwPDB-VP

5 Model quality [i](#)

5.1 Standard geometry [i](#)

Bond lengths and bond angles in the following residue types are not validated in this section: ZN, BMA, NAG, Z9N, GLA, GLC, CO3, SO4, MAN, FE

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	$\# Z > 5$	RMSZ	$\# Z > 5$
1	A	0.71	0/2641	0.90	9/3575 (0.3%)

There are no bond length outliers.

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed($^{\circ}$)	Ideal($^{\circ}$)
1	A	378	ASP	CB-CG-OD2	6.89	124.50	118.30
1	A	655	PRO	CA-N-CD	-6.22	102.79	111.50
1	A	643	ASP	CB-CG-OD2	5.75	123.47	118.30
1	A	627	ASP	CB-CG-OD2	5.68	123.41	118.30
1	A	536	ASP	CB-CG-OD2	5.60	123.34	118.30
1	A	509	ASP	CB-CG-OD2	5.36	123.12	118.30
1	A	602	ASP	CB-CG-OD2	5.11	122.90	118.30
1	A	635	GLU	CA-CB-CG	5.10	124.62	113.40
1	A	453	ASP	CB-CG-OD2	5.05	122.84	118.30

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts [i](#)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	2594	0	2510	41	0
2	B	39	0	34	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
3	C	50	0	43	0	0
4	D	34	0	20	1	0
5	A	14	0	13	0	0
6	A	1	0	0	0	0
7	A	4	0	0	0	0
8	A	2	0	0	0	0
9	A	5	0	0	0	0
10	A	324	0	0	8	0
All	All	3067	0	2620	42	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 8.

All (42) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:343:THR:HA	1:A:606:HIS:CE1	1.79	1.15
1:A:565:LYS:HD3	1:A:567:GLU:H	1.19	1.01
1:A:343:THR:HA	1:A:606:HIS:HE1	1.32	0.94
1:A:514:LYS:HD3	10:A:2105:HOH:O	1.71	0.90
1:A:359:GLN:HG2	10:A:2190:HOH:O	1.70	0.89
1:A:565:LYS:CD	1:A:567:GLU:H	1.91	0.83
1:A:682:GLU:HA	1:A:682:GLU:OE2	1.79	0.81
1:A:635:GLU:O	1:A:636:THR:HG22	1.84	0.78
1:A:638:ASN:HD22	1:A:643:ASP:H	1.31	0.76
1:A:343:THR:HA	1:A:606:HIS:NE2	2.03	0.74
1:A:565:LYS:HD3	1:A:567:GLU:N	2.00	0.74
1:A:565:LYS:HD2	1:A:567:GLU:HB2	1.72	0.71
1:A:416:LYS:HG2	10:A:2060:HOH:O	1.93	0.69
1:A:638:ASN:ND2	1:A:643:ASP:H	1.98	0.61
1:A:626:PRO:HA	1:A:630:CYS:SG	2.44	0.58
1:A:432:GLY:N	10:A:2257:HOH:O	2.39	0.55
1:A:461:VAL:O	1:A:462:ASP:HB2	2.06	0.55
1:A:513:ASP:OD2	1:A:520:LYS:NZ	2.29	0.55
1:A:344:ARG:HD3	10:A:2258:HOH:O	2.08	0.54
1:A:514:LYS:HB2	10:A:2308:HOH:O	2.07	0.53
1:A:471:MET:HE1	1:A:487:PHE:HE2	1.74	0.52
1:A:654:ARG:N	1:A:655:PRO:CD	2.73	0.52
1:A:343:THR:HG22	1:A:606:HIS:HE1	1.75	0.50
1:A:343:THR:CA	1:A:606:HIS:HE1	2.14	0.50
1:A:440:LYS:HE3	1:A:534:ALA:HA	1.95	0.49

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:633:LYS:NZ	10:A:2154:HOH:O	2.37	0.48
1:A:566:ARG:HG2	1:A:581:VAL:HG21	1.95	0.48
1:A:676:SER:HB2	10:A:2316:HOH:O	2.14	0.48
1:A:565:LYS:HD3	1:A:565:LYS:C	2.33	0.48
1:A:395:ASP:HA	1:A:595:HIS:CD2	2.48	0.47
1:A:565:LYS:HD3	1:A:566:ARG:N	2.31	0.46
1:A:665:TYR:CZ	1:A:669:ILE:HD11	2.52	0.45
1:A:553:ASN:OD1	1:A:565:LYS:HE2	2.18	0.43
1:A:632:PHE:O	1:A:633:LYS:HE3	2.20	0.42
4:D:1:GLC:H5	4:D:2:Z9N:C1	2.49	0.42
1:A:447:THR:HA	1:A:572:LEU:HD22	2.01	0.42
1:A:651:LEU:HA	1:A:651:LEU:HD23	1.85	0.42
1:A:428:ARG:HA	1:A:429:PRO:HD3	1.96	0.41
1:A:654:ARG:N	1:A:655:PRO:HD3	2.34	0.41
1:A:634:SER:O	1:A:635:GLU:HG2	2.21	0.40
1:A:400:TYR:CZ	1:A:404:LYS:HE3	2.57	0.40
1:A:460:ALA:HB3	1:A:463:ARG:HD3	2.03	0.40

There are no symmetry-related clashes.

5.3 Torsion angles [i](#)

5.3.1 Protein backbone [i](#)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	A	336/344 (98%)	319 (95%)	17 (5%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains [i](#)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	281/285 (99%)	267 (95%)	14 (5%)	24 9

All (14) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	367	GLN
1	A	414	ASN
1	A	421	SER
1	A	424	ASP
1	A	504	LEU
1	A	515	CYS
1	A	559	ASP
1	A	565	LYS
1	A	566	ARG
1	A	591	VAL
1	A	631	LEU
1	A	633	LYS
1	A	655	PRO
1	A	682	GLU

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (5) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	414	ASN
1	A	606	HIS
1	A	613	HIS
1	A	621	ASN
1	A	638	ASN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates ⓘ

10 monosaccharides are modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
2	NAG	B	1	1,2	14,14,15	0.66	0	17,19,21	1.21	3 (17%)
2	NAG	B	2	2	14,14,15	0.55	0	17,19,21	1.02	0
2	BMA	B	3	2	11,11,12	0.69	0	15,15,17	1.42	1 (6%)
3	NAG	C	1	1,3	14,14,15	0.77	0	17,19,21	1.13	2 (11%)
3	NAG	C	2	3	14,14,15	0.70	0	17,19,21	1.05	1 (5%)
3	BMA	C	3	3	11,11,12	0.57	0	15,15,17	1.28	2 (13%)
3	MAN	C	4	3	11,11,12	0.56	0	15,15,17	0.87	1 (6%)
4	GLC	D	1	4	11,11,12	0.69	0	15,15,17	1.20	1 (6%)
4	Z9N	D	2	4	11,12,12	1.29	1 (9%)	10,18,18	1.01	1 (10%)
4	GLA	D	3	4	11,11,12	0.79	0	15,15,17	1.40	3 (20%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	NAG	B	1	1,2	-	2/6/23/26	0/1/1/1
2	NAG	B	2	2	-	0/6/23/26	0/1/1/1
2	BMA	B	3	2	-	2/2/19/22	0/1/1/1
3	NAG	C	1	1,3	-	0/6/23/26	0/1/1/1
3	NAG	C	2	3	-	2/6/23/26	0/1/1/1
3	BMA	C	3	3	-	0/2/19/22	0/1/1/1
3	MAN	C	4	3	-	0/2/19/22	0/1/1/1
4	GLC	D	1	4	-	2/2/19/22	0/1/1/1
4	Z9N	D	2	4	-	1/5/24/24	0/1/1/1
4	GLA	D	3	4	-	0/2/19/22	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	D	2	Z9N	O2-C2	3.17	1.46	1.40

All (15) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	B	3	BMA	C3-C4-C5	3.55	116.57	110.24
3	C	3	BMA	O5-C5-C6	3.41	112.54	107.20
2	B	1	NAG	O5-C5-C6	2.77	111.54	107.20
4	D	3	GLA	C2-C3-C4	2.63	115.44	110.89
3	C	1	NAG	C1-O5-C5	2.58	115.69	112.19
3	C	1	NAG	O5-C1-C2	-2.52	107.31	111.29
4	D	3	GLA	C1-C2-C3	2.50	112.73	109.67
4	D	1	GLC	C1-C2-C3	2.32	112.52	109.67
3	C	2	NAG	C4-C3-C2	2.31	114.40	111.02
3	C	4	MAN	C1-C2-C3	2.29	112.48	109.67
3	C	3	BMA	C3-C4-C5	2.28	114.31	110.24
4	D	3	GLA	O2-C2-C1	2.21	113.67	109.15
4	D	2	Z9N	O1-C1-C2	-2.19	107.20	111.86
2	B	1	NAG	O5-C1-C2	-2.04	108.06	111.29
2	B	1	NAG	O5-C5-C4	-2.03	105.89	110.83

There are no chirality outliers.

All (9) torsion outliers are listed below:

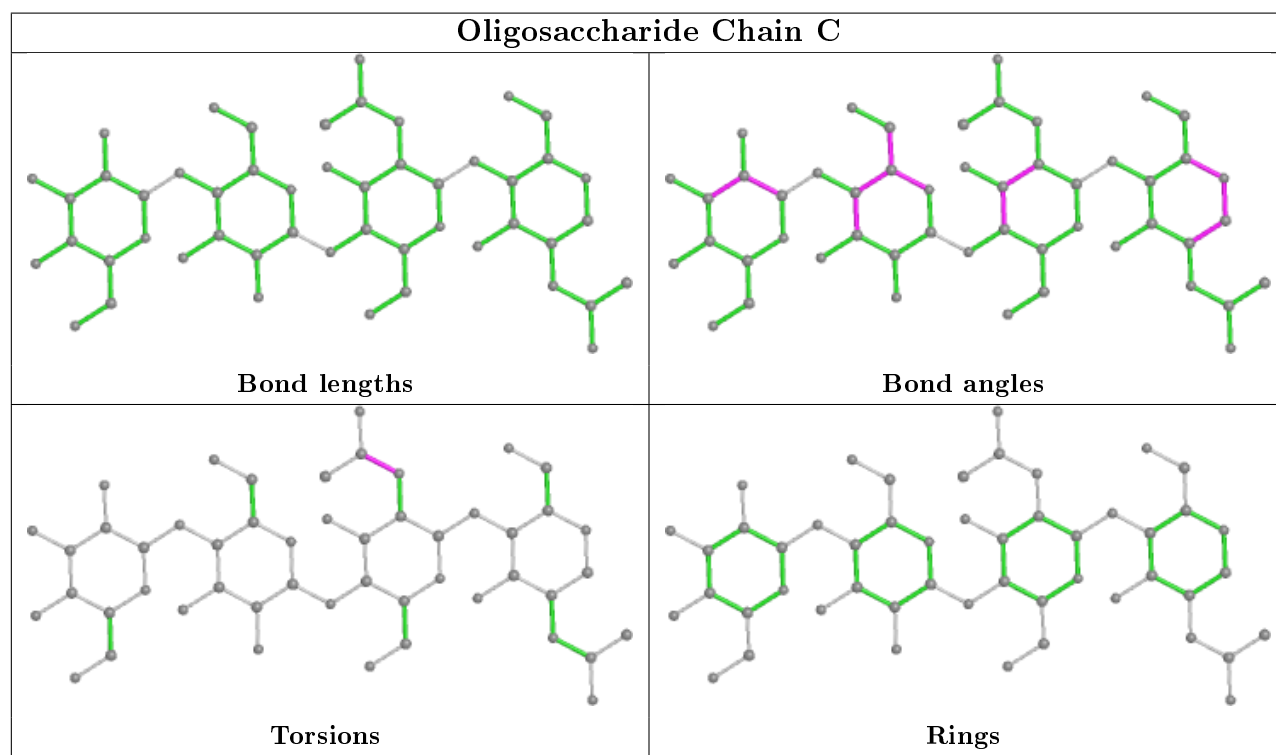
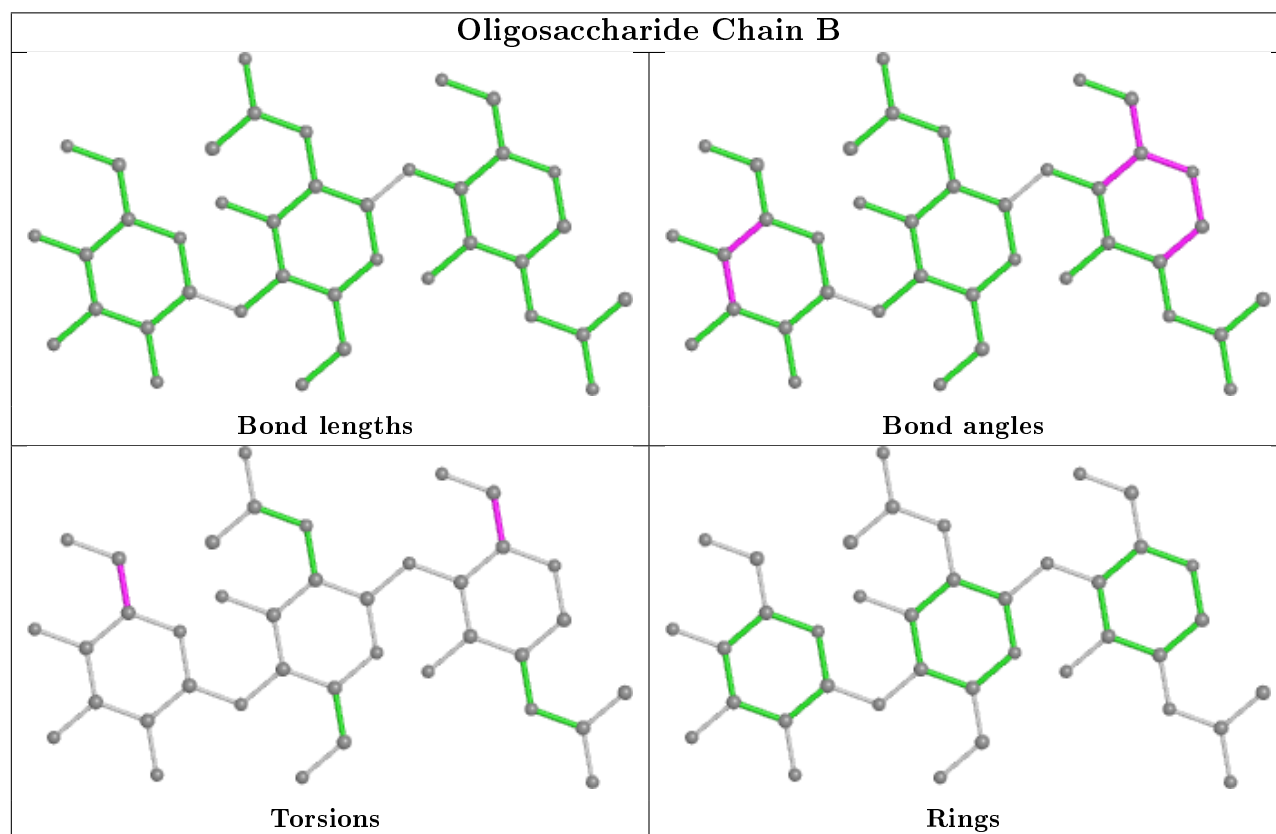
Mol	Chain	Res	Type	Atoms
2	B	1	NAG	O5-C5-C6-O6
2	B	3	BMA	O5-C5-C6-O6
2	B	1	NAG	C4-C5-C6-O6
2	B	3	BMA	C4-C5-C6-O6
3	C	2	NAG	C8-C7-N2-C2
4	D	1	GLC	C4-C5-C6-O6
3	C	2	NAG	O7-C7-N2-C2
4	D	1	GLC	O5-C5-C6-O6
4	D	2	Z9N	O1-C1-C2-C3

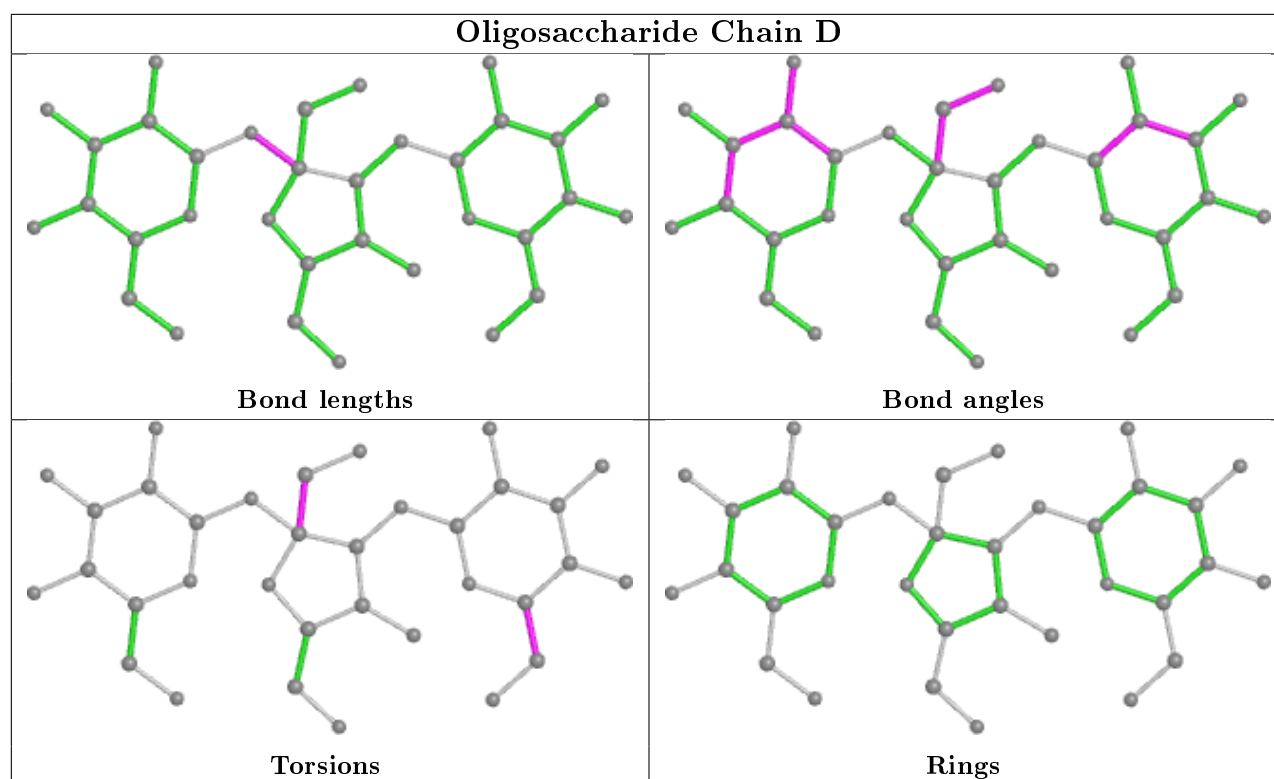
There are no ring outliers.

2 monomers are involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	D	1	GLC	1	0
4	D	2	Z9N	1	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for oligosaccharide.





5.6 Ligand geometry [i](#)

Of 6 ligands modelled in this entry, 3 are monoatomic - leaving 3 for Mogul analysis.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\# Z > 2$	Counts	RMSZ	$\# Z > 2$
7	CO3	A	687	6	0,3,3	0.00	-	0,3,3	0.00	-
5	NAG	A	1	1	14,14,15	0.75	1 (7%)	17,19,21	1.50	3 (17%)
9	SO4	A	2001	-	4,4,4	0.12	0	6,6,6	0.20	0

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
5	NAG	A	1	1	-	2/6/23/26	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
5	A	1	NAG	O5-C1	-2.20	1.40	1.43

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
5	A	1	NAG	O5-C1-C2	-4.13	104.76	111.29
5	A	1	NAG	C6-C5-C4	-2.42	107.34	113.00
5	A	1	NAG	C3-C4-C5	2.10	113.99	110.24

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
5	A	1	NAG	C4-C5-C6-O6
5	A	1	NAG	O5-C5-C6-O6

There are no ring outliers.

No monomer is involved in short contacts.

5.7 Other polymers [i](#)

There are no such residues in this entry.

5.8 Polymer linkage issues [i](#)

There are no chain breaks in this entry.

6 Fit of model and data ⓘ

6.1 Protein, DNA and RNA chains ⓘ

EDS was not executed - this section is therefore empty.

6.2 Non-standard residues in protein, DNA, RNA chains ⓘ

EDS was not executed - this section is therefore empty.

6.3 Carbohydrates ⓘ

EDS was not executed - this section is therefore empty.

6.4 Ligands ⓘ

EDS was not executed - this section is therefore empty.

6.5 Other polymers ⓘ

EDS was not executed - this section is therefore empty.