wwPDB X-ray Structure Validation Summary Report (i)

Feb 14, 2024 – 12:00 PM EST

PDB ID	:	3ML9
Title	:	Discovery of the Highly Potent PI3K/mTOR Dual Inhibitor PF-04691502 through Structure Based Drug Design
Authors	:	Knighton, D.R.
Deposited on	:	2010-04-16
Resolution	:	2.55 Å(reported)

This is a wwPDB X-ray Structure Validation Summary 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 (i) symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

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

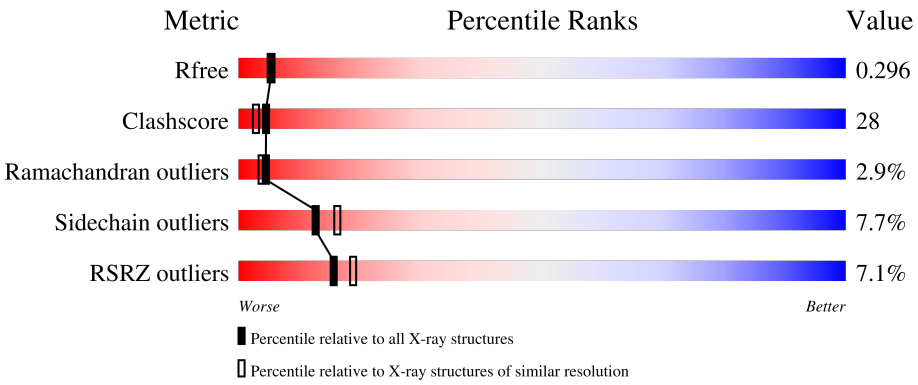
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

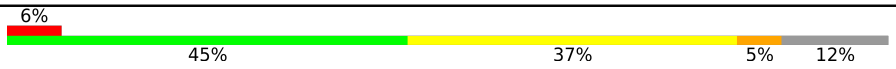
The reported resolution of this entry is 2.55 Å.

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))
R_{free}	130704	1284 (2.56-2.52)
Clashscore	141614	1332 (2.56-2.52)
Ramachandran outliers	138981	1315 (2.56-2.52)
Sidechain outliers	138945	1315 (2.56-2.52)
RSRZ outliers	127900	1272 (2.56-2.52)

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 of 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 <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	966	

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 6896 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.

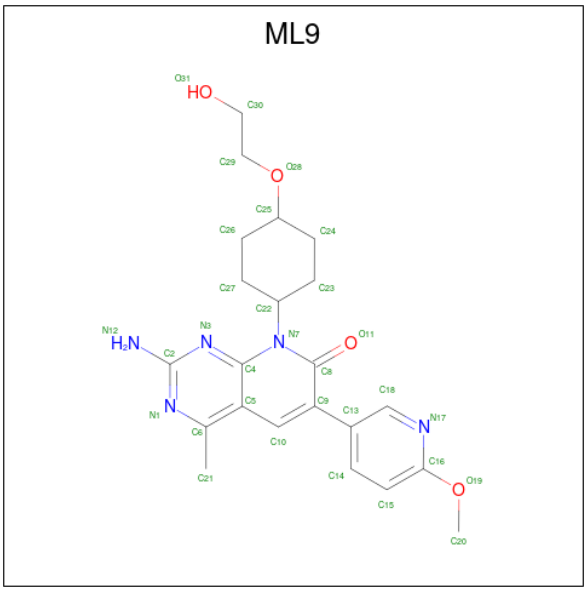
- Molecule 1 is a protein called Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	847	6864	4413	1170	1246	35	0	0	0

There are 8 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	143	MET	-	expression tag	UNP P48736
A	459	ARG	GLN	conflict	UNP P48736
A	1103	HIS	-	expression tag	UNP P48736
A	1104	HIS	-	expression tag	UNP P48736
A	1105	HIS	-	expression tag	UNP P48736
A	1106	HIS	-	expression tag	UNP P48736
A	1107	HIS	-	expression tag	UNP P48736
A	1108	HIS	-	expression tag	UNP P48736

- Molecule 2 is 2-amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxypyridin-3-yl)-4-methylpyrido[2,3-d]pyrimidin-7(8H)-one (three-letter code: ML9) (formula: $C_{22}H_{27}N_5O_4$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	N	O		
2	A	1	31	22	5	4	0	0

- Molecule 3 is water.

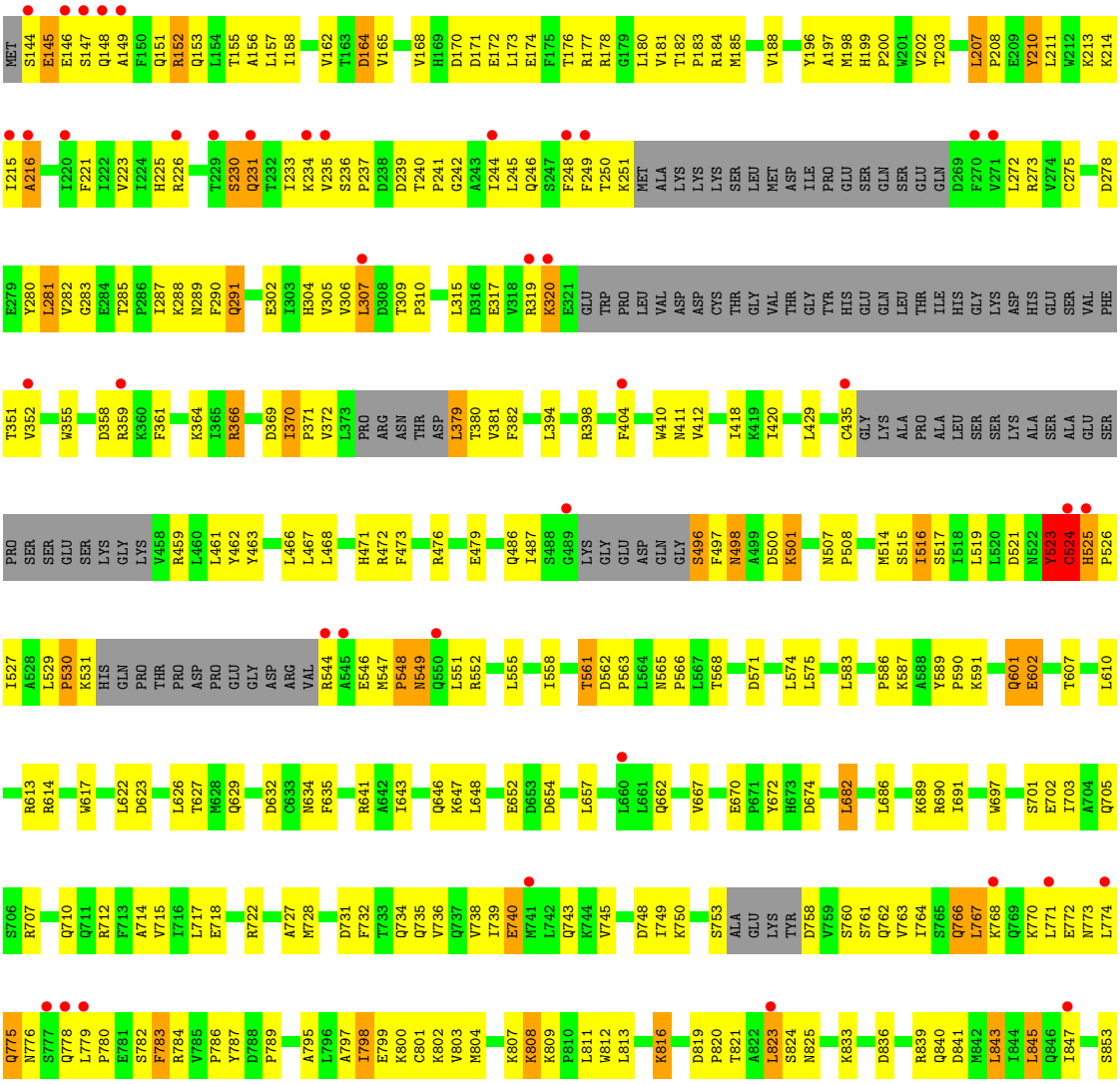
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
3	A	1	1	1	0	0

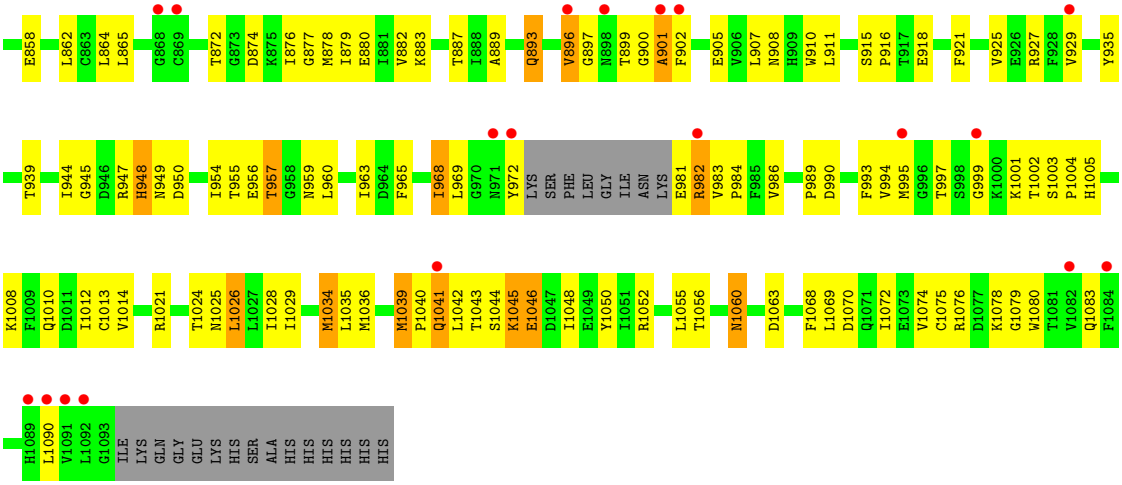
3 Residue-property plots (i)

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 and electron density. 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. A red dot above a residue indicates a poor fit to the electron density (RSRZ > 2). 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.

• Molecule 1: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform

Chain A:




E858
L862 C863 L864 L865
G868 C869
T872 G873 D874 K875 I876 G877 M878 I879 E880 I881 V882 K883
T887 I888 A889
Q893
V896 G897 N898 T899 G900 A901 F902
E905 V906 L907 N908 H909 W910 L911
S915 P916 T917 E918
F921
V925 E926 R927 F928 V929
Y935
T939
I944 G945 D946 R947 H948 N949 D950
I954 T955 E956 T957 G958 N959 L960
I963 D964 F965
I968 L969 G970 N971 Y972
LYS SER PHE LEU GLY ILE ASN LYS
E981 R982 V983 P984 F985 V986
P989 D990
F993 V994 M995 G996 T997 S998 G999 K1000 K1001 T1002 S1003 P1004 H1005
K1008 F1009 Q1010 D1011 I1012 C1013 V1014
R1021
T1024 N1025 L1026 L1027 I1028 I1029
M1034 L1035 M1036
M1039 P1040 Q1041 L1042 T1043 S1044 K1045 E1046 D1047 I1048 E1049 Y1050 I1051 R1052
L1055 T1056
N1060
D1063
F1068 L1069 D1070 Q1071 I1072 E1073 V1074 C1075 R1076 D1077 K1078 G1079 W1080 T1081 V1082 Q1083 F1084
H1089 L1090 V1091 L1092 G1093
ILE LYS GLN GLY GLU LYS HIS SER ALA HIS HIS HIS HIS HIS HIS

4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α, β, γ	143.93Å 67.29Å 106.84Å 90.00° 95.46° 90.00°	Depositor
Resolution (Å)	44.79 – 2.55 44.79 – 2.52	Depositor EDS
% Data completeness (in resolution range)	95.9 (44.79-2.55) 95.3 (44.79-2.52)	Depositor EDS
R_{merge}	0.05	Depositor
R_{sym}	0.05	Depositor
$< I/\sigma(I) >$ [1]	2.51 (at 2.51Å)	Xtriage
Refinement program	CNX	Depositor
R, R_{free}	0.242 , 0.298 0.240 , 0.296	Depositor DCC
R_{free} test set	1700 reflections (4.98%)	wwPDB-VP
Wilson B-factor (Å^2)	54.0	Xtriage
Anisotropy	0.304	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.34 , 49.4	EDS
L-test for twinning[2]	$< \|L\| > = 0.49$, $< L^2 > = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.93	EDS
Total number of atoms	6896	wwPDB-VP
Average B, all atoms (Å^2)	60.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.46% of the height of the origin peak. No significant pseudotranslation is detected.*

[1]Intensities estimated from amplitudes.

[2]Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

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: ML9

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	0.38	0/7012	0.54	0/9487

There are no bond length outliers.

There are no bond angle outliers.

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	6864	0	6913	394	0
2	A	31	0	27	4	0
3	A	1	0	0	0	0
All	All	6896	0	6940	394	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 28.

The worst 5 of 394 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:568:THR:HG23	1:A:571:ASP:H	1.17	1.06

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:558:ILE:HG21	1:A:575:LEU:HD21	1.47	0.96
1:A:523:TYR:HB2	1:A:525:HIS:HB3	1.48	0.94
1:A:887:THR:HG22	1:A:889:ALA:H	1.31	0.94
1:A:807:LYS:H	1:A:807:LYS:HD2	1.34	0.93

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	829/966 (86%)	748 (90%)	57 (7%)	24 (3%)	4 4

5 of 24 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	525	HIS
1	A	530	PRO
1	A	548	PRO
1	A	1045	LYS
1	A	164	ASP

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.

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	762/864 (88%)	703 (92%)	59 (8%)	13 16

5 of 59 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	602	GLU
1	A	1039	MET
1	A	766	GLN
1	A	1034	MET
1	A	957	THR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 18 such sidechains are listed below:

Mol	Chain	Res	Type
1	A	967	HIS
1	A	1083	GLN
1	A	1060	ASN
1	A	734	GLN
1	A	959	ASN

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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

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

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

1 ligand is 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	ML9	A	9999	-	33,34,34	1.89	9 (27%)	43,48,48	1.39	5 (11%)

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	ML9	A	9999	-	-	2/14/24/24	0/4/4/4

The worst 5 of 9 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	9999	ML9	C16-N17	4.52	1.39	1.32
2	A	9999	ML9	C9-C8	4.21	1.52	1.46
2	A	9999	ML9	C8-N7	3.74	1.43	1.38
2	A	9999	ML9	O19-C16	2.98	1.40	1.35
2	A	9999	ML9	C2-N12	2.77	1.39	1.33

All (5) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	9999	ML9	O11-C8-C9	-4.95	119.03	125.72
2	A	9999	ML9	C13-C9-C8	2.73	121.95	119.50
2	A	9999	ML9	C14-C13-C18	-2.29	115.03	117.63
2	A	9999	ML9	C2-N3-C4	2.04	119.52	113.89
2	A	9999	ML9	C2-N1-C6	2.00	118.43	116.79

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	9999	ML9	C24-C25-O28-C29

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Mol	Chain	Res	Type	Atoms
2	A	9999	ML9	C26-C25-O28-C29

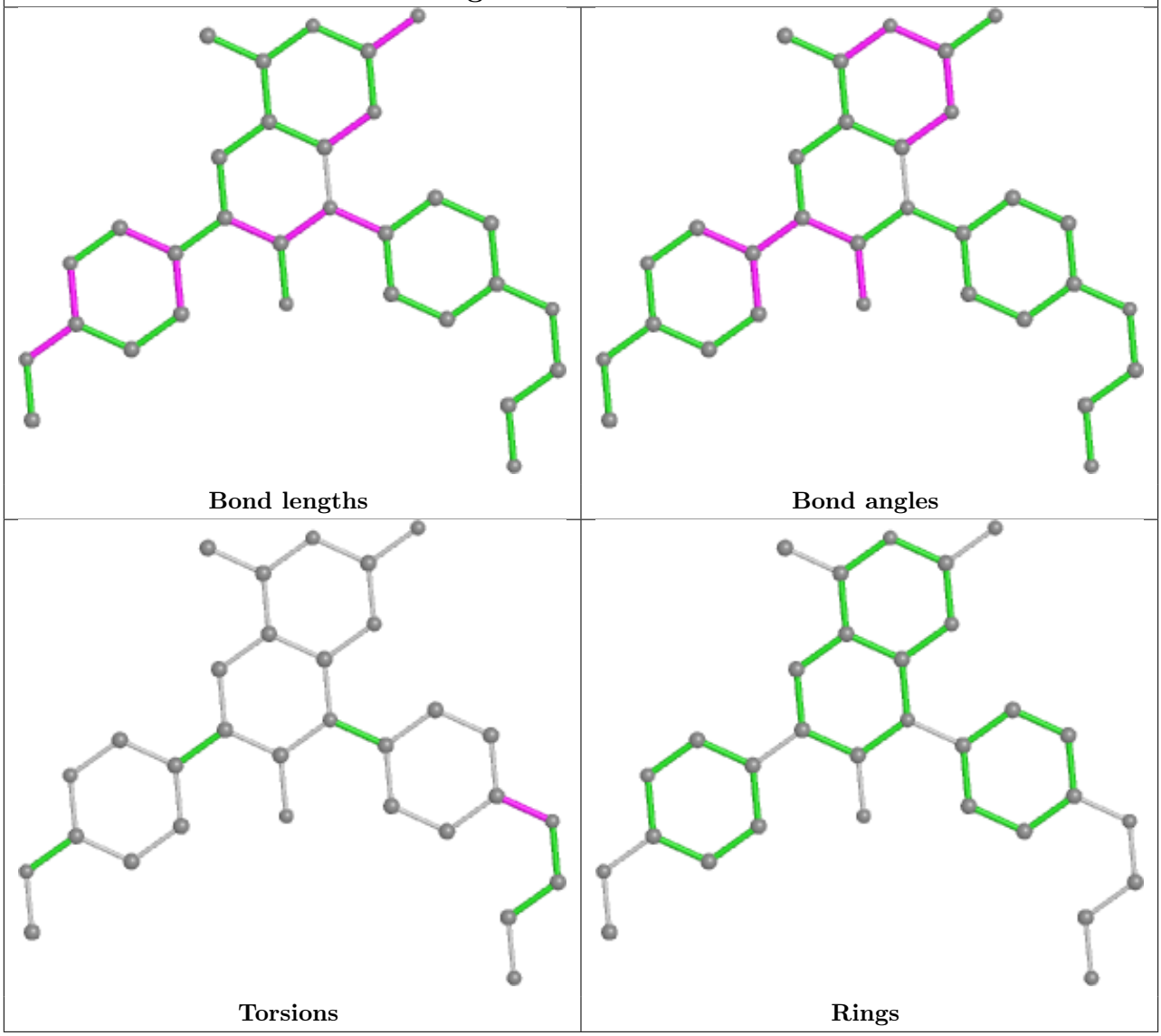
There are no ring outliers.

1 monomer is involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	9999	ML9	4	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand ML9 A 9999



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 (i)

6.1 Protein, DNA and RNA chains (i)

In the following table, the column labelled '#RSRZ> 2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95th percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å^2)	Q<0.9
1	A	847/966 (87%)	0.54	60 (7%) 16 19	20, 58, 96, 118	0

The worst 5 of 60 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	147	SER	6.3
1	A	235	VAL	5.3
1	A	525	HIS	5.3
1	A	524	CYS	4.6
1	A	545	ALA	4.4

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

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

6.3 Carbohydrates (i)

There are no monosaccharides in this entry.

6.4 Ligands (i)

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

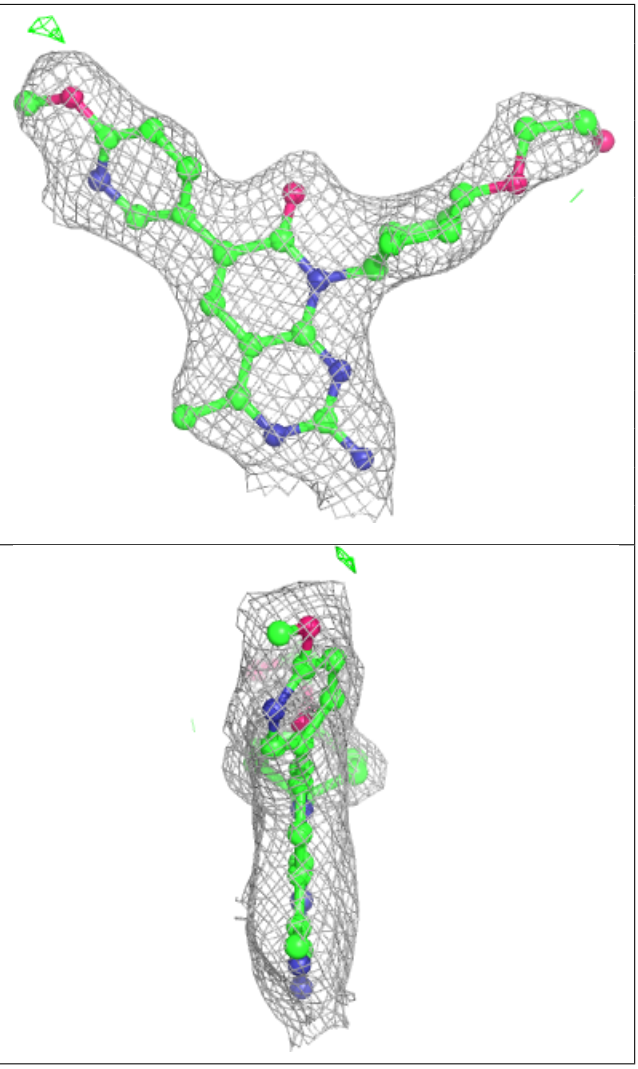
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
2	ML9	A	9999	31/31	0.93	0.20	42,48,68,73	0

The following is a graphical depiction of the model fit to experimental electron density of all

instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.

Electron density around ML9 A 9999:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



6.5 Other polymers (i)

There are no such residues in this entry.