



# Full wwPDB X-ray Structure Validation Report ⓘ

Feb 1, 2016 – 03:18 PM GMT

PDB ID : 4C4E  
Title : Structure-based design of orally bioavailable pyrrolopyridine inhibitors of the mitotic kinase MPS1  
Authors : Naud, S.; Westwood, I.M.; Faisal, A.; Sheldrake, P.; Bavetsias, V.; Atrash, B.; Liu, M.; Hayes, A.; Schmitt, J.; Wood, A.; Choi, V.; Boxall, K.; Mak, G.; Gurden, M.; Valenti, M.; de Haven Brandon, A.; Henley, A.; Baker, R.; McAndrew, C.; Matijssen, B.; Burke, R.; Eccles, S.A.; Raynaud, F.I.; Linardopoulos, S.; van Montfort, R.; Blagg, J.  
Deposited on : 2013-09-05  
Resolution : 2.60 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](mailto:validation@mail.wwpdb.org)  
A user guide is available at  
<http://wwpdb.org/validation/2016/XrayValidationReportHelp>  
with specific help available everywhere you see the ⓘ symbol.

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

MolProbity : 4.02b-467  
Mogul : 1.7 (RC4), CSD as536be (2015)  
Xtriage (Phenix) : 1.9-1692  
EDS : rb-20026688  
Percentile statistics : 20151230.v01 (using entries in the PDB archive December 30th 2015)  
Refmac : 5.8.0135  
CCP4 : 6.5.0  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)  
Validation Pipeline (wwPDB-VP) : trunk26865

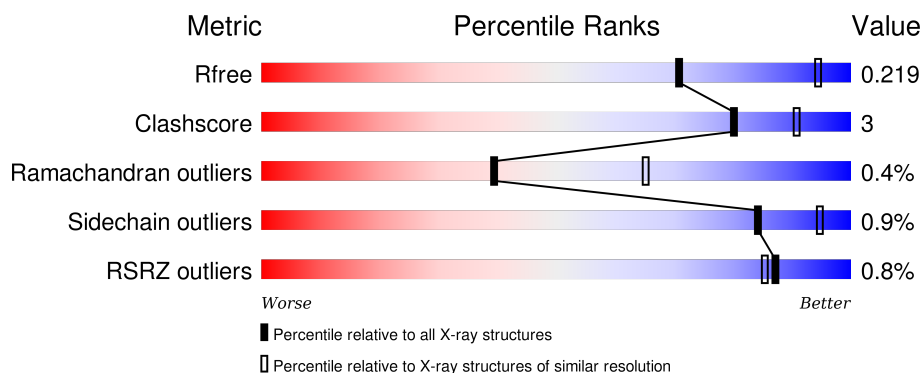
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

## *X-RAY DIFFRACTION*

The reported resolution of this entry is 2.60 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))
$R_{free}$	91344	2328 (2.60-2.60)
Clashscore	102246	2679 (2.60-2.60)
Ramachandran outliers	100387	2635 (2.60-2.60)
Sidechain outliers	100360	2635 (2.60-2.60)
RSRZ outliers	91569	2334 (2.60-2.60)

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  $\geq 3$ , 2, 1 and 0 types of geometric quality criteria. 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  $\leq 5\%$ . 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	313	<div> <div></div> <div>77%</div> <div>5%</div> <div>18%</div> </div>

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	4T9	A	1796[A]	-	-	-	X

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Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
4	7PE	A	1801	-	-	-	X

## 2 Entry composition

There are 5 unique types of molecules in this entry. The entry contains 2151 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.

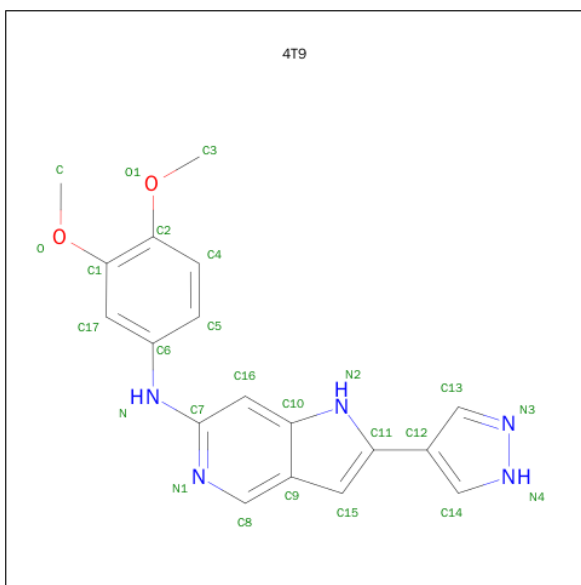
- Molecule 1 is a protein called DUAL SPECIFICITY PROTEIN KINASE TTK.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
1	A	257	Total	C	N	O	P	S	0	0	0
			2034	1308	335	378	1	12			

There are 23 discrepancies between the modelled and reference sequences:

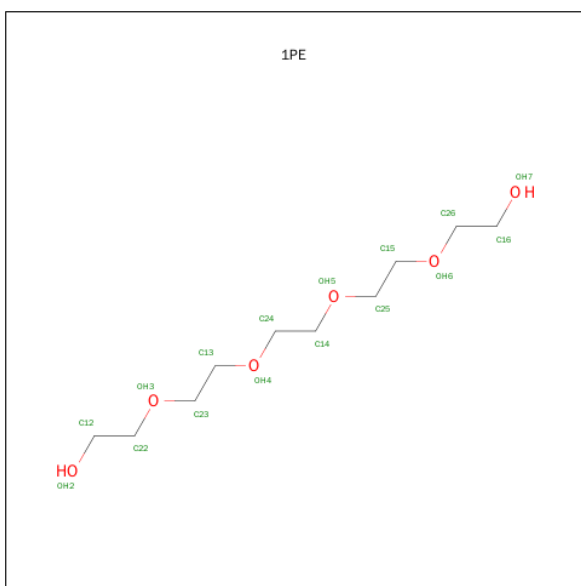
Chain	Residue	Modelled	Actual	Comment	Reference
A	496	MET	-	EXPRESSION TAG	UNP P33981
A	497	HIS	-	EXPRESSION TAG	UNP P33981
A	498	HIS	-	EXPRESSION TAG	UNP P33981
A	499	HIS	-	EXPRESSION TAG	UNP P33981
A	500	HIS	-	EXPRESSION TAG	UNP P33981
A	501	HIS	-	EXPRESSION TAG	UNP P33981
A	502	HIS	-	EXPRESSION TAG	UNP P33981
A	503	SER	-	EXPRESSION TAG	UNP P33981
A	504	SER	-	EXPRESSION TAG	UNP P33981
A	505	GLY	-	EXPRESSION TAG	UNP P33981
A	506	VAL	-	EXPRESSION TAG	UNP P33981
A	507	ASP	-	EXPRESSION TAG	UNP P33981
A	508	LEU	-	EXPRESSION TAG	UNP P33981
A	509	GLY	-	EXPRESSION TAG	UNP P33981
A	510	THR	-	EXPRESSION TAG	UNP P33981
A	511	GLU	-	EXPRESSION TAG	UNP P33981
A	512	ASN	-	EXPRESSION TAG	UNP P33981
A	513	LEU	-	EXPRESSION TAG	UNP P33981
A	514	TYR	-	EXPRESSION TAG	UNP P33981
A	515	PHE	-	EXPRESSION TAG	UNP P33981
A	516	GLN	-	EXPRESSION TAG	UNP P33981
A	517	SER	-	EXPRESSION TAG	UNP P33981
A	518	MET	-	EXPRESSION TAG	UNP P33981

- Molecule 2 is N-(3,4-DIMETHOXYPHENYL)-2-(1H-PYRAZOL-4-YL)-1H-PYRROLO[3,2-C]PYRIDIN-6-AMINE (three-letter code: 4T9) (formula: C<sub>18</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>).



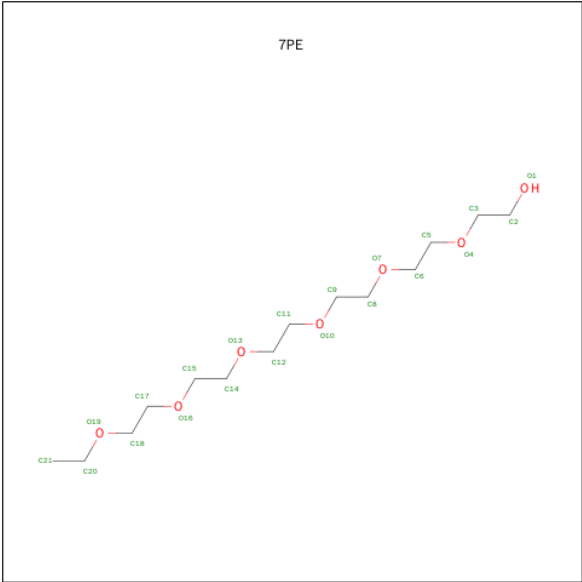
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
2	A	1	Total	C	N	O	0	1
			25	18	5	2		

- Molecule 3 is PENTAETHYLENE GLYCOL (three-letter code: 1PE) (formula: C<sub>10</sub>H<sub>22</sub>O<sub>6</sub>).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	A	1	Total	C	O	0	1
			26	16	10		

- Molecule 4 is 2-(2-(2-(2-(2-ETHOXYETHOXY)ETHOXY)ETHOXY)ETHOXY)ETHOXY)ETHANOL (three-letter code: 7PE) (formula: C<sub>14</sub>H<sub>30</sub>O<sub>7</sub>).



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

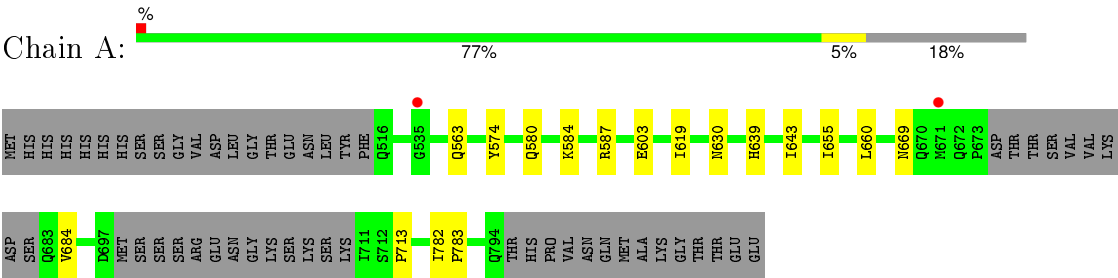
- Molecule 5 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	38	Total	O	0	0
			38	38		

### 3 Residue-property plots

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of errors 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: DUAL SPECIFICITY PROTEIN KINASE TTK



## 4 Data and refinement statistics

Property	Value	Source
Space group	I 2 2 2	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	70.94Å 111.79Å 113.40Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	22.68 – 2.60 60.14 – 2.60	Depositor EDS
% Data completeness (in resolution range)	99.6 (22.68-2.60) 99.5 (60.14-2.60)	Depositor EDS
$R_{merge}$	0.06	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.22 (at 2.61Å)	Xtriage
Refinement program	BUSTER 2.11.4	Depositor
R, $R_{free}$	0.187 , 0.212 0.195 , 0.219	Depositor DCC
$R_{free}$ test set	711 reflections (5.29%)	DCC
Wilson B-factor (Å <sup>2</sup> )	58.8	Xtriage
Anisotropy	0.460	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.36 , 64.4	EDS
Estimated twinning fraction	0.025 for -h,-l,-k	Xtriage
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.48$ , $\langle L^2 \rangle = 0.31$	Xtriage
Outliers	0 of 14181 reflections	Xtriage
$F_o, F_c$ correlation	0.95	EDS
Total number of atoms	2151	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	67.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 7.34% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

<sup>2</sup>Theoretical values of  $\langle |L| \rangle$ ,  $\langle L^2 \rangle$  for acentric reflections are 0.5, 0.375 respectively for untwinned datasets, and 0.333, 0.2 for perfectly twinned datasets.



## 5 Model quality

### 5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: TPO, 7PE, 4T9, 1PE

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	0.48	0/2068	0.67	0/2807

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

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	2034	0	1951	12	0
2	A	25	0	17	2	0
3	A	26	0	35	0	0
4	A	28	0	32	5	0
5	A	38	0	0	0	0
All	All	2151	0	2035	14	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 3.

All (14) 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:782:ILE:HG12	4:A:1801:7PE:H182	1.68	0.73
1:A:783:PRO:HD3	4:A:1801:7PE:H171	1.72	0.69
1:A:639:HIS:HB3	4:A:1801:7PE:H152	1.74	0.68
1:A:574:TYR:CD1	1:A:643:ILE:HD11	2.42	0.54
1:A:587:ARG:HB2	1:A:603:GLU:HB2	1.89	0.54
1:A:713:PRO:HB2	4:A:1801:7PE:H181	1.90	0.54
1:A:713:PRO:CB	4:A:1801:7PE:H181	2.41	0.51
2:A:1796[A]:4T9:C5	2:A:1796[A]:4T9:H16	2.41	0.51
1:A:584:LYS:HE2	1:A:630:ASN:ND2	2.29	0.47
1:A:563:GLN:NE2	1:A:669:ASN:HD21	2.12	0.46
1:A:563:GLN:HE22	1:A:669:ASN:HD21	1.64	0.46
2:A:1796[A]:4T9:H5	2:A:1796[A]:4T9:H16	1.98	0.45
1:A:655:ILE:HG12	1:A:660:LEU:HD23	1.99	0.45
1:A:655:ILE:HG12	1:A:660:LEU:CD2	2.48	0.43

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	250/313 (80%)	242 (97%)	7 (3%)	1 (0%)	39 65

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	684	VAL

### 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	213/287 (74%)	211 (99%)	2 (1%)	84	95

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	580	GLN
1	A	619	ILE

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	669	ASN

### 5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

1 non-standard protein/DNA/RNA residue 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	$\# Z  > 2$
1	TPO	A	686	1	8,10,11	1.21	1 (12%)	7,14,16	2.08	2 (28%)

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
1	TPO	A	686	1	-	1/8/11/13	0/0/0/0

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	686	TPO	CB-CA	2.59	1.58	1.54

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	686	TPO	O-C-CA	-2.03	120.07	125.44
1	A	686	TPO	OG1-P-O1P	3.60	116.09	107.11

There are no chirality outliers.

All (1) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	686	TPO	OG1-CB-CA-N

There are no ring outliers.

No monomer is involved in short contacts.

## 5.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

## 5.6 Ligand geometry [i](#)

7 ligands 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	4T9	A	1796[A]	-	25,28,28	1.69	6 (24%)	32,39,39	1.35	6 (18%)

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
3	1PE	A	1797[A]	-	9,9,15	0.15	0	8,8,14	0.15	0
3	1PE	A	1797[B]	-	15,15,15	0.19	0	14,14,14	0.15	0
4	7PE	A	1798	-	6,6,20	0.27	0	5,5,19	0.15	0
4	7PE	A	1799	-	6,6,20	0.11	0	5,5,19	0.19	0
4	7PE	A	1800	-	6,6,20	0.17	0	5,5,19	0.10	0
4	7PE	A	1801	-	6,6,20	0.20	0	5,5,19	0.33	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
2	4T9	A	1796[A]	-	-	0/8/12/12	0/4/4/4
3	1PE	A	1797[A]	-	-	0/7/7/13	0/0/0/0
3	1PE	A	1797[B]	-	-	0/13/13/13	0/0/0/0
4	7PE	A	1798	-	-	0/4/4/18	0/0/0/0
4	7PE	A	1799	-	-	0/4/4/18	0/0/0/0
4	7PE	A	1800	-	-	0/4/4/18	0/0/0/0
4	7PE	A	1801	-	-	0/4/4/18	0/0/0/0

All (6) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	1796[A]	4T9	C12-C11	-3.54	1.43	1.48
2	A	1796[A]	4T9	C8-N1	2.26	1.36	1.32
2	A	1796[A]	4T9	C7-N	2.39	1.42	1.38
2	A	1796[A]	4T9	C5-C6	2.48	1.43	1.39
2	A	1796[A]	4T9	C13-N3	2.74	1.39	1.33
2	A	1796[A]	4T9	C7-N1	3.62	1.40	1.34

All (6) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	1796[A]	4T9	O1-C2-C4	-2.69	119.83	124.35
2	A	1796[A]	4T9	C3-O1-C2	-2.27	114.09	117.54
2	A	1796[A]	4T9	C7-C16-C10	-2.20	116.35	120.05
2	A	1796[A]	4T9	O-C1-C17	-2.02	120.76	124.21
2	A	1796[A]	4T9	O-C1-C2	2.48	118.93	115.40
2	A	1796[A]	4T9	O1-C2-C1	3.36	120.18	115.40

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

2 monomers are involved in 7 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	1796[A]	4T9	2	0
4	A	1801	7PE	5	0

## 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, 95<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	256/313 (81%)	0.34	2 (0%) 87 85	42, 65, 98, 119	0

All (2) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	671	MET	4.6
1	A	535	GLY	2.6

### 6.2 Non-standard residues in protein, DNA, RNA chains [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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95<sup>th</sup> 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	LLDF	B-factors(Å <sup>2</sup> )	Q<0.9
1	TPO	A	686	11/12	0.82	0.25	-	79,87,100,104	0

### 6.3 Carbohydrates [i](#)

There are no carbohydrates 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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95<sup>th</sup> 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	LLDF	B-factors( $\text{\AA}^2$ )	Q<0.9
4	7PE	A	1801	7/21	0.83	0.46	14.67	73,75,79,79	0
2	4T9	A	1796[A]	25/25	0.78	0.34	5.86	50,57,66,67	25
3	1PE	A	1797[A]	10/16	0.89	0.30	1.03	66,67,73,73	10
3	1PE	A	1797[B]	16/16	0.89	0.30	1.02	63,65,67,67	16
4	7PE	A	1798	7/21	0.82	0.20	0.45	67,73,77,78	0
4	7PE	A	1800	7/21	0.76	0.18	-0.74	82,86,89,89	0
4	7PE	A	1799	7/21	0.80	0.29	-	89,90,94,95	0

## 6.5 Other polymers [i](#)

There are no such residues in this entry.