Supporting Information

Discovery of Pyrrolo[3,2-d]pyrimidin-4-one Derivatives as a New Class of Potent and Cell Active Inhibitors of P300/CBP-Associated Factor Bromodomain

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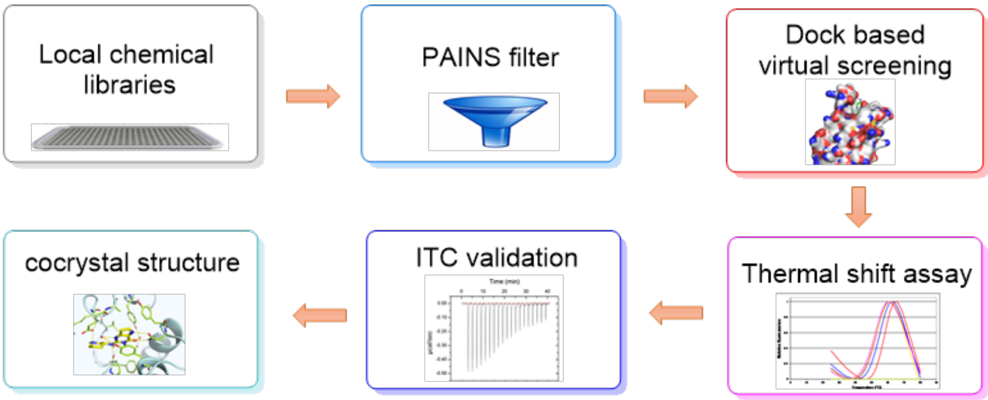
Figure S1. Flowchart of hits identification against PCAF bromodomain. [a]



[a] To identify new PCAF bromodomain inhibitors, we conducted a virtual screening against our in-house chemical library (over 20,000 compounds; **Figure S1**). First of all, “pan-assay interference compounds” (PAINS) filter was applied to discard molecular patterns known to react non-specifically with most proteins. Second, the crystallographic coordinate of the 1.77 Å human PCAF bromodomain (PDB ID, 5FE6) crystal structure was obtained from PDB. The preparation of protein and ligands were performed using the CHARMM force field on Discovery Studio 3.1 (Accelrys Inc.). The binding pocket was defined as a sphere with a radius of 8 Å, which is large enough to cover the KAc-binding pocket. The GOLD 5.3 program (CCDC) was adopted for the molecular docking. GoldScore was employed to evaluate and rank the binding poses. Finally, high-ranking candidates were carefully selected for DSF assays. The initial DSF assays were conducted using 2 μM PCAF and 20 μM tested compound. Three compounds showed a thermal shift ≥ 1 °C in DSF assay (**10**: ΔT_m = 1.0 °C, **11**: ΔT_m = 1.0 °C, and **12**: ΔT_m = 3.5 °C,). The active compounds were then validated by ITC assay, which gave K_D values of 45, 7.8 and 2.4 μM for **10**, **11** and **12**, respectively. To elucidate the interaction mode of hit with PCAF bromodomain, cocrystal structure of **12** in complex with PCAF BRD was solved.

Compounds **10**, **11**, and **12** were synthesized previously and stored in our in-house chemical library.

1-{1-[(2R)-3-Hydroxy-2-methylpropyl]-1H-indol-3-yl}ethan-1-one (**10**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.21 (d, *J*= 7.6 Hz, 1H), 7.58 (d, *J*= 8.0 Hz, 1H), 7.26 (t, *J*= 7.2 Hz, 1H), 7.21 (t, *J*= 7.2 Hz, 1H), 4.76 (t, *J*= 5.0 Hz, 1H), 4.30 (dd, *J*= 14.0, 6.4 Hz, 1H), 4.05 (dd, *J*= 14.0, 7.6 Hz, 1H), 3.34 –3.24 (m, 2H), 2.45 (s, 3H), 2.14 (dq, *J*= 12.6, 6.4 Hz, 1H), 0.84 (d, *J*= 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.68, 138.16, 137.43, 126.20, 123.22, 122.37, 122.02, 116.17, 111.28, 63.71, 49.53, 36.83, 27.74, 15.08. ESI-MS m/z: 232.1 $[M + H]^+$.

3-Methyl-4-oxo-N-[(1r,4r)-4-methylcyclohexyl]-3,4-dihydrophthalazine-1-carboxamide (**11**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, *J* = 8.0 Hz, 1H), 8.43 (dd, *J* = 8.0, 0.8 Hz, 1H), 8.30 (dd, *J* = 7.8, 0.8 Hz, 1H), 7.94 (td, *J* = 7.6, 1.6 Hz, 1H), 7.88 (td, *J* = 7.6, 1.2 Hz, 1H), 3.85 – 3.68 (m, 4H), 1.88 (d, *J* = 10.8 Hz, 2H), 1.71 (d, *J* = 12.4 Hz, 2H), 1.44 – 1.29 (m, 3H), 1.14 – 0.97 (m, 2H), 0.89 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.82, 159.03, 139.61, 133.95, 132.51, 127.84, 127.58, 126.81, 126.48, 48.70, 39.64, 34.09, 32.49, 31.94, 22.66. ESI-MS *m/z*: 300.2 $[M + H]^+$.

3-Methyl-2-{[(3R)-1-methylpiperidin-3-yl]amino}-3H,4H,5H-pyrrolo[3,2-d]pyrimidin-4-one (**12**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 7.18 (t, *J* = 2.9 Hz, 1H), 6.14 – 5.97 (m, 2H), 4.25 – 4.12 (m, 1H), 3.38 (s, 3H), 3.27 – 3.14 (m, 2H), 3.04 – 2.88 (m, 1H), 2.51 – 2.39 (m, 4H), 1.93 – 1.79 (m, 2H), 1.72 – 1.45 (m, 2H). HRMS *m*/*z* 262.1665 ($M + H^+$, $C_{13}H_{19}N_5O$, requires 261.1590).

Table S1. DSF results of test compounds against a panel of 12 bromodomains.[a]

Compd	PCAF ΔT_m (°C) [b]	GCN5 ΔT_m (°C) [c]	FALZ ΔT_m (°C) [c]	BRD4(1) ΔT_m (°C) [c]	ATAD2 ΔT_m (°C) [c]	BAZ2B ΔT_m (°C) [c]	BRD9 ΔT_m (°C) [c]	BRPF1 ΔT_m (°C) [c]	BRPF3 ΔT_m (°C) [c]	EP300 ΔT_m (°C) [c]	TAF1(1) ΔT_m (°C) [c]	TRIM24 ΔT_m (°C) [c]
(*R*,*R*)-24a	6.3 ± 0.6	4.6	3.5	0.1	-2.1	1.0	2.8	0.7	1.4	0.0	0.3	-1.6
(*S*,*S*)-24a	0.6 ± 0.2	-0.4	0.1	-0.1	-1.0	-0.1	0.8	-0.1	-1.0	-0.1	-0.7	-0.3
***cis*-24b**	3.2 ± 0.3	1.7	2.3	0.1	-0.7	0.3	0.2	1.2	0.6	-0.1	-0.3	-0.6
***endo*-24c**	7.3 ± 0.4	4.9	2.4	0.4	0.8	0.3	1.2	0.2	-1.0	0.5	-0.2	-0.1
***endo*-24d**	0.4 ± 0.4	-0.3	0.2	-1.3	0.8	0.0	-0.2	0.3	-1.6	0.2	-0.2	-0.9
***endo*-24e**	1.7 ± 0.5	0.7	N.D.	0.0	0.7	0.4	1.2	1.5	-2.1	-0.2	-0.1	-0.6
***endo*-25a**	6.6 ± 0.2	5.5	3.2	0.7	0.9	0.3	0.6	1.7	-1.0	-0.4	-0.1	-0.8
***exo*-25a**	1.0 ± 0.2	1.1	1.1	0.5	1.6	0.1	0.6	4.3	1.9	0.2	-0.1	0.0
***endo*-26a**	3.6 ± 1.0	2.7	1.3	-0.7	-0.6	0.2	0.5	1.3	-1.4	0.1	-0.1	-0.4
***endo*-26b**	4.6 ± 0.3	2.3	1.5	0.3	0.7	0.4	1.8	2.7	-1.5	-0.4	0.0	-0.4
(*R*,*R*)-32a	4.8 ± 0.2	3.1	1.4	-0.1	1.0	0.3	2.7	0.3	1.5	-0.2	-0.3	-0.5
(*R*,*R*)-32b	2.2 ± 0.6	1.3	0.3	-0.7	0.6	-0.3	0.9	-0.1	-0.7	0.0	-0.3	-1.9
(*R*,*R*)-32c	1.5 ± 0.4	-0.2	0.6	-0.1	0.9	0.1	0.5	-0.3	-1.9	-0.3	-0.4	0.1
(*R*,*R*)-32d	1.5 ± 0.4	1.3	0.7	-0.1	0.5	0.0	0.6	-0.1	-1.9	-0.7	-0.5	-0.2
(*R*,*R*)-32e	-0.2 ± 0.4	-0.6	1.3	-0.3	1.2	-0.1	-0.1	-0.3	-2.2	0.0	-0.3	-0.3
(*R*,*R*)-32f	4.6 ± 0.4	2.1	N.D.	-0.2	-0.3	0.2	-0.8	0.3	-1.2	-0.4	-0.3	-2.3
(*R*,*R*)-36a	4.4 ± 0.3	N.D.	3.7	0.9	1.2	N.D.	1.2	N.D.	-0.4	0.3	-0.1	-0.3
(*R*,*R*)-36b	4.7 ± 0.3	2.3	0.6	0.6	1.3	0.7	1.7	2.5	0.5	-0.2	-0.2	-0.2
(*R*,*R*)-36c	2.5 ± 0.7	0.3	2.8	0.5	0.8	0.1	0.8	0.7	-1.5	0.2	-0.1	-0.6
(*R*,*R*)-36d	7.5 ± 0.4	5.0	4.0	1.0	1.9	0.6	2.0	2.9	1.1	0.1	-0.1	0.3
(*R*,*R*)-36e	8.0 ± 0.4	5.4	4.2	0.5	1.3	1.5	3.2	1.1	1.9	0.2	-0.2	-0.2
(*R*,*R*)-36f	4.6 ± 0.9	3.1	2.3	-0.4	0.1	0.5	2.0	1.4	0.8	0.1	-0.2	-0.4
(*R*,*R*)-36g	5.9 ± 0.3	4.2	3.2	0.1	2.2	1.0	2.9	2.0	1.6	0.2	-0.3	-0.7
(*R*,*R*)-36h	6.6 ± 0.3	3.7	4.0	0.2	1.3	0.6	3.2	1.7	1.0	0.4	0.1	-0.3

(*R*,*R*)-36i	6.7 ± 0.9	4.4	4.2	0.2	2.9	0.5	1.4	2.2	0.3	0.2	0.0	-1.4
(*R*,*R*)-36j	8.4 ± 0.3	6.2	4.0	0.6	2.4	1.2	3.7	4.6	2.5	0.4	0.1	-0.9
(*R*,*R*)-*36k*	5.6 ± 0.3	3.9	4.8	0.9	2.4	0.7	0.9	2.8	0.4	-0.1	-0.2	-2.1
(*R*,*R*)-36l	6.9 ± 0.5	N.D.	5.4	0.8	3.0	N.D.	2.5	N.D.	2.0	0.1	-0.1	-0.2
(*R*,*R*)-36m	6.6 ± 0.4	4.7	N.D.	0.1	0.8	0.9	1.9	1.8	1.0	0.1	-0.2	-0.3
(*R*,*R*)-36n	9.5 ± 0.5	7.3	5.3	0.1	0.3	1.9	2.1	2.6	1.7	0.1	0.2	-0.3
(*S*,*S*)-36n	0.7 ± 0.3	-0.6	N.D.	-0.1	0.8	0.1	0.4	-0.1	-2.0	-0.3	-0.3	-0.3
L-Moses	7.6 ± 0.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] All assays were conducted using 2 µM protein and 10 µM tested compound. [b] Values shown are the average of three biological replicates and standard deviation. [c] Values are a single measurement. N.D.= not determined

Table S2. BROMOscan assay of (*R*,*R*)-**36n** against 32 bromodomains at 1000 nM.

Target	(*R*,*R*)-**36n**	Target	(*R*,*R*)-**36n**
Gene Symbol	%Ctrl @ 1000nM	Gene Symbol	%Ctrl @ 1000nM
ATAD2A	97	BRPF3	21
ATAD2B	100	CECR2	36
BAZ2A	34	CREBBP	78
BAZ2B	21	EP300	100
BRD1	12	FALZ	2.3
BRD2(1)	69	GCN5L2	0
BRD2(2)	100	PBRM1(2)	100
BRD3(1)	95	PBRM1(5)	100
BRD3(2)	68	PCAF	0
BRD4(1)	100	SMARCA2	100
BRD4(2)	100	SMARCA4	100
BRD7	60	TAF1(2)	79
BRD9	60	TAF1L(2)	82
BRDT(1)	85	TRIM24(PHD,Bromo.)	93
BRDT(2)	74	TRIM33(PHD,Bromo.)	69
BRPF1	22	WDR9(2)	100

Ctrl Legend

0≤x<0.1	0.1≤x<1	1≤x<10	10≤x<35	x≥35

Table S3. Kinase (422 kinases) inhibition profiles of (*R*,*R*)-**24a**, *endo*-**24c** and (*R*,*R*)-**36n**

Kinase	(*R*,*R*)-**24a**	*endo*-**24c**	(*R*,*R*)-**36n**	Kinase	(*R*,*R*)-**24a**	*endo*-**24c**	(*R*,*R*)-**36n**
	%Ctrl@10 μM				%Ctrl@10 μM		
AAK1(h)	83	88	108	MEKK3(h)	103	73	108
Abl(h)	88	85	100	MELK(h)	82	65	92
Abl(m)	105	106	104	Mer(h)	82	60	75
Abl (H396P) (h)	105	110	86	Met(h)	77	70	89
Abl (M351T)(h)	113	118	100	Met(D1246H)(h)	106	91	102
Abl (Q252H) (h)	113	116	104	Met(D1246N)(h)	107	100	89
Abl(T315I)(h)	94	102	109	Met(M1268T)(h)	109	103	108
Abl(Y253F)(h)	108	105	101	Met(Y1248C)(h)	92	87	99
ACK1(h)	91	93	105	Met(Y1248D)(h)	108	97	94
ACTR2(h)	100	94	91	Met(Y1248H)(h)	109	106	80
ALK(h)	84	74	94	MINK(h)	78	74	114
ALK1(h)	97	100	91	MKK3(h)	106	110	110
ALK2(h)	105	106	127	MKK4(m)	93	82	120
ALK4(h)	95	102	95	MKK6(h)	92	99	99
ALK6(h)	97	101	99	MLCK(h)	89	80	120
Arg(h)	82	75	98	MLK1(h)	73	78	139
AMPKα1(h)	112	98	85	MLK2(h)	89	84	56
AMPKα2(h)	90	79	121	MLK3(h)	87	86	101
A-Raf(h)	92	64	107	Mnk2(h)	86	90	97
Arg(m)	108	111	98	MOK(h)	106	99	107
ARK5(h)	92	75	103	MRCKα(h)	89	80	100
ASK1(h)	101	87	108	MRCKβ(h)	99	79	99
Aurora-A(h)	99	99	110	MRCKγ(h)	82	54	94
Aurora-B(h)	72	76	99	MSK1(h)	64	57	75
Aurora-C(h)	85	95	124	MSK2(h)	78	65	72
Axl(h)	99	84	93	MSSK1(h)	90	79	116
BIKe(h)	76	72	115	MST1(h)	85	72	107
Blk(h)	113	128	103	MST2(h)	74	59	106
Blk(m)	100	111	111	MST3(h)	81	71	109
BMPR2(h)	96	84	97	MST4(h)	96	77	102
Bmx(h)	85	93	107	mTOR(h)	125	120	113
BRK(h)	93	101	101	mTOR/FKBP12(h)	99	111	97
BrSK1(h)	89	83	114	MuSK(h)	88	86	95
BrSK2(h)	85	78	102	MYLK2(h)	77	76	17
BTK(h)	89	92	93	MYO3B(h)	78	49	112
BTK(R28H)(h)	95	91	89	NDR2(h)	64	74	96
B-Raf(h)	92	81	101	NEK1(h)	57	58	100
B-Raf(V599E)(h)	102	90	123	NEK2(h)	86	83	102
CaMKI(h)	93	84	114	NEK4(h)	94	90	113
CaMKIß(h)	102	91	81	NEK3(h)	79	77	110

CaMKIγ(h)	106	93	108	NEK6(h)	95	93	97
CaMKIIα(h)	98	95	100	NEK7(h)	96	93	96
CaMKIIβ(h)	81	77	101	NEK9(h)	85	78	57
CaMKIIγ(h)	88	79	94	NIM1(h)	92	91	119
CaMKIδ(h)	82	71	92	NEK11(h)	71	70	95
CaMKIIδ(h)	108	94	100	NLK(h)	92	94	97
CaMKIV(h)	101	87	99	NUAK2(h)	80	72	101
CaMKK1(h)	87	90	103	p70S6K(h)	82	81	100
CaMKK2(h)	84	80	103	PAK1(h)	95	78	107
Cdc7/cyclinB1(h)	85	72	113	PAK2(h)	94	87	101
CDK1/cyclinB(h)	97	87	108	PAK4(h)	81	77	83
CDK2/cyclinA(h)	90	83	105	PAK3(h)	90	76	87
CDK2/cyclinE(h)	66	59	94	PAK5(h)	82	67	91
CDK3/cyclinE(h)	82	64	103	PAK6(h)	87	75	113
CDK4/cyclinD3(h)	72	68	93	PAR-1Bα(h)	86	83	105
CDK5/p25(h)	97	69	100	PASK(h)	90	67	111
CDK5/p35(h)	85	72	96	PEK(h)	89	83	113
CDK6/cyclinD3(h)	73	68	101	PDGFRα(h)	85	98	100
CDK7/cyclinH/MAT1(h)	86	46	104	PDGFRα(D842V)(h)	94	101	102
CDK9/cyclin T1(h)	84	59	105	PDGFRα(V561D)(h)	141	147	113
CDK12/cyclinK(h)	100	92	95	PDGFRβ(h)	83	92	104
CDK13/cyclinK(h)	108	91	111	PDHK2(h)	93	73	118
CDK14/cyclinY(h)	84	80	107	PDHK4(h)	107	107	109
CDK16/cyclinY(h)	80	57	102	PDK1(h)	105	96	107
CDK17/cyclinY(h)	97	74	100	PhKγ1(h)	80	75	90
CDK18/cyclinY(h)	103	81	103	PhKγ2(h)	96	97	96
CDKL1(h)	89	88	101	Pim-1(h)	85	84	107
CDKL2(h)	74	74	110	Pim-2(h)	88	91	99
CDKL3(h)	85	80	91	Pim-3(h)	75	89	103
CDKL4(h)	87	85	98	PKA(h)	99	93	73
ChaK1(h)	100	100	111	PKAcβ(h)	91	86	100
CHK1(h)	82	90	99	PKBα(h)	85	81	97
CHK2(h)	82	82	101	PKBβ(h)	86	90	95
CHK2(I157T)(h)	110	103	102	PKBγ(h)	89	82	97
CHK2(R145W)(h)	101	103	103	PKCα(h)	93	81	94
CK1ε(h)	75	68	98	PKCβI(h)	95	86	99
CK1γ1(h)	87	69	99	PKCβII(h)	91	96	105
CK1γ2(h)	95	78	84	PKCγ(h)	93	96	90
CK1γ3(h)	82	62	93	PKCδ(h)	94	46	95
CK1δ(h)	89	77	99	PKCε(h)	85	77	102
CK1(y)	81	36	96	PKCη(h)	76	43	106
CK2(h)	98	87	104	PKCι(h)	79	70	105
CK2α1(h)	104	95	93	PKCμ(h)	100	52	102
CK2α2(h)	77	70	87	PKCθ(h)	91	71	100
CLIK1(h)	78	76	115	PKCζ(h)	73	73	95
CLK1(h)	68	51	131	PKD2(h)	84	58	98

CLK2(h)	89	70	115	PKD3(h)	85	48	94
CLK3(h)	91	87	91	PKG1α(h)	82	77	104
CLK4(h)	66	50	136	PKG1β(h)	84	71	96
cKit(h)	93	90	120	PKR(h)	84	79	105
cKit(D816V)(h)	111	105	70	Plk1(h)	88	85	101
cKit(D816H)(h)	93	89	83	Plk3(h)	109	106	94
cKit(V560G)(h)	101	103	83	Plk4(h)	88	89	96
cKit(V654A)(h)	106	102	102	PRAK(h)	88	69	110
CRIK(h)	103	96	114	PRKG2(h)	91	77	89
CSK(h)	101	100	115	PRK1(h)	89	64	104
c-RAF(h)	91	77	88	PRK2(h)	86	73	107
cSRC(h)	81	78	88	PrKX(h)	77	67	93
DAPK1(h)	85	93	96	PRP4(h)	86	84	109
DAPK2(h)	92	80	101	PTK5(h)	75	81	101
DCAMKL2(h)	70	62	93	Pyk2(h)	86	85	93
DCAMKL3(h)	81	64	104	Ret(h)	90	90	98
DDR1(h)	78	74	96	Ret (V804L)(h)	113	82	81
DDR2(h)	81	73	98	Ret(V804M)(h)	114	76	94
DMPK(h)	89	81	100	RIPK1(h)	101	99	104
DRAK1(h)	100	99	119	RIPK2(h)	93	91	100
DRAK2(h)	89	93	122	ROCK-I(h)	98	94	100
DYRK1A(h)	72	77	101	ROCK-II(h)	90	77	102
DYRK1B(h)	79	79	106	ROCK-II(r)	105	87	86
DYRK2(h)	83	80	97	Ron(h)	92	100	108
DYRK3(h)	94	81	97	Ros(h)	103	101	105
eEF-2K(h)	73	67	111	Rse(h)	69	76	76
EGFR(h)	106	97	103	Rsk1(h)	71	57	77
EGFR(L858R)(h)	109	122	93	Rsk1(r)	92	77	79
EGFR(L861Q)(h)	113	118	104	Rsk2(h)	76	43	61
EGFR(T790M)(h)	92	93	113	Rsk3(h)	82	44	71
EGFR(T790M,L858R)(h)	95	84	107	Rsk4(h)	72	30	59
EphA1(h)	72	79	118	SAPK2a(h)	99	100	97
EphA2(h)	93	85	96	SAPK2a(T106M)(h)	97	95	89
EphA3(h)	85	88	91	SAPK2b(h)	103	106	88
EphA4(h)	93	95	94	SAPK3(h)	104	72	99
EphA5(h)	98	96	89	SAPK4(h)	84	79	95
EphA7(h)	90	84	81	SBK1(h)	86	80	121
EphA8(h)	89	91	99	SGK(h)	81	85	114
EphB2(h)	103	99	100	SGK2(h)	90	83	114
EphB1(h)	81	86	83	SGK3(h)	80	67	98
EphB3(h)	93	90	106	SIK(h)	53	56	94
EphB4(h)	96	111	78	SIK2(h)	71	79	95
ErbB2(h)	102	106	100	SIK3(h)	81	85	107
ErbB4(h)	83	79	101	SLK(h)	89	76	117
FAK(h)	69	64	97	Snk(h)	93	87	101
Fer(h)	77	83	107	SNRK(h)	95	95	119

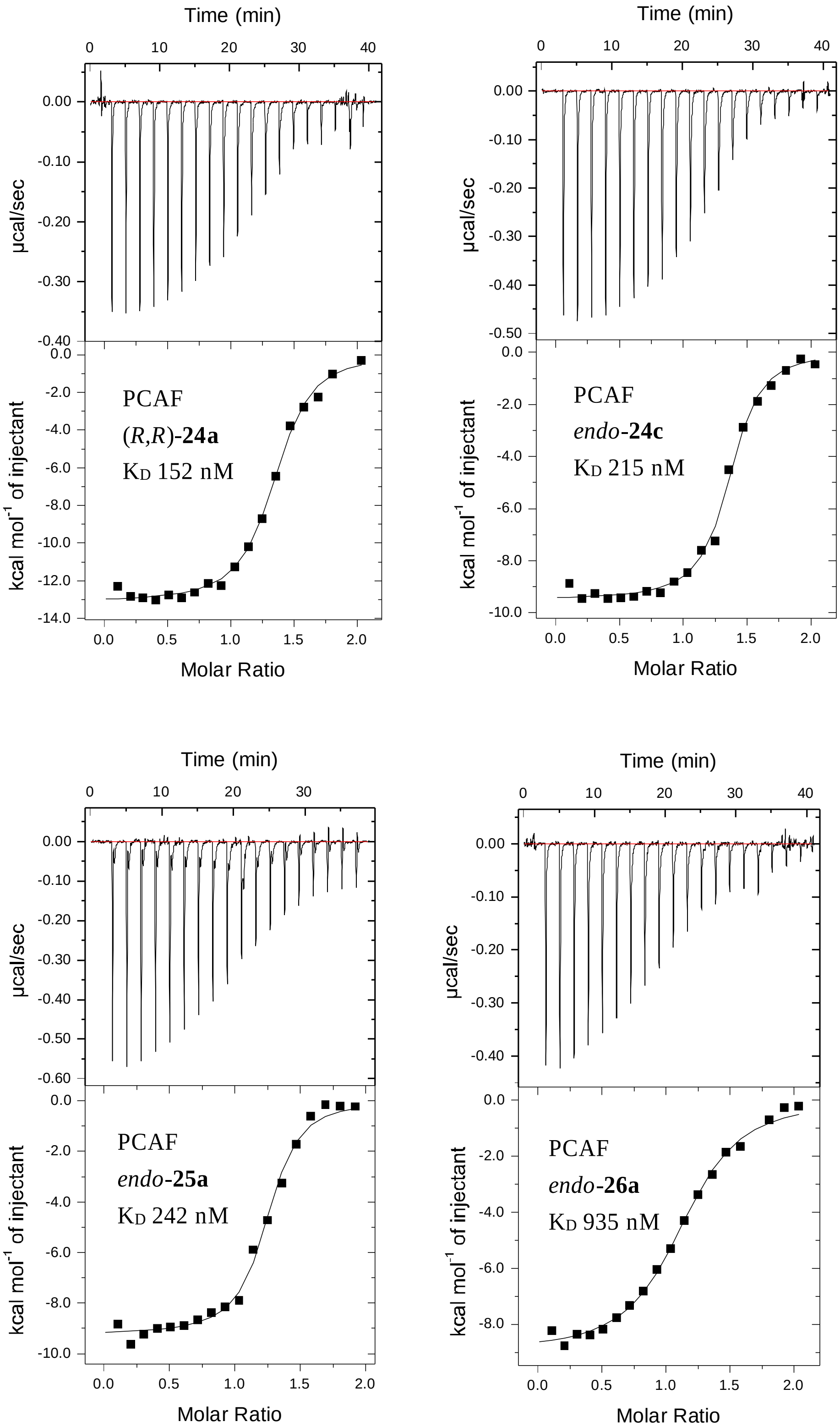
Fes(h)	84	80	87	Src(1-530)(h)	106	103	92
FGFR1(h)	58	63	85	Src(T341M)(h)	100	95	96
FGFR1(V561M)(h)	102	86	87	SRMS(h)	86	86	96
FGFR2(h)	96	89	93	SRPK1(h)	91	88	91
FGFR2(N549H)(h)	101	102	93	SRPK2(h)	99	99	104
FGFR3(h)	98	97	93	STK16(h)	93	91	94
FGFR4(h)	96	91	81	STK25(h)	77	48	99
Fgr(h)	83	96	106	STK32A(h)	98	100	107
Flt1(h)	75	67	86	STK32B(h)	100	93	105
Flt3(D835Y)(h)	96	36	89	STK32C(h)	94	98	104
Flt3(h)	71	18	96	STK33(h)	78	69	102
Flt4(h)	74	95	113	Syk(h)	83	81	(IC_{50} > 30 μM)
Fms(h)	113	120	103	TAF1L(h)	89	85	103
Fms(Y969C)(h)	95	95	103	TAK1(h)	90	54	100
Fyn(h)	91	83	104	TAO1(h)	89	87	101
GCK(h)	87	33	104	TAO2(h)	93	94	96
GCN2(h)	85	84	114	TAO3(h)	89	95	114
GRK1(h)	96	83	90	TBK1(h)	90	90	103
GRK2(h)	91	94	108	Tec(h) activated	66	53	107
GRK3(h)	97	94	93	TGFBR1(h)	105	100	94
GRK5(h)	98	77	100	TGFBR2(h)	98	95	101
GRK6(h)	84	65	107	Tie2 (h)	78	75	101
GRK7(h)	82	61	94	Tie2(R849W)(h)	97	108	85
GSK3α(h)	101	103	99	Tie2(Y897S)(h)	105	114	83
GSK3β(h)	83	94	87	TLK1(h)	85	78	115
Haspin(h)	98	53	84	TLK2(h)	82	68	106
Hck(h)	64	58	96	TNIK(h)	58	48	93
Hck(h) activated	95	91	111	TRB2(h)	89	87	116
HIPK1(h)	86	86	104	TrkA(h)	92	69	80
HIPK2(h)	85	95	101	TrkB(h)	68	96	103
HIPK3(h)	98	92	99	TrkC(h)	74	82	115
HIPK4(h)	97	101	105	TSSK1(h)	87	63	91
HPK1(h)	99	58	107	TSSK2(h)	93	88	95
HRI(h)	85	88	101	TSSK3(h)	108	92	100
ICK(h)	56	49	121	TSSK4(h)	91	84	96
IGF-1R(h)	101	100	86	TTBK1(h)	101	105	90
IGF-1R(h), activated	121	100	99	TTBK2(h)	97	84	107
IKKα(h)	111	91	92	TTK(h)	97	92	102
IKKβ(h)	93	93	85	Txk(h)	81	82	90
IKKε(h)	92	98	86	TYK2(h)	92	75	99
IR(h)	77	72	98	ULK1(h)	98	34	98
IR(h), activated	78	68	104	ULK2(h)	103	63	110
IRE1(h)	101	104	99	ULK3(h)	85	59	100
IRR(h)	101	99	100	VRK1(h)	93	87	104
IRAK1(h)	86	87	100	VRK2(h)	49	14	82

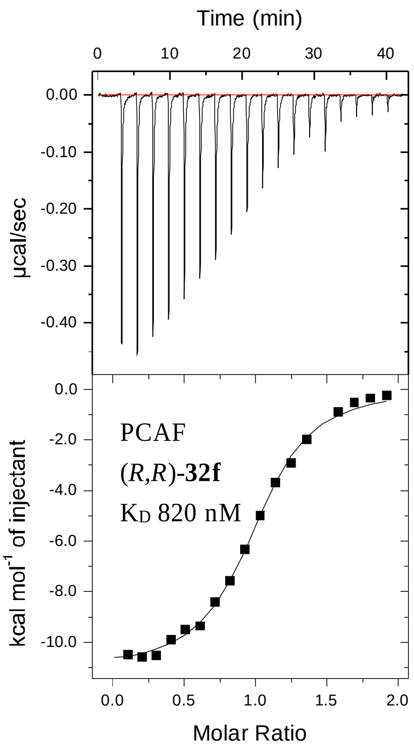
IRAK4(h)	92	97	117	Wee1(h)	87	83	87
Itk(h)	100	98	110	Wee1B(h)	87	91	99
JAK1(h)	80	79	84	WNK1(h)	100	96	105
JAK2(h)	94	89	101	WNK2(h)	74	66	100
JAK3(h)	93	89	102	WNK3(h)	83	80	108
JNK1α1(h)	104	99	112	WNK4(h)	97	94	110
JNK2α2(h)	106	115	97	Yes(h)	77	75	93
JNK3(h)	70	81	86	ZAK(h)	79	81	100
KDR(h)	69	64	103	ZAP-70(h)	99	107	111
Lck(h)	67	66	105	ZIPK(h)	96	97	109
Lck(h) activated	95	91	91	ATM(h)	88	72	104
LIMK1(h)	56	85	38	ATR/ATRIP(h)	91	98	104
LIMK2(h)	80	78	92	DNA-PK(h)	121	99	112
LKB1(h)	62	56	107	PI3 Kinase (p110β/p85α)(h)	100	100	99
LOK(h)	83	73	98	PI3 Kinase (p120γ)(h)	102	100	103
Lyn(h)	88	87	88	PI3 Kinase (p110δ/p85α)(h)	98	100	99
Lyn(m)	95	111	82	PI3 Kinase (p110α/p85α)(m)	98	98	98
LRRK2(h)	80	21	102	PI3 Kinase (p110α/p65α)(m)	98	98	92
LTK(h)	84	65	89	PI3 Kinase (p110α(E545K)/p85α)(m)	98	99	99
MAK(h)	75	76	97	PI3 Kinase (p110α(H1047R)/p85α)(m)	105	105	97
MAPK1(h)	87	71	99	PI3 Kinase (p110β/p85β)(m)	104	103	101
MAPK2(h)	92	85	104	PI3 Kinase (p110β/p85α)(m)	98	98	102
MAPK2(m)	100	105	90	PI3 Kinase (p110δ/p85α)(m)	102	101	96
MAP4K3(h)	75	45	96	PI3 Kinase (p110α(E542K)/p85α)(m)	102	100	100
MAP4K4(h)	66	66	107	PI3 Kinase (p110α/p85α)(h)	99	99	97
MAP4K5(h)	63	49	98	PI3 Kinase (p110α(E542K)/p85α)(h)	95	94	95
MAPKAP-K2(h)	90	81	111	PI3 Kinase (p110α(H1047R)/p85α)(h)	56	59	97
MAPKAP-K3(h)	86	73	98	PI3 Kinase (p110α(E545K)/p85α)(h)	97	97	100
MEK1(h)	87	90	113	PI3 Kinase (p110α/p65α)(h)	99	100	96
MEK2(h)	57	29	89	PI3KC2α(h)	96	95	102
MARK1(h)	92	81	111	PI3KC2γ(h)	86	95	99
MARK3(h)	75	76	104	PIP4K2α(h)	95	102	100
MARK4(h)	77	82	92	PIP5K1α(h)	99	94	102
MEKK2(h)	93	96	103	PIP5K1γ(h)	107	106	108

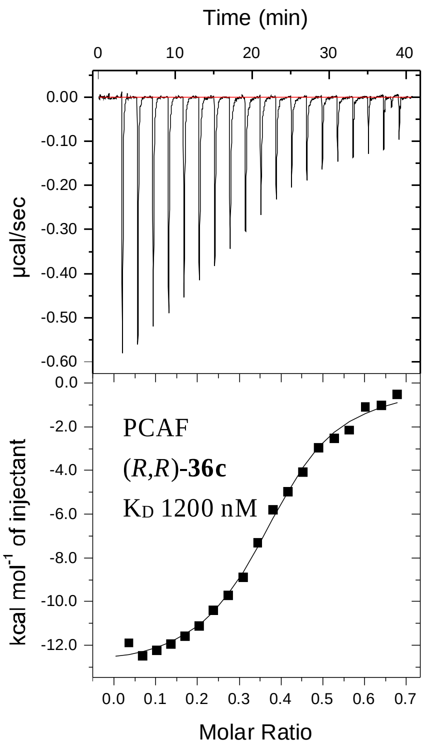
Ctrl Legend

$0 \le x < 1$	$1 \le x < 5$	$5 \le x < 20$	$20 \le x < 50$	$x \ge 50$

Figure S2. ITC curves for selected compounds (PCAF bromodomain).




Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
PCAF
(R,R)-32f
KD 820 nM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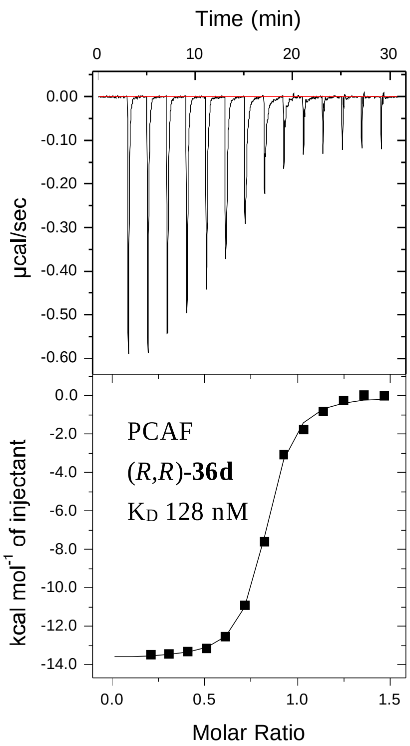
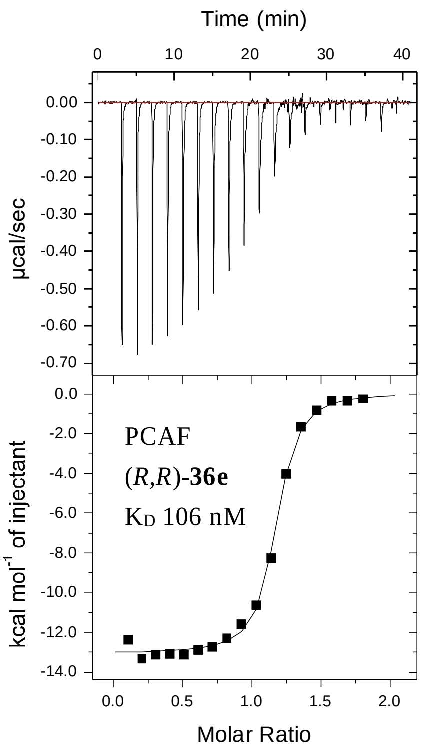
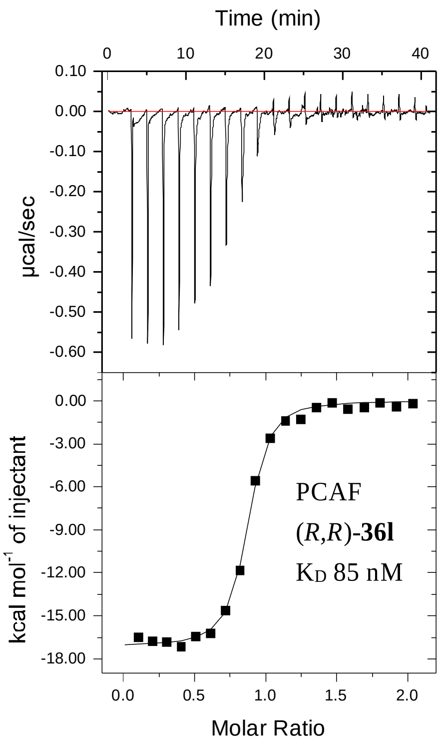
µcal/sec
kcal mol-1 of injectant
Molar Ratio
PCAF
(R,R)-36c
KD 1200 nM


Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
PCAF
(R,R)-36d
KD 128 nM


Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
PCAF
(R,R)-36e
KD 106 nM



Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
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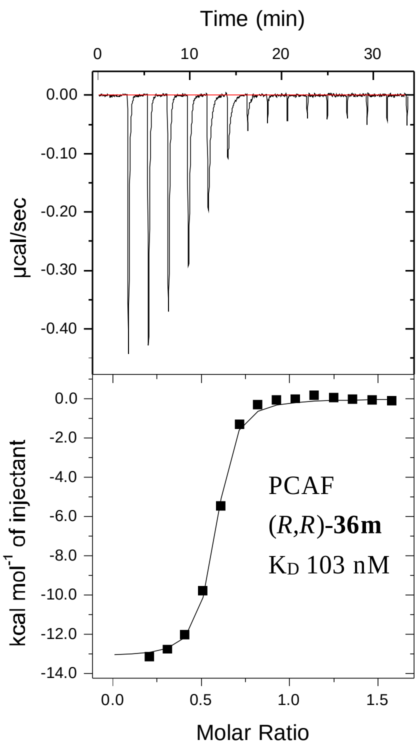
KD 85 nM



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µcal/sec
kcal mol-1 of injectant
Molar Ratio
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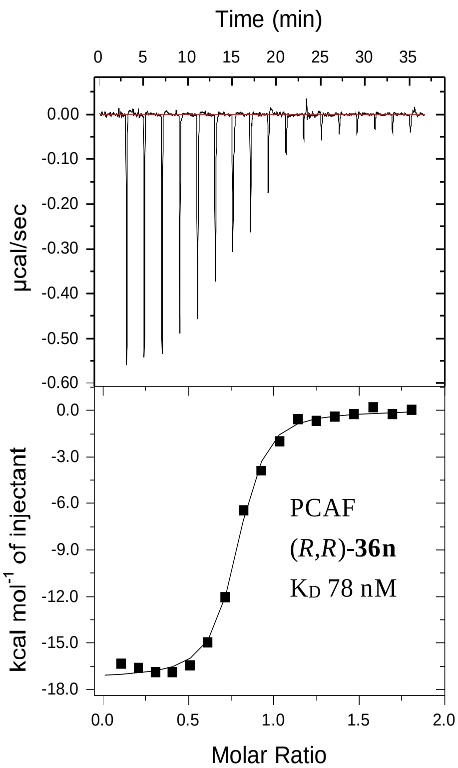
(R,R)-36m
KD 103 nM



Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
PCAF
(R,R)-36n
KD 78 nM

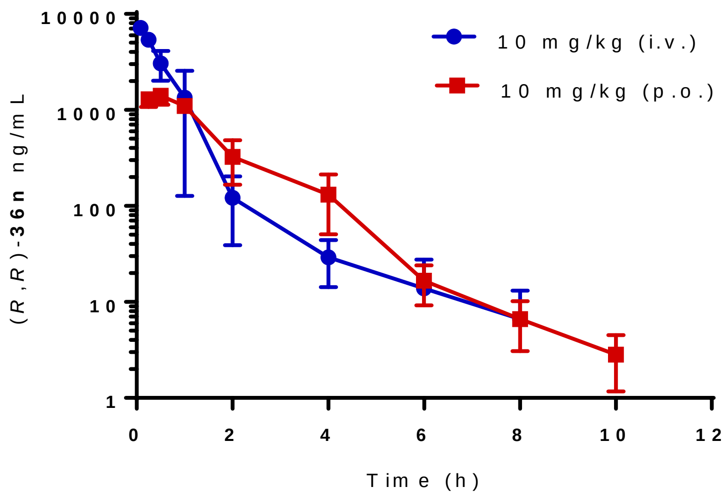
Table S4. Data collection and refinement statistics for X-ray structures.

(collection on a single crystal)	PCAF/1**2**	GCN5/(*R*,*R*)-**36n**
PDB ID	6J3O	6J3P
Data collection		
Space group	H3	P $2_1 2_1 2_1$
Cell dimension		
a, *b*, *c* (Å)	101.52 101.52 99.68	45.57 73.09 76.22
α, β, γ (°)	90 90 120	90 90 90
Resolution (Å)	50.76-2.11 (2.17-2.11)	50.0-1.6 (1.66-1.60)
R_{merge}	0.129 (1.553)	0.099 (0.463)
I/σ*I*	10.500 (1.600)	30.167 (6.667)
Completeness (%)	99.6（99.8)	99.9 (100)
Redundancy	5.3	12.8 (11.6)
Refinement		
No. unique reflections	21934	34345
R_{work}/ R_{free}	0.235/0.265	0.167/ 0.192
No. atoms	1843	2128
Protein	1761	1784
Ligand	38	58
Water	44	286
B-factors (Å^2)		
Protein	62.52	16.07
Ligand	65.51	16.71
Water	56.99	26.81
RMS bond length (Å)	0.002	0.015
RMS bond angle (°)	0.51	1.32

Table S5. Human liver microsomes stability of (R,R)-**36n**.

Human Liver Microsome Stability	
$T_{1/2}$ (minute)	38
CLint, in vitro (mL/min/mg)	0.04
Cl_{int} (mL/min/kg)	45

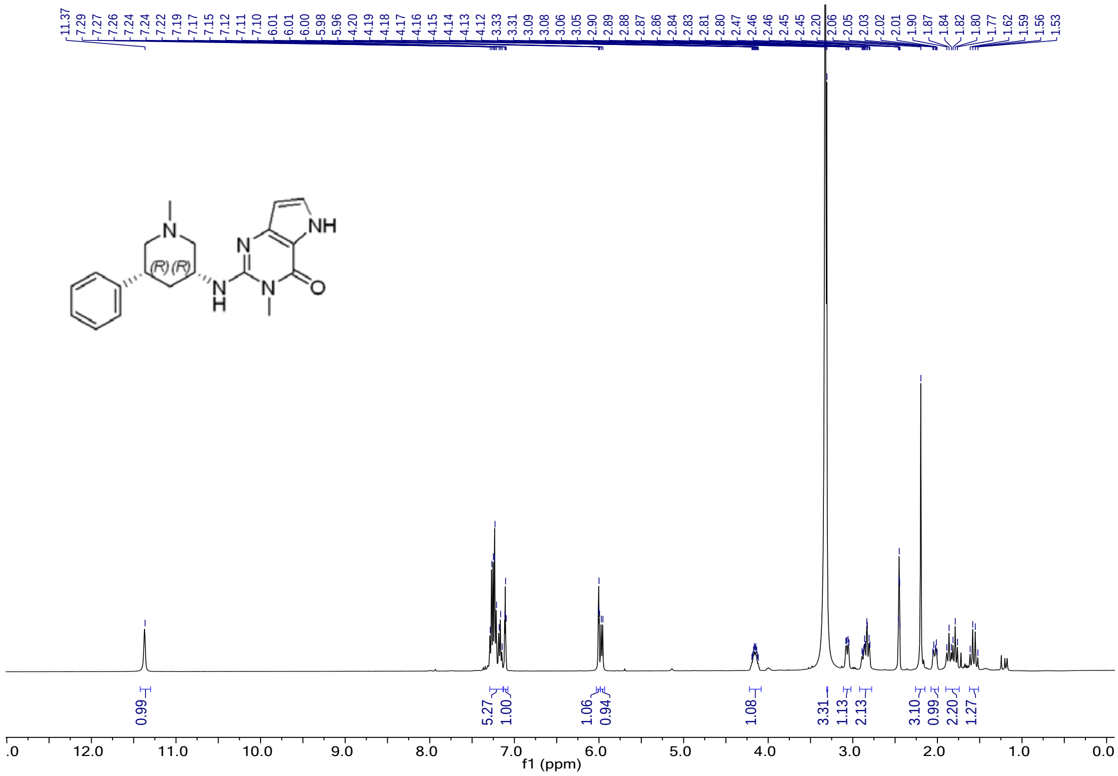
Figure S3. Mean total blood concentration-time profiles and pharmacokinetic parameters of (*R*,*R*)-**36n** after i.v. dose and p.o. dose of 10 mg/kg in male rats, n=3, mean ± SD.

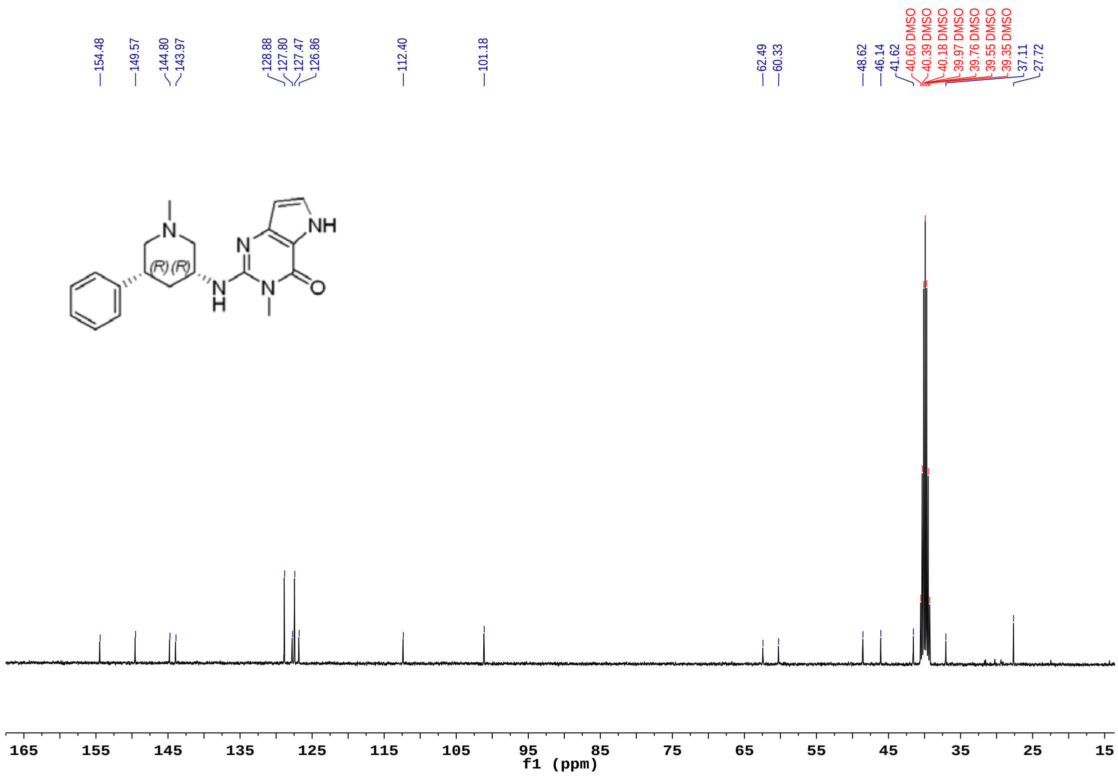


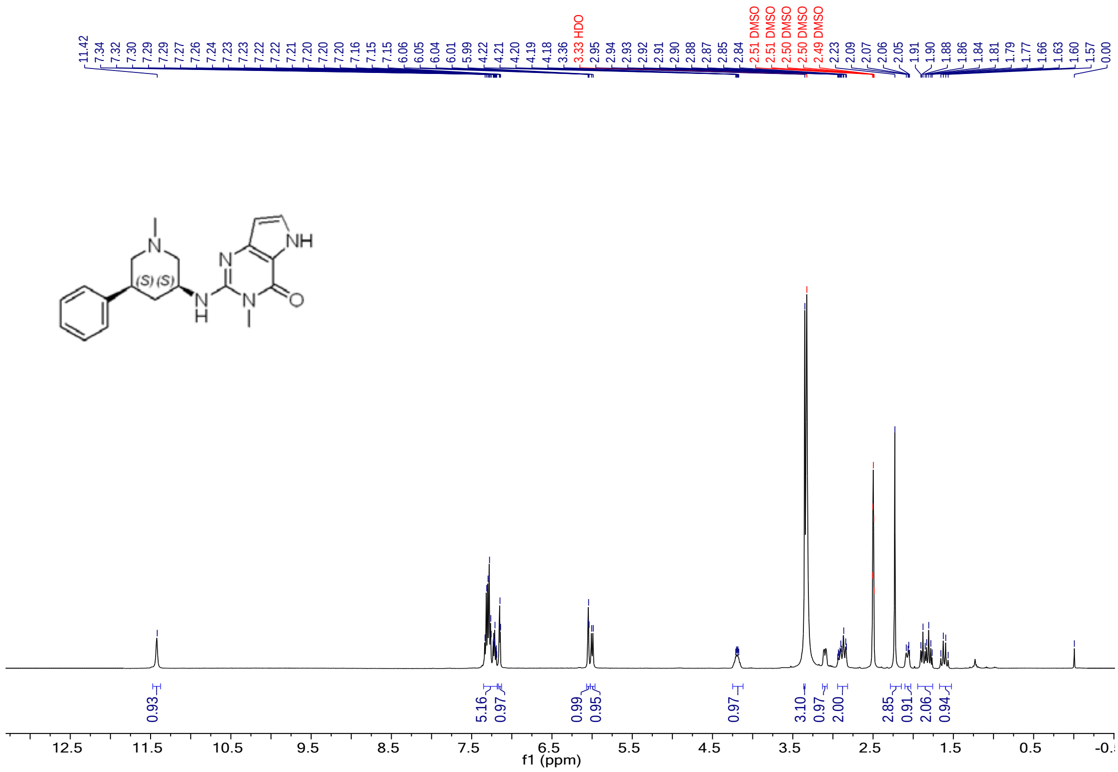
Pharmacokinetic parameter	10 mg/kg i.v. [a]	10 mg/kg p.o. [a]
CL (L/h/kg)	2.2 ± 0.6	-
V_{ss} (L/kg)	1.2 ± 0.09	-
$T_{1/2}$ (h)	1.62 ± 0.63	1.51 ± 0.23
T_{max} (h)	0.08 ± 0.00	0.67 ± 0.29
C_{max} (ng/mL)	7181 ± 611	1418 ± 252
$AUC_{(0-t)}$ (h·ng/mL)	4795 ± 1506	2479 ± 399
$AUC_{(0-\infty)}$ (h·ng/mL)	4815 ± 1530	2486 ± 403
F (%)	-	52 ± 8.3

[a] Expressed as Mean ± SD, n = 3

^{1}H and ^{13}C NMR spectrums of tested compounds.







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7.21
7.20
7.20
7.20
7.16
7.15
7.15
6.06
6.05
6.04
6.01
5.99
4.22
4.21
4.20
4.19
4.18
3.36
3.33 HDO
2.95
2.94
2.93
2.92
2.91
2.90
2.88
2.87
2.85
2.84
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
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2.07
2.06
2.05
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1.90
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H
N
O
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0.97
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0.95
0.97
3.10
0.97
2.00
2.85
0.91
2.06
0.94
12.5
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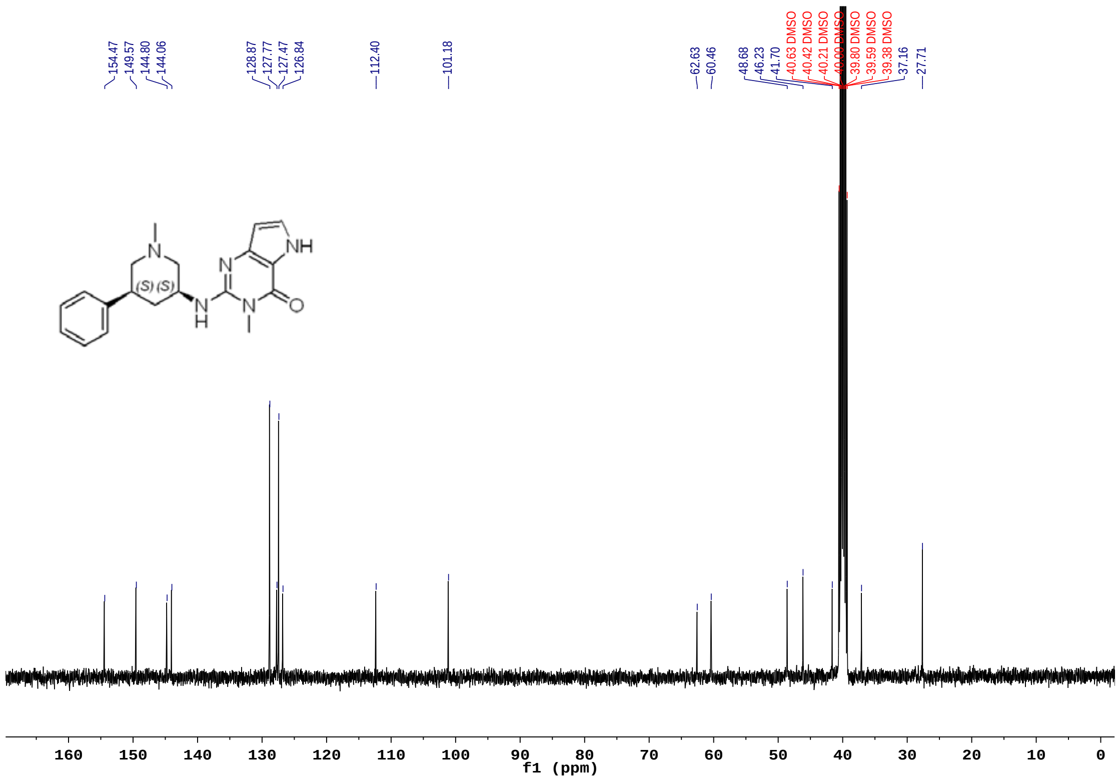
f1 (ppm)



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40.00 DMSO
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39.59 DMSO
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H
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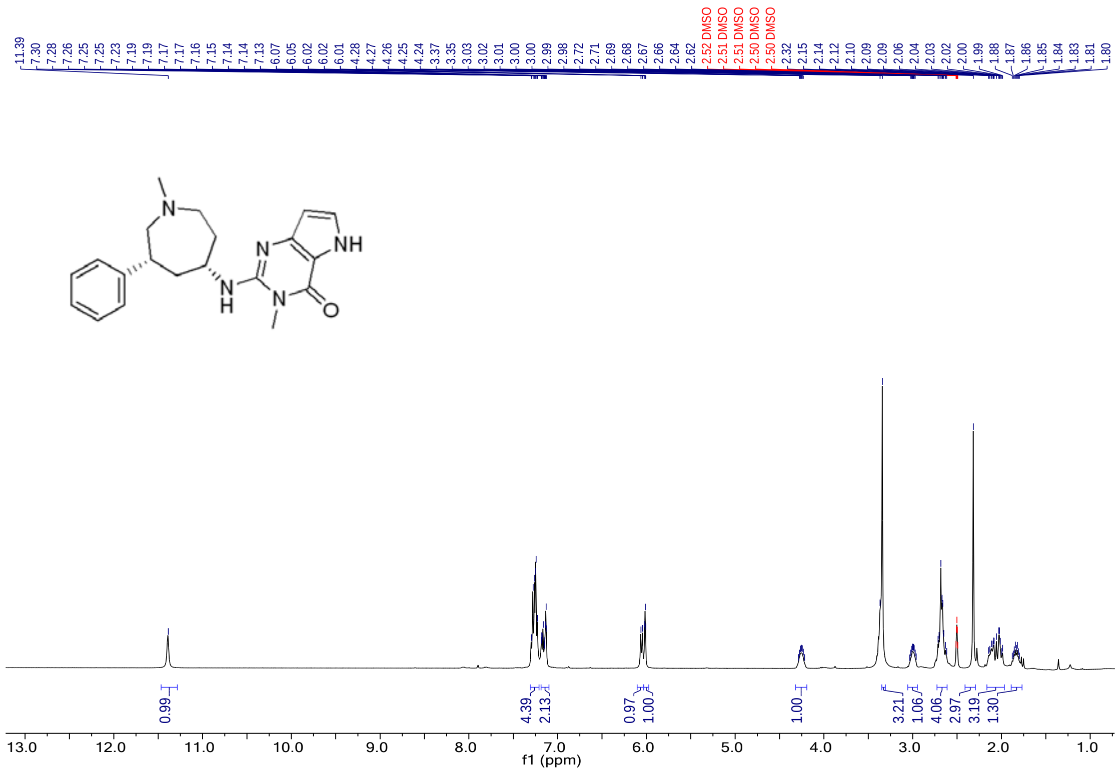
f1 (ppm)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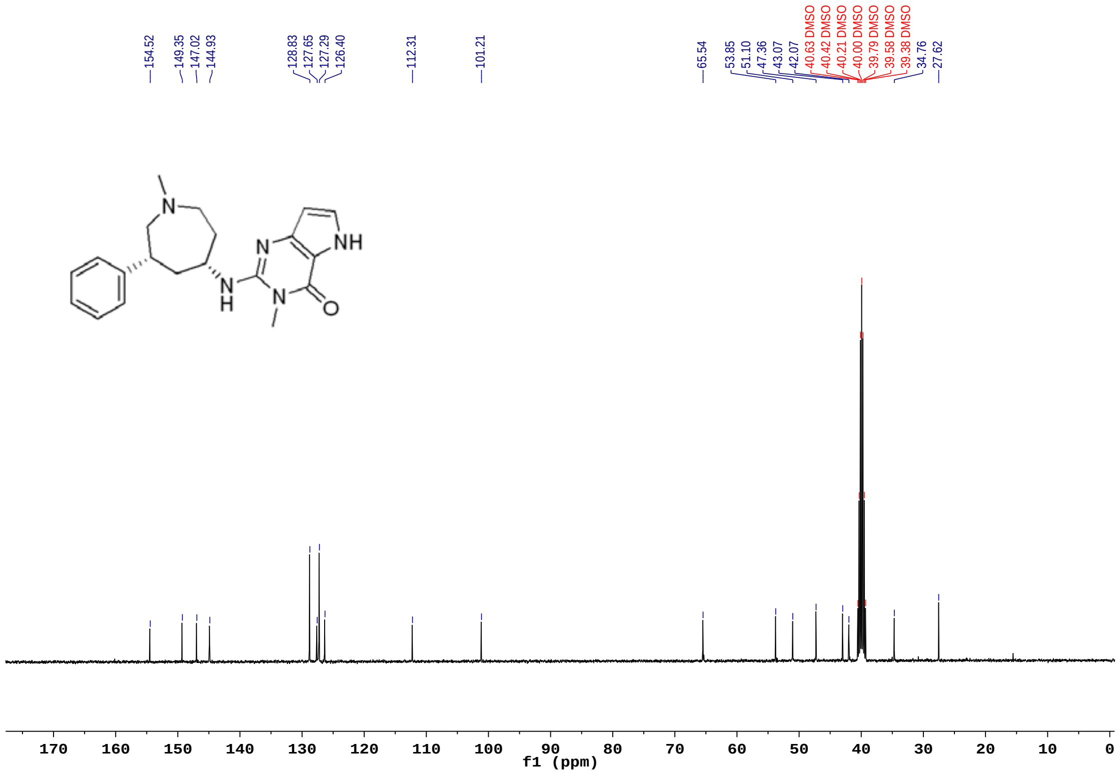
f1 (ppm)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42.07
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O
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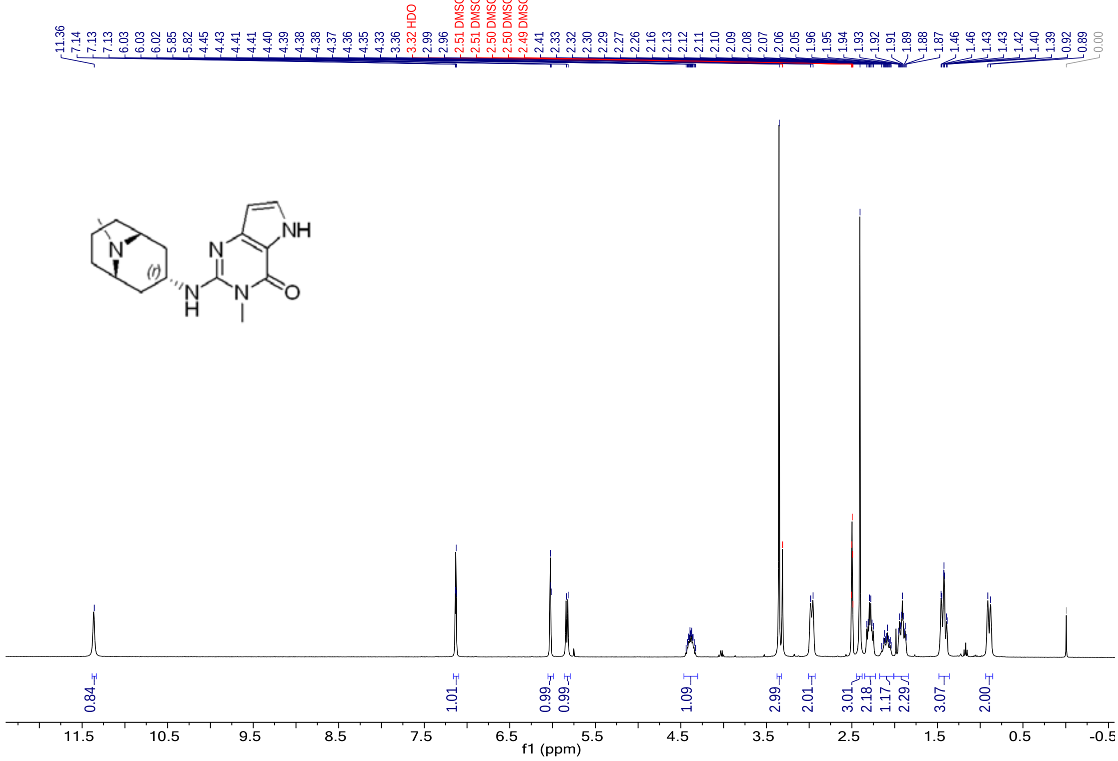
f1 (ppm)



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(r)
N
H
N
O
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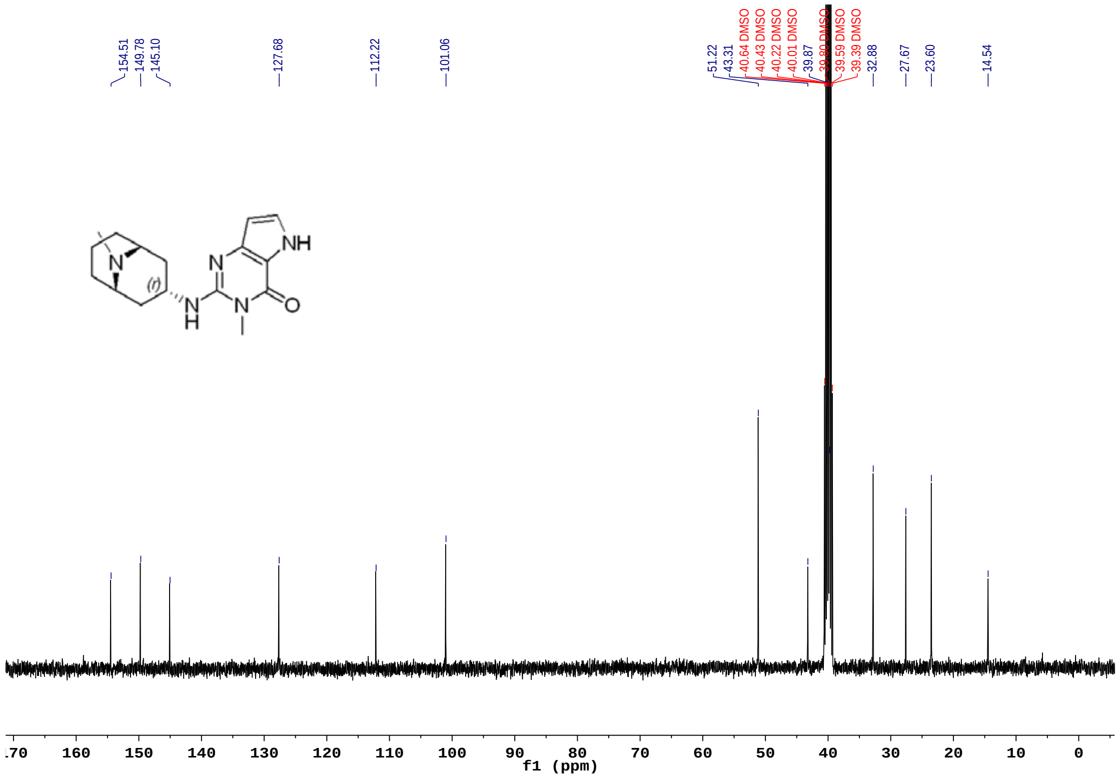
f1 (ppm)



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N
(r)
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H
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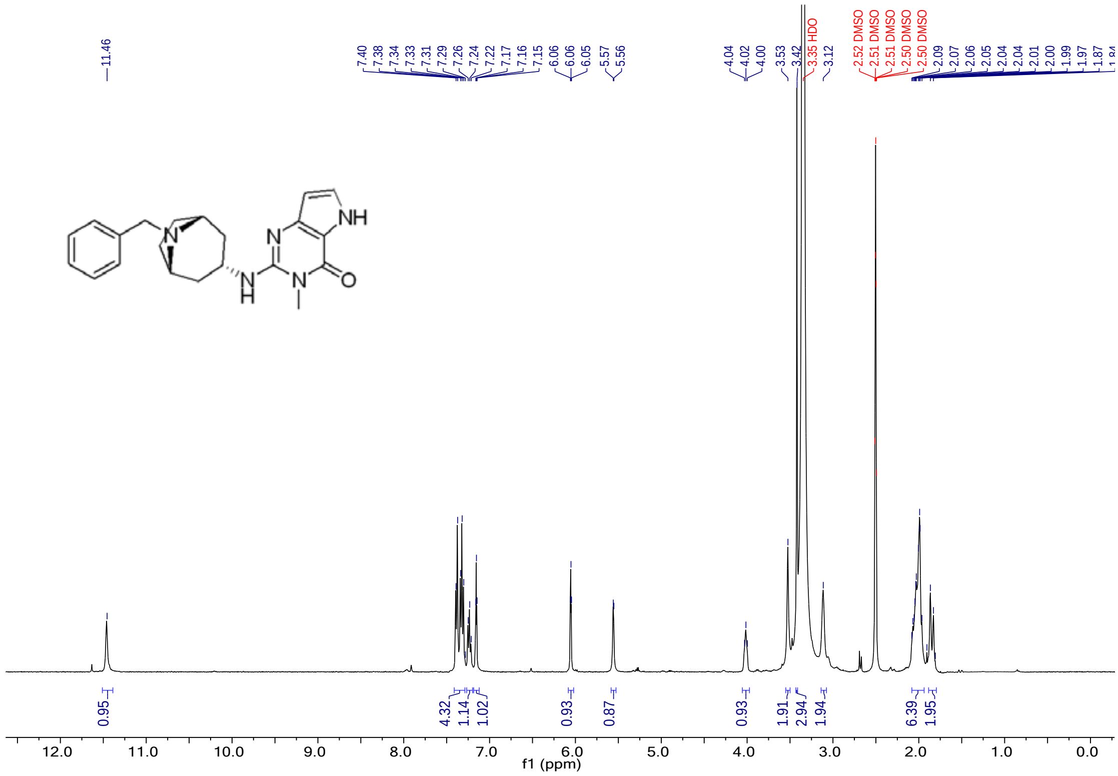

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2.09 2.07 2.06 2.05 2.04 2.04 2.01 2.00 1.99 1.97 1.87
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N
N
H
O
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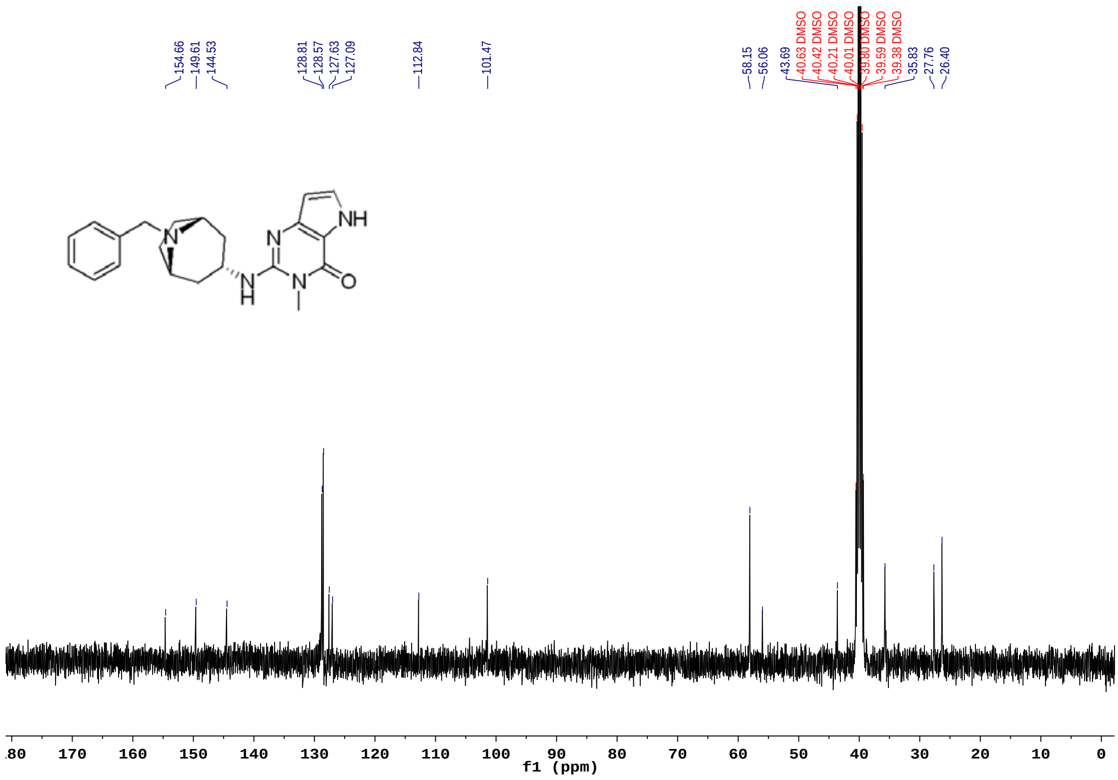

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N
N
H
O
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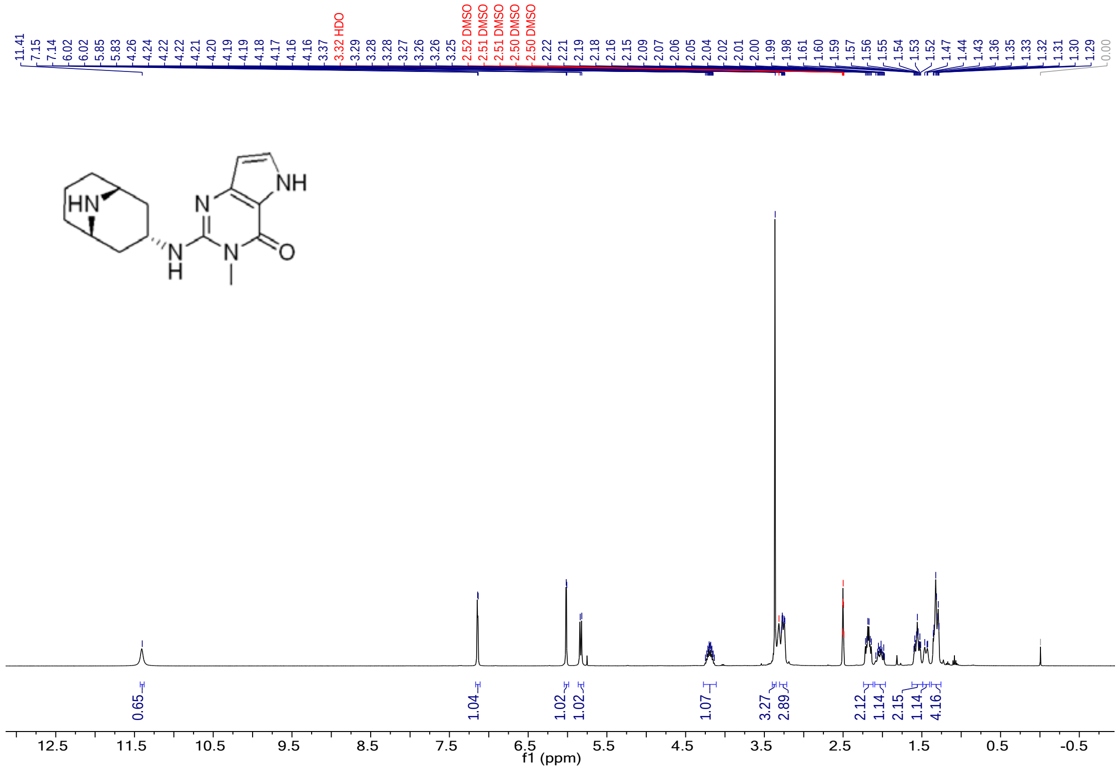
f1 (ppm)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4.19
4.19
4.18
4.17
4.16
4.16
3.37
3.32 HDO
3.29
3.28
3.28
3.27
3.26
3.26
3.25
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.22
2.21
2.19
2.18
2.16
2.15
2.09
2.07
2.06
2.05
2.04
2.02
2.01
2.00
1.99
1.98
1.61
1.60
1.59
1.57
1.56
1.55
1.54
1.53
1.52
1.47
1.44
1.43
1.36
1.35
1.33
1.32
1.31
1.30
1.29
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HN
N
NH
N
H
N
O
0.65
1.04
1.02
1.02
1.07
3.27
2.89
2.12
1.14
2.15
1.14
4.16
12.5
11.5
10.5
9.5
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4.5
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1.5
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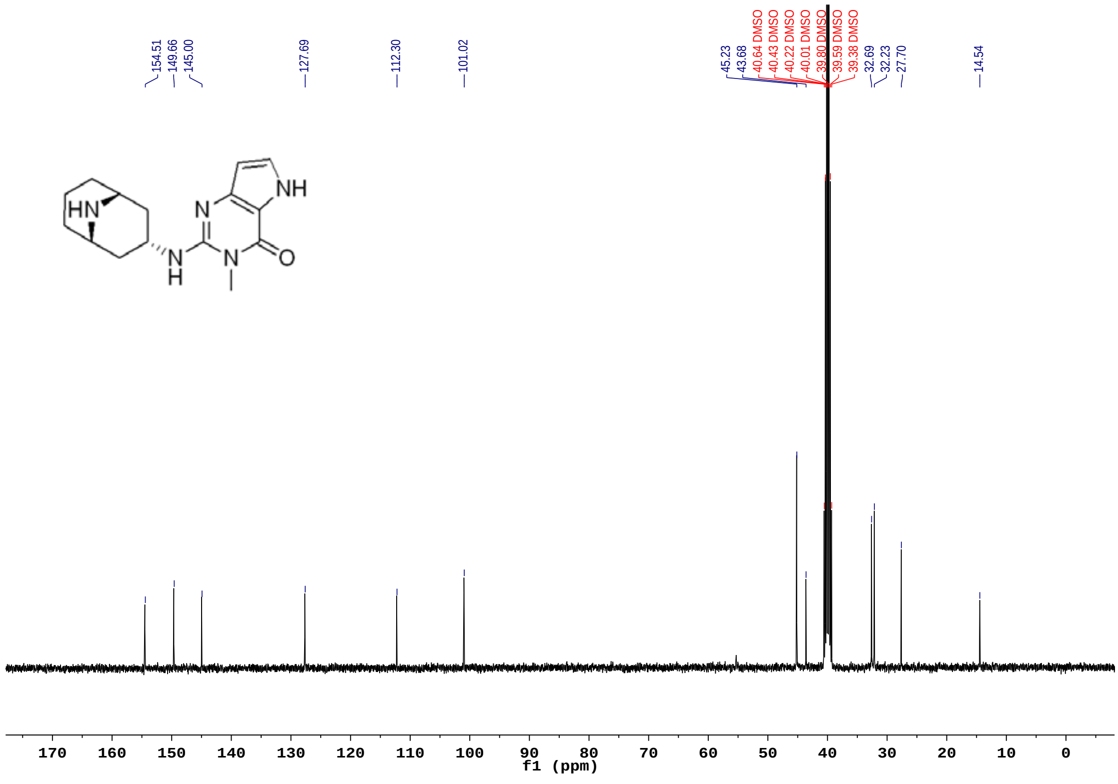
-0.5
f1 (ppm)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40.01 DMSO
39.80 DMSO
39.59 DMSO
39.38 DMSO
32.69
32.23
27.70
14.54
HN
N
NH
N
H
N
O
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130
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110
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90
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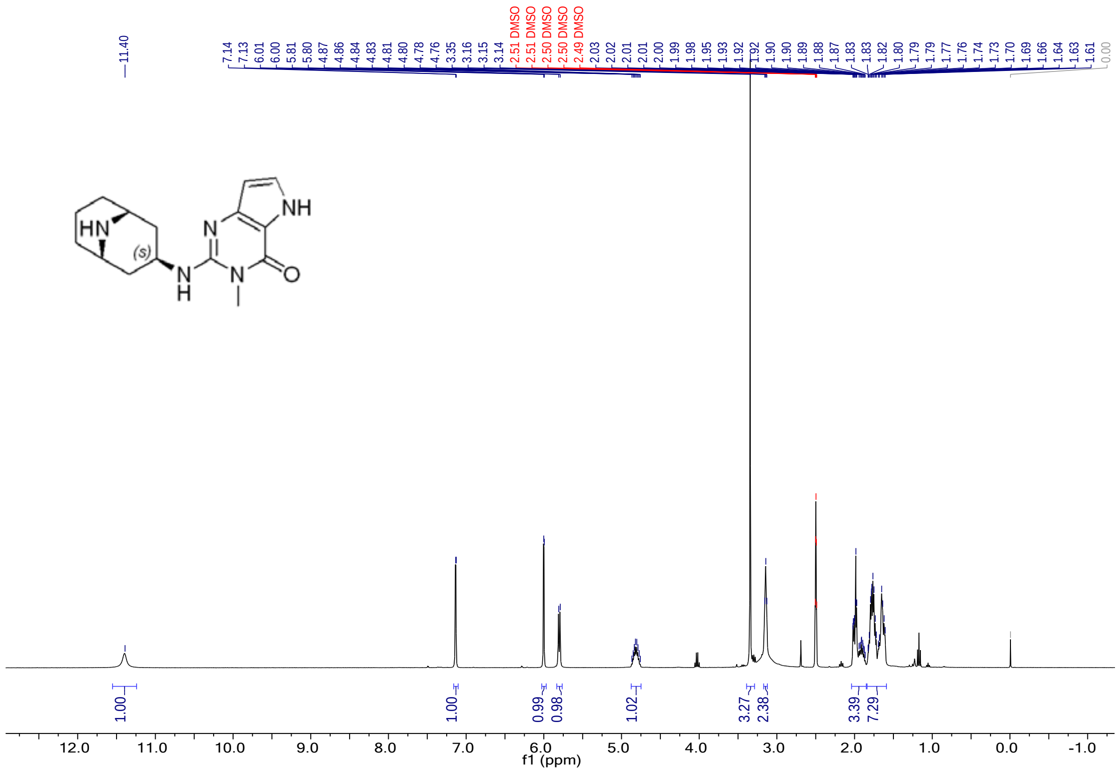
50
40
30
20
10
0
f1 (ppm)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4.81
4.80
4.78
4.76
3.35
3.16
3.15
3.14
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.03
2.02
2.01
2.01
2.00
1.99
1.98
1.95
1.93
1.92
1.92
1.90
1.90
1.89
1.88
1.87
1.83
1.83
1.82
1.80
1.79
1.79
1.77
1.76
1.74
1.73
1.70
1.69
1.66
1.64
1.63
1.61
0.00
HN
(s)
N
NH
N
H
N
O
1.00
1.00
0.99
0.98
1.02
3.27
2.38
3.39
7.29
12.0
11.0
10.0
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6.0
5.0
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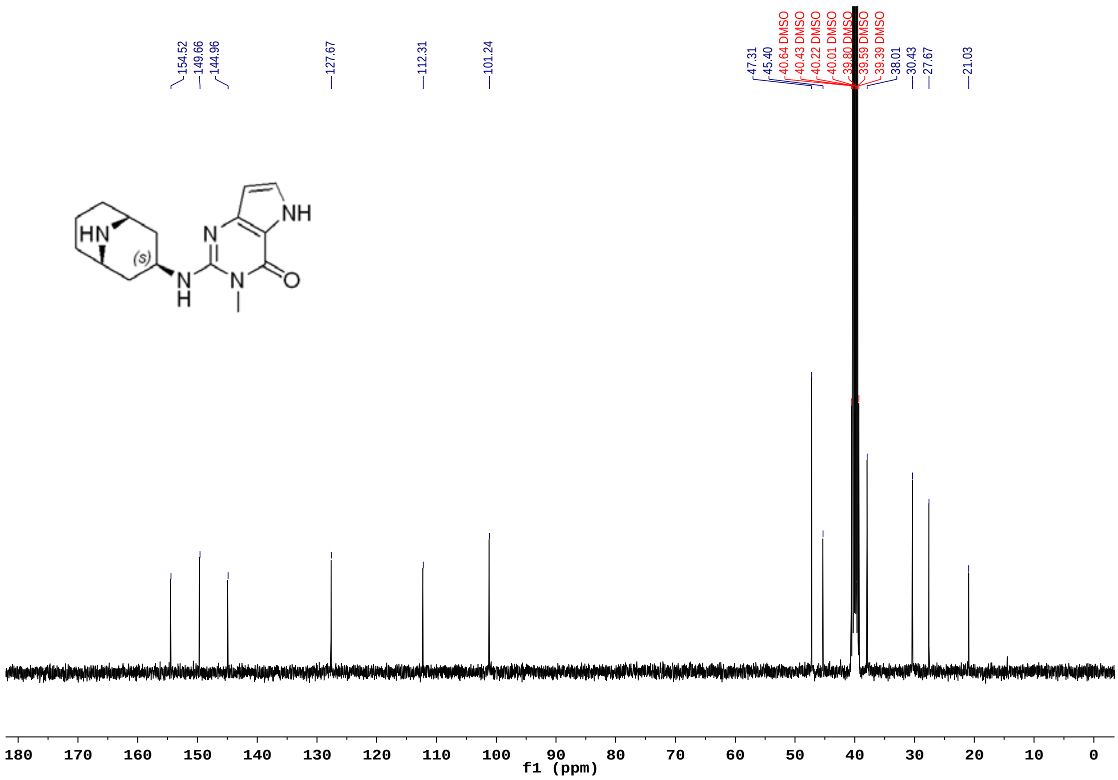
1.0
0.0
-1.0
f1 (ppm)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40.01 DMSO
39.80 DMSO
39.59 DMSO
39.39 DMSO
38.01
30.43
27.67
21.03
HN
(s)
N
NH
N
H
N
O
180
170
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110
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90
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50
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30
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10
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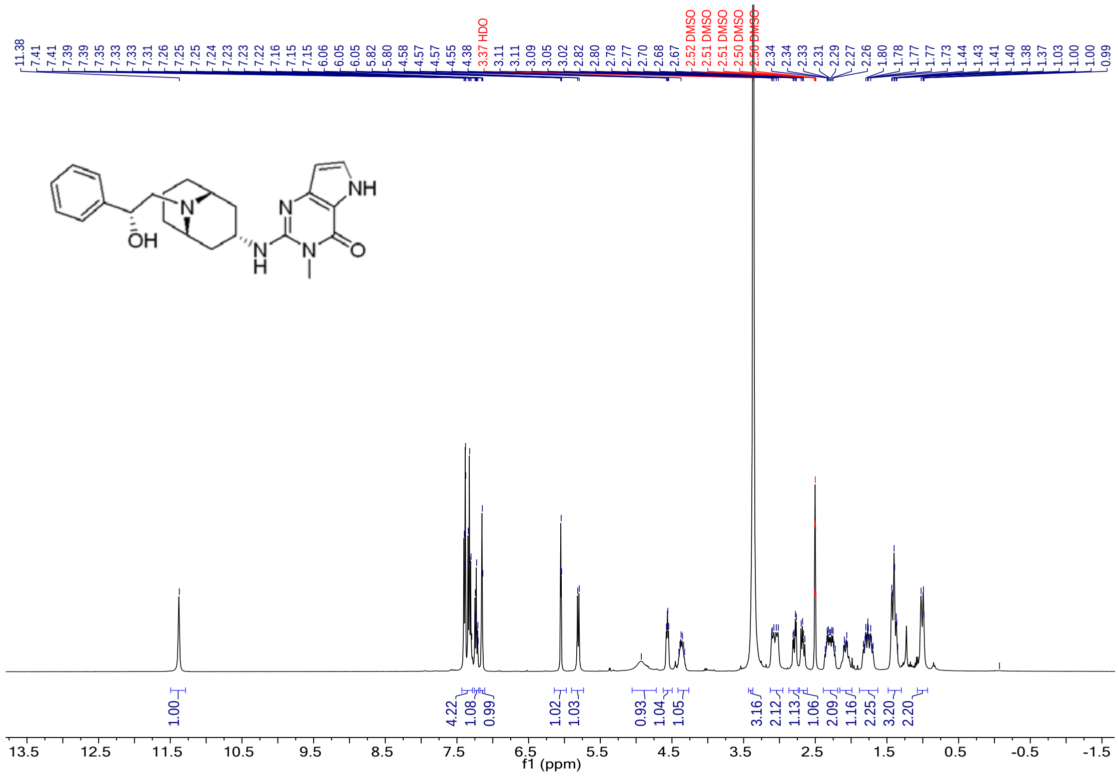
f1 (ppm)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7.25
7.24
7.23
7.23
7.22
7.16
7.15
7.15
6.06
6.05
6.05
5.82
5.80
4.58
4.57
4.57
4.55
4.38
3.37 HDO
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3.11
3.09
3.05
3.02
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2.80
2.78
2.77
2.70
2.68
2.67
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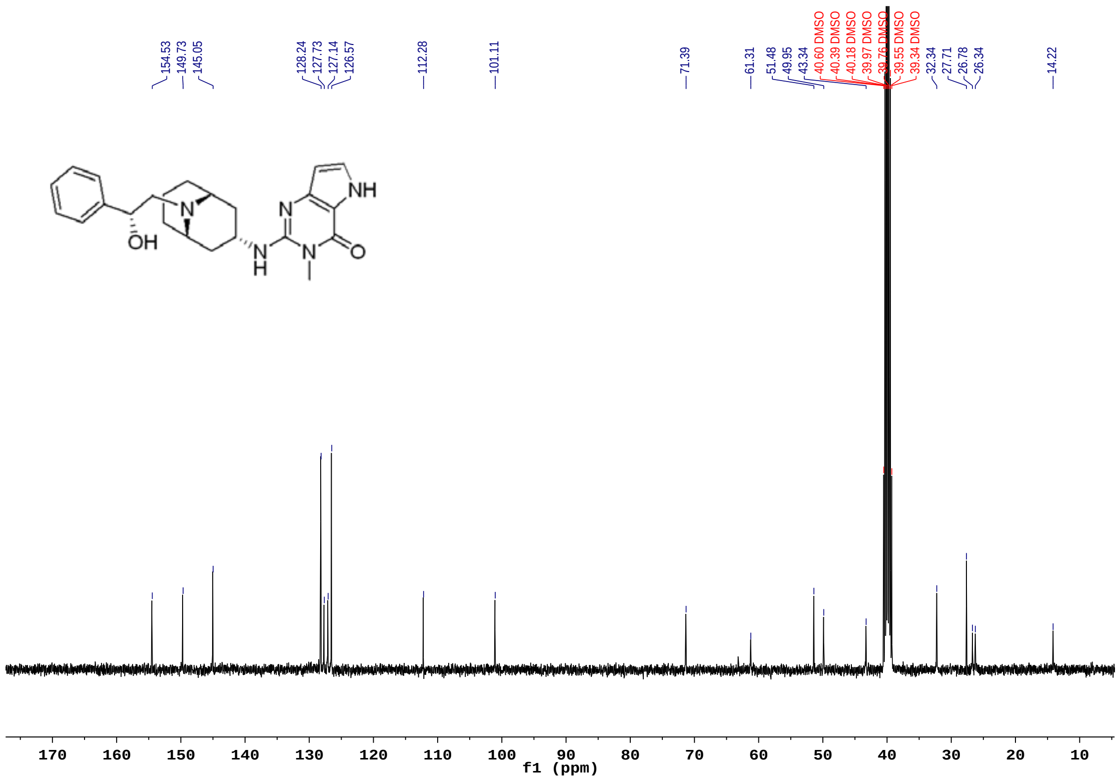
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.34
2.34
2.33
2.31
2.29
2.27
2.26
1.80
1.78
1.77
1.77
1.73
1.44
1.43
1.41
1.40
1.38
1.37
1.03
1.00
1.00
0.99
NH
N
N
OH
N
H
N
O
1.00
4.22
1.08
0.99
1.02
1.03
0.93
1.04
1.05
3.16
2.12
1.13
1.06
2.09
1.16
2.25
3.20
2.20
13.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)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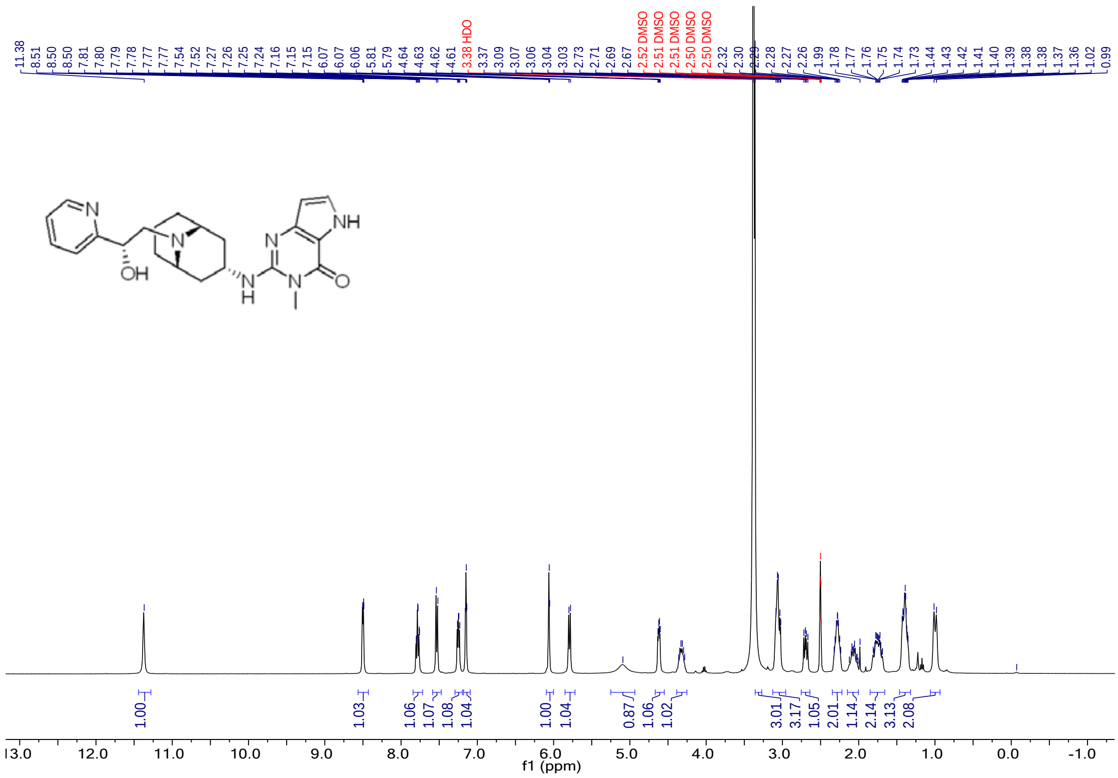
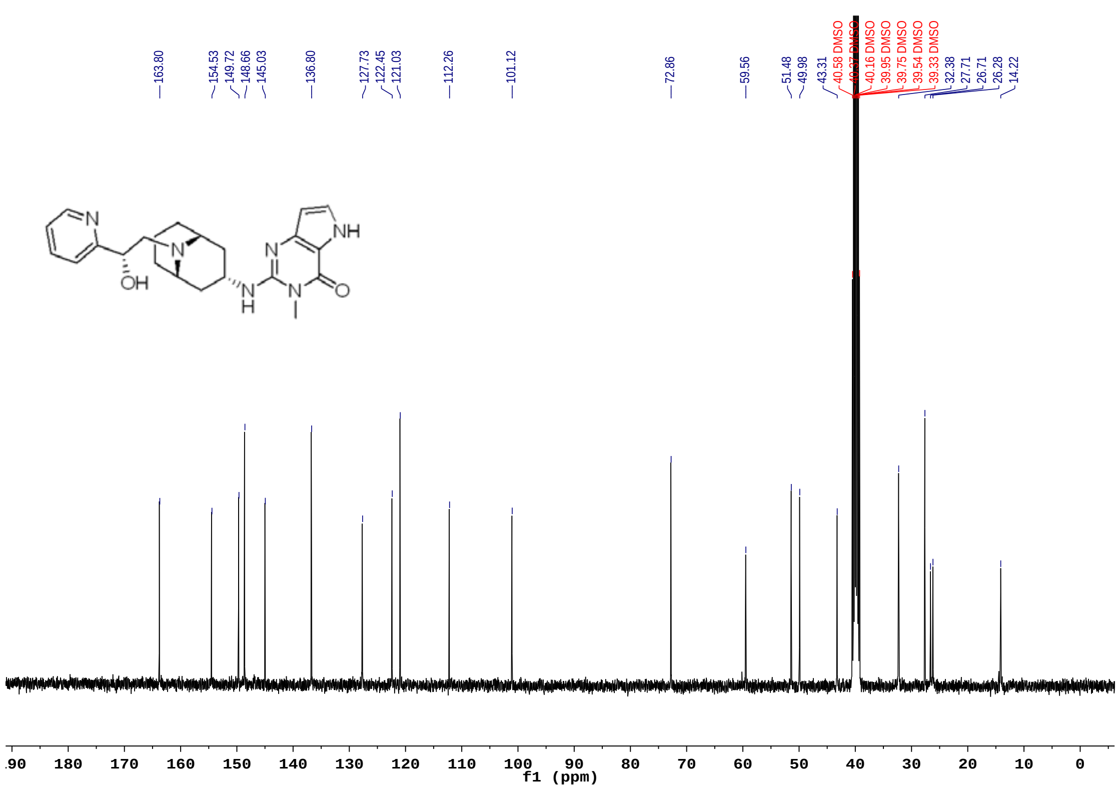
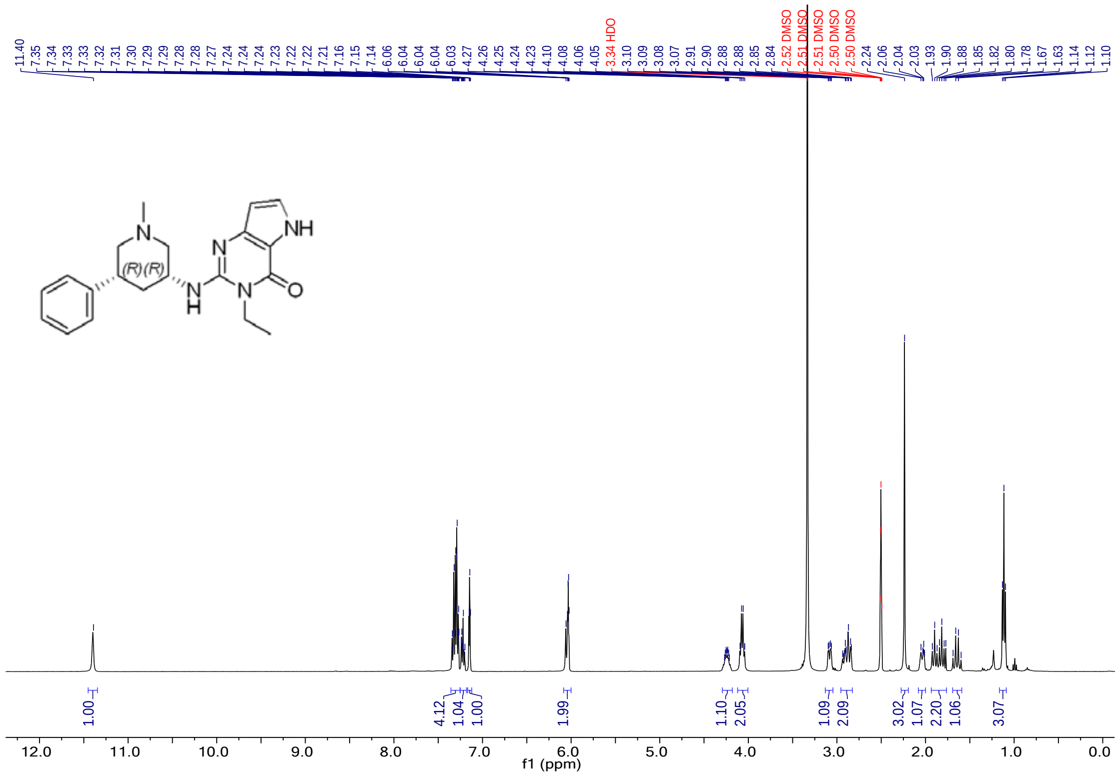
145.05
128.24
127.73
127.14
126.57
112.28
101.11
71.39
61.31
51.48
49.95
43.34
40.60 DMSO
40.39 DMSO
40.18 DMSO
39.97 DMSO
39.76 DMSO
39.55 DMSO
39.34 DMSO
32.34
27.71
26.78
26.34
14.22
NH
N
N
OH
N
H
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



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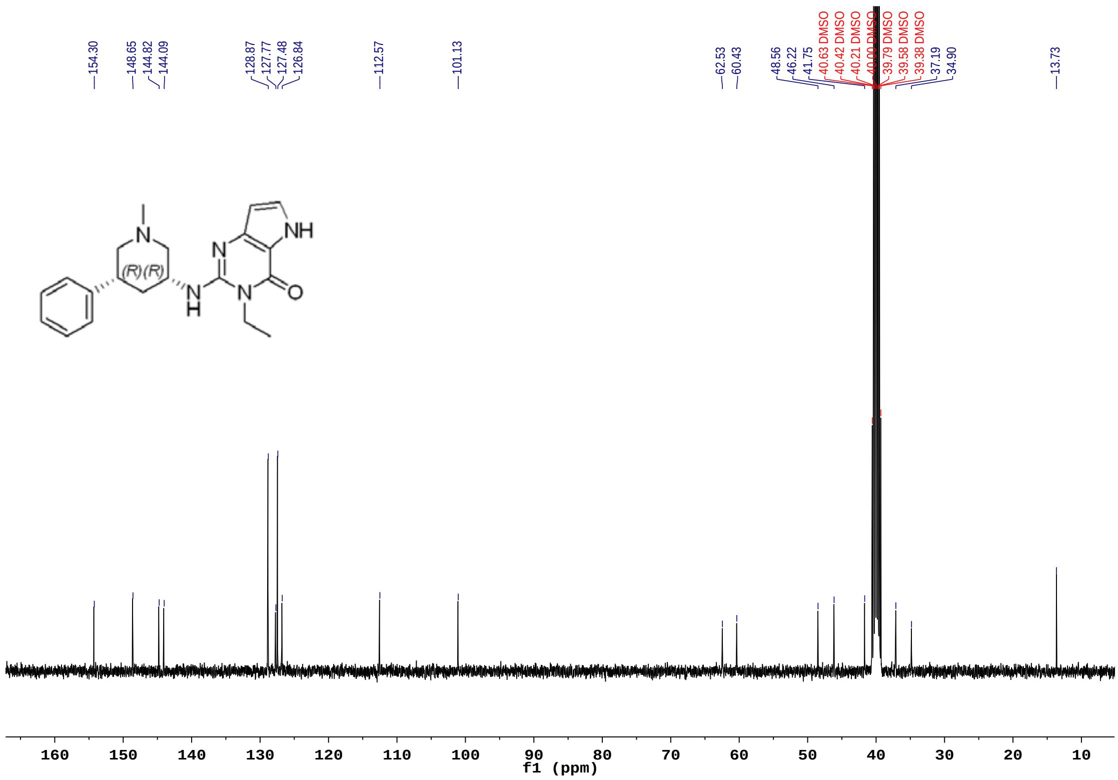
3.34 HDO
3.10 3.09 3.08 3.07 2.91 2.90 2.88 2.88 2.85 2.84
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.24 2.06 2.04 2.03 1.93 1.90 1.88 1.85 1.82 1.80 1.78 1.67 1.63 1.14 1.12 1.10
N
NH
N
(R)(R)
N
H
N
O
1.00
4.12
1.04
1.00
1.99
1.10
2.05
1.09
2.09
3.02
1.07
2.20
1.06
3.07
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)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148.65 144.82 144.09
128.87 127.77 127.48 126.84
112.57
101.13
62.53 60.43
48.56 46.22 41.75
40.63 DMSO
40.42 DMSO
40.21 DMSO
40.00 DMSO
39.79 DMSO
39.58 DMSO
39.38 DMSO
37.19 34.90
13.73
N
NH
N
(R)(R)
N
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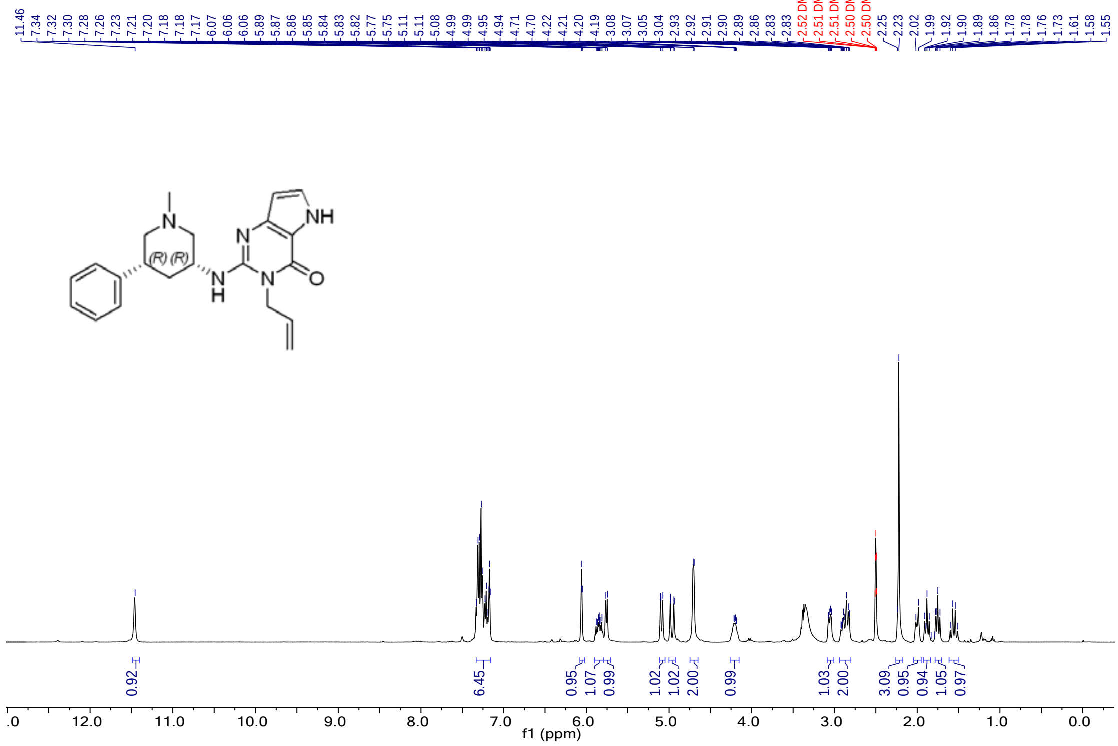
f1 (ppm)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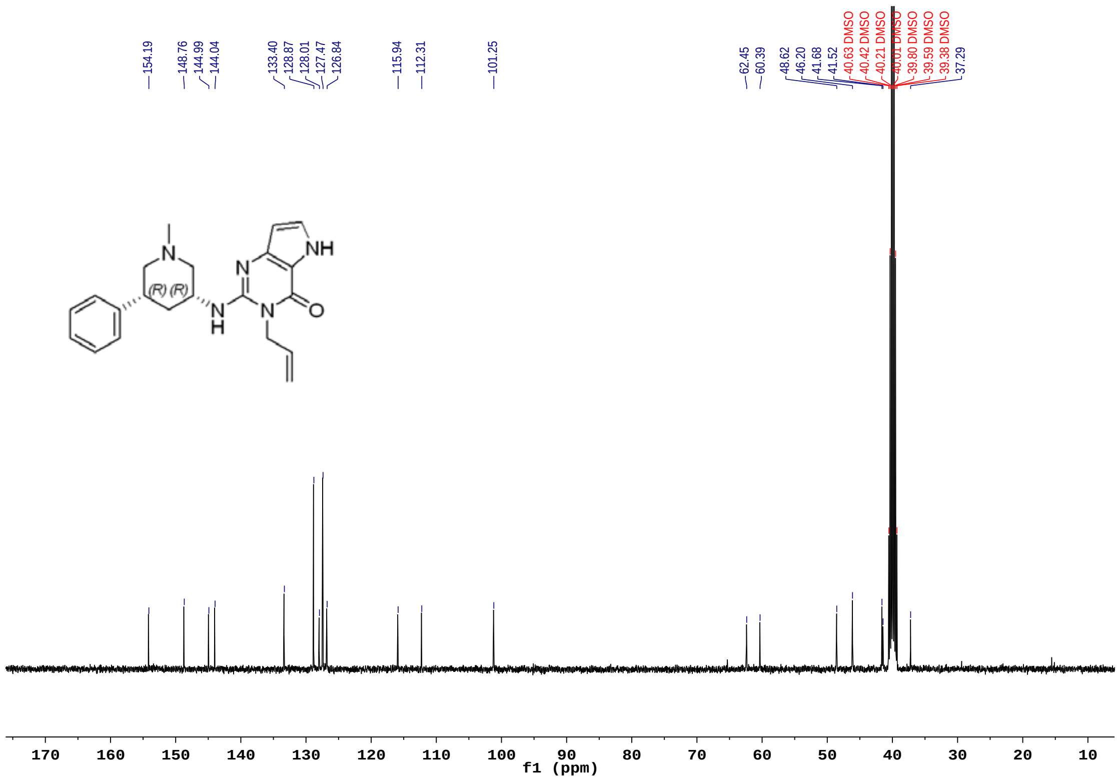
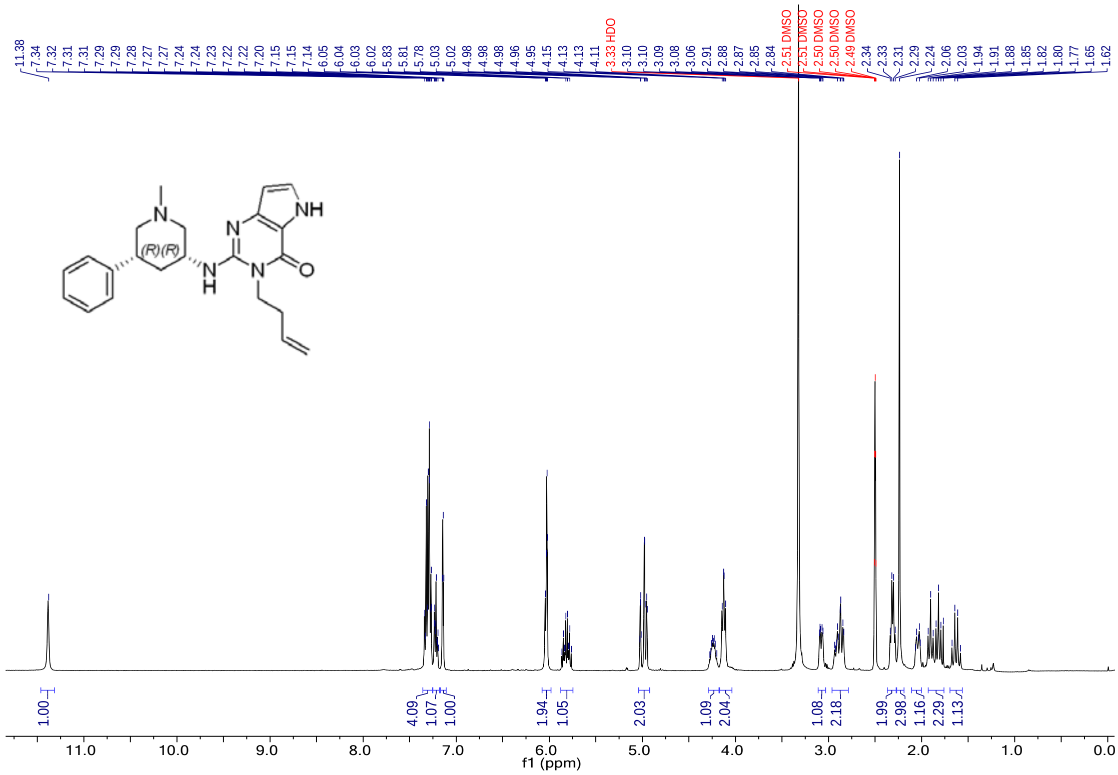
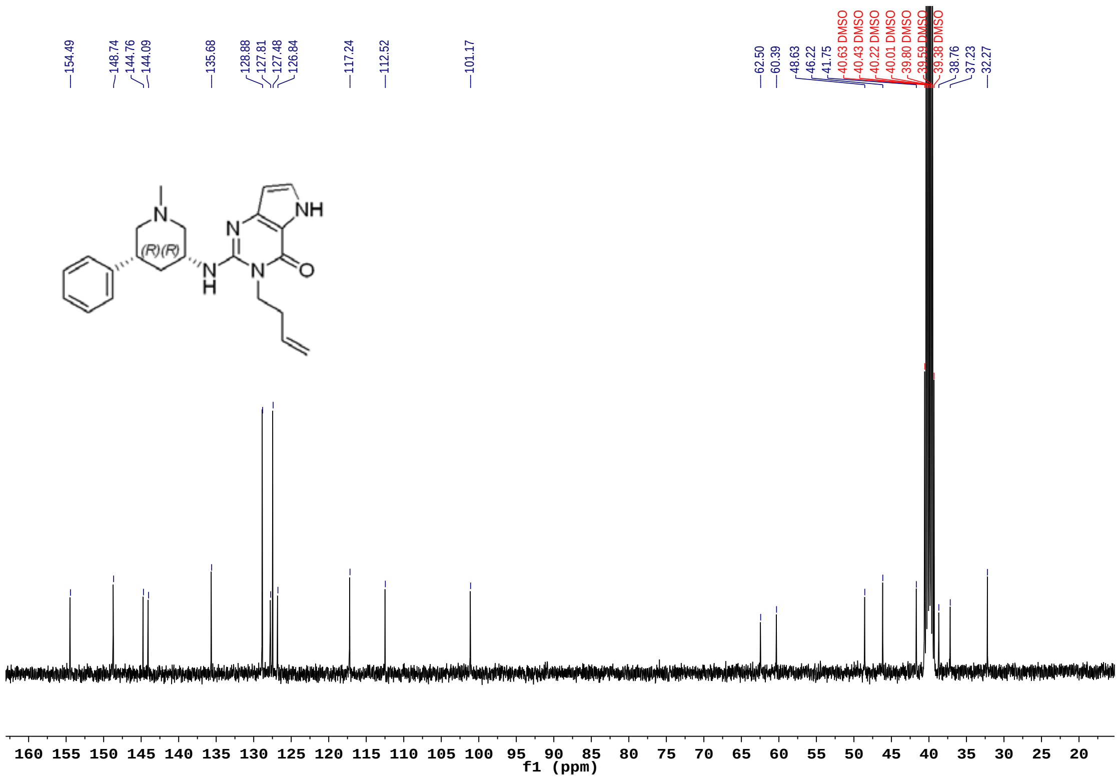
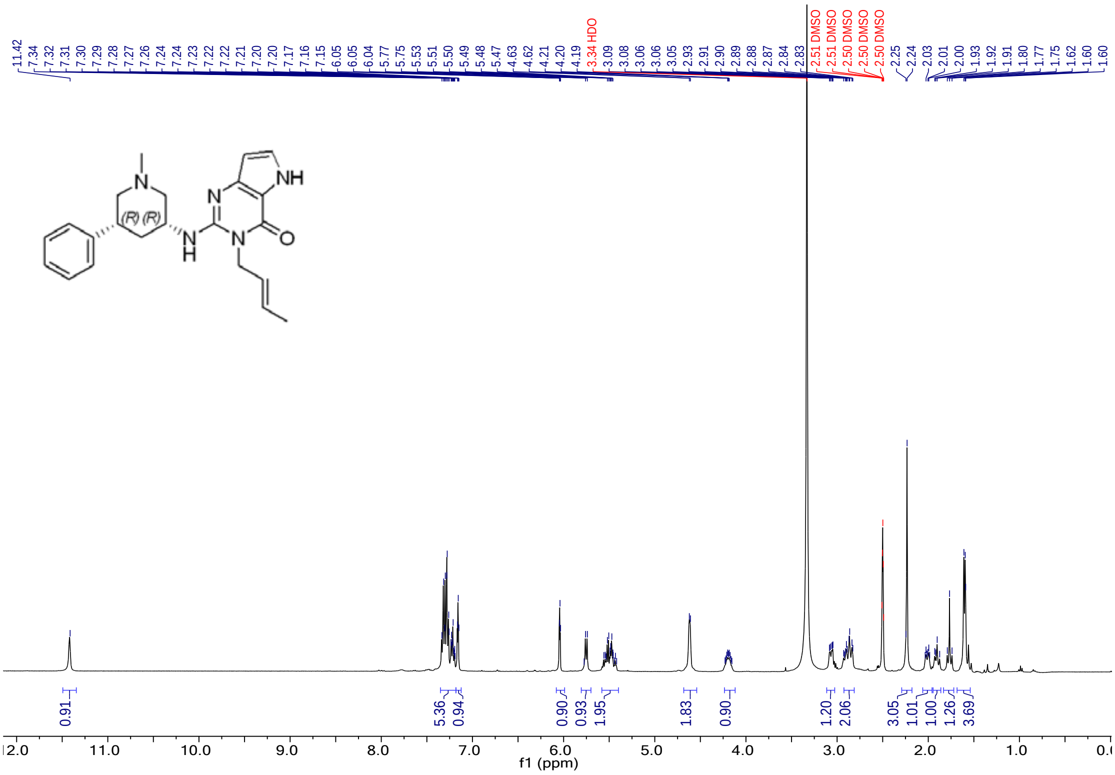
2.52 DMSO 2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO
2.25 2.23 2.02 1.99 1.92 1.90 1.89 1.86 1.78 1.78 1.76 1.73 1.61 1.58 1.55
N
NH
N
(R) (R)
N
H
N
O
0.92 6.45 0.95 1.07 0.99 1.02 1.02 2.00 0.99 1.03 2.00 3.09 0.95 0.94 1.05 0.97
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)


154.19 148.76 144.99 144.04 133.40 128.87 128.01 127.47 126.84 115.94 112.31 101.25 62.45 60.39 48.62 46.20 41.68 41.52
40.63 DMSO 40.42 DMSO 40.21 DMSO 40.01 DMSO 39.80 DMSO 39.59 DMSO 39.38 DMSO
37.29
N
NH
N
(R) (R)
N
H
N
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)



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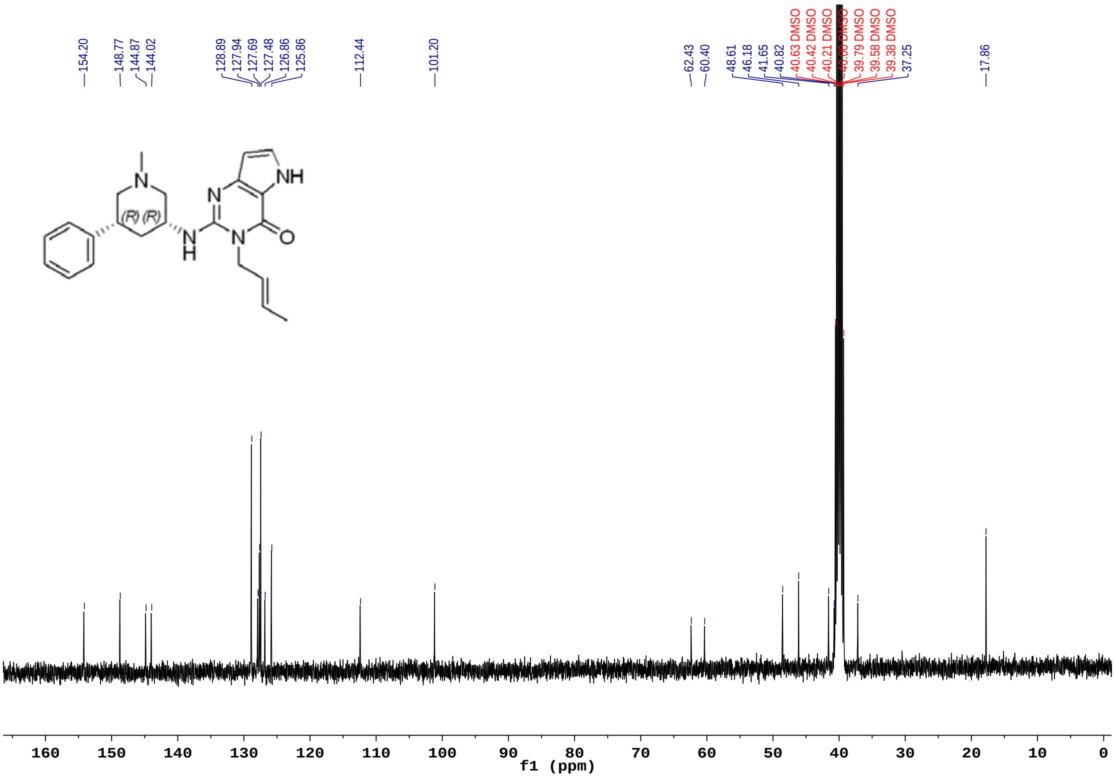
3.34 HDO
3.09 3.08 3.06 3.06 3.05 2.93 2.91 2.90 2.89 2.88 2.87 2.84 2.83
2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO 2.50 DMSO
2.25 2.24 2.03 2.01 2.00 1.93 1.92 1.91 1.80 1.77 1.75 1.62 1.60 1.60
NH
N
(R) (R)
O
0.91 5.36 0.94 0.90 0.93 1.95 1.83 0.90 1.20 2.06 3.05 1.01 1.00 1.26 3.69
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)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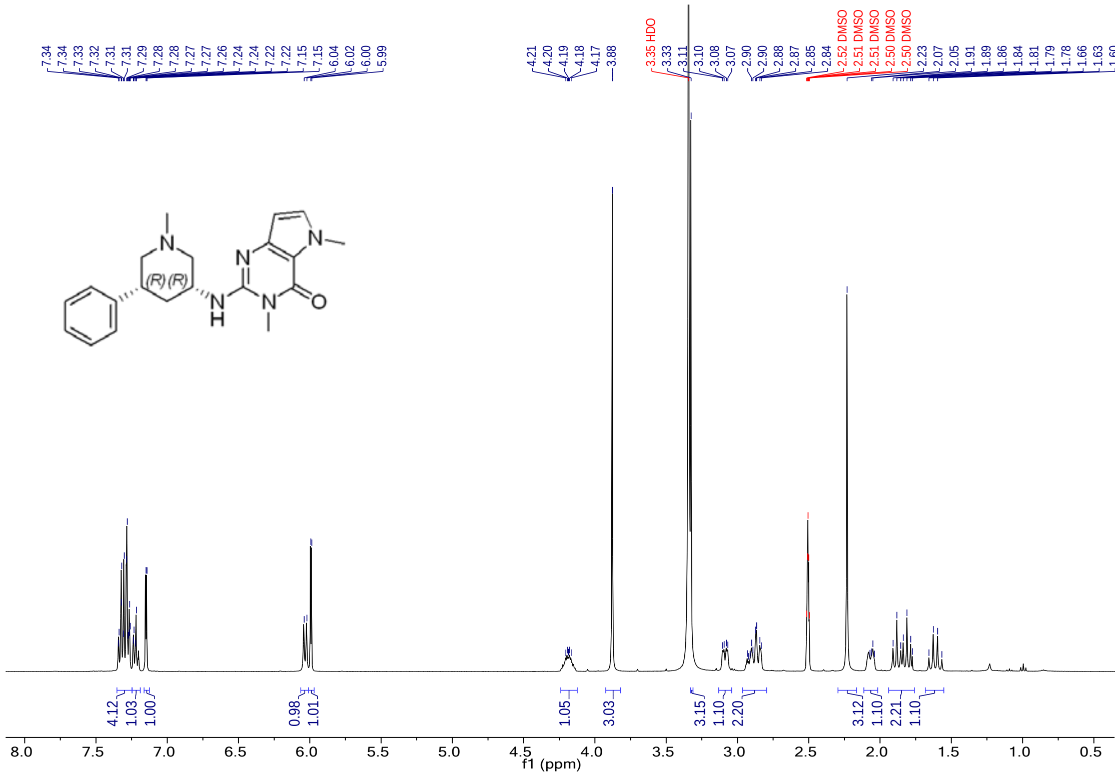
40.63 DMSO 40.42 DMSO 40.21 DMSO 40.00 DMSO 39.79 DMSO 39.58 DMSO 39.38 DMSO
37.25 17.86
NH
N
(R) (R)
O
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



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7.34
7.33
7.32
7.31
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7.28
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7.24
7.22
7.22
7.15
7.15
6.04
6.02
6.00
5.99
4.21
4.20
4.19
4.18
4.17
3.88
3.35 HDO
3.33
3.11
3.10
3.08
3.07
2.90
2.90
2.88
2.87
2.85
2.84
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.23
2.07
2.05
1.91
1.89
1.86
1.84
1.81
1.79
1.78
1.66
1.63
(R)(R)
H
4.12
1.03
1.00
0.98
1.01
1.05
3.03
3.15
1.10
2.20
3.12
1.10
2.21
1.10
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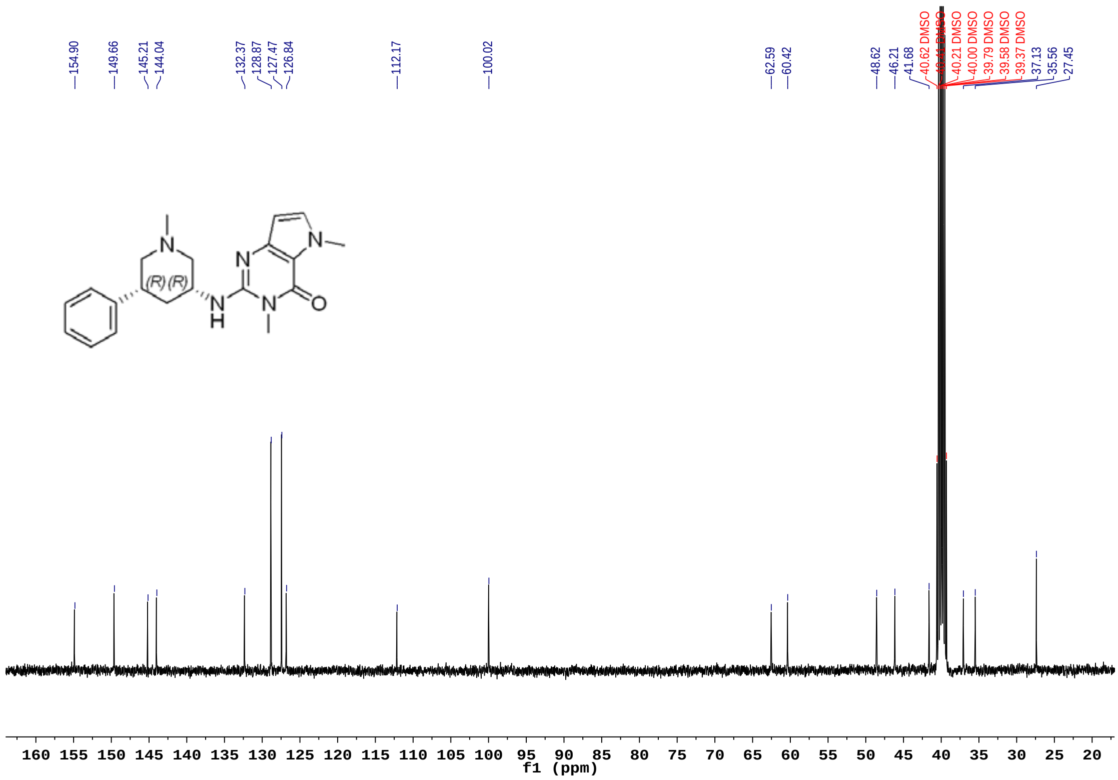
f1 (ppm)



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144.04
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128.87
127.47
126.84
112.17
100.02
62.59
60.42
48.62
46.21
41.68
40.62 DMSO
40.21 DMSO
40.00 DMSO
39.79 DMSO
39.58 DMSO
39.37 DMSO
37.13
35.56
27.45
(R)(R)
H
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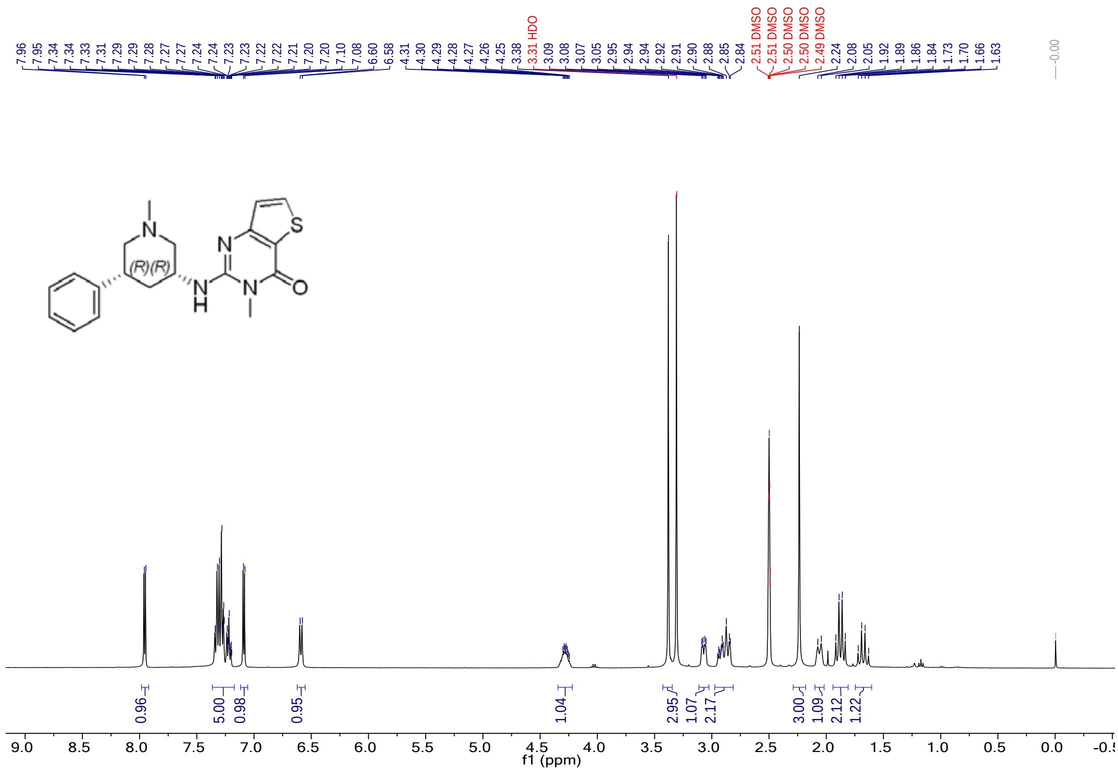
f1 (ppm)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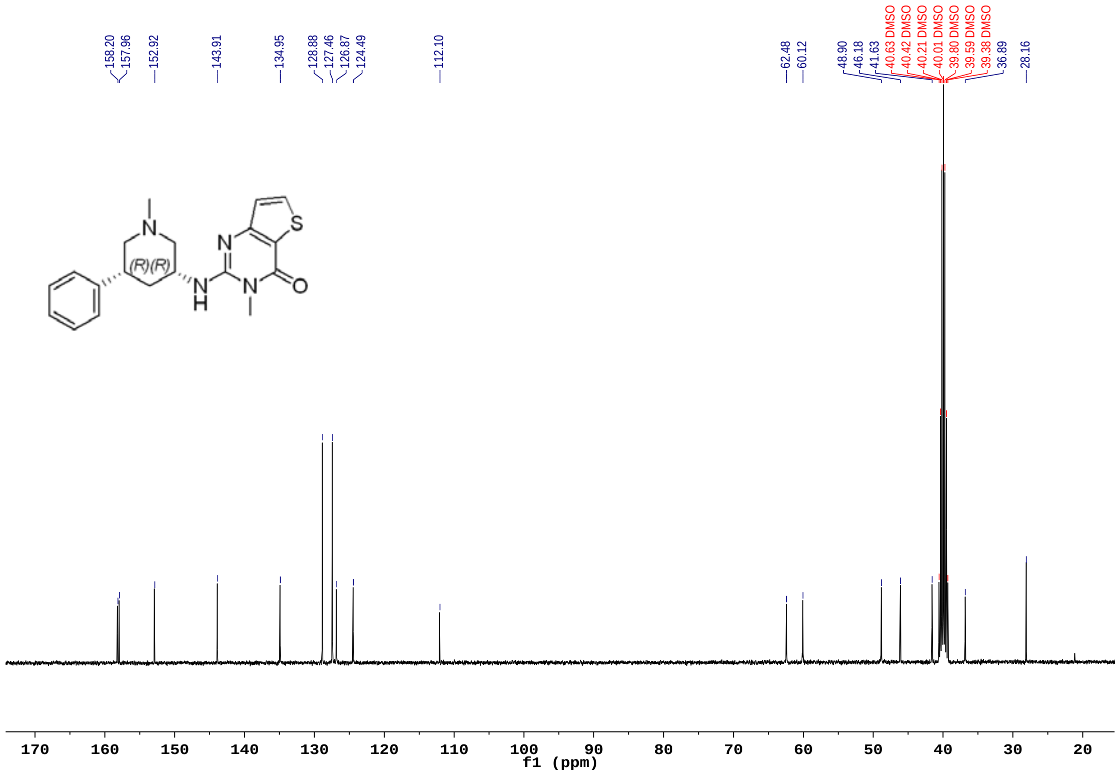
N
S
N
(R)(R)
N
H
N
O
0.96 5.00 0.98 0.95 1.04 2.95 1.07 2.17 3.00 1.09 2.12 1.22
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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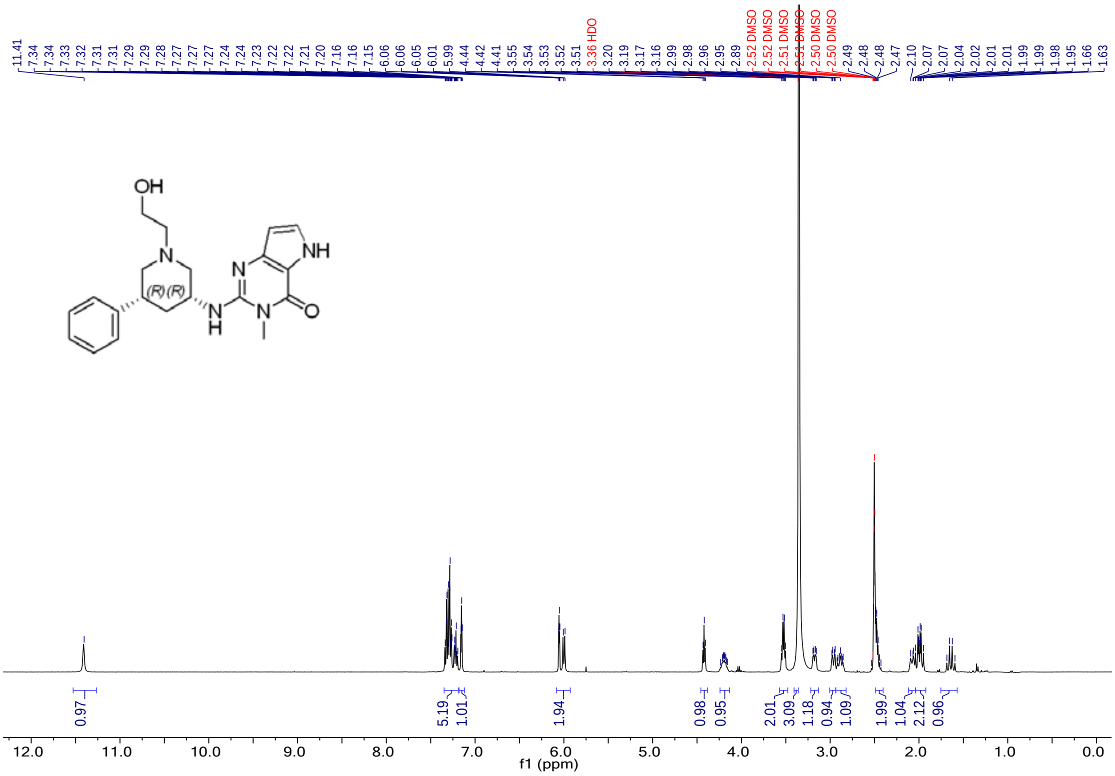
N
S
N
(R)(R)
N
H
N
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)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7.22
7.22
7.21
7.20
7.16
7.16
7.15
6.06
6.06
6.05
6.01
5.99
4.44
4.42
4.41
3.55
3.54
3.53
3.52
3.51
3.36 HDO
3.20
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2.98
2.96
2.95
2.89
2.52 DMSO
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2.07
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2.01
1.99
1.99
1.98
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1.66
1.63
OH
N
(R)
(R)
N
NH
N
H
N
O
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5.19
1.01
1.94
0.98
0.95
2.01
3.09
1.18
0.94
1.09
1.99
1.04
2.12
0.96
12.0
11.0
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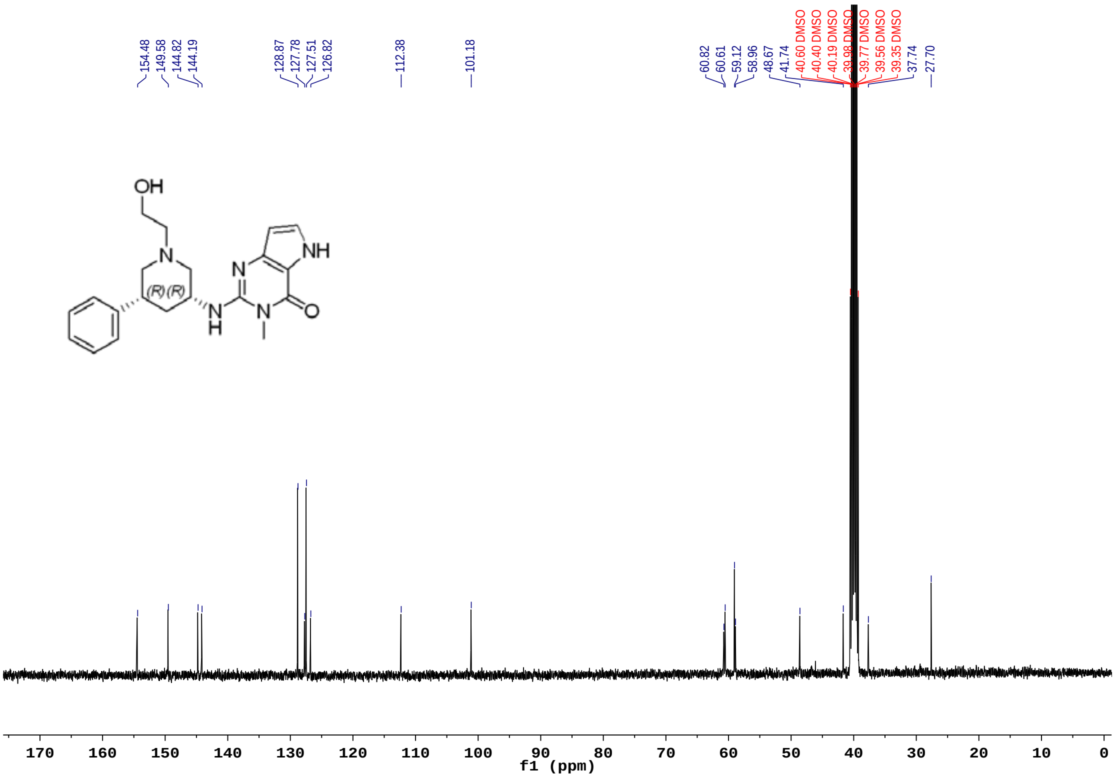
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)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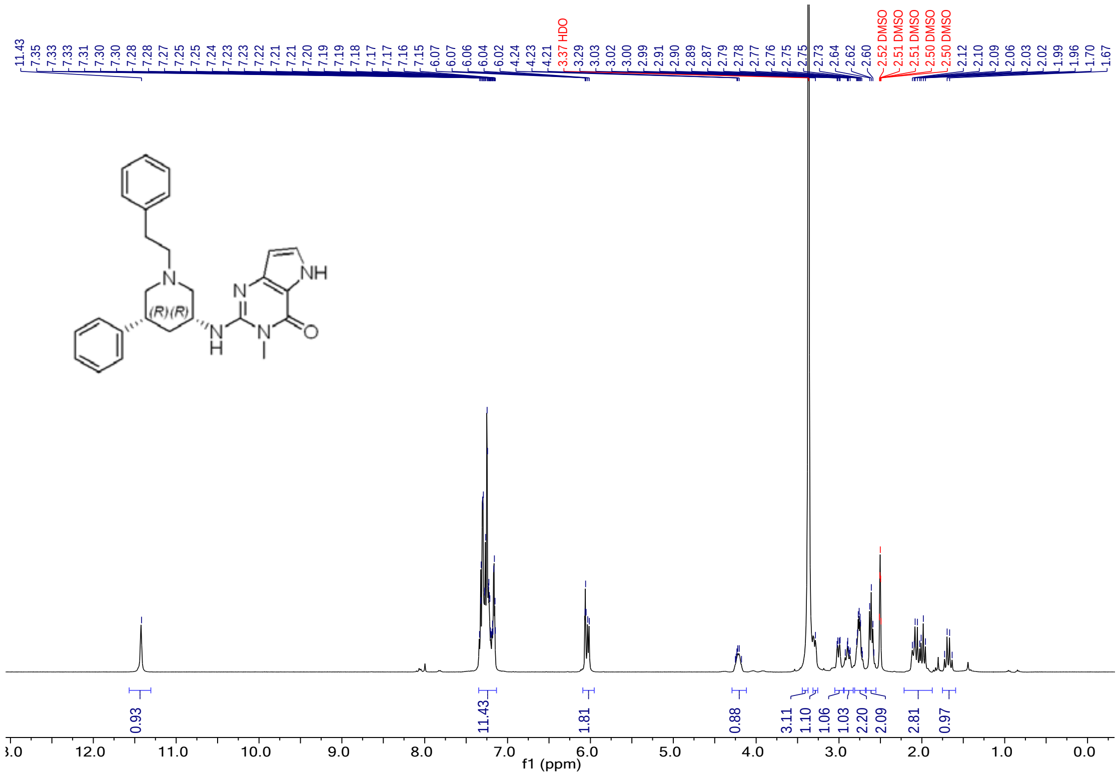
127.51
126.82
112.38
101.18
60.82
60.61
59.12
58.96
48.67
41.74
40.60 DMSO
40.40 DMSO
40.19 DMSO
39.98 DMSO
39.77 DMSO
39.56 DMSO
39.35 DMSO
37.74
27.70
OH
N
(R)
(R)
N
NH
N
H
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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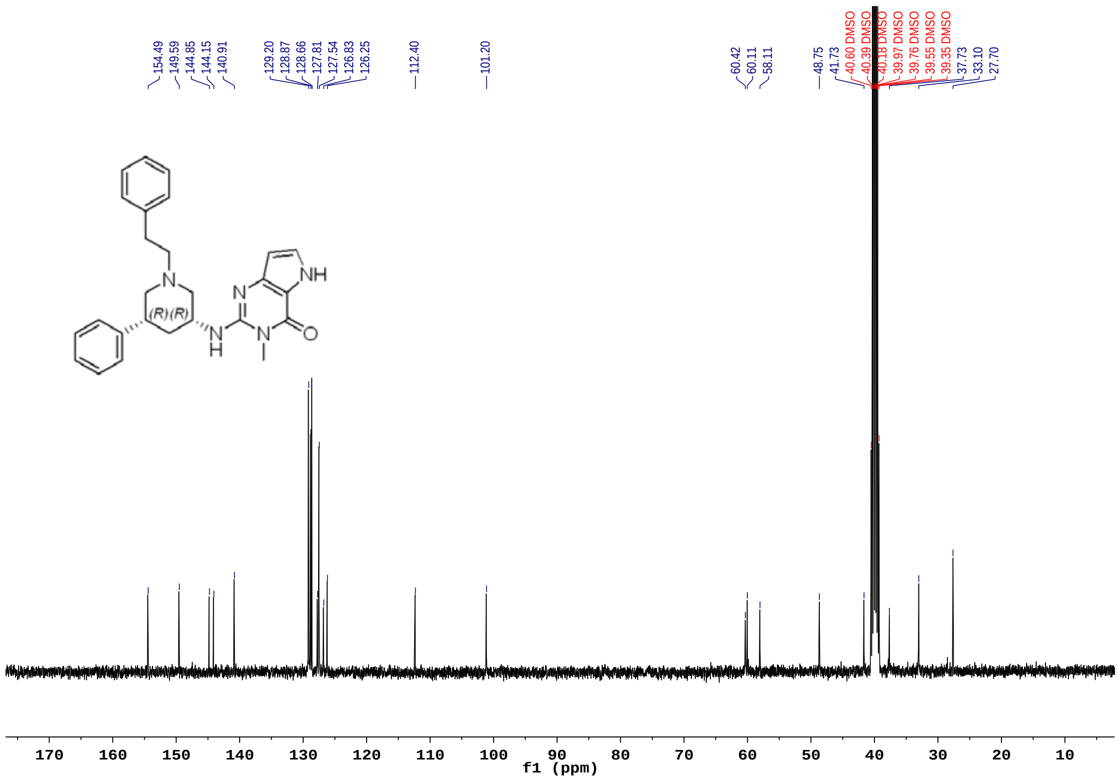
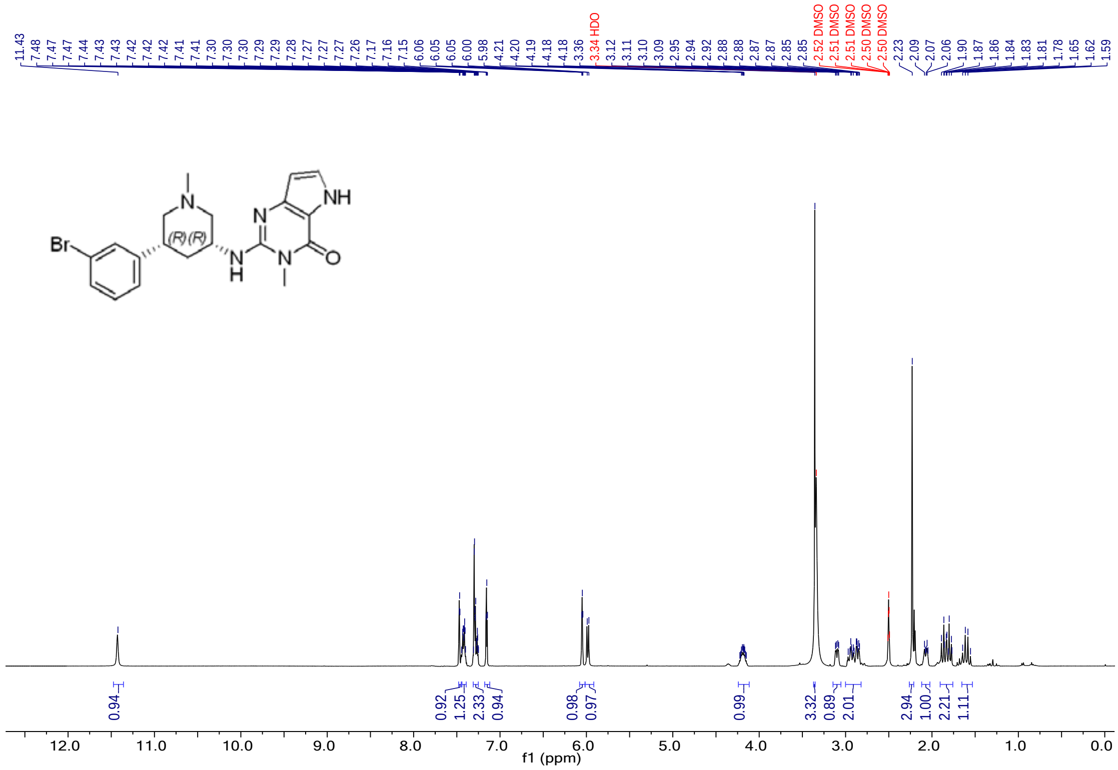
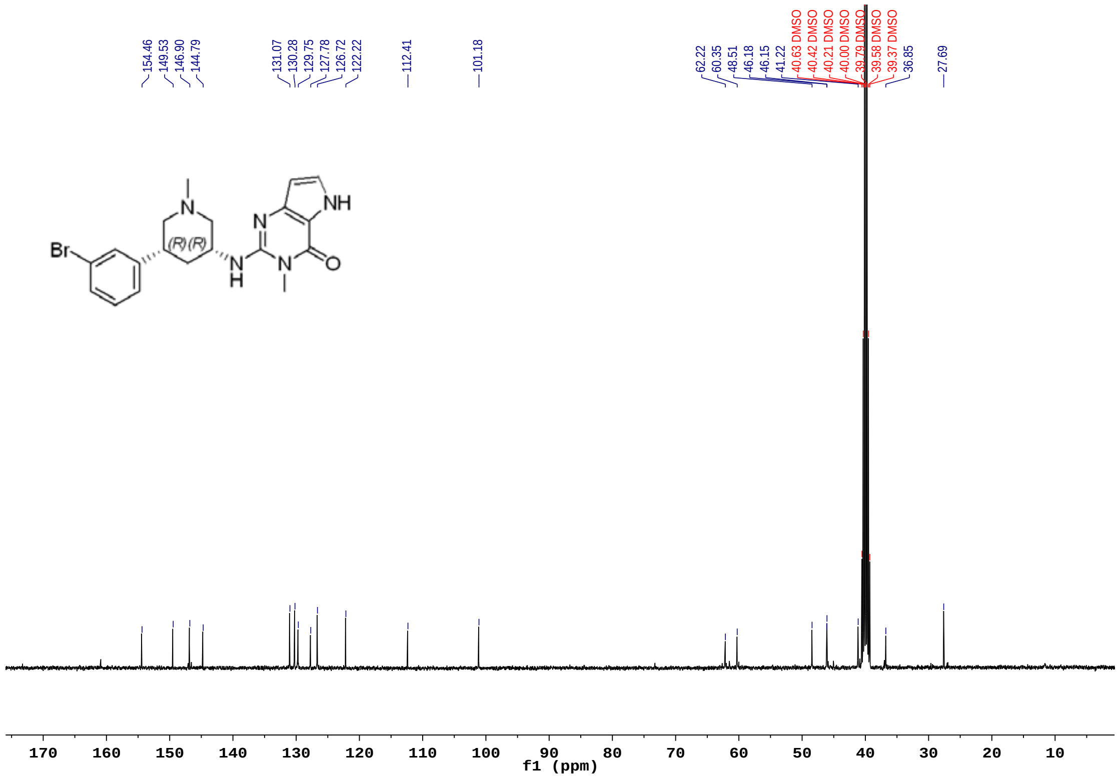
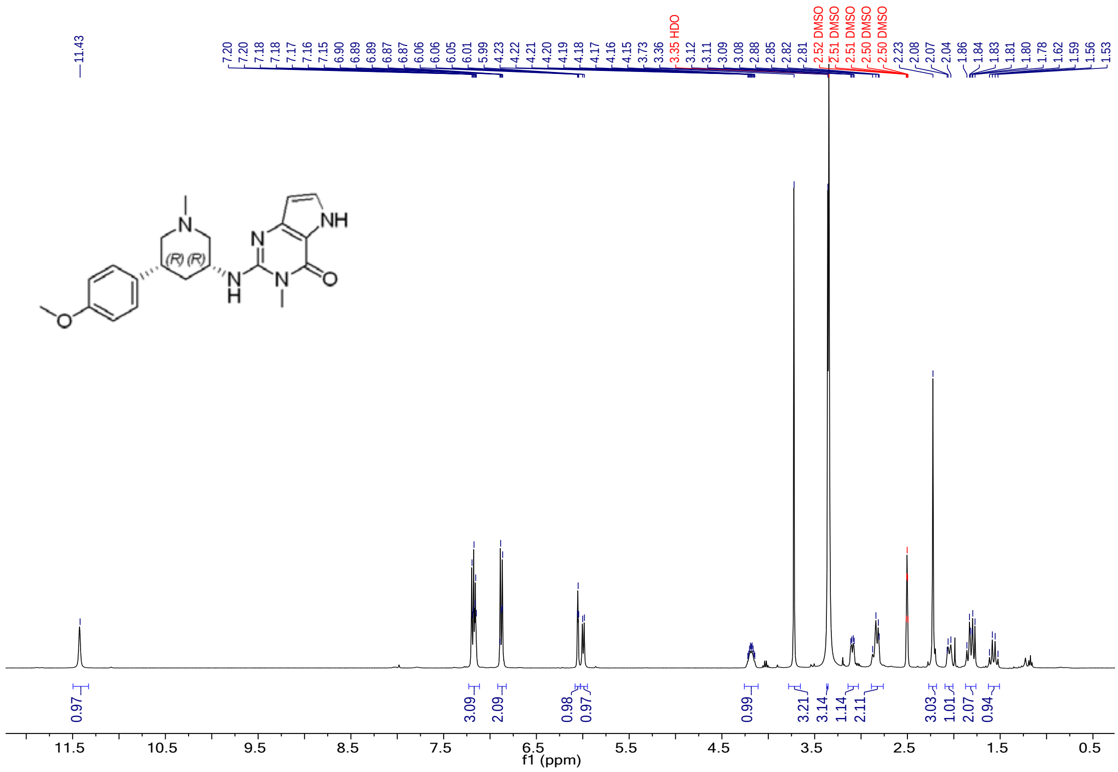
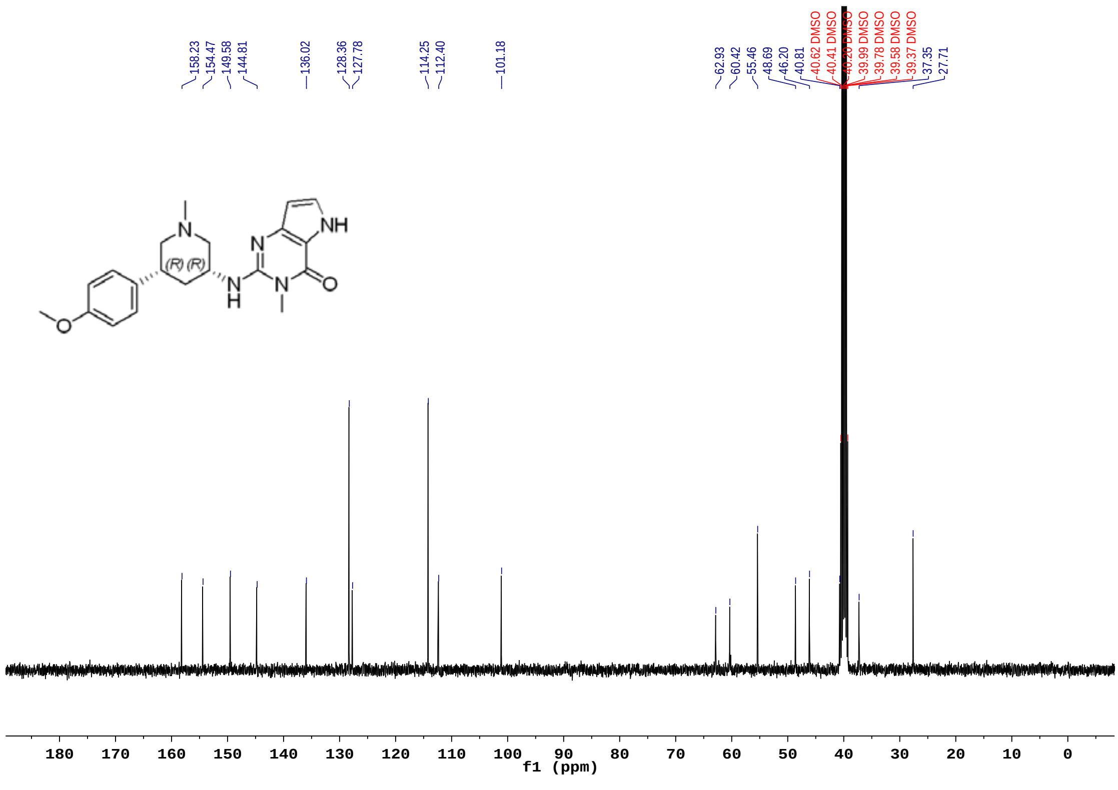
3.37 HDO
3.29 3.03 3.02 3.00 2.99 2.91 2.90 2.89 2.87 2.79 2.78 2.77 2.76 2.75 2.75 2.73 2.64 2.62 2.60
2.52 DMSO 2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO
2.12 2.10 2.09 2.06 2.03 2.02 1.99 1.96 1.70 1.67
N
NH
(R)
(R)
N
N
H
N
O
0.93 11.43 1.81 0.88 3.11 1.10 1.06 1.03 2.20 2.09 2.81 0.97
f1 (ppm)

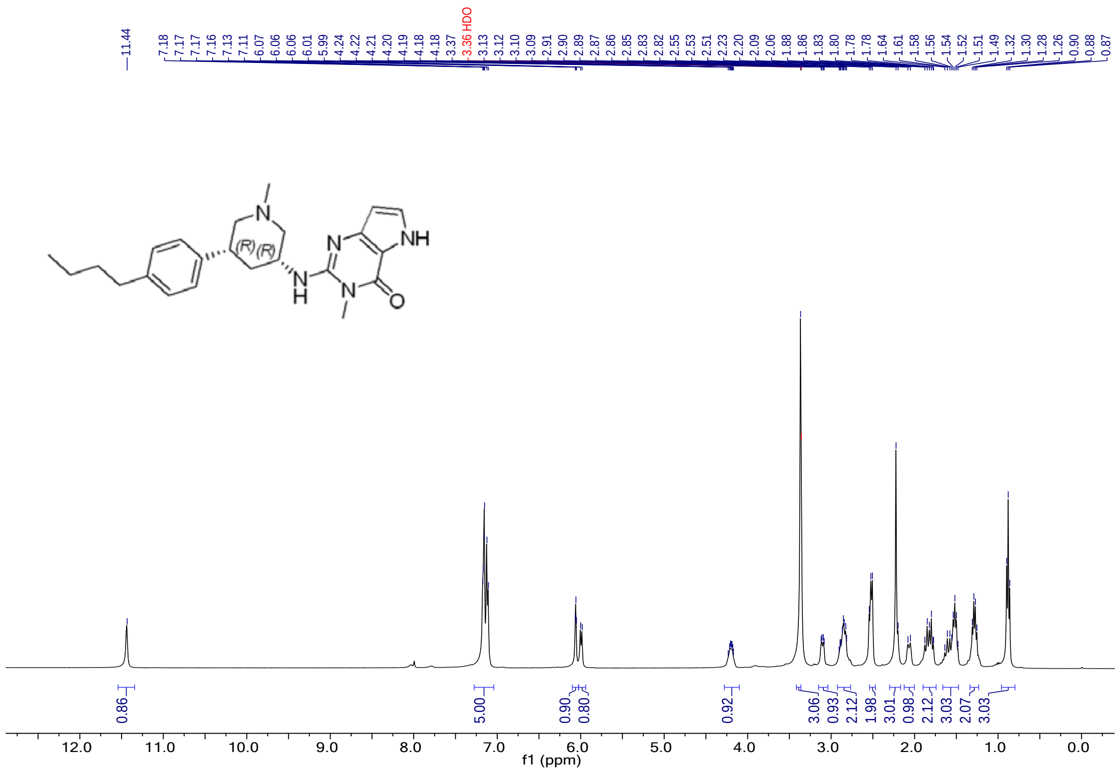

154.49 149.59 144.85 144.15 140.91 129.20 128.87 128.66 127.81 127.54 126.83 126.25 112.40 101.20 60.42 60.11 58.11 48.75 41.73
40.60 DMSO 40.39 DMSO 40.18 DMSO 39.97 DMSO 39.76 DMSO 39.55 DMSO 39.35 DMSO
37.73 33.10 27.70
N
NH
(R)
(R)
N
N
H
N
O
f1 (ppm)


11.43 7.48 7.47 7.47 7.44 7.43 7.43 7.42 7.42 7.42 7.41 7.41 7.30 7.30 7.30 7.29 7.29 7.28 7.27 7.27 7.27 7.26 7.17 7.16 7.15 6.06 6.05 6.00 5.98 4.21 4.20 4.19 4.18 4.18 3.36
3.34 HDO
3.12 3.11 3.10 3.09 2.95 2.94 2.92 2.88 2.88 2.87 2.87 2.85 2.85
2.52 DMSO 2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO
2.23 2.09 2.07 2.06 1.90 1.87 1.86 1.84 1.83 1.81 1.78 1.65 1.62 1.59
Br
N
(R) (R)
N
NH
N
H
O
0.94 0.92 1.25 2.33 0.94 0.98 0.97 0.99 3.32 0.89 2.01 2.94 1.00 2.21 1.11
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)

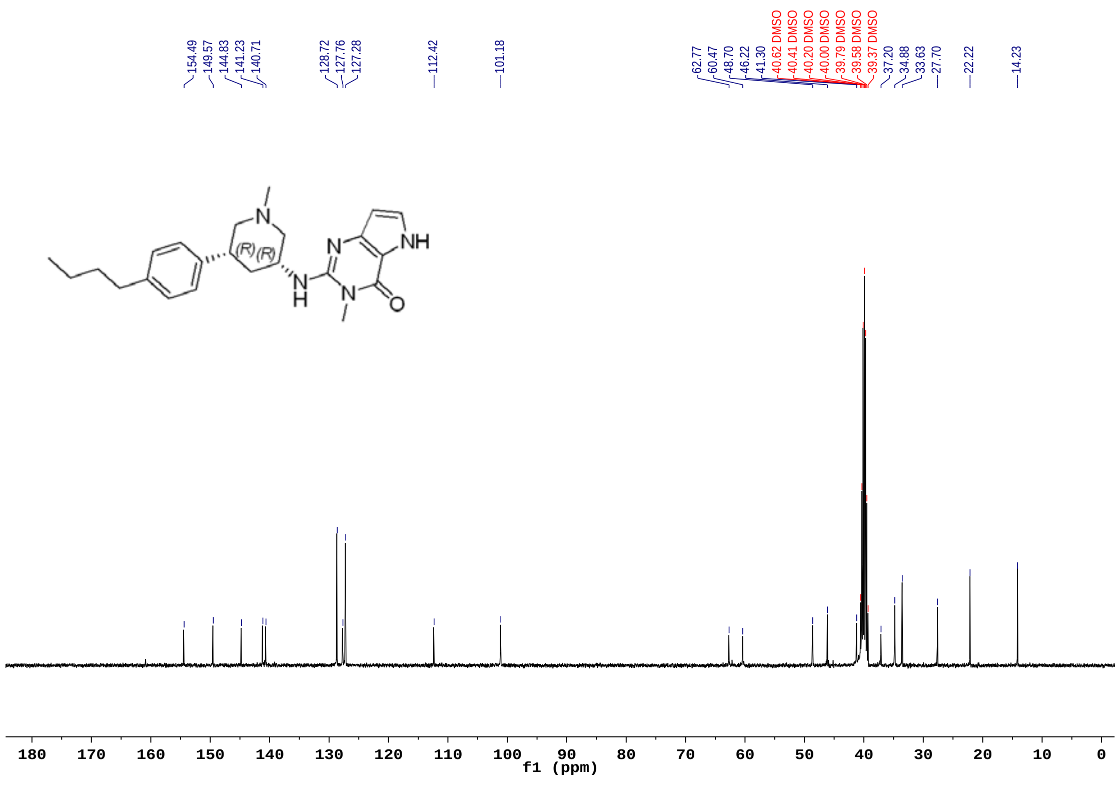

154.46 149.53 146.90 144.79 131.07 130.28 129.75 127.78 126.72 122.22 112.41 101.18 62.22 60.35 48.51 46.18 46.15 41.22
40.63 DMSO 40.42 DMSO 40.21 DMSO 40.00 DMSO 39.79 DMSO 39.58 DMSO 39.37 DMSO
36.85 27.69
Br
N
(R) (R)
N
NH
N
H
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)



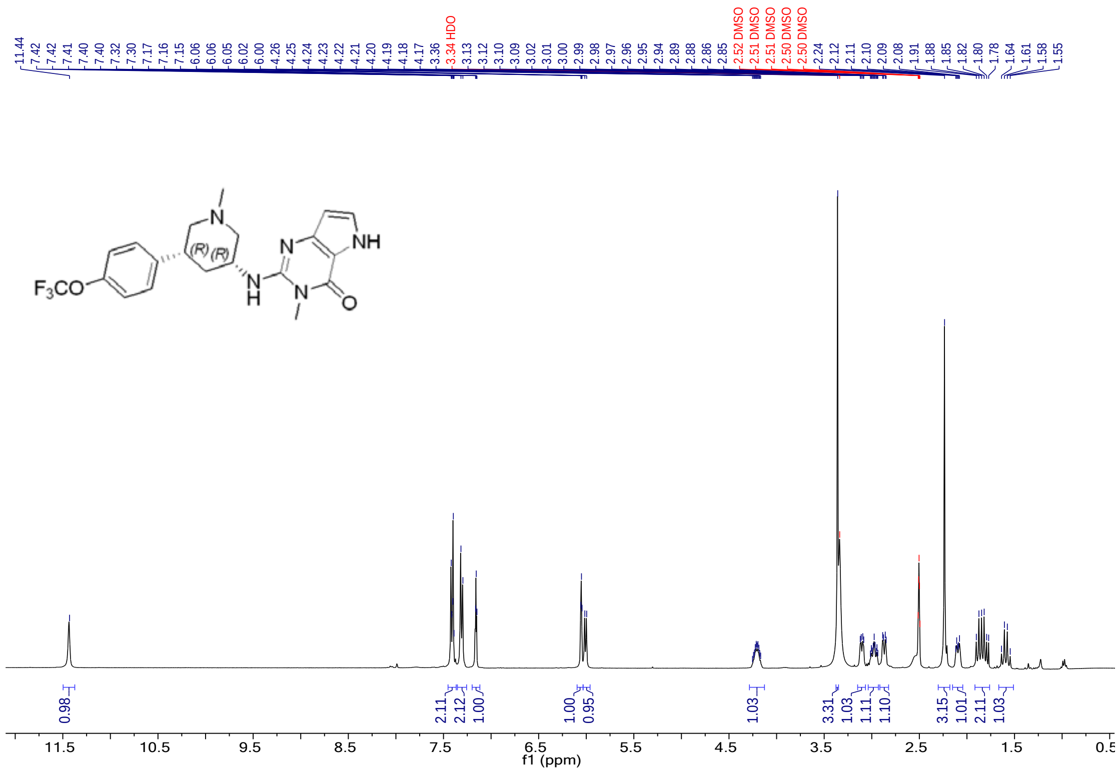


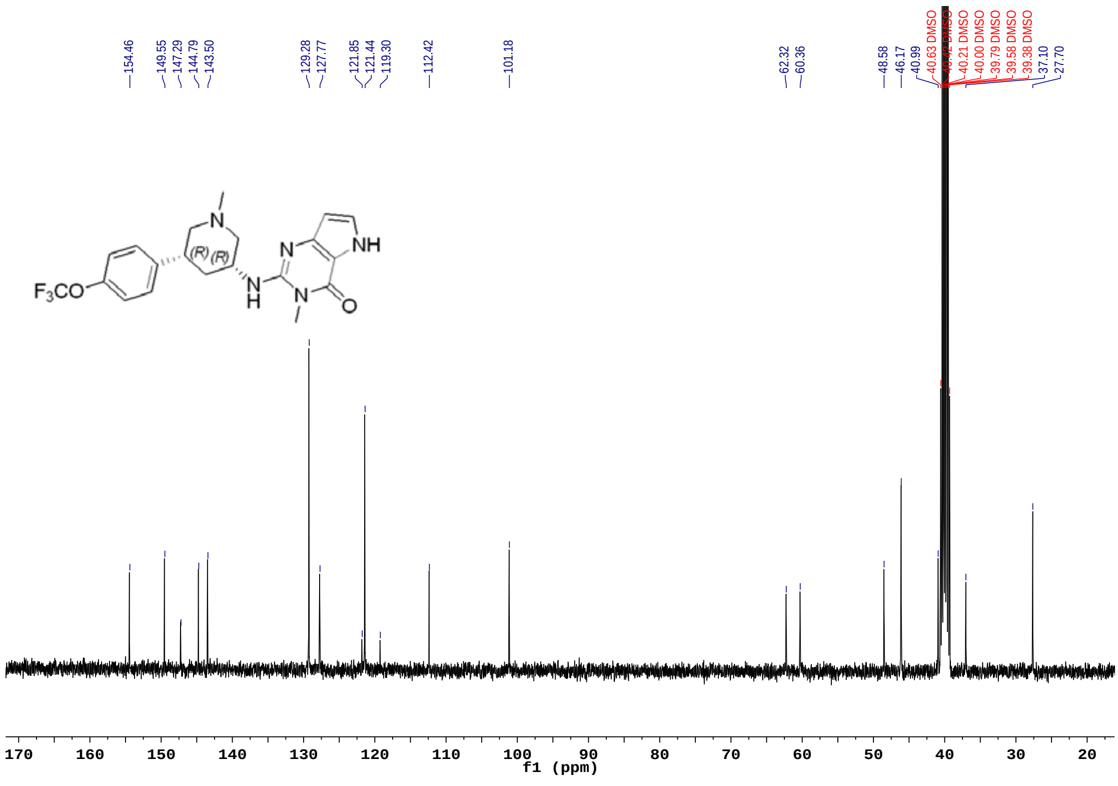


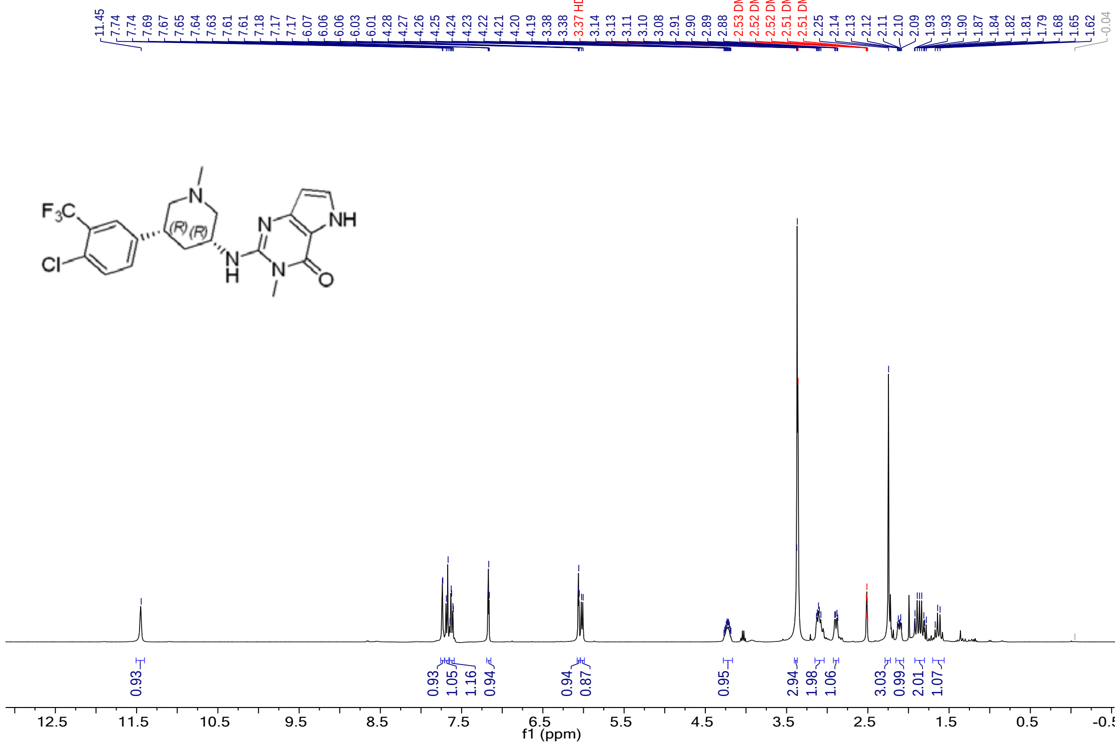
11.44
7.18 7.17 7.17 7.16 7.13 7.11 6.07 6.06 6.06 6.01 5.99 4.24 4.22 4.21 4.20 4.19 4.18 4.18 3.37 3.36 HDO 3.13 3.12 3.10 3.09 2.91 2.90 2.89 2.87 2.86 2.85 2.83 2.82 2.55 2.53 2.51 2.23 2.20 2.09 2.06 1.88 1.86 1.83 1.80 1.78 1.78 1.64 1.61 1.58 1.56 1.54 1.52 1.51 1.49 1.32 1.30 1.28 1.26 0.90 0.88 0.87
N
NH
(R)
(R)
N
H
N
O
0.86 5.00 0.90 0.80 0.92 3.06 0.93 2.12 1.98 3.01 0.98 2.12 3.03 2.07 3.03
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)

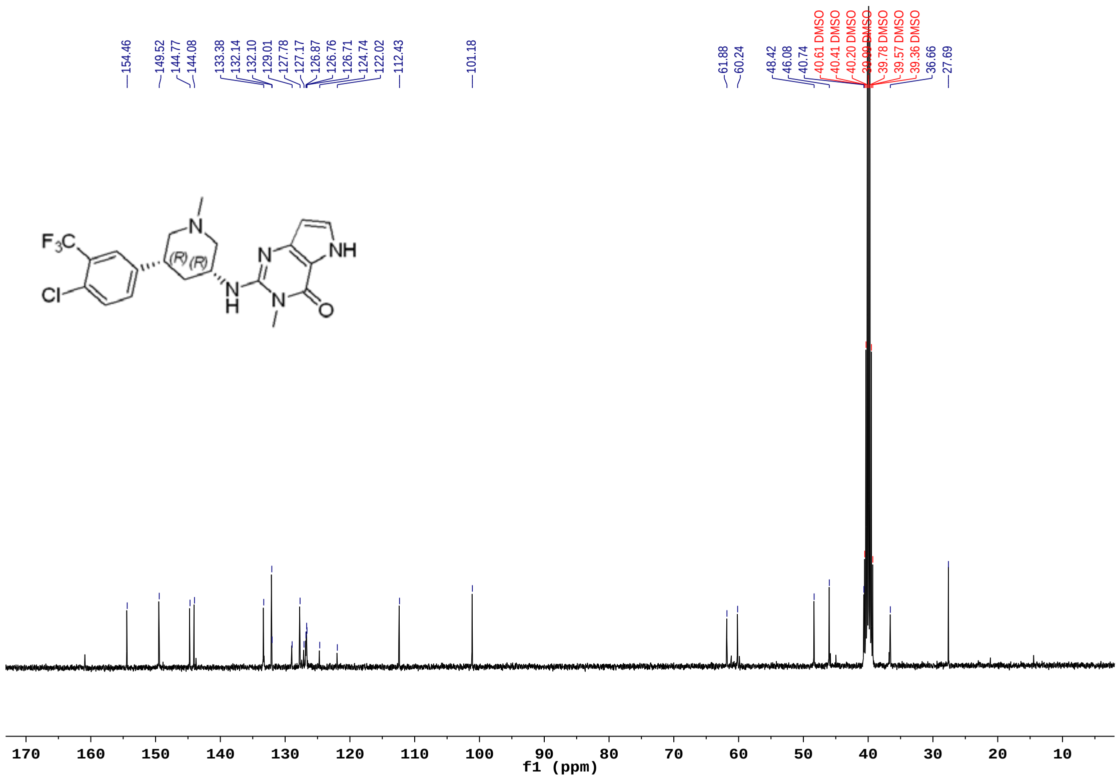


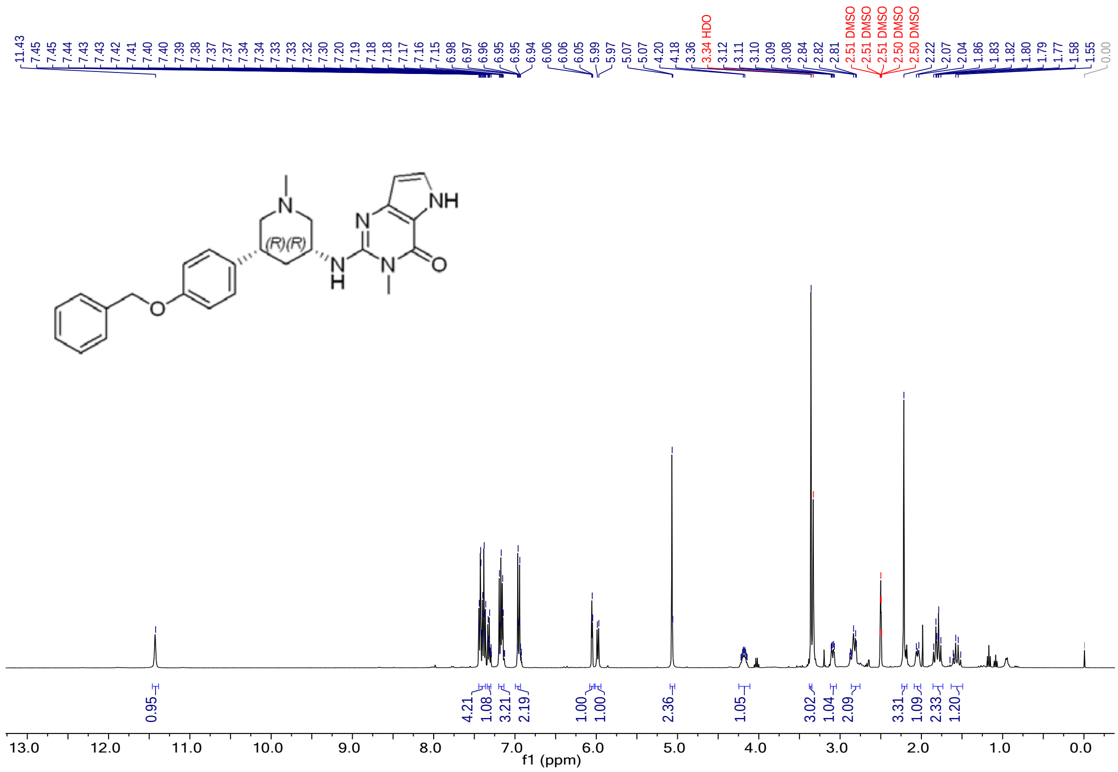
154.49 149.57 144.83 141.23 140.71 128.72 127.76 127.28 112.42 101.18
62.77 60.47 48.70 46.22 41.30 40.62 DMSO 40.41 DMSO 40.20 DMSO 40.00 DMSO 39.79 DMSO 39.58 DMSO 39.37 DMSO 37.20 34.88 33.63 27.70 22.22 14.23
N
NH
(R)
(R)
N
H
N
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

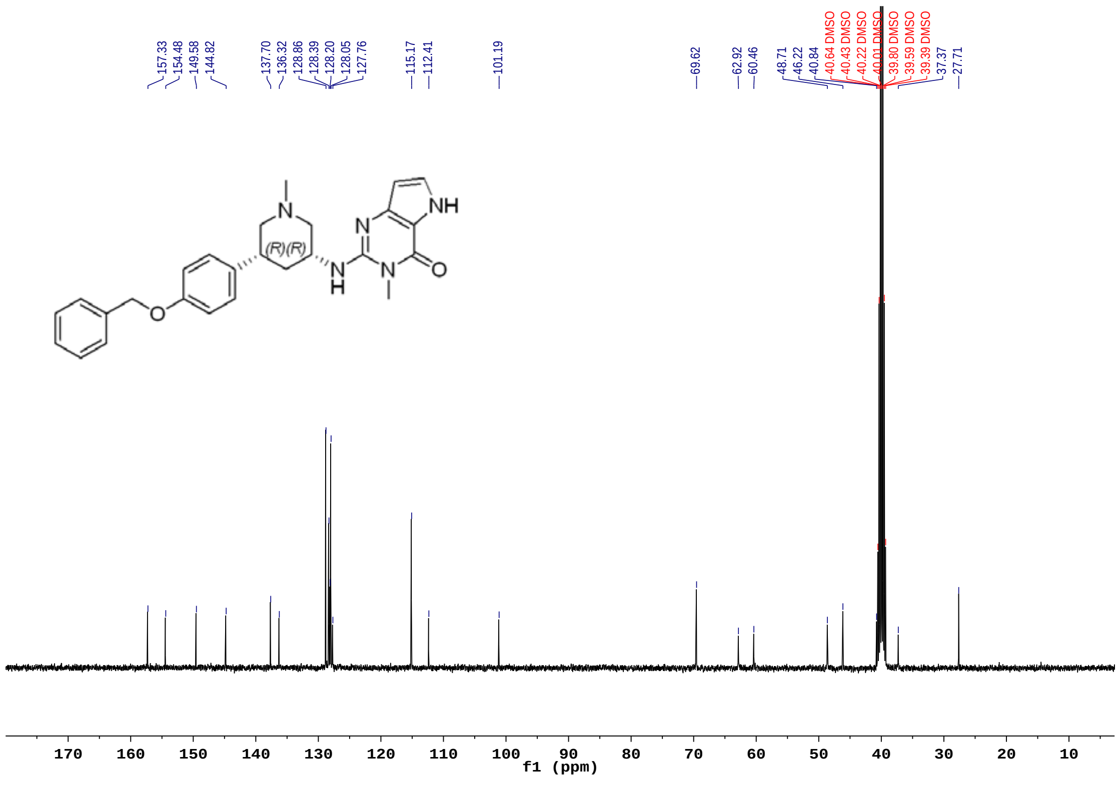


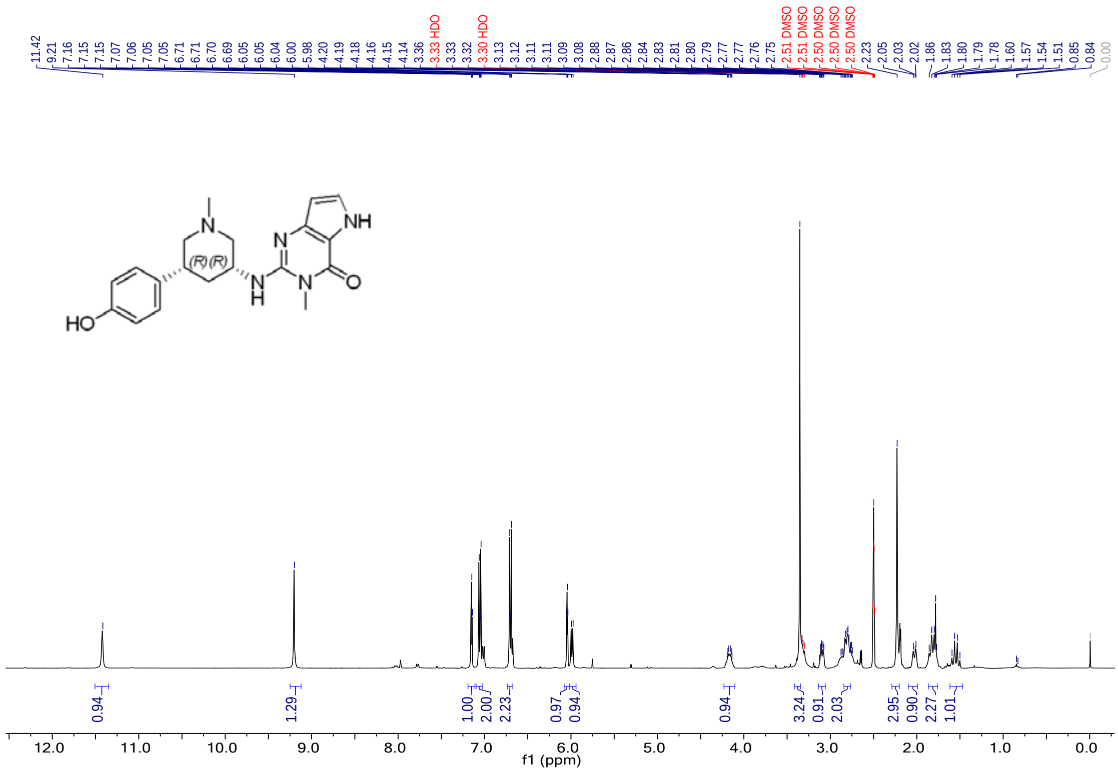



11.45 7.74 7.74 7.69 7.67 7.65 7.64 7.63 7.61 7.61 7.18 7.17 7.17 6.07 6.06 6.06 6.03 6.01 4.28 4.27 4.26 4.25 4.24 4.23 4.22 4.21 4.20 4.19 3.38 3.38 3.37 HDO 3.14 3.13 3.11 3.10 3.08 2.91 2.90 2.89 2.88 2.53 DMSO 2.52 DMSO 2.52 DMSO 2.51 DMSO 2.51 DMSO 2.25 2.14 2.13 2.12 2.11 2.10 2.09 1.93 1.93 1.90 1.87 1.84 1.82 1.81 1.79 1.68 1.65 1.62 -0.04
F3C
Cl
N
(R) (R)
N
H
N
N
NH
O
0.93 0.93 1.05 1.16 0.94 0.94 0.87 0.95 2.94 1.98 1.06 3.03 0.99 2.01 1.07
12.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 -0.5
f1 (ppm)

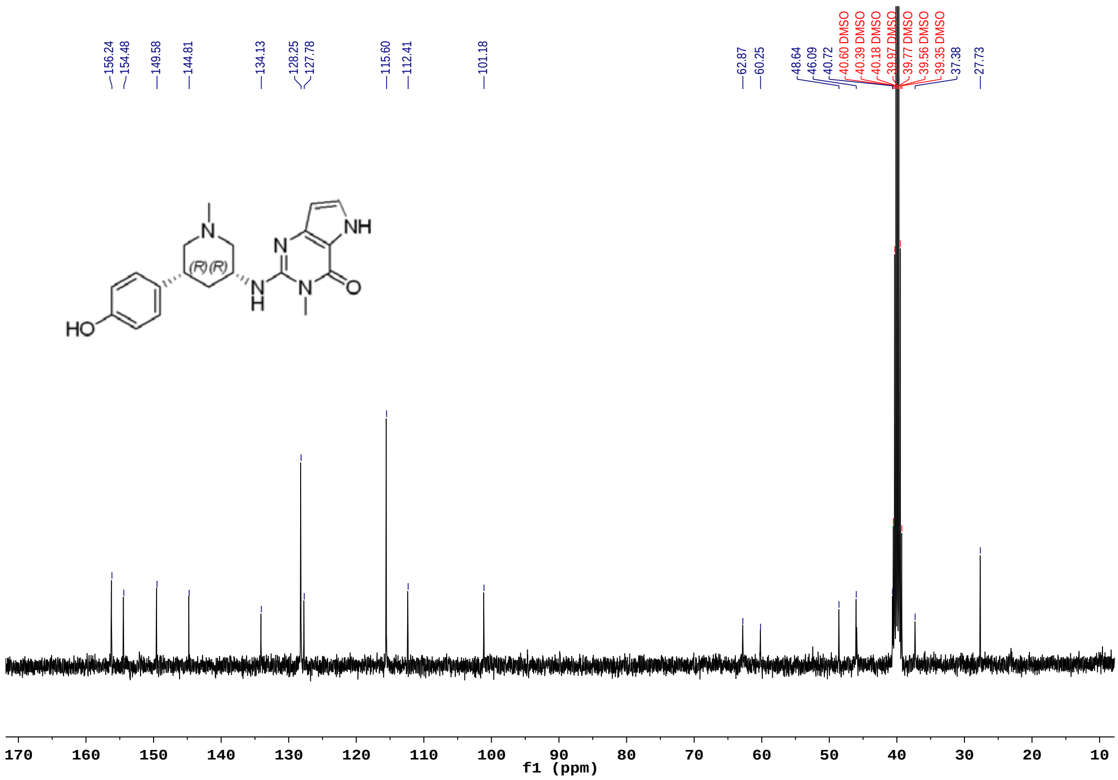

154.46 149.52 144.77 144.08 133.38 132.14 132.10 129.01 127.78 127.17 126.87 126.76 126.71 124.74 122.02 112.43 101.18 61.88 60.24 48.42 46.08 40.74 40.61 DMSO 40.41 DMSO 40.20 DMSO 40.00 DMSO 39.78 DMSO 39.57 DMSO 39.36 DMSO 36.66 27.69
F3C
Cl
N
(R) (R)
N
H
N
N
NH
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


11.43
7.45 7.45 7.44 7.43 7.43 7.42 7.41 7.40 7.40 7.39 7.38 7.37 7.37 7.34 7.34 7.33 7.33 7.32 7.30 7.20 7.19 7.18 7.18 7.17 7.16 7.15 6.98 6.97 6.96 6.95 6.95 6.94
6.06 6.06 6.05 5.99 5.97
5.07 5.07
4.20 4.18
3.36
3.34 HDO
3.12 3.11 3.10 3.09 3.08 2.84 2.82 2.81
2.51 DMSO 2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO
2.22 2.07 2.04 1.86 1.83 1.82 1.80 1.79 1.77 1.58 1.55
0.00
NH
N
(R)(R)
O
0.95
4.21 1.08 3.21 2.19
1.00 1.00
2.36
1.05
3.02 1.04 2.09
3.31 1.09 2.33 1.20
13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)

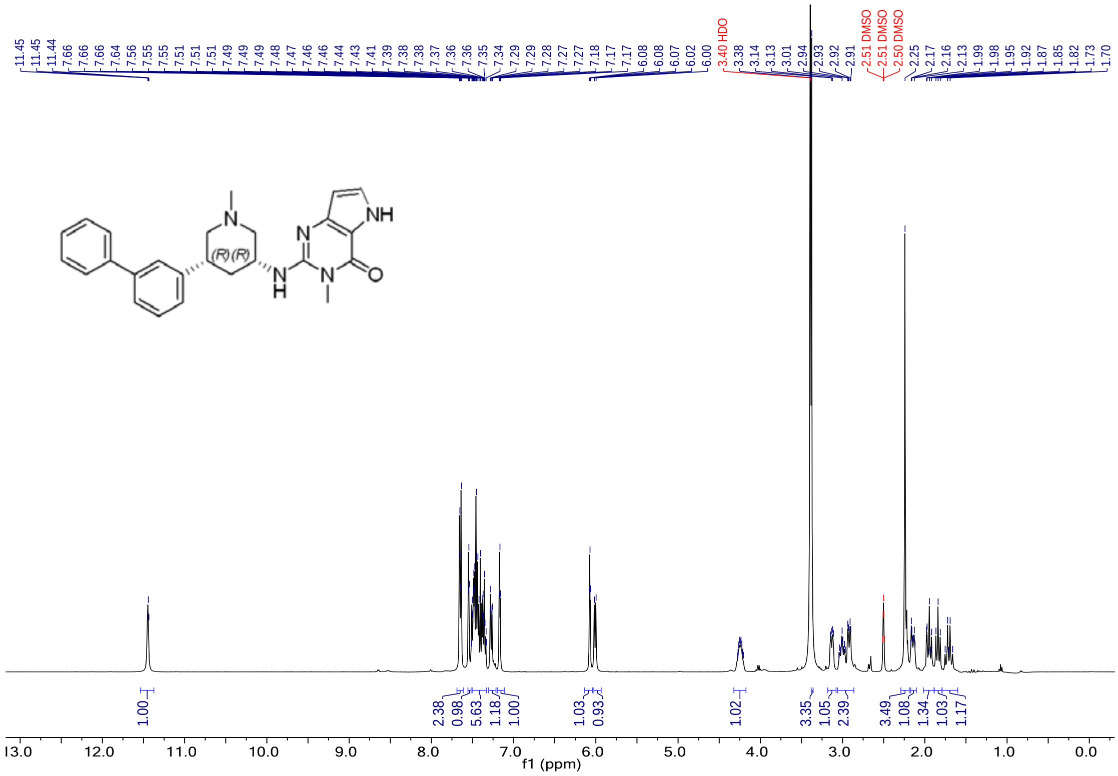

157.33 154.48 149.58 144.82
137.70 136.32 128.86 128.39 128.20 128.05 127.76
115.17 112.41
101.19
69.62
62.92 60.46
48.71 46.22 40.84
40.64 DMSO 40.43 DMSO 40.22 DMSO 40.01 DMSO 39.80 DMSO 39.59 DMSO 39.39 DMSO
37.37 27.71
NH
N
(R)(R)
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

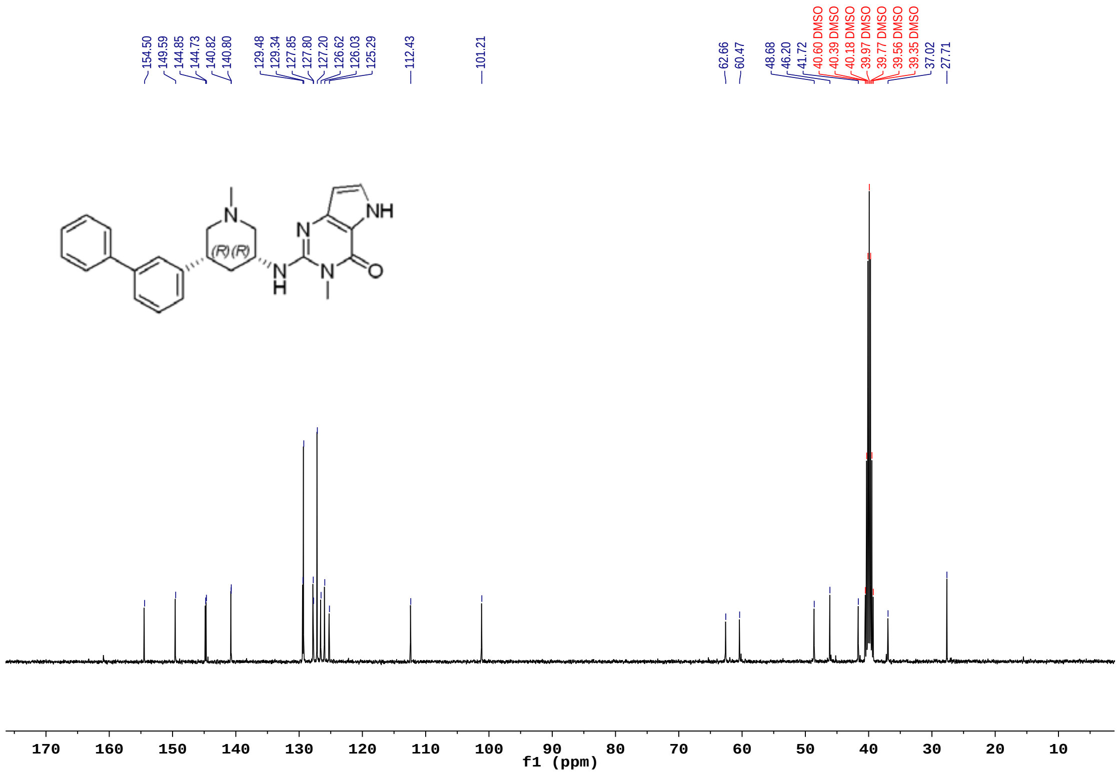


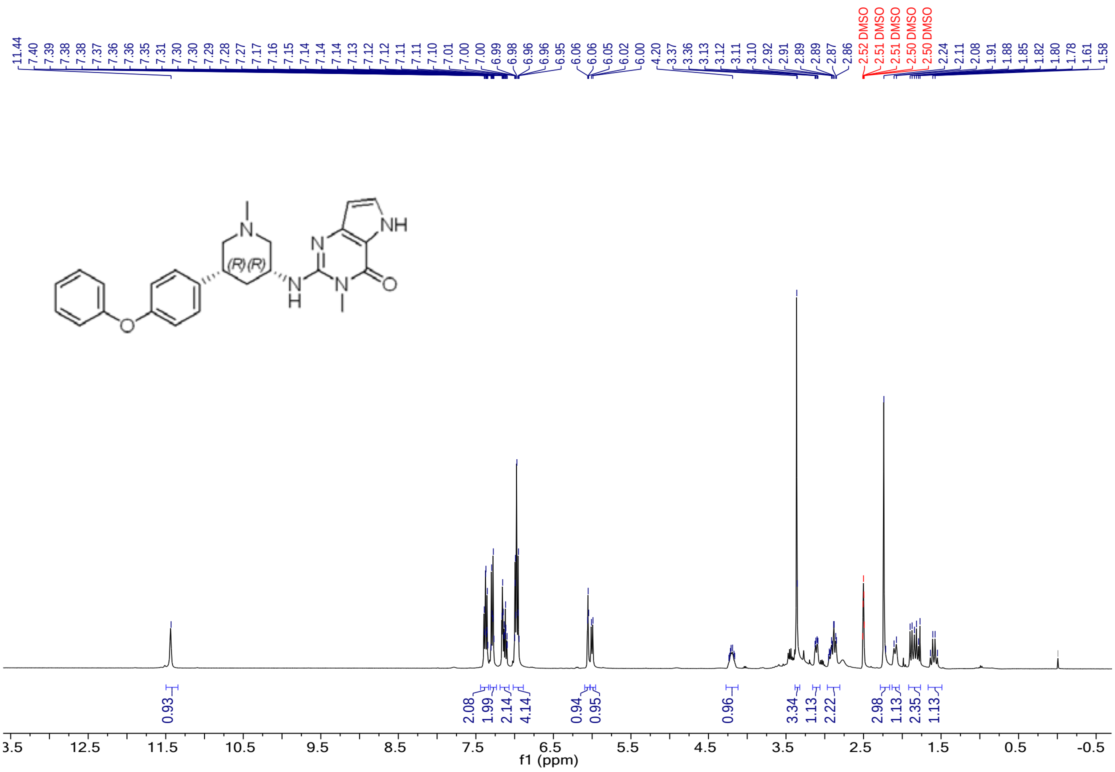
NH
N
N
N
H
N
O
HO
(R)
(R)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

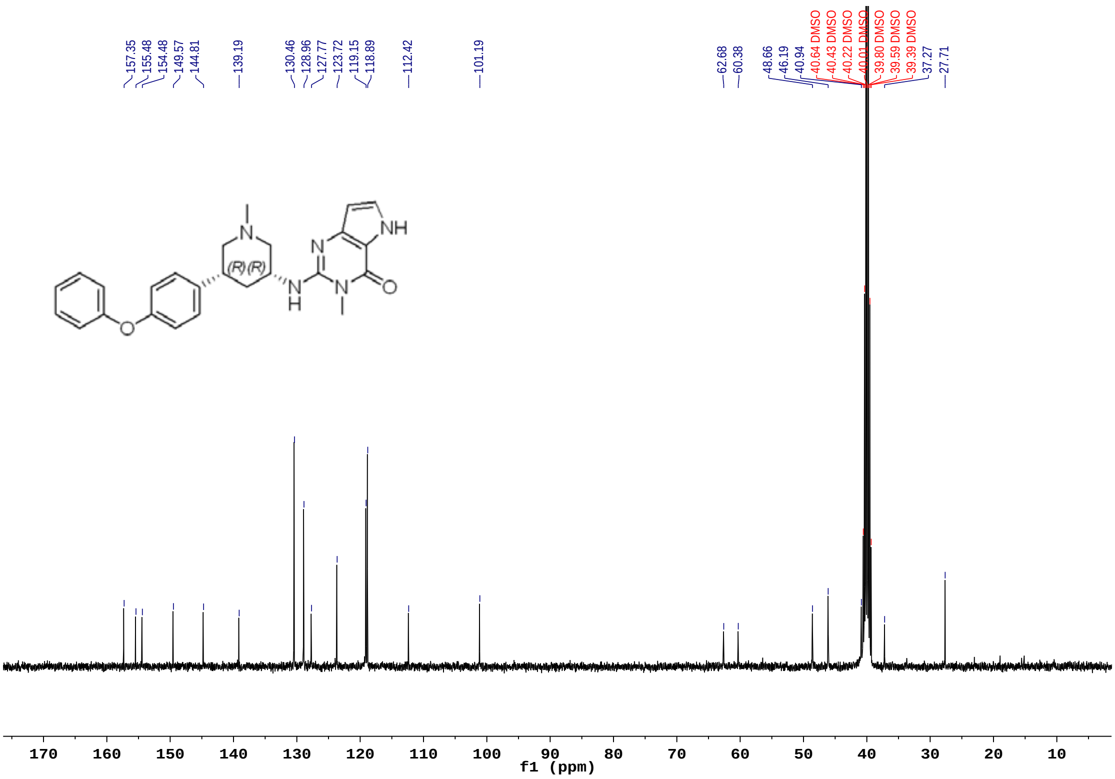


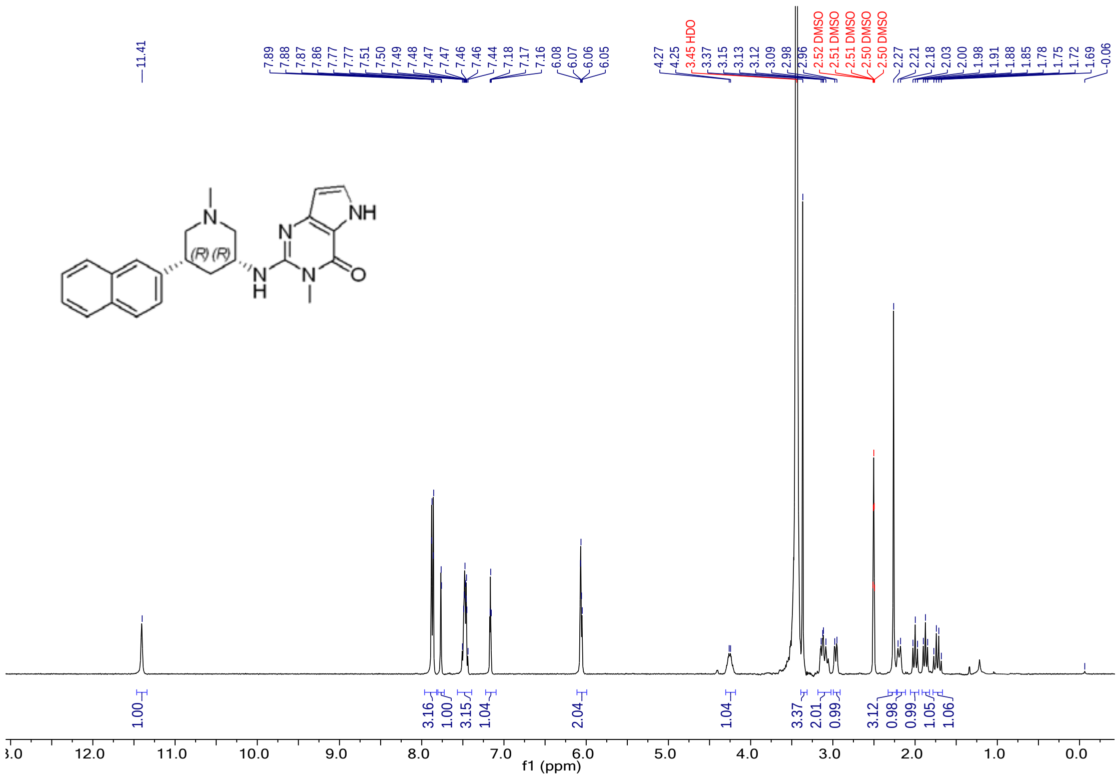
NH
N
N
N
H
N
O
HO
(R)
(R)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

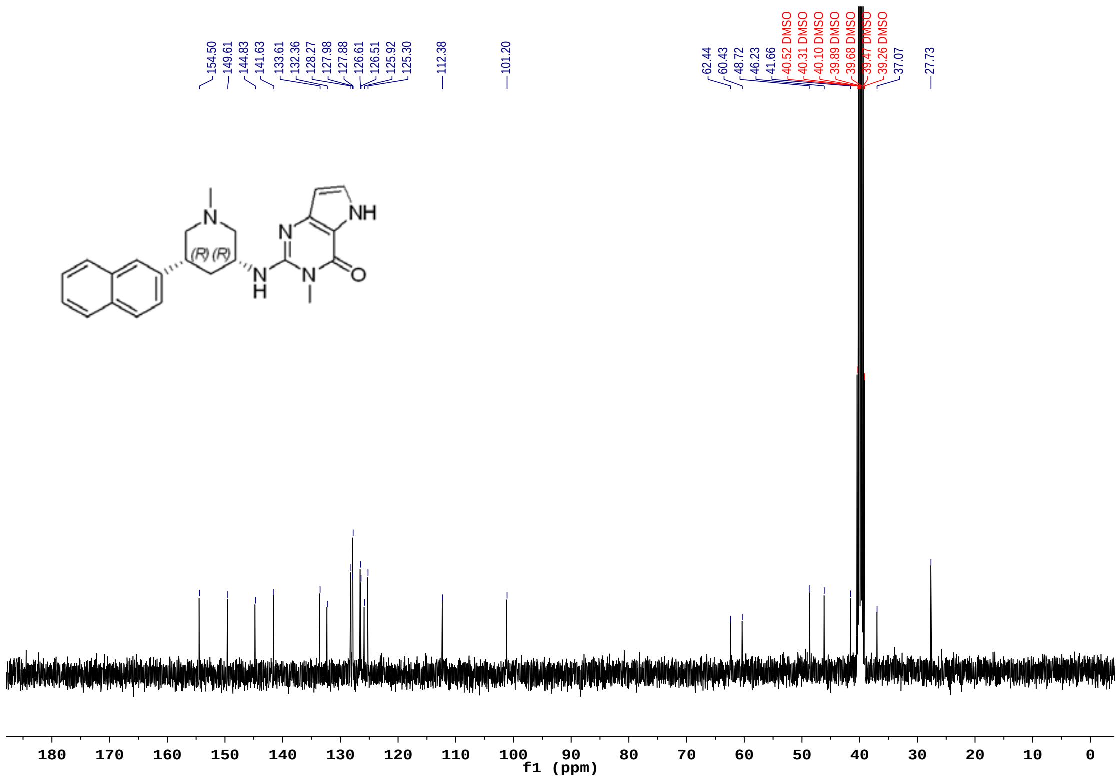


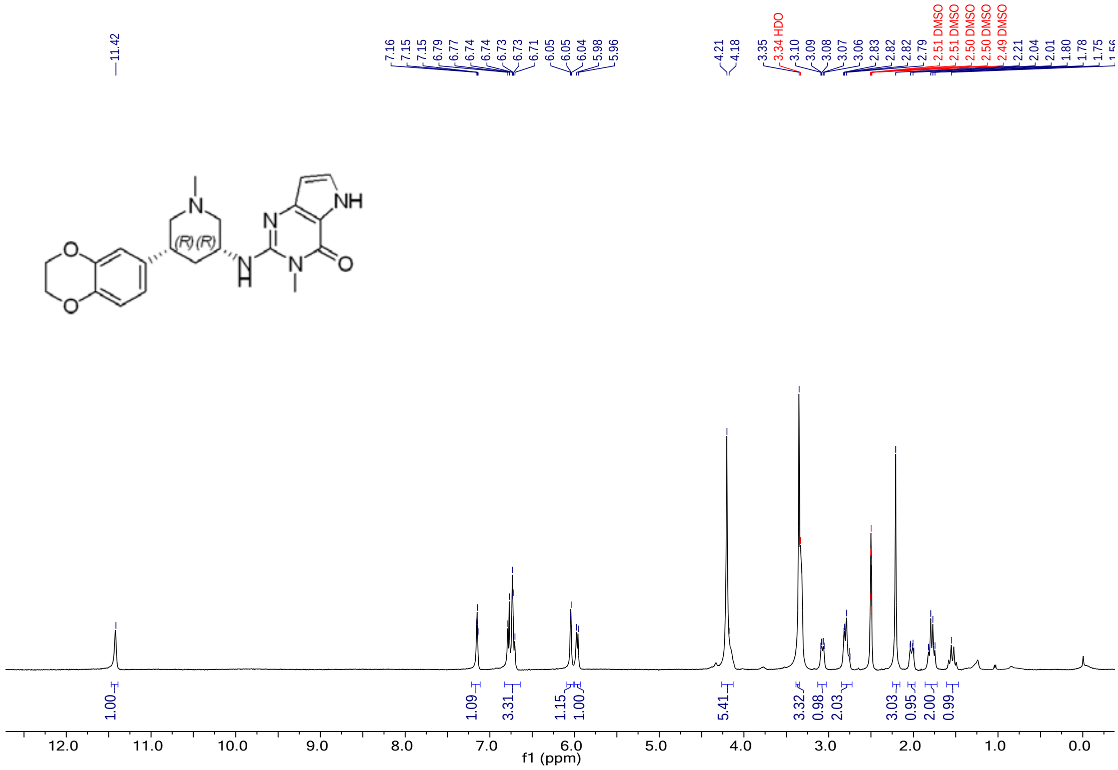


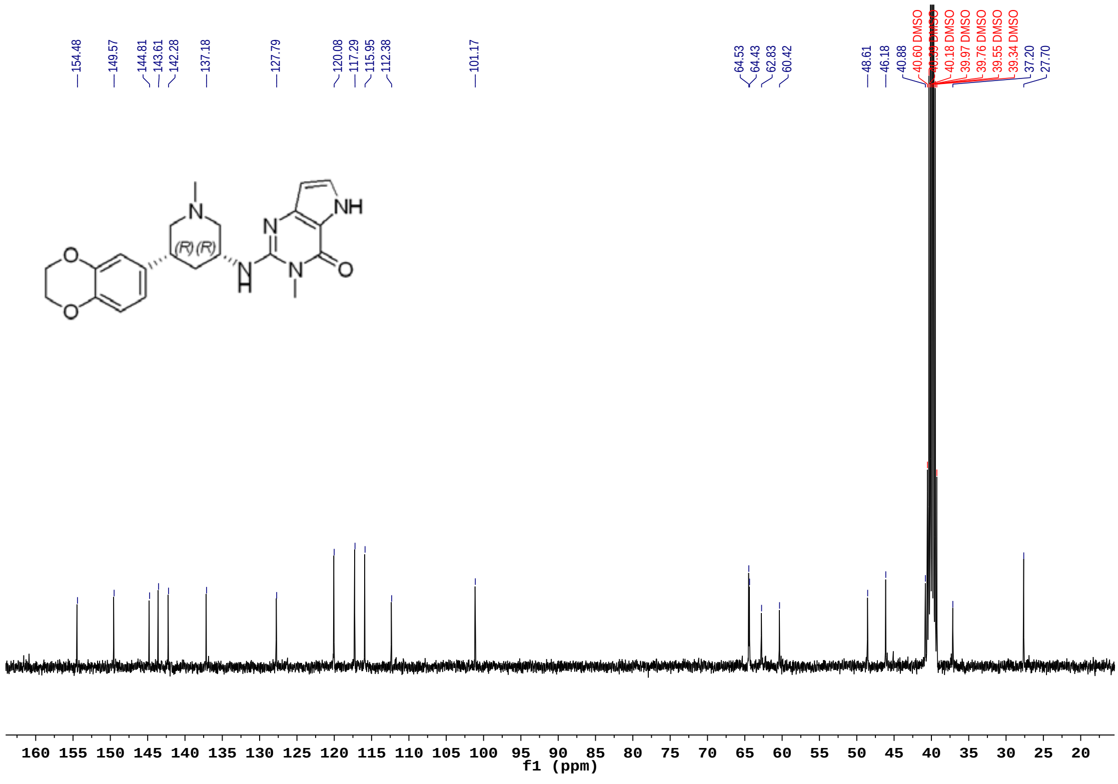


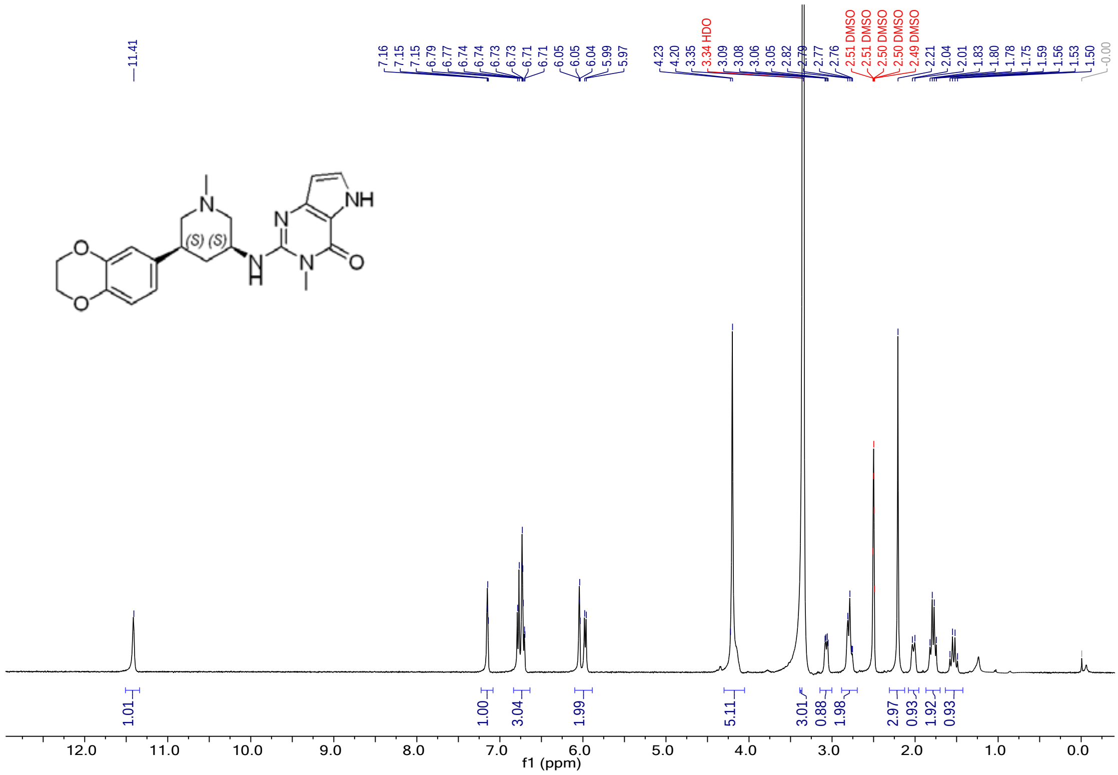


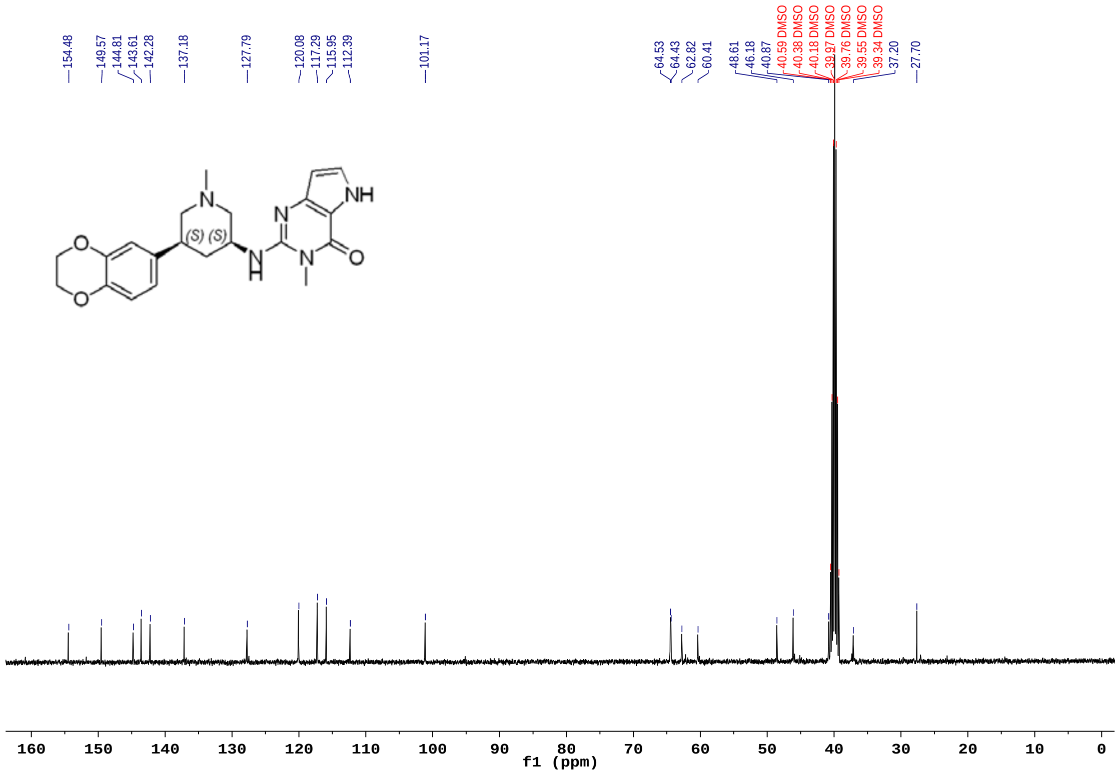




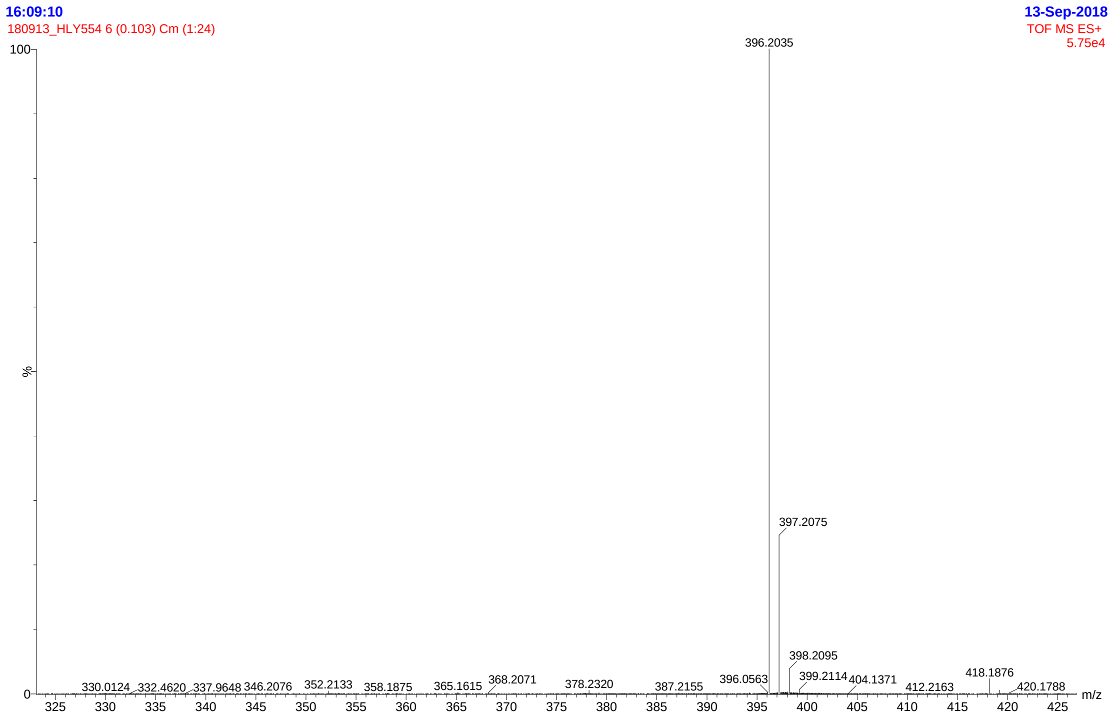

11.42
7.16 7.15 7.15 6.79 6.77 6.74 6.74 6.73 6.73 6.71 6.05 6.05 6.04 5.98 5.96
4.21 4.18
3.35 3.34 HDO 3.10 3.09 3.08 3.07 3.06 2.83 2.82 2.82 2.79 2.51 DMSO 2.51 DMSO 2.50 DMSO 2.50 DMSO 2.49 DMSO 2.21 2.04 2.01 1.80 1.78 1.75
(R) (R)
NH
N
O
1.00 1.09 3.31 1.15 1.00 5.41 3.32 0.98 2.03 3.03 0.95 2.00 0.99
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f1 (ppm)


154.48 149.57 144.81 143.61 142.28 137.18 127.79 120.08 117.29 115.95 112.38 101.17
64.53 64.43 62.83 60.42
48.61 46.18 40.88 40.60 DMSO 40.18 DMSO 39.97 DMSO 39.76 DMSO 39.55 DMSO 39.34 DMSO 37.20 27.70
(R) (R)
NH
N
O
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

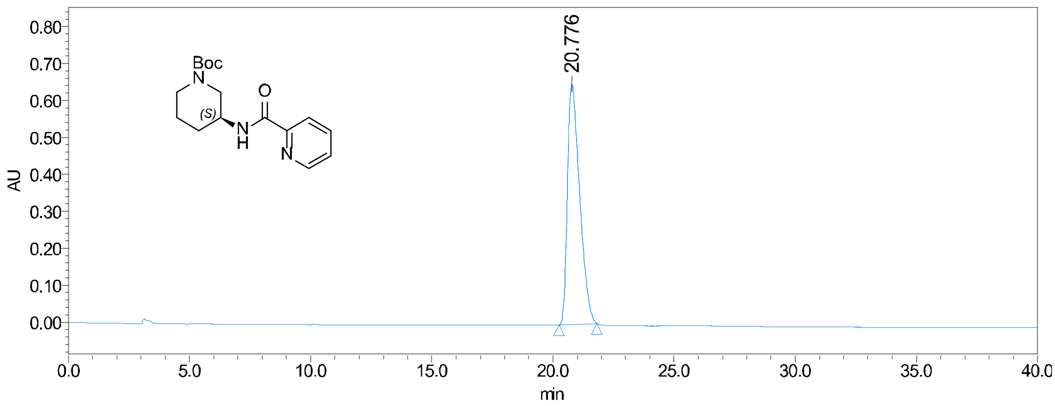

11.41
7.16
7.15
7.15
6.79
6.77
6.74
6.74
6.73
6.73
6.71
6.71
6.05
6.05
6.04
5.99
5.97
4.23
4.20
3.35
3.34 HDO
3.09
3.08
3.06
3.05
2.82
2.79
2.77
2.76
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.21
2.04
2.01
1.83
1.80
1.78
1.75
1.59
1.56
1.53
1.50
-0.00
NH
N
O
(S)
(S)
H
1.01
1.00
3.04
1.99
5.11
3.01
0.88
1.98
2.97
0.93
1.92
0.93
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

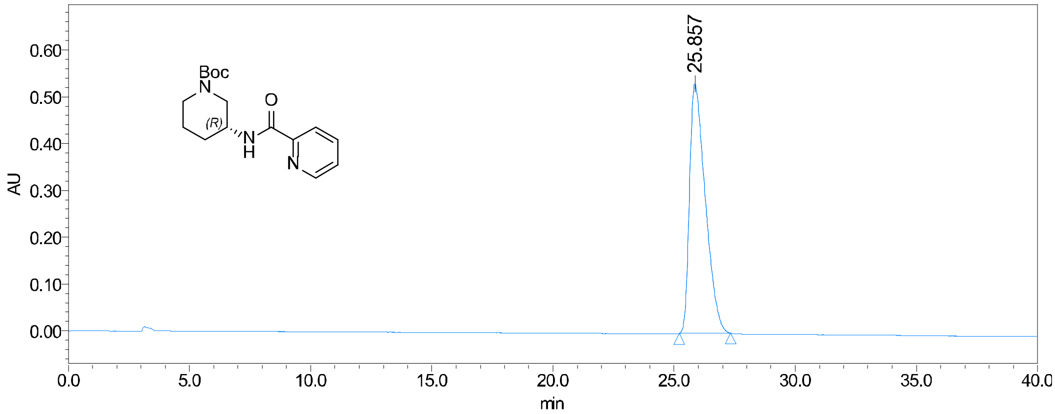

154.48
149.57
144.81
143.61
142.28
137.18
127.79
120.08
117.29
115.95
112.39
101.17
64.53
64.43
62.82
60.41
48.61
46.18
40.87
40.59 DMSO
40.38 DMSO
40.18 DMSO
39.97 DMSO
39.76 DMSO
39.55 DMSO
39.34 DMSO
37.20
27.70
NH
N
O
(S)
(S)
H
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

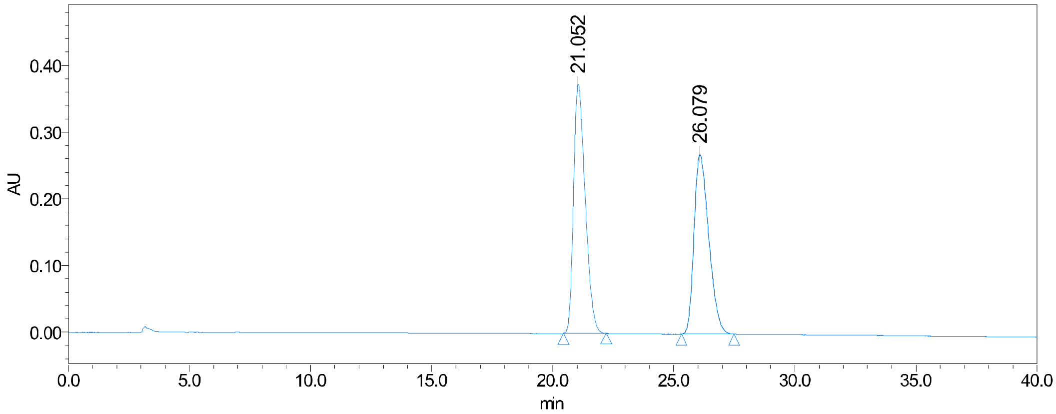
HRMS trace of compound (R,R)-**36n**:



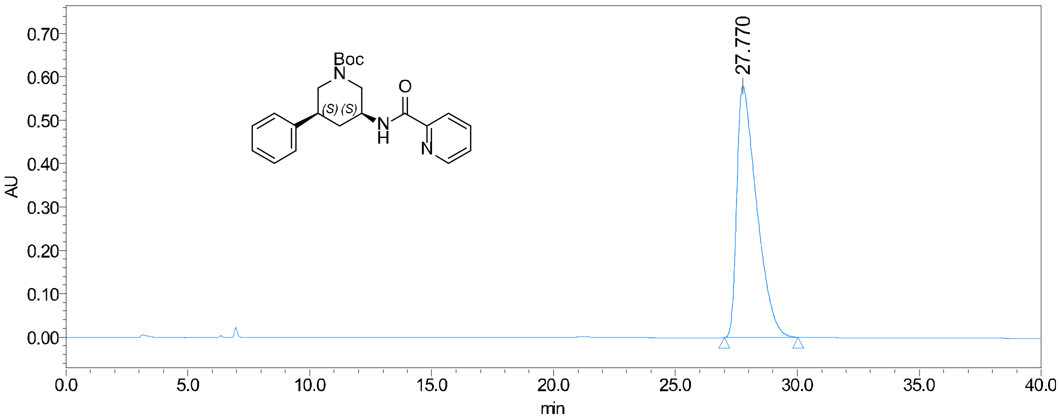
Chiral HPLC Traces

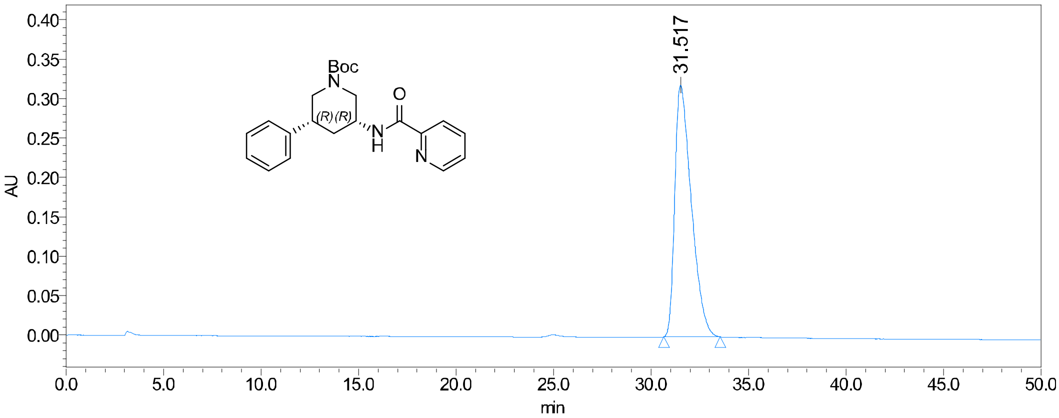


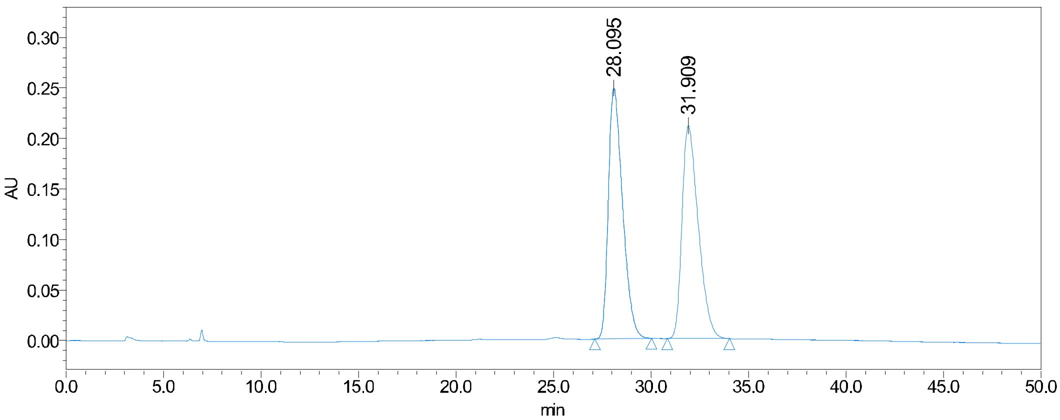




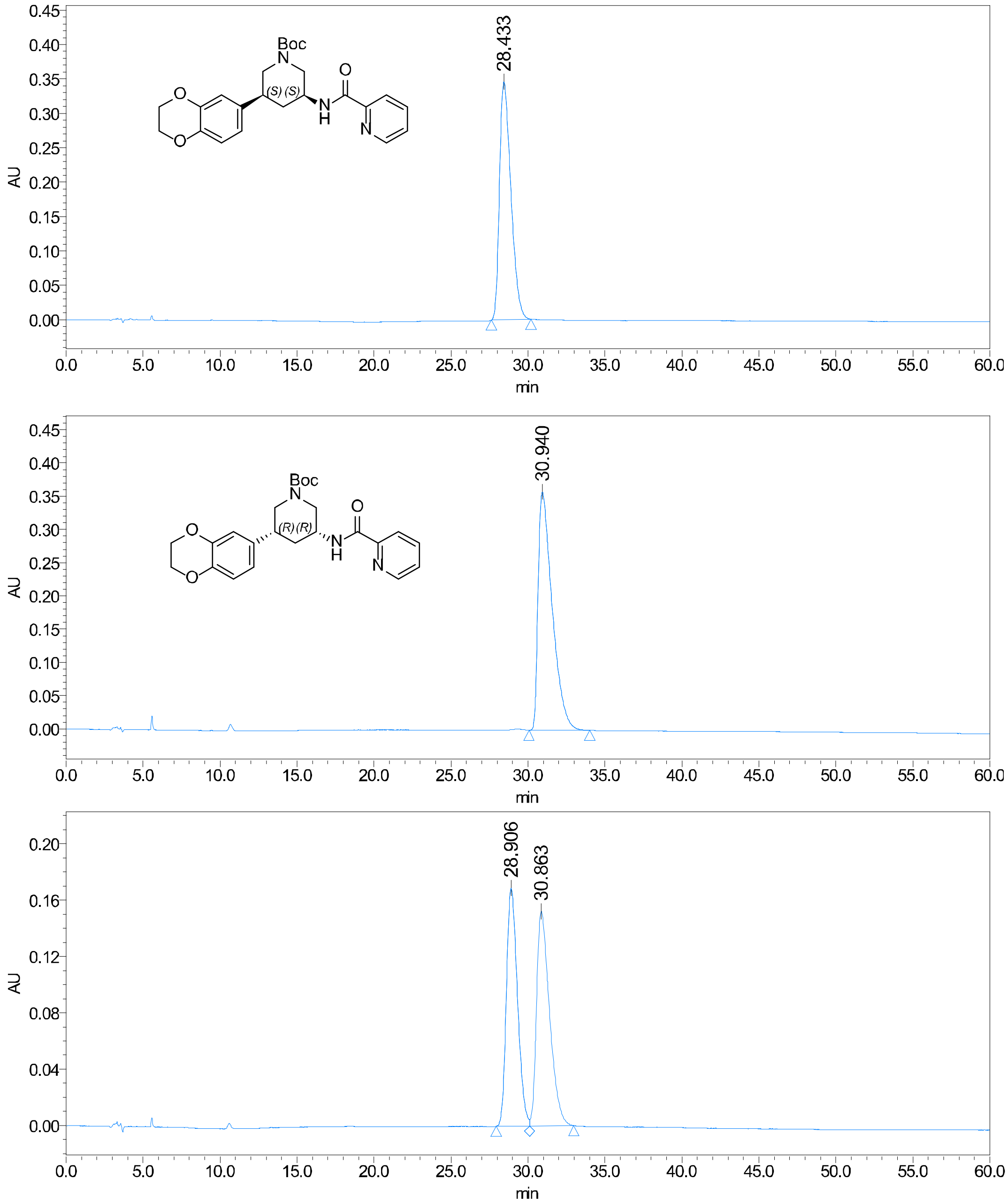
Chiral HPLC traces of (*S*)-**18**, (*R*)-**18**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, *i*-PrOH/Hexane 20: 80, 1 mL/min) R_t = 20.776 min, 25.857 min, >99% *ee*



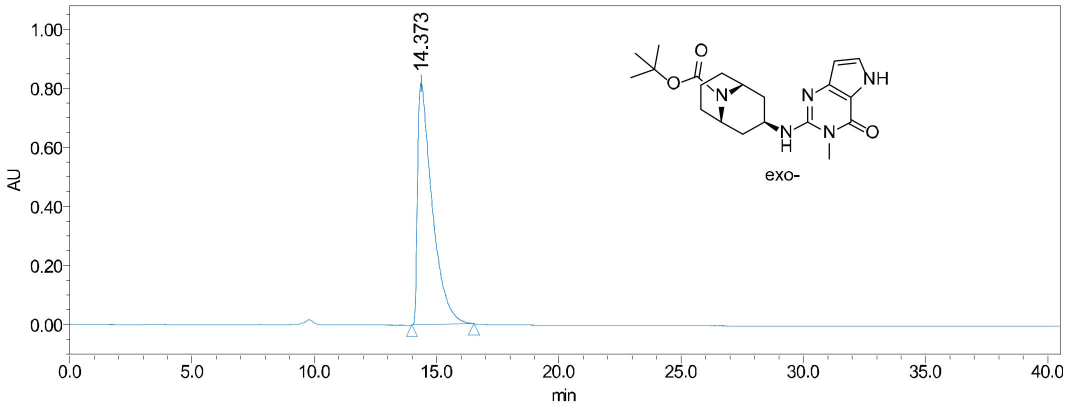


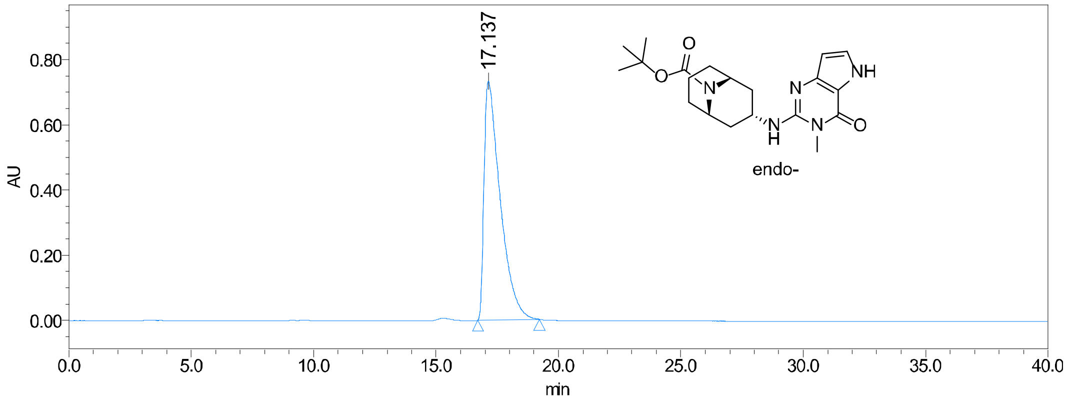


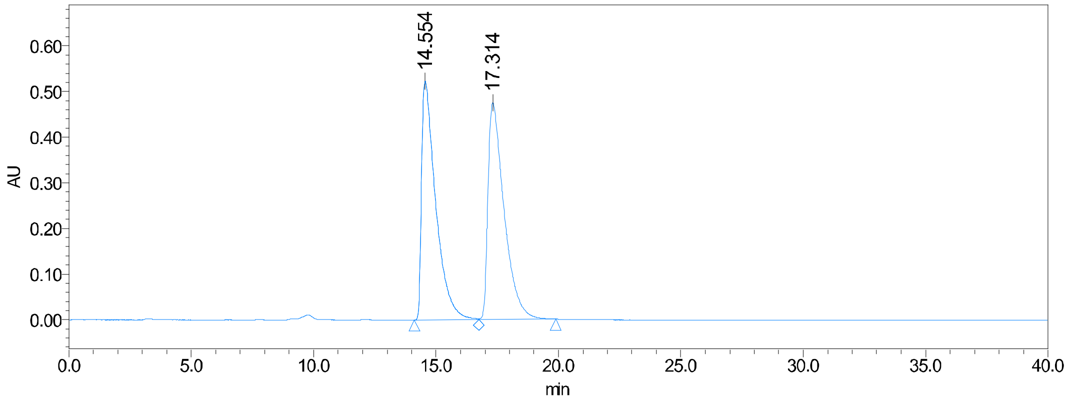
Chiral HPLC traces of (*S*,*S*)-**19a**, (*R*,*R*)-**19a**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, *i*-PrOH/Hexane 20: 80, 1 mL/min) R_t = 27.770 min, 31.517 min, >99% *ee*



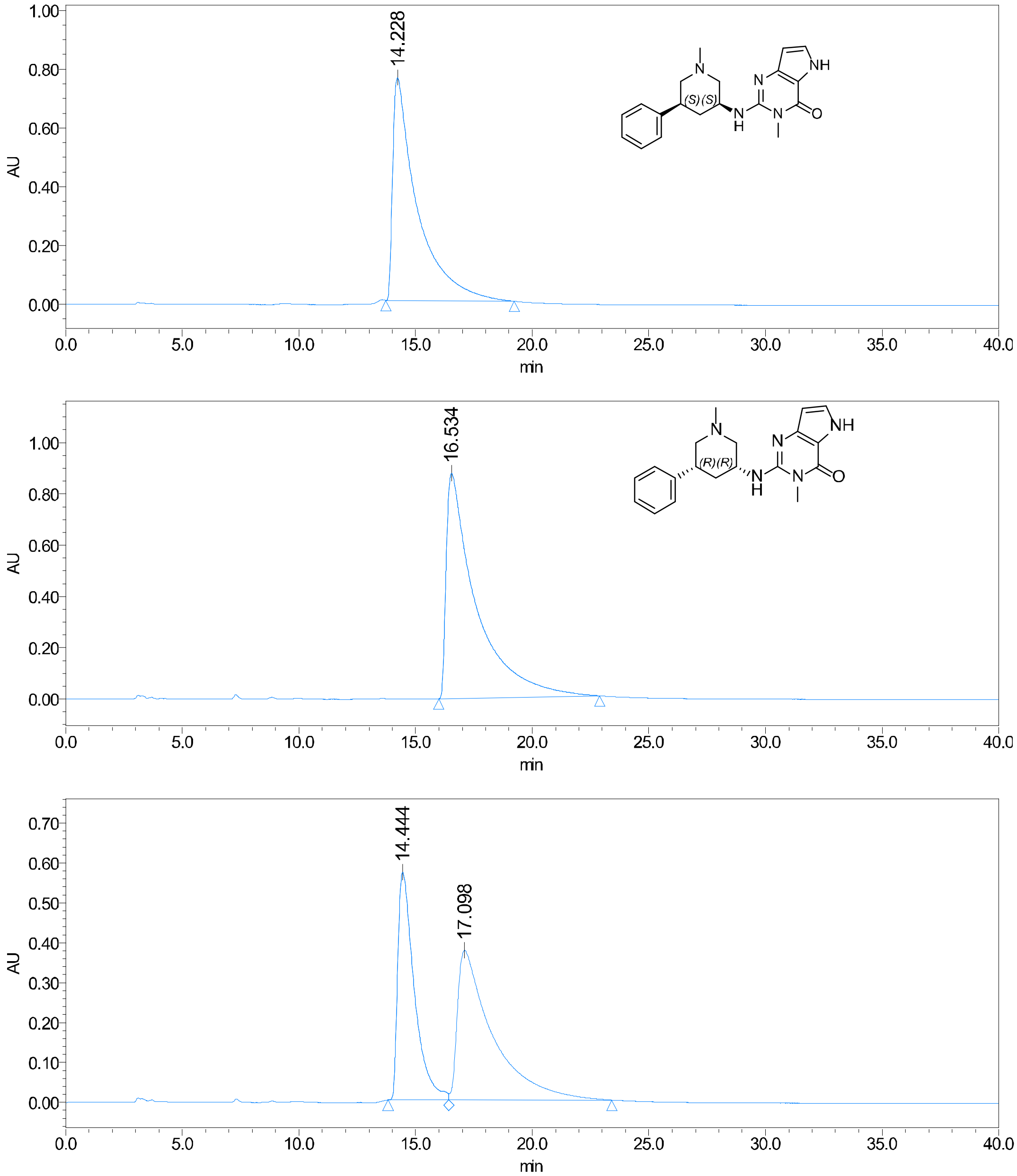
Chiral HPLC traces of (*S*,*S*)-**33j**, (*R*,*R*)-**33j**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, EtOH/Hexane 30: 70, 1 mL/min) R_t = 28.433 min, 30.940 min, >99% *ee*



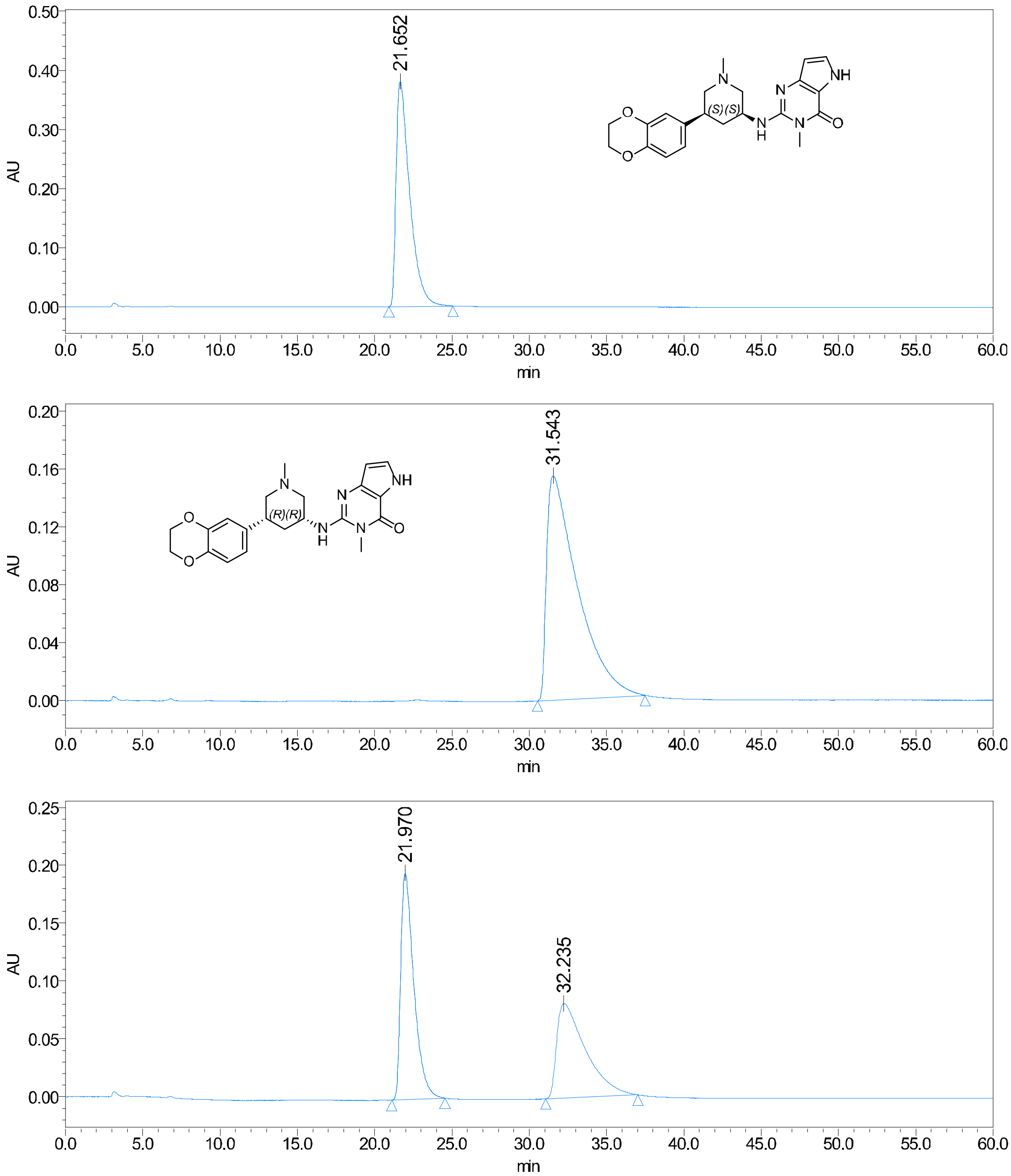




Chiral HPLC traces of *exo*-**24e**, *endo*-**24e**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, *i*-PrOH/Hexane 20: 80, 1 mL/min) R_t = 14.373 min, 17.137 min, >99% ee



Chiral HPLC traces of (*S*,*S*)-**24a**, (*R*,*R*)-**24a**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, *i*-PrOH/Hexane 25: 75, 1 mL/min) R_t = 14.228 min, 16.534 min, >99% ee



Chiral HPLC traces of (*S*,*S*)-**36n**, (*R*,*R*)-**36n**, and the mixture. (Chiralpak IE column, 4.6 mm × 150 mm, *i*-PrOH/Hexane/EtN_3 30: 70: 0.14, 1 mL/min) R_t = 21.652 min, 31.543 min, >99% ee