Supplementary materials

Design of novel small molecule base-pair recognizers of toxic CUG RNA transcripts characteristics of DM1

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Computer-aided molecular design

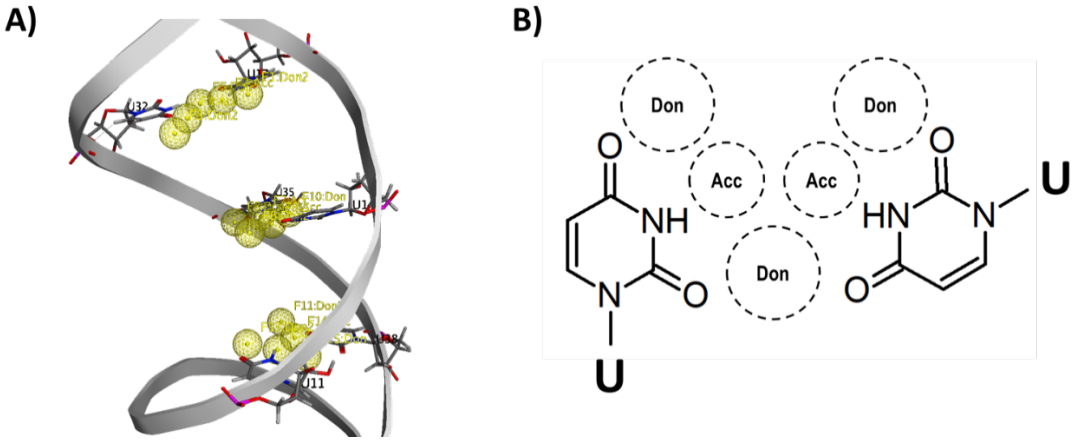
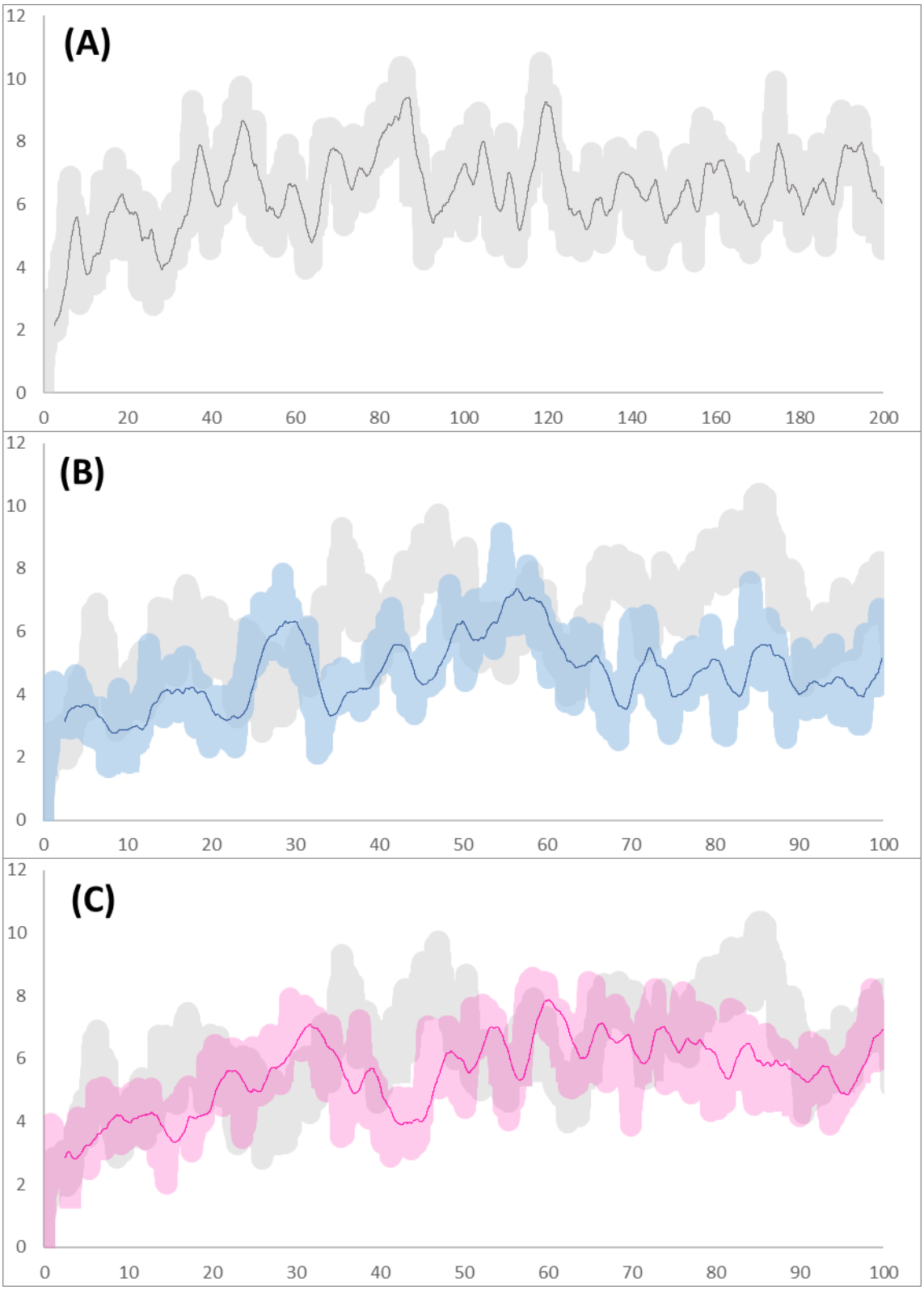


Figure S1. Graphical representation of the pharmacophore features within the RNA structure (A). Schematic view of the pharmacophore model generated for each U·U mismatch (B).


(A)
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
180
200
(B)
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
(C)
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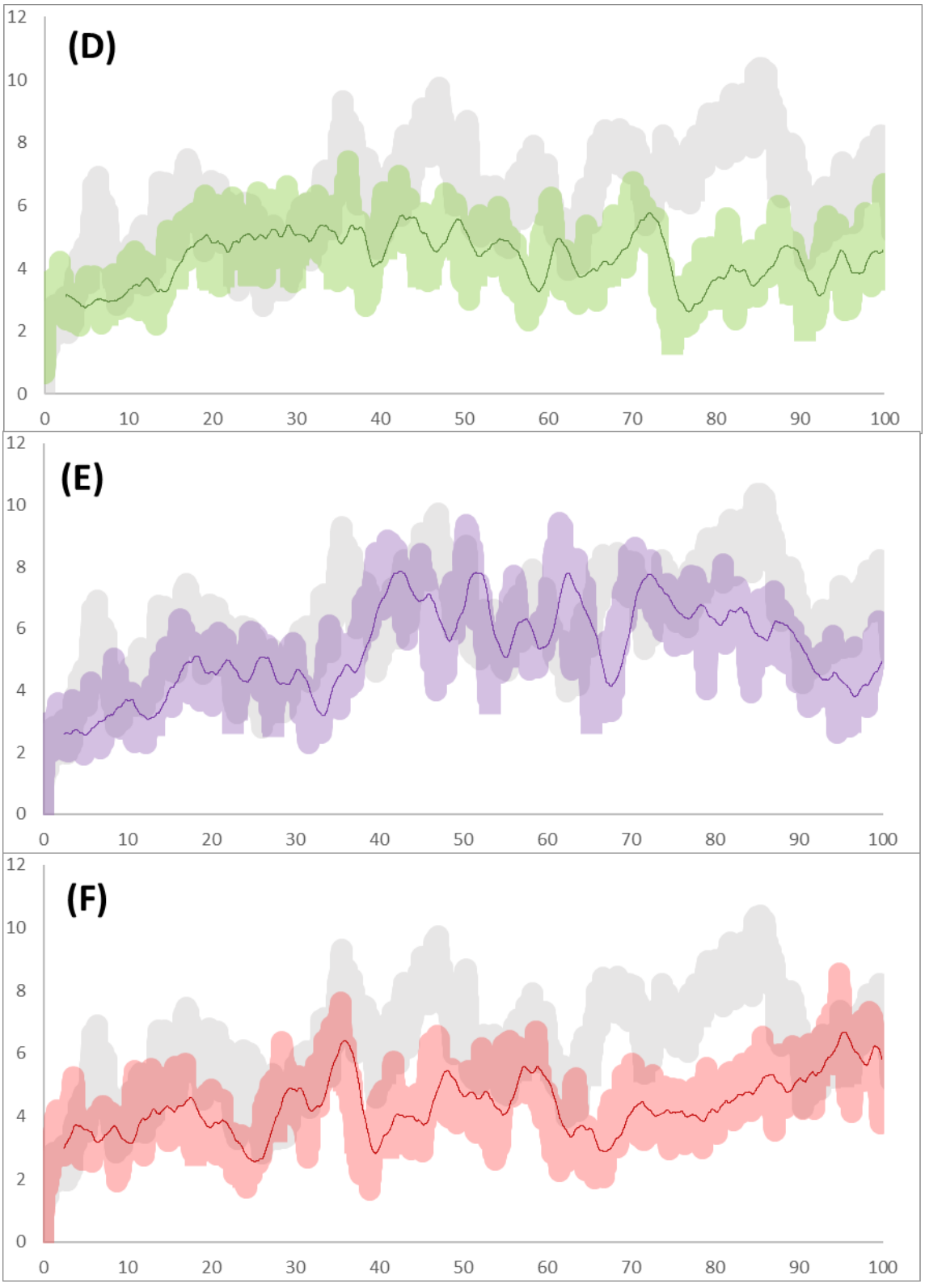
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100


(D)
(E)
(F)
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6
8
10
12
0
10
20
30
40
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60
70
80
90
100

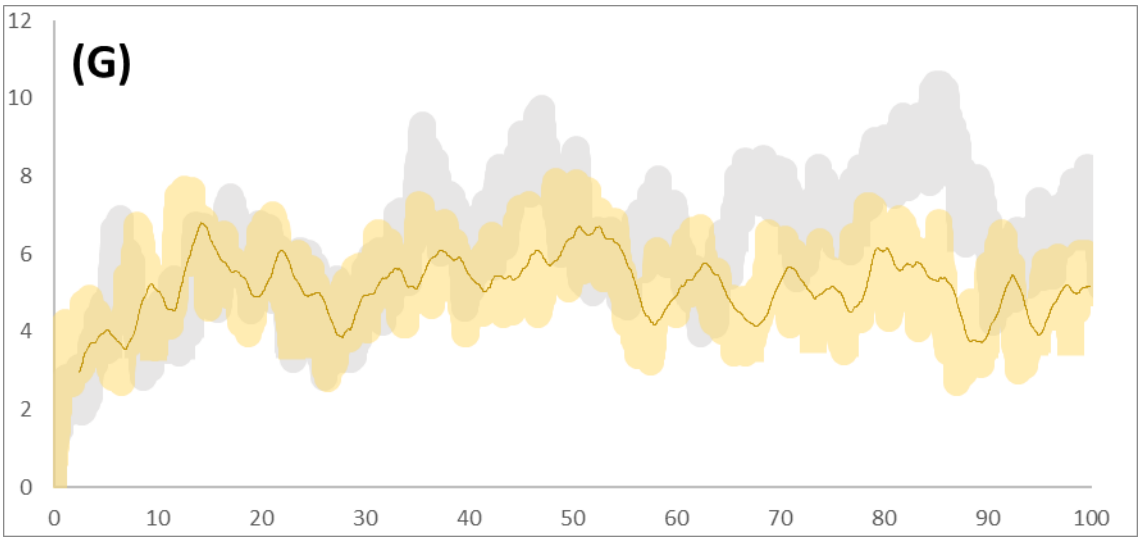

Figure S2. RMSD profile of the RNA structure along MD calculations performed considering the RNA structure only (A) and in complex with the compound of reference Z (B) and 1a-1e candidates (C-G). RMSD is represented in Armstrongs (Å) and the simulation time in ns.

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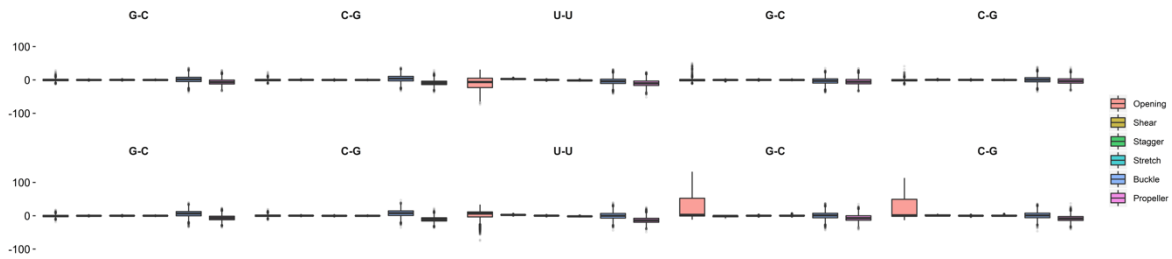
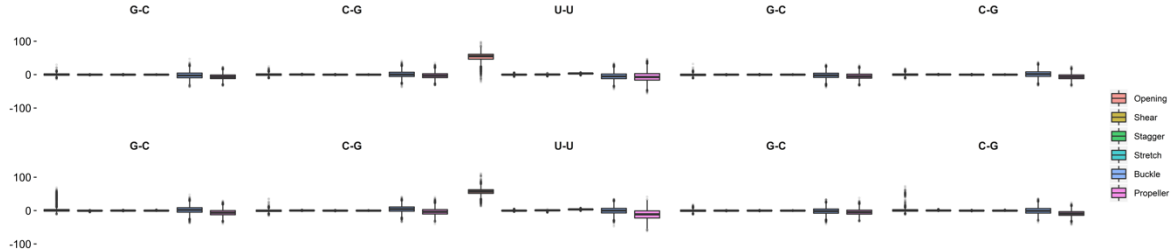
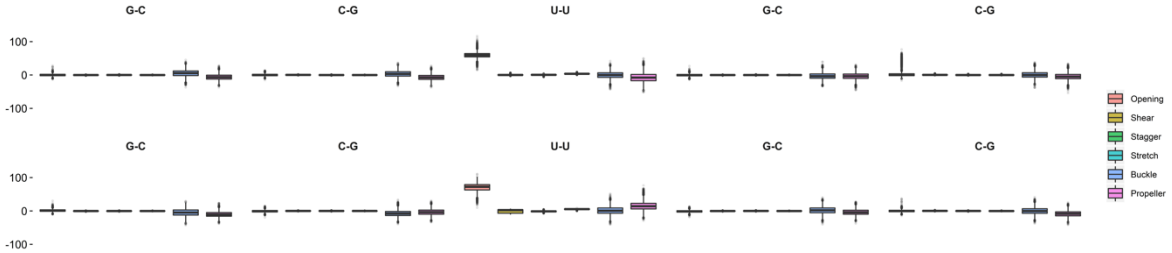

G-C
C-G
U-U
G-C
C-G
100
0
-100
Opening
Shear
Stagger
Stretch
Buckle
Propeller

Z:


G-C
C-G
U-U
G-C
C-G
100
0
-100
Opening
Shear
Stagger
Stretch
Buckle
Propeller

1a: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G-C
C-G
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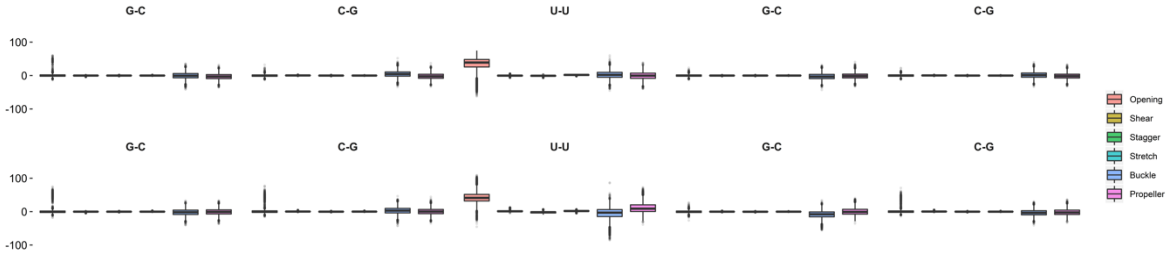
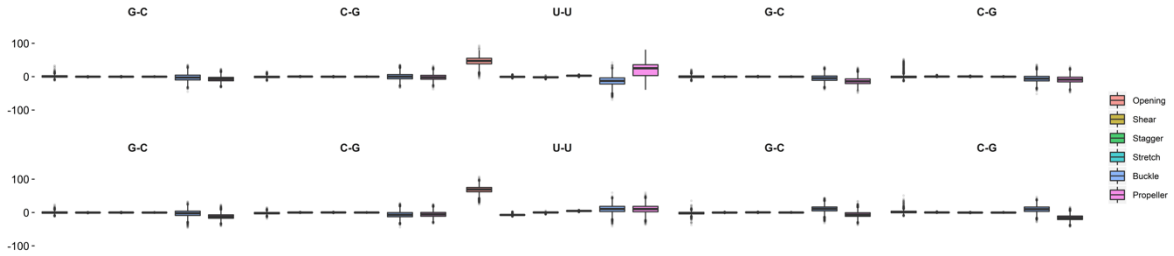
Shear
Stagger
Stretch
Buckle
Propeller

1b:


G-C
C-G
U-U
G-C
C-G
100
0
-100
Opening
Shear
Stagger
Stretch
Buckle
Propeller

1c:



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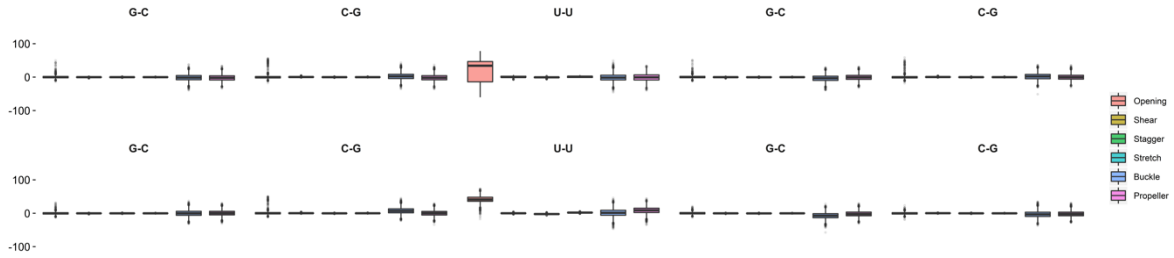


1e:

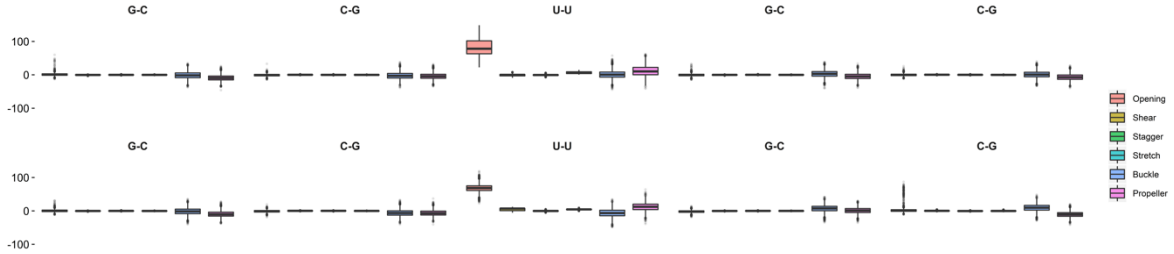
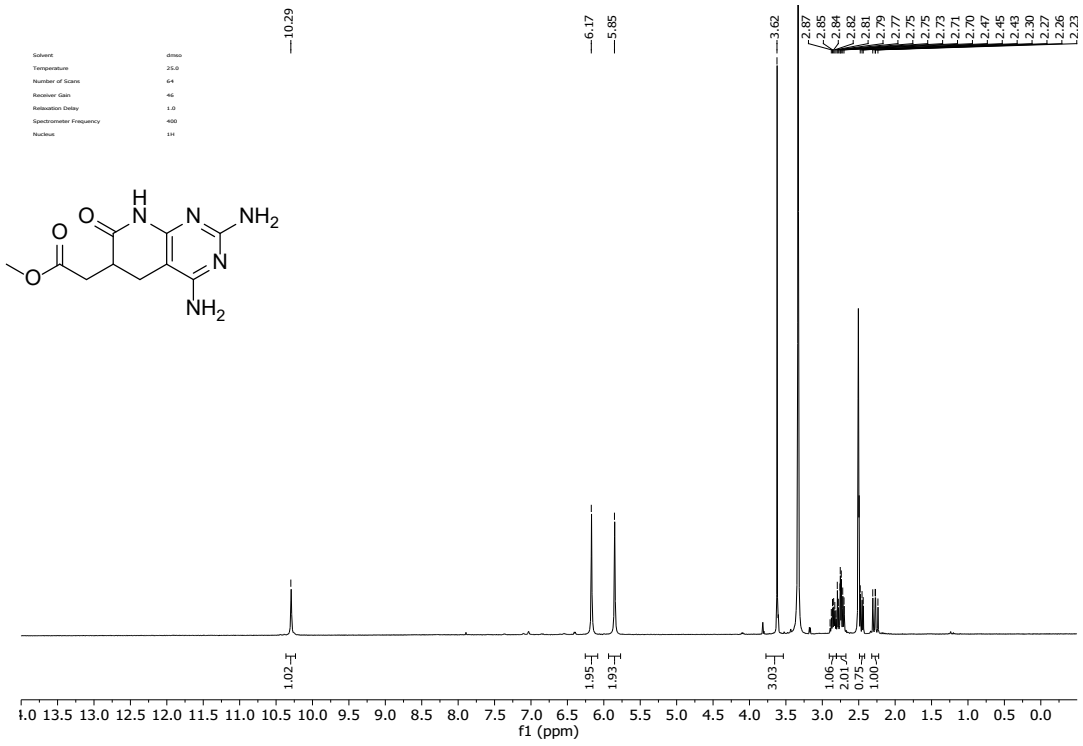
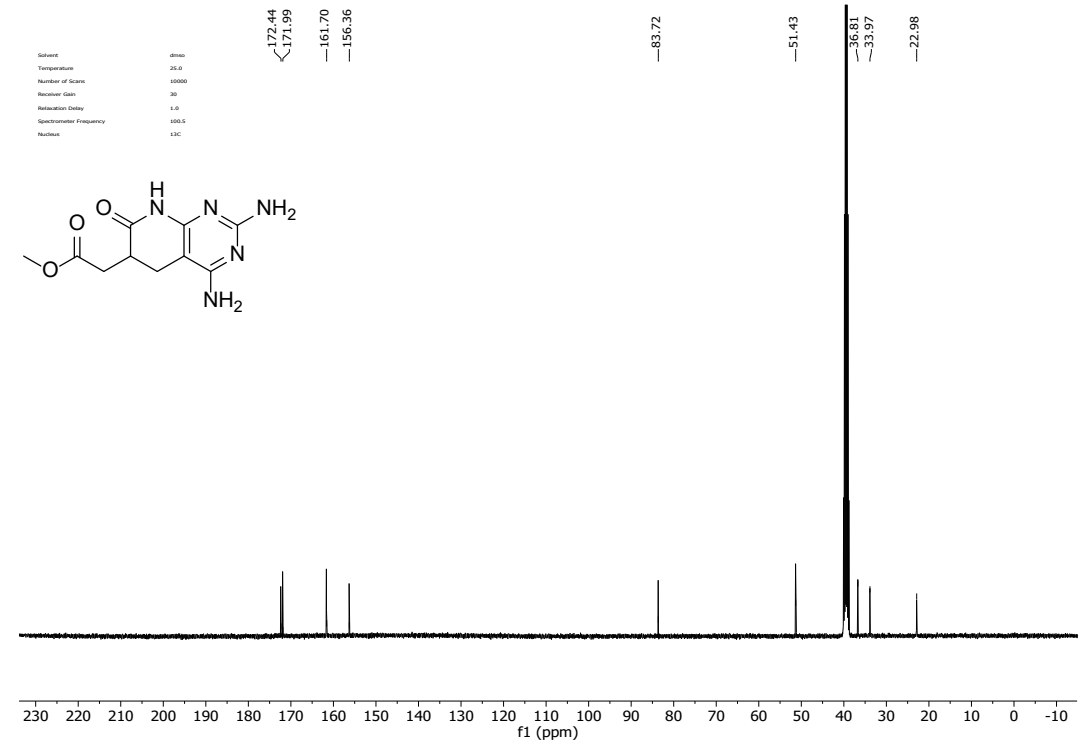


Figure S3. A) analysis of single base-pair structural parameters (Opening, Shear, Stagger, Stretch, Buckle, Propeller). Full-length MD is displayed in boxplot graph format.

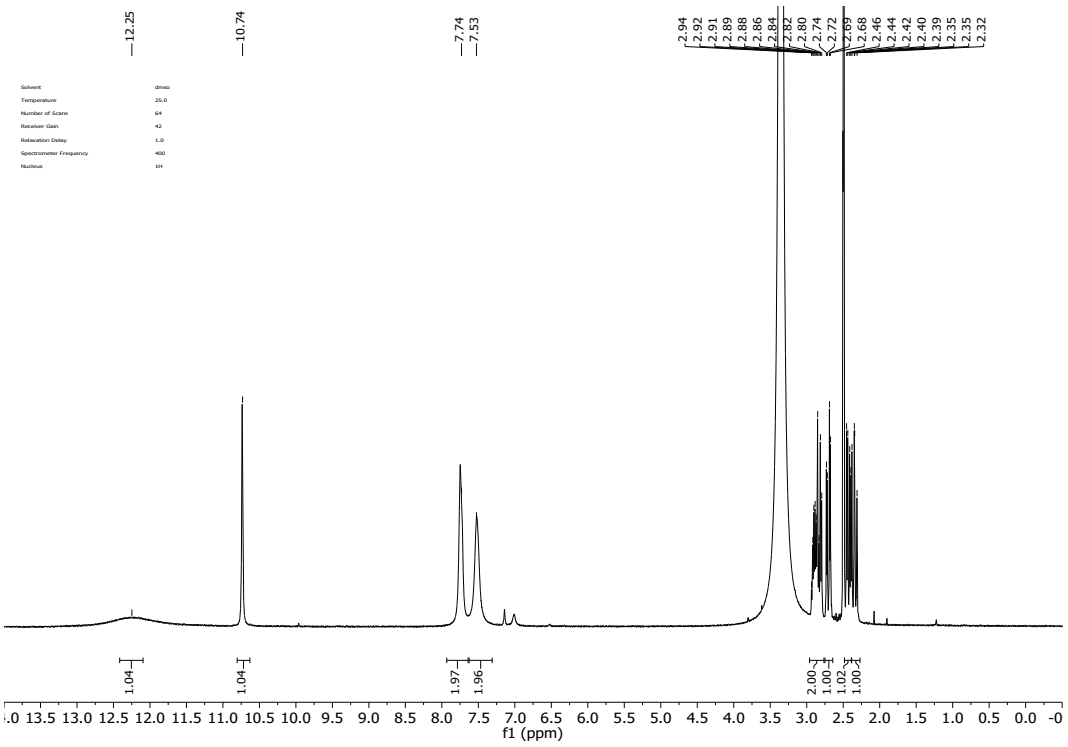
NMR Spectra

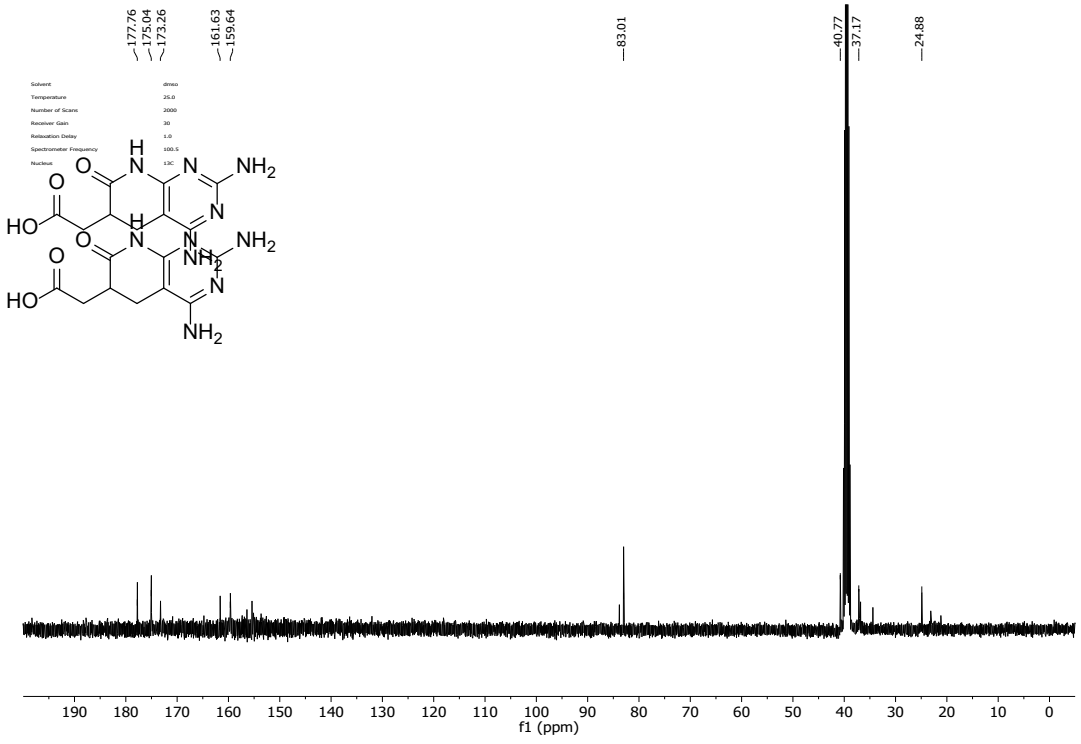
methyl 2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetate (4)



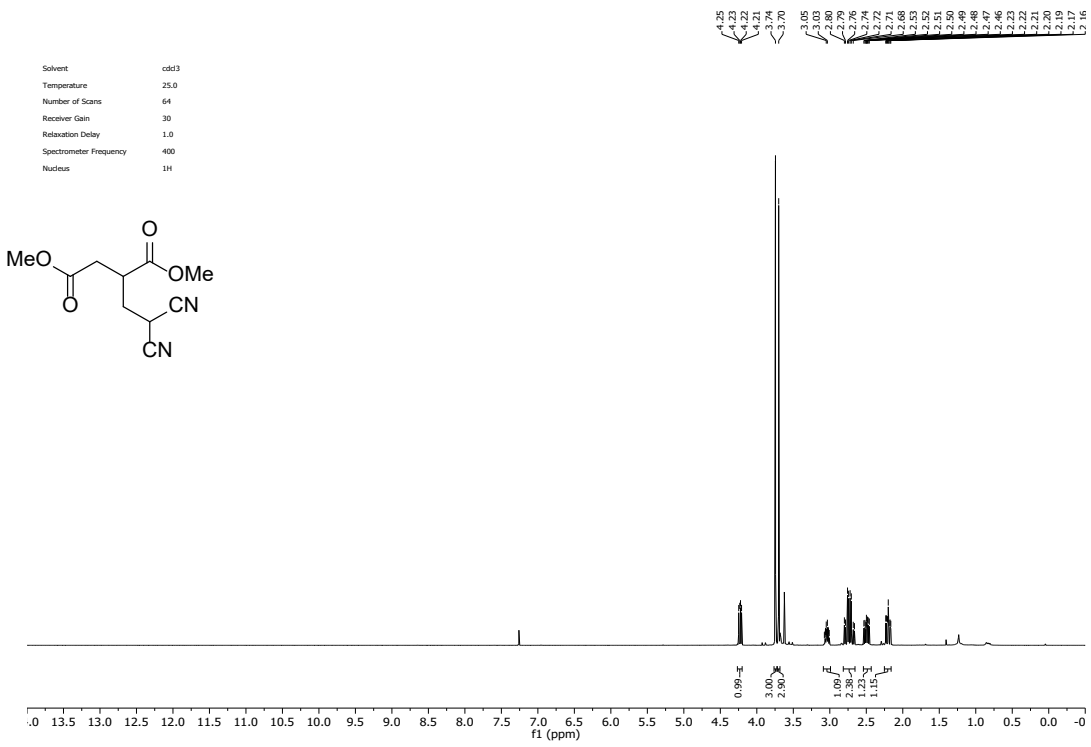


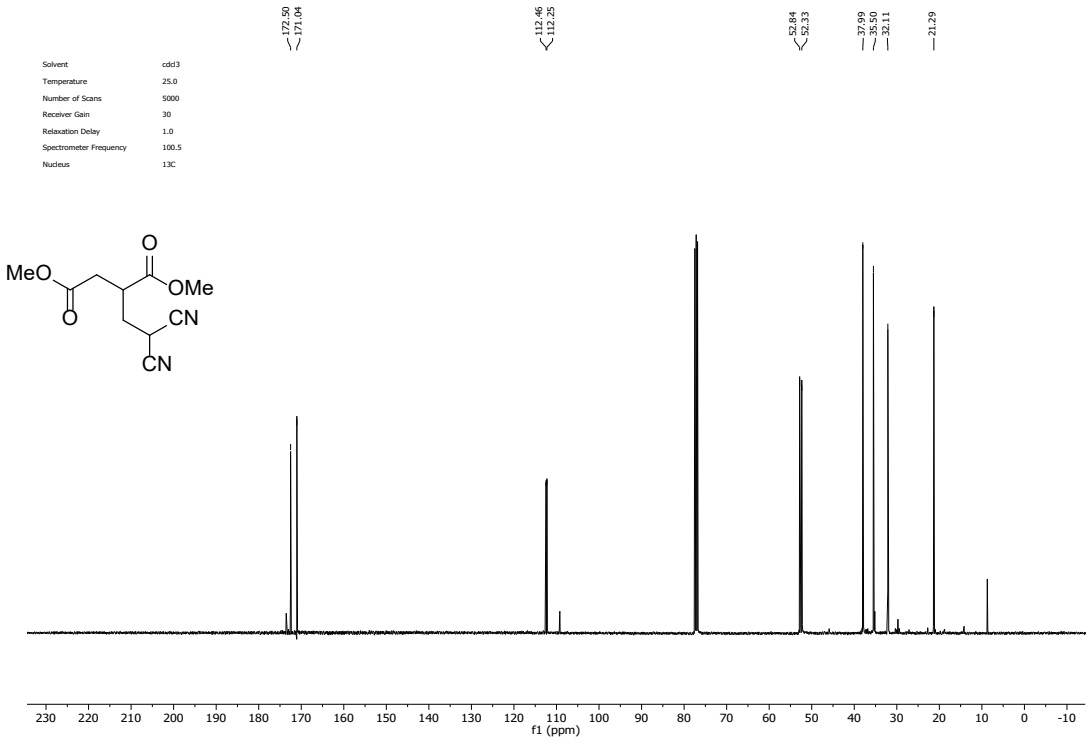
2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetic acid (5)



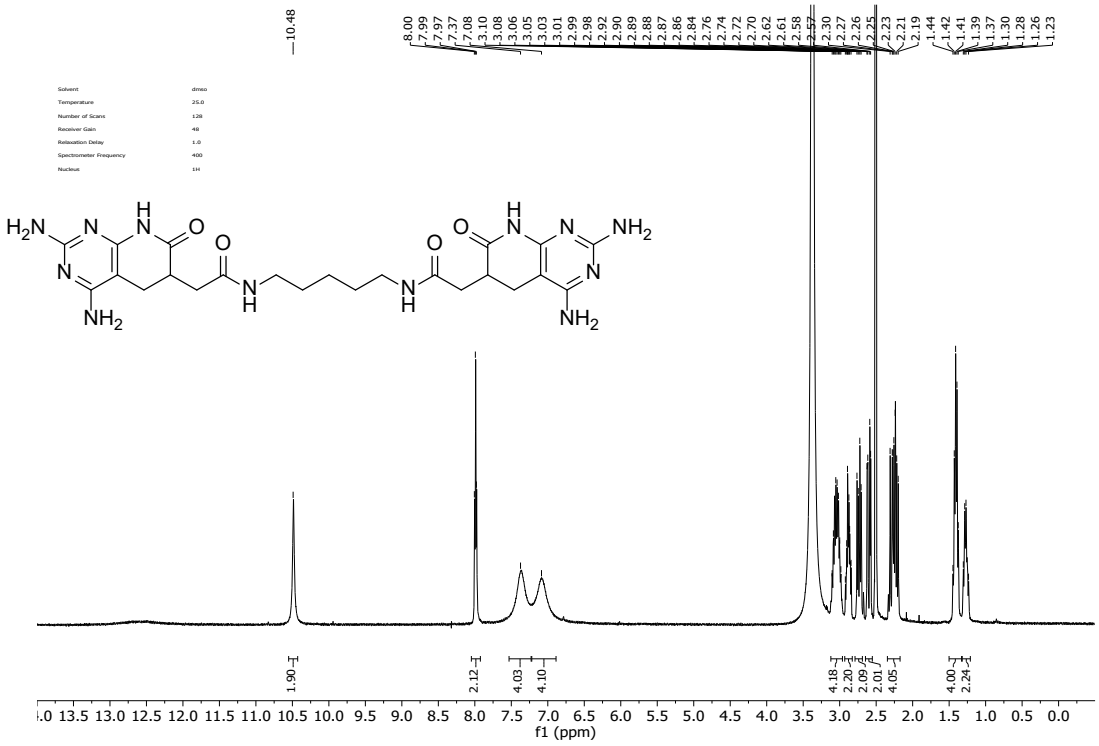


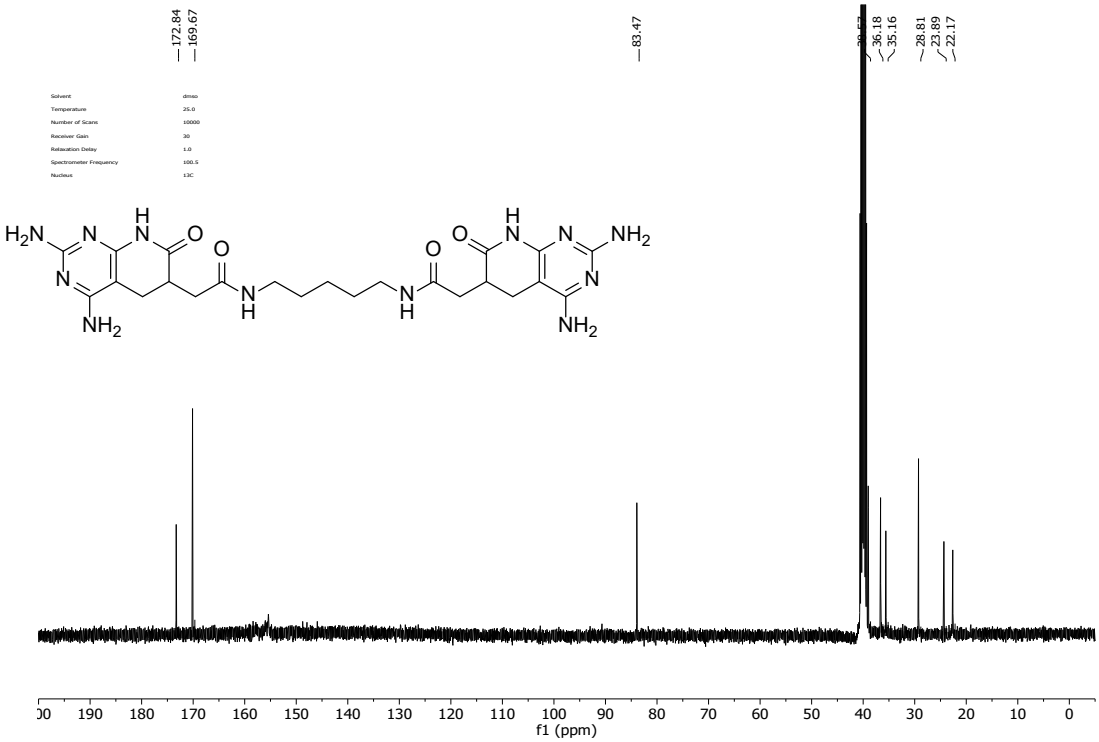
dimethyl 2-(2,2-dicyanoethyl)succinate (6)



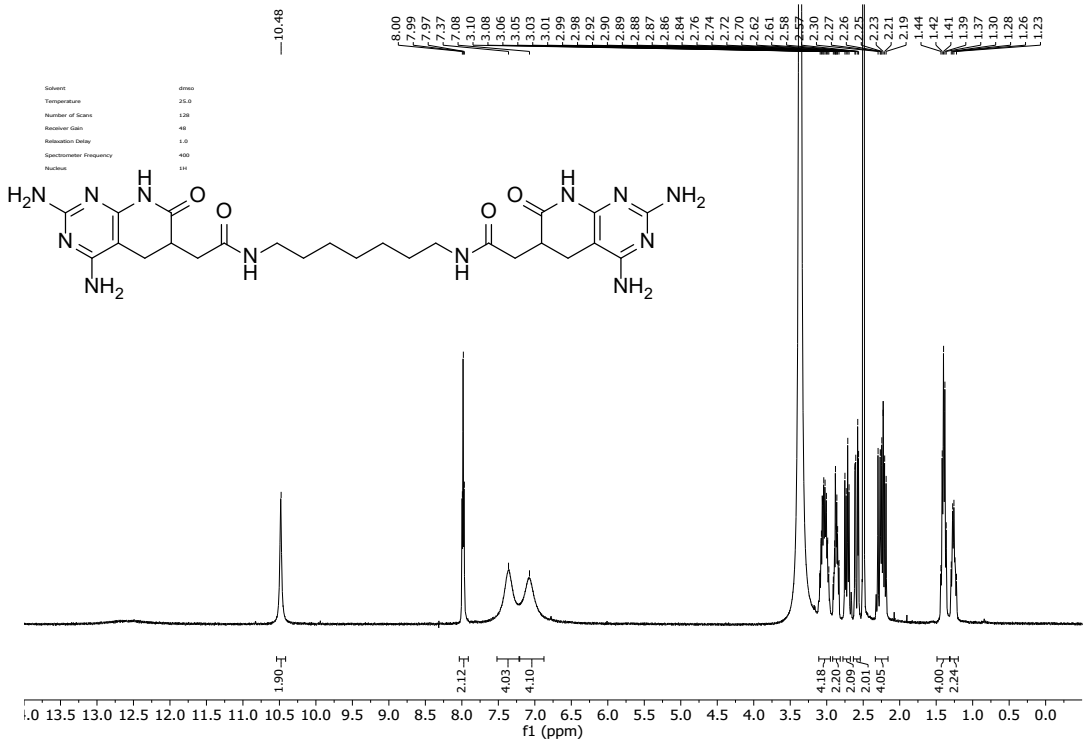


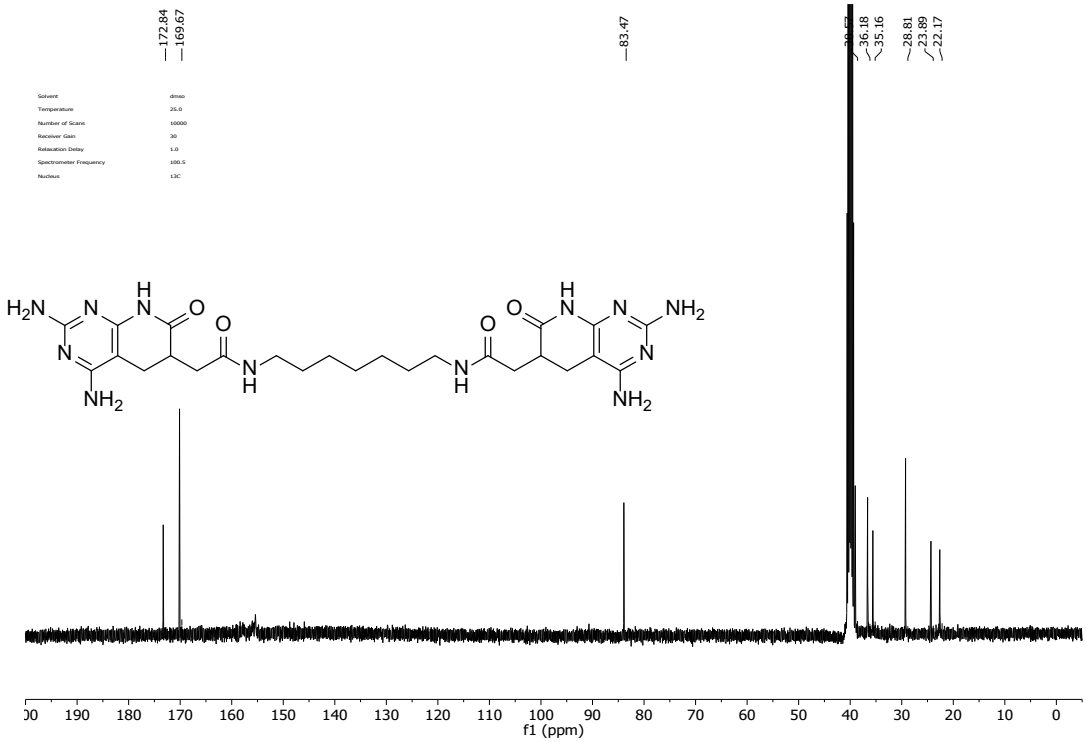
***N,N'*-(pentane-1,5-diyl)bis(2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide) (1a)**



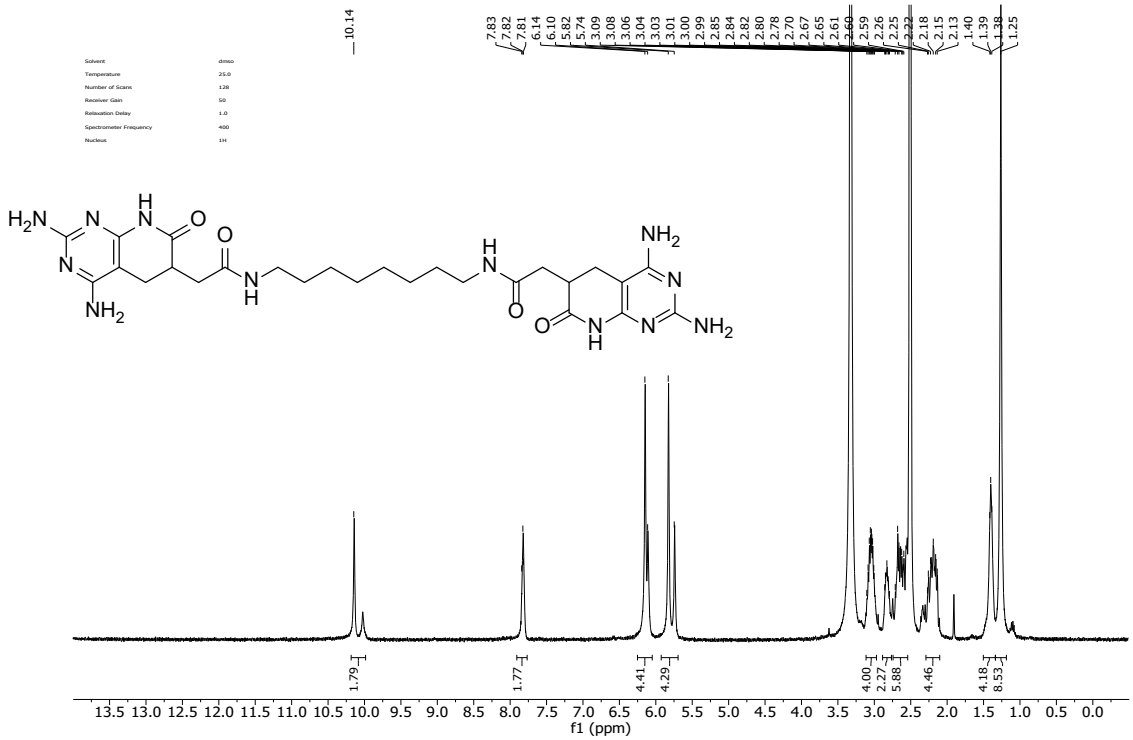


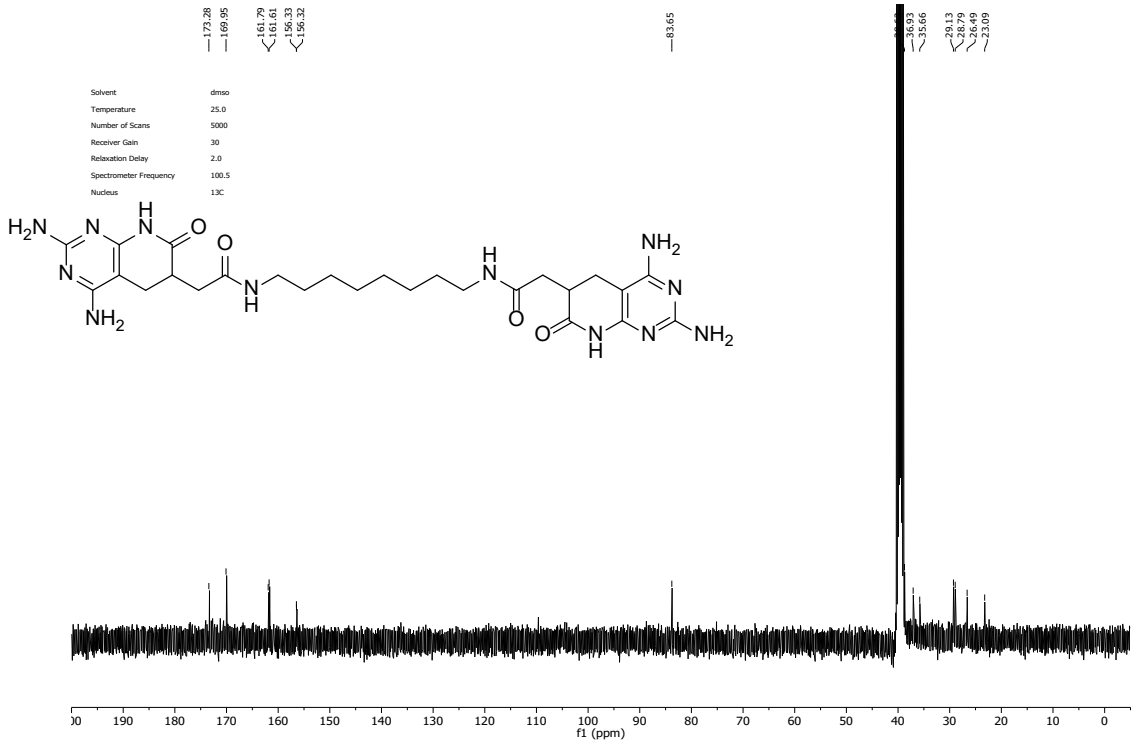
***N,N'*-(heptane-1,7-diyl)bis(2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide) (1b)**



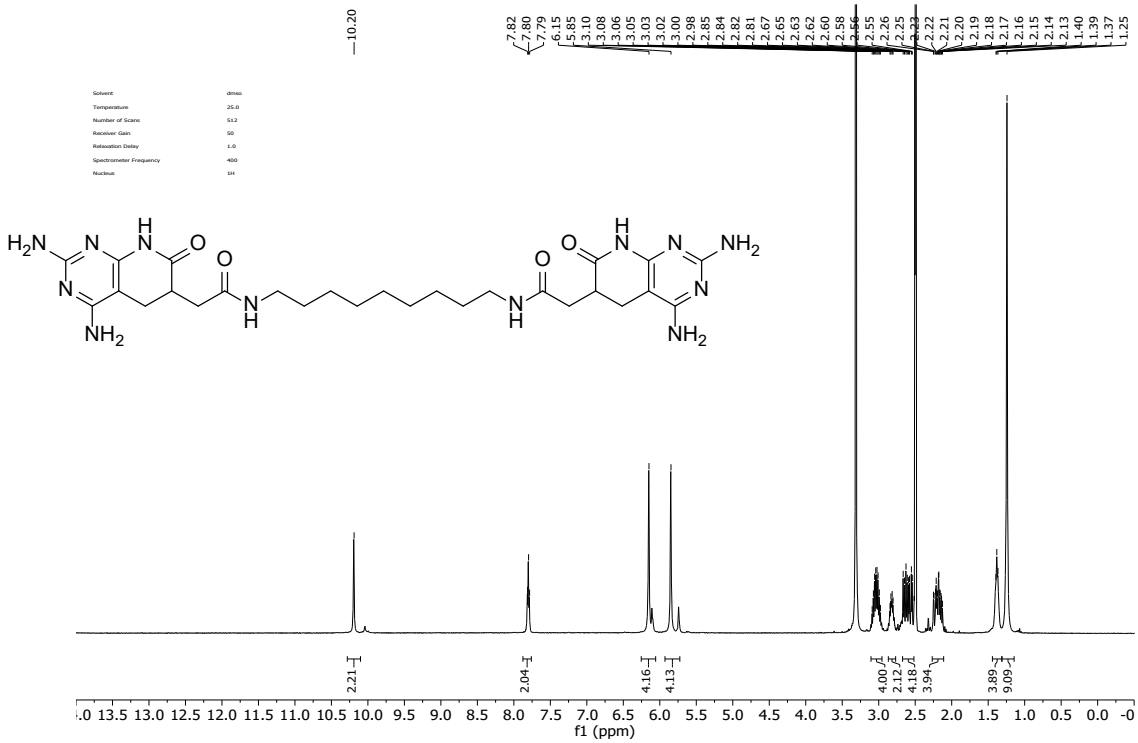


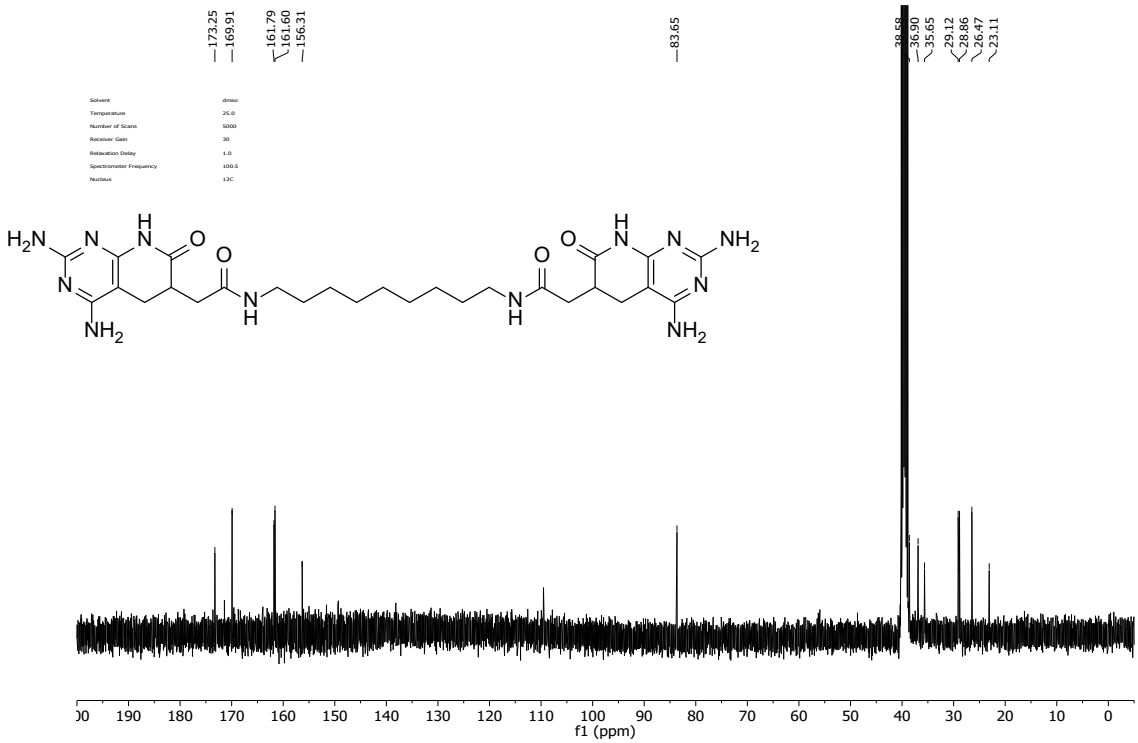
***N,N'*-(octane-1,8-diyl)bis(2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide) (1c).**



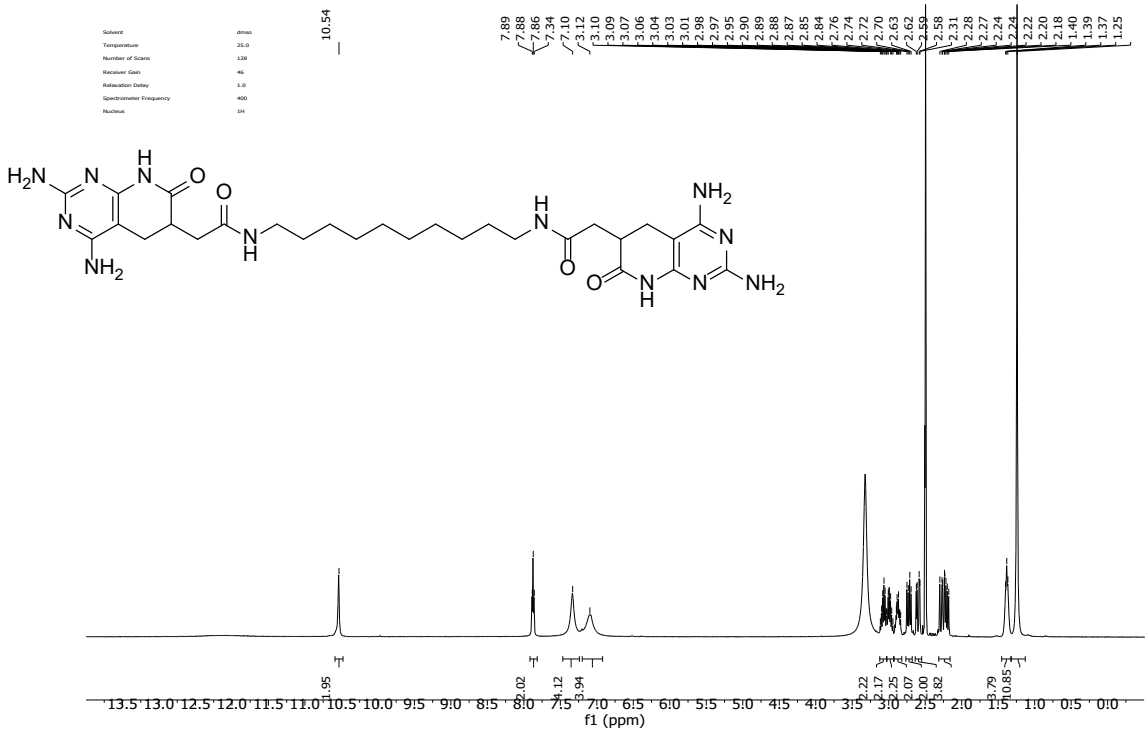


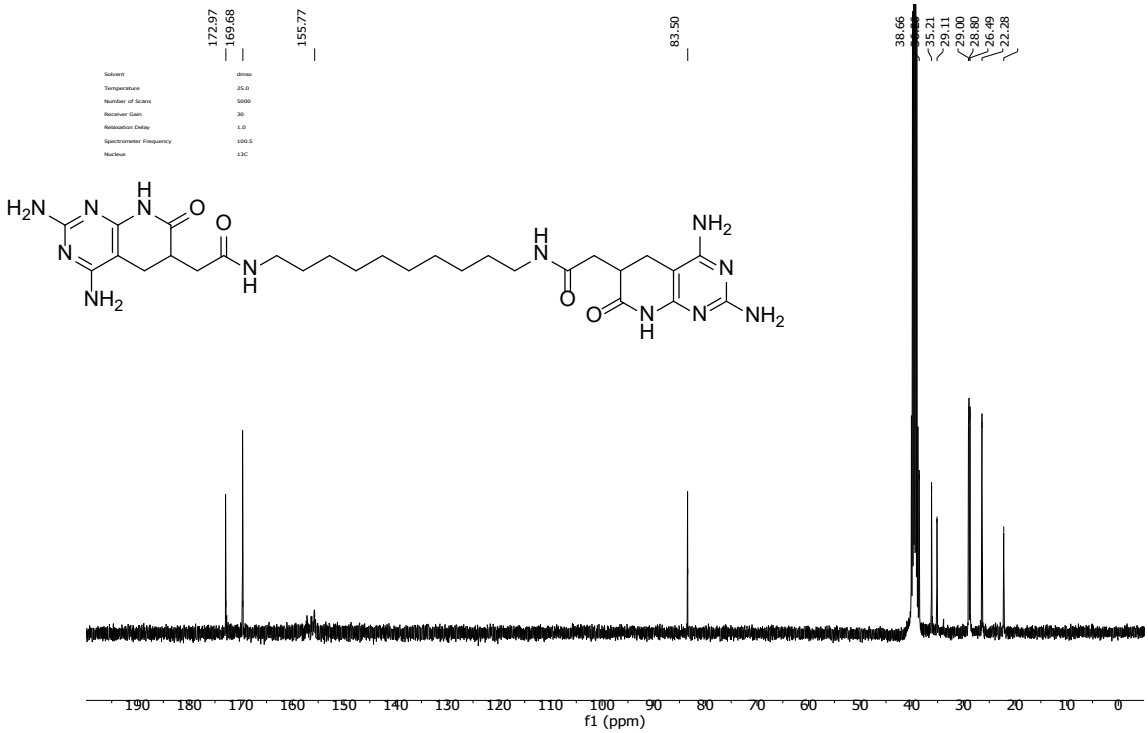
***N,N'*-(nonane-1,9-diyl)bis(2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide) (1d)**





***N,N'*-(decane-1,10-diyl)bis(2-(2,4-diamino-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide) (1e)**





Biochemistry

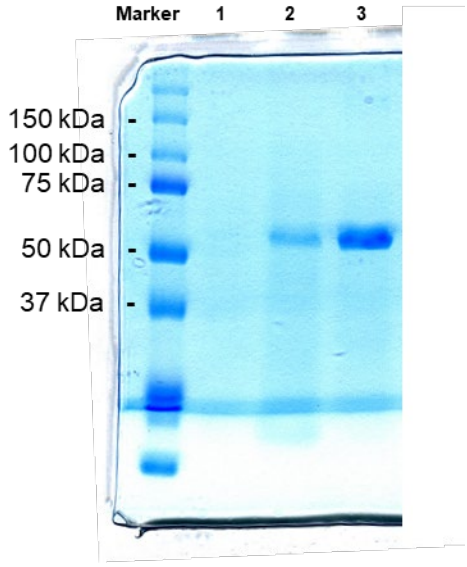


Figure S4. Comassie staining of SDS-PAGE gel to verify MBNL1 purity. First lane corresponds to the marker (Prescission Plus Protein Dual Color Marker), and next lanes correspond to the 3 eluate fractions collected from PD-10, being selected for the study **3**.

Cell Biology

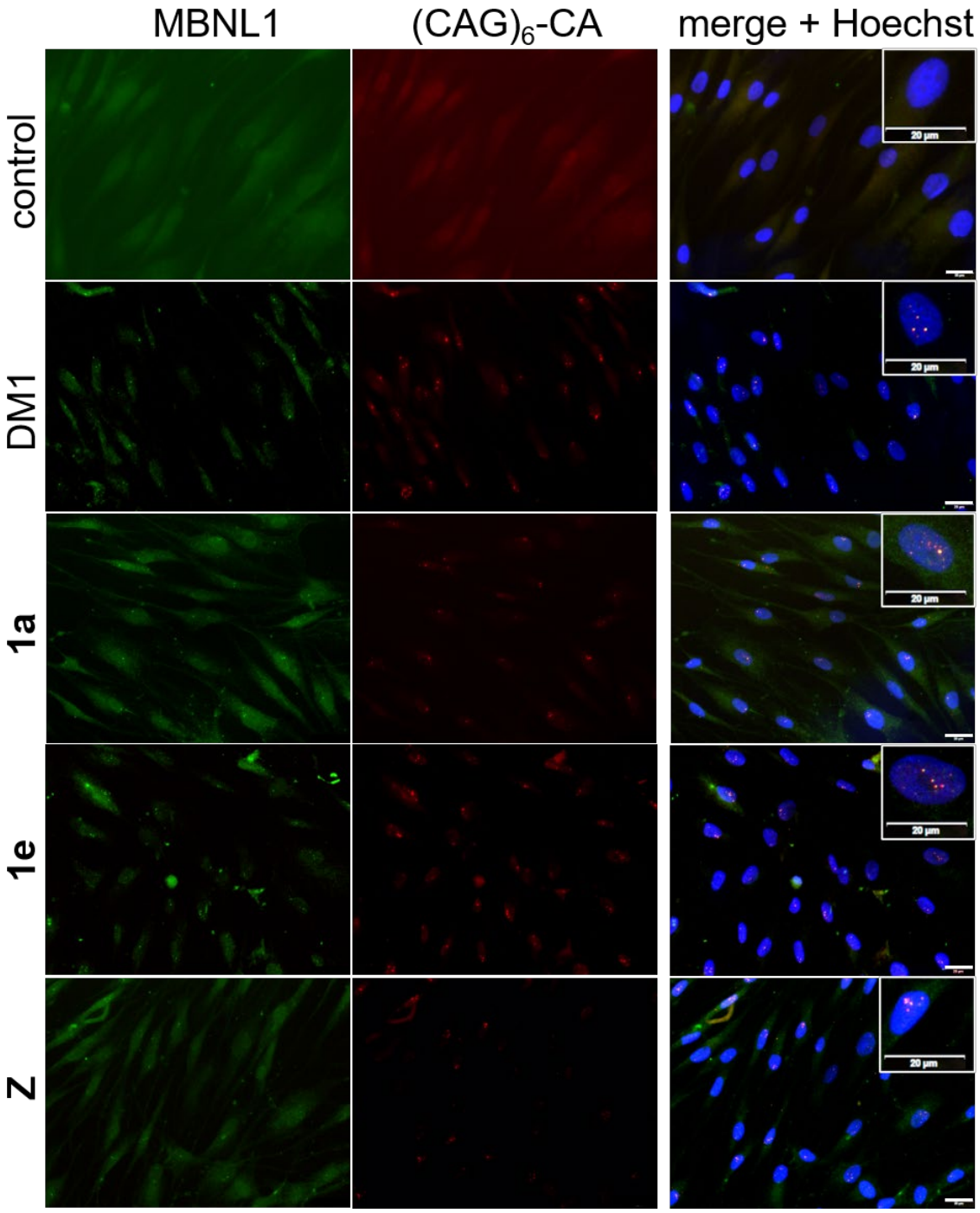


Figure S5. FISH using a (CAG)6 probe labelled with Texas Red at the 5' end in combination with immunofluorescence staining. To verify the co-localization of MBNL1 in ribonuclear inclusions, following the last post-hybridization wash, the indicated cells were stained sequentially with antibodies to MBNL1 and with goat anti-mouse antibody conjugated with Alexa Fluor 488. Nuclei were visualized with Hoechst 33258 dye. (scale bars 20 µm).

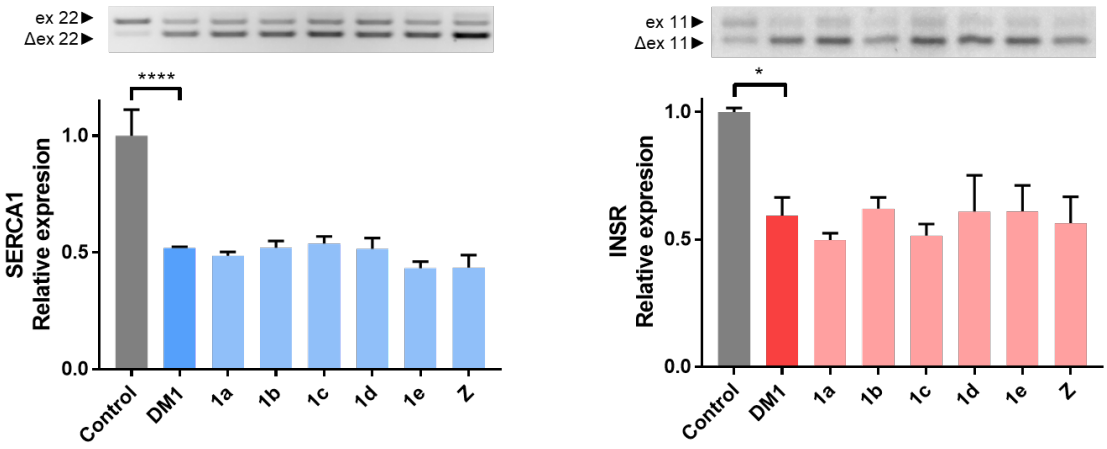


Figure S6. PCR study of SERCA1 and INSR alternative splicing. The results show the relative intensity of the upper band divided by the sum of intensities of both bands and normalised to control cells. All results are presented as the mean ± SD of three experiments. P values were assessed by two-tailed t test. Strong significance was observed between control and diseased cells. On the other hand, no significance was observed between treated cells and DM1 cells. (****$p<0.0001$, *$p<0.05$).

Table S1. List of primers used in PCR.

Primers	**Forward (F)**	**Reverse (R)**
hINSR ex11	CCAAAGACAGACTCTCAGAT	AACATCGCCAAGGGACCTGC
hSERCA1 ex22	ATCTTCAAGCTCCGGGCCCT	CAGCTCTGCCTGAAGATGTG

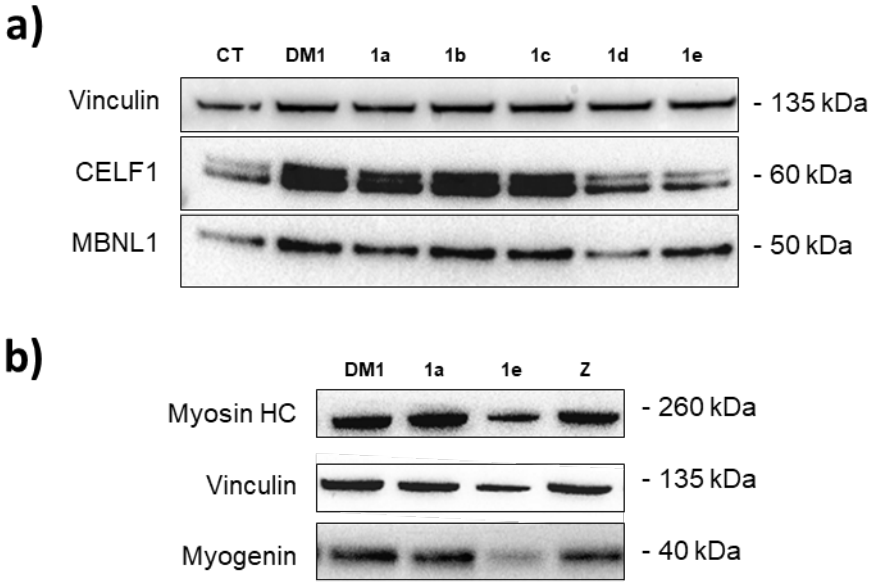


Figure S7. Western blot analysis of: a) CUGBP Elav-like family member 1 (CELF1), muscleblind like protein 1 (MBNL1) and constitutively expressed vinculin (Vinculin) in control, DM1 cells untreated or treated with the indicated drugs. a) muscle specific factors myogenin (Myogenin), myosin (Myosin HC) and constitutively expressed vinculin (Vinculin).

Table S2. List of antibodies used in both IF and WB experiments. Otherwise noticed all antibodies are obtained from mouse.

Antibody	**Source**	**Reference**
mAb anti-vinculin	Sigma-Aldrich	V4505
mAb anti-MBNL1 (3A4)	Santa Cruz Biotechnology	sc-47740
mAb anti-CUGBP1 (3B1)	Santa Cruz Biotechnology	sc-20003
mAb myosin HC (MF20)	D. Fischman	-
mAb myogenin (F5D)	G. Cossu	-
GAM IgG-HRP	Santa Cruz Biotechnology	sc-2005
Alexa Fluor 488 GAM	Invitrogen	A32723