Supporting Information For:

Rationally Designed Small Molecules Targeting the RNA that Causes Myotonic Dystrophy Type 1 Are Potently Bioactive

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Contents:

1. Full citations for references 1 & 2

(1) Venter, J. C.; Adams, M. D.; Myers, E. W.; Li, P. W.; Mural, R. J.; Sutton, G. G.; Smith, H. O.; Yandell, M.; Evans, C. A.; Holt, R. A.; Gocayne, J. D.; Amanatides, P.; Ballew, R. M.; Huson, D. H.; Wortman, J. R.; Zhang, Q.; Kodira, C. D.; Zheng, X. H.; Chen, L.; Skupski, M.; Subramanian, G.; Thomas, P. D.; Zhang, J.; Gabor Miklos, G. L.; Nelson, C.; Broder, S.; Clark, A. G.; Nadeau, J.; McKusick, V. A.; Zinder, N.; Levine, A. J.; Roberts, R. J.; Simon, M.; Slayman, C.; Hunkapiller, M.; Bolanos, R.; Delcher, A.; Dew, I.; Fasulo, D.; Flanigan, M.; Florea, L.; Halpern, A.; Hannenhalli, S.; Kravitz, S.; Levy, S.; Mobarry, C.; Reinert, K.; Remington, K.; Abu-Threideh, J.; Beasley, E.; Biddick, K.; Bonazzi, V.; Brandon, R.; Cargill, M.; Chandramouliswaran, I.; Charlab, R.; Chaturvedi, K.; Deng, Z.; Di Francesco, V.; Dunn, P.; Eilbeck, K.; Evangelista, C.; Gabrielian, A. E.; Gan, W.; Ge, W.; Gong, F.; Gu, Z.; Guan, P.; Heiman, T. J.; Higgins, M. E.; Ji, R. R.; Ke, Z.; Ketchum, K. A.; Lai, Z.; Lei, Y.; Li, Z.; Li, J.; Liang, Y.; Lin, X.; Lu, F.; Merkulov, G. V.; Milshina, N.; Moore, H. M.; Naik, A. K.; Narayan, V. A.; Neelam, B.; Nusskern, D.; Rusch, D. B.; Salzberg, S.; Shao, W.; Shue, B.; Sun, J.; Wang, Z.; Wang, A.; Wang, X.; Wang, J.; Wei, M.; Wides, R.; Xiao, C.; Yan, C. *Science* 2001, *291*, 1304.

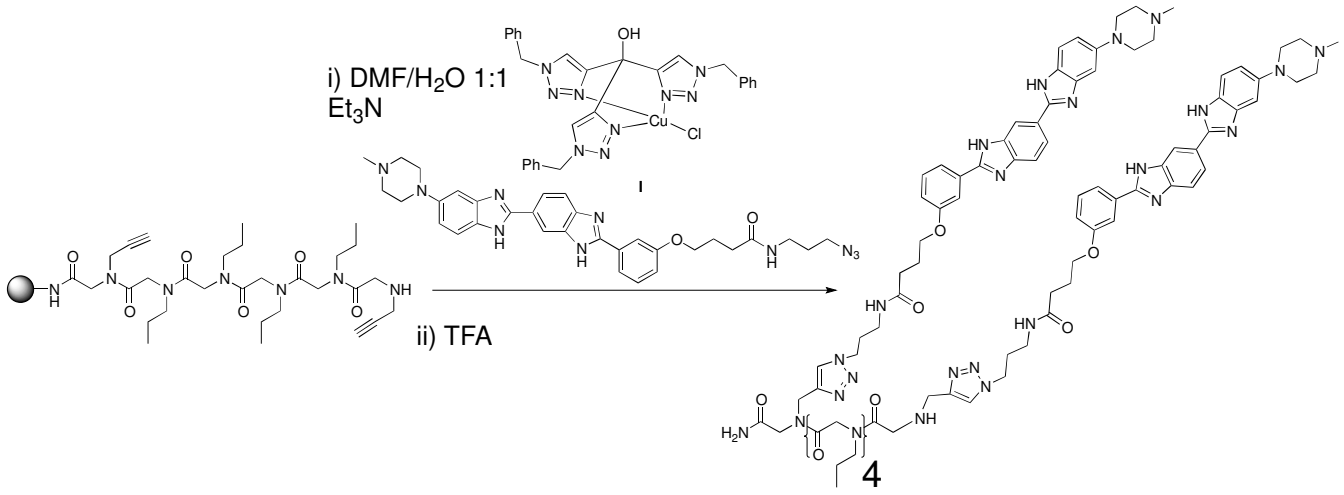
(2) Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J. P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, N.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J. C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R. H.; Wilson, R. K.; Hillier, L. W.; McPherson, J. D.; Marra, M. A.; Mardis, E. R.; Fulton, L. A.; Chinwalla, A. T.; Pepin, K. H.; Gish, W. R.; Chissoe, S. L.; Wendl, M. C.; Delehaunty, K. D.; Miner, T. L.; Delehaunty, A.; Kramer, J. B.; Cook, L. L.; Fulton, R. S.; Johnson, D. L.; Minx, P. J.; Clifton, S. W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J. F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M. *Nature* **2001**, *409*, 860.

2. Syntheses of nH-4 Compounds

The syntheses and characterization of the **nH-4** compounds has been previously reported. (*1*)

The **2H-4** compound was also synthesized via an optimized route:

The peptoid backbone, **2P-4**, was synthesized according to a previously reported route.(*1*)



2H-4

Scheme S-1: Optimized synthetic route for **2H-4**.

A mixture of resin bound **2P-4** (20 mg, 0.034 mmol), Hoechst azide (200 mg, 0.34 mmol), catalyst I (*2*) (3 mg, 0.01 mmol) and Et_3N (135 µl, 0.001 mmol) was suspended in *N*,*N*-dimethyl formamide (DMF; 0.5 mL) and water (0.5 mL) in a microwave reaction vessel. The reaction was microwaved at 110 ^{o}C for 2 h, after which the resin was isolated and washed with DMF. The clean resin was suspended in a 1:1 mixture of trifluoroacetic acid (TFA) : dichloromethane (DCM) (3 mL) and stirred for 30 min. The solution was drained and the sequence was repeated. The combined filtrate was concentrated and then purified using reverse phase HPLC (20–100% Methanol/H_2O + 0.1% TFA over 60 min). A total of 5.4 mg of **2H-4** was isolated; 9% yield.

3. Representative Autoradiograms for Improvement of Splicing Defects in a Cellular Model System

A. cTNT Splicing

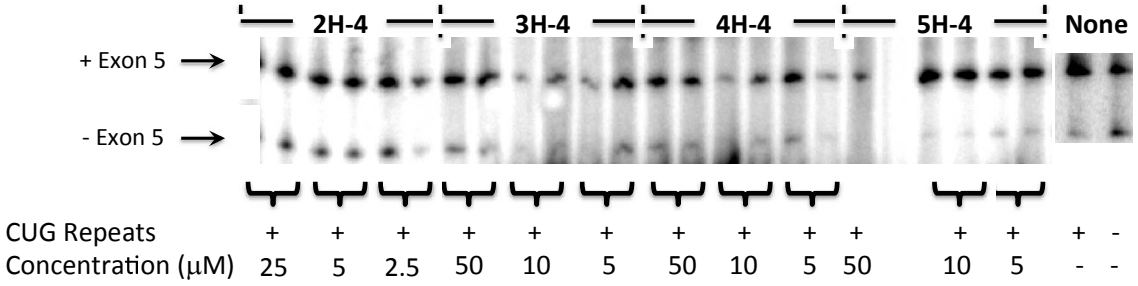


Figure S-1: Representative gel autoradiogram that shows the rationally designed **nH-4** compounds improve splicing defects in a cell culture model of DM1. HeLa cells were co-transfected with a DM1 mini-gene that encodes 960 interrupted CTG repeats (*3*) and a cTNT alternative splicing mini-gene.(*3*)

B. The **nH-4** compounds do not affect cTNT splicing in the absence of r(CUG) repeats

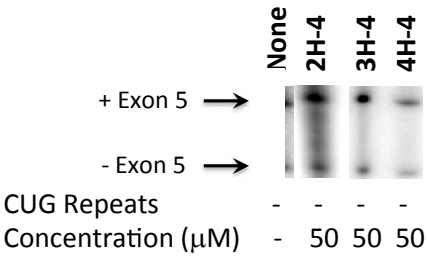


Figure S-2: Representative gel autoradiogram that shows the rationally designed **nH-4** compounds do not affect cTNT splicing when expanded r(CUG) repeats are absent. HeLa cells were co-transfected with a mini-gene that encodes five CTG repeats (*3*) and a cTNT alternative splicing mini-gene.(*3*)

C. The **nH-4** compounds do not affect the alternative splicing of a pre-mRNA that is not controlled by MBNL1 (*PLEKHH2*).

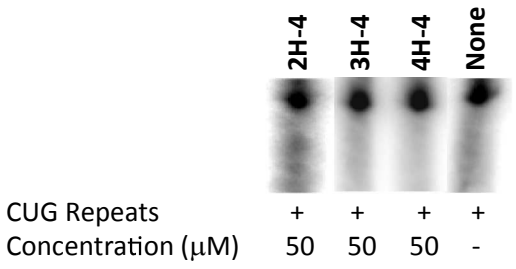


Figure S-3: Representative gel autoradiogram that shows the rationally designed **nH-4** compounds do not affect splicing of pre-mRNAs that are not controlled by MBNL1. HeLa cells were co-transfected with a DM1 mini-gene that encodes 960 interrupted CTG repeats (*3*) and a *PLEKHH2* alternative splicing mini-gene.(*4*)

REFERENCES

1. Pushechnikov, A., Lee, M. M., Childs-Disney, J. L., Sobczak, K., French, J. M., Thornton, C. A., and Disney, M. D. (2009) Rational design of ligands targeting triplet repeating transcripts that cause RNA dominant disease: application to myotonic muscular dystrophy type 1 and spinocerebellar ataxia type 3, *J. Am. Chem. Soc. 131*, 9767-9779.
2. Chan, T. R., Hilgraf, R., Sharpless, K. B., and Fokin, V. V. (2004) Polytriazoles as copper(I)-stabilizing ligands in catalysis, *Org. Lett. 6*, 2853-2855.
3. Philips, A. V., Timchenko, L. T., and Cooper, T. A. (1998) Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy, *Science 280*, 737-741.
4. Warf, M. B., Nakamori, M., Matthys, C. M., Thornton, C. A., and Berglund, J. A. (2009) Pentamidine reverses the splicing defects associated with myotonic dystrophy, *Proc. Natl. Acad. Sci. U. S. A. 106*, 18551-18556.