Supporting Information

Duplex-Forming Oligocarbamates with Tunable Nonbonding Sites

R. Kenton Weigel[1] and Christopher A. Alabi[1*]

[1]Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA

*Corresponding author
caa238@cornell.edu

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Materials

All solvents and aqueous ammonium hydroxide solution (28-30 w/w%) were obtained from Fisher Scientific and used as received. The following reagents were also used as received: vanillin (99%, BeanTown), 4-nitrophenyl chloroformate (97%, Oakwood), triethylamine (Acros Organics), guaiacol (TCI), pentafluorophenyl carbonate (Thermo Scientific), thymine (97%, Thermo Scientific), pyridine (Fisher), benzoyl chloride (BeanTown), 2-(boc-amino)ethyl bromide (MilliporeSigma), potassium carbonate (Fisher), trifluoroacetic acid (≥ 99%, BeanTown), 2-chloro-4,6-diamino-1,3,5-triazine (Aldrich), aminoethanethiol (TCI), di-tert-butyl dicarbonate (BeanTown), potassium hydroxide(Thermo Scientific), 3,4-dimethoxybenzaldehyde (Aldrich), methylamine (2 M in THF, Aldrich), glacial acetic acid (MilliporeSigma), chloroform-d (Cambridge Isotopes), dimethyl sulfoxide-d_6 (Cambridge Isotopes).

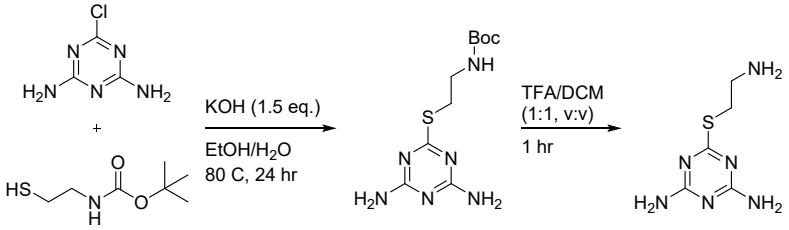
Methods

NMR spectroscopy was conducted on a Bruker 400 MHz NMR spectrometer equipped with a cryoprobe for ^{19}F experiments and a Varian INOVA 600 MHz spectrometer for DOSY and ^{1}H-NMR titration experiments. Column chromatography was carried out with a Teledyne CombiFlash® Rf+ Lumen automated flash chromatography system. ITC experiments were performed using a TA Instruments Low Volume NanoITC.

Liquid chromatography-mass spectrometry (LC-MS) experiments were carried out on an Agilent 1200 Series LC/Thermo Fisher LTQ XL MSD equipped with a Agilent EC C18, 2.7 μm, 120 Å LC column (3 x 100 mm, reversed phase), UV diode-array detector monitoring 210 nm, 230 nm, 260 nm, 360 nm, and 505 nm wavelengths, and Agilent multimode source. Water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) were used as LC-MS eluents. Compounds were eluted at a flow rate of 0.6 mL/min with a linear gradient of 5% to 100% solvent B over 10 minutes, then constant at 100% solvent B for 2 minutes before equilibrating the column back to 5% solvent B over 3 min. All masses were detected in positive ion mode.

Synthesis and Characterization

Synthesis of primary-amine-terminated diaminotriazine monomer (**2**).



2-chloro-4,6-diamino-1,3,5-triazine (2.00 g, 1 eq.) was suspended in acetonitrile. Potassium hydroxide (1.15 g, 1.5 eq.) was added to the mixture. 2-(boc-amino)ethanethiol (3.67 g, 1.5 eq.) was also added. The mixture was refluxed for 24 hours. The reaction was filtered and the filtrate was dried under reduced pressure. The filtration residue was extracted with dichloromethane (4 x 45 mL), and the organic portion was dried under reduced pressure. Once dried, the extract was combined with the filtrate and purified via silica-gel chromatography (hexanes, ethyl acetate). The purified intermediate was dissolved in trifluoroacetic acid/dichloromethane (1:1, v:v) and stirred at RT for 1 hour. The reaction was dried under reduced pressure to yield **7** as a white solid (overall yield = 50%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (s, 3H), 6.93 (s, 4H), 3.16 (h, J = 5.8 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 177.27, 162.63, 159.28, 159.00, 158.73, 158.45, 119.74, 117.40, 115.06, 112.73, 39.06, 27.05.

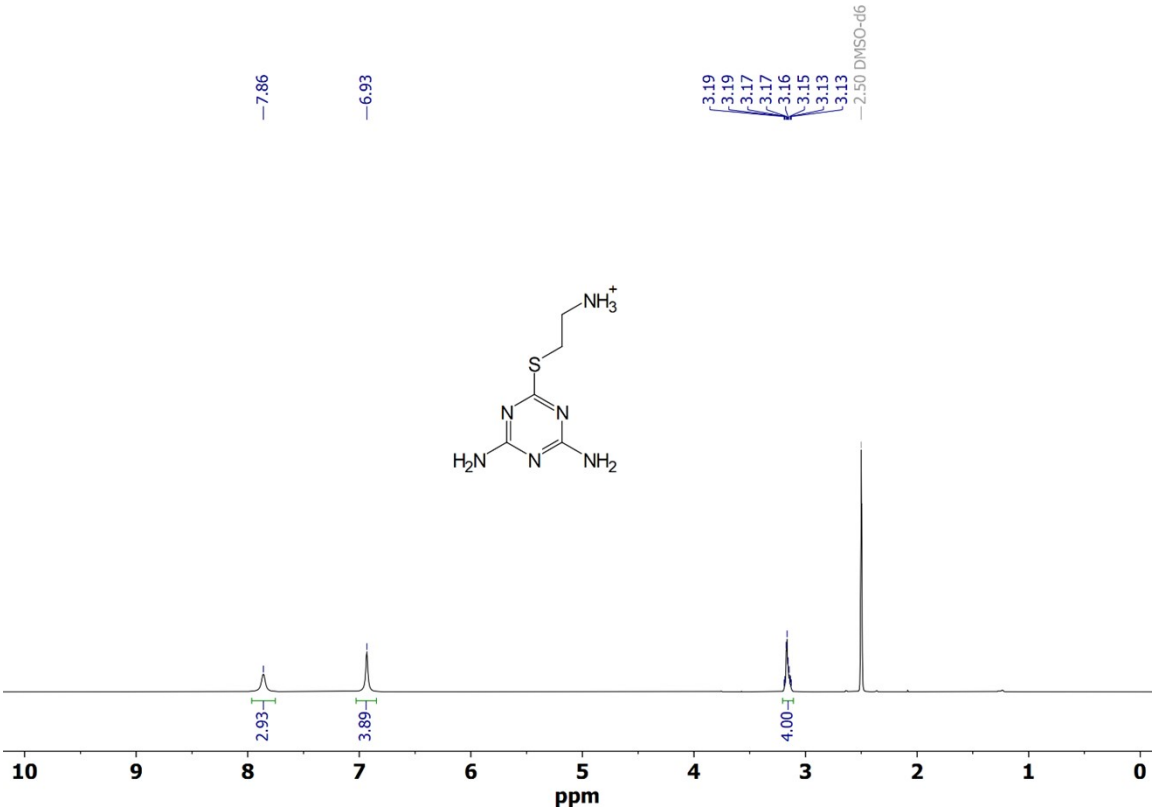


Figure S1. ^{1}H-NMR of primary-amine-terminated diaminotriazine (2) in DMSO-d_6.

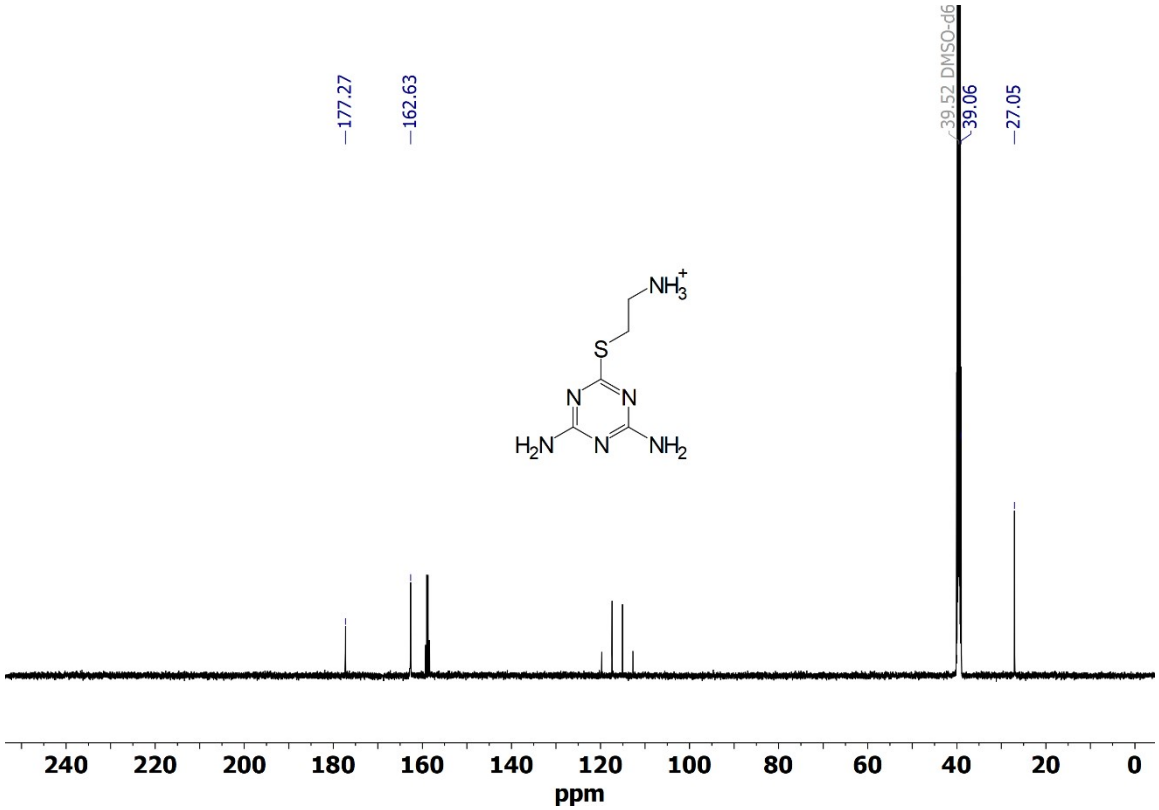
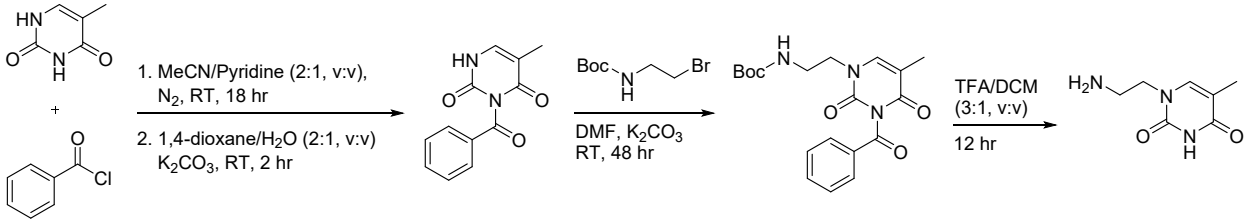


Figure S2. ^{13}C-NMR of primary-amine-terminated diaminotriazine (2) in DMSO-d_6. Additional peaks in this spectrum are due to presence of trifluoroacetic acid in sample.

Synthesis of primary-amine-terminated thymine monomer (**3**).



N_3-benzoyl thymine was synthesized following a procedure from Zhou and Shevlin.[1] Acetonitrile (50 mL) and pyridine (25 mL) were added to thymine (5.0 g, 1 eq.). The suspension was cooled to 0 °C in an ice bath and benzoyl chloride (10.0 mL, 2.5 eq.) was added to the mixture. The reaction was removed from the ice bath and stirred at RT for 18 hours. The reaction was partitioned between dichloromethane (125 mL) and water (75 mL). The organic portion was dried under reduced pressure and resuspended in 1,4-dioxane (75 mL) and water (40 mL). Potassium carbonate (3.0 g, 0.55 eq.) was added to solution and the reaction was stirred at RT for 3 hours. The reaction was dried under reduced pressure and partitioned between dichloromethane and water. The organic fraction was dried under reduced pressure to give N_3-benzoyl thymine (step 1 yield = 8.3205 g, 91%). N_3-benzoyl thymine (0.5 g, 1 eq.) was dissolved in 9 mL DMF. Potassium carbonate (0.33 g, 1.1 eq.) and 2-(boc-amino)ethyl bromide (0.73 g, 1.5 eq.) were added to solution, and the reaction was stirred at RT for 48 hours. 6 mL Ethyl acetate and 30 mL water were added to the reaction. The mixture was

refrigerated to precipitate the, which was isolated via vacuum filtration (step 2 yield = 1.8948 g, 81%). Finally, the doubly-protected thymine was dissolved in 12 mL trifluoroacetic acid/dichloromethane (3:1, v:v) and stirred for 12 hours. The reaction was dried by rotary evaporation. 10 mL water was added to the residue, and the organic was washed repeatedly with ethyl acetate (6 x 5 mL) to give **8.** The aqueous layer was lyophilized to yield a white solid (step 3 yield = 88%, overall yield = 65%). ^{1}H NMR (500 MHz, DMSO) δ 11.31 (s, 1H), 7.99 (s, 3H), 7.45 (s, *J* = 1.4 Hz, 1H), 3.87 (t, *J* = 5.8 Hz, 2H), 3.08 (d, *J* = 11.6 Hz, 1H), 1.75 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 164.48, 151.51, 141.18, 108.95, 45.22, 37.95, 12.07.

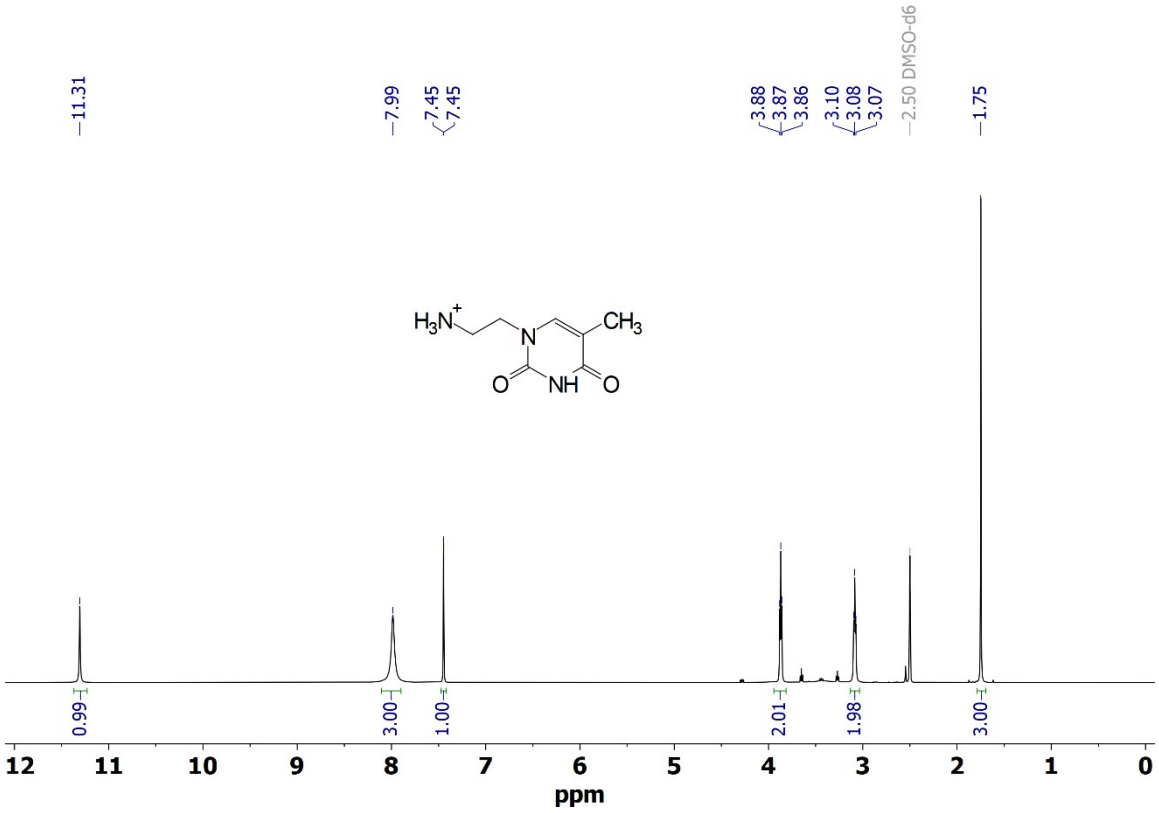


Figure S3. ^{1}H-NMR of primary amine-terminated thymine monomer (3) in DMSO-d_6.

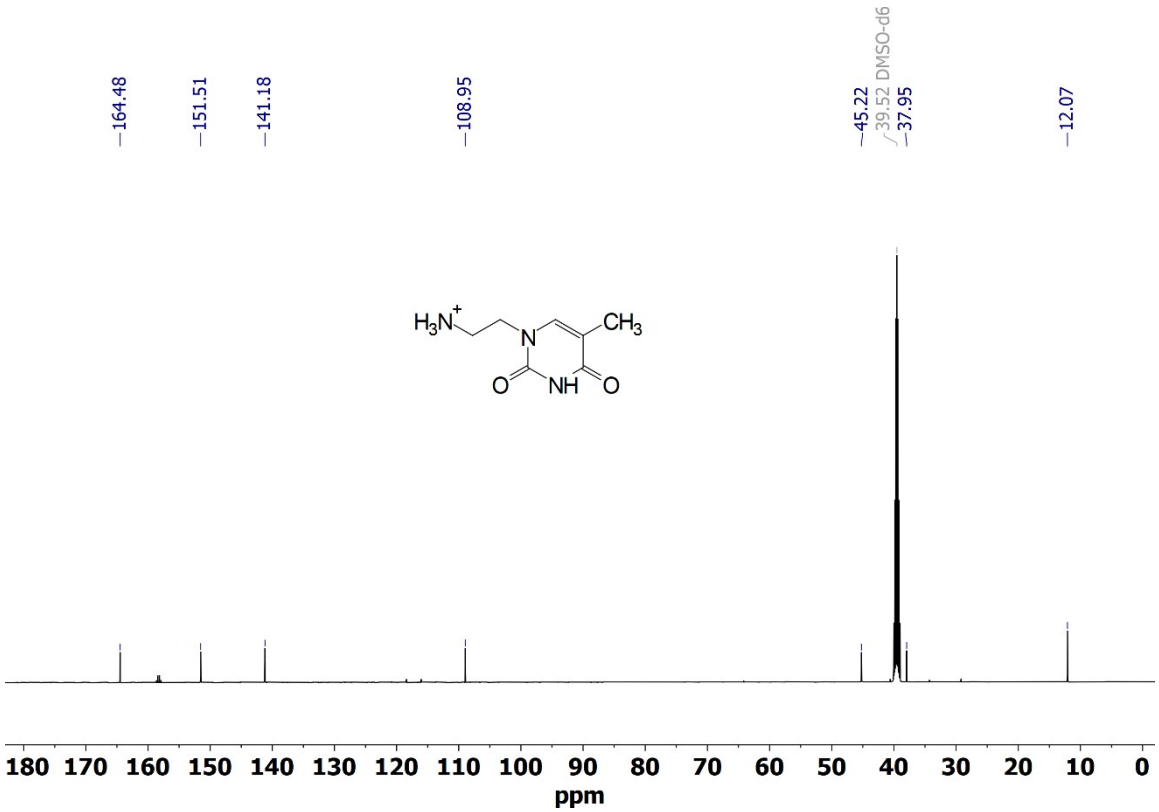
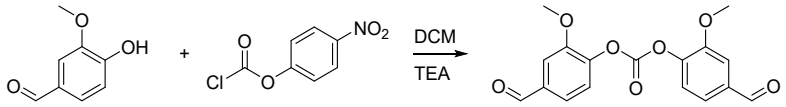


Figure S4. ^{13}C-NMR of primary amine-terminated thymine monomer (3) in DMSO-d_6.

Synthesis of divanillin carbonate (**6**).



Divanillin carbonate was synthesized according to the procedure developed by Hoff et al.[2] Vanillin (92.3 g, 0.61 mol, 5.0 equiv.) and triethylamine (25.3 mL, 0.18 mol, 1.5 equiv.) were dissolved in dichloromethane (250 mL) in a 1-L round-bottom flask equipped with an addition funnel. Once cooled to 0 °C, a solution of 4-Nitrophenyl chloroformate (24.4 g, 0.18 mol, 1.0 equiv.) dissolved in dichloromethane (100 mL) was added dropwise over 45 minutes. The reaction was stirred for 18 h and then washed with 1 M HCl (1 x 200 mL), saturated $NaHCO_3$ (4 x 250 mL), and brine (1 x 300 mL). The organic layer was dried over Na_2SO_4 before the solvent was removed by rotary evaporation. The yellow solid was then triturated with diethyl ether (4 x 250 mL) to yield divanillin carbonate (30.0 g, 75%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.60 – 7.42 (m, 6H), 3.99 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.99, 152.01, 149.96, 144.72, 135.78, 124.73, 122.96, 111.35, 56.46.

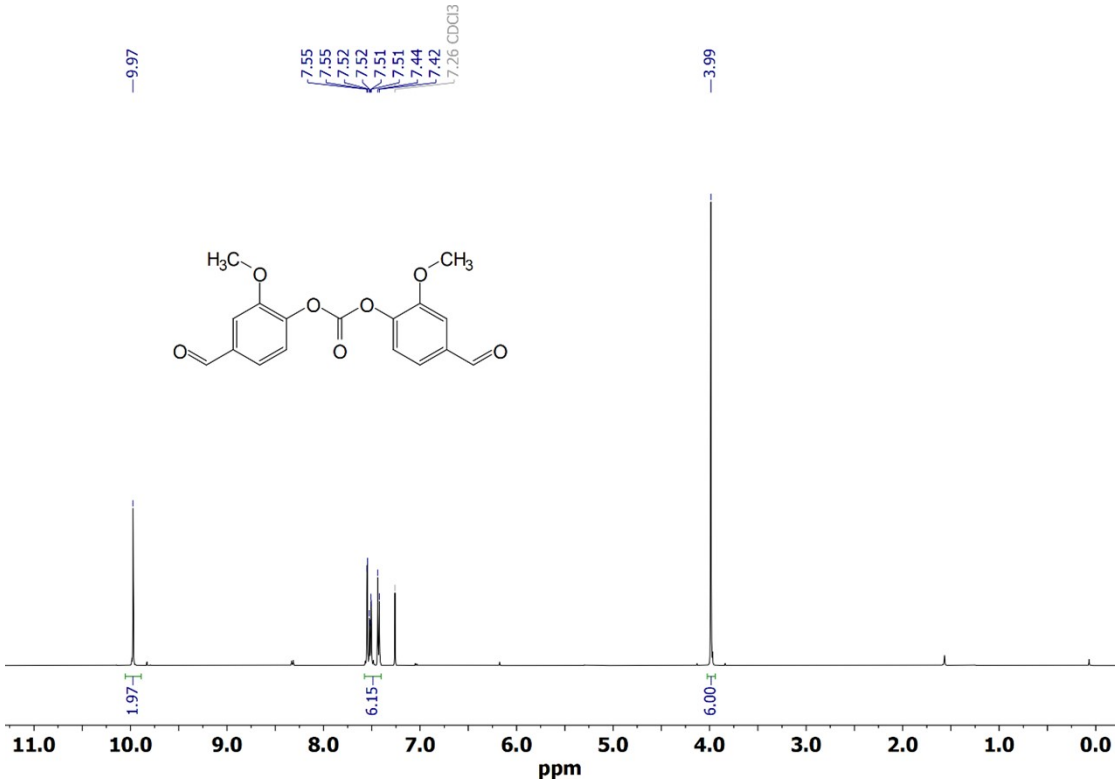


Figure S5. ^{1}H-NMR of divanillin carbonate in $CDCl_3$.

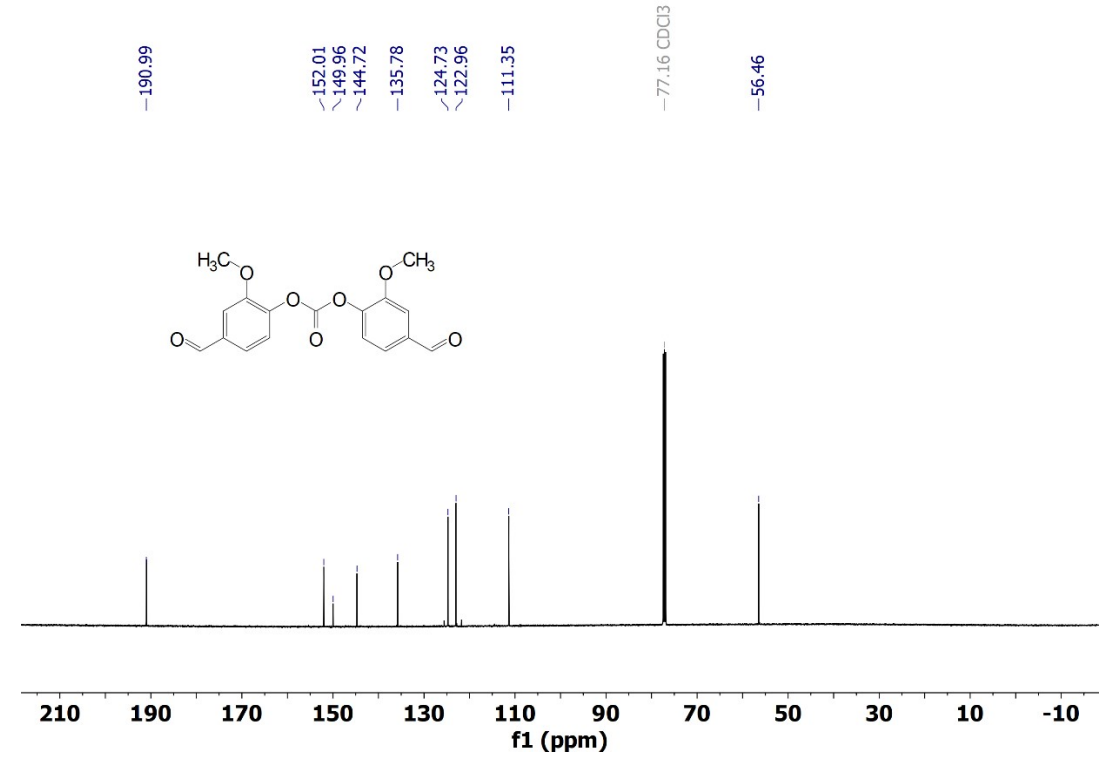
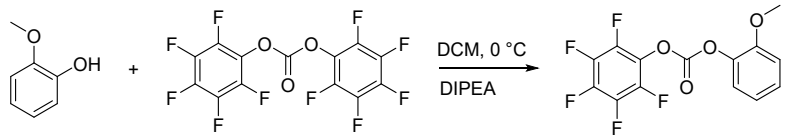


Figure S6. ^{13}C-NMR of divanillin carbonate in $CDCl_3$.

Synthesis of pentafluorophenyl guaiacol carbonate (**10**).



0.28 g guaiacol (1 eq.) was dissolved in 2 mL dichloromethane. N,N-diisopropylethylamine (0.594 mL, 1.5 eq.) was added to solution and the solution was cooled to 0° C. Bis(pentafluorophenyl) carbonate (3.6 g, 4 eq.) were dissolved in 5 mL dichloromethane and added to the guaiacol solution dropwise. The reaction was stirred at 0 °C for 15 minutes. The reaction was dried via rotary evaporation and purified via flash chromatography (hexanes/ethyl acetate). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.32 – 7.21 (m, 2H), 7.05 – 6.95 (m, 2H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.88, 149.61, 142.52, 140.54, 140.03, 139.07, 137.09, 128.14, 121.85, 120.93, 112.91, 56.13. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -161.96 – -161.91 (m), -161.87 (d, *J* = 4.8 Hz), -161.83 – -161.78 (m), -157.12 (t, *J* = 21.6 Hz), -153.01 – -152.91 (m), -152.90 – -152.84 (m).

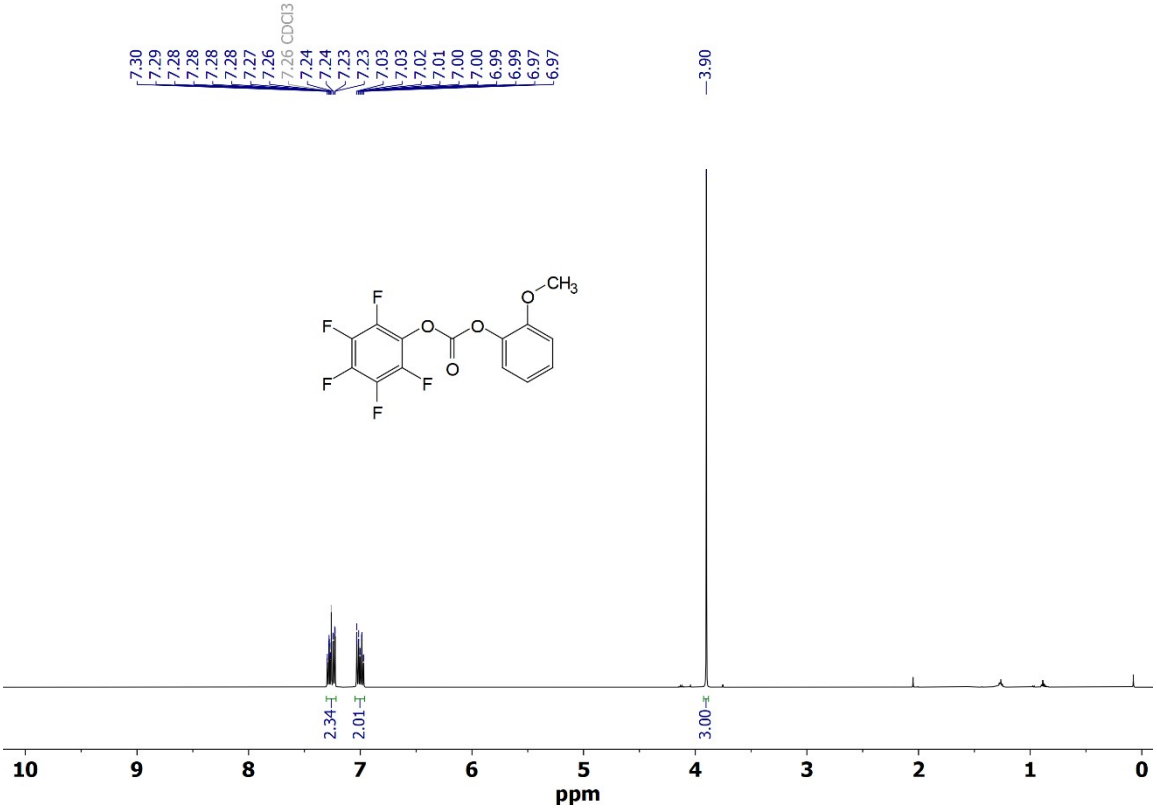


Figure S7. ^{1}H-NMR of pentafluorophenyl guaiacol carbonate in $CDCl_3$.

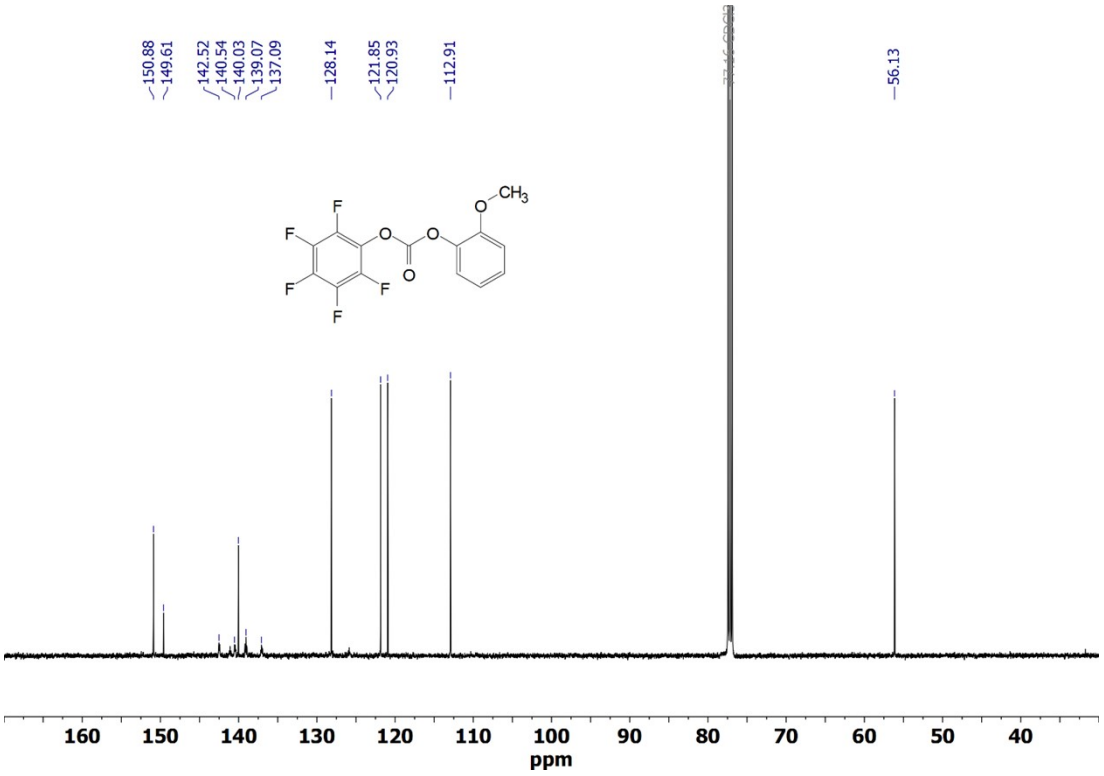


Figure S8. ^{13}C-NMR of pentafluorophenyl guaiacol carbonate in $CDCl_3$.

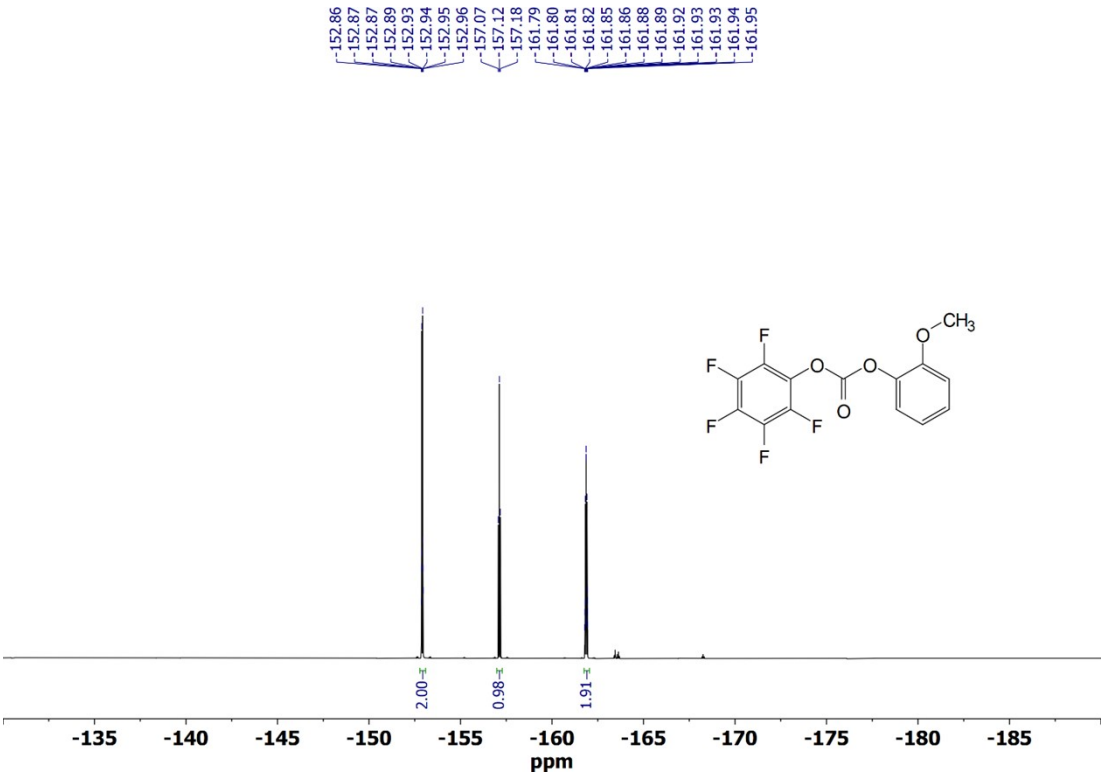


Figure S9. ^{19}F-NMR of pentafluorophenyl guaiacol carbonate in $CDCl_3$.

General Reductive Amination Procedure. Methanol was added to primary-amine terminated monomer (1.5 eq. if **2** or **3**, 3 eq. if methylamine). If the monomer was **2** or **3**, triethylamine (1.5 eq.) was added to the mixture. The solution was added to aldehyde-terminated oligomer precursor (1 eq.). Acetic acid (0.1 eq.) was added to the mixture. The reaction was stirred at 60 °C in the microwave reactor. Reactions were stirred for 20 minutes if the oligomer contained diaminotriazine pendant groups, and for 40 minutes if the oligomer contained thymine pendant groups. The reaction was allowed to cool to below 35 °C prior to reduction with sodium borohydride. Sodium borohydride (1 eq.) was added to solution and the reaction was stirred at RT for 1 hour. The reaction was then quenched with water and extracted into dichloromethane. The combined organic portions were washed with water and dried under rotary evaporation to yield secondary-amine-terminated oligomer precursor.

General Carbamation Procedure. Secondary-amine-terminated oligomer precursor and divanillin carbonate (1.1 eq.) were combined. N,N-dimethylformamide was added to the mixture (concentration of secondary-amine-terminated precursor was 300 mM). Triethylamine (1.1 eq.) was added to the mixture. The reaction was heated in the microwave reactor. The reaction was concentrated under reduced pressure. The dry reaction was resuspended in dichloromethane and ammonium hydroxide (1:1, v:v) and stirred at RT for 1 hour. The reaction was extracted with dichloromethane. The combined organic portions were washed with 1 M HCl and saturated sodium bicarbonate. The organic portion was dried under rotary evaporation to yield aldehyde terminated oligomer precursor.

General Procedure for End-Capping Reaction. The secondary-amine-terminated precursor was dissolved in acetonitrile and cooled to 0 °C. Triethylamine and pentafluorophenyl guaiacol carbonate were added to solution. The reaction was stirred at 0 °C for 15 minutes. The reaction was dried under reduced pressure and purified via flash chromatography (dichloromethane/methanol).

Deviations from the general procedures listed above are now described for each oligomer, where applicable.

Synthesis of **mDm**. Reductive amination reactions were stirred at room temperature for 6 hours instead of being heated in the microwave reactor. The first carbamation reaction used acetonitrile instead of DMF. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 6.81 (m, 13H), 5.86 (d, *J* = 64.2 Hz, 4H), 4.78 – 4.45 (m, 6H), 3.94 – 3.79 (m, 15H), 3.65 (dt, *J* = 28.7, 7.6 Hz, 2H), 3.30 (dt, *J* = 41.8, 7.4 Hz, 2H), 3.10 – 2.91 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.44, 155.29, 155.11, 154.66, 154.42, 151.84, 151.74, 149.32, 148.74, 140.71, 140.53, 139.84, 136.12, 129.69, 129.61, 126.57, 123.34, 123.15, 120.89, 120.43, 120.32, 120.20, 120.02, 119.83, 112.59, 112.48, 111.98, 111.47, 111.23, 56.25, 56.20, 56.09, 52.90, 52.84, 52.10, 47.42, 34.73, 34.54, 34.21, 28.21, 27.85.

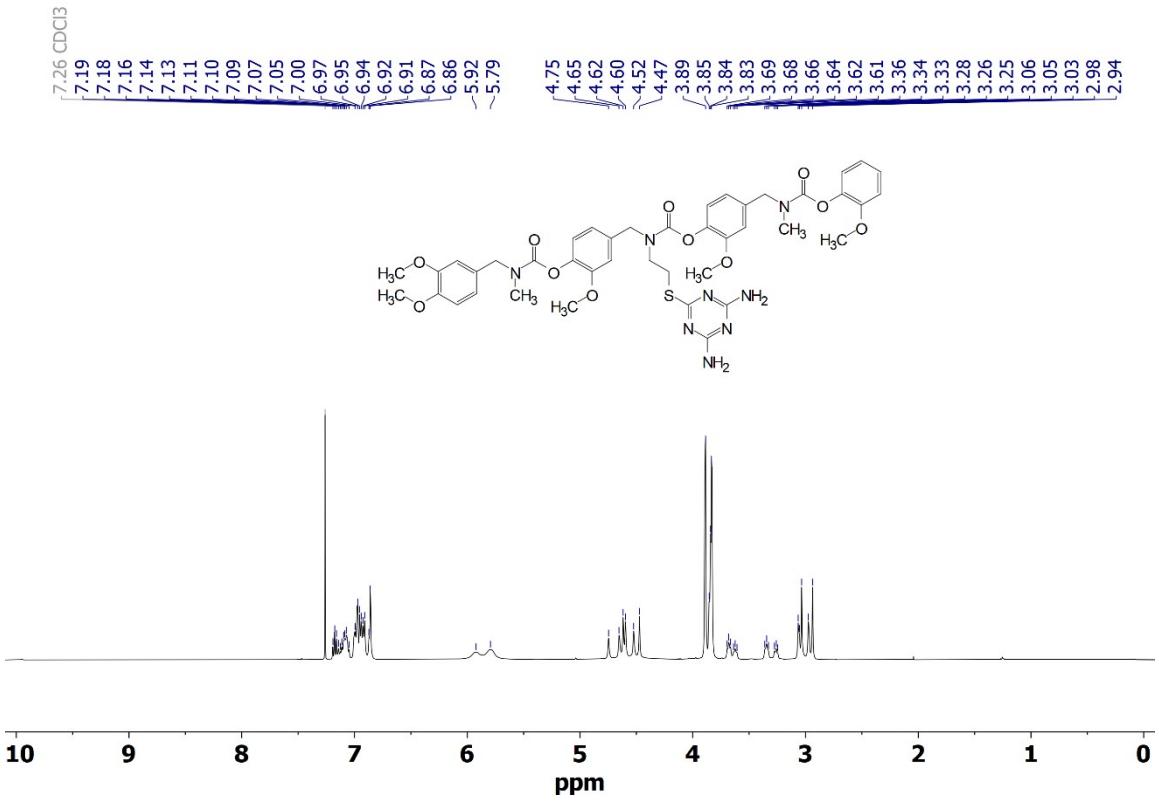


Figure S10. ^{1}H-NMR of **mDm** in $CDCl_3$.

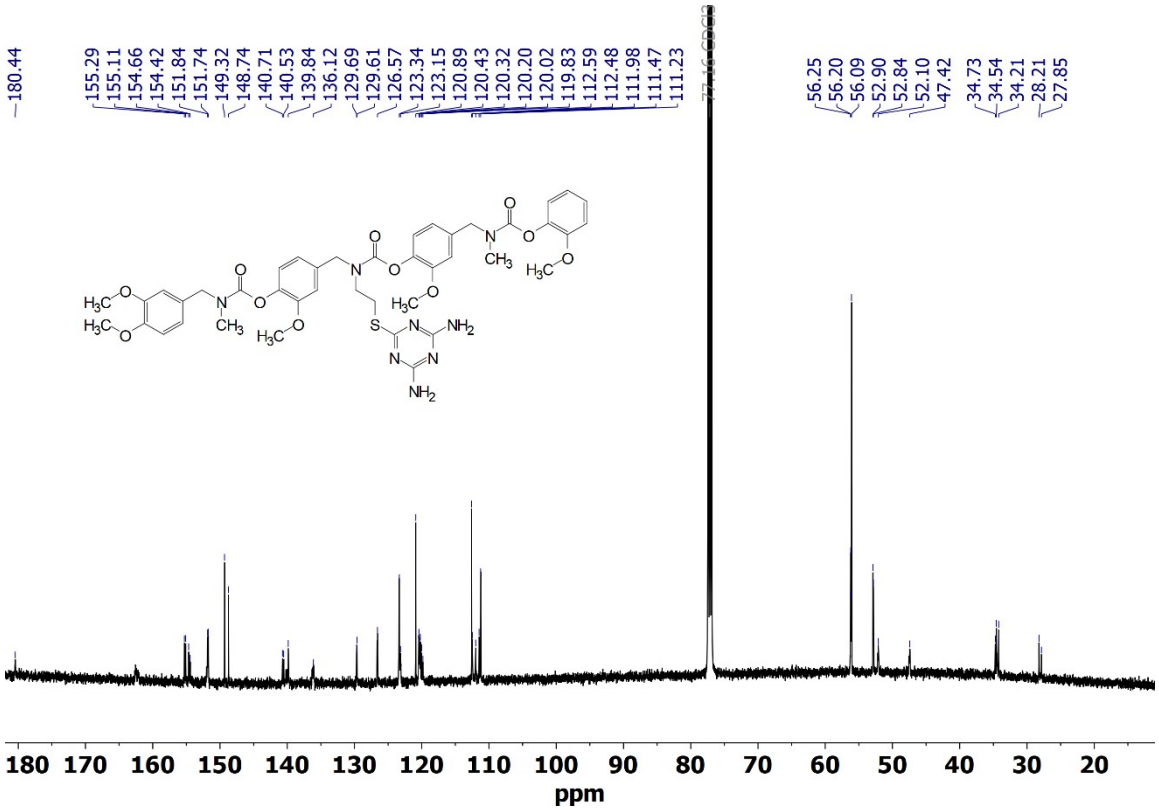


Figure S11. ^{13}C-NMR of **mDm** in $CDCl_3$.

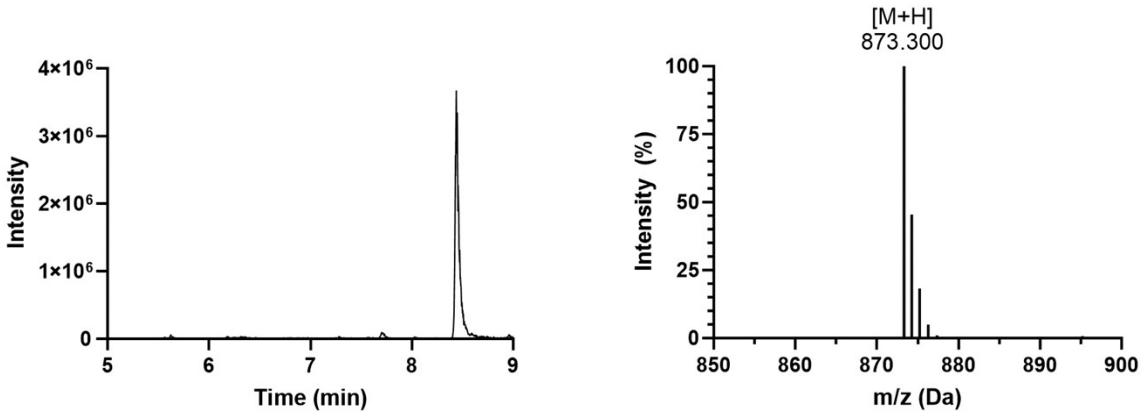


Figure S12. Total Ion Chromatogram and MS spectrum of **mDm**.

Synthesis of **mmD**. Reductive amination reactions were stirred at room temperature for 6 hours instead of being heated in the microwave reactor. Both carbamation reactions used acetonitrile instead of DMF. Triethylamine was not used in the end-capping reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 6.83 (m, 13H), 5.10 (d, J = 36.0 Hz, 4H), 4.78 – 4.46 (m, 6H), 3.94 – 3.80 (m, 15H), 3.67 (dt, J = 24.9, 7.5 Hz, 2H), 3.31 (dt, J = 41.5, 7.1 Hz, 2H), 3.00 (dd, J = 41.7, 9.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.60, 165.57, 165.48, 155.23, 154.60, 154.48, 152.05, 151.95, 151.75, 151.70, 149.32, 148.70, 140.57, 140.50, 140.10, 139.94, 136.31, 135.71, 135.62, 129.91, 129.78, 126.72, 123.31, 120.96, 120.60, 120.31, 120.22, 119.87, 119.80, 116.51, 112.64, 112.46, 111.92, 111.55, 111.21, 111.13, 56.19, 56.08, 52.94, 52.84, 52.02, 51.92, 50.97, 47.51, 47.37, 34.81, 34.50, 34.45, 34.14, 28.03, 27.59.

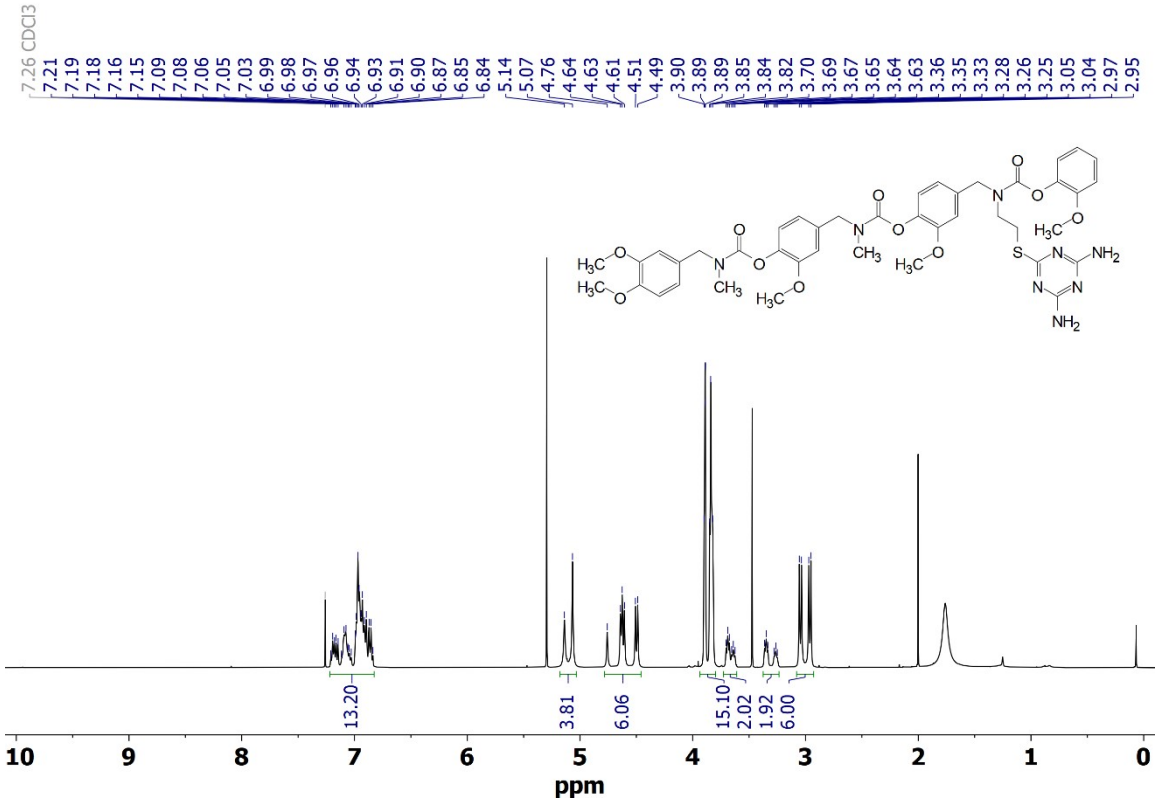


Figure S13. ^{1}H-NMR in $CDCl_3$ of **mmD**.

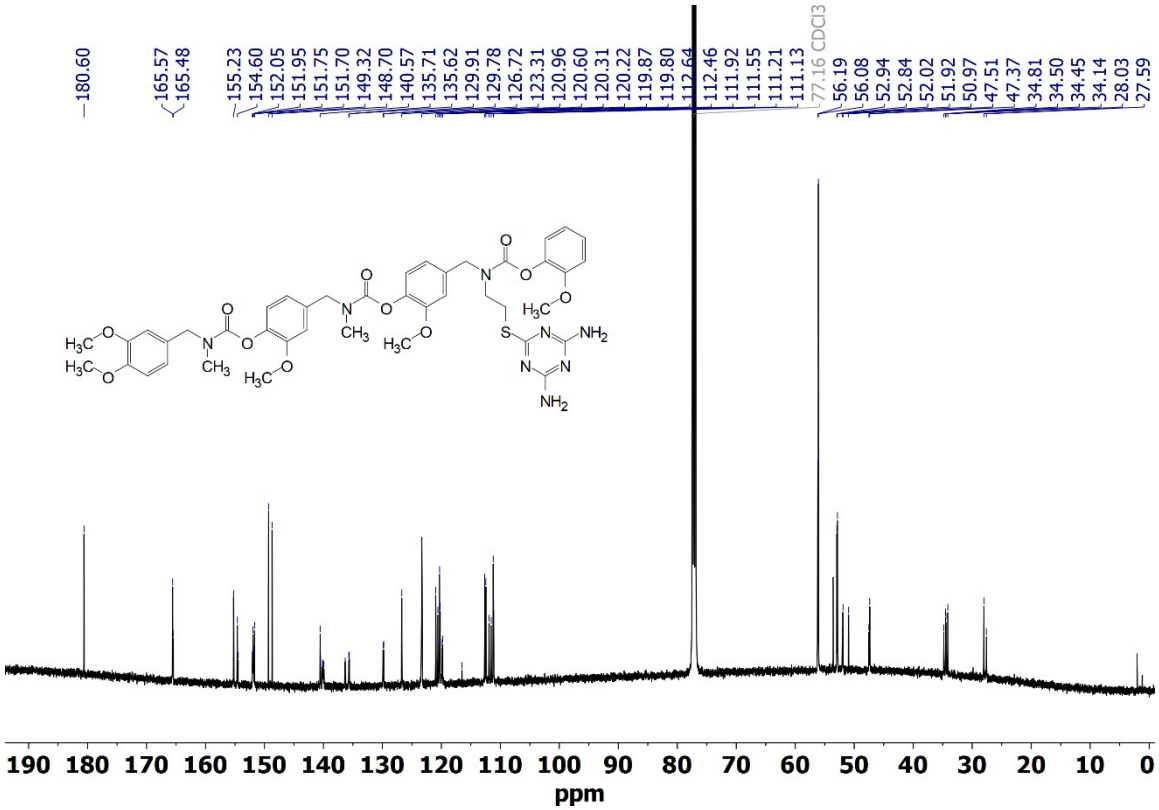


Figure S14. ^{13}C-NMR in $CDCl_3$ of **mmD**.

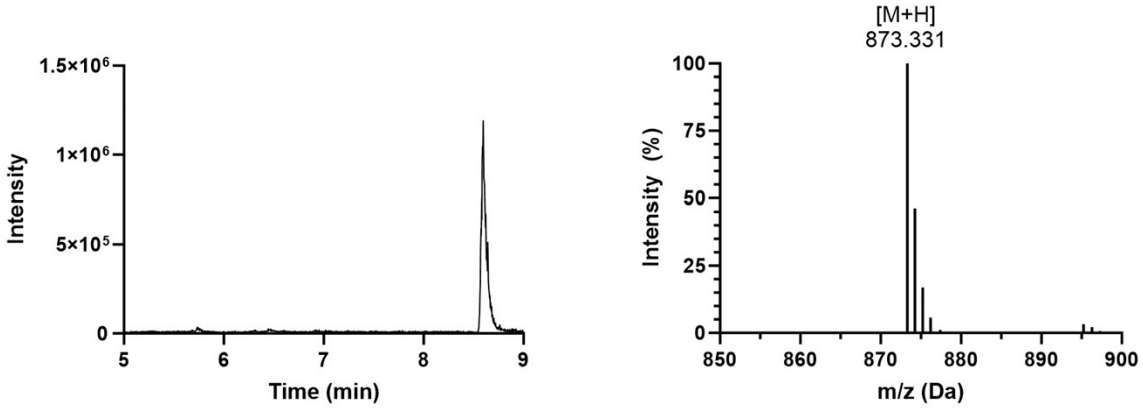


Figure S15. Total ion chromatogram and MS spectrum of **mmD**.

Synthesis of **mDD**. The first and second reductive amination reactions were stirred at room temperature for 6 hours instead of being heated in the microwave reactor. The first carbamation reaction used acetonitrile instead of DMF. The third reductive amination was done in methanol/DMSO (9:1, v:v).. The end-capping reaction was carried out in DMF rather than acetonitrile. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 – 6.79 (m, 13H), 5.24 – 4.99 (m, 8H), 4.81 – 4.43 (m, 6H), 3.92 – 3.78 (m, 15H), 3.67 (d, *J* = 26.9 Hz, 4H), 3.41 – 3.22 (m, 4H), 2.99 (d, *J* = 44.6 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 180.12, 180.08, 179.99, 165.30, 155.32, 155.20, 154.71, 151.85, 151.74, 151.63, 151.57, 151.49, 149.15, 148.56, 140.24, 140.12, 139.99, 139.79, 139.72, 139.62, 136.32, 136.16, 136.06, 135.95, 129.58, 129.51, 126.75, 123.13, 123.03, 120.81, 120.77, 120.39, 120.17, 119.88, 119.73, 112.49, 112.31, 111.89, 111.36, 111.22, 111.17, 55.96, 55.91, 55.85, 52.70, 52.61, 51.74, 51.54, 47.73, 47.42, 47.18, 46.98, 34.28, 34.00, 27.79, 27.14.

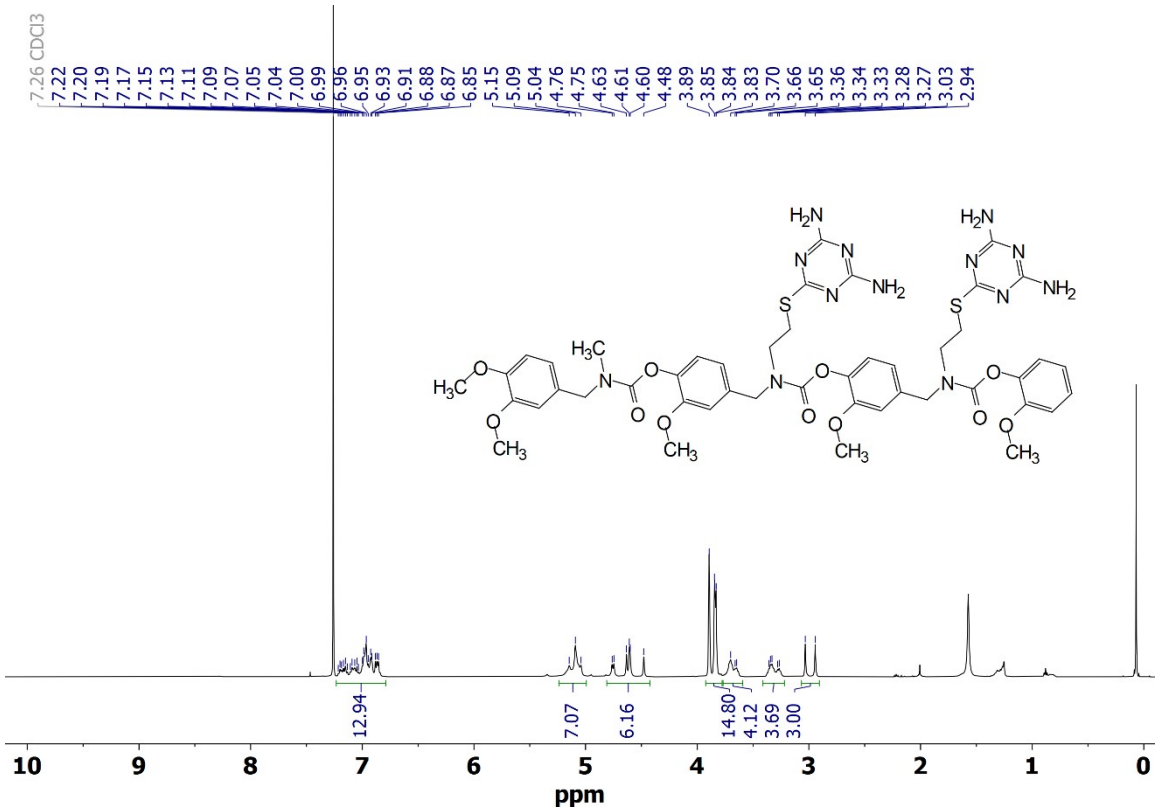


Figure S16. ^{1}H-NMR of **mDD** in $CDCl_3$.

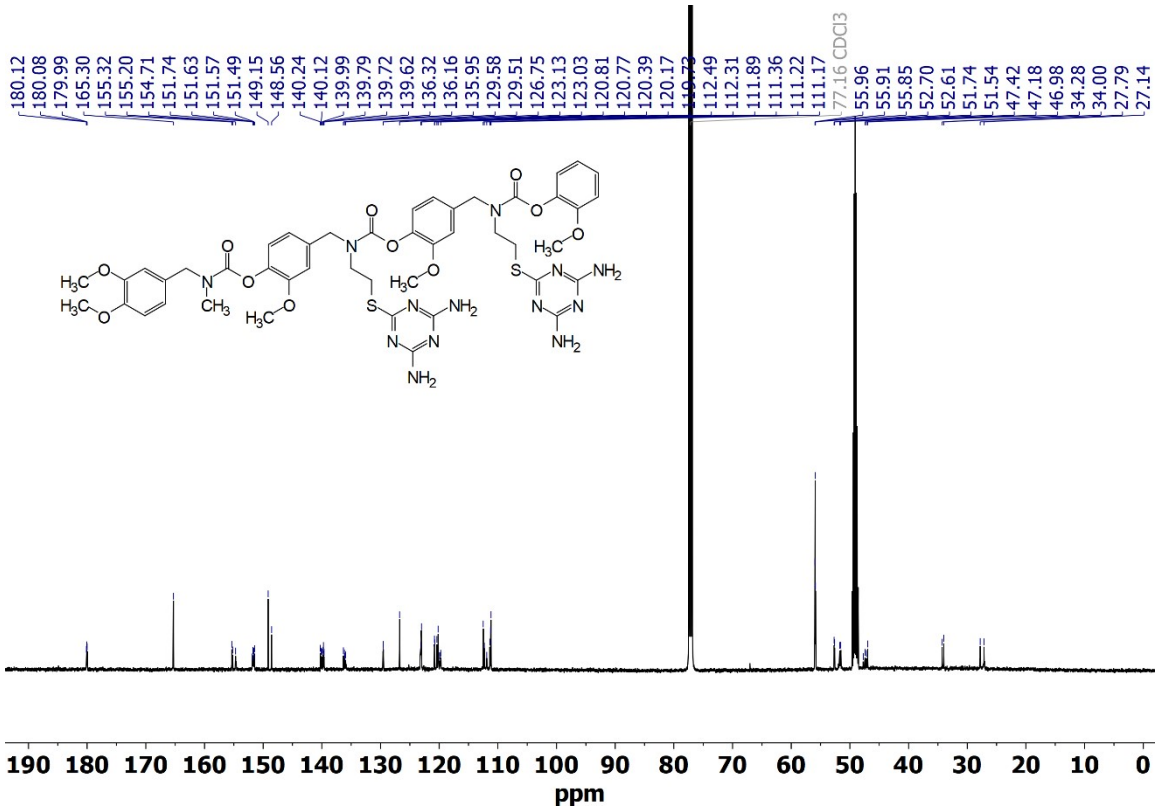


Figure S17. ^{13}C-NMR of **mDD** in 0.9:0.1 $CDCl_3$:MeOD.

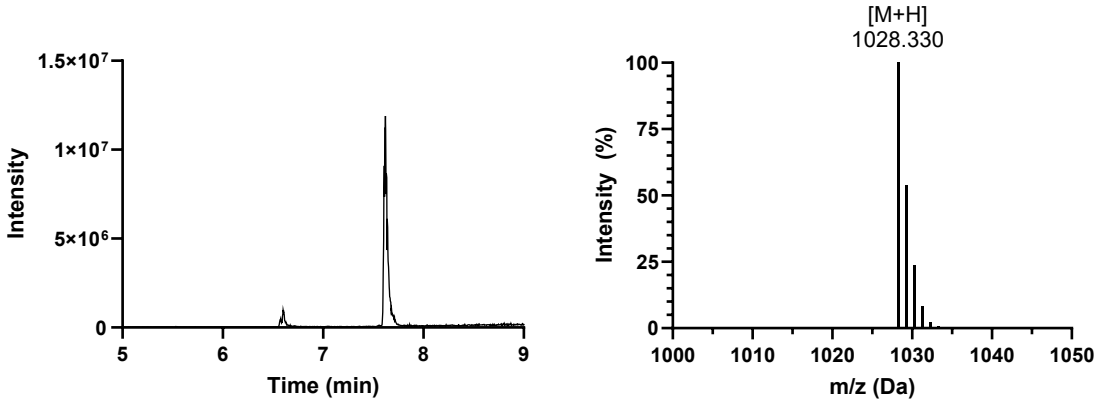


Figure S18. Total ion chromatogram and MS spectrum of **mDD**.

Synthesis of **DmD**. The first reductive amination reaction was not purified via aqueous washes; the crude reaction was taken directly to the first carbamation. The third reductive amination reaction was performed in methanol/DMSO (3:1, v:v) without acetic acid. Each carbamation reaction followed the general protocol. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 6.80 (m, 13H), 5.50 – 5.00 (m, 8H), 4.84 – 4.40 (m, 6H), 3.92 – 3.78 (m, 15H), 3.73 – 3.58 (m, 4H), 3.40 – 3.19 (m, 4H), 3.17 – 2.95 (m, 3H). ^{13}C NMR (126 MHz, MeOD) δ 180.19, 164.99, 164.88, 164.59, 155.31, 155.16, 154.69, 154.65, 154.56, 151.82, 151.71, 151.58, 151.49, 150.67, 149.11, 148.63, 140.26, 140.13, 139.91, 139.79, 139.69, 136.23, 136.03, 135.76, 130.03, 129.79, 126.74, 123.07, 120.81, 120.76, 120.58, 120.22, 120.04, 119.84, 112.49, 112.39, 112.20, 111.96, 111.83, 111.62, 111.29, 111.23, 111.16, 55.99, 55.96, 55.93, 55.91, 55.86, 52.78, 51.84, 51.73, 51.62, 51.48, 47.47, 47.31, 46.92, 46.82, 34.61, 34.30, 27.83, 27.09.

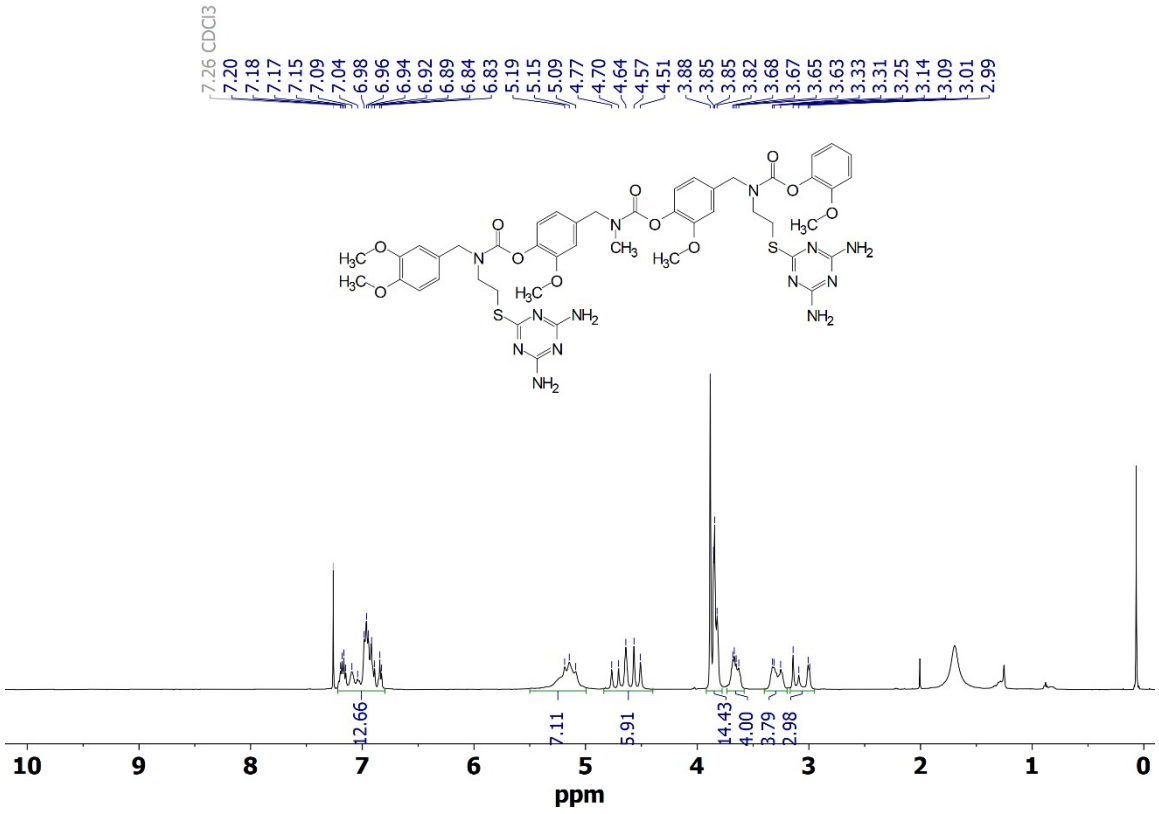


Figure S19. ^{1}H-NMR of **DmD** in $CDCl_3$.

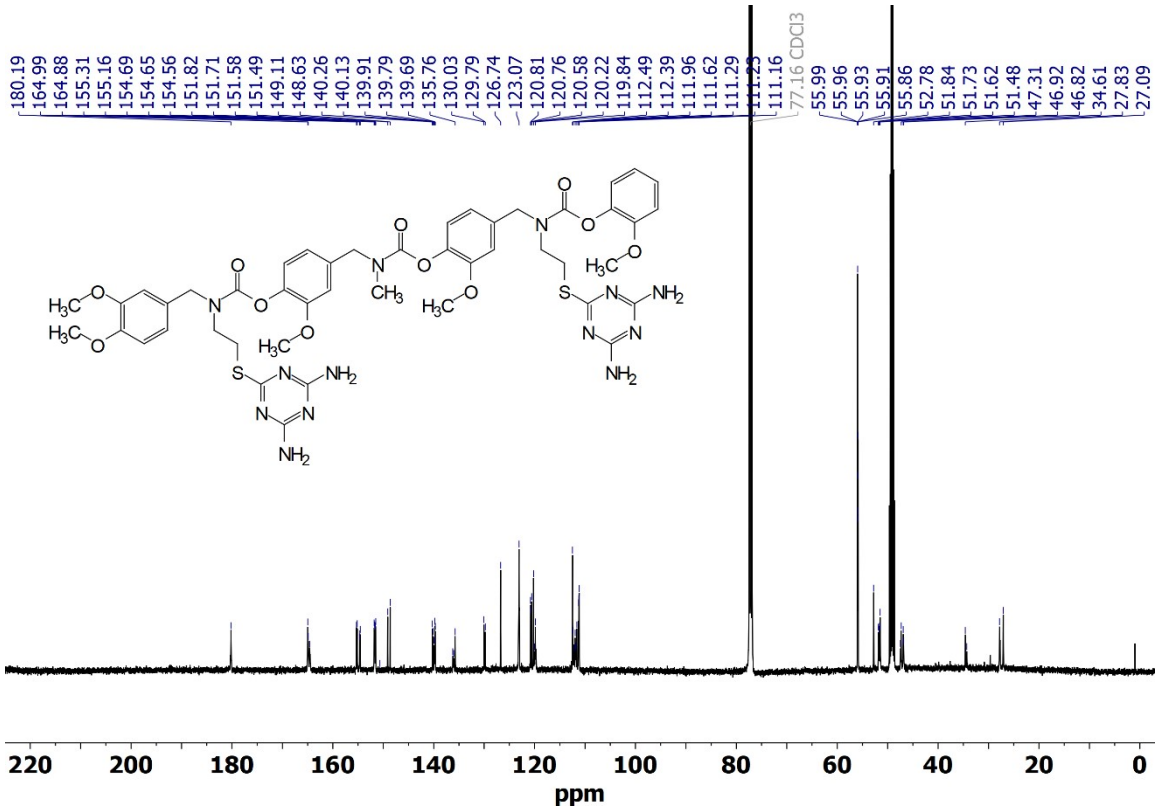


Figure S20. ^{13}C-NMR of **DmD** in 0.9:0.1 $CDCl_3$:MeOD.

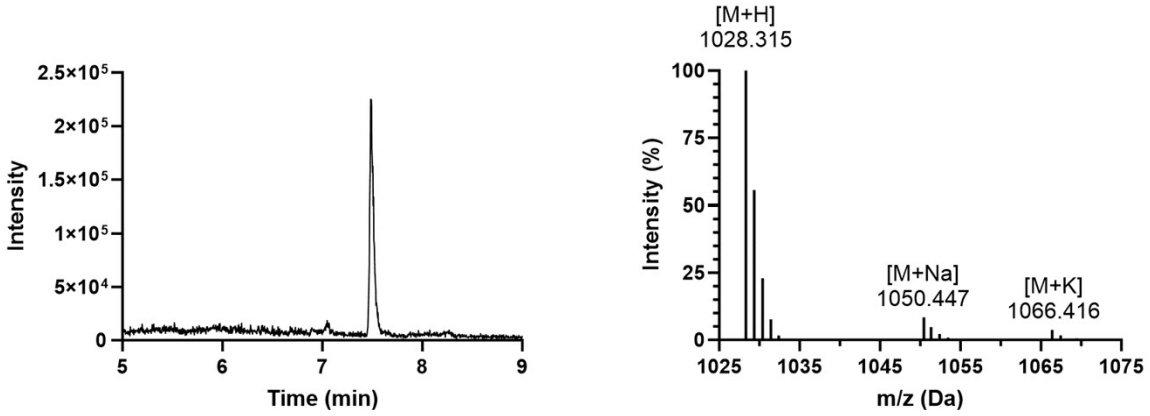


Figure S21. Total ion chromatogram and MS spectrum of **DmD**.

Synthesis of **DDD**. The first reductive amination reaction was not purified; the crude reaction was taken directly to the first carbamation. The second and third reductive aminations were performed in methanol/DMSO (3:1, v:v). The reactions were lyophilized and purified via trituration with water. Following trituration, the residue was dried under reduced pressure and taken to the next reaction. Each carbamation reaction followed the general protocol, except the reactions were suspended in pure ammonium hydroxide, not a mixture of dichloromethane and ammonium hydroxide. The reaction was trituration with water. ^{1}H NMR (500 MHz, DMSO) δ 7.25 – 6.82 (m, 12H), 6.73 (s, 11H), 4.62 (dd, *J* = 69.6, 36.4 Hz, 6H), 3.86 – 3.68 (m, 15H), 3.59 – 3.36 (m, 6H), 3.30 – 3.08 (m, 6H). ^{13}C NMR (126 MHz, MeOD) δ 180.21, 164.35, 163.56, 155.16, 154.61, 152.13, 151.64, 151.53, 151.44, 149.10, 148.62, 140.18, 140.04, 139.74, 139.65, 136.35, 136.21, 136.03, 129.90, 129.67, 126.77, 123.01, 120.76, 120.51, 120.22, 120.06, 112.47, 112.29, 111.98, 111.55, 111.29, 111.23, 111.17, 55.93, 55.87, 55.83, 51.97, 51.67, 51.40, 46.72, 27.84, 27.19.

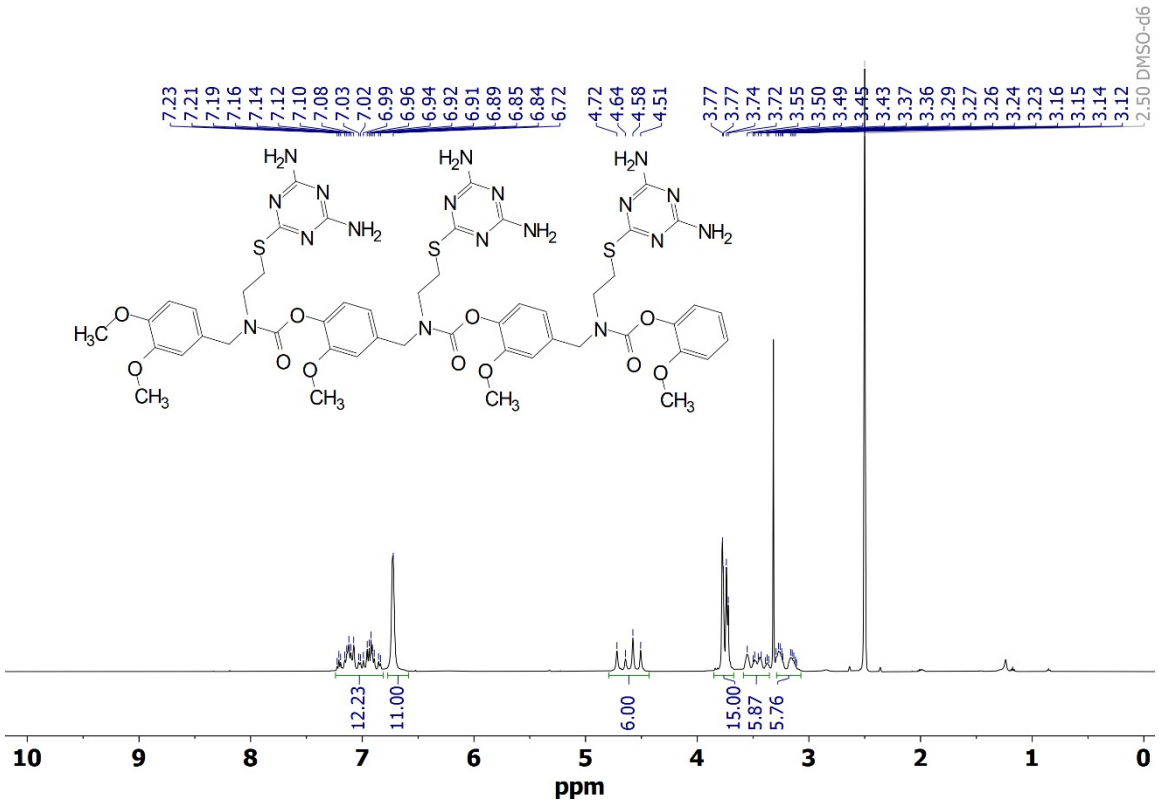


Figure S22. ^{1}H-NMR of **DDD** in DMSO-d_6.

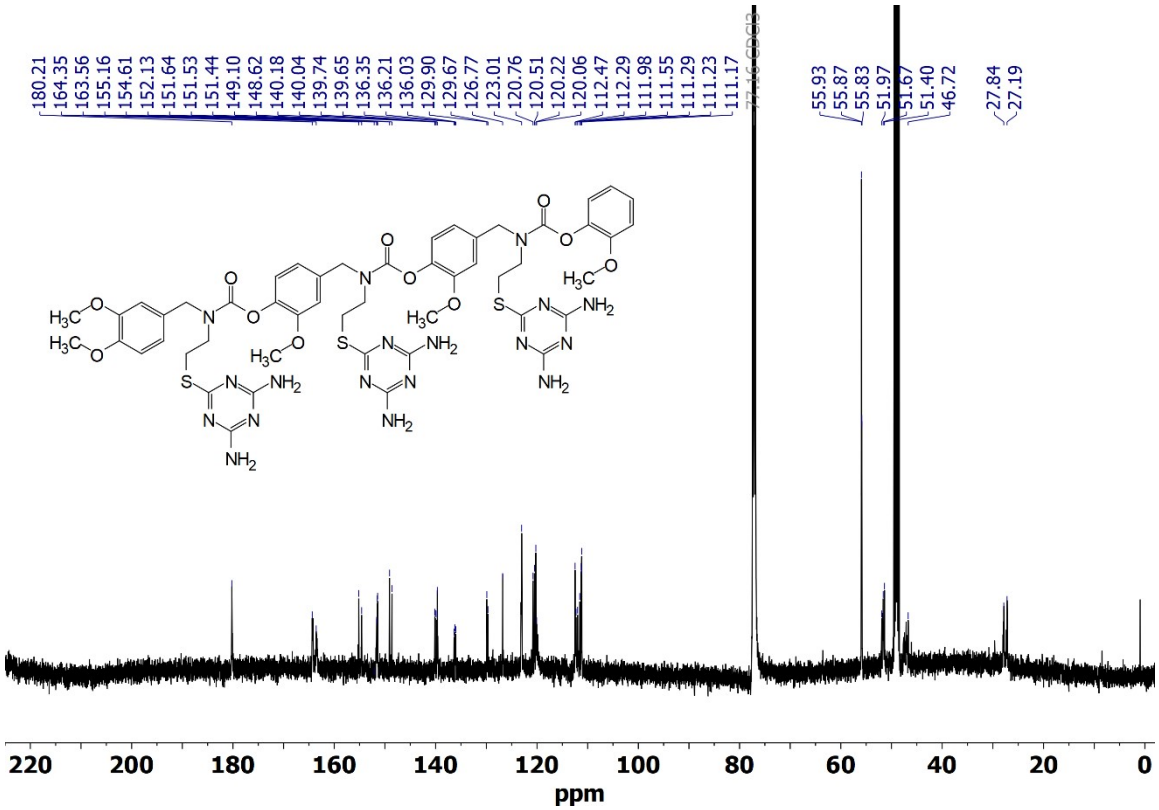


Figure S23. ^{13}C-NMR of **DDD** in 0.9:0.1 $CDCl_3$:MeOD.

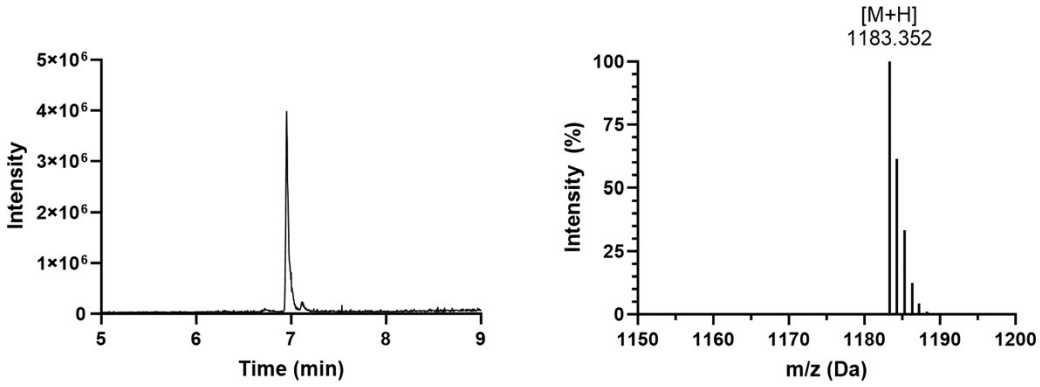


Figure S24. Total ion chromatogram and MS spectrum of **DDD**.

Synthesis of **mTm**. The first reductive amination reaction was stirred at room temperature for 6 hours instead of being heated in the microwave reactor. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (d, *J* = 34.2 Hz, 1H), 7.23 – 6.81 (m, 14H), 4.69 – 4.44 (m, 6H), 4.00 – 3.80 (m, 17H), 3.67 (m, *J* = 55.4 Hz, 2H), 3.12 – 2.93 (m, 6H), 1.87 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.14, 164.07, 155.29, 155.15, 154.98, 154.58, 152.09, 151.98, 151.85, 151.75, 151.56, 150.56, 149.34, 148.70, 141.04, 140.71, 140.53, 139.40, 136.36, 135.17, 129.84, 129.75, 126.65, 126.59, 123.56, 123.34, 121.14, 120.91, 120.61, 120.18, 113.61, 113.28, 112.60, 112.36, 111.99, 111.82, 111.57, 111.22, 111.14, 110.46, 110.38, 56.28, 56.09, 52.91, 52.86, 47.52, 46.45, 46.22, 45.95, 34.74, 34.54, 34.45, 34.11, 12.39.

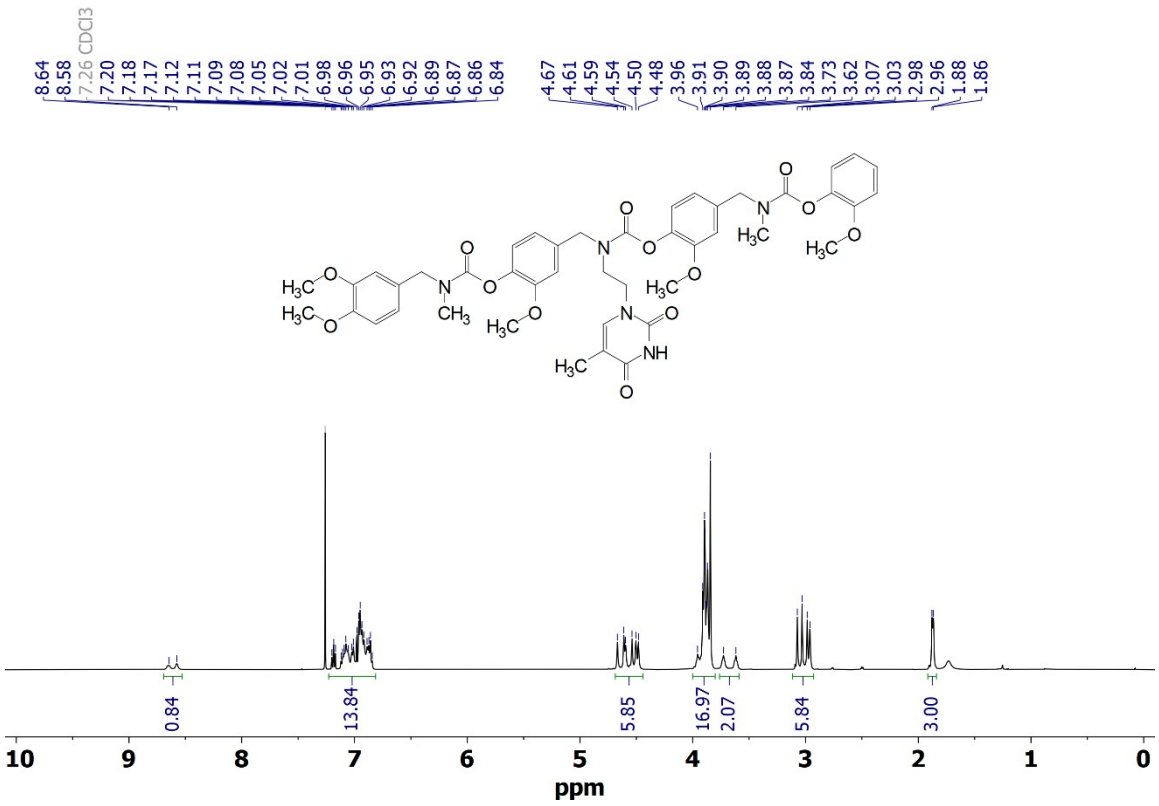


Figure S25. ^{1}H-NMR of **mTm** in $CDCl_3$.

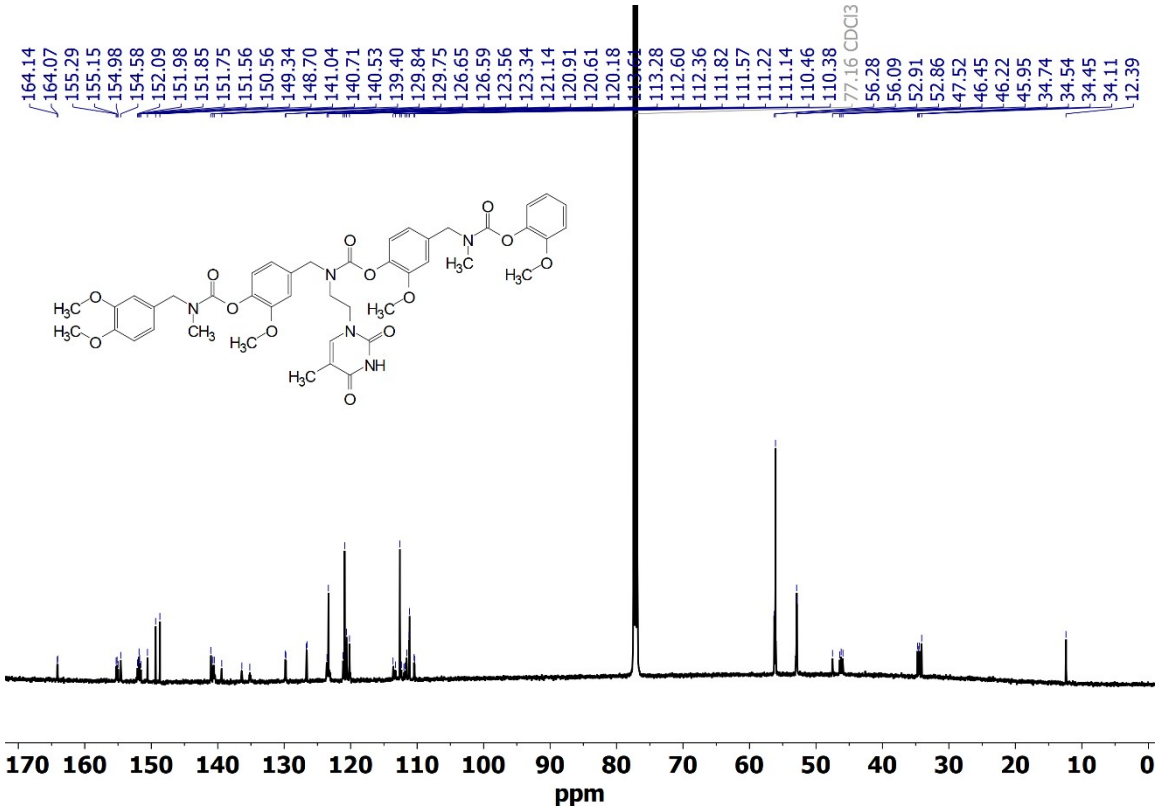


Figure S26. ^{13}C-NMR of **mTm** in $CDCl_3$.

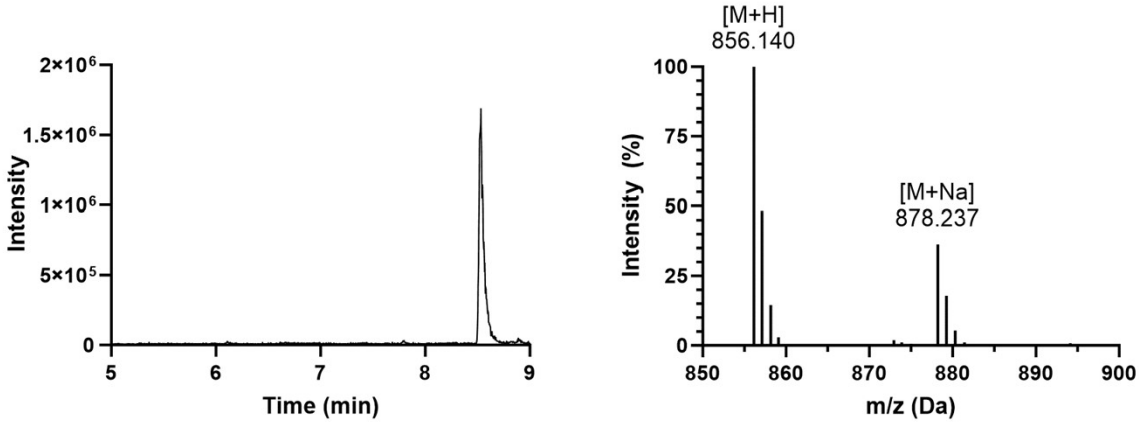


Figure S27. Total ion chromatogram and MS spectrum of **mTm**.

Synthesis of **mmT**. The first two reductive amination reactions are stirred at room temperature for 6 hours instead of being heated in the microwave reactor. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (d, J = 35.4 Hz, 1H), 7.25 – 6.82 (m, 14H), 4.72 – 4.44 (m, 6H), 4.01 – 3.80 (m, 17H), 3.67 (m, J = 55.5 Hz, 2H), 3.11 – 2.92 (m, 6H), 1.87 (d, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.10, 155.19, 155.01, 154.59, 152.05, 151.95, 151.67, 151.38, 150.56, 149.33, 148.70, 141.08, 140.55, 140.25, 140.06, 140.01, 135.69, 135.61, 135.25, 129.94, 129.82, 127.03, 126.97, 123.50, 123.40, 123.24, 121.13, 121.02, 120.60, 120.42, 120.23, 119.88, 113.56, 113.20, 112.68, 112.52, 111.87, 111.54, 111.20, 111.11, 110.44, 110.37, 56.24, 56.08, 55.93, 53.02, 52.94, 52.84, 34.78, 34.48, 34.35, 34.13, 12.38.

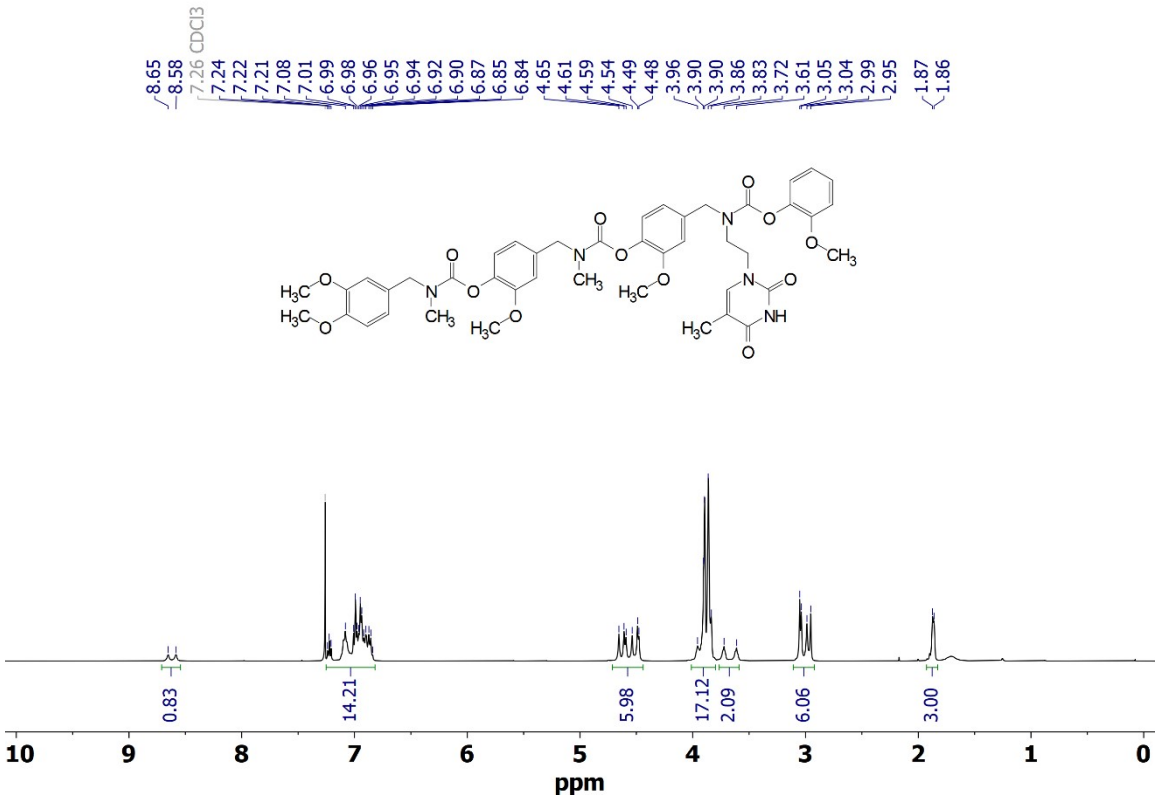


Figure S28. ^{1}H-NMR of **mmT** in $CDCl_3$.

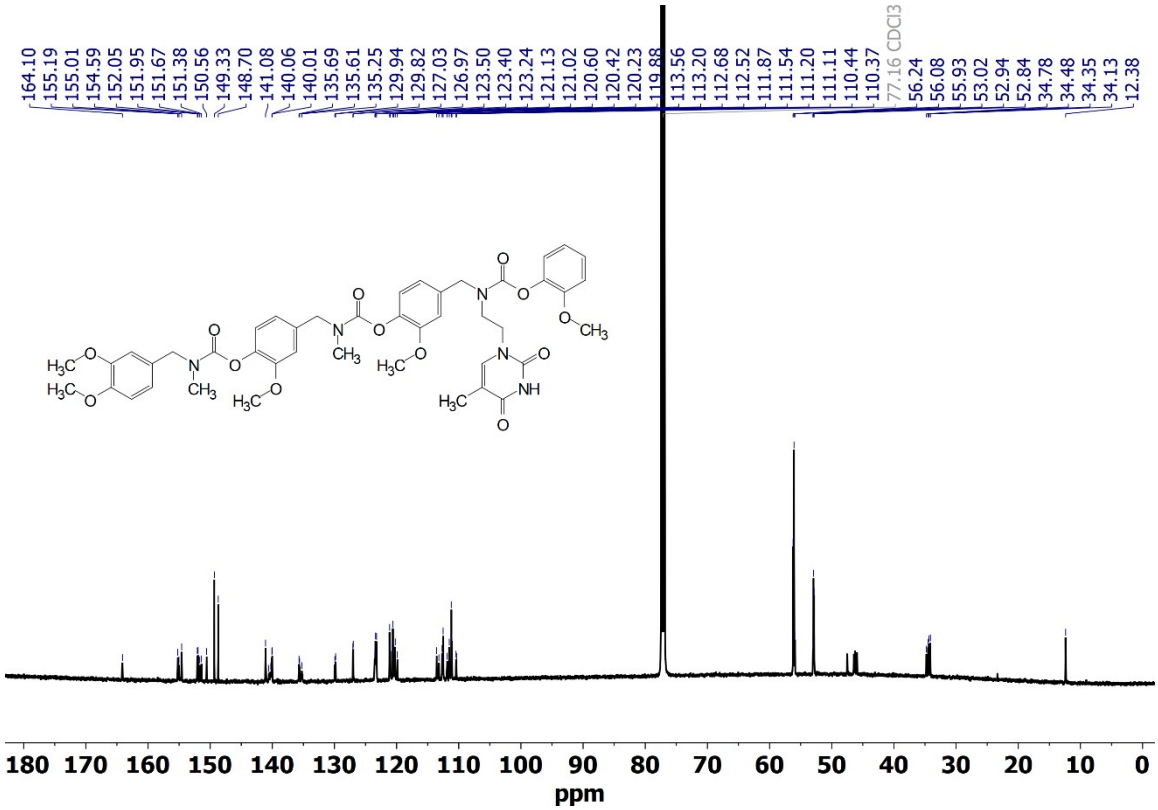


Figure S29. ^{13}C-NMR of **mmT** in $CDCl_3$.

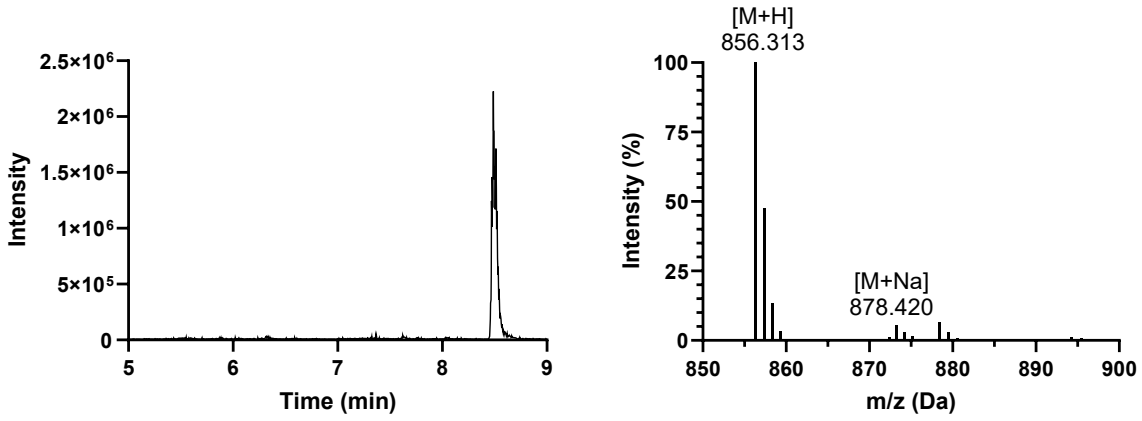


Figure S30. Total ion chromatogram and MS spectrum of **mmT**.

Synthesis of **mTT**. The first reductive amination reaction was stirred at room temperature for 6 hours instead of being heated in the microwave. The end-capping reaction was performed in DMF rather than acetonitrile. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.67 – 8.67 (m, 2H), 7.24 – 6.60 (m, 15H), 4.66 – 4.42 (m, 6H), 4.08 – 3.54 (m, 23H), 3.09 – 2.88 (m, 3H), 1.90 (d, J = 28.5 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 164.92, 155.23, 154.87, 154.70, 152.03, 151.91, 151.70, 151.51, 151.37, 151.20, 151.02, 149.16, 148.55, 141.54, 141.25, 140.19, 139.77, 135.62, 135.51, 129.61, 129.55, 127.03, 123.36, 123.32, 123.13, 120.95, 120.75, 120.44, 120.17, 113.29, 113.04, 112.60, 112.45, 111.41, 111.22, 111.16, 110.41, 110.31, 56.02, 55.97, 55.93, 55.77, 53.46, 53.02, 52.72, 52.63, 52.40, 47.67, 46.20, 45.92, 34.29, 33.99, 12.13, 12.01.

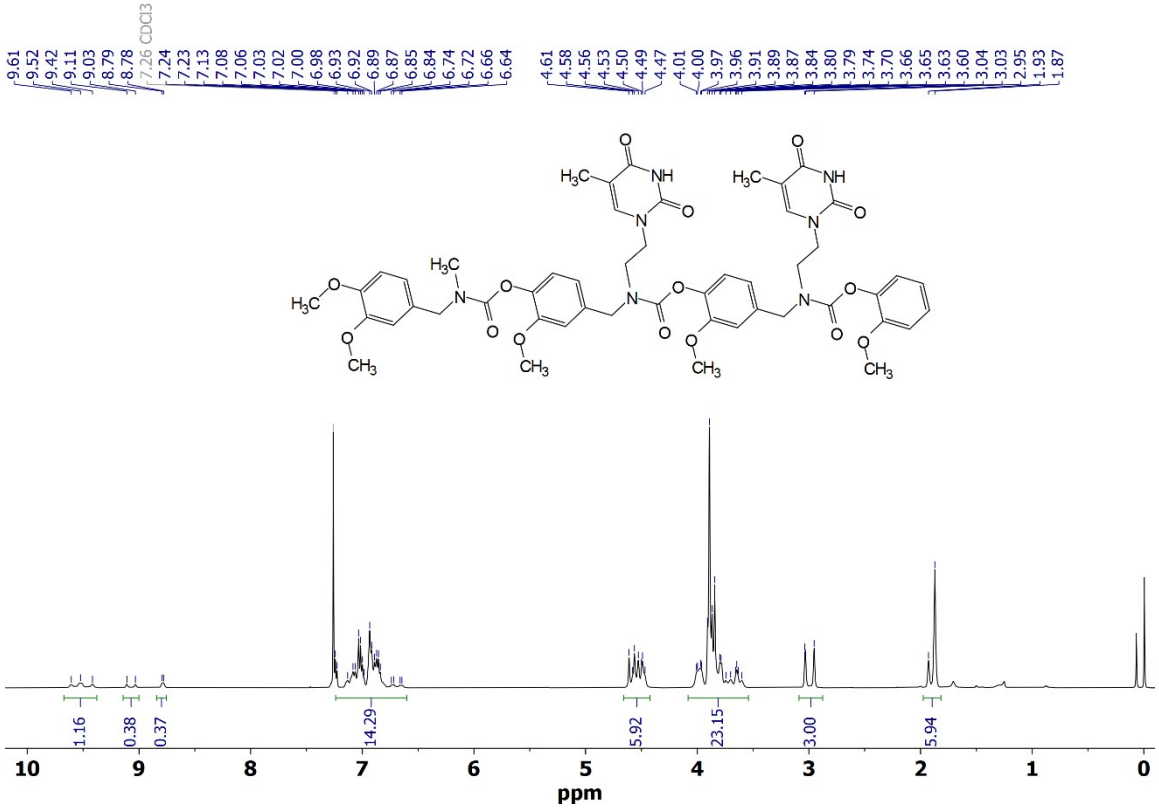


Figure S31. ^{1}H-NMR of **mTT** in $CDCl_3$.

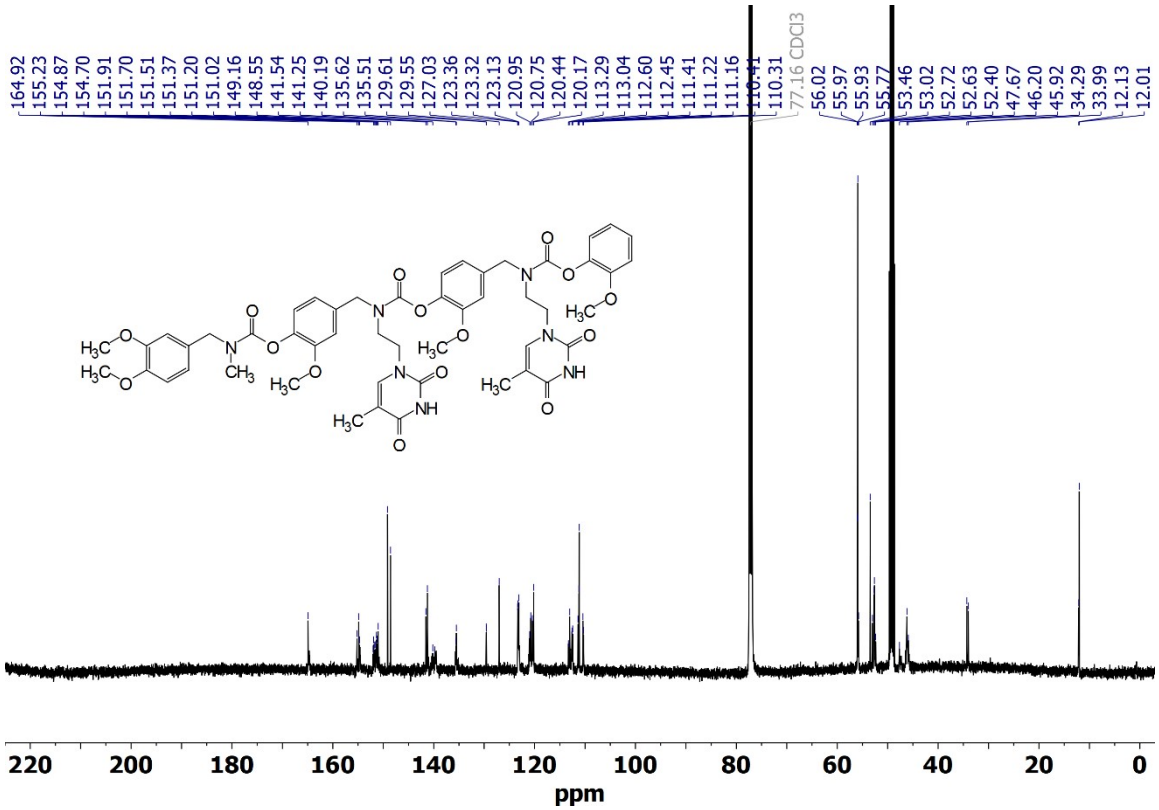


Figure S32. ^{13}C-NMR of **mTT** in 0.9:0.1 $CDCl_3$:MeOD.

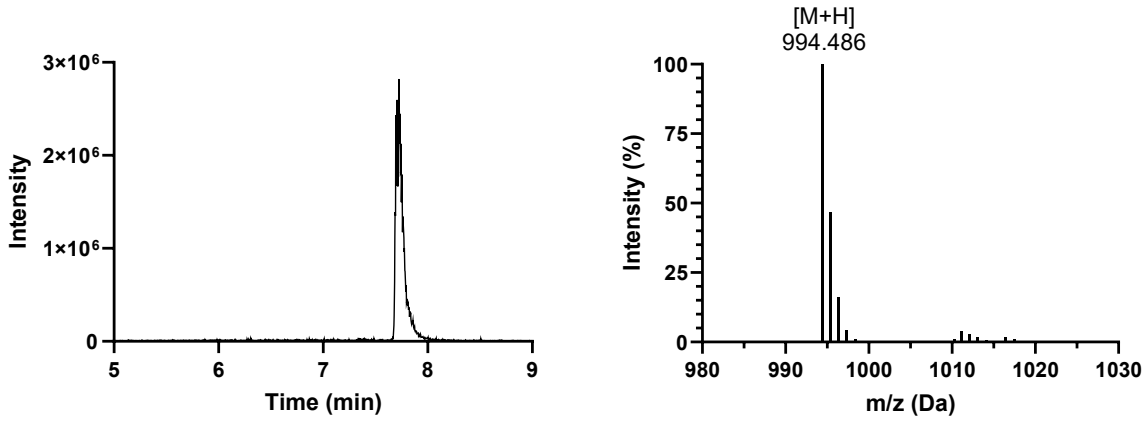


Figure S33. Total ion chromatogram and MS spectrum of **mTT**.

Synthesis of **TmT**. The product of the first and second reductive amination reactions were taken directly to the first carbamation without purification. The second reductive amination was stirred at 60 °C for 45 minutes in the microwave reactor. The first carbamation reaction was stirred at 75 °C for 1 hour in the microwave reactor. The second carbamation reaction was stirred at 65 °C for 10 minutes in the microwave reactor. The first carbamation reaction was purified by stirring in pure ammonium hydroxide at room temperature for 1 hour before triturating with water. The end-capping reaction was stirred at room temperature for 30 minutes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (m, J = 49.3, 32.7 Hz, 1H), 8.46 – 7.98 (m, 1H), 7.25 – 6.73 (m, 15H), 4.74 – 4.39 (m, 6H), 3.88 (m, J = 11.5, 4.8 Hz, 19H), 3.78 – 3.55 (m, 4H), 3.12 – 2.93 (m, 3H), 1.84 (m, J = 17.2 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 164.77, 155.28, 155.03, 154.92, 154.71, 154.64, 151.86, 151.74, 151.49, 151.25, 151.11, 151.00, 149.14, 148.83, 141.10, 141.02, 140.19, 140.02, 139.86, 139.33, 136.03, 135.39, 129.63, 129.37, 126.96, 123.29, 123.11, 122.99, 122.80, 120.95, 120.89, 120.77, 120.65, 120.36, 120.28, 119.97, 113.11, 112.76, 112.57, 112.40, 111.94, 111.66, 111.18, 111.06, 110.48, 110.42, 56.00, 55.90, 55.84, 55.79, 52.72, 52.46, 47.41, 47.23, 46.51, 46.28, 46.16, 46.06, 45.81, 34.59, 34.29, 12.09.

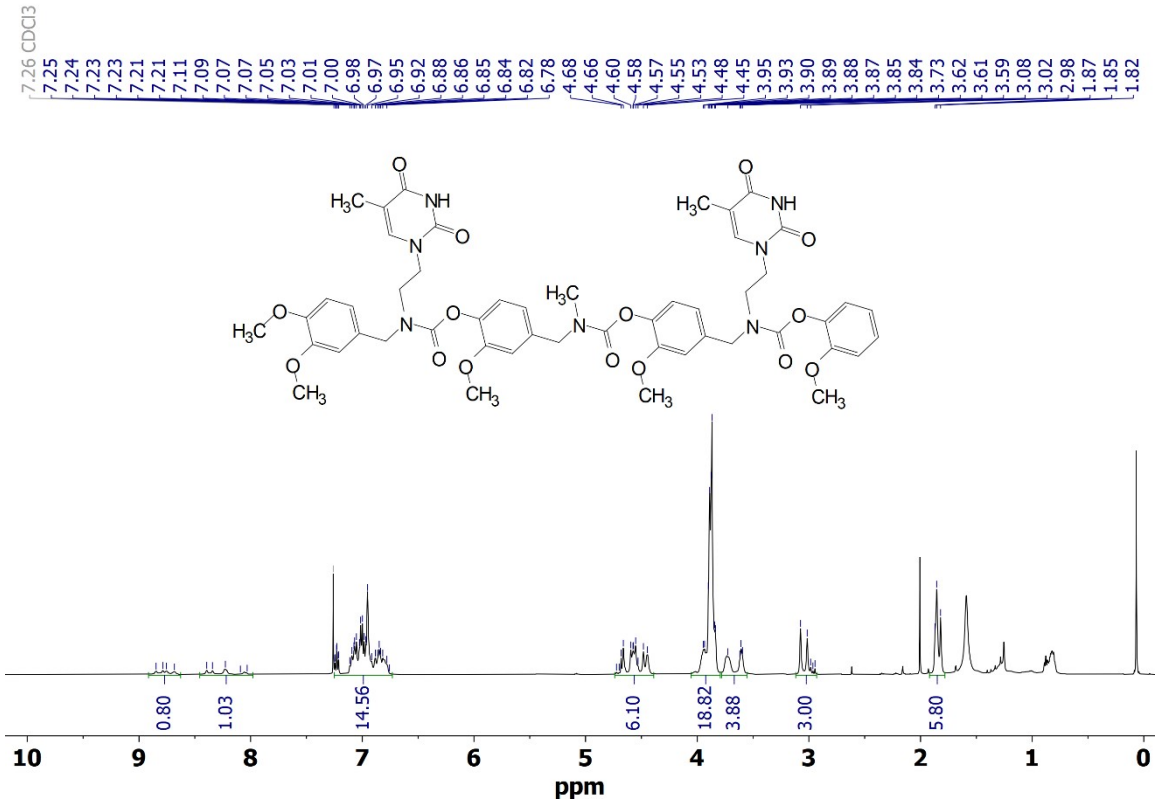


Figure S34. ^{1}H-NMR of **TmT** in $CDCl_3$

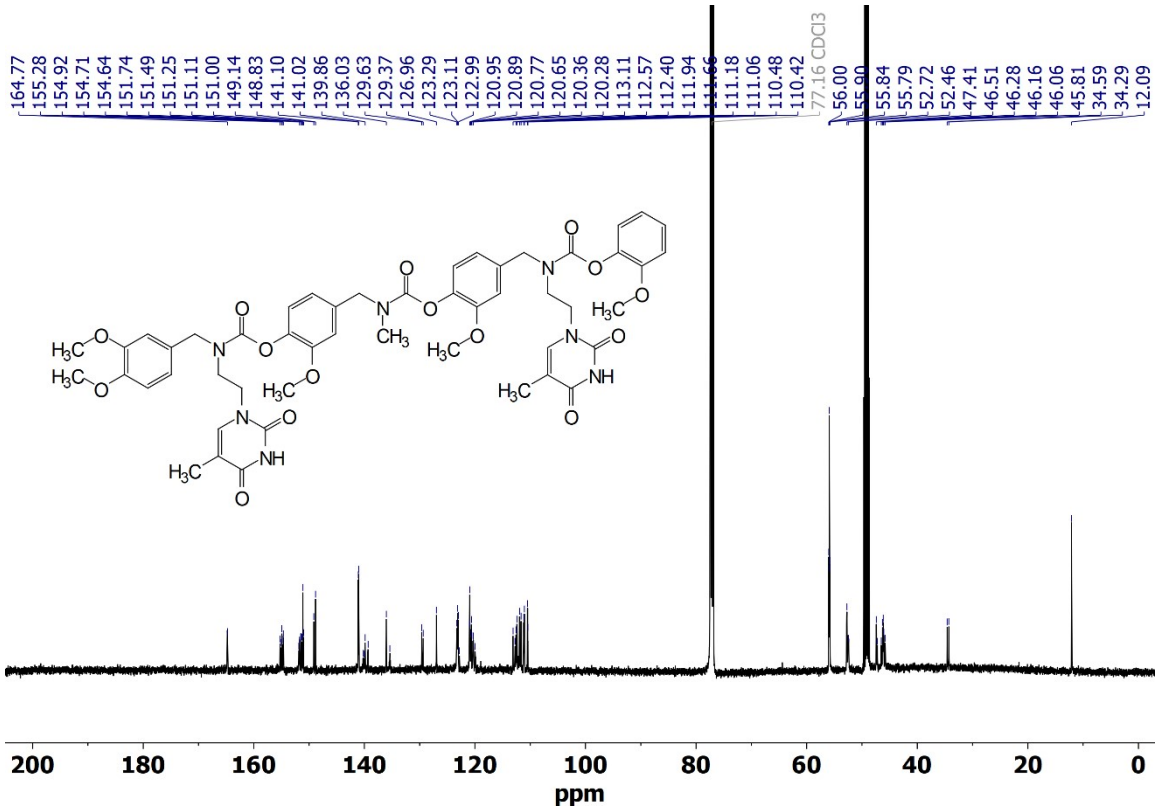


Figure S35. ^{13}C-NMR of **TmT** in 0.9:0.1 $CDCl_3$:MeOD

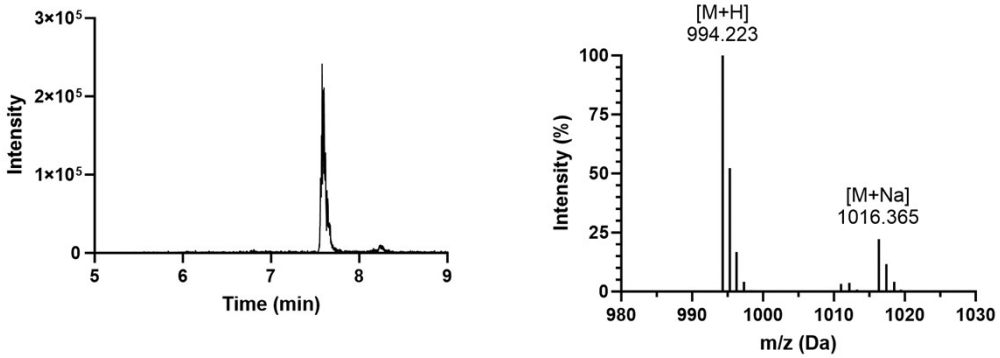


Figure S36. Total ion chromatogram and MS spectrum of **TmT**.

Synthesis of **TTT**. The product of the first reductive amination reaction was taken directly to the first carbamation without purification. The second reductive amination reaction used methanol/DMSO (4:1, v:v). The third reductive amination used mixture methanol/DMSO as the reaction solvent (1:1, v:v).The first carbamation reaction was stirred at 75 °C for 1 hour in the microwave reactor. The second carbamation reaction was stirred at 120 °C for 30 minutes in the microwave reactor. Both carbamation reactions were purified by stirring in pure ammonium hydroxide at room temperature for 1 hour before triturating with water. The end-capping reaction was stirred overnight at room temperature. 1H NMR (600 MHz, CD3OD) δ 7.17 – 6.66 (m, 16H), 4.55 – 4.27 (m, 6H), 3.94 – 3.70 (m, 21H), 3.63 (d, J = 30.8 Hz, 6H), 1.80 – 1.68 (m, 9H).

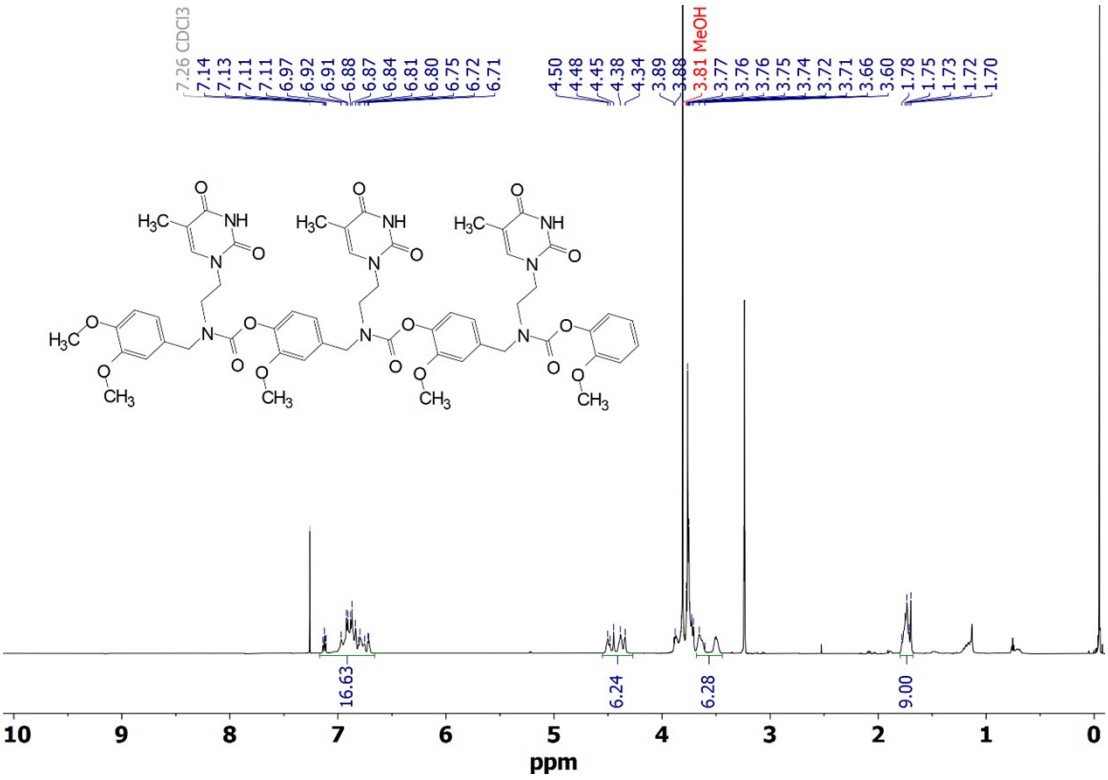


Figure S37. ^{1}H-NMR of **TTT** in MeOD/$CDCl_3$ (1:9, v:v)

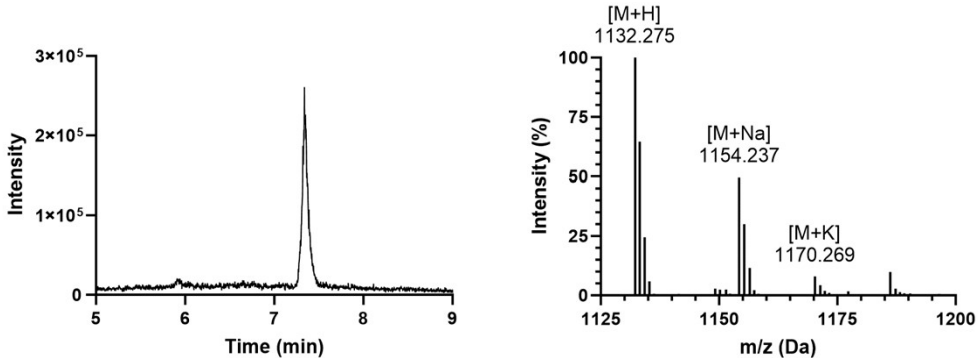


Figure S38. Total ion chromatogram and MS spectrum of **TTT**.

Synthesis of **12**. 12 mL acetonitrile were added to 0.4992 g 2-chloro-4,6-diamino-1,3,5-triazine (1 eq.). 0.289 g potassium hydroxide (1.5 eq.) were added to the suspension. 0.48 mL 1-propanethiol (1.5 eq.) were also added to the suspension. The reaction was stirred at 81 °C for 24 hours with attached reflux condenser. The reaction was filtered. The filtrate was dried under rotary evaporation and then purified via flash chromatography (dichlormethane/methanol). Yield = 0.05 g, 8%. ^{1}H NMR (500 MHz, DMSO) δ 6.64 (s, 4H), 2.96 (t, *J* = 7.2 Hz, 4H), 1.61 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H).

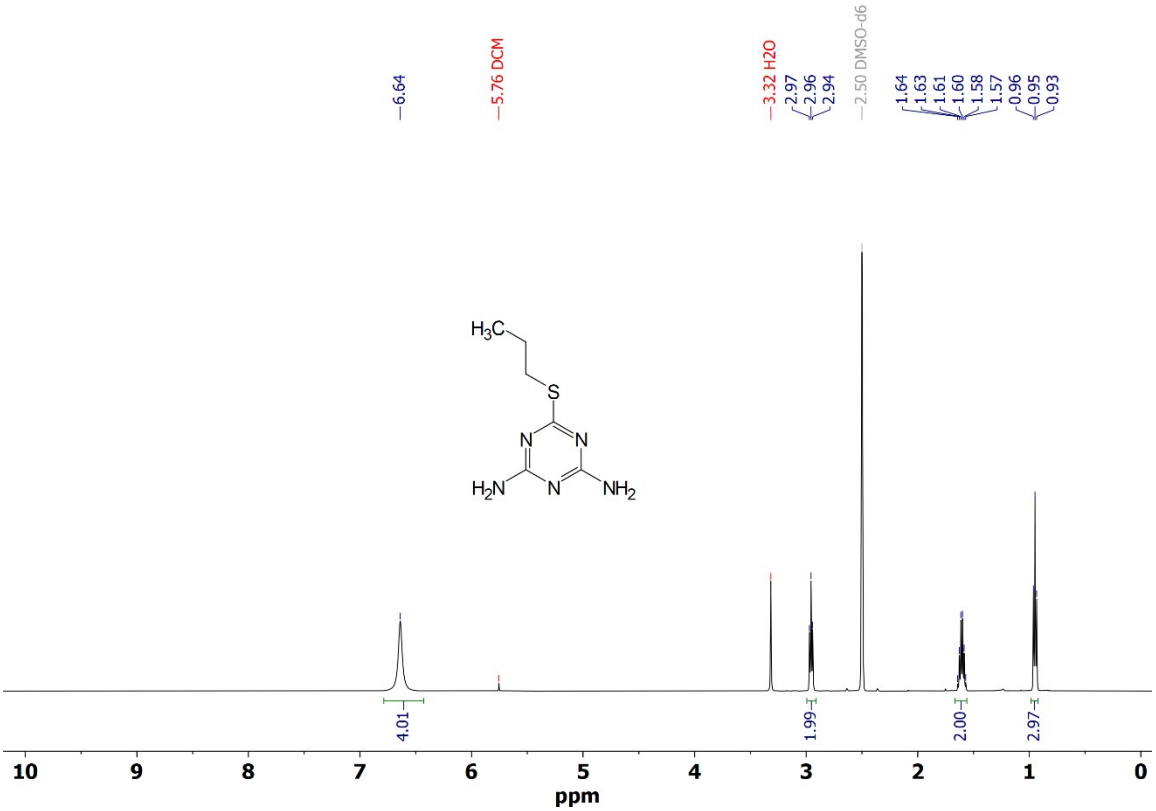


Figure S39. ^{1}H-NMR of stand-alone diaminotriazine monomer (**12**) in DMSO-d_6.

Synthesis of **13.** 0.5081 g N_3-benzoyl thymine (1 eq.) was dissolved in 4.4 mL N,N-dimethylformamide. 0.77 mL N,N-diisopropylethylamine (2 eq.) were added to solution. The solution was stirred for 10 minutes. 0.26 mL 1-iodobutane (1eq.) were added to solution. The reaction was stirred at 50 °C for 24 hours. The reaction was concentrated under reduced pressure and purified via flash chromatography (hexanes/ethyl acetate). N_3-benzoyl-N_1-butyl-thymine was dissolved in 5 mL of 200 mM potassium carbonate in methanol. The reaction was stirred at RT for 24 hours. The reaction was dried under rotary evaporation and purified via flash chromatography (hexanes/ethyl acetate). Yield = 0.1248 g, 31%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H), 6.97 (d, J = 1.4 Hz, 1H), 3.69 (t, J = 7.4 Hz, 2H), 1.66 (p, 2H), 1.36 (h, J = 7.5 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.06, 150.75, 140.41, 110.52, 48.32, 31.14, 19.71, 13.67, 12.35.

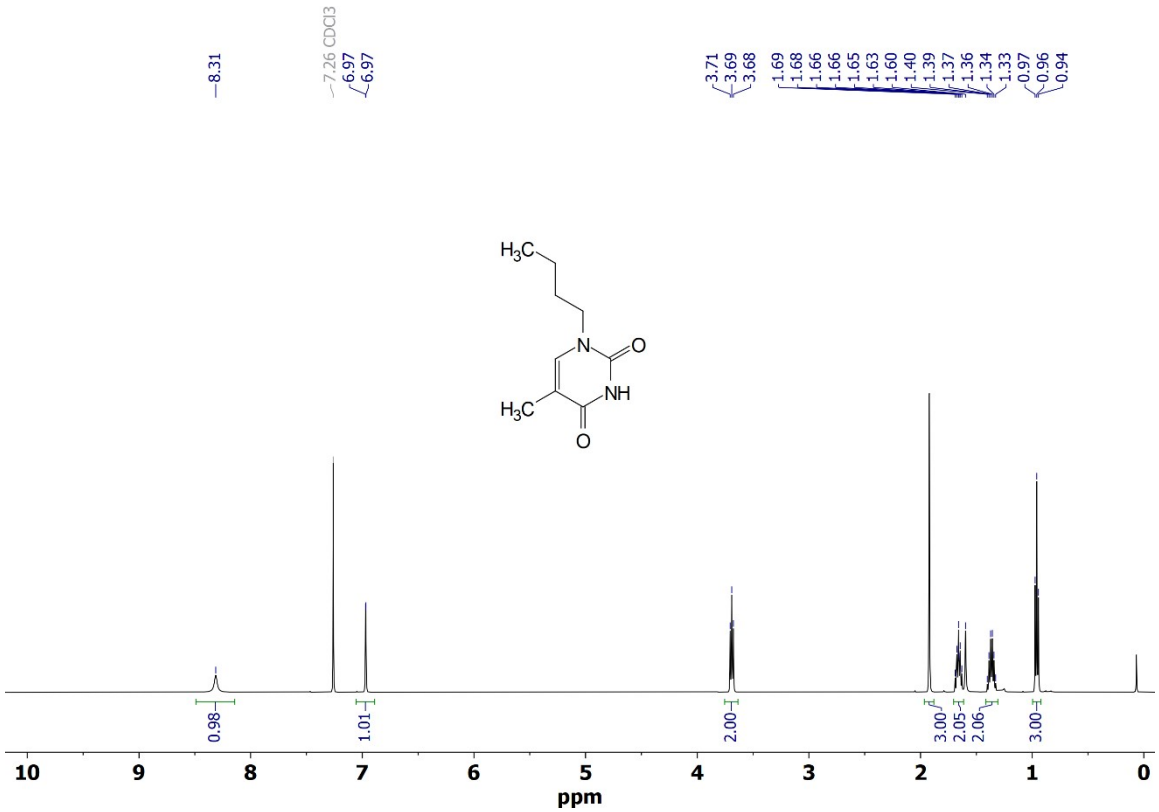


Figure S40. ^{1}H-NMR of stand-alone thymine monomer (**13**) in $CDCl_3$.

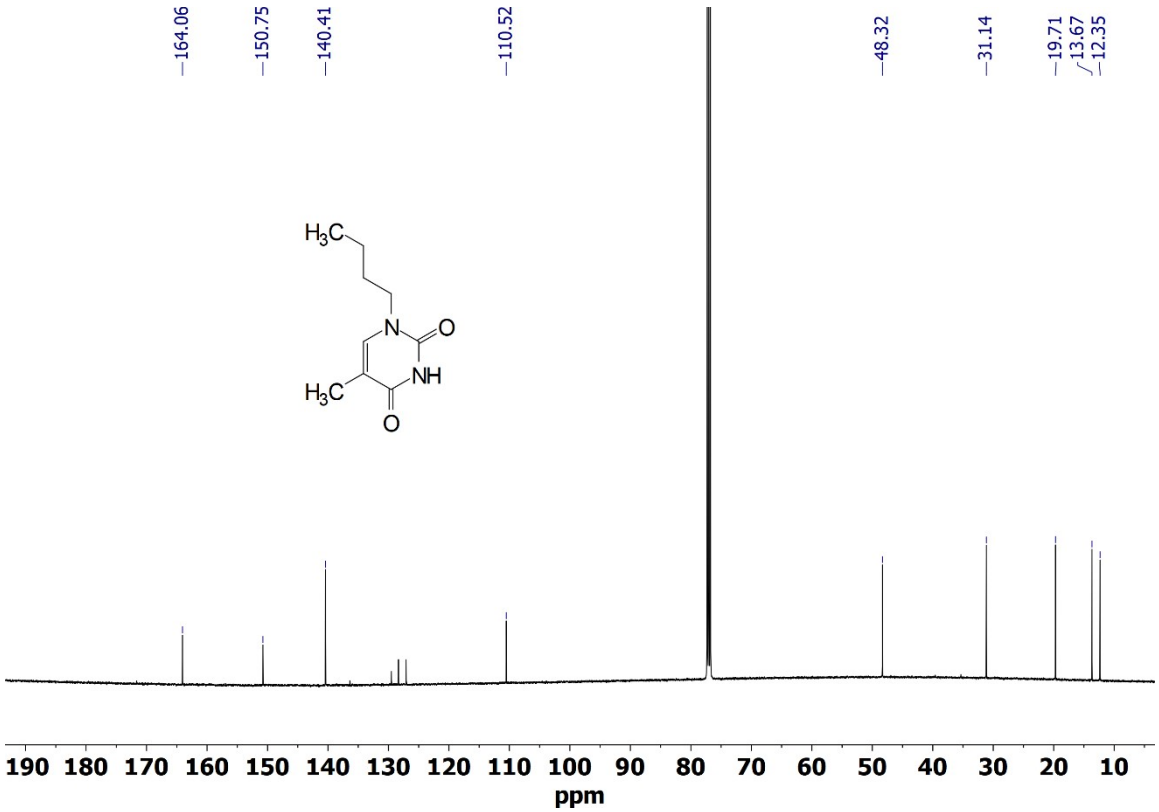


Figure S41. ^{13}C-NMR of stand-alone thymine monomer (**13**) in $CDCl_3$.

^{1}H-NMR Titration and Dilution Experiments

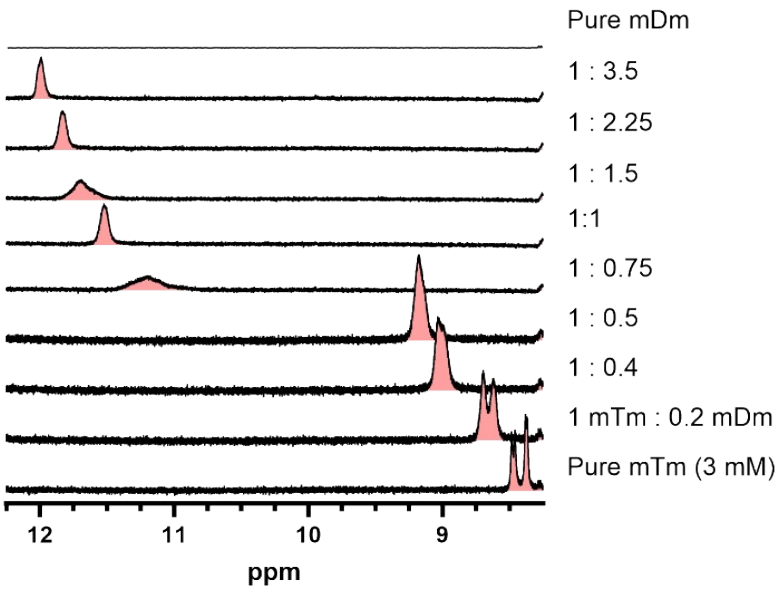


Figure S42. ^{1}H-NMR titration of **mDm** into **mTm** (3 mM) in $CDCl_3$, NH region.

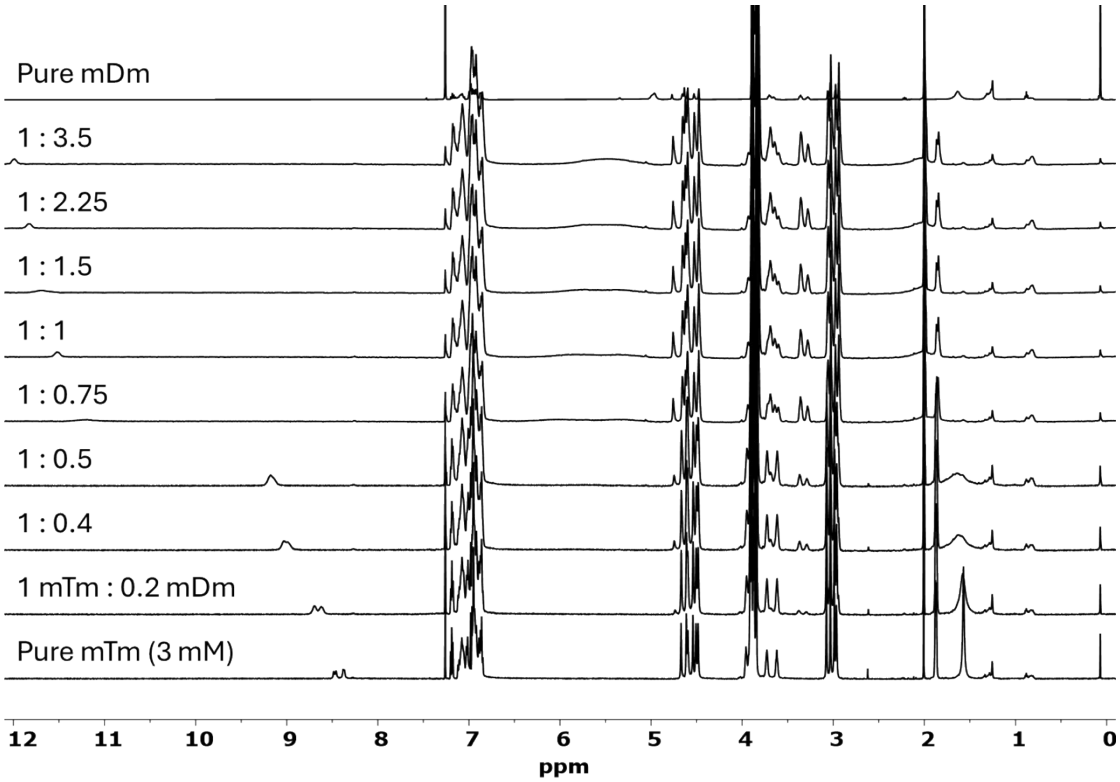


Figure S43. ^{1}H-NMR titration of **mDm** into **mTm** (3 mM) in $CDCl_3$ (full spectrum).

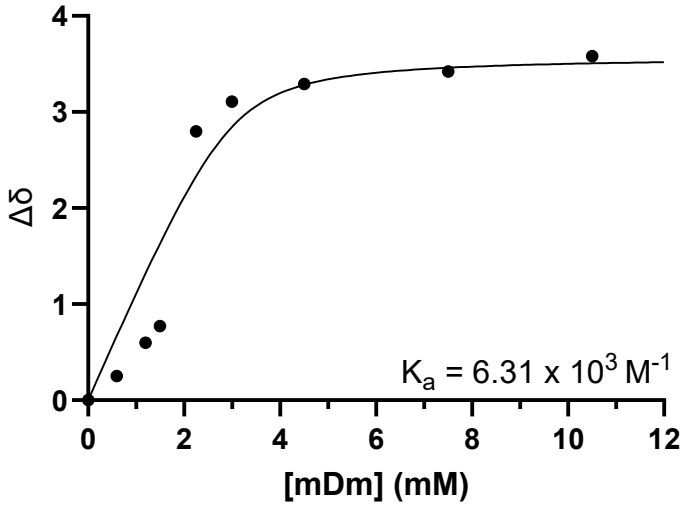


Figure S44. ^{1}H-NMR titration of **mDm** into **mTm** (3 mM) in $CDCl_3$ fitted to 1:1 isotherm.

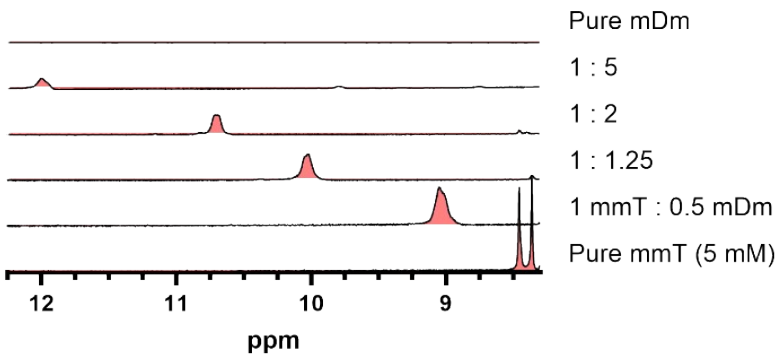


Figure S45. ^{1}H-NMR titration of **mDm** into **mmT** (5 mM) in $CDCl_3$, NH region.

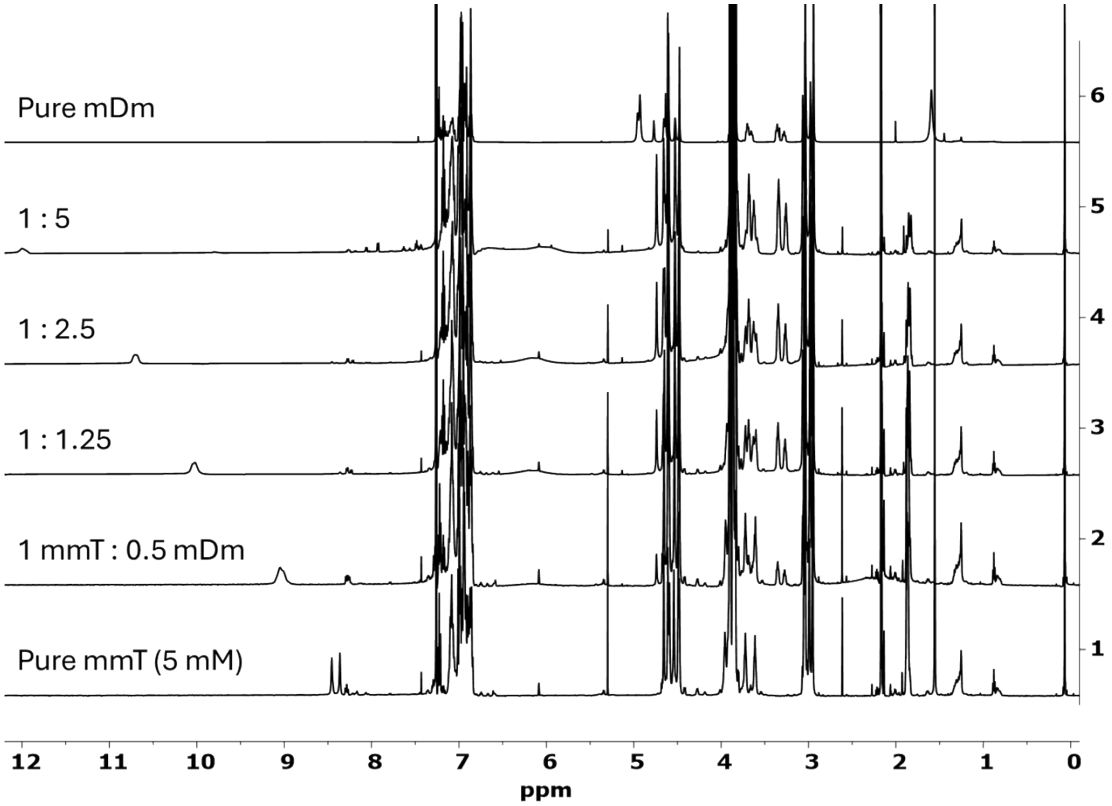


Figure S46. ^{1}H-NMR titration of **mDm** into **mmT** (5 mM) in $CDCl_3$ (full spectrum).

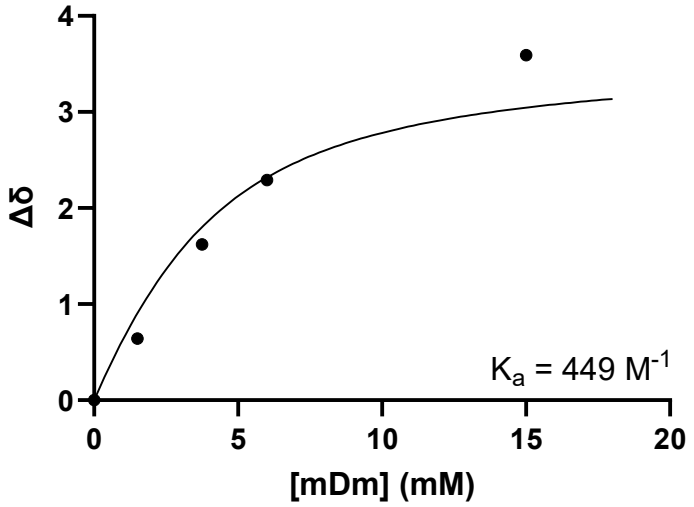


Figure S47. ^{1}H-NMR titration of **mDm** into **mmT** (3 mM) in $CDCl_3$ fitted to 1:1 isotherm.

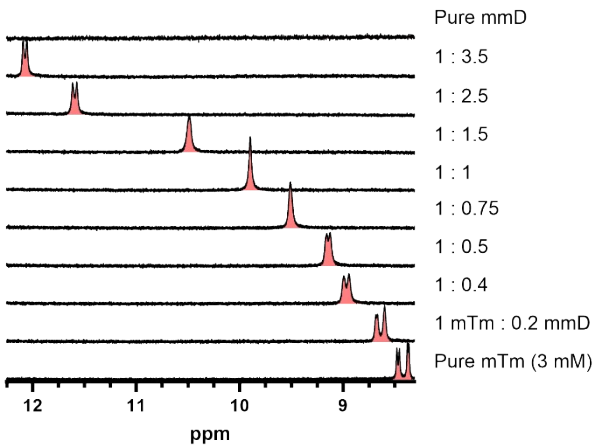


Figure S48. ^{1}H-NMR titration of **mmD** into **mTm** (3 mM) in $CDCl_3$, NH region.

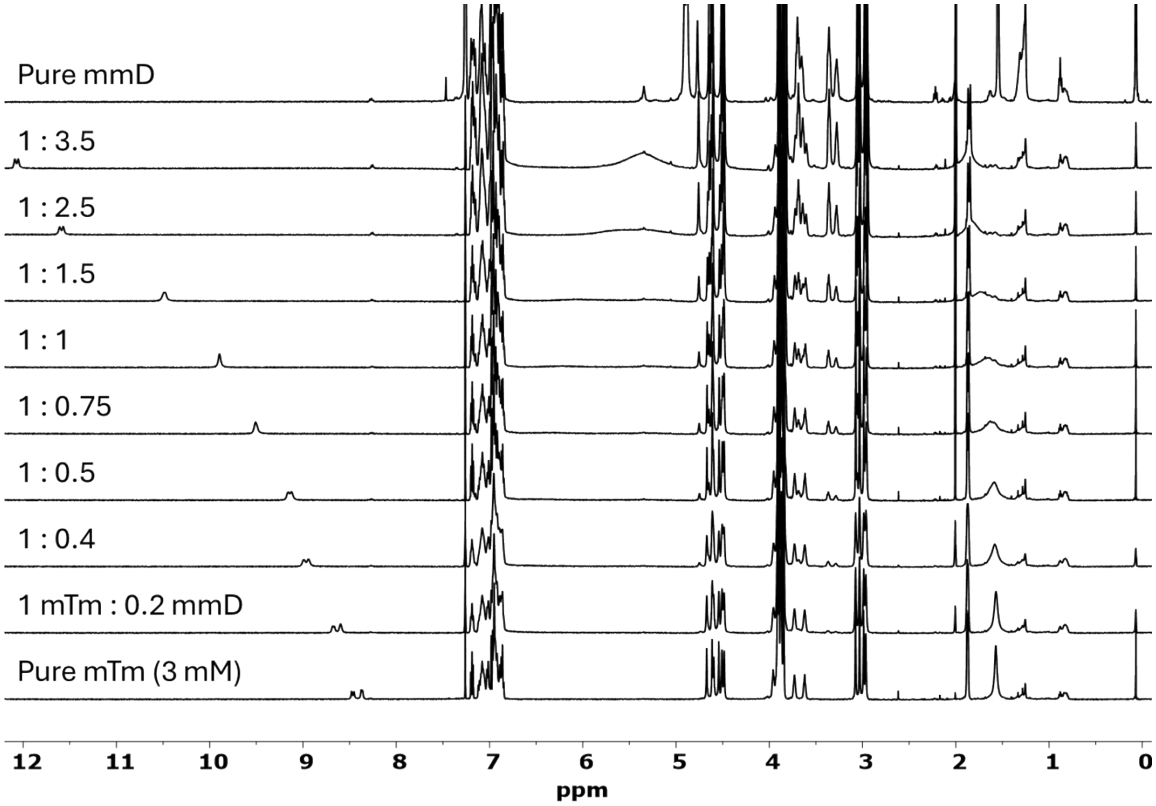


Figure S49. ^{1}H-NMR titration of **mmD** into **mTm** (3 mM) in $CDCl_3$ (full spectrum).

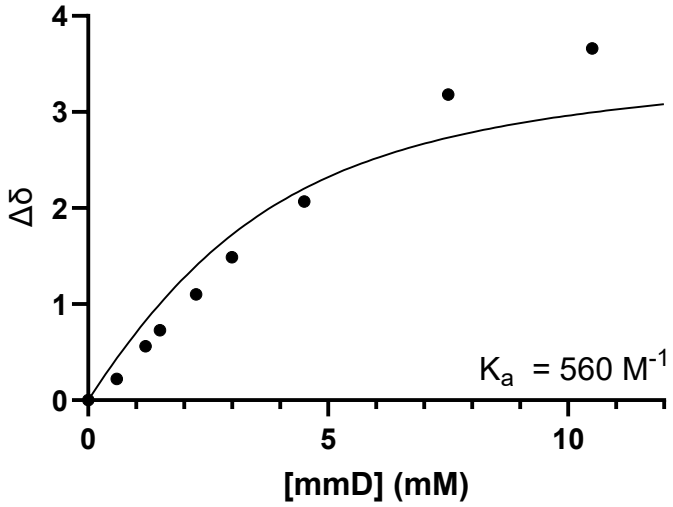


Figure S50. ^{1}H-NMR titration of **mmD** into **mTm** (3 mM) in $CDCl_3$ fitted to 1:1 isotherm.

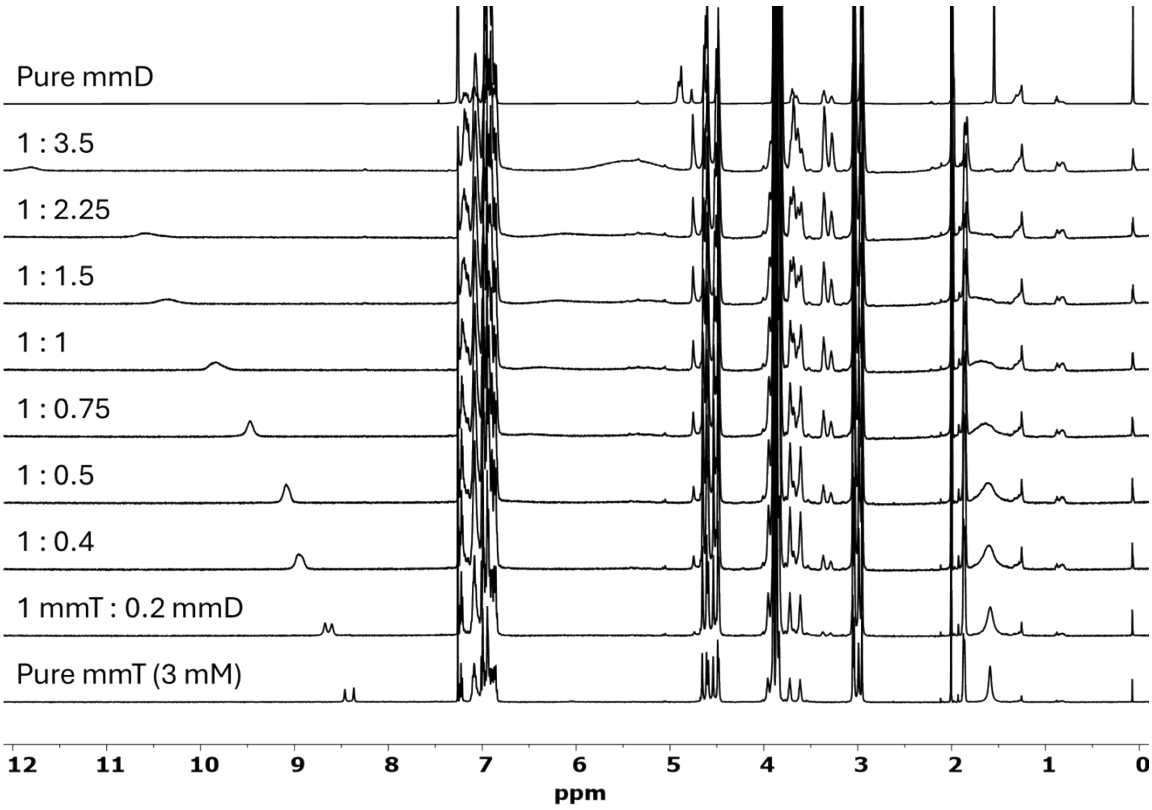


Figure S51. ^{1}H-NMR titration of **mmD** into **mmT** (3 mM) in $CDCl_3$ (full spectrum).

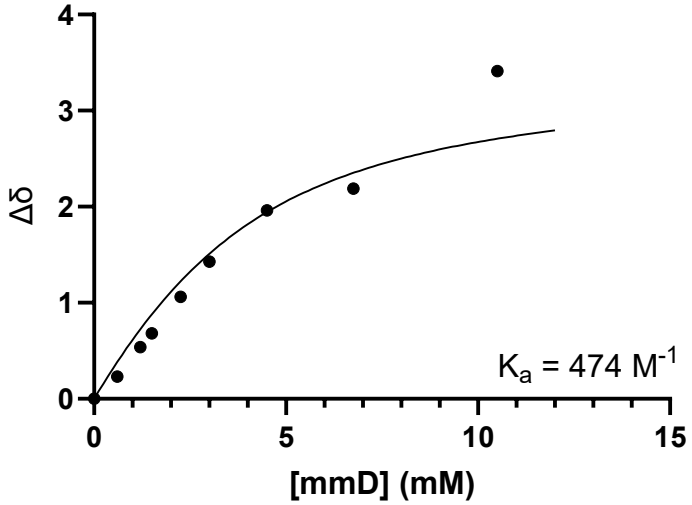


Figure S52. ^{1}H-NMR titration of **mmD** into **mmT** (3 mM) in $CDCl_3$ fitted to 1:1 isotherm.

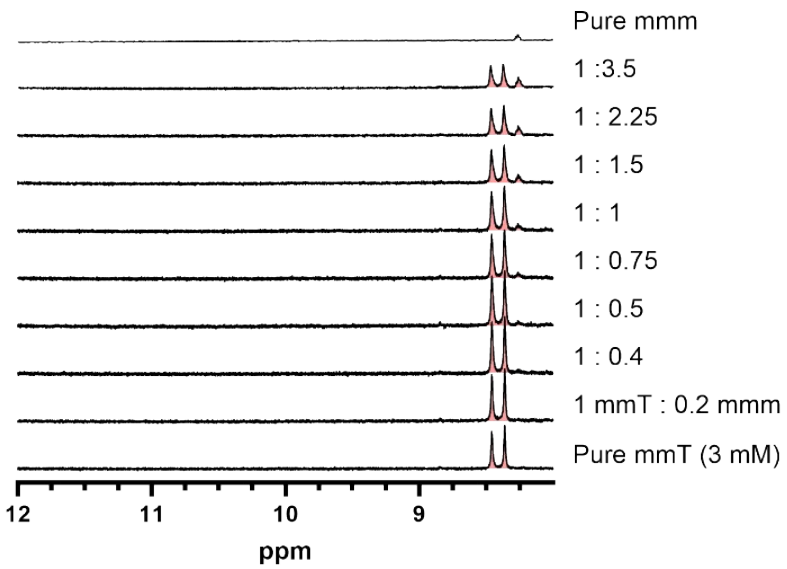


Figure S53. ^{1}H-NMR titration of **mmm** into **mmT** (3 mM) in $CDCl_3$, NH region.

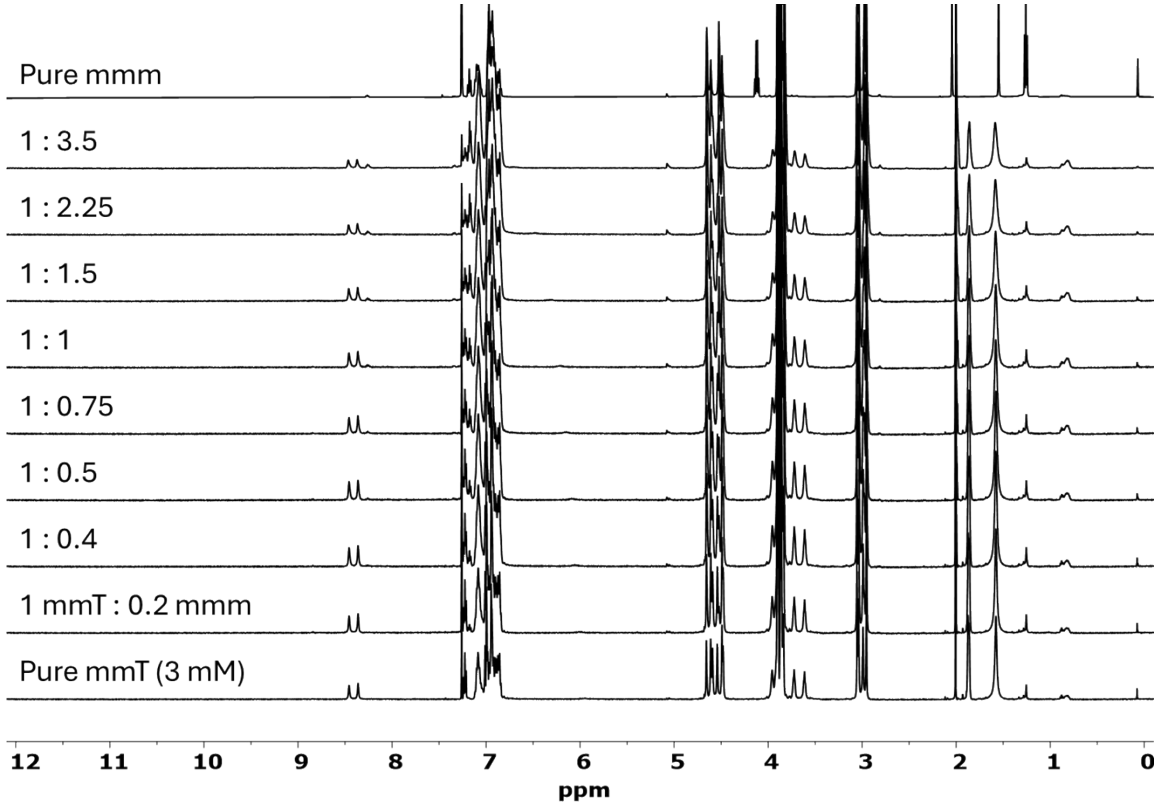


Figure S54. ^{1}H-NMR titration of **mmm** into **mmT** (3 mM) in $CDCl_3$ (full spectrum).

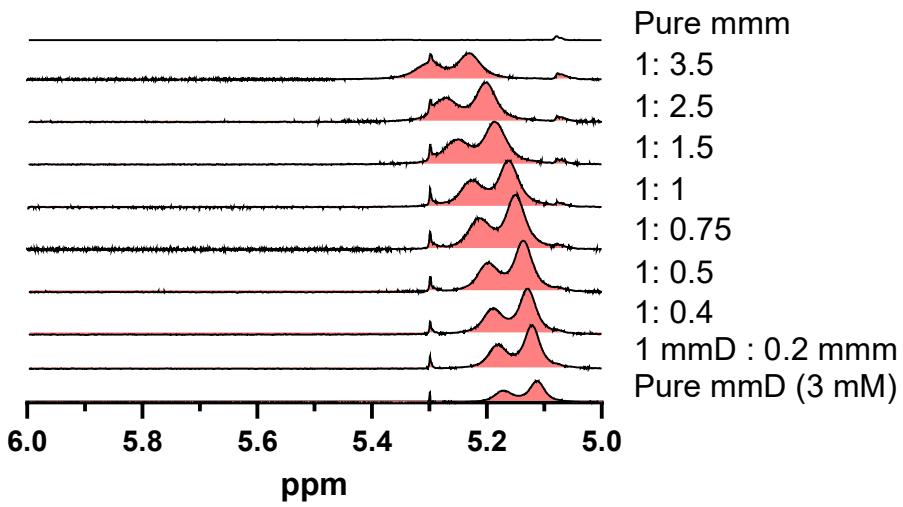


Figure S55. ^{1}H-NMR titration of **mmm** into **mmD** (3 mM) in $CDCl_3$, NH_2 region.

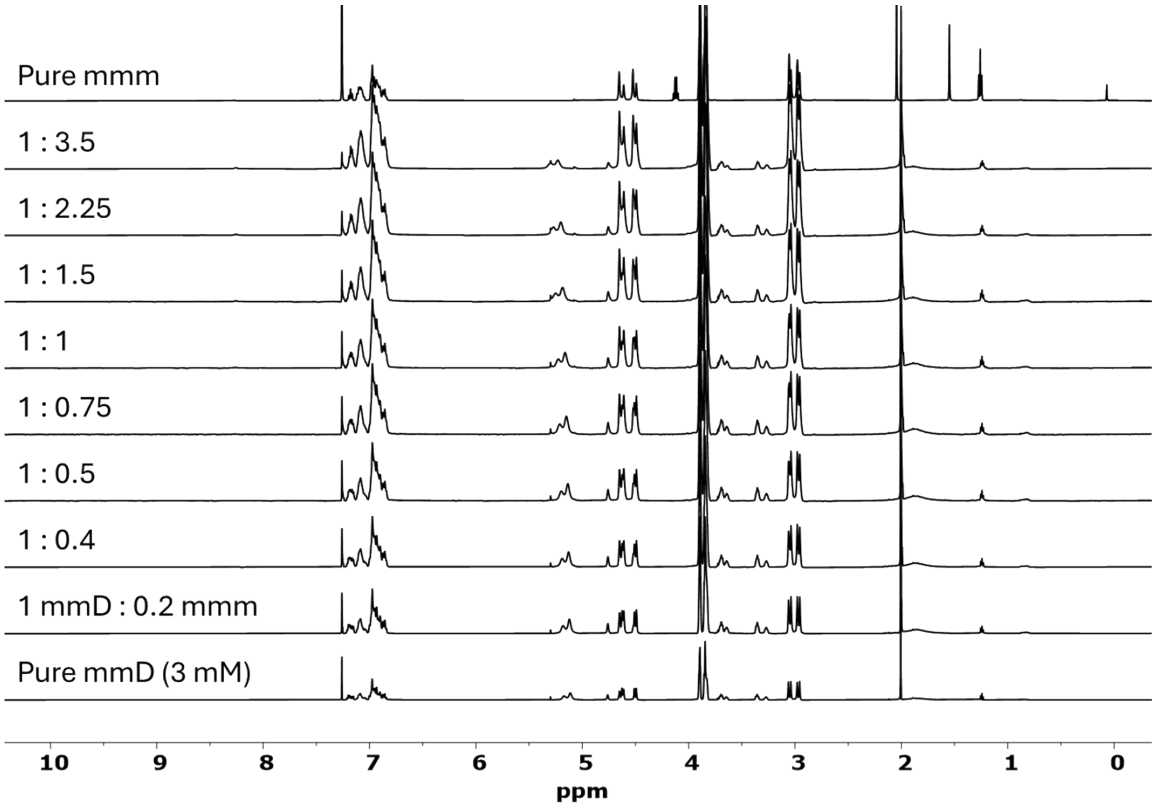


Figure S56. ^{1}H-NMR titration of **mmm** into **mmD** (3 mM) in $CDCl_3$ (full spectrum).

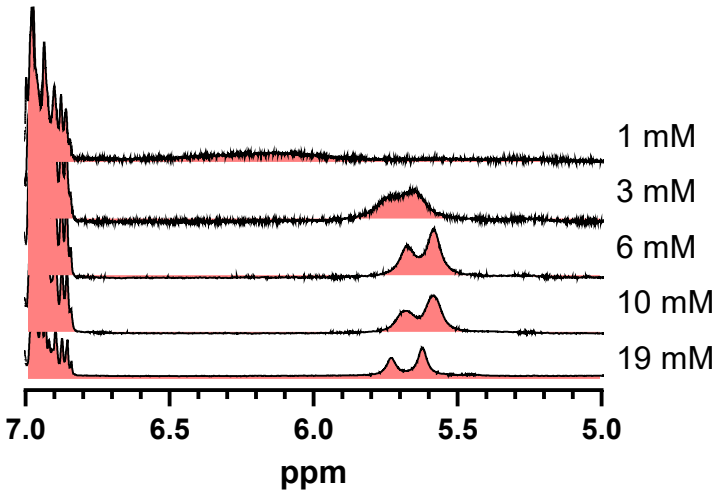


Figure S57. ^{1}H-NMR dilution of **mmD** in $CDCl_3$, NH_2 region.

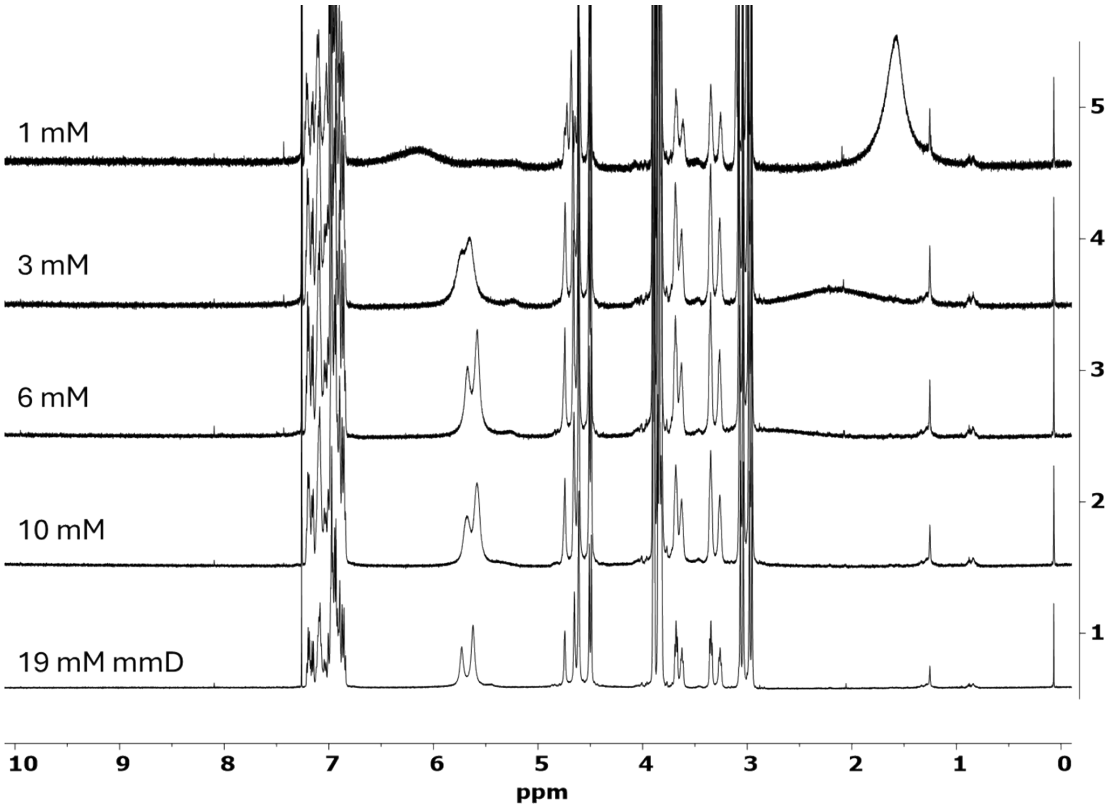


Figure S58. ^{1}H-NMR dilution of **mmD** in $CDCl_3$ (full spectrum).

ITC Experiments

ITC experiments were performed using a Low Volume NanoITC and an organic solvent buret handle (TA Instruments). Titrations with monovalent oligomers involved an initial 1.5 uL injection followed by 19 injections of 2 uL. Experiments with divalent or trivalent oligomers (including mixed sequence experiments) had 25 injections of 2 μL. Every experiment was stirred at 350 revolutions per minute and included a 5-minute equilibration period both prior to the first injection and after every injection. Thermodynamic binding parameters were calculated by using the TA Instruments NanoAnalyze software to integrate raw data from the thermogram and fit it to an independent binding model.

Monovalent SeDOC ITC Experiments

Table S1. Thermodynamic binding parameters for **mDm·mTm** measured by ITC.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.32	-3.2	-18.9	15.7	1.09
Trial 2	3.07	-2.6	-17.5	14.9	1.01
Trial 3	3.41	-3.7	-19.5	15.7	1.12

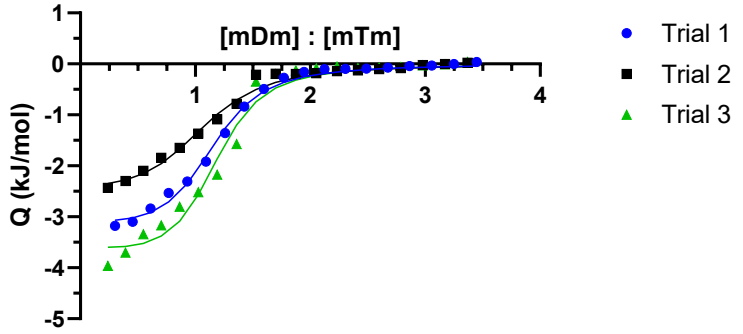


Figure S59. ITC titration of **mDm** (100 mM) into **mTm** (10 mM) in chloroform.
Samples contained residual TFA from HPLC.

Table S2. Thermodynamic binding parameters for **mDm·mmT** measured by ITC.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.37	-3.8	-19.2	15.5	1.10
Trial 2	3.04	-5.0	-17.3	12.3	0.97
Trial 3	3.23	-4.7	-18.4	13.7	0.94

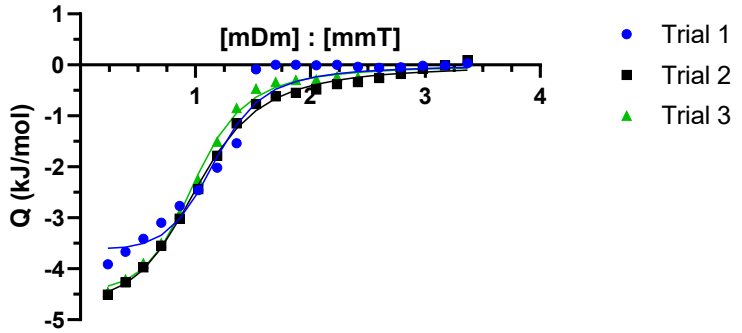


Figure S60. ITC titration of **mDm** (100 mM) into **mmT** (10 mM) in chloroform.
Samples contained residual TFA from HPLC.

Table S3. Thermodynamic binding parameters of **mmD·mTm** measured by ITC.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.06	-3.7	-17.4	13.8	1.14
Trial 2	3.70	-2.3	-21.1	18.8	0.99
Trial 3	3.33	-4.1	-19.0	14.9	0.97

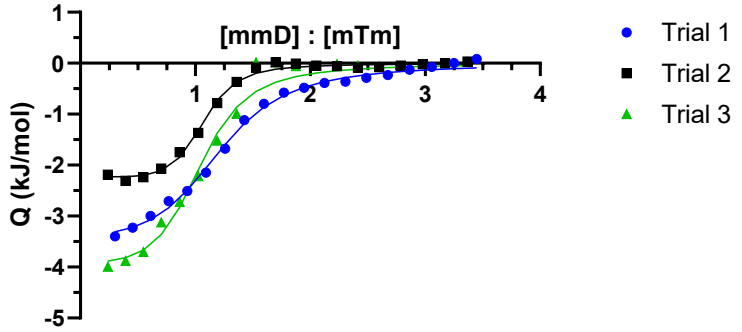


Figure S61. ITC titration of **mmD** (100 mM) into **mTm** (10 mM) in chloroform.
Samples contained residual TFA from HPLC.

Table S4. Thermodynamic binding parameters of **mmD·mmT** measured by ITC.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.21	-4.3	-18.3	14.0	0.92
Trial 2	2.82	-5.6	-16.1	10.5	0.77
Trial 3	2.68	-5.7	-15.3	9.6	0.98
Trial 4	3.49	-4.2	-19.9	15.8	1.00
Trial 5	3.61	-2.2	-20.6	18.4	1.36

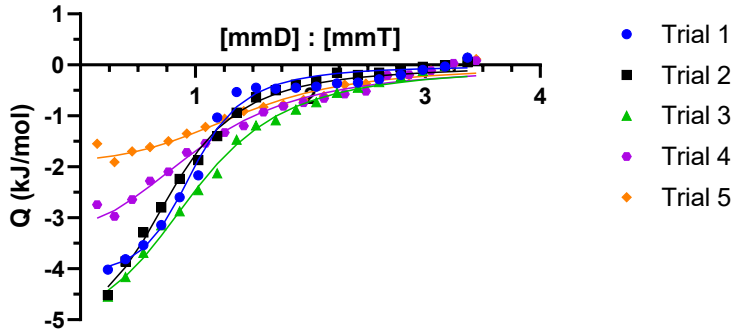


Figure S62. ITC titration of **mmD** into **mmT** in chloroform. In trials 1-3 of Fig S30, the samples contained TFA, the concentration of mmD in the syringe was 100 mM, and the concentration of mmT in the sample cell was 10 mM. In trials 4 and 5, the samples were TFA-free, the concentration of mmD in the syringe was 10 mM, and the concentration of mmT in the cell was 1 mM.

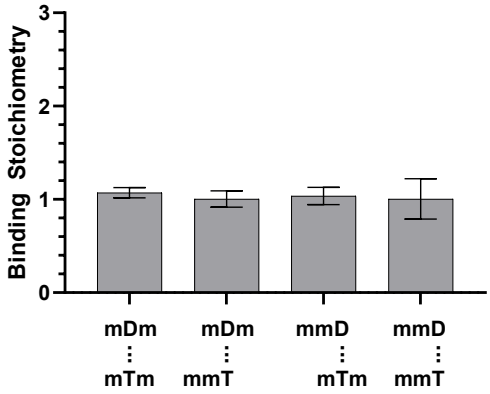


Figure S63. Binding stoichiometries of complexes of monovalent SeDOCs measured by ITC in chloroform.

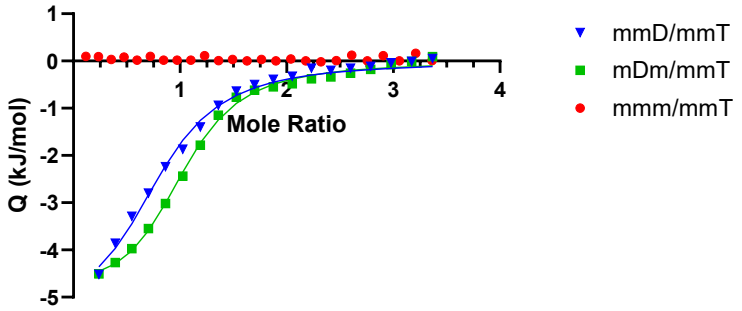


Figure S64. ITC titration of **mmm** (100 mM) into **mmT** (10 mM). The data is overlayed with representative trials of the titrations of mmD into mmT and mDm into mmT.

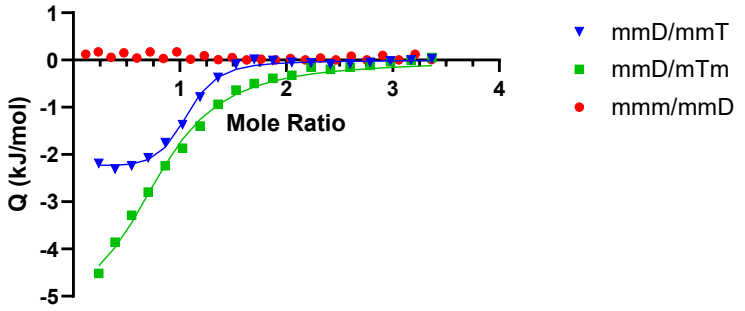


Figure S65. ITC titration of **mmm** (100 mM) into **mmD** (10 mM). The data is overlayed with representative trials of the titrations of mmD into mmT and mmD into mTm.

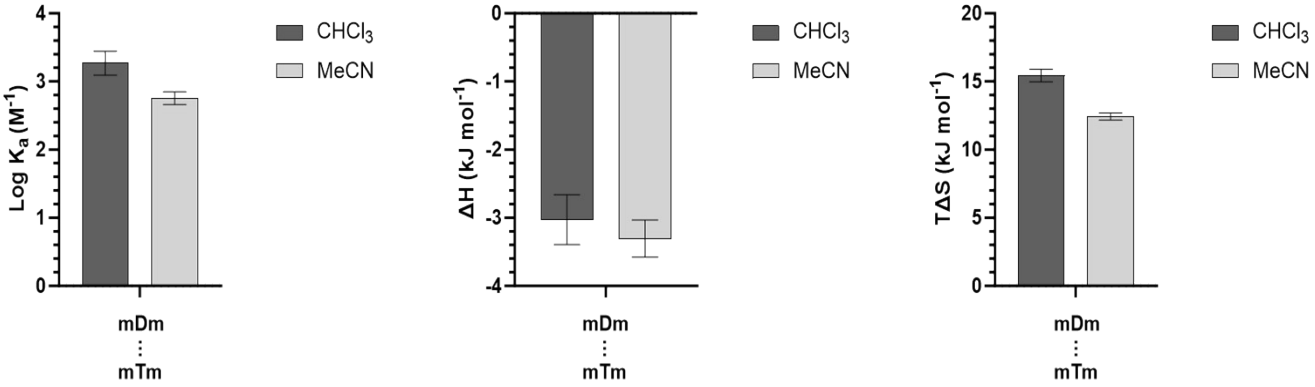


Figure S66. Comparison of thermodynamic binding parameters of **mDm·mTm** in chloroform and in acetonitrile.

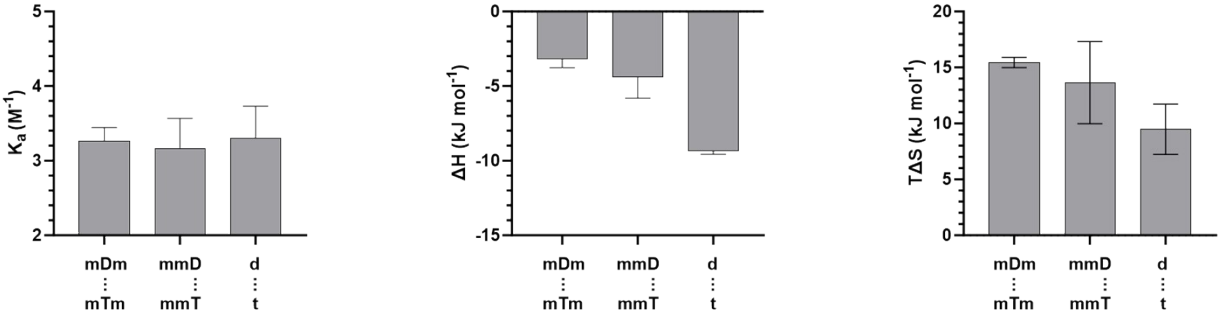


Figure S67. Comparison of thermodynamic parameters of **mDm·mTm**, **mmD·mmT**, and **d·t**.

Table S5. Thermodynamic binding parameters of **mDD·mTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.80	-19.8	-21.7	1.9	0.98
Trial 2	3.75	-22.7	-22.4	-1.3	0.93
Trial 3	3.84	-21.3	-21.9	0.62	0.81
Trial 4	3.80	-20.8	-20.8	0.85	0.95

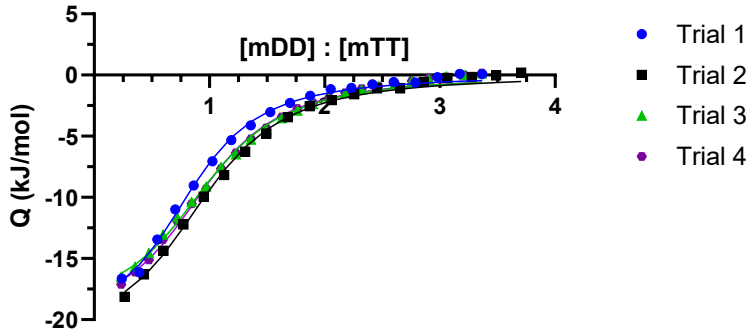


Figure S68. ITC titration of **mDD** (10 mM) into **mTT** (1 mM) in chloroform.
Samples were TFA-free.

Table S6. Thermodynamic binding parameters of **DmD·TmT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.79	-37.1	-21.6	-15.5	0.60
Trial 2	3.82	-37.9	-21.8	-16.1	0.64

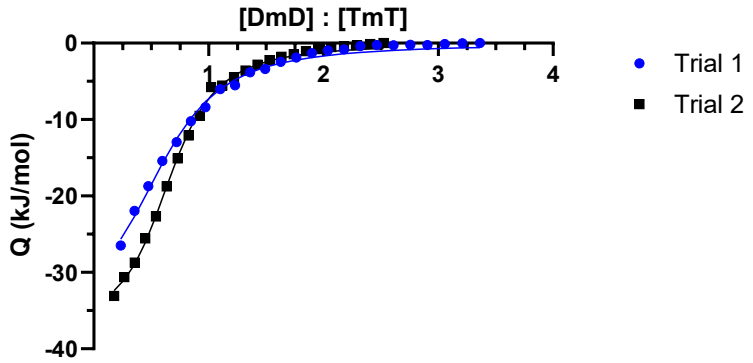


Figure S69. ITC titration of **DmD** into **TmT** in chloroform.
Trial 1: 10 mM **DmD** titrated into 1 mM **TmT.** Trial 2: 15 mM **DmD** titrated into 2 mM **TmT**. Samples were TFA-free.

Table S7. Thermodynamic binding parameters of **mDD·TmT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.82	-32.5	-21.8	-10.6	0.69
Trial 2	3.87	-36.0	-22.1	-13.9	0.66

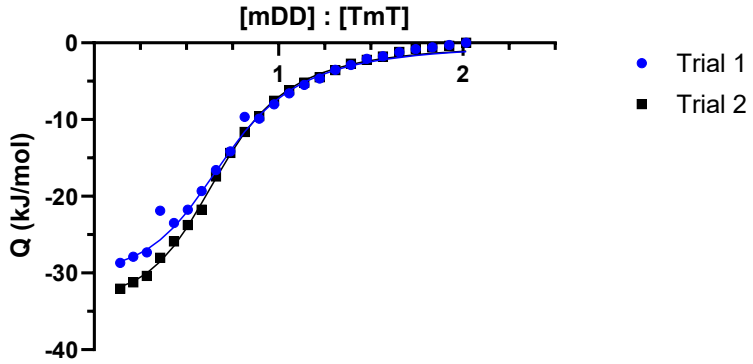


Figure S70. ITC titration of **mDD** (15 mM) into **TmT** (2 mM) in chloroform.
Samples were TFA-free.

Table S8. Thermodynamic binding parameters of **DmD·mTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.61	-27.4	-20.6	-6.8	0.59
Trial 2	3.69	-28.1	-21.0	-7.1	0.57

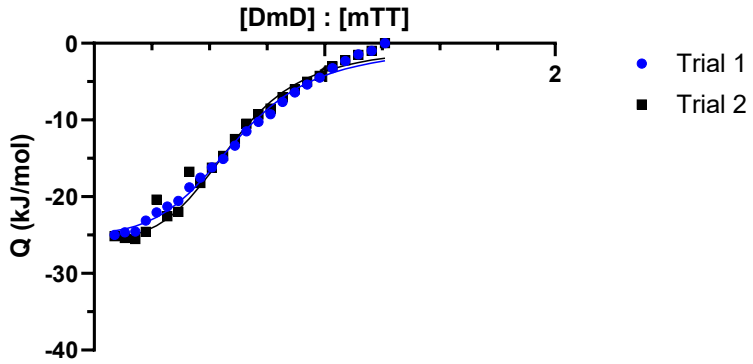


Figure S71. ITC titration of **DmD** (15 mM) into **mTT** (4 mM) in chloroform.
Samples were TFA-free.

Table S9. Thermodynamic binding parameters of **DDD·TTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	4.94	-77.5	-28.2	-49.3	0.62
Trial 2	4.76	-58.3	-27.1	-31.2	0.88
Trial 3	4.89	-52.9	-27.9	-25.0	0.86

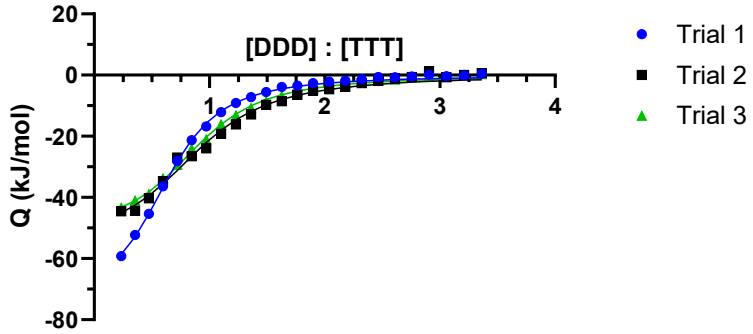


Figure S72. ITC titration of **TTT** (1 mM) into **DDD** (0.1 mM) in chloroform.
Samples were TFA-free.

Mixed Mode SeDOC ITC Experiments

Table S10. Thermodynamic binding parameters of **mmD·mTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.42	-12.4	-19.5	7.2	0.80
Trial 2	3.16	-12.7	-18.0	5.3	0.78

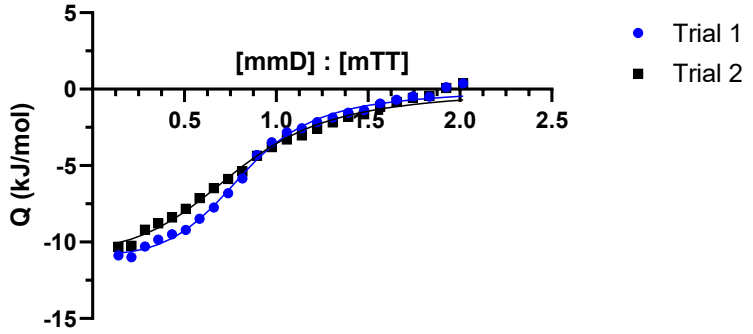


Figure S73. ITC titration of **mmD** (30 mM) into **mTT** (5 mM) in chloroform.
Samples were TFA-free.

Table S11. Thermodynamic binding parameters of **mmD·TTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	3.53	-9.1	-20.1	11.1	0.95
Trial 2	3.53	-10.9	-20.1	9.2	0.94

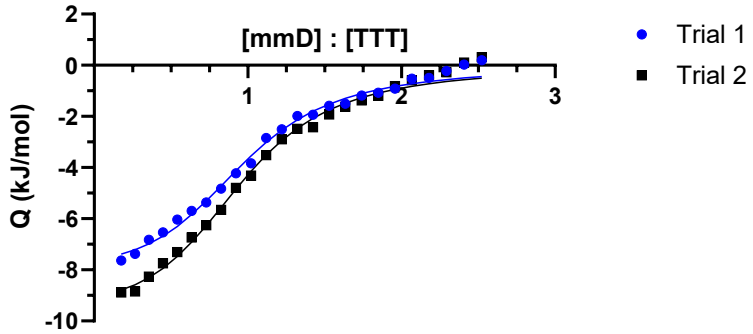


Figure S74. ITC titration of **mmD** (15 mM) into **TTT** (2 mM) in chloroform.
Samples were TFA-free.

Table S12. Thermodynamic binding parameters of **mDD·TTT** measured by ITC in chloroform.

	Log K_a (M^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	TΔS (kJ mol^{-1})	n
Trial 1	4.15	-33.5	-23.7	-9.9	1.54
Trial 2	4.11	-34.7	-23.5	-11.2	1.47

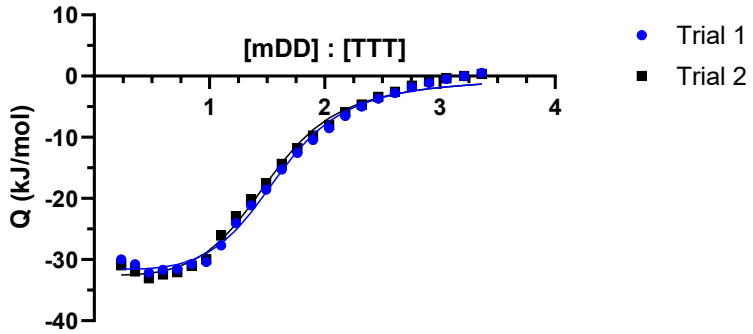


Figure S75. ITC titration of **mDD** (10 mM) into **TTT** (1 mM) in chloroform.
Samples were TFA-free.

References

1 X.-Y. Jin, C.-S. Wu, A.-D. Liu, L. Liu and L. Cheng, *Synthesis*, 2022, **54**, 1643–1651.
2 E. A. Hoff, G. X. De Hoe, C. M. Mulvaney, M. A. Hillmyer and C. A. Alabi, *J. Am. Chem. Soc.*, 2020, **142**, 6729–6736.