Electronic Supplementary Information

Aqueous Dynamic Covalent Assembly of Molecular Ladders and Grids Bearing Boronate Ester Rungs

Megan F. Dunn,[a] Tao Wei,[a] Ronald N. Zuckermann,[b] and Timothy F. Scott*[,a,c]

a: Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

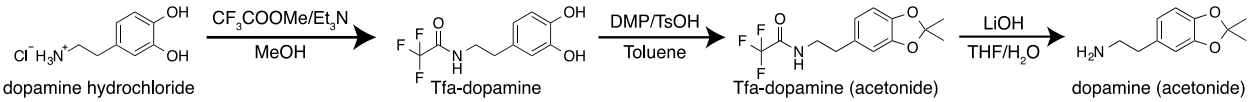
b: Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

c: Macromolecular Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA

General Experimental Techniques

^{1}H NMR spectra of the acetonide-protected dopamine were collected using a Varian VNMRS 500 spectrometer. Electrospray ionization (ESI) mass spectra were recorded using an Agilent Q-TOF 1200 series spectrometer in positive ion mode. Matrix-assisted laser desorption/ionization (MALDI-TOF) time-of-flight mass spectra were collected by utilizing a Bruker Autoflex mass spectrometer used in reflectron mode with both positive and negative ionizations as indicated. Reverse phase high performance liquid chromatography (RP-HPLC) was performed using both a preparative reversed phase Phenomenex Luna C18(2) columns with a linear gradient of water and acetonitrile as the eluent at 30°C as well as an analytical scale column. The RP-HPLC system was equipped with dual Shimadzu LC-6AD HPLC pump, Shimadzu FRC 70A fraction collector, and monitored using Shimadzu Prominence detector at 214 nm. Fluorescent and ultraviolet-visible (UV-vis) spectroscopy readings were collected on a BioTek Synergy H1 multi-mode microplate reader. Unless otherwise noted all reagents and materials were purchased from commercial sources including Sigma Aldrich, AK Scientific, Oakwood Chemical, and TCI Chemical.

Preparation of Acetonide-protected Dopamine



Scheme **S1**. Synthetic scheme for the preparation of acetonide-protected dopamine.

The acetonide-protected dopamine monomer for peptoid synthesis was synthesized *via* a three-step process (Scheme S1) adapted from a published protocol from Messersmith et al.[1] The synthesis of the Tfa-dopamine proceeded by treatment of dopamine hydrochloride (21.3 g) with methyl trifluoroacetate (23 mL) in 250 mL of methanol in the presence of triethylamine (64 mL) overnight at room temperature. The solvent was removed by rotary evaporation, and the pH of the resulting solid was adjusted to ~1 with 1 N HCl solution. A liquid extraction was then performed with ethyl acetate followed by water washes. The product was dried with sodium sulfate and rotary evaporation was used to remove the solvent. ^{1}H-

NMR of the Tfa-dopamine measured in δ (ppm) relative to residual solvent (DMSO-d_6 = 2.50) is shown in Figure S2.

Tfa-dopamine

δH(400 MHz; DMSO-d6; Tfa-dopamine) 2.6 (2 H, t, Me), 3.3 (2 H, q, Me), 3.4 (water), 6.5 (1 H, dd, Ph), 6.6 (1 H, d, Ph), 6.65 (1 H, d, Ph), 8.7–8.8 (2 H, br, catecholic proton), and 9.5 (1 H, s, amide)

Subsequently, Tfa-dopamine (5 g) was refluxed with 2,2-dimethoxypropane (10 mL) and a catalytic amount of *p*-toluenesulfonic acid (172 mg) in toluene. The reaction was outfitted with a Soxhlet extractor filled with granular anhydrous $CaCl_2$. The reaction was refluxed for 2 hours and then allowed to cool to room temperature before being filtered with a short silica gel column washed with dichloromethane (DCM). The solvent was then removed from the product by rotary evaporation before recrystallization in hexanes to afford Tfa-dopamine (acetonide) as confirmed by ^{1}H-NMR in δ (ppm) relative to residual solvent ($CDCl_3$ = 7.24), shown in Figure S3.

Tfa-dopamine(acetonide)

δH(400 MHz; $CDCl_3$; Tfa-dopamine (acetonide)) 1.7 (6 H, s, Me), 2.8 (2 H, t, Me), 3.6 (2 H, q, Me), 6.5 (1 H, br, amide), 6.6 (2 H, m, Ph), and 6.7 (1 H, d, Ph)

The acetonide-protected dopamine was obtained by hydrolysis of Tfa-dopamine (acetonide) in THF and an aqueous lithium hydroxide solution (2 equivalents), followed by a liquid-liquid extraction with ethyl acetate. The ethyl acetate was removed by rotary evaporation before the monomer was dried under high vacuum. The ^{1}H-NMR of dopamine(acetonide) in δ (ppm) relative to residual solvent ($CDCl_3$ = 7.24) is shown in Figure S4.

Dopamine(acetonide)

δH(400 MHz; $CDCl_3$; dopamine (acetonide)) 1.7 (6 H, s, Me), 2.6 (2 H, t, Me), 2.9 (2 H, t, Me), and 6.7 (3 H, m, Ph)

Preparation of Oligopeptoids

The majority of the peptoid-based oligomers were prepared using a microwave-assisted Liberty Blue peptide synthesizer (CEM Corporation) on Rink amide 4-methylbenzhydrylamine (MBHA) resin (ChemPep Inc.) using a submonomer solid phase synthesis scheme.[2] Four of the catechol functionalized peptoids ($(NmeNdop)_nNdop$ where n = 3-6) were synthesized on Rink amide MBHA resin using an AAPPTec Apex 396 Peptide Synthesizer at the Molecular Foundry at Lawrence Berkley National Laboratory following the same protocol. The resin contains a fluorenylmethyloxycarbonyl (Fmoc)-

protected amine that is initially deprotected before synthesis by treatment in 4-methylpiperidine: dimethylformamide (DMF) (20:80, volume ratio). The synthesis then proceeds by a sequential addition reaction whereby the *N*-terminal amine from the solid support is acetylated with 1 M bromoacetic acid using 1.2 M *N*,*N*'-diisopropylcarbodiimide (DIC) as an activating agent for 5 minutes at 75°C, to afford a terminal bromide which is subsequently displaced via nucleophilic substitution with a 0.5 M primary amine bearing the pendant group for 5 minutes at 75°C (see Figure S1A). This two-step process of acetylation and displacement is repeated until the desired chain length is achieved. The *N*-terminal of the complementary oligomers was capped with 1 M acetic anhydride activated with DIC to prevent further chain elongation. The pendant group-bearing primary amine monomers are shown in Figure S1B. These primary amines fall into two categories, those bearing dynamic covalent pendant groups and those acting as inert spacer residues. Monomers bearing protected dynamic covalent pendant groups include acetonide-protected dopamine (Nace) and the commercially available 4-(aminomethyl)phenylboronic acid, pinacol ester (Npbe) purchased from AccelaChem. Upon deprotection, these yield dopamine (Ndop) and 4-(aminomethyl)phenylboronic acid (Npba) residues, respectively. The inert spacer monomers were the commercially-available 2-methoxyethylamine (Nme) and 2-(2-ethoxyethoxy)ethylamine (Neee) prepared according to the protocol established in Wei et al.[3] All of the reagents were prepared in DMF with the exception of the Neee monomer that was prepared in *N*-methyl-2-pyrrolidone (NMP).

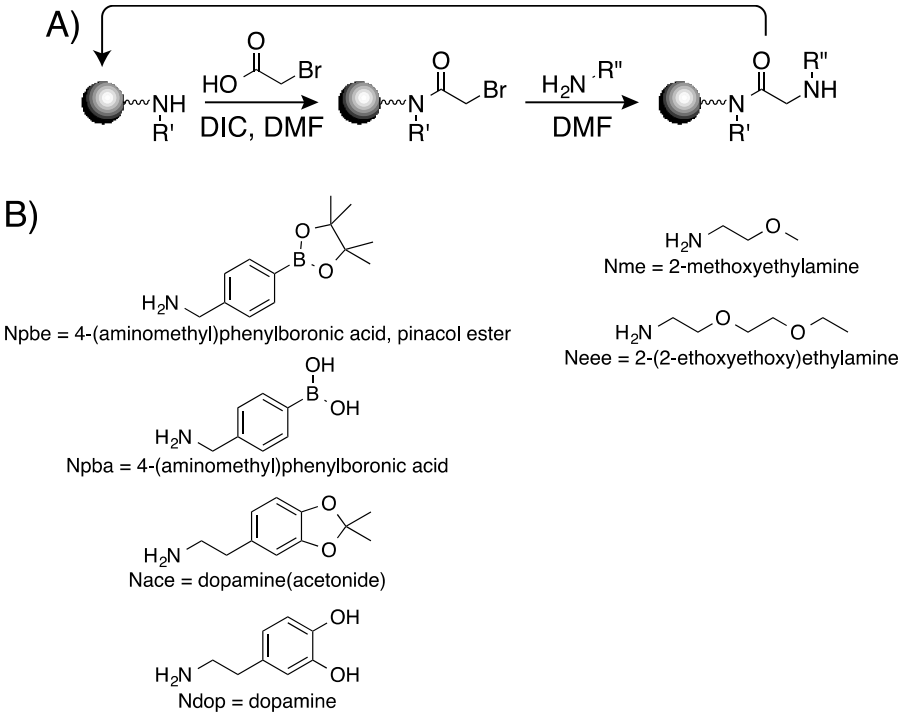


Figure **S1**. (A) Schematic diagram of solid-phase peptoid synthesis. The sequential addition of bromoacetic acid and primary amines yields sequence-specific peptoid oligomers without a deprotection step. Here, DIC is *N*,*N*'-diisopropylcarbodiimide and DMF is dimethylformamide. (B) Primary amine

monomers used in this study, including the pinacol-protected 4-(aminomethyl)phenylboronic acid (Npbe), 4-(aminomethyl)phenylboronic acid (Npba), acetonide-protected dopamine (Nace), dopamine (Ndop), 2-methoxyethylamine (Nme), and 2-(2-ethoxyethoxy)ethylamine (Neee).

The solid support resin of the pinacol-protected boronic acid-functionalized peptoids ($(NmeNpbe)_nNme$) underwent a two-step process to rapidly and efficiently prepare a peptoid with exposed boronic acid functionality. The first step is an esterification with a 1 M solution diethanolamine prepared in DMF (5 mL) for 30 minutes with the resin being mixed by a steady nitrogen stream. The second step is hydrolysis by immersion in a mixture of trifluoroacetic acid (TFA) and water (95:5 volume ratio) at room temperature for 5 minutes leaving an unbound and deprotected peptoid ($(NmeNpba)_nNme$) with a C-terminal primary amide. The catechol-functionalized peptoids ($(NmeNace)_nNme$) were cleaved from the resin by a TFA/water mixture (95:5 volume ratio) at room temperature for 25 minutes. In addition to the peptoid being cleaved from the resin, the acid-labile acetonide protecting group is removed leaving the exposed catechol peptoids ($(NmeNdop)_nNme$). The deprotected peptoids were then purified by preparative RP-HPLC at a linear gradient of 10%-acetonitrile-water to 90%-acetonitrile-water over 22 minutes. The molecular weight of the major peak was confirmed using ESI mass spectrometry in positive mode. The purified fractions were combined and lyophilized to afford a fine white powder. The peptoids sequences and characterization can be found below.

Hybridization of Oligopeptoids into Molecular Ladders

All hybridization experiments were performed in an anaerobic chamber to minimize the risk of oxidation of the individual strands. Stock solutions of the peptoids were prepare at a 10 mM concentration in water with the exception of the $Ndop_6$ and $Ndop_8$ peptoids which were prepared in 50:50 MeCN:water mixture due to a decrease in polarity with these strands. For the single molecular ladders, 160 μL of a sodium hydroxide solution adjusted to a pH of 9 was added to a vial with a magnetic stir bar. 20 μL (10 mM stock solution) of each of the complementary strands were added to the vial. For the double rung molecular ladders, 140 μL of the same alkaline aqueous solution was added to a vial with a magnetic stir bar. 20 μL (10 mM stock solution) of the catechol-functionalized peptoids and 40 μL of the corresponding boronic acid-functionalized peptoids were added to the vial. The solutions were stirred overnight before preparing a MALDI-TOF sample in α-cyano-4-hydroxycinnamic acid (CHCA). The MALDI-TOF samples were ran in negative reflectron mode. The molecular structures and the expected exact mass are shown in Scheme S4.

Dynamic Strand Rearrangement

A sample of the duplex hybrid-3 solution was prepared as described above. The solution was monitored in positive reflectron mode MALDI-TOF with 2-(4-hydroxyphenylazo)benzoic acid (HABA) as the

matrix after being allowed to stir overnight. 20 μL of the ((NeeeNdop)$_3$Neee) peptoid (10 mM stock solution) was added to the already-formed solution of hybrid-3. The solution was stirred overnight before taking a MALDI-TOF spot using HABA matrix and positive reflectron mode.

Determination of the Boronic Acid/Diol Binding Constant

In anaerobic conditions, 0.25 mM alizarin red S (ARS) was prepared in an aqueous NaOH solution (pH = 9), and 30 μL of the solution was added to each of the wells in a clear 96-well plate. Using the peptoid, Nme$_2$NpbaNme$_2$, bearing a single boronic acid pendant group, samples were made ranging from 1 to 100 equivalents of the peptoid. 30 μL of the different boronic acid samples were added to the wells containing the fixed amount of ARS. The solution was mixed with a micropipette. An absorbance scan in 400-800 nm wavelength region was performed as well as a fluorescent scan at excitation 485 nm and emission 620 nm. This data was fitted to a Benesi-Hildebrand equation to determine the equilibrium binding constant.

A solution 50% ARS (0.25 mM) and 50% Nme$_2$NpbaNme$_2$ (0.25 mM) in an aqueous NaOH solution (pH = 9) was prepared and 60 μL was added to each well. Nme$_2$NdopNme$_2$, a short peptoid with one catchol functional group was used to make samples ranging from 0.5 to 100 equivalents of the diol pendent group; 30 μL of each of these samples were added to the corresponding well. An absorbance scan in 400-800 nm wavelength region was performed as well as a fluorescent scan at excitation 485 nm and emission 620 nm. This data was used to calculate the binding constant between two different peptoid strands.

Qualitative demonstration of the competitive binding of tetramer peptoids

ARS (0.25 mM) was prepared in an aqueous NaOH solution (pH = 9), and 60 μL of the solution was added to each of the wells. Using the (NmeNpba)$_4$Nme as a model peptoid, samples were made ranging from 1 to 100 equivalents of the boronic acid functional group. 15 μL of the boronic acid samples of varying equivalence were added to each well. An absorbance scan in 400-800 nm wavelength region was performed as well as a fluorescent scan at excitation 485 nm and emission 620 nm.

Similarly, 60 μL of ARS (0.25 mM) and 15 μL of (NmeNpba)$_4$Nme (0.25 mM) was added to each well. (NmeNdop)$_4$Nme was used to make catechol samples ranging from 0.5 to 100 equivalents; 15 μL of each of these samples were added to the corresponding well. An absorbance scan in 400-800 nm wavelength region was performed as well as a fluorescent scan at excitation 485 nm and emission 620 nm.

Monomer Characterization

The ^{1}H NMR spectra for the different intermediate products in the 3-step process outlined in the experimental section above for preparing the acetonide-protected dopamine are presented here.

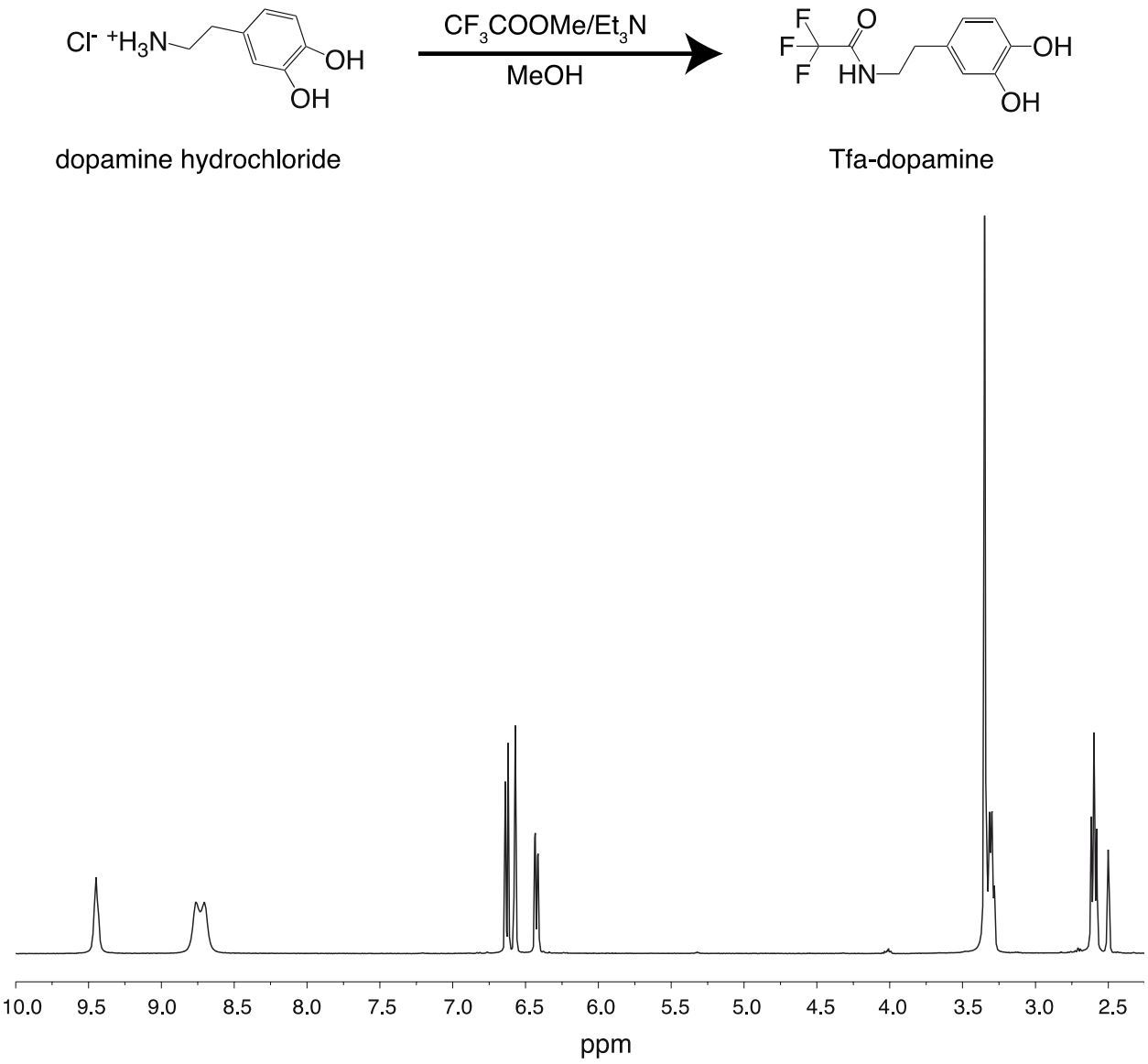


Figure **S2**. ^{1}H-NMR spectrum confirming the synthesis of Tfa-dopamine. δH(400 MHz; DMSO-d6; Tfa-dopamine) 2.6 (2 H, t, Me), 3.3 (2 H, q, Me), 3.4 (water), 6.5 (1H, dd, Ph), 6.6 (1H, d, Ph), 6.65 (1H, d, Ph), 8.7–8.8(2 H, br, catecholic proton), and 9.5 (1H, s, amide).

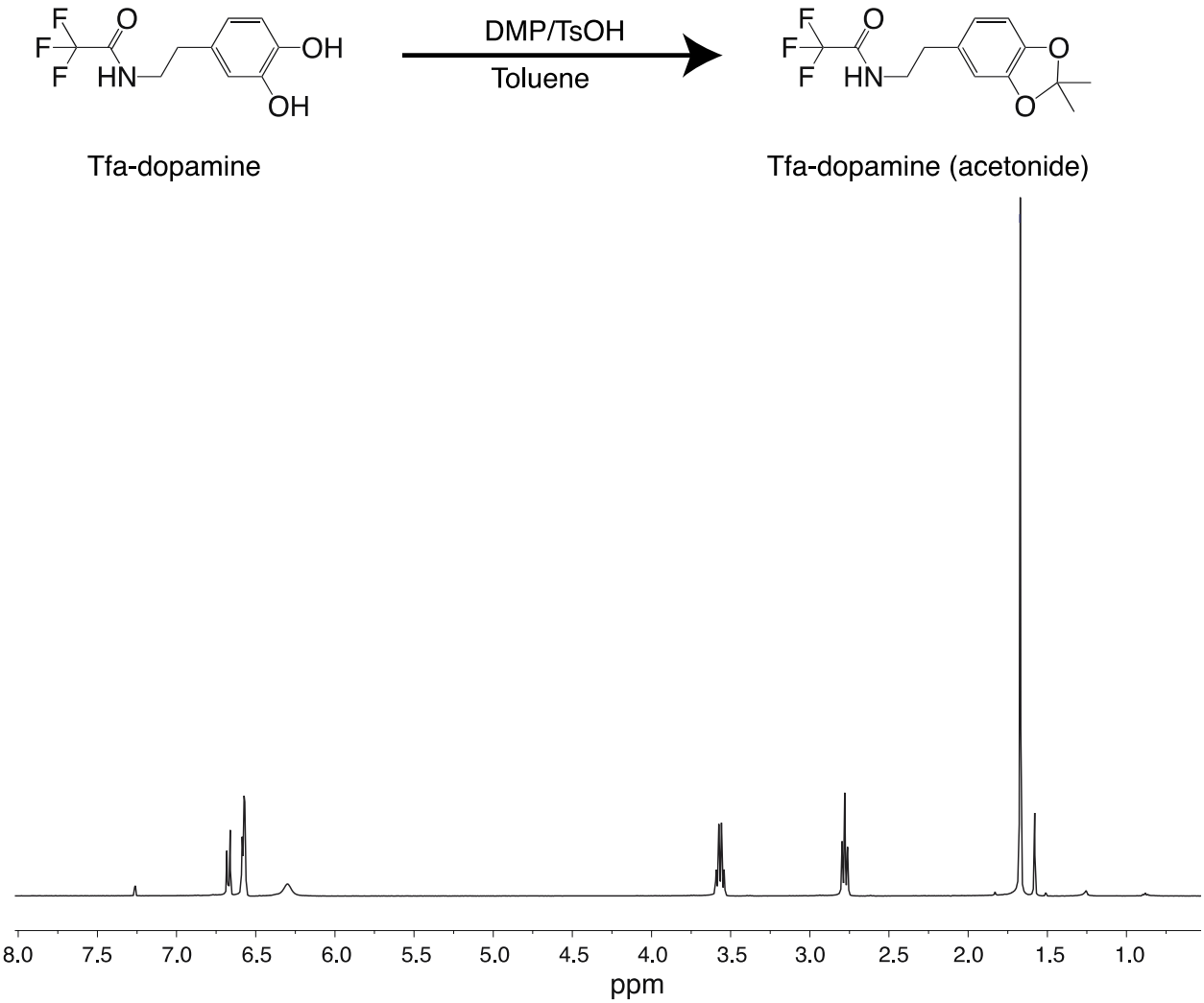


Figure **S3**. ^{1}H NMR spectrum confirming the synthesis of Tfa-dopamine(acetonide). δH(400 MHz; $CDCl_3$; Tfa-dopamine (acetonide)) 1.7 (6 H, s, Me), 2.8 (2 H, t, Me), 3.6 (2H, q, Me), 6.5 (1 H, br, amide), 6.6 (2 H, m, Ph), and 6.7 (1 H, d, Ph).

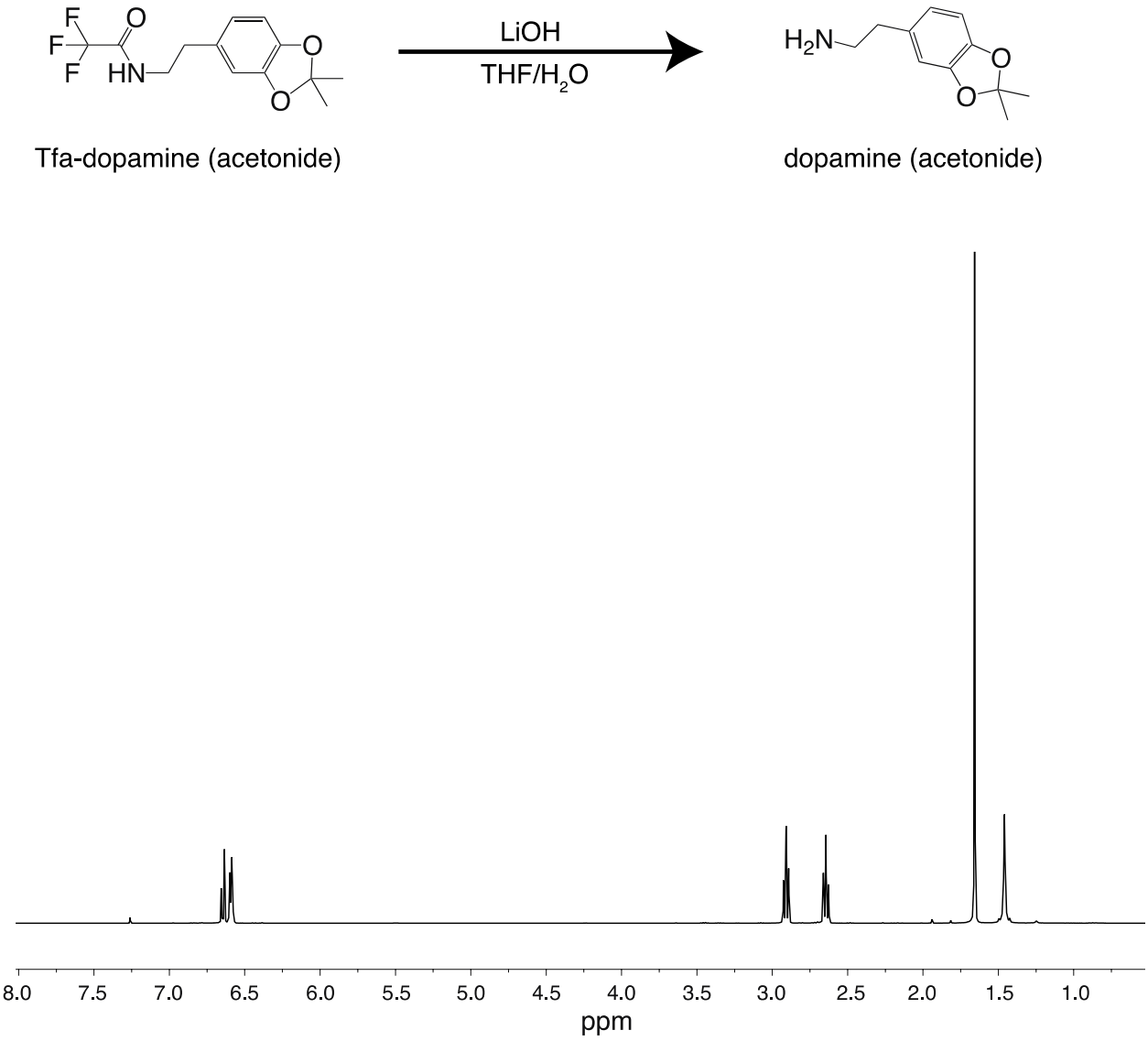
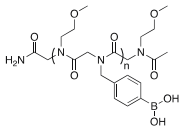


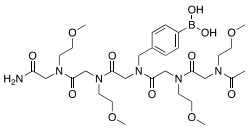
Figure **S4**. ^{1}H-NMR spectrum confirming the synthesis of acetonide-protected dopamine. δH(400 MHz; $CDCl_3$; dopamine (acetonide)) 1.7 (6 H, s, Me), 2.6 (2 H, t, Me), 2.9 (2 H, t, Me), and 6.7 (3H, m, Ph).

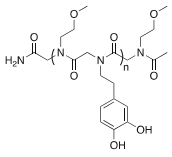
Oligomer Characterization

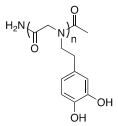
The ESI mass spectra and analytical HPLC traces confirming the synthesis of the oligomeric peptoid strands are shown here.

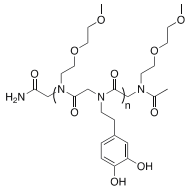
Table **S1**. Exact mass and nomenclature of the oligopeptoids used throughout this study.

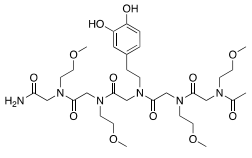
	n	exact mass (g/mol)
 $(NmeNpba)_nNme$	3	1092.5
	4	1398.7
	5	1704.8
	6	2010.9

	exact mass (g/mol)
 $Nme_2NpbaNme_2$	710.4

	n	exact mass (g/mol)
 $(NmeNdop)_nNme$	3	1098.5
	4	1406.6
	5	1714.8
	6	2022.9

	n	exact mass (g/mol)
 $Ndop_n$	6	1217.5
	8	1603.6

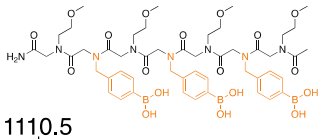
	exact mass (g/mol)
 $(NeeeNdop)_3Neee$	1217.5

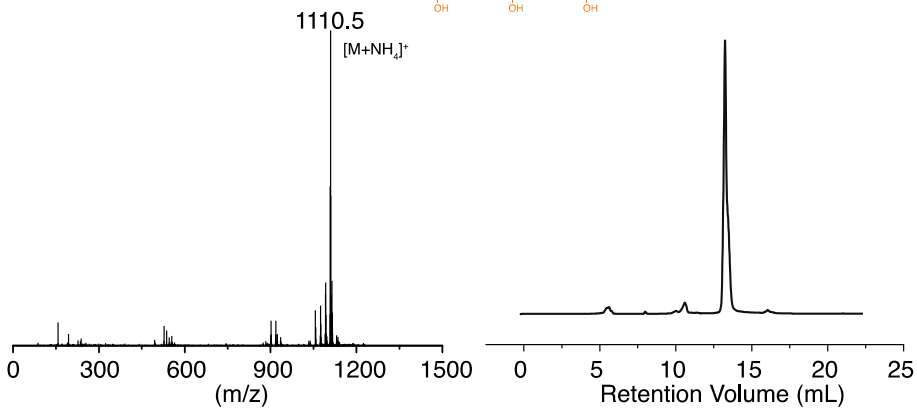
	exact mass (g/mol)
 $Nme_2NdopNme_2$	712.4

(A) $(NmeNpba)_3Nme$

$C_{49}H_{71}B_3N_8O_{18}$

Exact Mass: 1092.5

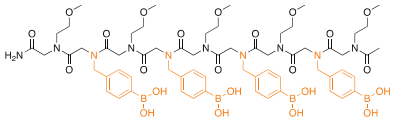


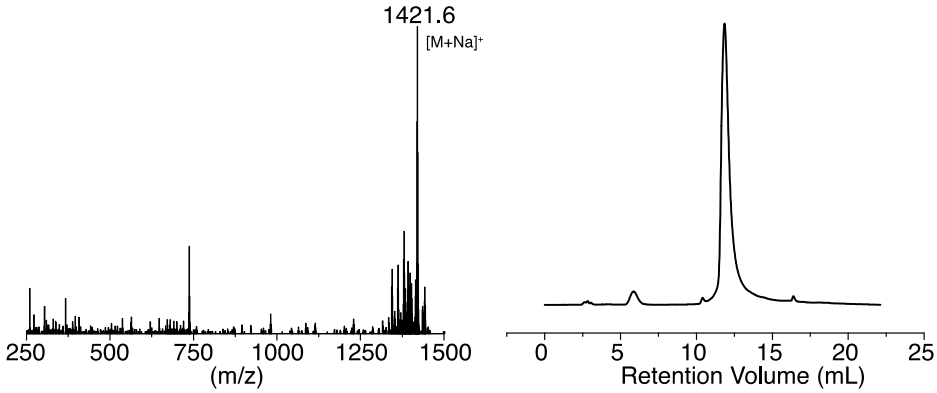


(B) $(NmeNpba)_4Nme$

$C_{63}H_{90}B_4N_{10}O_{23}$

Exact Mass: 1398.6

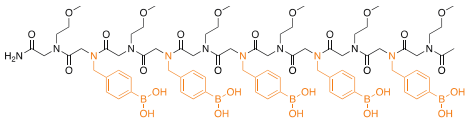


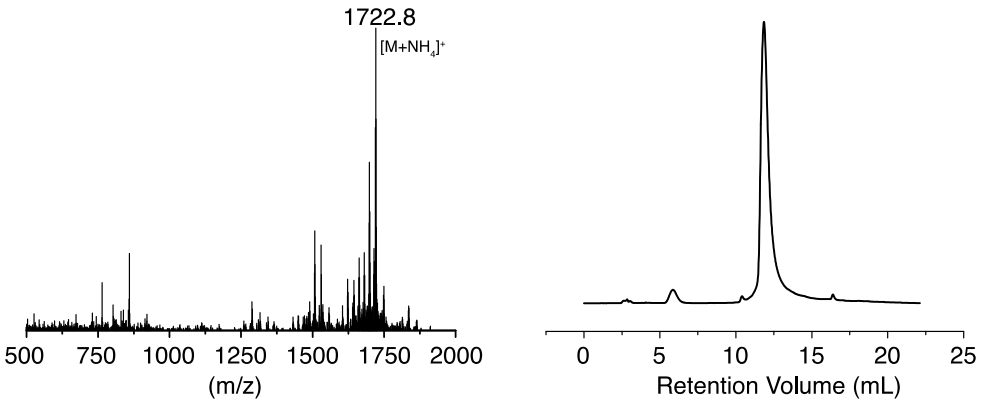


(C) $(NmeNpba)_5Nme$

$C_{77}H_{109}B_5N_{12}O_{28}$

Exact Mass: 1704.8





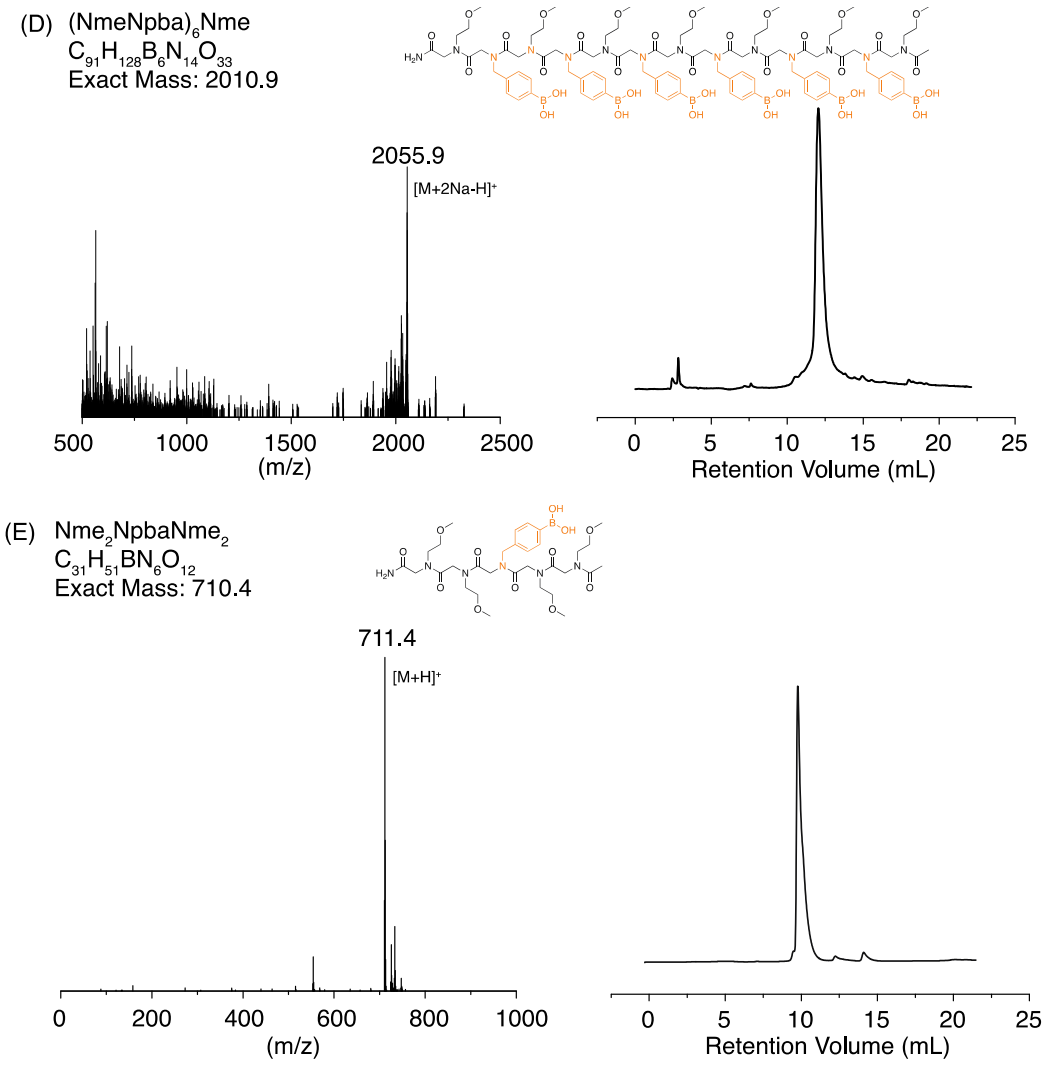
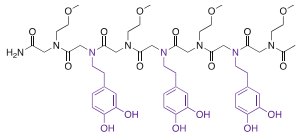
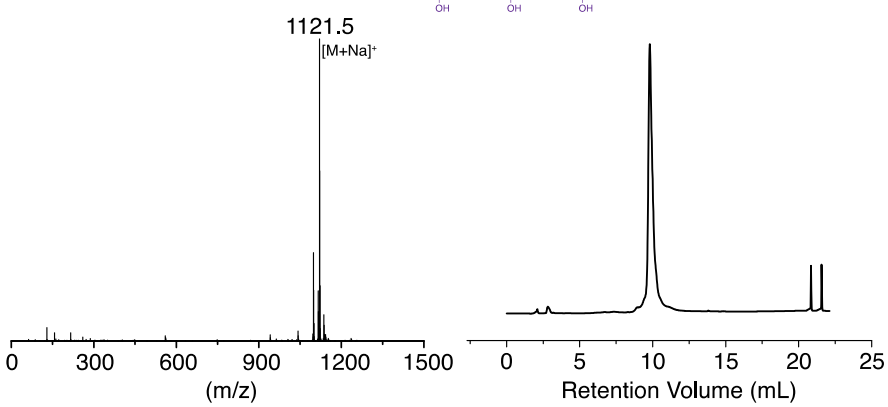


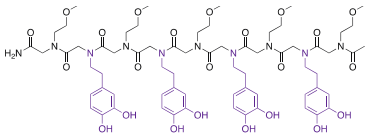
Figure **S5**. ESI mass spectra of boronic acid-functionalized oligopeptoids analyzed after cleavage from the solid support and HPLC purification, and analytical HPLC traces of the corresponding peptoids: (A) m/z = 1110.5 $[(Nme\text{-}Npba)_3Nme + NH_4]^+$, 97.2% purity; (B) m/z = 1421.6 $[(Nme\text{-}Npba)_4Nme + Na]^+$, 98.1% purity; (C) m/z = 1722.8 $[(Nme\text{-}Npba)_5Nme + NH_4]^+$, 96.5% purity; (D) m/z = 2055.9 $[(Nme\text{-}Npba)_6Nme + 2Na\text{-}H]^+$, 96.5% purity; and (E) m/z = 711.4 $[Nme_2NdpbaNme_2 + H]^+$, 95.5% purity.

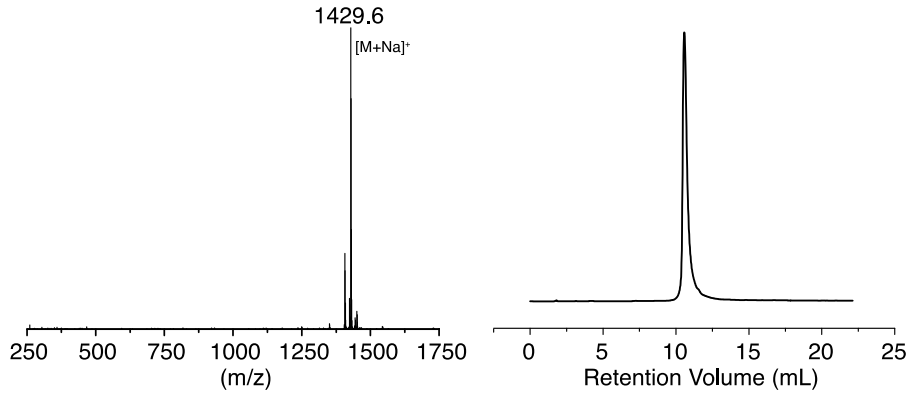
(A) $(NmeNdop)_3Nme$
$C_{52}H_{74}N_8O_{18}$
Exact Mass: 1098.5



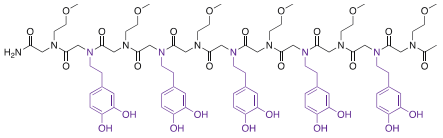


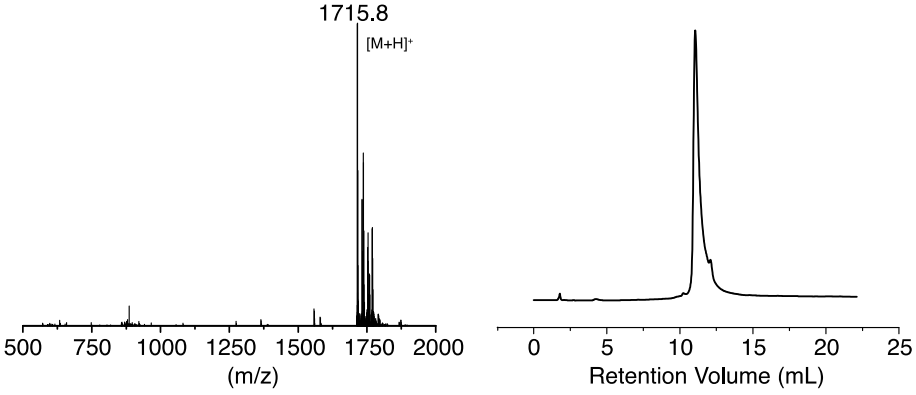
(B) $(NmeNdop)_4Nme$
$C_{67}H_{94}N_{10}O_{23}$
Exact Mass: 1406.7



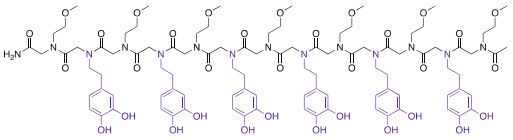


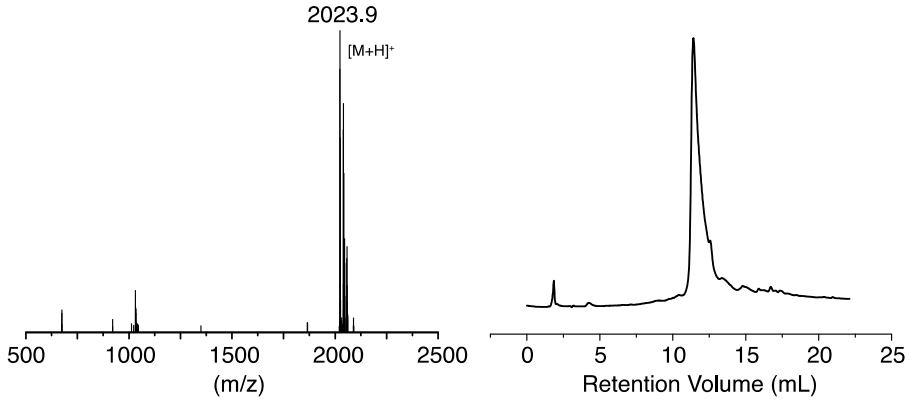
(C) $(NmeNdop)_5Nme$
$C_{82}H_{114}N_{12}O_{28}$
Exact Mass: 1714.8



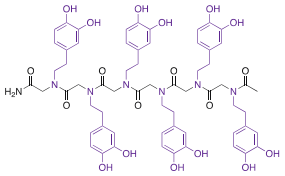


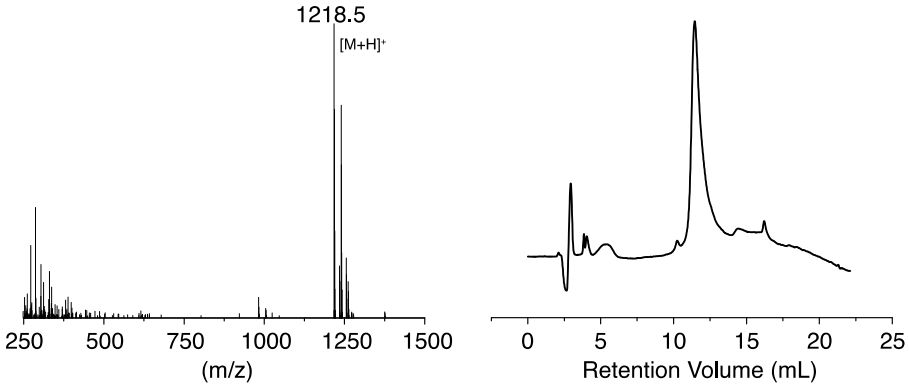
(D) $(NmeNdop)_6Nme$
$C_{97}H_{134}N_{14}O_{33}$
Exact Mass: 2022.9



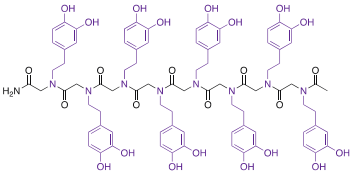


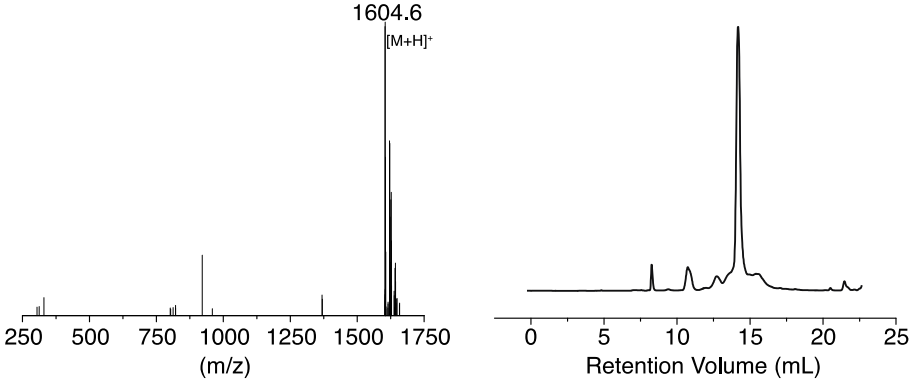
(E) $Ndop_6$
$C_{62}H_{71}N_7O_{19}$
Exact Mass: 1217.5





(F) $Ndop_8$
$C_{82}H_{93}N_9O_{25}$
Exact Mass: 1603.6





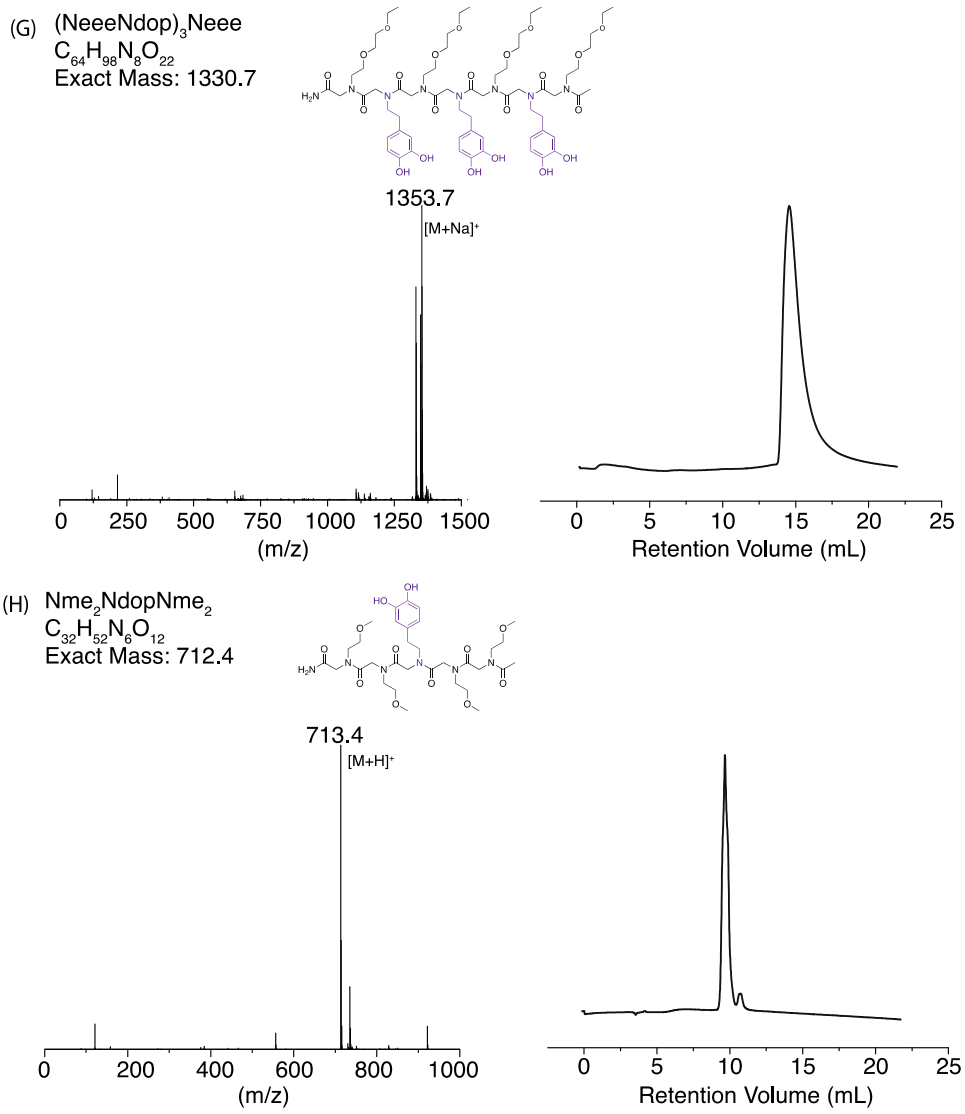


Figure **S6.** ESI mass spectra of catechol functionalized oligopeptoids analyzed after cleavage from the solid-support and HPLC purification and analytical HPLC traces of the corresponding peptoids: (A) m/z = 1121.5 $[(NmeNdop)_3Nme + Na]^+$, 95.2% purity; (B) m/z = 1429.6 $[(NmeNdop)_4Nme + Na]^+$, 99.2% purity; (C) m/z = 1715.8 $[(NmeNdop)_5Nme + H]^+$, 86.4% purity; (D) m/z = 2023.9 $[(NmeNdop)_6Nme + H]^+$, 97.5% purity; (E) m/z = 1218.5 $[Ndop_6 + H]^+$, 80.3% purity; (F) m/z = 1604.6 $[Ndop_8 + H]^+$, 89.4% purity; (G) m/z = 1353.7 $[(NeeeNdop)_3Neee + Na]^+$, 97.5% purity; and (H) m/z = 713.4 $[Nme_2NdopNme_2 + H]^+$, 99.4% purity.

Dimer Hybridization and Characterization

This section includes the structures and the exact mass of the hybrids formed in this study. In the main text of the article, the majority of the dimeric structures were characterized using negative ion mode MALDI-TOF; however, some of the structures were similarly characterized by positive ion mode MALDI-TOF using HABA as the matrix. The results of the positive mode mass spectra when available are shown in this section.

Table **S2**. Nomenclature and exact masses of hybridized structures.

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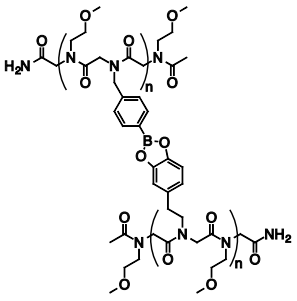
<tr><td rowspan="5"> 
Hybrid-n</td><td>n</td><td>exact mass
(g/mol)</td></tr>
<tr><td>3</td><td>2083.0</td></tr>
<tr><td>4</td><td>2661.2</td></tr>
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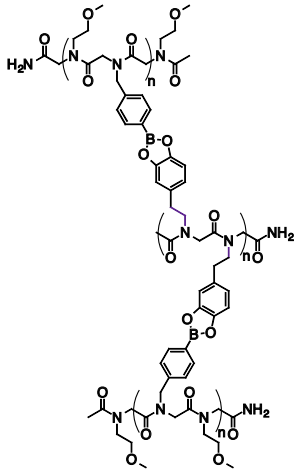
<tr><td rowspan="4"> 
Triplex-n</td><td>n</td><td>exact mass
(g/mol)</td></tr>
<tr><td>6</td><td>3186.4</td></tr>
<tr><td>8</td><td>4112.8</td></tr>
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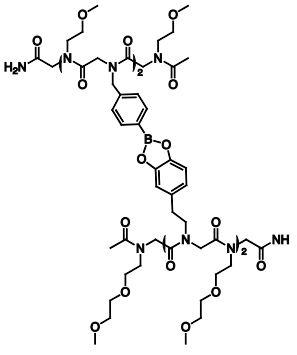
<tr><td rowspan="3"> 
Hybrid-E3</td><td colspan="2">exact mass
(g/mol)</td></tr>
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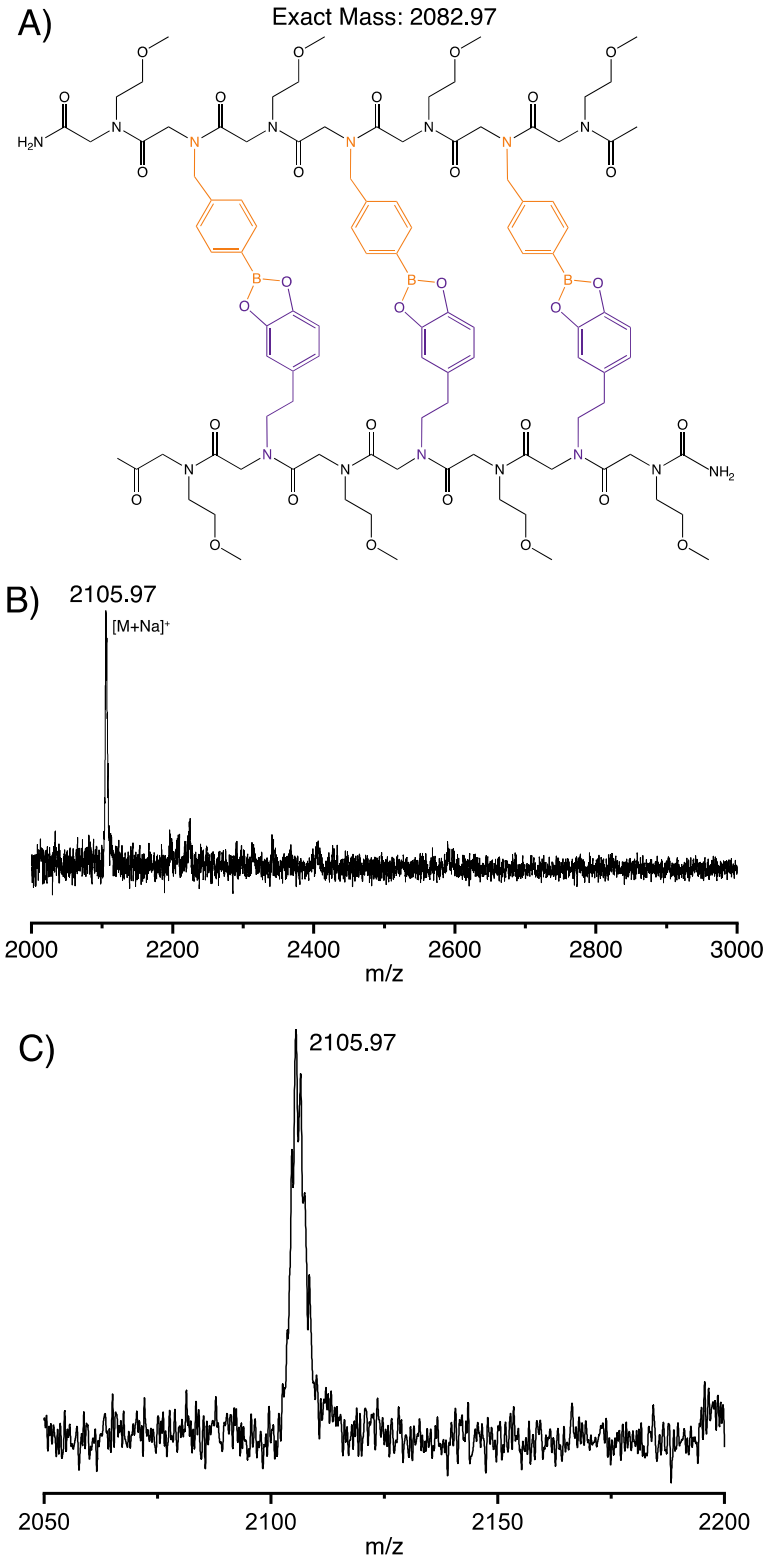


Figure **S7**. (A) Chemical structure of hybrid-3. (B) Positive ion mode MALDI-TOF spectrum confirming the formation of hybrid-3, expected m/z = 2105.95 [hybrid-3 + Na]$^+$, and (C) zoom-in of the region in the vicinity of the [hybrid-3 + Na]$^+$ peak.

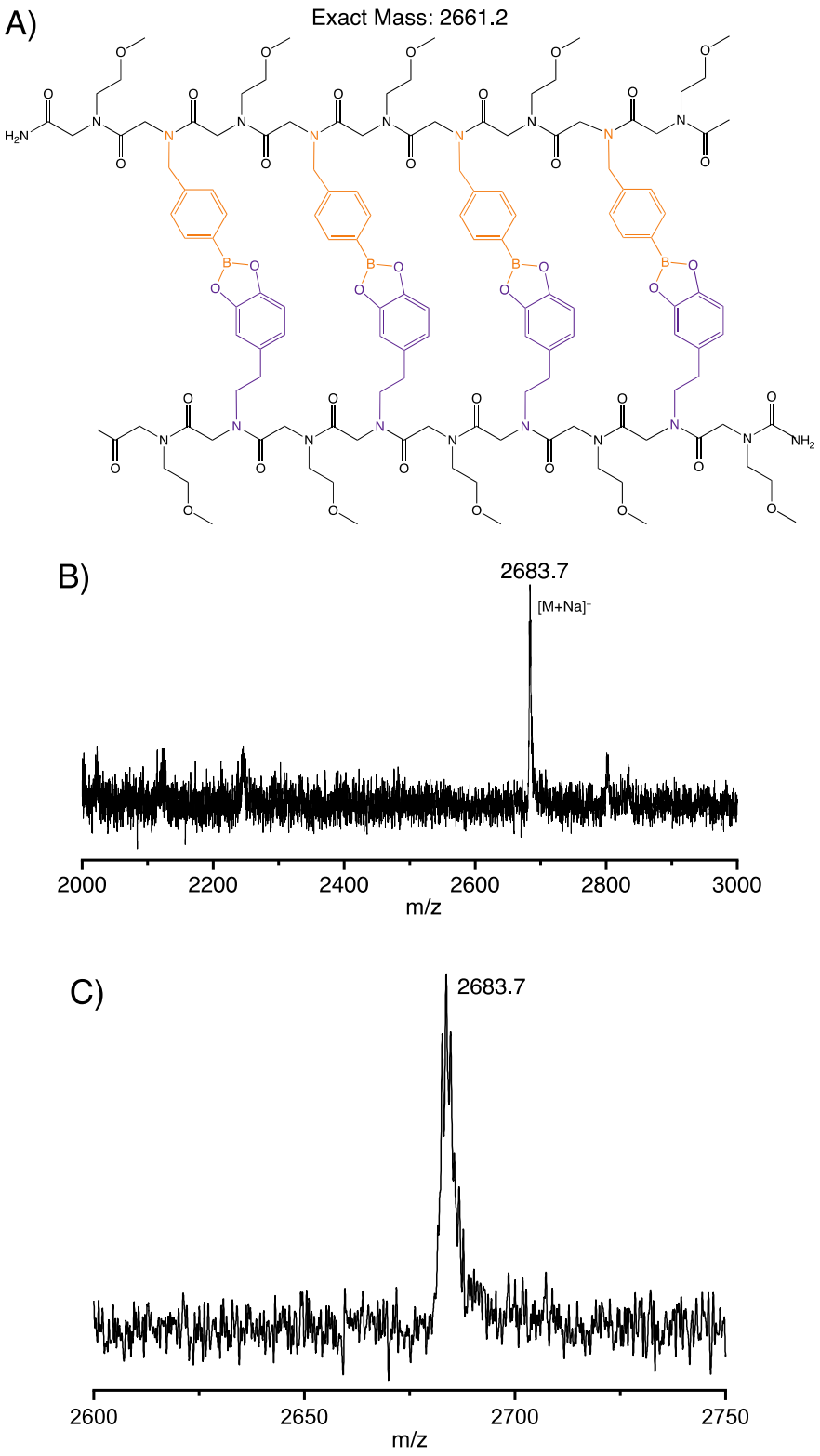


Figure **S8**. (A) Chemical structure of hybrid-4. (B) Positive ion mode MALDI-TOF spectrum confirming the formation of hybrid-4, expected m/z = 2684.2 [hybrid-4 + Na]$^+$, and (C) zoom-in of the region in the vicinity of the [hybrid-4 + Na]$^+$ peak.

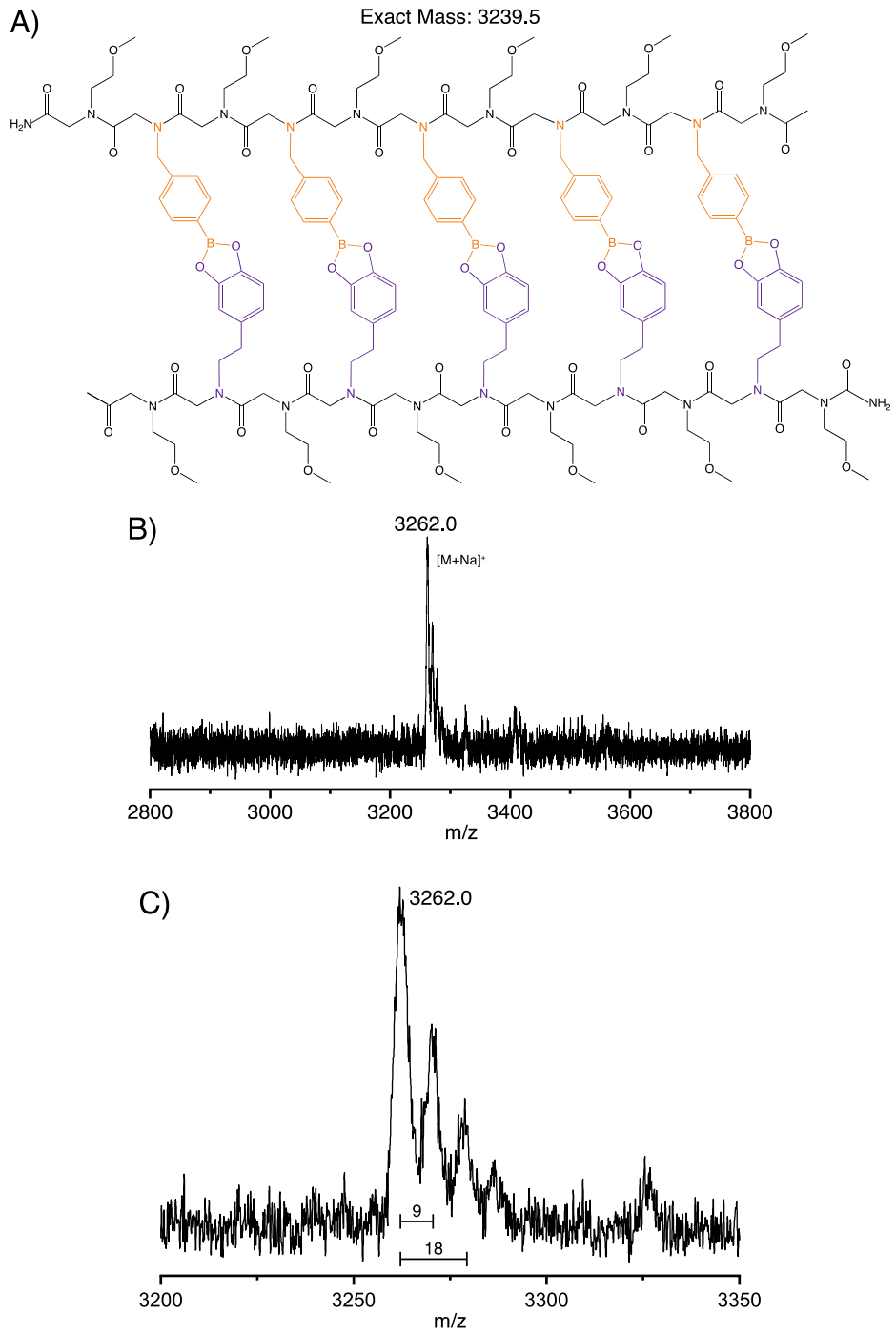


Figure **S9**. (A) Chemical structure of hybrid-5. (B) Positive ion mode MALDI-TOF spectrum confirming the formation of hybrid-5, expected m/z = 3262.5 [hybrid-5 + Na]$^+$, and (C) zoom-in of the region in the vicinity of the [hybrid-5 + Na]$^+$ peak, revealing additional peaks at +9 and +18 from the expected, in-registry peak.

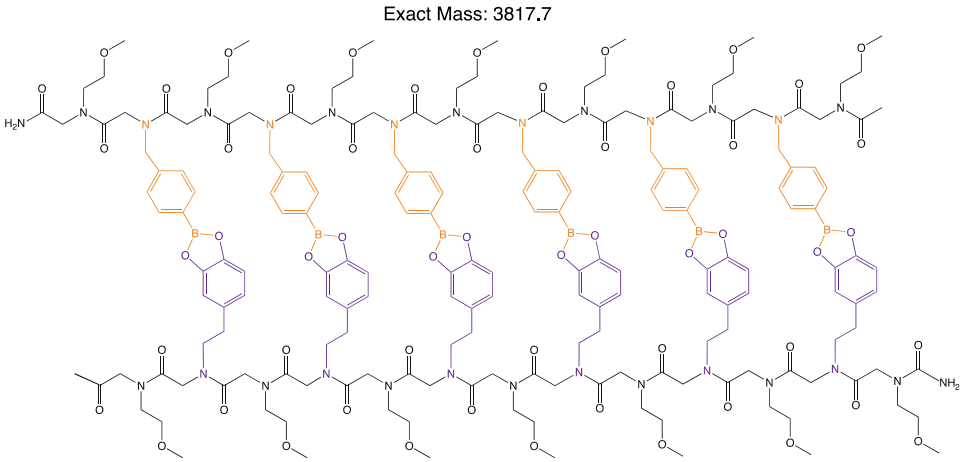


Figure **S10**. Structure of hybrid-6 with 6 boronate ester linkages.

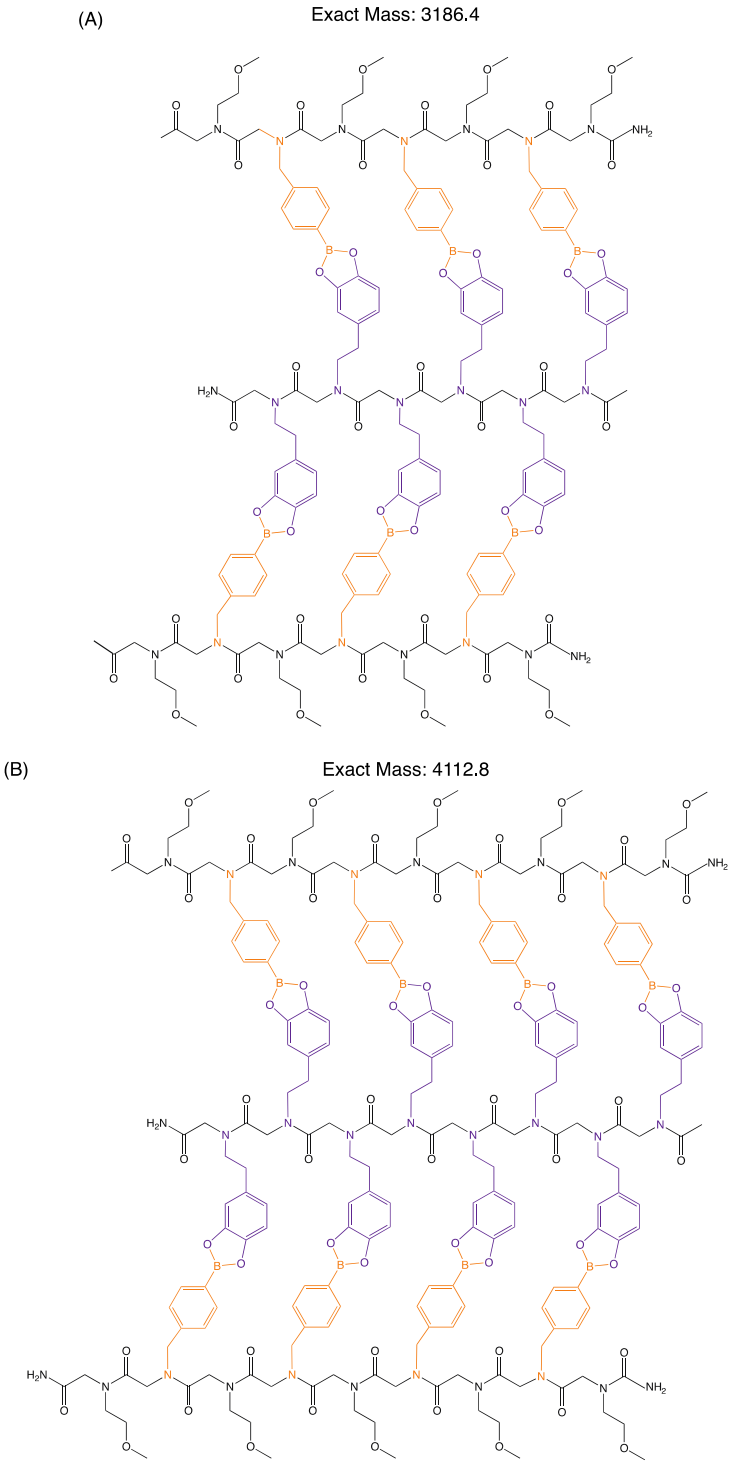
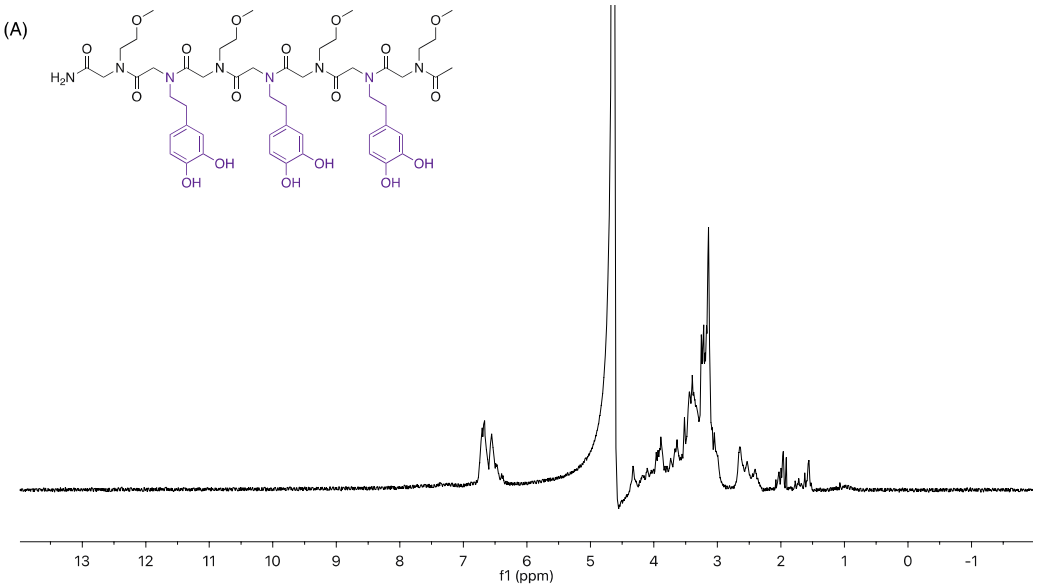
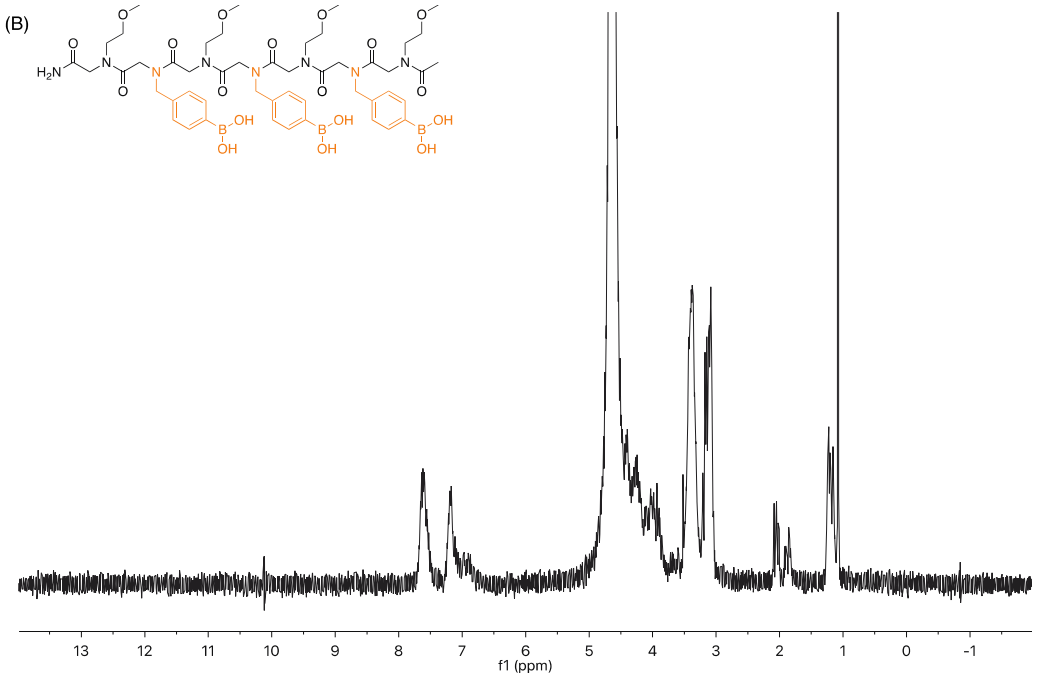


Figure **S11**. Structures and expected exact masses of assembled molecular grids bearing boronate ester linkages. (A) 3 × 3 grid, and (B) 3 × 4 grid.

^{1}H-NMR Characterization of Molecular Ladder





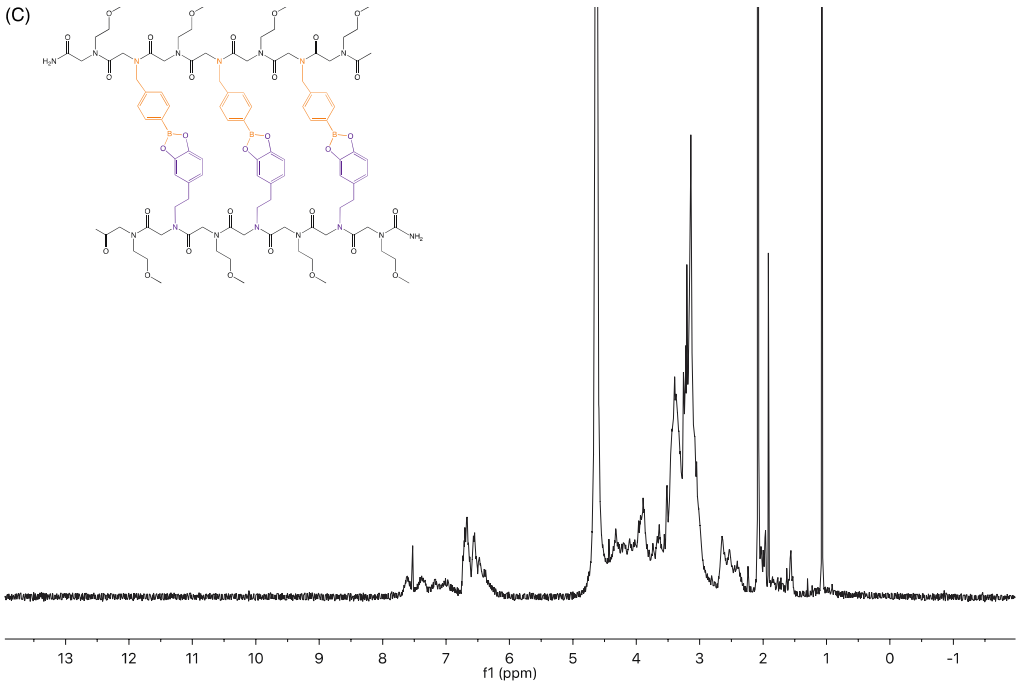


Figure **S12.** ^{1}H-NMR of three-rung molecular ladder system measured in δ (ppm) relative to residual solvent (D_2O = 4.79). (A) Oligomeric strand $(NmeNdop)_3Nme$, (B) oligomeric strand $(NmeNpba)_3Nme$, and (C) Hybrid-3.

Competitive Binding

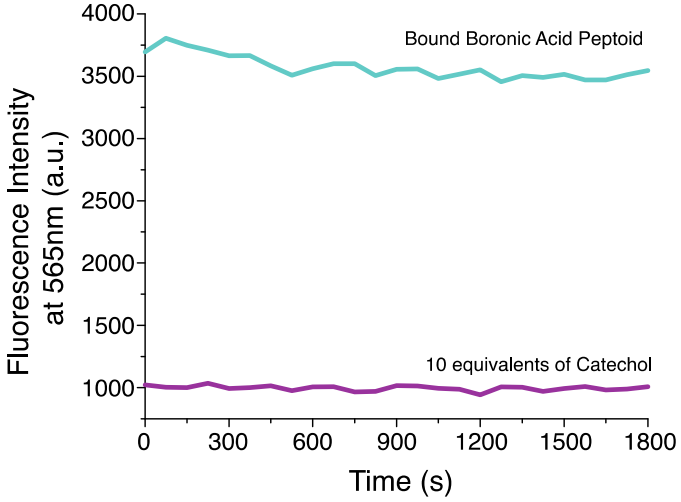


Figure **S13**. Kinetic study of the interaction between $(NmeNpba)_4Nme$ bound to ARS and $(NmeNdop)_4Nme$. Two samples were prepared with a stoichiometric ratio of ARS and $(NmeNpba)_4Nme$, where one sample was used as a control (blue line) with only water added and the other was treated with 10 equivalents of $(NmeNdop)_4Nme$ (purple) immediately before monitoring the sample with the plate reader. A kinetic scan monitored the fluorescent intensity of each of the samples for 30 minutes readings were taken every 75 seconds. It is noted that the samples had already reached equilibrium before the first scan. This demonstrates that the fluorescent intensity scans with the plate reader were at equilibrium before the readings were taken.

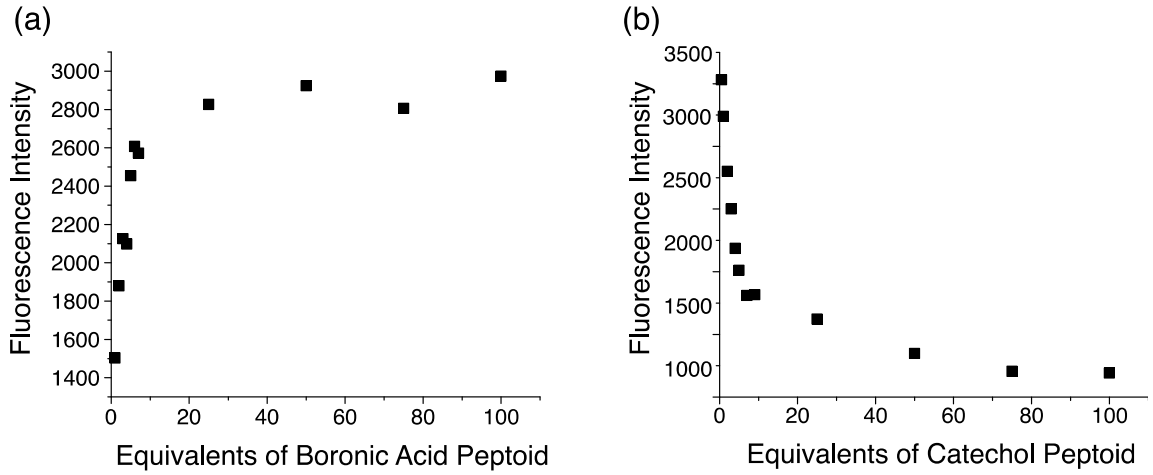


Figure **S14**. Competitive binding between $(NmeNpba)_4Nme$ and $(NmeNdop)_4Nme$ using ARS as a model diol. (A) The increase in fluorescent intensity experienced by adding increasing equivalents of boronic acid peptoid $(NmeNpba)_4Nme$ to ARS. The increase in intensity is a result of the ARS binding to the boronic acid peptoid. (B) The decrease in fluorescent intensity caused by increasing equivalents of catechol peptoid $(NmeNdop)_4Nme$ to $(NmeNpba)_4Nme$ bound to ARS. The decrease in intensity is a result of the catechol interaction with the boronic acid peptoid releasing the ARS into the system.

1. Z. Q. Liu, B. H. Hu and P. B. Messersmith, *Tetrahedron Lett.*, 2010, **51**, 2403-2405.
2. R. N. Zuckermann, J. M. Kerr, S. B. H. Kent and W. H. Moos, *J. Am. Chem. Soc.*, 1992, **114**, 10646-10647.
3. T. Wei, J. H. Jung and T. F. Scott, *J. Am. Chem. Soc.*, 2015, **137**, 16196-16202.