Supplementary Information

Self-association of aromatic oligoamide foldamers into double helices in water

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Table of contents

1. Materials and methods

All reactions were carried out under a dry nitrogen atmosphere. Low loading Wang resin was purchased from Novabiochem. Ghosez reagent (1-chloro-N,N,2-trimethyl-1-propenylamine) was purchased from Sigma Aldrich. *N,N*-diisopropylethylamine (DIPEA) was distilled over calcium hydride. Reactions requiring anhydrous conditions were performed under argon. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60-F254 plates and observed under UV light. Column chromatographies were carried out on Merck GEDURAN Si60 (40-63 µm). Analytical grade organic solvents were used for solid phase synthesis. Anhydrous THF and CH_2Cl_2 for solution and solid phase synthesis were dispensed from an MBRAUN SPS-800 solvent purification system. RP-HPLC-quality acetonitrile and MilliQ water were used for RP-HPLC analyses and purification. ESI mass spectra were obtained from the mass spectrometry service at the IECB (UMS3033 & US001). NMR spectra were recorded on 2 different NMR spectrometers: (1) an Avance II NMR spectrometer (Bruker Biospin) with a vertical 7,05T narrow-bore/ultrashield magnet operating at 300 MHz for ^{1}H observation and 75 MHz for ^{13}C observation by means of a 5-mm direct BBO H/X probe with Z gradient capabilities; (2) a DPX-400 NMR spectrometer (Bruker Biospin) with a vertical 9,4T narrow-bore/ultrashield magnet operating at 400 MHz for ^{1}H observation by means of a 5-mm direct QNP $^{1}H/^{13}C/^{31}P/^{19}F$ probe with gradient capabilities. ^{1}H-NMR spectra were measured at 300 or 400 MHz and ^{13}C-NMR spectra were measured at 75 MHz. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.2), DMSO-d_6 (δ 2.50, 39.4), or D_2O (δ 4.79). All coupling constants are reported in hertz (Hz). Signals were abbreviated as s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet, dd, doublet of doublets. Data processing was performed with Topspin 2.0 software.

- *Methods for NMR titrations*

Solutions of oligomers **1** and **2** (2mM) were made up in DMSO-d_6 (99.9%). Aliquots of a solution containing oligomers **1** or **2** in water at the same concentration (2 mM) were then added. The sample tube was shaken carefully after each addition of the aqueous solution and ^{1}H NMR spectra were recorded at 298 K using a water suppression sequence (watergate).

- *Methods for Mass Spectrometry*

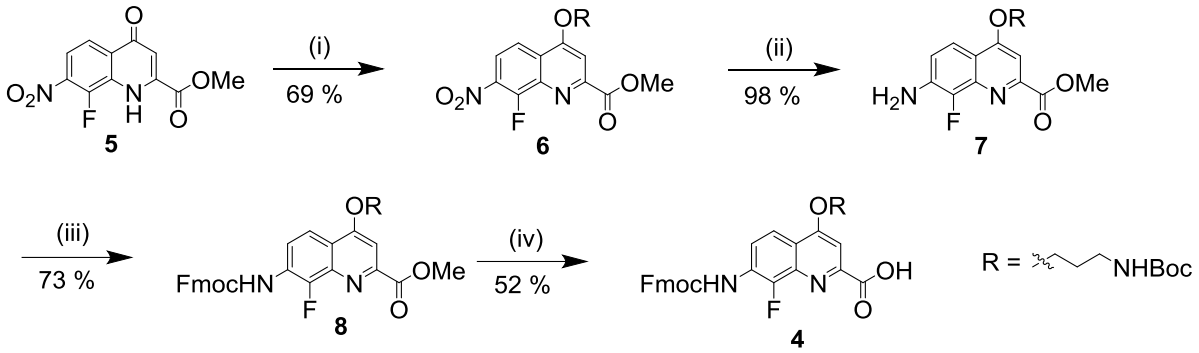
Mass spectrometry experiments were carried out on an Exactive orbitrap mass spectrometer (Thermo, Bremen, Germany) equipped with the HESI electrospray source. The experiments were performed in positive ion mode (capillary voltage=3.7 kV) in soft source conditions. The capillary temperature was 140 °C, the skimmer voltage was 10 V, the gate lens inject was 6.2 V and the tube lens offset was 195 V. The samples were disolved in water. In-source collision induced dissociation has been performed by increasing the source skimmer voltage. The duplex dissociates into a single strand when using a high skimmer voltage (Figure S3).

- *Methods for modeling*

MacroModel v6.5, with the GB/SA model for water was used to perform the modeling of the single and double helices. All calculations were achieved using Montecarlo conformational analyses. Minimizations were carried out using the TNCG method as implemented MacroModel v6.5, the energy gradient was fixed at 0.05 as the convergence criteria, and at least 1000 iterations. All Montecarlo calculations were performed with MCMM (Monte Carlo Multiple Minimum) method. The minimization method was TNCG with the same characteristics described above. In a typical Montecarlo run a MCMM is performed with a minimum of 10000 steps, to carry out the search torsional rotations are performed and for all the Montecarlo a cutoff is applied to Van der Waals, electrostatic and H-bond interactions with 8 Å, 20 Å and 4 Å, respectively. These calculations were carried out with the Merck Molecular Force Field static (MMFFs) as implemented in the version of the program.

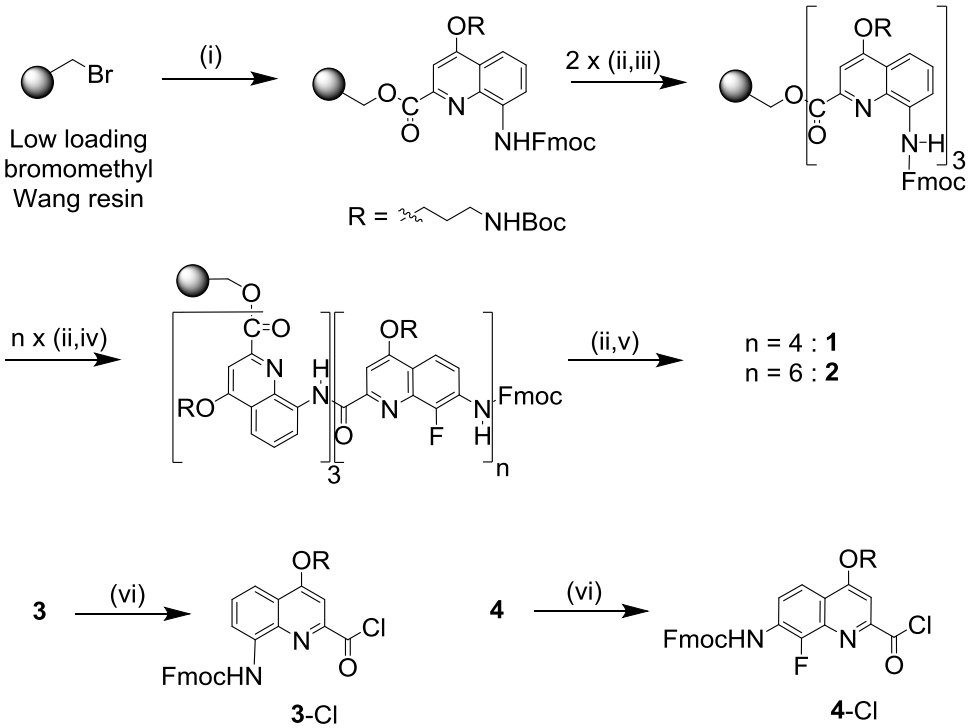
2. Synthetic schemes

- *Solution phase preparation of Q^F monomers*



Scheme S1. Synthesis of Q^F monomers: (i) diisopropylazodicarboxylate, N-(tert-butoxycarbonyl)-3-aminopropanol, PPh_3; (ii) Pd/C, H_2; (iii) Fmoc-Cl, $NaHCO_3$; (iv) LiI.

- *Solid phase synthesis of $Q_3(Q^F)_n$ oligomers*

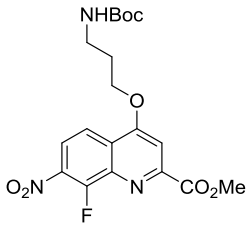


Scheme S1. Solid phase synthesis of $Q_3(Q^F)_n$ oligomers: (i) **3**, CsI, DIEA, DMF, microwaves; (ii) piperidine, DMF; (iii) **3-Cl**, DIEA, THF, microwaves; (iv) **4-Cl**, DIEA, THF, microwaves (v) TFA, iPr_3SiH, H2O; (vi) 1-Chloro-N,N,2-trimethyl-1-propenylamine, CH_2Cl_2.

3. Experimental section

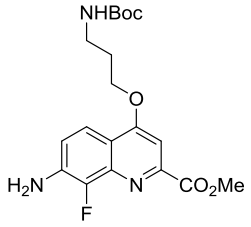
- *Experimental procedure for monomer synthesis and characterization*

Methyl 8-fluoro-4-(3-(tert-butoxycarbonylamino)propoxy)-7-nitro-2- quinolinecarboxylate **6**:



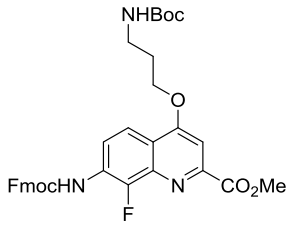
Methyl 8-fluoro-7-nitro-4-(1H)-quinolone-2-carboxylate (0.84 g, 3 mmol), triphenylphosphine (1.18 g, 4.5 mmol, 1.5 equiv.) and *N*-(*t*-butoxycarbonyl)-3-aminopropanol (0.78 g, 4.5 mmol, 1.5 equiv.) were partially dissolved in anhydrous THF (100 mL) and stirred at 0 °C under Ar. Diisopropylazaodicarboxylate (1.3 equiv., 8.25 mL, 41.93 mmol.) was added and the reaction mixture stirred at 0 °C for 30 min, then at room temperature for 15 h. Solvents were removed under reduced pressure, and the product purified by silica gel chromatography (100% DCM to DCM/EtOAc (20:1)) to afford the title compound as a pale brown solid (0.87 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.12 (m, 2H), 7.74 (s, 1H), 4.69 (brs, 1H), 4.40 (t, 2H, ^{3}J = 6.0), 4.10 (s, 3H), 3.43 (q, 2H, ^{3}J = 6.4), 2.20 (quin, 2H, ^{3}J = 6.4), 1.43 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 165.09, 162.42, 162.37, 156.02, 154.20, 151.41, 150.49, 139.35, 139.2, 136.47, 136.40, 126.17, 121.79, 117.96, 117.88, 103.87, 67.52, 53.56, 37.48, 29.39, 28.36. HRMS calculated for $C_{19}H_{23}FN_3O_7$: 424.1515, found: 424.1508 $[M+H]^+$.

Methyl 7-amino-8-fluoro-4-(3-(tert-butoxycarbonylamino)propoxy)-2- quinolinecarboxylate **7**:



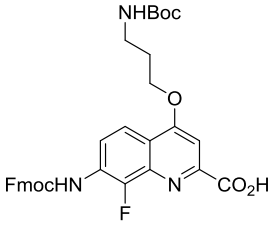
Methyl 8-fluoro-4-(3-(tert-butoxycarbonylamino)propoxy)-7-nitro-2-quinolinecarboxylate **6** (0.84 g, 2.0 mmol) was dissolved in 150 mL EtOAc, to which was added 10% Pd/C (84 mg, 10% by mass) and the mixture stirred under H_2 for 15 h at room temperature. The reaction mixture was filtered through celite, and solvents removed under reduced pressure to afford the title compound as a yellow solid (0.77 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.84 (dd, 1H, ^{3}J = 9.0, 1.5), 7.44 (s, 1H), 7.12 (dd, 1H, *J* = 8.9, 7.8), 4.74 (brs, 1H), 4.34 (t, 2H, ^{3}J = 6.4), 4.05 (s, 3H), 3.43 (q, 2H, *J* = 6.4), 2.15 (quin., 2H, *J* = 6.4), 1.44 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 166.24, 162.36, 162.31, 156.0, 149.57, 145.46, 142.22, 139.70, 139.57, 135.03, 134.88, 119.73, 119.67, 117.59, 117.53, 115.67, 98.94, 79.42, 66.60, 53.16, 37.80, 29.34, 28.39. HRMS calculated for $C_{19}H_{25}FN_3O_5$: 394.1773, found: 394.1774 $[M+H]^+$.

Methyl 7-(((9H-fluoren-9-yl)methoxy)carbonylamino)-8-fluoro-4-(3-(tert-butoxycarbonylamino) propoxy)-2-quinolinecarboxylate **8**:



Methyl 7-amino-8-fluoro-4-(3-(tert-butoxy carbonylamino)propoxy)-2-quinolinecarboxylate **7** (0.75 g, 1.9 mmol) was dissolved in a mixture of 5% $NaHCO_3$ and dioxane (100 mL/20mL) and cooled to 0 °C. Over one hour, a solution of 9-fluorenylmethylchloroformate (0.72 g, 2.8 mmol, 1.5 equiv) in 20 mL dioxane was added dropwise using an addition funnel. The solution was further stirred for one hour at 0 °C and subsequently stirred for 15 h at room temperature. The reaction mixture was then poured into 160 ml water and acidified to pH 2 with a 1 M HCl solution. The aqueous phase was extracted with DCM (3 x 100 ml). The collected organic layers were washed with brine (3 x 150 ml), dried over Na_2SO_4, solvents evaporated under reduced pressure and the product purified by silica gel chromatography to afford the title compound as a white solid (0.85 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (brs, 1H), 7.98 (d, 1H, *J* = 9.2), 7.80 (d, 2H, *J* = 7.3), 7.66 (d, 2H, *J* = 7.3), 7.54 (s, 1H), 7.44 (t, 2H, *J* = 7.3), 7.35 (t, 2H, *J* = 7.3), 4.74 (brs, 1H), 4.61 (d, 2H, *J* = 6.7), 4.36 (t, 2H, *J* = 6.7), 4.34 (t, 2H, *J* = 6.0), 4.07 (s, 3H), 3.42 (q, 2H, *J* = 6.3), 2.18 (quin, 2H, *J* = 6.3), 1.44 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 165.86, 162.36, 162.32, 156.03, 153.13, 149.91, 147.66, 144.31, 143.51, 141.35, 138.62, 138.48, 127.90, 127.19, 126.99, 126.88, 124.95, 120.42, 120.11, 118.83, 117.55, 117.48, 100.58, 79.46, 67.55, 66.91, 53.31, 46.99, 37.71, 29.35, 28.41. HRMS calculated for $C_{34}H_{35}FN_3O_7$: 616.2454, found: 616.2459 $[M+H]^+$.

7-(((9H-fluoren-9-yl)methoxy)carbonylamino)-8-fluoro-4-(3-(tert -butoxycarbonylamino)propoxy)-2-quinolinecarboxylic acid **4**:



Compound **8** (0.8 g, 1.3 mmol) was dissolved in 100 mL of dry and degassed EtOAc. To the solution, LiI (0.52 g, 3.9 mmol, 3.0 equiv) was added, and the mixture was stirred for 6 h at 80 ºC under inert atmosphere, then cooled to room temperature and concentrated under reduced pressure (to approximately 30 mL). The reaction mixture was then poured into 100 ml water and acidified to pH 2 with a 1 M HCl solution. The aqueous phase was extracted with DCM (3 x 30 mL), the combined organic

layers washed with brine (3 x 150 ml) and dried over Na_2SO_4. Solvents were removed under reduced pressure and product was precipitated from DCM/cyclohexane to yield the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.45 (brs, 1H), 9.97 (s, 1H), 7.92 (m, 4H), 7.80 (d, 2H, *J* = 6.7), 7.52 (s, 1H), 7.44 (t, 2H, *J* = 7.6), 7.35 (t, 2H, *J* = 7.6), 6.97 (t, 1H, *J* = 5.6), 4.51 (d, 2H, J = 6.5), 4.35 (m, 3H), 3.21 (q, 2H, *J* = 6.3), 2.02 (quin., 2H), 1.35 (s, 9H); ^{13}C NMR (300 MHz, DMSO-d_6): δ = 166.86, 162.39, 162.34, 156.15, 154.26, 151.57, 150.24, 146.85, 144.18, 141.29, 138.86, 138.71, 128.21, 127.62, 126.86, 126.73, 125.77, 124.10, 120.65, 119.57, 117.33, 101.35, 78.01, 67.28, 66.88, 47.04, 37.30, 29.34, 28.70. HRMS calculated for $C_{33}H_{33}FN_3O_7$: 602.2297, found: 602.2295 $[M+H]^+$.

- *Method and experimental procedures for SPS*

Loading of first monomer unit: Wang bromide resin (75 mg, 0.38 mmol g^{-1}, 0.0285 mmol) was swollen in anhydrous DMF (1.5 mL) for 1 h under N_2. Compound **3** (50 mg, 0.0855 mmol, 3.0 equiv) and CsI (22 mg, 0.0855 mmol, 3.0 equiv) were then added, followed by DIEA (28 uL, 0.171 mmol, 6.0 equiv). The mixture was then treated with microwaves (50 W, ramp to 50 ºC over 5 min, then hold at 50 ºC for 5 min). The resin was washed briefly with anhydrous DMF and the process was repeated. The resin was then washed thoroughly with DMF.

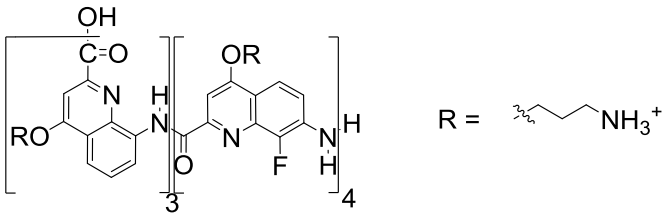
Fmoc deprotection: To Fmoc-Q-Wang was added a 20% vol/vol solution of piperidine in DMF (4 mL) and the resin was stirred for 10 min at room temperature. The resin was washed briefly with DMF and the process was repeated twice again. The resin was then washed thoroughly with DMF.

Conversion of Fmoc monomer acid to acid chloride exemplified by preparation of 3-Cl: Compound **3** (100 mg , 0.171 mmol) was dissolved in anhydrous DCM (3 mL) under N_2, and 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (70 µL, 0.513 mmol, 2 equiv) was added. The mixture was stirred for 1 h and then solvents evaporated on a vacuum manifold.

Coupling of Fmoc monomer acid chloride exemplified by preparation of Fmoc-$(Q)_2$-Wang: NH_2-Q-Wang resin was suspended in anhydrous THF (1 mL), to which was added DIEA (30 µL, 0.171 mmol, 6.0 equiv). Compound **3-Cl** was dissolved in anhydrous THF (1.5 mL) and added to the resin, which was then treated with microwaves (50 W, ramp to 50 ºC over 5 min, then hold at 50 ºC for 15 min). The resin was washed briefly with anhydrous THF, and the process repeated once. The resin was then washed thoroughly with THF and DMF.

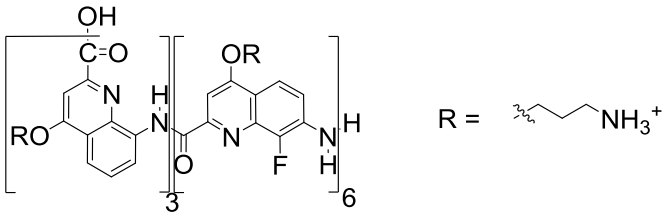
Resin cleavage: The completed resin-bound foldamer was washed with DMF, DCM and then DCM/MeOH (1:1), dried and desiccated. It was then suspended in a solution of TFA/*i*Pr_3SiH/H_2O (95:2.5:2.5 vol/vol/vol, 2 mL for 75 mg resin), and the mixture was stirred for 2 h at room temperature. The resin was then removed by filtration and washed with TFA, and the filtrate evaporated under reduced pressure. The resulting oily solid was precipitated with Et_2O, triturated, washed with Et_2O, filtered and dried under vacuum to obtain the crude foldamer.

- *Characterization of aromatic amide oligomers*



1

NH_2-(**Q^F**)$_4$-(**Q**)$_3$-OH **1**: Prepared on a 28.5 µmol scale (75 mg of resin, 0.38 mmol g^{-1}) using the general procedures reported above, affording the title compound as a pale orange solid (35 mg, 69%) after purification by preparative RP-HPLC (System C). RP-HPLC (System A): R_t = 4.25 min. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.24 (s, 1H), 11.93 (s, 1H), 11.74 (s, 1H), 11.11 (s, 1H), 11.03 (s, 1H), 9.63 (s, 1H), 8.93 (m, 2H), 8.77 (m, 2H), 8.35-7.66 (m, CHAr), 7.46 (m, 2H), 7.22 (m, 2H), 7.07-7.03 (m, 2H), 6.62 (s, 1H), 4.63-4.18 (m, 14H), 3.17 (m, 14H), 2.27 (m, 14H). HRMS calculated for $C_{91}H_{89}F_4N_{21}O_{15}$: 1791.6, found: 897.3 $[M+2H]^{2+}$.



2

NH_2-(**Q^F**)$_6$-(**Q**)$_3$-OH **2**: Prepared on a 28.5 µmol scale (75 mg of resin, 0.38 mmol g^{-1}) using the general procedures reported above, affording the title compound as a pale orange solid (7.4 mg, 11%) after purification by preparative RP-HPLC (System C). RP-HPLC (System B): R_t = 9.37 min. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.11 (s, 1H), 12.00 (s, 1H), 11.52 (s, 1H), 11.43 (s, 1H), 11.11 (s, 1H), 10.36 (s, 1H), 10.21 (s, 1H), 10.12 (s, 1H), 9.12 (m, 1H), 8.92 (m, 2H), 8.52 (m, 2H), 8.39-7.74 (m, CHAr), 7.70-7.28 (m, 7H), 7.10-6.96 (m, 4H), 6.70-6.36 (m, 7H), 4.71-4.46 (m, 18H), 3.25-3.17 (m, 18H), 2.37-2.21 (m, 18H). HRMS calculated for $C_{117}H_{113}F_6N_{27}O_{19}$: 2313.9, found : 1158.4 $[M + 2H]^{2+}$.

4. NMR studies

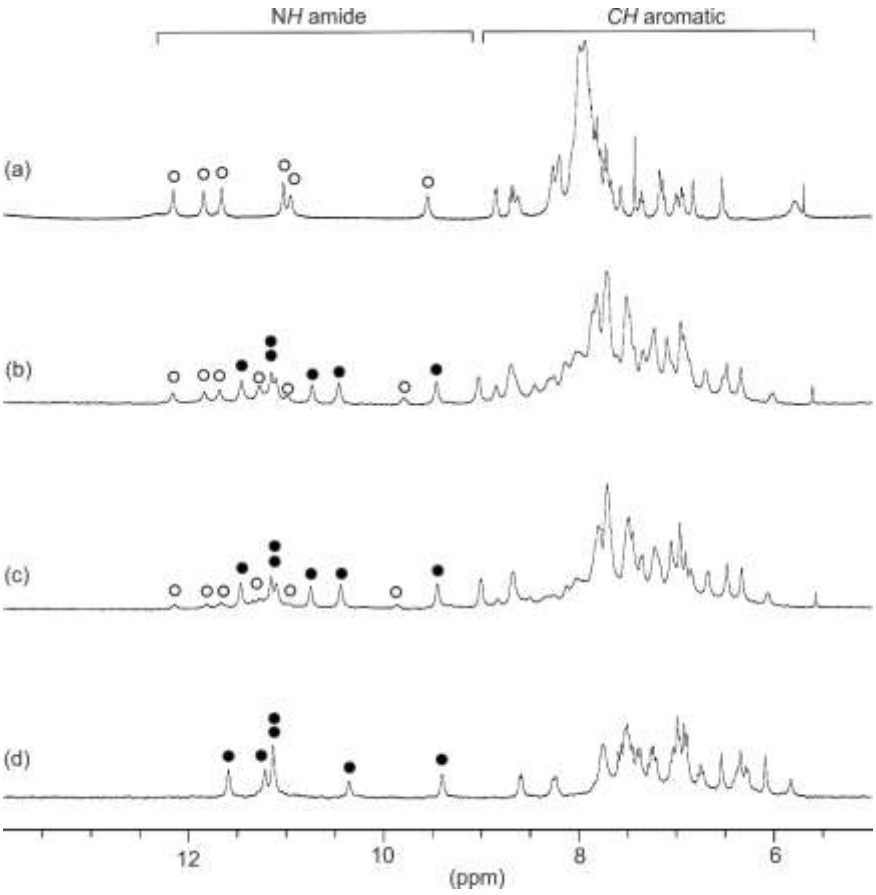


Figure S1. Excerpts of the ^{1}H NMR (400 MHz) titration at 298 K of **1** (2 mM) in varying proportions of d_6-DMSO:H2O vol/vol: (a) 100:0; (b) 80:20; (c) 76:24; (d) 0:100. Empty and black circles stand for the eight amide signals of either the single **1** or the double helix (**1**)$_2$, respectively. Aromatic resonances are visible between 6 and 9 ppm.

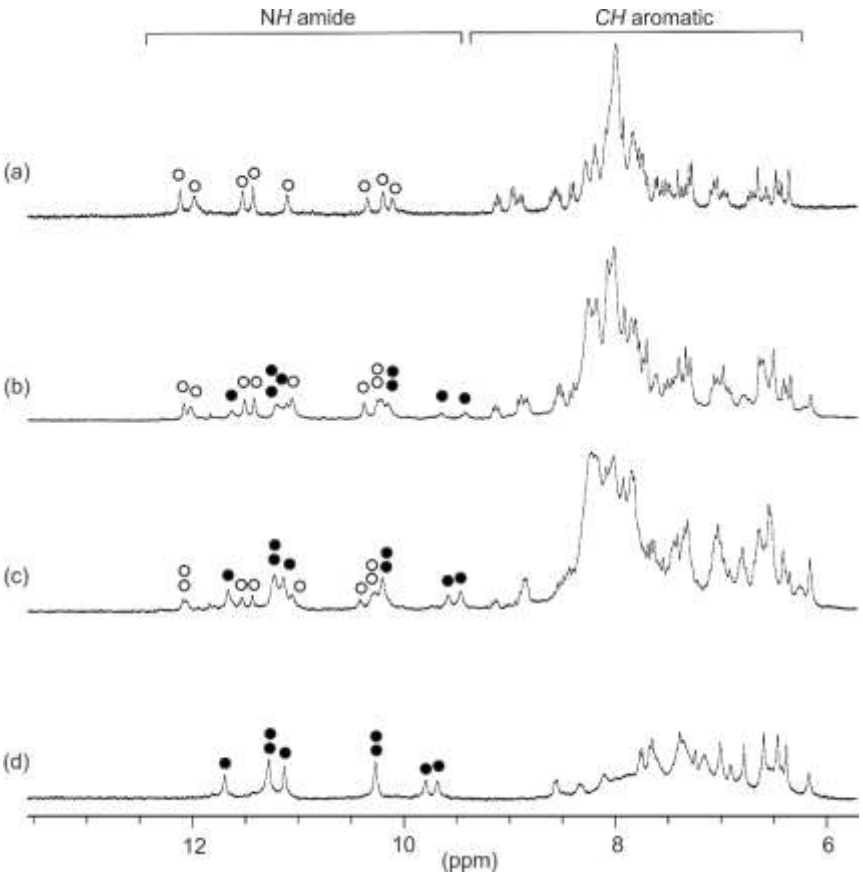


Figure S2. Excerpts of the ^{1}H NMR (400 MHz) titration at 298 K of **2** (2 mM) in varying proportions of d_6-DMSO:H2O vol/vol: (a) 100:0; (b) 84:16; (c) 80:20; (d) 0:100. Empty and black circles stand for the eight amide signals of either the single **2** or the double helix $(\mathbf{2})_2$, respectively. Aromatic resonances are visible between 6 and 9 ppm.

5. Mass spectrometry studies

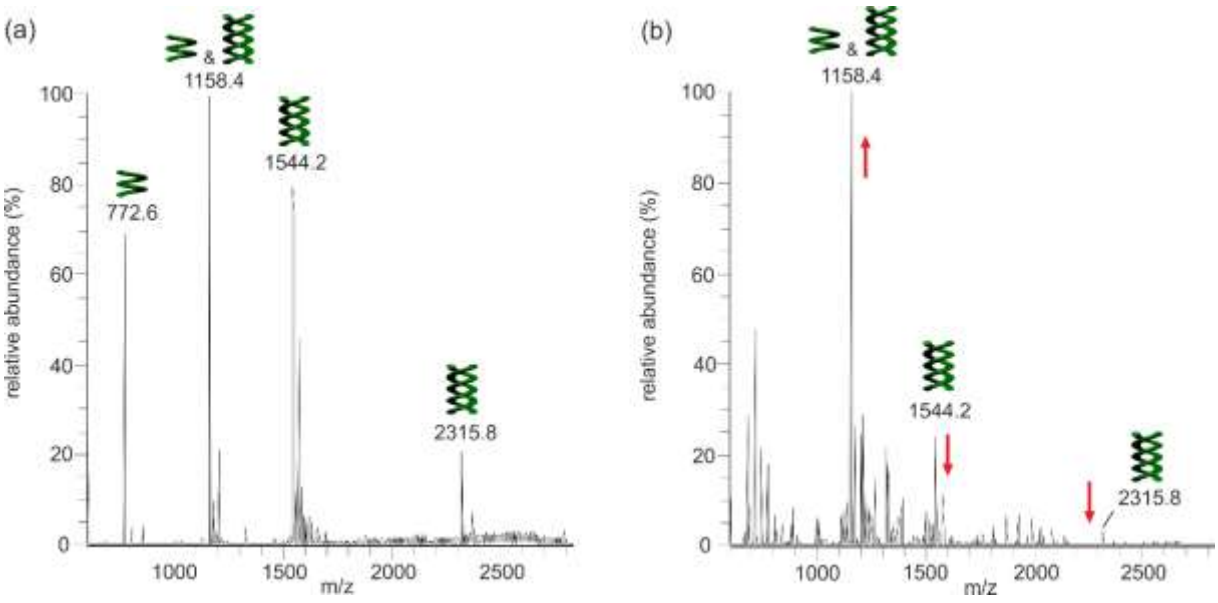


Figure S3. Electrospray ionization mass spectrum (ESI-MS) of a solution of 20 μM of oligomer **2** in: (a) H_2O and (b) 50:50 H_2O:MeCN vol/vol mixture. The red arrows indicate the decrease of the relative abundance of the duplex compared to the single helix in the presence of organic solvent in water.

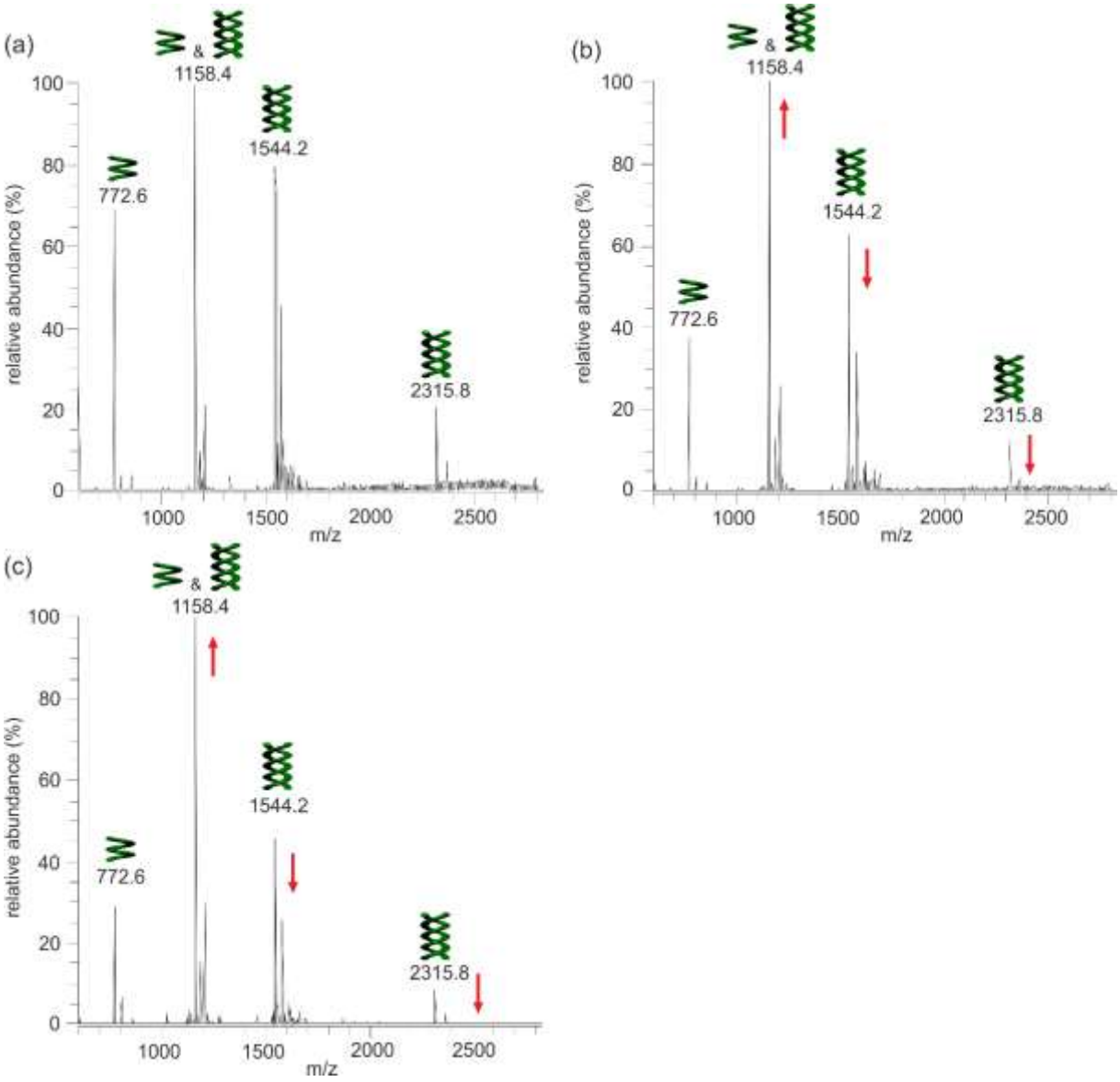


Figure S4. Electrospray ionization mass spectrum (ESI-MS) of a solution of 20μM of oligomer **2** with varying skimmer voltage: (a) 10V; (b) 17V and (c) 20V. The increase of experimental harshness (e.g. voltage increase) induced the dissociation of the duplex.

6. Modelling

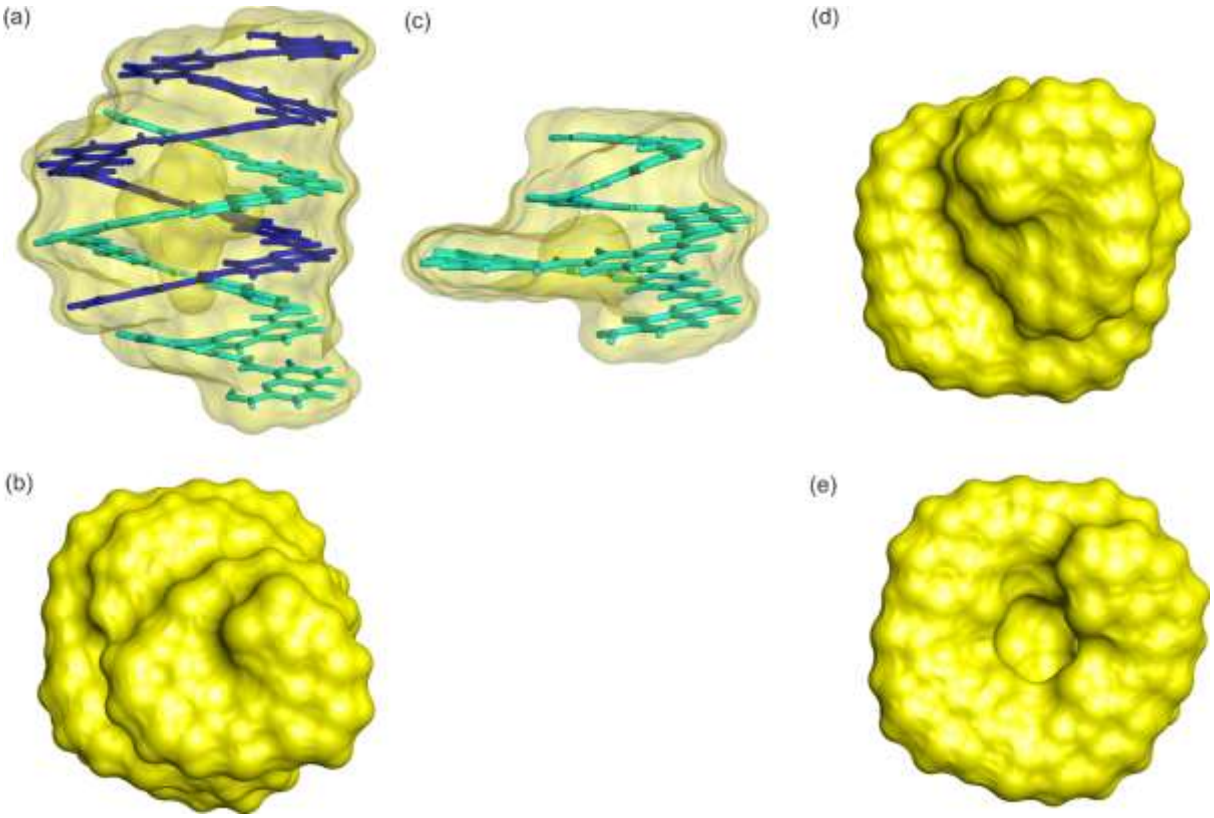
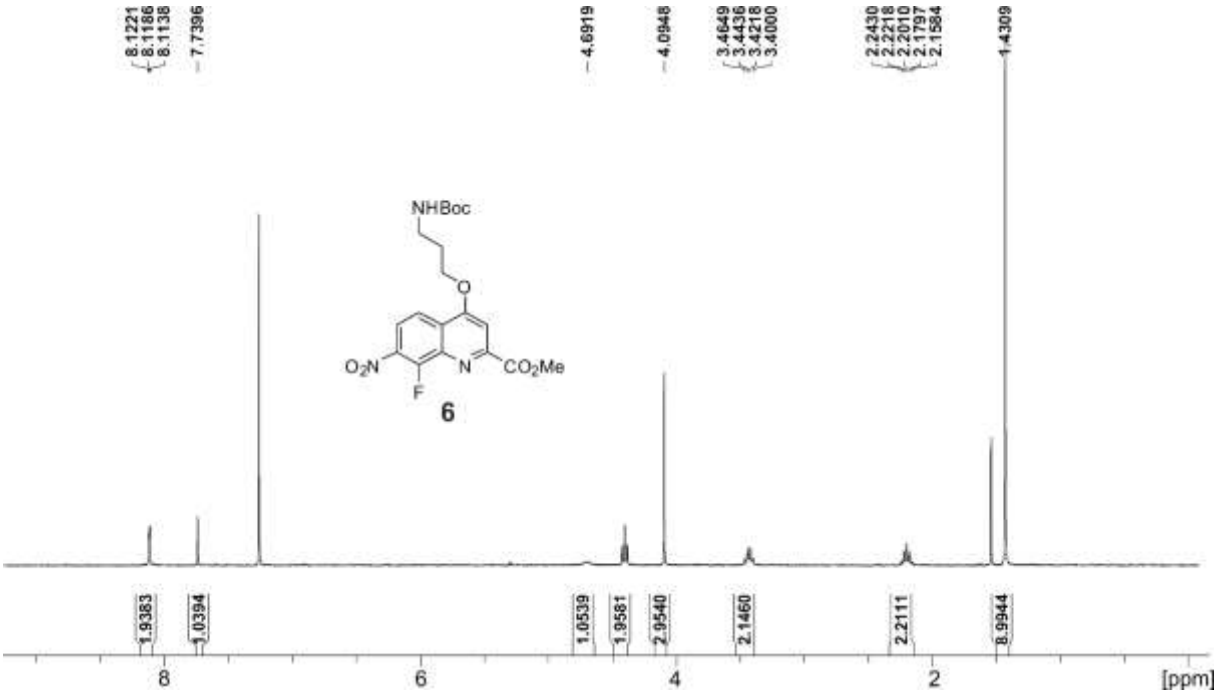
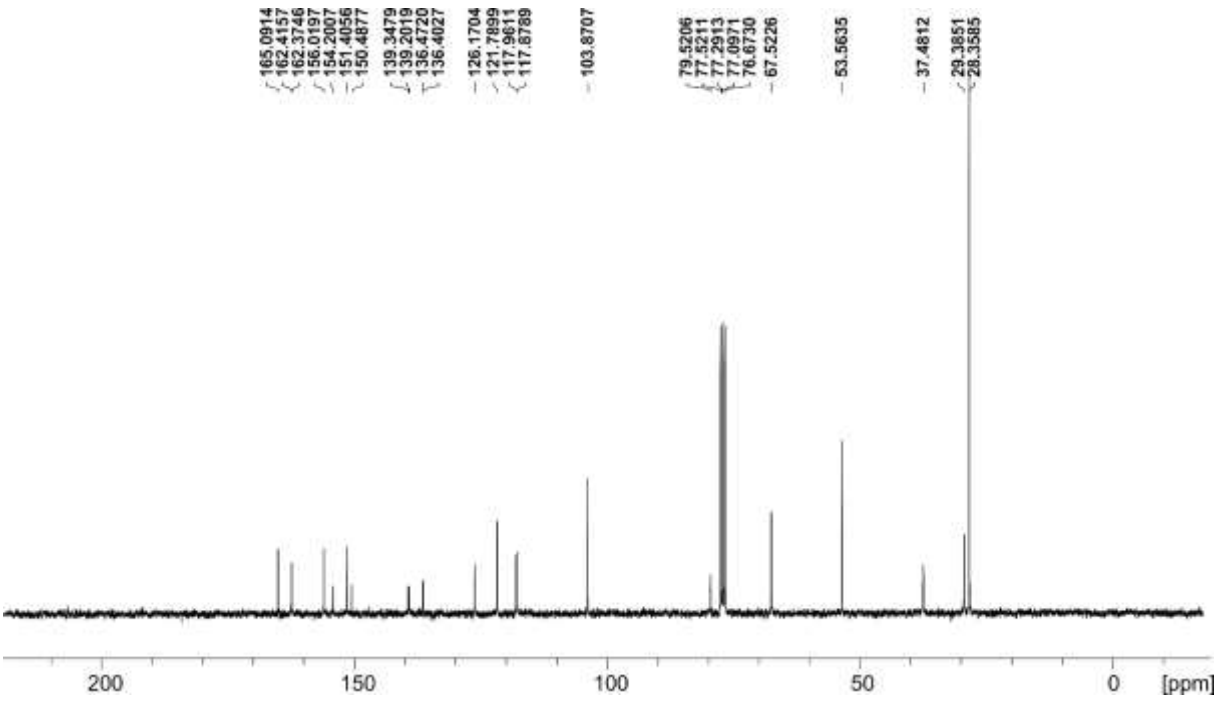
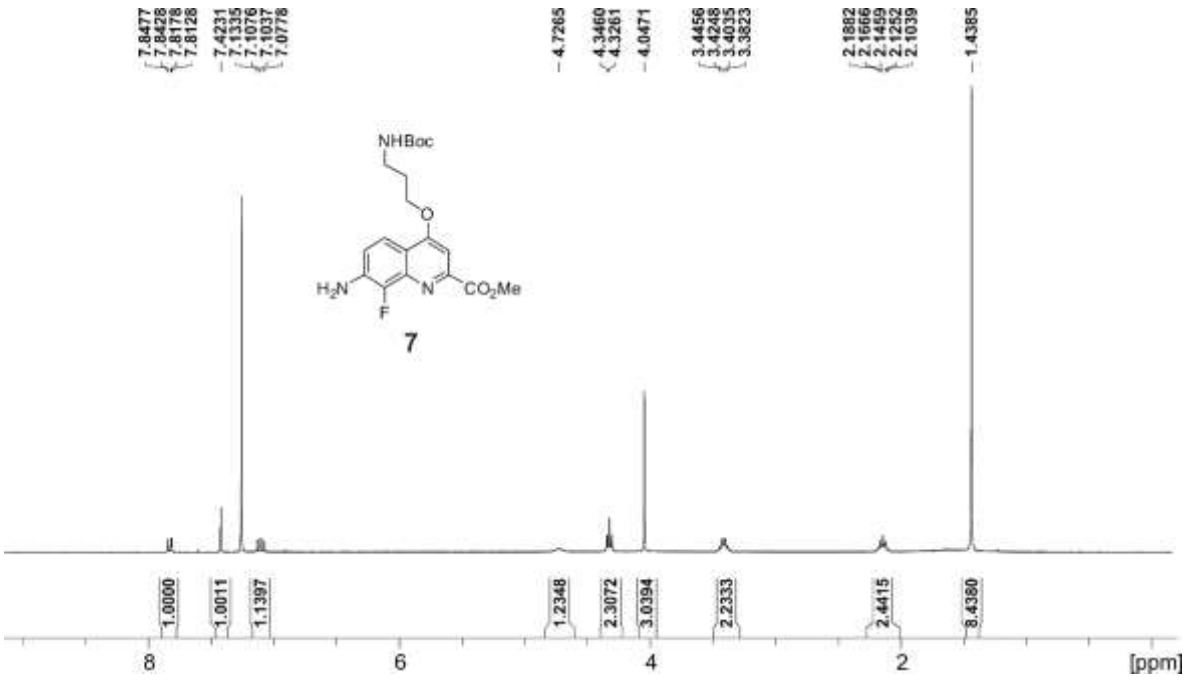


Figure S5. Solvent accessible surface of: (a) side view and (b) top view of duplex (**2**)$_2$. Solvent accessible surface of: (c) side view, (d) top view and (e) bottom view of single helix **2**. In (a) and (c) solvent accessible surfaces are shown as transparent yellow isosurfaces and helical backbones are shown as blue tube. In (b), (d) and (e) solvent accessible surfaces are shown as yellow opaque isosurfaces. Solvent-accessible surface area was calculated by rolling ball method with a radius of 1.4Å. Flexible side chains have been removed for the surface estimation. The calculated solvent accessible surface for (**2**)$_2$ and **2** were found to be 1655 Å^2 and 1021Å^2, respectively. Thus, one can estimate that a double helix (**2**)$_2$ presents to the solvent about 80% of the surface presented by two individual single helix **2**.

7. NMR spectra



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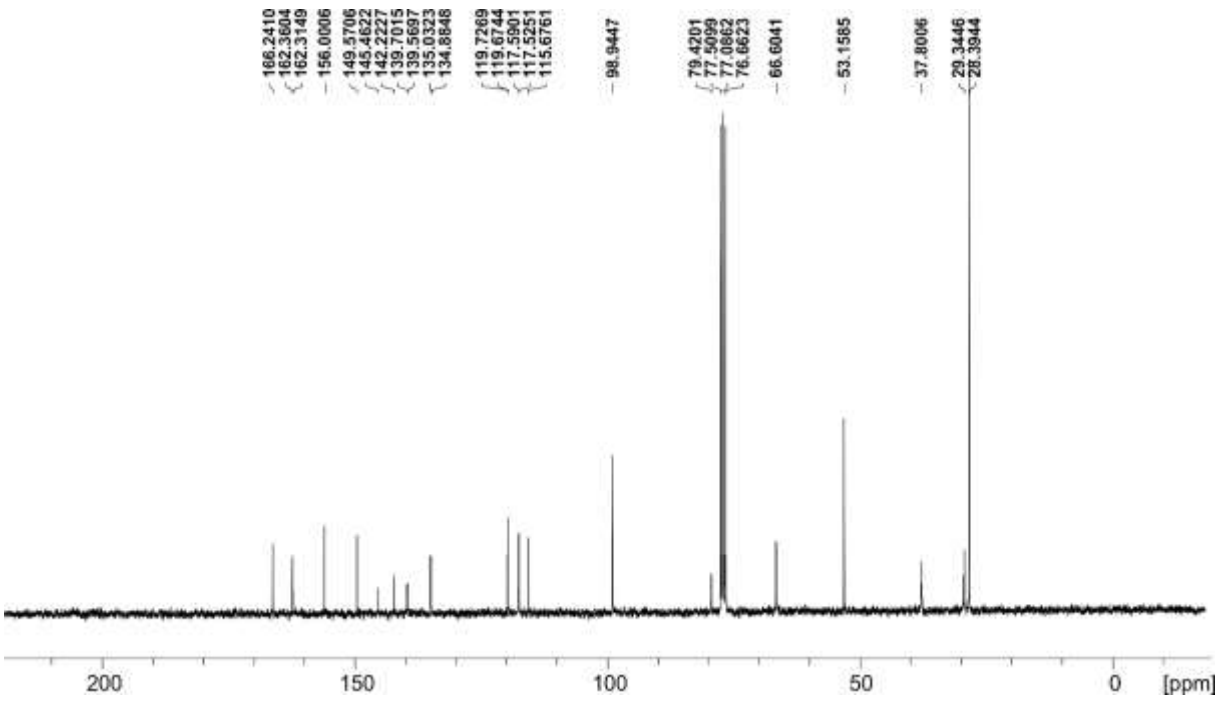
2
[ppm]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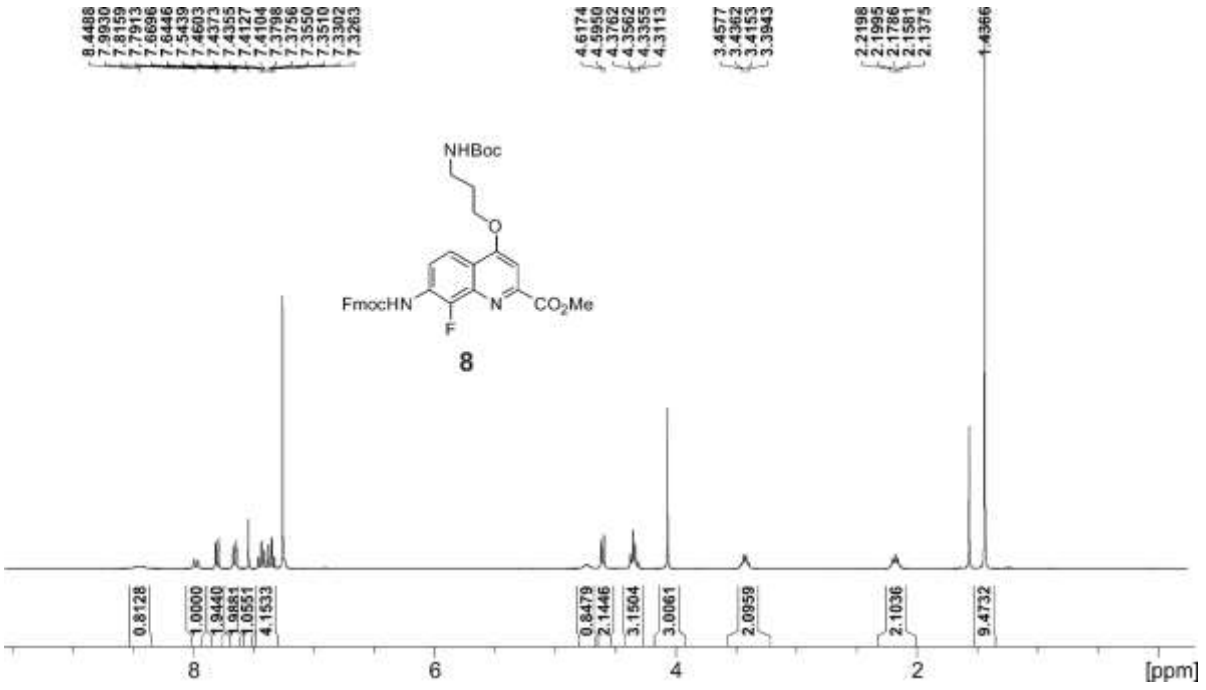
162.3604
162.3149
156.0006
149.5706
145.4622
142.2227
139.7015
139.5697
135.0323
134.8848
119.7269
119.6744
117.5901
117.5251
115.6761
98.9447
79.4201
77.5099
77.0862
76.6623
66.6041
53.1585
37.8006
29.3446
28.3944
200
150
100
50
0
[ppm]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7.9930
7.8159
7.7913
7.6696
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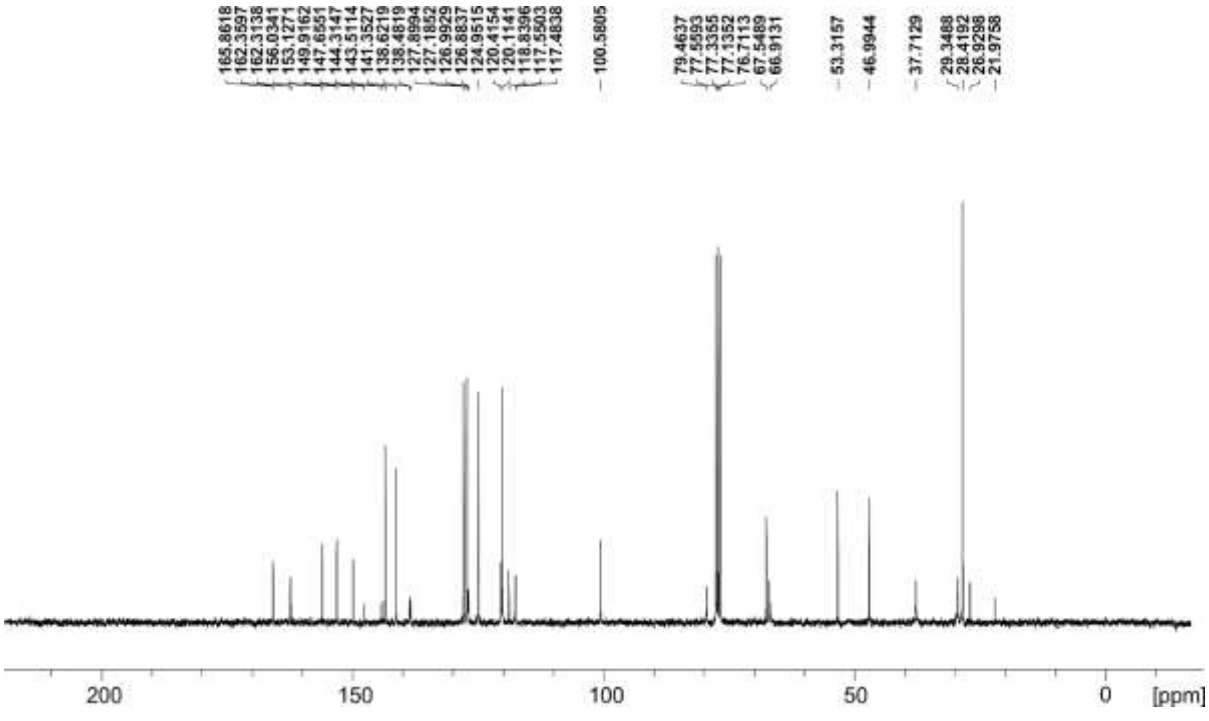
7.4373
7.4355
7.4127
7.4104
7.3798
7.3756
7.3550
7.3510
7.3302
7.3263
4.6174
4.5950
4.3762
4.3562
4.3355
4.3113
3.4577
3.4362
3.4153
3.3943
2.2198
2.1995
2.1786
2.1581
2.1375
1.4366
NHBoc
O
FmocHN
N
CO2Me
F
8
0.8128
1.0000
1.9440
1.9881
1.0551
4.1533
0.8479
2.1446
3.1504
3.0061
2.0959
2.1036
9.4732
8
6
4
2
[ppm]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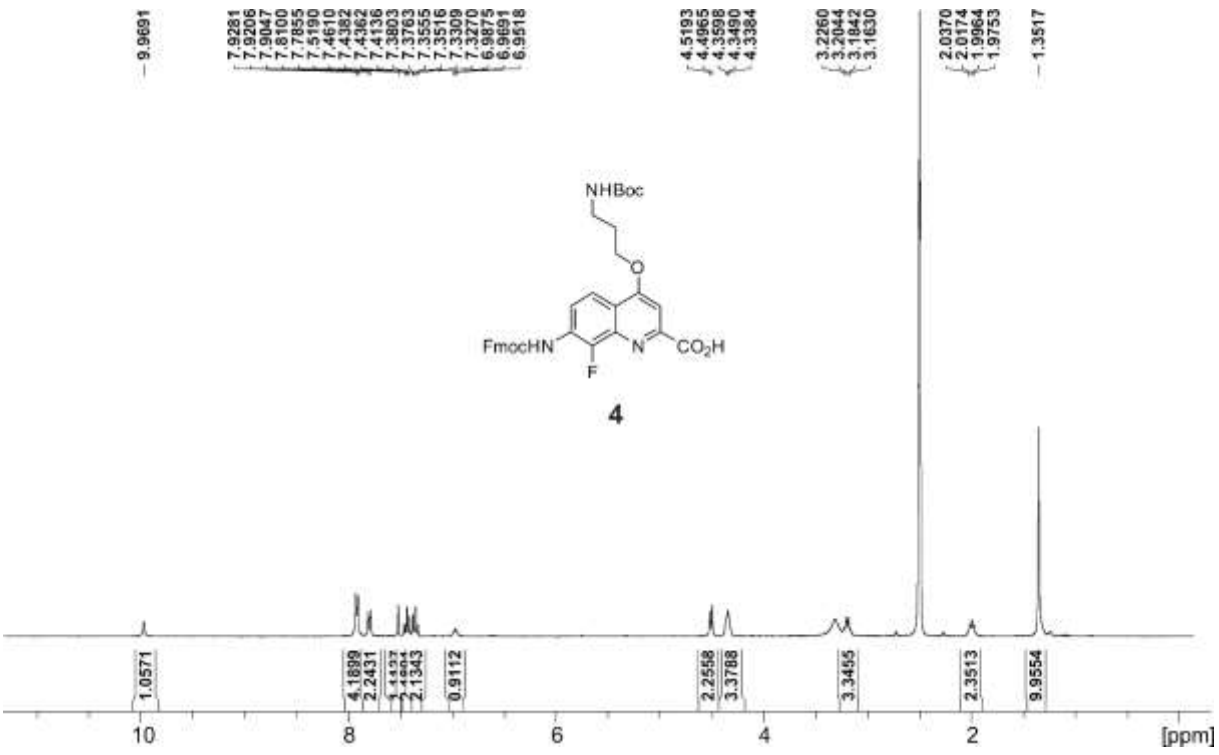
162.3597
162.3138
156.0341
153.1271
149.9162
147.6551
144.3147
143.5114
141.3527
138.6219
138.4819
127.8994
127.1852
126.9929
126.8837
124.9515
120.4154
120.1141
118.8396
117.5503
117.4838
100.5805
79.4637
77.5593
77.3355
77.1352
76.7113
67.5489
66.9131
53.3157
46.9944
37.7129
29.3488
28.4192
26.9298
21.9758
200
150
100
50
0
[ppm]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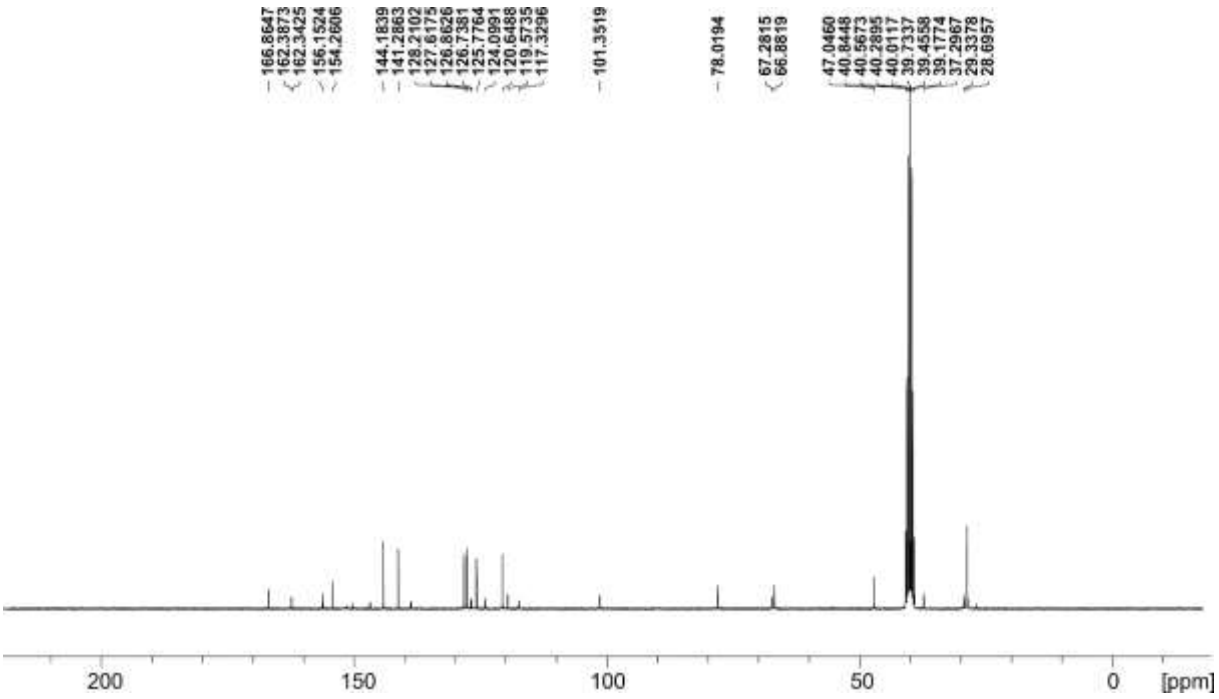
7.9281
7.9206
7.9047
7.8100
7.7855
7.5190
7.4610
7.4382
7.4362
7.4136
7.3803
7.3763
7.3555
7.3516
7.3309
7.3270
6.9875
6.9691
6.9518
4.5193
4.4965
4.3598
4.3490
4.3384
3.2260
3.2044
3.1842
3.1630
2.0370
2.0174
1.9964
1.9753
1.3517
NHBoc
O
FmocHN
N
CO2H
F
4
1.0571
4.1899
2.2431
2.1343
0.9112
2.2558
3.3788
3.3455
2.3513
9.9554
10
8
6
4
2
[ppm]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162.3425
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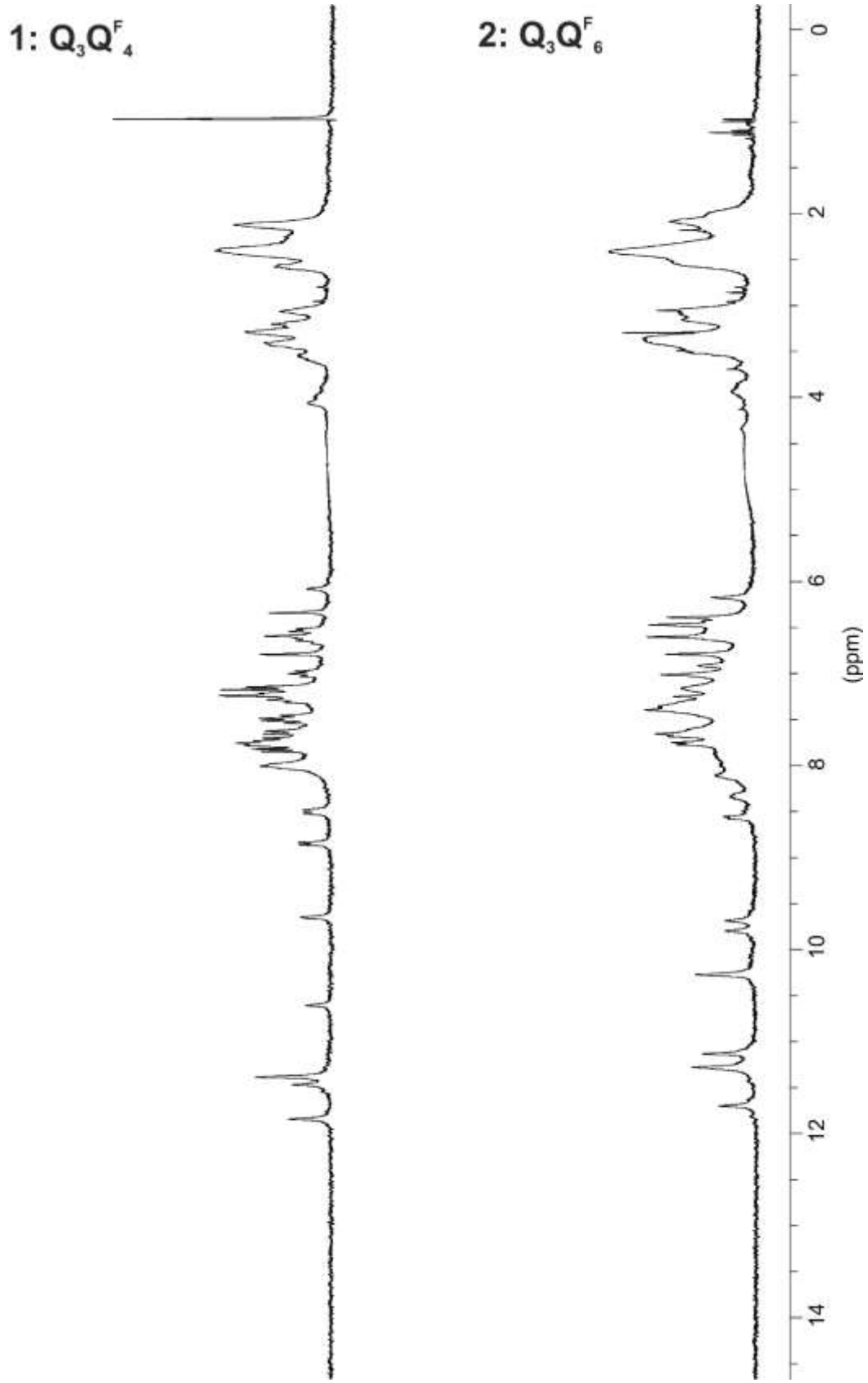
154.2606
144.1839
141.2863
128.2102
127.6175
126.8626
126.7381
125.7764
124.0991
120.6488
119.5735
117.3296
101.3519
78.0194
67.2815
66.8819
47.0460
40.8448
40.5673
40.2895
40.0117
39.7337
39.4558
39.1774
37.2967
29.3378
28.6957
200
150
100
50
0
[ppm]


1: $Q_3Q^F_4$
2: $Q_3Q^F_6$
0
2
4
6
8
10
12
14
(ppm)

6. RP-HPLC chromatograms