Constitutionally Selective Amplification of Multicomponent 84-Membered Macrocyclic Hosts for (–)-Cytidine·H^+

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Supplementary Information

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Experimental Details

General Methods. Chemicals were purchased from Aldrich, Chem-Impex International, Inc. and Berry & Associates, Inc., and used as received. Anhydrous acetonitrile was purchased from Acros. L,L-**2a** or **b** were prepared using literature methods.[1]

HPLC analysis was performed on a Hewlett-Packard Series 1100 instrument, using a Halo-C18 column (4.6 x 150 mm, 2.7 μm) with gradient elution (methanol/water) at a flow rate of 0.45 mL/min and at 55 ºC. The injection volume for a 5 mM DL was typically 3.5 μL. UV absorbance chromatograms were recorded at wavelengths of 220 nm and 289 nm. The data were analyzed using an HP chemstation.

Rapid resolution LC-MS analysis was performed on an Agilent Series 1200 instrument, using a Halo-C18 1.8 μm column (4.6 x 50 mm, 2.7 μm) with gradient elution (acetonitrile/water containing 0.2% formic acid or methanol/water containing 0.1% formic acid) at a flow rate of 1.0 mL/min (gradient column temperature: 50 °C to 45 °C). The injection volume for a 5 mM DL was typically 3.0 μL. The eluent was analyzed by an LC/MSD Quadrapole SL mass spectrometer in positive ion mode with an electrospray ionization source.

UPLC-MS analysis was performed on Acquity UPLC instrument, using an Acquity UPLC® BEH C18 1.7μm column (2.1 x 150 mm) with various gradient elution (17 to 30% acetonitrile/water containing 0.2 % formic acid, in 12.5 min) at a flow rate of 0.7 mL/min (column temperature: 55 °C). The injection volume for a 5 mM DL was typically 1.5 μL. The eluent was analyzed by a Micromass ZQ mass spectrometer in positive ion mode with the electrospray ionization source.

The isolation of cyclic hexamer was performed on the modified semi-prep HPLC Agilent Series 1200 instrument, using an Agilent Zorbax Eclipse XDB-C18 PrepHT Cartridge Column (21. 2 x 250 mm, 7 μm) with 62% methanol/water elution (no additive) at a flow rate of 9 mL/min (column temperature 55 ^{o}C). For the sample preparation, DLs were neutralized by adding Et_3N and then filtered and concentrated in vacuo. The concentrated DLs were re-dissolved in 20% $CHCl_3$/methanol and filtered before the injection.

MS/MS analyses of the isolated cyclic hexamer **3b** were preformed on an Accurate Mass LC-QTOF (Agilent Series 6520) equipped with an Agilent Series 1200 LC instrument without a column in positive ion mode with an electrospray ionization source. The sample was prepared by dissolving **3b** in methanol and eluted (50% methanol/water, water contained 0.2% formic acid) at a flow rate of 0.2 mL/min for 2 min. Injection volume was 1 μL. The MS source Nebulizer Gas, VCap and fragmentor parameters were set to 35psig, 3500V and 375V, respectively. The other MS source parameters were default

[1] M.-K. Chung, P. S. White, S. J. Lee, M. R. Gagné, *Angew. Chem. Int. Ed.* 2009, **48**, 8683.

settings. The Ramped Collusion Energy was adjusted by slope (typically 2) and offset (0 to 30) values. The MS1 and MS2 data were collected in the range of 100 to 3200 (m/z) and analyzed using Agilent MassHunter Workstation Software, Qualitative Analysis (V. B.02.00).

MS/MS analyses of DLs were also preformed on an Accurate Mass LC-QTOF (Agilent Series 6520) equipped with an Agilent Series 1200 LC instrument with a Halo-C18 1.8 μm column (2.1 x 50 mm, 2.7 μm). The sample was doubly diluted just before the injection and a gradient elution (methanol/water containing 0.1% formic acid) was used at a flow rate of 0.5 mL/min (column temperature: 50 °C). The injection volume for a doubly diluted 5 mM DL was typically 2.0 μL. For MS/MS analyses of the other isomer of **3b**, the injection volume increased to 5.0 μL due to its small quantity in the DL. The MS source Nebulizer Gas, VCap and fragmentor parameters were set to 50psig, 3500V and 375V, respectively. The other MS source parameters were default settings. The Ramped Collusion Energy was adjusted by slope (typically 2) and offset (0 to 30) values. The MS1 and MS2 data were collected in the range of 100 to 3200 (m/z) and analyzed using Agilent MassHunter Workstation Software, Qualitative Analysis (V. B.02.00).

^{1}H NMR, COSY and TOCSY spectra of **3b** were recorded on Varian Inova 600 spectrometer and processed ACD/SpecManager software (V.10.08).

Generation of DLs using D-1 and L,L-2a or L,L-2b. 5.0 mM DLs were prepared on a 1 mL scale. D-**1** (50 μmol) and L,L-**2a** or **b** (50 μmol) were separately dissolved in 10 mL of a solution of acetonitrile and chloroform (1:2 for L,L-**2a** and 1:3 for L,L-**2b**). 5.0 mL of D-**1** solution was mixed with 5.0 mL of the L,L-**2a** or **b** solution and TFA (50 eq., 2500 μmol, 187 μL). Aliquots of this solution (1.0 mL) were transferred to two vials [an empty vial and a vial containing a template: (–)-cytidine (3.6 mg, 15 μmol)]. The resultant solution was allowed to sit for more than a month (typically 30 – 35 days) until a steady state was reached prior to LC analyses. For solutions where a precipitate appeared, it was filtered using a syringe filter (0.2 μm) prior to LC or LC-MS analyses.

Generation of DLs from D-1 and L,L-2a for simulation. A series of DLs were prepared on a 1 mL scale using D-**1** and L,L-**2a** (1:1, 5.0 mM in a solution of MeCN and $CHCl_3$ (1:2)) in the presence of TFA (50 eq., 250 mM). The concentration of (–)-cytidine was varied from 0 to 15 mM. DLs were allowed to sit for more than 1 month, during which equilibrium was reached, and then analyzed by HPLC, monitoring the hydrazone absorption at 289 nm. Assuming that extinction coefficients in the UV trace (the hydrazone absorption at 289 nm) were equal for all oligomers, the concentration of each oligomer in a DL was calculated. Experiments were performed at least in duplicate and the average values were used for simulations. Oligomers were identified by rapid resolution LC-MS and UPLC-MS. Low concentration oligomers were further identified using various biased DLs and homo-DLs generated from D-**1** and L,L-**2a**, respectively. For poorly resolved

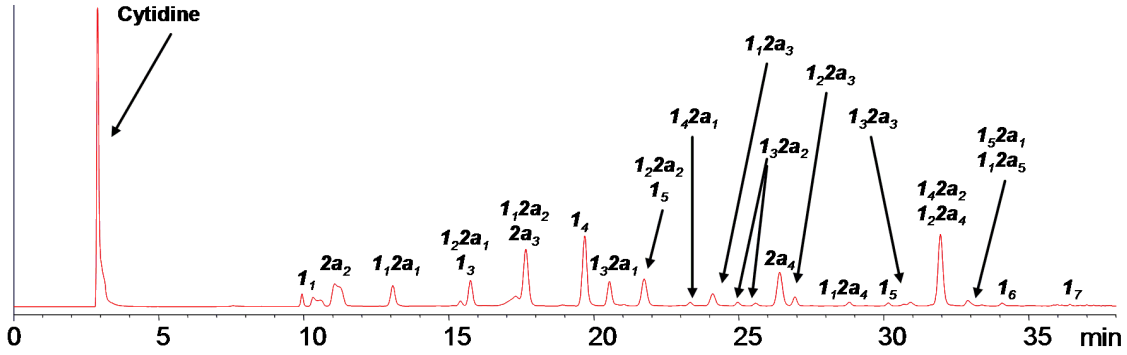
oligomers, their UV area (%) were obtained by incorporating the relative ratio of species calculated from UPLC-MS chromatograms.

Numerical Simulations. The computational studies were performed with the help of the program DCLFit.[2] The fitting was performed in a similar way as described in a previous report.[3] The oligomer association constant $K\mathbf{1}_2$ was fixed arbitrarily at 10^5 M^{-1}. The remaining oligomer association constants $K\mathbf{1}_x\mathbf{2a}_x$ (x = 0 – 6) for dimers, trimers, tetramers, pentamers and hexamers were fit to the equilibrium concentrations of the oligomers in the absence of template. The latter values were obtained by extrapolation of the oligomer distributions of (–)-cytidine-templated libraries to 0 mM template with the help of simple polynomial fits. The extrapolated values are believed to be more accurate than the experimentally determined values for 0 mM template (DL equilibrium without template was very slow and the beginnings of library decomposition hampered the precise determination of the 0 mM template values). The association constants $K\mathbf{1}_x\mathbf{2a}_x$ (x = 0 – 6) were then fixed, and the model was expanded to include the interactions with the template (–)-cytidine (1:1 host-guest complex) under the assumption that the experimentally determined concentrations corresponded to the sum of the free and the template complexed oligomers.

[2] R. F. Ludlow, J. Liu, H. Li, S. L. Roberts, J. M. K. Sanders, S. Otto, *Angew. Chem., Int. Ed.* 2007, **46**, 5762.
[3] M.-K. Chung, C. R. Hebling, J. W. Jorgenson, K. Severin, S. J. Lee, M. R. Gagné, *J. Am. Chem. Soc.* 2008, **130**, 11819.

SI Table 1. Raw data for simulations: UV trace peak area (%) of oligomers in DLs generated from D-**1** and L,L-**2a** (1:1, 5.0 mM in MeCN:$CHCl_3$ = 1:2, 250 mM TFA, (–)-cytidine). Data points were collected at day 35 – day 60. At low concentrations of (–)-cytidine (≤ 2.5 mM), the templated DLs were still adjusting prior to day 35.

(a)



(a) The UV trace (289 nm) of HPLC analysis for (–)-cytidine templated DL formed from D-**1** and L,L-**2a** (1:1, 5 mM). Oligomers were identified by rapid resolution LC-MS and UPLC-MS. Low concentration oligomers were identified using various biased DLs and homo-DLs generated from D-**1** and L,L-**2a**, respectively.

(b) Dimers

Cytidine conc. (mM)	UV area (%)[a]			Oligomer conc. (mM)[a]		
	$\mathbf{1}_2$	$\mathbf{2a}_2$	$\mathbf{1}_1\mathbf{2a}_1$	$\mathbf{1}_2$	$\mathbf{2a}_2$	$\mathbf{1}_1\mathbf{2a}_1$
15.0	4.29883	9.08277	4.08488	0.10747	0.22707	0.10212
12.5	3.89861	8.58112	4.13625	0.09747	0.21453	0.10341
10.0	3.94463	8.73604	4.06738	0.09862	0.21840	0.10168
7.50	3.847172	8.68032	4.11861	0.09618	0.21701	0.10297
5.00	3.66520	8.32276	4.26402	0.09163	0.20807	0.10660
3.75	3.66989	7.87956	4.73840	0.09175	0.19699	0.11846
2.50	3.64679	7.71260	4.95619	0.09117	0.19282	0.12390
1.25	3.68320	7.75716	5.00594	0.09208	0.19393	0.12515

[a]The average value of two separately prepared samples.

(c) Trimers

Cytidine conc. (mM)	UV area (%)[a]				Oligomer conc. (mM)[a]			
	$\mathbf{1}_3$[b]	$\mathbf{1}_2\mathbf{2a}_1$[b]	$\mathbf{1}_1\mathbf{2a}_2$[b]	$\mathbf{2a}_3$[b]	$\mathbf{1}_3$[b]	$\mathbf{1}_2\mathbf{2a}_1$[b]	$\mathbf{1}_1\mathbf{2a}_2$[b]	$\mathbf{2a}_3$[b]
15.0	1.20776	4.41586	12.58194	4.31908	0.02013	0.07360	0.20970	0.07198
12.5	1.40461	5.22778	13.48152	4.71845	0.02341	0.08713	0.22469	0.07864
10.0	1.26098	5.05731	13.25928	4.62585	0.02102	0.08429	0.22099	0.07710
7.50	1.42316	5.29291	13.78794	4.47969	0.02372	0.08822	0.22980	0.07466
5.00	1.77574	6.19380	14.98578	4.76200	0.02960	0.10323	0.24976	0.07937
3.75	2.54134	7.81320	16.31520	5.66157	0.04236	0.13022	0.27192	0.09436
2.50	2.87969	8.79864	17.20914	5.96705	0.04799	0.14664	0.28682	0.09945
1.25	3.31844	9.50631	17.61522	6.56879	0.05530	0.15844	0.29359	0.10948

[a]The average value of two separately prepared samples. [b]Due to the poor resolution of UV trace (see (a)), UV area (%) of each species were obtained by incorporating the relative ratio of species calculated from UPLC-MS chromatograms

(d) Tetramers

Cytidine conc. (mM)	UV area (%)[a]				
	$\mathbf{1}_4$	$\mathbf{1}_3\mathbf{2a}_1$	$\mathbf{1}_2\mathbf{2a}_2$[b,c]	$\mathbf{1}_1\mathbf{2a}_3$	$\mathbf{2a}_4$
15.0	13.80616	4.70524	6.35096	3.05195	7.68048
12.5	13.44512	4.93801	5.86280	2.86927	7.48640
10.0	13.30344	4.88358	5.88664	2.86470	7.45616
7.50	13.13768	4.95765	5.69880	2.84163	7.31568
5.00	13.01536	5.08823	5.42872	2.84955	6.83912
3.75	12.92112	5.23970	5.21968	2.80681	6.13616
2.50	12.93816	5.24820	5.12568	2.77885	5.81360
1.25	12.77432	5.13068	5.28304	2.86740	5.29136

Cytidine conc. (mM)	Oligomer conc. (mM)[a]				
	$\mathbf{1}_4$	$\mathbf{1}_3\mathbf{2a}_1$	$\mathbf{1}_2\mathbf{2a}_2$[b]	$\mathbf{1}_1\mathbf{2a}_3$	$\mathbf{2a}_4$
15.0	0.17258	0.05882	0.07939	0.03815	0.09601
12.5	0.16806	0.06173	0.07329	0.03587	0.09358
10.0	0.16629	0.06104	0.07358	0.03581	0.09320
7.50	0.16422	0.06197	0.07124	0.03552	0.09145
5.00	0.16269	0.06360	0.06786	0.03562	0.08549
3.75	0.16151	0.06550	0.06525	0.03509	0.07670
2.50	0.16173	0.06560	0.06407	0.03474	0.07267
1.25	0.15968	0.06413	0.06604	0.03584	0.06614

[a]The average value of two separately prepared samples. [b]Due to the poor resolution of UV trace with the species $\mathbf{1}_5$ (see (a)), UV area (%) of each species were obtained by incorporating the relative ratio of species calculated from UPLC-MS chromatograms. [c]Constitutional isomers were not distinguished.

(e) Pentamers

Cytidine conc. (mM)	UV area (%)[a]					
	$\mathbf{1}_5$[b]	$\mathbf{1}_4\mathbf{2a}_1$	$\mathbf{1}_3\mathbf{2a}_2$[b,c]	$\mathbf{1}_2\mathbf{2a}_3$[b,c]	$\mathbf{1}_1\mathbf{2a}_4$	$\mathbf{2a}_5$
15.0	0.24932	0.69870	1.34680	1.77540	0.86940	0.60770
12.5	0.17313	0.70020	1.31560	1.72320	0.83347	0.61420
10.0	0.17586	0.69810	1.31540	1.72610	0.84122	0.60900
7.50	0.15223	0.68990	1.29480	1.72400	0.83613	0.61050
5.00	0.13280	0.71910	1.32540	1.70180	0.83536	0.62370
3.75	0.09781	0.80300	1.33540	1.65920	0.82615	0.62610
2.50	0.09929	0.80580	1.32040	1.63920	0.81687	0.63190
1.25	0.04473	0.87390	1.38250	1.66190	0.87129	0.65990
Cytidine conc. (mM)	Oligomer conc. (mM)[a]					
	$\mathbf{1}_5$[b]	$\mathbf{1}_4\mathbf{2a}_1$	$\mathbf{1}_3\mathbf{2a}_2$[b,c]	$\mathbf{1}_2\mathbf{2a}_3$[b,c]	$\mathbf{1}_1\mathbf{2a}_4$	$\mathbf{2a}_5$
15.0	0.00249	0.00699	0.01347	0.01775	0.00869	0.00608
12.5	0.00173	0.00700	0.01316	0.01723	0.00833	0.00614
10.0	0.00176	0.00698	0.01315	0.01726	0.00841	0.00609
7.50	0.00152	0.00690	0.01295	0.01724	0.00836	0.00611
5.00	0.00133	0.00719	0.01325	0.01702	0.00835	0.00624
3.75	0.00098	0.00803	0.01335	0.01659	0.00826	0.00626
2.50	0.00099	0.00806	0.01320	0.01639	0.00817	0.00632
1.25	0.00045	0.00874	0.01383	0.01662	0.00871	0.00660

[a]The average value of two separately prepared samples. [b]Due to the poor resolution of UV traces (see (a)), UV area (%) of each species were obtained by incorporating the relative ratio of species calculated from UPLC-MS chromatograms. [c]Constitutional isomers were not distinguished.

(f) Hexamers

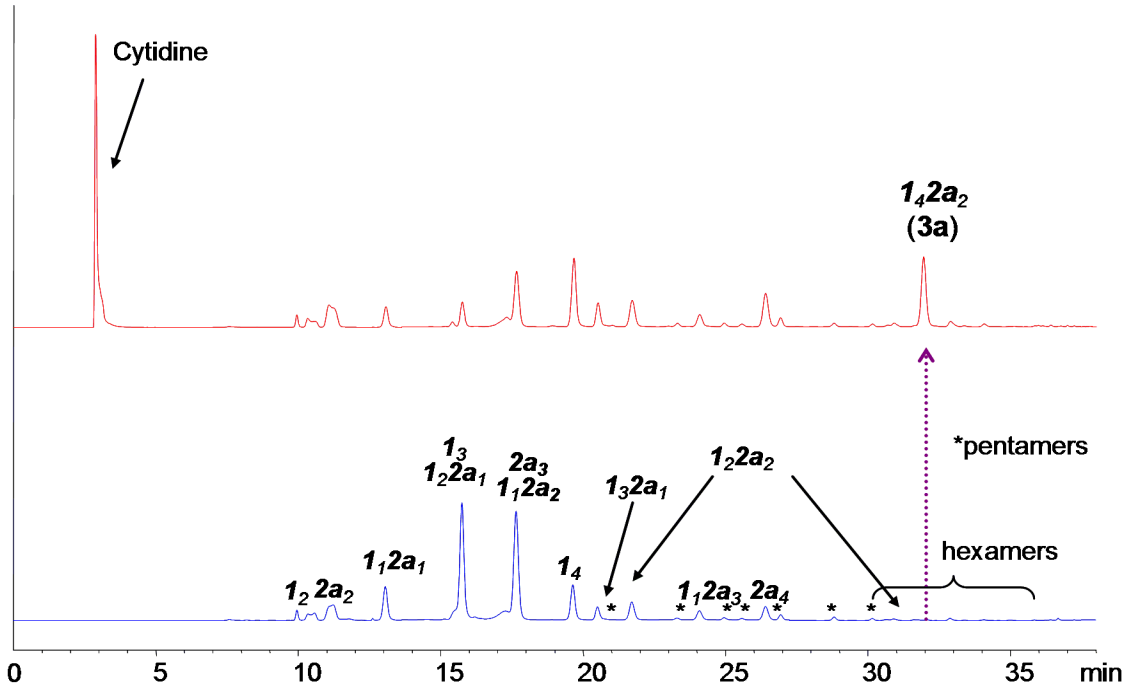
Cytidine conc. (mM)	UV area (%)[a]						
	$\mathbf{1}_6$	$\mathbf{1}_5\mathbf{2a}_1$[b]	$\mathbf{1}_4\mathbf{2a}_2$[b]	$\mathbf{1}_3\mathbf{2a}_3$[b,c]	$\mathbf{1}_2\mathbf{2a}_4$[b,c]	$\mathbf{1}_1\mathbf{2a}_5$	$\mathbf{2a}_6$
15.0	0.90315	0.38347	14.12652	0.34332	1.23792	0.92568	0.68729
12.5	0.86664	0.34853	14.13396	0.34320	1.11936	0.91608	0.59634
10.0	0.86532	0.33433	14.71236	0.33888	1.21968	0.94008	0.61533
7.50	0.86107	0.34643	14.58624	0.34056	1.25556	0.90672	0.58177
5.00	0.83984	0.31460	13.06176	0.34860	1.25388	0.84948	0.54140
3.75	0.80529	0.20318	9.68484	0.35688	1.14336	0.81576	0.46541
2.50	0.78800	0.20195	7.56084	0.35748	1.27392	0.75816	0.44182
1.25	0.79466	0.20507	5.68224	0.37500	1.26336	0.69864	0.43411
Cytidine conc. (mM)	Oligomer conc. (mM)[a]						
	$\mathbf{1}_6$	$\mathbf{1}_5\mathbf{2a}_1$[b]	$\mathbf{1}_4\mathbf{2a}_2$[b]	$\mathbf{1}_3\mathbf{2a}_3$[b,c]	$\mathbf{1}_2\mathbf{2a}_4$[b,c]	$\mathbf{1}_1\mathbf{2a}_5$	$\mathbf{2a}_6$
15.0	0.00753	0.00320	0.11772	0.00286	0.01032	0.00771	0.00573
12.5	0.00722	0.00290	0.11778	0.00286	0.00933	0.00763	0.00497
10.0	0.00721	0.00279	0.12260	0.00282	0.01016	0.00783	0.00513
7.50	0.00718	0.00289	0.12155	0.00291	0.01046	0.00756	0.00485
5.00	0.00700	0.00262	0.10885	0.00297	0.01045	0.00708	0.00451
3.75	0.00671	0.00169	0.08071	0.00298	0.00953	0.00680	0.00388
2.50	0.00657	0.00168	0.06301	0.00313	0.01062	0.00632	0.00368
1.25	0.00662	0.00171	0.04735	0.00408	0.01053	0.00582	0.00362

[a]The average value of two separately prepared samples. [b]Due to the poor resolution of UV traces (see (a)), UV area (%) of each species were obtained by incorporating the relative ratio of species calculated from UPLC-MS chromatograms. [c]Constitutional isomers were not distinguished.

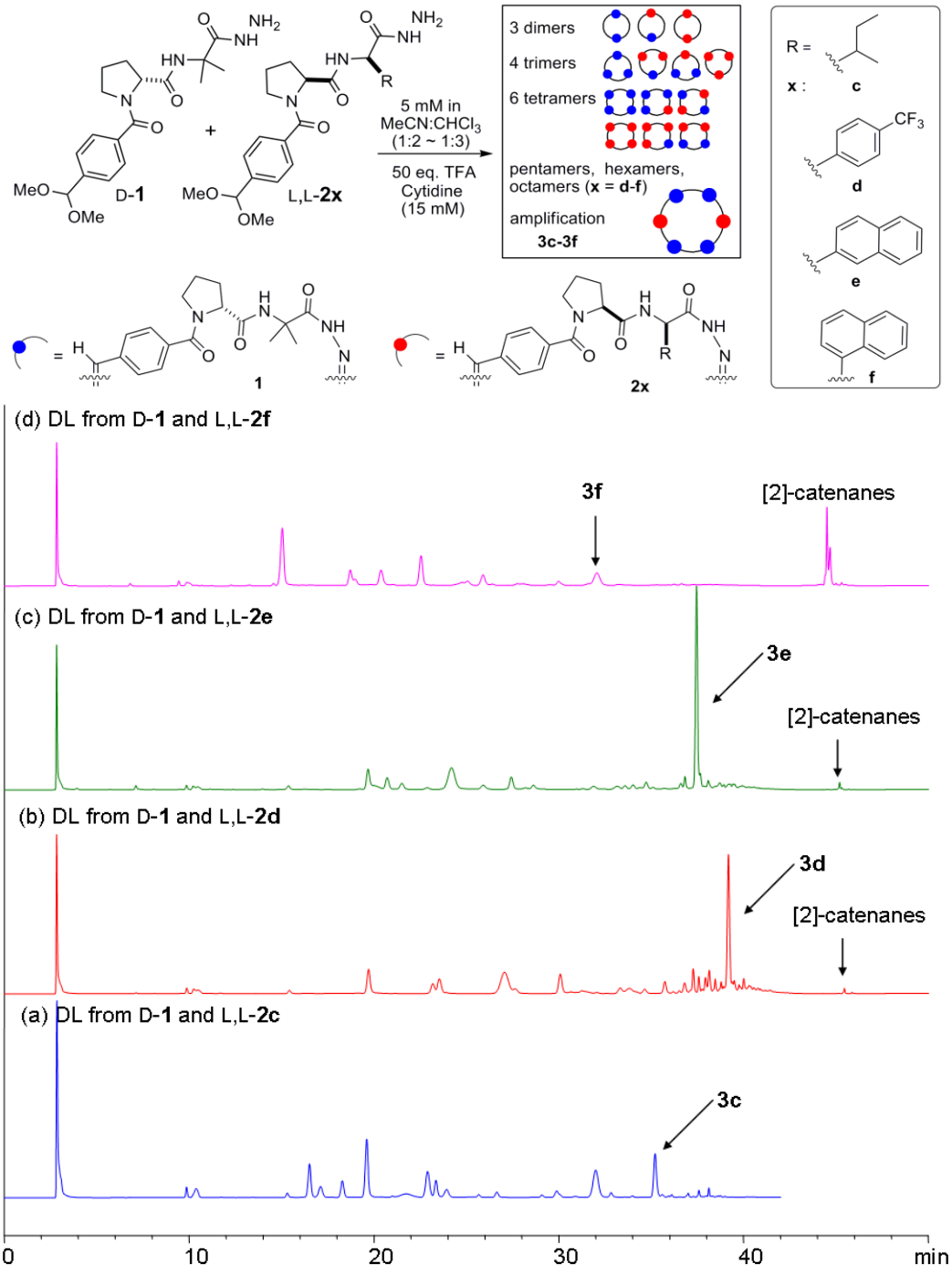
(g) Heptamers: $\mathbf{1}_7$ was detected as a sole heptamer (0.0015 – 0.0019 mM in each DL) but omitted for the simulations.

SI Table 2. Binding constants *K* and Gibbs free energies Δ*G*° (at 25 °C) for binding of oligomers in DLs generated from D-**1** and L,L-**2a** to (–)-cytidine.

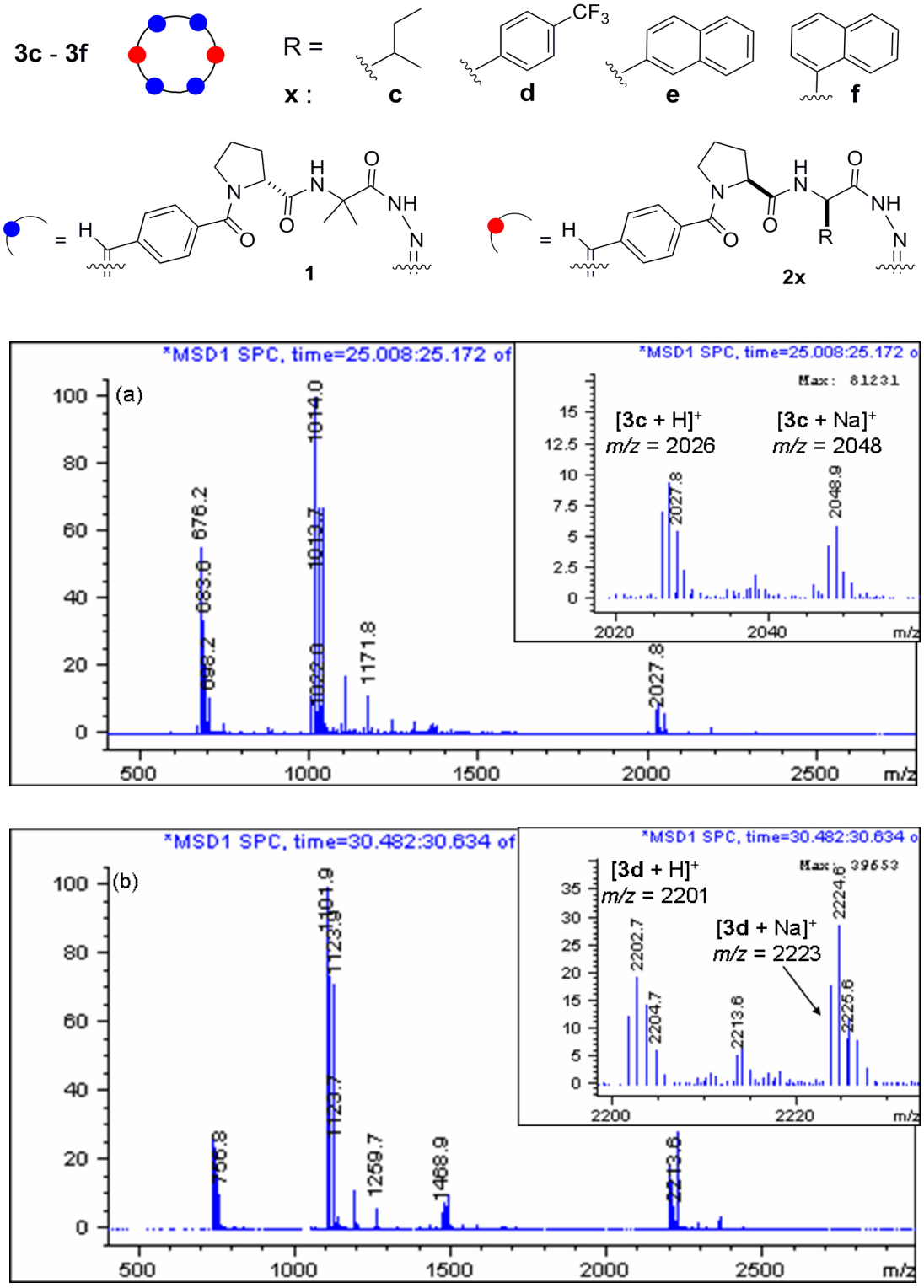
Oligomer	*K* (M^{-1})	Δ*G*° (kJ•mol^{-1})
$\mathbf{1}_6$	624.2	16.0
$\mathbf{1}_5$	7664.6	22.2
$\mathbf{1}_4$	263.4	13.8
$\mathbf{1}_3$	1.0	0.0
$\mathbf{1}_2$	103.0	11.5
$\mathbf{1}_5\mathbf{2a}_1$	1248.4	17.7
$\mathbf{1}_4\mathbf{2a}_1$	202.2	13.2
$\mathbf{1}_3\mathbf{2a}_1$	186.7	13.0
$\mathbf{1}_2\mathbf{2a}_1$	1.0	0.0
$\mathbf{1}_1\mathbf{2a}_1$	1.0	0.0
$\mathbf{1}_4\mathbf{2a}_2$	**4864.5**	**21.0**
$\mathbf{1}_3\mathbf{2a}_2$	233.9	13.5
$\mathbf{1}_2\mathbf{2a}_2$	197.8	13.1
$\mathbf{1}_1\mathbf{2a}_2$	32.3	8.6
$\mathbf{2a}_2$	69.9	10.5
$\mathbf{1}_3\mathbf{2a}_3$	269.1	13.9
$\mathbf{1}_2\mathbf{2a}_3$	227.7	13.5
$\mathbf{1}_1\mathbf{2a}_3$	130.9	12.1
$\mathbf{2a}_3$	1.0	0.0
$\mathbf{1}_2\mathbf{2a}_4$	250.6	13.7
$\mathbf{1}_1\mathbf{2a}_4$	153.8	12.5
$\mathbf{2a}_4$	234.0	13.5
$\mathbf{1}_1\mathbf{2a}_5$	366.3	14.6
$\mathbf{2a}_5$	102.5	11.5
$\mathbf{2a}_6$	348.8	14.5



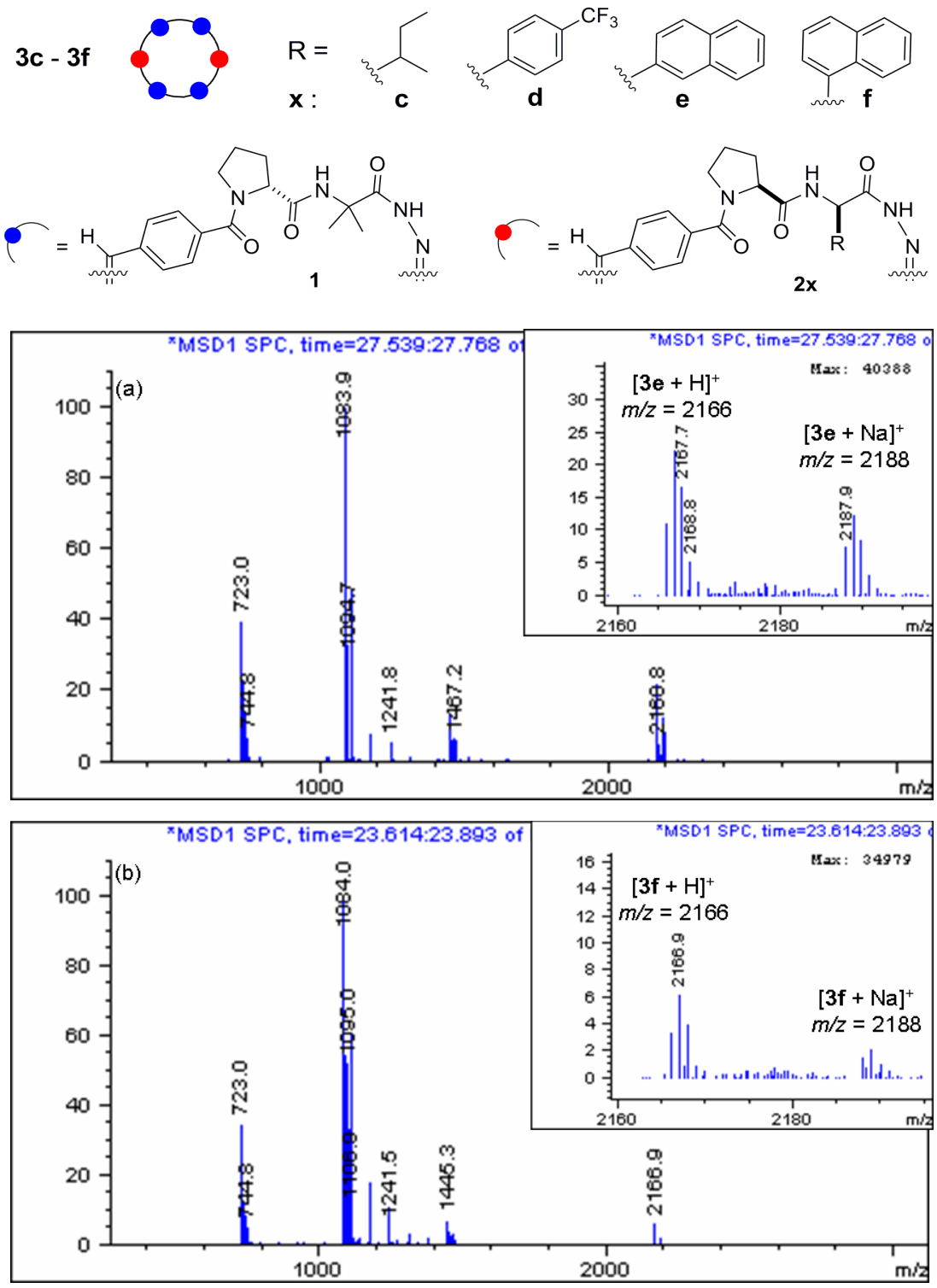
SI Fig. 1. HPLC-UV trace (289 nm) at day 35 of untemplated (5.0 mM, blue (bottom)) and templated (15 mM (–)-cytidine, red (top)) DLs formed from D-**1** and L,L-**2a** (1:1 in MeCN:$CHCl_3$ = 1:2). The oligomers in DLs were identified by Rapid resolution LC-MS (Agilent) and UPLC-MS (Waters). **3a** was amplified with 14 % total amount of monomers (AF 70 – 80; AF = $[\mathbf{3a}]_{templated}/[\mathbf{3a}]_{untemplated}$). The **3a** concentration was produced by the following equation: 5 mM (monomer concentration) x HPLC-UV trace integrations of the oligomer as fractions of the total (i.e. %UV area of **3a**) / number of monomer units (it is 6 for the haxamer). The y-axis is the normalized absorbance.



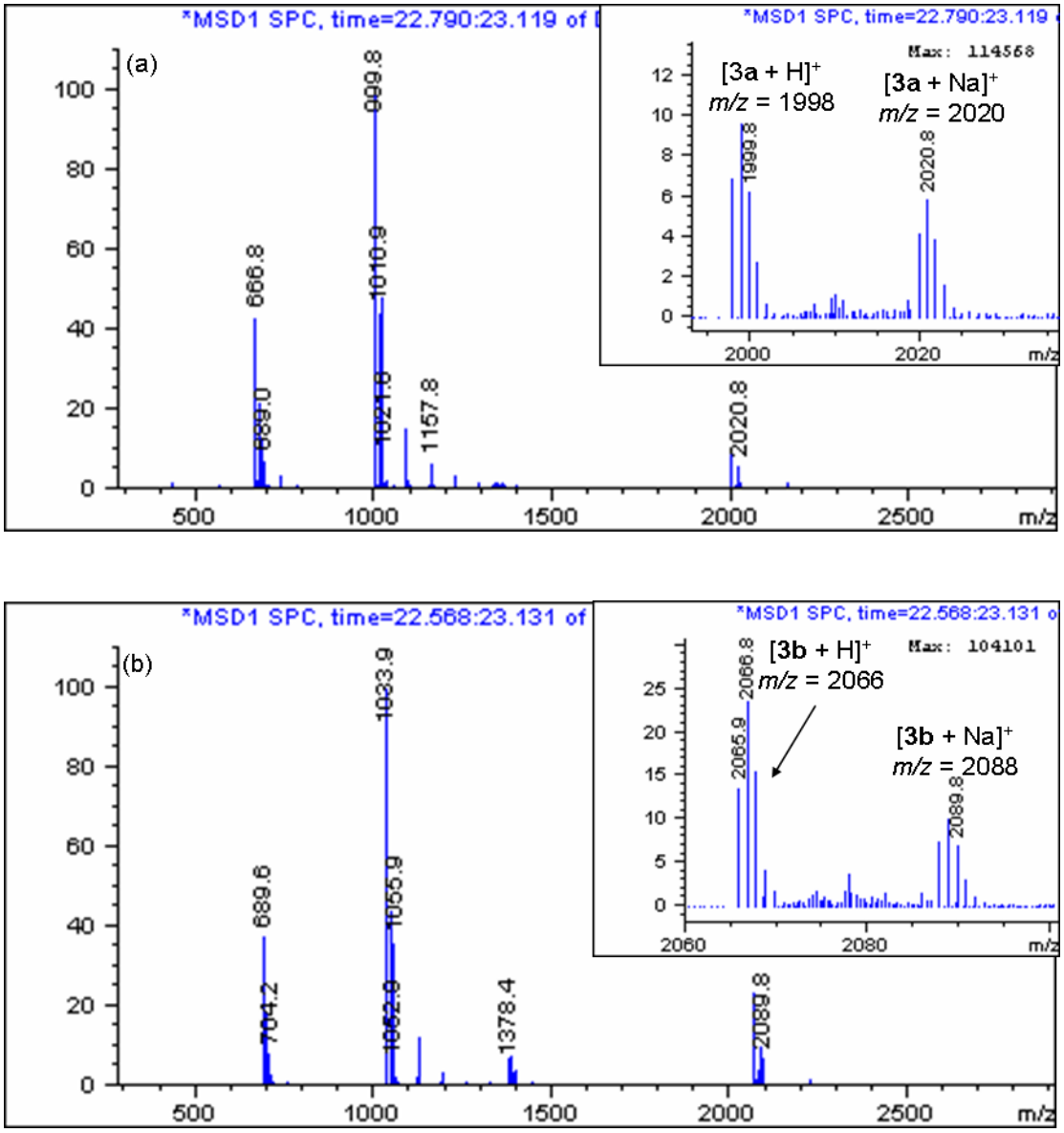
SI Fig. 2. HPLC-UV trace (289 nm) at day 35 (**x**=**c**) or at day 30 (**x**=**d**-**f**) of templated DLs formed from D-**1** and L,L-**2x** (1:1, 5.0 mM in MeCN:$CHCl_3$ =1:2 (**x**=**c**) or 1:3 (**x**=**d**-**f**), 50 eq. TFA, 3.0 eq. of (–)-cytidine). The oligomers in DLs were identified by Rapid resolution LC-MS (Agilent); for hexamers, please see SI Fig. 3 and 4. The amplified hexamers accounted for (a) 12% (b) 24% (c) 34% (d) 8% of total monomers, respectively. The y-axes are the normalized absorbance.



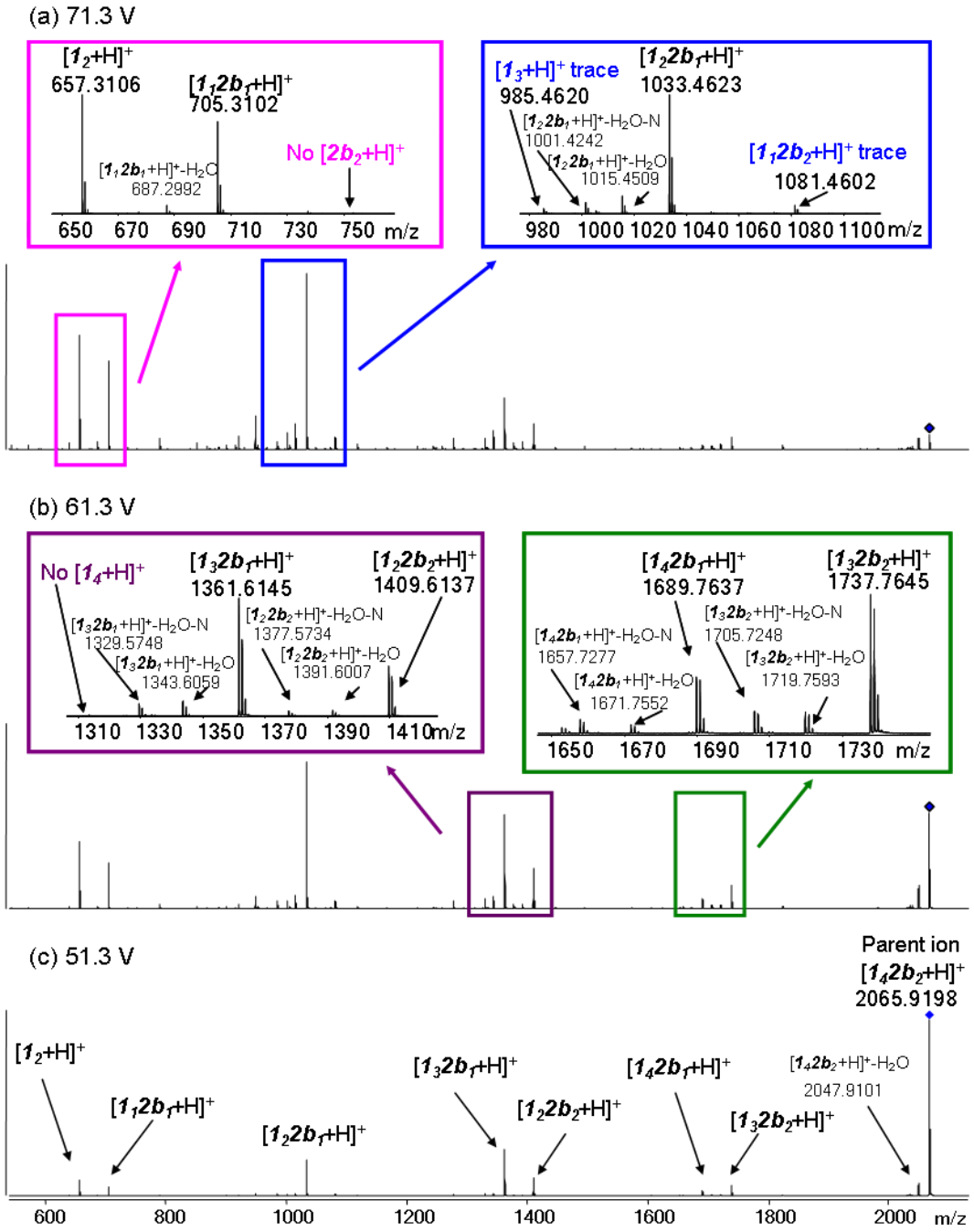
SI Fig. 3. MS spectra of (a) **3c** and (b) **3d** observed by Rapid resolution LC-MS (Agilent). Insets are the extended regions ((a) *m/z* = 2017-2060, (b) *m/z* = 2197-2223).



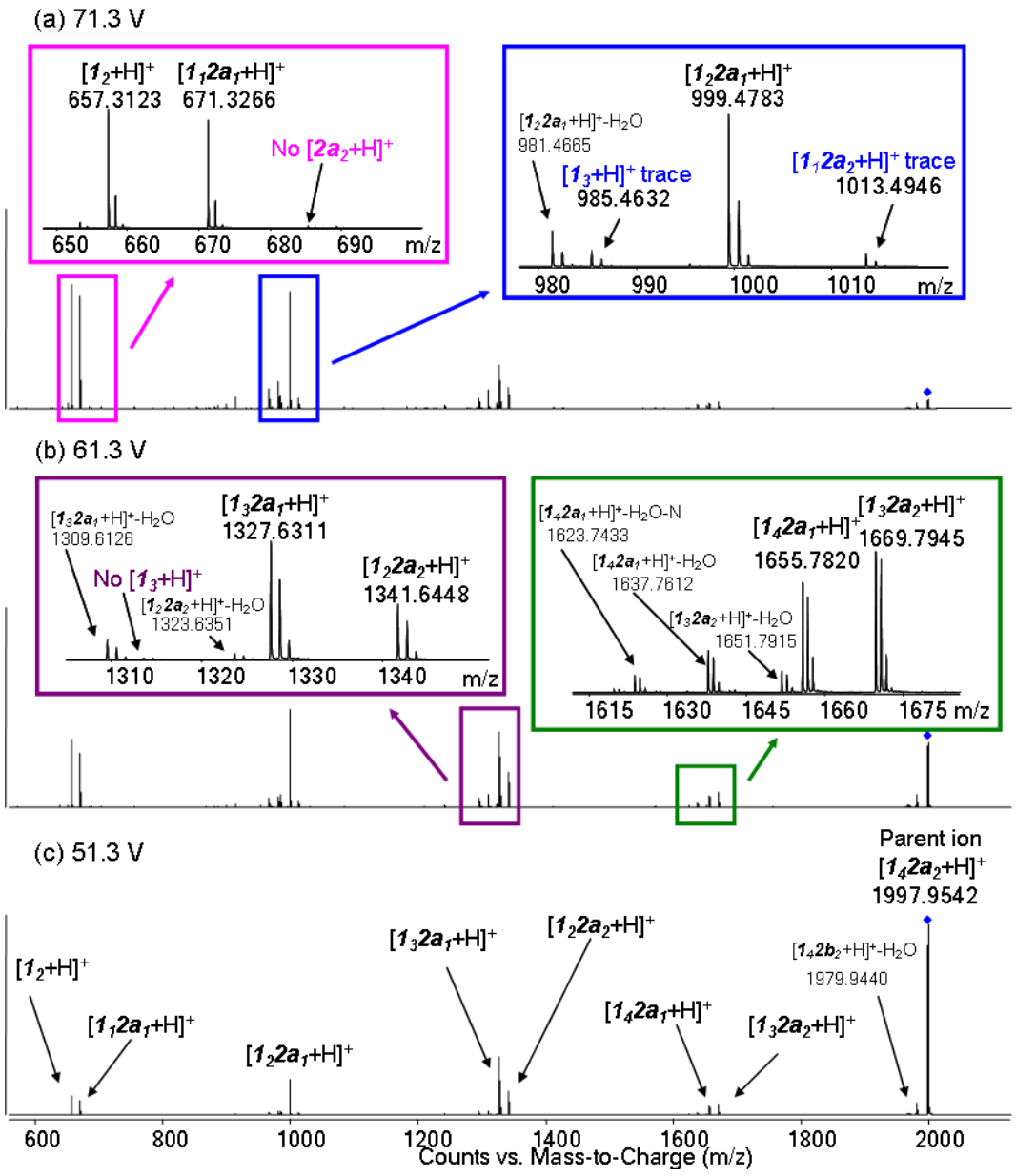
SI Fig. 4. MS spectra of (a) **3e** and (b) **3f** observed by Rapid resolution LC-MS (Agilent). Insets are the extended regions ((a) *m/z* = 2157-2197, (b) *m/z* = 2157-2197).



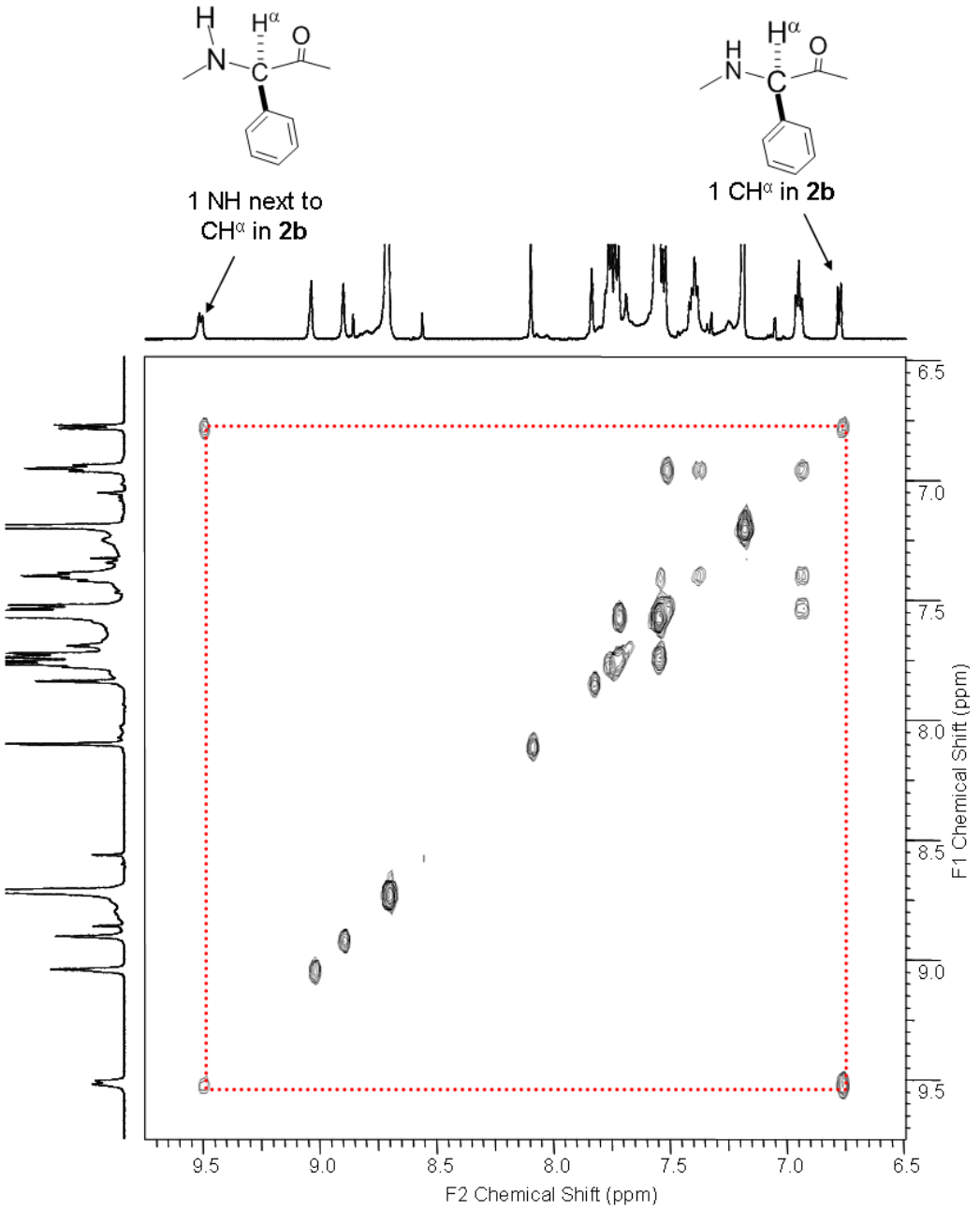
SI Fig. 5. MS spectra of (a) **3a** and (b) **3b** observed by Rapid resolution LC-MS (Agilent). Insets are the extended regions ((a) *m/z* = 1993-2037, (b) *m/z* = 2060-2113).



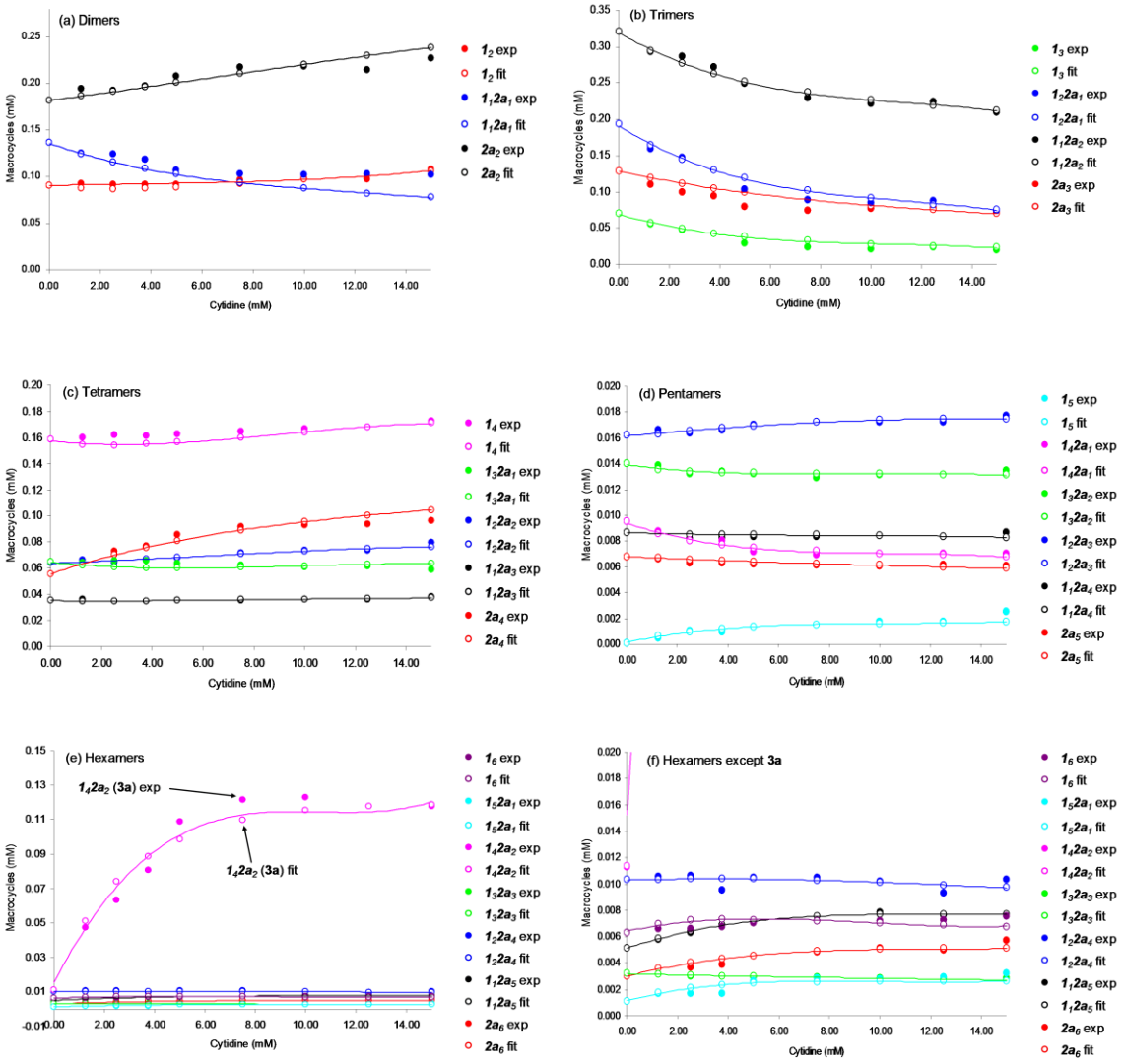
SI Fig. 6. Accurate Mass LC-QTOF CID MS/MS spectra of **3b** in a DL formed from D-**1** and L,L-**2b** at a collision energy of (a) 71.3 V, (b) 61.3 V and (c) 51.3 V. The lack of the $[\boldsymbol{2b}_2 + H]^+$ dimeric daughter ion and the detection of $[\boldsymbol{1}_1\boldsymbol{2b}_2 + H]^+$ as the dominant trimeric daughter ion along with the observation of tetrameric and pentameric daughter ions implied that the structure of **3b** is the symmetric constitutional isomer. The detected trace amounts of $[\boldsymbol{1}_3 + H]^+$ and $[\boldsymbol{1}_2\boldsymbol{2a}_1 + H]^+$ daughter ions might be generated during ESI process due to ion oligomerization and fragmentization.



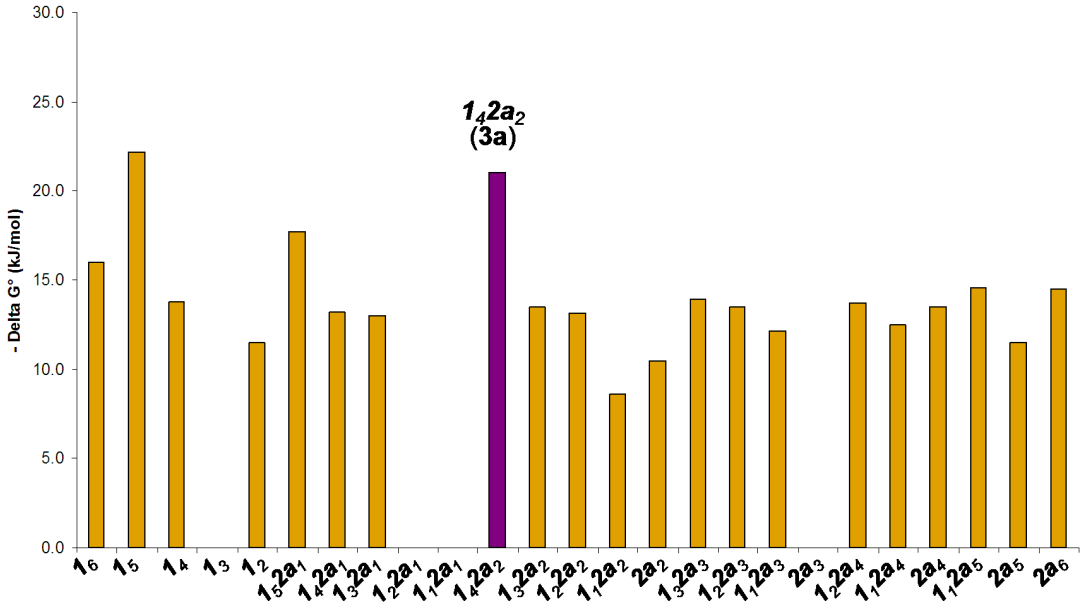
SI Fig. 7. Accurate Mass LC-QTOF CID MS/MS spectra of **3a** in a DL formed from D-**1** and L,L-**2a** at a collision energy of (a) 71.3 V, (b) 61.3 V and (c) 51.3 V. The lack of the $[\boldsymbol{2a}_2 + H]^+$ dimeric daughter ion and the detection of $[\boldsymbol{1}_1\boldsymbol{2a}_2 + H]^+$ as the dominant trimeric daughter ion along with the observation of tetrameric and pentameric daughter ions implied that the structure of **3a** is the symmetric constitutional isomer. The detected trace amounts of $[\boldsymbol{1}_3 + H]^+$ and $[\boldsymbol{1}_2\boldsymbol{2a}_1 + H]^+$ daughter ions might be generated during ESI process due to ion oligomerization and fragmentization.



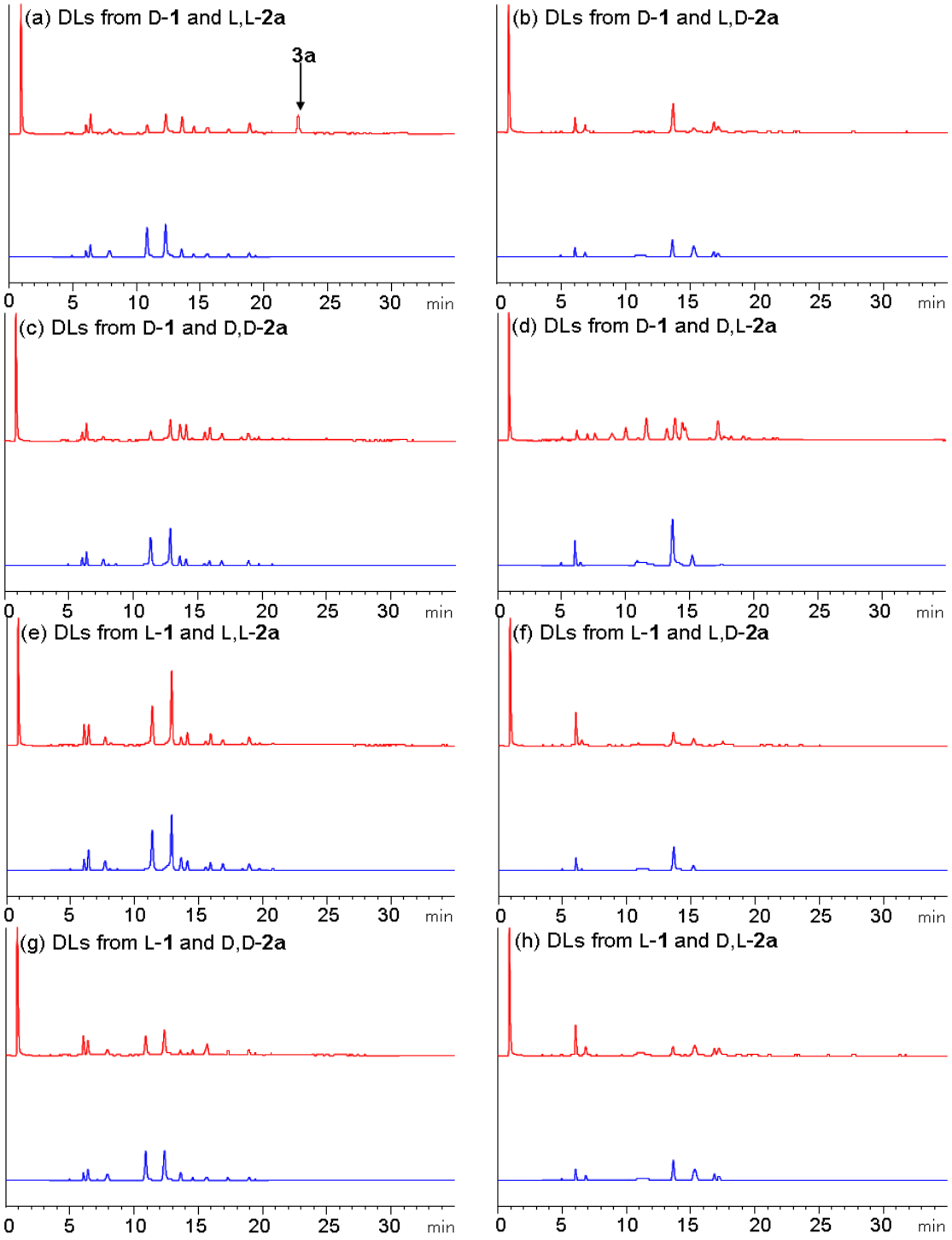
SI Fig. 8. A portion (δ = 6.5 to 10.0) of 599.8 MHz COSY spectrum of **3b** at 20 ^{o}C in pyridine-d_5. It showed the correlation between a single phenylglycine CH^{α} group and an adjacent amide NH group.



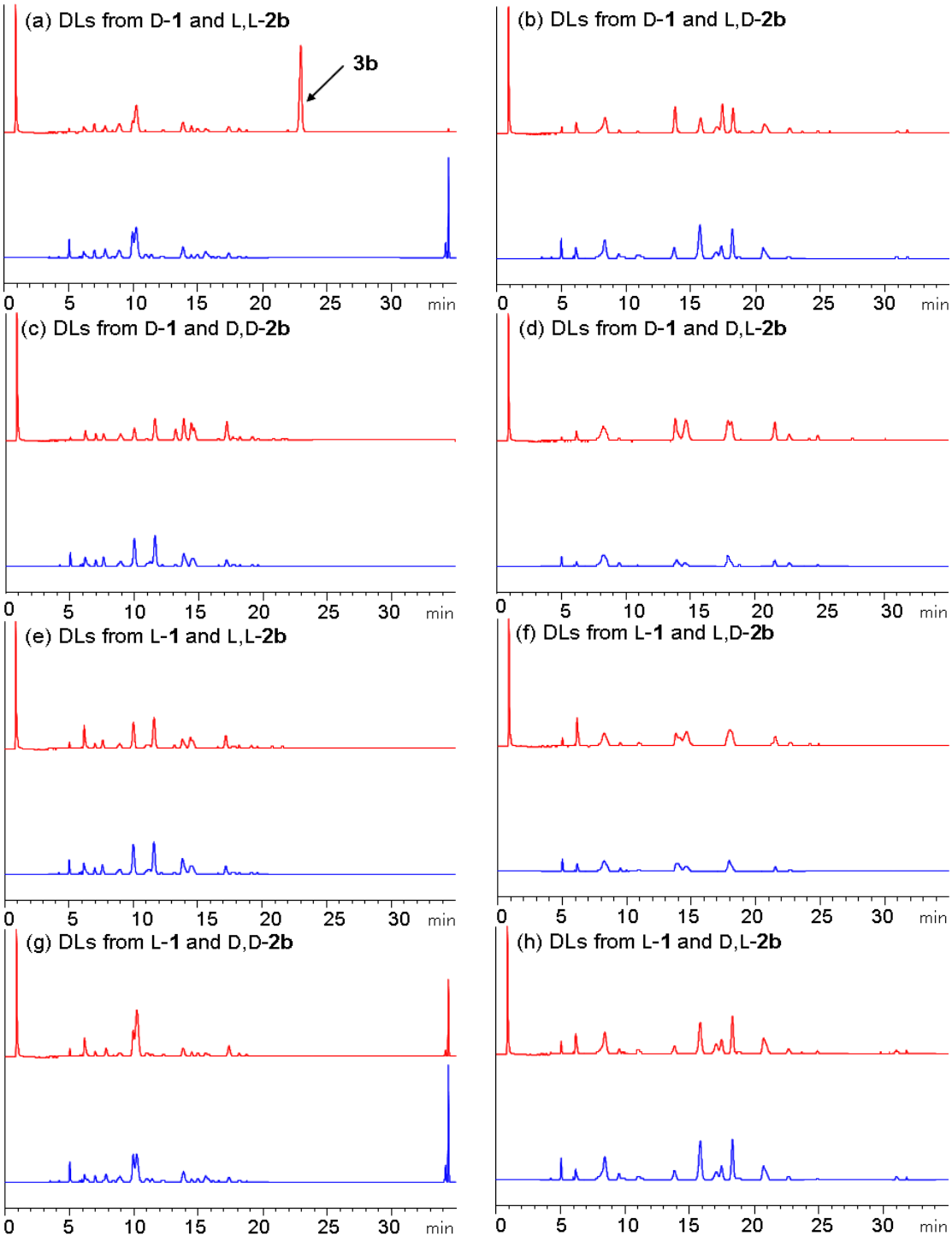
SI Fig. 9. Experimental data (full circle) and data obtained by the fitting procedure (empty circle) for (–)-cytidine-templated DLs generated from D-**1** and L,L-**2a**: (a) dimers, (b) trimers, (c) tetramers, (d) pentamers, (e) hexamers and (f) hexamers except **3a**. The lines are simple polynomial fits to the calculated values. They were added to guide the eye.



SI Fig. 10. The Gibbs free energies Δ*G*° (at 25 °C) for binding of oligomers in DLs formed from D-**1** and L,L-**2a** to (–)-cytidine.



SI Fig. 11. Rapid resolution LC-MS-UV trace (289 nm) at day 24 of untemplated (blue (bottom)) and templated (15 mM (–)-cytidine, red (top)) DLs generated from stereochemical combinations of **1** and **2a** (1:1, 5.0 mM in MeCN:$CHCl_3$ = 1:2, 50 eq. TFA). Since precipitation was observed in untemplated/templated DLs of (b), (d), (f) and (h) within 7 days, only the supernatant was analyzed in these DLs. Only D-**1**/L,L-**2a** in (a) led to a hexamer amplification.



SI Fig. 12. Rapid resolution LC-MS-UV trace (289 nm) at day 24 of untemplated (blue (bottom)) and templated (15 mM (–)-cytidine, red (top)) DLs generated from stereochemical combinations of **1** and **2b** (1:1, 5.0 mM in MeCN:$CHCl_3$ = 1:3, 50 eq. TFA). Since precipitation was observed in untemplated/templated DLs of (b), (d), (f) and (h) within 7 days, only the supernatant was analyzed in these DLs. Only D-**1**/L,L-**2b** in (a) led to a hexamer amplification.