Supplementary Material

Biological Evaluation and Conformational Preferences of Ferrocene Dipeptides with Hydrophobic Amino Acids

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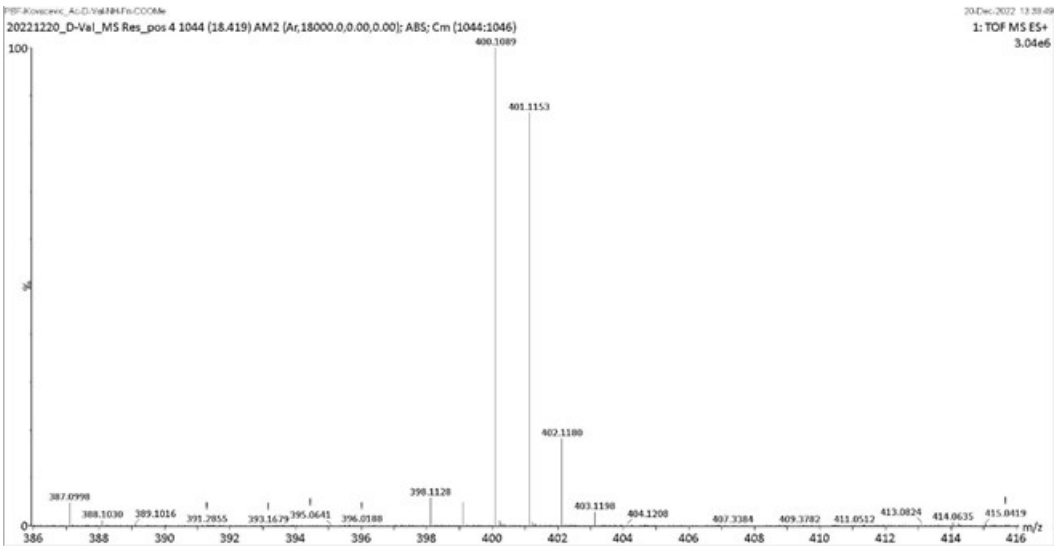
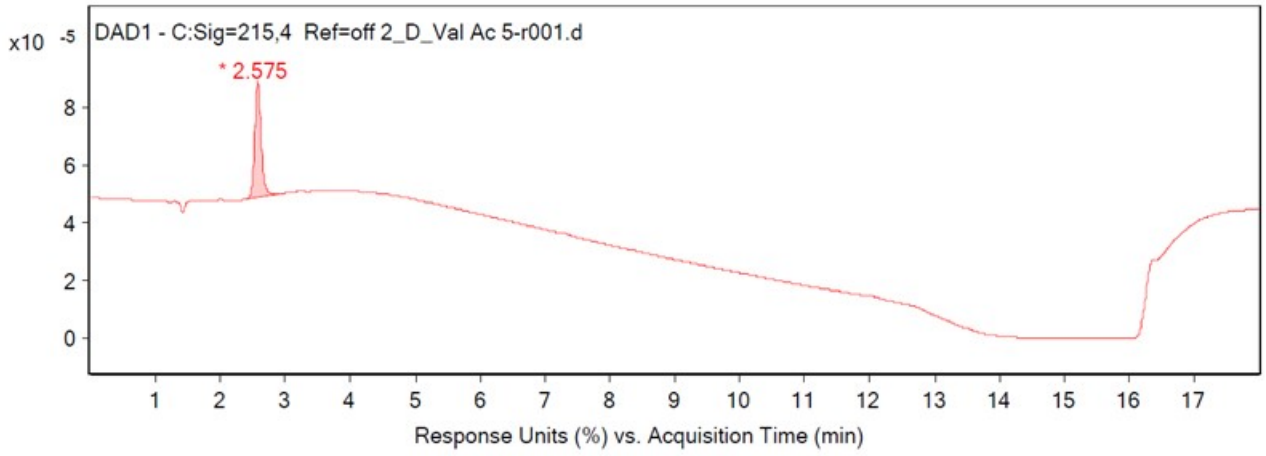
Table S1. Melting points of L-/D-**1a-3a** and L-/D-**1b-3b**

	Peptide	Melting point (°C)
L-**1b**	Boc-L-Val-NH-Fn-COOMe	112.3
L-**2b**	Boc-L-Leu-NH-Fn-COOMe	123
L-**3b**	Boc-L-Phe-NH-Fn-COOMe	130.6
D-**1b**	Boc-D-Val-NH-Fn-COOMe	112.5
D-**2b**	Boc-D-Leu-NH-Fn-COOMe	123.1
D-**3b**	Boc-D-Phe-NH-Fn-COOMe	130.7
L-**1a**	Ac-L-Val-NH-Fn-COOMe	156
L-**2a**	Ac-L-Leu-NH-Fn-COOMe	145.2
L-**3a**	Ac-L-Phe-NH-Fn-COOMe	198.6
D-**1a**	Ac-D-Val-NH-Fn-COOMe	156.1
D-**2a**	Ac-D-Leu-NH-Fn-COOMe	145.3
D-**3a**	Ac-D-Phe-NH-Fn-COOMe	198.7



	Peptide	Molecular formula	Ion	Calculated mass	Measured mass
D-**1a**	Ac-D-Val-NH-Fn-COOMe	$C_{19}H_{24}FeN_2O_4$	$[M]^+$	400.1085	400.1089

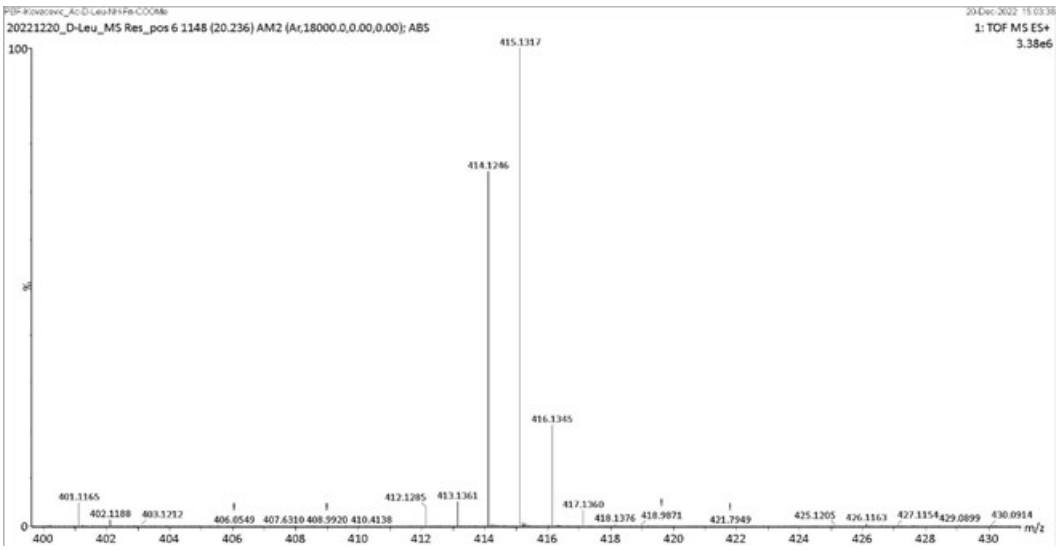
Figure S1. HRMS spectrum of compound D-**1a**



Integration Peak List

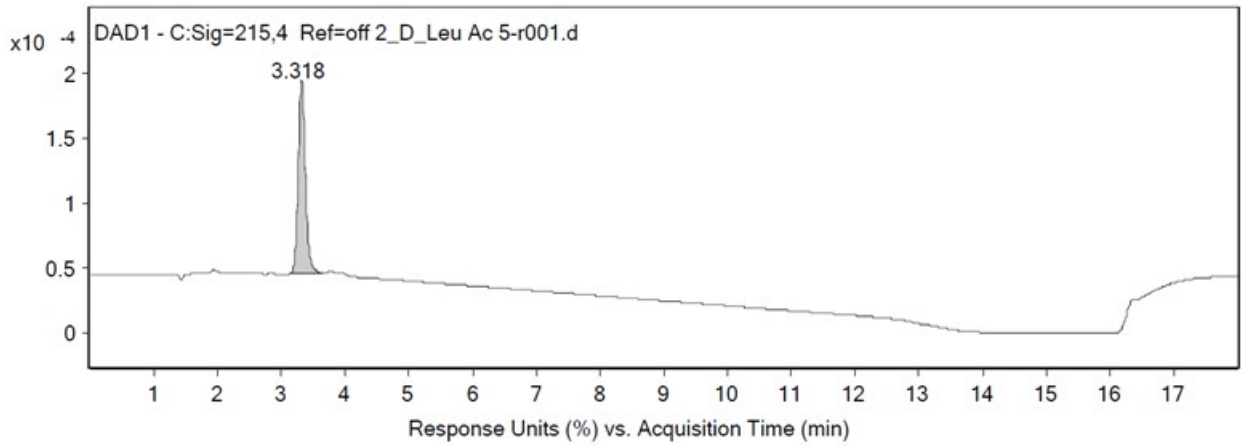
Peak	Start	RT	End	Height	Area	Area %
1	2.382	2.575	2.922	108.73	769.81	100

Figure S2. HPLC spectrum of compound D-**1a**



	Peptide	Molecular formula	Ion	Calculated mass	Measured mass
D-**2a**	Ac-D-Leu-NH-Fn-COOMe	$C_{20}H_{26}FeN_2O_4$	$[M+H]^+$	415.1320	415.1317

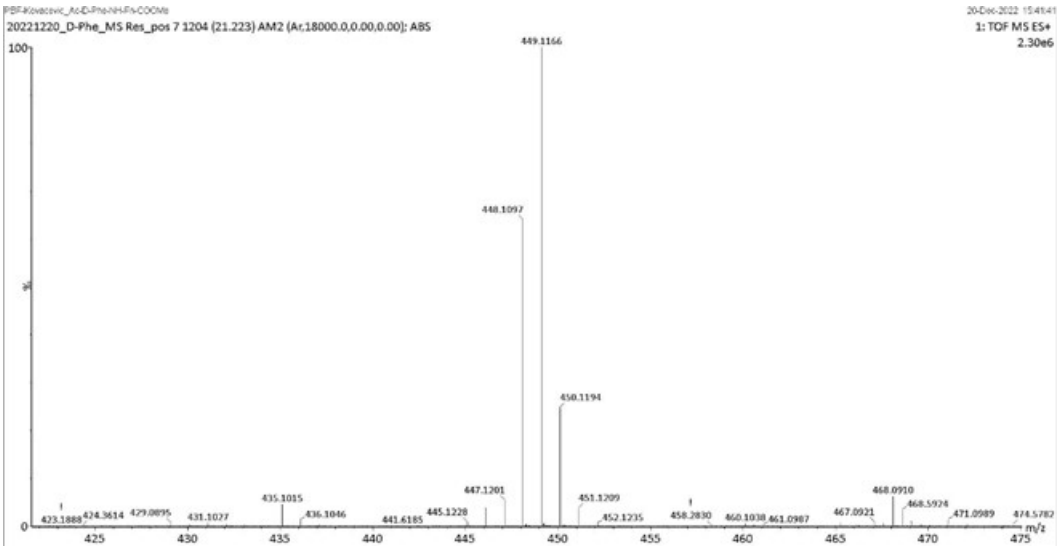
Figure S3. HRMS spectrum of compound D-**2a**



Integration Peak List

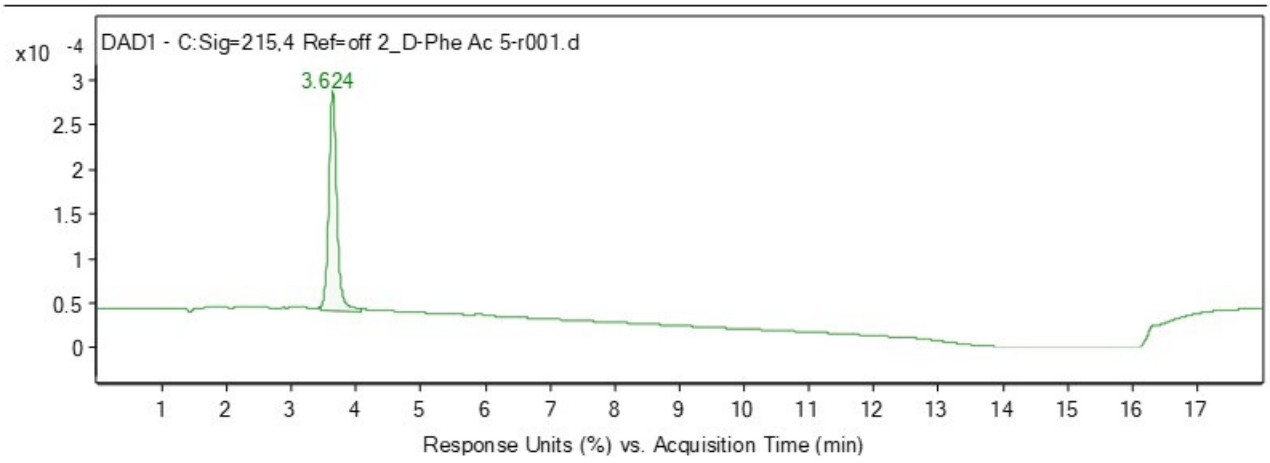
Peak	Start	RT	End	Height	Area	Area %
1	3.146	3.318	3.647	399.87	3184.69	100

Figure S4. HPLC spectrum of compound D-**2a**



	Peptide	Molecular formula	Ion	Calculated mass	Measured mass
D-**3a**	Ac-D-Phe-NH-Fn-COOMe	$C_{23}H_{24}FeN_2O_4$	$[M+H]^+$	449.1164	449.1166

Figure S5. HRMS spectrum of compound D-**3a**



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	3.43	3.624	4.07	663.75	5882.13	100

Figure S6. HPLC spectrum of compound D-**3a**

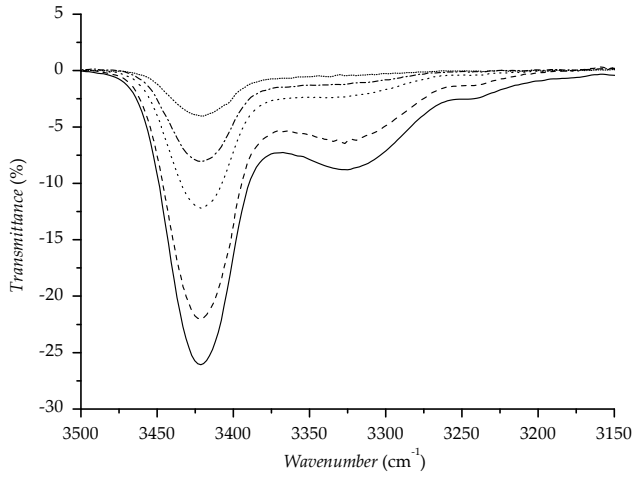


Figure S7. The NH stretching vibrations in concentration-dependent IR spectra of D-**1b** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

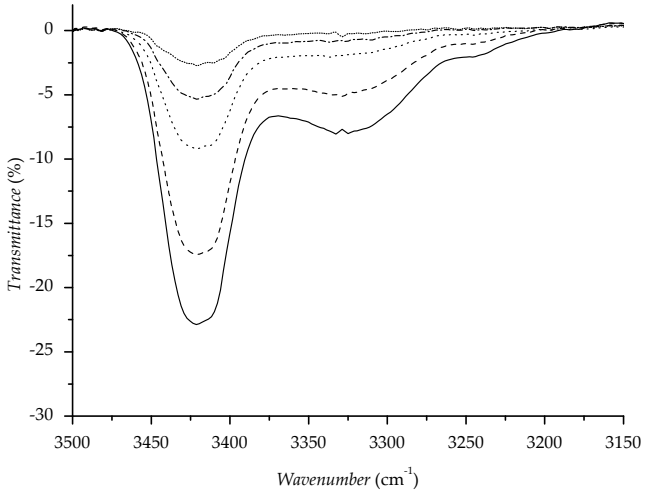


Figure S8. The NH stretching vibrations in concentration-dependent IR spectra of D-**2b** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

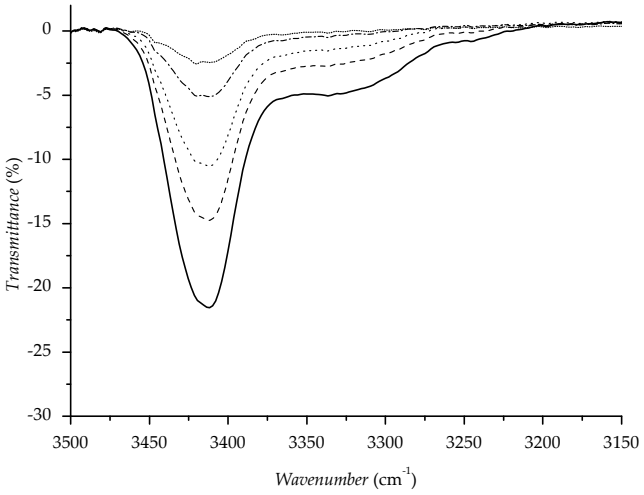


Figure S9. The NH stretching vibrations in concentration-dependent IR spectra of D-**3b** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

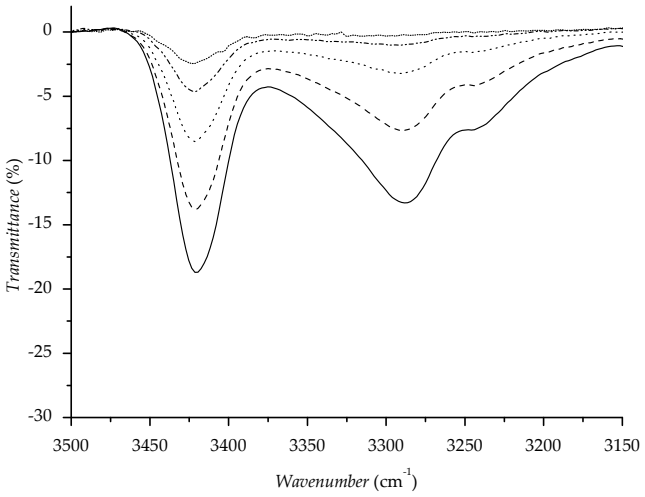


Figure S10. The NH stretching vibrations in concentration-dependent IR spectra of D-**1a** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

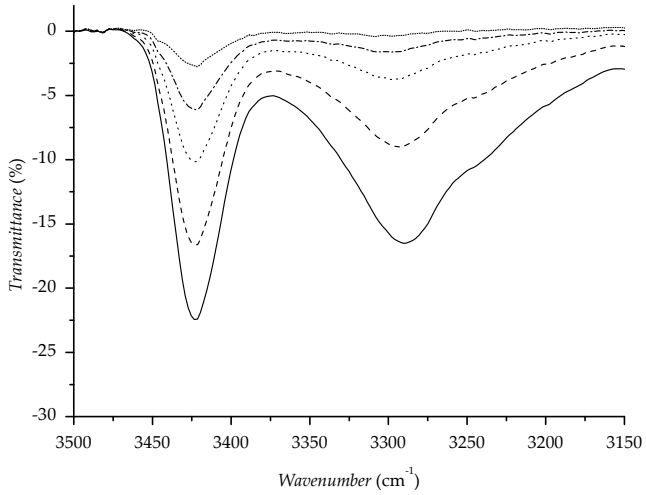


Figure S11. The NH stretching vibrations in concentration-dependent IR spectra of D-**2a** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

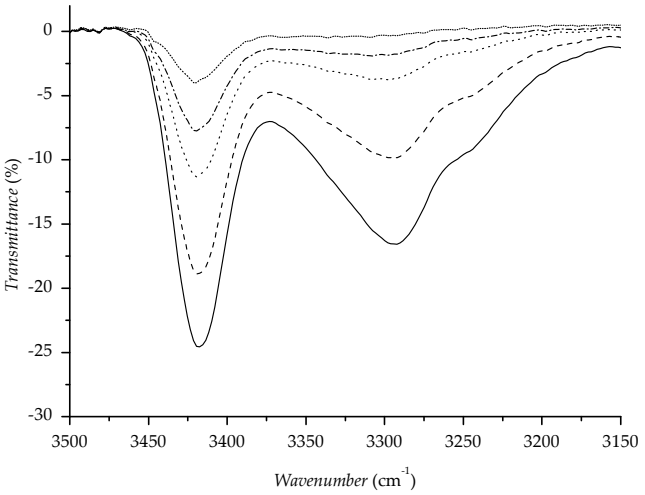


Figure S12. The NH stretching vibrations in concentration-dependent IR spectra of D-**3a** in DCM [(—) c = 5 × 10^{-2} M, (– – –) c = 2.5 × 10^{-2} M, (· · ·) c = 1.25 × 10^{-2} M, (-·-·-·) c = 0.6 × 10^{-2} M, (·····) c = 0.3 × 10^{-2} M.

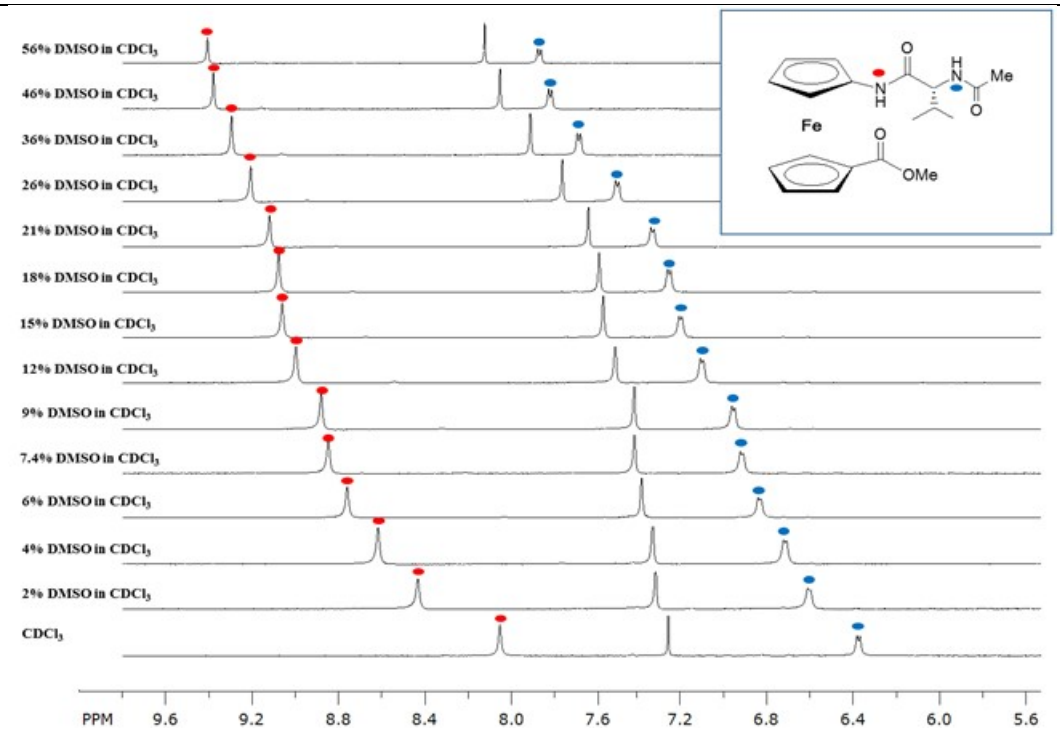
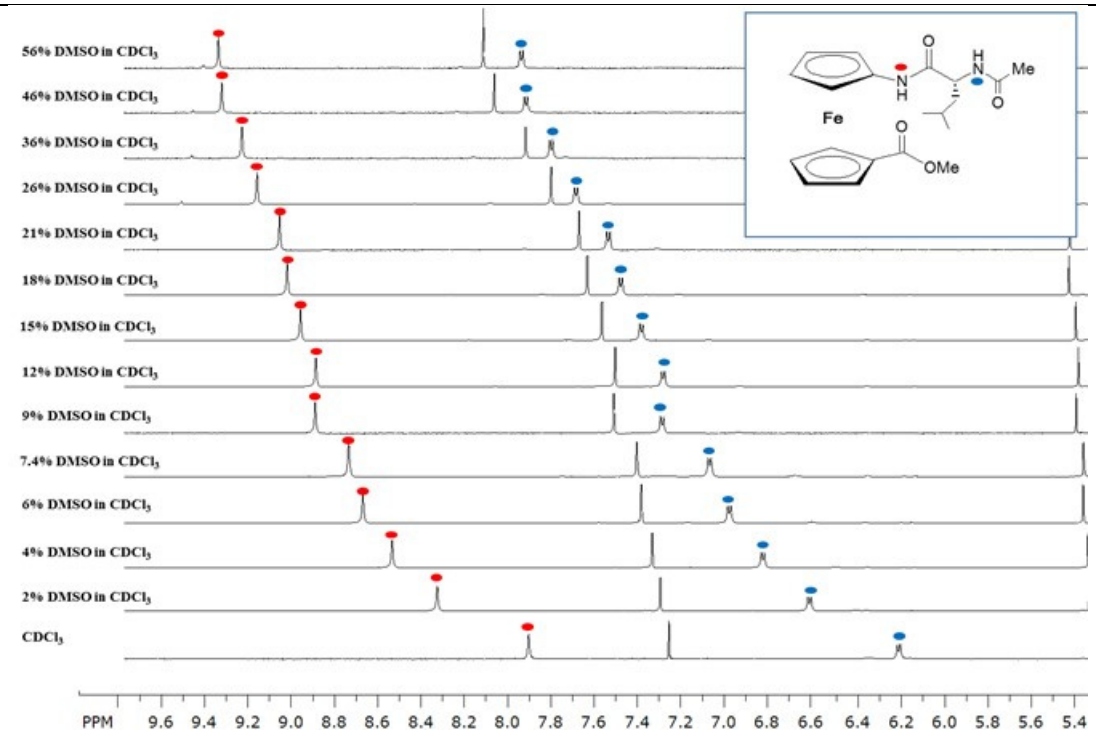
Table S2. Concentration dependence of NH chemical shifts of derivatives D-**1a**, D-**2a** and D-**3a**

c (mM)	δ (ppm)					
	NH_{Fca}	NH_{Val}	NH_{Fca}	NH_{Leu}	NH_{Fca}	NH_{Phe}
50	8.19	6.46	8.15	6.38	7.73	6.44
25	7.93	6.33	7.89	6.17	7.56	6.35
12.5	7.69	6.23	7.73	6.05	7.37	6.24
6.25	7.56	6.19	7.63	5.98	7.3	6.18
$\Delta\delta$	**0.63**	**0.27**	**0.52**	**0.4**	**0.43**	**0.26**

Table S3. Temperature dependence of chemical shifts of ferrocene peptides D-**1a**, D-**2a** and D-**3a** [NMR-spectra are recorded in $CDCl_3$ (c = 2.5·10^{-2} M)]

	δ (ppm)					
T (K)	NH_{Fca}	NH_{Val}	NH_{Fca}	NH_{Leu}	NH_{Fca}	NH_{Phe}
258	8.8	6.74	8.68	6.76	8.3	6.81
268	8.59	6.63	8.39	6.53	8.04	6.65
278	8.43	6.55	8.16	6.36	7.84	6.53
288	8.24	6.46	8.00	6.24	7.69	6.42
298	8.06	6.38	7.86	6.15	7.55	6.35
308	7.9	6.31	7.75	6.08	7.46	6.3
318	7.75	6.25	7.68	6.03	7.37	6.23
328	7.62	6.19	7.6	5.97	7.31	6.17
$\Delta\delta$	**1.18**	**0.55**	**1.08**	**0.79**	**0.99**	**0.64**

Table S4. DMSO titration of ferrocene peptides D-**1a**, D-**2a** and D-**3a**

D-**1a**	
D-**2a**	

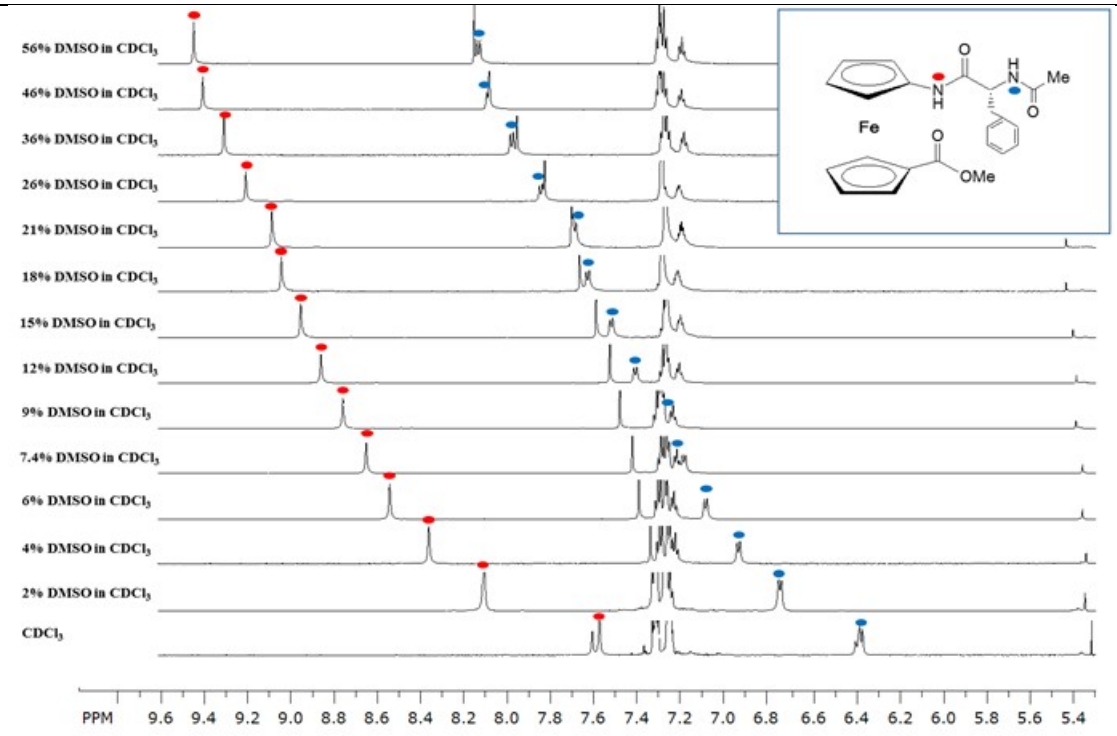
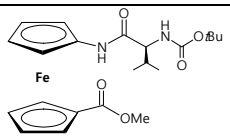
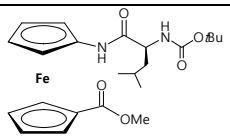
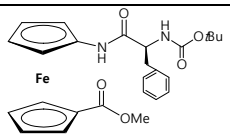
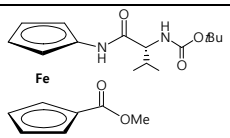
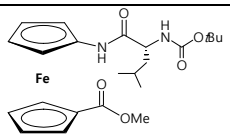
D-**3a**	

Table S5. UV/Vis-signals and Cottons efects in peptides L-/D-**1a**-**3a** and L-/D-**1b**-**3b**

	Peptide		λ_{max} / nm	[θ]/ deg cm² dmol⁻¹
L-**1b**	Boc-L-Val-NH-Fn-COOMe		480	1227,62624
L-**2b**	Boc-L-Leu-NH-Fn-COOMe		479,2	959,55305
L-**3b**	Boc-L-Phe-NH-Fn-COOMe		480	678,77391
D-**1b**	Boc-D-Val-NH-Fn-COOMe		482,2	-1205,79523
D-**2b**	Boc-D-Leu-NH-Fn-COOMe		478,3	-957,2157

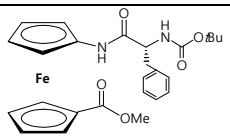
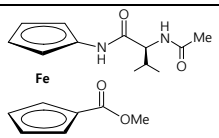
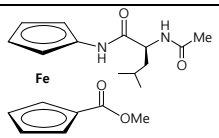
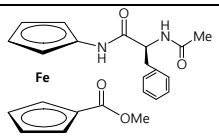
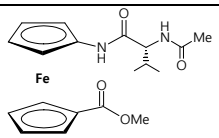
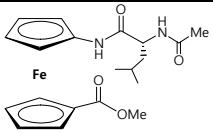
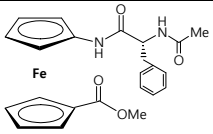
D-**3b**	Boc-D-Phe-NH-Fn-COOMe		479,9	-676,39962
L-**1a**	Ac-L-Val-NH-Fn-COOMe		480,2	675,26792
L-**2a**	Ac-L-Leu-NH-Fn-COOMe		474,2	455,83704
L-**3a**	Ac-L-Phe-NH-Fn-COOMe		478,3	672,45099
D-**1a**	Ac-D-Val-NH-Fn-COOMe		483,9	-687,37927
D-**2a**	Ac-D-Leu-NH-Fn-COOMe		475,1	-479,18576
D-**3a**	Ac-D-Phe-NH-Fn-COOMe		476,6	-643,06888

Table S6. Relative energies (reported energies refer to standard Gibbs free energies at 298 K in kJ mol^{-1}) of the most stable conformers of D-series of compounds **1a–3a** (Ac-protected) and **1b–3b** (Boc-protected), AA=Val, Leu, Phe. Optimizations were performed at the B3LYP-D3/6-311+G(d,p), LanL2DZ for Fe, level of theory, SMD model for solvent effects. Stereochemical descriptors and helicity determined from the value of pseudo-torsion angles, X–Y distances (in Å) of the selected X–H···Y hydrogen bonds connecting the *n*-membered rings.

type	Stereochemical descriptors	Δ*E* / kJ mol^{-1}	ω / ° pseudotorsion angle	$NH_{Fca}\cdots O{=}C_{Ac/Boc}$ 7-membered	$NH_{Fca}\cdots N_{AA}$ 5-membered	$NH_{AA}\cdots O{=}C_{COOMe}$ 9-membered
1a-1	*P-1,2'*	0.00	+39.9	2.99	-	2.95
1a-2	*P-1,2'*	1.73	+43.4	3.00	-	2.99
1a-3	*M-1,1'*	1.95	−32.1	2.83	-	2.96
1a-4	*P-1,1'*	2.61	+17.4	2.93	-	-
1a-5	*P-1,1'*	5.19	+17.0	2.92	-	-
1b-1	*M-1,1'*	0.00	−30.4	-	2.70	2.96
1b-2	*P-1,2'*	6.11	+41.7	3.07	-	2.92
2a-1	*P-1,2'*	0.00	+38.7	2.96	-	2.96
2a-2	*P-1,2'*	0.73	+39.1	2.97	-	2.94
2a-3	*P-1,1'*	3.41	+15.9	2.92	-	-
2b-1	*M-1,1'*	0.00	−29.3	-	2.69	2.93
2b-2	*P-1,1'*	3.87	+31.6	-	2.71	2.97
2b-3	*M-1,1'*	4.22	−28.6	-	2.69	2.94
3a-1	*P-1,2'*	0.00	+40.2	2.98	-	2.93
3a-2	*M-1,5'*	1.71	−50.7	-	2.70	2.96
3a-3	*P-1,1'*	4.91	+17.9	-	-	3.03
3b-1	*M-1,1'*	0.00	−32.0	-	2.70	2.93
3b-2	*M-1,1'*	1.29	−28.1	-	2.69	2.93
3b-3	*P-1,1'*	2.30	+31.8	-	2.70	2.97

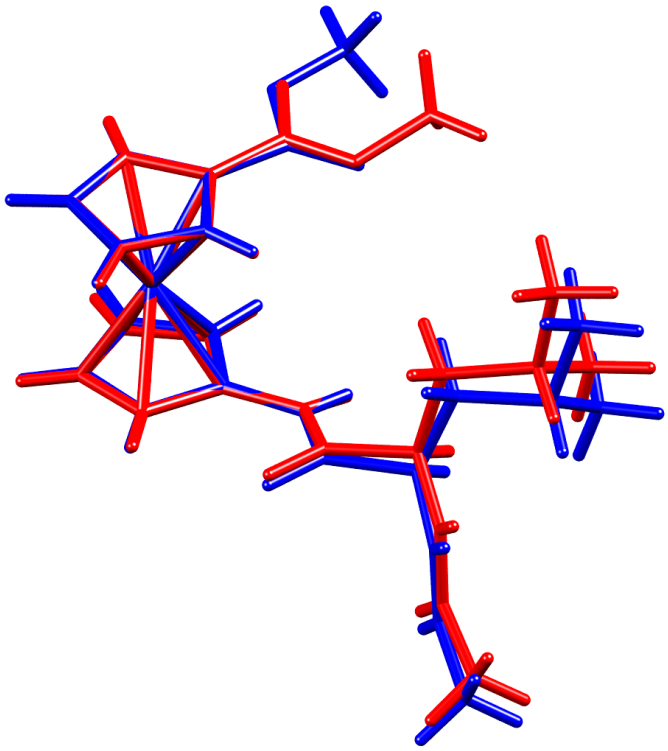


Figure S13. Overlay of two symmetry-independent molecules in crystal structure of D-**2a**: A is red and B is blue.

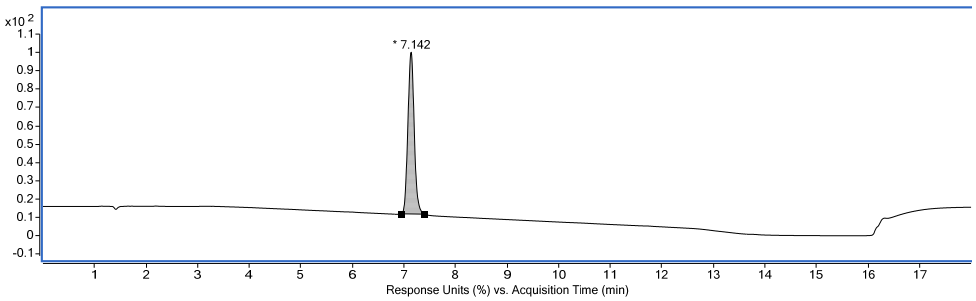
Table S7. Geometric parameters of hydrogen bonding (Å, °).

	D–H / Å	H···*A* / Å	*D*···*A* / Å	*D*–H···*A* / °	Symm. op. on *A*
N1–H1···O5	0.86	2.02	2.876(4)	171	1–*x*, –1/2+*y*, 3/2–*z*
N2–H2A···O6	0.86	2.11	2.964(5)	171	*x*, *y*, *z*
N3–H3A···O1	0.86	2.03	2.881(5)	169	*x*, *y*, *z*
N4–H4A···O2	0.86	2.06	2.912(5)	170	1–*x*, 1/2+*y*, 3/2–*z*
C5–H5···O1	0.93	2.49	2.921(5)	109	*x*, *y*, *z*
C14–H14C···O8	0.96	2.70	3.351(7)	125	1/2–*x*, 1–*y*, 1/2+*z*
C25–H25···O5	0.93	2.53	2.964(7)	108	*x*, *y*, *z*
C32–H32···O1	0.98	2.70	3.475(7)	136	*x*, *y*, *z*

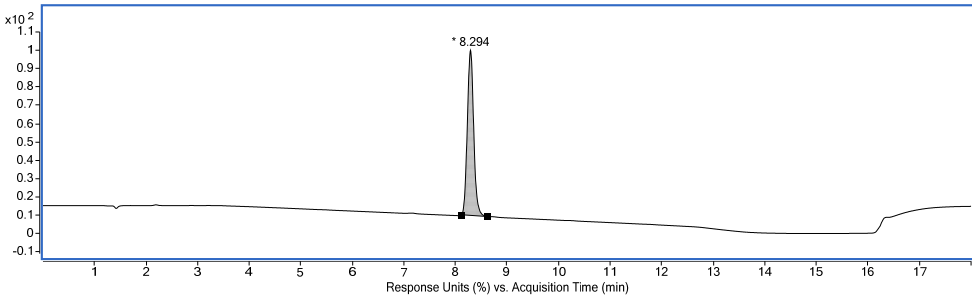
Table S8. Crystallographic, data collection and refinement data.

D-**2a**	
Empirical formula	$C_{20}H_{26}FeN_2O_4$
Formula wt. / g mol^{-1}	414.28
Colour	yellow
Crystal dimensions / mm	0.10 x 0.03 x 0.02
Space group	$P\ 2_12_12_1$
a / Å	12.1234(2)
b / Å	16.8527(3)
c / Å	21.1094(4)
α / °	90
β / °	90
γ / °	90
Z	8
V / Å^3	4312.90(13)
D_{calc} / g cm^{-3}	1.276
λ / Å	1.54184
μ / mm^{-1}	5.820
Θ range / °	3.36 - 79.86
T / K	296(2)
Diffractometer type	Synergy S
Range of h, k, l	$-15 < h < 15$; $-21 < k < 19$; $-26 < l < 25$
Reflections collected	41552
Independent reflections	9214
Observed reflections ($I \geq 2\sigma$)	6983
Absorption correction	multi-scan
T_{min}, T_{max}	0.2708; 1.0000
R_{int}	0.0679
R (F)	0.0425
R_w (F^2)	0.1278
Goodness of fit	1.032
H atom treatment	riding
No. of parameters	488
No. of restraints	0
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (eÅ^{-3})	0.467; -0.294

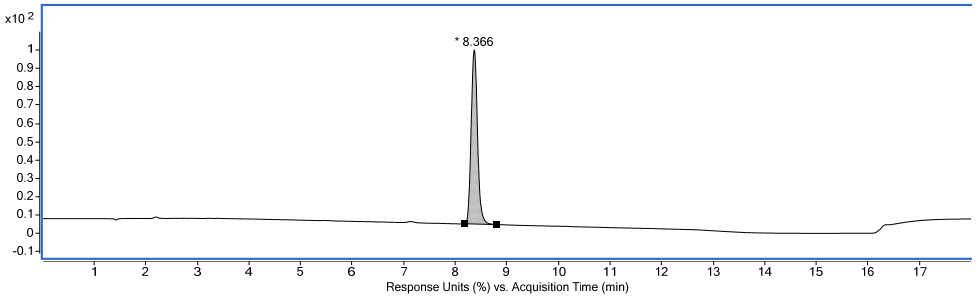
L-**1b**



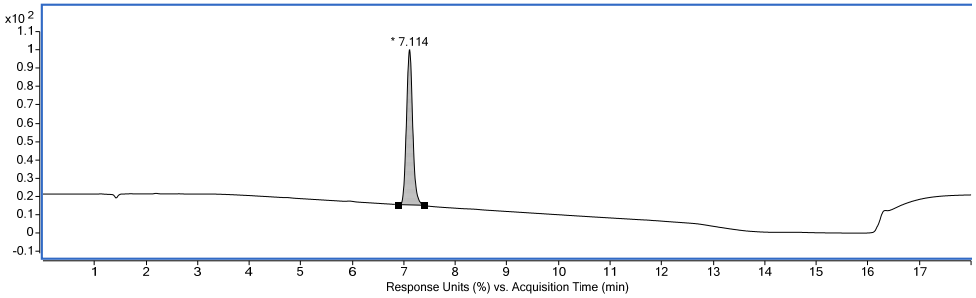
L-**2b**



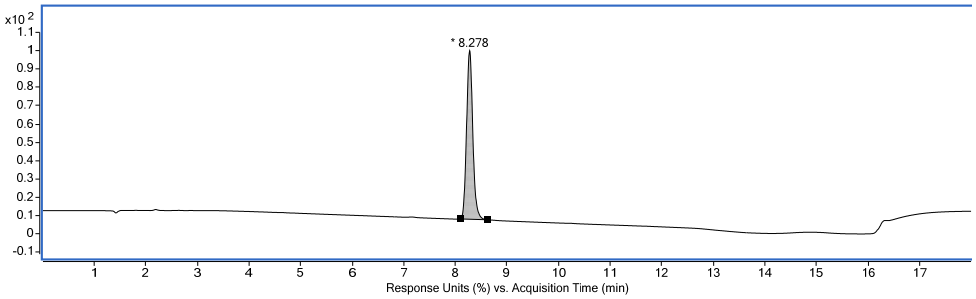
L-**3b**



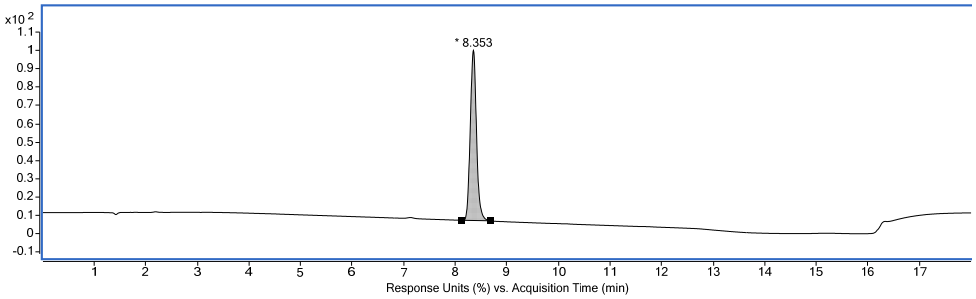
D-**1b**



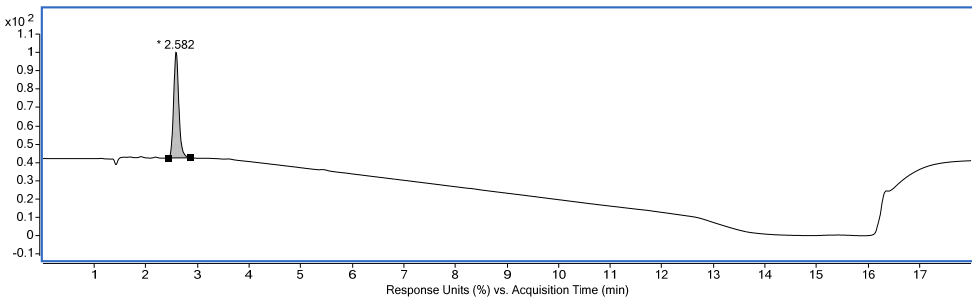
D-**2b**



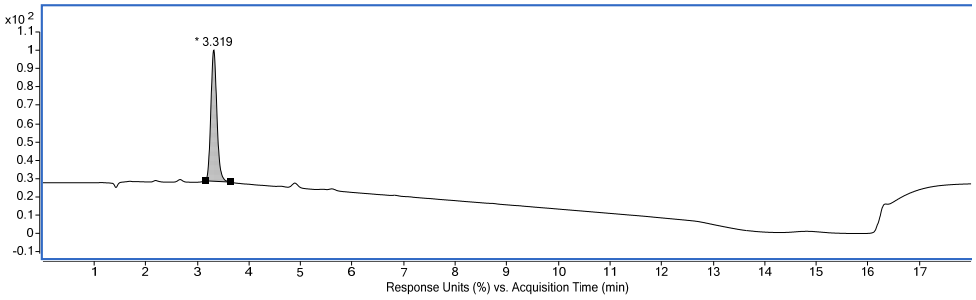
D-**3b**



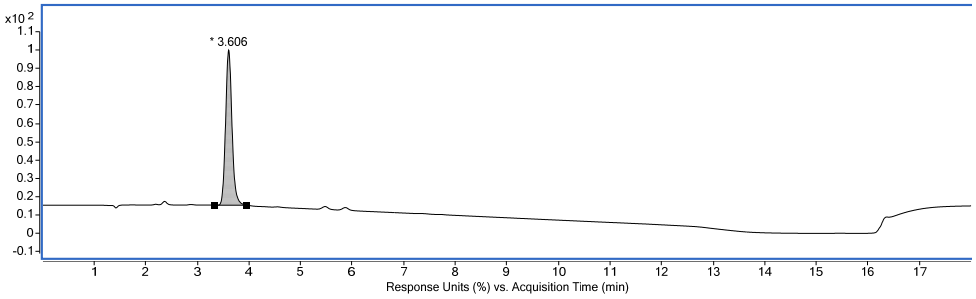
L-**1a**



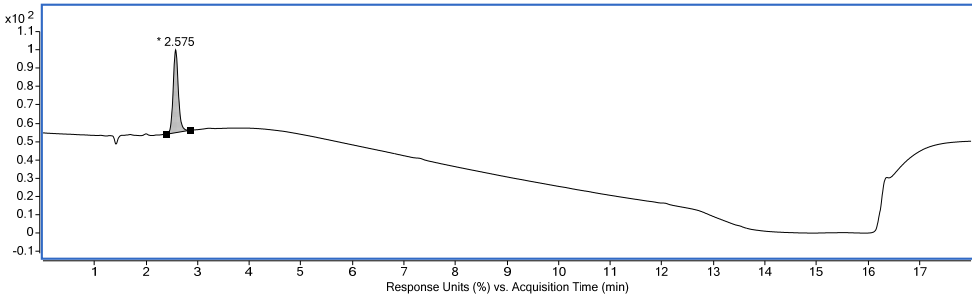
L-**2a**



L-**3a**



D-**1a**



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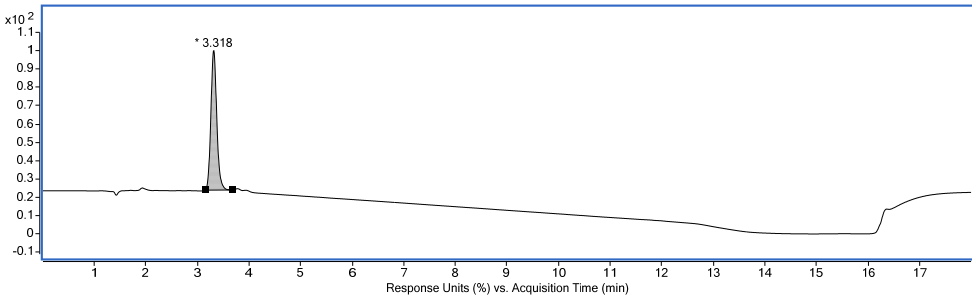

D-**3a**

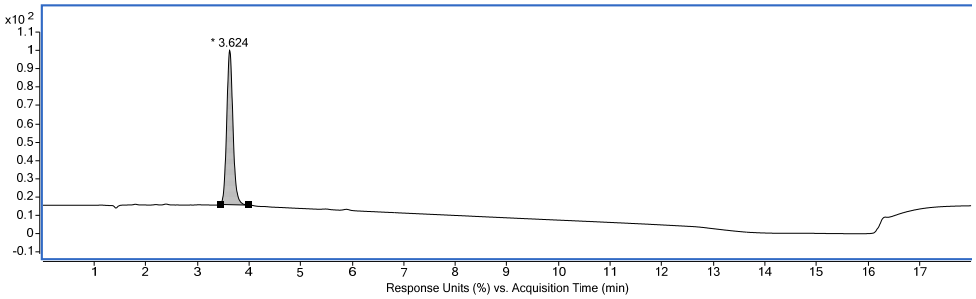

Figure S14. HPLC chromatograms of L-/D-**1a-3a** and L-/D-**1b-3b**