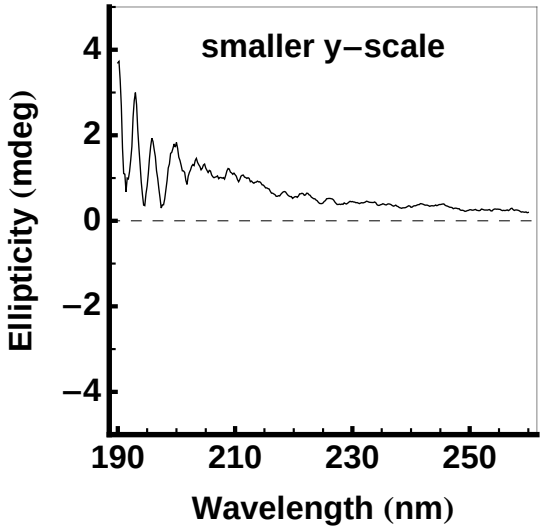
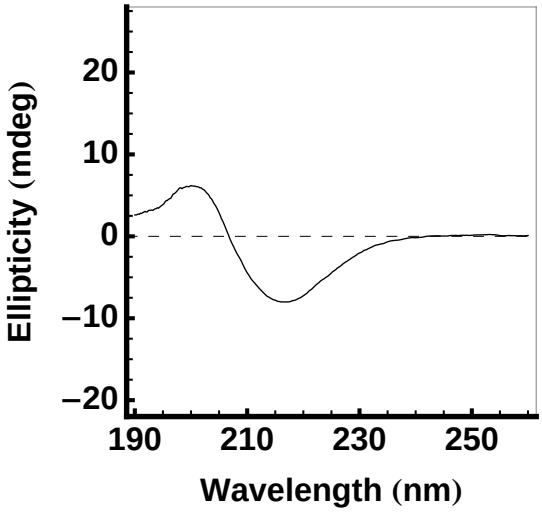
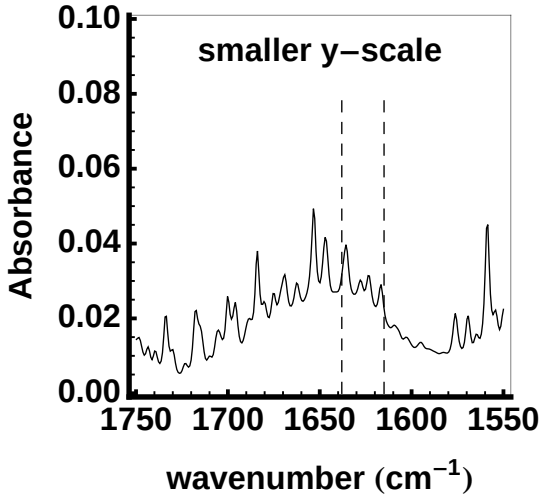
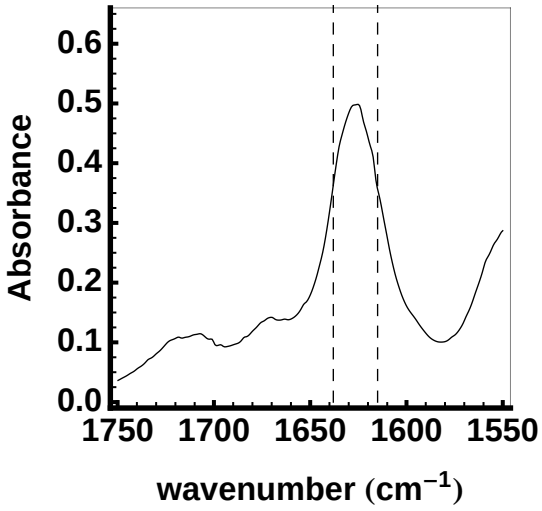
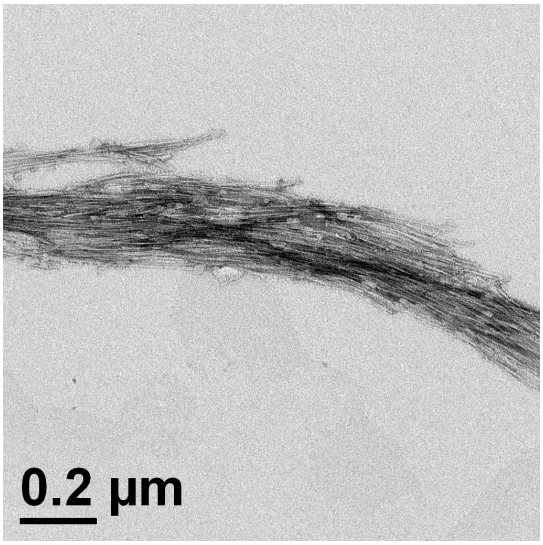
Table A and associated data: Biophysical characterization of library peptides.

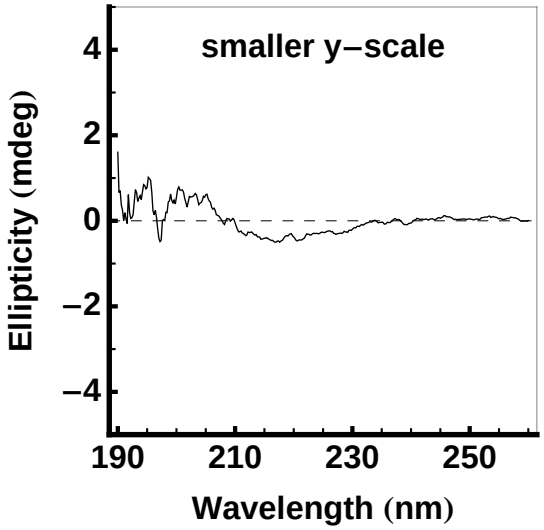
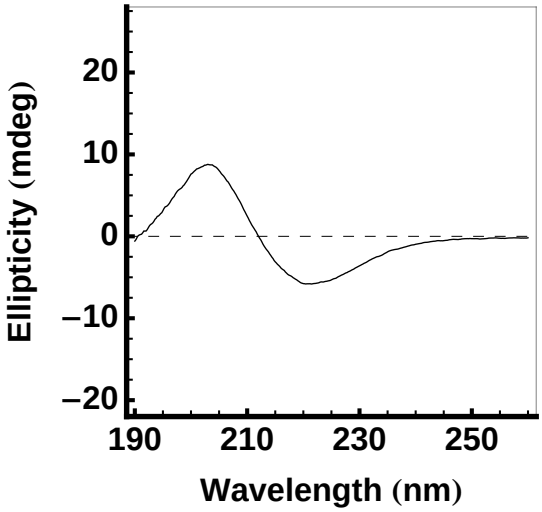
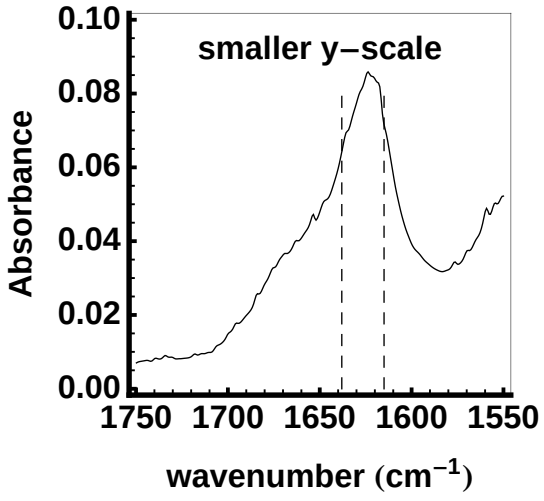
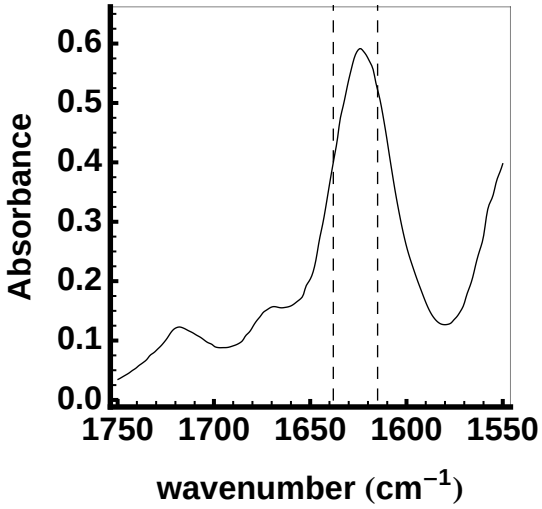
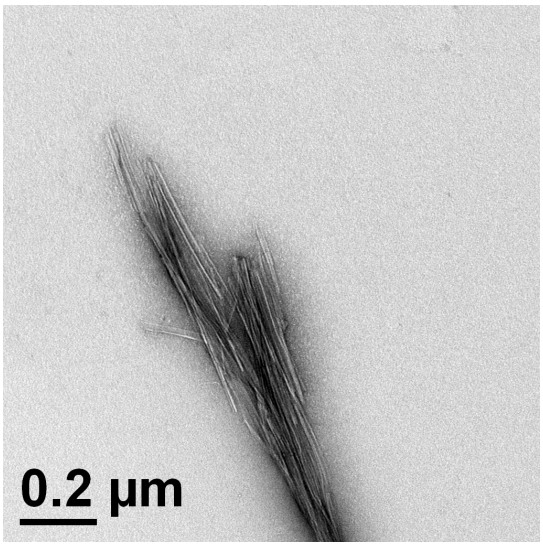
	Sequence	pH 7.3 ThT[1]	pH 7.3 CD[2]	pH 7.3 FTIR[3]	pH 7.3 EM[4]	pH 4.0 ThT	pH 4.0 CD	pH 4.0 FTIR	pH 4.0 EM	X-ray[5]
1	Ac–YV**D**V**D**V**D**V–$CONH_2$	1	–	–		89	β^{++}	1625	+	
2	Ac–YV**S**V**D**V**D**V–$CONH_2$	24	–	1624		322	β^{++}	1624	+	cross-β
3	Ac–YV**D**V**S**V**D**V–$CONH_2$	2	–	–		120	β^{++}	1623	+	
4	Ac–YV**D**V**D**V**S**V–$CONH_2$	3	–	–		160	β^{++}	1618	+	
5	Ac–YV**S**V**S**V**D**V–$CONH_2$	322	β^{+}	1619		322	β^{++}	1618	+	
6	Ac–YV**S**V**D**V**S**V–$CONH_2$	309	β^{+}	1618		322	β^{++}	1622	+	
7	Ac–YV**D**V**S**V**S**V–$CONH_2$	208	–	–		128	β^{++}	1619	+	
8	Ac–YV**H**V**H**V**H**V–$CONH_2$	126	β^{+}	1627	+	1	–	–	–	cross-β
9	Ac–YV**S**V**H**V**H**V–$CONH_2$	82	β^{++}	1624	+	1	–	–		
10	Ac–YV**H**V**S**V**H**V–$CONH_2$	29	β^{+}	1623	+	1	–	–		
11	Ac–YV**H**V**H**V**S**V–$CONH_2$	18	β^{++}	1624	+	1	–	–		
12	Ac–YV**S**V**S**V**H**V–$CONH_2$	183	β^{++}	1623		5	?	1621	+	cross-β
13	Ac–YV**S**V**H**V**S**V–$CONH_2$	0	β^{+}	1622	+	11	β^{++}	1621		
14	Ac–YV**H**V**S**V**S**V–$CONH_2$	322	β^{+}	1624	+	2	β^{+}	1623		cross-β
15	Ac–YV**D**V**H**V**S**V–$CONH_2$	114	β^{+}	1623	+	13	β^{+}	1622	+	
16	Ac–YV**D**V**S**V**H**V–$CONH_2$	177	β^{++}	1618	+	7	β^{++}	1619	+	
17	Ac–YV**H**V**D**V**S**V–$CONH_2$	163	β^{+}	1622	+	5	β^{++}	1622		
18	Ac–YV**S**V**D**V**H**V–$CONH_2$	31	β^{++}	1622	+	1	β^{++}	1622		
19	Ac–YV**S**V**H**V**D**V–$CONH_2$	142	β^{++}	1624	+	9	β^{++}	1624		
20	Ac–YV**H**V**S**V**D**V–$CONH_2$	98	β^{+}	–	+	23	β^{++}	1622	+	
21	Ac–YV**H**V**H**V**D**V–$CONH_2$	133	β^{+}	1626	+	1	?	–	+	
22	Ac–YV**H**V**D**V**H**V–$CONH_2$	44	β^{+}	1624	+	1	?	–	+	
23	Ac–YV**D**V**H**V**H**V–$CONH_2$	21	β^{+}	1624	+	1	–	1626	+	
24	Ac–YV**D**V**D**V**H**V–$CONH_2$	2	–	–	+	1	β^{++}	1626	+	
25	Ac–YV**D**V**H**V**D**V–$CONH_2$	2	–	–		9	β^{++}	1623	+	
26	Ac–YV**H**V**D**V**D**V–$CONH_2$	5	–	–		5	β^{++}	1624	+	
27	Ac–YV**A**V**H**V**H**V–$CONH_2$	129	β^{+}	1625	+	1	–	–		cross-β
28	Ac–YV**H**V**A**V**H**V–$CONH_2$	114	β^{+}	1624	+	1	–	–		
29	Ac–YV**D**V**H**V**A**V–$CONH_2$	183	β^{+}	1622	+	25	?	1618		
30	Ac–YV**A**V**D**V**H**V–$CONH_2$	42	–	1623	+	10	β^{++}	1624		
31	Ac–YV**H**V**D**V**A**V–$CONH_2$	259	β^{+}	1622		32	β^{++}	1621	+	
32	Ac–YV**H**V**A**V**D**V–$CONH_2$	322	β^{+}	1621		111	β^{++}	1621	+	
33	Ac–YV**A**V**H**V**D**V–$CONH_2$	67	β^{+}	1624		2	β^{++}	1623	+	

1. Fold increase in ThT fluorescence over background.
2. CD spectra classified as follows: (β^{+}) has minimum near 217 nm, (β^{++}) has minimum near 217 nm and a maximum near 197 nm, (–) signal too weak to interpret, (?) spectra not typical of any secondary structure.
3. The peak position in amide I region is given in cm^{-1}. (–) indicates that no peak was observed within the typical range of stretching frequencies for β-structure (between dashed lines at 1638 cm^{-1} and 1615 cm^{-1})[1].
4. (+) fibrils present or (–) absent in EM micrographs.
5. Diffraction images recorded for the aligned fibrils of 5 different peptides (see materials and methods).

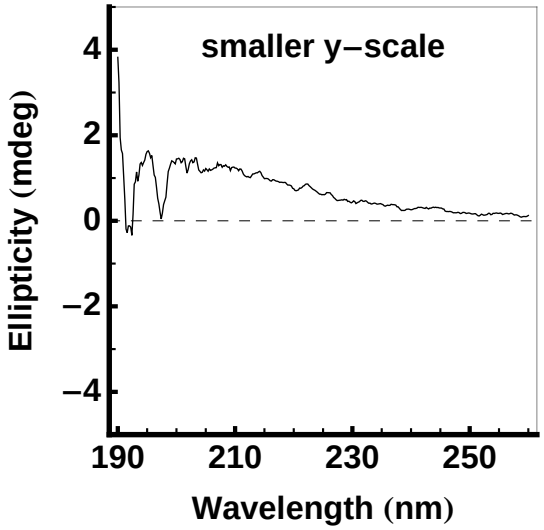
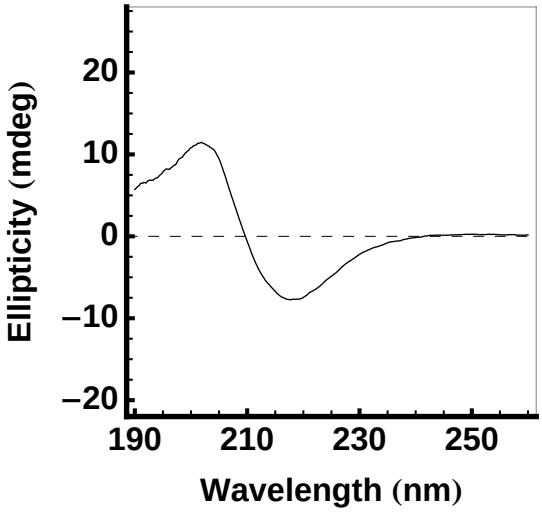
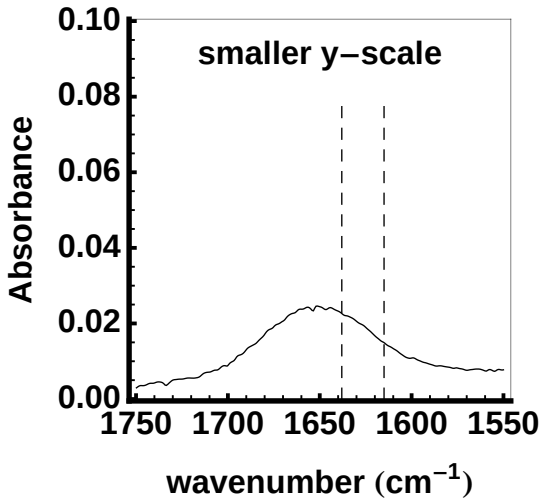
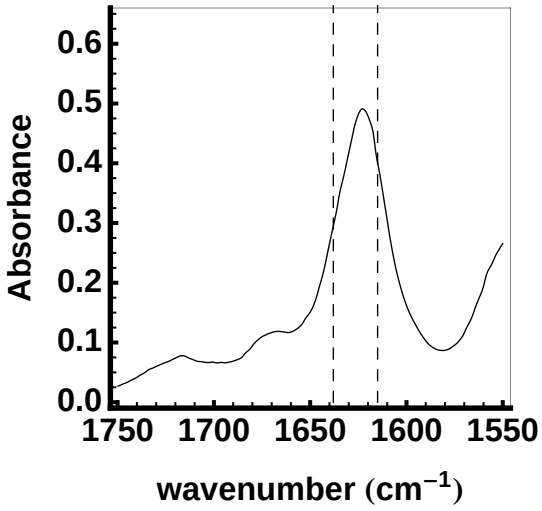
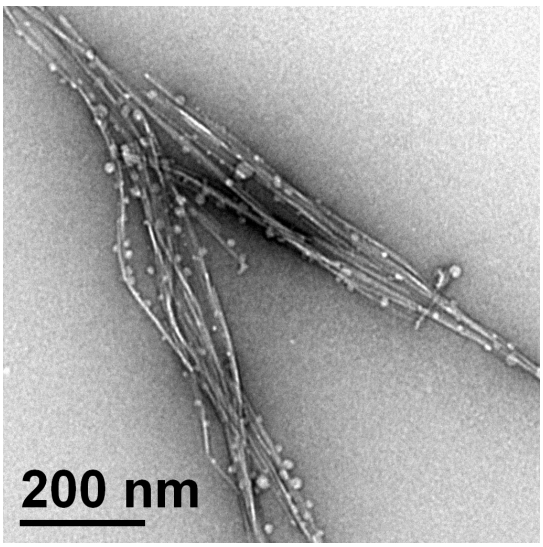
Ac–YV**D**V**D**V**D**V–$CONH_2$ (1)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

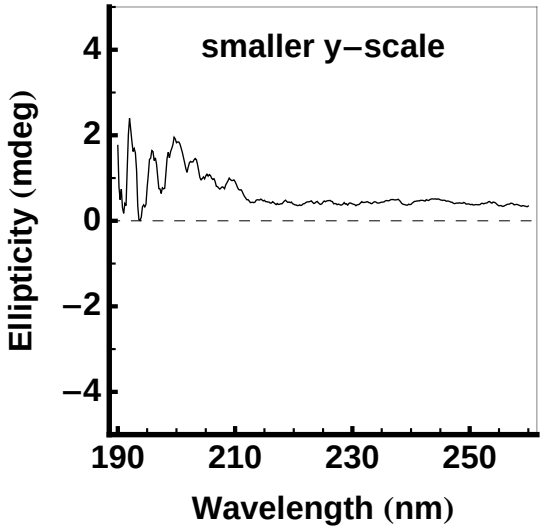
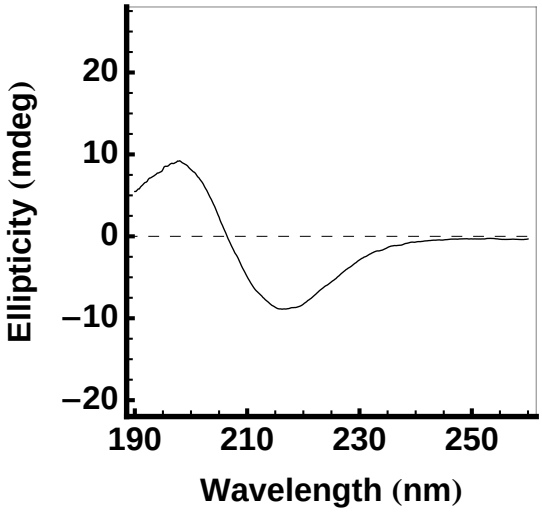
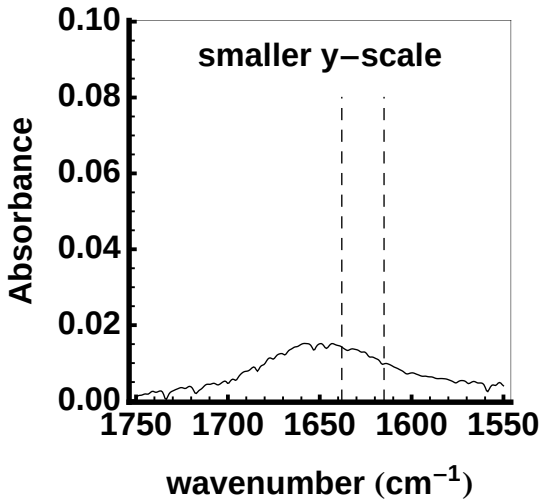
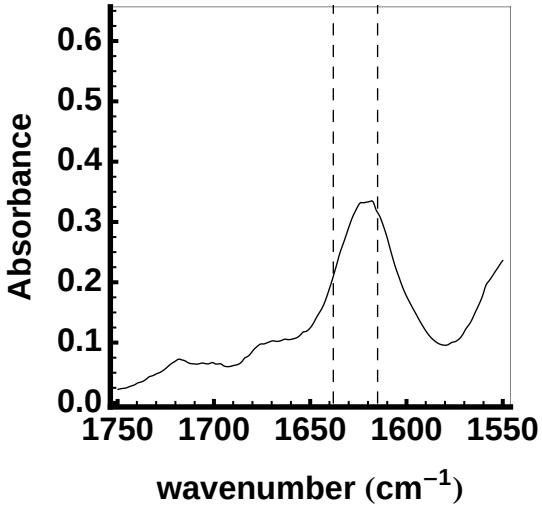
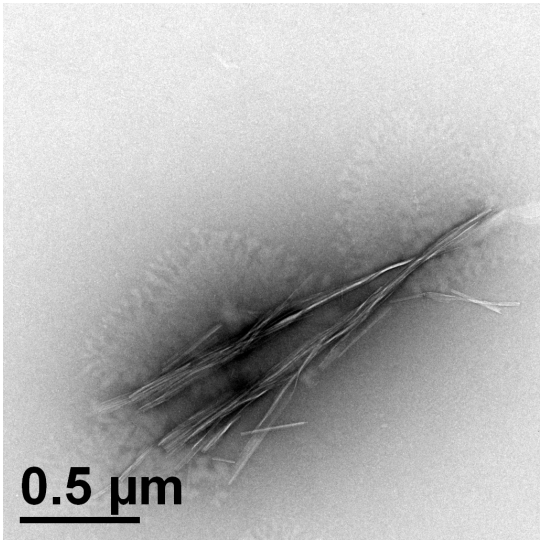
Ac–YV**S**V**D**V**D**V–$CONH_2$ (**2**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

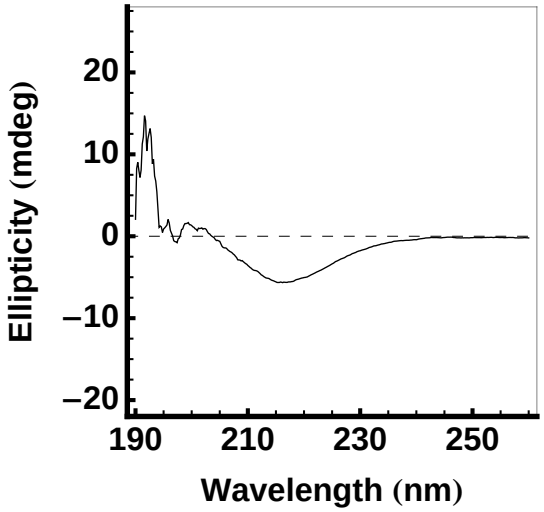
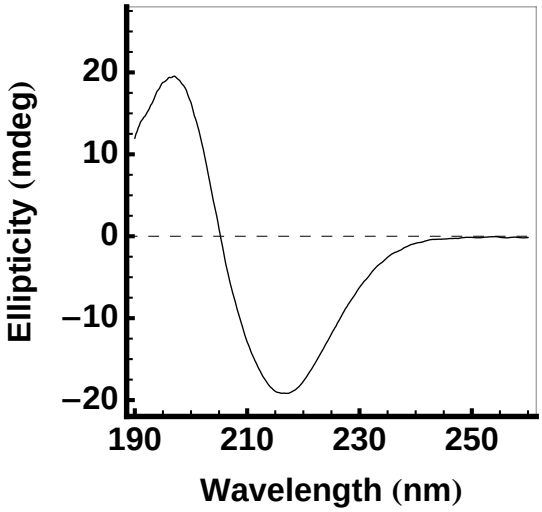
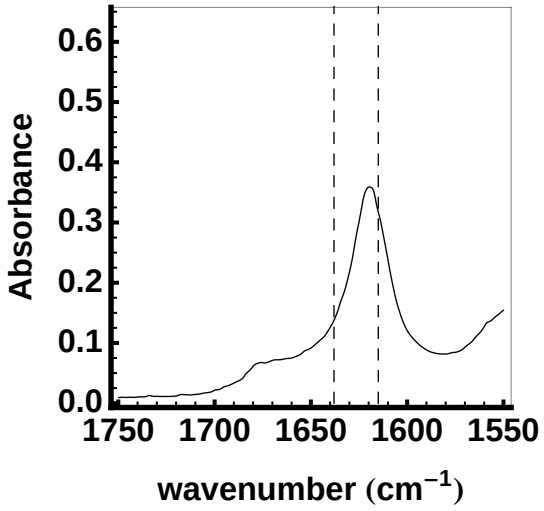
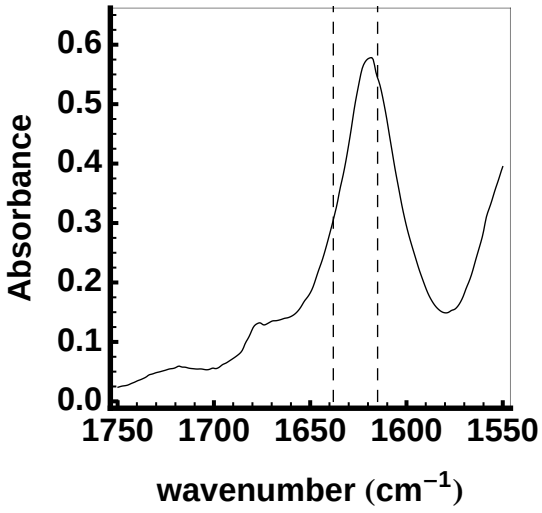
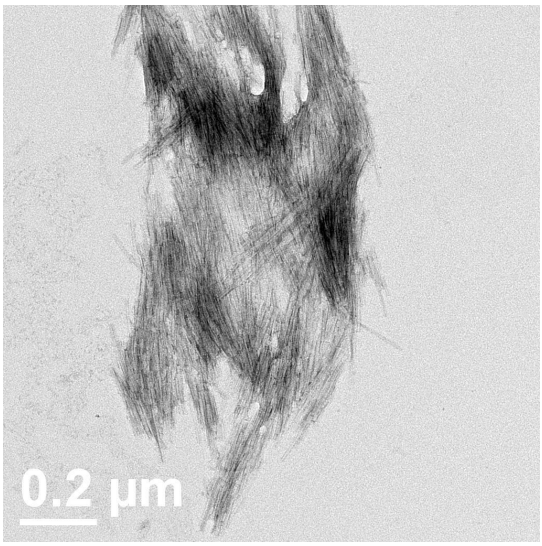
Ac–YV**D**V**S**V**D**V–$CONH_2$ (**3**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

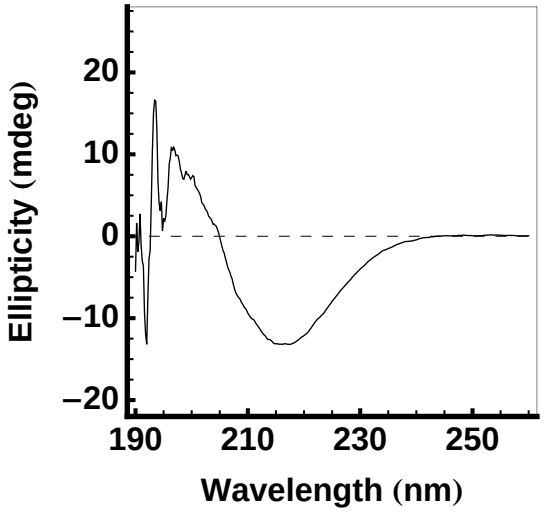
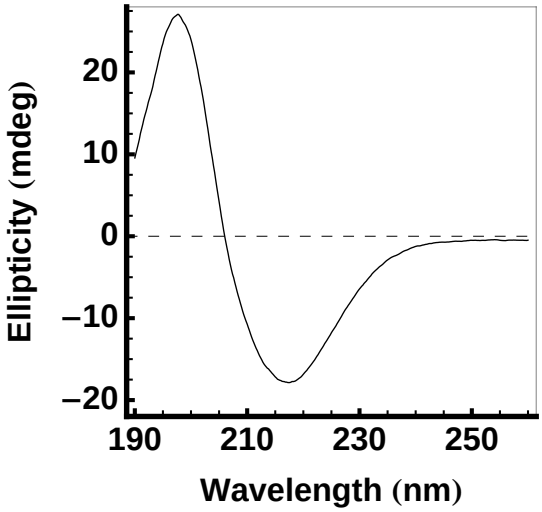
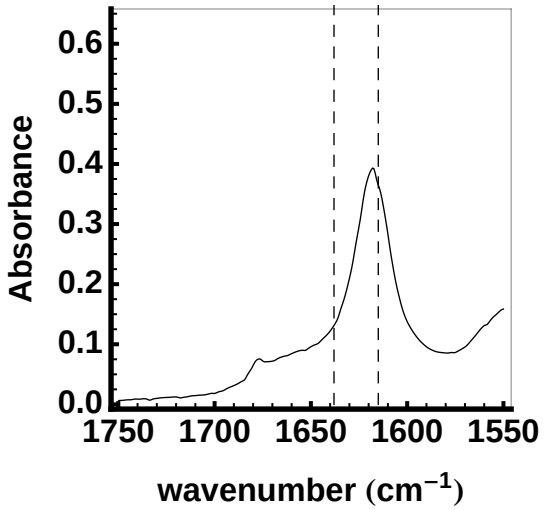
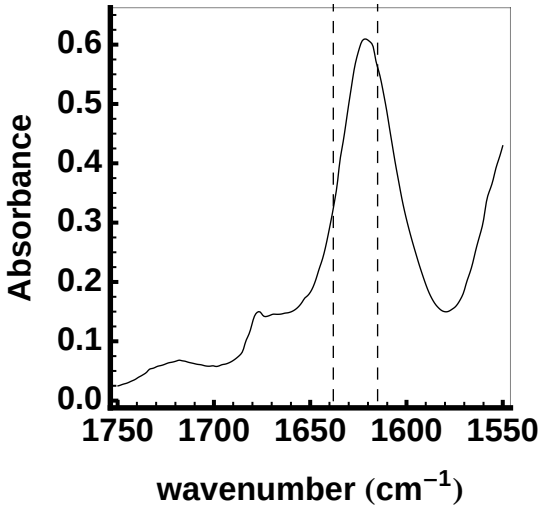
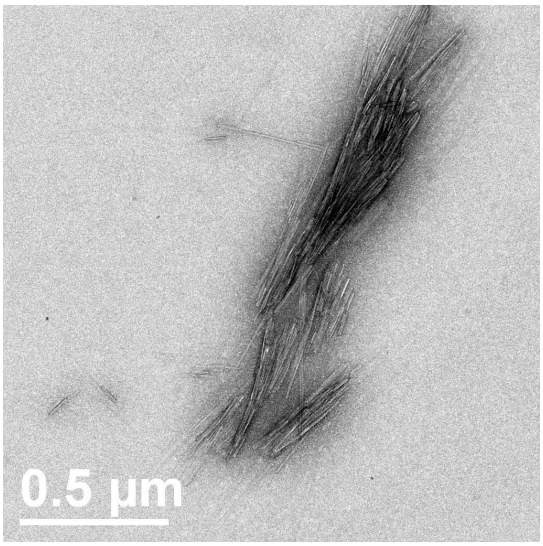
Ac–YV**D**V**D**V**S**V–$CONH_2$ (4)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

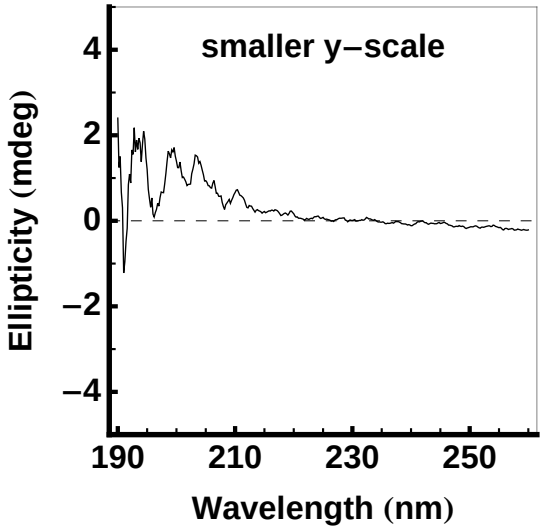
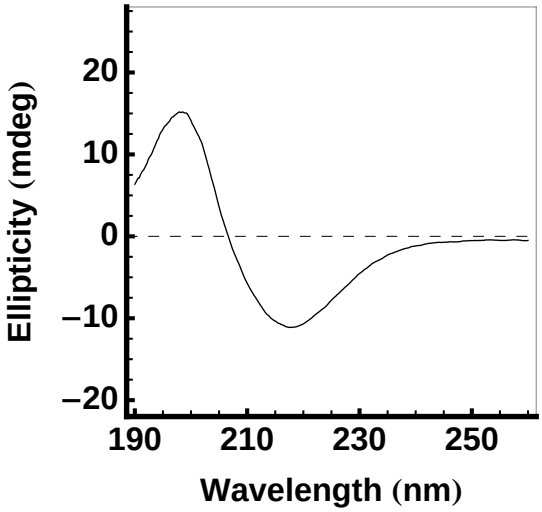
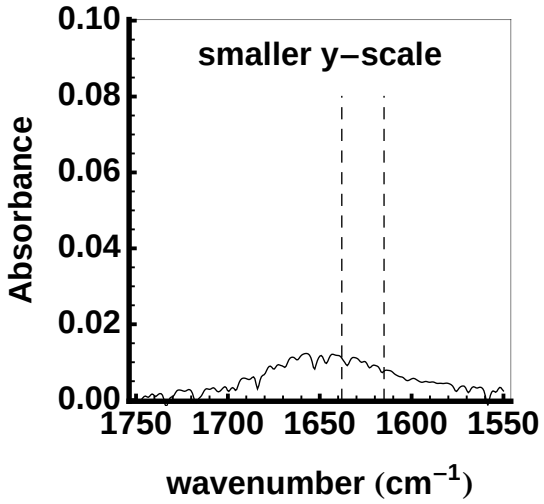
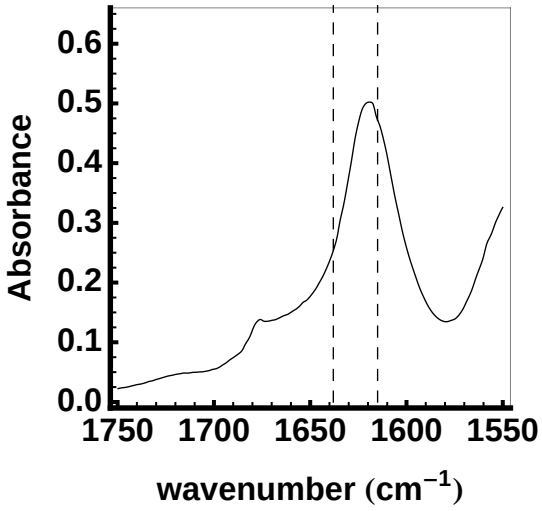
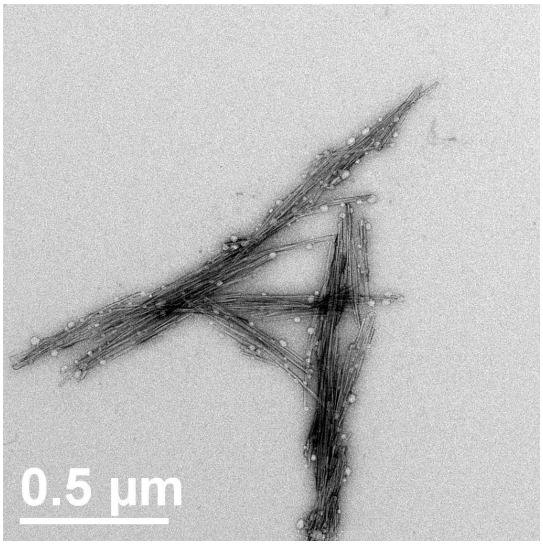
Ac–YV**S**V**S**V**D**V–$CONH_2$ (**5**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

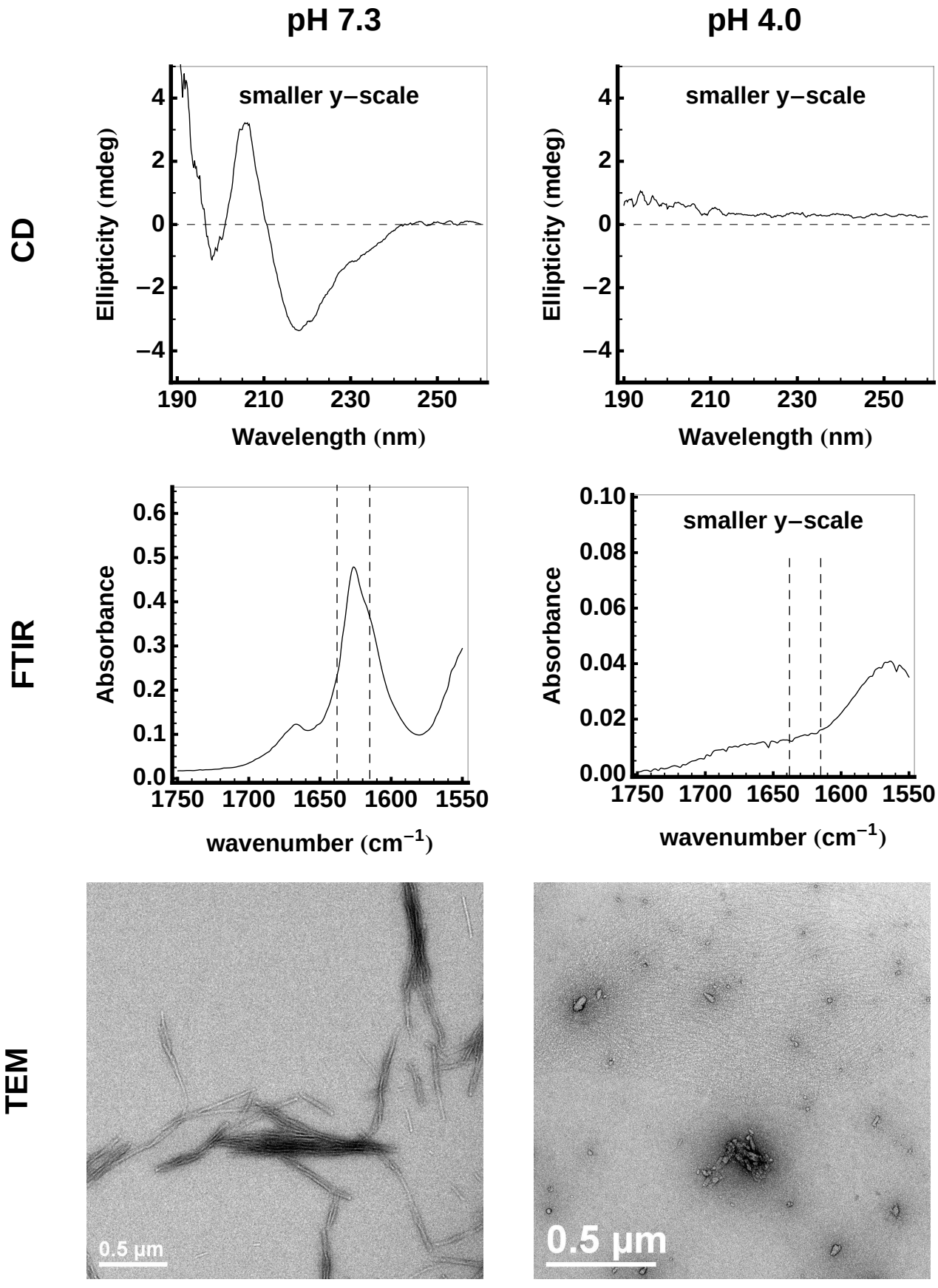
Ac–YV**S**V**D**V**S**V–$CONH_2$ (**6**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

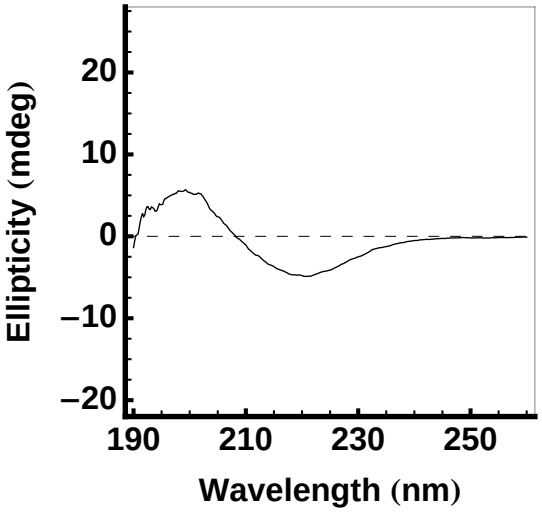
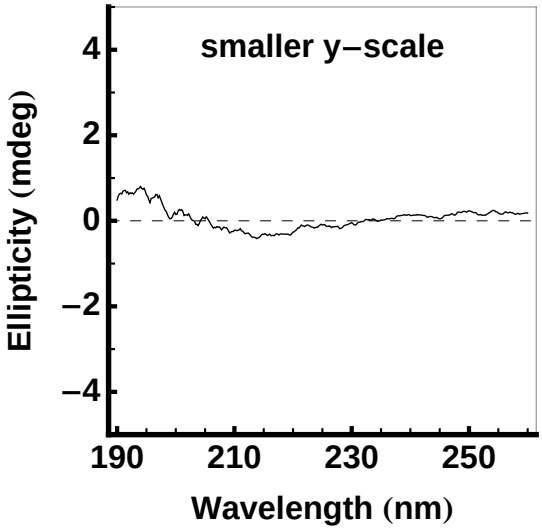
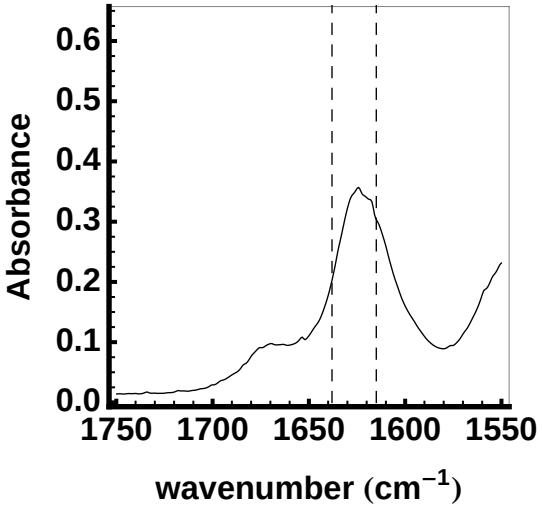
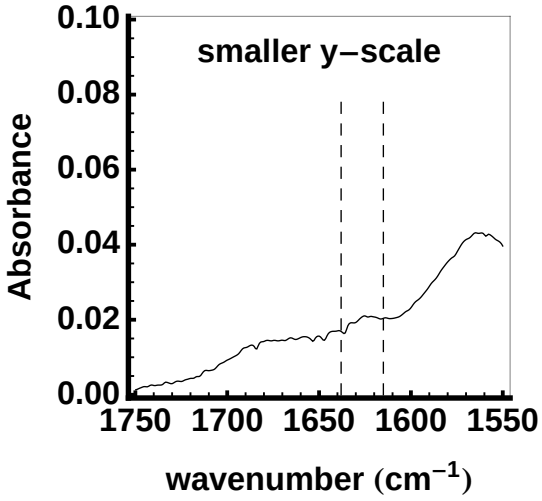
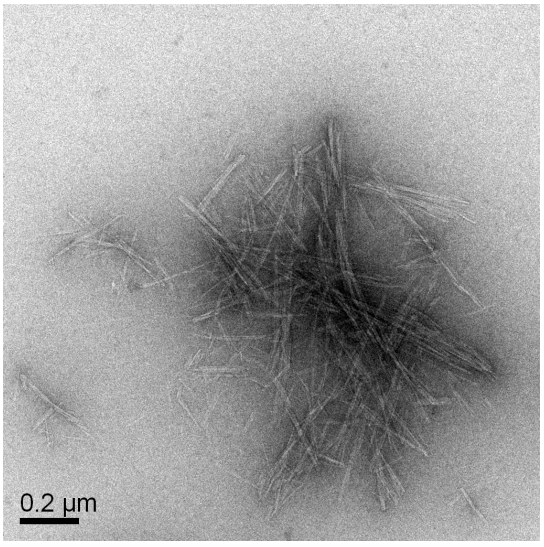
Ac–YV**D**V**S**V**S**V–$CONH_2$ (**7**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

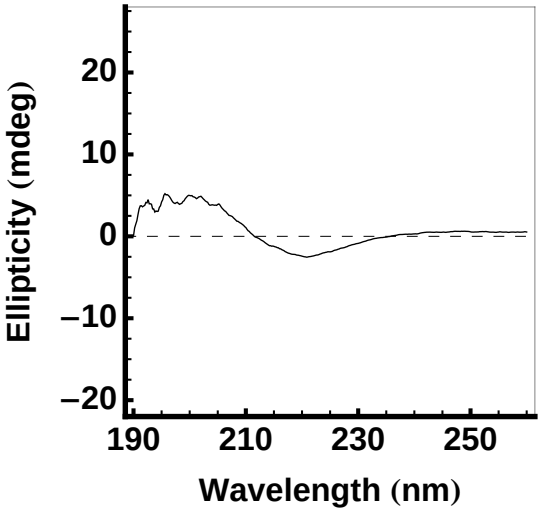
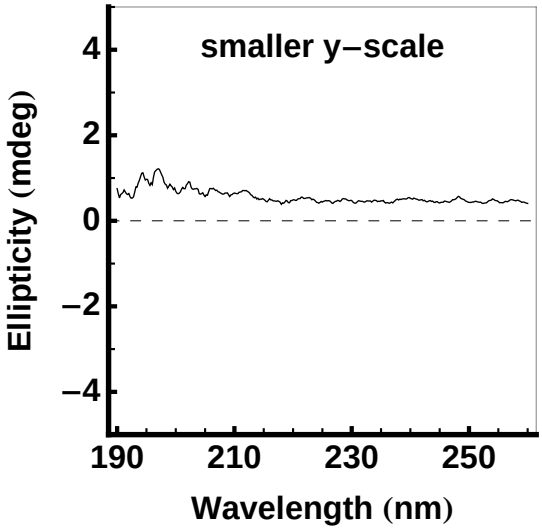
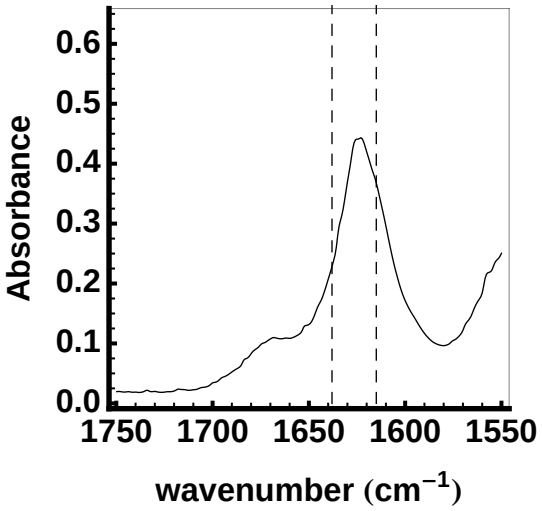
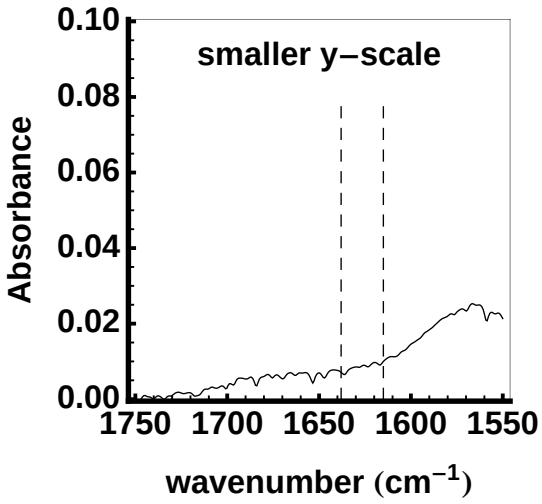
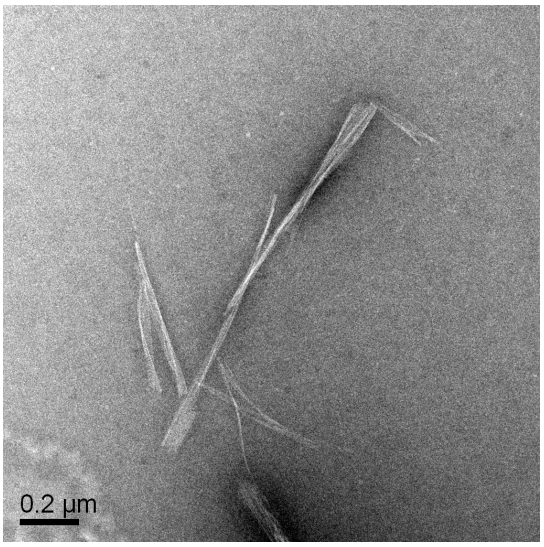
Ac–YV**H**V**H**V**H**V–$CONH_2$ (**8**)



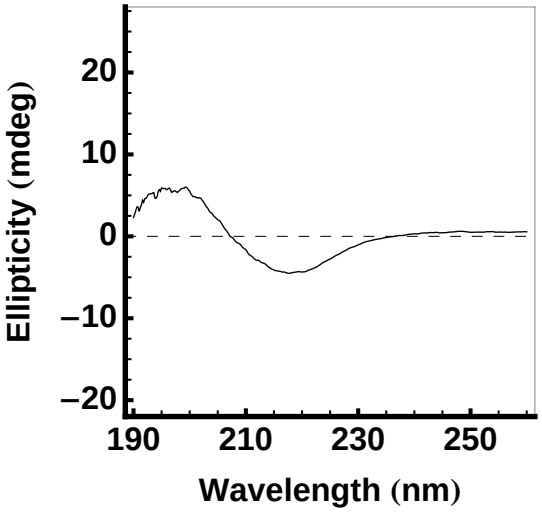
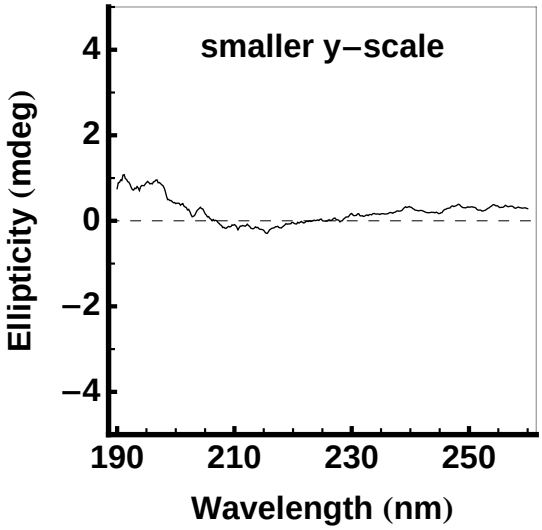
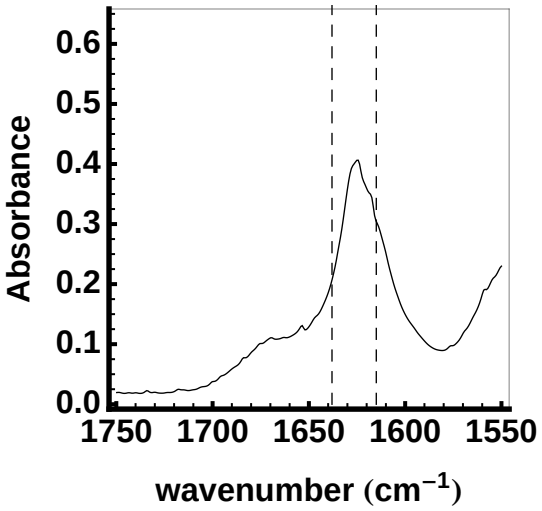
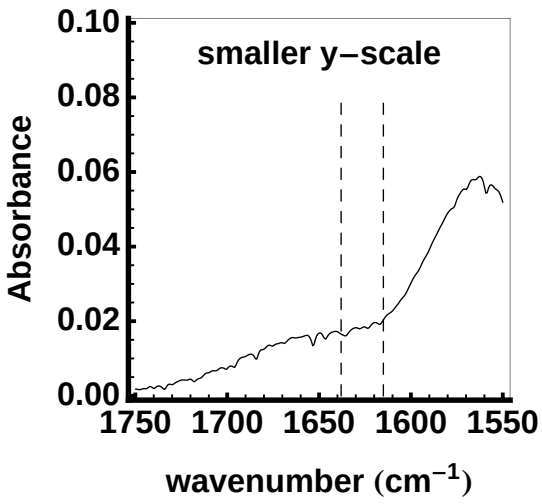
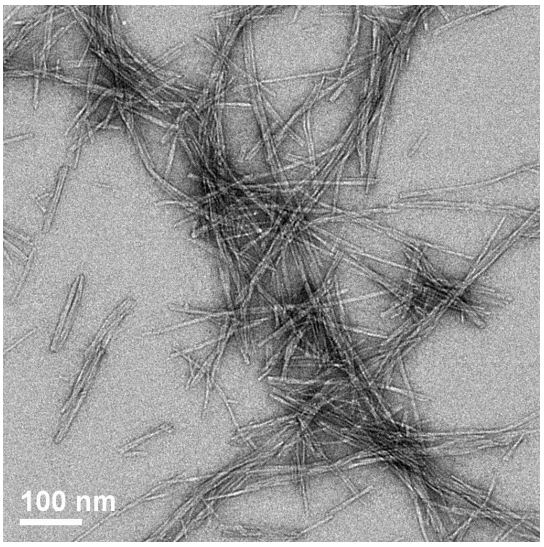
Ac–YV**S**V**H**V**H**V–$CONH_2$ (**9**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

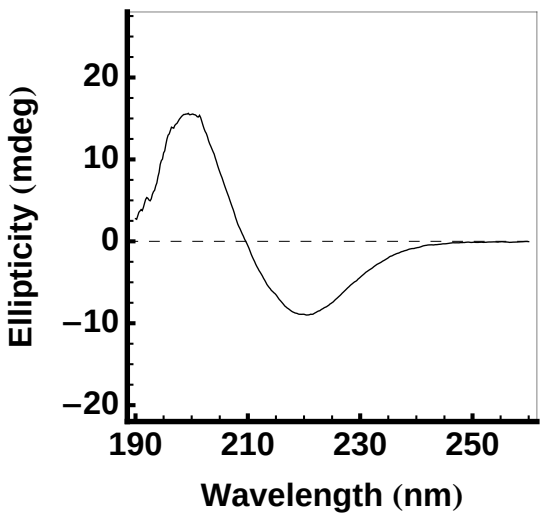
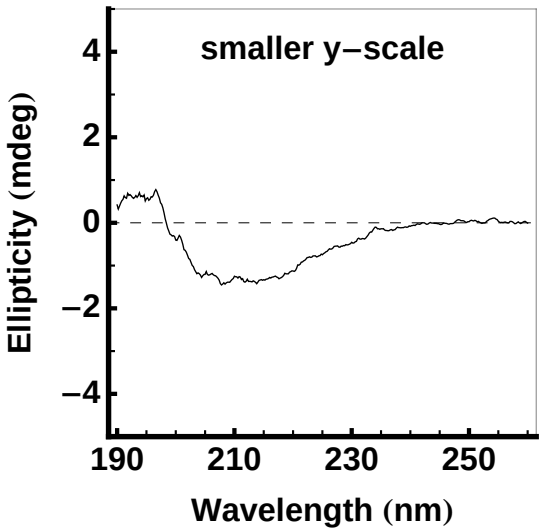
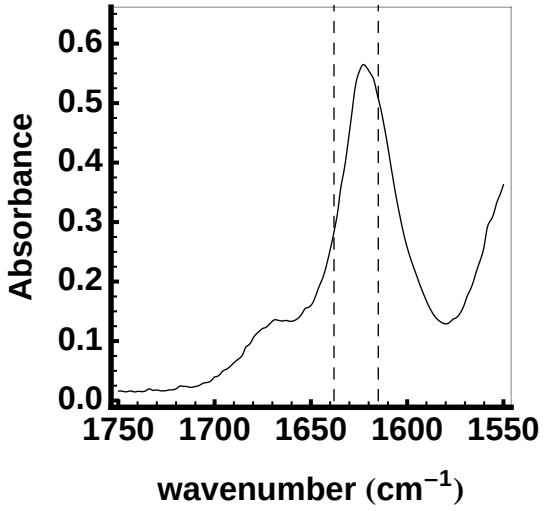
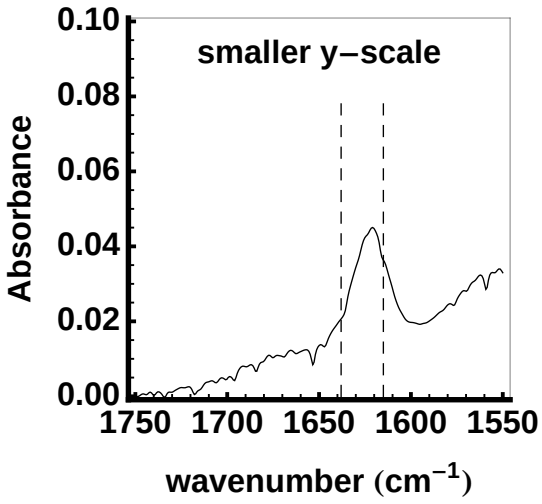
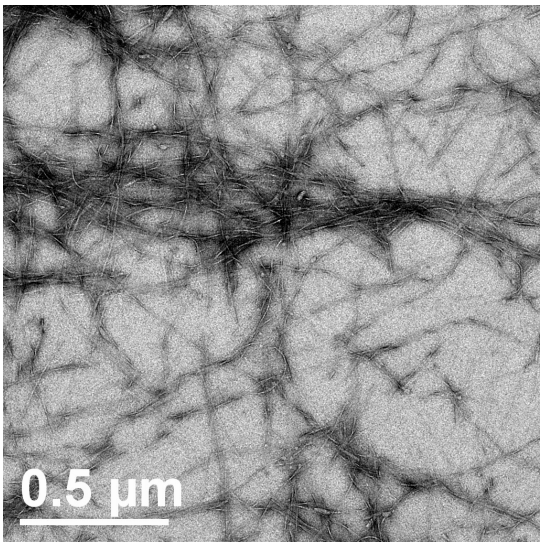
Ac–YV**H**V**S**V**H**V–$CONH_2$ (**10**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

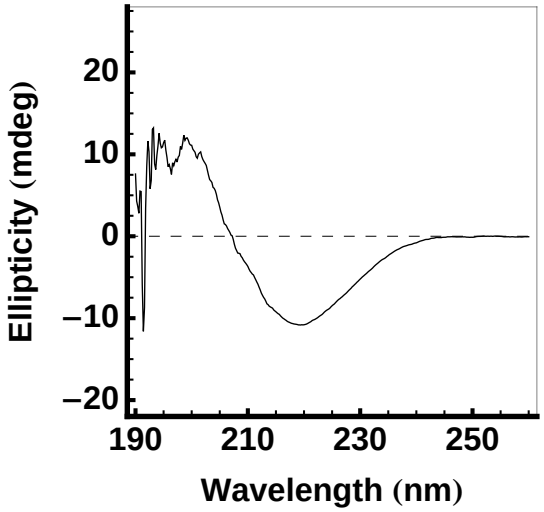
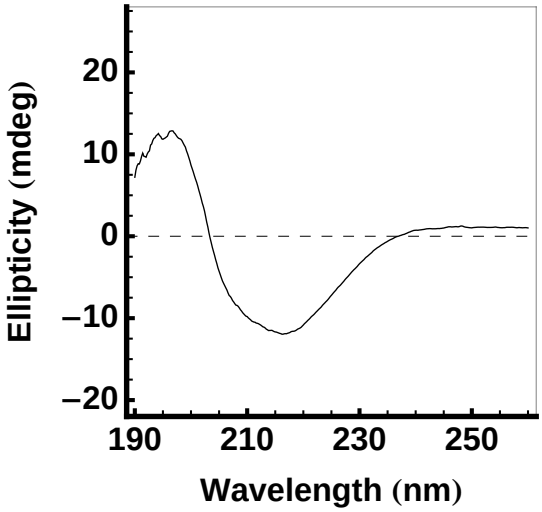
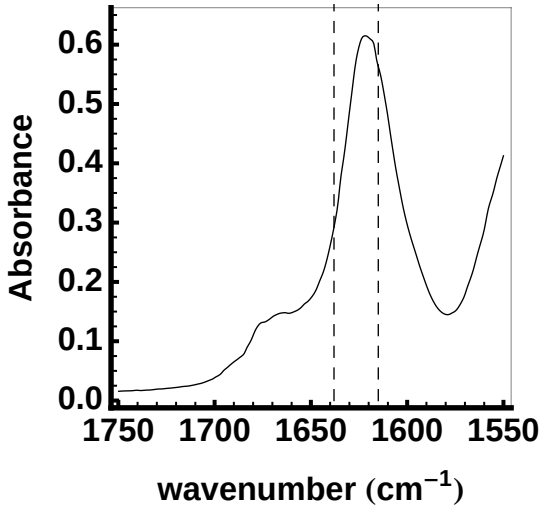
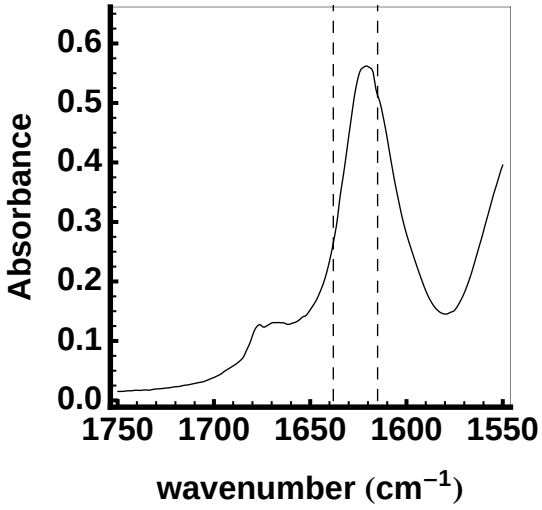
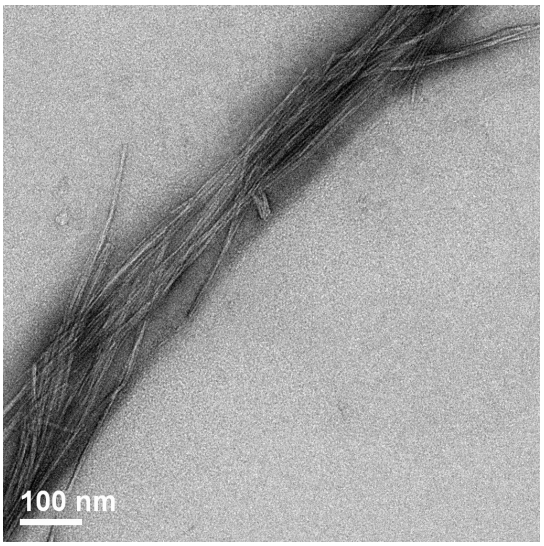
Ac–YV**H**V**H**V**S**V–$CONH_2$ (11)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

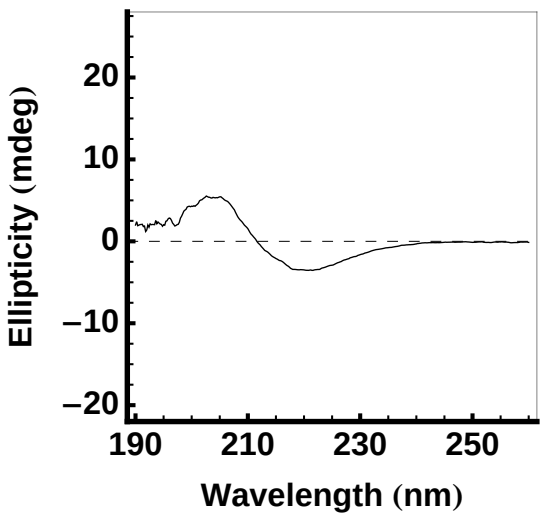
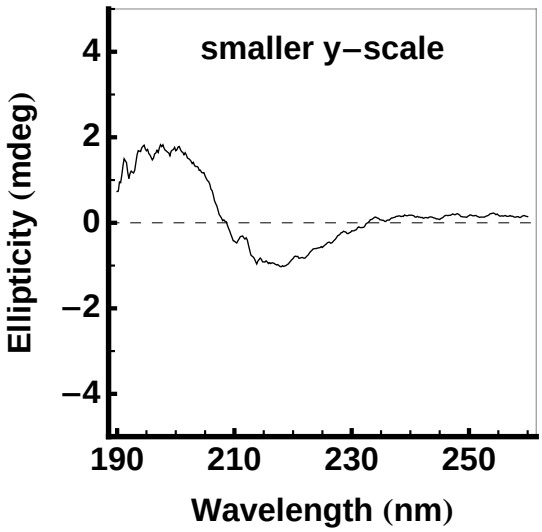
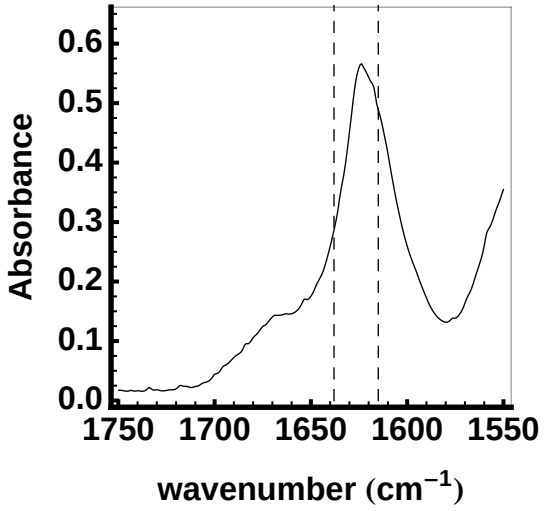
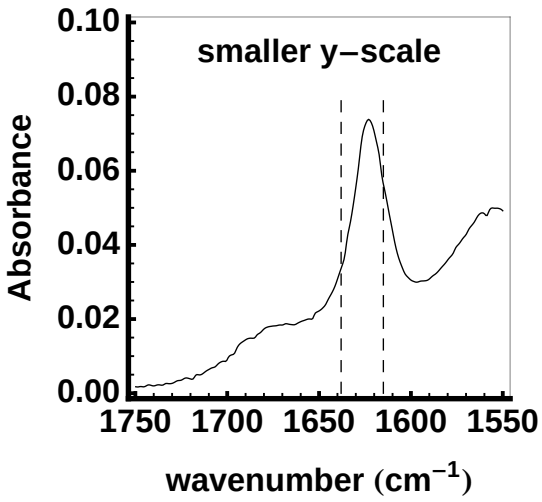
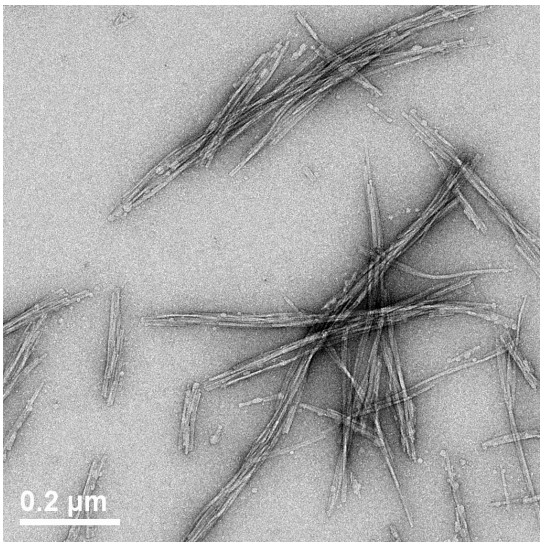
Ac–YV**S**V**S**V**H**V–$CONH_2$ (**12**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

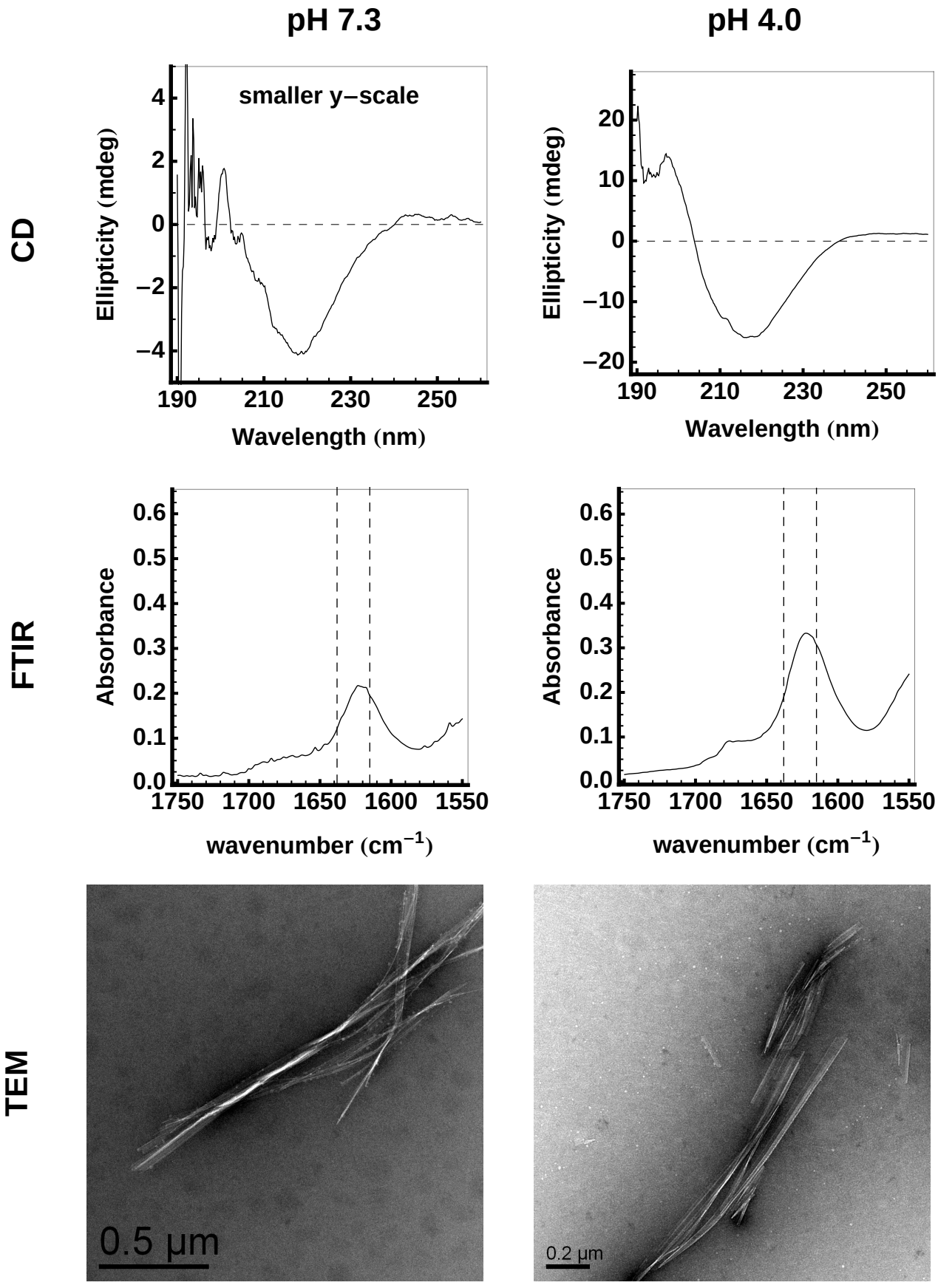
Ac–YV**S**V**H**V**S**V–$CONH_2$ (**13**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

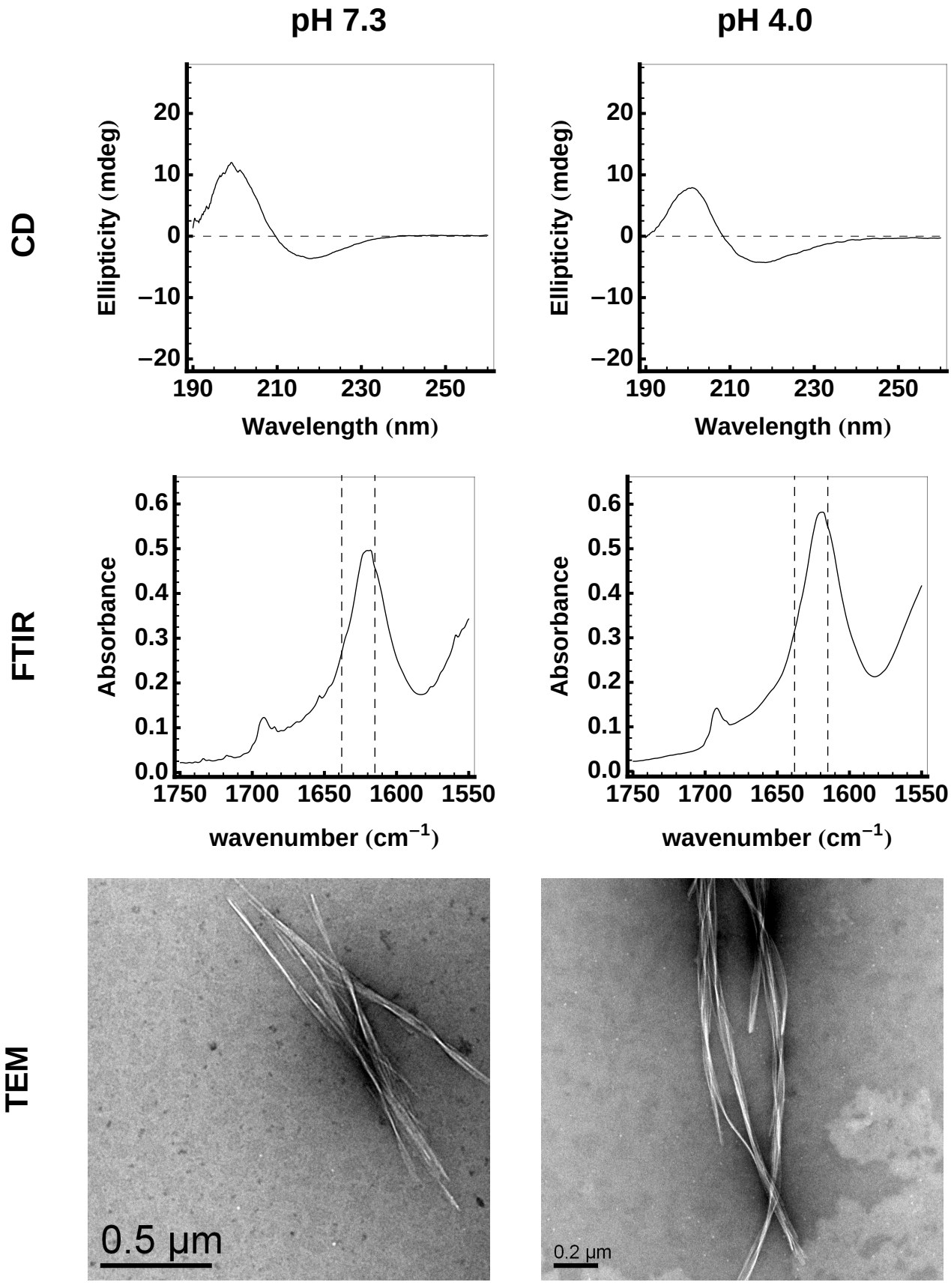
Ac–YV**H**V**S**V**S**V–$CONH_2$ (14)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

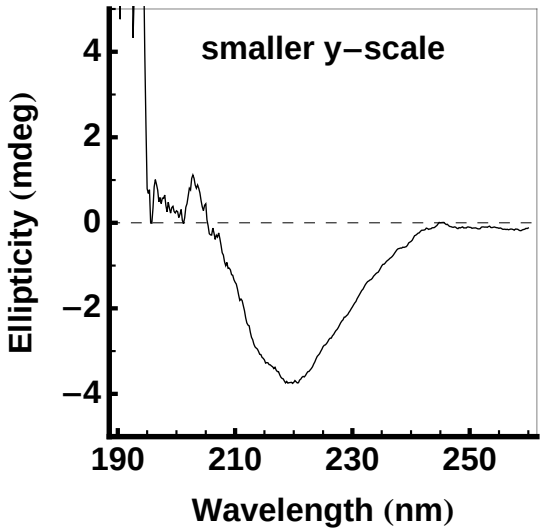
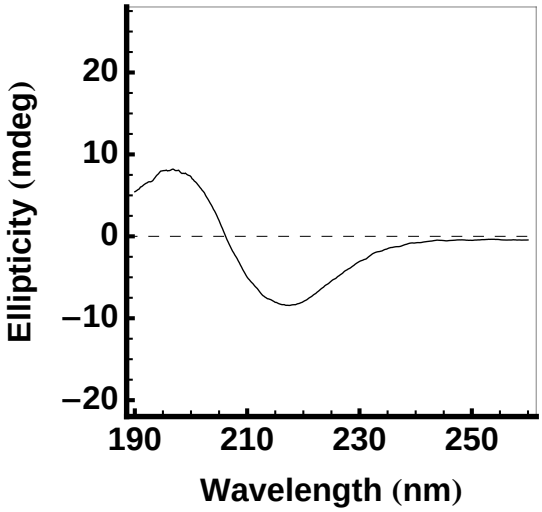
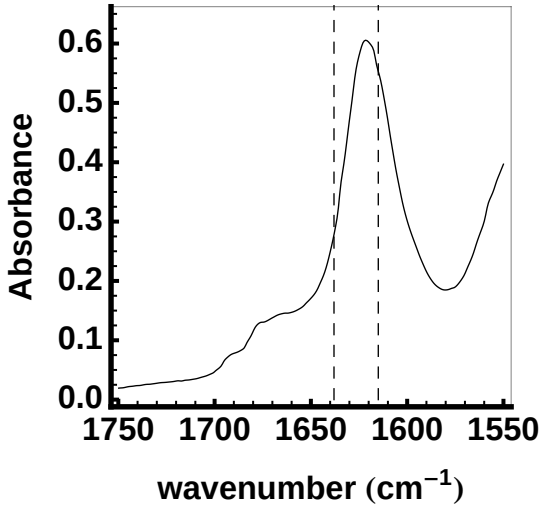
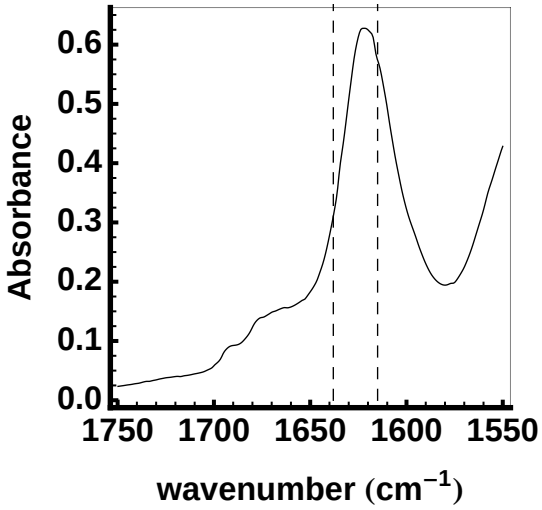
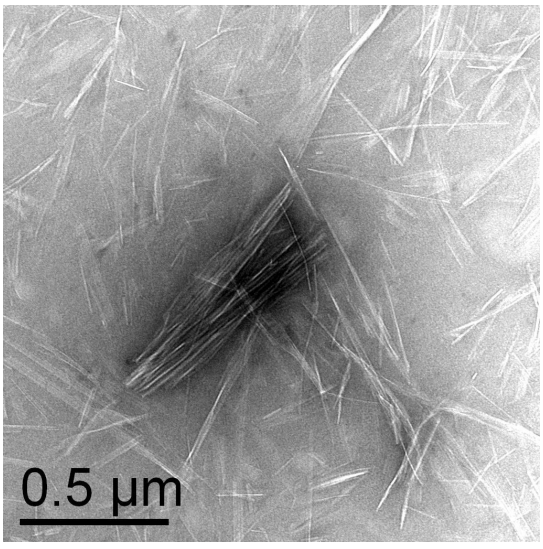
Ac–YV**D**V**H**V**S**V–$CONH_2$ (**15**)



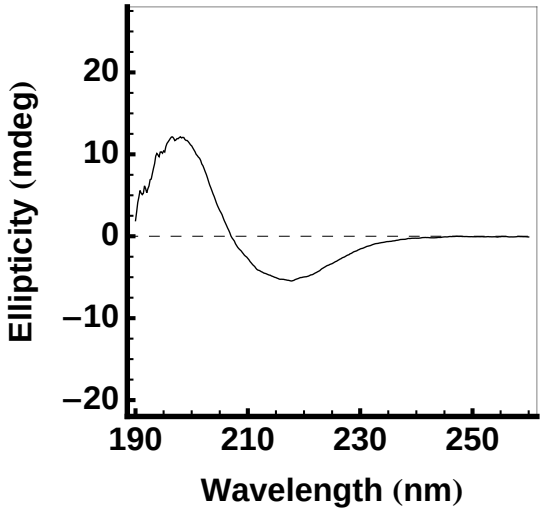
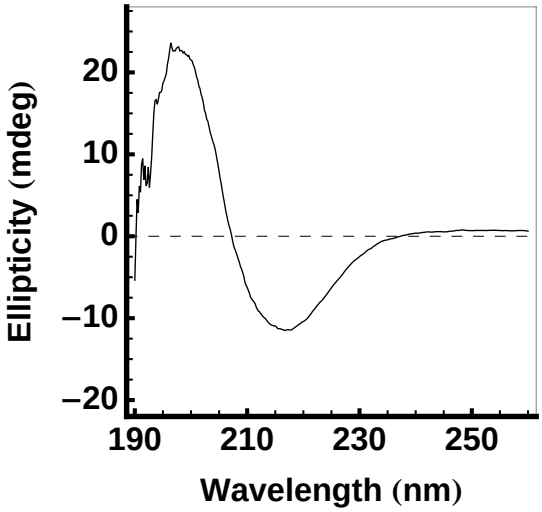
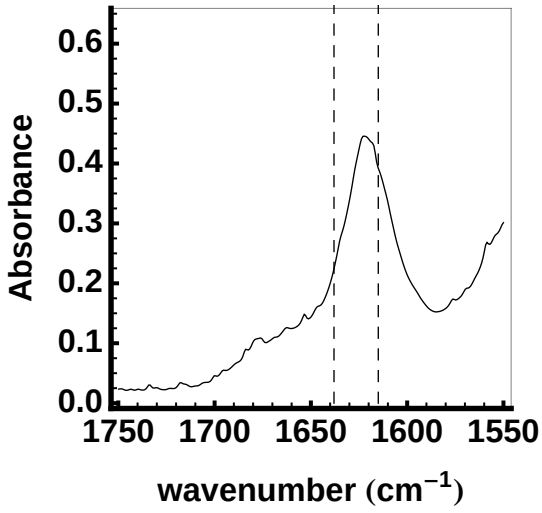
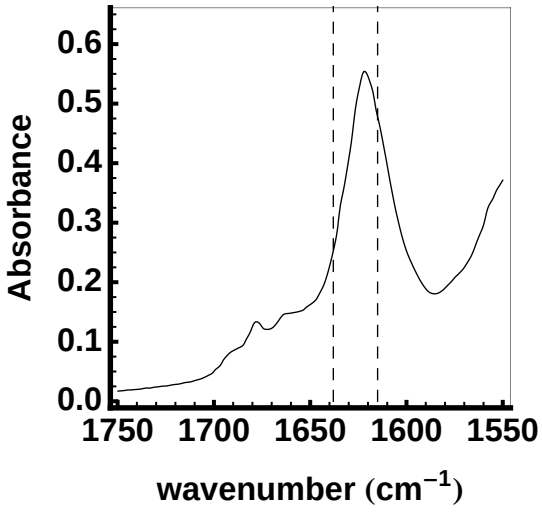
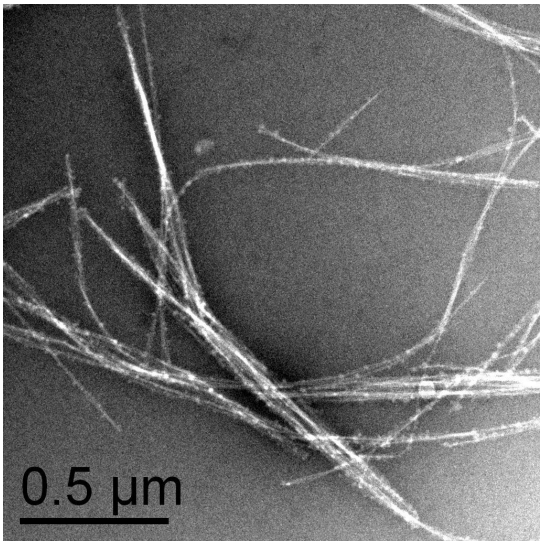
Ac–YV**D**V**S**V**H**V–$CONH_2$ (**16**)



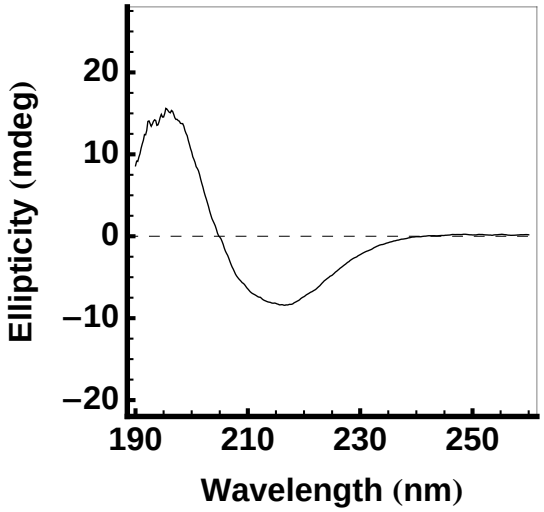
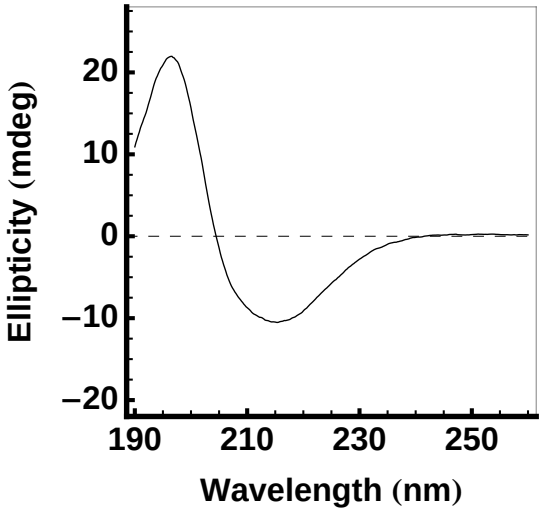
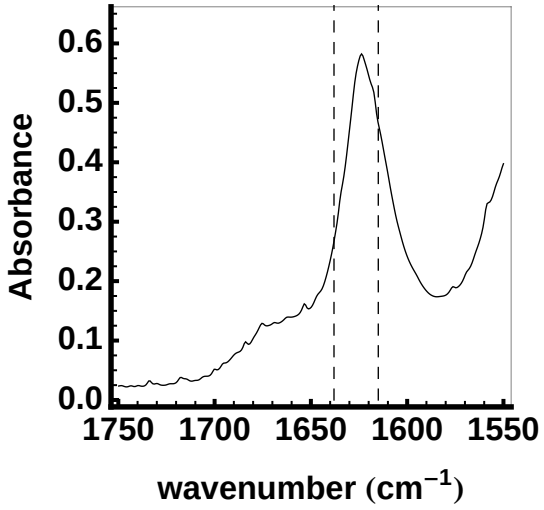
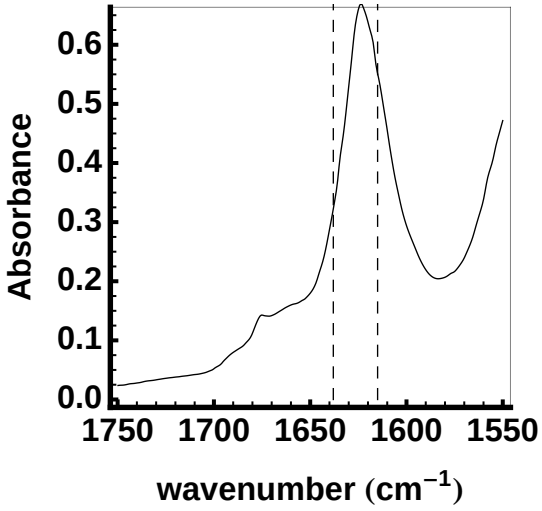
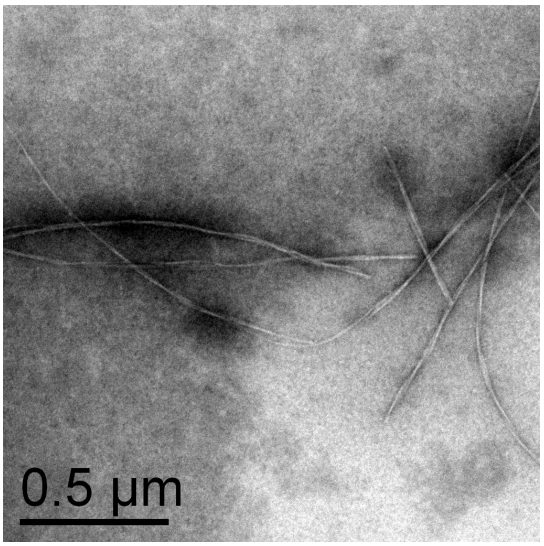
Ac–YV**H**V**D**V**S**V–$CONH_2$ (**17**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

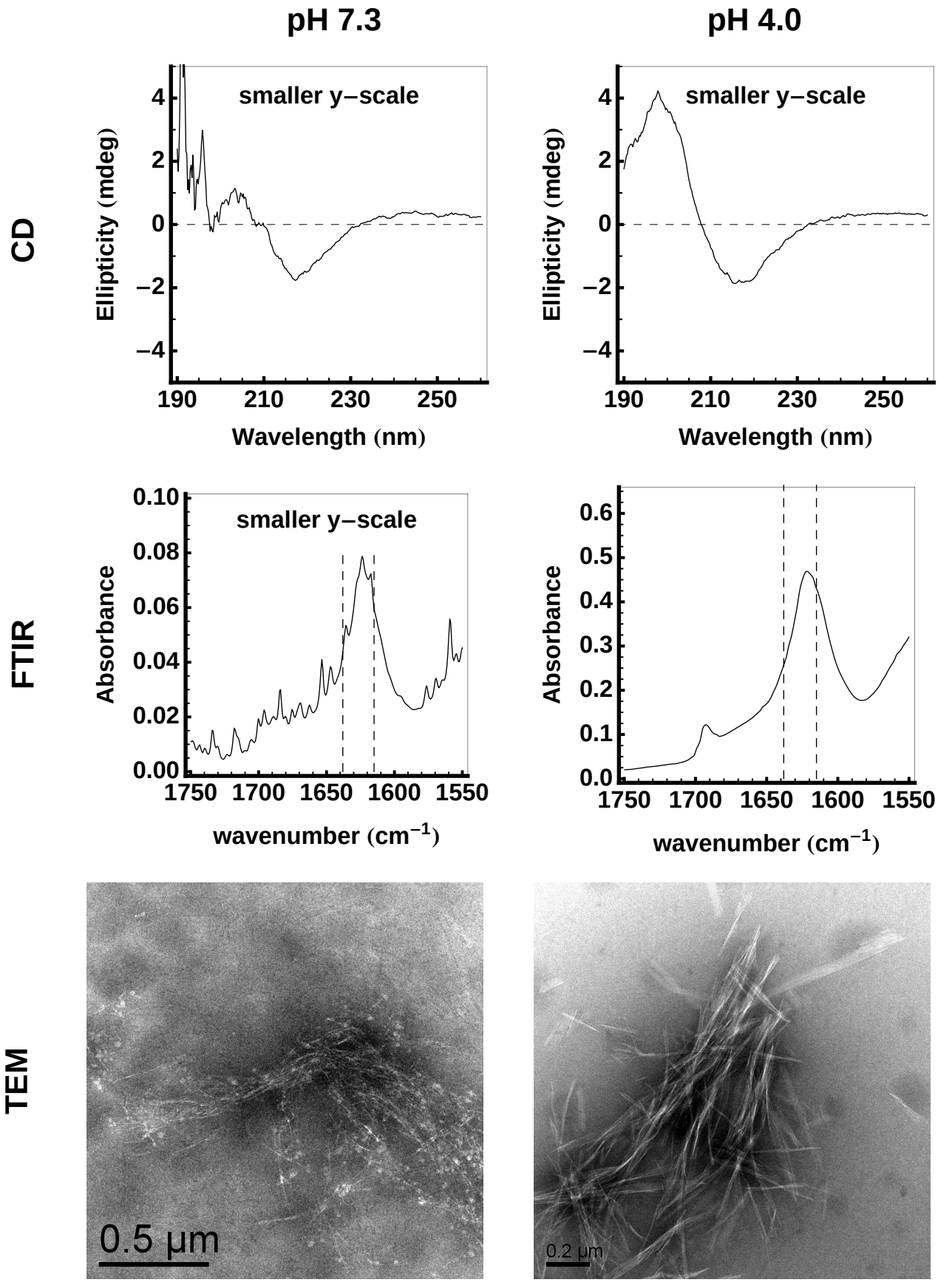
Ac–YV**S**V**D**V**H**V–$CONH_2$ (18)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

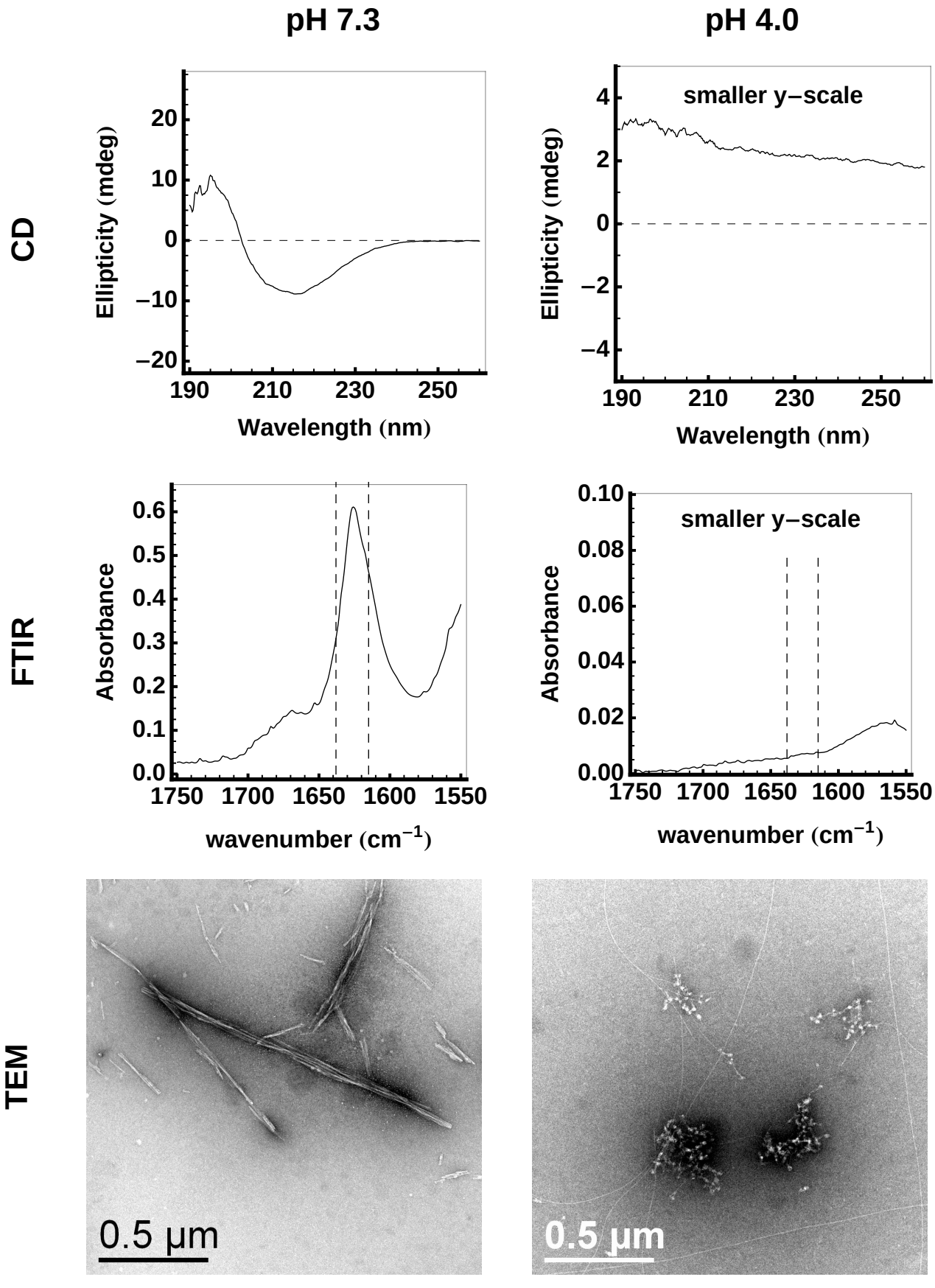
Ac–YV**S**V**H**V**D**V–$CONH_2$ (**19**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

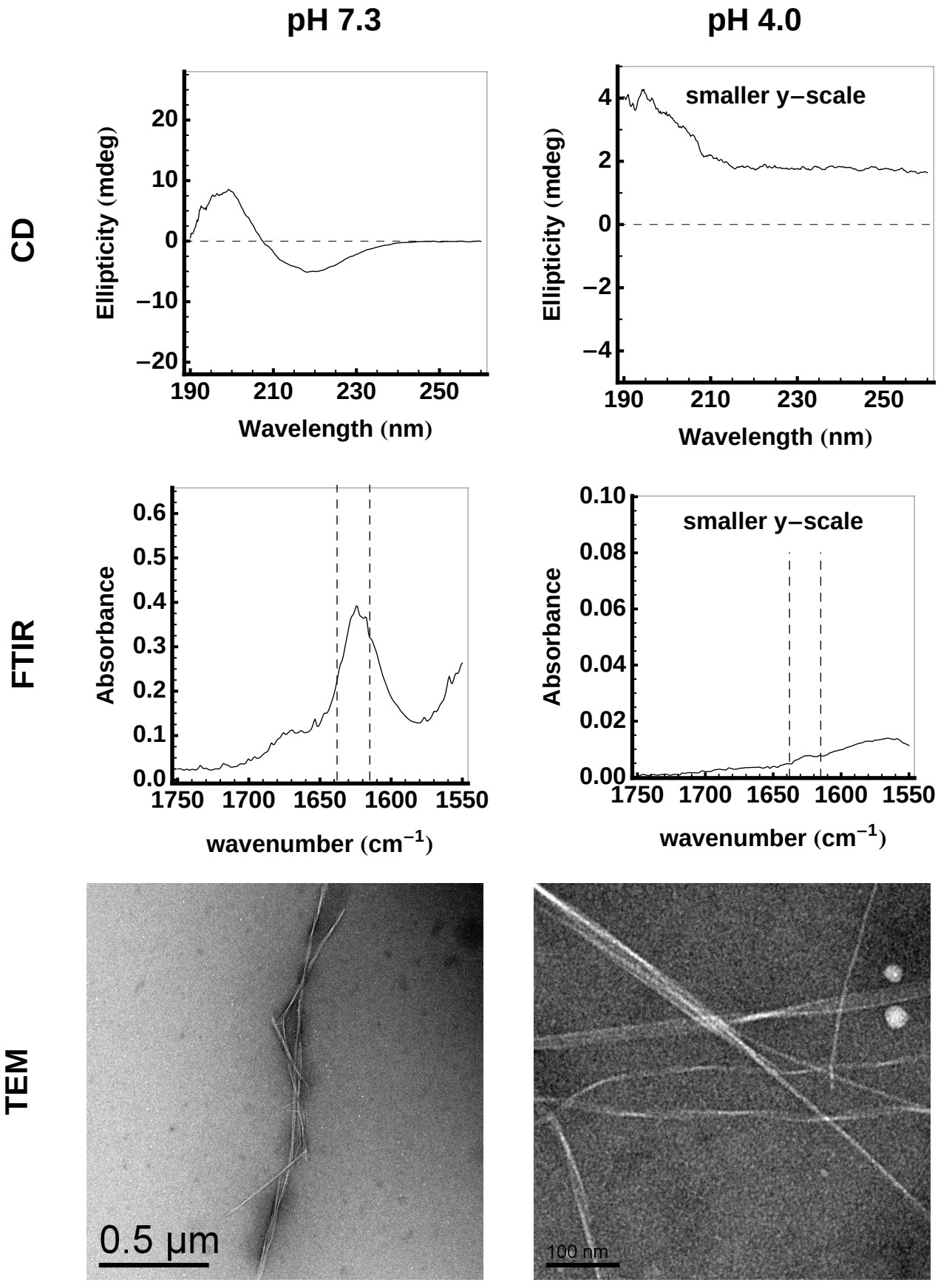
Ac–YV**H**V**S**V**D**V–$CONH_2$ (**20**)



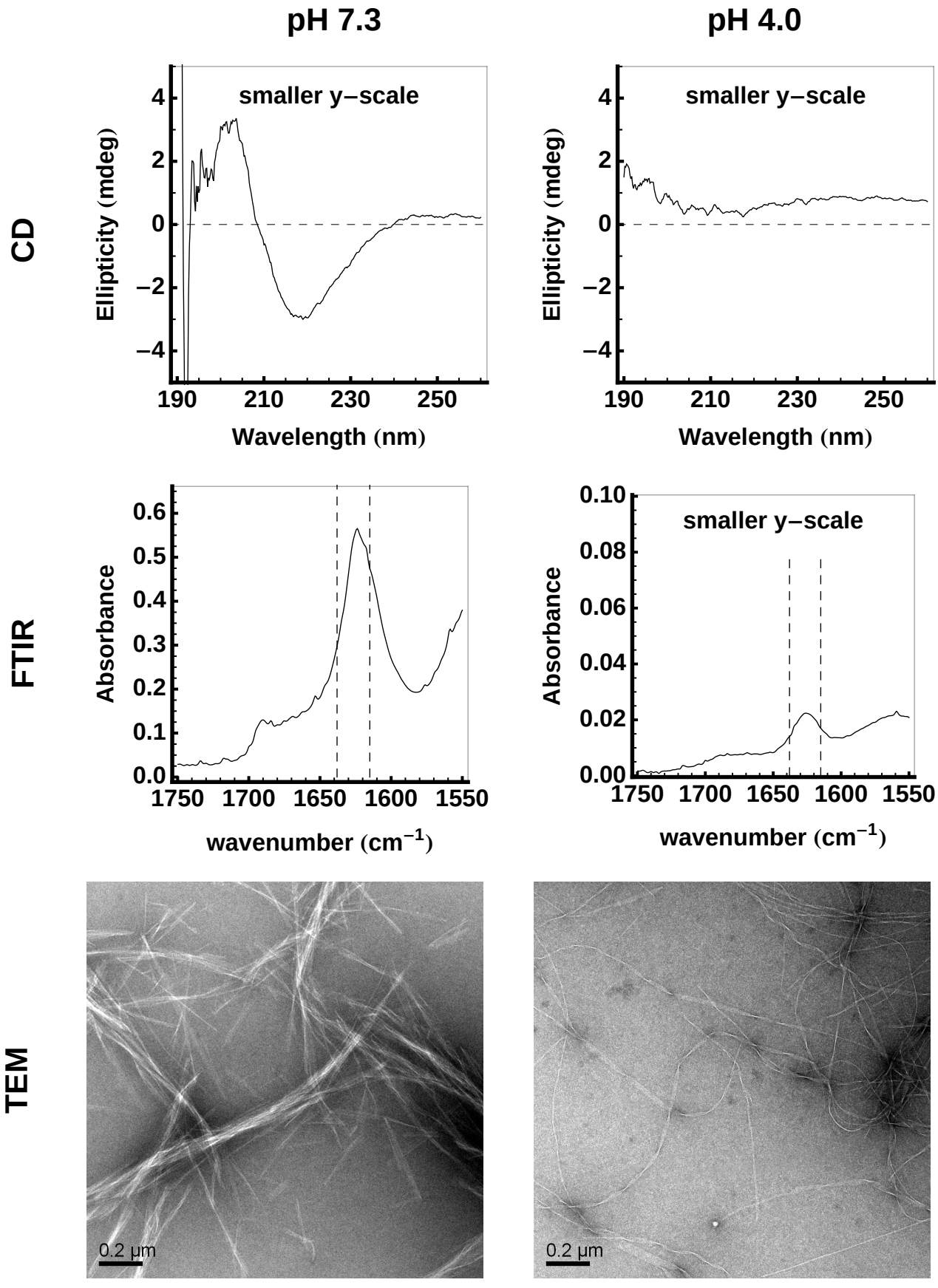
Ac–YV**H**V**H**V**D**V–$CONH_2$ (**21**)



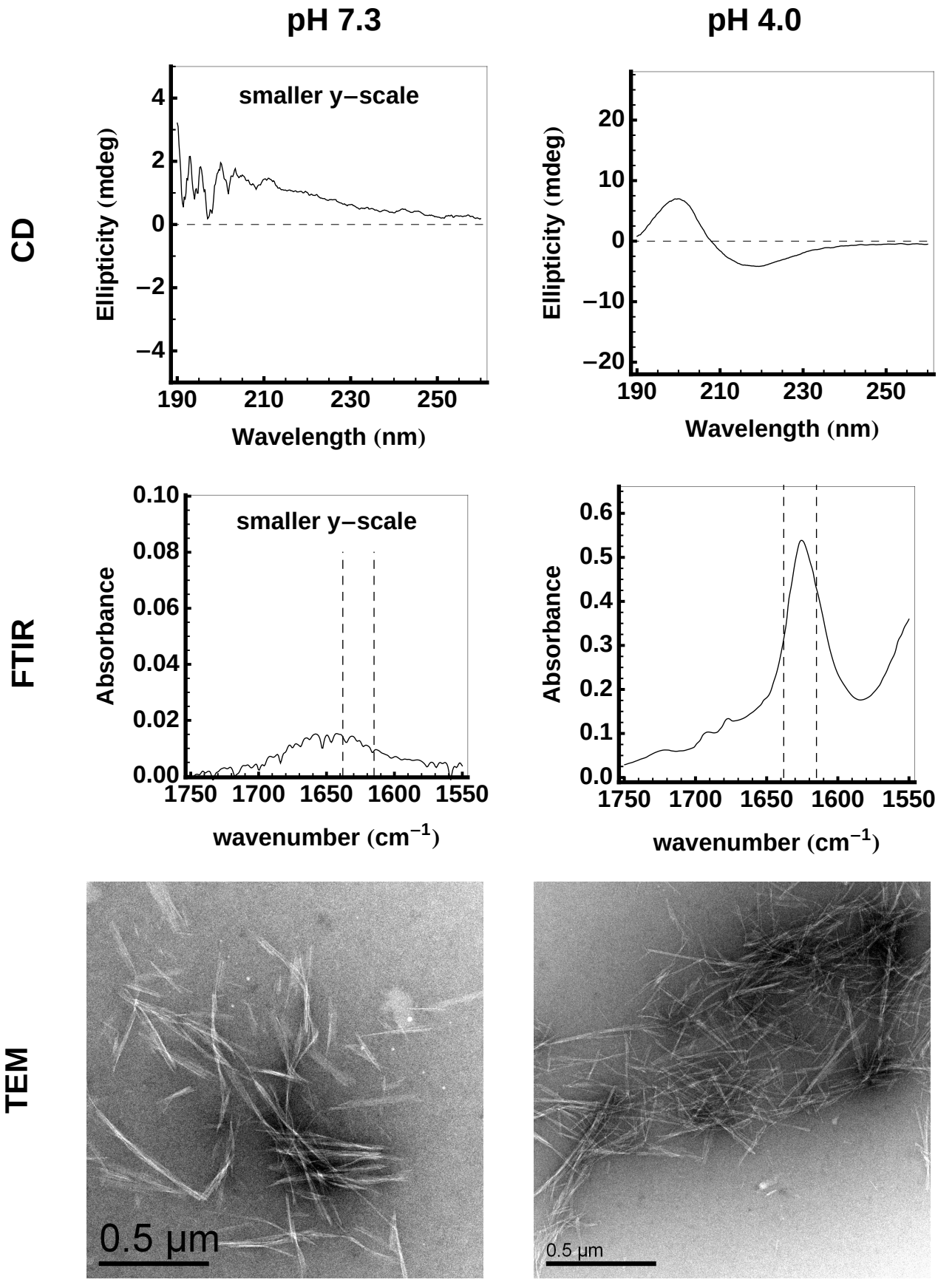
Ac–YV**H**V**D**V**H**V–$CONH_2$ (**22**)



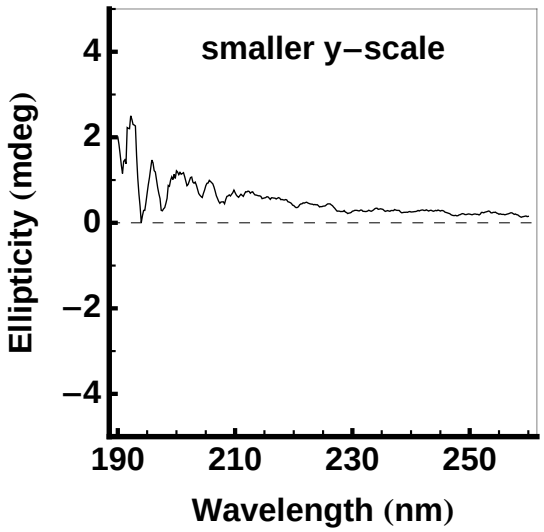
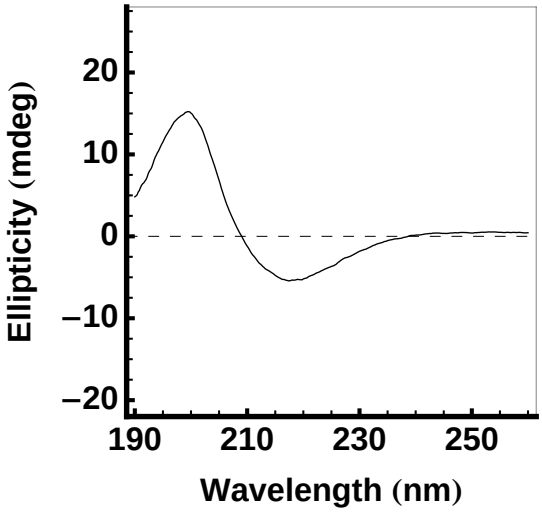
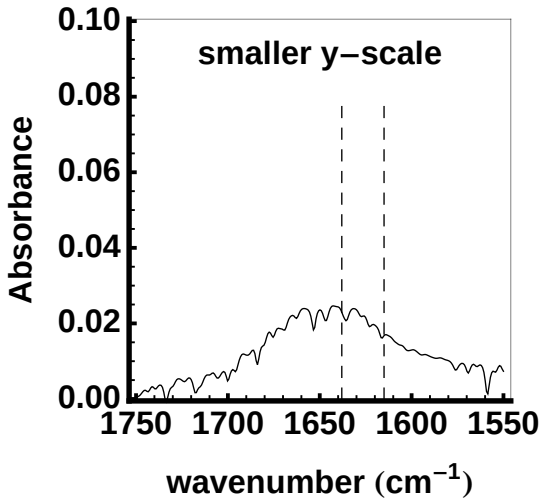
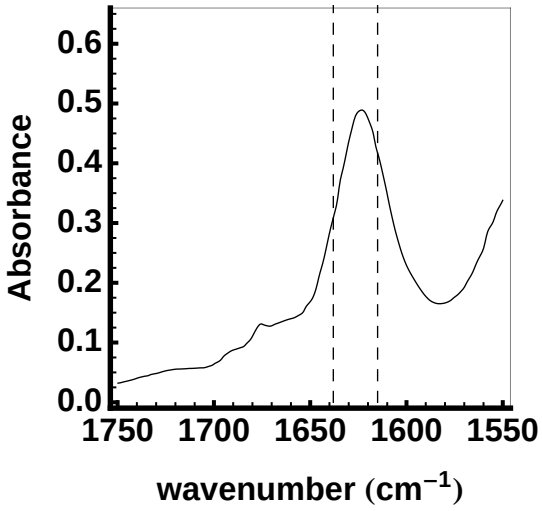
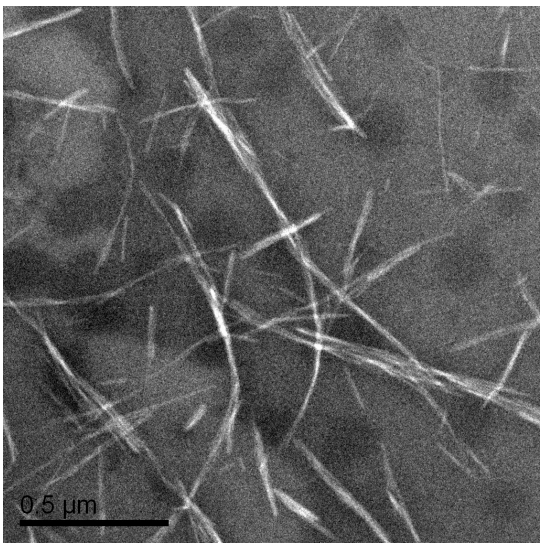
Ac–YV**D**V**H**V**H**V–$CONH_2$ (**23**)



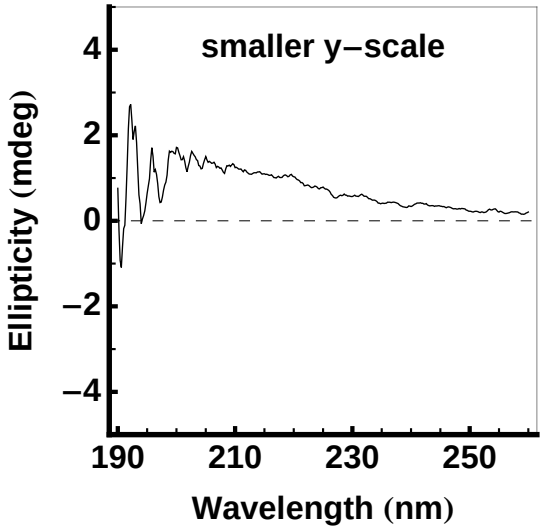
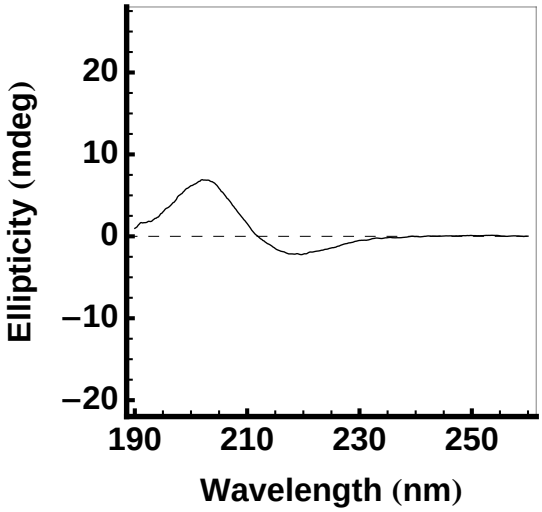
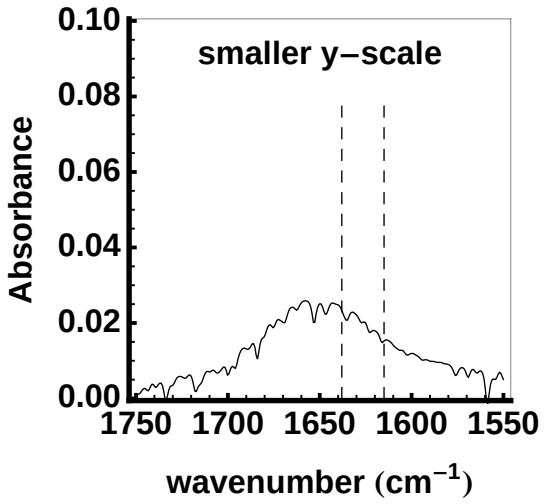
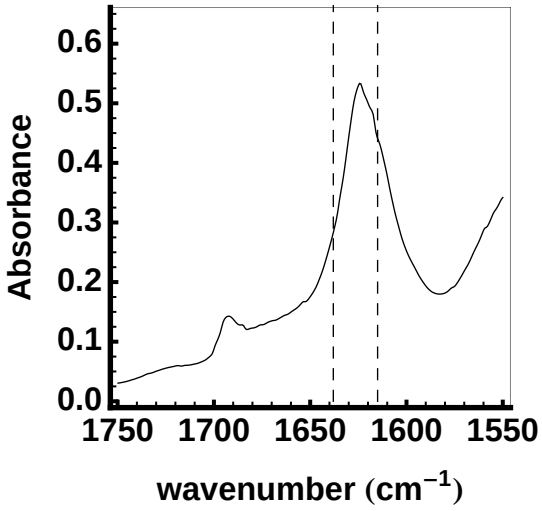
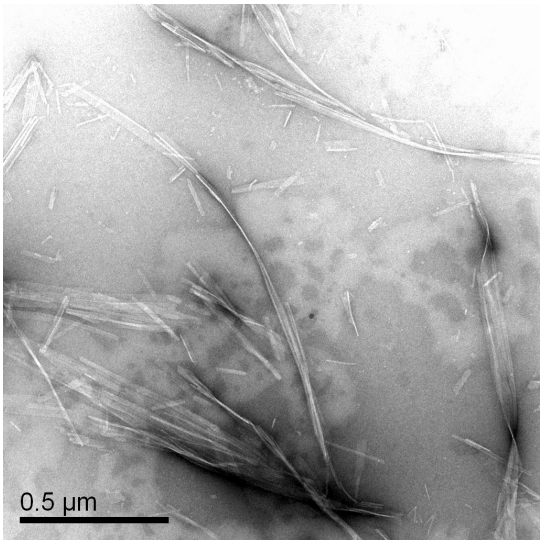
Ac–YV**D**V**D**V**H**V–$CONH_2$ (**24**)



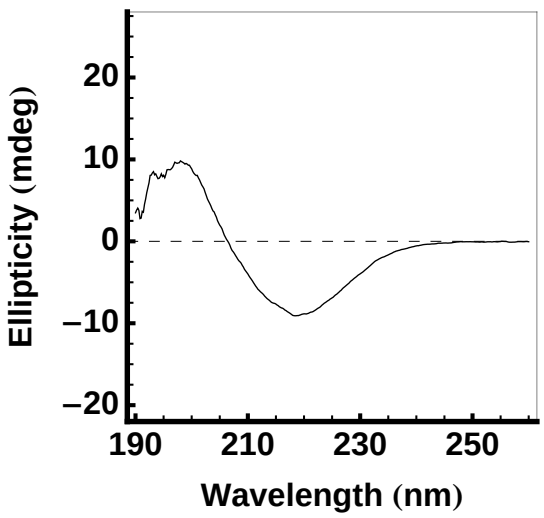
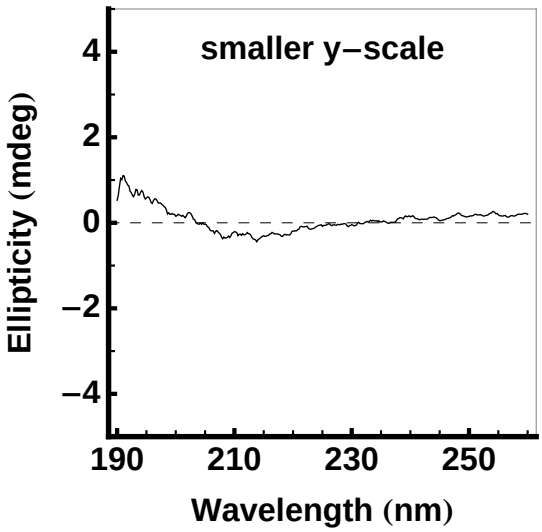
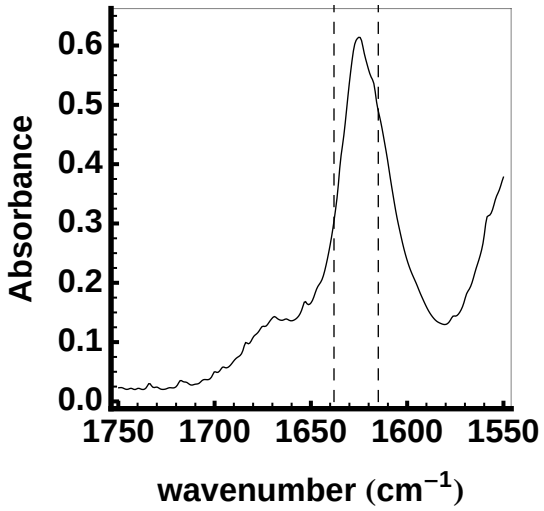
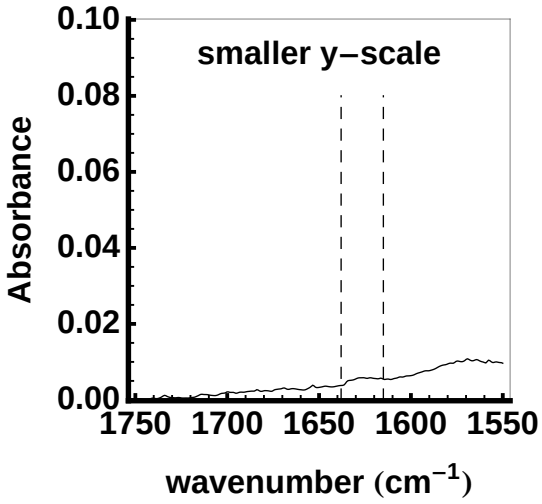
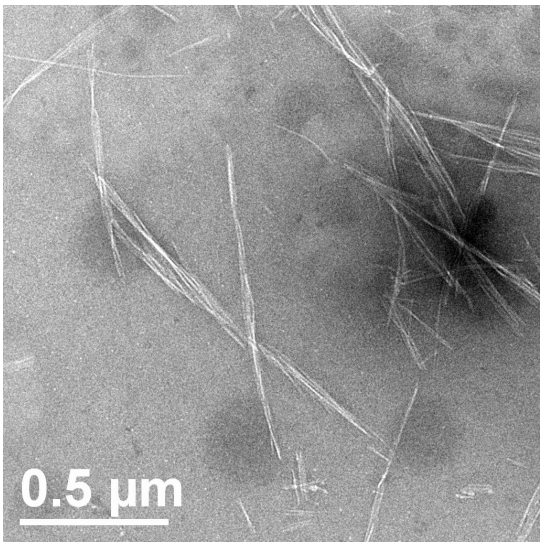
Ac–YV**D**V**H**V**D**V–$CONH_2$ (**25**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

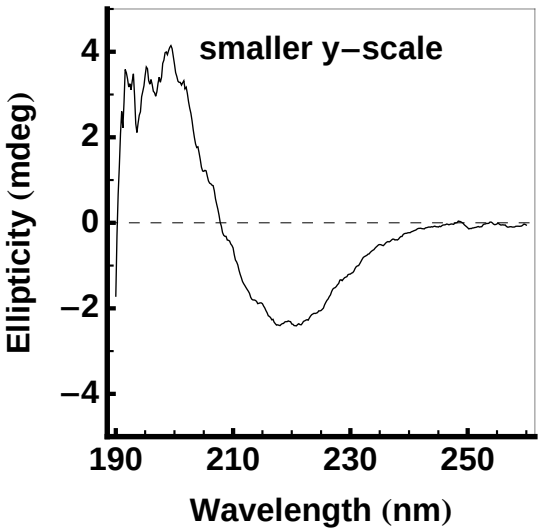
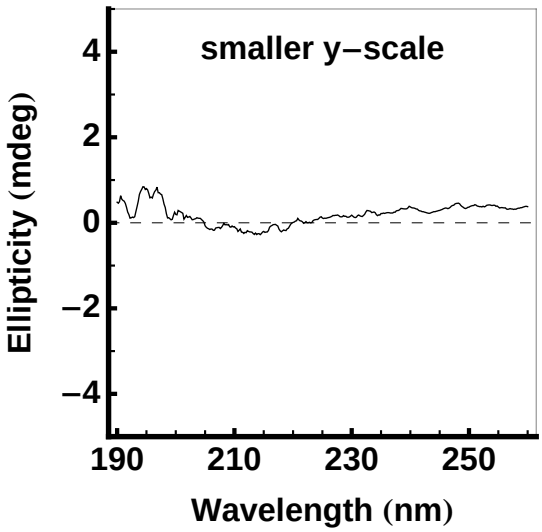
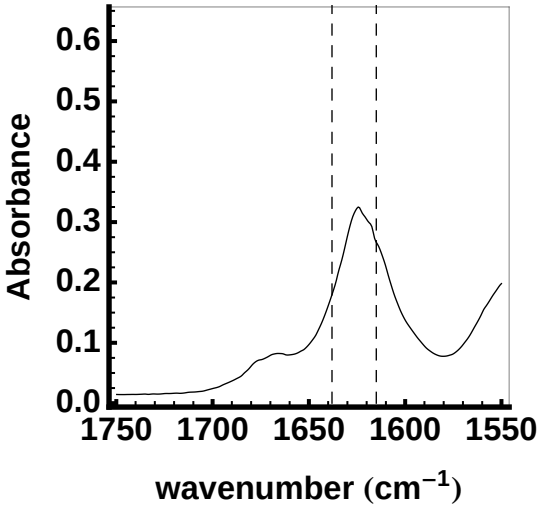
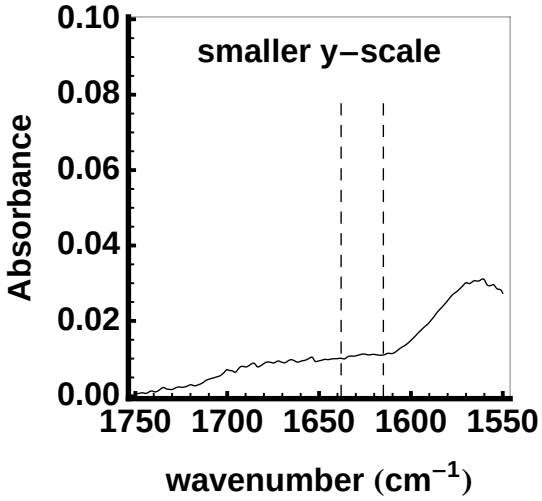
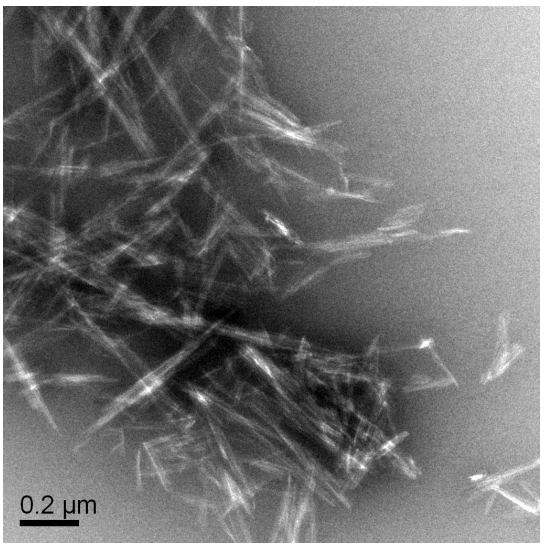
Ac–YV**H**V**D**V**D**V–$CONH_2$ (**26**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	**n/a**	

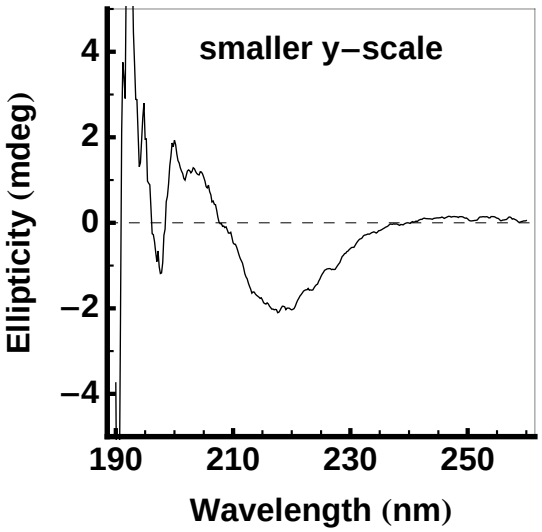
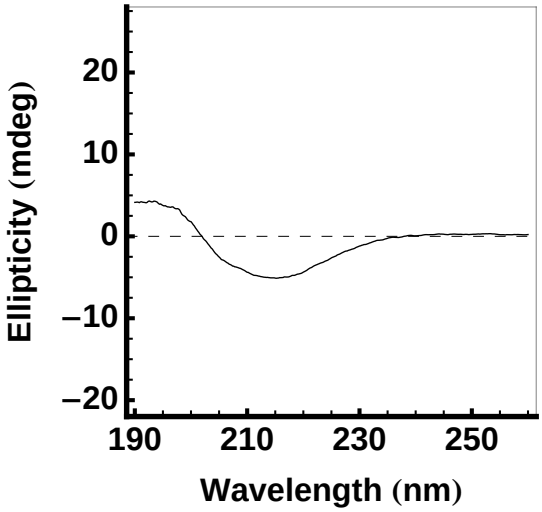
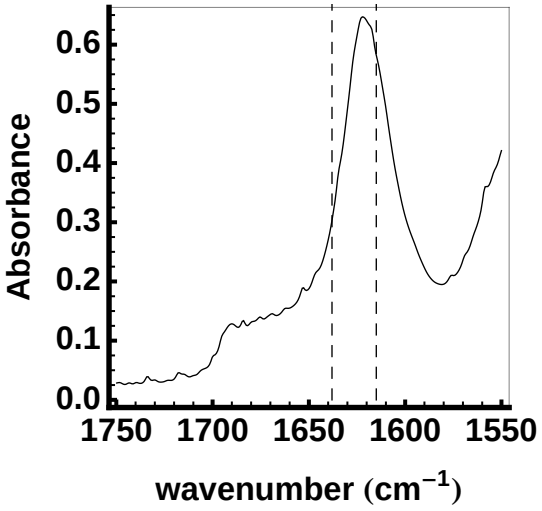
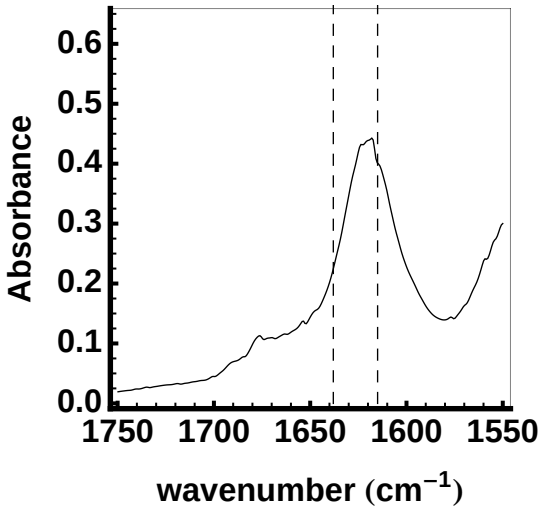
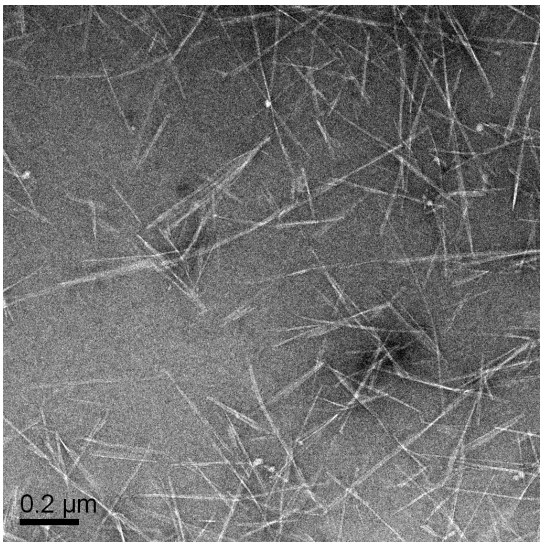
Ac–YV**A**V**H**V**H**V–$CONH_2$ (**27**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

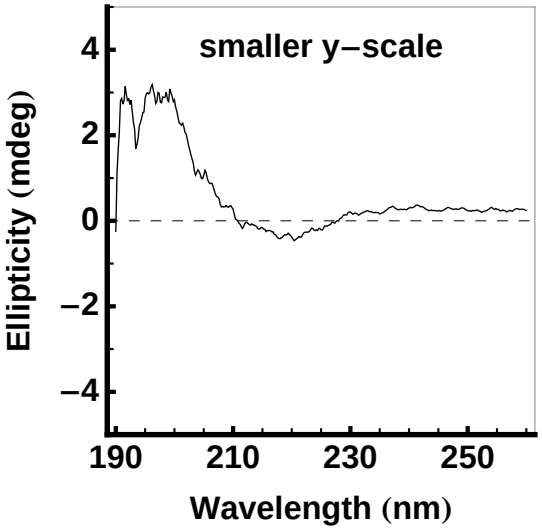
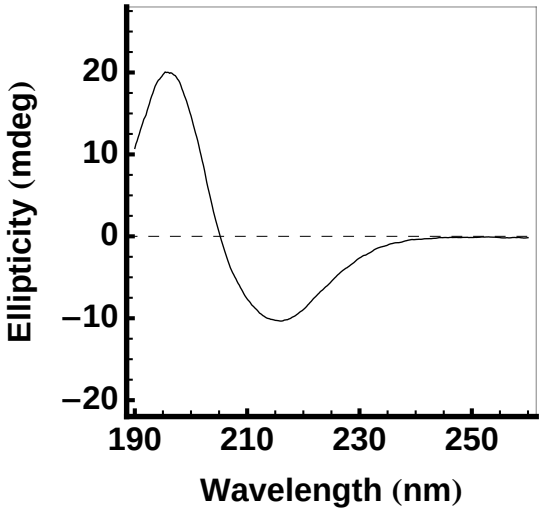
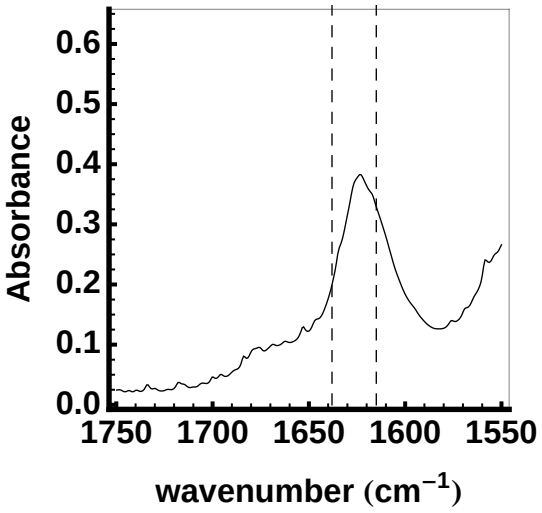
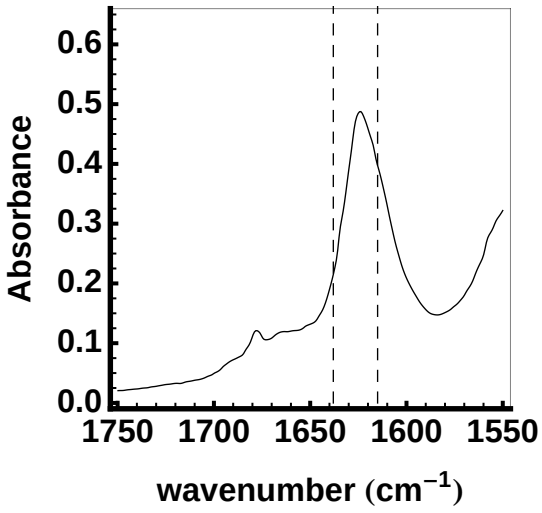
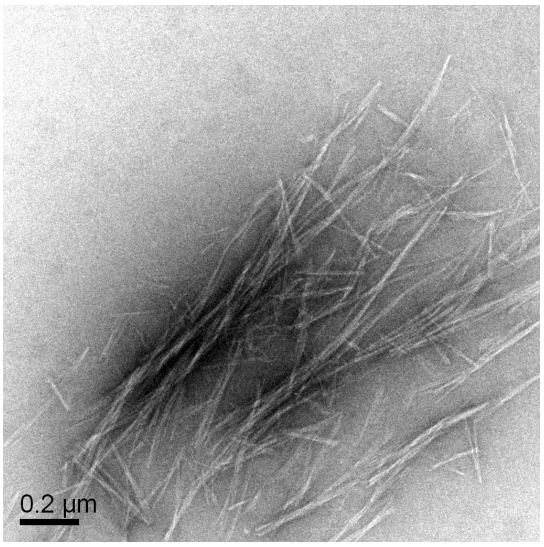
Ac–YV**H**V**A**V**H**V–$CONH_2$ (**28**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		**n/a**

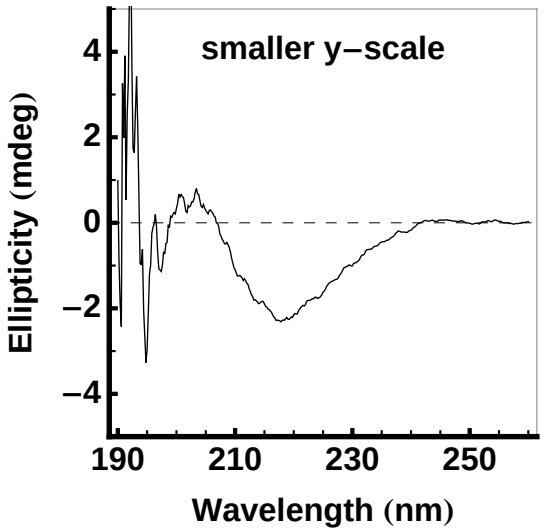
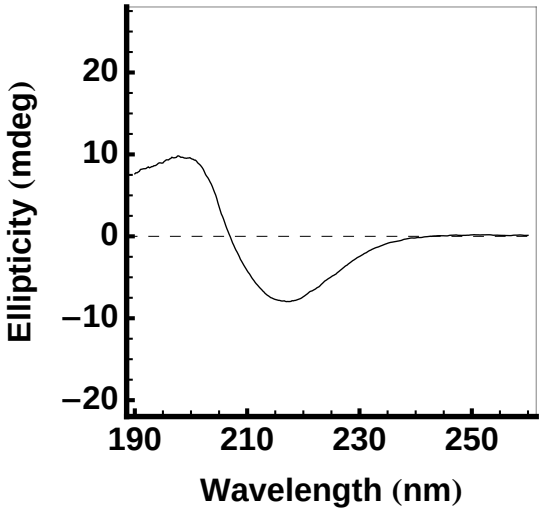
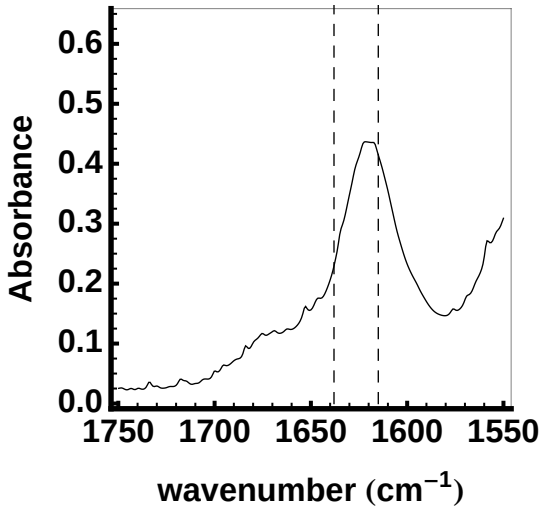
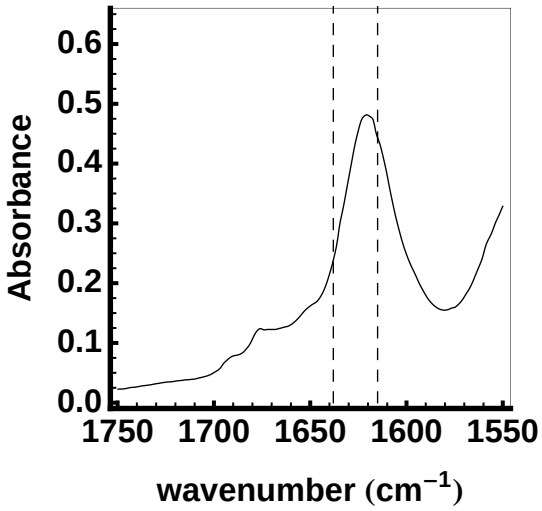
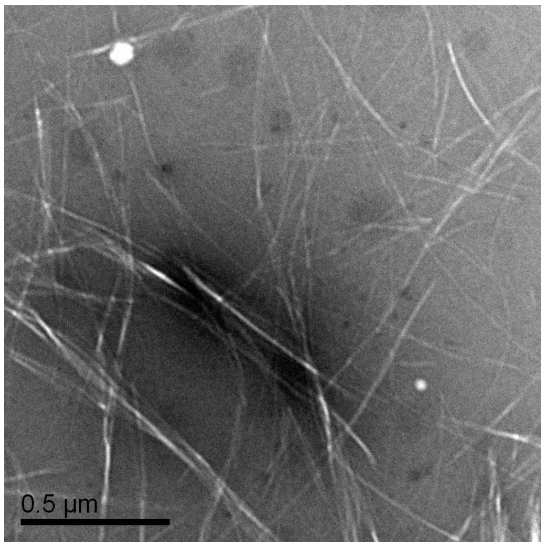
Ac–YV**D**V**H**V**A**V–$CONH_2$ (**29**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

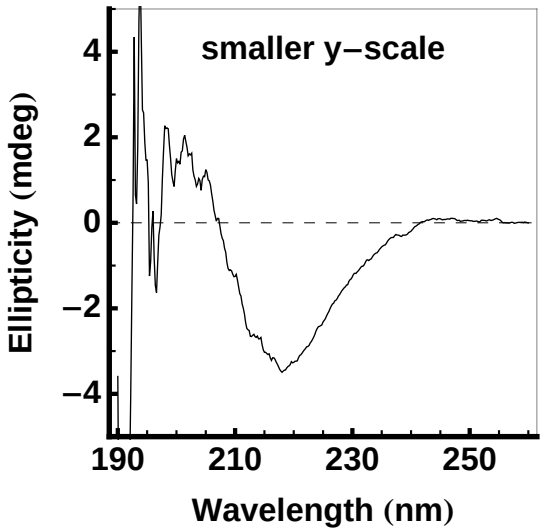
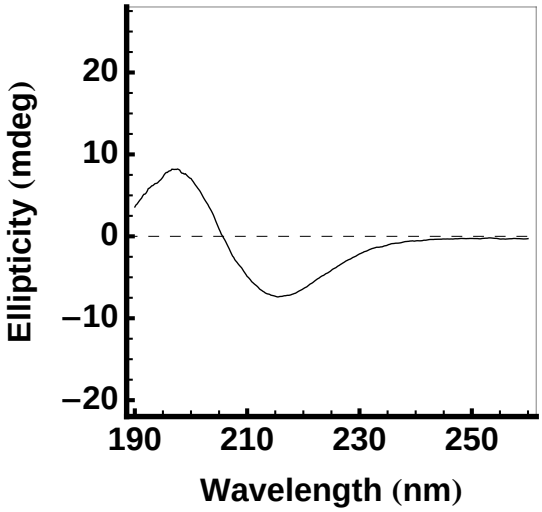
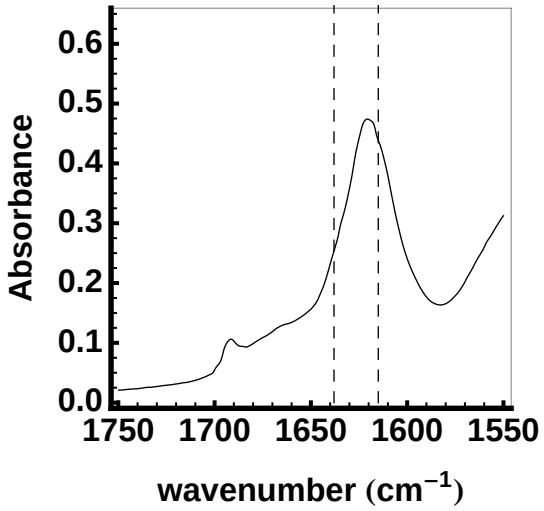
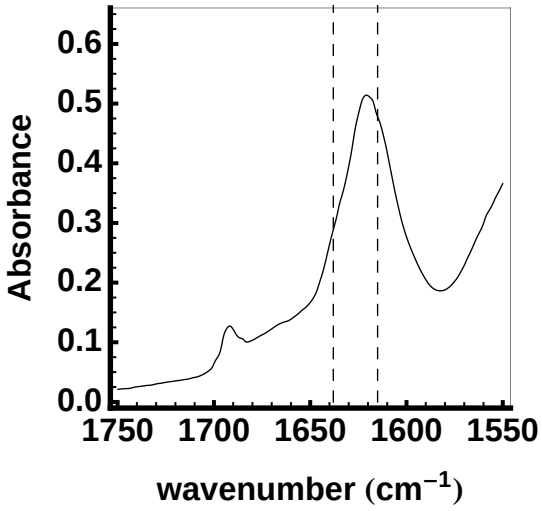
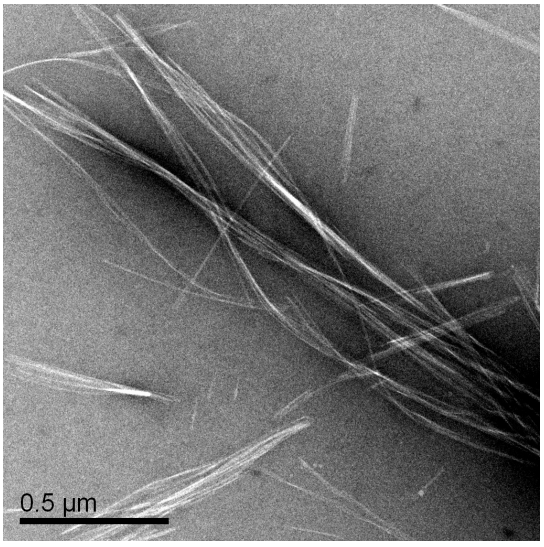
Ac–YV**A**V**D**V**H**V–$CONH_2$ (**30**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM		n/a

Ac–YV**H**V**D**V**A**V–$CONH_2$ (**31**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

Ac–YV**H**V**A**V**D**V–$CONH_2$ (**32**)

	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

Ac–YV**A**V**H**V**D**V–$CONH_2$ (**33**)

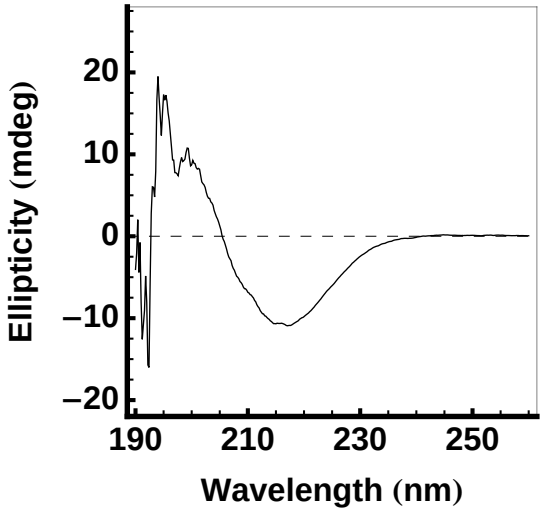
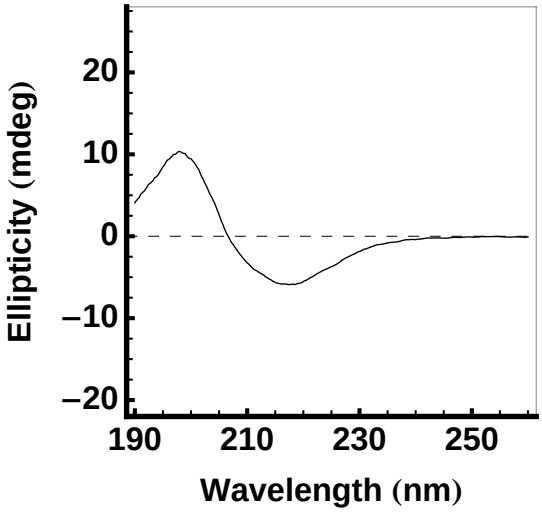
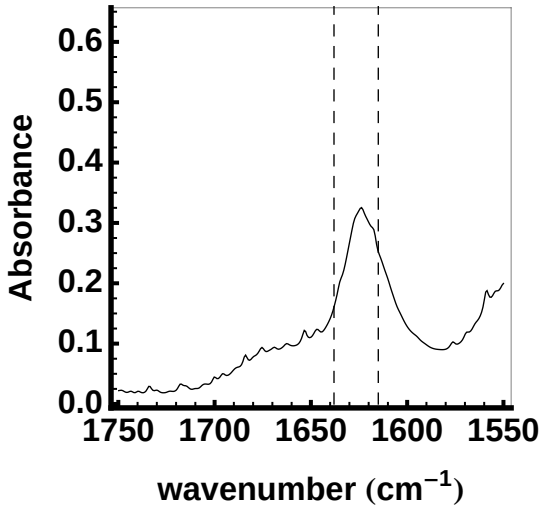
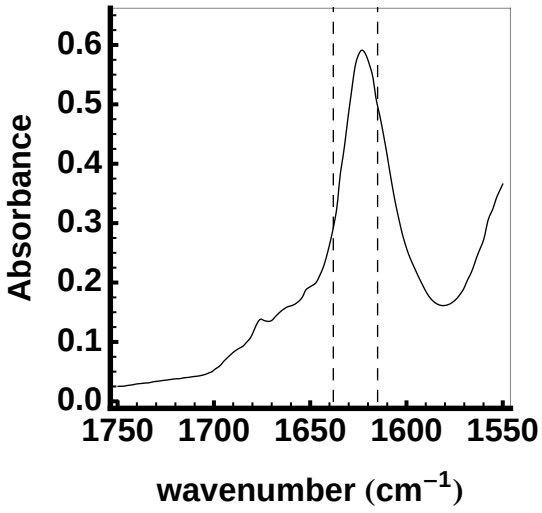
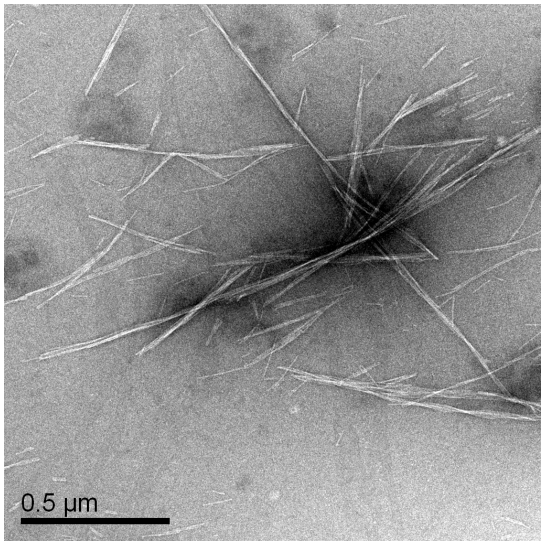
	pH 7.3	pH 4.0
CD		
FTIR		
TEM	n/a	

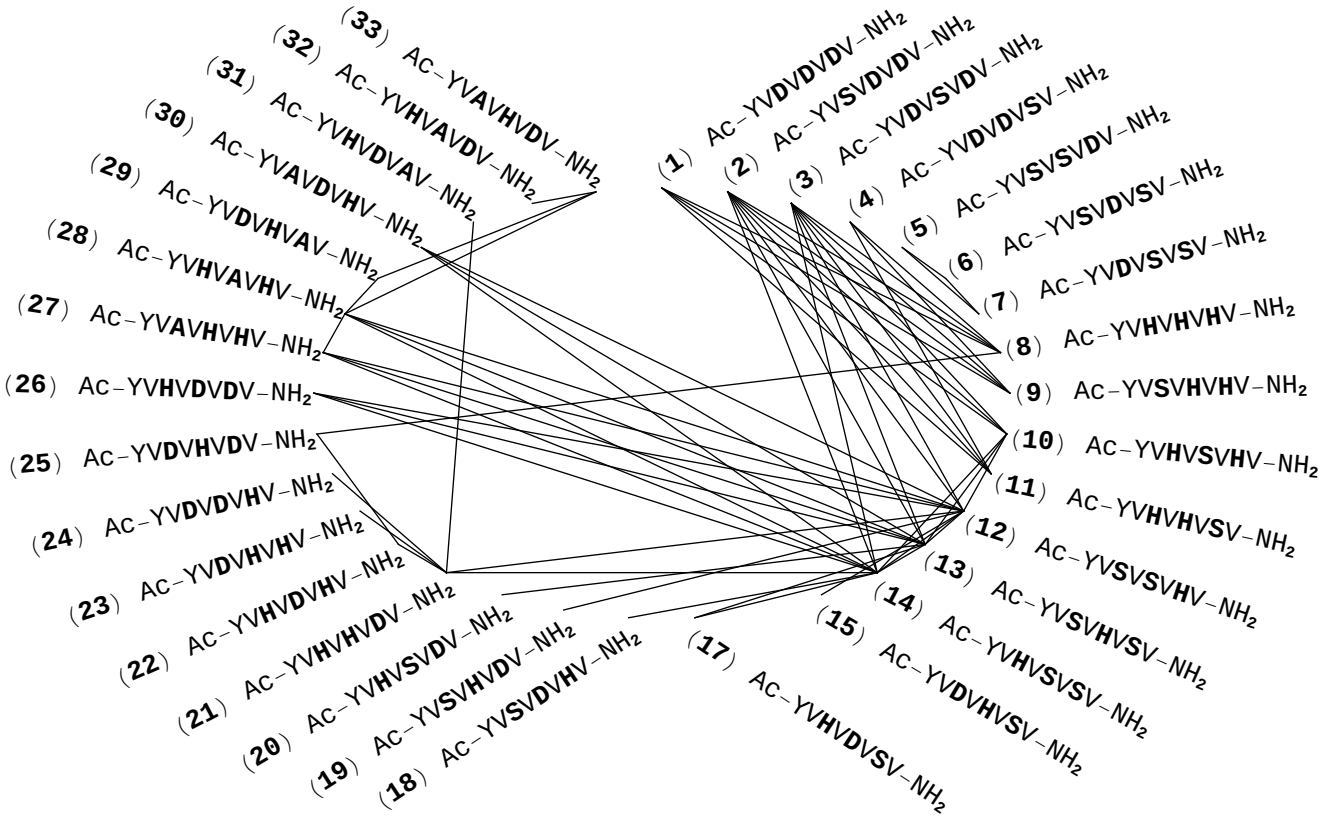
Table B: Amyloid growth and measurement conditions.

	Growth = Measurement[1]			
	Divalent[2]	pH[3]	T (°C)	Additive
1	-	7.3	20	-
2	$ZnCl_2$	7.3	20	-
3	$MgCl_2$	7.3	20	-
4	$CoCl_2$	7.3	20	-
5	$CuCl_2$	7.3	20	-
6	$NiCl_2$	7.3	20	-
7	EDTA	7.3	20	-
8	-	4.0	20	-
9	$ZnCl_2$	4.0	20	-
10	-	2.2	20	-
11	-	7.3	4	-
12	$ZnCl_2$	7.3	4	-
13	-	7.3	20	75 % *i*PrOH
14	-	7.3	20	64 % CH_3CN
15	-	7.3	20	50 % EtOH
16	-	7.3	20	90 % EtOH
17	$ZnCl_2$	7.3	20	75 % *i*PrOH
18	$ZnCl_2$	7.3	20	64 % CH_3CN
19	$ZnCl_2$	7.3	20	50 % EtOH
20	$ZnCl_2$	7.3	20	90 % EtOH
21	-	7.3	20	0.5 M NaCl
22	-	7.3	20	4.75 M NaCl
23	-	7.3	20	0.2 M $MgCl_2$
24	-	7.3	20	2 M $MgCl_2$
25	$ZnCl_2$	7.3	20	0.5 M NaCl
26	$ZnCl_2$	7.3	20	4.75 M NaCl
27	$ZnCl_2$	7.3	20	0.2 M $MgCl_2$
28	$ZnCl_2$	7.3	20	2 M $MgCl_2$
29	-	7.3	20	1 M Na_2SO_4
30	$ZnCl_2$	7.3	20	1 M Na_2SO_4
31	-	4.0	20	2 M $(NH_4)_2SO_4$
32	-	2.2	20	2 M $(NH_4)_2SO_4$

	Growth ≠ Measurement[1]			
	Divalent	pH	T (°C)	Additive
33	$ZnCl_2$	7.3	95	-
34	-	7.3	20	0.5 M NaCl
35	-	7.3	20	4.75 M NaCl
36	-	7.3	20	0.2 M $MgCl_2$
37	-	7.3	20	2 M $MgCl_2$
38	$ZnCl_2$	7.3	20	0.5 M NaCl
39	$ZnCl_2$	7.3	20	4.75 M NaCl
40	$ZnCl_2$	7.3	20	0.2 M $MgCl_2$
41	$ZnCl_2$	7.3	20	2 M $MgCl_2$
42	$ZnCl_2$	7.3	20	75 % *i*PrOH
43	$ZnCl_2$	7.3	20	64 % CH_3CN
44	$ZnCl_2$	7.3	20	50 % EtOH
45	$ZnCl_2$	7.3	20	90 % EtOH
46	$ZnCl_2$	7.3	20	50 % DMSO

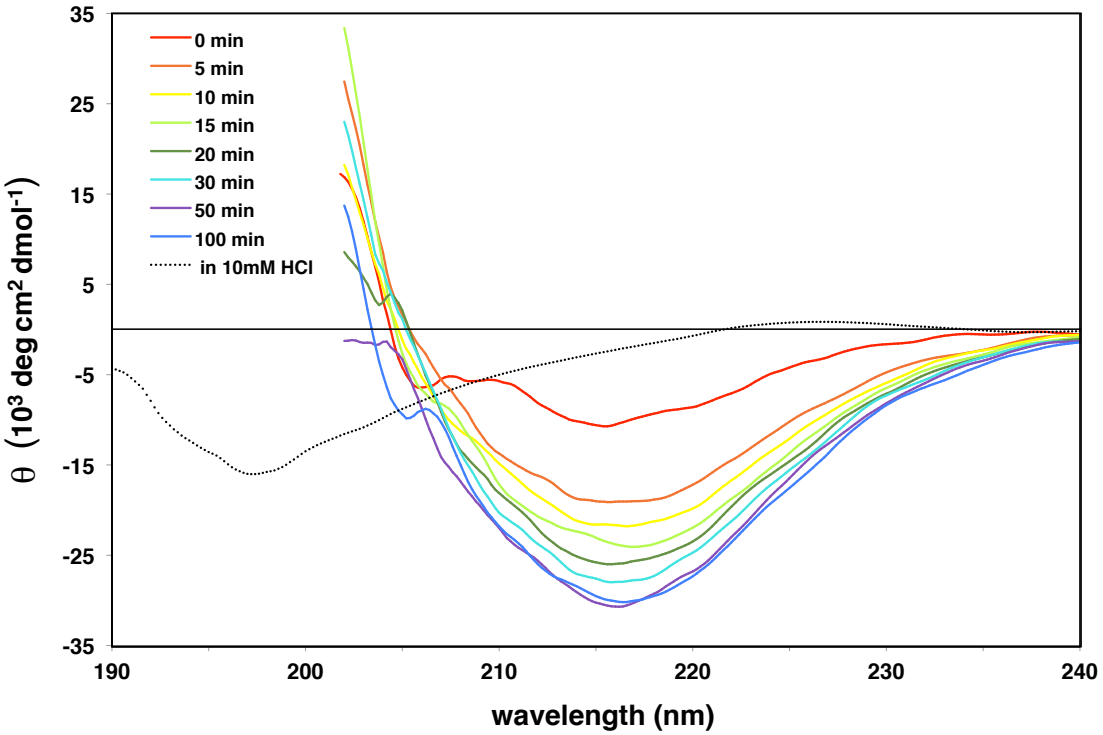
1. The conditions for fibrillization and 4NPA assay are the same for 1-32 except that the 4NPA assay was always at 30 °C and the divalent concentration was one-fourth as high. For condition 33, the sample was heated to 95 °C for 1 hour prior to the 4NPA assay and for 34-46 the samples were fibrillized in condition 1 or 2 and then transferred to the new conditions with additive just for the 4NPA assay.
2. Divalent concentration is 1 mM for growth and 250 μM for 4NPA assay.
3. Grown and measured in 50 mM buffer: HEPES at pH 7.3, NaOAc at pH 4.0, or phthalate at pH 2.2.

Figure A: Binary peptide mixtures.



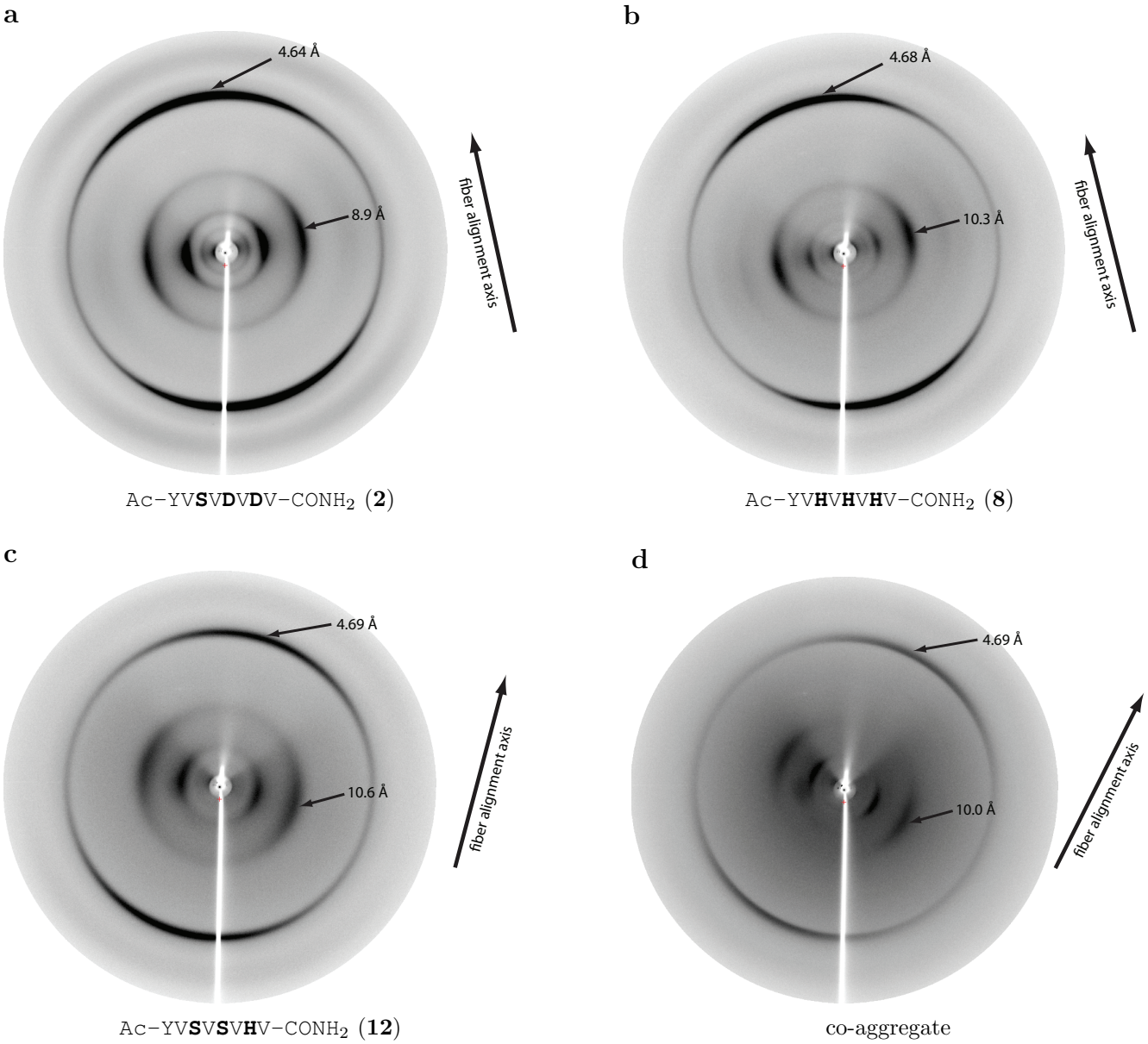
A line connecting the two peptides represents each of the 61 combinations.

Figure B:



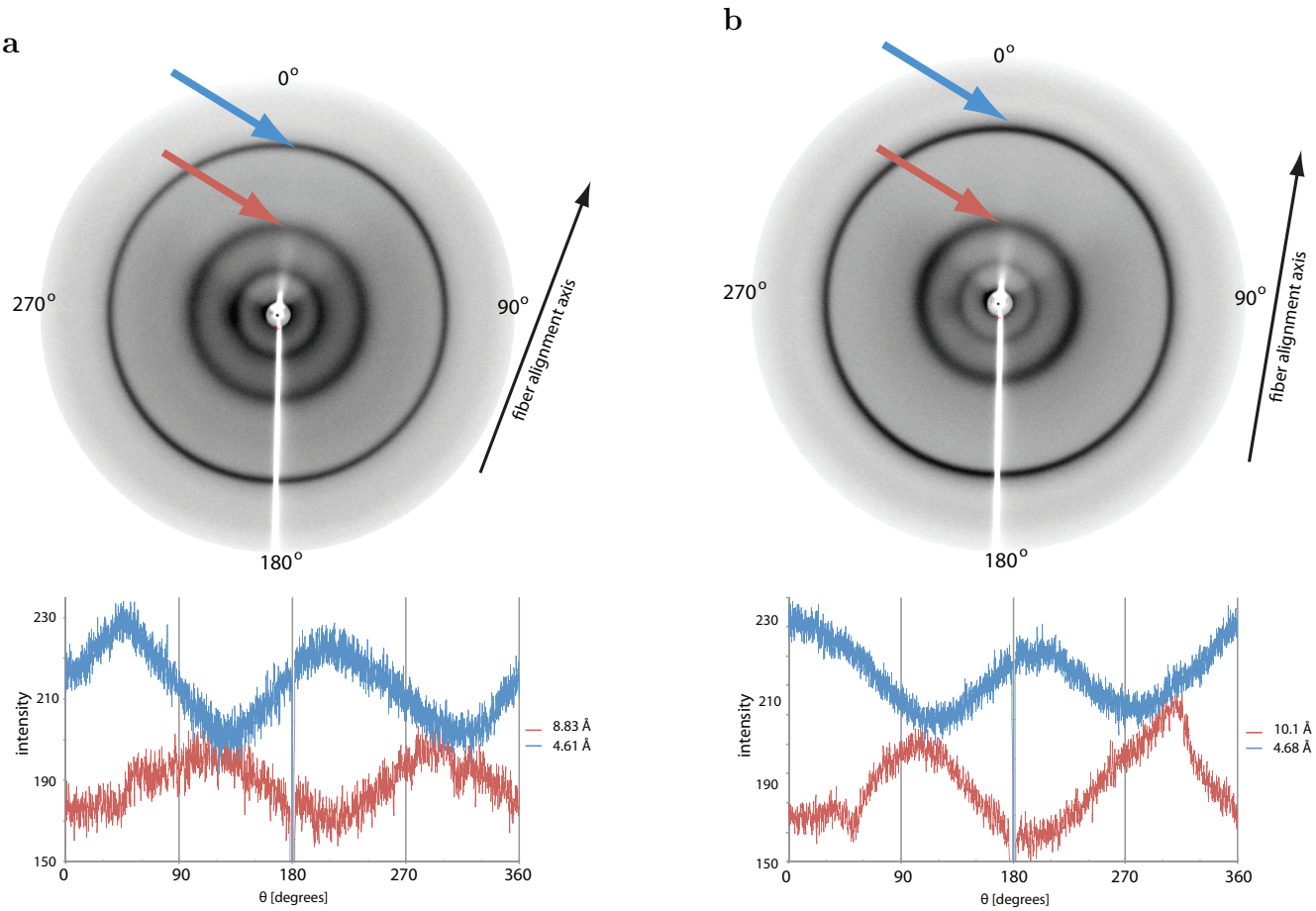
Appearance of β-structure for (**35**) at neutral pH. A 200 μM stock of **35** in 10 mM HCl was diluted to a final concentration of 50 μM into 10 mM HEPES pH 7.3 with 0.5 mM $ZnCl_2$ and the CD spectrum was measured every 5 min. The selected spectra (plotted in rainbow colors) show that the degree of β-structure increases over the first hour of aggregation. The spectrum of 50 μM **35** in 10 mM HCl (dotted line) is time-stable and appears to be mostly random coil.

Figure C:



X-ray diffraction images from aligned fibrils. The peptides **8** and **12** were fibrillized at pH 7.3, washed once in water, and then aligned by drying them between the ends of two glass rods. The peptide **2** in **a** and the co-aggregate of **12** and **21** (Ac–YV**H**V**H**V**D**V–CONH$_2$) in **d** were fibrillized at pH 4 and then washed once in 10 mM NaOAc pH 4 before being aligned by drying.

Figure D:



Ac–YV**H**V**S**V**S**V–CONH$_2$ (**14**)

Ac–YV**A**V**H**V**H**V–CONH$_2$ (**27**)

X-ray diffraction images from aligned fibrils with the plot of angular intensity. The fibrils were aligned similarly as in Figure C in S1 File. The diffraction pattern for fibrils of **14** and **27** at pH 7.3 were less clearly cross-β, possibly due to poor alignment. However, the cross-β pattern was present and is more easily visualized in the angular intensity distribution for the two major spacings.

References

[1] Tatulian, S. A. (2013). *Structural characterization of membrane proteins and peptides by FTIR and ATR-FTIR spectroscopy.* Methods in Molecular Biology (Clifton, N.J.), 974(Chapter 9), 177218. http://doi.org/10.1007/978-1-62703-275-9_9