

# Improving solid state properties of the side-chain peptide building blocks for the synthesis of GLP-1 analogs through their complexation with metal cations

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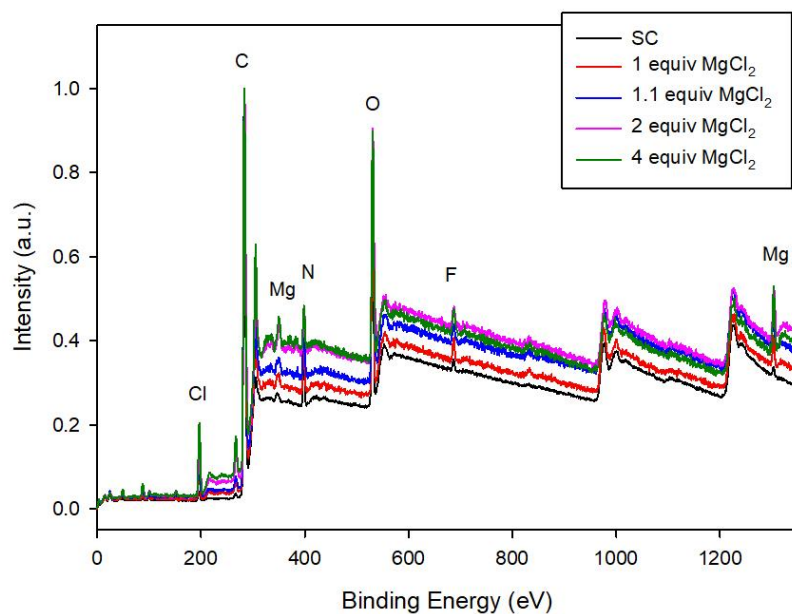


Figure S1. XPS total spectra of Tirzepatide SCBB and its complexes with  $\text{MgCl}_2$ .

Table S1. Atomic composition of the complexes Tirzepatide SCBB: $\text{MgCl}_2$ .

SCBB: $\text{MgCl}_2$ (equiv)		C, %	O, %	Mg, %	Cl, %	F, %	N, %	C/Mg	O/C	N/C	Cl/Mg
1:0	exp	75.18	17.73	-	0.87	0.80	5.42	-	0.24	0.07	-
	teor	64.73	20.90	-	-	-	5.72	-	0.32	0.09	-
1:1	exp	71.76	17.92	1.19	1.65	1.89	5.59	60.30	0.25	0.08	1.39
	teor	73.33	17.78	1.11	2.22	-	5.56	66.06	0.24	0.08	2
1:1.1	exp	72.74	17.36	1.22	1.48	1.61	5.59	59.62	0.24	0.08	1.21
	teor	73.09	17.72	1.22	2.44	-	5.54	59.91	0.24	0.08	2
1:2	exp	69.21	17.52	2.3	3.36	2.13	5.48	30.09	0.25	0.08	1.46
	teor	70.97	17.20	2.15	4.30	-	5.38	33.01	0.24	0.08	2
1:4	exp	68.09	17.16	3.28	4.55	2.23	4.69	20.76	0.25	0.07	1.39
	teor	66.67	16.16	4.04	8.08	-	5.05	16.50	0.24	0.08	2

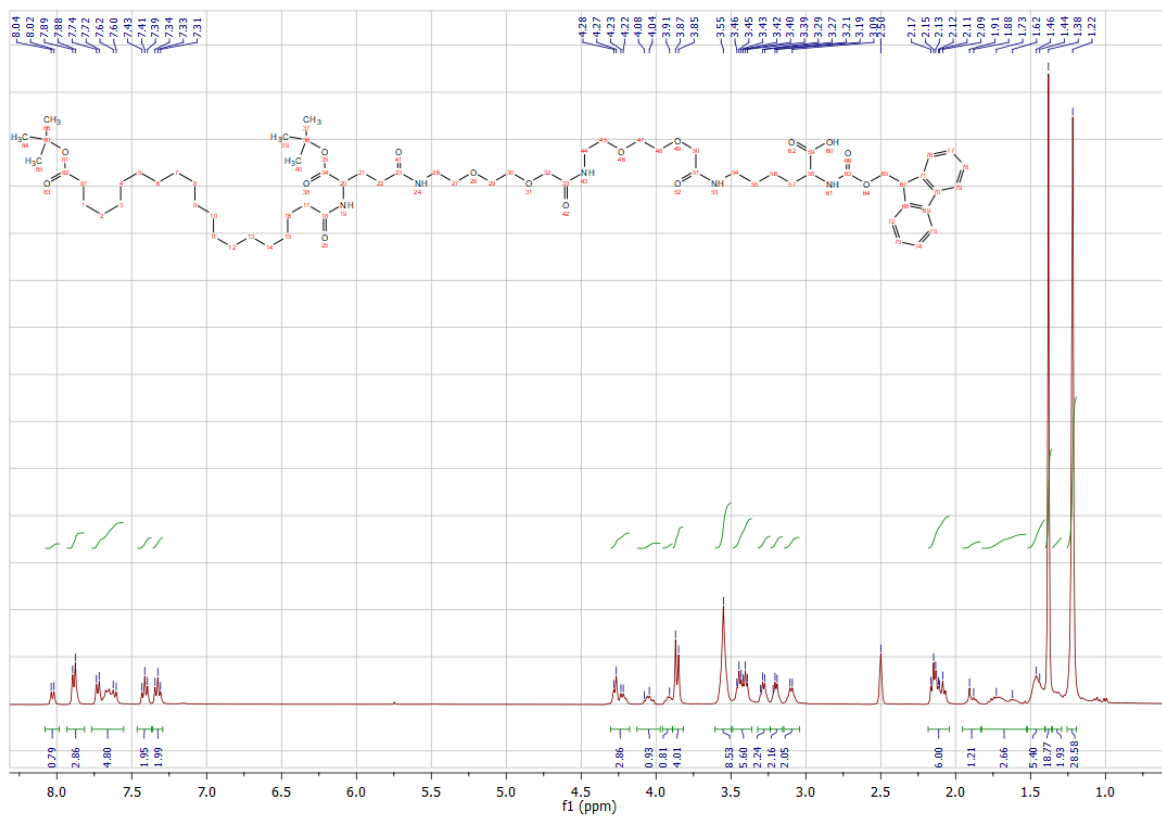


Figure S2.  $^1\text{H}$  NMR spectrum of Tirzepatide SCBB (400 MHz,  $\text{DMSO}-d_6$ ).

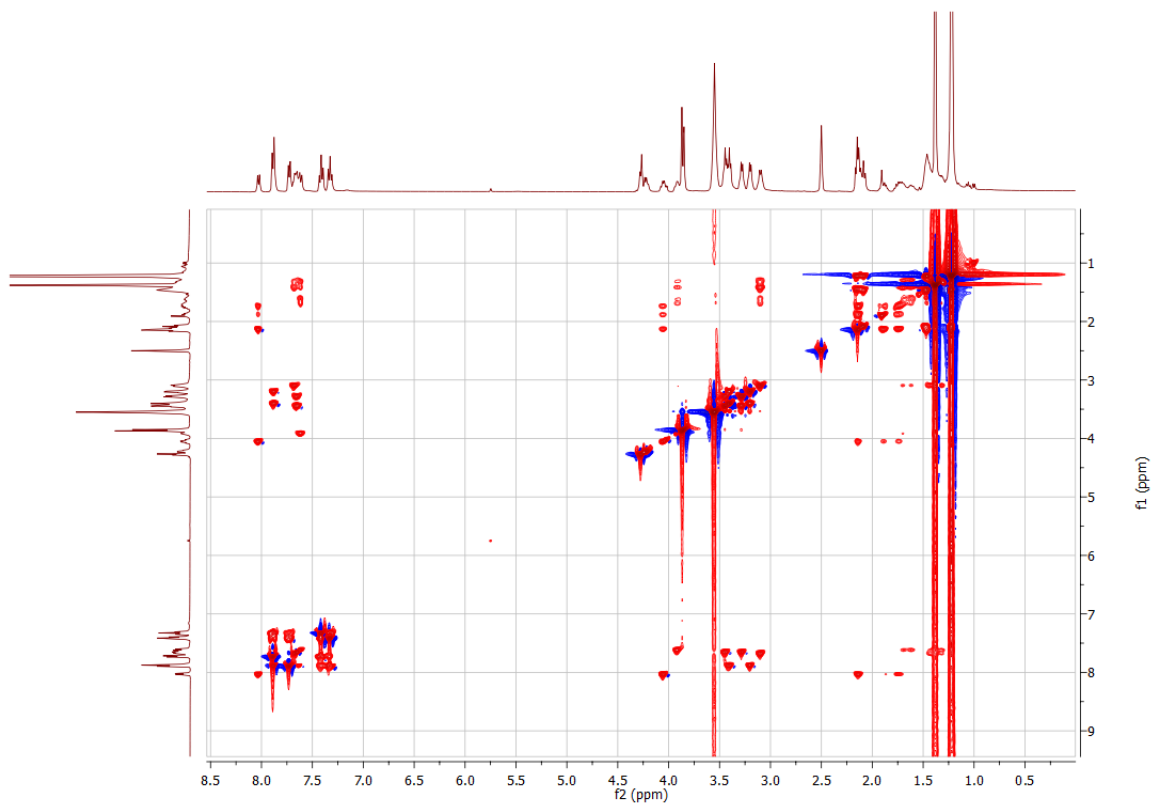


Figure S3. TOCSY spectrum of Tirzepatide SCBB (400 MHz,  $\text{DMSO}-d_6$ ).

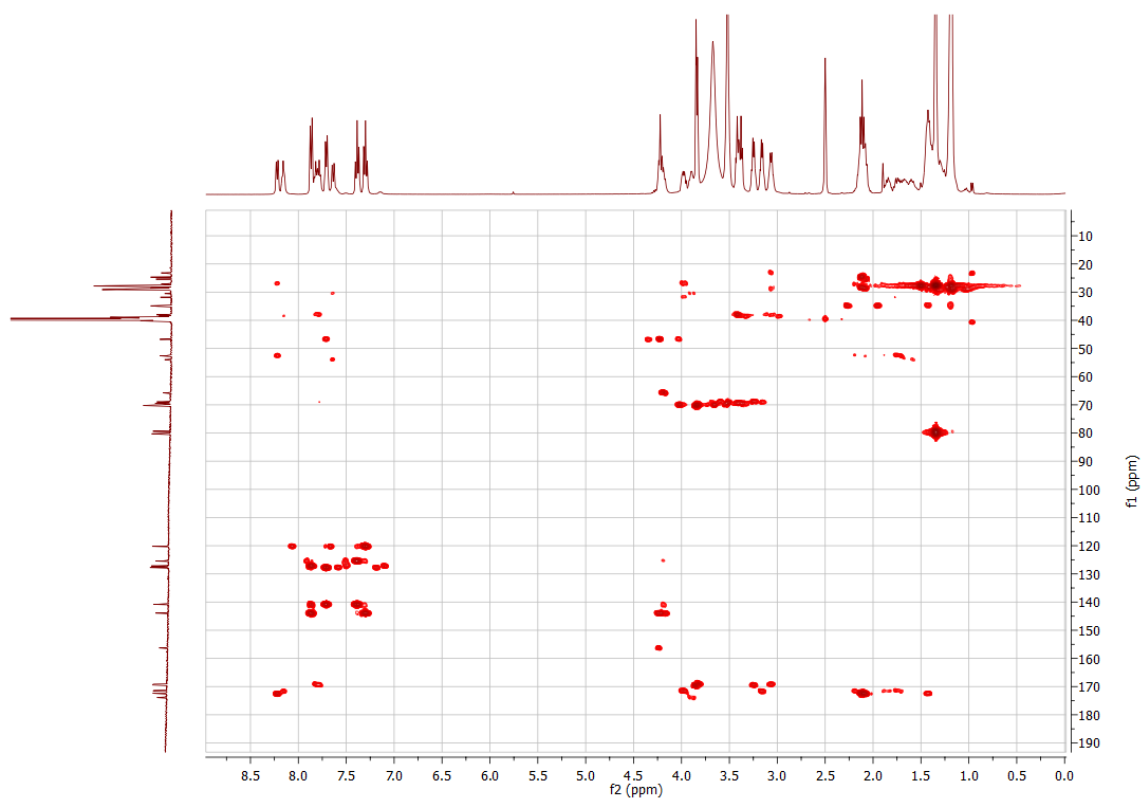


Figure S4. HMBC spectrum of Tirzepatide SCBB (400 MHz,  $\text{DMSO-}d_6$ ).

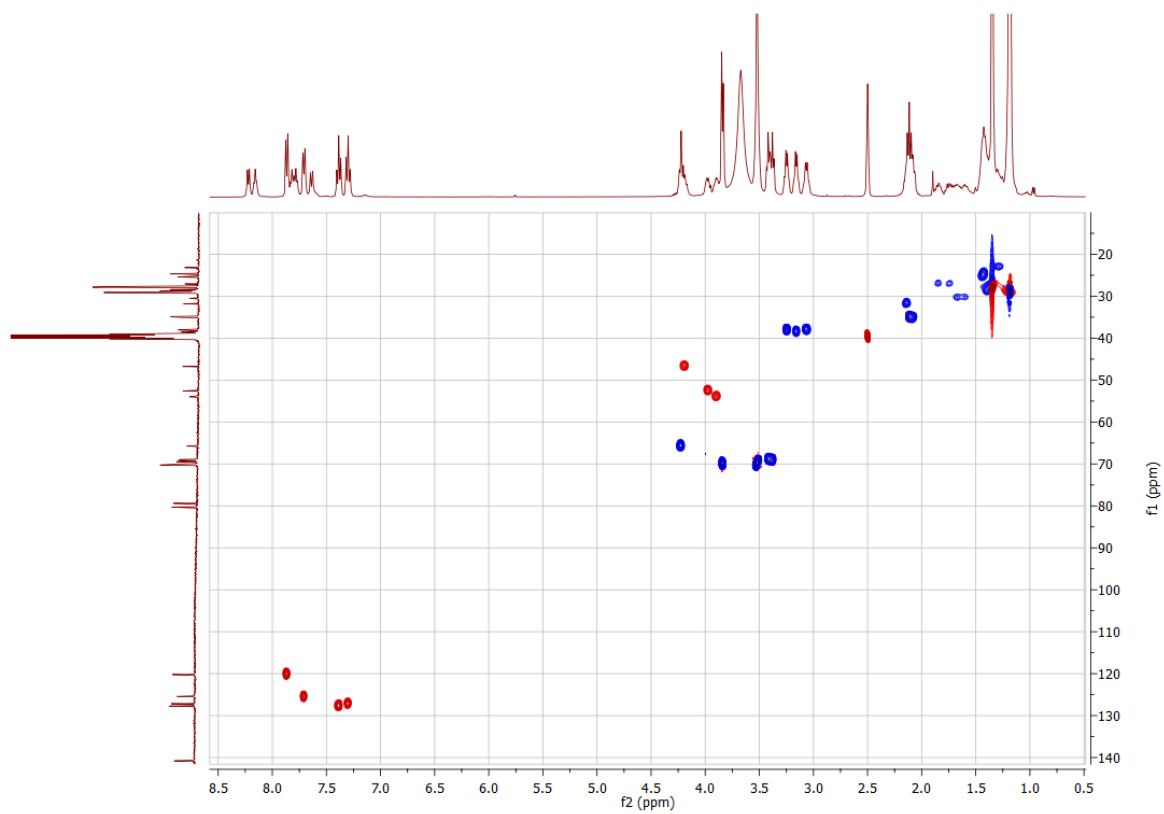
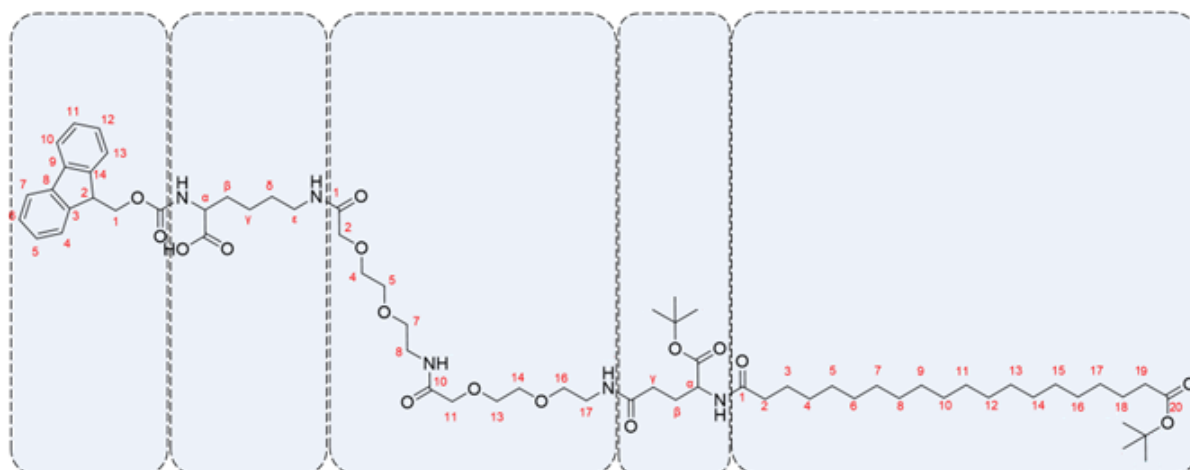


Figure S5. HSQC spectrum of Tirzepatide SCBB (400 MHz,  $\text{DMSO-}d_6$ ).

Table S2. <sup>1</sup>H and <sup>13</sup>C chemical shifts of Tirzepatide SCBB in DMSO-*d*<sub>6</sub>



	NH	C $\alpha$	H $\alpha$	C $\beta$	H $\beta$	C $\gamma$	H $\gamma$	C $_{other}$	H $_{other}$
Fmoc								C1: 65.6 C2: 46.6 C3, C14: 144.0 C4, C13: 125.6 C5, C12: 127.3 C6, C11: 127.7 C7, C10: 120.2 C8, C9: 140.1 C $_{CO}$ : 156.2	H1: 4.27 H2: 4.22 H4, H13: 7.73 H5, H12: 7.33 H6, H11: 7.42 H7, H10: 7.88
Lys	$\alpha$ NH: 7.61 $\epsilon$ NH: 7.67	53.9	3.91	30.23	1.62 1.70	23.05	1.46 1.38	C $\delta$ : 24.8 C $\epsilon$ : 38.1 $\alpha$ C $_{CO}$ : 173.9	H $\delta$ : 1.42 H $\epsilon$ : 3.10
AEEA1	7.87							C11: 70.5 C13: 69.6 C14: 69.6 C16: 69.0 C17: 38.6 C10: 169.0	H11: 3.85 H13: 3.55 H14: 3.55 H16: 3.40 H17: 3.21
AEEA2	7.65							C2: 70.5 C4: 69.6 C5: 69.6 C7: 69.0 C8: 38.1 C1: 169.8	H2: 3.87 H4: 3.55 H5: 3.55 H7: 3.45 H8: 3.29
Glu	8.03	52.4	4.05	27.0	1.75 1.90	31.7	2.15	C $_{tBu(Me)}$ : 28.1 $\alpha$ C $_{CO}$ : 171.5 $\gamma$ C $_{CO}$ : 171.7 C $_{tBu}$ : 80.0	H $_{tBu}$ : 1.38
C20								C2: 35.2 C3: 24.8 C4-17: 28.9 C18: 24.8 C19: 35.2 C $_{tBu(Me)}$ : 28.1 C1: 172.5 C20: 172.5 C $_{tBu}$ : 80.0	H2: 2.15 H3: 1.46 H4-17: 1.22 H18: 1.46 H19: 2.08 H $_{tBu}$ : 1.38

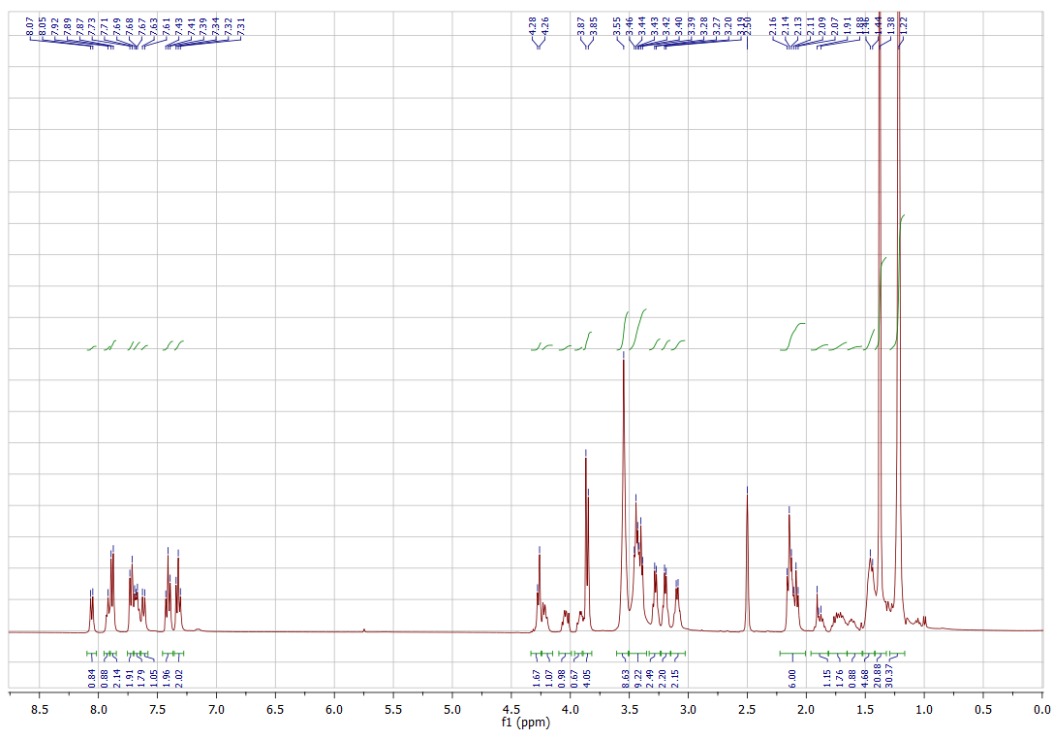


Figure S6. <sup>1</sup>H NMR spectrum of Tirzepatide SCBB after addition of 0.5 equiv. of MgCl<sub>2</sub> (400 MHz, DMSO-d<sub>6</sub>).

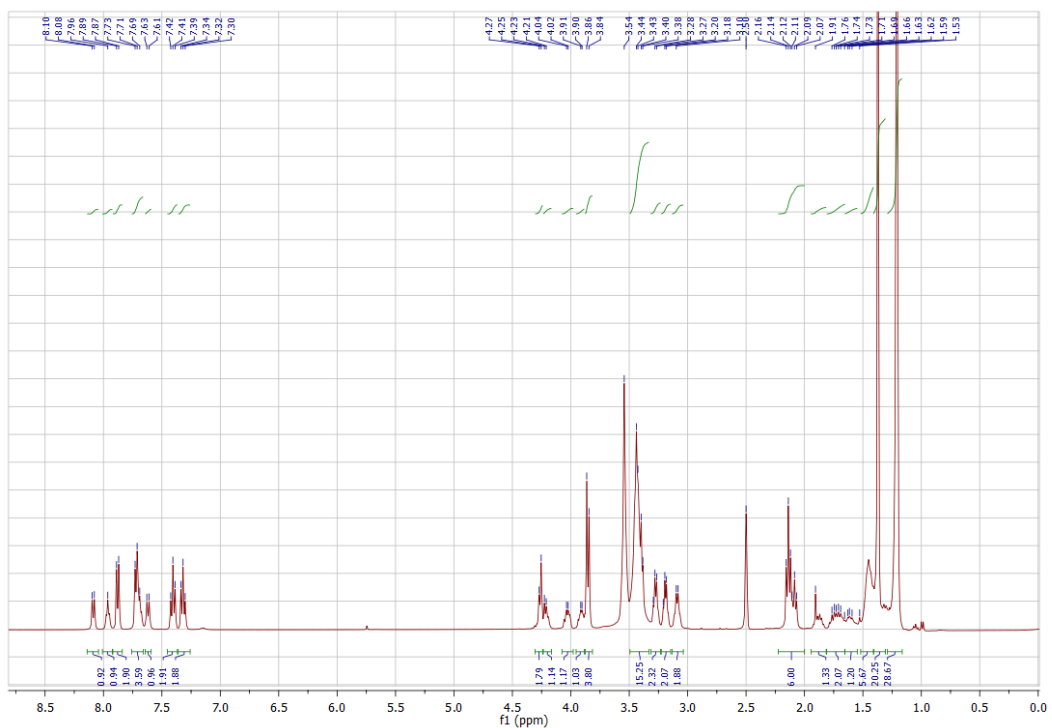


Figure S7. <sup>1</sup>H NMR spectrum of Tirzepatide SCBB after addition of 1 equiv. of MgCl<sub>2</sub> (400 MHz, DMSO-d<sub>6</sub>).

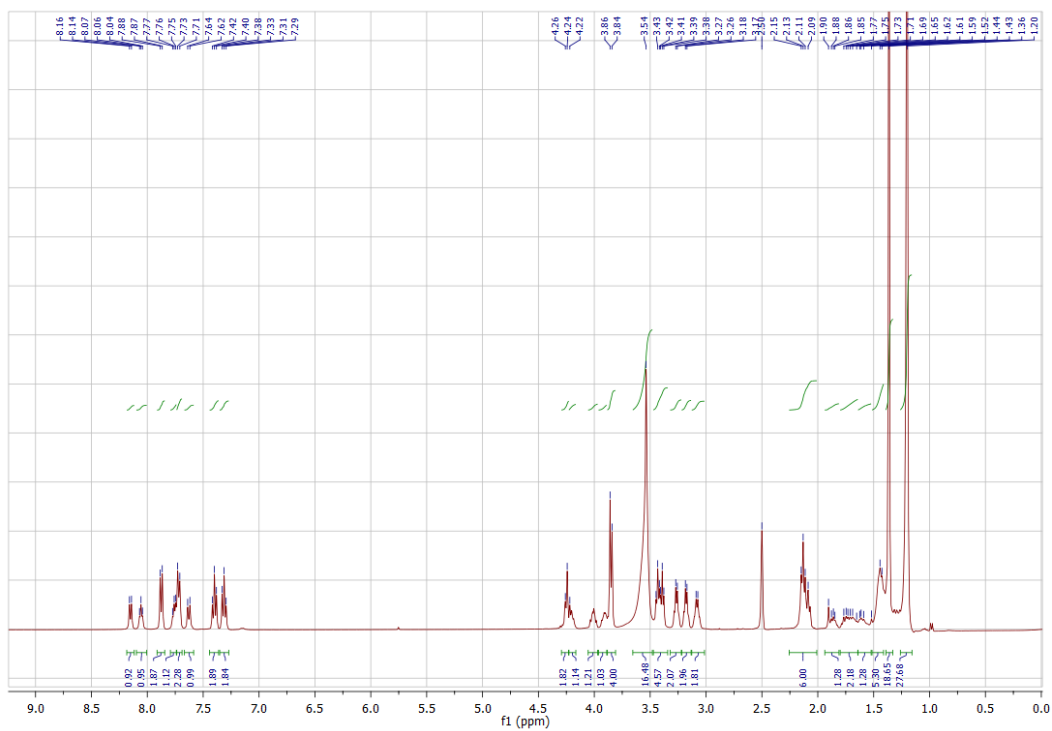


Figure S8. <sup>1</sup>H NMR spectrum of Tirzepatide SCBB after addition of 2 equiv. of MgCl<sub>2</sub> (400 MHz, DMSO-*d*<sub>6</sub>).

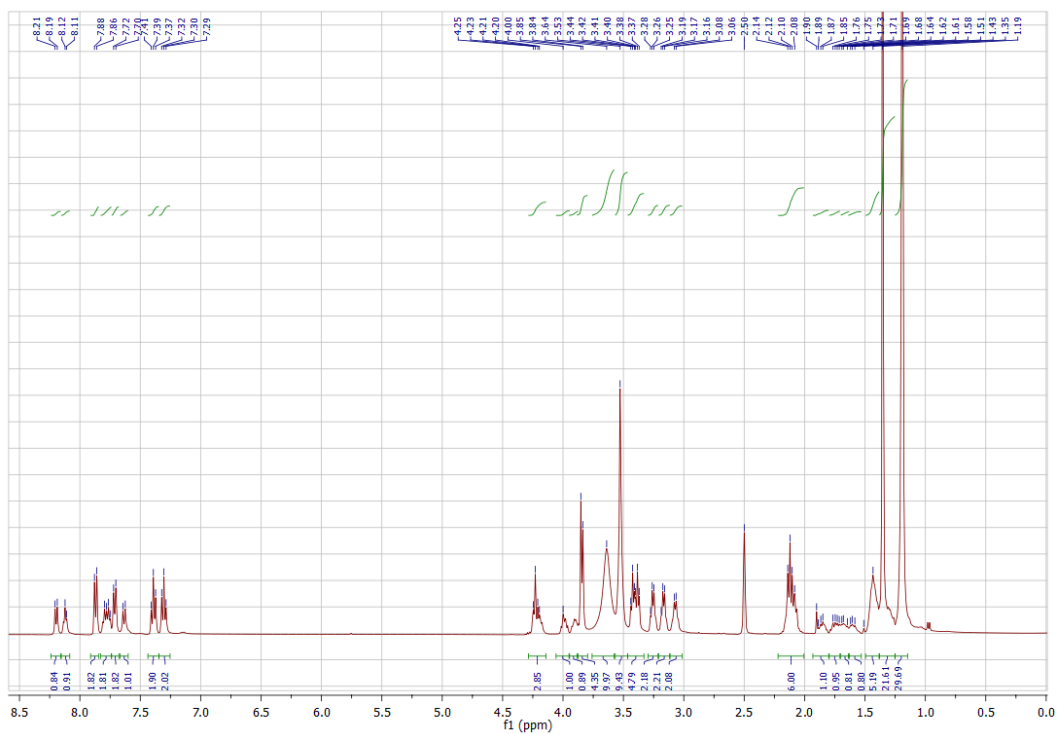


Figure S9. <sup>1</sup>H NMR spectrum of Tirzepatide SCBB after addition of 3 equiv. of MgCl<sub>2</sub> (400 MHz, DMSO-*d*<sub>6</sub>).

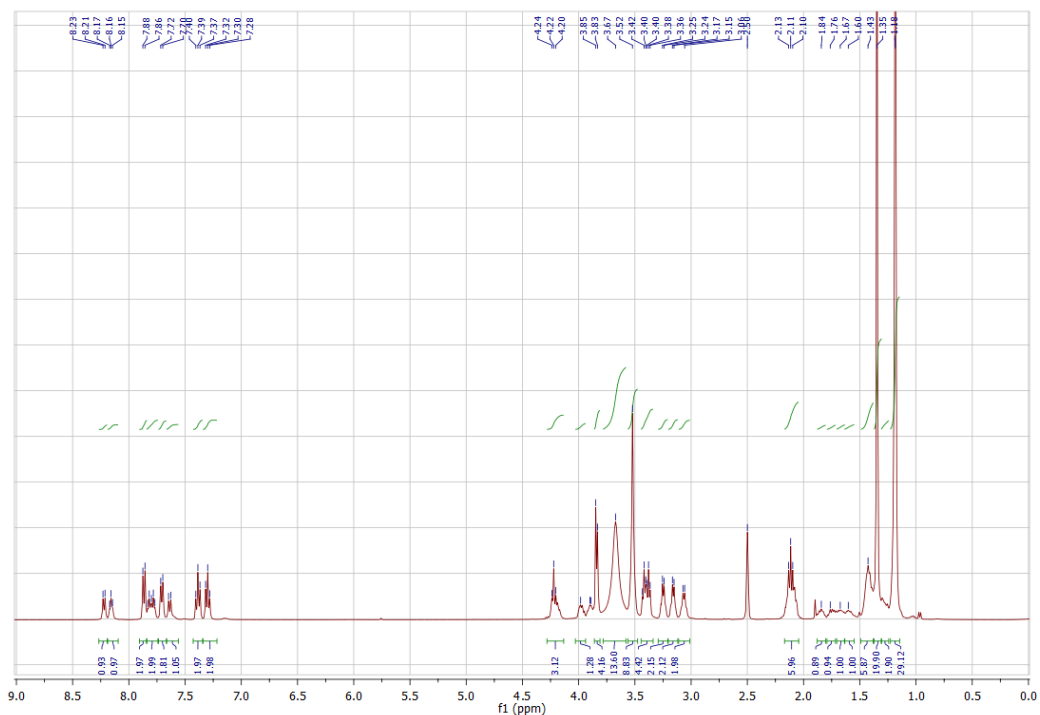


Figure S10.  $^1\text{H}$  NMR spectrum of Tirzepatide SCBB after addition of 4 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).

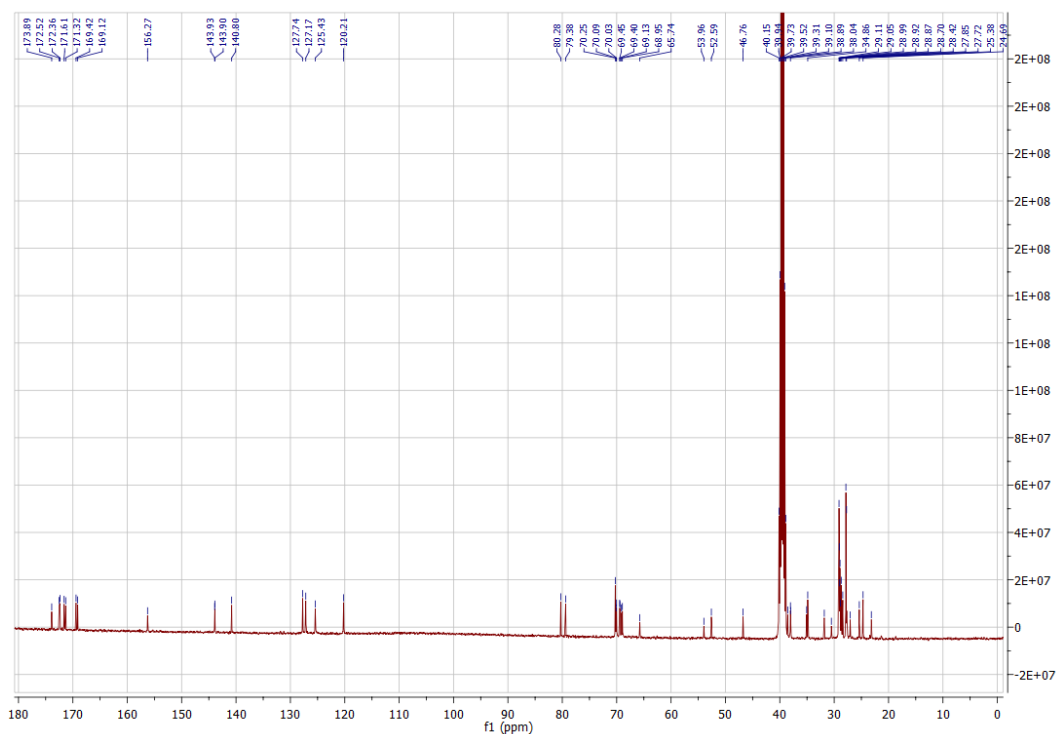


Figure S11.  $^{13}\text{C}$  NMR spectrum of Tirzepatide SCBB after addition of 4 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).



Figure S12. TOCSY spectrum of Tirzepatide SCBB after addition of 4 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).

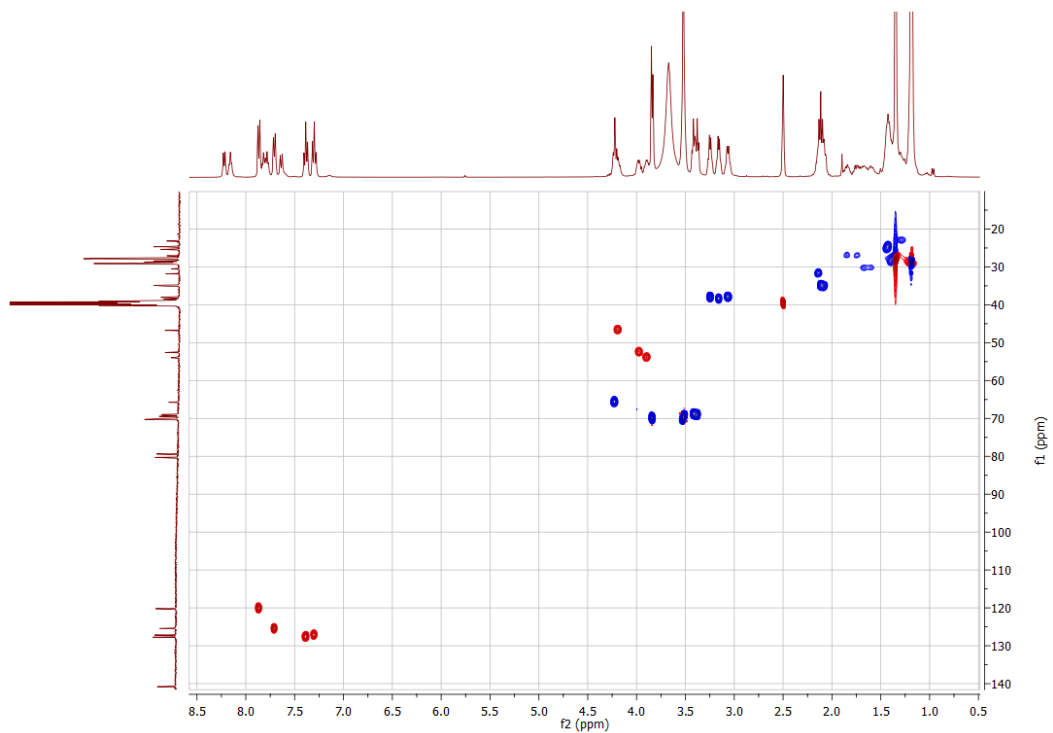


Figure S13. HSQC spectrum of Tirzepatide SCBB after addition of 4 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).

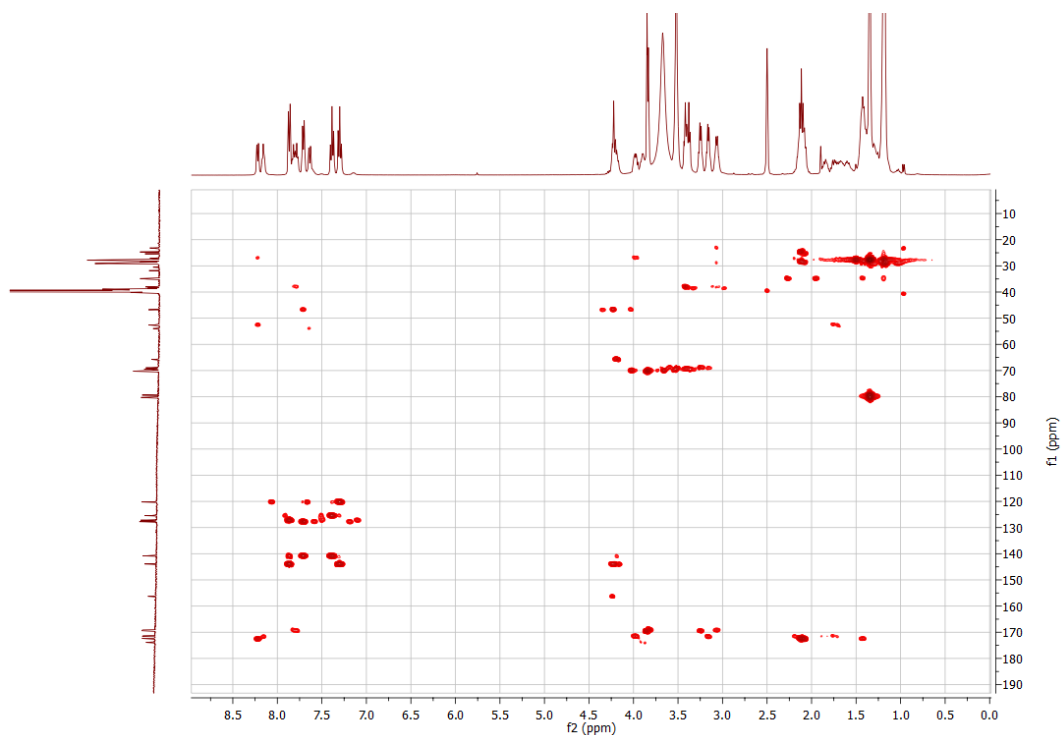


Figure S14. HMBC spectrum of Tirzepatide SCBB after addition of 4 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).

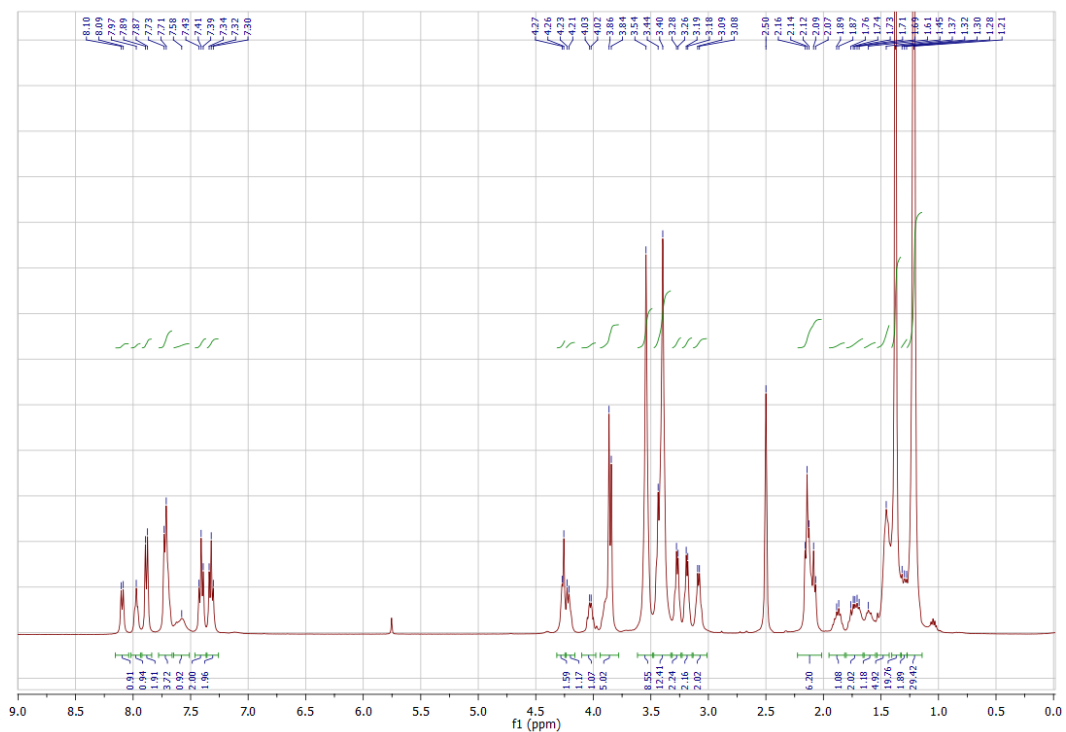


Figure S15.  $^1\text{H}$  NMR spectrum of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  (400 MHz,  $\text{DMSO-}d_6$ ).







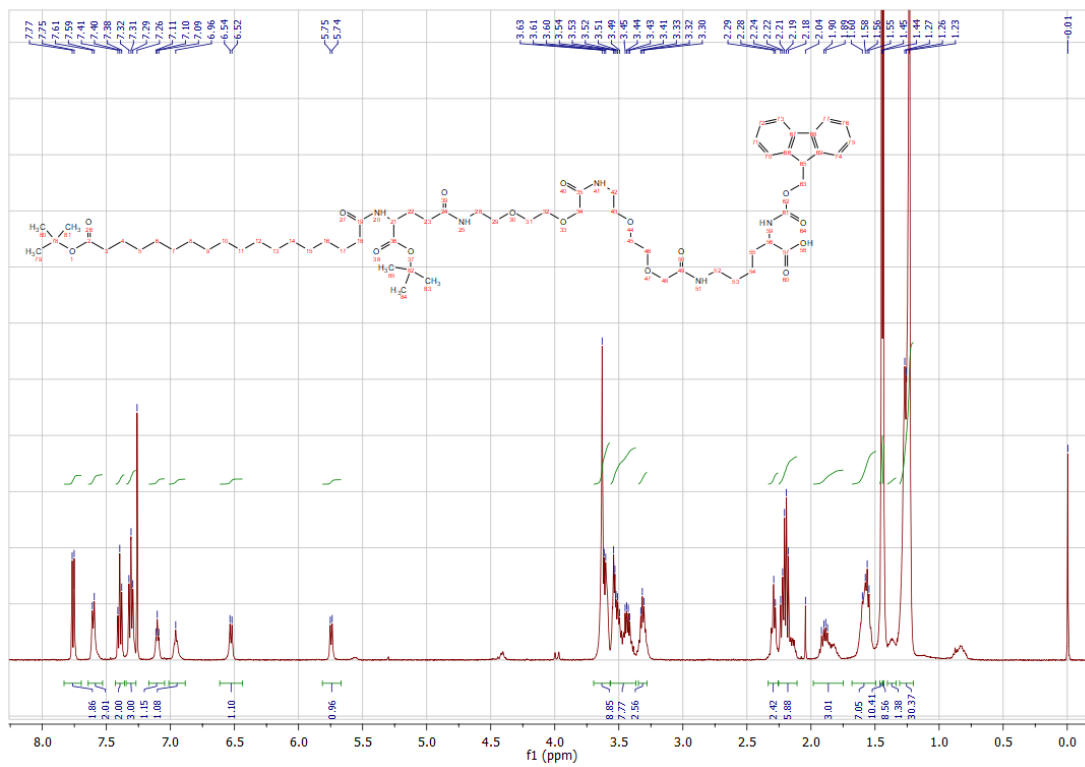


Figure S22.  $^1\text{H}$  NMR spectrum of Semaglutide SCBB (600 MHz,  $\text{CDCl}_3$ ).

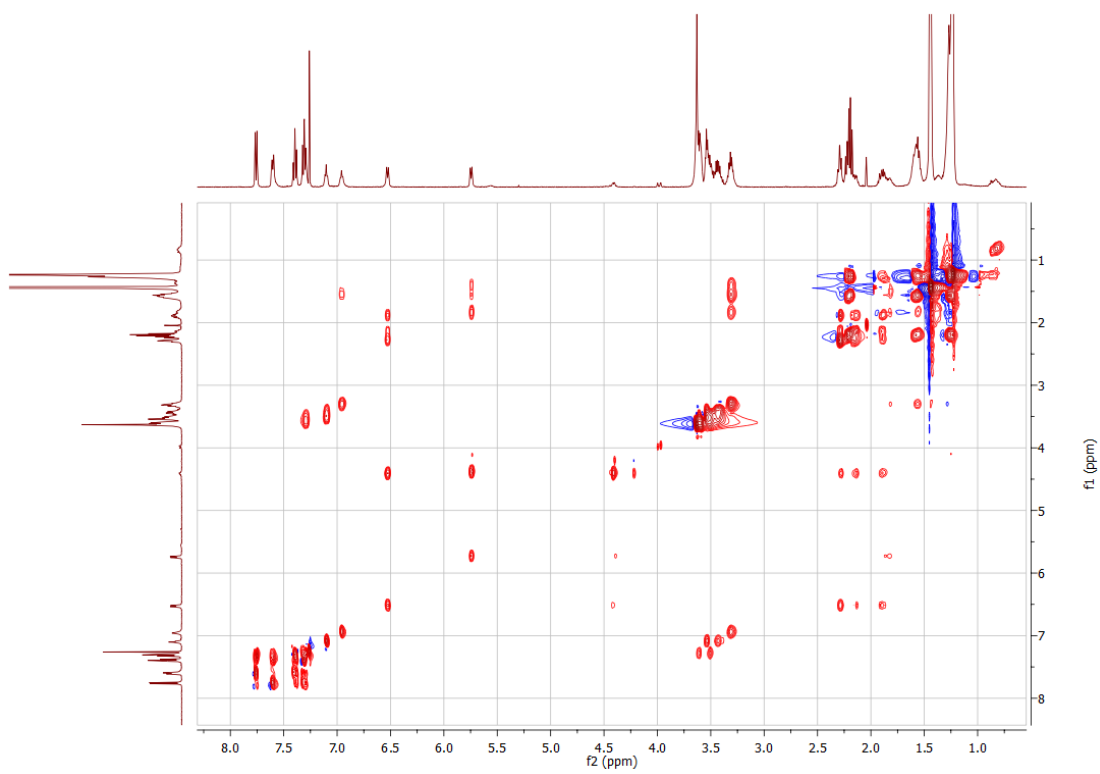


Figure S23. TOCSY spectrum of Semaglutide SCBB (600 MHz,  $\text{CDCl}_3$ ).

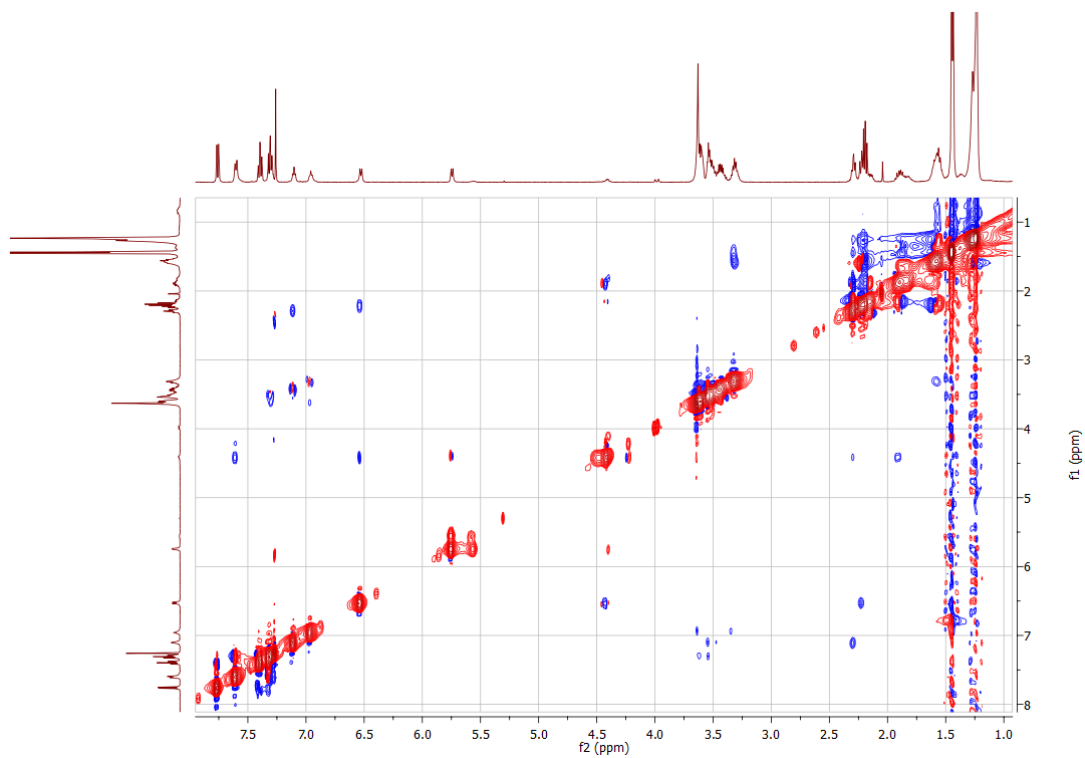


Figure S24. NOESY spectrum of Semaglutide SCBB (600 MHz,  $\text{CDCl}_3$ ).

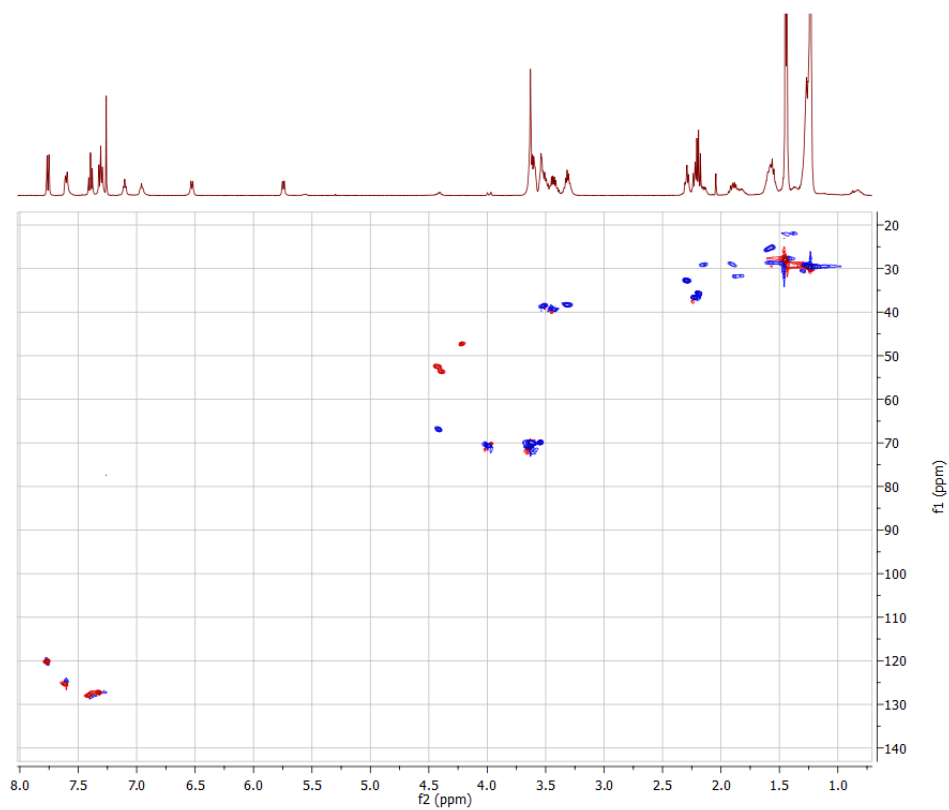
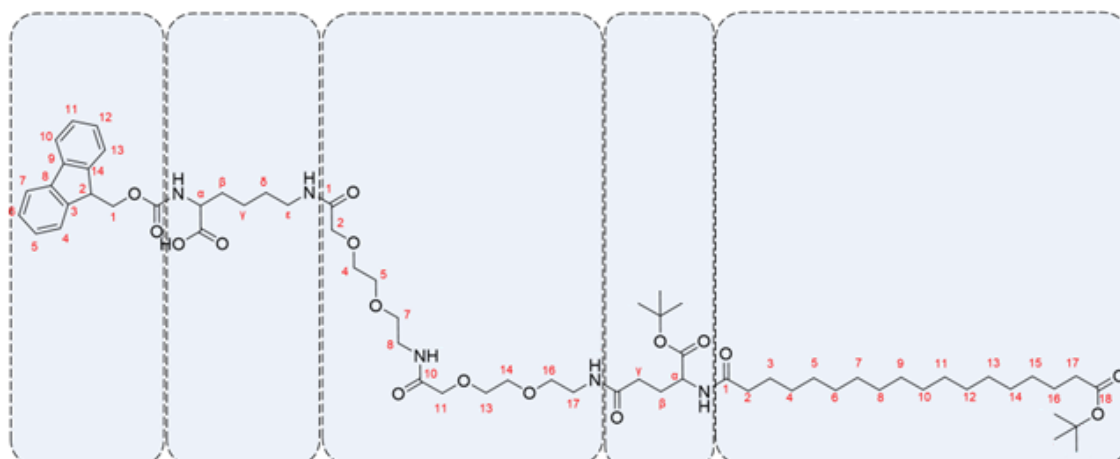


Figure S25. HSQC spectrum of Semaglutide SCBB (600 MHz,  $\text{CDCl}_3$ ).

Table S3.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of Semaglutide SCBB in  $\text{CDCl}_3$



	NH	C $\alpha$	H $\alpha$	C $\beta$	H $\beta$	C $\gamma$	H $\gamma$	C $\text{other}$	H $\text{other}$
Fmoc								C1: 66.8 C2: 47.2 C4, C13: 125.2 C5, C12: 127.1 C6, C11: 127.7 C7, C10: 120.0	H1: 4.42 H2: 4.22 H4: 7.61 H5: 7.32 H6: 7.40 H7: 7.76
Lys	$\alpha\text{NH}$ : 5.75 $\epsilon\text{NH}$ : 6.97	53.5	4.38	31.7	1.87 1.82	21.9	1.44 1.38	C $\delta$ : 28.6 C $\epsilon$ : 38.3	H $\delta$ : 1.57 H $\epsilon$ : 3.32
AEEA1	7.11							C11: 70.4 C13: 69.9 C14: 70.9 C16: 69.9 C17: 39.1	H11: 4.00 H13: 3.61 H14: 3.65 H16: 3.54 H17: 3.43
AEEA2	7.30							C2: 70.5 C4: 69.8 C5: 70.9 C7: 69.9 C8: 38.5	H2: 3.97 H4: 3.64 H5: 3.65 H7: 3.55 H8: 3.51
Glu	6.53	52.4	4.42	29.1	2.15 1.90	32.7	2.29	C $\text{tBu(Me)}$ : 27.8	H $\text{tBu}$ : 1.46
C18								C2: 36.6 C3: 25.6 C4: 29.4 C5: 29.2 C6: 29.6 C7-13: 29.6 C14: 29.1 C15: 29.4 C16: 25.5 C17: 35.6 C $\text{tBu(Me)}$ : 28.2	H2: 2.23 H3: 1.60 H4: 1.25 H5: 1.28 H6: 1.24 H7-13: 1.24 H14: 1.27 H15: 1.26 H16: 1.57 H17: 2.20 H $\text{tBu}$ : 1.44

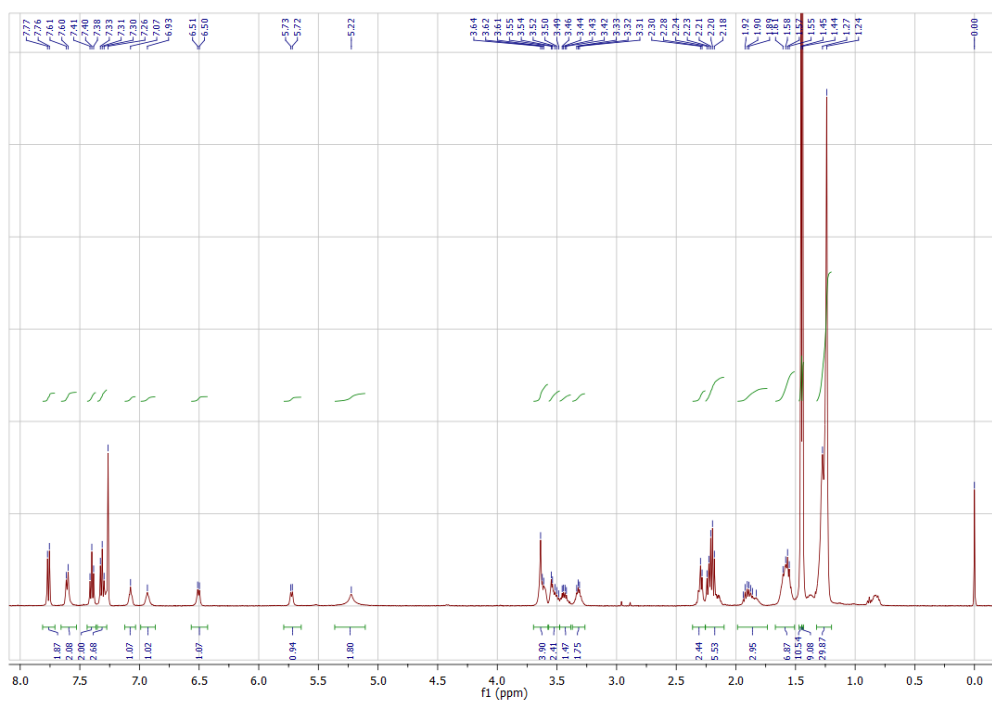


Figure S26.  $^1\text{H}$  NMR spectrum of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

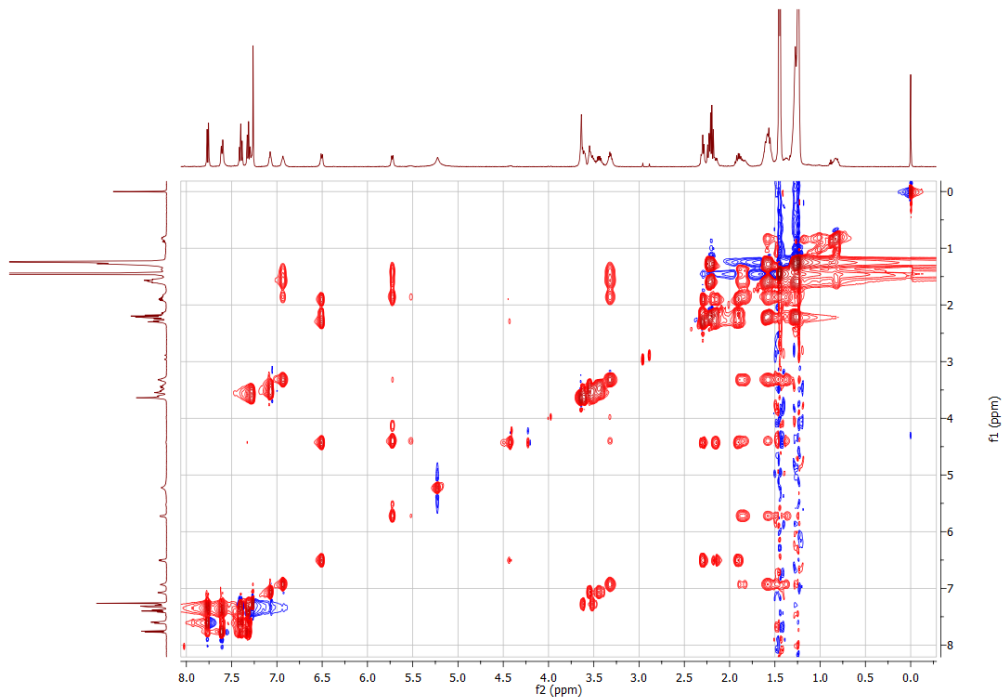


Figure S27. TOCSY spectrum of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

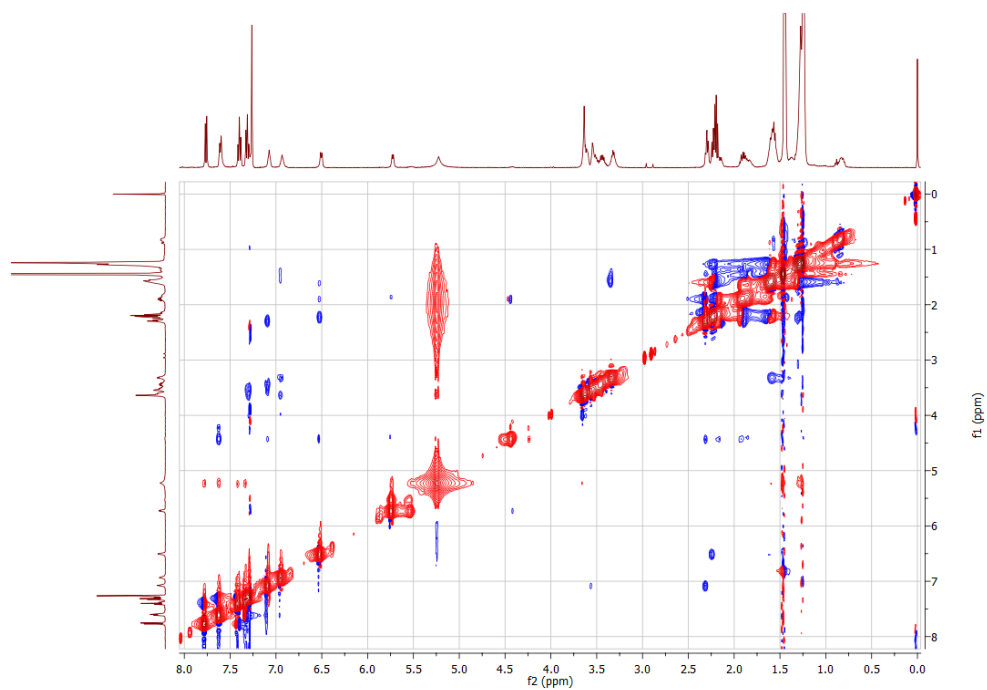


Figure S28. NOESY spectrum of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

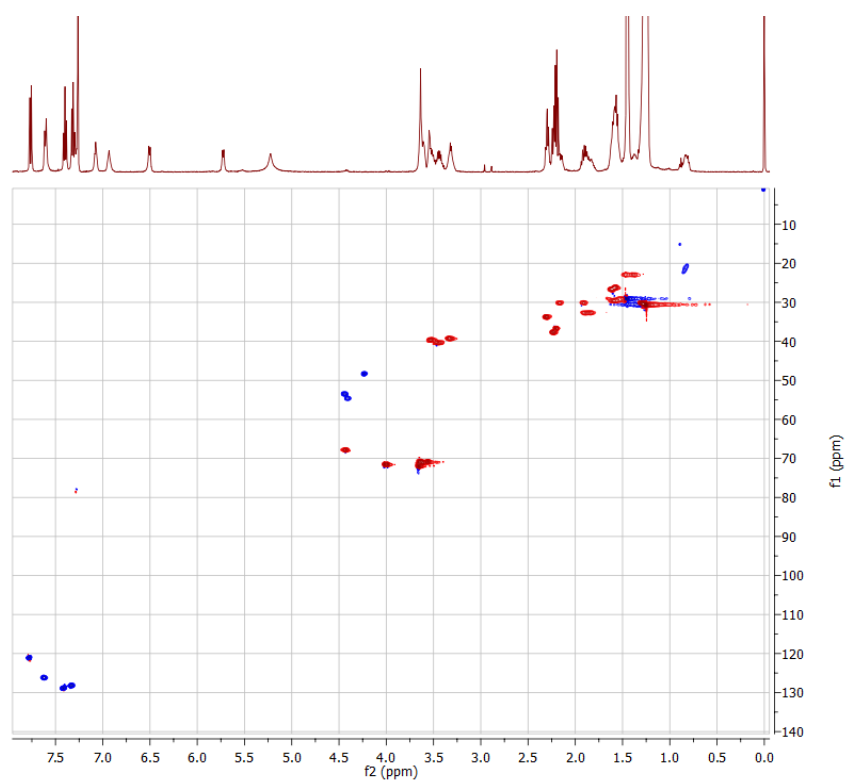
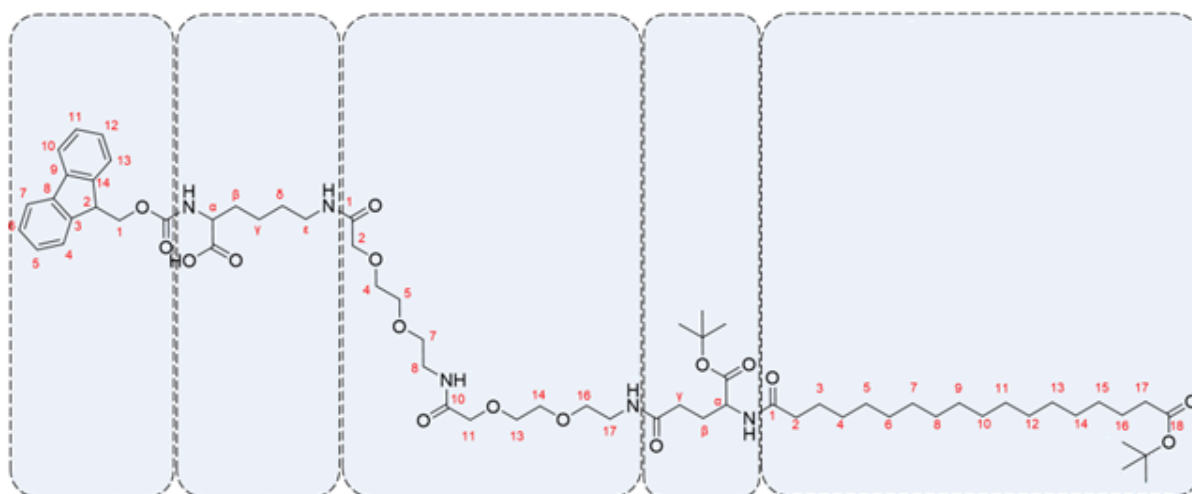


Figure S29. HSQC spectrum of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

Table S4.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of the complex of Semaglutide SCBB with 1 equiv. of  $\text{MgCl}_2$  in  $\text{CDCl}_3$



	NH	C $\alpha$	H $\alpha$	C $\beta$	H $\beta$	C $\gamma$	H $\gamma$	C $_{other}$	H $_{other}$
Fmoc								C1: 66.8 C2: 47.3 C4, C13: 125.0 C5, C12: 127.1 C6, C11: 127.7 C7, C10: 120.0	H1: 4.42 H2: 4.22 H4: 7.60 H5: 7.31 H6: 7.40 H7: 7.76
Lys	$\alpha\text{NH}$ : 5.73 $\epsilon\text{NH}$ : 6.93	53.5	4.41	31.7	1.89 1.83	21.9	1.46 1.37	C $\delta$ : 28.5 C $\epsilon$ : 38.2	H $\delta$ : 1.57 H $\epsilon$ : 3.32
AEEA1	7.27							C11: 70.5 C13: 70.0 C14: 70.9 C16: 69.8 C17: 39.3	H11: 4.00 H13: 3.61 H14: 3.64 H16: 3.55 H17: 3.44
AEEA2	7.07							C2: 70.5 C4: 69.9 C5: 70.9 C7: 69.9 C8: 38.6	H2: 3.97 H4: 3.64 H5: 3.64 H7: 3.55 H8: 3.51
Glu	6.51	52.4	4.43	29.1	2.16 1.90	32.7	2.30	C $_{t\text{Bu(Me)}}$ : 28.0	H $_{t\text{Bu}}$ : 1.46
C18								C2: 36.6 C3: 25.7 C4: 29.6 C5: 29.4 C6: 29.8 C7-13: 29.8 C14: 29.3 C15: 29.5 C16: 25.2 C17: 35.7 C $_{t\text{Bu(Me)}}$ : 28.2	H2: 2.22 H3: 1.61 H4: 1.23 H5: 1.27 H6: 1.24 H7-13: 1.24 H14: 1.28 H15: 1.25 H16: 1.57 H17: 2.19 H $_{t\text{Bu}}$ : 1.43

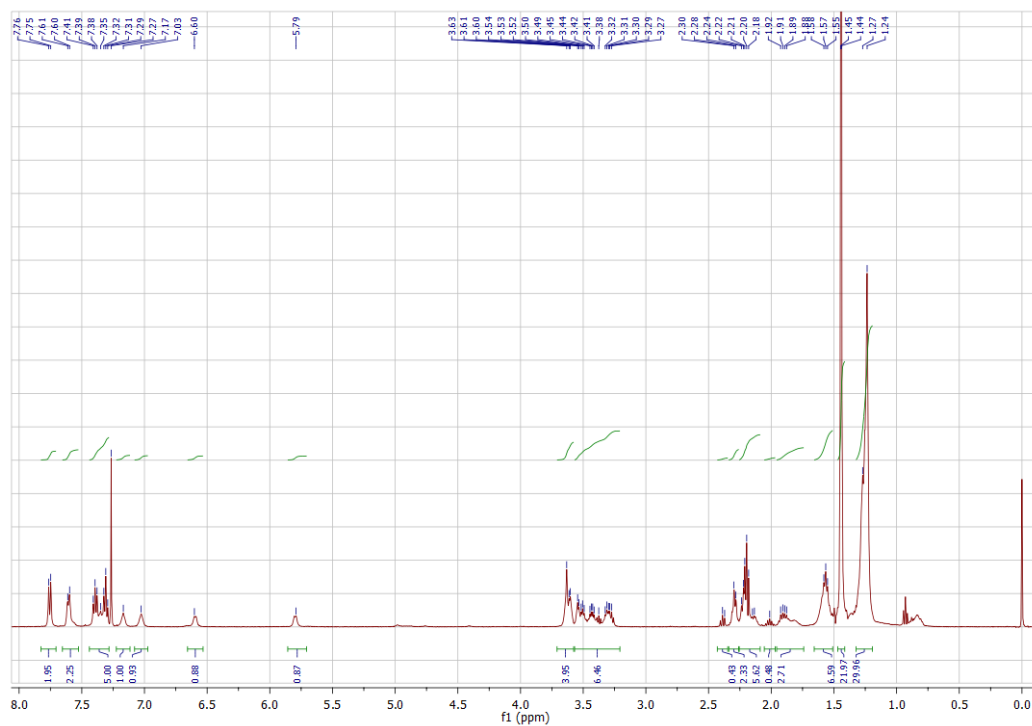


Figure S30.  $^1\text{H}$  NMR spectrum of the complex of Semaglutide SCBB with 2 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

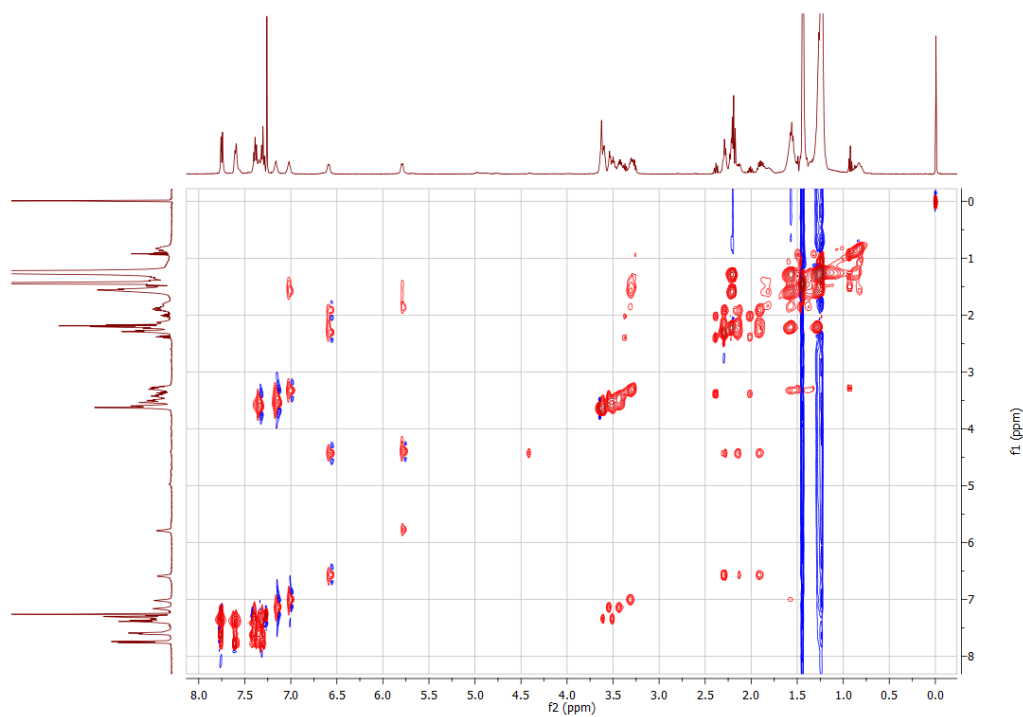


Figure S31. TOCSY spectrum of the complex of Semaglutide SCBB with 2 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

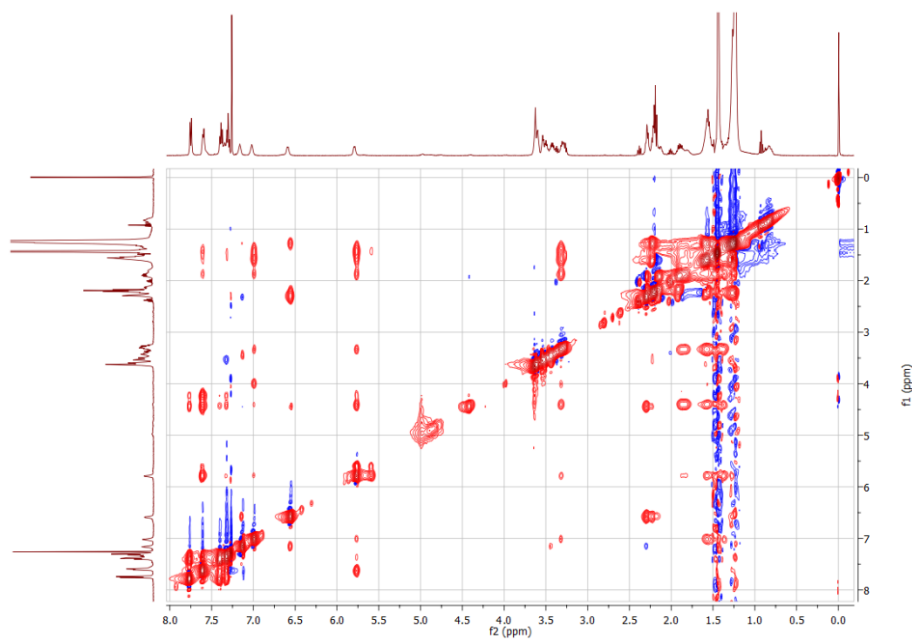


Figure S32. NOESY spectrum of the complex of Semaglutide SCBB with 2 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

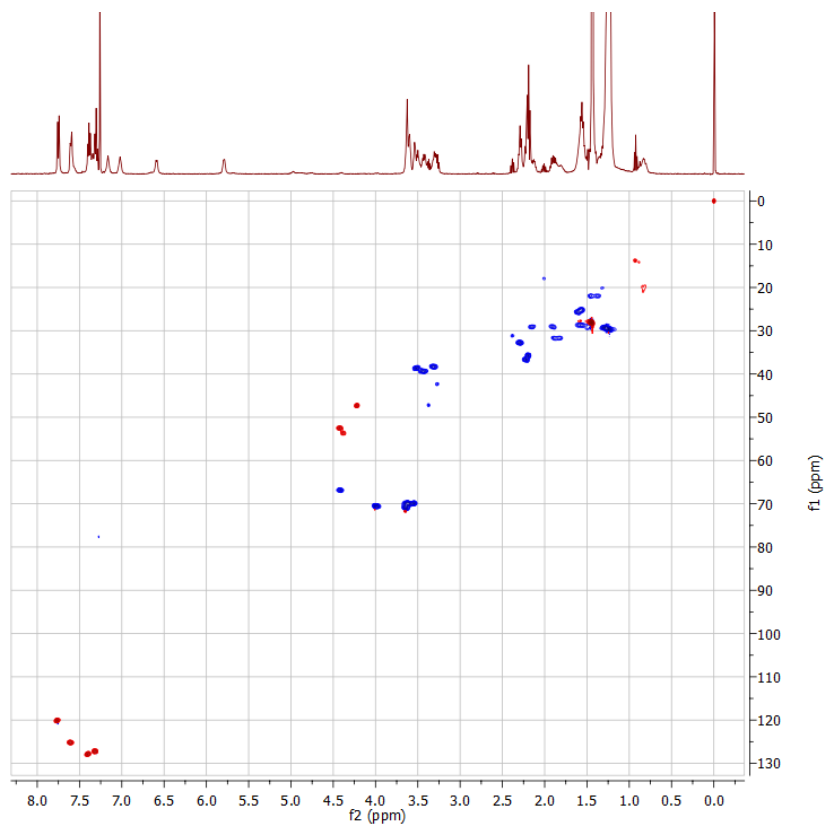
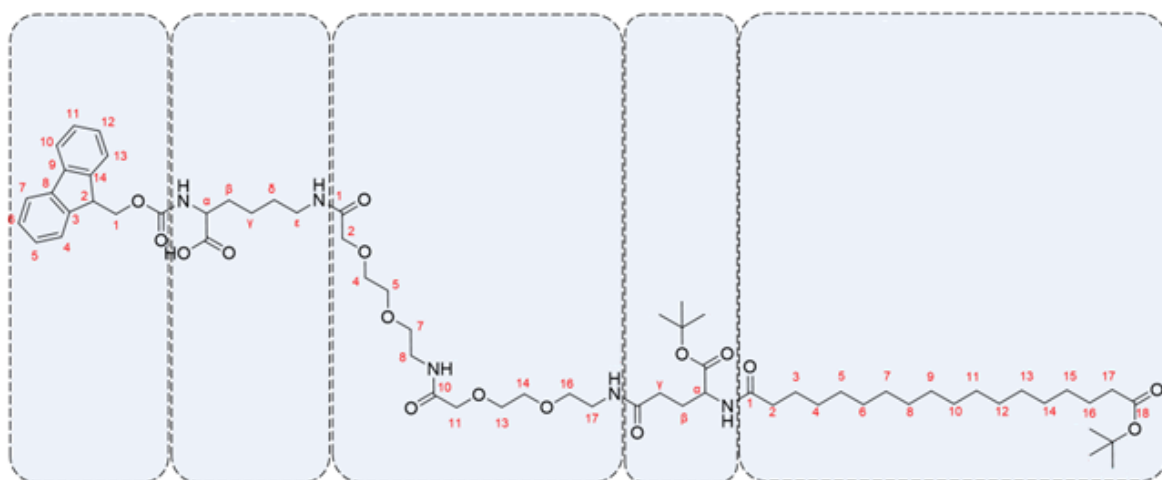


Figure S33. HSQC spectrum of the complex of Semaglutide SCBB with 2 equiv. of  $\text{MgCl}_2$  (600 MHz,  $\text{CDCl}_3$ ).

Table S5.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of the complex of Semaglutide SCBB with 2 equiv. of  $\text{MgCl}_2$  in  $\text{CDCl}_3$



	NH	C $\alpha$	H $\alpha$	C $\beta$	H $\beta$	C $\gamma$	H $\gamma$	C $_{other}$	H $_{other}$
Fmoc								C1: 66.8 C2: 47.2 C4, C13: 125.0 C5, C12: 127.1 C6, C11: 127.7 C7, C10: 120.0	H1: 4.43 H2: 4.22 H4: 7.60 H5: 7.32 H6: 7.40 H7: 7.76
Lys	$\alpha\text{NH}$ : 5.80 $\epsilon\text{NH}$ : 7.03	53.6	4.39	31.7	1.87 1.83	21.9	1.46 1.38	C $\delta$ : 28.7 C $\epsilon$ : 38.3	H $\delta$ : 1.58 H $\epsilon$ : 3.32
AEEA1	7.36							C11: 70.5 C13: 69.9 C14: 70.8 C16: 69.7 C17: 39.3	H11: 4.00 H13: 3.60 H14: 3.63 H16: 3.54 H17: 3.43
AEEA1	7.17							C2: 70.5 C4: 69.9 C5: 70.8 C7: 69.9 C8: 38.6	H2: 3.98 H4: 3.63 H5: 3.64 H7: 3.55 H8: 3.49
Glu	6.60	52.4	4.42	29.1	2.16 1.91	32.7	2.30	C $_{t\text{Bu(Me)}}$ : 28.0	H $_{t\text{Bu}}$ : 1.46
C18								C2: 36.6 C3: 25.6 C4: 29.4 C5: 29.2 C6: 29.6 C7-13: 29.6 C14: 29.2 C15: 29.4 C16: 25.2 C17: 35.6 C $_{t\text{Bu(Me)}}$ : 28.2	H2: 2.23 H3: 1.59 H4: 1.27 H5: 1.28 H6: 1.24 H7-13: 1.24 H14: 1.29 H15: 1.26 H16: 1.57 H17: 2.20 H $_{t\text{Bu}}$ : 1.43

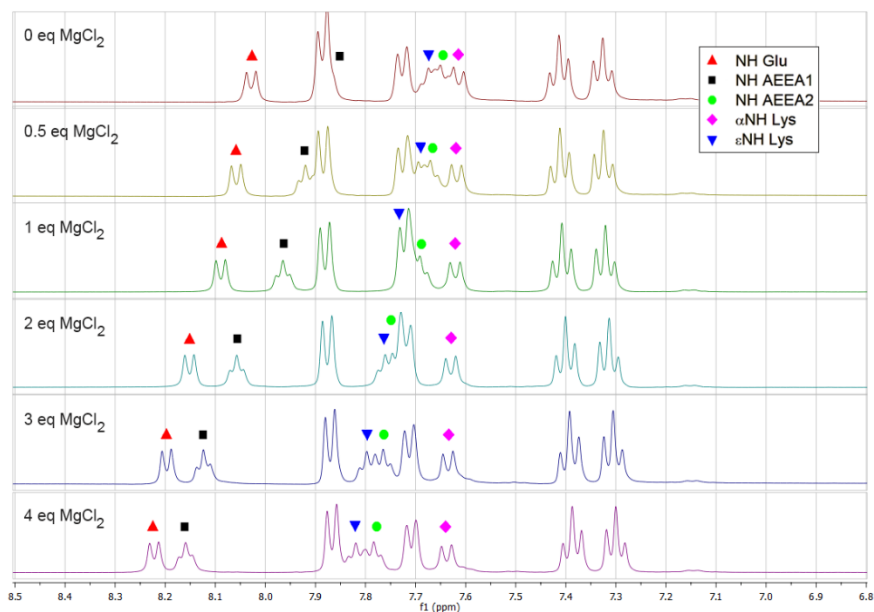


Figure S34. A part of <sup>1</sup>H NMR spectrum of Tirzepatide SCBB in the range 8.5-6.8 ppm in presence of various amounts of magnesium chloride (400 MHz, DMSO-*d*<sub>6</sub>).