Supplementary Material (ESI) for *Soft Matter*

Site-specific *N*-terminus conjugation of poly(mPEG$_{1100}$) methacrylates to salmon calcitonin: synthesis and preliminary biological evaluation.

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1.0 Conjugating Polymers.

M_n(NMR) of p(mPEG$_{1100}$)MA **(1a-d)** were determined by ^{1}H NMR, Table S1.Those values were in agreement with those previously determined by ^{1}H NMR analysis of **(1a-d)** precursors, in which the α-aldehyde unit was protected as dimethoxyacetal(*1*). Polydispersity index (PDi) of **(1a-d)** was determined by GPC using $CHCl_3$/triethylamine 95:5 (vol/vol) as the eluent (Table S1).

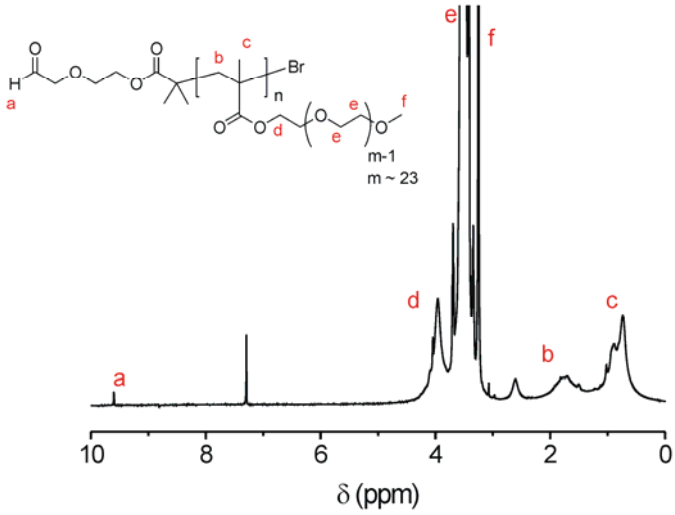


Figure S1: ^{1}H NMR spectrum of p(mPEG$_{1100}$)MA **(1c)**.

1.1 Characterization of (1a-d)

Table S1

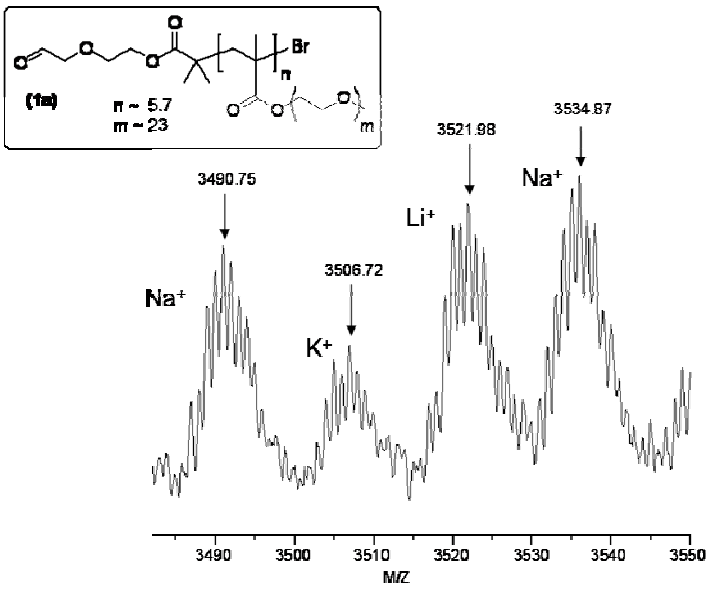
Polymers	Analysis	M_n (kDa)	M_w (kDa)	M_w/M_n
(1a)	Aqueous GPC[a]	nd	nd	nd
(1b)		14.3	22.8	1.56
(1c)		49.7	74.2	1.38
(1d)		97.6	186.4	1.91
(1a)	Chloroform GPC[b]	5.2	6.1	1.17
(1b)		10.2	10.9	1.06
(1c)		17.9	20.0	1.11
(1d)		nd	nd	nd
(1a)	^{1}H NMR[c]	6.5	-	-
(1b)		26.0	-	-
(1c)		50.6	-	-
(1d)		109	-	-

[a] Aqueous GPC: the system was equipped with two PL Aquagel-OH 8 µm mixed columns (300 x 7.5 mm) with differential refractive index detection using water (containing $NaNO_3$ (53 g), NaH_2PO_4 (3 g) and NaN_3 (25 mg) per 2.5 L) at 1.0 mL min^{-1} as the eluent. p(mPEG$_{1100}$)MA standards (10.7 – 130 kDa) were used to calibrate the SEC.

[b] Chloroform GPC: the system was equipped with two PL gel 5 μm mixed D-columns (300 x 7.5 mm) (Polymer Laboratories, suitable for molecular weights between 200 and 400,000 g mol^{-1}) and one PL gel 5 mm guard column (50 x 7.5 mm) with differential refractive index detection using $CHCl_3$/triethylamine 95:5 (vol/vol) at 1.0 mL min^{-1} as the eluent. Poly(MMA) standards ($1 \cdot 10^6$-200 g mol^{-1}) were used to calibrate the SEC.

[c] M_n(NMR) was calculated by comparing the aldehyde C(O)H chain-end signal at 9.6 ppm (chain end) with the OCH_3 singlet at around 3.3 ppm (3H per $mPEG_{1100}MA$ repeating unit)

1.2 MALDI-TOF MS of (1a).



Formula	Calc. mass (100% abundance)	Calc. mass (monoisotopic)	Calc. mass (average)	Ion
$C_{159}H_{308}O_{78}$	3490.01	3489.00	3491.10	Na^+
$C_{159}H_{308}O_{78}$	3506.98	3504.98	3507.20	K^+
$C_{161}H_{312}O_{79}$	3512.05	3511.05	3513.16	H^+
$C_{161}H_{312}O_{79}$	3518.06	3517.06	3519.09	Li^+

Figure S2 Partial MALDI-TOF MS spectrum (reflectron mode, showing isotopic resolution) of α-aldehyde functionalised (6.5 kDa) p($mPEG_{1100}$)MA **(1a)**.

2.0 Influence of the p(mPEG$_{1100}$)MA polymers on the conjugation rate.

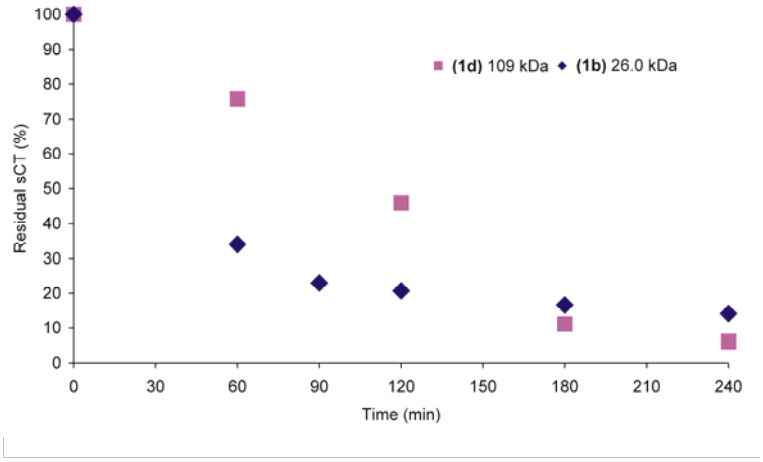


Figure S3: Rate of conjugation monitored by RP-HPLC (with UV detection at 215 nm) determined by following the disappearance of sCT and by the increase in the formed conjugate. Conjugates were formed by reacting sCT with α-aldehyde functionalised 26.0 kDa **(1b)** and 109 kDa **(1d)** p(mPEG$_{1100}$)MA at a 1:5 sCT:polymer molar ratio. Amount of residual unreacted sCT vs. time is reported here for both 26.0 kDa **(1b)** (blue diamonds) and 109 kDa **(1d)** (purple squares) p(mPEG$_{1100}$)MA.

3.0 FPLC purification of sCT conjugates

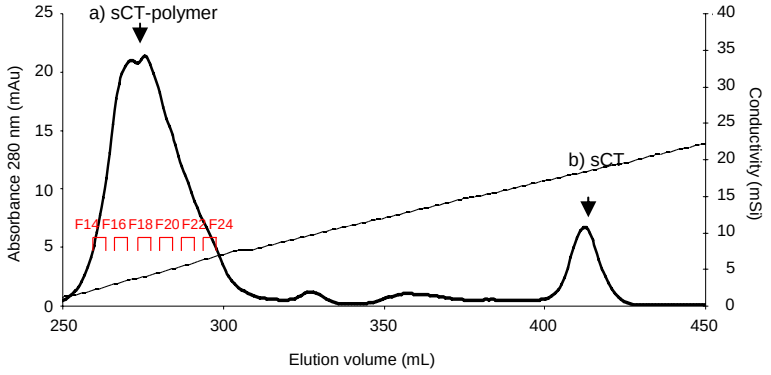


Figure S4: Purification of sCT-**(1a-d)** conjugates: typical IE-FPLC chromatogram (online UV detection at λ = 280 nm). In this case **(1d)** was employed, at a 1:10 sCT : p(mPEG$_{1100}$)MA molar ratio.

4.0 RP-HPLC and SEC-HPLC analysis of conjugates sCT-(1a), sCT-(1c) and sCT-(1d).

*Note: the synthesis of sCT-**(1b)** was not monitored by RP-HPLC and SEC-HPLC.*

4.1 Analysis of sCT-(1a)

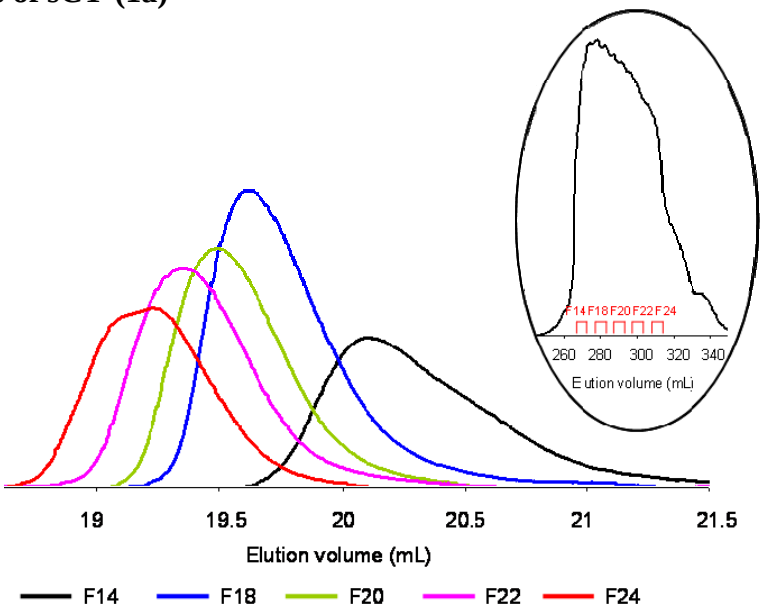


Figure S5. RP-HPLC of fractions purified using IE-FPLC using 6.5 kDa aldehyde-functionalised p(mPEG$_{1100}$)MA **(1a)** conjugated to sCT (at a 1:10 molar ratio), with online UV detection at λ = 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset).

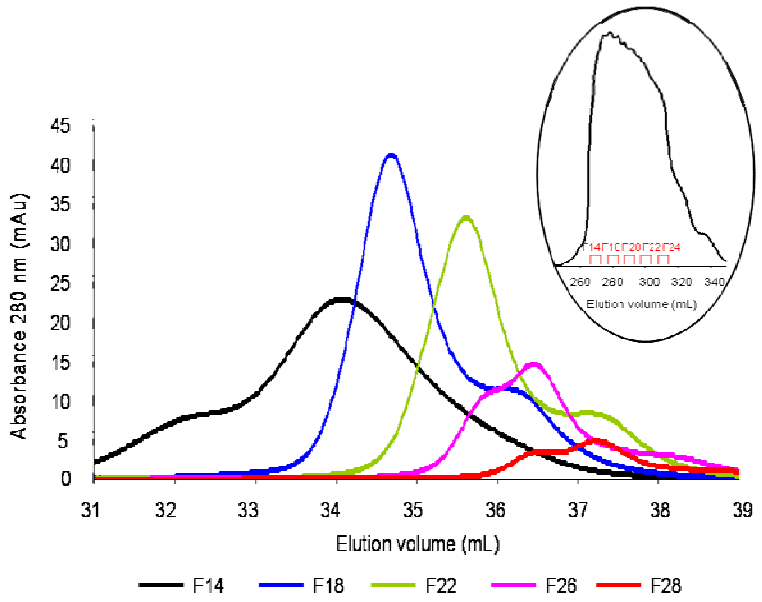


Figure S6. SEC-HPLC of fractions purified using IE-FPLC using 6.5 kDa aldehyde-functionalised p(mPEG$_{1100}$)MA **(1a)** to sCT, with online UV detection at 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset). SEC-HPLC was

carried out using two BIOSEP S3000 columns connected in series with detection at λ = 215 nm. The mobile phase was 30.9 % acetonitrile, 69 % water and 0.1 % TFA and the flow rate was 0.5 mL min^{-1}. The shape of the chromatogram relative to each IE-FPLC fraction confirmed the presence of conjugates with different DP in their polymer part in each of these fractions.

4.2 Analysis of sCT-(1c)

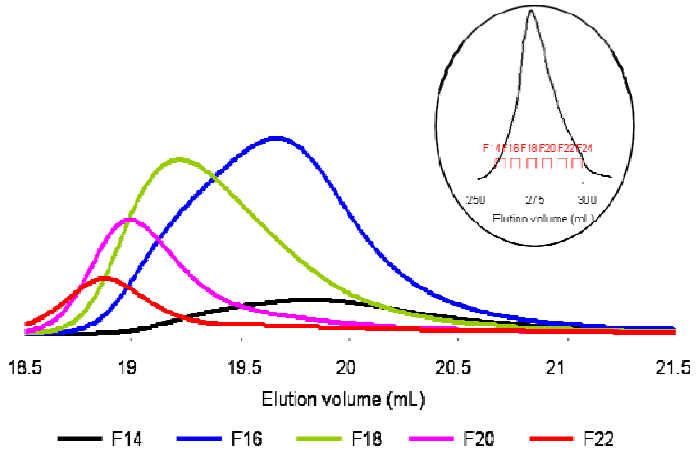


Figure S7. RP-HPLC of fractions purified using IE-FPLC using 49.7 kDa aldehyde-functionalised p($mPEG_{1100}$)MA **(1c)** conjugated to sCT (at a 1:10 molar ratio), with online UV detection at λ = 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset).

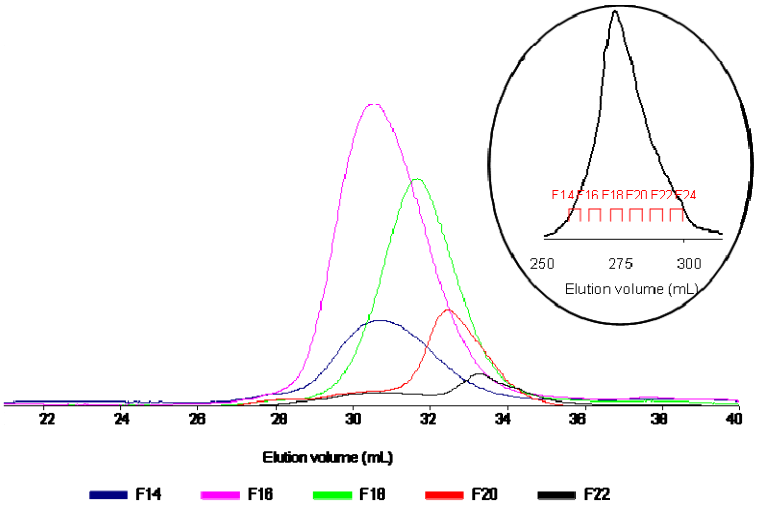


Figure S8. SEC-HPLC of fractions purified using IE-FPLC using 49.7 kDa aldehyde-functionalised p($mPEG_{1100}$)MA **(1c)** to sCT, with online UV detection at 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset). SEC-HPLC was carried out using two BIOSEP S3000 columns connected in series with detection at λ = 215 nm. The mobile phase was 30.9 % acetonitrile, 69 % water and 0.1 % TFA and the flow rate was 0.5 mL min^{-1}.

4.3 Analysis of sCT-(1d)

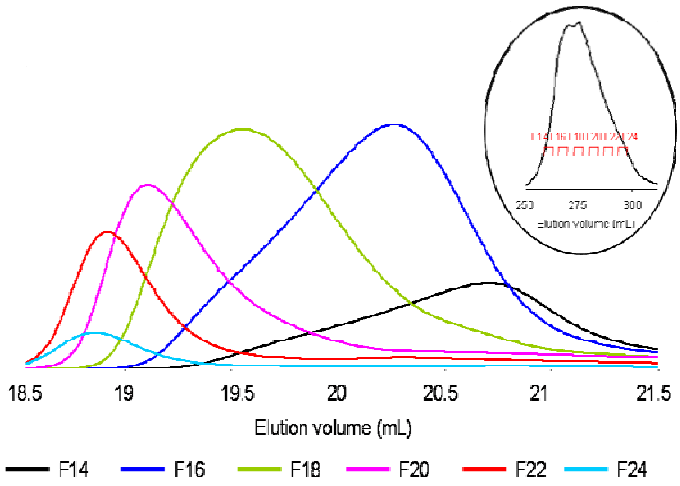


Figure S9. RP-HPLC of fractions purified using IE-FPLC using 109 kDa aldehyde-functionalised p(mPEG$_{1100}$)MA **(1d)** conjugated to sCT (at a 1:10 molar ratio), with online UV detection at λ = 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset).

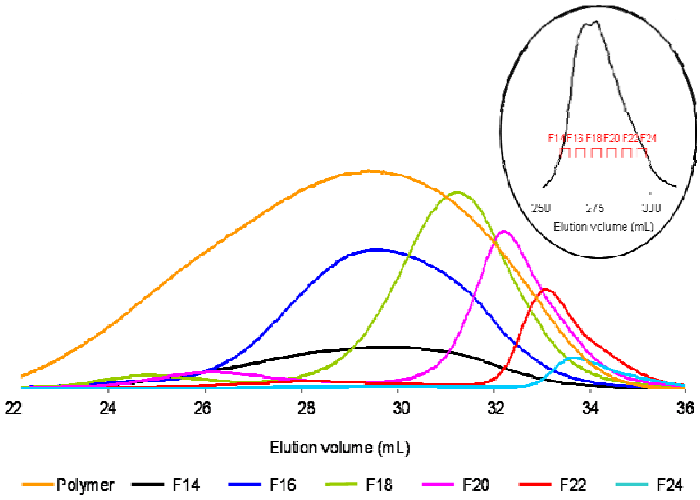


Figure S10. SEC-HPLC of fractions purified using IE-FPLC using 109 kDa aldehyde-functionalised p(mPEG$_{1100}$)MA **(1d)** to sCT, with online UV detection at 280 nm. The corresponding IE-FPLC chromatogram is also shown for reference (inset). SEC-HPLC was carried out using two BIOSEP S3000 columns connected in series with detection at λ = 215 nm. The mobile phase was 30.9 % acetonitrile, 69 % water and 0.1 % TFA and the flow rate was 0.5 mL min^{-1}.

5.0 TOCSY NMR of native sCT and sCT-(1a)

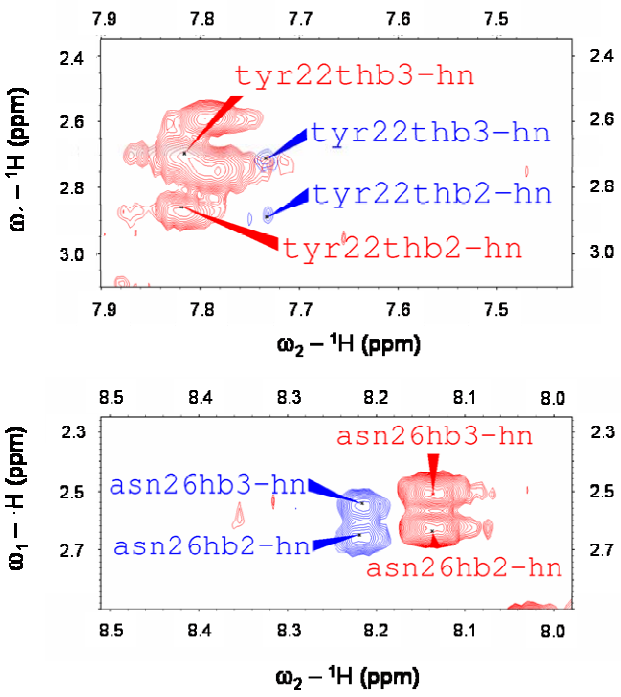


Figure S11. TOCSY of native sCT (red) and sCT-**(1a)** (blue): partial spectra showing the change in chemical shift of Tyr^{22} and Asn^{26} residues that occurred upon conjugation.

Table S2. Native sCT, table of chemical shifts as observed by 2D ^{1}H NMR (500 MHz, 100% DMSO-d_6, 308 K).[a,b]

Residue	^{1}H chemical shift (ppm)					
	NH	αH	βH	γH	δH	εH
Ser^{5}	8.44	4.20	3.71	-	-	-
Thr^{6}	7.46	4.22	4.08	1.04	-	-
Cys^{7}	8.07	4.58	3.33;2.98	-	-	-
Val^{8}	7.80	4.15	2.04	0.87	-	-
Leu^{9}	8.04	4.28	1.66	1.56	0.87	-
Gly^{10}	8.13	3.72	-	-	-	-
Lys^{11}	7.92	4.30	1.71	1.34	1.56	2.78
Leu^{12}	8.14	4.35	1.55	n.a.	0.89	-
Gln^{14}	8.12	4.22	1.83	2.15	6.77;7.27	-
Glu^{15}	8.30	4.20	1.82	2.14	-	-
Leu^{16}	7.95	4.21	1.63	1.52	0.86	-
His^{17}	7.98	4.43	2.98	-	6.86	7.54
Gln^{20}	7.87	4.28	1.95;1.83	2.14	6.79;7.30	-
Thr^{21}	7.68	4.19	3.97	1.00	-	-
Tyr^{22}	7.85	4.63	2.90;2.73	-	7.12	6.67
Tyr$^{22(s)}$	7.82	4.55	2.78;2.62	-	6.99	6.68
Arg^{24}	8.26	4.39	1.82	1.61	n.a	n.a
Thr^{25}	7.74	4.30	4.05	1.07	-	-
Asn^{26}	8.17	4.70	2.66;2.53	-	7.02;7.50	-
Thr^{27}	7.80	4.29	n.a.	1.08	-	-
Gly^{28}	8.13	3.81	-	-	-	-
Ser^{29}	7.96	4.31	3.63	-	-	-
Gly^{30}	8.19	3.80	-	-	-	-
Thr^{31}	7.89	4.55	3.93	1.14	-	--
Thr$^{31(s)}$	7.62	4.50	-	1.03	-	

n.a.: not assignable due to lack of intra-residual crosspeaks or heavy overlap

[a] Residues without resonances for backbone amide protons are not listed

[b] (s) refers to a second isomer, due to cis-trans isomerism of Pro23 and Pro32.

Table S3. sCT-**(1a)**, table of chemical shifts as observed by 2D NMR (500 MHz, 100% DMSO-d_6, 308 K).[a,b,c]

Residue	^{1}H chemical shift (ppm)					
	NH	αH	βH	γH	δH	εH
Thr^{21}	7.52	4.11	3.93	0.94		
Tyr^{22}	7.76	4.59	2.91;2.75		6.65	7.07
$Tyr^{22(s)}$					6.69	6.98
Arg^{24}	8.17	4.32	-	-	-	
Thr^{25}	7.75	4.23	4.05	1.06		
Asn^{26}	8.25	4.65	2.69;2.58		n.a.	
Thr^{27}	7.80	4.14	-	1.07		
Gly^{28}	8.19	3.82	-	-	-	
Ser^{29}	7.94	4.28	3.67		-	
Gly^{30}	8.24	3.80	-	-	-	
Thr^{31}	7.74	4.48	3.95	1.10		
$Thr^{31(s)}$	7.54	4.40	3.88	1.01	-	

[a] Residues without resonances for backbone amide protons are not listed
[b] (s) refers to a second isomer, due to cis-trans isomerism of Pro23 and Pro32
[c] Crosspeaks for side-chain resonances for leucine residues are present in the methyl-CH(γ) region, and those for glutamine NH_2 groups are observable in the amide region, but sequential assignment was not possible due to the lack of crosspeaks to backbone protons. The side chain resonances of Val^8 (βH, 2.07; γH, 0.86 and 0.90 ppm) are also observable, as are some of Lys^{11} (γH,1.37; δH,1.56; εH,2.80 ppm), which can be assigned due to absence of other signals in this region of the spectrum.

6.0 References

(1) Tao, L., Mantovani, G., Lecolley, F., and Haddleton, D. M. (2004) alpha-Aldehyde Terminally Functional Methacrylic Polymers from Living Radical Polymerization: Application in Protein Conjugation "Pegylation". *J. Amer. Chem. Soc. 126*, 13220-13221.