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The effect of the chromophoric group modification on the optical properties of retinal proteins

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List of Abbrevations

AR retinal analog; BO bacterioopsin; BR bacteriorhodopsin; DDM *n*-dodecyl-β-D-maltopyranoside; DIBAL diisobutylaluminum hydride; ESRh proteorhodopsin from microorganism *E. sibiricum*; HWE olefination is Horner-Wadsworth-Emmons olefination; NBS *N*-bromosuccimide; NC noncovalent complex; OS opsin shift; PCC pyridinium chlorochromate; PRS percent red shift; CHO, SB, SBH^+ aldehyde, non-protonated and protonated Schiff base, respectively; THF tetrahydrofuran; LA light-adapted; DA dark-adapted; (P) - pigment of which is unknown to what form (dark or light) states of pigments, considered in the Table S3.

Materials and Methods

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III-500 spectrometer in $CDCl_3$ with the working frequency of 500 MHz and 125 MHz, respectively. Chemical shifts are reported in ppm relative to tetramethylsilane or $CDCl_3$ as the internal standards (δ 0.00 ppm and δ 7.25 ppm, respectively). The spin–spin coupling constants are expressed in Hz. Electron impact (EI, IE 70 eV) mass spectra were recorded on a Kratos model MS-30 (England). Mass-spectra of all the new retinal analogs contained a characteristic decay with $[M^+]$ ion corresponded to their molecular masses.

The qualitative composition of the reaction mixtures and the homogeneity of the compounds were monitored by TLC on Kieselgel 60 F_{254} plates (Merck) using hexane–ethyl acetate mixture (4:1) as the eluent. The spots were visualized under UV light or in the iodine vapors. All operations with oxygen-sensitive reagents were carried out under argon atmosphere.

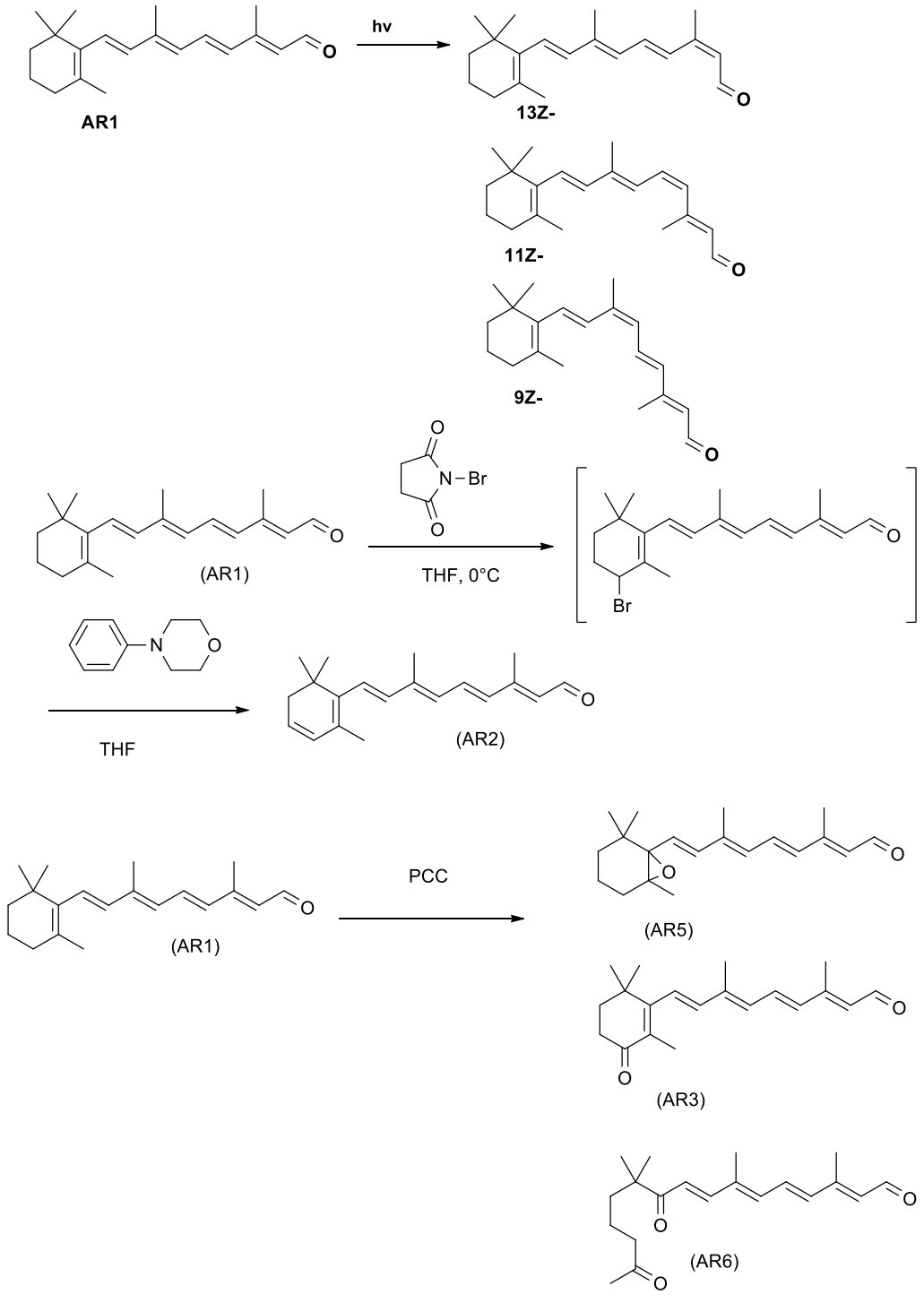
The preparative column chromatography was performed on Kieselgel 60 (0.040–0.063 mm, Merck), or on a HPLC-chromatograph Smart Line 1000 (Knauer). All solvents were purified and dried according the standard procedures. The used 1.5 M toluene solution of DIBAL was obtained from Aldrich.

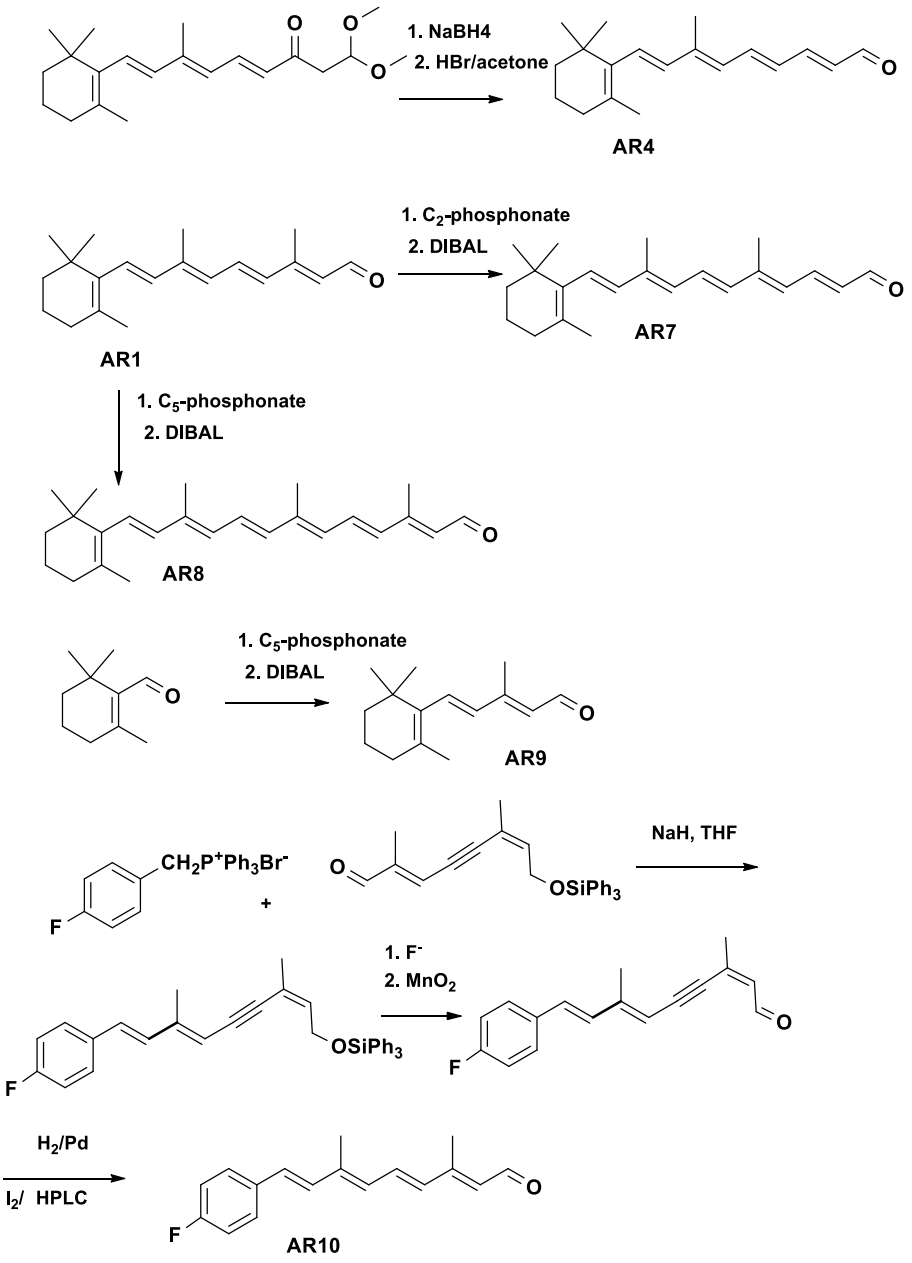
The spectral-kinetic studies were performed on solutions in quartz cells of 1 cm thickness. The following solvents were used: 1) methanol of the high purity grade for retinoid derivatives and models SB and SBH^+; 2) 50mM MES, 5mM EDTA, pH 6.5 for BR analogs; and 3) 50 mM

NaH_2PO_4 buffer, 200 mM NaCl, 0.2% DDM, 300 mM imidazole, pH 8.0 for ESRh analogs at 20°C. The solution concentrations of compounds were in the interval of 1×10^{-4}–1×10^{-5} M. The UV–VIS spectra were recorded on a Shimadzu 2140PC instrument with Peltier thermostatic cell holder in 250–700 nm range. Photo-induced absorption spectra were obtained after the irradiation with filtered light of 100W halogen lamp (ThorLabs, USA).

S1. Synthesis of retinal analogues (AR).

We used the general synthetic approaches depicted in Scheme S1 to prepare a library of retinal derivatives (**AR2**–**AR9**). The synthesis of some of the retinal analogues has been described previously.[S1,S3-S10,S13] ^{1}H and ^{13}C NMR data are given in Tables S1 and S2, and also in Figures S2–S9.





Scheme S1 Synthesis of modified retinal chromophores.

Z-isomers of retinal **AR1** (13*Z*-; 11*Z*-; and 9*Z*-) were prepared by a photo-isomerization of the all-*E*-isomer with the subsequent isolation of desired isomers from the reaction mixture by preparative HPLC. Two-step synthetic procedure for 3,4-didehydroretinal **AR2** included bromination of retinal **AR1** by NBS/THF system and following dehydrobromination of 4-bromoderivative by *N*-phenylmorpholine. The total yield of pure 3,4-didehydroretinal **AR2** was 47%.

Direct one-pot oxidation of all-*E*-isomer retinal by PCC/CH_2Cl_2 system gave a mixture of the following retinals: 4-oxoretinal **AR3**, 5,6-dihydro-5,6-epoxyretinal **AR5**, all-*E*-5,6-dioxo-5,6-secoretinal **AR6**. Target retinoids (**AR3**, **AR5**, and **AR6**) were isolated by the preparative HPLC from this mixture.[S4]

Analogs containing chain modifications were synthesized *via* selected reaction sequences depicted in Scheme S1. Thus, 13-desmethylretinal **AR4** was prepared by reduction with $NaBH_4$ of C_{19}-ketoacetal with the subsequent dehydration of resulted secondary alcohol by HBr/acetone.[S5-S9] Novel retinoids with alterated polyenic chain length (**AR7**–**AR9**) were synthesized *via* a two-step synthetic procedure, which included HWE olefination of

corresponded carbonyl precursor by either C_2-phosphonate or C_5-phosphonate; and then following DIBAL/CH_2Cl_2 reduction of intermediate nitriles.
4-Fluorophenylretinal (**AR10**) was synthesized with selected reaction sequences depicted in Scheme S1. Key step included Wittig olefination of carbonyl precursor by an ylide, generated from 4-fluorobenzyltriphenylphosphonium bromide.[S12-S13]

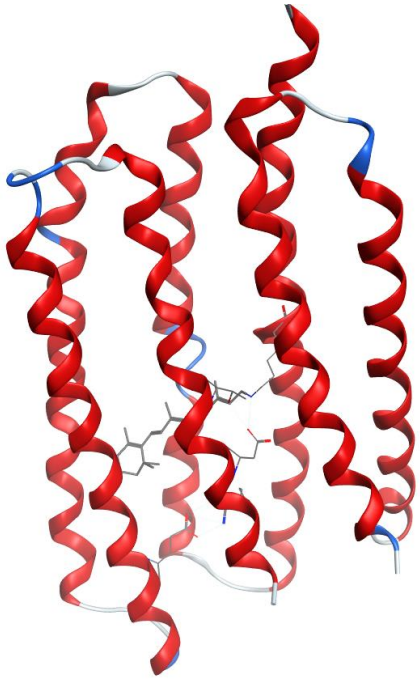


Figure S1 3D-structure of the ESRh molecule, the PDB code is 4hyj.

S2. Apoproteins and artificial pigments preparation procedures.

Apomembranes with bacterioopsin were prepared from purple membranes obtained from *H. salinarum* str. 353P by hydroxylaminolysis at pH 7.0 and 0–5 °C and intensive illumination, according to the previously published procedures.[S1,S6,S10,S11,S16]

ESRh opsin from *E. sibiricum*. ESRh purification

E. coli BL21(DE3)pLysS cells freshly transformed with pET-ESRh were grown for 2 days at 30 °C in 2XZY5052 autoinduction media[S21] supplied with ampicillin (100 μg ml^{-1}) and chloramphenicol (34 μg ml^{-1}).

Upon harvesting, the cells were resuspended in 100 mM Tris–HCl buffer, pH 8.0, 20% sucrose, and 5 mM EDTA, incubated with 0.2 mg ml^{-1} lysozyme and diluted with 5 volumes of cold deionized water. After ultrasonication, the insoluble material was removed by centrifugation at 6000×g for 30 min. Membrane fraction was sedimented at 100 000×g for 1 h and after addition of 5 μM, all-*E*-retinal ESRh was extracted by incubation with 50 mM Tris–HCl, 200 mM NaCl, 1% n-dodecyl-β-D-maltopyranoside (DDM), 10 mM imidazole buffer, pH 8.0, for 18 h at 4 °C. Solubilized fraction was applied on Ni Sepharose FastFlow resin (GE Healthcare) and extensively washed with buffer containing 50 mM NaH_2PO_4, 200 mM NaCl, 0.1% DDM, and 30 mM imidazole at pH 6.0. ESRh was eluted with buffer containing 50 mM NaH_2PO_4, 200 mM NaCl, 0.2% DDM, and 300 mM imidazole at pH 7.4 and concentrated using Amicon centrifugal filter device (10 kDa MWCO).

References

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Table S1 ^{1}H NMR spectral data.

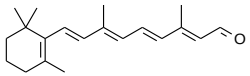
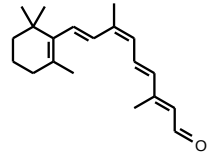
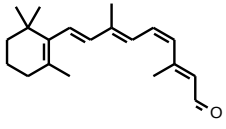
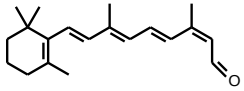
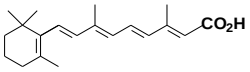
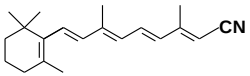
No.	Structure		1,1-CH_3	2-CH_2	3-CH_2	4-CH_2	5-CH_3	7H	8H	10H	11H	12H	14H	15H	9-CH_3	13-CH_3	others
AR1		δ	1.03	1.48	1.61	2.03	1.72	6.36	6.18	6.20	7.15	6.37	5.98	10.12	2.03	2.33	
		J/Hz	-	-	-	6.5	-	16.5	16.5	12.0	12.0/ 15.4	15.4	8.0	8.0	-	-	
		mode	s	m	m	t	s	d	d	d	dd	d	d	d	s	s	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR1 9Z-		δ	1.01	1.48	1.63	2.04	1.74	6.31	6.65	6.08	7.21	6.29	5.96	10.09	2.01	2.30	
		J/Hz	-	-	-	5.5	0.5	16.0	16.0	11.8	11.8/ 15.0	15.0	8.2	8.2	-	1.2	
		mode	s	m	m	t	d	d	d	d	dd	d	d	d	s	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR1 11Z-		δ	1.01	1.45	1.58	2.00	1.69	6.31	6.12	6.51	6.67	5.91	6.06	10.06	1.96	2.34	
		J/Hz	-	-	-	6.0	-	16.0	16.0	12.5	11.5/ 12.5	11.5	8.2	8.2	-	1.2	
		mode	s	m	m	t	s	d	d	d	dd	d	d	d	s	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR1 13Z-		δ	1.06	1.48	1.62	2.01	1.74	6.34	6.15	6.21	7.02	7.27	5.63	10.19	2.02	2.14	
		J/Hz	-	-	-	6.0	-	16.0	16.0	11.1	11.1/ 15.0	15.0	8.0	8.0	-	-	
		mode	s	m	m	t	s	d	d	d	dd	d	d	d	s	s	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR11		δ	1.03	1.48	1.61	2.03	1.70	6.22	6.13	6.15	7.04	6.31	5.79	-	2.00	2.36	
		J/Hz	-	-	-	-	-	16.0	16.0	11.5	11.5/ 15.0	15.0	-	-	-	1.2	
		mode	s	m	m	m	s	d	d	d	dd	d	s	-	s	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H		3H	3H	
AR12		δ	1.03	1.45	1.63	1.97	1.72	6.32	6.19	6.10	6.94	6.28	5.20	-	1.99	2.21	
		J/Hz	-	-	-	-	0.8	16.2	16.2	11.5	11.5/ 15.0	15.0	-		1.0	1.2	
		mode	s	m	m	m	d	d	d	d	dd	d	s		d	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H		3H	3H	

Table S1 ^{1}H NMR spectral data.

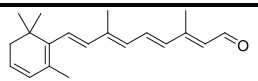
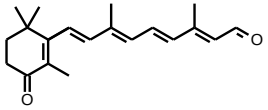
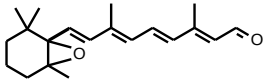
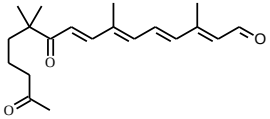
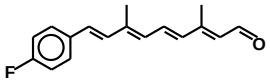
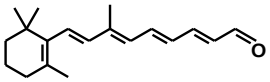
No.	Structure		1,1-CH_3	2-CH_2	3-CH_2	4-CH_2	5-CH_3	7H	8H	10H	11H	12H	14H	15H	9-CH_3	13-CH_3	others
AR2		δ	1.04	2.08	5.75	5.85	1.87	6.34	6.29	6.21	7.13	6.37	5.96	10.10	2.03	2.32	
		J/Hz	-	4.4/ 1.6	9.5/4.4	9.5/1.6	-	16.0	16.0	11.5	11.5/ 15.0	15.0	8.0	8.0	0.6	0.9	
		mode	s	dd	dt	dt	s	d	d	d	dd	dd	dd	d	d	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR3		δ	1.17	1.84	2.49	-	1.83	6.36	6.31	6.27	7.10	6.42	5.98	10.10	2.04	2.32	
		J/Hz	-	6.8	6.8	-	-	16.3	16.3	11.5	11.5/ 15.*0*	15.0	8.1	8.1	-	-	
		mode	s	t	t	-	s	d	d	d	dd	d	d	d	s	s	
		intensity	6H	2H	2H	-	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR5		δ	1.11 1.16	1.40	1.75	1.90	0.93	6.32	6.05	6.22	7.09	6.38	5.97	10.10	2.00	2.31	
		J/Hz	-	-	-	-	-	15.6	15.6	11.5	11.5/ 15.1	15.1	8.1	8.1	0.8	1.0	
		mode	2 s	m	m	m	s	d	d	d	dd	d	d	d	d	d	
		intensity	3H+3H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR6		δ	1.16	1.54	1.47	2.39	2.09	7.35	6.64	6.55	7.07	6.51	6.02	10.12	2.03	2.32	
		J/Hz	-	-	-	6.8	-	15.2	15.2	11.5	11.5/ 15.2	15.2	7.9	7.9	-	0.8	
		mode	s	m	m	t	s	d	d	d	dd	d	d	d	s	d	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR10		δ	-	-	-	-	-	6.82	6.68	6.36	7.15	6.43	6.05	10.12	2.10	2.35	$J_{1(5)H,F}$ (*J* 5.5 Hz) $J_{2(4)H,F}$ (*J* 8.5 Hz) 1,5-H (7.42, *J* 8.5 Hz, dd) 2,4-H (7.03, *J* 8.5 Hz, dd)
		J/Hz						16.2	16.2	11.5	11.5/ 15.5	15.5	8.2	8.2	-	1.5	
		mode						d	d	d	dd	d	d	d	s	d	
		intensity						1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR4		δ	1.02	1.61	1.46	2.03	1.71	6.37	6.155	6.20	7.06	6.45	6.12	9.55	2.01	7.19	
		J/Hz	-	-	-	6.8	-	16.1	16.1	11.8	11.8/ 14.5	11.3/ 14.5	8.0/ 15.1	8.0	-	11.3/ 15.1	
		mode	s	m	m	t	s	d	d	d	dd	dd	dd	d	s	dd	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	1H	

Table S1 ^{1}H NMR spectral data.

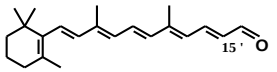
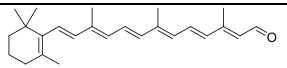
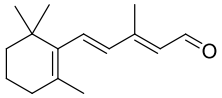
No.	Structure		1,1-CH_3	2-CH_2	3-CH_2	4-CH_2	5-CH_3	7H	8H	10H	11H	12H	14H	15H	9-CH_3	13-CH_3	others
AR7		δ	1.02	1.47	1.61	2.02	1.71	6.28	6.145	6.18	6.91	6.365	6.34	7.50	2.00	2.10	15'H (6.15, *J* 15.0/8.0 Hz, dd)
		J/Hz	-	-	-	6.8	-	15.9	15.9	12.0	11.6/ 14.9	15.7	12.0	11.9/ 14.8	-	-	14'H (9.60, *J* 8.0 Hz, d)
		mode	s	m	m	t	s	d	d	d	dd	d	d	dd	s	s	
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	
AR8		δ	1.02	1.46	1.60	2.01	1.71	6.23	6.13	6.24	6.78	6.35	6.15	7.11	1.98	2.04	12'H (10.09, *J* 8.0 Hz, d)
		J/Hz	-	-	-	6.8	-	15.9	15.9	11.9	11.9/ 14.6	14.5	11.9	12.0/ 14.6	-	-	13'H (5.97, *J* 8.0 Hz, d)
		mode	s	m	m	t	s	d	d	d	dd	d	d	dd	s	s	14'CH_3 (2.31, s)
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H	1H	1H	1H	3H	3H	15'H (6.38, *J* 14.6 Hz, d)
AR9		δ	1.03	1.46	1.61	2.03	1.71	6.72	6.19	5.91	10.11	-	-	-	2.29	-	-
		J/Hz	-	-	-	6.0	-	16.1	16.1	8.2	8.2				-		
		mode	s	m	m	t	s	d	d	d	d				s		
		intensity	6H	2H	2H	2H	3H	1H	1H	1H	1H				3H		

Table S2 ^{13}C NMR spectral data.

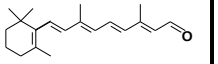
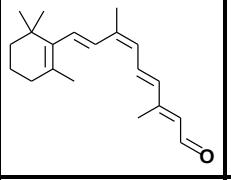
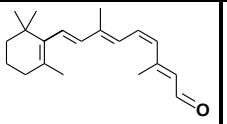
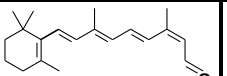
No.	Structure	1-C	2-C	3-C	4-C	5-C	6-C	7-C	8-C	9-C	10-C	11-C	12-C	13-C	14-C	15-C	16-C	17-C	18-C	19-C	20-C	others
AR1		34.18	39.69	19.15	33.04	130.18	137.61	129.52	136.91	140.88	129.19	132.21	134.45	154.08	128.71	190.35	28.81	28.81	21.46	12.79	12.97	
AR1 9*Z*-		34.1	39.7	19.3	33.2	130.4	138.1	131.1	129.4	140.0	127.9	131.2	133.8	154.3	128.9	190.6	29.0	29.0	21.8	20.9	13.2	
AR1 11*Z*-		32.9	38.4	17.9	31.6	128.5	136.5	127.9	136.5	139.7	125.0	129.6	129.6	153.9	128.7	189.2	27.3	27.3	19.9	10.4	15.9	
AR1 13*Z*-		34.3	39.7	19.3	33.2	130.3	137.6	129.6	137.0	141.3	129.4	134.4	126.5	154.2	127.7	189.6	29.0	29.0	21.7	13.0	21.1	

Table S2 ^{13}C NMR spectral data.

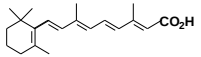
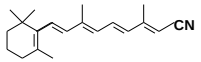
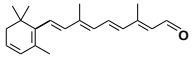
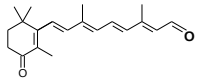
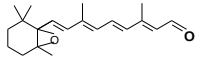
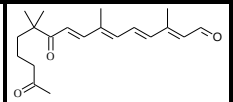
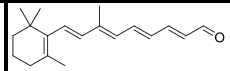
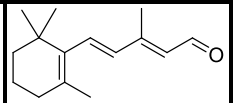
No.	Structure	1-C	2-C	3-C	4-C	5-C	6-C	7-C	8-C	9-C	10-C	11-C	12-C	13-C	14-C	15-C	16-C	17-C	18-C	19-C	20-C	others
AR11		34.33	39.77	19.29	33.19	130.18	137.79	129.12	137.24	140.25	129.41	131.84	134.96	155.22	117.54	171.72	28.99	28.99	21.68	12.93	14.15	
AR12		34.22	39.65	19.18	33.08	130.29	137.61	128.68* 129.60*	136.87	141.25	128.68* 129.60*	131.21	132.29	156.80	96.41	117.94	28.89	28.89	21.57	12.82	16.50	
AR2		34.2	40.1	125.8	129.3	128.0	138.4	128.7	136.7	141.3	130.1	132.6	135.0	154.8	129.3	191.2	27.0	27.0	20.5	13.1	13.3	
AR3		35.9	37.5	34.4	199.3	130.5	160.7	127.0	140.4	139.6	132.7	131.8	136.7	154.2	129.9	191.2	27.8	27.59	13.9	13.0	13.3	
AR5		34.0	35.90	17.2	30.2	65.8	71.4	127.6	136.9	139.7	130.5	132.3,	135.5	154.6	129.4	191.2	26.09	26.14	21.3	13.3	13.4	
AR6		46.7	39.4	19.3	44.1	208.7	204.0	146.6	121.7	138.6	138.1	131.2	139.0	153.6	130.8	191.2	24.5	24.5	30.0	13.3	13.3	
AR4		34.5	39.8	19.4	33.4	131.0	137.8	130.7	137.1	142.8	129.2	139.4	129.6	152.5	130.5	193.6	29.2	29.2	21.9	13.1	-	
AR9		34.4	39.7	19.2	33.4	132.9	137.2	135.7	135.9	155.1	128.9	191.5	-	-	-	-	29.1	29.1	21.9	13.1	-	

Table S3 Properties of the artificial retinal proteins.[S2]

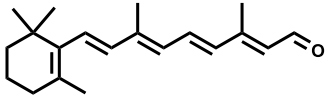
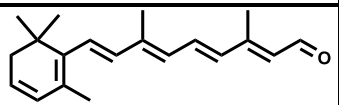
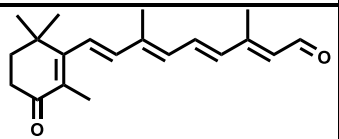
No.	Structure	Isomer	λ_{max} AR (nm); ε (M^{-1} cm^{-1}) in CH_3OH			Target	λ_{max} Rh analogs (nm)			Photocycle Rh analogs	H^+-pump %	Isomer ratio all-E/13Z-	OS Rh analogs cm^{-1} S17,S18	PRS Rh analogs (%) S19,S20	Reactions Rh analogs with		Remarks	Ref.
			"CHO"	SB	SBH^+		(P)	DA	LA	M					NH_2OH	all-E-RET		
AR1	 **natural *all-E*-RetCHO**	all-E-	381	360	440	BR		558	568	+ 412	100 (+++)	50/50	4810	31.7	stable	-	pK_a 13.3-+0.3 str.353P, pH 6.5, 20°C	S1,S2
		13Z-	375				548				0	100/0	5120	32.9				
		11Z-	254, 290sh 377				NO 400 (NC)					0/100	(3900)					
		9Z-	372				NO 388 390/ 470 (NC)											
		all-E-	381	360	440	ESRh	531	531	531	+ 400	+		3895	28.3	Relatively stable	-	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C	
		13Z-	375				528											
		11Z-	254, 290sh 377				NO											
		9Z-	372				NO											
AR2	 ***all-E*-3,4-didehydroRetCHO**	all-E-	401 ε 45700	381	463	BR		592	603 ε 52000	+	+++ 70	95/5	4710 5010	32.3 33.5	stable	stable	str.353P, pH 6.5, 20°C	S1,S2
		all-E-	401	381	463	ESRh	558 525sh	558 525sh	558 525sh	+			3677 3677 3677	28.1 28.1 28.1	stable	stable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C. Light adaptation absent.	
AR3	 ***all-E*-4-oxoRetCHO**	all-E-	374	369	425	BR	527	506	502	+410	++ 25-70		4550 3770 3610	29.0 26.1 25.5	stable	stable	str.353P, pH 6.5, 20°C. BRA cycle biphasic with drastically slowed intermediates	S10, S11, S14
		all-E-	374	369	425	ESRh	494 475sh	494		+			3286 3286	24.3 24.3	Relatively stable	stable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200	

Table S3 Properties of the artificial retinal proteins.[S2]

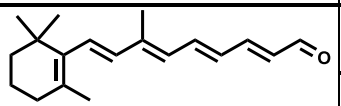
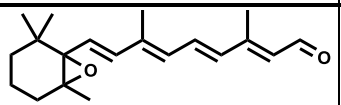
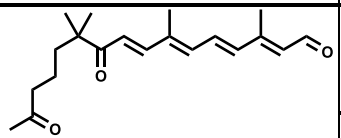
No.	Structure	Isomer	λ_{max} AR (nm); ε (M^{-1} cm^{-1}) in CH_3OH			Target	λ_{max} Rh analogs (nm)			Photocycle Rh analogs	H^+-pump %	Isomer ratio all-E/13Z-	OS Rh analogs cm^{-1} S17,S18	PRS Rh analogs (%) S19,S20	Reactions Rh analogs with		Remarks	Ref.
			"CHO"	SB	SBH^+		(P)	DA	LA	M					NH_2OH	all-E-RET		
								475sh	494 475sh 436 410				3286	24.3			mM NaCl, buffer, pH 8.0, 20°C	
AR4	 ***all-E*-13-desmethylRetCHO**	all-E-	377	362	449	BR	565		565	+	++		5080 5080	33.3 33.3			str.353P, pH 6.5, 20°C	S5-S9
		all-E-	377	362	449	ESRh	518	518	511				2967 2967 2702	27.2 27.2 26.2	stable	stable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C. Light adaptation shift in short-wave region, back reaction in 30 min.	
AR5	 ***all-E*-5,6-dihydro-5,6-epoxyRetCHO**	all-E- rac all-E- (5S,6R) all-E- (5R,6S)	362 ε 53500		420	BR	452 485 445		452 478 445	+ 375	+ 2.8-16		1690 1690 3100 2830 1300 1300	19.9 19.9	nt	stable	str.353P, pH 6.5, 20°C	S10, S14-S15
		all-E-	362 ε 53500		420	ESRh	476	476	469	+			2802 2802 2488	24.0 24.0 22.8	nt	stable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C	
AR6	 ***all-E*-5,6-dioxo-5,6-secoRetCHO**	all-E-	269 364 ε 49900	270 364 381	399 409	BR	450		450	NO			2228 2228	19.1 19.1			str.353P, pH 6.5, 20°C Destroy under action of light.	S1,S4
		all-E-	269 364 ε 49900	270 364 381	399 409	ESRh	426 440sh		442sh	NO			976 1825	14.6 17.7	unstable	Relatively stable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C. Light adaptation shift in long-wave	

Table S3 Properties of the artificial retinal proteins.[S2]

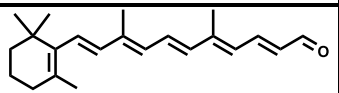
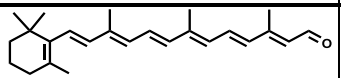
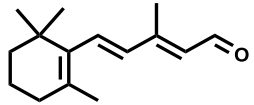
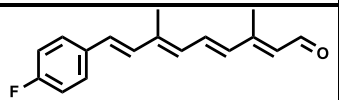
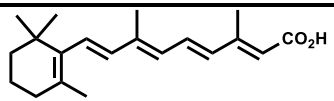
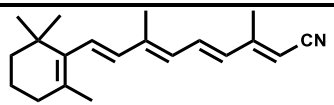
No.	Structure	Isomer	λ_{max} AR (nm); ε (M^{-1} cm^{-1}) in CH_3OH			Target	λ_{max} Rh analogs (nm)			Photocycle Rh analogs	H^+-pump %	Isomer ratio all-E/13Z-	OS Rh analogs cm^{-1} S17,S18	PRS Rh analogs (%) S19,S20	Reactions Rh analogs with		Remarks	Ref.
			"CHO"	SB	SBH^+		(P)	DA	LA	M					NH_2OH	all-E-RET		
																	region, back reaction in 3.5 h.	
AR7	 ***all-E*-C22-RetCHO**	all-E-	398 ε 45000	376	458	BR	555	555	555	+ 400	++		3810 3810 3810	28.3 28.3 28.3	unstable	stable	str.353P, pH 6.5, 20°C	S1
		all-E-	398 ε 45000	376	458	ESRh	522	522	546	?			2677 2677 3519	23.8 23.8 27.1	unstable	unstable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C. Light adaptation shift in long-wave region, back reaction in 30 min.	
AR8	 ***all-E*-C25-RetCHO**	all-E-	400			BR	460sh 523		460sh 527	NO				23.5 24.1	unstable		str.353P, pH 6.5, 20°C	S1
		all-E-	400			ESRh	NO										In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C	
AR9	 ***all-E*-C15-RetCHO**	all-E-	326			BR	NO 364 (NC)										str.353P, pH 6.5, 20°C	S16
		all-E-	326			ESRh	NO										In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20°C	
AR10	 ***all-E*-4-Fluorophenyl-RetCHO**	all-E-	387,5 ε 47700	369	452	BR	524		510	+ 395			3040 2520	26.1 24.0			str.353P, pH 6.5, 20°C. L-D-adaptation drastically retarded	S1, S12, S13, S14
		all-E-	387,5 ε 47700	369	452	ESRh	527	527	521 377?	+			3149 3149 2930	26.5 26.5 25.6	Relatively stable	unstable	In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0,	

Table S3 Properties of the artificial retinal proteins.[S2]

No.	Structure	Isomer	λ_{max} AR (nm); ε (M^{-1} cm^{-1}) in CH_3OH			Target	λ_{max} Rh analogs (nm)			Photocycle Rh analogs	H^+-pump %	Isomer ratio all-E/13Z-	OS Rh analogs cm^{-1} S17,S18	PRS Rh analogs (%) S19,S20	Reactions Rh analogs with		Remarks	Ref.
			"CHO"	SB	SBH^+		(P)	DA	LA	M					NH_2OH	all-E-RET		
																	20^oC. Light adaptation shift in short-wave region, back reaction in 30 min. Formation light-induced reverse hydrolysis of aldimine bond (377 nm product).	
AR11	 ***all-E*-RetCO$_2$H**	all-E-	354 ε 44500			BR	NO										str.353P, pH 6.5, 20^oC	S1
		all-E-	354 ε 44500			ESRh	NO										In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20^oC	
AR12	 ***all-E*-RetCN**	all-E-	356 ε 43000			BR	NO										str.353P, pH 6.5, 20^oC	S1
		all-E-	356 ε 43000			ESRh	NO										In micells: 0.2% DDM, 50 mM NaH_2PO_4, 200 mM NaCl, buffer, pH 8.0, 20^oC	

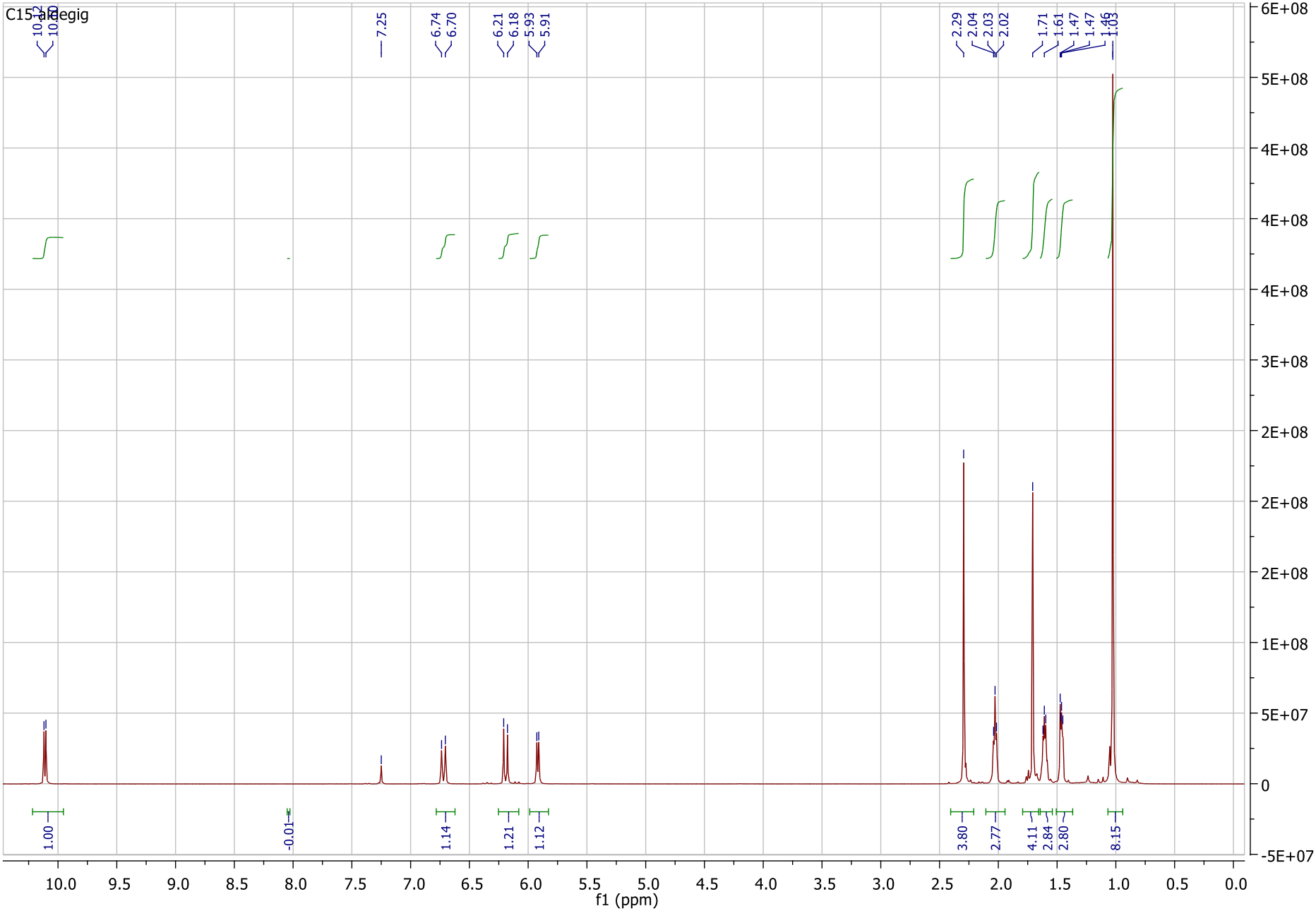


Figure S2 ^{1}H NMR spectrum of C15-aldehyde (**AR9**).

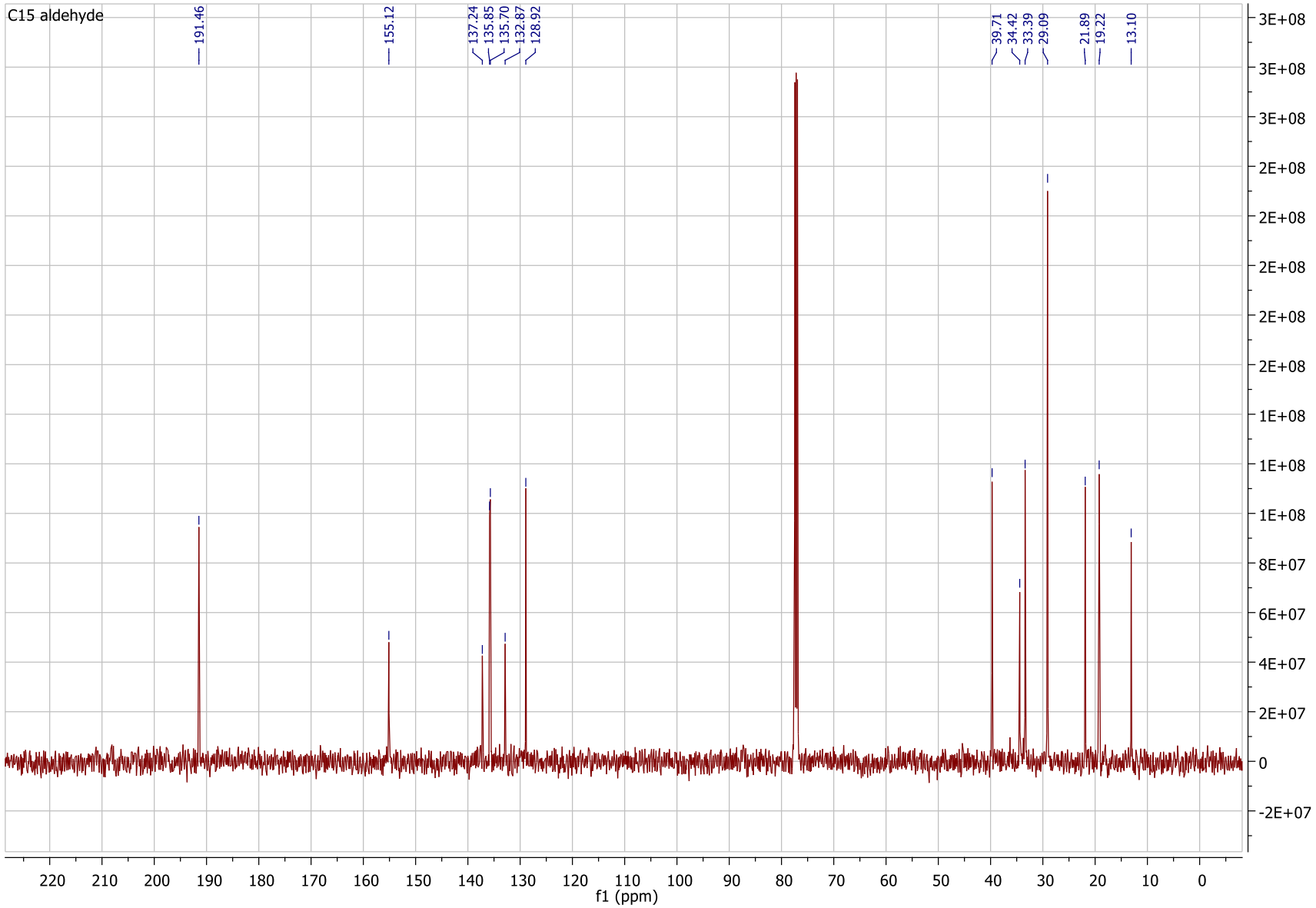


Figure S3 ^{13}C NMR spectrum of C15-aldehyde (**AR9**).

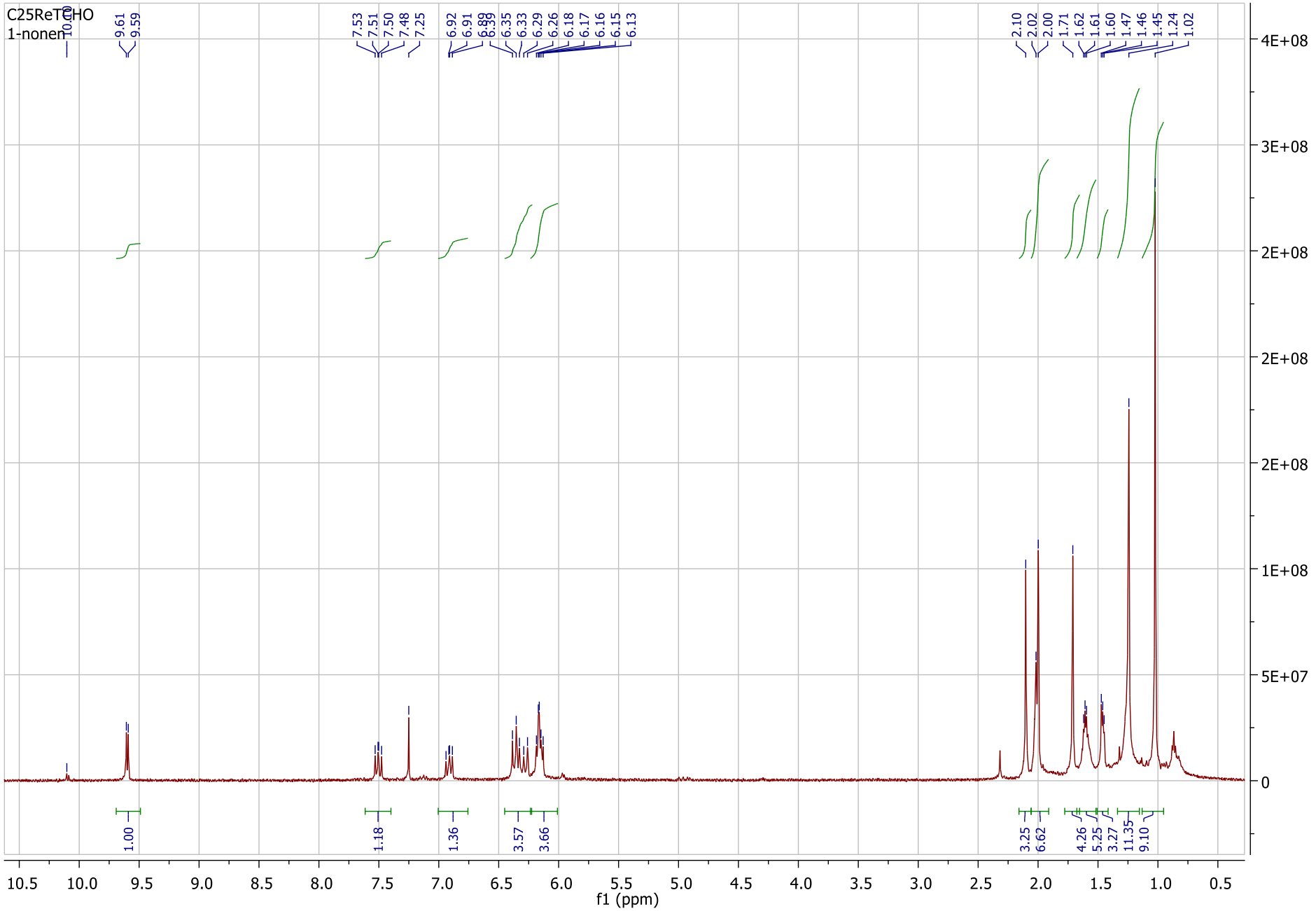


Figure S4 ^{1}H NMR spectrum of C22-aldehyde (**AR7**).

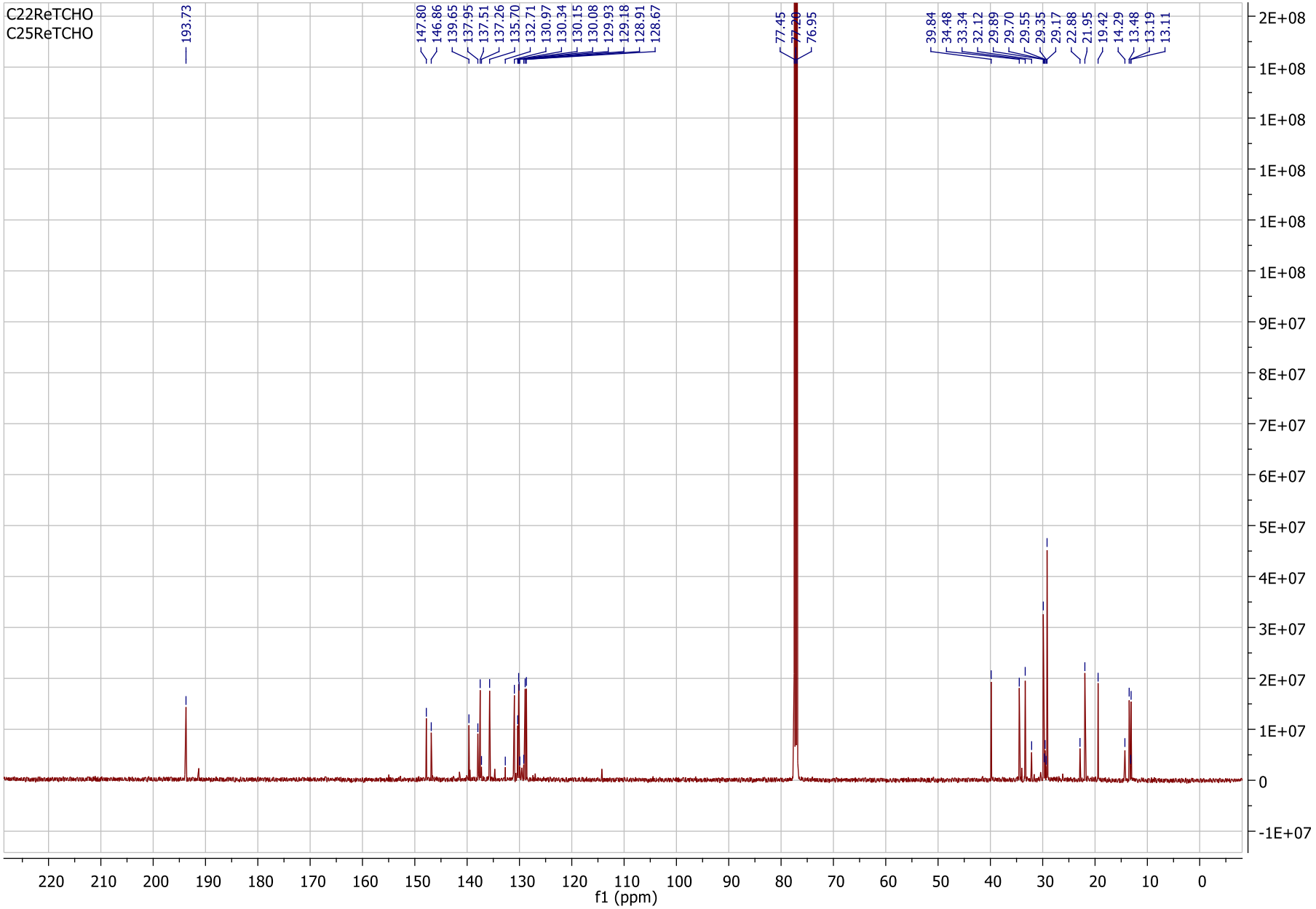


Figure S5 ^{13}C NMR spectrum of C22-aldehyde (**AR7**).

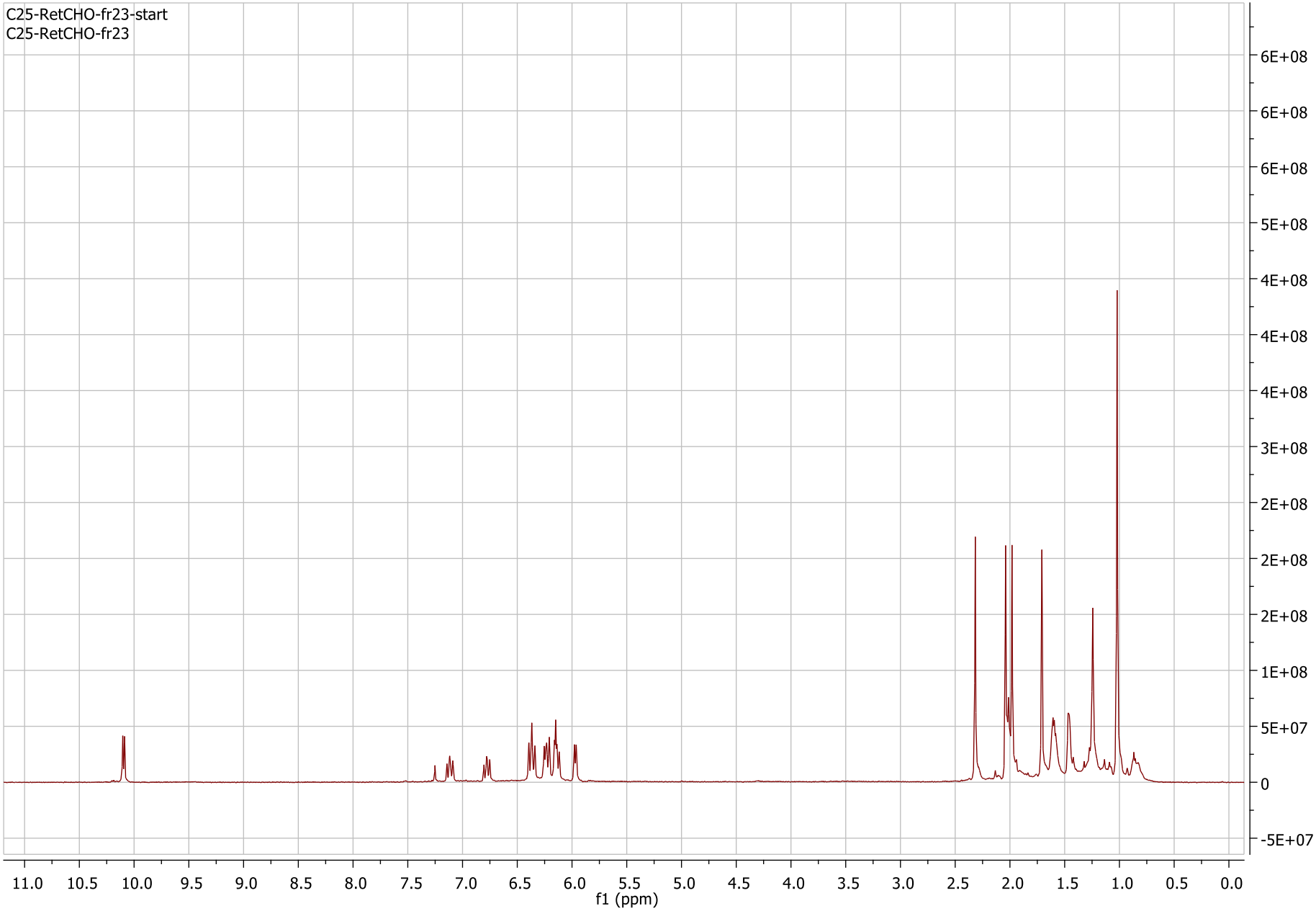


Figure S6 ^{1}H NMR spectrum of C25-aldehyde (**AR8**).

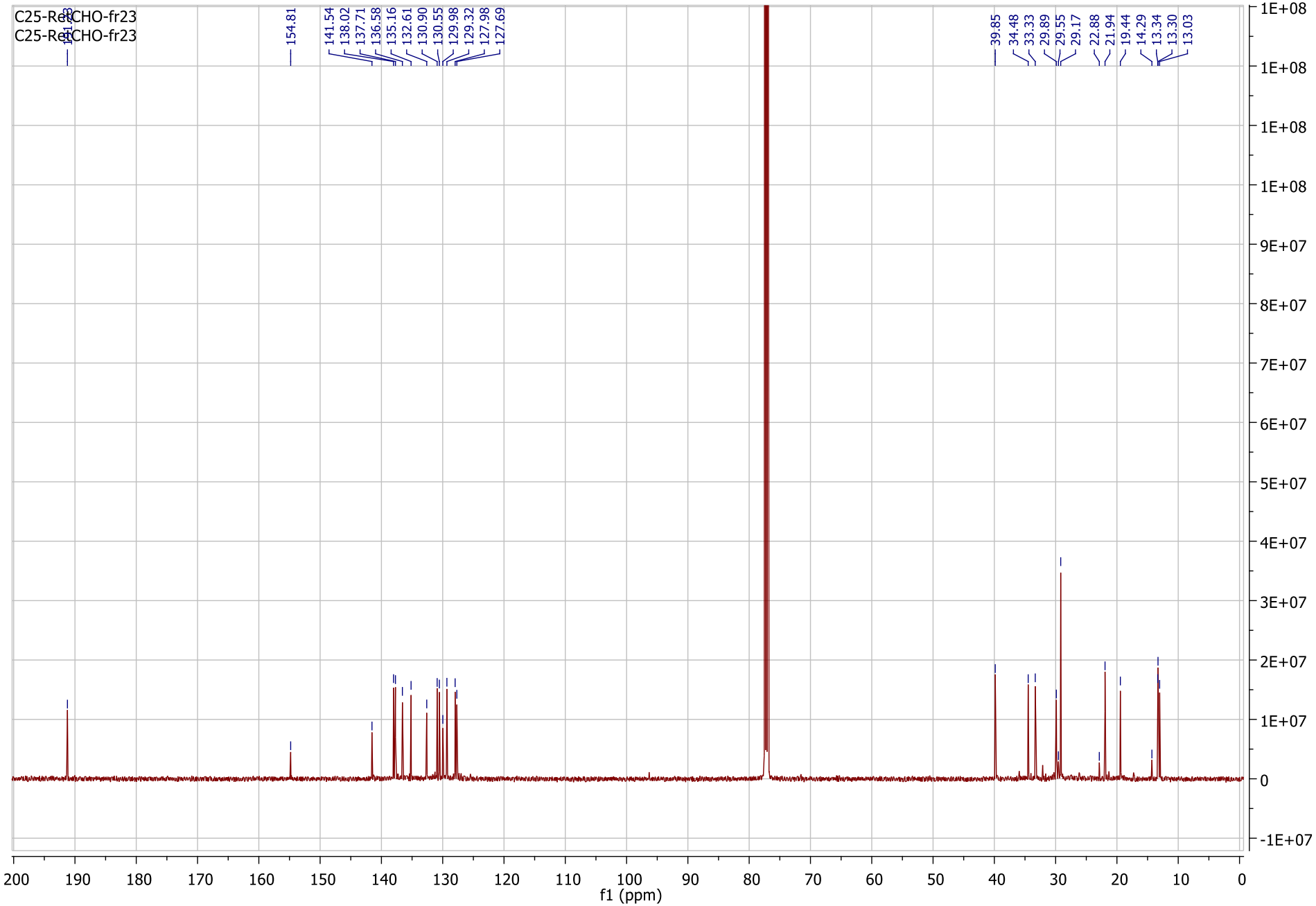


Figure S7 ^{13}C NMR spectrum of C25-aldehyde (**AR8**).

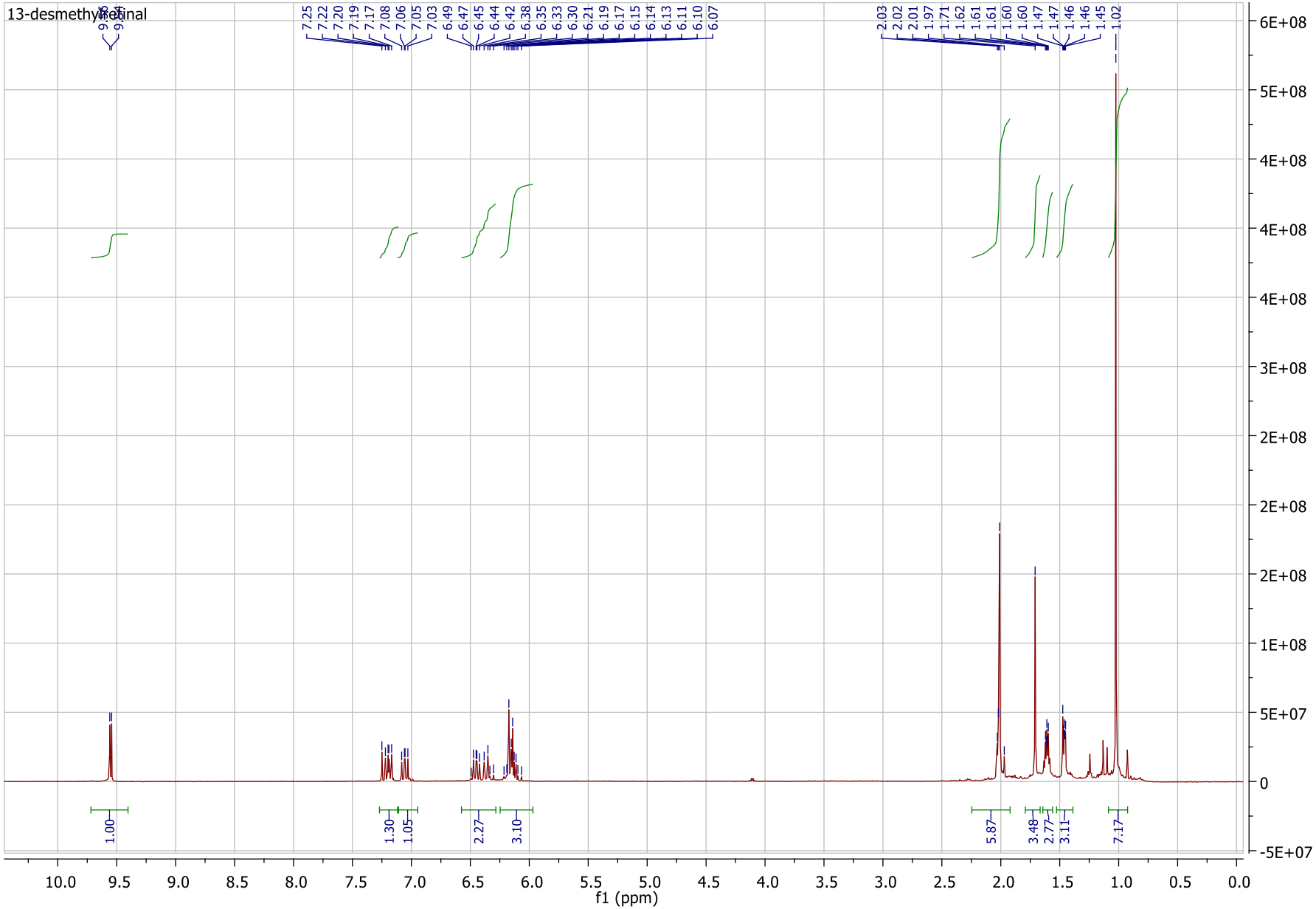


Figure S8 ^{1}H NMR spectrum of 13-desmethylretinal (**AR4**).

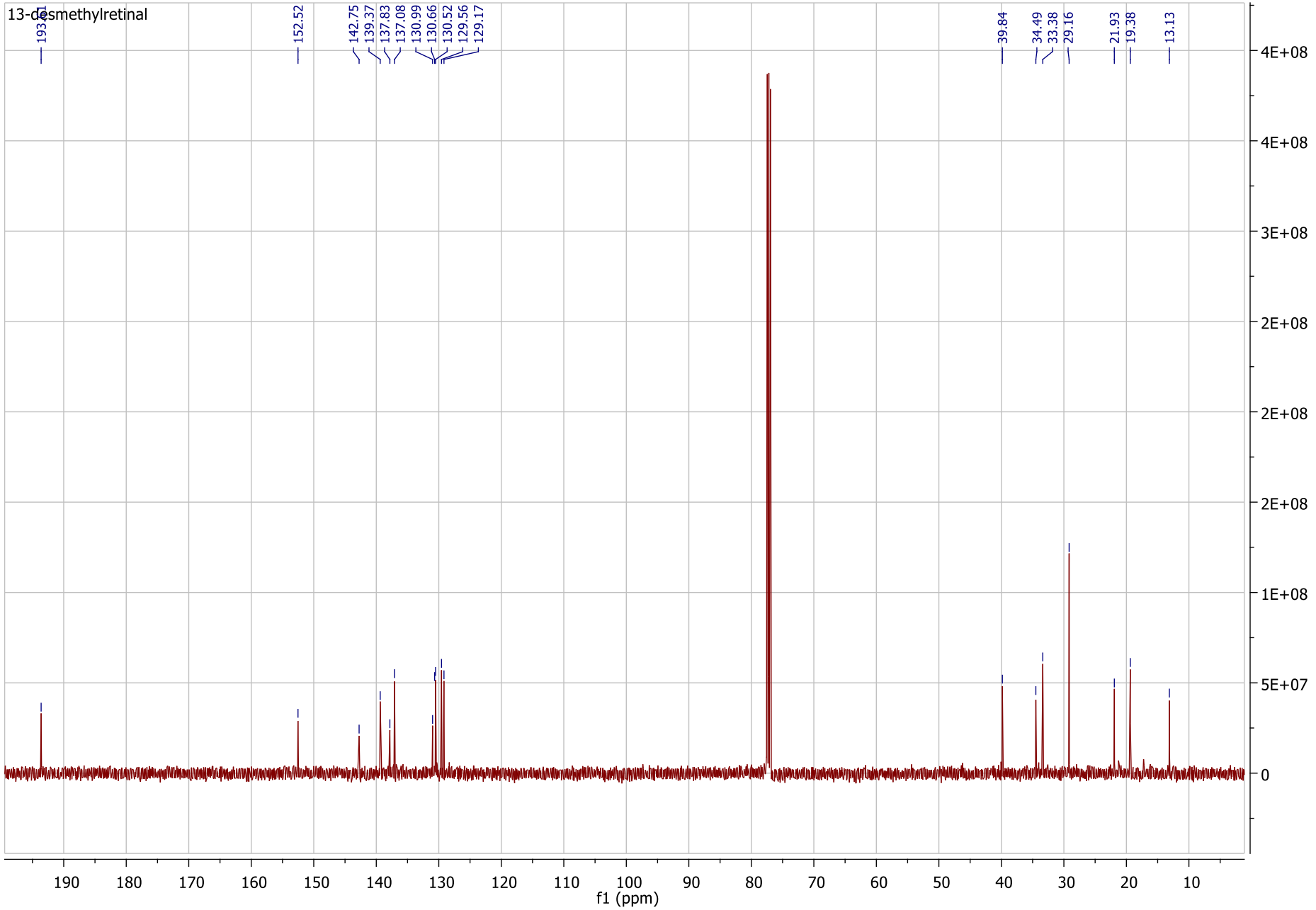


Figure S9 ^{13}C NMR spectrum of 13-desmethylretinal (**AR4**).