Sequence- and stereospecific assignment of methyl groups using paramagnetic lanthanides

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Supporting Information

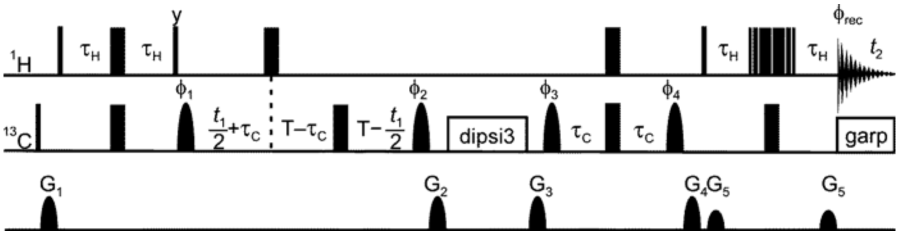


Figure S1. Pulse scheme of the 2D (H)C(C)H-TOCSY experiment used in this study. Parameters are as for the pulse schemes of Figure 1. Efficient magnetization transfer between the methyl groups of isopropyl groups was obtained by applying DIPSI3 mixing for 12 ms with a radiofrequency amplitude of 8.6 kHz. The Bruker pulse programs of this pulse sequence and of the pulse sequences of Figure 1 can be downloaded from http://rsc.anu.edu.au/~go/.

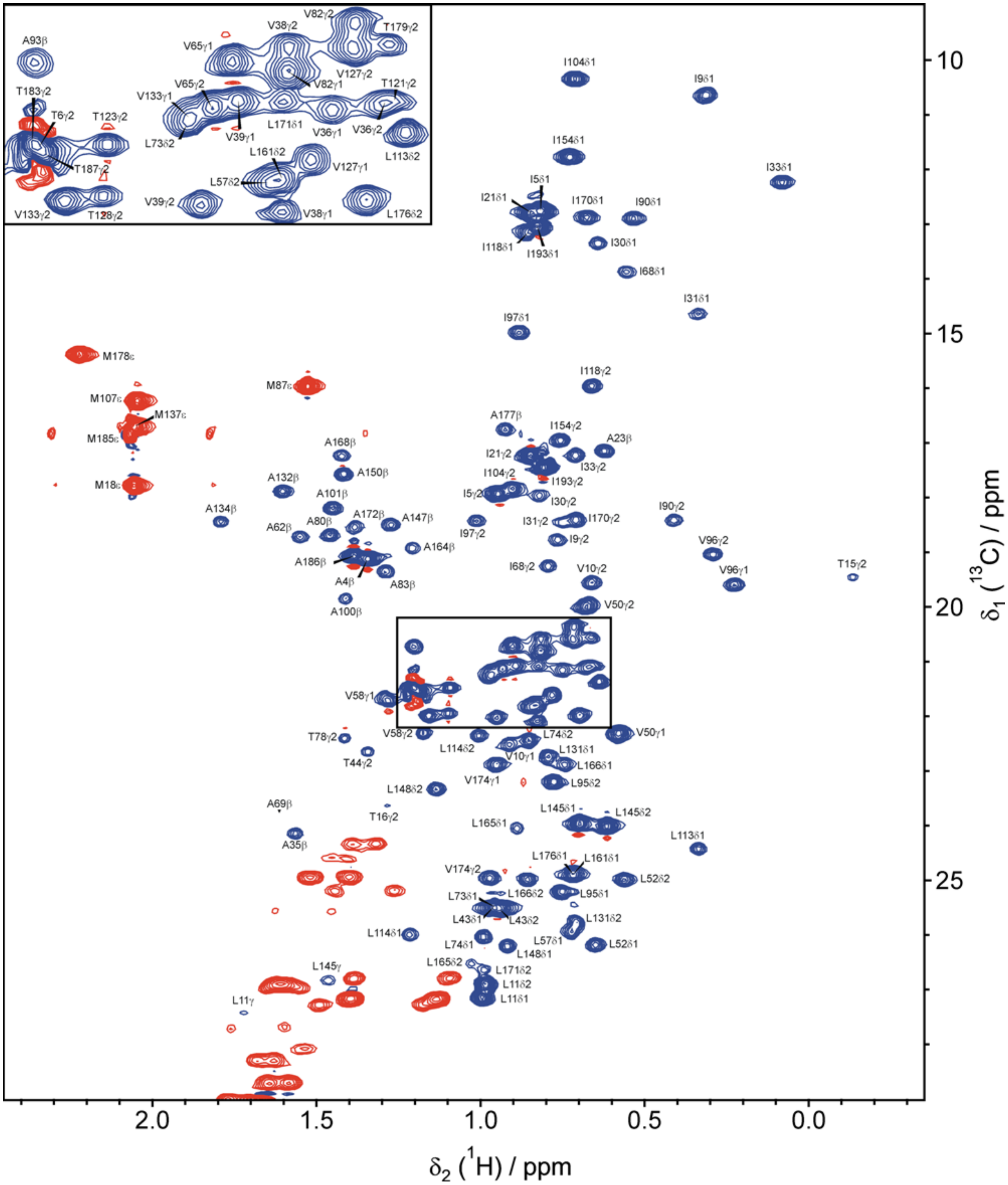


Figure S2. Assigned constant-time (28 ms) ^{13}C-HSQC spectrum of the cz-ε186/θ/La^{3+} complex (^{13}C/^{15}N labeled cz-ε186) at pH 7.2 and 25 ^{o}C. Only the region containing the methyl cross-peaks is shown. Cross-peaks from methyl groups of Val, Leu, Ile, Ala and Thr appear as positive peaks (blue), whereas cross-peaks from Met εCH$_3$ and all CH$_2$ groups appear as negative peaks (red).

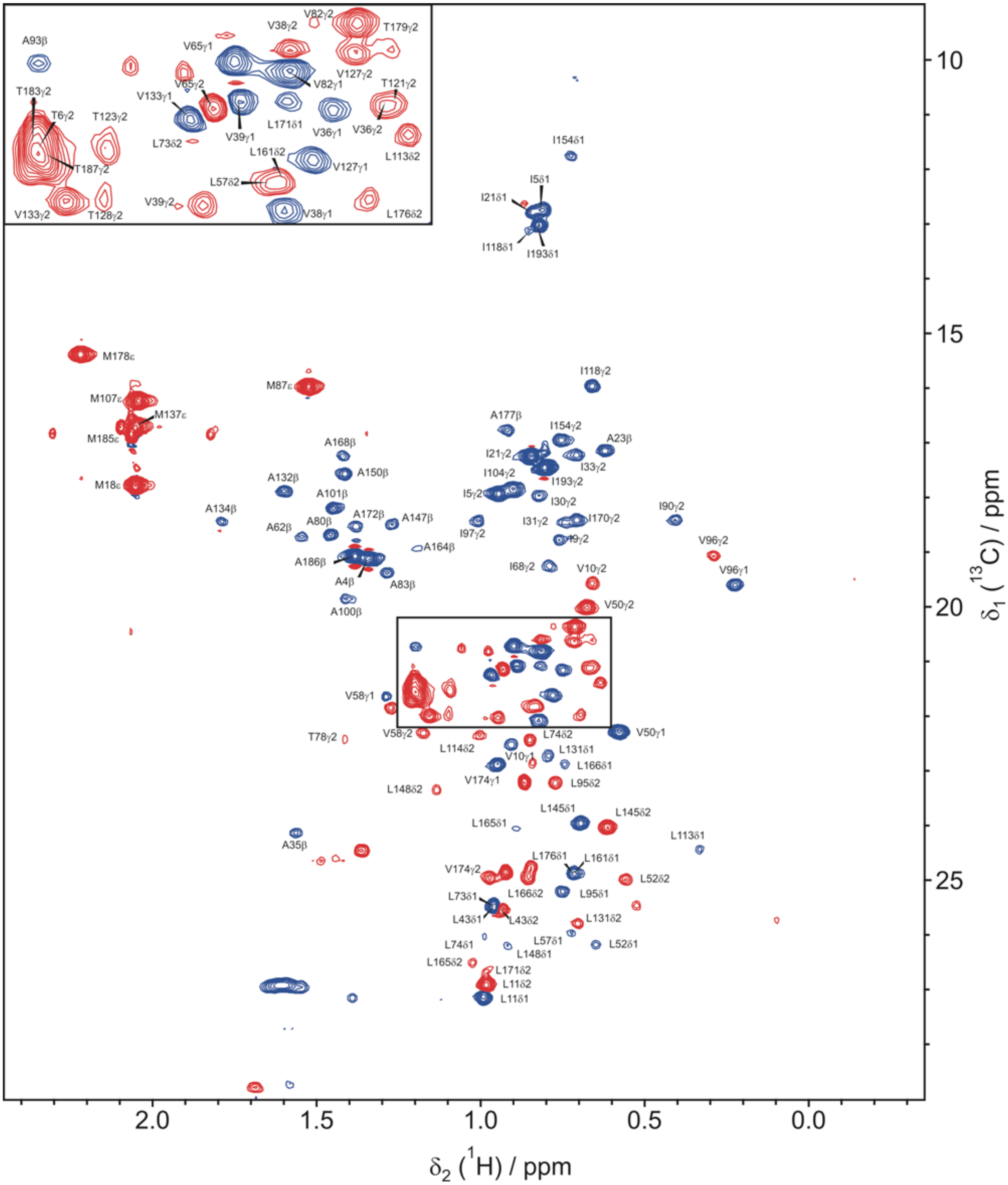


Figure S3. Assigned constant-time ^{13}C-HSQC spectrum of the cz-ε186/θ/La^{3+} complex, where cz-ε186 was biosynthetically fractionally ^{13}C-labeled using 20% uniformly ^{13}C-labeled glucose. Parameters and plot region as in Figure S2. Cross-peaks from Val γ1, Leu δ1, and Ala β methyl groups are positive (blue). Cross-peaks from Val γ2, Leu δ2, Thr γ2 and Met ε methyl groups are negative (red). Cross-peaks from Ile δ1 and γ2 methyl groups are mostly invisible due to scrambling of ^{13}C during Ile biosynthesis.

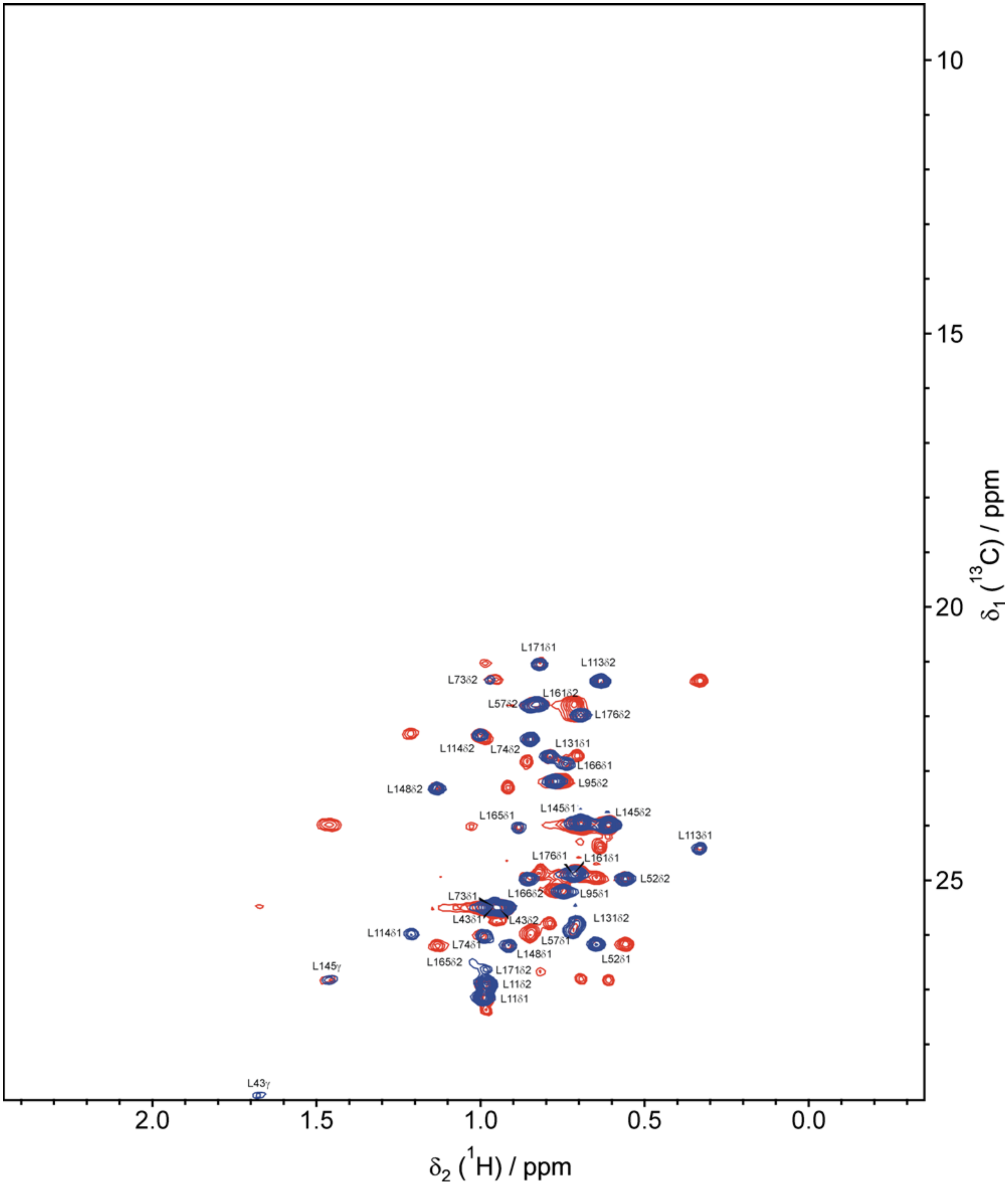


Figure S4. Assigned constant-time ^{13}C-HSQC spectrum of the cz-ε186/θ/La^{3+} complex containing ^{13}C/^{15}N-Leu labeled cz-ε186 (blue) superimposed onto a 2D (H)C(C)H-TOCSY spectrum of the same sample (red). The assignments of the ^{13}C-HSQC cross-peaks are indicated. The three mobile residues Leu11, Leu43 and Leu145 also show one-bond correlations between δCH$_3$ and γCH groups.

Figure S5. Comparisons of calculated and experimental PCS in the cz-ε186/θ/Dy^{3+} complex for methyl groups of (a) Met, (b) Ala, (c) Thr, (d) Val, (e) Leu, and (f) Ile. ^{13}C and ^{1}H PCS are plotted with filled and open bars, respectively, in the sequence $C^{\gamma1}/H^{\gamma1}/C^{\gamma2}/H^{\gamma2}$ (Val), $C^{\delta1}/H^{\delta1}/C^{\delta2}/H^{\delta2}$ (Leu), and $C^{\gamma2}/H^{\gamma2}/C^{\delta1}/H^{\delta1}$ (Ile). The distances $r_{\text{C-Ln}}$ are indicated in Å at the top of each plot. For residues with two methyl groups, the distance value shown at the top refers to the $C^{\gamma1}$ (Val), $C^{\delta1}$ (Leu), or $C^{\delta1}$ (Ile) atom.

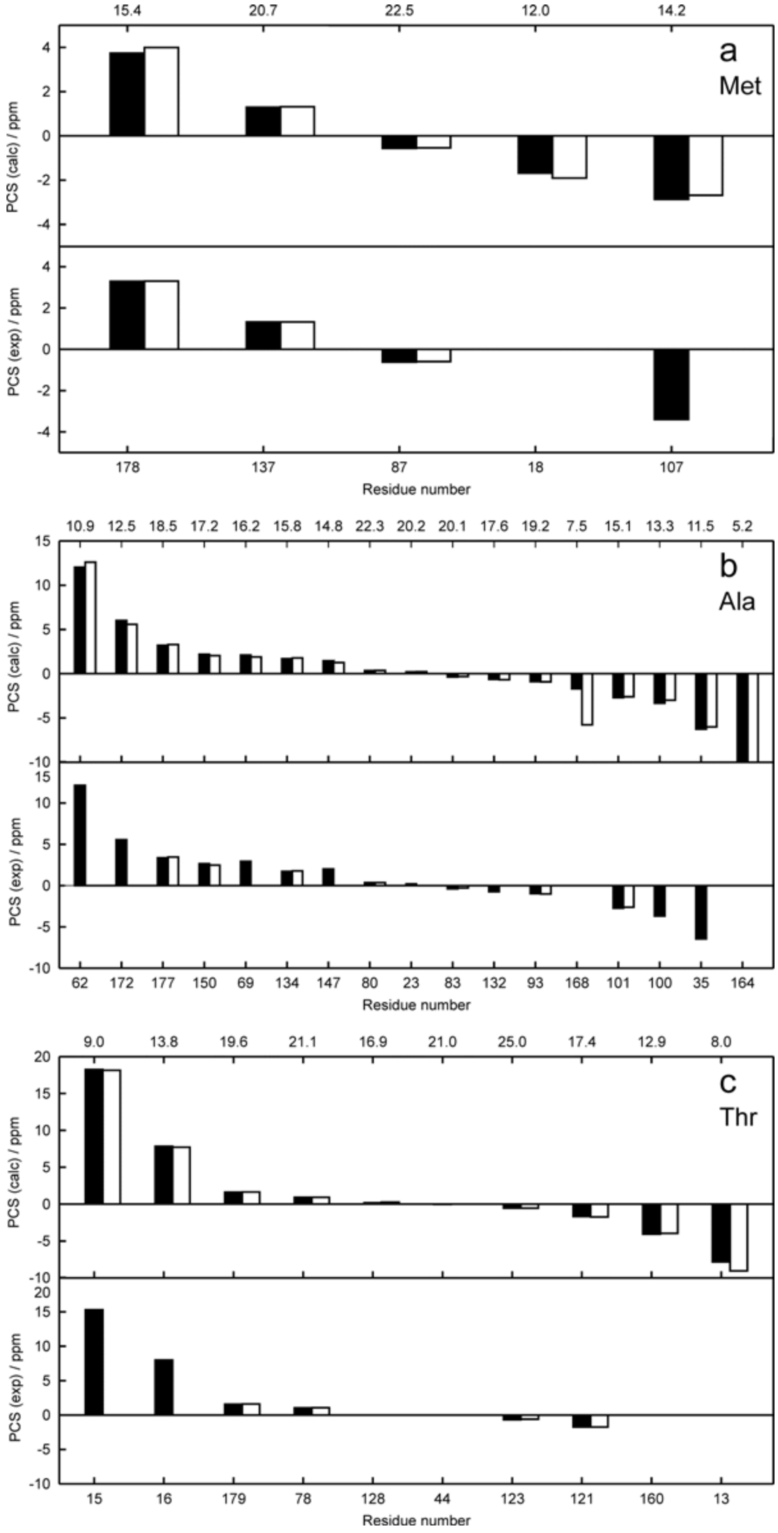


Figure S5 continued

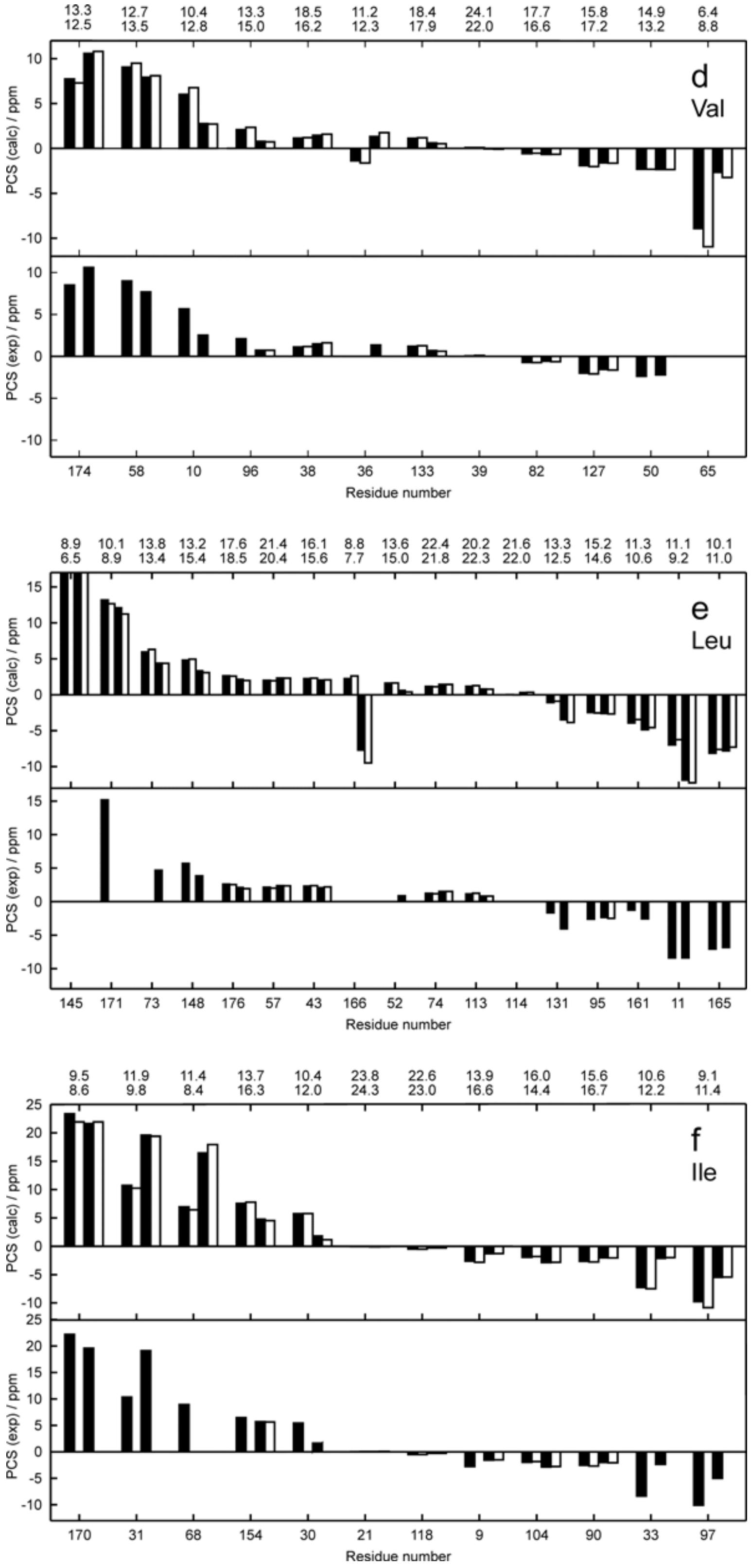


Figure S6. Comparisons of calculated and experimental ^{13}C and ^{1}H PCS as in Figure S5, but for the cz-ε186/θ/Yb^{3+} complex.

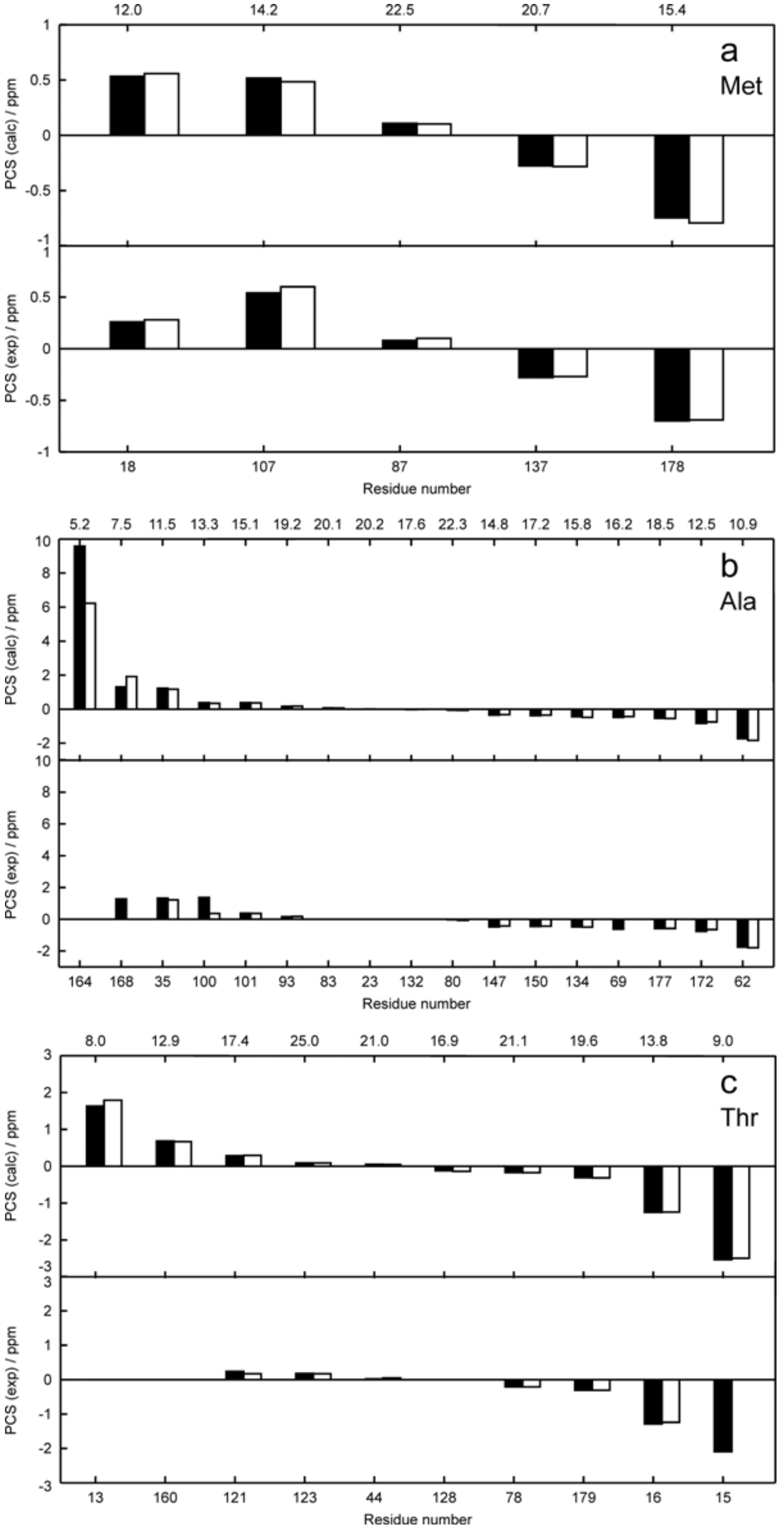
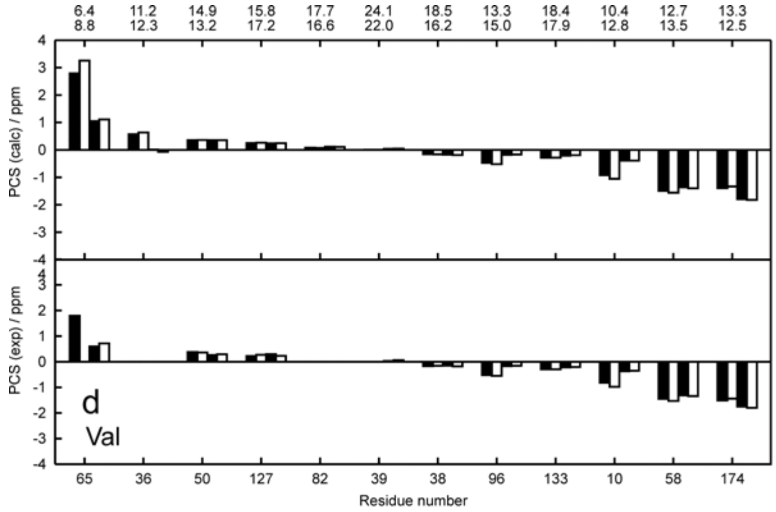
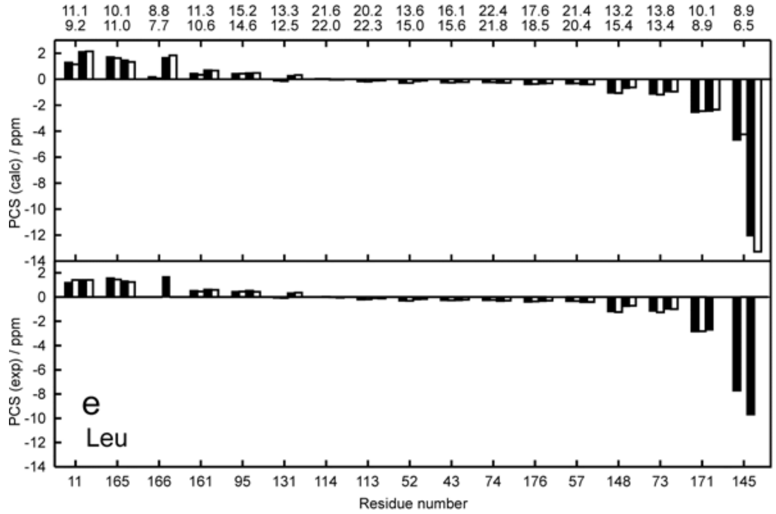


Figure S6 continued





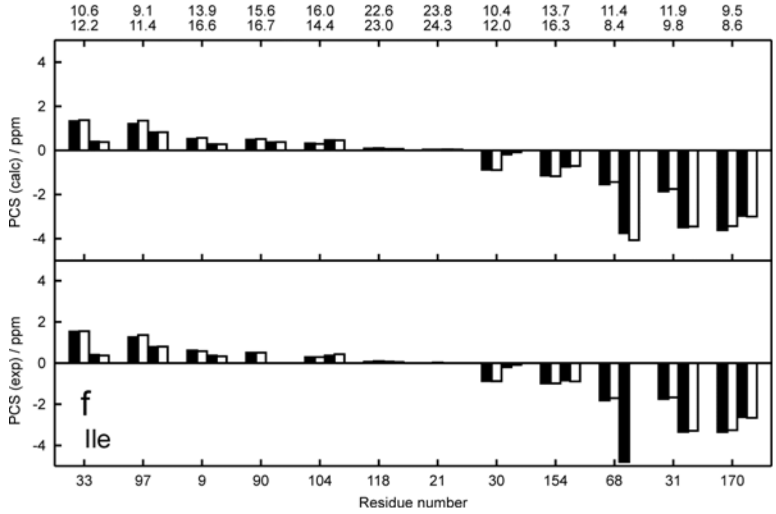


Table S1. ^{13}C and ^{1}H chemical shifts (ppm) of methyl groups of cz-ε186 in the cz-ε186/θ/Ln^{3+} complexes used in this study.[a]

Group	$r_{\mathrm{C-Ln}}$ (Å)	cz-ε186/θ/La^{3+}		cz-ε186/θ/Dy^{3+}		cz-ε186/θ/Yb^{3+}	
		^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Methionine							
M18 ε	12.0	17.78	2.06			18.04	2.34
M87 ε	22.5	15.97	1.53	15.32	0.93	16.05	1.63
M107 ε	14.2	16.23	2.04	12.84		16.77	2.64
M137 ε	20.7	16.69	2.05	18.00	3.38	16.41	1.78
M178 ε	15.4	15.39	2.22	18.67	5.53	14.69	1.53
M185 ε		16.83	2.07	17.32	2.55	16.75	1.98
Alanine							
A4 β		19.14	1.35				
A23 β	20.2	17.15	0.62	17.35			
A35 β	11.5	24.14	1.57	17.70		25.47	2.79
A62 β	10.9	18.72	1.55	30.88		16.97	-0.24
A69 β	16.2	23.77	1.61	26.74		23.15	
A80 β	22.3	18.68	1.46	19.04	1.84	18.66	1.39
A83 β	20.1	19.35	1.29	18.97	1.00		
A93 β	19.2	20.74	1.21	19.78	0.22	20.90	1.39
A100 β	13.3	19.85	1.41	16.19		21.22	1.77
A101 β	15.1	18.20	1.45	15.48	-1.16	18.58	1.82
A132 β	17.6	17.89	1.60	17.16			
A134 β	15.8	18.44	1.79	20.16	3.58	17.96	1.30
A147 β	14.8	18.49	1.28	20.54		18.01	0.86
A150 β	17.2	17.57	1.42	20.22	3.91	17.12	0.99
A164 β	5.2	18.93	1.21				
A168 β	7.5	17.24	1.42			18.52	
A172 β	12.5	18.54	1.38	24.10		17.77	0.73
A177 β	18.5	16.75	0.92	20.11	4.39	16.17	0.34
A186 β		19.07	1.39	19.45	1.77	19.02	1.32
Threonine							
T3 γ2							
T6 γ2		21.50	1.20	21.53	1.25		
T13 γ2	8.0						
T15 γ2	9.0	19.45	-0.13	34.77		17.36	
T16 γ2	13.8	23.64	1.28	31.65		22.35	0.04
T44 γ2	21.0	22.65	1.34	22.64	1.35	22.67	1.39
T78 γ2	21.1	22.40	1.42	23.44	2.48	22.19	1.21
T121 γ2	17.4	21.07	0.66	19.34	-1.07	21.31	0.83
T123 γ2	25.0	21.48	1.09	20.80	0.50	21.66	1.26
T128 γ2	16.9	21.93	1.10				
T160 γ2	12.9						
T179 γ2	19.6	20.56	0.66	22.15	2.28	20.25	0.35
T183 γ2		21.44	1.21	22.16	1.91	21.33	1.08
T187 γ2		21.57	1.19	21.88	1.51	21.50	1.13
Valine							
V10 γ1	10.4	22.52	0.91	28.21		21.70	-0.07
γ2	12.8	19.56	0.66	22.13		19.19	0.31
V36 γ1	11.2	21.16	0.75				
γ2	12.3	21.09	0.67	21.97			
V38 γ1	18.5	22.09	0.83	23.20	2.03	21.92	0.67
γ2	16.2	20.58	0.82	22.08	2.42	20.43	0.64

V39	γ1	24.1	21.08	0.89	21.14	1.00		
	γ2	22.0	22.03	0.95			22.06	1.01
V50	γ1	14.9	22.32	0.58	19.95		22.70	0.94
	γ2	13.2	20.00	0.68	17.80		20.26	0.97
V58	γ1	12.7	21.64	1.29	30.67		20.19	-0.24
	γ2	13.5	22.31	1.18	30.02		21.00	-0.16
V65	γ1	6.4	20.72	0.90			22.51	
	γ2	8.8	21.14	0.93			21.74	1.65
V82	γ1	17.7	20.81	0.82	20.09	0.08		
	γ2	16.6	20.37	0.72	19.79	0.10		
V96	γ1	13.3	19.60	0.23	21.73		19.08	-0.32
	γ2	15.0	19.04	0.29	19.83	1.04	18.87	0.13
V127	γ1	15.8	21.62	0.78	19.64	-1.32	21.84	1.05
	γ2	17.2	20.59	0.72	19.02	-0.93	20.89	0.95
V133	γ1	17.9	21.22	0.97	22.43	2.24	20.92	0.67
	γ2	18.4	21.99	1.16	22.69	1.76	21.77	0.95
V174	γ1	13.3	22.88	0.95	31.40		21.37	-0.49
	γ2	12.5	24.96	0.97	35.55		23.21	-0.83
Leucine								
L11	δ1	11.1	27.14	1.00	18.89		28.30	2.40
	δ2	9.2	26.90	0.99	18.65		28.30	2.39
L43	δ1	16.1	25.49	0.96	27.78	3.35	25.24	0.69
	δ2	15.6	25.52	0.94	27.63	3.15	25.26	0.72
L52	δ1	13.6	26.18	0.65			25.88	0.34
	δ2	15.0	24.99	0.56	25.93		24.81	0.39
L57	δ1	21.4	25.94	0.72	28.07	2.74	25.61	0.39
	δ2	20.4	21.82	0.85	24.23	3.21	21.40	0.44
L73	δ1	13.8	25.49	0.96			24.36	-0.30
	δ2	13.4	21.30	0.97	25.98		20.34	-0.02
L74	δ1	22.4	26.03	0.99	27.27	2.17	25.78	0.75
	δ2	21.8	22.44	0.85	23.95	2.39	22.12	0.55
L95	δ1	15.2	25.21	0.75	22.60		25.64	1.20
	δ2	14.6	23.20	0.78	20.84	-1.73	23.72	1.21
L113	δ1	20.2	24.42	0.34	25.63	1.59	24.22	0.15
	δ2	22.3	21.37	0.64	22.17	1.45	21.27	0.53
L114	δ1	21.6	26.00	1.22			26.00	1.24
	δ2	22.0	22.35	1.01			22.33	0.95
L131	δ1	13.3	22.74	0.79	21.14		22.69	0.70
	δ2	12.5	25.78	0.71	21.89		26.09	1.07
L145	δ1	8.9	23.96	0.70			16.25	
	δ2	6.5	24.01	0.62			14.34	
L148	δ1	13.2	26.21	0.92	31.96		25.03	-0.32
	δ2	15.4	23.33	1.14	27.21		22.59	0.42
L161	δ1	11.3	24.89	0.72	23.66		25.39	1.18
	δ2	10.6	21.78	0.83	19.26		22.39	1.42
L165	δ1	10.1	24.05	0.89	17.02		25.60	2.34
	δ2	11.0	26.53	1.03	19.74		27.83	2.26
L166	δ1	8.8	22.89	0.74				
	δ2	7.7	24.99	0.86			26.66	
L171	δ1	10.1	21.06	0.82	36.23		18.23	-2.00
	δ2	8.9	26.65	0.99			23.97	
L176	δ1	17.6	24.89	0.72	27.48	3.25	24.51	0.36
	δ2	18.5	21.98	0.70	24.08	2.64	21.66	0.40
Isoleucine								
I5	γ2		17.94	0.95	17.86	0.87	17.96	0.97
	δ1		12.76	0.82	12.66	0.71		
I9	γ2	13.9	18.78	0.77	16.02		19.39	1.35
	δ1	16.6	10.64	0.31	9.09	-1.20	11.00	0.64

I21	γ2	23.8	17.24	0.85	17.29	0.91	17.24	0.87
	δ1	24.3	12.79	0.85	12.86	0.93		
I30	γ2	10.4	17.96	0.82	23.37		17.09	-0.06
	δ1	12.0	13.35	0.64	15.00		13.15	0.55
I31	γ2	11.9	18.45	0.75	28.85		16.71	-0.92
	δ1	9.8	14.65	0.34	33.74		11.31	-2.95
I33	γ2	10.6	17.24	0.71	8.89		18.77	2.26
	δ1	12.2	12.24	0.08	9.89		12.65	0.45
I68	γ2	11.4	19.25	0.79	28.19		17.44	-0.91
	δ1	8.4	13.87	0.56			9.07	
I90	γ2	15.6	18.42	0.41	15.93	-2.28	18.93	0.92
	δ1	16.7	12.90	0.53	10.88	-1.52		
I97	γ2	9.1	18.43	1.01	8.34		19.69	2.37
	δ1	11.4	14.99	0.88	9.50		15.78	1.68
I104	γ2	16.0	17.84	0.90	15.86	-2.05	18.13	1.19
	δ1	14.4	10.34	0.71	7.50		10.70	1.14
I118	γ2	22.6	15.96	0.66	15.47	0.17	16.02	0.75
	δ1	23.0	13.16	0.86	12.89	0.58	13.23	0.91
I154	γ2	13.7	16.96	0.76	23.43		15.97	-0.23
	δ1	16.3	11.78	0.73	17.48	6.40	10.94	-0.16
I170	γ2	9.5	18.41	0.71	40.65		15.06	-2.55
	δ1	8.6	12.88	0.68	32.52		10.26	-1.98
I193	γ2		17.46	0.81	17.55	0.91	17.42	0.78
	δ1		13.04	0.83	13.15	0.94	13.00	0.80

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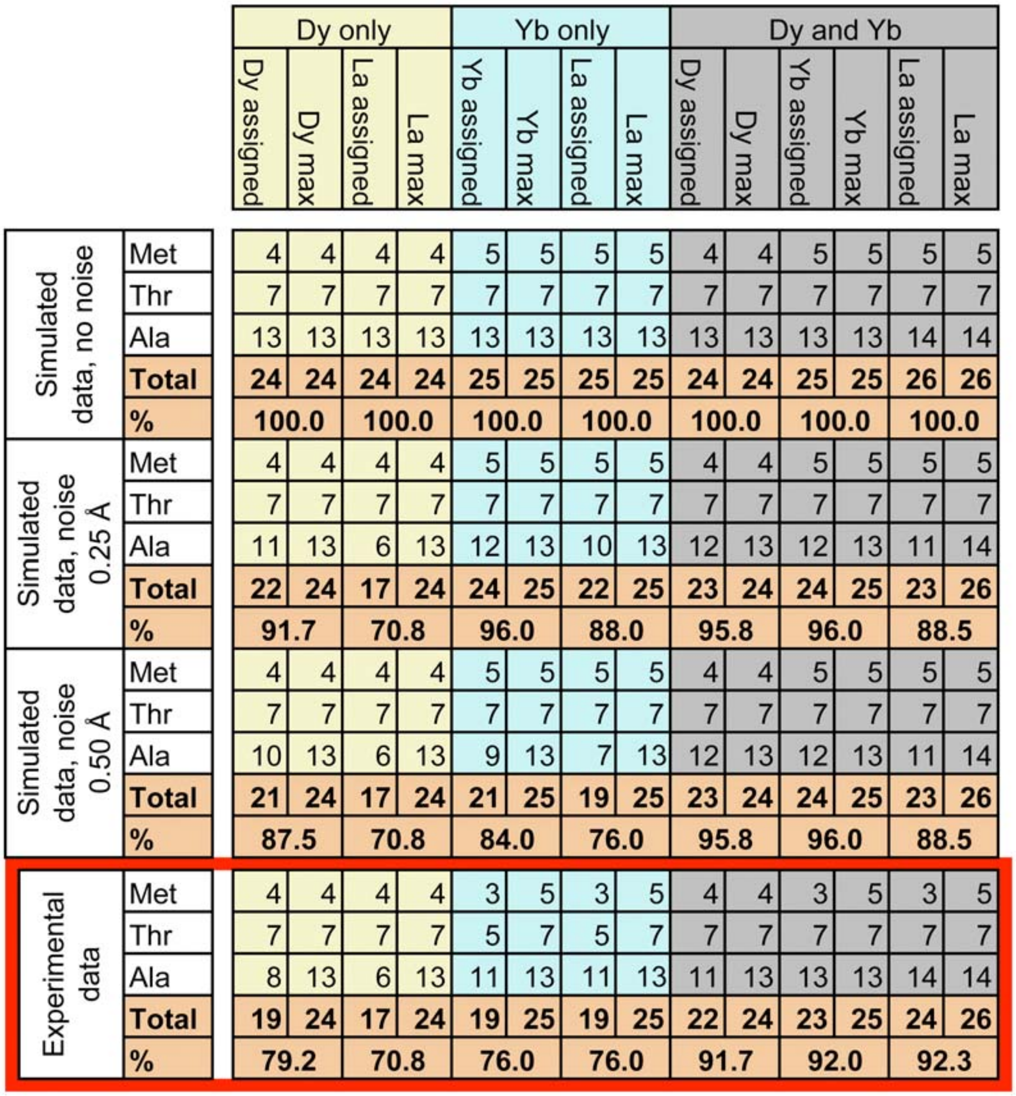
[a] Conditions: 25 °C, pH 7.2. The chemical shifts in the cz-ε186/θ/La^{3+} complex were measured from ^{13}C-HSQC spectra of the sample containing ^{13}C/^{15}N labeled cz-ε186 in the presence of 1 equivalent La^{3+}. Whenever possible, chemical shifts of the cz-ε186/θ/Dy^{3+} and cz-ε186/θ/Yb^{3+} complexes were measured from ^{13}C-HSQC spectra of samples prepared with 1:1 mixtures of La^{3+} and Dy^{3+}, or La^{3+} and Yb^{3+}, respectively. ^{13}C chemical shifts of methyl groups for which no ^{1}H chemical shift is reported were measured from the pd exchange peaks in 2D or 3D methyl C$_z$-EXSY spectra, whichever gave better resolution. When neither ^{13}C nor ^{1}H chemical shifts are indicated, the expected cross-peak could not be identified either because of spectral overlap (e.g. in the case of vanishing PCS) or strong PRE.

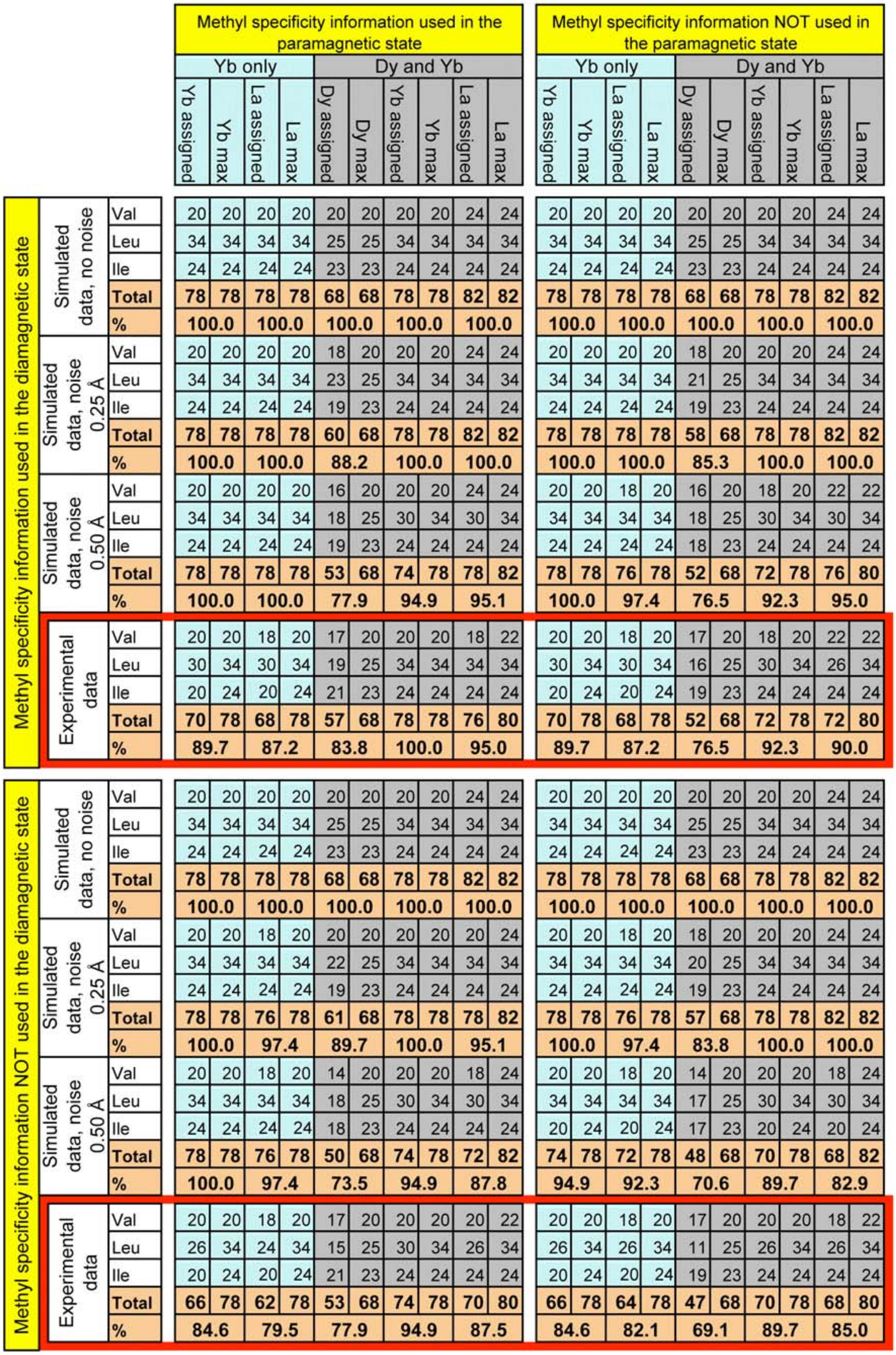
Table S2. Number of correctly assigned methyl groups of Met, Thr, and Ala residues of cz-ε186 using the program Possum.[a]



		Dy only				Yb only				Dy and Yb					
		Dy assigned	Dy max	La assigned	La max	Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	Yb assigned	Yb max	La assigned	La max
Simulated data, no noise	Met	4	4	4	4	5	5	5	5	4	4	5	5	5	5
	Thr	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Ala	13	13	13	13	13	13	13	13	13	13	13	13	14	14
	Total	**24**	**24**	**24**	**24**	**25**	**25**	**25**	**25**	**24**	**24**	**25**	**25**	**26**	**26**
	%	**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**	
Simulated data, noise 0.25 Å	Met	4	4	4	4	5	5	5	5	4	4	5	5	5	5
	Thr	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Ala	11	13	6	13	12	13	10	13	12	13	12	13	11	14
	Total	**22**	**24**	**17**	**24**	**24**	**25**	**22**	**25**	**23**	**24**	**24**	**25**	**23**	**26**
	%	**91.7**		**70.8**		**96.0**		**88.0**		**95.8**		**96.0**		**88.5**	
Simulated data, noise 0.50 Å	Met	4	4	4	4	5	5	5	5	4	4	5	5	5	5
	Thr	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	Ala	10	13	6	13	9	13	7	13	12	13	12	13	11	14
	Total	**21**	**24**	**17**	**24**	**21**	**25**	**19**	**25**	**23**	**24**	**24**	**25**	**23**	**26**
	%	**87.5**		**70.8**		**84.0**		**76.0**		**95.8**		**96.0**		**88.5**	
Experimental data	Met	4	4	4	4	3	5	3	5	4	4	3	5	3	5
	Thr	7	7	7	7	5	7	5	7	7	7	7	7	7	7
	Ala	8	13	6	13	11	13	11	13	11	13	13	13	14	14
	Total	**19**	**24**	**17**	**24**	**19**	**25**	**19**	**25**	**22**	**24**	**23**	**25**	**24**	**26**
	%	**79.2**		**70.8**		**76.0**		**76.0**		**91.7**		**92.0**		**92.3**	

[a] Calculations were performed using the experimental data of Table S1 and simulated data, where the paramagnetic chemical shifts of Table S1 were replaced by chemical shifts back-calculated from the crystal structure of ε186 and the $\Delta\chi$ tensors used in the present study. Two additional sets of simulated data were generated by addition of structural noise to the PDB coordinates of ε186. The structural noise followed a Gaussian distribution of 0.25 and 0.5 Å standard deviation, resulting in a Maxwell-Boltzmann distribution of atomic displacements with maxima at 0.35 and 0.7 Å, respectively. The columns marked “Dy max”, “Yb max”, and “La max” report the number of methyl groups for which data in the paramagnetic state were available to the program. (Additional peaks observed in the diamagnetic state remained unassigned.) The results are reported for calculations where the diamagnetic chemical shifts were supplemented only with data from Dy^{3+} (light yellow), Yb^{3+} (light blue) or both (grey). The rows marked with the % symbol display the percentage of correctly assigned methyl groups for all three residues. The program Possum is available from http://compbio.chemistry.uq.edu.au/bmmg/christophe.

Table S3. Number of correctly assigned methyl groups of Val, Leu, and Ile residues of cz-ε186 using the program Possum *with* methyl connectivity information in the Yb^{3+} complex.[a]



			Methyl specificity information used in the paramagnetic state										Methyl specificity information NOT used in the paramagnetic state									
			Yb only				Dy and Yb						Yb only				Dy and Yb					
			Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	Yb assigned	Yb max	La assigned	La max	Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	Yb assigned	Yb max	La assigned	La max
Methyl specificity information used in the diamagnetic state	Simulated data, no noise	Val	20	20	20	20	20	20	20	20	24	24	20	20	20	20	20	20	20	20	24	24
		Leu	34	34	34	34	25	25	34	34	34	34	34	34	34	34	25	25	34	34	34	34
		Ile	24	24	24	24	23	23	24	24	24	24	24	24	24	24	23	23	24	24	24	24
		Total	**78**	**78**	**78**	**78**	**68**	**68**	**78**	**78**	**82**	**82**	**78**	**78**	**78**	**78**	**68**	**68**	**78**	**78**	**82**	**82**
		%	**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**	
	Simulated data, noise 0.25 Å	Val	20	20	20	20	18	20	20	20	24	24	20	20	20	20	18	20	20	20	24	24
		Leu	34	34	34	34	23	25	34	34	34	34	34	34	34	34	21	25	34	34	34	34
		Ile	24	24	24	24	19	23	24	24	24	24	24	24	24	24	19	23	24	24	24	24
		Total	**78**	**78**	**78**	**78**	**60**	**68**	**78**	**78**	**82**	**82**	**78**	**78**	**78**	**78**	**58**	**68**	**78**	**78**	**82**	**82**
		%	**100.0**		**100.0**		**88.2**		**100.0**		**100.0**		**100.0**		**100.0**		**85.3**		**100.0**		**100.0**	
	Simulated data, noise 0.50 Å	Val	20	20	20	20	16	20	20	20	24	24	20	20	18	20	16	20	18	20	22	22
		Leu	34	34	34	34	18	25	30	34	30	34	34	34	34	34	18	25	30	34	30	34
		Ile	24	24	24	24	19	23	24	24	24	24	24	24	24	24	18	23	24	24	24	24
		Total	**78**	**78**	**78**	**78**	**53**	**68**	**74**	**78**	**78**	**82**	**78**	**78**	**76**	**78**	**52**	**68**	**72**	**78**	**76**	**80**
		%	**100.0**		**100.0**		**77.9**		**94.9**		**95.1**		**100.0**		**97.4**		**76.5**		**92.3**		**95.0**	
	Experimental data	Val	20	20	18	20	17	20	20	20	18	22	20	20	18	20	17	20	18	20	22	22
		Leu	30	34	30	34	19	25	34	34	34	34	30	34	30	34	16	25	30	34	26	34
		Ile	20	24	20	24	21	23	24	24	24	24	20	24	20	24	19	23	24	24	24	24
		Total	**70**	**78**	**68**	**78**	**57**	**68**	**78**	**78**	**76**	**80**	**70**	**78**	**68**	**78**	**52**	**68**	**72**	**78**	**72**	**80**
		%	**89.7**		**87.2**		**83.8**		**100.0**		**95.0**		**89.7**		**87.2**		**76.5**		**92.3**		**90.0**	
Methyl specificity information NOT used in the diamagnetic state	Simulated data, no noise	Val	20	20	20	20	20	20	20	20	24	24	20	20	20	20	20	20	20	20	24	24
		Leu	34	34	34	34	25	25	34	34	34	34	34	34	34	34	25	25	34	34	34	34
		Ile	24	24	24	24	23	23	24	24	24	24	24	24	24	24	23	23	24	24	24	24
		Total	**78**	**78**	**78**	**78**	**68**	**68**	**78**	**78**	**82**	**82**	**78**	**78**	**78**	**78**	**68**	**68**	**78**	**78**	**82**	**82**
		%	**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**	
	Simulated data, noise 0.25 Å	Val	20	20	18	20	20	20	20	20	20	24	20	20	18	20	18	20	20	20	24	24
		Leu	34	34	34	34	22	25	34	34	34	34	34	34	34	34	20	25	34	34	34	34
		Ile	24	24	24	24	19	23	24	24	24	24	24	24	24	24	19	23	24	24	24	24
		Total	**78**	**78**	**76**	**78**	**61**	**68**	**78**	**78**	**78**	**82**	**78**	**78**	**76**	**78**	**57**	**68**	**78**	**78**	**82**	**82**
		%	**100.0**		**97.4**		**89.7**		**100.0**		**95.1**		**100.0**		**97.4**		**83.8**		**100.0**		**100.0**	
	Simulated data, noise 0.50 Å	Val	20	20	18	20	14	20	20	20	18	24	20	20	18	20	14	20	20	20	18	24
		Leu	34	34	34	34	18	25	30	34	30	34	34	34	34	34	17	25	30	34	30	34
		Ile	24	24	24	24	18	23	24	24	24	24	20	24	20	24	17	23	20	24	20	24
		Total	**78**	**78**	**76**	**78**	**50**	**68**	**74**	**78**	**72**	**82**	**74**	**78**	**72**	**78**	**48**	**68**	**70**	**78**	**68**	**82**
		%	**100.0**		**97.4**		**73.5**		**94.9**		**87.8**		**94.9**		**92.3**		**70.6**		**89.7**		**82.9**	
	Experimental data	Val	20	20	18	20	17	20	20	20	20	22	20	20	18	20	17	20	20	20	18	22
		Leu	26	34	24	34	15	25	30	34	26	34	26	34	26	34	11	25	26	34	26	34
		Ile	20	24	20	24	21	23	24	24	24	24	20	24	20	24	19	23	24	24	24	24
		Total	**66**	**78**	**62**	**78**	**53**	**68**	**74**	**78**	**70**	**80**	**66**	**78**	**64**	**78**	**47**	**68**	**70**	**78**	**68**	**80**
		%	**84.6**		**79.5**		**77.9**		**94.9**		**87.5**		**84.6**		**82.1**		**69.1**		**89.7**		**85.0**	

[a] Calculations were performed using the experimental data of Table S1 and simulated data as described in the footnote of Table S2. As each Val, Leu and Ile residue contains two methyl groups, methyl specificity and methyl connectivity information can be used as additional information to support the resonance assignment. (Methyl specificity information refers to stereospecific assignments of the methyl groups of Val and Leu and the a priori distinction of γ_2 and δ_1 methyl groups of Ile. Methyl connectivity information refers to the knowledge of which peaks arise from the same residue.) The results of four different combinations are shown, with and without methyl specificity information in the paramagnetic complexes, and with and without methyl specificity information in the diamagnetic complex. It was assumed that no methyl connectivity information can be established for the Dy^{3+} complex because of strong PRE. The data are presented in the same format as in Table S2. Assignments were counted as correct whenever a methyl cross-peak was assigned to the correct residue, disregarding the stereospecific correctness of the assignment. Note that the maximum number of assignable methyl groups reported in the column marked “La max” can vary when both Dy^{3+} and Yb^{3+} data are used, because Possum has the freedom not to assign every HSQC cross-peak observed for the Dy^{3+} complex to a peak observed for the Yb^{3+} complex. This results in a small variation of the number of residues for which the program has paramagnetic information available and can attempt an assignment of the diamagnetic data.

Table S4. Number of correctly assigned methyl groups of valine, leucine, and isoleucine residues of cz-ε186 using the program Possum *without* methyl connectivity information in the Yb^{3+} complex.[a]

			Methyl specificity information used in the paramagnetic state														Methyl specificity information NOT used in the paramagnetic state													
			Dy only				Yb only				Dy and Yb						Dy only				Yb only				Dy and Yb					
			Dy assigned	Dy max	La assigned	La max	Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	La assigned	La max	Yb assigned	Yb max	La assigned	La max	Dy assigned	Dy max	Yb assigned	Yb max	La assigned	La max
Methyl specificity information used in the diamagnetic state	Simulated data, no noise	Val	20	20	22	22	19	19	20	20	20	20	19	19	24	24	20	20	22	22	19	19	20	20	20	20	19	19	24	24
		Leu	25	25	28	28	33	33	34	34	25	25	33	33	34	34	25	25	26	28	33	33	34	34	25	25	33	33	34	34
		Ile	23	23	24	24	22	22	24	24	23	23	22	22	24	24	23	23	24	24	22	22	24	24	23	23	22	22	24	24
		Total	**68**	**68**	**74**	**74**	**74**	**74**	**78**	**78**	**68**	**68**	**74**	**74**	**82**	**82**	**68**	**68**	**72**	**74**	**74**	**74**	**78**	**78**	**68**	**68**	**74**	**74**	**82**	**82**
		%	**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**97.3**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**	
	Simulated data, noise 0.25 Å	Val	18	20	20	22	19	19	20	20	18	20	19	19	24	24	18	20	20	22	19	19	20	20	18	20	19	19	24	24
		Leu	21	25	24	30	33	33	34	34	22	25	33	33	34	34	21	25	18	30	33	33	34	34	20	25	33	33	34	34
		Ile	22	23	14	24	22	22	24	24	19	23	22	22	24	24	18	23	12	24	22	22	24	24	19	23	20	22	24	24
		Total	**61**	**68**	**58**	**76**	**74**	**74**	**78**	**78**	**59**	**68**	**74**	**74**	**82**	**82**	**57**	**68**	**50**	**76**	**74**	**74**	**78**	**78**	**57**	**68**	**72**	**74**	**82**	**82**
		%	**89.7**		**76.3**		**100.0**		**100.0**		**86.8**		**100.0**		**100.0**		**83.8**		**65.8**		**100.0**		**100.0**		**83.8**		**97.3**		**100.0**	
	Simulated data, noise 0.50 Å	Val	16	20	20	22	19	19	20	20	16	20	18	19	24	24	13	20	16	22	18	19	18	22	15	20	18	19	20	24
		Leu	14	25	14	30	33	33	34	34	18	25	29	33	30	34	9	25	8	28	27	33	24	34	15	25	25	33	24	34
		Ile	16	23	6	24	22	22	24	24	19	23	22	22	24	24	16	23	10	24	22	22	24	24	19	23	22	22	24	24
		Total	**46**	**68**	**40**	**76**	**74**	**74**	**78**	**78**	**53**	**68**	**69**	**74**	**78**	**82**	**38**	**68**	**34**	**74**	**67**	**74**	**66**	**80**	**49**	**68**	**65**	**74**	**68**	**82**
		%	**67.6**		**52.6**		**100.0**		**100.0**		**77.9**		**93.2**		**95.1**		**55.9**		**45.9**		**90.5**		**82.5**		**72.1**		**87.8**		**82.9**	
	Experimental data	Val	17	20	20	20	18	19	20	22	17	20	18	19	20	24	17	20	20	20	18	19	22	22	17	20	18	19	24	24
		Leu	14	25	10	30	25	33	24	34	19	25	33	33	34	34	12	25	12	30	25	33	24	34	16	25	29	33	26	34
		Ile	19	23	18	24	19	22	20	24	21	23	22	22	24	24	17	23	14	24	19	22	20	24	17	23	22	22	24	24
		Total	**50**	**68**	**48**	**74**	**62**	**74**	**64**	**80**	**57**	**68**	**73**	**74**	**78**	**82**	**46**	**68**	**46**	**74**	**62**	**74**	**66**	**80**	**50**	**68**	**69**	**74**	**74**	**82**
		%	**73.5**		**64.9**		**83.8**		**80.0**		**83.8**		**98.6**		**95.1**		**67.6**		**62.2**		**83.8**		**82.5**		**73.5**		**93.2**		**90.2**	
Methyl specificity information NOT used in the diamagnetic state	Simulated data, no noise	Val	20	20	22	22	19	19	20	20	20	20	19	19	24	24	20	20	22	22	19	19	20	20	20	20	19	19	24	24
		Leu	25	25	26	28	33	33	34	34	25	25	33	33	34	34	25	25	26	28	33	33	34	34	25	25	33	33	34	34
		Ile	23	23	24	24	22	22	24	24	23	23	22	22	24	24	23	23	24	24	22	22	24	24	23	23	22	22	24	24
		Total	**68**	**68**	**72**	**74**	**74**	**74**	**78**	**78**	**68**	**68**	**74**	**74**	**82**	**82**	**68**	**68**	**72**	**74**	**74**	**74**	**78**	**78**	**68**	**68**	**74**	**74**	**82**	**82**
		%	**100.0**		**97.3**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**		**97.3**		**100.0**		**100.0**		**100.0**		**100.0**		**100.0**	
	Simulated data, noise 0.25 Å	Val	18	20	20	24	19	19	18	20	20	20	19	19	20	24	18	20	20	22	19	19	18	20	18	20	19	19	24	24
		Leu	16	25	16	30	33	33	34	34	22	25	33	33	34	34	13	25	14	30	33	33	34	34	20	25	33	33	34	34
		Ile	22	23	14	24	22	22	24	24	19	23	22	22	24	24	18	23	12	24	22	22	24	24	17	23	21	22	24	24
		Total	**56**	**68**	**50**	**78**	**74**	**74**	**76**	**78**	**61**	**68**	**74**	**74**	**78**	**82**	**49**	**68**	**46**	**76**	**74**	**74**	**76**	**78**	**55**	**68**	**73**	**74**	**82**	**82**
		%	**82.4**		**64.1**		**100.0**		**97.4**		**89.7**		**100.0**		**95.1**		**72.1**		**60.5**		**100.0**		**97.4**		**80.9**		**98.6**		**100.0**	
	Simulated data, noise 0.50 Å	Val	17	20	16	22	15	19	14	22	14	20	17	19	18	22	13	20	16	22	14	19	14	22	11	20	18	19	20	24
		Leu	14	25	14	30	31	33	28	34	13	25	27	33	24	34	12	25	10	30	27	33	26	34	15	25	24	33	24	34
		Ile	17	23	6	24	22	22	24	24	18	23	22	22	24	24	16	23	6	24	22	22	20	24	16	23	18	22	20	24
		Total	**48**	**68**	**36**	**76**	**68**	**74**	**66**	**80**	**45**	**68**	**66**	**74**	**66**	**80**	**41**	**68**	**32**	**76**	**63**	**74**	**60**	**80**	**42**	**68**	**60**	**74**	**64**	**82**
		%	**70.6**		**47.4**		**91.9**		**82.5**		**66.2**		**89.2**		**82.5**		**60.3**		**42.1**		**85.1**		**75.0**		**61.8**		**81.1**		**78.0**	
	Experimental data	Val	17	20	20	22	19	19	18	20	17	20	19	19	18	22	16	20	20	22	18	19	18	22	16	20	18	19	20	24
		Leu	15	25	10	30	26	33	20	34	11	25	26	33	20	34	12	25	12	30	22	33	18	34	11	25	25	33	20	34
		Ile	18	23	18	24	19	22	20	24	21	23	22	22	24	24	17	23	14	24	19	22	20	24	17	23	21	22	24	24
		Total	**50**	**68**	**48**	**76**	**64**	**74**	**58**	**78**	**49**	**68**	**67**	**74**	**62**	**80**	**45**	**68**	**46**	**76**	**59**	**74**	**56**	**80**	**44**	**68**	**64**	**74**	**64**	**82**
		%	**73.5**		**63.2**		**86.5**		**74.4**		**72.1**		**90.5**		**77.5**		**66.2**		**60.5**		**79.7**		**70.0**		**64.7**		**86.5**		**78.0**	

[a] The data are presented as in Table S3.