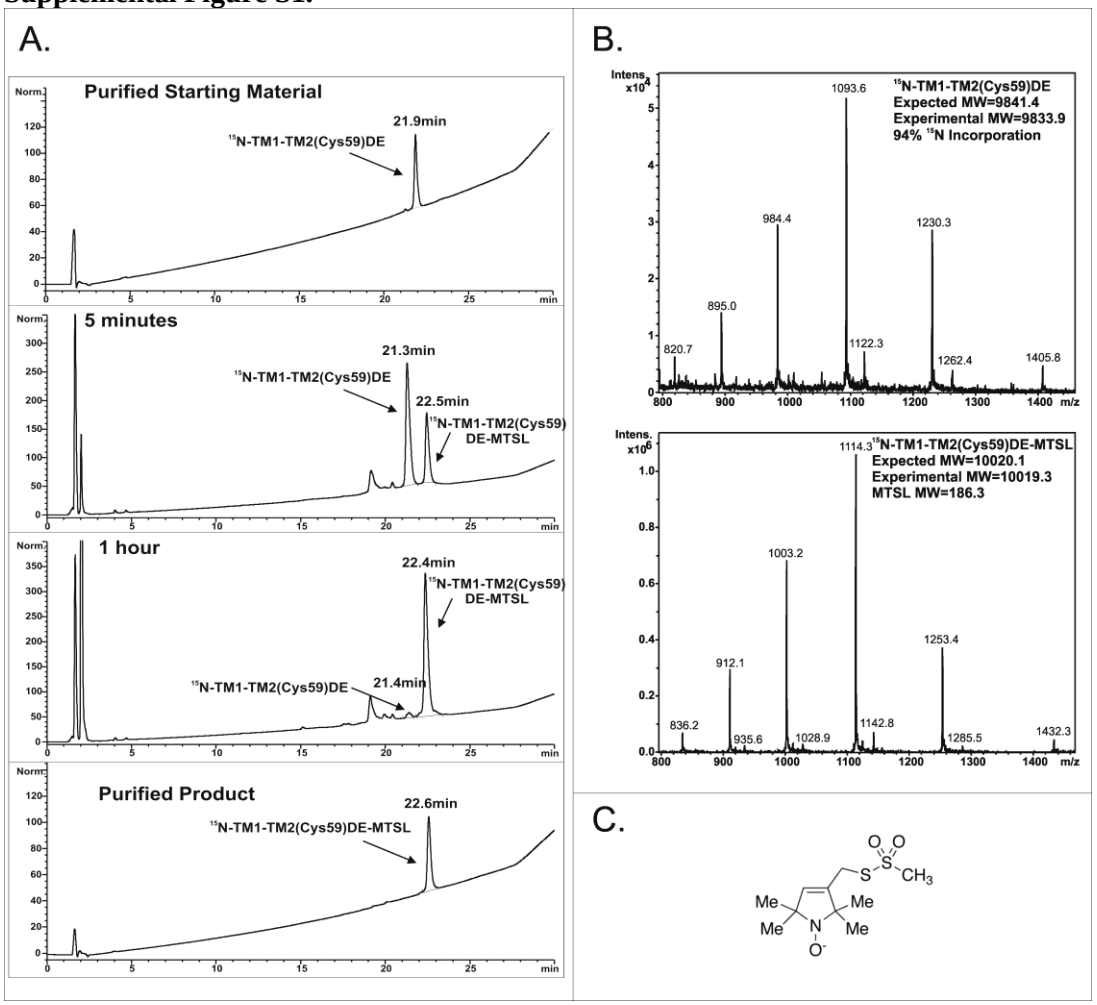
Supplementary Data

Table S1. NMR experiemtnal parameters used in structure determination of TM1-TM2 in TFE:water(0.1%TFA) (1:1, v:v)

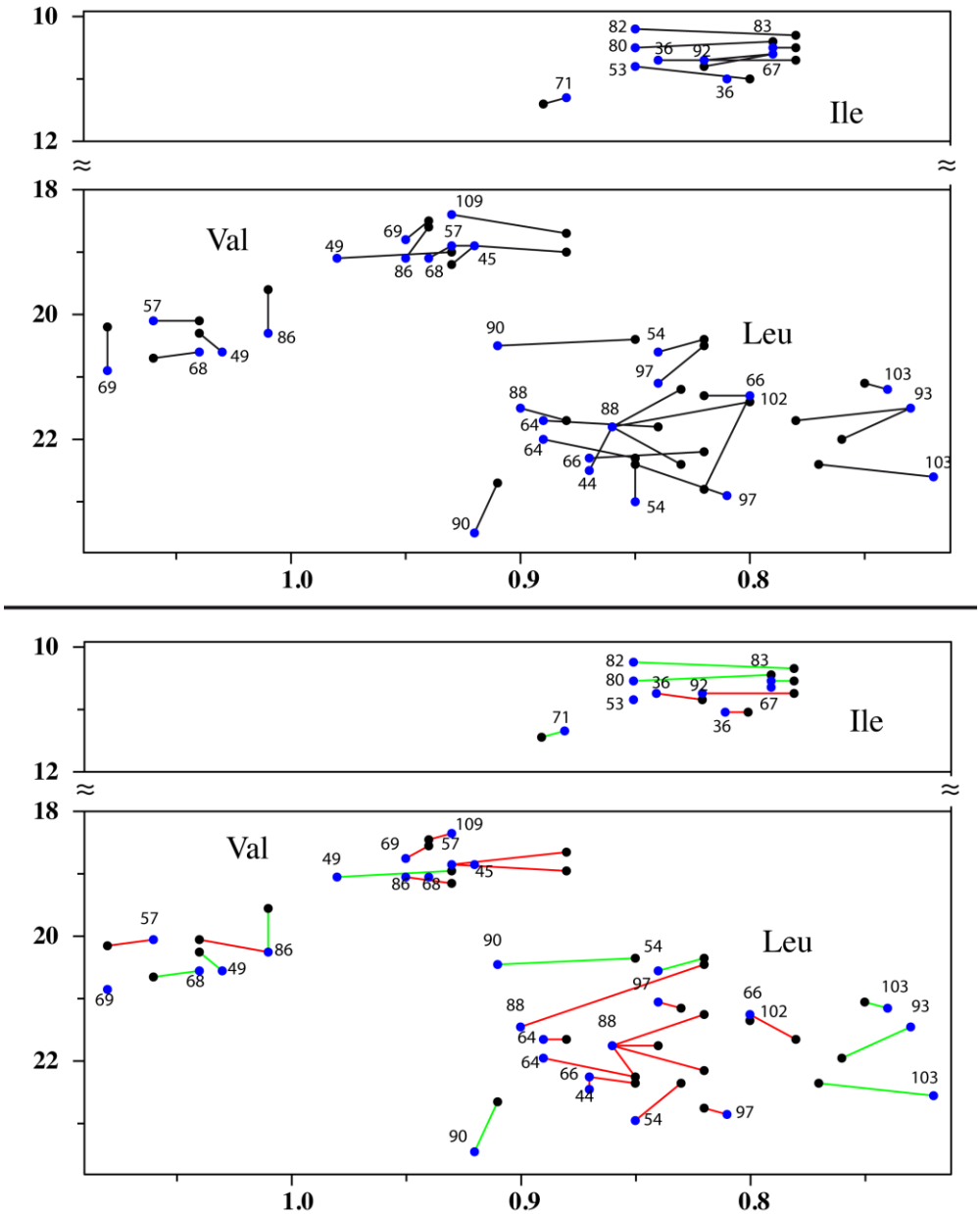
NMR Experiment	**Complex Points**	**Sweep Width**	**Instrument**
[^{15}N,^{1}H]-HSQC at 45ºC	2048 (^{1}H)*256 (^{15}N)	6010 (^{1}H)*900(^{15}N)	600 MHz
[^{15}N,^{1}H]-HSQC at 25ºC	4096 (^{1}H)*512 (^{15}N)	6010 (^{1}H)*1500(^{15}N)	600 MHz
NOESY-[^{15}N,^{1}H]-HSQC	2048(^{1}H)*256(^{1}H)*64(^{15}N)	8012(^{1}H)*6600(^{1}H)*12068(^{15}N)	600 MHz
TOCSY-[^{15}N,^{1}H]-HSQC	5195(^{1}H)*7299(^{15}N)*2838(^{1}H)	6010(^{1}H)*6000(^{13}C)*900(^{1}H)	600 MHz
HNCA	2048(^{1}H)*512(^{13}C)*128(^{15}N)	6010(^{1}H)*4526(^{13}C)*900(^{15}N)	600 MHz
HNCACB	2048(^{1}H)*512(^{13}C)*128(^{15}N)	6010(^{1}H)*8000(^{13}C)*900(^{15}N)	600 MHz
HN(CA)CO	2048(^{1}H)*256(^{13}C)*32(^{15}N)	6010(^{1}H)*2500(^{13}C)*900(^{15}N)	600 MHz
HNCO	2048(^{1}H)*512(^{13}C)*128(^{15}N)	6010(^{1}H)*2000(^{13}C)*900(^{15}N)	600 MHz
HCCH-TOCSY	2048(^{1}H)*256(^{13}C)*256(^{15}N)	3613(^{1}H)*3600(^{13}C)*12001(^{15}N)	600 MHz
^{13}C-edited TOCSY	2048(^{1}H)*256(^{13}C)*512(^{13}C)	7225(^{1}H)*12019(^{13}C)*1199(^{13}C)	600 MHz
^{15}N-edited NOESY	2048(^{1}H)*256(^{15}N)*256(^{1}H)	8012(^{1}H)*6000(^{15}N)*10200(^{1}H)	600 MHz
NOESY (300 and 150 msec)	256(^{1}H)*2048(^{1}H)	6010(^{1}H)	600 MHz
TOCSY (60 and 25 msec)	256(^{1}H)*2048(^{1}H)	6010(^{1}H)	600 MHz
NOESY-[^{13}C,^{1}H]-HSQC	2048(^{1}H)*256(^{1}H)*256(^{13}C)	8012(^{1}H)*6000(^{1}H)*10200	600 MHz
[^{15}N,^{1}H]-HSQC for T2 relaxation	2048(^{1}H)*512(^{15}N)	6009(^{1}H)*900(^{15}N)	600 MHz
NOE-[^{15}N,^{1}H]-HSQC	2048(^{1}H)*256(^{15}N)	6010(^{1}H)*900(^{15}N)	600 MHz
[^{15}N,^{1}H]-HSQC for H-D exchange	4096(^{1}H)*512(^{15}N)	6010(^{1}H)*1500(^{15}N)	600 MHz
NOESY-ct-[^{13}C,^{1}H]-HSQC	256(^{1}H)*128(^{13}C)*128(^{1}H)	8091(^{1}H)*3622(^{13}C)*3622(^{1}H)	900 MHz
ct-[^{13}C,^{1}H]-HSQC-NOESY-ct-[^{13}C,^{1}H]-HSQC	1024(^{1}H)*106(^{13}C)*104(^{1}H)	1796(^{1}H)*3622(^{13}C)*1800(^{1}H)	900 MHz
HMCMCBCANH_val/ile	2048(^{1}H)*40(^{15}N)*36(^{13}C)	9765(^{1}H)*1998(^{15}N)*2641(^{13}C)	700 MHz
HMCMCBCANH_leu	2048(^{1}H)*40(^{15}N)*60(^{13}C)	9765(^{1}H)*1998(^{15}N)*2641(^{13}C)	700 MHz
ct-[^{13}C,^{1}H]-HSQC	2048(^{1}H)*400(^{13}C)	9328(^{1}H)*8803(^{13}C)	700 MHz

Supplemental Figure S1.



Supplemental Figure S1. Addition of the S-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothionate (MTSL) paramagentic label. A) The labeling reaction of [^{15}N]Met-His$_6$-TM1-TM2-C59 with the paramagnetic spin label was performed in 6M GnHCl:TFE (1:1) with 10 mM MTSL. The reaction was followed over time by analytical RP-HPLC as described and the peaks were collected and analyzed by ESI-MS. B) The ESI-MS analysis for the starting material (top panel) and product of the labeling reaction (bottom panel). The MWs are given in each panel. C) The MTSL paramagnetic label.

Supplemental Figure S2:



Supplemental Figure S2: Top: Correlation of ^{1}H and ^{13}C chemical shifts of [^{15}N,^{13}C,^{2}H(^{1}H(methyl)-ILV)]-TM1-TM2 in TFE:water (black spheres) and LPPG (blue spheres). The assignments are annotated close to the TFE:water data. Bottom: Transfer of assignments by an automatic procedure (see text). Correct and erroneous transfers are indicated by green and red lines, respectively.