Supporting Information

Drug-Induced Conformational and Dynamical Changes of the S31N Mutant of the Influenza M2 Proton Channel Investigated by Solid-State NMR

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Synthesis of deuterated WJ352 compounds

The synthesis of unlabeled **M2WJ332** and **M2WJ352** has been reported recently [1]. We describe below the modified procedure to synthesize two deuterated forms of WJ352. All chemicals were purchased from commercial vendors and used without further purification unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-300 NMR spectrometer. Chemical shifts are reported in parts per million with respect to residual solvent, CD_3OD = 3.31 ppm and CD_2Cl_2 = 5.30 ppm, or to the internal standard, tetramethylsilane = 0.00 ppm. The following abbreviations were used in reporting the NMR spectra: singlet (s), doublet (d), and triplet (t). All reactions were carried out under N_2 atmosphere unless otherwise stated. HPLC grade solvents were used for all the reactions. Flash column chromatography was performed using silica gel (230–400 mesh; Merck). Low-resolution mass spectrometry data were obtained using an electrospray ionization (ESI) technique on a 3200 Q Trap LC tandem MS system (Applied Biosystems).

*Synthesis of **D5-M2WJ352***

A solution of phenyl-D5-acetylene (1mmol) in THF-H_2O mixture (1:1) was cooled down to 0˚C. Na_2CO_3 (12 mmol) was added in one portion. A solution of 2-chloro-2-hydroxyliminoacetic acid ethyl ester (2.5mmol) in THF was added dropwise using a dropping funnel. The solution was stirred overnight and diluted with ethyl acetate. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (10% ethyl acetate/hexane) to give the isoxazole ester as a yellow solid (45% yield).

Ethyl 5-D5-phenyl-1,2-oxazole-3-carboxylate

^{1}H NMR (300 MHz, CD_2Cl_2): δ 6.96 (s, 1H), 4.45 (q, J = 7.10 Hz, 2H), 1.44 (t, J = 7.10 Hz, 3H). ^{13}CNMR (75 MHz, CD_2Cl_2): 171.58, 159.91, 157.11, 130.29 (t, J = 23.56 Hz), 128.64 (t, J = 25.58 Hz), 126.51, 125.43 (t, J = 24.32 Hz), 99.93, 62.16, 13.95. EI-MS: m/z ($M+H^+$): 223.1 (calculated), 223.3 (found).

Ethyl 5-D5-phenyl-1,2-oxazole-3-carboxylate (1 mmol) was dissolved in CH_3OH. $NaBH_4$ (2 mmol) was added portionwise to avoid solvent boiling. The resulting solution was stirred overnight at room temperature. 1N HCl was added to quench the reaction. Solvent was

removed under reduced pressure and the residue was extracted with CH_2Cl_2 and H_2O. The alcohol intermediate, (5-D5-phenyl-1,2-oxazol-3-yl)methanol, was used for the next step bromination without further purification.

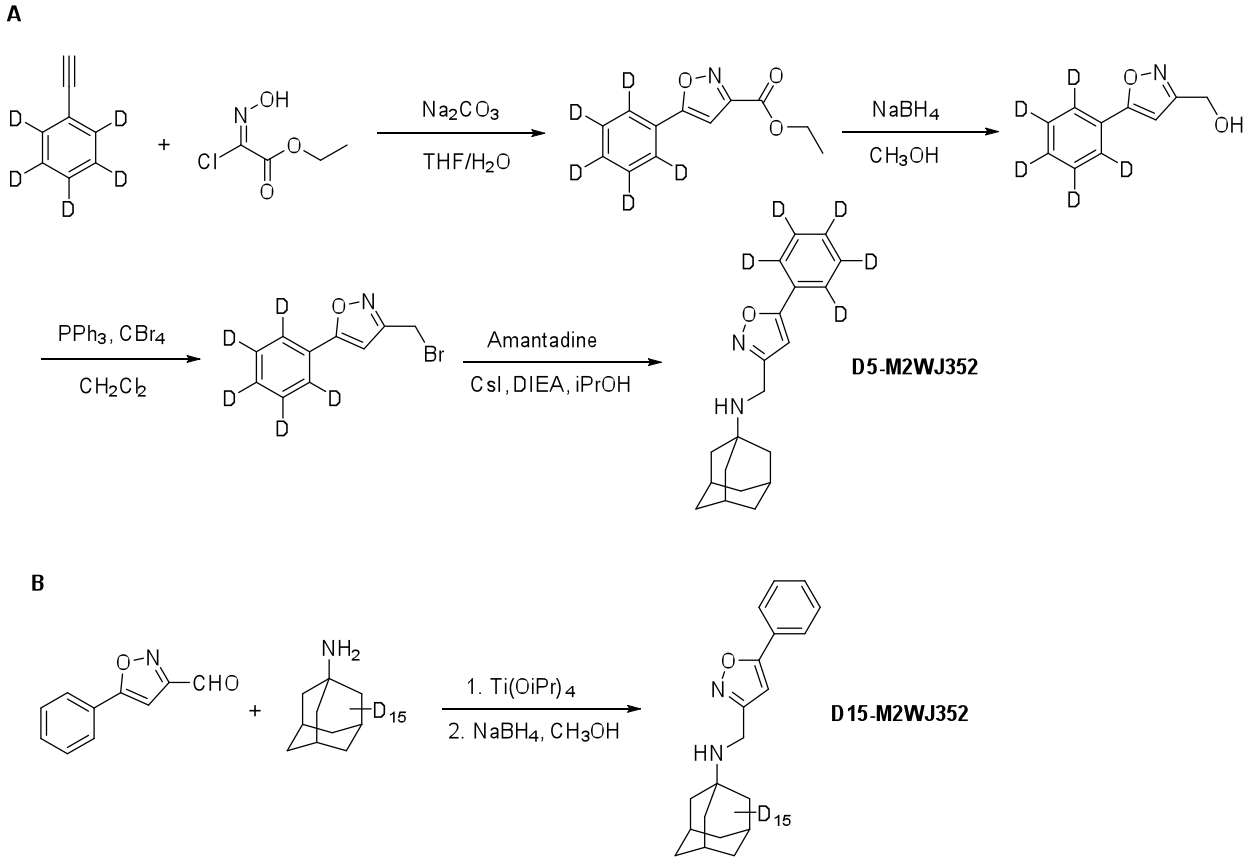

Figure S1. Synthesis scheme of deuterium-labeled **M2WJ352**. (A) Synthesis of **D5-M2WJ352**. Phenyl-D_5-acetylene (Cat. # D-1086) was purchased from C.D.N isotopes Inc. (B) Synthesis of **D15-M2WJ352**. D_{15}-labeled amantadine was prepared according to the previous published procedure [2].

(5-D5-phenyl-1,2-oxazol-3-yl)methanol (1 mmol) was dissolved in CH_2Cl_2. CBr_4 (1.5 mmol) and PPh_3 (1.5 mmol) were added sequentially. The mixture was stirred at room temperature for 3 hrs. Then solvent was removed and the mixture was extracted with CH_2Cl_2 and H_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The bromide (1mmol) was added to a solution of amantadine (1.5mmol) in iPrOH. Then CsI (0.2 mmol) and DIEA (1.5 eq) were added. The resulting solution was heated to reflux overnight. Solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and extracted with H_2O. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (10-15% CH_3OH/CH_2Cl_2) to give D5-M2WJ352 as a white solid (yield over three steps 21%).

*N-[(5-D5-phenyl-1,2-oxazol-3-yl)methyl]adamantan-1-amine (**D5-M2WJ352**)*

^{1}H NMR (300 MHz, CD_3OD): δ 6.83 (s, 1H), 3.88 (s, 2H), 2.10-2.08 (m, 3H), 1.77-1.70 (m, 12H). ^{13}CNMR (75 MHz, CD_3OD): 171.22, 165.38, 129.68 (t, J = 24.71 Hz), 128.55, 126.32 (t, J = 24.17 Hz), 100.45, 52.46, 42.77, 37.63, 37.16, 31.01. EI-MS: m/z ($M+H^+$): 314.2 (calculated), 314.8 (found).

*Synthesis of **D15-M2WJ352** (N-[(5-phenyl-1,2-oxazol-3-yl)methyl]D15-adamantan-1-amine).*

Synthesis of **D15-M2WJ352** started with D15-amantadine and 5-phenyl-1,2-oxazole-3-carbaldehyde using reductive amination as reported [3]. ^{1}H NMR (300 MHz, CD_3OD): δ 7.84-7.81 (m, 2H), 7.50-7.47 (m, 3H), 6.80 (s, 1H), 4.68 (s, 2H). ^{13}CNMR (75 MHz, CD_3OD): 171.37, 166.29, 131.41, 130.19, 128.71, 126.76, 100.46, 99.71, 56.83. EI-MS: m/z ($M+H^+$): 324.2 (calculated), 324.6 (found).

Synthesis of selectively isotope labeled M2(19-49) S31N peptide followed the optimized solid phase synthesis protocol as reported [4].

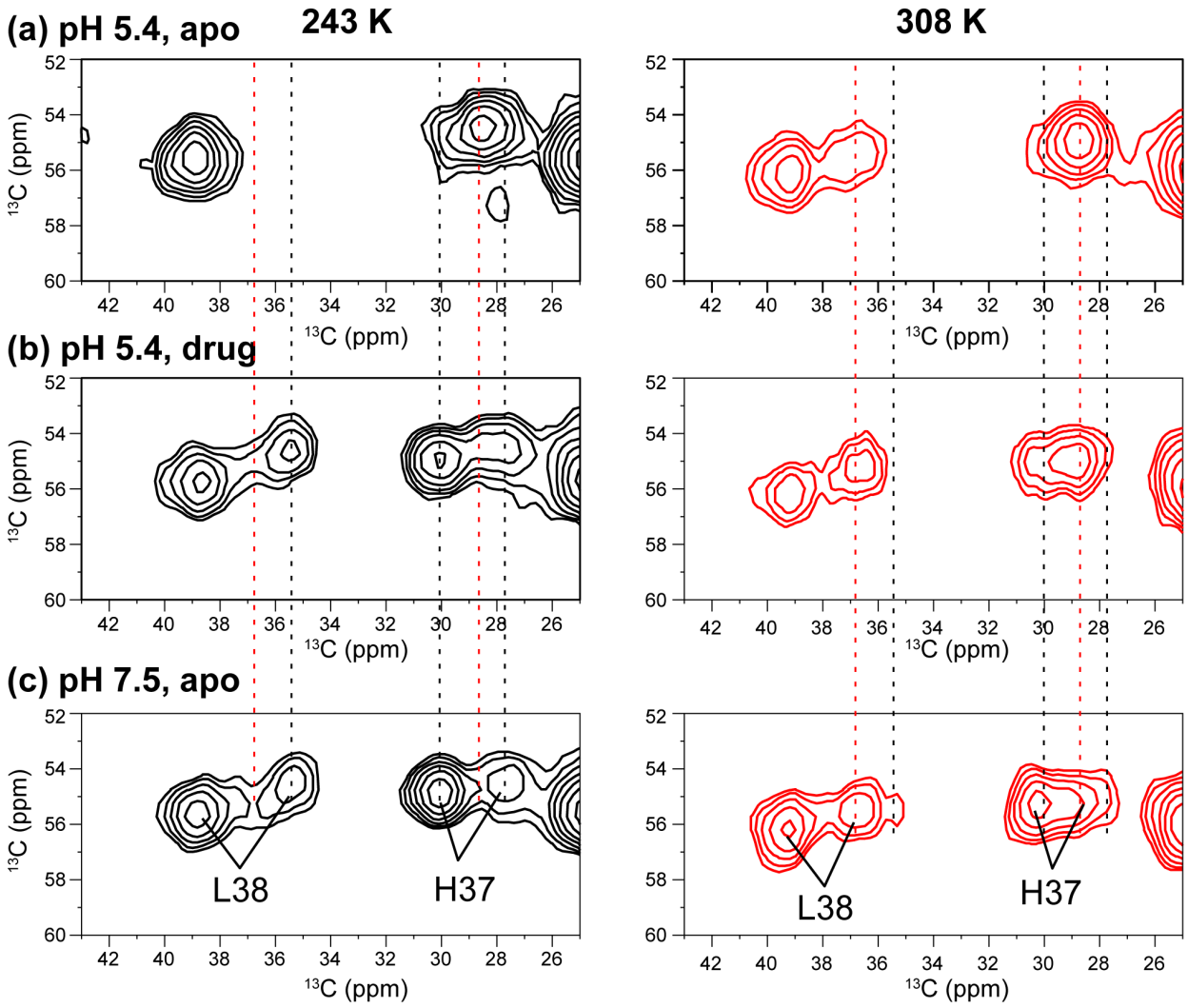

Figure S2. 2D ^{13}C-^{13}C correlation spectra showing the influence of pH, temperature and drug binding on the H37 and L38 chemical shifts in VGIHL-S31N-M2(19-49). (a) Apo peptide at pH 5.4. (b) Drug-bound peptide at pH 5.4. (c) Apo peptide at pH 7.5. Left column: 243 K spectra. Right column: 308 K spectra. L38 Cβ exhibits two conformations. At low temperature, the minor conformation is absent in the low-pH apo peptide but significantly present in the other two samples. At high temperature, the minor conformer is populated in the low-pH apo peptide, but the chemical shift is changed by 1.2 ppm, suggesting conformational exchange with the major conformation. H37 exhibits a similar trend.

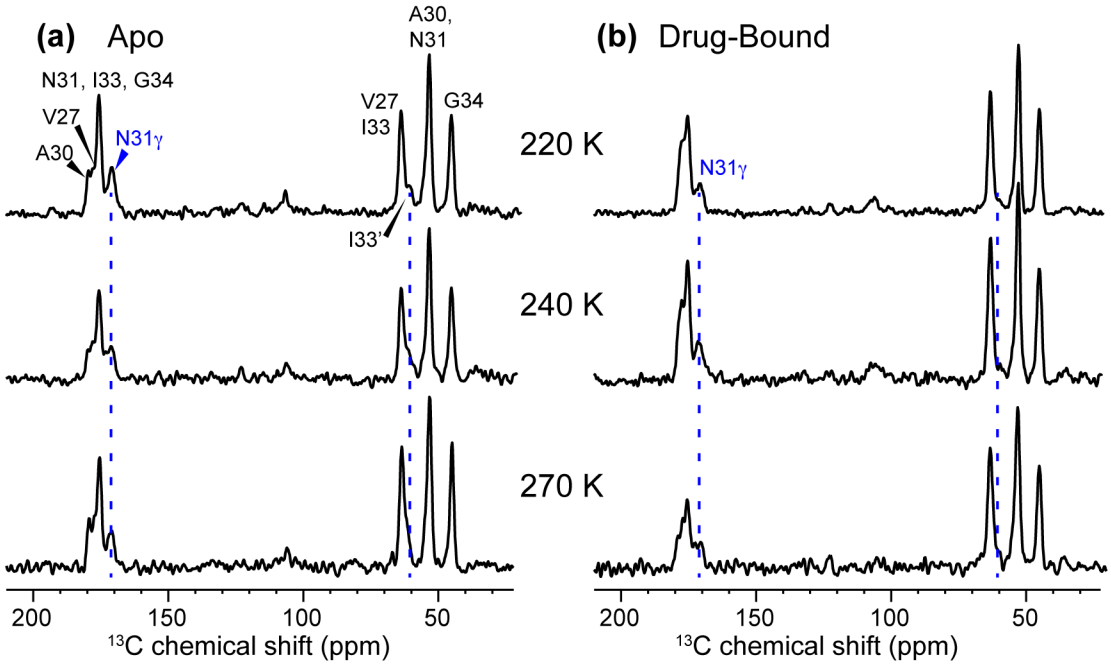

Figure S3. 1D ^{15}N-^{13}C dipolar filtered ^{13}C spectra of (a) apo and (b) drug-bound VANIG-S31N-M2(19-49) from 220 K to 270 K. The N31 sidechain Cγ chemical shift is unaffected by the drug.

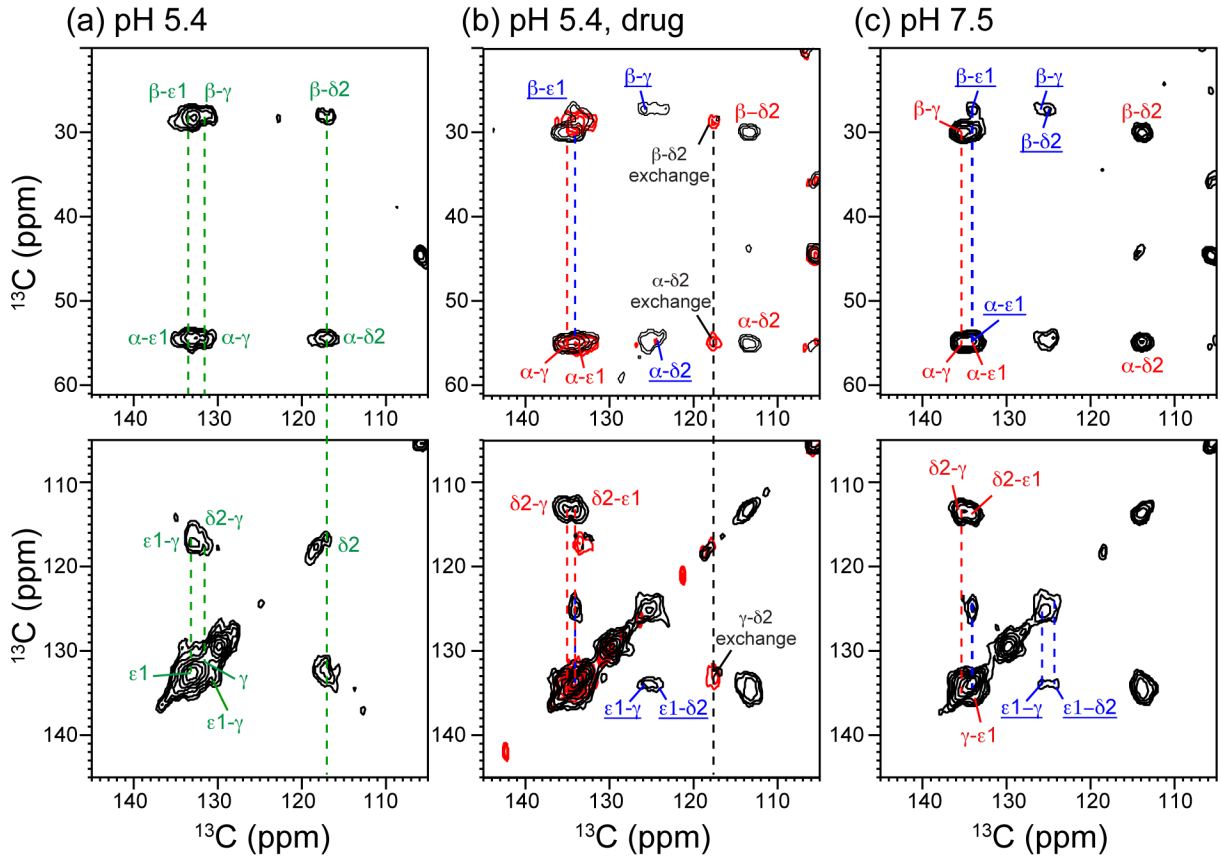

Figure S4. Aromatic region of the 2D ^{13}C-^{13}C correlation spectra of His37 in VGIHL-S31N-M2(19-49). The spectra were measured at 243 K under 7 kHz MAS. (a) Apo peptide at pH 5.4. (b) Drug-bound peptide at pH 5.4. (c) Apo peptide at pH 7.5. Superimposed in red in (b) is the 308 K spectrum of the drug-bound sample, showing Cδ2 exchange peaks.

Table S1. Different NMR samples used in this study.

Peptide	^{13}C, ^{15}N labeled residues	Membrane	pH	Drug : Tetramer ratio
$GIHL_{22-46}$	G34, I35, H37, L38	DMPC	7.5	Apo
				1 : 1 d_5-WJ352
				8 : 1 d_5-WJ352
$VANIG_{19-49}$	V27, A30, N31, I33, G34	DMPC	6.5	Apo
				1 : 1 d_{15}-WJ352
				10 : 1 d_{15}-WJ352
$VGIHL_{19-49}$	V28, G34, I35, H37, L38	VM+	5.4	Apo
				10 : 1 WJ352
		VM+	7.5	Apo

Table S2. ^{15}N and ^{13}C (referenced to TMS) chemical shifts of S31N-M2 in lipid membranes under various pH and drug binding conditions. Bold indicates sites with > 0.5 ppm chemical shift perturbation by the drug.

	Membrane	State	Nα	CO	Cα	Cβ	Cγ,γ1	Cγ2	Cδ,δ1	Cδ2	Cε, ε1	Nδ1,2	Nε2
V27	DMPC	Apo pH 6.5	119.8	177.3	63.7	29.4	20.7	**19.8**					
		Drug pH 6.5	120.1	177.3	63.4	29.4	20.4	**19.3**					
V28	VM+	Apo pH 5.4	**122.9**	**176.3**	65.2	29.2	**20.3**	19.5					
		Drug pH 5.4	122.6	**175.8**	65.0	28.9	**21.0**	19.3					
		Apo pH 7.5	122.0	176.0	65.2	28.9	20.7	19.3					
A30	DMPC	Apo pH 6.5	119.4	**179.5**	**53.2**	**15.5**							
		Drug pH 6.5	119.0	**177.9**	**52.7**	**16.2**							
N31	DMPC	Apo pH 6.5	117.7	**175.6** **174.8**	**53.5s** **55.1w**	**35.4s** **38.7w**	**171.3** **170.5**					106.0	
		Drug pH 6.5	117.4	**175.4**	**53.0**	**35.3**	**171.0**					106.0	
I33	DMPC	Apo pH 6.5	**119.0**	175.4	63.7s **61.4w**	35.7	28.4	**14.8**	12.0				
		Drug pH 6.5	**119.8**	175.6	63.5s **62.4w**	35.4	28.8	**15.6**	11.7				
G34	VM+	Apo pH 5.4	105.1 109.5	175.5 173.1	44.9								
		Drug pH 5.4	105.6 109.7	175.0 173.0	44.5								
		Apo pH 7.5	105.1 109.0	175.3 173.4	44.8								
	DMPC	Apo pH 6.5	**105.0**	**174.9s** **173.2w**	45.0								
		Drug pH 6.5	**106.7**	**175.1w** **173.1s**	45.4								
	DMPC	Apo pH 7.5	107.3 104.7	175.0s 173.0w	44.9								
		Drug pH 7.5	107.3	175.0s	45.2								

				173.2s									
I35	VM+	Apo pH 5.4	123.2 118.2	174.8	63.3	35.2	**28.6**	**15.6**	11.8				
		Drug pH 5.4	122.5 118.8	174.7 175.3	63.4	35.6	**26.6** **28.8**	**15.0** **16.4**	11.4 11.6				
		Apo pH 7.5	122.1 119.0	174.7 175.4	63.6	35.3 35.6	26.7 28.7	15.0 16.1	11.2 11.9				
	DMPC	Apo pH 7.5	122.8	175.1	63.5	35.4	28.0	15.8	11.8				
		Drug pH 7.5	122.2	175.4	63.5	35.5	27.8	15.7	11.9				
H37	VM+	Apo pH 5.4	--- 117.4 ---	--- 174.2 ---	--- 54.8 ---	--- 28.4 ---							
		Drug pH 5.4	117.2	174.2	**54.8** **54.8** **55.4**	**27.6** **28.5** **30.1**							
		Apo pH 7.5	117.6	174.3	54.8 --- 55.3	27.9 --- 30.1							
	DMPC	Apo pH 7.5	117.7	174.5	55.1	29.9	135.2			113.3	134.2	251.2τ 170.2π	159.0τ 251.2π
		Drug pH 7.5	118.0	174.5	**55.5** **58.8**	**30.0** **29.1**	135.4			113.5	134.0	165.9π 252.0τ	165.9τ 252.0π
L38	VM+	Apo pH 5.4	117.4	175.2	55.5	38.7	24.3		19.8				
		Drug pH 5.4	117.2	**175.3** **175.9**	**55.7** **54.7**	**38.6** **35.4**	24.8 24.7		**19.8** **21.3**				
		Apo pH 7.5	117.6	174.4 175.3	55.6 54.6	38.7 35.4	24.7 24.7		19.7 21.1				
	DMPC	Apo pH 7.5	117.7	175.4	55.8	38.9	24.6	**19.9**	**19.9**				
		Drug pH 7.5	118.0	175.7	55.9	39.3	24.7	**20.5**	**20.5**				

References

(1) Wang, J.; Wu, Y.; Ma, C.; Fiorin, G.; Wang, J.; Pinto, L. H.; Lamb, R. A.; Klein, M. L.; Degrado, W. F. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 1315-1320.
(2) Cady, S. D.; Schmidt-Rohr, K.; Wang, J.; Soto, C. S.; DeGrado, W. F.; Hong, M. *Nature* **2010**, *463*, 689-692.
(3) Wang, J.; Ma, C.; Wang, J.; Jo, H.; Canturk, B.; Fiorin, G.; Pinto, L. H.; Lamb, R. A.; Klein, M. L.; DeGrado, W. F. *J. Med. Chem.* **2013**, *56*, 2804-2812.
(4) Cady, S. D.; Wang, J.; Wu, Y.; DeGrado, W. F.; Hong, M. *J. Am. Chem. Soc.* **2011**, *133*, 4274-4284.