Supporting Information to:

Structure and Mechanism of the Influenza-A $M2_{18-60}$ Dimer of Dimers

Loren B. Andreas[1], Marcel Reese[1], Matthew T. Eddy[1], Vladimir Gelev[2], Qing Zhe Ni[1], Eric A. Miller[1], Lyndon Emsley[3], Guido Pintacuda[3], James J. Chou[4], Robert G. Griffin[1]*

Synthesis of DPhPC

Dideuterophytol

Dideuterophytol was synthesized using Raney-nickel hydrogenation of phytol purchased from Sigma-Aldrich. The procedure has been described in the literature[1] and is briefly reproduced here. To an 85 mL solution of 20% phytol in ethanol was added 4 mL of Raney Ni Slurry, which was purged with N_2 in a round bottomed flask. The flask was left for 24 hrs under a stream of D_2 at 1 atm pressure, and the product was separated from the catalyst by filtration and rotary evaporation.

Phytanic Acid

Phytanic Acid was synthesized from dideuterophytol by oxidation with chromium trioxide as previously described.[2] This procedure is described briefly here. To 5 g of dideuterophytol in 300 mL 2:1 acetone/acetic acid solvent mixture was added 0.8 g of chromium trioxide in 5 mL of water. The chromium trioxide was added dropwise at room temperature, with stirring for over 1 hr. 250 mL of water was then added, and excess sodium bisulfate was used to destroy excess oxidant. Extraction in diethyl ether and purification on silica followed the previously described protocol. The product was verified with ^{1}H and ^{13}C NMR.

^{2}H Phytanic Acid

Perdeuteration of phytanic acid was performed using a simple method of hydrogenation conditions at an elevated temperature of 195 °C with palladium on charcoal (Pd/C) catalyst (Sigma-Aldrich).[3] 4 to 5 grams of Pd/C (10% Pd) and 25g of phytanic acid were heated to 195 °C and 1 atm. of D_2 with stirring in a 250 mL round bottom flask. A continual flow of D_2 gas was generated from D_2O using a Parker-Balston hydrogen generator with a flow rate exceeding 30 cubic centimeters per minute. The reaction was tracked by the loss of proton NMR signal using benzene as an internal standard, and reached 97-98% deuteration after 16 days.

DPhPC

Coupling of phytanic acid to GPC was performed by FBReagents (Cambridge MA) using DCC and DMAP, and purified with silica gel chromatography. The ^{1}H NMR spectrum of DPhPC in deuterated chloroform is shown in ***Figure 1a***. The choline methyl (9 ^{1}H) at 3.2 ppm and the

choline and glycero methylene and methine groups (9 ^{1}H) around 4 ppm are the major signals expected. In addition, residual signal from the deuterated acyl chains (~3% of 78 ^{1}H) appears between 0 and 3 ppm. The remaining signal intensity between 0 and 3 ppm and at 5.2 ppm arises from impurity. With this analysis, the purity by NMR is above 85 %. In ***Figure 1b***, a thin layer chromatogram shows a single spot with the same retention time as commercial protonated DPhPC (Avanti). The running condition for the TLC was 65:24:4 chloroform:methanol:4M Ammonium Hydroxide.

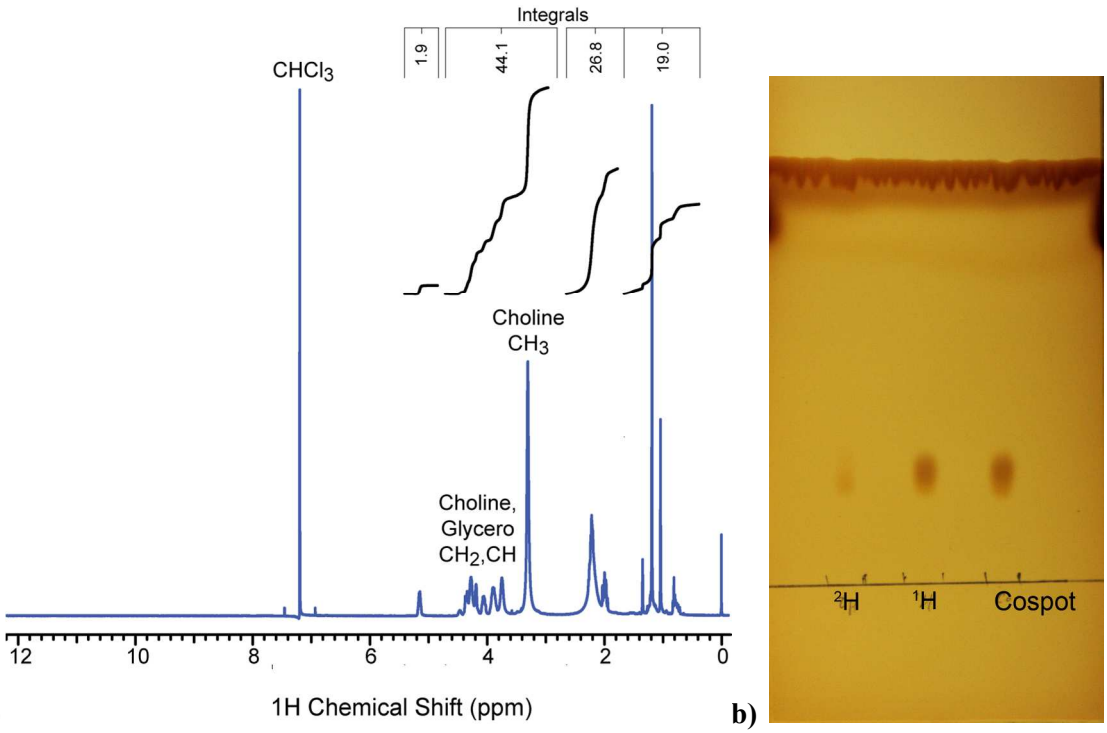

Figure 1. Proton spectrum (a) of acyl chain deuterated (d78) DPhPC demonstrating a purity by NMR of above 85%. In (b), a thin layer chromatogram shows a single spot with the same retention time as commercial protonated DPhPC.

Assignments

Seq	H	N	Co	Ca	Cb	Cg,	Cg2,	Cd,	Cd2	Ce,	Ce3	Cz,	Cz3
D24′	8.47	124.2	175.0	52.0	41.9	179.1	-	-	-	-	-	-	-
P25	-	135.2	177.9	65.4	32.7	27.5	-	50.7	-	-	-	-	-
P25′	-	136.2	177.5	65.3	32.6	27.3	-	50.7	-	-	-	-	-
L26	8.69	115.7	178.2	58.1	40.8	28.2	-	25.8	23.3	-	-	-	-
L26′	8.64	116.2	178.3	57.9	41.3	26.5	-	25.3	22.6	-	-	-	-
V27	7.51	119.3	178.6	66.1	31.9	**21.6**	**23.1**	-	-	-	-	-	-
V27′	7.43	119.1	177.7	66.6	31.5	**22.4**	**21.6**	-	-	-	-	-	-
V28	8.19	120.0	178.3	66.7	31.3	**21.9**	**22.5**	-	-	-	-	-	-
V28′	7.98	118.7	178.7	65.6	31.7	**21.5**	**22.7**	-	-	-	-	-	-
A29	8.44	118.4	179.4	55.2	18.5	-	-	-	-	-	-	-	-
A29′	8.53	119.9	178.6	55.4	18.4	-	-	-	-	-	-	-	-
A30	8.43	118.9	176.9	55.0	18.6	-	-	-	-	-	-	-	-
A30′	8.36	117.9	178.3	55.4	18.7	-	-	-	-	-	-	-	-
N31	7.69	110.8	175.1	54.7	40.6	177.5	-	-	-	-	-	-	-
N31′	8.73	115.6	177.5	58.0	39.9	174.8	-	-	-	-	-	-	-
I32	7.71	110.4	177.7	61.4	41.3	26.3	17.2	14.3	-	-	-	-	-
I32′	8.69	118.8	177.2	65.6	37.2	29.6	17.6	14.1	-	-	-	-	-
I33	7.62	121.3	175.3	64.3	37.6	28.9	17.2	14.6	-	-	-	-	-
I33′	8.46	120.2	177.7	66.0	37.1	30.2	19.6	13.6	-	-	-	-	-
G34	9.15	109.8	176.0	47.6	-	-	-	-	-	-	-	-	-
G34′	8.72	106.8	175.2	47.9	-	-	-	-	-	-	-	-	-
I35	8.27	121.4	176.9	64.1	37.1	28.8	17.7	12.7	-	-	-	-	-
I35′	8.62	121.9	177.2	66.1	37.6	30.7	18.0	14.2	-	-	-	-	-
L36	8.46	118.6	177.4	57.6	41.7	26.8	-	26.1	24.7	-	-	-	-
L36′	8.80	119.9	177.3	58.1	41.3	26.5	-	24.7	24.4	-	-	-	-
H37	8.60	116.5	175.6	59.3	30.3	136.3	-	-	119.1	136.9	-	-	-
H37′	8.74	118.9	177.3	62.3	31.3	135.8	-	-	117.2	135.7	-	-	-
L38	7.98	116.3	178.7	58.4	42.3	26.4	-	26.5	22.5	-	-	-	-
L38′	8.32	120.5	178.3	58.3	40.4	26.5	-	22.0	26.0	-	-	-	-
I39	8.29	114.3	175.9	60.4	37.2	30.2	19.7	13.5	-	-	-	-	-
I39′	8.98	118.7	177.4	65.5	37.3	29.6	17.2	13.9	-	-	-	-	-
L40	8.52	126.3	178.5	58.5	41.3	26.9	-	25.0	24.4	-	-	-	-
L40′	8.70	118.8	179.3	58.1	40.8	27.0	-	26.2	23.2	-	-	-	-
W41	9.78	121.1	178.6	60.7	28.2	111.8	124.4	125.1	129.3	138.7	119.7	114.0	121.7
W41′	8.40	122.7	177.8	62.6	27.4	111.1	123.8	127.5	130.9	138.8	121.0	114.9	120.9
I42	8.35	116.8	178.4	64.2	37.9	28.8	18.6	14.2	-	-	-	-	-
I42′	9.04	119.6	178.1	65.8	37.8	29.4	17.1	14.4	-	-	-	-	-
L43	8.86	119.3	179.9	57.7	41.7	26.5	-	23.2	22.6	-	-	-	-
L43′	8.91	117.9	177.6	58.1	41.3	*	-	*	*	-	-	-	-
D44	9.47	121.0	178.5	57.9	42.7	180.2	-	-	-	-	-	-	-
D44′	*	117.7	*	*	*	*	-	-	-	-	-	-	-
R45	8.57	116.7	178.5	57.8	29.5	25.9	-	42.8	-	-	-	159.6	-
L46	8.13	114.6	177.5	56.3	43.0	26.7	-	25.5	22.9	-	-	-	-
F47	7.95	112.6	175.6	58.4	41.5	-	-	131.8	-	-	-	-	-
F48	7.76	116.6	175.8	61.0	39.1	-	-	131.8	-	-	-	-	-
K49	10.27	120.3	175.6	56.7	30.4	25.2	-	29.3	-	42.5	-	-	-
S50	9.69	111.0	174.4	60.6	66.9	-	-	-	-	-	-	-	-
I51	10.41	126.2	176.0	65.8	38.2	28.5	17.2	13.8	-	-	-	-	-
Y52	-	118.0	-	-	-	-	-	-	-	-	-	-	-

Seq	Hd1	Hd2	He1	He2	Hg2	Nd1	Nd2	Ne	Ne1	Ne2	Nz
D24′	-	-	-	-	-	-	-	-	-	-	-
P25	-	-	-	-	-	-	-	-	-	-	-
P25′	-	-	-	-	-	-	-	-	-	-	-
L26	-	-	-	-	-	-	-	-	-	-	-
L26′	-	-	-	-	-	-	-	-	-	-	-
V27	-	-	-	-	1.16	-	-	-	-	-	-
V27′	-	-	-	-	1.11	-	-	-	-	-	-
V28	-	-	-	-	1.12	-	-	-	-	-	-
V28′	-	-	-	-	1.24	-	-	-	-	-	-
A29	-	-	-	-	-	-	-	-	-	-	-
A29′	-	-	-	-	-	-	-	-	-	-	-
A30	-	-	-	-	-	-	-	-	-	-	-
A30′	-	-	-	-	-	-	-	-	-	-	-
N31	-	-	-	-	-	-	112.9	-	-	-	-
N31′	-	-	-	-	-	-	115.3	-	-	-	-
I32	0.85	-	-	-	-	-	-	-	-	-	-
I32′	0.95	-	-	-	-	-	-	-	-	-	-
I33	1.00	-	-	-	-	-	-	-	-	-	-
I33′	0.86	-	-	-	-	-	-	-	-	-	-
G34	-	-	-	-	-	-	-	-	-	-	-
G34′	-	-	-	-	-	-	-	-	-	-	-
I35	1.11	-	-	-	-	-	-	-	-	-	-
I35′	0.99	-	-	-	-	-	-	-	-	-	-
L36	-	-	-	-	-	-	-	-	-	-	-
L36′	-	-	-	-	-	-	-	-	-	-	-
H37	-	-	-	14.84	-	251.1	-	-	-	173.4	-
H37′	-	-	-	12.17	-	251.6	-	-	-	167.0	-
L38	-	1.06	-	-	-	-	-	-	-	-	-
L38′	-	0.87	-	-	-	-	-	-	-	-	-
I39	1.05	-	-	-	-	-	-	-	-	-	-
I39′	0.93	-	-	-	-	-	-	-	-	-	-
L40	-	1.09	-	-	-	-	-	-	-	-	-
L40′	-	-	-	-	-	-	-	-	-	-	-
W41	-	-	10.79	-	-	-	-	-	128.8	-	-
W41′	-	-	11.21	-	-	-	-	-	129.9	-	-
I42	1.02	-	-	-	-	-	-	-	-	-	-
I42′	1.00	-	-	-	-	-	-	-	-	-	-
L43	-	-	-	-	-	-	-	-	-	-	-
L43′	-	-	-	-	-	-	-	-	-	-	-
D44	-	-	-	-	-	-	-	-	-	-	-
D44′	-	-	-	-	-	-	-	-	-	-	-
R45	-	-	-	-	-	-	-	84.47	-	-	-
L46	-	-	-	-	-	-	-	-	-	-	-
F47	-	-	-	-	-	-	-	-	-	-	-
F48	-	-	-	-	-	-	-	-	-	-	-
K49	-	-	-	-	-	-	-	-	-	-	32.2
S50	-	-	-	-	-	-	-	-	-	-	-
I51	0.92	-	-	-	-	-	-	-	-	-	-
Y52	-	-	-	-	-	-	-	-	-	-	-

Table S1. Assignments of S31N M2 determined from TEDOR-CC spectra, (H)CaNH, and HCa(Co)NH spectra, as well as TEDOR, RFDR, CH, CCH, and NH spectra. In bold are stereo specifically assigned. Missing resonances are indicated by *. In addition, Nh1 and Nh2 of R45 were not observed.

Restraint Summary:

Restraints are summarized below for spectrally unambiguous inter- and intra-helical contacts. A complete list of restraints can be found in the PDB entry on the web.

Table S2. Spectrally unambiguous Inter-Helical Contacts

Residue-Atom	Residue-Atom	Dist (Å)	Method
G34′-N	L38-Cδ1	2-5.5	TEDOR
G35-N	I35′-Cδ1	2-5.5	TEDOR
H37′-Nε2	L38-Cδ2	2-5.5	TEDOR
H37-Nε2	L38′-Cδ2	2-5.5	TEDOR
H37-Nε2	L38′-Cδ1	2-5.5	TEDOR
N31′-N	I35-Cγ2	2-5.5	TEDOR
N31′-N	I35-Cδ1	2-5.5	TEDOR
N31′-C β	G34-Cα	<8.4	PDSD 400 ms
G34′-C α	H37-Cα	<8.3	PDSD 400 ms
G34-C α	N31′-Cα	<7.3	PDSD 400 ms
G34-C α	N31′-Cβ	<8.0	PDSD 400 ms
H37′-Cδ2	H37′Cδ2	<10.3	PDSD 400 ms
H37′-Cδ2	H37-Cε1	<8.5	PDSD 400 ms
W41-Cη2	W41′-Cδ1	<11	PDSD 400 ms
G34-Cα	N31′-Cα	<7.3	PAR
H37-Cα	H37′-Cα	<9.2	PAR
H37′-Cε1	H37-Cε1	<6.2	RFDR 8 ms
H37′-Cε1	W41-Cε2	<6.4	RFDR 8 ms
H37-Cε1	W41′-Cε2	<6.7	RFDR 8 ms
L38′-Cδ1	H37-Cδ2	<6.3	RFDR 8 ms
L38′-Cδ1	W41-Cε2	<5.7	RFDR 8 ms
L38′-Cδ2	H37-Cδ2	<4.6	RFDR 8 ms
L38′-Cδ2*	H37-Cε1	<4.3	RFDR 8 ms
L38-Cδ2	H37′-Cδ2	<4.9	RFDR 8 ms
L38-Cδ2	H37′-Cε1	<3.9	RFDR 8 ms
W41′-Cβ	H37-Cε1	<7.6	RFDR 8 ms
W41-Cβ	H37′-Cε1	<6.5	RFDR 8 ms
I42-Cδ1	H37′-C	<8.9	RFDR 8 ms
I51-Cγ2	W41′-Cε2	<8.0	RFDR 8 ms
H37′-C**	H37-Cε1	<6.8	RFDR 8 ms
H37′-C	H37-Cδ2	<8.4	RFDR 8 ms
I32′-Cδ1	I33-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)
L38′-Cδ2	I33-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)
I39-Cδ1	I33′-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)
H37′-Hε2	W41-Hε1	0.5-5.0	^{1}H RFDR (^{15}N-^{1}H)

Residue-Atom	Residue-Atom	Dist (Å)	Method
L38′-H^N	H37-Hε2	2.5-7.0	^{1}H RFDR (^{15}N-^{1}H)
I42-H^N	W41′-Hε1	2.5-7.0	^{1}H RFDR (^{15}N-^{1}H)
W41-H^N	W41′-Hε1	2.5-7.0	^{1}H RFDR (^{15}N-^{1}H)
H37-Hε2	H37′-Hε2	2.5-7.0	^{1}H RFDR (^{15}N-^{1}H)

*the restraint was violating, added 1Å to distance

**restraint was violating, added 0.5 Å to distance

Table S3. Spectrally unambiguous intra-Helical Contacts

Residue-Atom	Residue-Atom	Dist (Å)	Method
N31′-C α	V27′-Cα	<7.9	PDSD 400 ms
N31′-Cα	V28′-Cα	<7.4	PDSD 400 ms
N31′-Cβ	G34′-Cα	<9.2	PDSD 400 ms
G34′-Cα	N31′-Cα	<8.1	PDSD 400 ms
G34′-Cα	N31′-Cβ	<9.3	PDSD 400 ms
G34′-Cα	H37′-Cα	<8.2	PDSD 400 ms
G34-Cα	H37-Cα	<5.4	PDSD 400 ms
P25-Cα	V28-Cα	<8.3	PAR
V27′-Cα	A30′-Cα	<7	PAR
V27′-Cβ	N31′-Cβ	<8.9	PAR
V27′-Cγ1	N31′-Cβ	<7.1	PAR
V28-Cγ2	P25-Cγ	<7.0	PAR
V28′-Cβ	N31′-Cα	<7.6	PAR
V28′-Cγ2	N31′-Cα	<6.6	PAR
V28′-Cγ2	N31′-Cβ	<7.1	PAR
V28′-Cγ1	N31′-Cα	<6.3	PAR
A29′-Cβ	V28′-Cα	<5.3	PAR
A30-Cβ	V27-Cα	<5.9	PAR
A30′-Cβ	V27′-Cα	<5.3	PAR
N31′-Cβ	G34′-Cα	<8.9	PAR
N31′-Cα	V28′-Cα	<7.7	PAR
G34′-Cα	N31′-Cα	<8.2	PAR
G34′-Cα	N31′-Cβ	<8.8	PAR
G34′-Cα	H37′-Cα	<6.8	PAR
G34-Cα	H37-Cβ	<8.0	PAR
G34-Cα	H37-Cα	<7.7	PAR
H37′-Cβ	G34′-Cα	<7.9	PAR
H37-Cβ	G34-Cα	<8.8	PAR
H37-Cβ	W41-Cα	<9.3	PAR
W41-Cα	H37-Cα	<8.6	PAR
D44-Cβ	W41-Cα	<8.7	PAR

Residue-Atom	Residue-Atom	Dist (Å)	Method
D44-Cα	W41-Cα	<7.7	PAR
K49-Cα	D44-Cα	<8.2	PAR
I32′-Cγ1	N31′-C	<5.9	RFDR 8 ms
I32-Cδ1	N31-Cγ	<8.14	RFDR 8 ms
I32-Cδ1	N31-C	<10.3	RFDR 8 ms
I32-Cγ2	N31-Cγ	<5.7	RFDR 8 ms
I33′-Cγ2	N31′-C	<6.0	RFDR 8 ms
I36′-Cδ2	I36′-C	<8.7	RFDR 8 ms
H37-Cα	H37-Cδ2	<5.6	RFDR 8 ms
L38′-Cδ1	W41′-Cε3	<7.0	RFDR 8 ms
L38′-Cδ2*	H37′-C	<6.4	RFDR 8 ms
L38′-Cδ2	W41′-Cε2	<6.5	RFDR 8 ms
L38′-Cδ2	W41′-Cε3	<5.1	RFDR 8 ms
L38′-Cδ2	I42′-Cα	<6.1	RFDR 8 ms
L38-Cα	H37-Cε1	<5.1	RFDR 8 ms
L38-Cδ1	H37-Cδ2	<7.6	RFDR 8 ms
L38-Cδ1	H37-Cε1	<7.4	RFDR 8 ms
L38-Cδ2	H37-Cδ2	<5.8	RFDR 8 ms
L38-Cδ2	W41-Cδ1	<5.8	RFDR 8 ms
L38-Cδ2	W41-Cε2	<5.8	RFDR 8 ms
W41′-Cβ	H37′-Cε1	<7.3	RFDR 8 ms
W41-Cβ	H37-Cε1	<5.0	RFDR 8 ms
I42′-Cα	W41′-Cε3	<6.7	RFDR 8 ms
I42′-Cδ1	W41′-Cε3	<7.8	RFDR 8 ms
I42′-Cγ1	W41′-Cε3	<6.1	RFDR 8 ms
L43-Cδ2	F47-Cδ1/ Cδ2	<5.4	RFDR 8 ms
D44-Cα	F47-Cδ1/ Cδ2	<5.9	RFDR 8 ms
R45-Cα	W41-Cε2	<6.6	RFDR 8 ms
L46-Cα	F47-Cδ1/ Cδ2	<6.2	RFDR 8 ms
L46-Cδ1	F47-Cδ1/ Cδ2	<6.1	RFDR 8 ms
L46-Cδ2	F47-Cδ1/ Cδ2	<7.2	RFDR 8 ms
L46-Cγ	F47-Cδ1/ Cδ2	<6.5	RFDR 8 ms
I51-Cγ2	W41′-Cε3	<7.2	RFDR 8 ms
I51-Cγ2	D44-Cγ	<7.9	RFDR 8 ms
H37′-C	H37′-Cε1	<7.0	RFDR 8 ms
H37-C	H37-Cδ2	<6.2	RFDR 8 ms
H37-C	H37-Cε1	<7.2	RFDR 8 ms
L40′-Cδ2	H37′-Cε1	<7.3	RFDR 8 ms
L40′-Cδ2	H37′-Cδ2	<8.1	RFDR 8 ms
I39′-Cδ1	I42′-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)
L40-Cδ2	I51-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)

Residue-Atom	Residue-Atom	Dist (Å)	Method
I35-Cδ1	I39-Cδ1	2.5-7.0	^{1}H RFDR (^{13}C-^{1}H)
H37 ′-Hε2	W41′-Hε1	0.5-6.5	^{1}H RFDR (^{15}N-^{1}H)

*the restraint was violating, added 1Å to distance

Table S4. Restraints summary by experiment.

Method	Interhelical		Intrahelical		Highly
	Unambig.	Ambig	Unambig.	Ambig.	Ambig.
TEDOR	7	3	–	–	–
PDSD	7	–	7	–	–
PAR	2	–	26	–	–
^{13}C RFDR 8 ms	22	–	31	2	1
^{13}C RFDR 19 ms	–	–	–	–	70
^{1}H RFDR (^{13}C-	3	–	3	–	–
^{1}H RFDR (^{15}N-	5	–	1	–	1
TOTAL	46	3	68	2	72

Table S5. Structure Statistics. Calculated over structured residues corresponding to those observed in spectra: P25′ to D44, and P25 to S50

Number of Restraints		
Interhelical	unambiguous	46
	ambiguous	3
Intrahelical	unambiguous	68
	ambiguous	2
	Highly Ambiguous	72
	Dihedral (TALOS+)	92
	TOTAL	283
Coordinate RMSD		
	Backbone (Å)	0.7
	All Heavy (Å)	1.1
Ramachandran Analysis		
	Core	98%
	Allowed	2%
	Disallowed	0%

The tetramer structure was calculated with four copies of the sequence distinguished by adding multiples of 100 to the residue numbers of each molecule. Residue numbers 118-160 and 318-360 correspond to the first set of resonances, and residue numbers 218-260 and 418-460 correspond to the second set of assignments (designated with a prime in the assignment tables). The complete set of restraints can be found on the web associated with the deposited PDB entry.

References:

(1) Jellum, E.; Eldjarn, L.; Try, K. *Acta Chemica Scandinavica* **1966**, *20*, 2535.
(2) Sita, L. R. *Journal of Organic Chemistry* **1993**, *58*, 5285.
(3) Hsiao, C. Y. Y.; Ottaway, C. A.; Wetlaufe.Db *Lipids* **1974**, *9*, 913.