Supplementary Information

Materials, cells and growth conditions

CsA was purchased from LC labs (Woburn, MA, USA). The subgenomic genotype 1b HCV replicon systems used consisted of hepatocyte cells (generally Huh7 or Huh5-2 cells) carrying replicons of different design. Huh 9-13 HCV replicons have the same genetic make-up as Huh 5-2 replicons, but differ in their composition of cell culture adaptive mutations, the absence of a luciferase reporter gene and the higher HCV RNA content as compared to Huh 5-2 cells (Lohmann et al 1999. Science 285:110-113). The infectious genotype 2a hepatitis C virus strain JFH-1/CS-N6 was kindly provided by Prof. Zoulim (INSERM Unit 871, Lyon, France).

Production of sanglifehrin A (1) in 15-L stirred bioreactors with secondary seed

Vegetative cultures were prepared by inoculating 0.2 mL from a spore stock of *Streptomyces* sp. A92-308110 into 400mL seed medium SGS in 2-L Erlenmeyer flasks with foam plugs. The culture flasks were incubated at 27°C, 250 rpm (2.5 cm throw) for 24 h.

From the seed culture, 300 mL was transferred into 15 litres of primary seed medium SGS containing 0.02% antifoam SAG 471, in a 15 L Braun fermentor. The fermentation was carried out for 24 hours at 27 ^{o}C, with starting agitation set at ≥ 300rpm aeration rate at 0.5 V/V/M and dissolved oxygen (DO) level controlled with the agitation cascade at ≥30% air saturation.

From the secondary seed culture prepared in the fermentor, 600 mL was taken under aseptic conditions and transferred into 15 litres of production medium SGP-2 containing 0.02% antifoam SAG 471, in 15 L Braun fermentor. The fermentation was carried out for 5 days at 24 ^{o}C, with starting agitation set at 300 rpm, aeration rate at 0.5 V/V/M and dissolved oxygen (DO) level controlled with the agitation cascade at ≥30% air saturation.

Extraction and purification of sanglifehrin A (1)

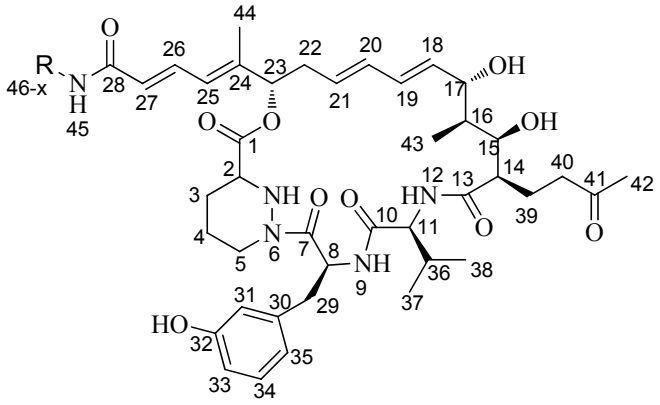
The whole broth (30 L) was clarified by centrifugation. The resulting cell pellet was extracted twice with ethyl acetate (2 x 10 L), each by stirring for 1 hour with overhead paddle stirrer and leaving to settle before pumping off solvent. The ethyl acetate layers were then combined (~20 L) and the solvent removed under reduced pressure at 40 ^{o}C to obtain an oily residue (18.3 g). This oily residue was then suspended in 80:20 methanol:water (total volume of 500 mL), and twice extracted with hexane (2 x 500 mL). The 80:20 methanol:water fraction was then dried under reduced pressure to yield a crude dry extract. This extract was dissolved in methanol (100 ml), mixed with 15 g Silica gel and dried to a powder. The powder was loaded into a silica gel column (5 x 20 cm) packed in 100% $CHCl_3$. For every one litre of elution solvent the methanol concentration was increased stepwise by 1% and 250 ml fractions collected. After three litres of solvent elution the methanol concentration was increased stepwise by 2% up to 8%. Fractions containing sanglifehrins were combined appropriately and reduced *in vacuo* to dryness and SfA purified by preparative HPLC. Preparative HPLC was achieved by multiple injections over a Waters Xterra Prep MS C18 OBD 10mm (19 x 250 mm) column running with solvent A (water) and solvent B (acetonitrile) at 20 ml/min (t = 0 mins, 55% B; t = 4 mins, 55% B; t = 30mins, 100% B; t = 32mins, 100% B; t = 36mins, 55% B).

Generation of sanglifehrin analogues

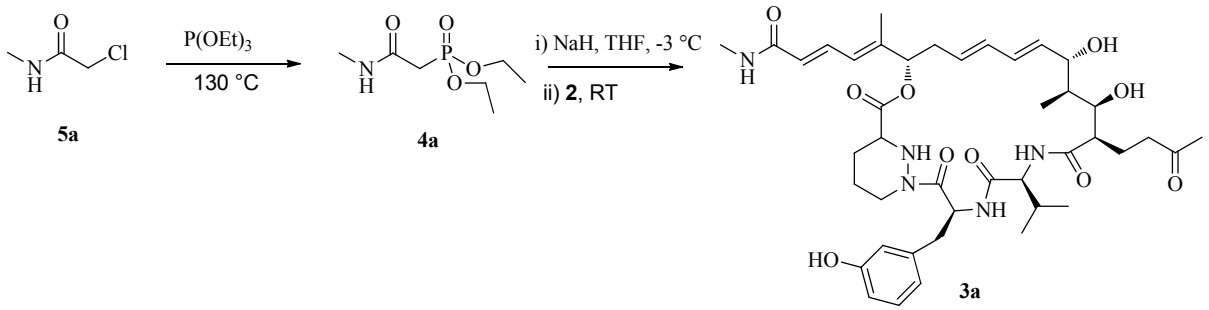
General methods

All purchased chemicals and solvents were of reagent or HPLC grade unless otherwise stated and were used directly as obtained from the manufacturer unless stated otherwise. Tetrahydrofuran was freshly distilled from sodium under an atmosphere of N_2. LC-MS of intermediates was conducted on an Agilent HP1100 or HP1200 operating in positive mode. Chromatography was achieved on a Waters Sunfire C18 column (length 50 mm, diameter 4.6 mm, 3.5 µm). Solvent A was water (0.05% TFA) and solvent B was acetonitrile (0.05% TFA). LC-timetable was T = 0 min; 1% B; T = 1.7 min, 99% B; T = 2.6 min, 99% B; T = 2.7 min, 1% B. NMR of intermediates was recorded on a Bruker Avance500 spectrometer at 500 MHz (1H NMR). NMR of final products were acquired on a Bruker Avance500 spectrometer fitted with a 5 mm triple resonance inverse automatic tuning and matching (TCI ATM) cryoprobe with Z gradients running at 298 K and operating at 500 MHz and125 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are reported in parts per million and are referenced relative to the solvent resonance. Coupling constants are given in hertz. LC-MS of final products was recorded on an integrated Agilent HP1100 HPLC system in combination with a Bruker Daltonics Esquire 3000+ spectrometer fitted with an electrospray source. LC-MS analysis was performed on an Agilent HP1100 equipped with a Gemini NX C18 110 Å column (150 mm × 4.6 mm, 3 µm, Phenomenex) heated to 40 °C. The gradient elution was from 10 % B held for 2 min followed by a linear increase to 100% mobile phase B over 13 min and held at 100% B for a further 2 minutes at a flow rate of 1 mL⁄ min. Mobile phase A was water containing 0.1% formic acid; mobile phase B

was acetonitrile containing 0.1% formic acid. The HPLC system described above was coupled to a Bruker Daltonics Esquire 3000+ electrospray mass spectrometer, scanning from 50 to 1,500 amu in switching mode. High-resolution MS were measured on a Bruker BioApex II 4.7e Fourier Transform Ion Cyclotron Resonance spectrometer fitted with an electrospray source and operating in positive ion mode. Final compounds were shown to be greater than 95% pure by NMR and the LC-MS method described above on two solid phases (C18 and phenyl-hexyl). The major confirmer of each sangamide was elucidated using HRMS and 1D and 2D NMR experiments (1H, 13C, APT, COSY, HMBC and HMQC), structures were numbered using the following scheme:



Preparation of 3a



A mixture of *N*-methylchloroacetamide (**5a**, 200 mg, 1.87 mmol) and triethyl phosphite (0.67 mL, 3.74 mmol) was stirred at 130 °C for 8 h. The reaction mixture was cooled to room temperature and was purified by preparative HPLC to give intermediate **4a** (60 mg, 15%) as a colourless oil.

LC-MS: retention time 0.97 min; observed $[M+H]^+$ m/z = 210.1

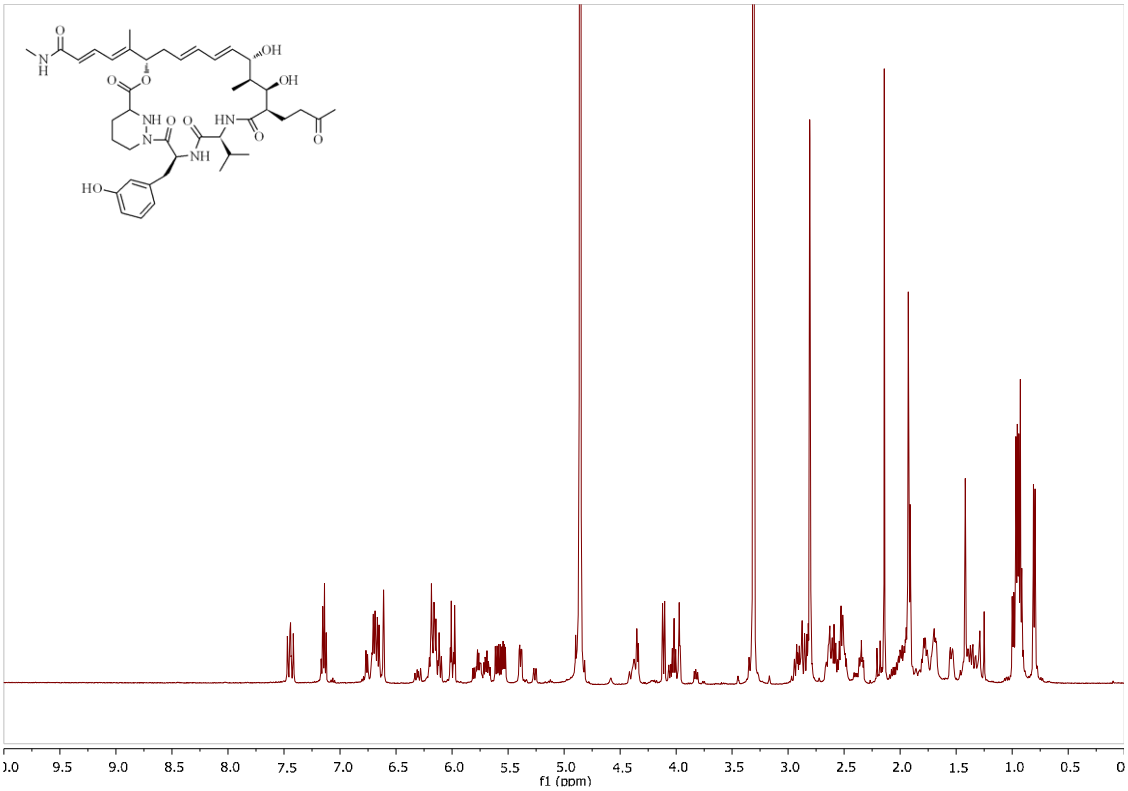
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 7.10 (1H, bs, N*H*), 4.05 (4H, m, OCH_2), 2.80 (1H, s, C*H*P), 2.76 (1H, s, C*H*P), 2.70 (3H, d, NCH_3), 1.24 (6H, m, OCH_2CH_3).

To a suspension of NaH (0.972 mg, 0.0405 mmol) in anhydrous THF (1.0 ml) was added dropwise a solution of **4a** (23 mg, 0.108 mmol) in anhydrous THF (0.2 ml) under N_2 atmosphere at -3 ^oC with stirring. The solution was then stirred at room temperature until it became clear. A solution of **2** (20 mg, 0.027 mmol) in anhydrous THF (0.2 ml) was added dropwise to the clear solution and the mixture stirred at room temperature for 30 min. The mixture was quenched with water and THF was evaporated under reduced pressure. The residue was extracted with ethyl acetate, the organic layer was washed with brine then dried over sodium sulfate. The solvent was removed in vacuo, giving a residue of 14 mg which was purified by preparative TLC. The crude compound (dissolved in acetone) was loaded on TLC plate (1 mm, 20*20 cm) and developed with acetone/petroleum ether = 3:2.The target band (visualized by UV) was collected and mixed

with acetone, then filtered through a pad of silica gel (2-3 cm height, pre-rinsed with acetone to remove impurities). The filtrate was concentrated *in vacuo*. Finally, acetonitrile and water were added to the obtained sample, and the solution was freeze-dried to give **3a** as white solid powder (7.0 mg, 37%).

LC-MS: retention time 9.2 min; observed $[M+H]^+$ m/z = 794.4

HRMS: $[M+H]^+$ $C_{42}H_{60}N_5O_{10}$ calc. 794.4340, obs. 794.4372, Δ 4.3 ppm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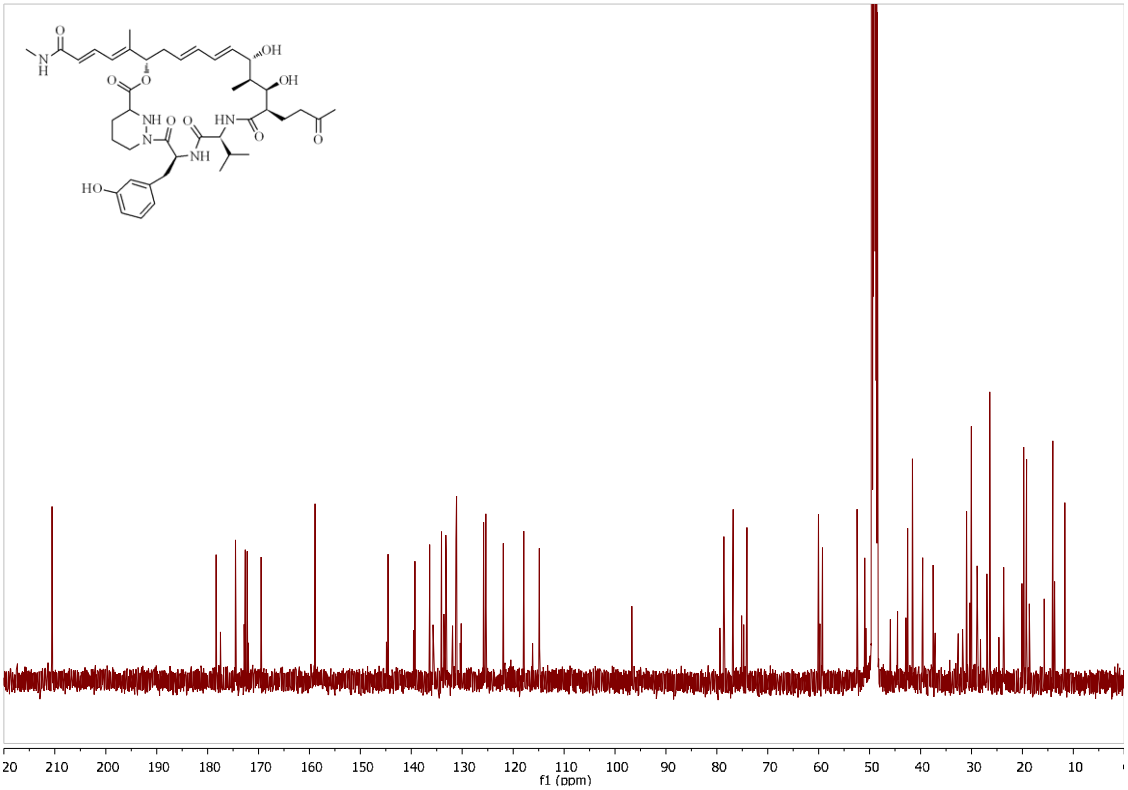
NH
HO
f1 (ppm)


OH
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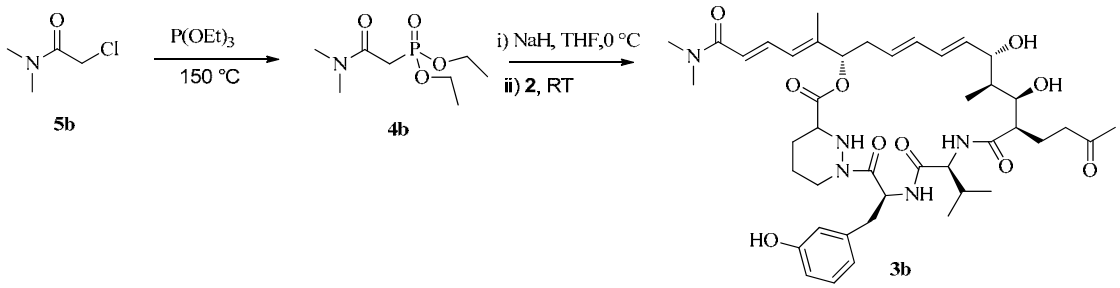
NH
HN
NH
HO
f1 (ppm)

NMR data of **3a** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.2		
2	1.78	m	59.3		3-H_2
3	1.55, 1.34	m x2	28.9		2-H
4	1.67, 1.39	m x2	23.7		5-H_2
5	4.38, 2.62	dd (21.0, 13.0), m	42.5		4-H_2
7			174.5		
8	5.54	dd (10.7, 5.4)	50.9	C-7, 29	29-H_2
10			172.6		
11	4.11	d (9.3)	60.0	C-10, 13, 36, 37, 38	36-H
13			178.3		
14	2.35	ddd (9.6, 6.3, 3.6)	52.5	C-13	15-H, 39-H_2
15	3.97	t (3.4)	74.1	C-13, 17	14-H, 16-H
16	1.79	m	44.5	C-17	15-H, 17-H, 43-H_3
17	4.02	t (7.6)	76.8	C-19, 16, 15	16-H, 18-H
18	5.59	dd (14.6, 7.7)	134.1	C-17, 19	17-H, 19-H
19	6.12	dd (14.5, 10.2)	133.2	C-17	18-H
20	6.16	m	133.2	C-22, 23	21-H
21	5.69	ddd (14.5, 8.3, 5.7)	131.1	C-19, 20, 22, 23	20-H, 22-H_2
22	2.50	m	37.5	C-20, 21, 23	21-H, 23-H
23	5.39	dd (9.0, 3.4)	78.6	C-1, 22, 25	22-H_2
24			144.6		
25	6.17	d (11.3)	125.4	C-23, 27, 44	26-H, 44-H_3
26	7.44	dd (14.1, 11.4)	136.4	C-24, 27, 28	25-H, 27-H
27	5.99	d (15.2)	125.8	C-25, 28	26-H
28			169.5		
29	2.89, 2.81	m x2	39.6	C-7, 8, 30, 31, 35	8-H
30			139.3		
31	6.61	t (2.0)	117.9	C-29, 33, 35	33-H
32			158.9		
33	6.70	dd (8.2, 2.4)	114.8	C-31, 32, 35	31-H, 34-H
34	7.14	t (7.6)	131.2	C-30, 32	33-H, 35-H
35	6.66	d (7.7)	121.9	C-29, 31, 33	34-H
36	1.98	m	31.0		11-H, 37-H_3, 38-H_3
37	0.96	d (6.8)	19.2	C-11, 36, 38	36-H
38	0.93	d (6.6)	19.7	C-11, 36, 37	36-H
39	1.92	m	27.0	C-14, 40	14-H, 40-H_2
40	2.61, 2.48	m x2	41.6	C-14, 39, 41	39-H_2
41			210.5		
42	2.14	s	30.0	C-39, 40, 41	
43	0.80	d (7.2)	11.7	C-15, 16, 17	16-H
44	1.93	s	14.0	C-23, 24, 25	25-H
46	2.81	s	26.4	C-28	

Preparation of 3b



A mixture of 2-chloro-N,N-dimethylacetamide (**5b**, 300 mg, 2.47 mmol) and triethyl phosphite (820 mg, 4.94 mmol) was stirred at 150 °C overnight. The reaction mixture was cooled to room temperature and was purified by preparative HPLC to give intermediate **4b** (105 mg, 20%).

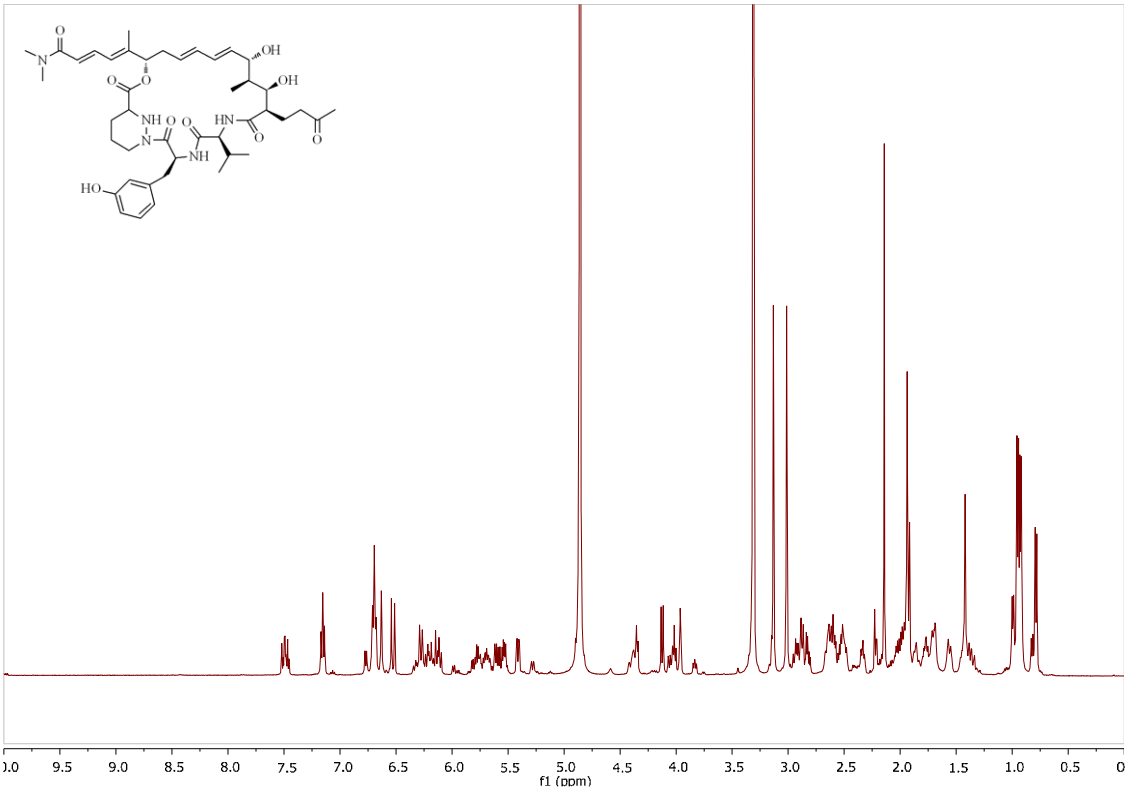
LC-MS: retention time 1.31 min; observed $[M+H]^+$ m/z = 224.2

NMR δ_H/ppm (d_6-DMSO, 500 MHz): 3.99 (4H, m, OC*H*$_2$), 3.10 (1H, s, C*H*P), 3.05 (1H, s, C*H*P), 2.99 (3H, d, NC*H*$_3$), 2.79 (3H, d, NC*H*$_3$), 1.19 (6H, m, OCH$_2$C*H*$_3$).

To a solution of **4b** (50 mg, 0.224 mmol) in THF (1.0 mL) was added NaH (1.6 mg, 0.068 mmol) in anhydrous THF (0.2 mL) at 0 °C with stirring. The solution was then stirred at room temperature until it became clear. **2** (40 mg, 0.054 mmol) was added to the clear solution and the mixture stirred at room temperature for 1 hour. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC [Column: Spring C18 (diameter 25 mm, length 250mm, particle size 10µm), Mobile phase: A = H_2O, B = Acetonitrile. Gradient: T = 0 min, 30% B; T = 10 min, 40% B] to obtained **3b** as a white amorphous solid (12.4 mg, 28%).

LC-MS: retention time 9.6 min; observed $[M+Na]^+$ m/z = 830.6

HRMS: $[M+H]^+$ $C_{43}H_{62}N_5O_{10}$ calc. 808.4497, obs. 808.4521, Δ 3.0 ppm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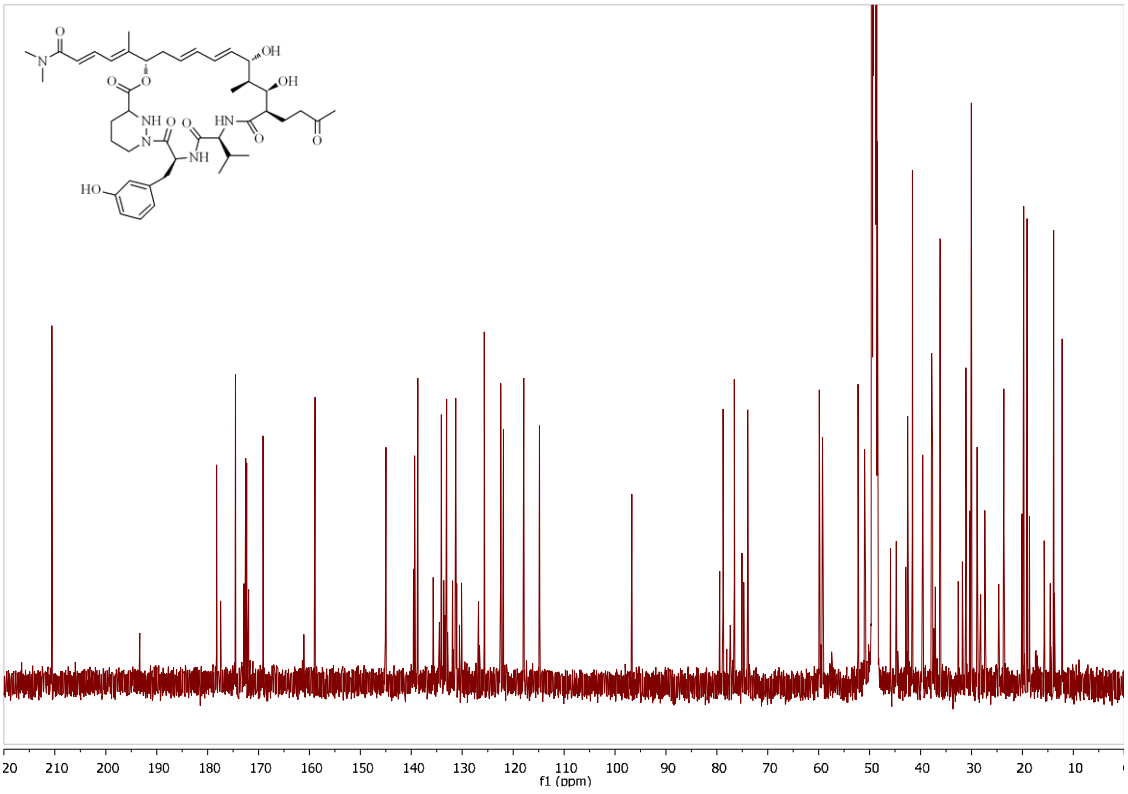
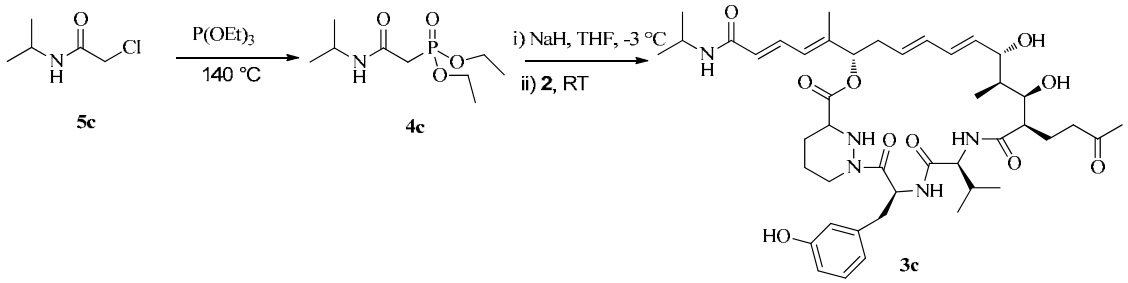

f1 (ppm)

NMR data of **3b** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.3		
2	1.86	m	59.3		3-H_2
3	1.56, 1.34	m x2	28.9		2-H
4	1.69, 1.38	m x2	23.6		5-H_2
5	4.37, 2.65	dd (20.0, 13.5), m	42.5		4-H_2
7			174.5		
8	5.53	dd (10.5, 5.6)	51.0	C-7, 29	29-H_2
10			172.5		
11	4.12	d (9.2)	59.9	C-10, 13, 36, 37, 38	36-H
13			178.2		
14	2.33	ddd (9.8, 6.3, 2.7)	52.3	C-13	15-H, 39-H_2
15	3.96	t (2.4)	73.9	C-13, 17, 39	14-H, 16-H
16	1.77	m	44.8	C-17	15-H, 17-H, 43-H_3
17	4.02	t (7.6)	76.5	C-19, 16, 15	16-H, 18-H
18	5.60	dd (14.9, 7.5)	134.1	C-16, 17, 19	17-H, 19-H
19	6.13	dd (14.9, 9.9)	133.2	C-17	18-H, 20-H
20	6.20	m	133.1	C-22	19-H, 21-H
21	5.69	ddd (15.0, 9.0, 5.6)	131.2	C-19, 20, 22, 23	20-H, 22-H_2
22	2.58, 2.48	m	37.6	C-20, 21, 23	21-H, 23-H
23	4.35	dd (10.0, 2.8)	78.8	C-1, 22, 25	22-H_2
24			145.0		
25	6.28	d (11.8)	125.7	C-23, 27, 44	26-H, 44-H_3
26	7.49	dd (14.7, 11.3)	138.7	C-24, 25, 27, 28	25-H, 27-H
27	6.53	d (14.7)	122.4	C-25, 28	26-H
28			169.1		
29	2.88, 2.82	m, dd (12.6, 5.5)	39.6	C-7, 8, 30, 31, 35	8-H
30			139.3		
31	6.63	bs	117.9	C-29, 32, 33, 35	33-H
32			158.9		
33	6.70	d (8.0)	114.8	C-31, 32, 35	31-H, 34-H
34	7.15	t (7.8)	131.3	C-30, 32	33-H, 35-H
35	6.69	d (7.2)	121.9	C-29, 31, 33	34-H
36	1.98	m	31.1		11-H, 37-H_3, 38-H_3
37	0.95	d (6.8)	19.1	C-11, 36, 38	36-H
38	0.93	d (6.6)	19.7	C-11, 36, 37	36-H
39	1.93	m	27.3	C-14, 40	14-H, 40-H_2
40	2.62, 2.50	m x2	41.6	C-14, 39, 41	39-H_2
41			210.6		
42	2.14	s	30.02	C-40, 41	
43	0.79	d (7.2)	12.2	C-15, 16, 17	16-H
44	1.94	s	13.9	C-23, 24, 25	25-H
46	3.13	s	37.8	C-28, C-47	

47	3.01	s	36.1	C-28, C-46	

Preparation of 3c



A mixture of *N*-isopropylchloroacetamide (**5c**, 1 g, 7.41 mmol) and triethyl phosphite (1.6 mL, 9.09 mmol) was stirred at 140 ℃ for 8 hours. The reaction mixture was cooled to room temperature and 200 mg sample was purified by preparative HPLC to give intermediate **4c** (60mg, 34%) as a colorless oil.

LC-MS: retention time 1.12 min; observed $[M+H]^+$ m/z = 238.2

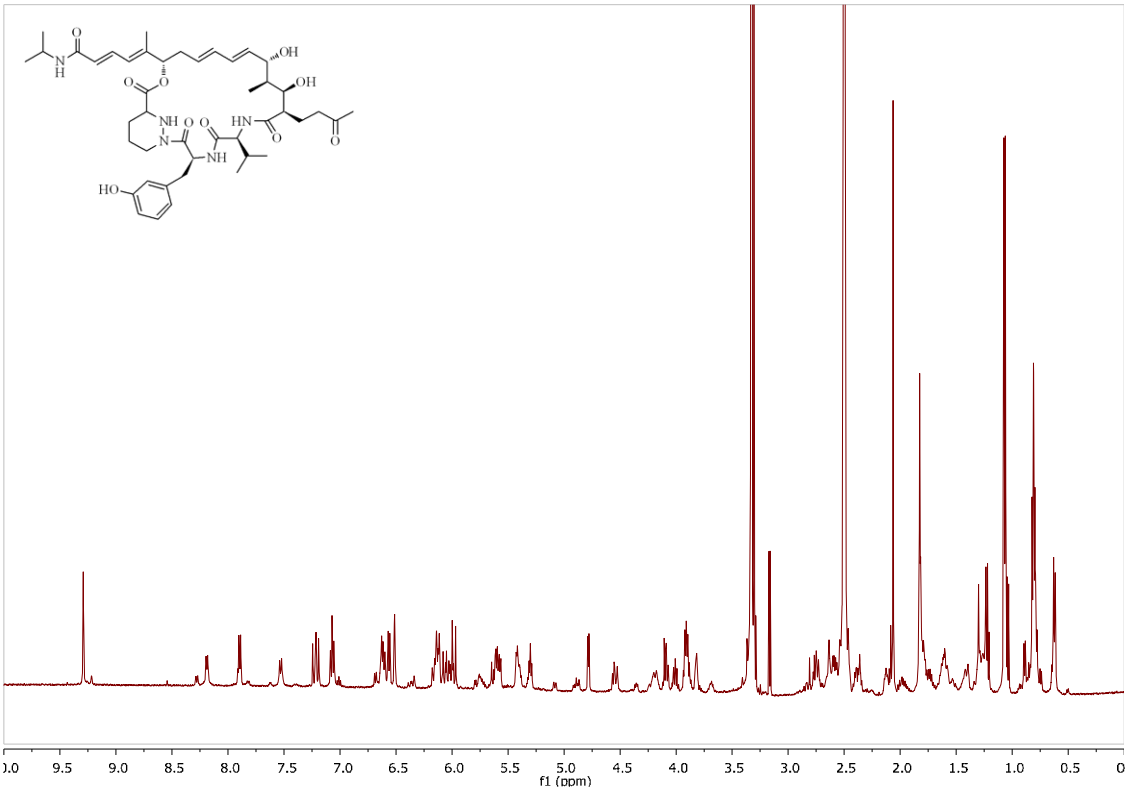
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 6.61 (1H, bs, N*H*), 4.10 (4H, m, OCH_2), 3.87 (1H, m, NC*H*), 2.83 (1H, s, C*H*P), 2.79 (1H, s, C*H*P), 1.37 (6H, m, OCH_2CH_3), 1.16 (6H, d, $NCHCH_3$)

To a suspension of NaH (0.972 mg, 0.0405 mmol) in anhydrous THF (1.0 mL) was added dropwise a solution of **4c** (26 mg, 0.108 mmol) in anhydrous THF (0.2 mL) under N_2 atmosphere at -3 ^oC with stirring. The solution was then stirred at room temperature until it became clear. A solution of **2** (20 mg, 0.027 mmol) in anhydrous THF (0.2 ml) was added dropwise to the clear solution and the mixture stirred at room temperature for 30 min. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The crude mixture (dissolved in acetone) was loaded on TLC plate (1mm, 20 x 20 cm) and developed with acetone/petroleum ether = 3:2. The target band (visualized by UV) was collected and mixed with acetone, then

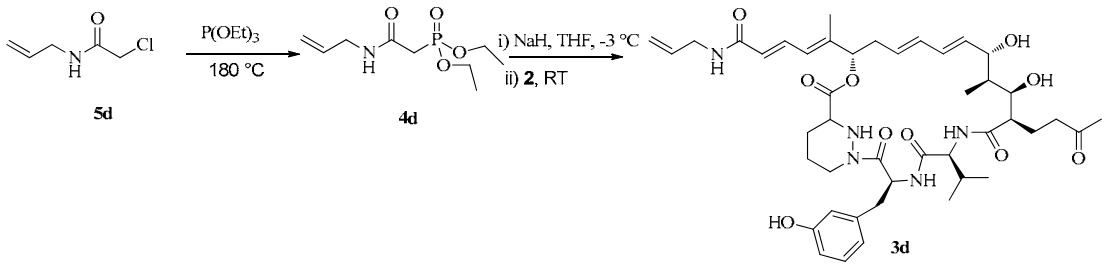
filtered through a pad of silica gel (2-3 cm height, pre-rinsed with acetone to remove impurities). The filtrate was concentrated *in vacuo*. Finally, acetonitrile and water were added to the obtained sample, and the solution was freeze-dried to give **3c** as white solid powder (8.5 mg, 38%).

LC-MS: retention time 10.0 min; observed $[M+H]^+$ m/z = 822.3

HRMS: $[M+H]^+$ $C_{44}H_{64}N_5O_{10}$ calc. 822.4653, obs. 822.4643, Δ 1.2 ppm



Preparation of 3d



A mixture of triethyl phosphite (0.63 ml, 3.75 mmol) and **5d** (500 mg, 3.74 mmol) were stirred at 180°C for 6 h. The reaction was cooled and purified by preparative HPLC to obtain intermediate **4d** as colorless oil (100 mg, 11%).

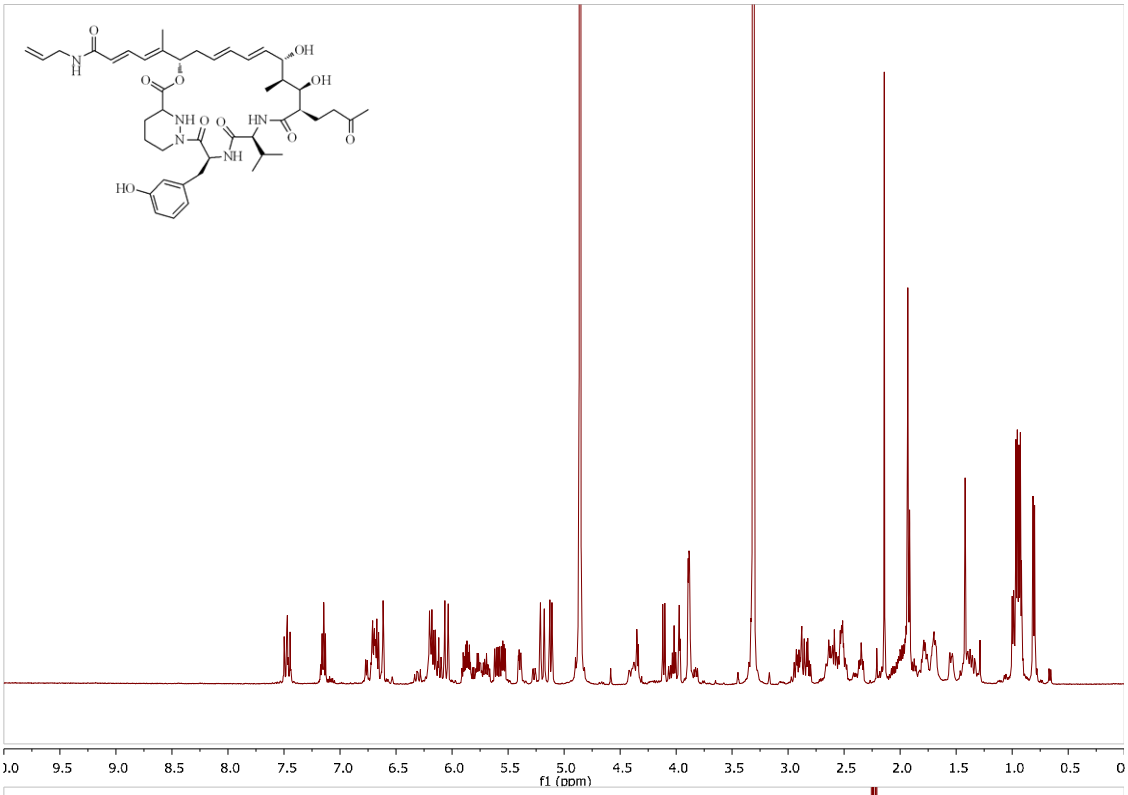
LC-MS: retention time 1.16 min; observed $[M+H]^+$ m/z = 236.1

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 6.87 (1H, bs, N*H*), 5.82 (1H, m, NCH_2CH), 5.23 (1H, d, NCH_2CHCH), 5.12 (1H, d, NCH_2CHCH), 4.13 (4H, m, OCH_2), 3.90 (2H, m, NCH_2), 2.89 (1H, s, C*H*P), 2.84 (1H, s, C*H*P), 1.34 (6H, m, OCH_2CH_3).

A solution of **4d** (38 mg, 0.1624 mmol) dissolved in THF (1 mL) was added to NaH (1.5 mg, 0.0625 mmol) at 0°C and stirred for 10 min. The solution was allowed to warm to room temperature and **2** (30 mg, 0.0406 mmol) was added was stirred at room temperature for afurther 30 minutes. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3d** as white solid (7.4 mg, 22%).

LC-MS: retention time 9.9 min; observed $[M+H]^+$ m/z = 820.3

HRMS: $[M+H]^+$ $C_{44}H_{62}N_5O_{10}$ calc. 820.4497, obs. 820.4490, Δ 0.9 ppm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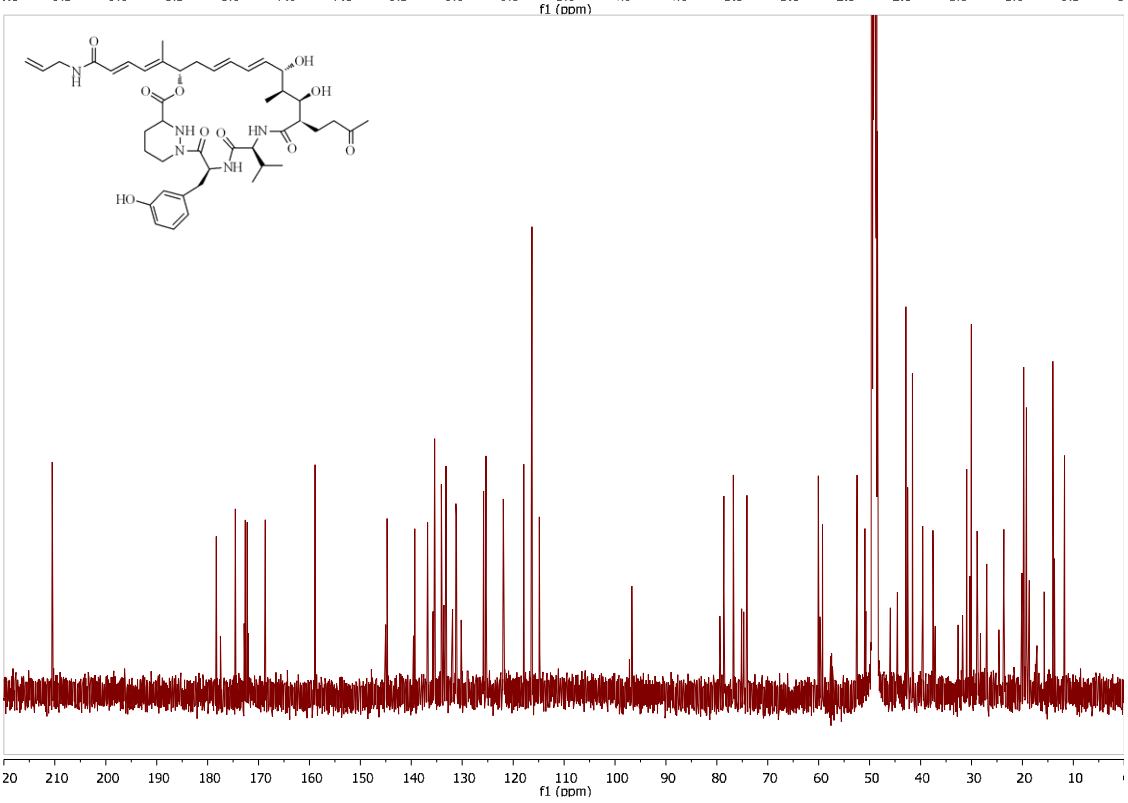
f1 (ppm)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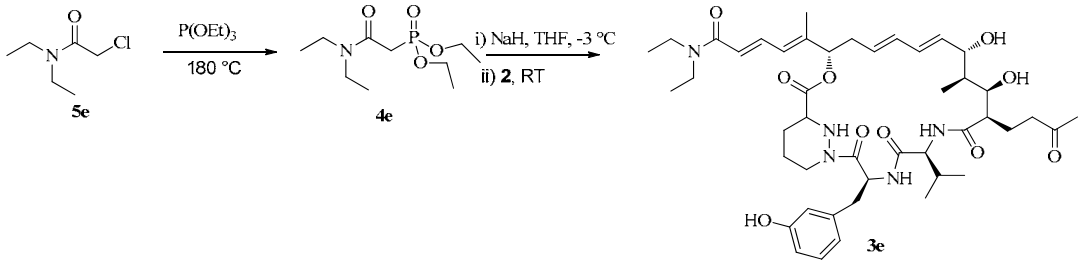
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

NMR data of **3d** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.3		
2	1.76	m	59.2		3-H_2
3	1.54, 1.34	m x2	28.9		2-H
4	1.69, 1.39	m x2	23.6		5-H_2
5	4.39, 2.62	dd (22.3, 11.9), m	42.5		4-H_2
7			174.6		
8	5.54	dd (10.7, 5.3)	50.9	C-7, 29	29-H_2
10			172.6		
11	4.11	d (9.4)	60.1	C-10, 13, 36, 37	36-H
13			178.3		
14	2.35	ddd (9.8, 6.2, 3.5)	52.4	C-13	15-H, 39-H_2
15	3.97	t (3.3)	74.1	C-13, 17	14-H, 16-H
16	1.80	m	44.6	C-17	15-H, 17-H, 43-H_3
17	4.02	t (7.6)	76.8	C-19, 16, 15	16-H, 18-H
18	5.60	dd (14.8, 7.7)	134.1	C-17, 19	17-H, 19-H
19	6.12	dd (14.4, 10.4)	133.2	C-17, 21	18-H, 20-H
20	6.17	m	133.2	C-18, 22	19-H, 21-H
21	5.68	ddd (14.5, 8.4, 5.7)	131.2	C-19, 20, 22, 23	20-H, 22-H_2
22	2.51	m	37.6	C-20, 21	21-H, 23-H
23	5.40	dd (8.8, 3.3)	78.6	C-1, 22, 25	22-H_2
24			144.8		
25	6.19	d (10.8)	125.3	C-22, 23, 27, 44	26-H, 44-H_3
26	7.47	dd (14.8, 11.5)	136.8	C-24, 27, 28	25-H, 27-H
27	6.05	d (14.9)	125.8	C-25, 28	26-H
28			168.7		
29	2.89, 2.82	m, dd (12.6, 5.4)	39.6	C-7, 8, 30, 31, 35	8-H
30			139.3		
31	6.62	t (2.0)	117.9	C-35	33-H
32			158.9		
33	6.70	dd (8.0, 2.4)	114.8	C-31, 32, 35	31-H, 34-H
34	7.14	t (7.8)	131.2	C-30, 32	33-H, 35-H
35	6.66	d (7.4)	121.9	C-29, 31, 33	34-H
36	1.99	m	31.0		11-H, 37-H_3, 38-H_3
37	0.96	d (6.8)	19.2	C-11, 36, 38	36-H
38	0.93	d (6.7)	19.7	C-11, 36, 37	36-H
39	1.92	m	27.0	C-14, 40, 41	14-H, 40-H_2
40	2.62, 2.48	m x2	41.6	C-14, 39, 41	39-H_2
41			210.5		
42	2.14	s	30.0	C-40, 41	
43	0.81	d (7.1)	11.7	C-15, 16, 17	16-H
44	1.93	d (1.5)	14.0	C-23, 24, 25	25-H
46	3.88	m	42.9	C-28, 47, 48	47-H, 48-H_2

47	5.87	m	135.4	C-46	46-H_2, 48-H_2
48	5.19, 5.12	d (17.2), d (10.3)	116.3	C-46, 47	46-H_2, 47-H

Preparation of 3e



A mixture of **5e** (17.5 mL, 0.127 mmol) and triethyl phosphite (22 mL, 0.1309 mmol) was stirred at 180 ℃ for 8 h. The reaction mixture was cooled to room temperature and distilled to give intermediate **4e** (15 g, 47 %) as a colorless oil.

LC-MS: retention time 1.18 min; observed $[M+H]^+$ m/z = 252.1

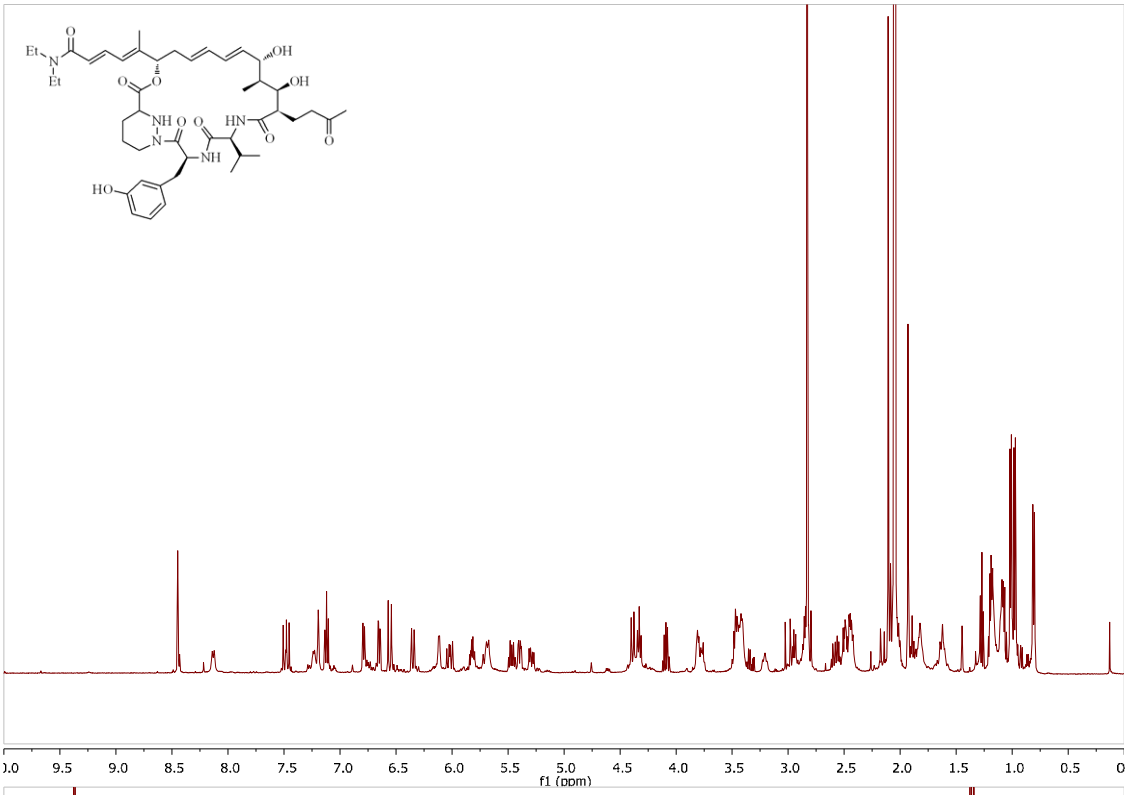
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 4.16 (4H, m, OC*H*$_2$), 3.42 (2H, m, NC*H*$_2$), 3.37 (2H, m, NC*H*$_2$), 3.02 (1H, s, C*H*P), 2.98 (1H, s, C*H*P), 1.32 (6H, m, OCH_2CH_3), 1.18 (3H, d, $NCHCH_3$), 1.15 (3H, d, $NCHCH_3$)

To a suspension of NaH (0.776 mg, 0.0324 mmol) in anhydrous THF (1.0 mL) was added dropwise a solution of **4e** (28.5 mg, 0.1134 mol) in anhydrous THF (0.2 mL) under N_2 atmosphere at -3 ^oC with stirring. The solution was then stirred at room temperature until it became clear. A solution of **2** (20 mg, 0.027 mmol) in anhydrous THF (0.2 mL) was added dropwise to the clear solution and the mixture stirred at room temperature for 30 min. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The crude mixture (dissolved in acetone) was loaded on TLC plate (1mm, 20 x 20 cm) and developed with acetone/petroleum

ether = 3:2. The target band (visualized by UV) was collected and mixed with acetone, then filtered through a pad of silica gel (2-3 cm height, pre-rinsed with acetone to remove impurities). The filtrate was concentrated *in vacuo*. Finally, acetonitrile and water were added to the obtained sample, and the solution was freeze-dried to give **3e** as white solid powder (9.0 mg, 40 %).

LC-MS: retention time 10.6 min; observed $[M+H]^+$ m/z = 836.6

HRMS: $[M+H]^+$ $C_{45}H_{66}N_5O_{10}$ calc. 836.4810, obs. 836.4822, Δ 1.4 ppm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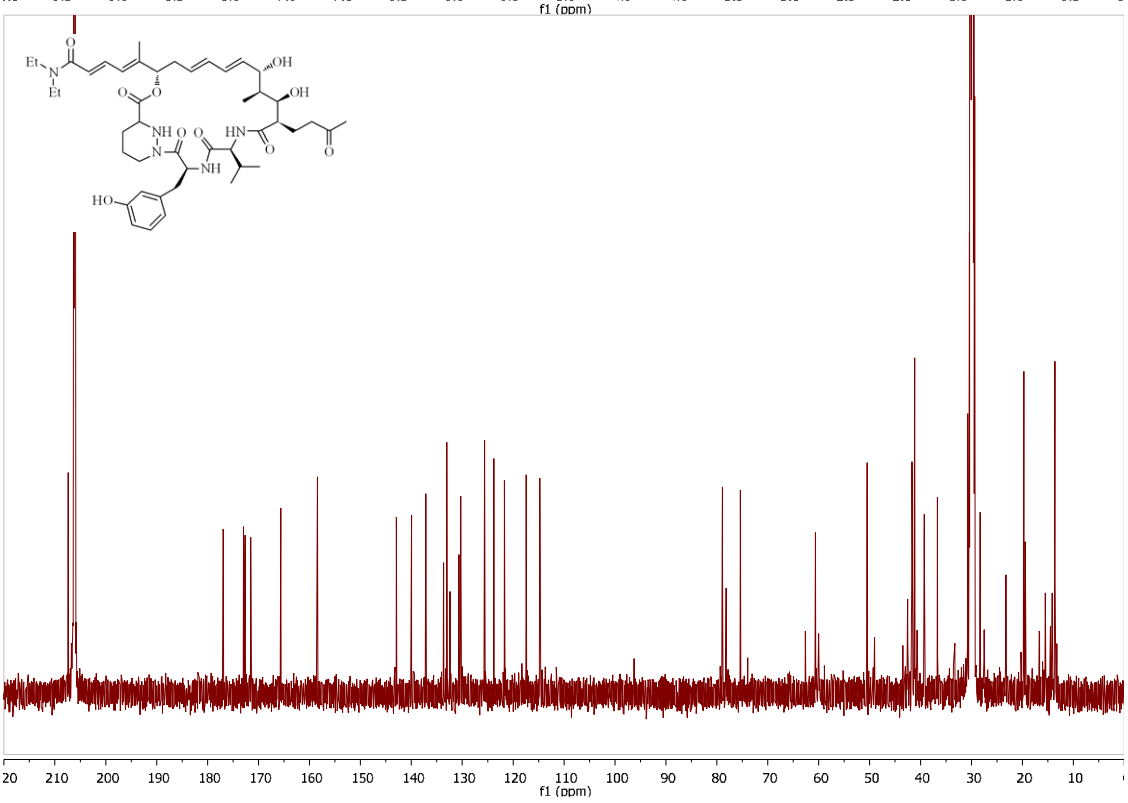
f1 (ppm)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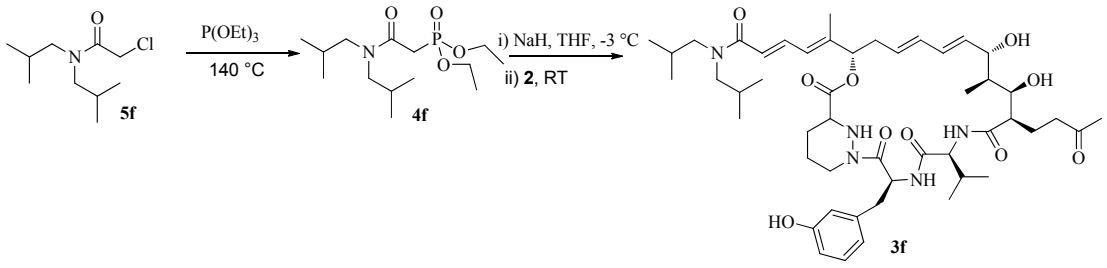
O
NH
HO
O
f1 (ppm)

NMR data of **3e** in $(CD_3)_2CO$ at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			171.6		
2	3.21	t (10.2)	60.0	C-1	3-H_2, 6'-NH
3	1.85, 1.62	m x2	28.3		2-H
4	1.82, 1.62	m x2	23.3		5-H_2
5	4.35, 2.81	m x2	41.7		4-H_2
6'-NH	4.39	d (11.9)		C-7	
7			172.6		
8	5.82	td (8.4, 5.1)	50.5	C-7, 29, 30	29-H_2, 9-NH
9-NH	8.13	d (9.1)			8-H
10			173.0		
11	4.33	t (8.2)	60.6	C-10, 13, 36, 37, 38	36-H, 12-NH
12-NH	7.24	d (7.4)			11-H
13			176.9		
14	2.44	m	49.1	C-13, 39, 40	15-H
15	3.81	m	78.2		14-H, 16-H, 15-OH
15-OH	6.12	d (5.4)		C-16	15-H
16	2.02	m	41.7		15-H, 17-H, 43-H_3
17	3.76	m	75.4		16-H, 18-H, 17-OH
17-OH	5.68	bs			17-H
18	5.29	dd (15.2, 6.5)	133.7	C-17, 20	17-H, 19-H
19	5.70	dd (14.9, 10.2)	132.4	C-17, 21	18-H, 20-H
20	6.02	dd (15.3, 10.6)	133.0	C-19, 22	19-H, 21-H
21	5.47	dt (15.0, 7.3)	130.6	C-19, 22, 23	20-H, 22-H_2
22	2.53, 2.45	m x2	36.7	C-20, 21, 23	21-H, 23-H
23	5.40	dd (10.7, 3.6)	78.9	C-1, 22, 25	22-H_2
24			142.9		
25	6.33	d (11.5)	125.6	C-23, 27, 44	26-H, 44-H_3
26	7.48	dd (14.5, 11.6)	137.2	C-24, 25, 27, 28	25-H, 27-H
27	6.56	d (14.6)	123.8	C-25, 28	26-H
28			165.6		
29	2.94, 2.84	dd (13.6, 5.5), m	39.3	C-7, 8, 30, 31, 35	8-H
30			140.0		
31	7.19	bs	117.4	C-29, 32, 33, 35	33-H
32			158.4		
33	6.66	ddd (8.2, 2.5, 1.1)	114.8	C-31, 32, 35	31-H, 34-H
34	7.12	t (7.9)	130.3	C-30, 32	33-H, 35-H
35	6.79	d (7.5)	121.7	C-29, 31, 33	34-H
36	2.02	m	30.8	C-11	11-H, 37-H_3, 38-H_3
37	1.01	d (6.6)	19.4	C-11, 36, 38	36-H
38	0.98	d (6.7)	19.7	C-11, 36, 37	36-H
39	1.89	m	27.5	C-14, 40	40-H_2

40	2.58, 2.44	m x2	41.1	C-39, 41	39-H_2
41			207.4		
42	2.11	s	30.4	C-40, 41	
43	0.81	d (7.4)	14.5	C-15, 16, 17	16-H
44	1.93	d (1.1)	13.6	C-23, 24, 25	25-H
46	3.46	m	42.5	C-28, 47, 46'	47-H_3
46'	3.43	m	41.2	C-28, 47', 46	47'-H_3
47	1.19	m	15.5	C-46	46-H_2
47'	1.09	m	13.5	C-46'	46'-H_2

Preparation of 3f



A mixture of **5f** (206 mg, 1.00 mmol) and triethyl phosphite (332 mg, 2.00 mmol) was stirred at 140°C for 6 h. The reaction mixture was cooled to room temperature and was purified by flash chromatography to give intermediate **4f** (222 mg, 20%).

LC-MS: retention time 1.61 min; observed $[M+H]^+$ m/z = 308.3

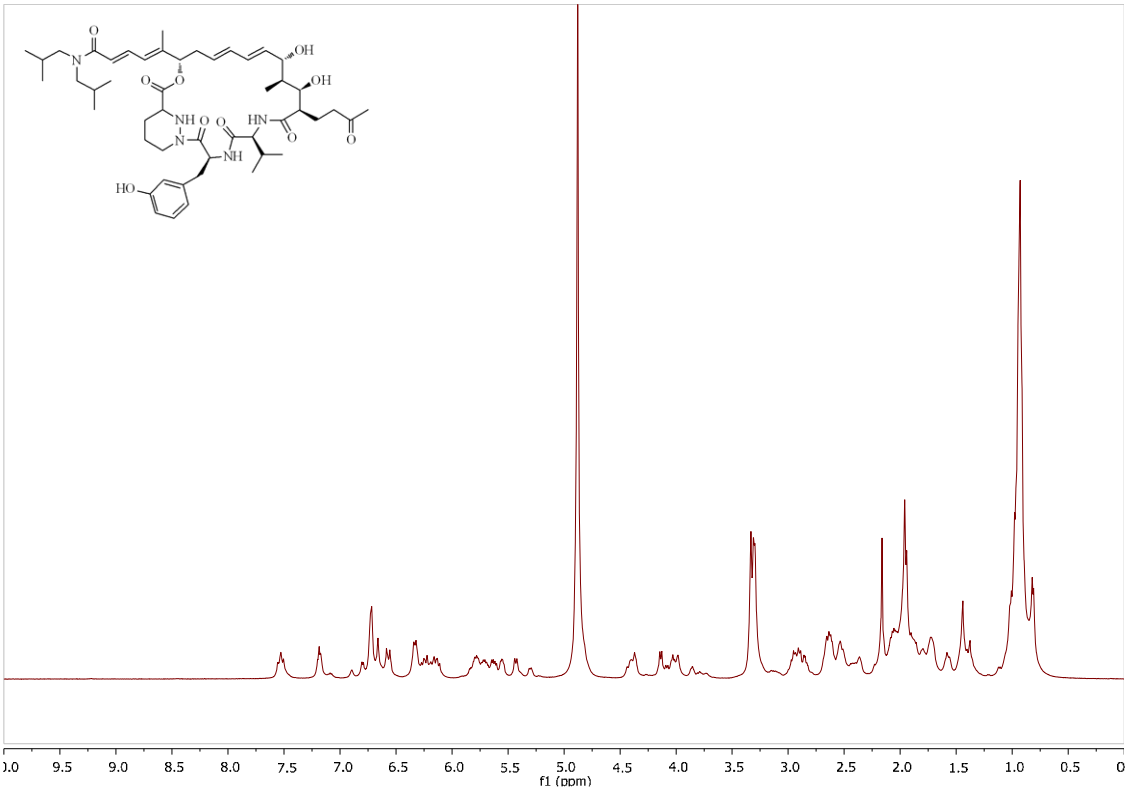
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 4.01 (4H, m, OC*H*$_2$), 3.20 (4H, m, NC*H*$_2$), 3.09 (1H, s, C*H*P), 3.04 (1H, s, C*H*P), 2.05 (1H, m, $NHCH_2CH$), 1.95 (1H, m, $NHCH_2CH$), 1.34 (6H, m, OCH_2CH_3), 0.91 (12H, d, NCH_2CHCH_3)

To a solution of **4f** (58 mg, 0.188 mmol) in THF (1.0 mL) was added NaH (1.4 mg, 0.0564 mmol) in anhydrous THF (0.2 mL) at rt with stirring. **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture stirred at room temperature for 3 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with

brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3f** as white solid (16.2 mg, 38%).

LC-MS: retention time 12.6 min; observed $[M+H]^+$ m/z = 892.6

HRMS: $[M+H]^+$ $C_{49}H_{74}N_5O_{10}$ calc. 892.5438, obs. 892.5436, Δ 0.2 ppm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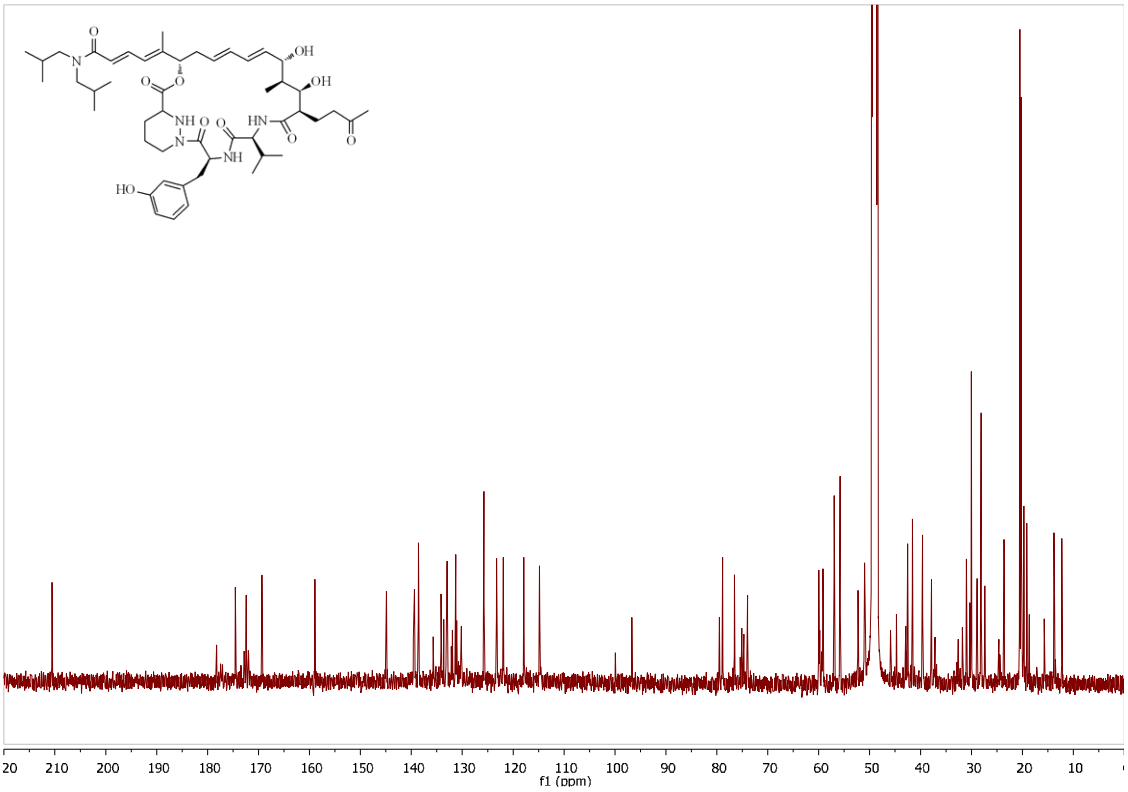
f1 (ppm)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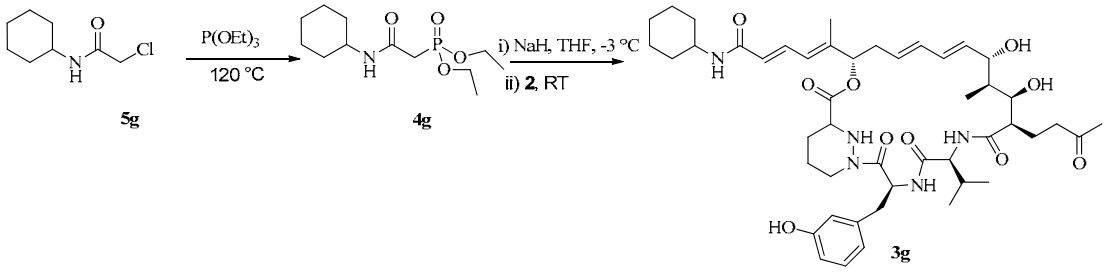
70
60
50
40
30
20
10
f1 (ppm)

NMR data of **3f** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.4		
2	1.84	m	59.2		3-H_2
3	1.55, 1.34	m x2	28.9		2-H
4	1.70, 1.38	m x2	23.6		5-H_2
5	4.40, 2.62	dd (20.0, 11.2), m	42.5		4-H_2
7			174.5		
8	5.54	dd (10.4, 5.5)	50.8	C-7, 29	29-H_2
10			172.5		
11	4.13	d (8.2)	60.0	C-10, 13, 36, 37, 38	36-H
13			178.2		
14	1.75	m	52.3		15-H
15	3.98	bs	74.0	C13	14-H
16	1.75	m	44.7	C-17	17-H, 43-H_3
17	4.02	t (7.6)	76.5	C-19, 16, 15	16-H, 18-H
18	5.62	dd (14.8, 7.2)	134.2	C-17, 19, 20	17-H, 19-H
19	6.13	dd (14.8, 9.8)	133.1	C-21, 17	18-H, 20-H
20	6.21	m	133.0	C-19, 22	19-H. 21-H
21	5.71	m	131.3	C-19, 20, 22	20-H, 22-H_2
22	2.62, 2.48	m x2	37.8	C-20, 21, 23	21-H, 23-H
23	5.42	d (10.0))	78.9	C-1, 21, 22, 24, 25, 44	22-H_2
24			145.0		
25	6.32	d (9.4)	125.7	C-23, 27, 44	26-H, 44-H_3
26	7.52	t (12.0)	138.6	C-24, 25, 27, 28	25-H, 27-H
27	6.56	d (14.0)	123.3	C-25, 28	26-H
28			169.3		
29	2.89, 2.83	m, dd (13.5, 6.2)	39.6	C-7, 8, 30, 31, 35	8-H
30			139.4		
31	6.65	bs	117.9	C-29, 32, 33, 35	
32			158.9		
33	6.72	m	114.8	C-31, 32, 35	34-H
34	7.18	t (6.8)	131.3	C-30, 32	33-H, 35-H
35	6.71	m	122.0	C-29, 31, 33	34-H
36	1.98	m	31.0	C-11	11-H, 37-H_3, 38-H_3
37	0.98	d (6.1)	19.1	C-11, 36	36-H
38	0.94	d (7.0)	19.7	C-11, 36	36-H
39	1.95	m	27.4	C-14, 40	14-H, 40-H_2
40	2.61, 2.48	m x2	41.6	C-14, 39, 41	39-H_2
41			210.6		
42	2.15	s	30.0	C-40, 41	
43	0.82	d (6.9)	12.2	C-15, 16, 17	16-H

44	1.95	s	13.8	C-23, 24, 25	25-H
46	3.29	m	57.0	C-28, 47, 48, 49, 46’	47-H
46’	3.29	m	55.8	C-28, 47’, 48’, 49’, 46	47’-H
47	1.95	m	30.0	C-46, 48, 49	46-H_2, 48-H_3, 49-H_3
47’	2.05	m	28.2	C-46’, 48’, 49’	46’-H_2, 48’-H_3, 49’-H_3
48	0.93	m	20.2	C-46, 47	47-H
48’	0.92	m	20.5	C-46’, 47’	47’H
49	0.93	m	20.2	C-46, 47	47-H
49’	0.92	m	20.5	C-46’, 47’	47’-H

Preparation of 3g



A mixture of **5g** (500 mg, 2.85 mmol) and triethyl phosphite (1 mL, 5.96 mmol) was stirred at 120 °C overnight. The reaction mixture was cooled to room temperature and white solid precipitated. The solution was filtered and the solid recrystallized from petroleum ether / ethyl acetate (3:2, 4 mL) to give the intermediate **4g** (300 mg, 38 %) as a white solid.

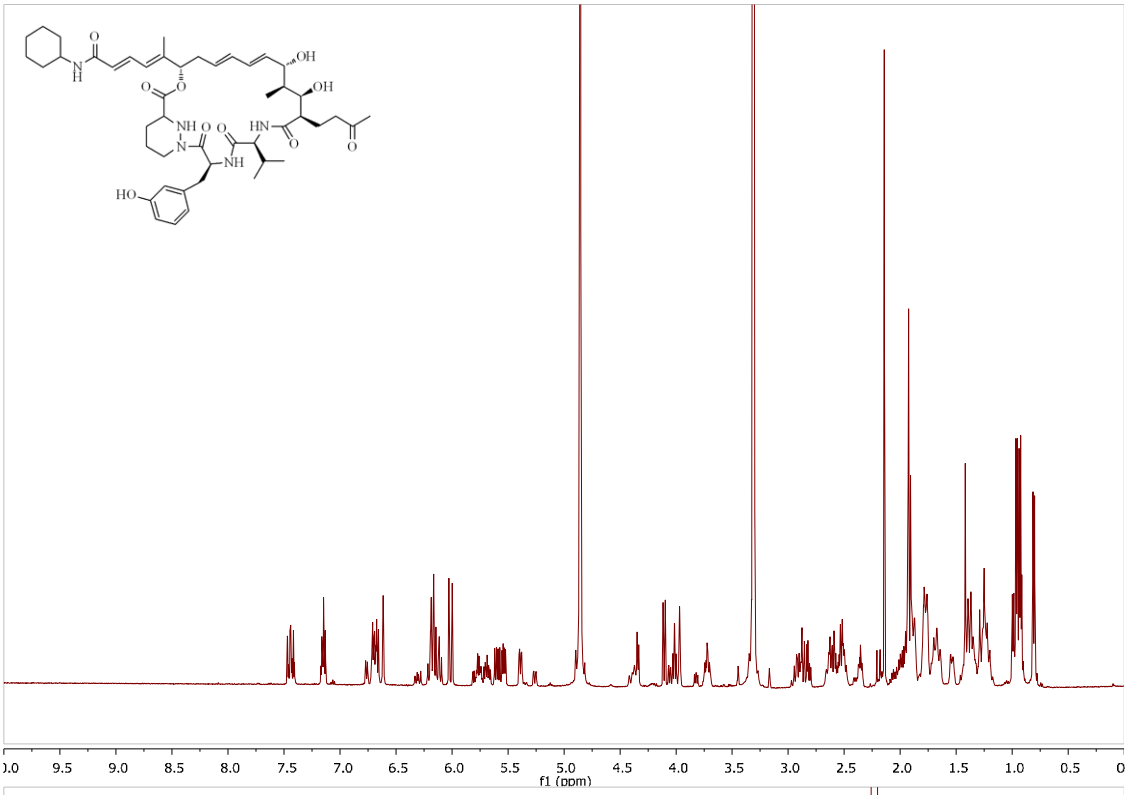
LC-MS: retention time 1.51 min; observed $[M+H]^+$ m/z = 278.2

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 6.64 (1H, bs, N*H*), 4.21 (4H, m, OCH_2), 3.78 (1H, m, NC*H*), 2.83 (1H, s, C*H*P), 2.79 (1H, s, C*H*P), 1.89 (2H, m, NHCHC*H*), 1.71 (2H, m), 1.59 (2H, m), 1.40 (2H, m), 1.38 (6H, m, OCH_2CH_3), 1.21 (2H, m)

To a solution of **4g** (45 mg, 0.164 mmol) in THF (2.0 mL) was added NaH (1.2 mg, 0.049 mmol) in anhydrous THF (0.2 mL) at 0 ^{o}C with stirring. The solution was then stirred at 20 ^{o}C until it became clear. Then **2** (30 mg, 0.041 mmol) was added to the clear solution and the mixture stirred at 20 ^{o}C for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 ml). The organic layer was washed with brine and dried over Na_2SO_4, filtered and evaporated. The residue was purified by preparative-TLC and preparative-HPLC to obtain **51** (2.4 mg, 7%) as a white solid.

LC-MS: retention time 11.1 min; observed $[M+H]^+$ m/z = 862.8

HRMS: $[M+H]^+$ $C_{47}H_{68}N_5O_{10}$ calc. 862.4966, obs. 862.5001, Δ 4.1 ppm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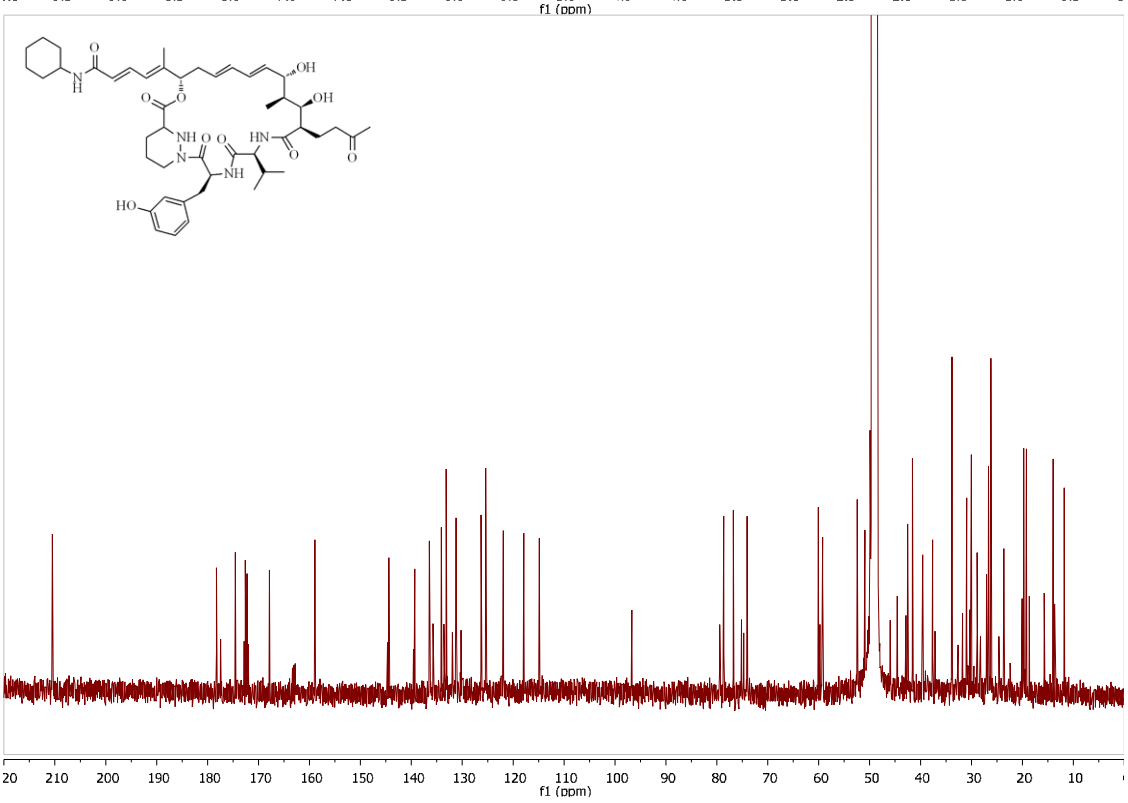
f1 (ppm)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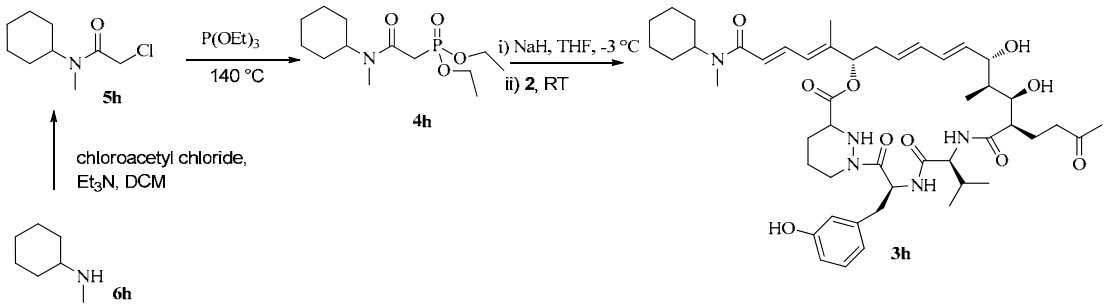
HO
f1 (ppm)

NMR data of **3g** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.3		
2	1.76	m	59.2		3-H_2
3	1.54, 1.30	m x2	28.9		2-H
4	1.67, 1.36	m x2	23.6		5-H_2
5	4.39, 2.61	dd (22.8, 12.7), m	42.5		4-H_2
7			174.6		
8	5.54	dd (10.6, 5.3)	50.9		29-H_2
10			172.6		
11	4.11	d (9.4)	60.1	C-10, 13, 36	36-H
13			178.3		
14	2.35	ddd (9.7, 6.2, 3.2)	52.4	C-13	15-H, 39-H_2
15	3.97	t (3.3)	74.1		14-H, 16-H
16	1.79	m	44.6		15-H, 43-H_3
17	4.01	t (7.6)	76.8	C-19, 16, 15	16-H, 18-H
18	5.60	dd (14.8, 7.6)	134.1	C-19	17-H, 19-H
19	6.12	dd (14.8, 10.2)	133.2		18-H
20	6.16	m	133.2		21-H
21	5.69	ddd (14.5, 8.5, 5.7)	131.2		20-H, 22-H_2
22	2.52	m	37.6	C-20, 21	21-H, 23-H
23	5.39	dd (9.2, 3.2)	78.6		22-H_2
24			144.4		
25	6.18	d (11.1)	125.4	C-27, 44	26-H, 44-H_3
26	7.44	dd (14.9, 11.3)	136.4	C-28	25-H, 27-H
27	6.01	d (14.9)	126.3	C-25, 28	26-H
28			167.8		
29	2.88, 2.82	dd (12.7, 10.7), dd (12.6, 5.3)	39.6	C-7, 8, 30, 31	8-H
30			139.3		
31	6.61	t (2.0)	117.9	C-35	33-H
32			158.9		
33	6.70	dd (8.0, 2.4)	114.8	C-31, 35	31-H, 34-H
34	7.15	t (7.8)	131.2	C-30, 32	33-H, 35-H
35	6.67	d (7.6)	121.9	C-29, 31, 33	34-H
36	1.98	m	30.9		11-H, 37-H_3, 38-H_3
37	0.96	d (6.6)	19.2	C-11, 36, 38	36-H
38	0.93	d (6.6)	19.7	C-11, 36, 37	36-H
39	1.92	m	27.0		14-H, 40-H_2
40	2.61, 2.52	m x2	41.6		39-H_2
41			210.5		
42	2.14	s	30.0	C-40, 41	
43	0.81	d (7.1)	11.8	C-15, 16, 17	16-H
44	1.93	d (1.4)	14.0	C-23, 24, 25	

46	3.72	tt (10.9, 3.7)	49.9		47-H_2, 47'-H_2
47, 47'	1.87, 1.21	m x2	33.8		46-H, 48,48'-H_2
48, 48'	1.75, 1.38	m x2	26.2		49-H_2, 47,47'-H_2
49	1.64, 1.19	m x2	26.7		48-H_2, 48'-H_2

Preparation of 3h



To a solution of **6h** (1 g, 8.834 mmol), Et_3N (0.983 g, 9.724 mmol) in dry DCM (10 mL) was added dropwise chloroacetyl chloride (1.088 g, 9.724 mmol). The reaction mixture was stirred at 0 °C for 3 h, poured into ice water, and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, filtered, concentrated *in vacuo* to give a light yellow liquid (1.95 g) which was used to the next step without any further purification.

LC-MS: retention time 1.54 min; observed $[M+H]^+$ m/z = 190.1

A mixture of **5h** (crude, 400 mg, 2.11 mmol) and triethyl phosphite (701 mg, 4.22 mmol) was stirred at 140℃ overnight. The reaction mixture was cooled to room temperature and purified by flash chromatography to give intermediate **4h** (265 mg, 43 %) as a light yellow liquid.

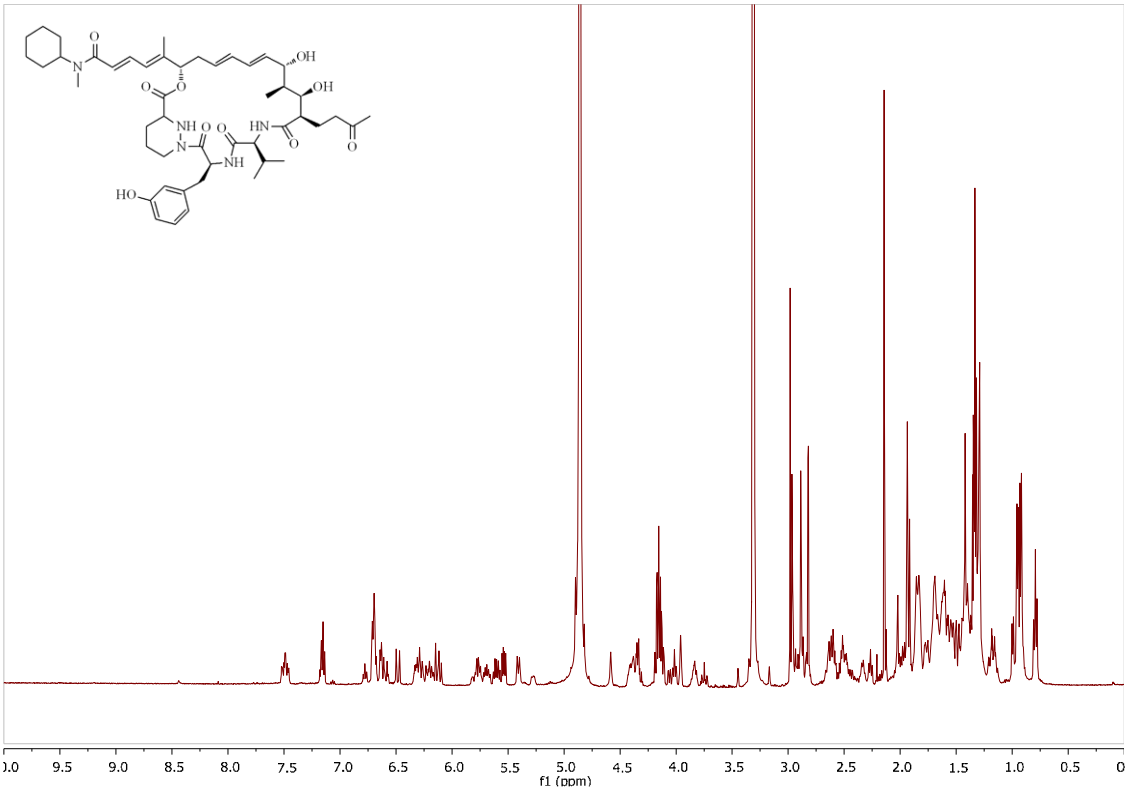
LC-MS: retention time 1.59 min; observed $[M+H]^+$ m/z = 292.2

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 5.84 (1H, bs, N*H*), 4.40 (1H, m, NC*H*), 4.17 (4H, m, OCH_2), 3.11 (1H, s, C*H*P), 3.06 (1H, s, C*H*P), 2.92 (3H, s, NCH_3), 1.86-1.55 (5H, m, cyclohexyl), 1.49 (1H, m, cyclohexyl), 1.35 (3H, m, cyclohexyl), 1.32 (6H, m, OCH_2CH_3), 1.08 (1H, m, cyclohexyl)

To a solution of **4h** (55 mg, 0.188 mmol) in THF (1.0 mL) was added NaH (1.4 mg, 0.056 mmol) in anhydrous THF (0.2 mL) at 0 ^{o}C with stirring. The solution was then stirred at room temperature until it became clear. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture stirred at room temperature for 3 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3h** as white solid (2.5 mg, 6%).

LC-MS: retention time 11.7 min; observed $[M+H]^+$ m/z = 877.9

HRMS: $[M+H]^+$ $C_{48}H_{70}N_5O_{10}$ calc. 876.5123, obs. 876.5153, Δ 3.4 ppm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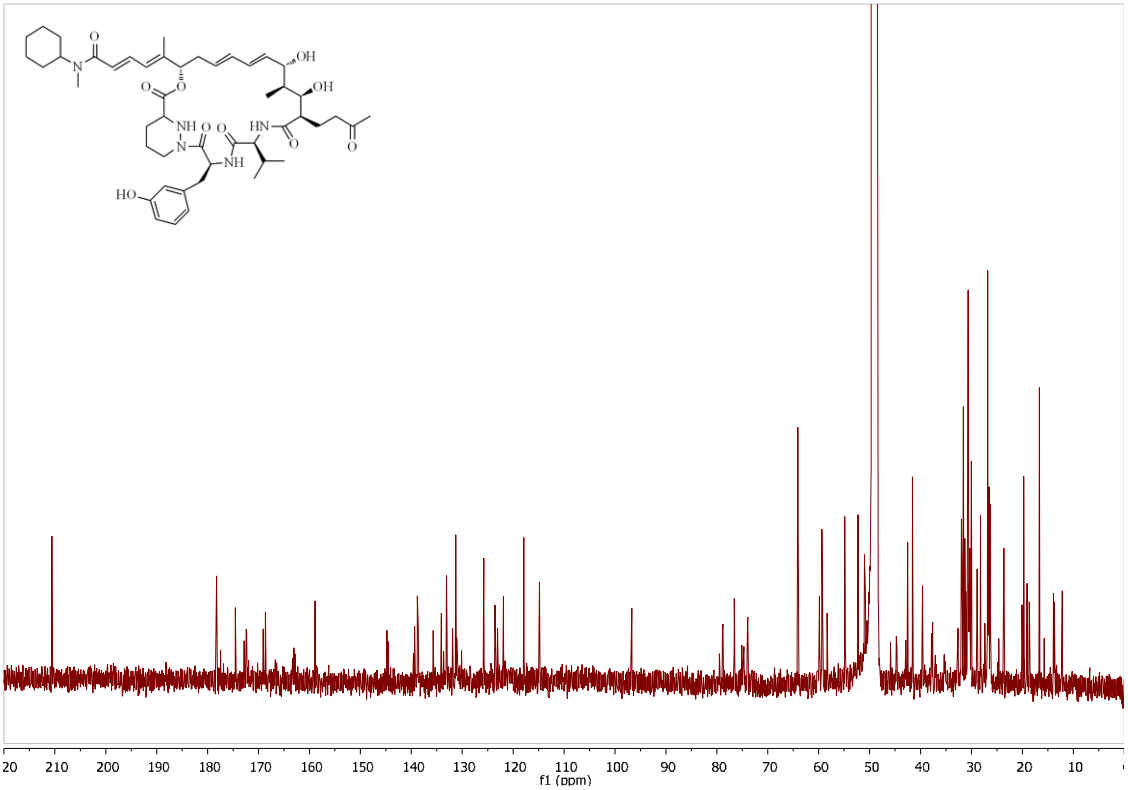
f1 (ppm)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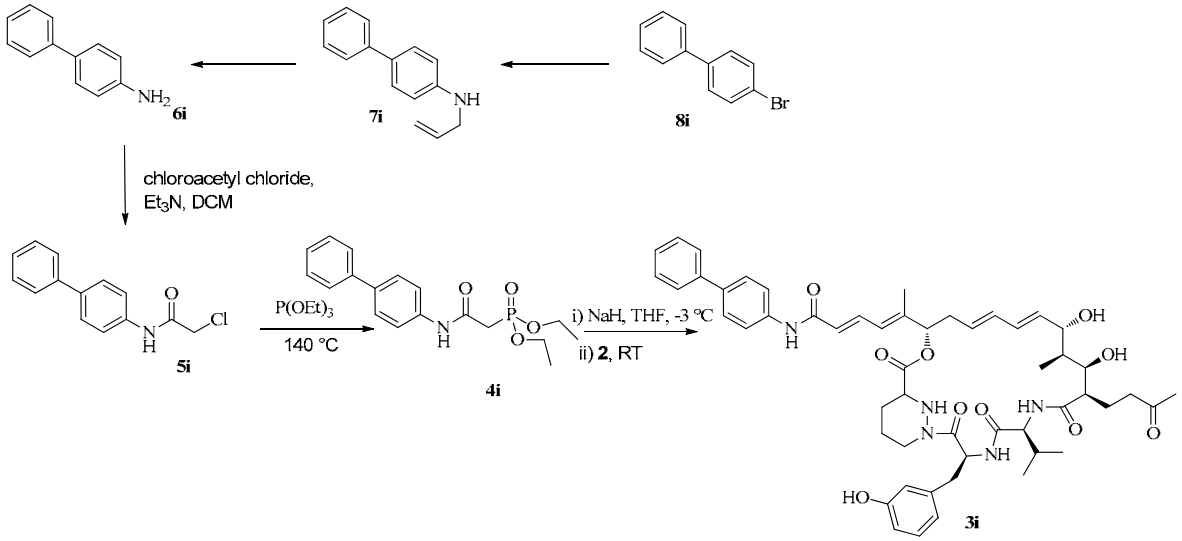
30
20
10
f1 (ppm)

Preparation of 3i



A mixture of 4-bromobiphenyl **8i** (5 g, 21.55 mmol), allylamine (2.4 mL, 32.33 mmol), sodium tert-butoxide (3.11 g, 32.33 mmol), Pd(DPPF)Cl_2 (0.79 g, 1.08 mmol) and DPPF (1.79 g, 3.23 mmol) in 20 mL anhydrous THF was heated to 80 °C for 4 h. The dark red reaction mixture was filtered through Celite and concentrated *in vacuo*. The resultant oil was by flash chromatography over silica gel using petroleum ether / ethyl acetate (5:1) to give **7i** (3.5 g, 60% yield) as a yellow solid.

LC-MS: retention time 1.82 min; observed $[M+H]^+$ m/z = 210.2

A mixture of **7i** (3 g, 14.35 mmol), 10% Pd/C (0.3 g) and methanesulfonic acid (922 uL, 14.35 mmol) in 50 mL of absolute ethanol was heated under reflux for 2 h. The reaction mixture was filtered through a Celite pad, washed with aq. NaOH (10 %), and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, filtered, concentrated *in vacuo* to give 1.6 g of the crude product **6i** which was used to the next step without any further purification.

LC-MS: retention time 1.36 min; observed $[M+H]^+$ m/z = 170.1

NMR δ_H/ppm (d_6-DMSO, 500 MHz): 7.52 (2H, d), 7.36 (2H, d), 7.35 (2H, d), 7.21 (1H, t), 6.65 (2H, d)

To a solution of crude **6i** (1.6 g, 9.462 mmol), Et_3N (1.052 g, 10.408 mmol) in dry DCM (50 mL) was added dropwise chloroacetyl chloride (1.165 g, 10.408 mmol). The reaction mixture was stirred at 0 ^oC for 3 h, poured into ice water, and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, filtered, concentrated *in vacuo* to give a light yellow solid. The solid was purified by preparative-TLC with petroleum ether/ethyl acetate (4:1), and give the desired compound **5i** (200 mg, 6% yield for two steps).

LC-MS: retention time 1.72 min; observed $[M+H]^+$ m/z = 246.0

NMR δ_H/ppm (d_6-DMSO, 500 MHz): 10.53 (1H, bs, N*H*), 7.71 (2H, d), 7.66 (2H, d), 7.64 (2H, d), 7.44 (2H, t), 7.33 (1H, t), 4.30 (2H, s, COCH_2Cl)

A mixture of **5i** (200 mg, 0.8161 mmol) and triethyl phosphite (271 mg, 1.6323 mmol) was stirred at 140 °C overnight. The reaction mixture was cooled to room temperature and was purified by flash chromatography to give intermediate **4i** (77 mg, 27%) as a light yellow solid.

LC-MS: retention time 1.62 min, $[M+H]^+$ m/z did not ionise

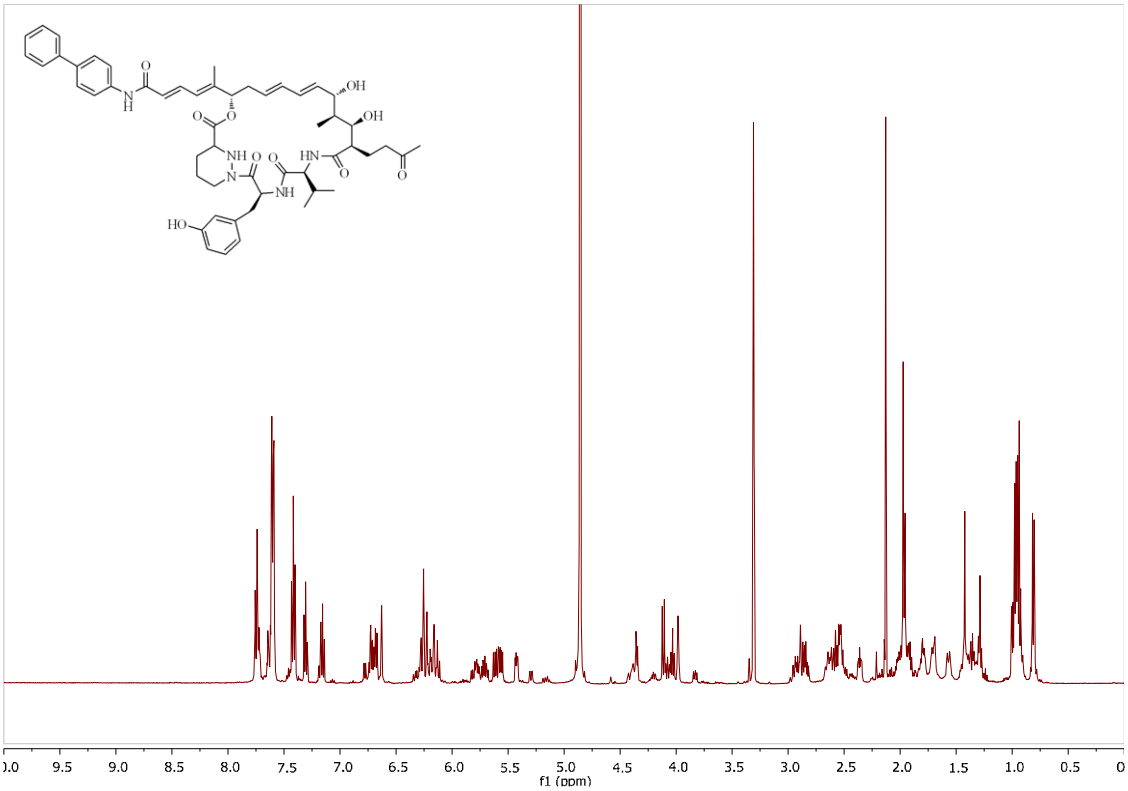
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 8.92 (1H, bs, N*H*), 7.60 (2H, d), 7.56 (2H, d), 7.53 (2H, d), 7.42 (2H, t), 7.32 (1H, t), 4.20 (4H, m, OCH_2), 3.05 (1H, s, C*H*P), 3.01 (1H, s, C*H*P), 1.32 (6H, m, OCH_2CH_3)

To a solution of **4i** (65 mg, 0.188 mmol) in THF (1.0 mL) was added NaH (1.4 mg, 0.056 mmol) in anhydrous THF (0.2 mL) at 0 ^oC with stirring. The solution was then stirred at room temperature until it became clear. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the

mixture stirred at room temperature for 3 hours. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3i** as white solid (9.4 mg, 21%).

LC-MS: retention time 12.7 min; observed $[M+H]^+$ m/z = 932.6

HRMS: $[M+H]^+$ $C_{53}H_{66}N_5O_{10}$ calc. 932.4810, obs. 932.4781, Δ 3.1 ppm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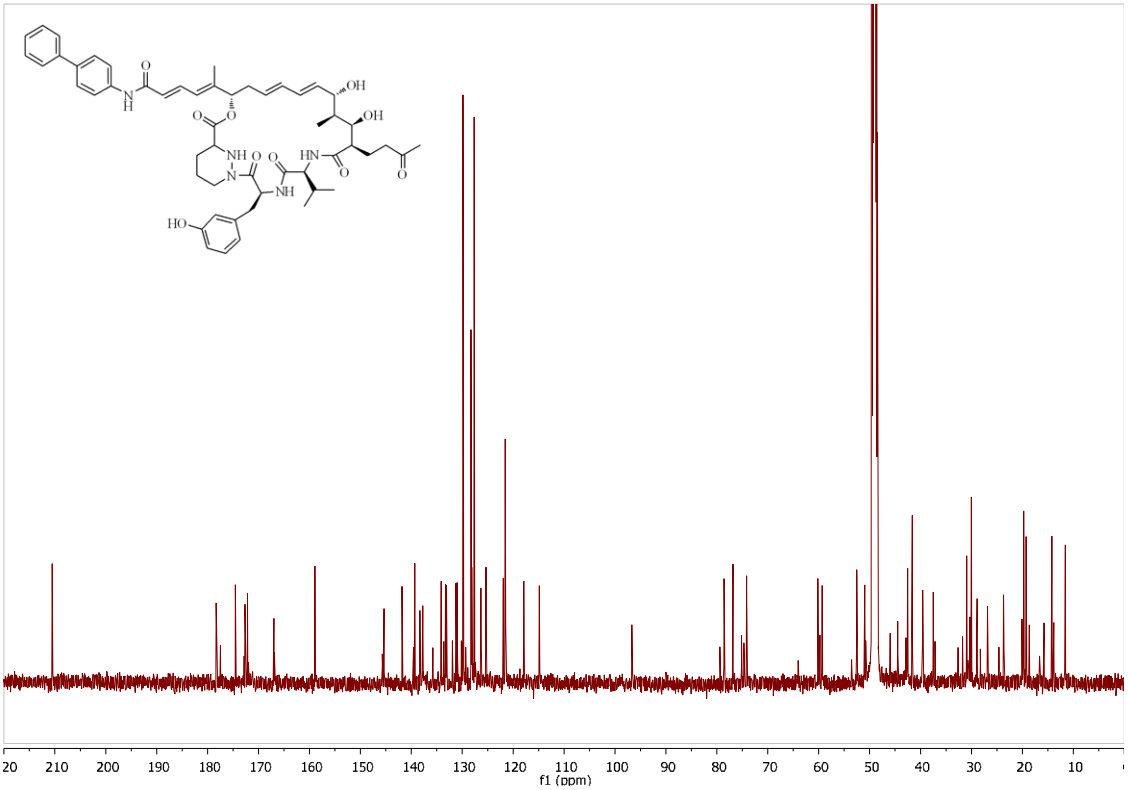
f1 (ppm)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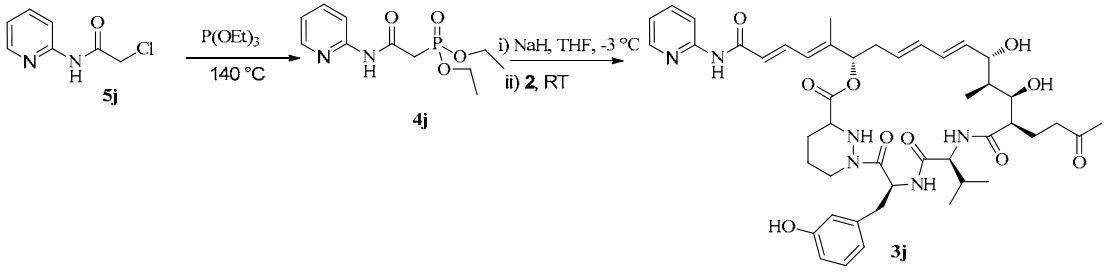
OH
HO
f1 (ppm)

NMR data of **3i** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.2		
2	1.79	m	59.3		3-H_2
3	1.57, 1.35	m x2	28.9		2-H
4	1.71, 1.38	m x2	23.7		5-H_2
5	4.39,2.65	dd (21.4, 11.8), m	42.5		4-H_2
7			174.6		
8	5.56	dd (10.6, 5.6)	51.0	C-7, 29	29-H_2
10			172.7		
11	4.11	d (9.3)	60.2	C-10, 13, 36	36-H
13			178.3		
14	2.36	ddd (9.9, 6.0, 3.5)	52.5	C-13, 39	15-H, 39-H_2, 40-H_2
15	3.98	t (3.4)	74.7	C13, 17	14-H, 16-H
16	1.80	m	44.5	C-17	17-H, 43-H_3
17	4.03	t (7.5)	76.8	C-19, 16, 15	16-H, 18-H
18	5.61	dd (14.4, 7.6)	134.1	C-17, 19, 20	17-H, 19-H
19	6.14	dd (14.3, 10.3)	133.3	C-21, 17	18-H, 20-H
20	6.19	m	133.1	C-19, 22	19-H. 21-H
21	5.71	ddd (14.6, 8.2, 5.8)	131.0	C-19, 20, 22	20-H, 22-H_2
22	2.53, 2.49	m x2	37.5	C-20, 21, 23	21-H, 23-H
23	5.42	dd (8.3, 3.9))	78.6	C-1, 21, 22, 24, 25	22-H_2
24			145.4		
25	6.27	d (11.3)	125.3	C-23, 27, 44	26-H, 44-H_3
26	7.62	m	137.8	C-28	25-H, 27-H
27	6.24	d (14.7)	126.3	C-25, 28	26-H
28			167.0		
29	2.88, 2.84	m, dd (12.6, 5.4)	39.6	C-7, 8, 30, 31, 35	8-H
30			139.3		
31	6.63	t (1.8)	117.9	C-29, 32, 33, 35	33-H
32			158.9		
33	6.72	dd (7.7, 2.3)	114.7	C-31, 32, 35	31-H, 34-H
34	7.16	t (7.9)	131.2	C-30, 32	33-H, 35-H
35	6.68	d (7.5))	122.0	C-29, 31, 33	34-H
36	2.00	m	30.9	C-11, 37, 38	11-H, 37-H_3, 38-H_3
37	0.97	d (6.5)	19.3	C-11, 36, 38	36-H
38	0.93	d (6.7)	19.8	C-11, 36, 37	36-H
39	1.94	m	26.9	C-14, 40, 41	14-H, 40-H_2
40	2.62, 2.49	m x2	41.6	C-14, 39, 41	39-H_2
41			210.5		
42	2.13	s	30.0	C-40, 41	
43	0.81	d (7.1)	11.6	C-15, 16, 17	16-H
44	1.97	d (1.4)	14.2	C-23, 24, 25	25-H

46			139.4	’	
47, 47’	7.75	d (8.6)	121.6	C-47, 47’, 49	48-H, 48’-H
48, 48’	7.60	d 8.7)	128.3	C-46, 47, 47’, 48, 48’,50	47-H, 47’-H
49			138.3		
50			141.8		
51, 51’	7.61	d (8.5)	127.7	C-53	52-H, 52’-H
52, 52’	7.42	t (7.8)	129.9	C-50, 51, 51’, 52, 52’	51-H, 51’-H, 53-H
53	7.31	tt (7.4, 1.2)	128.1	C-50, 50’	52-H, 52’-H

Preparation of 3j



A mixture of **5j** (170 mg, 1.00 mmol) and triethyl phosphite (332 mg, 2.00 mmol) was stirred at 140 ℃ for 6 h. The reaction mixture was cooled to room temperature and was purified by flash chromatography to give intermediate **4j** (48 mg, 18%).

LC-MS: retention time 1.17 min; observed $[M+H]^+$ m/z =273.0

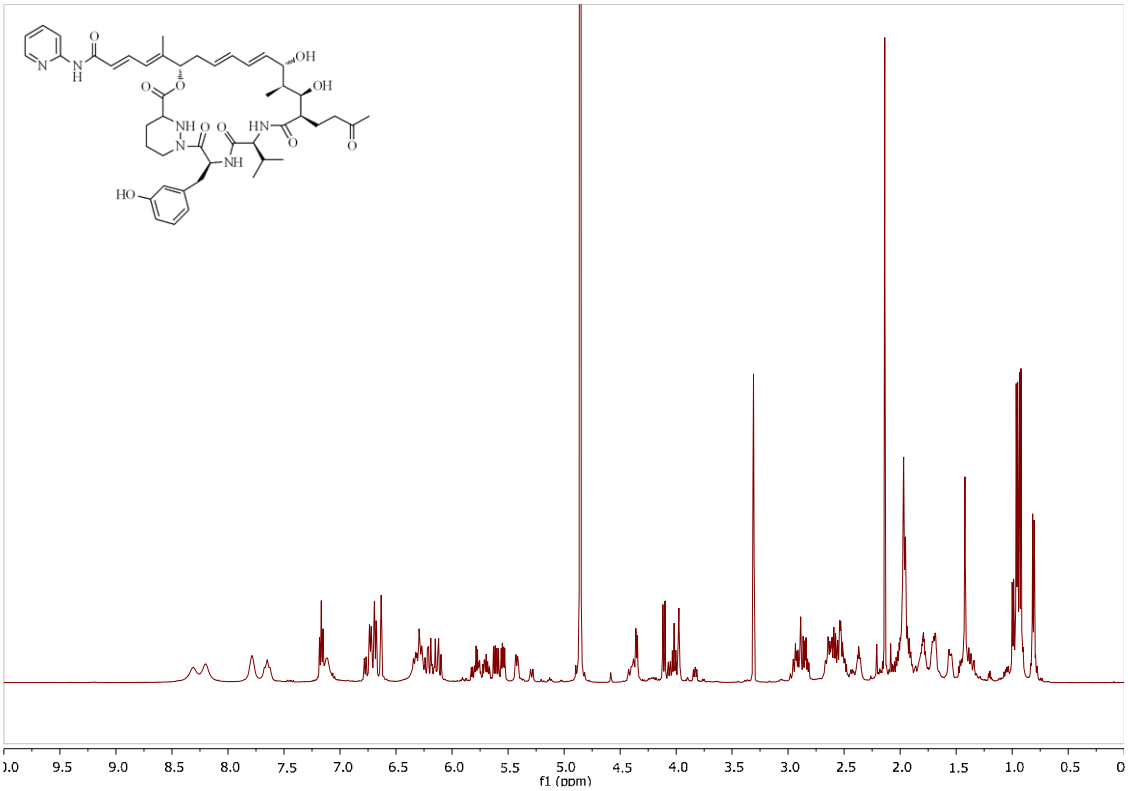
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 9.22 (1H, bs, N*H*), 8.27 (1H, d), 7.56 (2H, d), 8.13 (1H, d), 7.69 (1H, t), 7.03 (1H, t), 4.20 (4H, m, OCH_2), 3.09 (1H, s, C*H*P), 3.05 (1H, s, C*H*P), 1.32 (6H, m, OCH_2CH_3)

To a solution of **4j** (51 mg, 0.188 mmol) in THF (1.0 mL) was added NaH (1.4 mg, 0.0564 mmol) in anhydrous THF (0.2 mL) at rt with stirring. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture was stirred at rt for 3 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried

over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3h** as white solid (19.7 mg, 48.5%).

LC-MS: retention time 10.6 min; observed $[M+H]^+$ m/z = 857.5

HRMS: $[M+H]^+$ $C_{46}H_{61}N_6O_{10}$ calc. 857.4444, obs. 857.4446, Δ 0.26 ppm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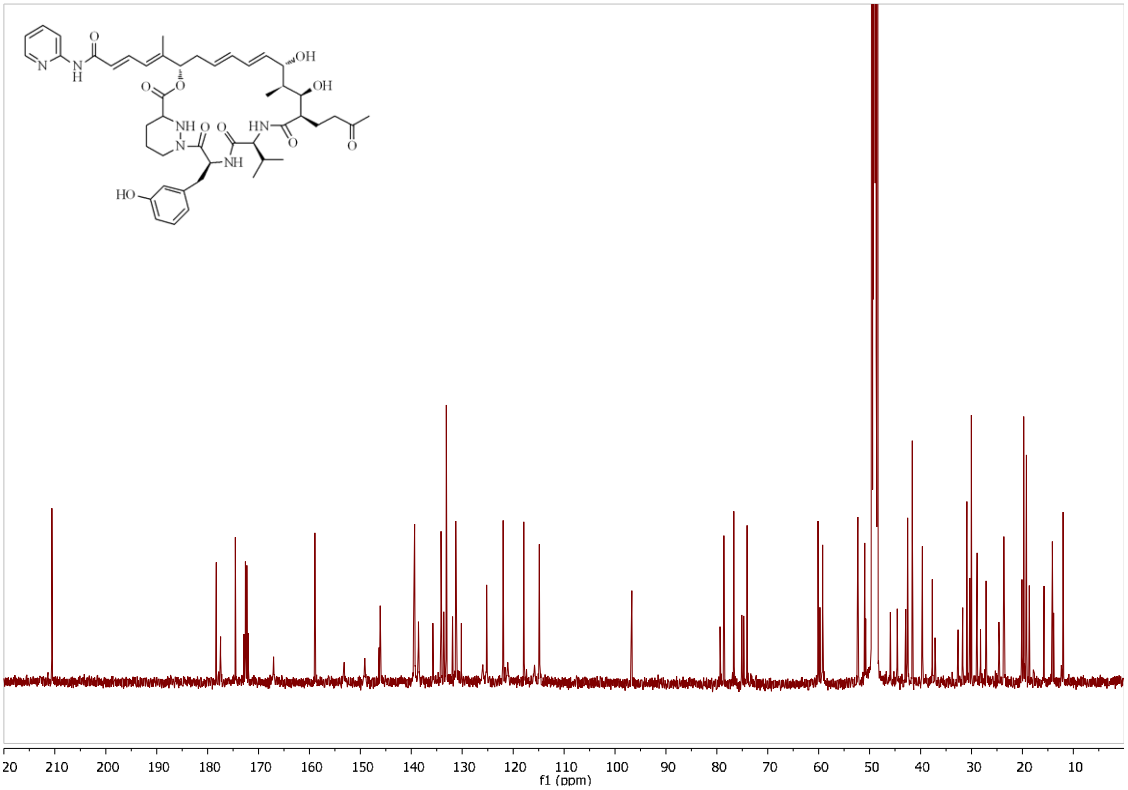
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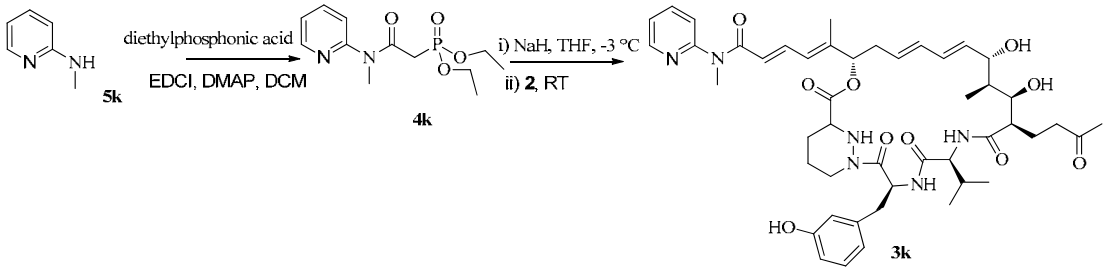
90
80
70
60
50
40
30
20
10
f1 (ppm)

NMR data of **3j** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.3		
2	1.79	m	59.2		3-H_2
3	1.55, 1.37	m x2	28.9		2-H
4	1.68, 1.38	m x2	23.6		5-H_2
5	4.39,2.62	dd (19.7, 11.4), m	42.5		4-H_2
7			174.6		
8	5.55	dd (10.5, 5.4)	60.0	C-7, 29, 30	29-H_2
10			172.6		
11	4.11	d (9.4)	60.1	C-10, 13, 36	36-H
13			178.3		
14	2.37	ddd (9.7, 6.1, 3.1)	52.3	C-13, 39	15-H, 39-H_2
15	3.98	t (3.3)	74.0	C13, 17, 39	14-H, 16-H
16	1.78	m	44.6	C-17	43-H_3
17	4.02	t (7.7)	76.7	C-19, 16, 15	16-H, 18-H
18	5.61	dd (15.0, 7.6)	134.2	C-16, 17, 19	17-H, 19-H
19	6.13	dd (14.9, 10.5)	133.1	C-21, 17	18-H, 20-H
20	6.20	dd (15.1, 10.4)	133.1	C-18, 22	19-H. 21-H
21	5.59	ddd (14.6, 8.3, 5.7)	131.2	C-19, 20, 22	20-H, 22-H_2
22	2.52	m	37.7	C-20, 21, 23	21-H, 23-H
23	5.42	d (9.0))	78.6	C-1, 24, 25	22-H_2
24			146.1		
25	6.27	d (11.4)	125.2	C-23, 44	26-H, 44-H_3
26	7.63	t (12.0)	138.6		25-H, 27-H
27	6.28	m	125.9		26-H
28			167.0		
29	2.90, 2.83	m, dd (12.5, 5.4)	39.7	C-7, 8, 30, 31, 35	8-H
30			139.3		
31	6.63	bs	117.9	C-29, 32, 33, 35	33-H
32			158.9		
33	6.73	dd (8.4, 2.3)	114.9	C-31, 32, 35	31-H, 34-H
34	7.17	t (7.7)	131.3	C-30, 32	33-H, 35-H
35	6.68	d (8.1)	122.0	C-29, 31, 33	34-H
36	1.99	m	30.9	C-11, 37, 38	11-H, 37-H_3, 38-H_3
37	0.96	d (6.5)	19.2	C-11, 36, 38	36-H
38	0.93	d (6.7)	19.7	C-11, 36, 37	36-H
39	1.93	m	27.1	C-14, 40, 41	14-H, 40-H_2
40	2.60, 2.47	m x2	41.6	C-14, 39, 41	39-H_2
41			210.6		
42	2.14	s	30.0	C-40, 41	
43	0.81	d (7.1)	12.0	C-15, 16, 17	16-H

44	1.97	s	14.1	C-23, 24, 25	25-H
46			153.2		
48	8.31	bs	149.1		
49	7.12	bs	121.1		48-H
50	7.79	bs	139.6		49-H, 51-H
51	8.20	bs	115.8		50-H

Preparation of 3k



To a stirred solution of diethylphosphonoacetic acid (200 mg, 1.02 mmol) in CH_2Cl_2 (10 mL) was added EDCI (270 mg, 1.4 mmol), **5k** (100 mg, 0.925 mmol) and DMAP (6 mg, 0.05 mmol). The reaction mixture was stirred at room temperature overnight. The reaction was quenched with aq. NH_4Cl (5 mL) and extracted with CH_2Cl_2 (3 x 30 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by reversed-phase flash chromatography to yield intermediate **4k** (100 mg, 38 %).

LC-MS: retention time 1.05 min; observed $[M+H]^+$ m/z = 287.1

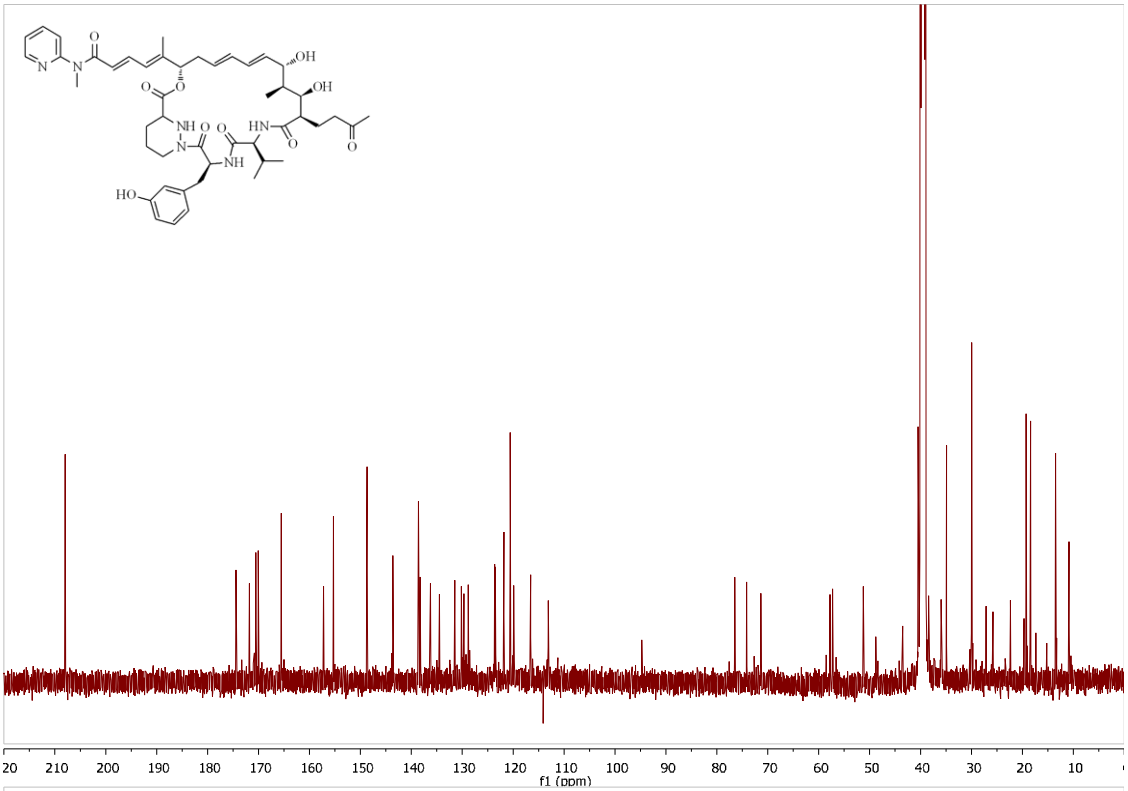
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 8.46 (1H, d), 7.75 (1H, t), 7.40 (1H, b), 7.20 (1H, bt), 4.12 (4H, m, OCH_2), 3.39 (3H, bs, NCH_3), 3.15 (1H, s, C*H*P), 3.10 (1H, s, C*H*P), 1.28 (6H, m, OCH_2CH_3)

To a solution of **4k** (100 mg, 0.352 mmol) in THF (5.0 mL) was added NaH (2.6 mg, 0.106 mmol) in anhydrous THF (0.2 mL) at 0℃ with stirring. The solution was then stirred at 20℃ until it became clear. Then **2** (65 mg, 0.088 mmol) was added to the clear solution and the mixture stirred at 20 °C for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl

acetate (3 x 40 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and evaporated. The residue was purified by preparative HPLC to obtained of **3k** as a white solid (26 mg, 34%).

LC-MS: retention time 9.5 min; observed $[M+H]^+$ m/z = 871.3

HRMS: $[M+H]^+$ $C_{47}H_{63}N_6O_{10}$ calc. 871.4606, obs. 871.4612, Δ 0.7 ppm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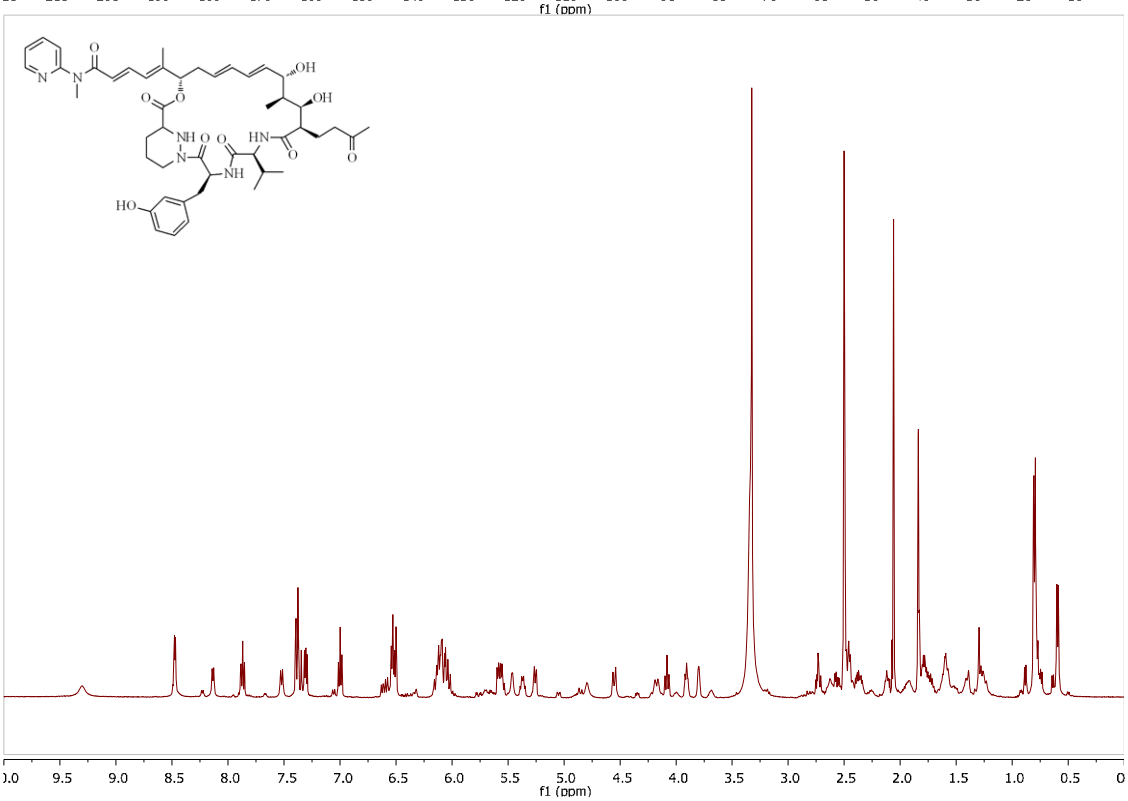
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HO
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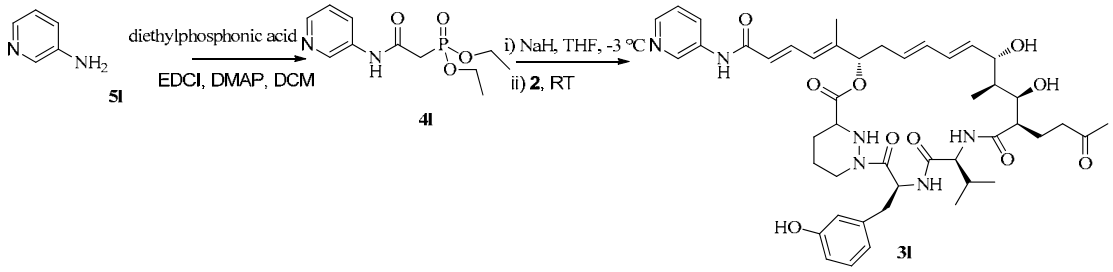
f1 (ppm)

NMR data of **3k** in d_6-DMSO at 500 MHz (^{1}H NMR) & 126 MHz (^{13}C NMR)

Position	δH ppm	Multiplicity, Hz	δC ppm	HMBC correlation (H → C)	COSY correlation
1			170.5		
2	1.91		57.8		N6'-H
3	1.43, 1.28		27.1		
4	1.62, 1.27		22.3		
5	4.16, 2.64		40.3		
6'-NH	4.54	d (11.7)			C2-H
7			171.8		
8	5.36		48.8		C29-H, N9-H
9-NH	8.12	d (6.9)			C8-H
10			170.0		
11	4.07	t	57.2	C10, C13	N12-H
12-NH	7.51	d (8.8)			C11-H
13			174.4		
14	2.10		51.2		C15-H
15	3.79		71.3		C14-H, C16-H
15-OH	5.45				
16	1.58		43.5		C15-H, C17-H
17	3.90		74.1		C16-H, C18-H
17-OH	4.79				
18	5.56		134.5		C17-H, C19-H
19	6.04		130.1		C18-H
20	6.10		131.4		
21	5.54		128.8		
22	2.44		36.0		
23	5.24		76.5	C24, C25	
24			143.6		
25	6.09		123.6		
26	7.36		136.2	C28, C24	C27-H
27	6.07		123.5	C28	C26-H
29	2.72, 2.57		38.4		C8-H
30			138.2		
31	6.49	s	116.6		
32			157.2		
32-OH	9.29	bs			
33	6.51		113.1		
34	6.99		129.6	C32, C30	
35	6.51		119.9		
36	1.76		30.0		
37	0.79	d	19.3		
38	0.79	d	18.4		
39	1.69, 1.82		25.8		
40	2.45, 2.36		40.5		
41			208.0		

42	2.05	s	29.9	C41, C40	
43	0.58	d (7.0)	10.8	C16, C14, C15	
44	1.83	s	13.46	C24, C23, C25	
45			165.5		
45-Me	3.31	s	34.9	C28, C46	
46			155.3		
48	8.46		148.7		C49-H
49	7.29		121.8		C48-H, C50-H
50	7.86		138.6		C49-H, C51-H
51	7.37		120.6		C50-H

Preparation of 3l



To a stirred solution of diethylphosphonoacetic acid (0.98 g, 5.0 mmol) in CH_2Cl_2 (10 mL) was added EDCI (1.34 g, 7 mmol), **5l** (0.5 g, 5.5 mmol) and DMAP (30 mg, 0.25 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with aq. NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 x 30 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography to give intermediate **4l** (160 mg, 10 %).

LC-MS: retention time 1.16 min; observed $[M+H]^+$ m/z = 273.1

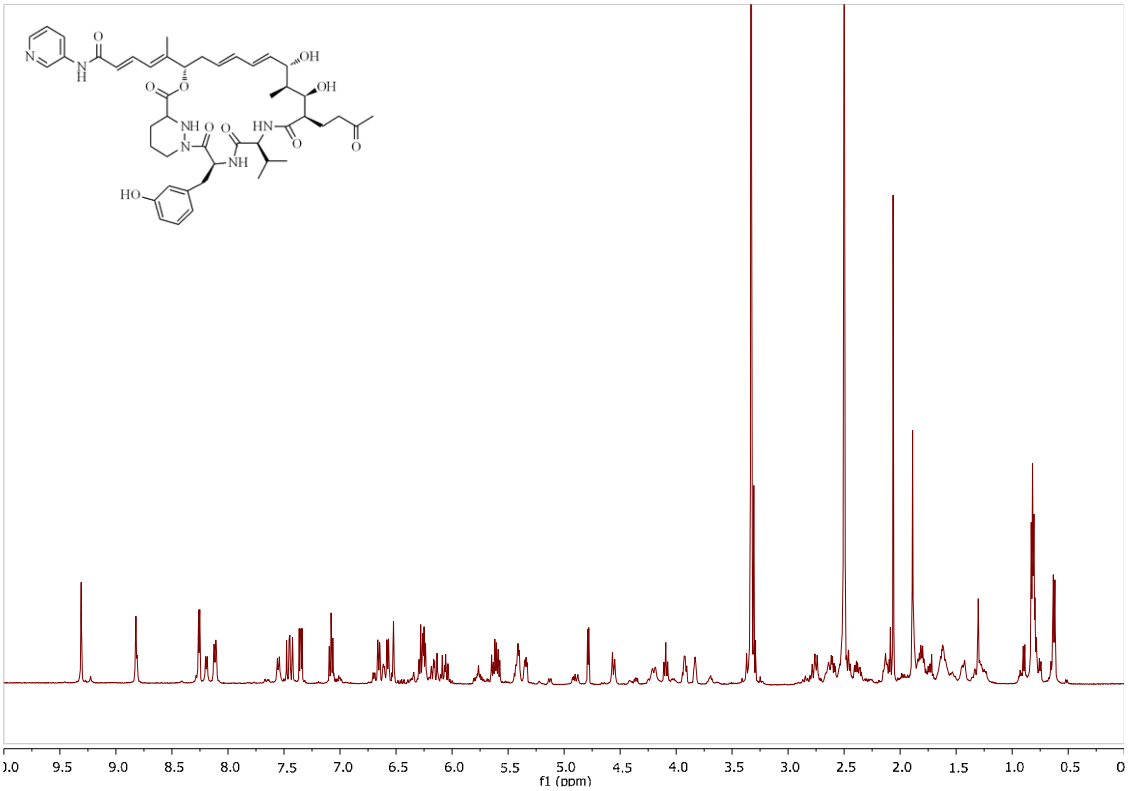
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 9.56 (1H, bs, N*H*), 8.56 (1H, d), 8.25 (1H, d), 8.02 (1H, d), 7.14 (1H, dd), 4.20 (4H, m, OCH_2), 3.10 (1H, s, C*H*P), 3.06 (1H, s, C*H*P), 1.37 (6H, m, OCH_2CH_3)

To a solution of **4l** (52 mg, 0.188 mmol) in THF (2.0 mL) was added NaH (1.4 mg, 0.0564 mmol) in anhydrous THF (0.2 mL) at 0 ℃ with stirring. The solution was then stirred at 20 ℃ until it became clear. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture

stirred at 20 °C for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered, evaporated. The residue was purified by preparative HPLC to yield **3l** as a white solid (7 mg, 17%).

LC-MS: retention time 9.5 min; observed $[M+H]^+$ m/z = 857.4

HRMS: $[M+H]^+$ $C_{46}H_{61}N_6O_{10}$ calc. 857.4449, obs. 857.4485, Δ 4.2 ppm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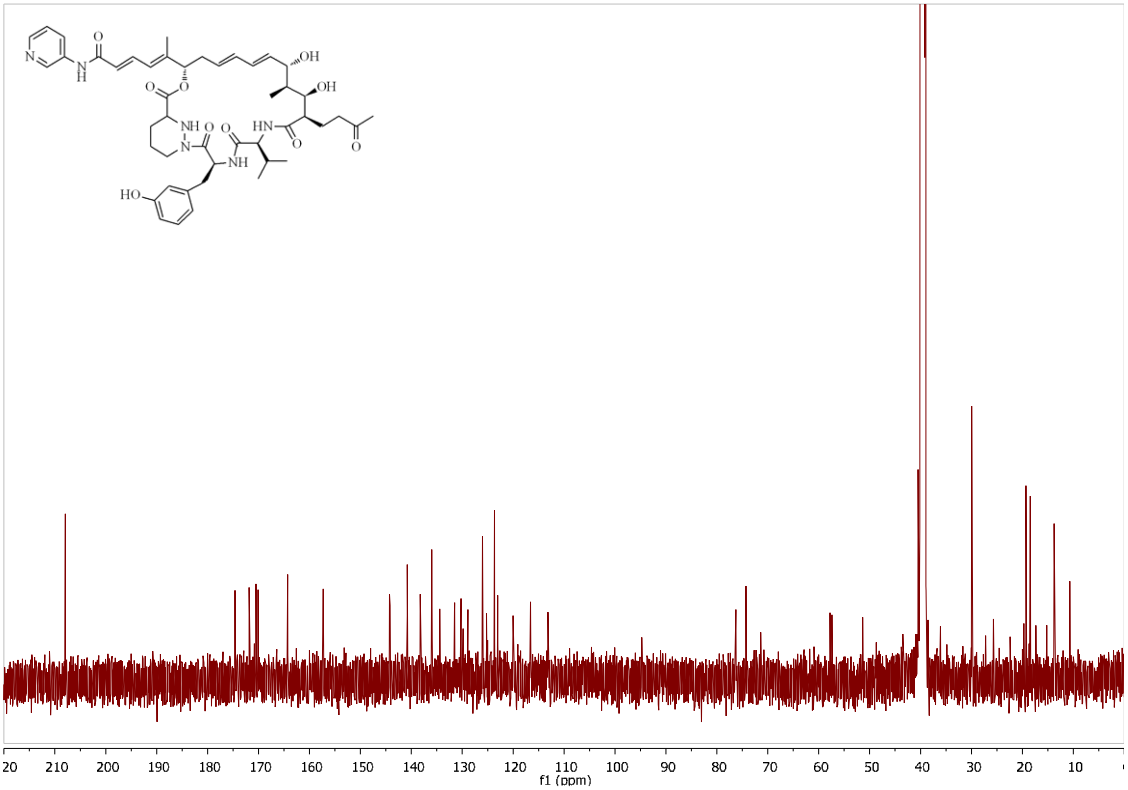
f1 (ppm)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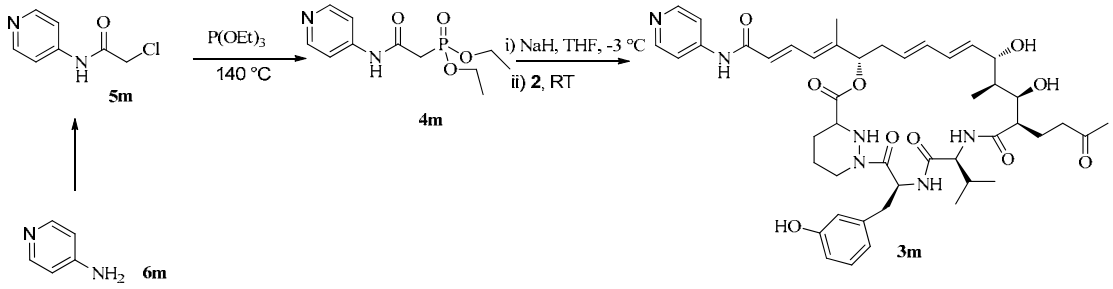
HN
NH
HO
f1 (ppm)

NMR data of **3l** in d_6-DMSO at 500 MHz (^{1}H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			170.5		
2	1.83	m	57.8		N6'-H
3	1.28, 1.43		27.2		
4	1.26, 1.59		22.4		
5	2.64, 4.18		40.3		
6'-NH	4.55	d (11.1)			C2-H
7			171.8		
8	5.41		48.9		C29-H, N9-H
9-NH	8.18	d		C10	C8-H
10			170.1		
11	4.08	t	57.4	C10, 13	N12-H
12-NH	7.53	d (8.9)		C13, 39	C11-H
13			174.6		
14	2.13	m			C15-H
15	3.82	m	71.4		C14-H
15-OH	5.40	d (5.5)			
16	1.61	m	43.4		C17-H
17	3.91	m	74.3		C16-H, C18-H
17-OH	4.77	d (4.5)			
18	5.59		134.4		C17-H
19	6.05		130.2		
20	6.15		131.5		
21	5.61		128.9		
22	2.49	m	36.1		
23	5.53	m	76.2	C22, C24, C1	
24			144.1		
25	6.24		123.0		
26	7.44	m (14.9, 11.6)	136.0	C28, C24	
27	6.26	d (14.9)	125.2	C28	
28			164.3		
29	2.73, 2.59	m	38.5		C8-H
30			138.4		
31	6.51	s	116.6		
32			157.3		
32-OH	9.30	s		C31, C32, C33	
33	6.64	dd (8.2, 1.4)	113.2		
34	7.07	m	129.8	C32, C30	
35	6.56	d (8.4)	120.0		
36	1.78		29.9		
37	0.81	d	18.4		
38	0.80	d	19.3		
39	1.71, 1.81		25.7		
40	2.47, 2.38		40.5		

41			207.9		
42	2.05	s	29.9	C41, C40	
43	0.62	d (7.0)	10.7		
44	1.88		13.8	C24, C23, C25	
N-45	10.38			C28, C47, C51	
46			136.0		
47	8.81		140.8	C49, C51, C46	
48					
49	8.25	dd (4.7, 1.4)	144.3		
50	7.34	m (8.2, 4.7)	123.6	C46	
51	8.10	m (8.2, 1.4)	126.0		

Preparation of 3m



To a solution of **6m** (3 g, 30 mmol), Et_3N (4.5 mL, 33 mmol) in dry DCM (60 mL) was added dropwise chloroacetyl chloride (2.7 mL, 33 mmol). The reaction mixture was stirred at 0 ^{o}C for 3 hours, poured into ice water; DCM was removed under reduced pressure. The residue was purified by reversed-phase flash chromatography to yield **5m** (2.7 g, 50%)

LC-MS: retention time 1.38 min; observed $[M+H]^+$ m/z = 171.1

To a solution of **5m** (200 mg, 1.17 mmol) in DMF (10 mL) was added triethyl phosphite (389 mg, 2.34 mmol), the reaction mixture was stirred at 130^{o}C overnight. The reaction mixture was cooled to room temperature and purified by reversed-phase flash chromatography to yield intermediate **4m** (20 mg, 10 %).

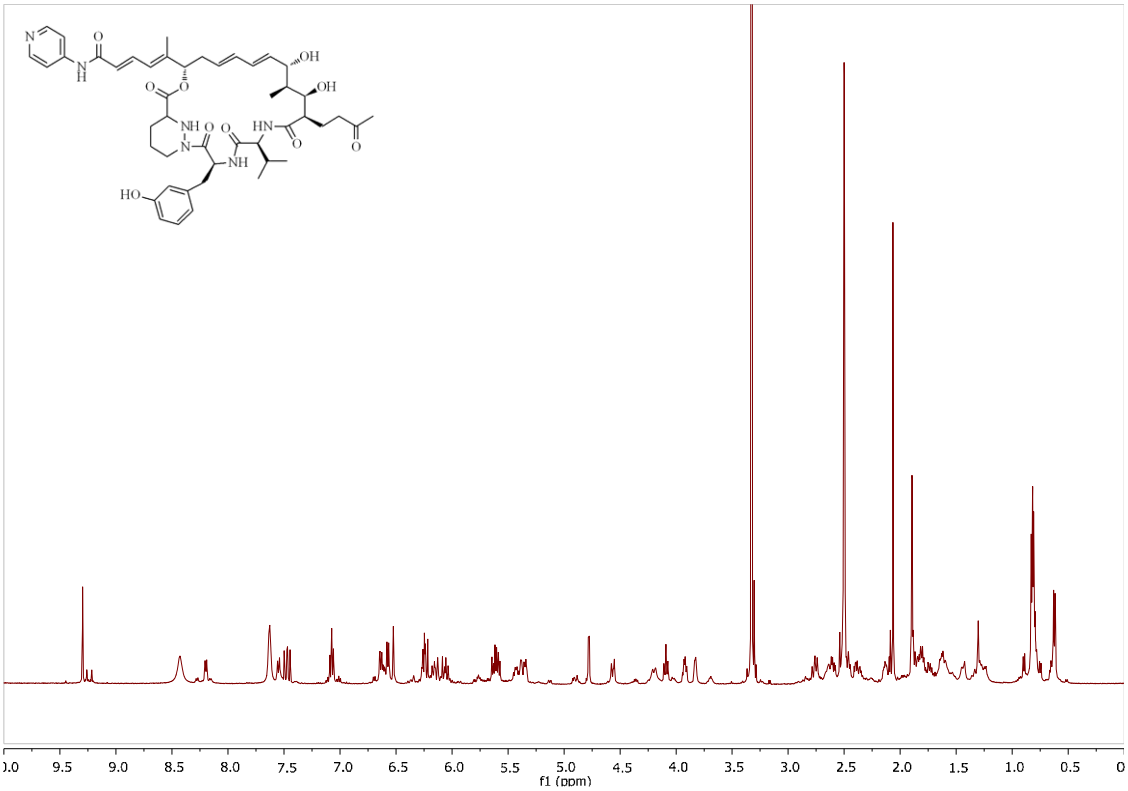
LC-MS: retention time 0.85 min; observed $[M+H]^+$ m/z = 273.1

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 8.66 (1H, bs, N*H*), 8.43 (2H, b), 7.47 (2H, d), 7.20 (1H, bt), 4.23 (4H, m, OCH_2), 3.11 (1H, s, C*H*P), 3.06 (1H, s, C*H*P), 1.37 (6H, m, OCH_2CH_3)

To a solution of **4m** (52 mg, 0.188 mmol) in THF (2.0 mL) was added NaH (1.4 mg, 0.0564 mmol) in anhydrous THF (0.2 mL) at 0℃ with stirring. The solution was then stirred at 20℃ until it became clear. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture stirred at 20℃ for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered, evaporated. The residue was purified by preparative HPLC to yield **3m** as a white solid (7 mg, 17%).

LC-MS: retention time 9.7 min; observed $[M+H]^+$ m/z = 857.3

HRMS: $[M+H]^+$ $C_{46}H_{61}N_6O_{10}$ calc. 857.4449, obs. 857.4464, Δ 1.7 ppm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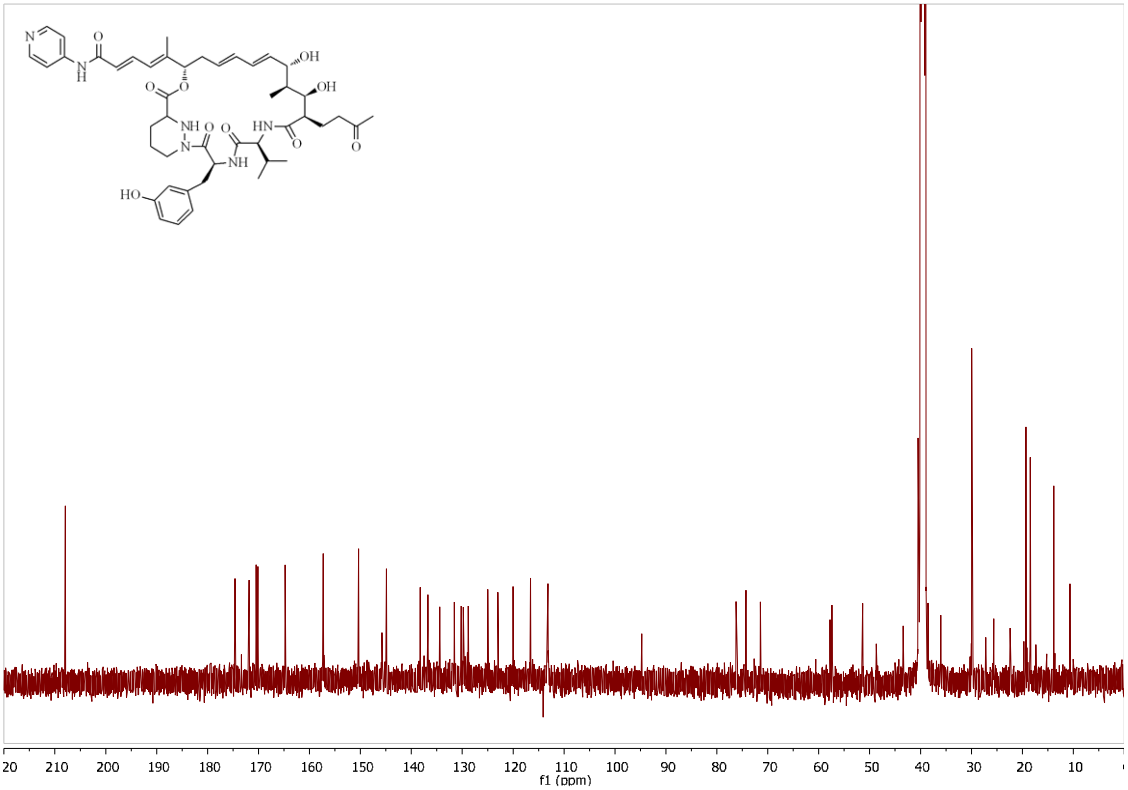
f1 (ppm)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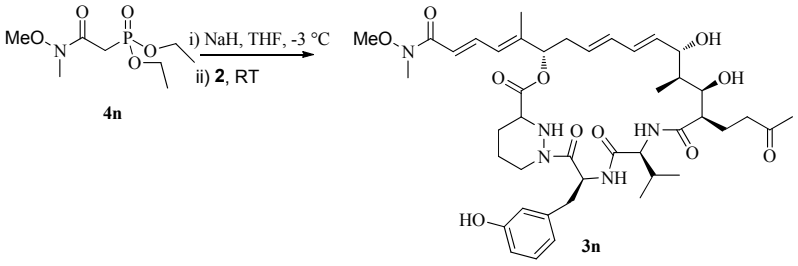
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

NMR data of **3m** in d_6-DMSO at 500 MHz (^{1}H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			170.5		
2	1.83	m	57.8		N6'-H
3	1.28, 1.43		27.2		
4	1.62, 1.27		22.4		
5	2.64, 4.18		40.3		
6'-NH	4.55	d (11.4)			C2-H
7			171.8		
8	5.41		48.9	C29	N9-H
9-NH	8.18	d		C10	C8-H
10			170.1	C10, 13, 36	
11	4.08	t	57.3	C10, C13	N12-H
12-NH	7.54	d (8.9)		C13, 39	C11
13			174.6		
14	2.12	m	51.4		C15-H
15	3.82	m	71.4		C14-H, C16-H
15-OH	5.37	d (5.1)			
16	1.62	m	43.4		C15-H, C17-H
17	3.91	m	74.3		C16-H, C18-H
17-OH	4.77	d (4.8)			
18	5.59		134.4		C17-H
19	6.05		130.2		
20	6.15		131.5		
21	5.61		128.9		
22	2.49	m	36.0		
23	5.33	m	76.2	C22, C24, C1, C25	
24			144.9		
25	6.24		123.0		
26	7.46	m (14.7, 11.5)	136.7	C24, C28	
27	6.22	d (14.7)	125.0	C28	
28			164.7		
29	2.73, 2.59	m	38.5		
30			138.4		
31	6.51	s	116.6		
32			157.3		
32-OH	9.29	s		C31, C33	
33	6.64	dd	113.2		
34	7.06	m	129.8	C32, C30	
35	6.56	d (7.6)	120.0		
36	1.78		29.9		
37	0.81	d	18.4		
38	0.80	d	19.3		
39	1.69, 1.82		25.6		
40	2.47, 2.38		40.5		

41			207.9		
42	2.05	s	29.9	C41, C40	
43	0.61	d (7.0)	10.6	C16, C15, C17	
44	1.88	s	13.8	C23, C24, C25	
N-45	10.48	s			
46			145.7		
47	7.62	b	113.2		C48-H
48	8.42	b	150.4		C47-H
49					
50	8.42		150.4		
51	7.62		113.2		

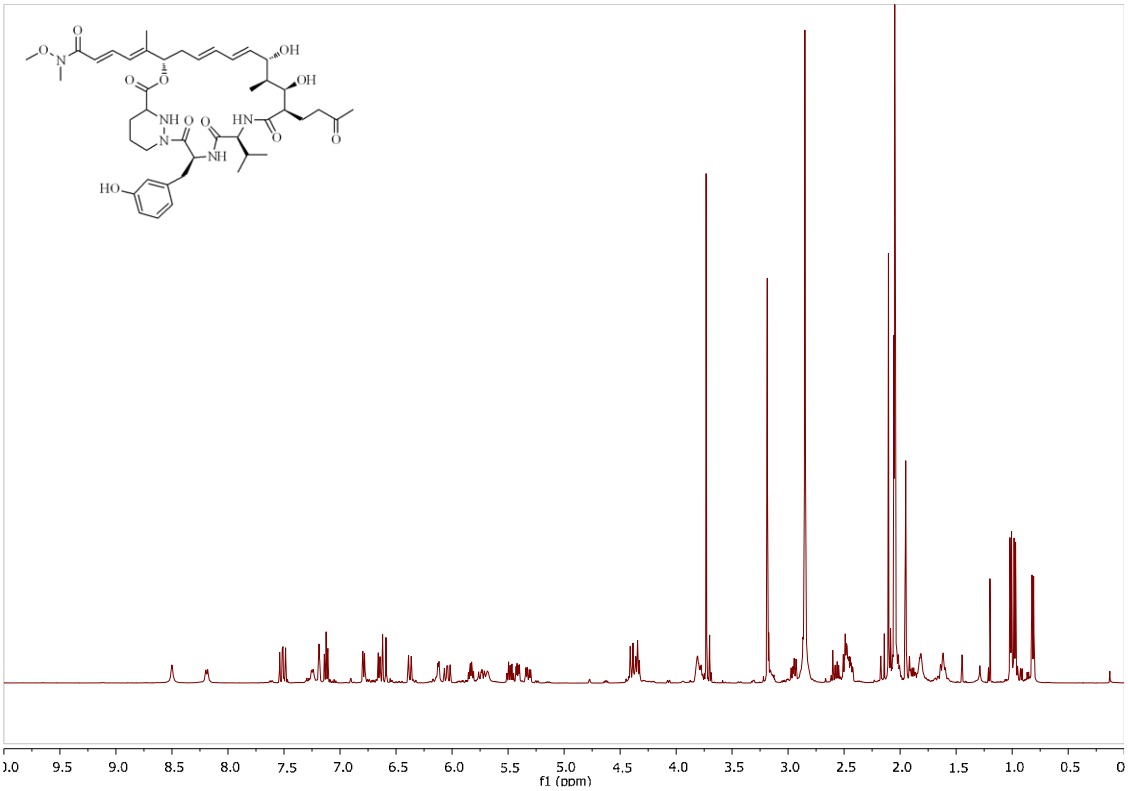
Preparation of 3n

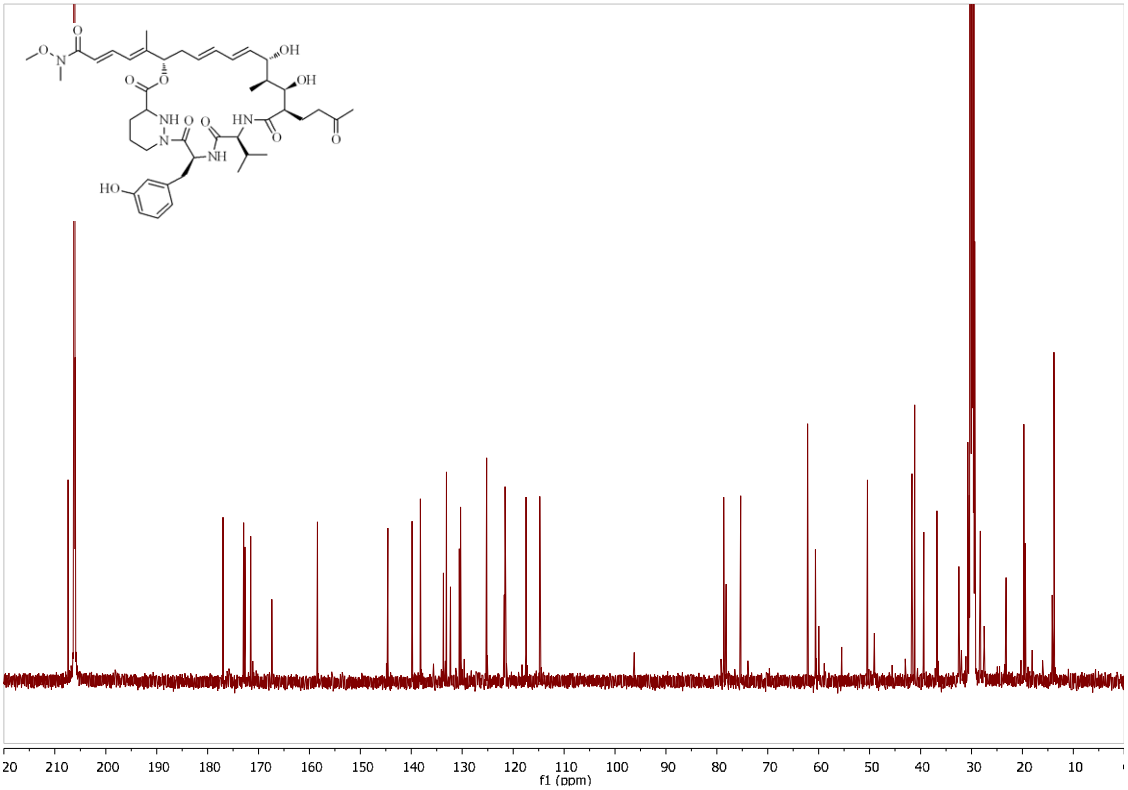


To a suspension of NaH (1.950 mg, 0.081 mmol) in anhydrous THF (0.6 ml) was added dropwise a solution of diethyl 2-(methoxy(methyl)amino)-2-oxoethylphosphonate (**4n**, 19.4 mg, 0.081 mmol) in anhydrous THF (0.2 ml) under N_2 atmosphere at -3 ^{o}C with stirring. The solution was then stirred at 20^{o}C until it became clear. A solution of **2** (30 mg, 0.040 mmol) in anhydrous THF (0.2 ml) was added dropwise to the clear solution and the mixture stirred at 20^{o}C for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative-HPLC to give **3n** as white solid powder (15.8 mg, 47 %).

LC-MS: retention time 10.2 min; observed $[M+Na]^+$ m/z = 847.0

HRMS: $[M+H]^+$ $C_{43}H_{62}N_5O_{10}$ calc. 824.4446, obs. 824.4447, Δ 0.1 ppm


f1 (ppm)

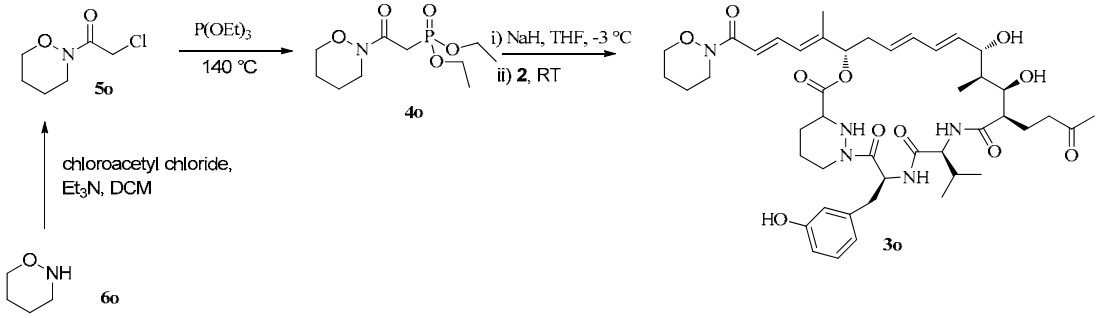

f1 (ppm)

NMR data of **3n** in $(CD_3)_2CO$ at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			171.5		
2	3.18	m	60.0		3-H_2, 6'-NH
3	1.84, 1.64	m x2	28.3		2-H
4	1.83, 1.61	m x2	23.2		5-H_2
5	4.38, 2.58	m x2	41.7		4-H_2
6'-NH	4.40	d (12)			
7			172.7		
8	5.83	td (8.4, 5.4)	50.4	C-7, 29, 30	29-H_2, 9-NH
9-NH	8.19	d (8.0)			8-H
10			172.9		
11	4.34	t (8.0)	60.6	C-10, 13, 36, 37, 38	36-H, 12-NH
12-NH	7.25	d (8.1)			11-H
13			177.0		
14	2.43	m	49.1	C-13, 39, 40	15-H, 39-H_2
15	3.81	m	78.2		14-H, 15-OH
15-OH	6.12	d (5)			15-H
16	2.03	m	41.7		43-H_3
17	3.79	m	75.3		16-H, 18-H, 17-OH
17-OH	5.68	bs			17-H
18	5.32	dd (15.2, 5.5)	133.7	C-17, 20	17-H, 19-H
19	5.74	dd (15.0, 11.0)	132.3	C-17	18-H, 20-H
20	6.04	dd (15.2, 10.5)	133.1	C-19, 22	19-H, 21-H
21	5.48	dt (15.5, 7.0)	130.6	C-19, 22, 23	20-H, 22-H_2
22	2.49, 2.46	m x2	36.8	C-20, 21, 23	21-H, 23-H
23	5.42	dd (10.0, 4.5)	78.7	C-1, 22, 24, 25	22-H_2
24			144.6		
25	6.38	d (11.5)	125.2	C-23, 26, 27, 44	26-H, 44-H_3
26	7.51	dd (15.0, 11.5)	138.2	C-24, 25, 27, 28	25-H, 27-H
27	6.62	d (14.5)	121.6	C-25, 28	26-H
28			167.4		
29	2.96, 2.83	dd (13.8, 8.2), m	39.4	C-7, 8, 30, 31, 35	8-H
30			139.9		
31	7.19	bs	117.5	C-29, 32, 33, 35	33-H
32			158.4		
33	6.66	dd (7.0, 2.0)	114.8	C-31, 32, 35	31-H, 34-H
34	7.12	t (7.8)	130.3	C-29, 30, 32	33-H, 35-H
35	6.79	d (7.0)	121.7	C-29, 31, 33	34-H
36	2.03	m	31.0		11-H, 37-H_3, 38-H_3
37	1.01	d (7.0)	19.4	C-11, 36, 38	36-H
38	0.98	d (7.0)	19.7	C-11, 36, 37	36-H
39	1.89	m	27.5	C-14, 40, 41	14-H, 40-H_2
40	2.56, 2.43	m x2	41.1	C-39, 41	39-H_2

41			207.4		
42	2.10	s	30.7	C-40, 41	
43	0.81	d (7.5)	13.8	C-15, 16, 17	16-H
44	1.95	d (1.1)	14.2	C-23, 24, 25	25-H
46	3.19	s	32.5	C-28	
48	3.73	s	62.2		

Preparation of 3o



To a solution of **6o** (100 mg, 0.81 mmol), Et_3N (246 mg, 2.43 mmol) in dry DCM (5 mL) was added dropwise chloroacetyl chloride (138 mg, 1.22 mmol). The reaction mixture was stirred at room temperature for 3 hours, poured into ice water, and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, filtered, concentrated in vacuo. The residue was used to the next step without any further purification. (123 mg, 90% yield).

LC-MS: retention time 1.04 min; observed $[M+H]^+$ m/z = 166.1

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 4.18 (2H, s, $COCH_2Cl$), 4.00 (2H, m), 3.75 (2H, m), 7.20 (1H, bt), 1.82 (2H, m), 1.73 (2H, m)

A mixture of crude **5o** (123 mg, 0.75 mmol) and triethyl phosphite (250 mg, 1.50 mmol) was stirred at 140 ℃ for 6 hours. The reaction mixture was cooled to room temperature and was purified by flash chromatography to give intermediate **4o** (70 mg, 36%).

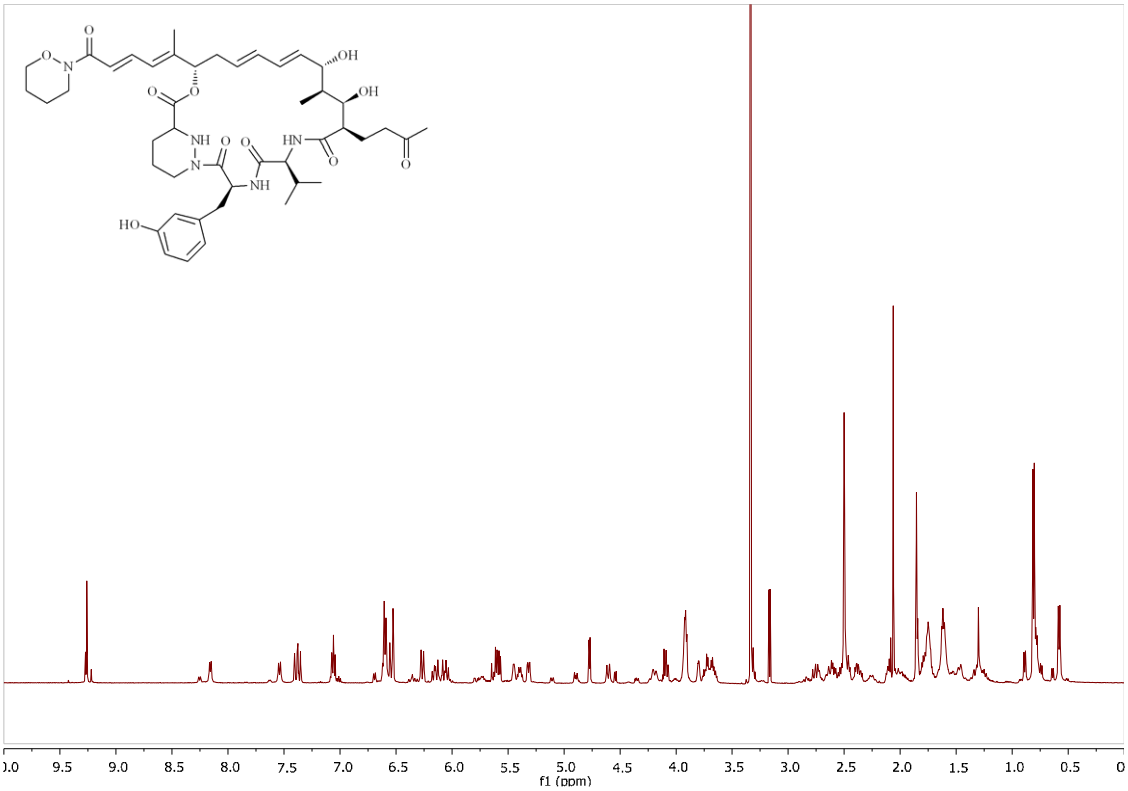
LC-MS: retention time 1.32 min; observed $[M+H]^+$ m/z = 266.2

NMR δ_H/ppm ($CDCl_3$, 500 MHz): 4.17 (4H, m, POC*H*$_2$), 4.07 (2H, t, NOC*H*$_2$), 3.78 (2H, t, NC*H*$_2$)3.17 (1H, s, C*H*P), 3.13 (1H, s, C*H*P), 1.80 (2H, m), 1.72 (2H, m), 1.33 (6H, t, OCH_2CH_3)

To a solution of **4o** (75mg, 0.282 mmol) in THF (0.2 mL) was added NaH (6.8mg, 0.282 mmol) in anhydrous THF (0.6 mL) at 0 ^oC with stirring. The solution was then stirred at 20 ^oC until it became clear. Then **2** (70mg, 0.094 mmol) was added to the clear solution and the mixture stirred at 20 ^oC for 2 h. The mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and evaporated. The residue was purified by preparative-HPLC to obtained **3o** as a white solid (44.5 mg, yield 47%).

LC-MS: retention time 13.0 min; observed $[M+Na]^+$ m/z = 872.5

HRMS: $[M+H]^+$ $C_{45}H_{64}N_5O_{10}$ calc. 850.4602, obs. 850.4577, Δ 2.9 ppm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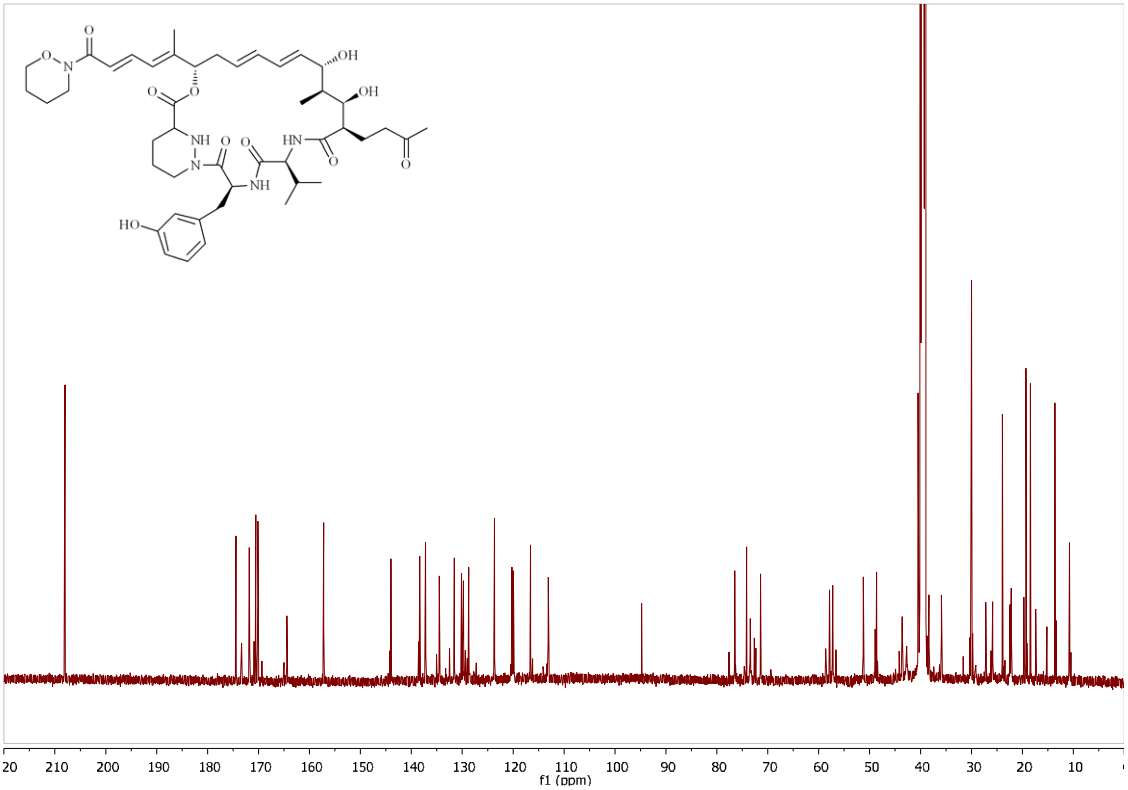
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

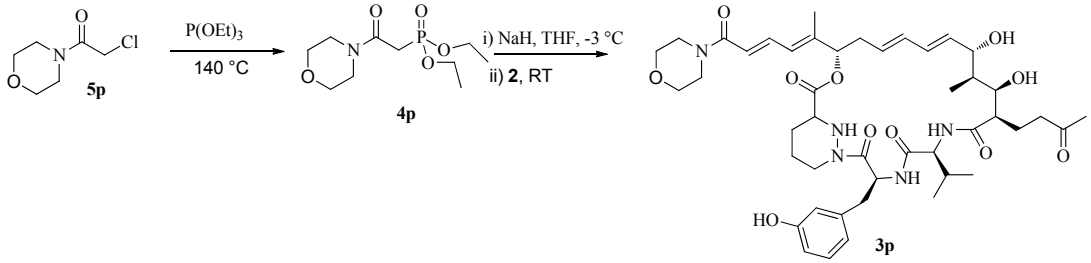

OH
OH
NH
HN
NH
HO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

NMR data of **3o** in d_6-DMSO at 500 MHz (^{1}H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			170.5		
2	2.01	m (11.6)	57.8		6'-NH
3	1.28, 1.47		27.2		
4	1.26, 1.64		22.4		
5	2.66, 4.20		40.4		
6'-NH	4.60	d (11.6)		C7	C2-H
7			171.8		
8	5.39		48.9		N9-H
9-NH	8.15	d (7.4)		C10	C8-H
10			170.1	C10, C13	N12-H
11	4.09	m	57.2		
12-NH	7.53	d (8.8)			C10-H
13			174.4		
14	2.10		51.2		C15-H
15	3.80		71.4		C14-H
15-OH	5.44	d (5.0)			
16	1.61		43.6		
17	3.92		74.2		
17-OH	4.76	d (4.7)			
18	5.58		134.5		
19	6.06		130.1		
20	6.15		131.5		
21	5.59		128.7		
22	2.49		35.9		
23	5.31		76.5	C1	
24			144.0		
25	6.25	d (11.6)	123.6		
26	7.38	m (14.7, 11.6)	137.2	C28	
27	6.54	d	120.2	C28	
28			164.4		
29	2.73, 2.59		38.3		
30			138.3		
31	6.53	s	116.6		
32			157.2		
32-OH	9.25	s		C31, C33	
33	6.60		113.1		
34	7.05		129.8		
35	6.60		119.9		
36	1.77		30.1		
37	0.80	d	18.4		
38	0.80	d	19.3		
39	1.73, 1.82		25.8		

40	2.38, 2.47		40.5		
41			208.0		
42	2.05	s	30.0		
43	0.57	d (7.0)	10.7		
44	1.85	s	13.6		
46	3.66, 3.74		42.7		
47	1.62		23.9		
48	1.74		22.2		
49	3.91		73.4		

Preparation of 3p



A mixture of 2-chloro-1-morpholinoethanone **5p** (327 mg, 2 mmol) and triethyl phosphite (665 mg, 4 mmol) was stirred at 140°C overnight. The reaction mixture was cooled to room temperature and was purified by flash chromatography to give intermediate **4p** as a colourless oil (190 mg, 36%).

LC-MS: retention time 1.08min; observed $[M+H]^+$ m/z = 266.0

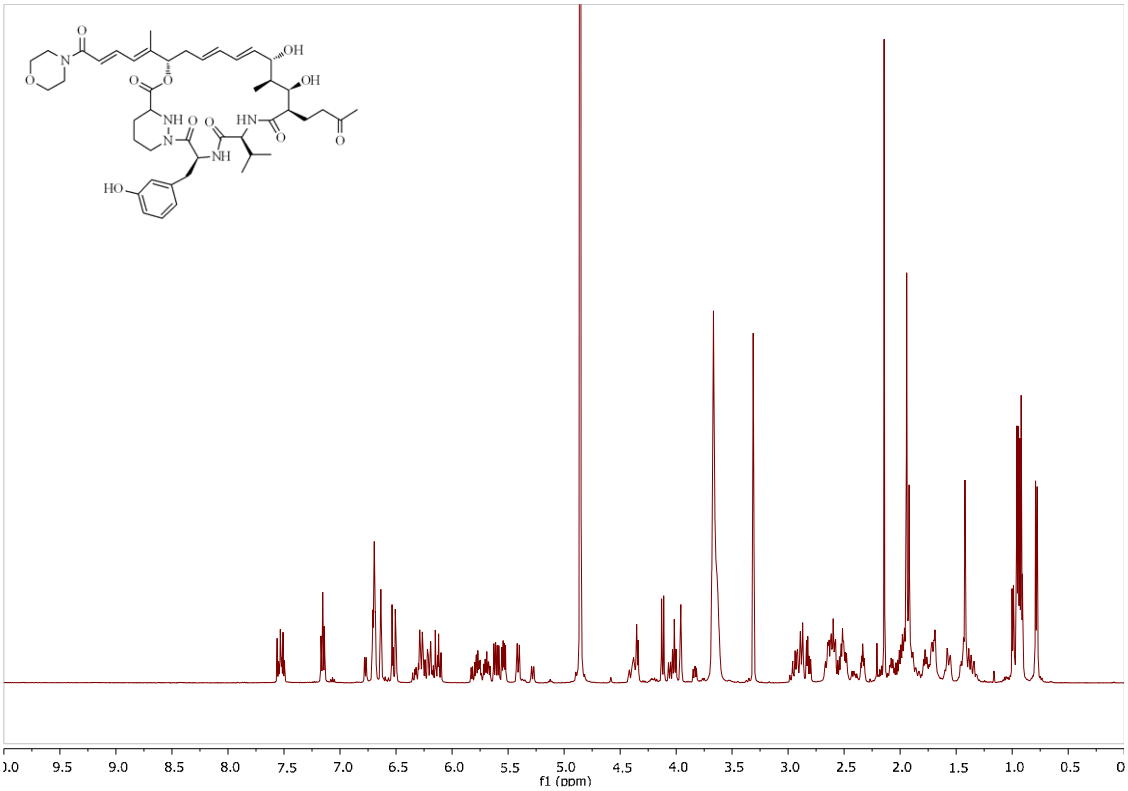
NMR δ_H/ppm ($CDCl_3$, 500 MHz): 4.12 (4H, m, POC*H*$_2$), 3.67 (2H, m), 3.62 (2H, m) 3.58 (2H, m), 3.53 (2H, m), 3.02 (1H, s, C*H*P), 2.98 (1H, s, C*H*P), 1.29 (6H, t, OCH_2CH_3)

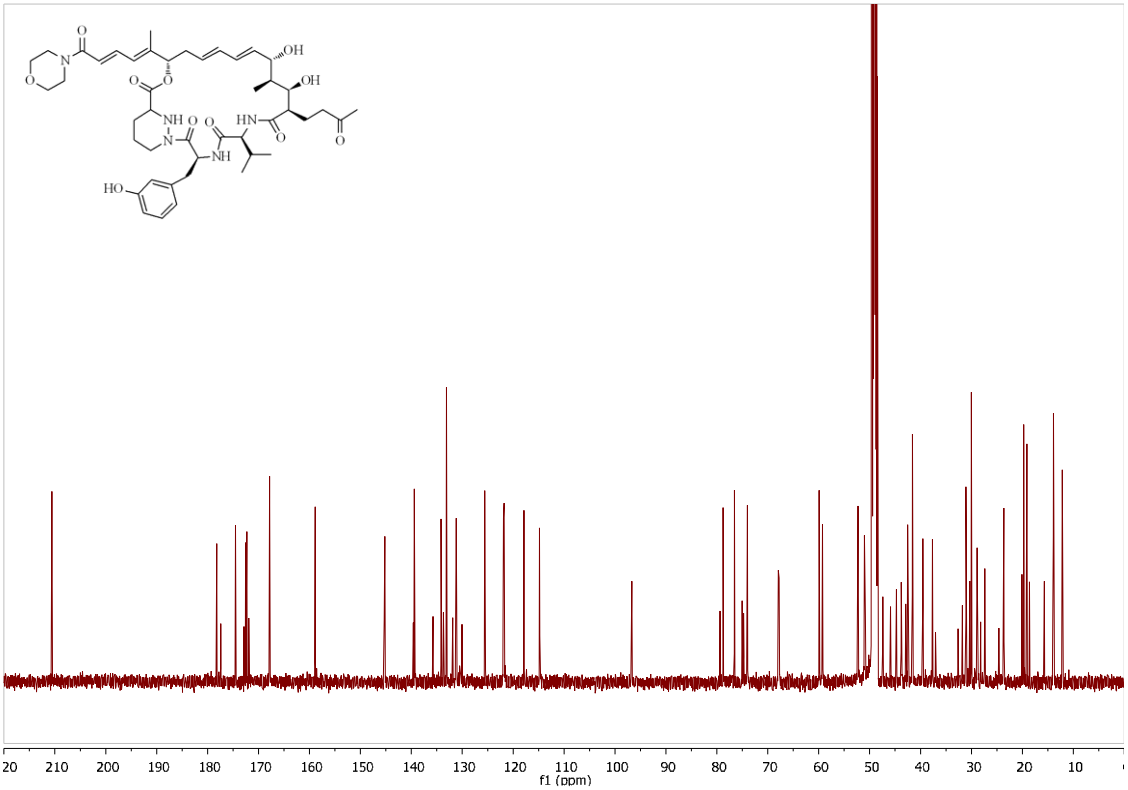
To a solution of **4p** (50 mg, 0.188 mmol) in THF (1.0 mL) was added NaH (1.4 mg, 0.056 mmol) in anhydrous THF (0.2 mL) at 0 °C with stirring. The solution was then stirred at room temperature until it became clear. Then **2** (35 mg, 0.047 mmol) was added to the clear solution and the mixture stirred at room temperature for 3 h. The mixture was quenched with water (10 mL) and

extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine and dried over Na_2SO_4, filtered and reduced *in vacuo*. The residue was purified by preparative HPLC to obtain **3p** as white solid (14 mg, 35%).

LC-MS: retention time 9.5 min; observed $[M+H]^+$ m/z = 850.5

HRMS: $[M+H]^+$ $C_{45}H_{64}N_5O_{11}$ calc. 850.4602, obs. 850.4601, Δ 0.1 ppm


f1 (ppm)


f1 (ppm)

NMR data of **3p** in CD_3OD at 500 MHz (^1H NMR) & 126 MHz (^{13}C NMR)

Position	δ_H ppm	Multiplicity, Hz	δ_C ppm	HMBC correlation (H → C)	COSY correlation
1			172.3		
2	1.90	m	59.3		3-H_2
3	1.56, 1.36	m x2	28.9		2-H
4	1.70, 1.38	m x2	23.65		5-H_2
5	4.38, 2.64	dd (22.8, 12.7), m	42.5		4-H_2
7			174.5		
8	5.54	dd (10.3, 5.5)	51.0	C-7, 29	29-H_2
10			172.5		
11	4.12	d (9.3)	59.9	C-10, 13, 36	36-H
13			178.2		
14	2.33	ddd (9.2, 6.1, 2.7)	52.3	C-13, 39	15-H, 39-H_2
15	3.96	t (3.1)	74.0	C13, 14, 16, 17, 39, 43	14-H, 16-H
16	1.77	m	44.8	C-17	15-H, 43-H_3
17	4.01	t (7.6)	76.6	C-19, 16, 15, 43	16-H, 18-H
18	5.60	dd (15.0, 7.5)	134.2	C-17, 19	17-H, 19-H
19	6.13	dd (14.9, 10.1)	133.1	C-21, 17	18-H, 20-H
20	6.20	m	133.1	C-18, 22	19-H. 21-H
21	5.69	ddd (14.7, 8.4, 5.6)	131.2	C-19, 20, 22	20-H, 22-H_2
22	2.60, 2.48	m x2	37.6	C-20, 21, 23	21-H, 23-H
23	5.41	dd (9.8, 2.7)	78.8	C-1, 21, 22, 24, 25	22-H_2
24			145.2		
25	6.28	d (11.9)	125.6	C-23, 26, 27, 44	26-H, 44-H_3
26	7.53	dd (14.5, 11.4)	139.4	C-28, 24, 25, 27	25-H, 27-H
27	6.52	d (14.6)	121.9	C-25, 26, 28	26-H
28			167.8		
29	2.88, 2.82	m, dd (12.6, 5.3)	39.6	C-7, 8, 30, 31, 35	8-H
30			139.4		
31	6.63	t (1.9)	117.9	C-29, 32, 33, 35	33-H
32			158.9		
33	6.70	m	114.8		34-H
34	7.15	t (7.7)	131.3	C-30, 32	33-H, 35-H
35	6.69	m	121.9	C-29, 31, 33	34-H
36	1.98	m	31.1	C-11	11-H, 37-H_3, 38-H_3
37	0.95	d (6.7)	19.1	C-11, 36, 38	36-H

38	0.92	d (6.5)	19.7	C-11, 36, 37	36-H
39	1.92	m	27.4	C-13, 14,40	14-H, 40-H_2
40	2.60, 2.48	m x2	41.6	C-14, 39, 41	39-H_2
41			210.6		
42	2.14	s	30.0	C-40, 41	
43	0.78	d (7.0)	12.2	C-15, 16, 17	16-H
44	1.94	d (1.3)	13.9	C-23, 24, 25	
46	3.66	m	47.4	C47, 47’	
46’	3.66	m	43.8	C47, 47’	
47	3.66	m	67.9	C46, 46’	
47’	3.66	m	67.8	C46, 46’	

ELISA Analysis of CypA-NS5A Interactions.

Test articles were tested for their capacities to block the interaction between CypA and HCV NS5A by ELISA. Specifically, we produced and purified recombinant GST, GST-CypA and Con1 NS5A-His proteins and conducted ELISA. Nunc MaxiSorb 8-well strip plates were coated with GST or GST-CypA for 16 h at 4˚C and blocked. Recombinant NS5A-His (1 ng/mL) was added to wells in 50 µL of binding buffer (20 mM Tris pH 7.9, 0.5 M NaCl, 10% glycerol, 10 mM DTT and 1% NP-40) for 16 h at 4˚C. Captured NS5A-His was subsequently detected using mouse anti-His antibodies (1 µg/mL) (anti-6xHis, Clontech) and rabbit anti-mouse-horseradish peroxidase phosphatase (HRP) antibodies (1:1000 dilution). To test the effect of Biotica compounds on the CypA-NS5A complex formation, increasing concentrations of each Biotica compounds were added to GST-CypA together with recombinant NS5A. All experiments were conducted twice. Data (triplicate) are presented (IC_{50}).

PPIase Inhibition Analysis

The inhibition of the PPIase activity of CypA was used to compare the inhibitory potential of sanglifehrin derivates as an indication of their binding affinity to CypA. The PPIase activity of recombinant CypA, produced by thrombin cleavage of GST-CypA, is determined by following the rate of hydrolysis of N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide by chymotrypsin. Chymotrypsin only hydrolyzes the trans form of the peptide, and hydrolysis of the cis form, the concentration of which is maximized by using a stock dissolved in trifluoroethanol containing 470 mM LiCl, is limited by the rate of cis-trans isomerization. CypA was equilibrated for 1 h at 5°C with selected sanglifehrin derivatives using a drug concentration range from 0.1 to 20 nM. The reaction was started by addition of the peptide, and the change in absorbance was monitored

spectrophotometrically at 10 data points per second. The blank rates of hydrolysis (in the absence of CypA) were subtracted from the rates in the presence of CypA. The initial rates of the enzymatic reaction were analyzed by first-order regression analysis of the time course of the change in absorbance. All sanglifehrin derivatives exhibited anti-PPIase activity that correlated well with their capacities to prevent CypA-NS5A interactions analyzed by ELISA or pulldown.

Analysis of HCV clearance and rebound by 3o.

Huh7-Con1 cells were passaged 7 consecutive times without G418 in the presence or absence of two concentrations of CsA or **3o** (0.5 and 1 μM). At each passage, clones (10) were collected, RNA extracted and analyzed by RT-qPCR for replicon content. Under drug selection, the presence of viral RNA was diminished after the first passage and was maintained at a low level for seven consecutive passages . After seven passages, drugs were removed and cells were analyzed for viral RNA content by qPCR to determine if a viral rebound occurs or not. No viral rebound was observed when the virus was placed under **3o** selection pressure. In sharp contrast, rapid viral rebound occurred when HCV was initially placed under CsA pressure.

Anti-HCV activity of 3o when used in combination with anti-HCV drugs.

We asked whether **3o** exhibits an additive effect when combined with anti-HCV drugs such as telaprevir, R-1479 and BMS-790052. To address this issue, we first determined the IC_{50} of telaprevir, R-1479 and BMS-790052 on the replication of the subgenomic Con1 (genotype 1b) replicon. We calculated IC_{50}s of 0.48 μM for telaprevir, 1.3 μM for R-1479, and 0.055 μM for BMS-790052 (Table S1). We also examined the cytotoxicity of these compounds on parental

Huh7 cells. We calculated CC50 of 83 µM for telaprevir, >200 µM for R1479 and >200 µM for BMS. We also measured an IC_{50} and CC_{50} for **3o** of 0.06 and >200 µM, respectively (Table S1).

	Telaprevir	**R-1479**	**BMS-790052**	**3o**
IC_{50}	0.48	1.3	0.055	0.06
CC_{50}	83	>200	>200	>200

Table S1: Antiviral and cytotoxicity analyses of selected anti-HCV drugs. Huh cells containing subgenomic genotype 1b were seeded at a density of 5 x 10^3 per well in a tissue culture–treated white 96-well plate in complete DMEM supplemented with 250 µg/mL G418. Medium was removed after 24 h and 3-fold serial dilutions in complete DMEM (without G418) of compounds were added in a total volume of 100 µL. After 4 days of incubation at 37°C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system. The luciferase signal was measured using a Luminoskan Ascent. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of compound that reduced the luciferase signal by 50%. The data are representative of 3 independent experiments. The toxicity of each compound was examined on parental Huh7 cells using the Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced the MTT signal by 50%. The data are representative of 2 independent experiments. These IC_{50}s were then used as fixed concentrations of telaprevir, R-1479 and BMS-790052 to examine the additive, synergistic or antagonistic effects of **3o**. Specifically, Huh7-Con1 cells were incubated with 0.48 µM for telaprevir, 1.3 µM for R-1479, and

0.055 µM for BMS-790052 (this higher than anticipated value was confirmed at this concentration of cells, subsequent analysis showed that lower assay cell concentrations of gave lower EC_{50} values) together with increasing concentrations (0.01 to 0.81 µM) of **3o**. We found that **3o** exhibits an additive inhibitory effect with each of the anti-HCV drugs (Fig. S2). This additive effect is very similar to that observed for another cyclophilin inhibitor, Debio-025 (alisporivir).

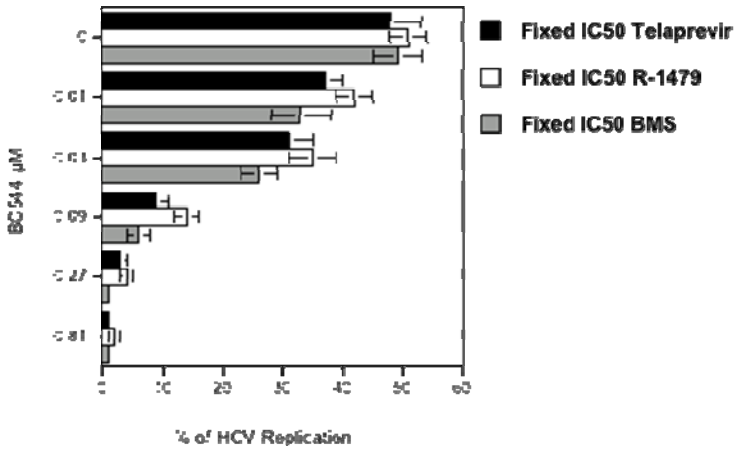


Figure S2: The combination of **3o** with anti-HCV drugs results in an additive antiviral effect. Huh7 cells containing subgenomic genotype 1b were seeded at a density of 5 x 10^3 per well in a tissue culture–treated white 96-well plate in complete DMEM supplemented with 250 µg/mL G418. Medium was removed after 24 h and fixed amounts of telaprevir (0.48 µM), R-1479 (1.3 µM) or BMS-790052 (0.055 µM) were added to cells together with increasing concentrations (0.01 to 0.81 µM) of **3o**. After 4 days of incubation at 37°C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system. The luciferase signal was measured using a Luminoskan Ascent. Percentage of replication in the absence of drug was arbitrarily fixed at 100. Data (triplicates) are representative of 2 independent experiments.

Antiviral Assay Huh 5-2

Anti-HCV assay in Huh 5-2 cells was performed by seeding 6.5 x 10^3 cells per well in a tissue culture treated white 96-well view plate (Packard, Canberra, Canada) in complete DMEM supplemented with 250 µg/ml G418. Following incubation for 24 h at 37°C (5% CO_2) medium was removed and 3-fold serial dilutions in complete DMEM (without G418) of the test compounds were added in a total volume of 100 µl. After 4 days of incubation at 37°C, cell culture medium was removed and luciferase activity was determined using the luciferase assay system (Promega, Leiden, The Netherlands); the luciferase signal was measured using a Safire^2 (Tecan, Switzerland). Relative luminescence units were converted to percentage of untreated controls. The 50% effective concentration (EC_{50}) was defined as the concentration of compound that reduced the luciferase signal by 50%.

Cytostatic Assay for Huh 5-2

For the assessment of the potential cytostatic effect of the evaluated inhibitor, Huh 5-2 cells were seeded at a density of 6.5 x 10^3 cells per well of a 96-well plate in complete DMEM. Serial dilutions of the test compounds in complete DMEM were added 24 h after seeding. Cells were allowed to proliferate for 3 days at 37°C, after which the cell number was determined by means of the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium)/phenazinemethosulfate (MTS/PMS) method (Promega). The CC_{50} (value derived from the dose-response curve) represents the concentration at which the metabolic activity of the cells would be reduced to 50% of the metabolic activity of untreated cells. The selectivity index (SI), indicative of the therapeutic window of the compound, was calculated as the CC_{50}/EC_{50}.

Compound name	Huh5.2 1b Replicon								
	EC_{50} (nM)	SD	EC_{90} (nM)	SD	CC_{50} (μM)	SD	SI_{50}	n	replicates
1	318	56	5500	N/A	9.1	3.3	29	1	6
CsA	306	141	1000	500	4.4	19.1	14	5	30
3a	8000	1700	60200	2400	>100	N/A	>13	1	6
3b	349	328	62000	2047	>100	N/A	>287	1	6
3c	560	442	4600	1200	>100	N/A	>179	1	6
3d	1600	400	8500	2000	48	1.8	30	1	6
3e	204	90	1200	400	>100	N/A	>490	1	6
3f	309	52	1100	200	2.1	0.9	6.8	1	6
3g	336	166	4230	12000	>100	N/A	>298	1	6
3h	148	49	1900	500	66	4.3	446	1	6
3i	208	78	1150	500	>100	N/A	481	1	6
3j	167	32	740	119	40	5.9	240	1	6
3k	2013	435	11500	3630	>100	N/A	>50	1	6
3l	2483	516	5410	850	>100	N/A	>40	1	6
3m	8065	904	31900	900	>100	N/A	>12.4	1	6
3n	162	85	920	190	>100	N/A	>617	1	6
3o	125	22	691	174	>100	N/A	>800	1	6
3p	1400	400	7400	2100	>100	N/A	>72	2	12
Telaprivir	235	191	838	294	>74	N/A	>315	1	6
DMSO	20480	2560	N/A	N/A	30720	2560	1.5	1	6

Table S2: Huh5.2 replicon data including standard deviations, n number and number of replicates.

1a, 1b and 2a replicon antiviral assays

The replicon cells (subgenomic replicons of genotype 1a (H77), 1b (Huh7) and 2a (JFH-1)) were grown in Dulbecco's modified essential media (DMEM), 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (pen-strep), 1% glutamine, 1% non-essential amino acids, 250 μg/ml

G418 in a 5% CO_2 incubator at 37ºC. All cell culture reagents were purchased from Mediatech (Herndon, VA). The replicon cells were trypsinized and seeded at 5 x 10^3 cells per well in 96-well plates with the above media without G418. On the following day, the culture medium was replaced with DMEM containing compounds serially diluted in the presence of 5% FBS. The cells containing the HCV replicon were seeded into 96-well plates and test articles were serially diluted with DMEM plus 5% FBS. The diluted compound was then applied to appropriate wells in the plate. After 72 hr incubation at 37°C, the cells were processed. The intracellular RNA from each well was extracted with an RNeasy 96 kit (Qiagen). The level of HCV RNA was determined by a reverse transcriptase-real time PCR assay using TaqMan® One-Step RT-PCR Master Mix Reagents (Applied Biosystems, Foster City, CA) and an ABI Prism 7900 sequence detection system (Applied Biosystems). The cytotoxic effects were measured with TaqMan® Ribosomal RNA Control Reagents (Applied Biosystems) as an indication of cell numbers. The amount of the HCV RNA and ribosomal RNA were then used to derive applicable EC_{50} values (concentration that would inhibit the replicon replication by 50%).

Mouse intravenous and oral pharmacokinetic analysis

Compounds were formulated in 5% ethanol, 5% cremophor EL, 90% saline for both *p.o.* and *i.v.* administration. Groups of 3 male CD1 mice were dosed with either 1 mg/kg *i.v.* or 10 mg/kg *p.o.* Whole blood samples (40 µL) were taken *via* tail or saphenous vein, pre-dose and at 0.25, 0.5, 2, 8, and 24 hours, diluted with an equal amount of dH_20 and kept on dry ice immediately. Samples were stored at -70 °C until analysis. The concentration of the test article in the sample was then determined by LC-MS/MS. The time-course of blood concentrations was plotted and used to derive area under the whole blood concentration-time curve (AUC), which is directly proportional to the total amount of unchanged drug that reaches the systemic circulation).

These values were used to generate PK parameters (with a non-compartmental model) using WinNonlin (version 5.2, Pharsight Corporation, California, USA).

Compound	Clearance (L/hr/kg)	Vss (L/kg)	i.v. $t_{1/2}$ (hr)	i.v. AUC_{last} $t_{1/2}$ (mins) AUC_{last}	p.o. AUC_{last} $t_{1/2}$ (mins) AUC_{last}
SfA	0.054	0.522	8.0	16473	2332
3n	0.0623	0.575	7.7	14333	2203
3o	0.055	0.678	10.2	15100	4837

Table S3: PK parameters after iv (1mg/kg) and po (10mg/kg) dosing to male CD-1 mice

Solubility analysis

Solubility was measured by diluting test compounds in DMSO (10 mM) into PBS at pH 7.4 to a target concentration of 100 µM with a final DMSO concentration of 1%. Sample tubes were gently shaken for 4 hours at room temperature, centrifuged and supernatants diluted into PBS. Diluted samples were mixed with the same volume (1:1) of methanol, then the same volume (1:1) of acetonitrile containing internal standard for LC-MS/MS analysis.

Log D analysis

Log D was measured at pH 7.4 using a miniaturised shake flask method. Partition of test compounds was measured in a mixture *n*-octanol and potassium phosphate buffer (pH 7.4) after shaking for 1 h at 25 ˚C. After centrifugation, water phase samples were diluted with additional water, then 50% ethanol with internal standard to achieve a 1:60 dilution. Samples of the *n*-

octanol phase were diluted in 50% ethanol with internal standard to achieve 1:2400 dilution. Samples were then analysed by LC-MS.

Hepatocyte stability analysis

Cryopreserved hepatocytes (2×10^6 cells/mL) were seeded in Krebs-Henseleit bicarbonate (KHB) buffer and test compound in 1% DMSO in KHB buffer added to a level of 1 µM. Plates were incubated at 37 °C and samples taken after 0, 15, 30, 60 and 120 min. Samples were added to an equal volume of acetonitrile containing internal standard, centrifuged and compound levels analysed by LC-MS/MS.

Microsome stability analysis

Mouse or human liver microsomes (2.5 mg/mL) were prepared in 0.1 M potassium phosphate buffer, 1.0 mM EDTA, pH 7.4. Test article was added to 1 µM. Following pre-incubation, the reaction was initiated by adding NADPH solution. Aliquots were removed at 0, 15, 30, 45 and 60 min and quenched with acetonitrile containing internal standard. Protein was removed by centrifugation and the sample plate analysed for compound concentration by LC-MS/MS.

Plasma Protein Binding analysis

Human plasma protein binding was analysed using a dialysis chamber methodology, with test article dosed at 1µM. Equilibrium was allowed for 5 hours at 37^{o}C, and levels of test article in each compartment were analysed by LCMS/MS.

MRP2 inhibition analysis

Inhibition of MRP2 was determined *in vitro* by measuring inhibition of ATPase activity in MRP2-containing membranes during stimulation with probenecid, an MRP2 substrate. Phosphate released was measured by use of a molybdenum blue assay and amounts released determined by comparison to a phosphate standard curve. Test article was incubated at 6 concentrations (0.2, 0.6, 1.9, 5.6, 16.7 and 50 µM for **3o** and 0.1, 0.3, 0.9, 2.8, 8.3 and 25 µM for CsA).

MDR1 inhibition analysis

Inhibition of MDR1 (P-glycoprotein) was determined *in vitro* by measuring inhibition of permeability of loperamide (at 5 µM), a Pgp substrate, through MDR1-MDCK cells, via LCMS/MS, at 7 concentrations of test article (0, 0.1, 0.3, 1, 3, 10, 30 and 50 µM). Incubations were carried out in an atmosphere of 5% CO_2 with a relative humidity of 95% at 37°C for 60 minutes.

Analysis of liver and blood levels after intravenous administration of 3o to CD1 mice

3o was formulated in 5% ethanol, 5% cremophor EL, 90% saline. A group of 18 male CD1 mice were dosed with 1 mg/kg *i.v.* Whole blood samples (300 µL) were transferred immediately into K2EDTA tubes. Livers were removed and rinsed with cold saline, dried on filtrate paper, placed in a screw top tube, weighed and snap frozen. Samples were taken pre-dose and at 0.25, 0.5, 2, 8, and 24 h. Samples were stored at -70 °C until analysis, with liver samples homogenised for 2 minutes with 3 volumes of PBS (pH7.4) by mini-bead-beater before smaple extraction. The concentration of the test article in the sample was then determined by LC-MS/MS. The time-course of blood concentrations was plotted and used to derive area under the whole blood

concentration-time curve (AUC). These values were used to generate PK parameters (with a non-compartmental model) using WinNonlin (version 5.2, Pharsight Corporation, California, USA).

Figure S3 – Mean blood and liver concentration-time profiles of 3o after a PO dose of 5 mg/kg to CD1 mice.

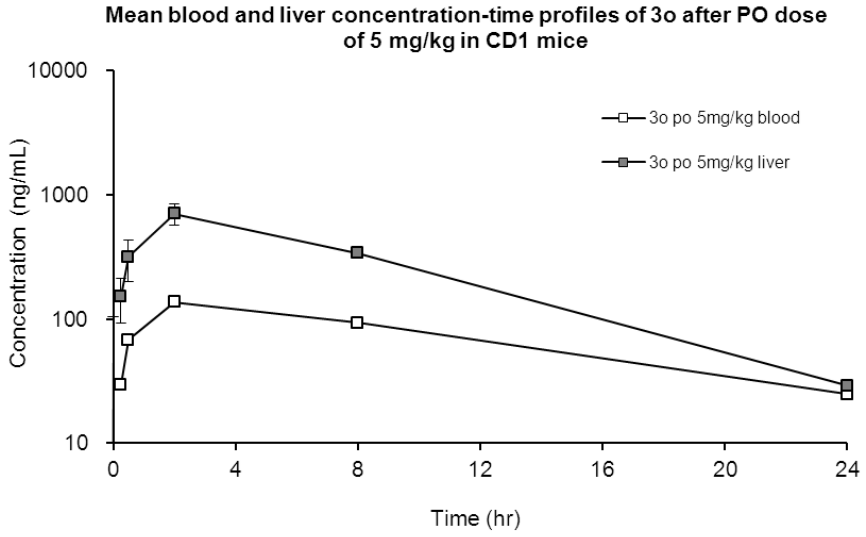


Table S4 – Comparison of 3o exposures in liver and whole bood in CD1 mice after a single 5mg/kg oral dose.

Organ	AUC_{inf}	$T_{1/2}$ (hrs)
Blood	2120	8.82
Liver	7130	4.73

Inhibition of human Mixed Lymphocyte Reaction (MLR)

Immunosuppressant activity was tested as follows: Peripheral blood mononuclear cell (PBMC) populations were purified from the blood of two normal, unrelated volunteer donors (A & B), using centrifugation over histopaque. Cells were counted and plated out at 1 x 105 cells per well in 96 well plates in RPMI media, with supplements and 2% Human AB serum.

Culture conditions included: cell populations A & B alone and a mixed population of cells A&B in the absence or presence of test compounds, each at 6 different concentrations. Compounds were tested at doses ranging from 10µM to 0.0001 µM in 1-log increments. Control wells contained a comparable concentration of vehicle (0.5% DMSO) to that present in the test compound wells. Cultures were established in triplicate in a 96 well plate and incubated at 37°C in 5% CO2 in a humidified atmosphere. 3H-thymidine was added on day 6 after assay set up and harvested 24hrs later. The levels of proliferation between the different culture conditions were then compared.

The ability of each dilution of test compound to inhibit proliferation in the MLR was calculated as percentage inhibition. This allowed estimation of the IC50 (concentration of test compound which resulted in a 50% reduction of counts per minute). In order to calculate the IC50, the X axis was transformed to a log scale. Non-linear regression was used to fit to the mean data points. A sigmoidal variable slope was selected.