A Natural Solution to Photoprotection and Isolation of the Potent Polyene Antibiotic, Marinomycin A.

Christopher S. Bailey[a], Joseph S. Zarins-Tutt[a,b], Matthias Agbo[a,b], Hong Gao[a,b], Alberto Diego-Taboada[c], Maoluo Gan[a,b], Refaat B. Hamed[a,b], Emily R. Abraham[a], Grahame Mackenzie[c]*, P. Andrew Evans[d]* and Rebecca J. M. Goss[a]*

School of Chemistry, University of St Andrews, St Andrews, KY16 9ST (UK) [a]

rjmg@st-andrews.ac.uk

School of Mathematics and Physical Sciences, University of Hull, Hull HU6 7RX (UK)

Sporomex Ltd, Medina House, 2 Station Ave, Bridlington YO16 4LZ (UK)[b]

G.Mackenzie@hull.ac.uk

Department of Chemistry, Queens University, 90 Bader Lane, Kingston, ON, K7M 3N6

(Canada) [c]

andrew.evans@chem.queensu.ca

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1 General Experimental

All procedures were carried out in dim light unless otherwise stated. Photoprotection experiments were carried out in Greiner Bio-One International 1.5 mL polypropylene reaction tubes. The reaction tubes had a height of 39 mm. The diameter of the tubes was 9.12 mm between 0.5 and 1.5 mL volume. The diameter of the tubes tapered from 9.12 mm to a point between the bottom of the tube and 0.5 mL. The thickness of the tube walls was 0.8 mm. Irradiation of samples was carried out using a Uvitec uvivue fitted with 6 x 8 watt UV bulbs. The bulbs are placed behind a Uvitec BXT 20M dual intensity UV filter allowing UV light of 312 nm to pass though. The intensity was set at 100% on the filter for all experiments. For irradiation the microcentrifuge tubes were places on their side in direct contact with the UV filter of the transilluminator.

UPLC analysis was carried out on an Acquity UPLC H class system using a Phenomenex kinetex 2.6 μm Phenyl –Hexyl 75 x 2.10 mm column. The column was held at 60 °C and an injection volume of 5 μL and a flow rate of 600 μL/min was used for all samples. The following conditions were used for the Marinomycin studies: UPLC analysis was carried out with 0.1% formic acid in water and methanol. The following gradient was used: 0.00 min – 0.20 min 5% methanol, 0.2 min – 1.00 min 5 – 65 % methanol, 1.00 min – 2.00 min 65 – 80 % methanol, 2.00 min – 4.00 min 80 % methanol, 4.00 – 4.45 min 80 – 5 % methanol, 4:45 – 5.00 min 5 % methanol. UV absorbance was measured at 360 nm at a resolution of 2.4 nm.

Microbiology work was carried out in a Faster BH-EN 2004 Laminar flow cabinet. Cultures were grown from spore stocks in 25 % glycerol stored at -20 °C or mycelial stocks in 25 % glycerol stored at -80 °C. Media was autoclaved before use at 121 °C load temperature and 124 °C chamber temperature for 20 minutes. Cultures were incubated in a New Brunswick Scientific I 26 or Innova 44 incubator shaker with a 2.5 cm orbit diameter.

LC-HRMS was carried out on a Termo Scientific Velos Pro / Orbitrap Velos Pro with a H-ESI source and a Themo Scientific Diones UltiMate 3000 RS chromatography system. The HPLC system was equipped with a Waters XBridge C_{18} 3.5 µm 2.1 x 100 mm column at 40 °C. 5 µL injection volume was used for all samples. HPLC analysis was carried out with 0.1 % formic acid in water and acetonitrile with a flow rate of 350 µL / min. The following gradient was used: 0.00 – 1.50 min 5 % acetonitrile, 1.50 min – 8.00 min 5 % - 95 % acetonitrile, 8.00 min – 10.00 min 95 % acetonitrile, 10.00 – 10.50 min 95 – 5 % acetonitrile. UV absorbance was measured between 220 – 800 nm at 2 nm resolution. The first minute of the run was diverted to waste. After 1 minute the eluent was passed to the H-ESI source. The HRMS was set up with the following parameters: Positive ionisation mode using 250 °C heater temperature, 350 °C capillary temperature, 40 U sheath gas flow, 20 U aux gas flow, 2 U sweep gas flow, 3.5 kV ionization voltage and 50 % RF lens power. A background ion corresponding to the $[M+H]^+$ of *n*-butly benzenesulfonamide was used as a lock-mass for internal scan-by-scan calibration.

All other chemicals were purchased from Sigma Aldrich, Acros Organics, Formedium or Alfa Aesar and used as supplied unless otherwise stated.

2 Preparation of Sporopollenin Exine Capsules (AT1862) (SpECs)

Lycopodium clavatum (club moss) SpECs were purchased from Tibrewala International (Nepal), acetone, potassium hydroxide, ethanol, *ortho*-phosphoric acid, hydrochloric acid, and sodium hydroxide from Fisher Scientific UK Ltd.

Sporopollenin exine capsules (SpECs) were extracted from *L. clavatum* SpECs as follows. SpECs (300 g) were heated at 80 °C for 12 h in an aqueous solution of potassium hydroxide (54 g in 900 mL), the solution being renewed after 6 h, filtered, washed with water (5 x 300 mL) and ethanol (5x300 mL), and dried overnight in open air. The particles were heated at 60 °C for 5 days in *ortho*-phosphoric acid 85% (900 mL), filtered, washed with water (5 x 300 mL), 2 M NaOH (2 x 300 mL), water (6 x 500 mL), PBS (2 x 250 mL), water (2x500 mL), ethanol (2x250 mL) then refluxed in ethanol (900 mL) for 4 h, filtered and washed with ethanol (2 x 250 mL). After this, the SpECs were suspended in acetone (500-700 mL) and sonicated for 30 min, filtered and dried to afford SpECs.

Typical elemental analysis of sporopollenin (g/100 g) was: carbon 68.90, hydrogen 7.90, nitrogen 0.00, as determined on a Fisons Instruments Carlo Erba EA 100 C H N S analyzer.

3 Isolation of Marinomycin

3.1 Media Screen for Marinomycin

In order to access the quantities of marinomycin that we would require for our photoprotection studies we first undertook to improve the fermentative production of marinomycin by Marinospora CNQ 140.[1] Initially four common media, routinely utilized for the fermentative production of natural products by actinomycetes were screened. Media included the carbohydrate rich media Tryptone Casitone Glucose marine(TCG marine) , used in the original isolation by Fenical *et al*.[1] and a variant, Tryptone Casitone Soluble Starch marine (TCSS marine). The minimal media marine broth and standard actinomycetes media ISP2 marine were also used. It was found that TCSS marine provided the highest titre of marinomycin with TCG marine and ISP2 marine providing approximately 10 times less marinomycin. Only trace quantities of marinomycin was detected in marine broth, as judged by LC-HRMS analysis. There were comparable productions of marinomycin in TCG marine and ISP2 marine. These results suggest a need for a carbohydrate rich medium for appreciable marinomycin production. This is unsurprising taking into consideration the polyketide biosynthetic origins of marinomycin.

To investigate the role of carbohydrate concentration and carbohydrate source on marinomycin production, 12 new media were designed and tested, six derived from TCG marine and six derived from TCSS marine (in all cases, the carbohydrate concentration was between 1-25 g/L). The autoclaved media were inoculated with *Marinospora* CNQ-140 and after growth for 6 days were submitted for LC-HRMS analysis to determine the relative quantities of marinomycin A produced. It was found that for equivalent quantities

of glucose and soluble starch used soluble starch consistently gave higher titres of marinomycin A. It was also observed that the peak of marinomycin A production occurred at a carbohydrate concentration of 15 g/L. Higher concentration resulted in a lower titre. This could potentially be a result of deregulation of the marinomycin biosynthetic pathway in very rich media. In such conditions, more effort may be put into growth than defense using secondary metabolites.

Marinospora CNQ-140 was grown on ISP2-marine agar at 28 °C for 18 days. Single colonies were picked and used to inoculate 4 mL of appropriate media. The cultures were incubated for 6 days (28 °C, 0.90 g) before pelleting by centrifugation (2844 g, 10 °C, 30 min). The supernatant was removed and added to XAD7-HP resin (0.6 g) and incubated with shaking overnight (4 °C, 0.90 g). The XAD7-HP resin was filtered through cotton wool and washed with MilliQ water until clear. The resin was eluted with acetone (5 mL). The extracts was dried *in vacuo* before analysis by LC-HRMS.

Table 1:

Media	Peak Area	Average Peak Area
ISP2-marine	10147733	11671492
	13195252	
TCG-marine	18145899	18145899
	-	
TCSS-marine	89077671	90837338
	92597005	
Marine Broth	823246	534583
	245919	

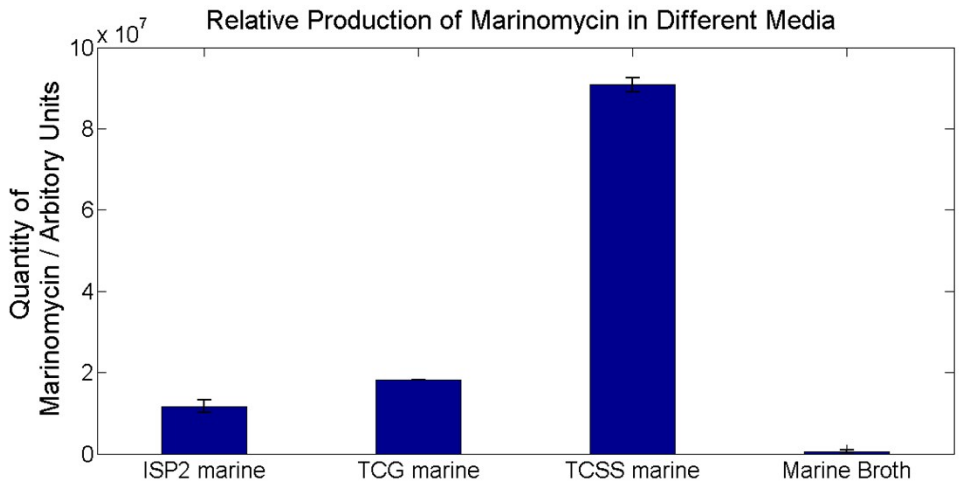


Figure 3.1: Relative production of Marinomycin in Different Media

The following media were made up with either glucose or soluble starch (SS) or glucose (G) as the carbohydrate source.

Table 2:

Media	Carbohydrate Concentration (g/L)	Tryptone (g/L)	Casein (g/L)
TCSS / TCG (1) marine	1	4	4
TCSS / TCG (5) marine	5	4	4
TCSS / TCG (10) marine	10	4	4
TCSS / TCG (15) marine	15	4	4
TCSS / TCG (20) marine	20	4	4
TCSS / TCG (25) marine	25	4	4

Culturing, sample preparation and analysis was carried out in duplicate for each media in the same manner as outlined previously.

Table 3:

Media	Peak Area	Average Peak Area
TCSS (1) marine	1093716	2492968
	3892219	
TCSS (5) marine	2146142	11408660
	20671178	
TCSS (10) marine	13441661	13441661
	-	
TCSS(15) marine	24123011	26424559
	28726107	
TCSS(20) marine	6556577	6556577
	-	
TCSS(20) marine	6556577	6556577
	-	
TCSS(25) marine	2364848	2223942
	2083035	
TCG(1) marine	2036964	2036969
	-	
TCG(5) marine	2448879	4030329
	5611779	
TCG(10) marine	9462239	9462239
	-	
TCG(15) marine	9928276	6524327
	3120378	
TCG(20) marine	9580416	8340448
	7100479	
TCG(25) marine	1829566	3978883
	6128199	

3.2 Isolation of Marinomycin A

Due to the photo instability of marinomycin A, all manipulations were conducted in dim light.

The strain was cultured in 20 × 0.5 L volumes of medium TCSS (15) (180 rpm, 14 days). At the end of the fermentation period, 10% (w/v) adsorbent resin (XAD-7HP and XAD-16N, 1:1) were added to each flask, and shake continuously at a reduced speed for 7 hours. The resin was then collected by filtration through sintered glass funnel and washed with MilliQ water (10 L), and then eluted with methanol (5.0 L) and finally washed with acetone (2.5 L). The combined extracts were evaporated *in vacuo* at reduced temperature and pressure to obtain the dry extract (30.7 g).

The combined extracts were fractionated on a Diaion HP-20 column eluting with methanol in water (25% -100%, 1.0 L each) and then 100% acetone (2.0 L) to afford twenty-six fractions ($F_1 - F_{26}$). $F_{18} - F_{25}$ which showed good antibiotic activity and by LC-MS analysis were rich in marinomycin were pooled together and concentrated *in vacuo* to yield approximately 3.70 g marinomycin rich fraction.

The marinomycin rich fraction was further purified using reversed-phase HPLC employing acetonitrile-MilliQ H_2O (0.05% formic acid) as mobile phase to obtain eighteen sub-fractions ($F_{18\text{-}25}A - F_{18\text{-}25}R$). Based on the antibiotic assay and LC-MS analysis, $F_{18\text{-}25}K$ and $F_{18\text{-}25}L$ were pooled together and concentrated to give 61.60 mg impure marinomycin A. This impure sample was further purified by reverse phase HPLC with acetonitrile-MilliQ H_2O (0.05% formic acid) (60: 40, t_R = 6.90 mins) as mobile phase to obtain marinomycin A (14.80 mg).

3.3 Disc Diffusion Bioassays:

During the purification of marinomycin A, the HPLC marinomycin fractions were tested for bioactivity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, to ascertain which fractions should be carried forward for further purification. To do this,

Staphylococcus aureus and *Pseudomonas aeruginosa* were first streaked onto LB agar and incubated overnight at 37°C. A single colony forming unit was then used to inoculate 10 mL of LB broth and this 10 mL culture was incubated in an orbital shaker overnight at 37°C. The next day, 10% of the inoculum volume was used to inoculate 0.5% LB agar which had been cooled to 40°C. This inoculated molten agar was then poured onto a LB agar plate and filter discs (6 mm diameter) were placed on the agar. The fractions were then loaded onto each disc in 10 µL volumes; apramycin was used as a positive control and the negative control was a 50:50 solution of methanol and water. These plates were then incubated overnight at 37°C and the zones of inhibition produced by each fraction were assessed the next day. The fractions with the most activity were taken forward for further purification.

4 Photoprotection experiments

4.1 Standard Curve for Marinomycin A

A 10 µM solution of marinomycin was made up from dilution of 10 mM marinomycin stock solution in DMSO diluted in MilliQ water. The following concentrations were made up in triplicate by serial dilution in 1:1 HPLC methanol in water: 20µM, 18µM, 16µM, 14µM, 12µM, 10µM, 8µM, 6µM, 4µM, 2µM, 1µM, 0.5µM, 0.1µM. The samples were submitted for UPLC analysis with the PDA detector set to 305 nm. The resulting peak areas were plotted and the line of best fit was calculated by least square fitting using Matlab polyfit function.. Absorbance was measured at 360 nm. An extinction coefficient of 3.91×10^3 UV*Sec/µM was calculated from the slope.

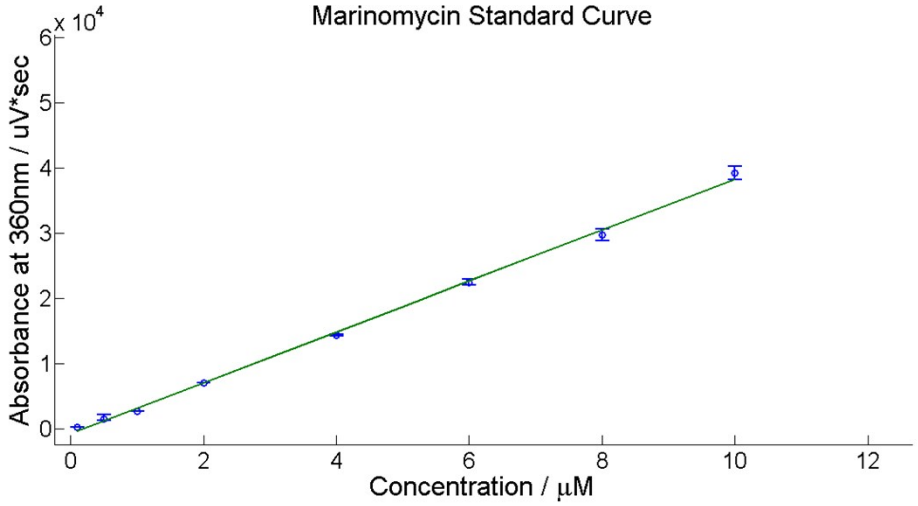


Figure 4.1: Marinomycin A Standard Curve

4.2 Photoprotection of Marinomycin A in Aqueous solution

A 10 μM solution of marinomycin was made up from dilution of 10 mM marinomycin stock in DMSO diluted in MilliQ water. 1 mL of the marinomycin solution was added to 50 mg of SpECs in triplicate for each time point. The suspensions were vortexed briefly before the SpECs were pelleted by centrifugation (16060 g, 5 min, R.T.). 600 μL of supernatant was removed and 600 μL of MilliQ water was added. The samples were pelleted by centrifugation (16060 g, 5 min, R.T.). 600 μL of supernatant was removed and 600μL of MilliQ water was added.

The samples were irradiated with UV light for between 0 to 180 minutes taking 30 minute time points. A final time point at 25 minutes was also taken. The samples were than pelleted by centrifugation (16060 g, 5 min, R.T.) and 600 μL of the supernatant was discarded. DMSO (600 μL) was added to the SpECs and vortexed briefly. The SpECs were pelleted by centrifugation (16060 g, 5 min, R.T.) and 200 μL of the supernatant was added to equi-volume HPLC methanol and submitted for UPLC analysis.

A 10 μM solution of marinomycin was made up from dilution of 10 mM marinomycin stock in DMSO diluted in MilliQ water. Unprotected control experiment consisted of 1mL of the 1μM solution being irradiated for between 0 to 2 minutes taking 20 second time points. 200 μL of the sample was then added to equi-volume HPLC methanol and submitted for UPLC analysis.

Table 4:

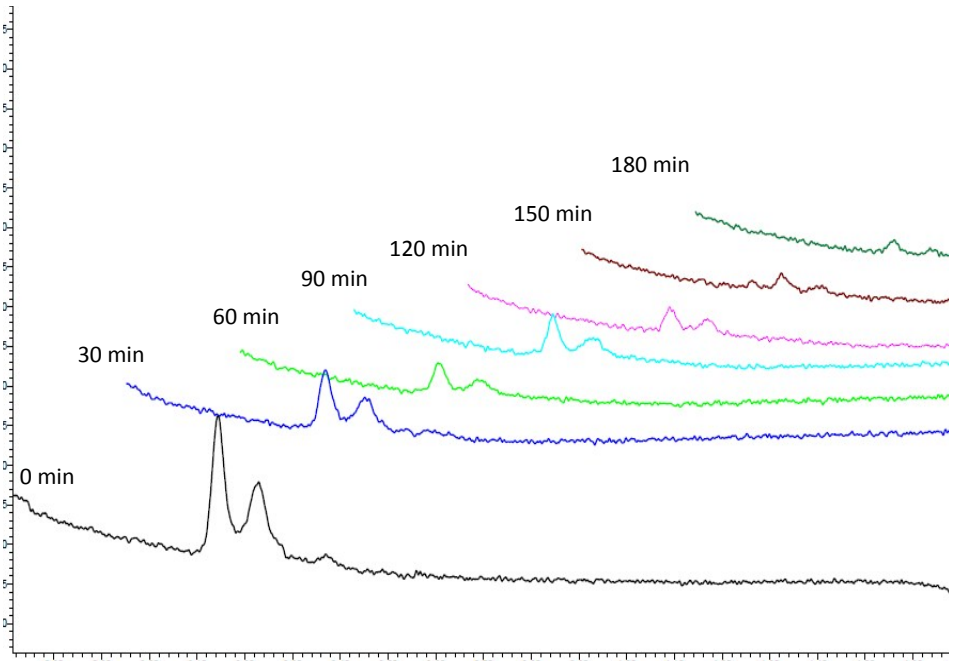
Time / min	Remaining Marinomycin (Protected) / μM	Average Remaining / μM	Remaining Marinomycin (Unprotected)/	Average Remaining / μM

			μM	
0	1.12	0.99	14.62	12.76
	0.93		12.05	
	0.93		11.61	
0.3	1.13	1.08	2.07	2.22
	0.97		1.97	
	1.12		2.62	
0.6	0.86	0.83	0.54	1.69
	0.84		1.93	
	0.78		2.61	
1.0	0.89	0.81	0.09	0.11
	0.72		0.09	
	0.81		0.14	
1.5	0.86	0.77	0	0
	0.71		0	
	0.73		0	
30	0.29	0.30		
	0.26			
	0.35			
60	0.14	0.17		
	0.20			
	0.16			
90	0.18	0.18		
	0.24			
	0.12			
120	0.15	0.12		
	0.05			
	0.15			
150	0.06	0.05		
	0.03			
	0.07			
180	0.02	0.04		
	0.05			
	0.03			

Table 5:

Protected half-life / min	Unprotected half-life / min	Protective ratio (Protected $t_{1/2}$ / Unprotected $t_{1/2}$)
33.98	0.13	261.38

(A)



(B)

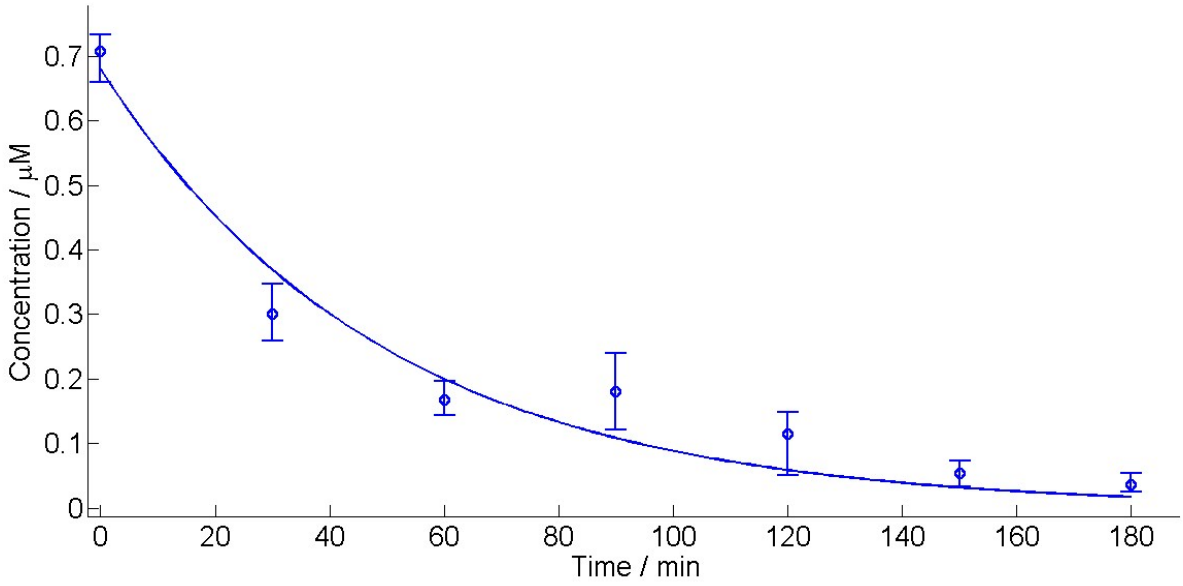


Figure 4.2: UV detection of SpECs protected marinomycin A, in aqueous solution, after varying length of exposure to UV light, showing marinomycin still present after 2 hours. (B) Concentration of marinomycin A after varying length of exposure of SpEC protected marinomycin, in aqueous solution to UV light.

4.3 Photoprotection of Marinomycin A using Pre-Irradiated SpECs

SpECs (50 mg) were exposed to 0, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 hrs of UV light in the same transilluminator as used in the initial photoprotection studies. The SpECs were loaded with marinomycin A using the procedure previously discussed. The loaded SpECs were suspended in MilliQ water (600 μL) and exposed to UV light for 5 minutes. The SpECs were extracted in the same manor previously described and submitted for UPLC analysis.

Table 6:

UV exposure	Protected Marinomycin / μM	Average Protected Marinomycin/ μM
0 hr	1.14	1.04
	1.19	

	0.78	
30 min	0.94	1.26
	1.70	
	1.15	
1 hr	1.26	1.09
	0.97	
	1.03	
1:30 hr	2.16	1.79
	1.95	
	1.26	
2:00 hr	1.54	1.15
	0.88	
	1.04	
2:30 hr	1.47	1.56
	1.88	
	1.33	
3:00 hr	2.17	1.49
	0.81	
	1.49	

4.4 Thermal Stability of SpECs Loaded Marinomycin A

During the photoprotection experiments the samples were heated as a byproduct of the UV bulb directly adjacent to the samples. We set about testing the thermal stability of marinomycin when loaded onto the SpECs to see if the decomposition we saw over 3 hours was a result of the heating.

The temperature of 1mL of water in a microcentrifuge was measured when exposed to UV light for 3 hrs. A standard microcentrifuge tube had a small hole drilled in the side to fit a thermal couple in. 1mL of water was added to the microcentrifuge tube and the tube was placed in the transilluminator in the same manner as described for the photoprotection experiments of marinomycin. The UV light was switched on and left to

run for 3 hours continuously. The temperature was recorded every 30 minutes. A maximum temperature of 48 °C was reached after 90 minutes.

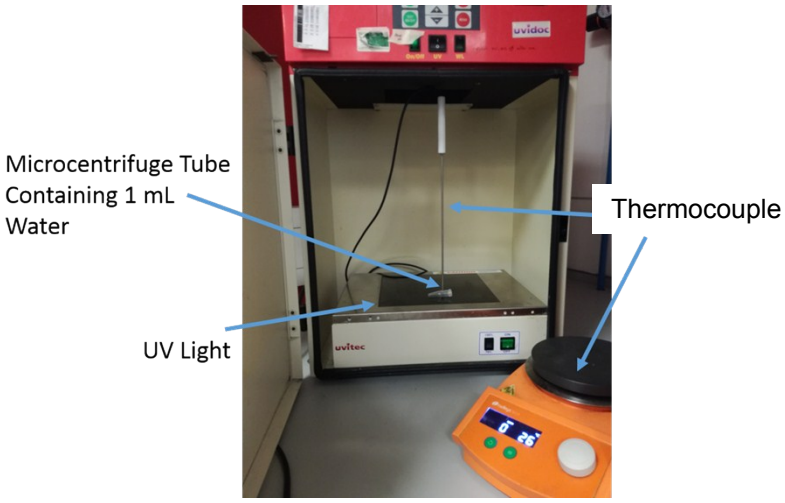


Figure 4.3:

SpECs were loaded with 10μM solution and washed with water in the same manner as in the photoprotection experiments of marinomycin. The loaded SpECs were heated at 50 °C in the dark. 3 Samples were removed every 30 minutes for 3 hours. The samples were eluted with DMSO and submitted for UPLC analysis in the same manner as in the photoprotection experiments of marinomycin. No degradation could be seen of the sample.

4.5 Photoprotection of dry SpEC Encapsulated Marinomycin

A 10 μM solution of marinomycin was made up from dilution of 10 mM marinomycin stock in DMSO diluted in MilliQ water. 1 mL of the marinomycin solution was added to 50 mg of SpECs in triplicate for each time point. The suspensions were vortexed briefly before the SpECs were pelleted by centrifugation (16060 g, 5 min, R.T.). 600 μL of

supernatant was removed and 600 µL of MilliQ water was added. The samples were pelleted by centrifugation (16060 g, 5 min, R.T.). 600 µL of supernatant was removed and the remaining solvent was removed by lyophilisation. Once dry the samples were exposed to UV light for for the specified time before elution with 1 mL DMSO.

The eluted SpEC's were vortexed briefly before being pelleted by centrifugation (16060 g, 5 min, R.T.). 200 µL of the resulting supernatant was added to equi-volume HPLC methanol and submitted for UPLC analysis. A decay of 30 % was observed between the t=0 hrs and t=7hrs samples.

Table 7:

UV exposure	Marinomycin Concentration / µM	Average Marinomycin Concentration/ µM
0	32.2	31.8
	33.5	
	29.8	
20 s	30.7	31.1
	32.8	
	29.9	
40 s	33.8	32.9
	32.4	
	32.6	
60 s	32.4	31.8
	32.1	
	31.0	
90 s	34.0	30.8
	28.8	
	29.6	
0.5 hrs	27.2	26.3
	25.0	
	26.8	
1.0 hr	26.5	26.9
	27.4	
	26.9	

1.5 hrs	24.3	25.4
	25.5	
	26.4	
2.0 hrs	27.0	25.9
	24.9	
	25.9	
2.5 hrs	22.9	23.4
	21.2	
	26.2	
3.0 hrs	29.3	26.1
	25.5	
	23.5	
4.0 hrs	21.3	23.3
	24.6	
	24.0	
5.0 hrs	21.7	23.6
	24.2	
	25.0	
6.0 hrs	22.8	24.0
	24.7	
	24.7	
7.0 hrs	24.3	23.7
	21.5	
	25.2	

4.6 Photoisomerization of Dry, Unprotected Marinomycin

A 10 μM solution of marinomycin was made up from dilution of 10 mM marinomycin stock in DMSO diluted in MilliQ water. 1 mL of the marinomycin solution was added to microcentrifuge tubes and dried *in vacuo* overnight. The microcentrifuge tubes where closed and exposed to UV light for the allocated amount of time before being removed

and stored in the dark. 1 mL HPLC methanol: water (1:1) was added to each microcentrifuge tube and vortexed. The Solution was analyzed by UPLC.

Table 8:

UV exposure / S	Marinomycin Concentration / μM	Average Marinomycin Concentration/ μM
0	93.1	86.1
	90.4	
	74.9	
20	72.8	73.3
	74.3	
	72.7	
40	67.4	63.7
	64.6	
	59.0	
60	24.4	28.2
	40.7	
	19.4	
80	47.2	43.1
	46.9	
	35.2	
100	22.0	19.8
	21.1	
	16.2	
120	4.5	5.0
	6.3	
	4.2	
180	0.0	0.0
	0.0	
	0.0	

5 Exploring the Utilization of the SpECs for Marinomycin Uptake and Purification

5.1 Uptake of Marinomycin Compared to XAD 16N and XAD 7HP

XAD resins were washed with 2 volumes water and 2 volumes methanol and dried at 70 °C before use.

A 10 μM solution of marinomycin A was made up from dilution of 10 mM marinomycin A stock in DMSO diluted in MilliQ water. 1 mL of the marinomycin A solution was added to 50 mg of XAD 16N, XAD 7HP and SpECs in triplicate. The suspensions were vortexed briefly before the resins and SpECs were pelleted by centrifugation (16060 g, 5 min, R.T.). 600 μL of supernatant was removed and MilliQ water (600 μL) was added. The samples were washed in the same manner as previously described. DMSO (600 μL) was added to the samples before being vortexed. The resins and SpECs were pelleted by centrifugation (16060 g, 5 min, R.T.). 200 μL of the supernatant was added to HPLC methanol (200 μL) and submitted for UPLC and LCMS analysis.

Table 9:

Resin	Marinomycin Uptake / μM	Average Uptake / μM	Marinomycin Uptake / Arbitrary Units (LCMS)	Average Uptake / Arbitrary Units (LCMS)
XAD 16N	1.83	1.78	2817072	2135734
	1.36		1484120	
	2.14		2106011	
XAD 7HP	1.33	1.02	1999494	2047866
	1.02		2006247	
	0.72		2137858	
SpECs	0.73	0.71	1627636	1502612
	0.66		1250978	
	0.73		1629223	

5.2 Explored Photoprotection with XAD 16N and XAD 7HP

XAD resins were washed with 2 volumes water and 2 volumes methanol and dried at 70 °C before use.

Photoprotection of marinomycin A with XAD 16N and XAD 7HP was carried out in the same manner as the photoprotection of marinomycin. 50 mg of each resin was used. UV irradiation was carried out for 15 minutes and time points were recorded at 5 minute intervals. No photoprotection was observed.

Table 10:

Time / min	Remaining Marinomycin for XAD 16N / μM	Average for XAD 16N / μM	Remaining Marinomycin for XAD 16N / μM	Average for XAD 7HP / μM
0	1.83	1.78	1.33	1.02
	1.36		1.02	
	2.14		0.72	
5	0	0	0	0
	0		0	
	0		0	
10	0	0	0	0
	0		0	
	0		0	
15	0	0	0	0
	0		0	
	0		0	

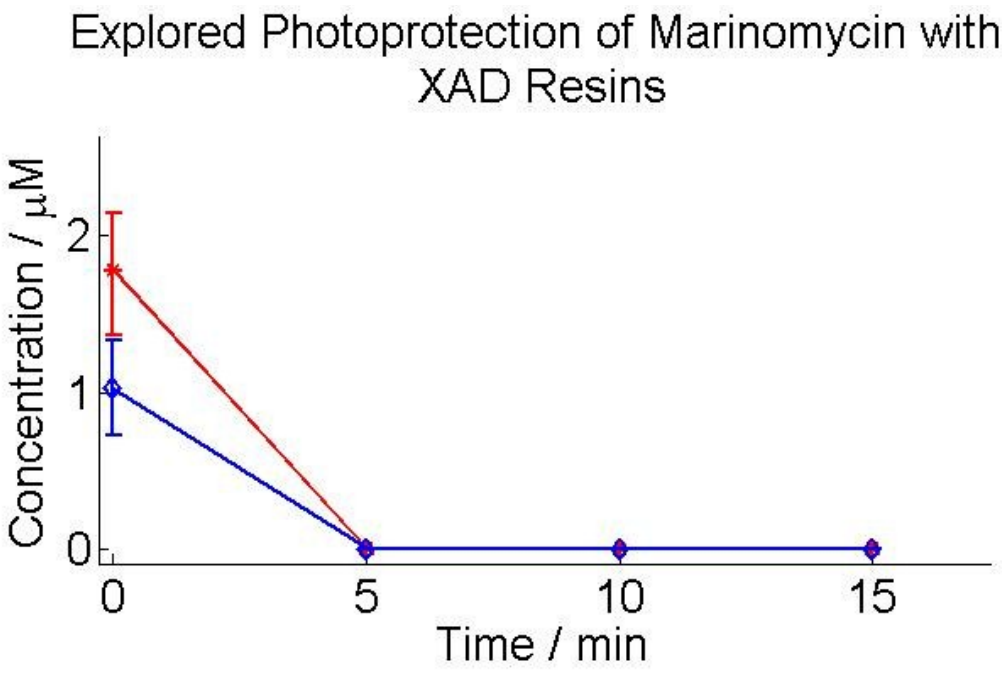


Figure 5.1: Remaining eluted marinomycin from XAD 16N (Red) and XAD 7HP (Blue) after exposure to UV light showing no detectable photoprotection.

5.3 Uptake of Marinomycin A from Culture Broth by SpECs, XAD 16N and XAD 7HP

50 mL of TCSS(15) was inoculated with 200 µL of *Marinospora* CNQ140 mycelial stocks. The cultures were incubated in the dark for 7 days (200 r.p.m., 28°C). The culture was pelleted by centrifugation (2844 g, 4 °C, 20 min). 1 mL of the resulting supernatant was added to 50 mg of SpECs, XAD 7HP resin or XAD 16N resin in triplicate. The resins and SpECs were vortexed briefly and pelleted by centrifugation (16060 g, R.T., 5 min). 600 µL of the supernatant was discarded and 600 µL of MilliQ water was added. The resins and SpECs were vortexed briefly and pelleted by centrifugation (16060 g, R.T., 5 min). 600 µL of the supernatant was discarded and 600 µL of DMSO was added. The samples were vortexed, pelleted by centrifugation (16060 g, R.T., 5 min) and 200 µL

of the supernatant was added to 200 μL of HPLC methanol and submitted for LC-HRMS analysis. The XIC for marinomycin A was integrated to give relative quantification of marinomycin A.

Table 11:

Resin	Marinomycin Uptake / Arbitrary Units	Average Uptake / Arbitrary Units	TIC / Arbitrary Units	Average TIC / Arbitrary Units	Ratio TIC / Marinomycin Uptake
XAD 16N	7228715	8654988	5892214658	5718184384	661
	9609540		5822704598		
	9126711		5439633895		
XAD 7HP	2250963	2896962	4387486039	4397237996	1517
	3285545		4399695836		
	3154379		4404532113		
SpECs	52456722	55109101	6264109549	6286295209	114
	56876198		6227444445		
	55994384		6367331632		

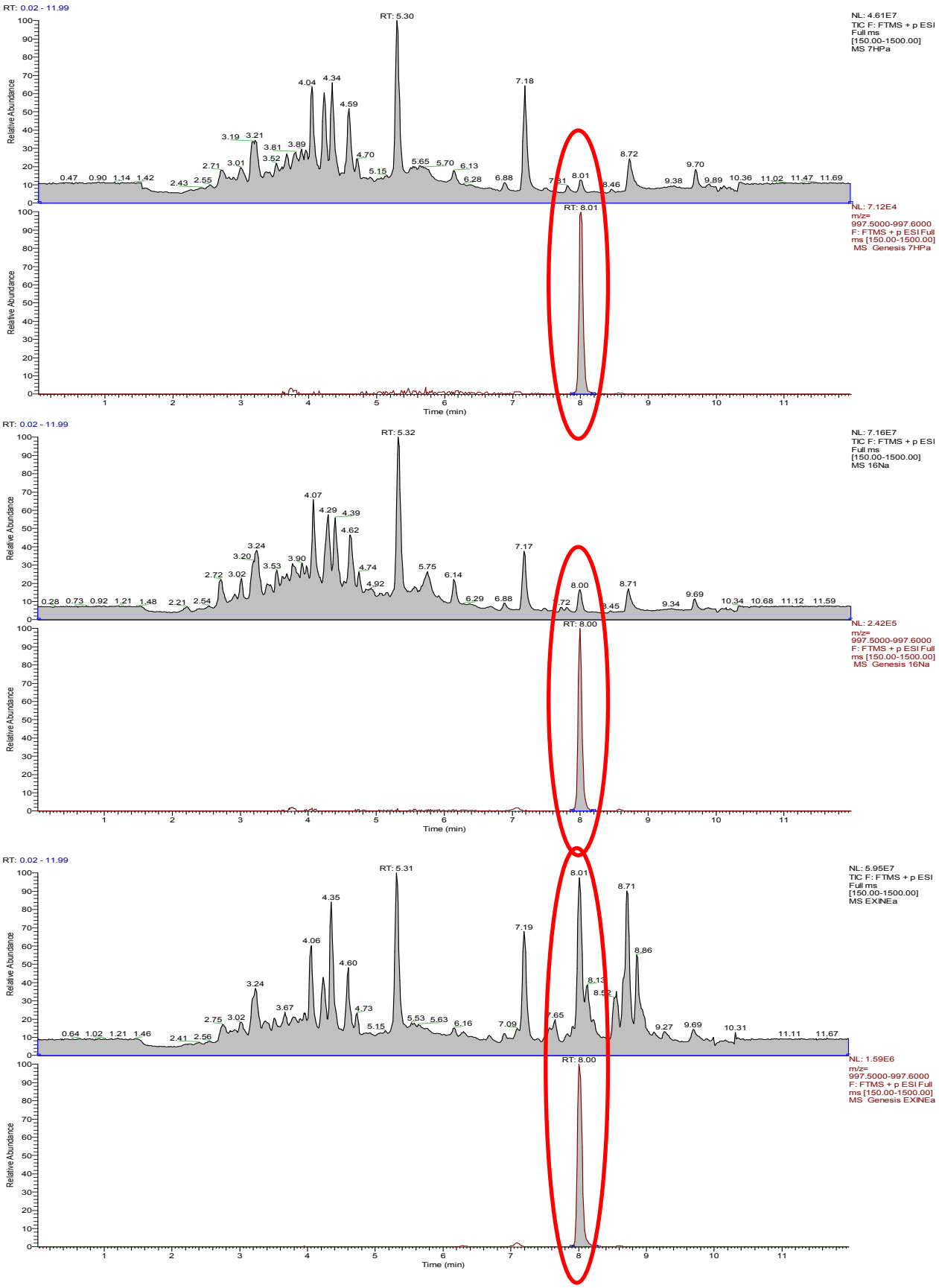


Figure 5.2: Total Ion Count (TIC) and Extracted Mass Chromatogram (XIC) for extracts from XAD 7HP (top), XAD 16N (middle) and SpECs (bottom).

5.4 Uptake of Marinomycin A at Different pH's

Marinospora CNQ140 was grown in an analogous manner to that described in section 5.3. The culture was pelleted by centrifugation (2844 g,, 4 °C, 20 min). The supernatant was divided into 15 mL portions. The pH of the supernatant was measured (pH 5.3). One of the portions was adjusted to pH 3.8 with 1 M HCl. Another portion was adjusted to pH 6.8 with 1 M NaOH. Each supernatant was used mixed with SpECs, XAD-16N or XAD-7HP followed by washing with water and elution with DMSO in the same manner as described in section 5.3. The eluted samples where submitted for LCMS analysis and the XIC for marinomycin A was integrated to give relative quantification of marinomycin.

Table 12:

Resin	pH	Marinomycin Uptake / Arbitrary Units	Average Uptake / Arbitrary Units
SpECs	3.8	46851	46733
		46355	
		46993	
	5.3 (Unaltered Culture)	42280	44455
		44440	
		46646	
	6.8	51605	51705
		53523	
		49987	
XAD-7HP	3.8	6646	6367
		5059	
		7398	
	5.3 (Unaltered Culture)	6134	8449
		9246	
		9968	
	6.8	9580	11133
		12152	

		11668	
XAD-16N	3.8	7436	8476
		7766	
		10227	
	5.3 (Unaltered Culture)	11752	11170
		10044	
		11714	
	6.8	12838	11545
		11598	
		10198	

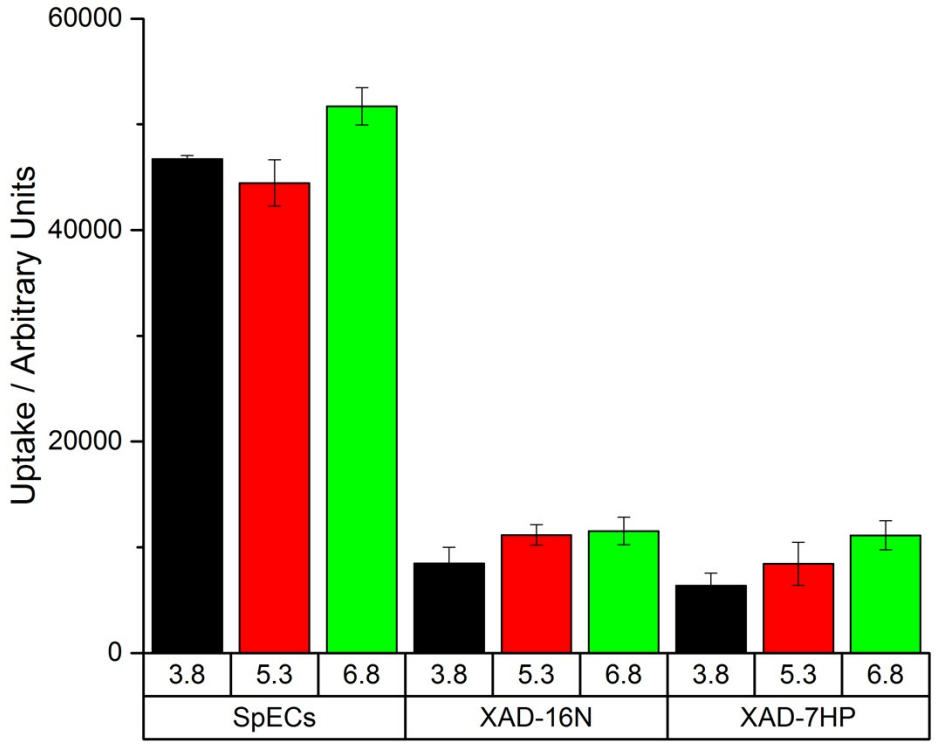


Figure 5.3: Marinomycin A Uptake at Different pH's. Error bars show one standard deviation.