# Characterization of rhamnolipids produced by an Arctic marine bacterium from the *Pseudomonas fluorescence* group

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**Figure S1.** Phylogenetic tree of *Pseudomonas* species in the *P. fluorescence* group based on 16S rRNA gene sequences. The studied isolate M10B774 is shown in bold. Branch labels represent identification and accession codes of the Pseudomonas sequences in GenBank, node support is given as posterior probabilities (PP). *P. jessenii* was used as an outgroup for the other taxa. The accurate identity and placement of M10B774 remains unresolved, because of the adjacent polytomy and lack of node support at nearby nodes of the tree. However, the study isolate seems to be most closely related, if not conspesific, with *P. gessardii*.



**Figure S2.** Antibacterial growth inhibition assay of compounds **1-6** tested at three concentrations against Gramnegative *P. aeruginosa* and *E.coli*. Bacteria cells and medium (50:50) was used as negative control (0μM). Values are mean of two parallels.

	1	2	3	4	5	6
	(dmso-d <sub>6</sub> )	(methanol-	(dmso-d <sub>6</sub> )	(dmso-d <sub>6</sub> )	(dmso-d <sub>6</sub> )	(methanol-
		<i>d</i> 3)				<i>d</i> 3)
1		100.62	98.7	98.75	98.69	99.82
2		72.29	70.3	70.54	70.97	72.79
3		72.19	69.3	70.47	70.31	72.03
4		74.04	71.9	71.85	71.87	74.24
5		70.00	69.0	68.76	68.93	70.14
6		17.79	17.8	17.77	17.83	17.92
1′	170.59	172.31	170.3	170.03	170.32	172.77
2′	42.76	41.18	40.1	39.54	40.06	41.25
3′	67.06	75.45	72.9	72.47	72.94	74.73
4'	36.69	34.25	32.1	30.38	32.47	33.51
5′	24.49	25.78	24.3	124.37	24.66	25.89
6'	28.70*	30.58	26.3	132.34	28.63*	28.08
7′	28.70*	30.25*	129.9	26.80	28.71*	130.29
8'	31.28*	32.86*	129.3	28.73	28.84*	131.46
9′	22.10*	23.59*	26.6	26.80	28.98*	28.22
10′	13.94*	14.32*	31.3	31.17*	31.22*	30.83
11′			21.7	22.09*	22.11*	30.08
12′			13.8	13.94*	13.96*	32.99*
13′						23.73*
14′						14.46*
1''	171.62	174.27	170.6	171.65	172.56	177.07
2''	38.81	39.88	40.4	38.79	40.43	42.31
3''	69.97	72.60	71.0	70.73	71.39	73.59
4''	33.36	34.96	33.7	33.29	33.61	35.40
5''	24.97	26.11	24.7	24.54	24.11	26.33
6''	28.70*	30.30	28.6	28.37	28.99*	30.56
7''	28.70*	30.18*	28.8	28.55	29.07*	30.36
8''	31.19*	32.82*	31.2	31.19*	31.30*	32.95*
9''	22.10*	23.58*	22.1	22.08*	22.10*	23.73*
10''	13.94*	14.32*	14.0	13.93*	13.95*	14.45*

 Table S3. <sup>13</sup>C NMR assignments for compounds 1-6.

\* Carbon resonances are not chain specifically assigned due to near identical shifts







Figure S6. 2D superimposed <sup>13</sup>C-HSQC and HMBC of 1 in DMSO-d<sub>6</sub>, T=298 K.



Figure S7. 2D DQF-COSY of 1 in DMSO-d<sub>6</sub>, T=298 K.



**Figure S8**. 2D ROESY (300 ms) of **1** in DMSO-d<sub>6</sub>, T=298 K.



**Figure S9**. 1D proton of **2** in methanol-d<sub>4</sub>, T=298 K.



Figure S10. 1D carbon of 2 in methanol-d<sub>4</sub>, T=298 K.



Figure S11. 2D superimposed <sup>13</sup>C-HSQC and HMBC of 2 in methanol-d<sub>4</sub>, T=298 K.



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Figure S14. 1D proton of 3 in DMSO-d<sub>6</sub>, T=298 K.



**Figure S15.** 1D carbon of **3** in DMSO-d<sub>6</sub>, T=298 K.



Figure S16. 2D superimposed <sup>13</sup>C-HSQC and HMBC of 3 in DMSO-d<sub>6</sub>, T=298 K.



Figure S17. Blown up aliphatic region of superimposed 2D <sup>13</sup>C-HSQC and HMBC of 3 in DMSO-d<sub>6</sub>, T=298 K.



Figure S18. Blown up aliphatic region of superimposed 2D <sup>13</sup>C-HSQC and H2BC of 3 in DMSO-d<sub>6</sub>, T=298 K.



Figure S19. 1D proton of 4 in DMSO-d<sub>6</sub>, T=298 K.



Figure S20. 1D carbon of 4 in DMSO-d<sub>6</sub>, T=298 K.



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**Figure S25.** 2D DQF-COSY of **4** in DMSO-d<sub>6</sub>, T=298 K.



**Figure S27.** Comparison between experimental and simulated multiplets of the olefinic protons of **4**. The fit was made using a qsine<sup>2</sup>(12°) window function (upper panel), and then compared to the raw multiplets (lower panel). The best fit was found to be a dtt, 10.9, 7.3, 1.5 Hz multiplet, which indicates a *cis* configuration (expected  ${}^{3}J_{5'6'} \sim 11$  Hz). The right panel shows the simulated peak for the expected *trans* coupling ~19 Hz with shows a poor fit.



**Figure S28.** The <sup>3</sup>*J*<sub>CH</sub> couplings of the olefinic protons are estimated using a selective CLIP-HSQMBC experiment. Values of 9.3 and 10.1 Hz is found for <sup>3</sup>*J*<sub>H6</sub><sup>+</sup>C4' and <sup>3</sup>*J*<sub>H5</sub><sup>+</sup>C7' respectively, suggesting anti configurations of the C and H.



**Figure S29.** *The* <sup>3</sup>*J*<sub>CH</sub> couplings of the olefinic protons are estimated using a SJS-HSQC experiment (j-resolved in f1). Values of 9.0 and 9.4 Hz is found for <sup>3</sup>*J*<sub>H6'C4'</sub> and <sup>3</sup>*J*<sub>H5'C7'</sub> respectively, suggesting anti configurations of the C and H.





**Figure S31.** 1D carbon of **5** in DMSO-d<sub>6</sub>, T=298 K.



Figure S32. 2D superimposed <sup>13</sup>C-HSQC and HMBC of 5 in DMSO-d<sub>6</sub>, T=298 K.



Figure S33. 2D DQF-COSY of 5 in DMSO-d<sub>6</sub>, T=298 K.



Figure S34. 2D ROESY (300 ms) of 5 in DMSO-d<sub>6</sub>, T=298 K.



Figure S35. 1D proton of 6 in methanol-d<sub>4</sub>, T=298 K.





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Figure S37. 2D superimposed <sup>13</sup>C-HSQC and HMBC of 6 in methanol-d<sub>4</sub>, T=298 K.



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**Figure S42**. 2D ROESY (300 ms) of **6** in methanol-d<sub>4</sub>, T=298 K.



Figure S43. Blown up cyclic region of 2D ROESY (300 ms) of 6 in methanol-d<sub>4</sub>, T=298 K.



**Figure S44**. Expansion of the nearly overlapped olefinic protons in a 2D NOESY (500 ms) of **3** in methanol-d<sub>4</sub>, T=298 K. The absence of NOE6',8' and NOE9',7' follows the *cis*-pattern observed for 4, which could be reliably determined to be in *cis* configuration.



**Figure S45.** Comparison between experimental and simulated multiplets of the nearly overlapped olefinic protons of **3**. The fit was made using a qsine<sup>2</sup>(12°) window function (upper panel), and then compared to the raw multiplets (lower panel). The best fit was found to be a dtt, 10.9, 6.6, 0.6 Hz multiplet, which indicates a cis configuration (expected  ${}^{3}J_{7'8'} \sim 11 \text{ Hz}$ ). The right panel shows the simulated peak for the expected trans coupling ~19 Hz with shows a poor fit.



**Figure S46.** MS/MS spectrum of **1** [M + Na]<sup>+</sup>. Ion mode ESI+.



Figure S47. MS/MS spectrum of 2 [M + Na]<sup>+</sup>. Ion mode ESI+.



Figure S48. MS/MS spectrum of 3 [M + Na]<sup>+</sup>. Ion mode ESI+.



Figure S49. MS/MS spectrum of 4 [M + Na]<sup>+</sup>. Ion mode ESI+.



Figure S50. MS/MS spectrum of 5 [M + Na]<sup>+</sup>. Ion mode ESI+.



Figure S51. MS/MS spectrum of 6 [M + Na]<sup>+</sup>. Ion mode ESI+.