

SUPPLEMENTARY MATERIAL

Punigratane, a novel pyrrolidine alkaloid from *Punica granatum* rind with putative efflux inhibition activity.

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Abstract

A new pyrrolidine alkaloid named Punigratane was isolated from the rind of *Punica granatum*. This is the first report of a pyrrolidine like structure from the rind. The activity of this compound was tested in a representative MDR *Klebsiella pneumoniae* strain which exhibited high efflux pump activity. At a concentration of 6 mg, this compound Punigratane was found to have efflux inhibition activity.

Keywords: Pyrrolidine alkaloid, Punigratane, *Punica granatum*, pomegranate rind, RND efflux pump inhibitor, multidrug resistance, *Klebsiella pneumoniae*.

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Figure S1: Mass Spectra of Punigratane

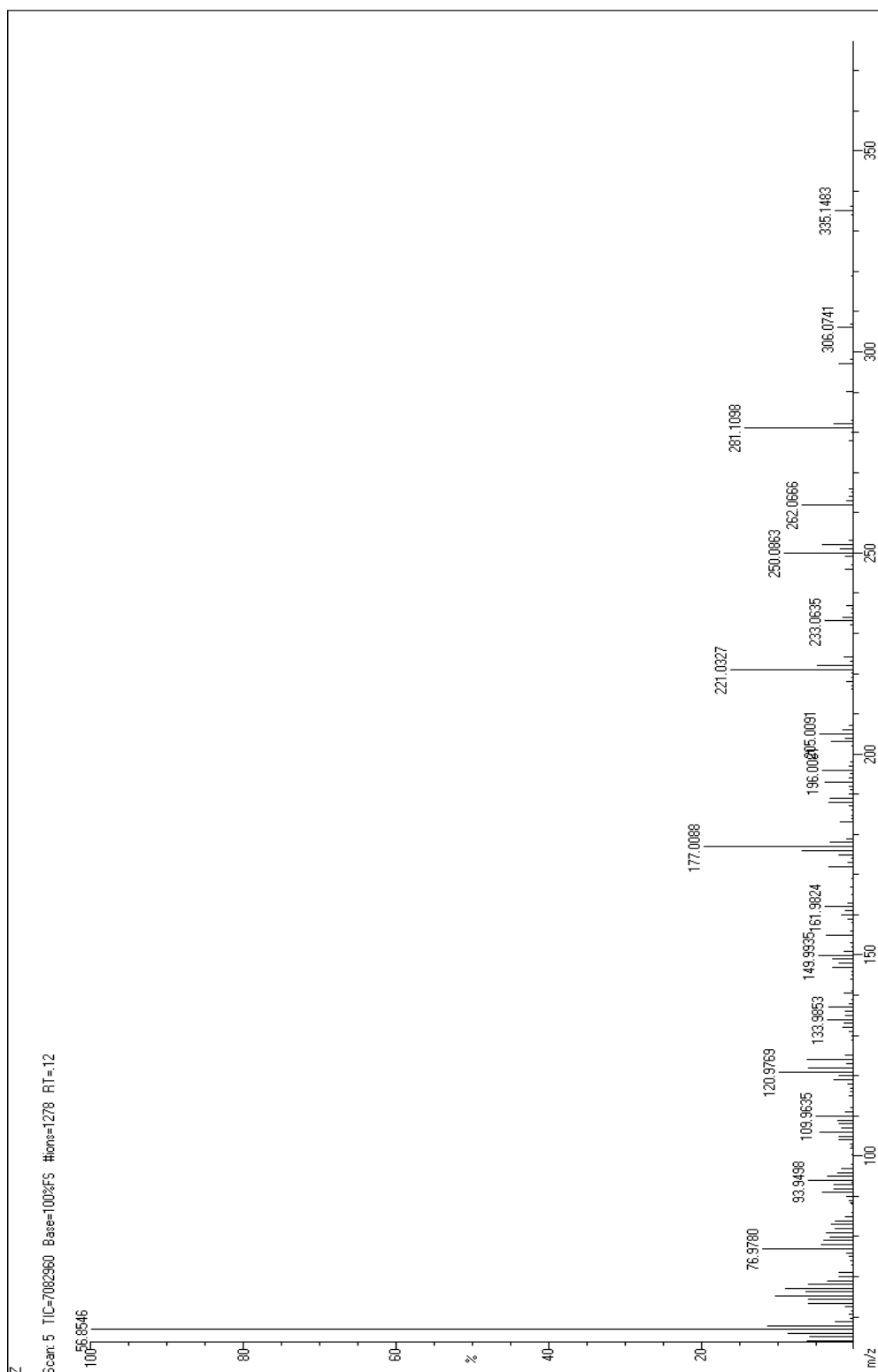


Figure S2: Infrared Spectra of Punigratane

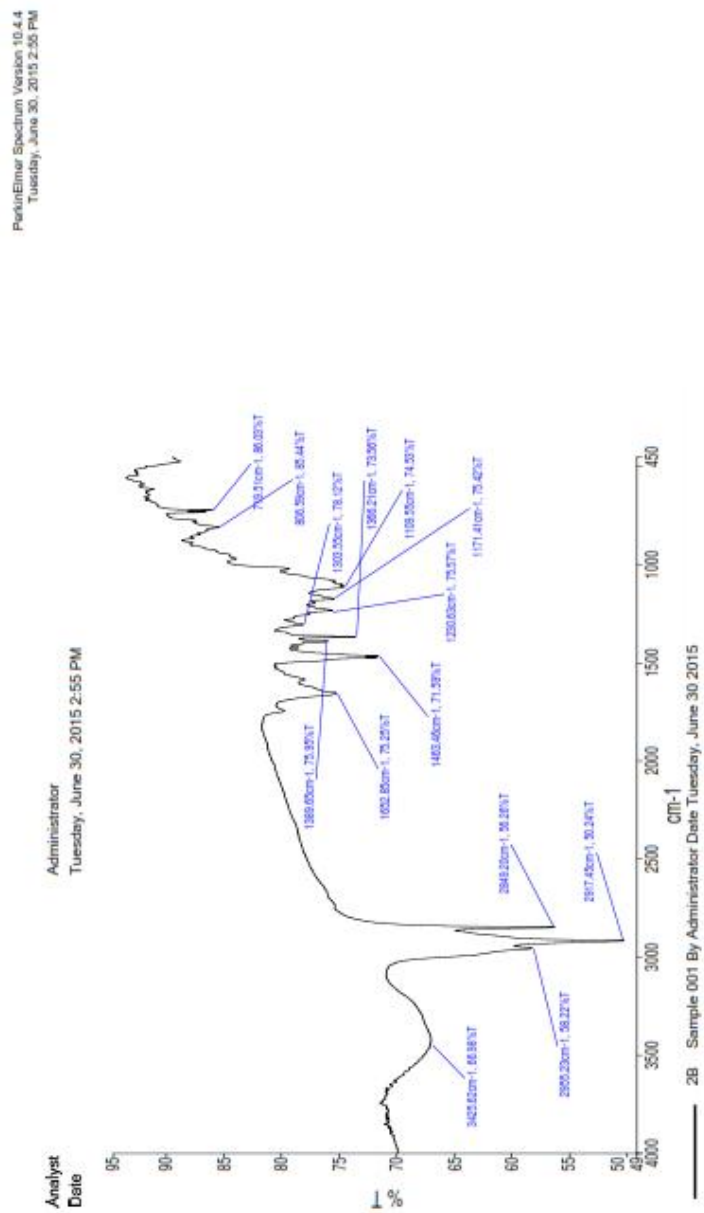


Figure S3: ^{13}C NMR of Punigratane

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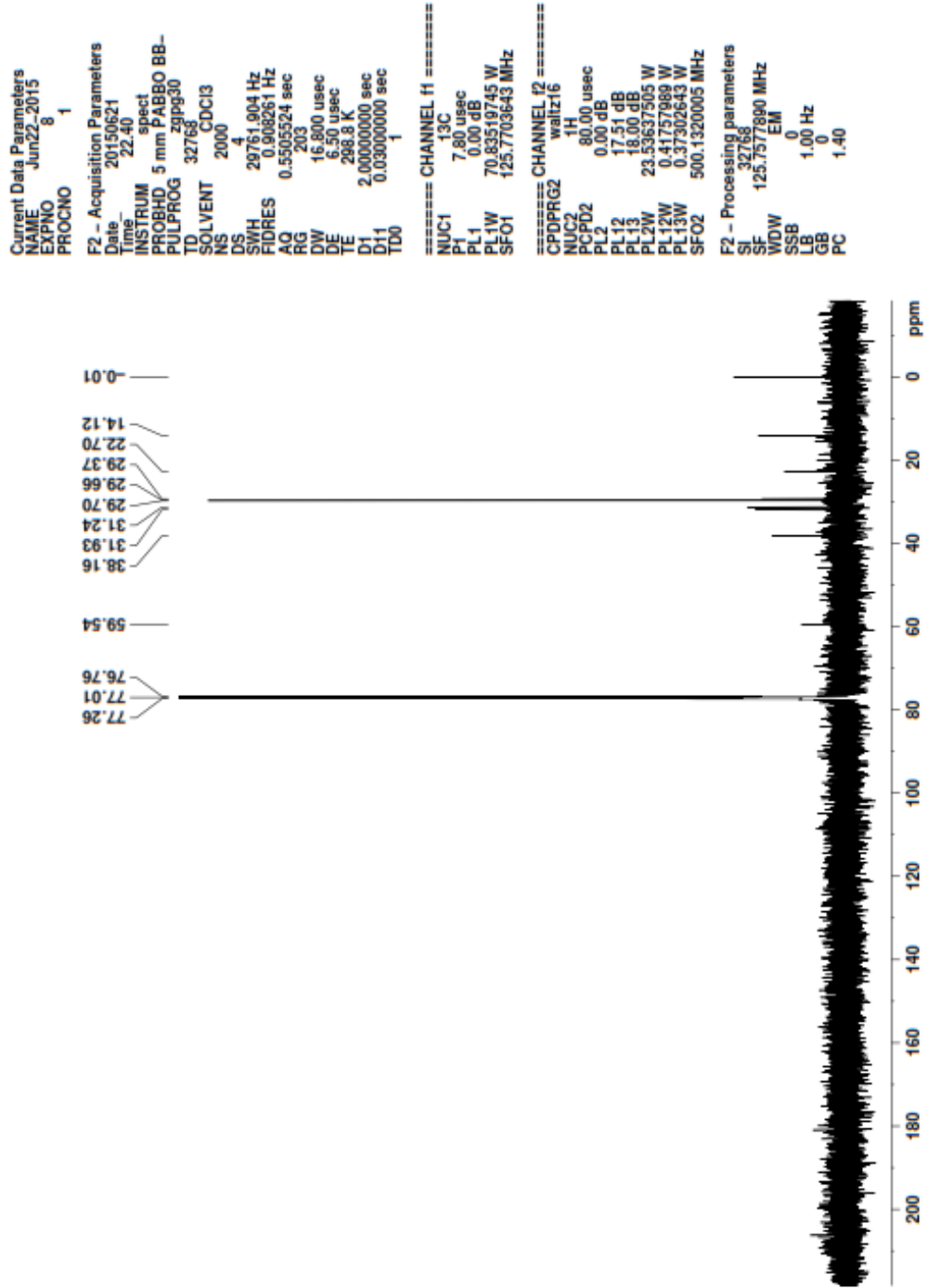


Figure S4: ¹H NMR of Punigratane

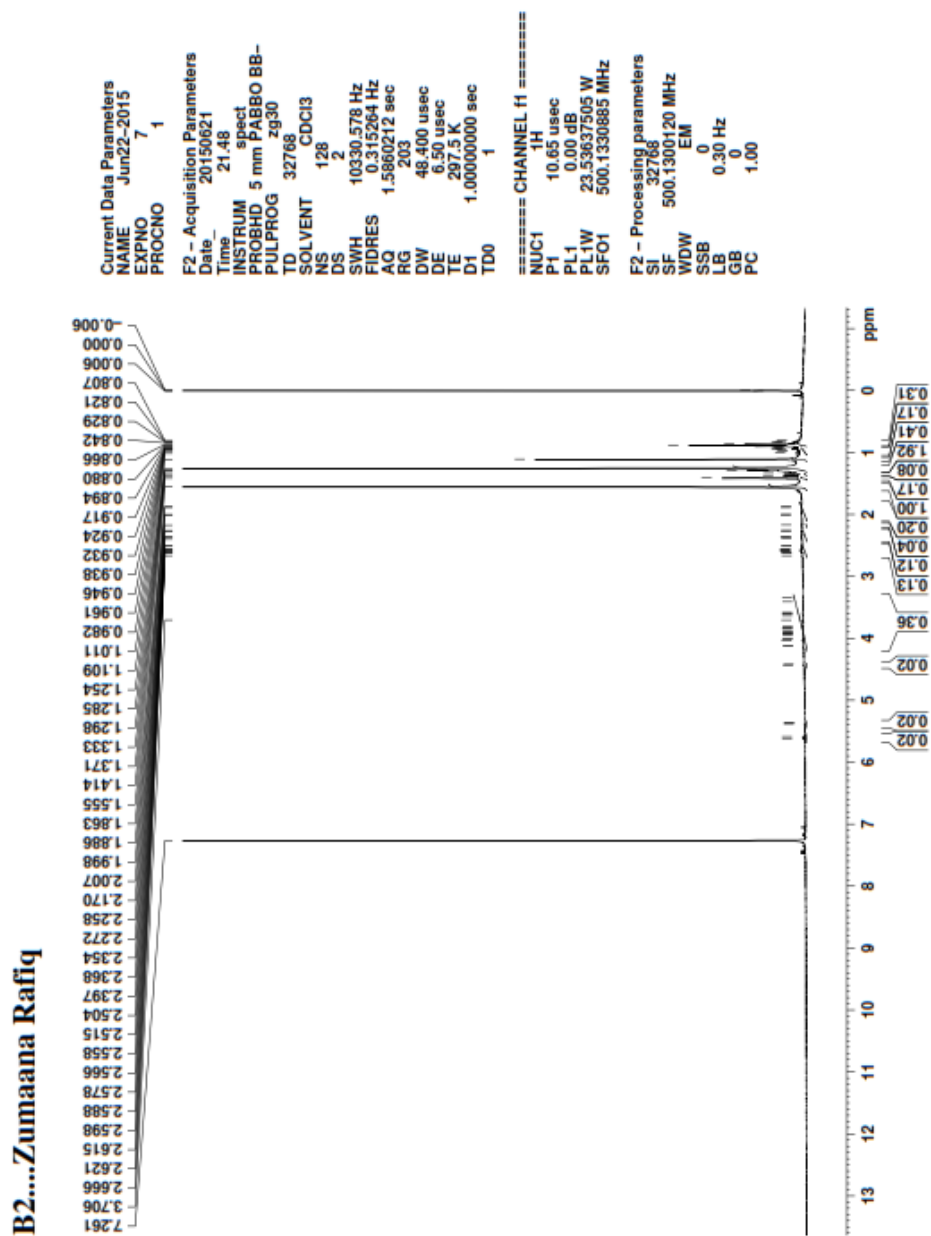


Figure S5: ^1H NMR of Punigratane – Expansion

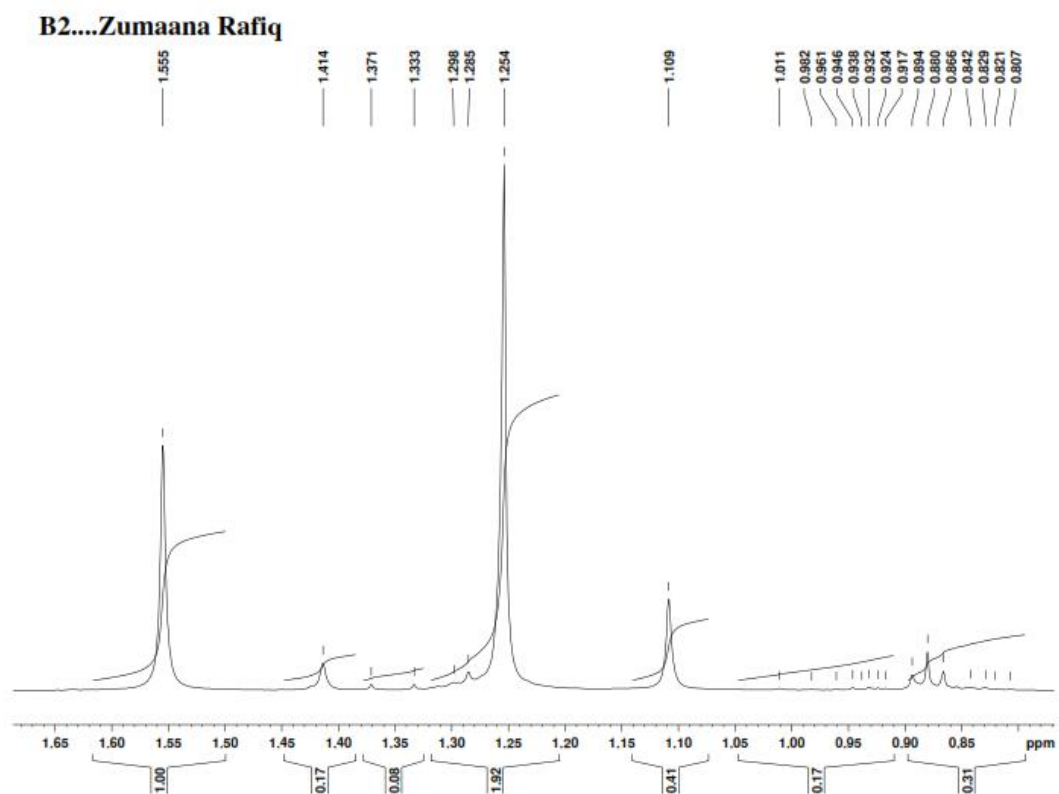
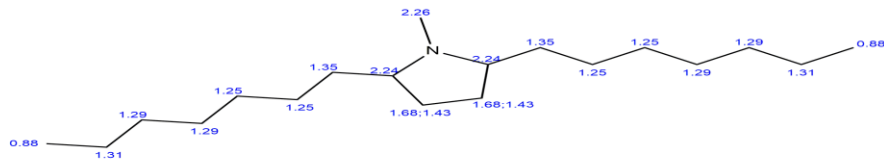
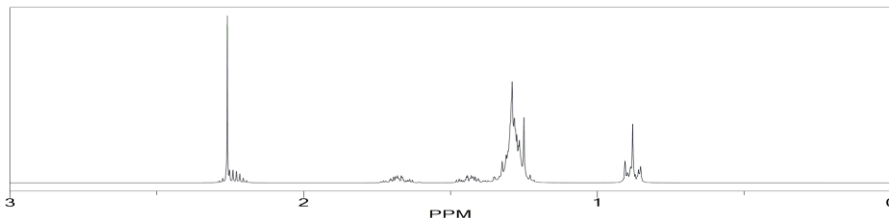


Figure S6: Chemdraw Simulated ^1H NMR of Punigratane

ChemNMR ¹H Estimation



Estimation quality is indicated by color: **good**, **medium**, **rough**



Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	2.24	2.75 -0.01 ?	pyrrolidine 1 beta -C from methine 1 unknown substituent(s)
CH	2.24	2.75 -0.01 ?	pyrrolidine 1 beta -C from methine 1 unknown substituent(s)
CH2	1.68,1.425000	-0.50 1.59 ?	1 -C from N-CHx pyrrolidine 1 beta -C from methylene 1 unknown substituent(s)
CH2	1.68,1.425000	-0.50 1.59 ?	1 -C from N-CHx pyrrolidine 1 beta -C from methylene 1 unknown substituent(s)
CH3	2.26	0.86 -1.41 -0.01	methyl 1 alpha -N(C)C general corrections
CH2	1.35	1.37 -0.06 -0.04 -0.04	methylene 1 beta -N(C)C 1 beta -C 1 beta -C
CH2	1.35	1.37 0.06 -0.04 -0.04	methylene 1 beta -N(C)C 1 beta -C 1 beta -C
CH2	1.25	1.37 -0.04 -0.04 -0.04	methylene 1 beta -C 1 beta -C general corrections
CH2	1.25	1.37 -0.04 -0.04 -0.04	methylene 1 beta -C 1 beta -C general corrections
CH2	1.25	1.37 -0.04 -0.04 -0.04	methylene 1 beta -C 1 beta -C general corrections
CH2	1.25	1.37 -0.04 -0.04 -0.04	methylene 1 beta -C 1 beta -C general corrections
CH2	1.29	1.37 -0.04 -0.04 0.00	methylene 1 beta -C 1 beta -C general corrections
CH2	1.29	1.37 -0.04 -0.04 0.00	methylene 1 beta -C 1 beta -C general corrections
CH2	1.29	1.37 -0.04 -0.04 0.00	methylene 1 beta -C 1 beta -C general corrections
CH2	1.29	1.37 -0.04 -0.04 0.00	methylene 1 beta -C 1 beta -C general corrections
CH2	1.31	1.37 0.00 -0.04 -0.02	methylene 1 alpha -C 1 beta -C general corrections
CH2	1.31	1.37 0.00 -0.04 -0.02	methylene 1 alpha -C 1 beta -C general corrections
CH3	0.88	0.86 -0.10 -0.08	methyl 1 beta -C-R general corrections
CH3	0.88	0.86 -0.10 -0.08	methyl 1 beta -C-R general corrections

¹H NMR Coupling Constant Prediction

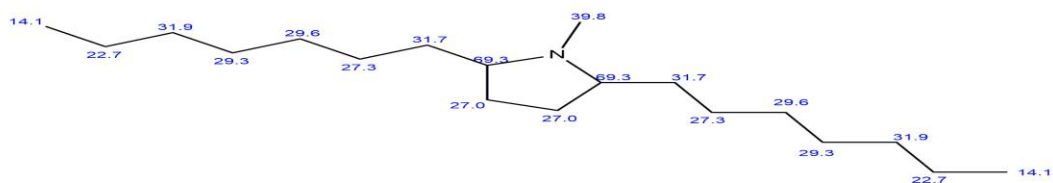
shift	atom index	coupling partner,	constant and vector
2.24	2	3 7.0 H-C-CH-H	
		13 7.0 H-C-CH-H	
2.24	5	4 7.0 H-C-CH-H	
		6 7.0 H-C-CH-H	
1.55	3 diastereotopic	-12.4 H-C-H	
		2 7.0 H-CH-C-H	
		4 7.1 H-CH-CH-H	
1.55	4 diastereotopic	-12.4 H-C-H	
		5 7.0 H-CH-C-H	
		3 7.1 H-CH-CH-H	
2.26	20		
1.35	6	5 7.0 H-CH-C-H	
		7 7.1 H-CH-CH-H	
1.35	13	2 7.0 H-CH-C-H	
		14 7.1 H-CH-CH-H	
1.25	7	6 7.1 H-CH-CH-H	
		8 7.1 H-CH-CH-H	
1.25	14	13 7.1 H-CH-CH-H	
		15 7.1 H-CH-CH-H	
1.25	8	7 7.1 H-CH-CH-H	
		9 7.1 H-CH-CH-H	
1.25	15	14 7.1 H-CH-CH-H	
		16 7.1 H-CH-CH-H	
1.29	9	8 7.1 H-CH-CH-H	
		10 7.1 H-CH-CH-H	
1.29	16	15 7.1 H-CH-CH-H	
		17 7.1 H-CH-CH-H	
1.29	10	9 7.1 H-CH-CH-H	
		11 7.1 H-CH-CH-H	
1.29	17	16 7.1 H-CH-CH-H	
		18 7.1 H-CH-CH-H	
1.31	11	10 7.1 H-CH-CH-H	
		12 8.0 H-CH-CH2-H	
1.31	18	17 7.1 H-CH-CH-H	
		19 8.0 H-CH-CH2-H	
0.88	12	11 8.0 H-CH2-CH-H	
0.88	19	18 8.0 H-CH2-CH-H	

Figure S7: Simulated ^1H NMR of Punigratane- nmrdb.org

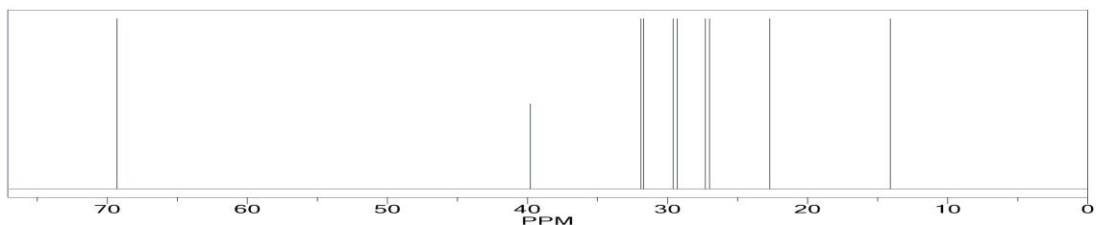
Spinus predicted chemical s...		
AtomID	From ▲	To ▲
54	0.866	0.866
38	0.866	0.866
40	0.866	0.866
39	0.866	0.866
55	0.866	0.866
53	0.866	0.866
47	1.233	1.233
48	1.233	1.233
32	1.233	1.233
33	1.233	1.233
50	1.24	1.24
34	1.24	1.24
49	1.24	1.24
35	1.24	1.24
46	1.251	1.251
31	1.251	1.251
30	1.251	1.251
45	1.251	1.251
29	1.255	1.255
44	1.255	1.255
28	1.255	1.255
43	1.255	1.255
51	1.278	1.278
52	1.278	1.278
36	1.278	1.278
37	1.278	1.278
26	1.33	1.33
27	1.33	1.33
42	1.33	1.33
41	1.33	1.33
24	1.7435	1.7435
20	1.7435	1.7435
25	1.7435	1.7435
21	1.7435	1.7435
58	2.364	2.364
57	2.364	2.364
56	2.364	2.364
22	2.818	2.818
23	2.818	2.818

Figure S8: Simulated ^{13}C NMR of Punigratane

ChemNMR ¹³C Estimation



Estimation quality is indicated by color: **good**, **medium**, **rough**



Protocol of the C-13 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH 69.3	69.3	-9.1	pyrrolidine
		18.2	2 alpha -C from aliphatic
		28.3	1 alpha -N from aliphatic
		37.6	4 beta -C from aliphatic
		-5.0	2 gamma -C from aliphatic
CH 69.3	69.3	0.6	2 delta -C from aliphatic
		-1.3	general corrections
		18.2	pyrrolidine
		28.3	2 alpha -C from aliphatic
		37.6	1 alpha -N from aliphatic
CH2 27.0	27.0	5.0	4 beta -C from aliphatic
		0.6	2 gamma -C from aliphatic
		-1.3	2 delta -C from aliphatic
		-13.2	general corrections
		18.2	pyrrolidine
CH2 27.0	27.0	18.8	2 alpha -C from aliphatic
		11.3	2 beta -C from aliphatic
		-7.5	1 beta -N from aliphatic
		0.6	3 gamma -C from aliphatic
		-1.2	2 delta -C from aliphatic
CH3 39.8	39.8	-2.3	general corrections
		28.3	aliphatic
		18.8	2 alpha -N
		-10.0	2 beta -C
		0.6	4 gamma -C
CH2 31.7	31.7	4.4	2 delta -C
		-2.3	general corrections
		18.2	aliphatic
		18.8	2 alpha -C
		11.3	2 beta -C
CH2 31.7	31.7	11.3	1 beta -N
		-10.0	4 gamma -C
		0.6	2 delta -C
		-4.9	general corrections
		-2.3	aliphatic
CH2 31.7	31.7	18.2	2 alpha -C
		18.8	2 beta -C
		11.3	1 beta -N
		-10.0	4 gamma -C
		0.6	2 delta -C
CH2 27.3	27.3	-4.9	general corrections
		-2.3	aliphatic
		18.2	2 alpha -C
		18.8	2 beta -C
		-5.0	2 gamma -C
CH2 27.3	27.3	-5.1	1 gamma -N
		1.2	4 delta -C
		1.5	general corrections
		-2.3	aliphatic
		18.2	2 alpha -C
CH2 29.6	29.6	18.8	2 beta -C
		-5.0	2 gamma -C
		0.6	2 delta -C
		0.0	1 delta -N
		-0.7	general corrections
CH2 29.6	29.6	-2.3	aliphatic
		18.2	2 alpha -C
		18.8	2 beta -C
		-5.0	2 gamma -C
		0.6	2 delta -C
CH2 29.3	29.3	0.0	1 delta -N
		-0.7	general corrections
		-2.3	aliphatic
		18.2	2 alpha -C
		18.8	2 beta -C
CH2 29.3	29.3	5.0	2 gamma -C
		0.3	1 delta -C
		-0.7	general corrections
		-2.3	aliphatic
		18.2	2 alpha -C
CH2 31.9	31.9	18.8	2 beta -C
		-2.5	1 gamma -C
		0.3	1 delta -C
		-0.6	general corrections
		-2.3	aliphatic
CH2 31.9	31.9	18.2	2 alpha -C
		18.8	2 beta -C
		-2.5	1 gamma -C
		0.3	1 delta -C
		-0.6	general corrections
CH2 22.7	22.7	-2.3	aliphatic
		18.2	2 alpha -C
		9.4	1 beta -C
		-2.5	1 gamma -C
		0.3	1 delta -C
CH2 22.7	22.7	-0.4	general corrections
		-2.3	aliphatic
		18.2	2 alpha -C
		9.4	1 beta -C
		-2.5	1 gamma -C
CH3 14.1	14.1	0.3	1 delta -C
		-0.4	general corrections
		-2.3	aliphatic
		9.1	1 alpha -C
		9.4	1 beta -C
CH3 14.1	14.1	-2.5	1 gamma -C
		0.3	1 delta -C
		-0.1	general corrections
		-2.3	aliphatic
		9.1	1 alpha -C

Figure S9: Simulated ^{13}C NMR of Punigratane – nmrdB.org

