10.1071/CH19227_AC

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SUPPLEMENTARY MATERIAL

A Sesquiterpene Isonitrile with a New Tricyclic Skeleton from the Indo-Pacific Nudibranch *Phyllidiella pustulosa*: Spectroscopic and Computational Studies

Desmond C.-M. Sim[A], *Natasha L. Hungerford*[A], *Elizabeth H. Krenske*[A], *Gregory K. Pierens*[B], *Katherine T. Andrews*[C], *Tina S. Skinner-Adams*[C], *and Mary J. Garson*[A,D]

[A]School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD 4072, Australia

[B]Centre for Advanced Imaging, The University of Queensland, Brisbane QLD 4072, Australia

[C]Griffith Institute for Drug Discovery, Griffith University, Nathan QLD 4111, Australia

[D]Corresponding author. Email: m.garson@uq.edu.au

Supplementary Material

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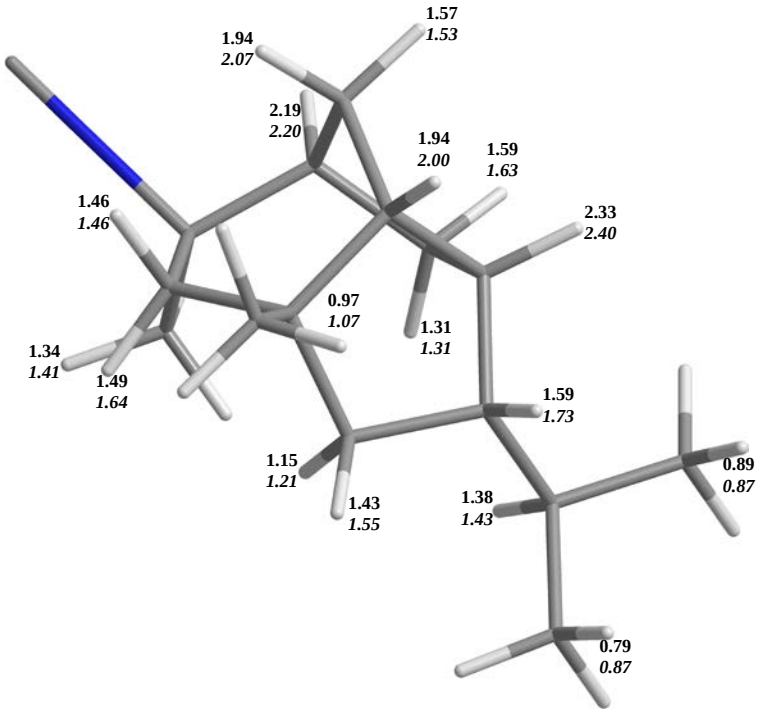


Figure S1. Theoretical (top) and experimental (bottom, underlined and italicized) ^{1}H and ^{13}C chemical shifts for 9-isocyanoneoallopupukeanane (**1**)

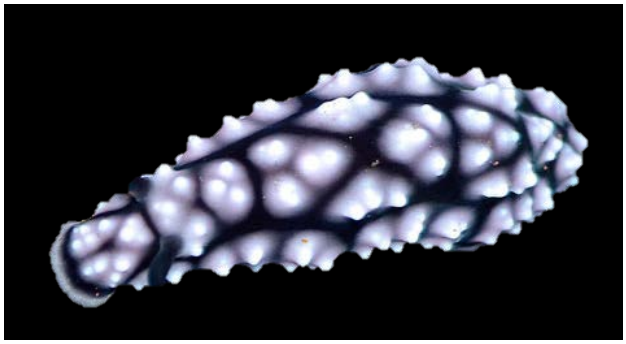
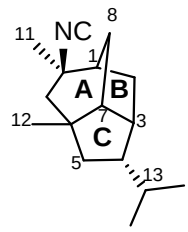
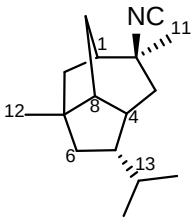


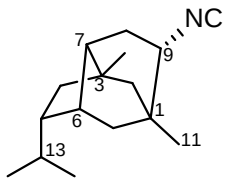
Figure S2. Image of *Phyllidiella pustulosa*



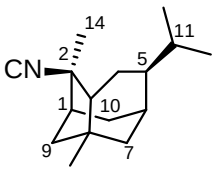
9-isocyanoneoallopupukeanane (**1**)



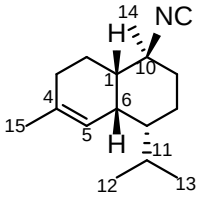
2-isocyanoallopupukeanane (**2**)



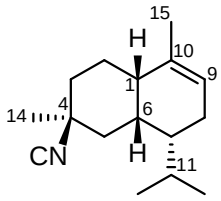
9-isocyanopupukeanane (**3**)



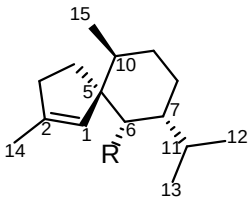
2-isocyanotrachyopsane



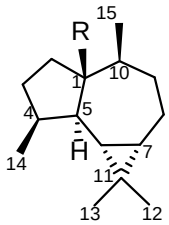
(1S*,6R*,7R*,10S*)-
10-isocyano-4-amorphene



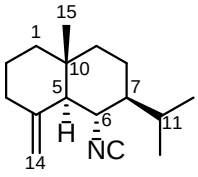
(1S*,4S*,6S*,7R*)-
4-isocyano-9-amorphene



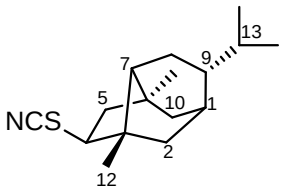
axisonitrile-3; R= NC
axisothiocyanate-3; R= NCS



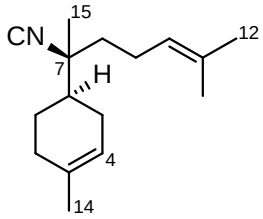
1-isocyanoaromadendrane; R = NC
1-isothiocyanatoaromadendrane; R = NCS
1-isocyanatoaromadendrane; R = NCO



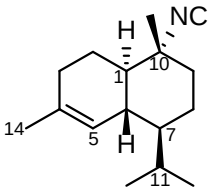
halichonadin C



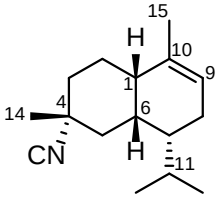
4-thiocyanatoneopupukeanane



7-isocyano-7,8-dihydro-$^{\alpha}$-bisabolene



10-isocyano-4-cadinene



4$^{\alpha}$-isocyano-9-amorphene

Figure S3. Structures of isolated isonitrile sesquiterpenes

Associated spectra

Figure S4. ^{1}H NMR spectrum of 9-isocyanoneoallopupukeanane (**1**) ($CDCl_3$)

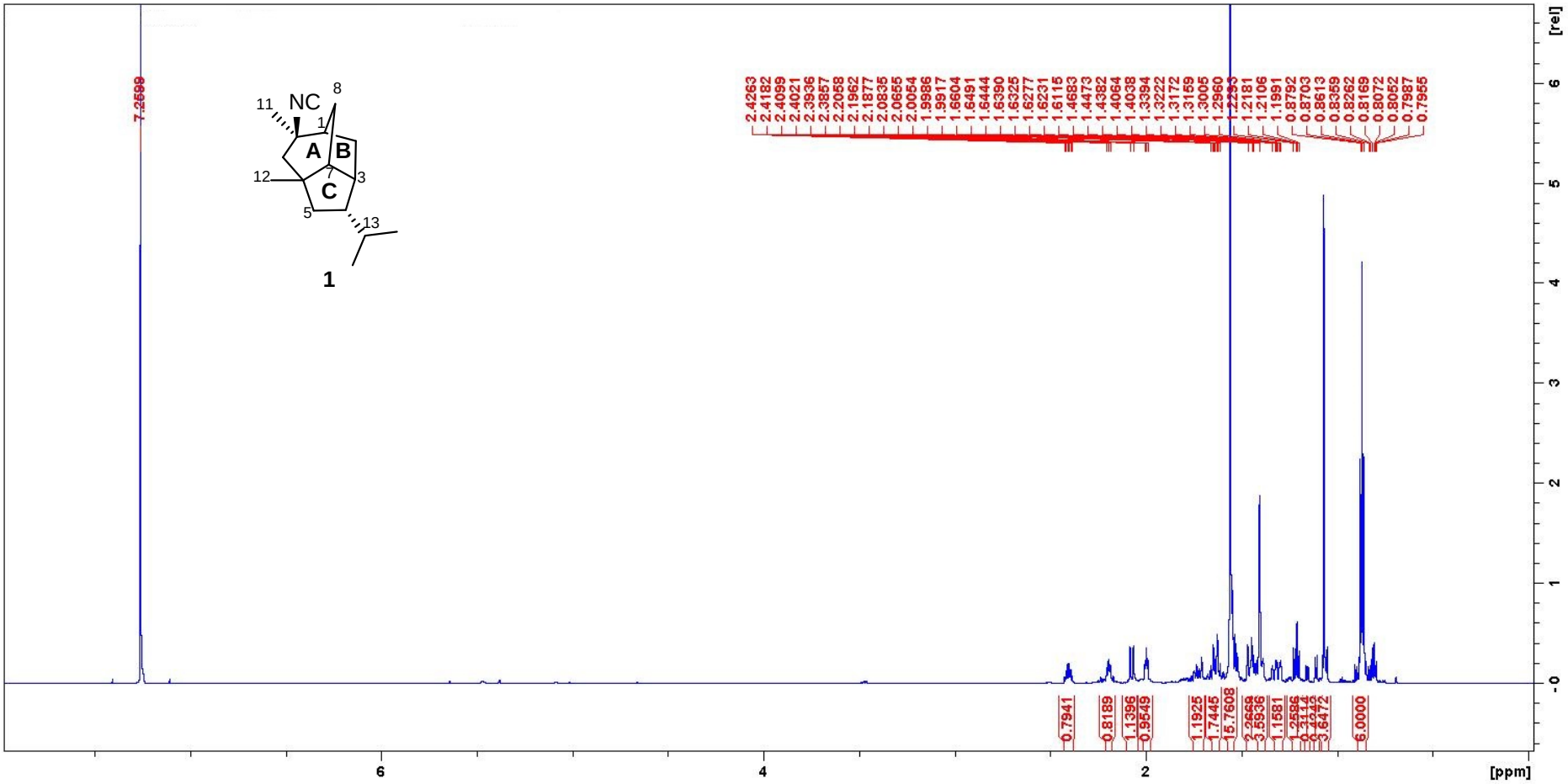


Figure S5. ^{1}H NMR spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$) up field region (0 - 3 ppm)

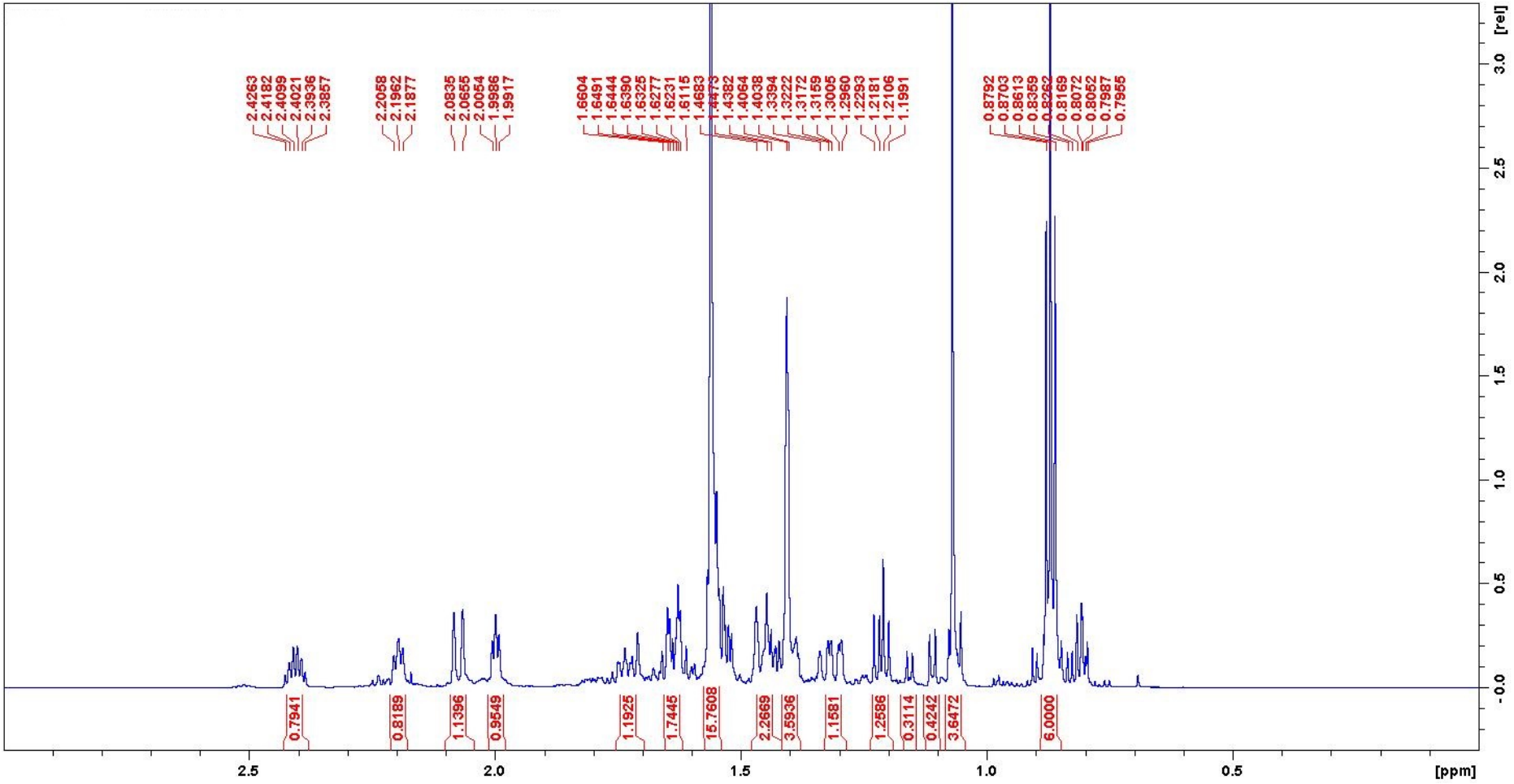
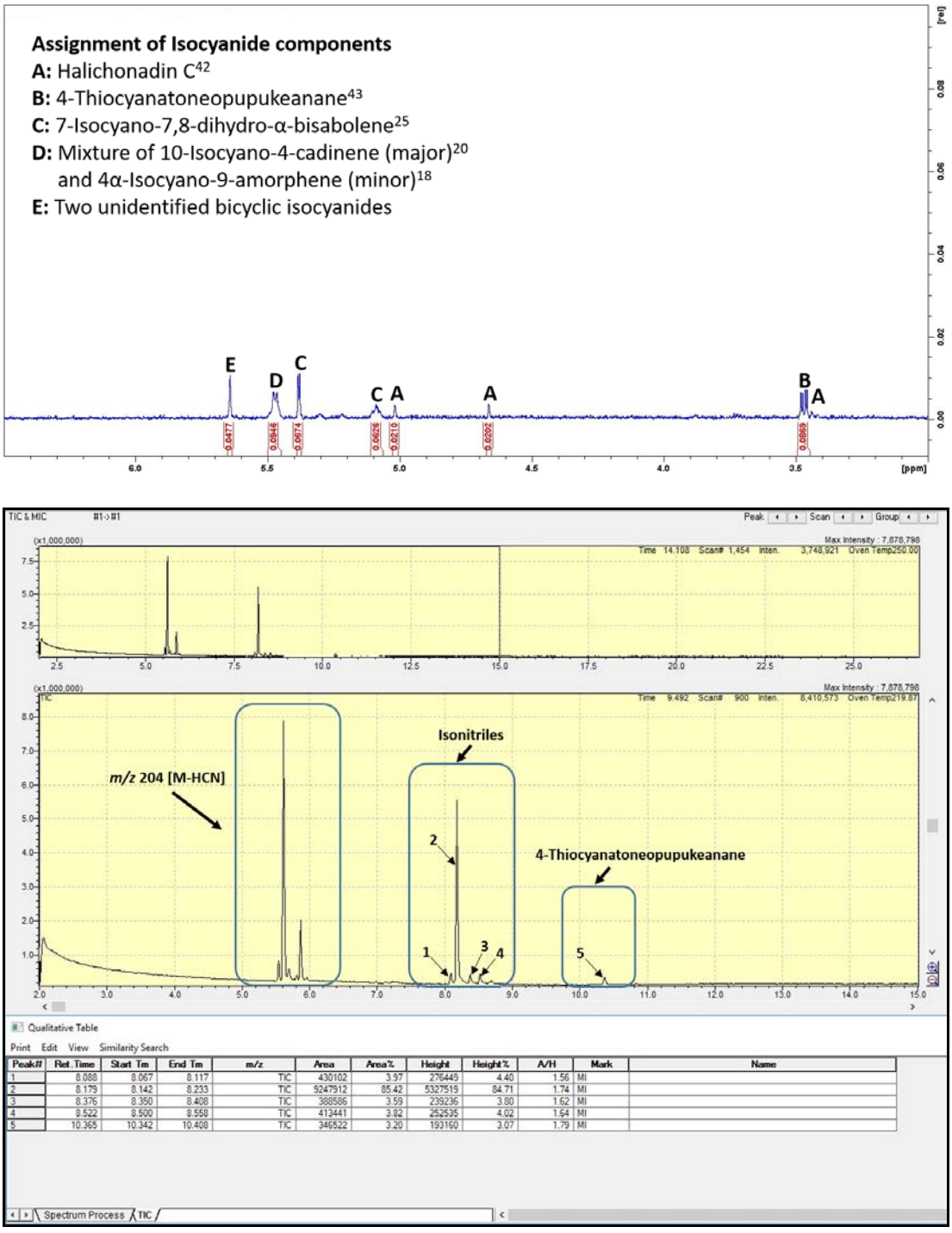


Figure S6. ^{1}H NMR spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$) down field region (3 – 6.5 ppm) showing putative identification of trace isocyanide and thiocyanate components and GC-MS chromatogram of the 9-isocyanoneoallopupukeanane (**1**) fraction



[42] H. Ishiyama, A. Hashimoto, J. Fromont, Y. Hoshino, Y. Mikami, J. Kobayashi, *Tetrahedron* **2005**, *61*, 1101.

[43] A. T. Pham, T. Ichiba, W. Yoshida, P. J. Scheuer, T. Uchida, J. Tanaka, T. Higa, *Tetrahedron Lett.* **1991**, *32*, 4843.

[25] P. Jumaryatno, B. L. Stapleton, J. N. A. Hooper, D. J. Brecknell, J. T. Blanchfield, M. J. Garson, *J. Nat. Prod.* **2007**, *70*, 1725.

[20] T. Okino, E. Yoshimura, H. Hirota, N. Fusetani, *Tetrahedron* **1996**, *52*, 9447.

[18] N. Fusetani, H. J. Wolstenholme, S. Matsunaga, H. Hirota, *Tetrahedron Lett.* **1991**, *32*, 7291.

Figure S7. HSQC spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$)

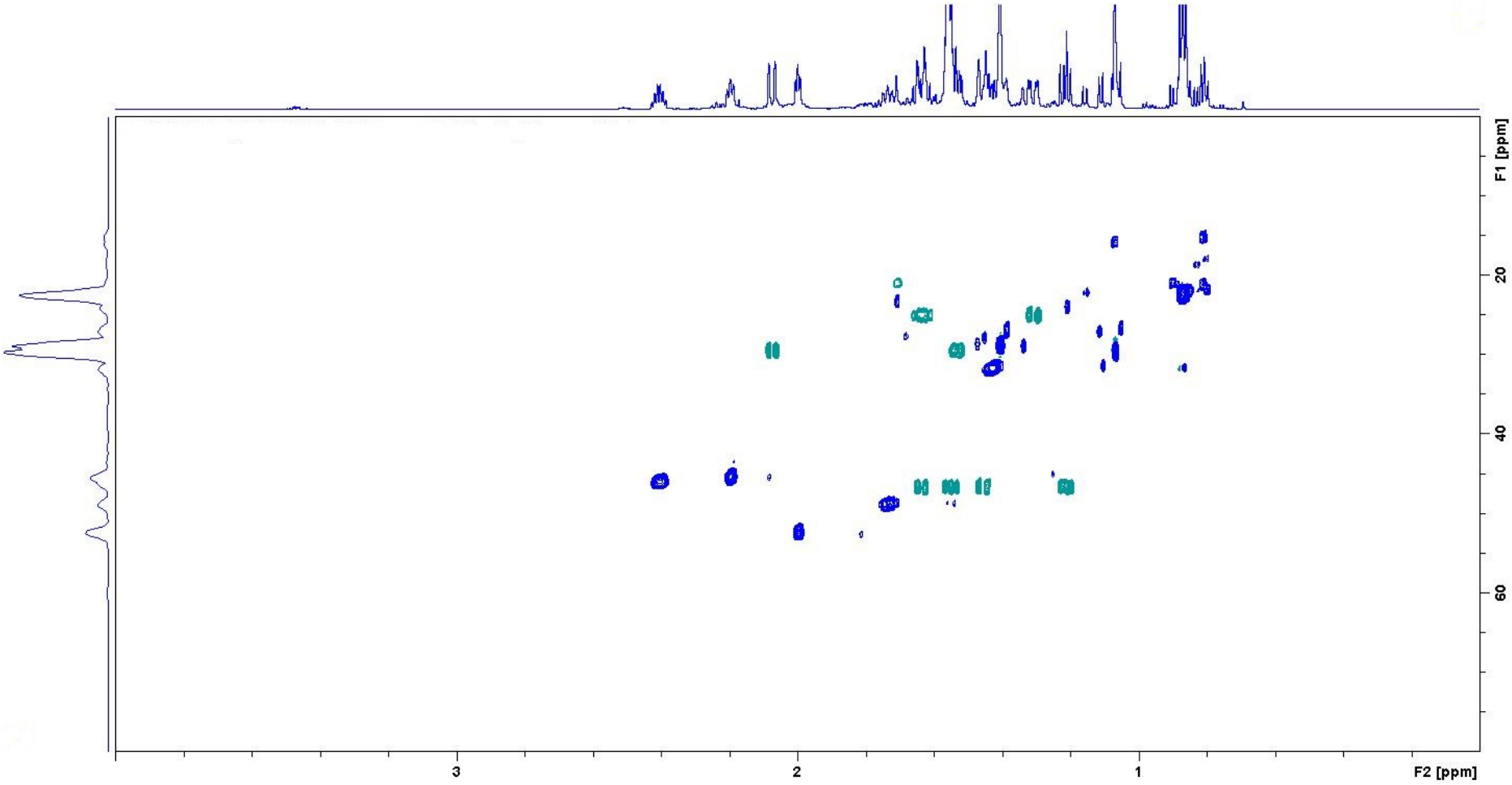


Figure S8. HMBC spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$)

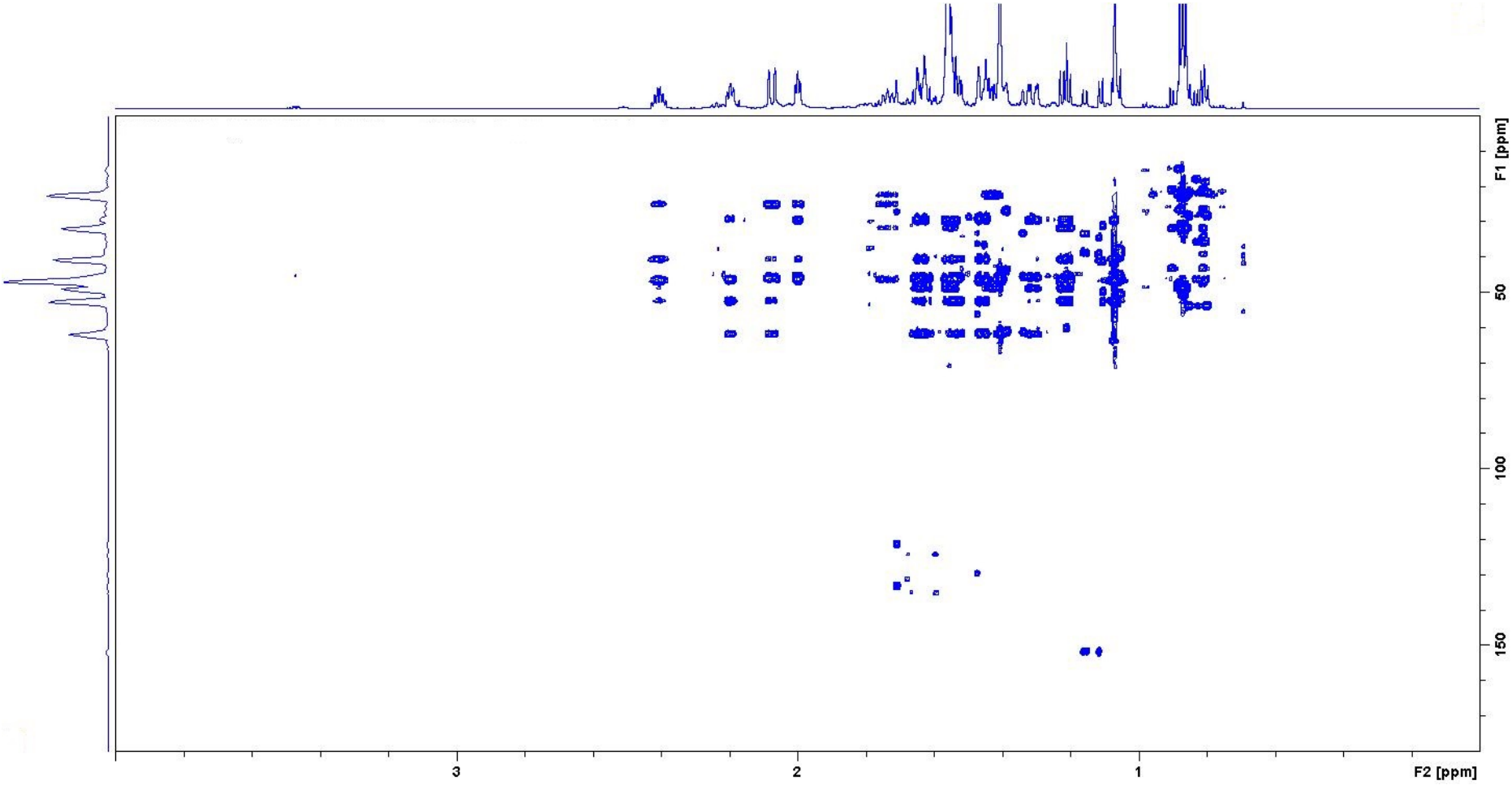


Figure S9. H2BC spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$)

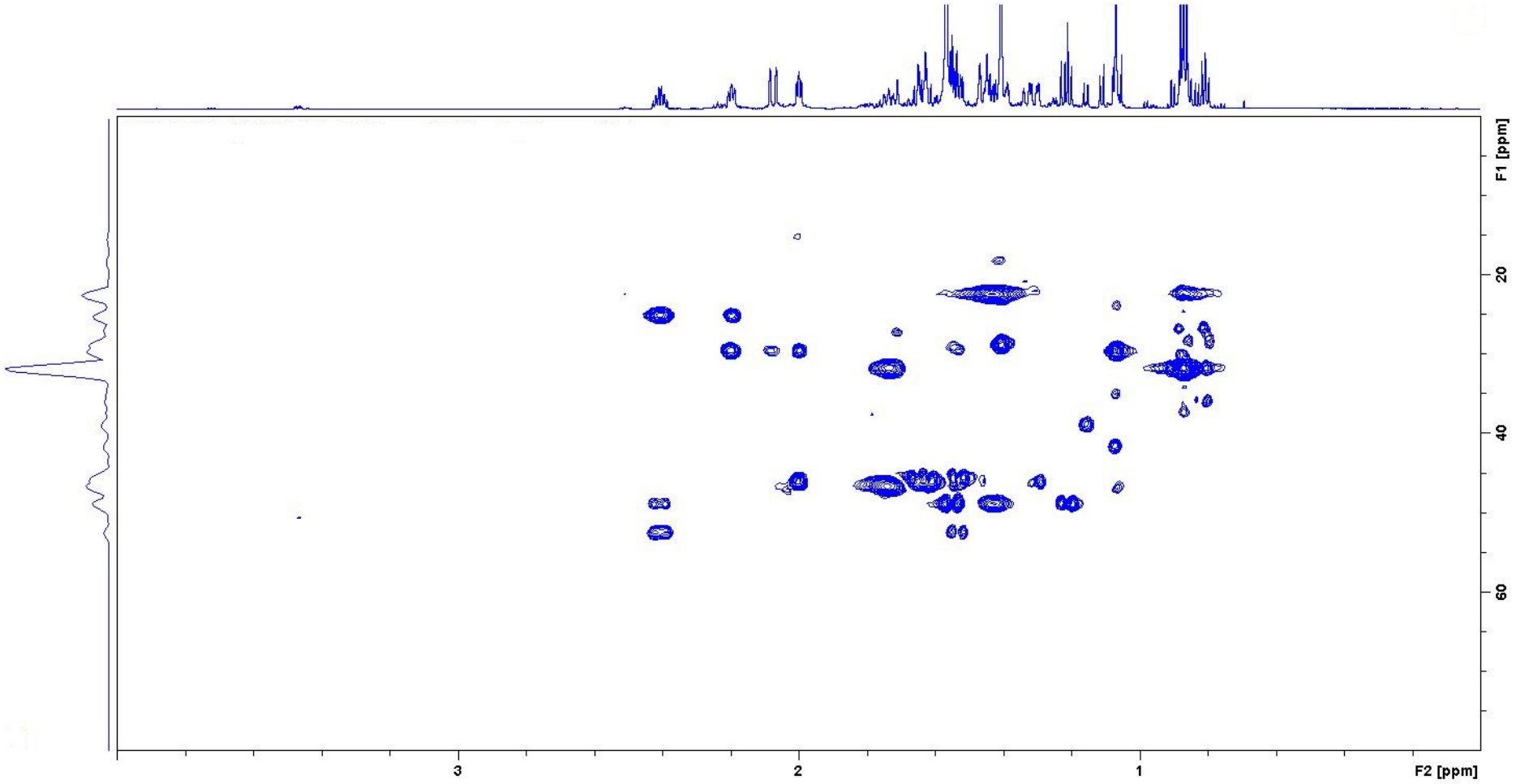


Figure S10. gCOSY spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, CDCl3)

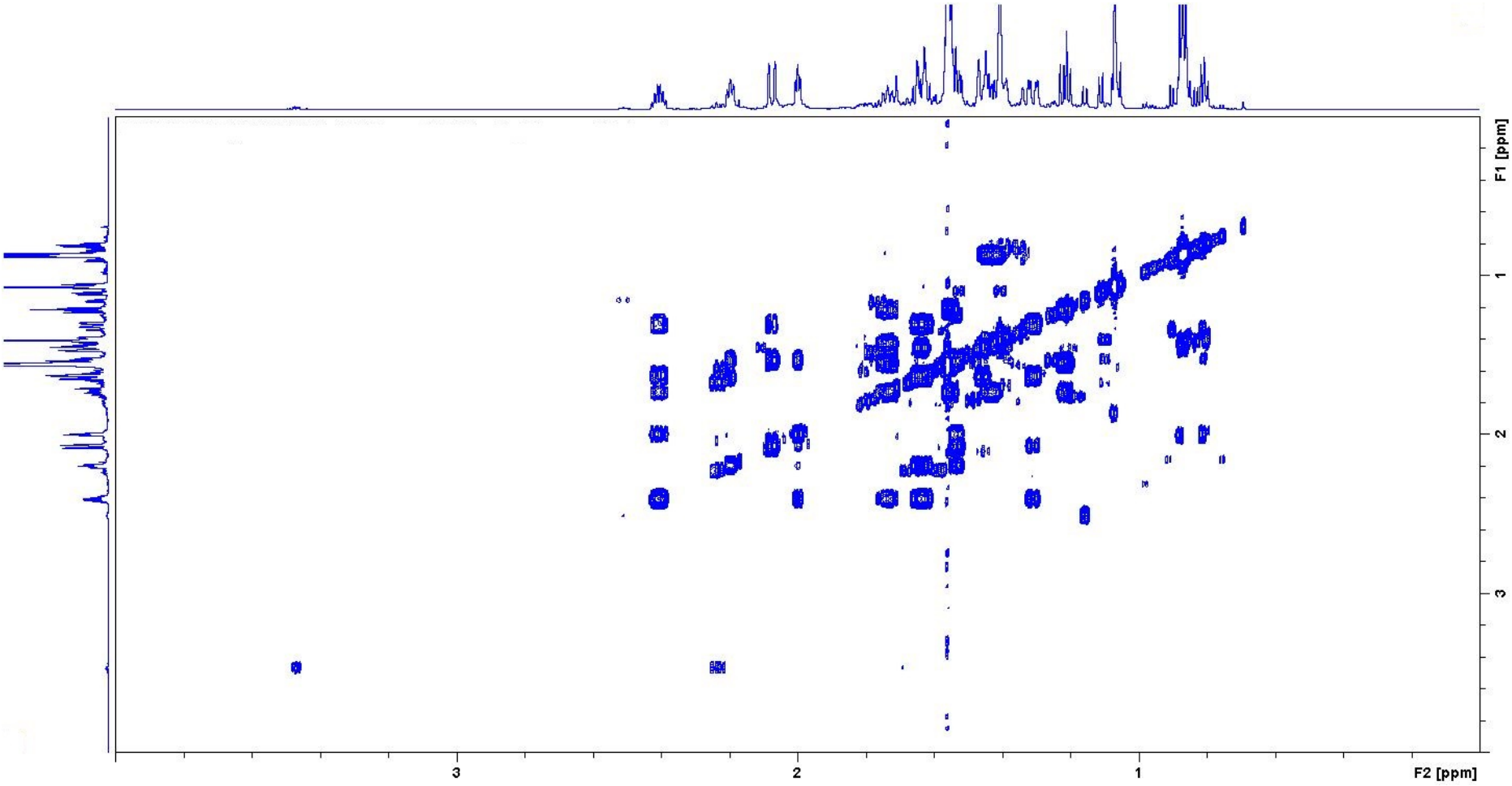
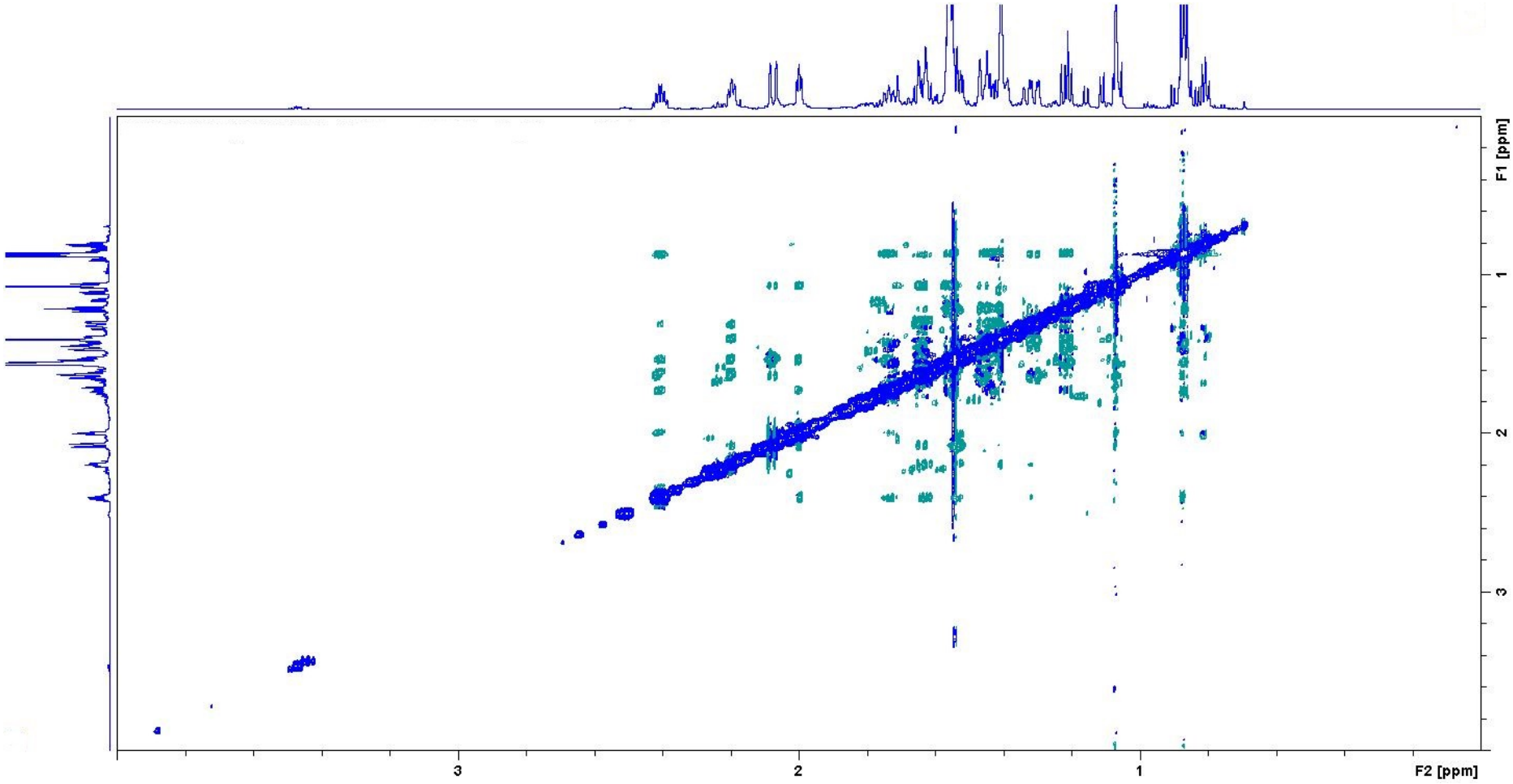


Figure S11. NOESY spectrum of 9-isocyanoneoallopupukeanane (**1**) (700 MHz, $CDCl_3$)



Molecular modeling and DFT calculations

A conformational search was with the Monte Carlo Multiple Minimum (MCMM) using Macromodel (Schrodinger Inc). The search provided five conformers (< 5 kcal/mol), including two lowest energy conformers (< 3 kcal/mol) in which ring A was either in a boat (80%) or in a chair (20%) conformation (**Figure S11**). All conformers were then further optimized by DFT calculations with Gaussian09w (Revision D.01) using B3LYP/6-31G(d) method which generated four unique candidate conformers which were further optimized using B3LYP/6-311+G(2d,p) and free energies calculated. The free energies of these four conformers were then used to calculate the Boltzmann-weighted ^{1}H and ^{13}C NMR chemical shifts using a single point calculation (mpw1pw91/6-311+g(2d,p)) in chloroform solvent (IEF-PCM). The calculation verified that the major conformer had a twist boat conformation in ring A.

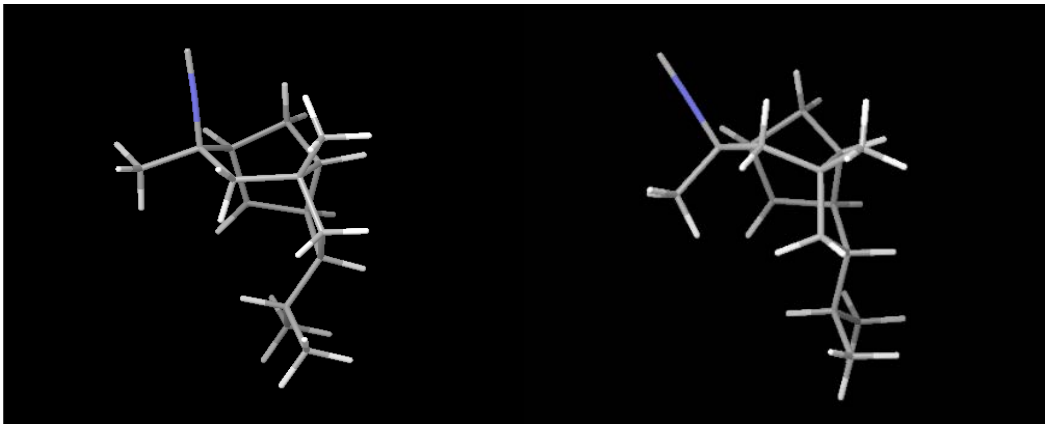


Figure S12. Overlay of two lowest energy conformers of 9-isocyanoneoallopupukeanane (**1**): Chair (left, 20 %) and twist boat (right, 80%) low energy conformers of ring A in 9-isocyanoneoallopupukeanane (**1**) (< 3 kcal/mol of the global minimum)

Table S1. ^{1}H NMR experimental and calculated chemical shifts for 9-isocyanoneoallopupukeanane (**1**)

Proton	Expt	Caculated
1	2.2	2.19
2a	1.63	1.59
2b	1.31	1.31
3	2.4	2.33
4	1.73	1.59
5a	1.55	1.43
5b	1.21	1.15
7	2	1.94
8a	2.07	1.94
8b	1.53	1.57
10a	1.64	1.49
10b	1.46	1.46
11	1.41	1.34
12	1.07	0.97
13	1.43	1.38
14	0.87	0.79
15	0.87	0.89
	MAE	**0.07**

Table S2. ^{13}C NMR experimental and calculated chemical shifts for 9-isocyanoneoallopupukeanane (**1**)

Carbon	Expt	Calculated
1	45.5	48.3
2	25.1	25.3
3	46.1	48.1
4	48.8	48.6
5	46.6	44.9
6	40.6	42.8
7	52.4	53.3
8	29.5	30.5
9	61.9	62.4
10	46.6	46.4
11	28.8	27.4
12	29.5	29.0
13	31.7	33.4
14	22.5	20.2
15	22.5	20.5
-NC	151.9	155.1
	MAE	**1.31**

XYZ coordinates for the four conformers of 9-isocyanoneoallopupukeanane (**1**)

Compound (**1**): Conformer 1, Energy:-679.333882 Hartree, Solvent: chloroform, Boltzmann %: 81.438

Zero-point correction=	0.378434 (Hartree/Particle)
Thermal correction to Energy=	0.395004
Thermal correction to Enthalpy=	0.395949
Thermal correction to Gibbs Free Energy=	0.336448
Sum of electronic and zero-point Energies=	-679.291896
Sum of electronic and thermal Energies=	-679.275326
Sum of electronic and thermal Enthalpies=	-679.274381
Sum of electronic and thermal Free Energies=	-679.333882

C	0.318118	1.461065	-0.020287
C	1.616985	0.982422	-0.713914
C	1.979389	-0.530583	-0.550618
C	1.401119	-1.108440	0.782484
H	2.446908	1.555206	-0.298464
H	1.579754	1.230086	-1.778208
C	-0.916200	-0.279093	1.208529
C	0.163985	0.802786	1.401868
C	1.402615	0.013208	1.833525
C	-0.095460	-1.517769	0.731376
H	-0.134379	1.558336	2.132280
H	2.328251	0.592098	1.851959
H	-0.243542	-2.350280	1.421831
H	-0.403696	-1.882798	-0.246948
C	-0.963215	1.037032	-0.807664
C	-1.899140	0.338011	0.196853
H	-0.711960	0.348300	-1.618448
H	-1.434932	1.899952	-1.281468
H	-2.451849	1.115493	0.743654
C	0.389365	2.990152	0.101845
H	1.212494	3.297307	0.753332
H	0.541113	3.459461	-0.874694
H	-0.537662	3.387848	0.523300
C	-2.949129	-0.597577	-0.428535
H	-2.418648	-1.363803	-1.008190
C	-3.867565	0.158859	-1.398744
H	-4.410062	0.955433	-0.879100
H	-3.310551	0.615399	-2.219260
H	-4.609025	-0.514471	-1.836517
C	-3.787091	-1.313334	0.639060
H	-3.173362	-1.935807	1.293200
H	-4.317689	-0.590668	1.267519
H	-4.536700	-1.959600	0.175075
C	1.632505	-1.371129	-1.788718
H	0.574138	-1.298745	-2.029385
H	2.199687	-1.012737	-2.648994
H	1.880062	-2.421686	-1.624225

H	2.023499	-1.960587	1.059548
H	1.256524	-0.399667	2.835489
N	3.427566	-0.599983	-0.425179
C	4.586016	-0.667631	-0.335098
H	-1.415124	-0.512049	2.151199

Compound (**1**): Conformer 2, Energy:-679.332465 Hartree, Solvent: chloroform, Boltzmann %: 18.157

Zero-point correction= 0.378491 (Hartree/Particle)
Thermal correction to Energy= 0.395090
Thermal correction to Enthalpy= 0.396034
Thermal correction to Gibbs Free Energy= 0.336671
Sum of electronic and zero-point Energies= -679.290646
Sum of electronic and thermal Energies= -679.274047
Sum of electronic and thermal Enthalpies= -679.273102
Sum of electronic and thermal Free Energies= -679.332465

C	0.349108	1.398961	-0.078301
C	0.876415	0.334506	-1.098531
C	1.809431	-0.775448	-0.565957
C	1.348134	-1.193958	0.855876
H	0.023358	-0.190189	-1.528932
H	1.364440	0.839749	-1.935383
C	-0.887394	-0.159373	1.309318
C	0.278178	0.845329	1.373260
C	1.476888	0.008049	1.802106
C	-0.178761	-1.495065	0.890339
H	0.071351	1.677209	2.053043
H	2.436280	0.520132	1.729094
H	-0.366776	-2.263195	1.642453
H	-0.544692	-1.899947	-0.052208
C	-1.148628	1.629133	-0.387156
C	-1.943986	0.519993	0.379917
H	-1.334907	1.622972	-1.463669
H	-1.450521	2.616447	-0.026720
H	-2.642362	1.032452	1.051695
C	1.173160	2.686891	-0.168085
H	0.798864	3.436402	0.534350
H	2.226948	2.509751	0.059589
H	1.122045	3.115703	-1.173102
C	-2.822483	-0.404342	-0.488108
H	-2.183901	-0.925801	-1.212480
C	-3.864831	0.395736	-1.282868
H	-4.478296	-0.269676	-1.895826
H	-4.535733	0.934047	-0.605538
H	-3.407237	1.129118	-1.948929
C	-3.537863	-1.461468	0.365064
H	-2.840274	-2.100138	0.908712
H	-4.191604	-0.981630	1.100600

H	-4.162010	-2.107015	-0.258353
C	1.850656	-1.963137	-1.540064
H	2.567655	-2.711349	-1.198258
H	0.868756	-2.430266	-1.611960
H	2.146359	-1.629570	-2.536456
H	1.941497	-2.053736	1.174772
H	1.357179	-0.315651	2.840163
N	3.171959	-0.278336	-0.485253
C	4.277479	0.084434	-0.458247
H	-1.323151	-0.308910	2.298732

Compound (**1**): Conformer 3, Energy:-679.328325 Hartree, Solvent: chloroform, Boltzmann %: 0.226

Zero-point correction= 0.378664 (Hartree/Particle)
Thermal correction to Energy= 0.395186
Thermal correction to Enthalpy= 0.396131
Thermal correction to Gibbs Free Energy= 0.336794
Sum of electronic and zero-point Energies= -679.286455
Sum of electronic and thermal Energies= -679.269932
Sum of electronic and thermal Enthalpies= -679.268988
Sum of electronic and thermal Free Energies= -679.328325

C	0.354957	1.430873	-0.454418
C	1.668883	0.747477	-0.904460
C	1.959706	-0.668838	-0.306303
C	1.264877	-0.848202	1.082993
H	2.497683	1.393580	-0.612712
H	1.698912	0.689393	-1.995900
C	-1.033750	0.142998	1.118019
C	0.093418	1.196405	1.084190
C	1.262344	0.512810	1.794823
C	-0.245980	-1.203301	1.018602
H	-0.203896	2.133822	1.559523
H	2.213565	1.043877	1.721610
H	-0.487709	-1.838968	1.872657
H	-0.497724	-1.780603	0.134216
C	-0.901838	0.839544	-1.174024
C	-1.927527	0.559033	-0.065453
H	-0.647746	-0.079676	-1.704897
H	-1.291971	1.527968	-1.928122
H	-2.336455	1.535166	0.231222
C	0.481100	2.931825	-0.752446
H	1.274596	3.388475	-0.153986
H	0.711890	3.108632	-1.807148
H	-0.452482	3.452194	-0.522730
C	-3.171577	-0.269709	-0.458775
H	-3.627558	0.281435	-1.292191
C	-4.200718	-0.290987	0.680292
H	-3.828129	-0.853648	1.541101

H	-4.441003	0.719780	1.020851
H	-5.131502	-0.764434	0.357232
C	-2.920593	-1.697377	-0.964048
H	-3.846308	-2.113107	-1.370815
H	-2.174319	-1.733870	-1.760732
H	-2.595377	-2.362318	-0.160966
C	1.670322	-1.809767	-1.293371
H	1.857155	-2.780027	-0.828958
H	0.637980	-1.782439	-1.634827
H	2.316371	-1.714377	-2.167281
H	1.819491	-1.617628	1.622280
H	1.037619	0.391637	2.858119
N	3.391201	-0.729016	-0.050891
C	4.536370	-0.792825	0.147115
H	-1.581467	0.178503	2.062277

Compound (**1**): Conformer 4, Energy:-679.328099 Hartree, Solvent: chloroform, Boltzmann %: 0.178

Zero-point correction= 0.378616 (Hartree/Particle)
Thermal correction to Energy= 0.395189
Thermal correction to Enthalpy= 0.396133
Thermal correction to Gibbs Free Energy= 0.336489
Sum of electronic and zero-point Energies= -679.285972
Sum of electronic and thermal Energies= -679.269400
Sum of electronic and thermal Enthalpies= -679.268455
Sum of electronic and thermal Free Energies= -679.328099

C	0.206560	1.391124	-0.348439
C	1.485358	0.800139	-0.989075
C	1.955901	-0.591892	-0.451053
C	1.480681	-0.816762	1.021374
H	2.301202	1.502549	-0.814297
H	1.361256	0.742739	-2.073891
C	-0.851979	0.016450	1.392931
C	0.186387	1.142551	1.210251
C	1.488739	0.540902	1.739266
C	0.003082	-1.272587	1.167564
H	-0.101792	2.058439	1.730943
H	2.381555	1.135580	1.535613
H	-0.072562	-1.925541	2.039159
H	-0.330229	-1.863420	0.320013
C	-1.098448	0.714862	-0.887626
C	-1.933414	0.377921	0.358342
H	-0.863626	-0.191381	-1.449186
H	-1.625435	1.374395	-1.580107
H	-2.344460	1.329988	0.722210
C	0.184811	2.896867	-0.648514
H	1.021855	3.408316	-0.164700
H	0.250629	3.087883	-1.723728

H	-0.740295	3.351323	-0.284152
C	-3.173937	-0.529366	0.188839
H	-3.603870	-0.637597	1.193160
C	-2.924045	-1.946619	-0.345823
H	-2.426841	-1.934450	-1.319975
H	-2.323761	-2.549413	0.335731
H	-3.877523	-2.464586	-0.479642
C	-4.231316	0.172260	-0.678740
H	-4.452076	1.176417	-0.307011
H	-3.896373	0.266623	-1.715675
H	-5.165964	-0.394171	-0.686808
C	1.606627	-1.751337	-1.396509
H	0.536486	-1.795939	-1.586550
H	2.112643	-1.612487	-2.352999
H	1.924318	-2.705798	-0.972145
H	2.156213	-1.547333	1.469009
H	1.427826	0.404865	2.822591
N	3.409847	-0.552764	-0.403599
C	4.573237	-0.537453	-0.372206
H	-1.267573	0.009981	2.404122

DFT computed carbocation geometries and energies

Cartesian coordinates for carbocations computed with B3LYP/6-31+G(d,p) are listed below, together with the following energies:

E: B3LYP/6-31+G(d,p) electronic potential energy

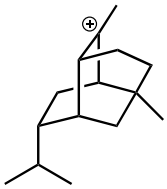
G: B3LYP/6-31+G(d,p) Gibbs free energy at 298.15 K and 1 atm

$E_{mPW1PW91}$: mPW1PW91/6-31+G(d,p) single-point electronic potential energy

G_{tot}: mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) Gibbs free energy at 298.15 K and 1 atm

All energies are reported in Hartree.

5



C	2.213551	0.457464	1.516755
C	2.634256	-0.692692	0.577443
C	1.396586	-1.153660	-0.234729
H	3.024595	-1.533010	1.159855
H	3.433962	-0.374381	-0.102604
C	-0.327801	0.021158	1.102286
C	0.807491	1.091396	1.021609
H	2.969491	1.241194	1.596519
H	1.992661	0.107515	2.529738
C	1.173186	1.307754	-0.366652
H	0.608729	2.004708	1.586873
C	0.984025	0.091292	-1.144786
C	-0.602494	0.091310	-1.360132
C	-1.342065	0.378371	-0.026211
H	-0.838452	-0.907302	-1.742269
H	-0.857620	0.807629	-2.143846
C	1.687416	2.575992	-0.908010
H	0.865318	3.021204	-1.495950
H	2.508554	2.421552	-1.615682
H	1.957749	3.295375	-0.132271
C	1.680452	-2.380093	-1.098692
H	0.818737	-2.646570	-1.719777
H	1.903075	-3.243587	-0.463188
H	2.539735	-2.219176	-1.758242
C	-2.721624	-0.322158	0.059257
H	-1.539562	1.460064	0.041607
C	-3.435079	-0.034417	1.390765
H	-4.428270	-0.493026	1.393458
H	-2.896720	-0.432257	2.256262

H -3.572426 1.043593 1.540902
C -3.618927 0.099444 -1.119223
H -2.562950 -1.407738 -0.015543
H -3.187303 -0.158582 -2.091629
H -3.802862 1.180913 -1.105774
H -4.590666 -0.398540 -1.054882
C 0.266455 -1.370660 0.797148
H -0.781223 0.065708 2.093880
H 0.674094 -1.851641 1.692458
H -0.509918 -2.040451 0.417855
H 1.498702 0.093809 -2.108724
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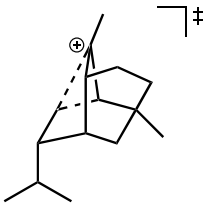
G = -586.110620
$E_{mPW1PW91}$ = -586.320124
G_{tot} = -585.991889

TSA

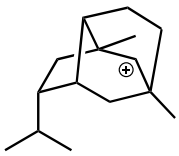


C 2.142816 0.171985 1.648344
C 2.691329 -0.716028 0.503079
C 1.547877 -1.012584 -0.538196
H 3.046408 -1.682721 0.874415
H 3.544272 -0.243951 0.002175
C -0.311196 -0.344535 1.072606
C 0.764806 0.761290 1.245746
H 2.844924 0.975132 1.889981
H 2.010343 -0.409375 2.565931
C 0.889348 1.360442 -0.157215
H 0.434895 1.518562 1.961991
C 1.286390 0.372519 -1.074436
C -0.617485 0.894358 -0.973417
C -1.405701 0.362591 0.236944
C 1.185601 2.831444 -0.342341
H 0.403202 3.449381 0.106878
H 1.298226 3.104667 -1.395077
H 2.124615 3.065514 0.170713
C 1.979752 -2.001785 -1.621752
H 1.187445 -2.154612 -2.362835
H 2.206654 -2.974929 -1.176977
H 2.878119 -1.659037 -2.146094
C -2.668033 -0.441832 -0.152351
H -1.740463 1.237009 0.813013
C -3.633885 0.420223 -0.985363
H -4.524197 -0.155390 -1.254135

H	-3.185108	0.772783	-1.920934
H	-3.967801	1.297658	-0.418543
C	-3.378656	-0.992810	1.094725
H	-2.362188	-1.295072	-0.774705
H	-3.665574	-0.183186	1.776510
H	-2.757911	-1.701316	1.651372
H	-4.293914	-1.517924	0.806215
C	0.332343	-1.517722	0.291041
H	-0.695470	-0.679149	2.038048
H	0.697975	-2.291477	0.972718
H	-0.396010	-2.002946	-0.365760
H	1.589904	0.643196	-2.086413
H	-0.617236	0.155637	-1.788159
H	-0.892936	1.846210	-1.424812

1 imaginary frequency
E = -586.429443
G = -586.100096
$E_{mPW1PW91}$ = -586.311966
G_{tot} = -585.982619

6

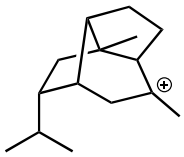


C	2.137804	-0.168332	-1.648248
C	2.689068	0.717645	-0.503532
C	1.545945	1.016652	0.544446
H	3.039134	1.688242	-0.869884
H	3.545475	0.247881	-0.006375
C	-0.316125	0.348600	-1.072136
C	0.760512	-0.754716	-1.245537
H	2.839088	-0.972388	-1.889353
H	2.008166	0.414483	-2.565331
C	0.873560	-1.355460	0.162303
H	0.430543	-1.512334	-1.961511
C	1.303972	-0.366685	1.072164
C	-0.622194	-0.940475	0.941277
C	-1.414165	-0.368527	-0.249485
C	1.215849	-2.821551	0.338139
H	0.453580	-3.456617	-0.121495
H	1.321603	-3.099174	1.390728
H	2.166473	-3.028697	-0.164049
C	1.981426	2.009280	1.623411
H	1.192157	2.161708	2.367560
H	2.203799	2.981718	1.174957
H	2.883180	1.669912	2.144039
C	-2.668955	0.434038	0.163225
H	-1.755604	-1.226677	-0.846068
C	-3.640500	-0.442695	0.974049

H	-4.525522	0.132623	1.260375
H	-3.192747	-0.824674	1.898452
H	-3.982464	-1.301419	0.383892
C	-3.376844	1.025114	-1.067058
H	-2.355861	1.267426	0.808687
H	-3.671541	0.236903	-1.770249
H	-2.750276	1.742762	-1.605324
H	-4.287033	1.550190	-0.762916
C	0.325236	1.517440	-0.281300
H	-0.695936	0.691269	-2.036592
H	0.687265	2.297750	-0.957442
H	-0.402147	1.994664	0.382201
H	1.609132	-0.639557	2.083697
H	-0.615394	-0.221445	1.775810
H	-0.912600	-1.896527	1.375882

0 imaginary frequencies
E = -586.429452
G = -586.101329
$E_{mPW1PW91}$ = -586.311842
G_{tot} = -585.983719

10

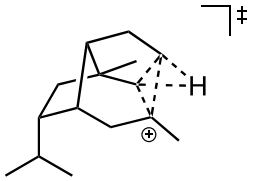


C	2.834419	-0.377381	0.358154
C	1.717190	-0.212337	-0.802064
C	0.978009	1.108622	-0.397286
C	0.865295	0.885994	1.134289
C	2.219422	0.319563	1.597955
C	-0.341463	-0.086057	1.192418
C	0.077923	-1.468469	0.641024
C	0.962662	-1.421790	-0.545745
C	-1.369344	0.624520	0.271190
C	-0.490748	1.136208	-0.912735
C	1.794415	2.327670	-0.832081
C	-2.635911	-0.155102	-0.141357
C	-3.562130	0.723477	-1.000848
C	1.111643	-2.614169	-1.398019
C	-3.400728	-0.698464	1.077169
H	0.603302	1.808616	1.661894
H	-0.719931	-0.219405	2.209287
H	-0.764533	-2.142199	0.440731
H	0.669712	-2.023763	1.394946
H	2.116978	-0.364636	2.446004
H	2.884132	1.121831	1.930801
H	3.713746	0.132983	-0.044178
H	3.138234	-1.413275	0.540406
H	1.860400	2.390080	-1.923788

H	2.813837	2.322878	-0.433369
H	1.308282	3.242636	-0.478896
H	-1.711953	1.503064	0.836163
H	-2.806757	-1.398853	1.675020
H	-4.305964	-1.225777	0.761099
H	-3.710865	0.119173	1.738634
H	-2.330712	-1.010786	-0.766374
H	-3.070309	1.080870	-1.910974
H	-3.901436	1.599698	-0.435670
H	-4.451202	0.163098	-1.305632
H	0.890881	-3.550229	-0.879058
H	2.075150	-2.652834	-1.912568
H	0.344171	-2.497326	-2.185871
H	2.184304	-0.204050	-1.790183
H	-0.777076	2.148355	-1.210154
H	-0.616311	0.516139	-1.810651

0 imaginary frequencies
E = -586.437892
G = -586.110699
$E_{mPW1PW91}$ = -586.318593
G_{tot} = -585.991401

TSB



C	2.587441	-0.549050	0.384395
C	1.670112	0.248648	-0.938642
C	0.801619	1.308072	-0.253274
C	0.721515	0.764638	1.215464
C	2.095284	0.180834	1.614866
C	-0.376668	-0.313211	1.079408
C	0.224142	-1.529395	0.322900
C	1.517077	-1.198054	-0.442175
C	-1.471430	0.445617	0.286176
C	-0.659863	1.203575	-0.799125
C	1.413714	2.706128	-0.396853
C	-2.674950	-0.360434	-0.244017
C	-3.689923	0.567060	-0.935783
C	1.975712	-2.278490	-1.414910
C	-3.361228	-1.166309	0.871026
H	0.413602	1.551640	1.908629
H	-0.744591	-0.636329	2.056678
H	-0.493520	-1.949214	-0.386242
H	0.462678	-2.339901	1.020564
H	1.997160	-0.547057	2.429739
H	2.811424	0.940063	1.941864
H	2.788804	0.511887	-0.448712

H	3.619457	-0.884205	0.264724
H	1.482351	3.006866	-1.447855
H	2.420196	2.767598	0.036592
H	0.791452	3.443472	0.118229
H	-1.876999	1.195541	0.981838
H	-2.695581	-1.909477	1.322950
H	-4.229784	-1.703501	0.478049
H	-3.717817	-0.505238	1.670273
H	-2.311665	-1.068668	-1.004485
H	-3.250546	1.110725	-1.778490
H	-4.089849	1.305099	-0.229928
H	-4.535149	-0.009373	-1.323867
H	2.103563	-3.229784	-0.889925
H	2.927338	-2.027349	-1.895442
H	1.227899	-2.426394	-2.199204
H	2.012074	0.353011	-1.971883
H	-1.057894	2.202445	-0.992859
H	-0.689937	0.670371	-1.757984

1 imaginary frequency

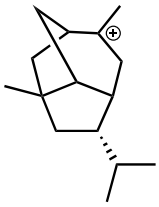
E = -586.412994

G = -586.085304

$E_{mPW1PW91}$ = -586.300366

G_{tot} = -585.972676

11



C	2.395288	-0.625418	0.054427
C	2.069249	0.617121	-0.928433
C	0.906461	1.382226	-0.237769
C	0.843440	0.754628	1.207560
C	2.190603	0.045548	1.418172
C	-0.335847	-0.244591	1.142570
C	0.143542	-1.618925	0.555830
C	1.360147	-1.596256	-0.269164
C	-1.361894	0.482030	0.235742
C	-0.475880	1.057780	-0.885456
C	1.197910	2.890254	-0.218849
C	-2.608535	-0.300500	-0.228305
C	-3.539848	0.594183	-1.065761
C	1.543423	-2.568227	-1.364660
C	-3.380402	-0.908599	0.955148
H	0.644919	1.512410	1.969805
H	-0.748030	-0.446558	2.133664
H	-0.656852	-2.210405	0.107172
H	0.509549	-2.211948	1.421252

H	2.190221	-0.663017	2.253698
H	3.000407	0.759419	1.594384
H	3.004609	1.186037	-0.931203
H	3.394974	-1.003312	-0.181474
H	1.329783	3.281184	-1.233768
H	2.106941	3.117187	0.349271
H	0.369321	3.437666	0.242219
H	-1.723323	1.323766	0.847404
H	-2.782765	-1.624680	1.530160
H	-4.270893	-1.437982	0.602953
H	-3.715513	-0.125989	1.646298
H	-2.278235	-1.122651	-0.883424
H	-3.050119	0.975872	-1.966743
H	-3.887121	1.453650	-0.479831
H	-4.424115	0.034451	-1.385221
H	1.639848	-3.564088	-0.899526
H	2.437360	-2.384173	-1.962533
H	0.651348	-2.632889	-1.997679
H	1.858788	0.321211	-1.958816
H	-0.916186	1.942213	-1.353095
H	-0.370861	0.315108	-1.691268

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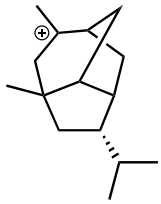
G = -586.112150
$E_{mPW1PW91}$ = -586.319326
G_{tot} = -585.992302

8



C	-1.897022	-0.249807	-1.695280
C	-1.824290	-1.325799	-0.606110
C	-2.041360	-0.810430	0.725971
H	-2.334133	-2.272114	-0.795736
C	0.467731	-0.371286	-1.134970
C	-0.687883	0.627534	-1.351624
H	-2.857527	0.277314	-1.695808
H	-1.777630	-0.711486	-2.679578
C	-0.821125	1.358139	0.034086
H	-0.459858	1.356362	-2.134017
C	-1.903741	0.644978	0.917845
C	0.583387	1.198519	0.712040
C	1.483170	0.406555	-0.270340
C	-1.226295	2.830265	-0.129328
H	-0.445862	3.378907	-0.665424
H	-1.366727	3.317758	0.841653

H	-2.157287	2.932818	-0.698602
C	-2.405723	-1.691411	1.853850
H	-1.814206	-1.471697	2.749393
H	-2.366644	-2.753288	1.605937
H	-3.445792	-1.440665	2.123633
C	2.623935	-0.397932	0.388528
H	1.953768	1.129277	-0.953218
C	3.569328	0.526623	1.175344
H	4.369650	-0.051688	1.646985
H	4.040315	1.260360	0.510045
H	3.050454	1.075543	1.967580
C	3.416922	-1.218491	-0.642157
H	2.176338	-1.099646	1.110886
H	2.794708	-1.954735	-1.162639
H	3.867890	-0.566212	-1.399603
H	4.229496	-1.766200	-0.155114
C	-0.206789	-1.596135	-0.461090
H	0.901001	-0.694041	-2.086099
H	-0.044885	-2.545547	-0.977289
H	0.119109	-1.750564	0.572384
H	-1.823690	0.924481	1.971901
H	0.501996	0.661809	1.666727
H	1.007793	2.175916	0.955715
H	-2.899754	0.999411	0.579701

0 imaginary frequencies
E = -586.439073
G = -586.111996
$E_{mPW1PW91}$ = -586.319194
G_{tot} = -585.992116

P. falciparum growth inhibition assays

P. falciparum in vitro growth inhibition assays were carried out as previously described.[59] Assays were carried out using asynchronous asexual stage *P. falciparum* infected erythrocytes (1% parasitemia; 1% hematocrit) seeded in triplicate wells in 96 well tissue culture plates containing the positive antimalarial control drug chloroquine (Sigma Aldrich, C6628) or test compounds. Following addition of 0.5 μCi [^{3}H]-hypoxanthine per well, culture plates were incubated under standard *P. falciparum* culture conditions for 48 h. Plates were then frozen, thawed and harvested onto 1450 MicroBeta filter mats (Wallac). [^{3}H]-hypoxanthine incorporation was determined using a 1450 MicroBeta liquid scintillation counter and percentage growth inhibition determined as compared to vehicle only (DMSO; 0.5%) controls. The concentrations of each fraction required to inhibit parasite growth by 50% (IC_{50}), were calculated using linear interpolation of inhibition curves[58] for three independent experiments, each carried out in triplicate.

Table S16. *In vitro* activity of fractions containing isonitrile compounds against asexual stage *P. falciparum* infected erythrocytes

Major isonitrile	*P. falciparum* IC_{50} (μM)[a]		P value
	3D7[b]	Dd2[c]	
(-)-**1**	0.80 (±0.53)	1.01 (±0.40)	
(+)-**2**	0.72 (±0.02)	1.20 (±0.30)	
Reference Compounds			
7,20-diisocyanoadociane*	0.01 (±0.01)	0.40 (±0.03)	<0.01
Chloroquine	0.01 (±0.003)	0.04 (±0.018)	<0.05

[a] *In vitro* assays carried out over 48 h using asynchronous asexual stage *P. falciparum* infected erythrocytes. Data show average IC_{50} (±SD) for three independent experiments, each carried out in triplicate wells; [b] *P. falciparum* line 3D7 is sensitive to chloroquine and other antimalarial drugs; [c] *P. falciparum* line Dd2 is resistant to chloroquine, pyrimethamine, cycloguanil and other antimalarial drugs.*NOTE: there were some solubility issues with this compound; stored 4^{o}C >2y)

References

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