Supporting Information

Acremolin from *Acremonium strictum* is N^2,3-Etheno-2'-isopropyl-1-methylguanine, not a 1*H*-Azirine. Synthesis and Structural Revision.

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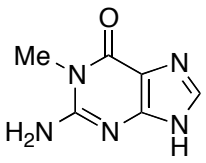
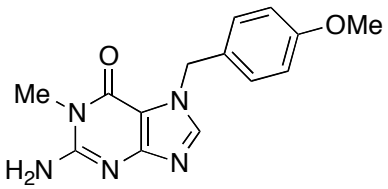


6

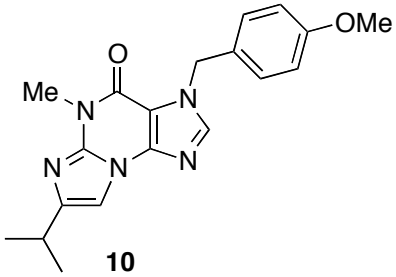
1-Methylguanine (**6**). A solution of guanosine (5.97 g, 21.1 mmol in 40.0 mL anhydrous DMSO) was treated with NaH (511 mg, 21.3 mmol) under N_2 at room temperature. After stirring the mixture for 75 minutes, a solution of iodomethane (3.00 g, 21.1 mmol) in DMSO (1.0 mL) was added dropwise. The reaction mixture was allowed to stir for 5 hours at room temperature then poured into 400 mL of isopropanol and combined with washings (40 mL). The combined *i*-PrOH solution was placed in a freezer (–20 ˚C) overnight and the precipitated solid material collected by filtration and washed with acetone (200 mL). The solid was quickly resuspended in acetone and filtered again. The filtered solid was dried under reduced to pressure to give pure *1-methylguanosine* (4.24 g, 14.3 mmol). A second crop was obtained by cooling the filtrate in a –80° C freezer and purified as described above (102 mg, 0.343 mmol). The combined yield **1** was 69%; ^{1}H-NMR (400 MHz, D_2O) δ 7.83 (1H, s), 5.79 (1H, d, J_H=5.6 Hz), 4.67 (1H, t, J_H=5.4 Hz), 4.35 (1H, t, J_H= 4.2 Hz), 4.16 (1H, q, J_H= 3.2 Hz), 3.85 (1H, dd, J_H=2.4, 12.8 Hz), 3.77 (1H, dd, J_H=3.6, 12.4 Hz), 3.32 (3H, s).[1][2][3]

A solution of *1-methylguanosine* (1.97 g, 6.63 mmol in 1M HCl (10.0 mL) was heated at reflux for 1.5 hours. The reaction mixture was allowed to cool to room temperature, diluted with deionized H_2O (10.0 mL), and made alkaline with 2M NaOH aqu. Upon cooling to 3 ˚C. the solution deposited a solid precipitate, which was collected by filtration, washed with deionized H_2O and dried under reduced pressure to give pure **6**[5] (617 mg, 61%; 42% over two steps) as an off-white amorphous solid; UV-vis (MeOH) λ_{max} 273, 249 nm. ^{1}H-NMR (500 MHz, CF_3COOD) δ 8.79 (1H, s), 3.58 (3H, s); ^{13}C-NMR (125 MHz, CF_3COOD) δ 156.5 (C-2), 155.1 (C-2), 144.4 (C-4), 140.5 (C-8), 109.9 (C-5), 30.9 (C-10).



7a

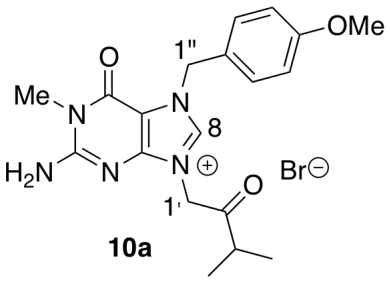
7-*N*-*p*-methoxybenzyl-1-methylguanine (7a). A suspension of **6** (300 mg, 1.82 mmol) and K_2CO_3 (303 mg, 2.19 mmol) in anhydrous DMSO (10.0 mL) was vigorously stirred under an atmosphere of N_2. *p*-Methoxybenzylchloride (512 mg, 3.27 mmol) was added and the solution was allowed to stir at room temperature for 26 hours. Deionized H_2O (100 mL) was added to the reaction mixture and the pH of the solution was adjusted to ~8 using 1M NH_4OH followed by extraction with EtOAc (5x100 mL). The EtOAc layers were combined, washed with deionized H_2O (50 mL) and concentrated under reduced pressure, during which **7a** precipitated as a while solid (87 mg) and recrystallized from EtOH to give pure **7a** (61.8 mg). The combined EtOAc layers were concentrated and passed through a short SiO_2 column (0-10% CH_3OH in CH_2Cl_2) to obtain an additional 30.0 mg of pure **7a** (total yield, 91.8 mg, 31%) along with a mixture of **7a**,**b** (200 mg, 0.702 mmol). ^{1}H-NMR analysis of the mixture showed a 1:2 isomeric ratio of **7a** and its N^9 isomer, **7b** (1.2:1 N^7:N^9 isomers, 56%); **7a**: UV (CH_3OH) λ_{max} (log ε) 215 (4.03), 282 (3.45); FTIR (ATR): ν 3338, 3171, 2959, 2930, 1688, 1642, 1614, 1556, 1513, 1248 cm^{-1}; ^{1}H-NMR (500 MHz, $(CD_3)_2SO$) δ 8.08 (1H, s), 7.29 (2H, d, *J*=8.6 Hz), 6.87 (2H, d, *J*=8.6 Hz), 6.71 (1H, s), 5.35 (2H, s), 3.70 (3H, s), 3.36 (3H, s); ^{13}C-NMR (125 MHz, $(CD_3)_2SO$) ^{13}C-NMR (125 MHz, $(CD_3)_2SO$) δ 158.8 (C-6), 157.9 (C-5''), 154.3 (C-2), 153.5 (C-4), 143.4 (C-8), 129.2 (C-2''), 129.8 (C-3''), 113.9 (C-4''), 107.0 (C-5), 55.1 (C-6''), 48.3 (C-1''), 27.8 (C-10); HRMS *m/z* 286.1299 $[M+H]^+$ calc. for $C_{14}H_{16}O_2N_5$, 286.1299



10

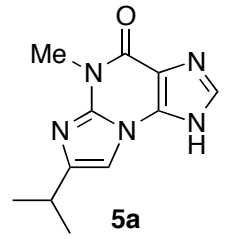
7-*N*-(*p*-Methoxybenzyl)acremolin (10). To a suspension of **7a** (51.4 mg, 0.180 mmol) and 4Å molecular sieves (400 mg) in anhydrous CH_3CN (5.0 mL) was added 3-methyl-1-bromo-2-butanone (**8**, 38.9 mg, 0.236 mmol) under an atmosphere of N_2. The reaction mixture was heated and stirred at 40 °C for 21 hours. Additional bromoketone (15.4 mg, 0.0933 mmol) was added and the stirring continued until TLC showed absence of starting material (23 h). The mixture was neutralized using 1M NH_4OH aqu. and the solid material removed by filtration. The filtrate was dried to give a white solid (64 mg) that was purified on a short SiO_2 column (0-10% CH_3OH-CH_2Cl_2) to yield crude material (20.8 mg) containing **10** and the mono-alkylation product **10a** (43.4 mg, 54%). Repurification of the former by silica chromatography (1:1 EtOAc-hexanes) gave pure **10** (16.9 mg, 27%). UV (CH_3OH) λ_{max}

(log ε): 225 (4.65), 273 (4.11); FTIR (ATR): ν 2954, 2935, 1671, 1613, 1516, 1246 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 8.14 (1H, s), 7.37 (2H, d, J_{H-H}=8.8 Hz), 7.33 (1H, s), 6.88 (2H, d, J_{H-H}=8.8 Hz), 5.58 (2H, s), 3.75 (3H, s), 3.66 (3H, s), 2.94 (1H, m), 1.31 (3H, d, J_{H-H}=6.8 Hz); ^{13}C-NMR (125 MHz, CD_3OD) 159.8 (C-5''), 153.4(C-6), 148.9(C-2'), 142.7 (C-2), 142.3 (C-4), 141.7 (C-8), 129.2 (C-3''), 128.2 (C-2''), 113.8 (C-4''), 108.4 (C-5), 102.9 (C-1''), 54.3 (C-6''), 49.2 (C-1''), 27.9 (C-10), 27.7 (C-3'), 21.1 (C-4' and C-5'); HRMS *m/z* 352.1769 $[M+H]^+$ calcd for $C_{19}H_{22}N_5O_2$, 352.1768.



10a

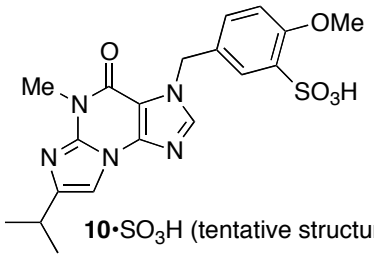
Monoalkylation product 10a. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$) δ 9.25 (1H, br s, H-8), 7.80 (2H, br s, N-H_2), 7.44 (2H, d, J_{H-H}=8.7 Hz, H-3''), 6.98 (2H, d, J_{H-H}=8.7 Hz, H-4''), 5.62 (2H, s, H-1'), 5.31 (2H, s, H-1''), 3.75 (3H, s, H-6''), 3.33 (3H, s, H-10), 2.89 (1H, septet, J_{H-H}=6.9 Hz, H-3'), 1.32 (3H, d, J_{H-H}=6.9 Hz, H-4' and H-5'); ^{13}C-NMR (125 MHz, $(CD_3)_2SO$) δ 205.3 (C-2'), 159.6 (C-5''), 156.1 (C-6), 152.9 (C-2), 148.1 (C-4), 138.54 (C-8), 130.2 (C-3''), 126.3 (C-2''), 114.3 (C-4''), 104.9 (C-5), 55.2 (C-6''), 51.0 (C-1''), 50.8 (C-1'), 37.8 (C-3'), 28.6 (C-10), 17.7 (C-4' and C-5'). In CD_3OD, H-1' and H-8 underwent rapid deuterium exchange (23 ˚C), and the corresponding ^{1}H and ^{13}C NMR signals were attenuated. HRMS *m/z* 370.1873 $[M]^+$ calcd for $C_{19}H_{24}N_5O_3$, 370.1874



5a

Acremolin (5a). Compound **10** was deprotected to give acremolin (**5a**) using two methods. *Method A*: To a solution of **10** (16.9 mg, 0.0481 mmol in 0.5 mL toluene) was added 90% H_2SO_4 (50 µL). The mixture was vigorously stirred at 50 °C for 3 days. The reaction mixture was then diluted with toluene (1.5 mL) and deionized H_2O (2 mL). The toluene layer was removed and the aqueous layer was extracted with a second portion of toluene (2 mL). The aqueous layer was neutralized to pH 7-8 using 1M aqueous NH_4OH and extracted with EtOAc (3x3 mL). The EtOAc layers were combined and concentrated to yield pure **5a (**4.3 mg, 39%) as a colorless amorphous solid with spectroscopic properties identical to those reported for acremolin.[4]: UV (CH_3OH) λ_{max} (log ε) pH 3: 219 (4.57), 254 (4.06); pH 7: 223 (4.49), 269 (4.05); pH 9: 225 (4.61), 270 (4.16); Fluorescence λ_{ex} 296 nm, λ_{em} 421 nm; FTIR (ATR): ν 3099, 2958, 2925, 1672, 1617, 1568, 1518, 1464 cm^{-1}; ^{1}H-NMR (500 MHz, $(CD_3)_2SO$) δ 13.87 (1H, s, br), 8.16 (1H, s), 7.38 (1H, s), 3.57 (3H, s), 2.88 (1H, septet, J_{H-H}=6.9 Hz), 1.25 (6H, d, J_{H-H}=6.9 Hz); ^{13}C-NMR (125 MHz, $(CD_3)_2SO$) δ 152.8 (C-6), 148.0(C-2'), 142.3 (C-2), 141.6 (C-4), 140.5 (C-8, $^1J_{CH}$ = 212.7 Hz), 108.9 (C-5), 103.2 (C-1', $^1J_{CH}$ = 195.5 Hz),), 28.9 (C-10), 27.7 (C-3'), 22.1 (C-4' and C-5'); HRMS *m/z* 232.1191 $[M+H]^+$ calcd for $C_{11}H_{14}O_2N_5$, 232.1193.

The aqueous phase from the workup of **5a** was examined by LCMS (see Figure S11) and showed a single major *water-soluble* by-product, **10**•SO_3H (*m/z* 432.2).

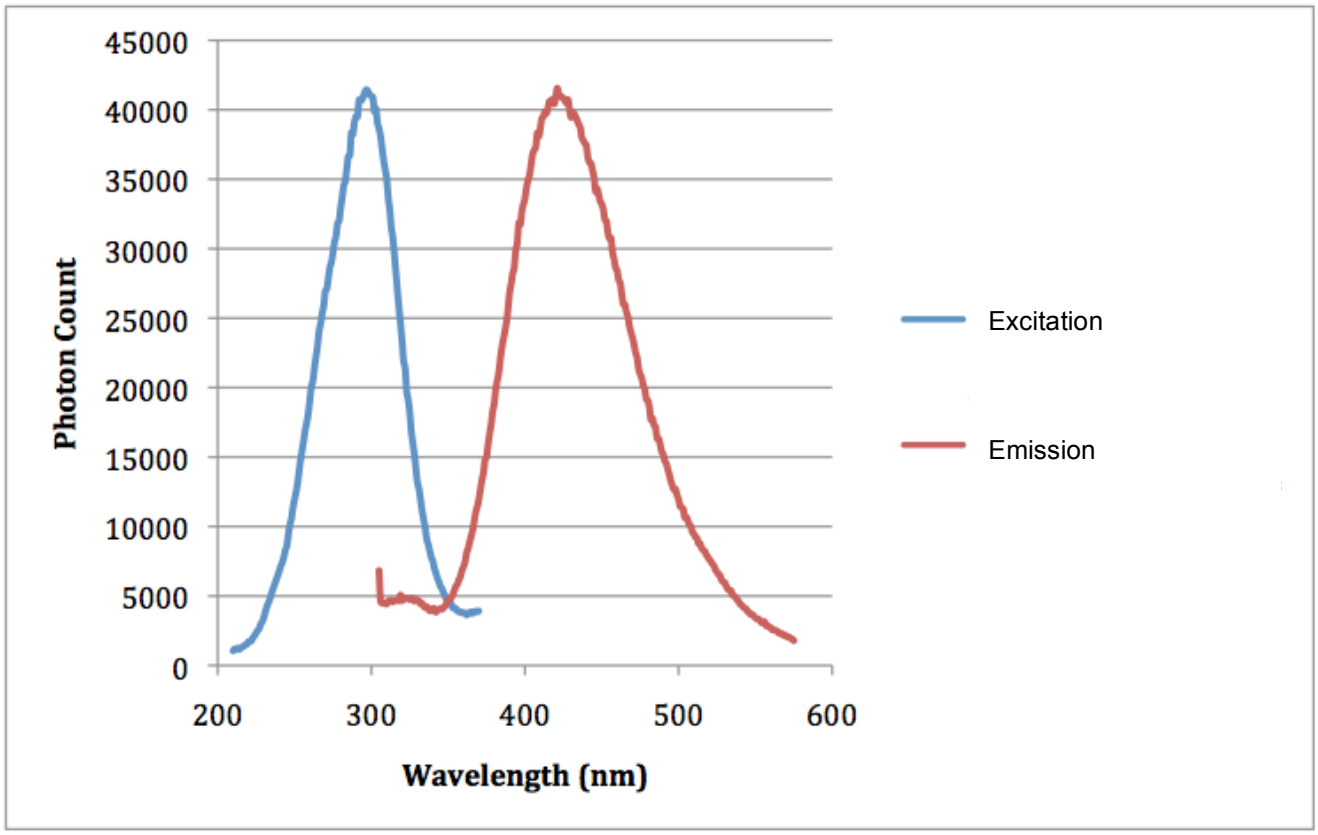


10•SO_3H (tentative structure)

Method B: A solution of **10** (2.8 mg 7.9 µmol) in TFA (1.0 mL) in a sealed vial was heated to 40 ˚C with vigorous stirring for 24 h, then at 80 °C for an additional 15 h. The mixture was cooled to room temperature and TFA was removed under reduced pressure to give a brown residue. The residue was dissolved in deionized H_2O (1.0 mL) and the solution neutralized (1M NH_4OH) before extraction with EtOAc (4 x 4 mL). The combined organic layers were dried to give crude **5a** that was passed through a short SiO_2 column (0-5% MeOH in CH_2Cl_2) to yield pure **5a** (1.4 mg, 76%) identical by ^{1}H NMR with the product from *Method A*.

[1] Hobartner, C., Kreutz, C.; Flecker, E.; Ottenschlager, E.; Pils, W.; Grubmayr, K.; Micura R. *Monatsh. Chem.* **2003**, *134*, 851–873.
[2] Gladkaya, V. A.; Levitskaya, Z. V.; Shalamai, A. S.; Usenko, L. S.; T.; Dashevskaya A. *Chem. Nat. Compd.*, **1989**, *25*, 488.
[3] Chang, C.; Ashworth, D. J.; Chern, L.; Gomes, J. D.; Lee, C.; Mou, P. W.; Narayan, R. *Mag. Reson. Chem.* **1984**, *22*, 671.
[4] Julianti, E.; Oh, H.; Lee, H.-S.; Oh, D.-C.; Oh, K.-B.; Shin, J. *Tetrahedron Lett.* **2012**, *53*, 2885-2886.
[5] Bredereck, H.; Martini, A. *Ber.* **1947**, *80*, 401-405

Figure S1. Fluorescence spectrum of synthetic acremolin (**5a**) (0.10 μM MeOH, *T* = 23 ˚C).[a]



[a] 1.0 nm resolution, nm; λ_{ex} = 296 nm, λ_{em} = 420, collected on a Photon Technology International QuantaMaster Fluorospectrometer (Birmingham, NJ, USA)

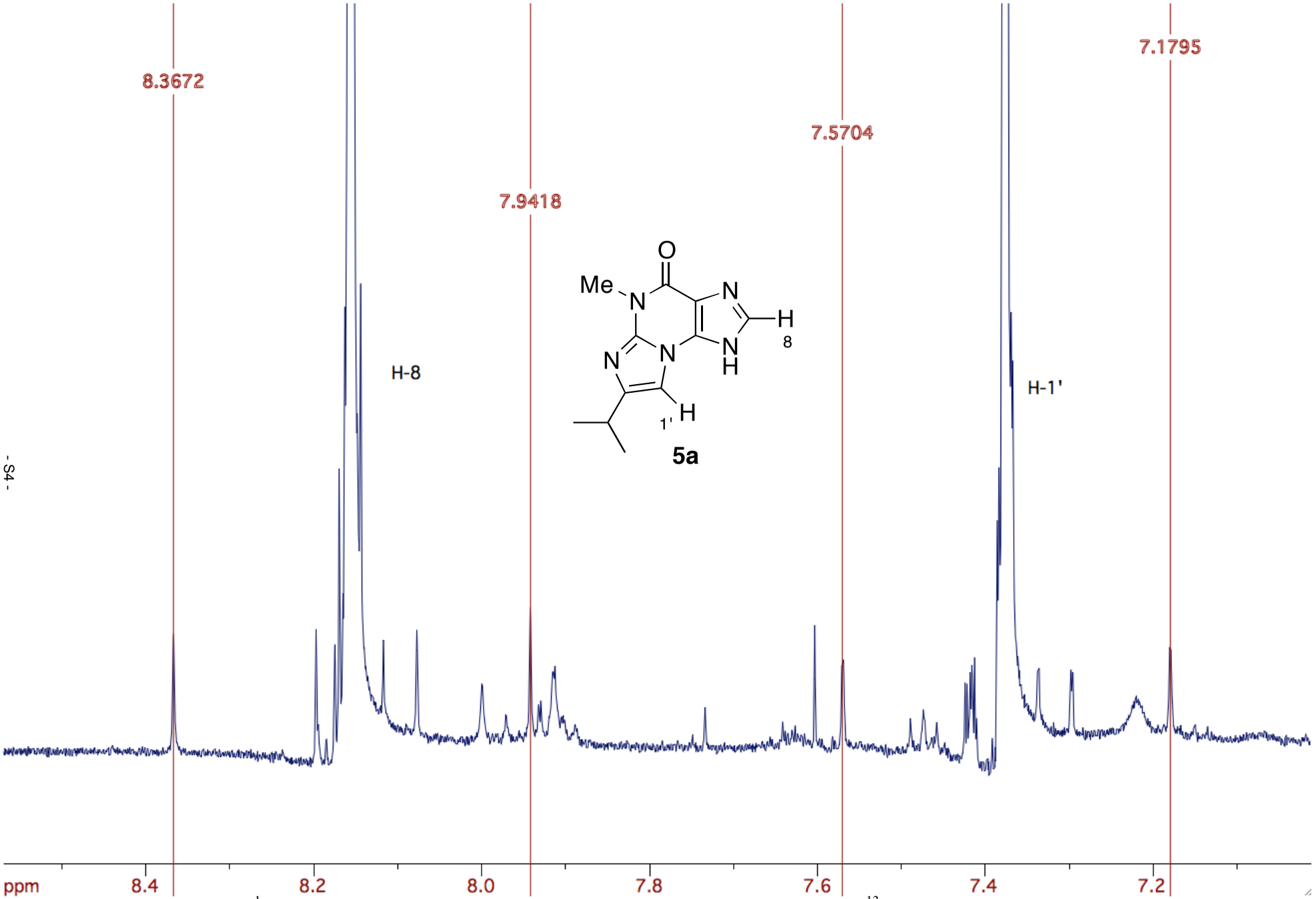
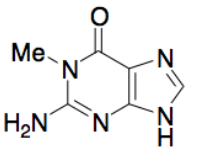


Figure S2: Expansion of the ^{1}H NMR spectrum of acremolin (**5a**, DMSO-d_6, 500 MHz, $T = 23$ °C). '^{13}C satellite' peaks of H-8 and H-1' are marked by red cursors.

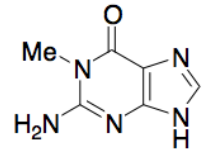
Fig. S3. ^{1}H NMR of **6** (CF_3COOD, 500 MHz)



(CF_3COOD)

6

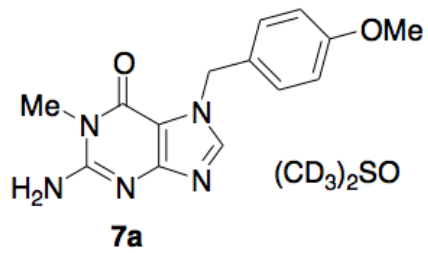
Fig. S4. ^{13}C NMR of **6** (CF_3COOD, 125 MHz)



6

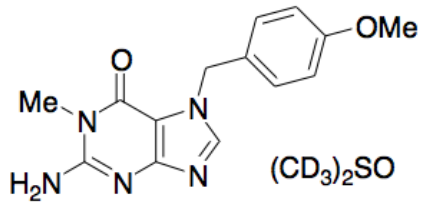
(CF_3COOD)

Fig. S5. ^{1}H NMR of **7a** ($(CD_3)_2SO$, 500 MHz)



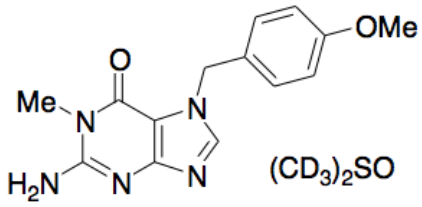
7a

Fig. S6. ^{13}C NMR of **7a** ($(CD_3)_2SO$, 125 MHz)



7a

Fig. S7. DEPT-135 of **7a** ($(CD_3)_2SO$, 125 MHz)



7a

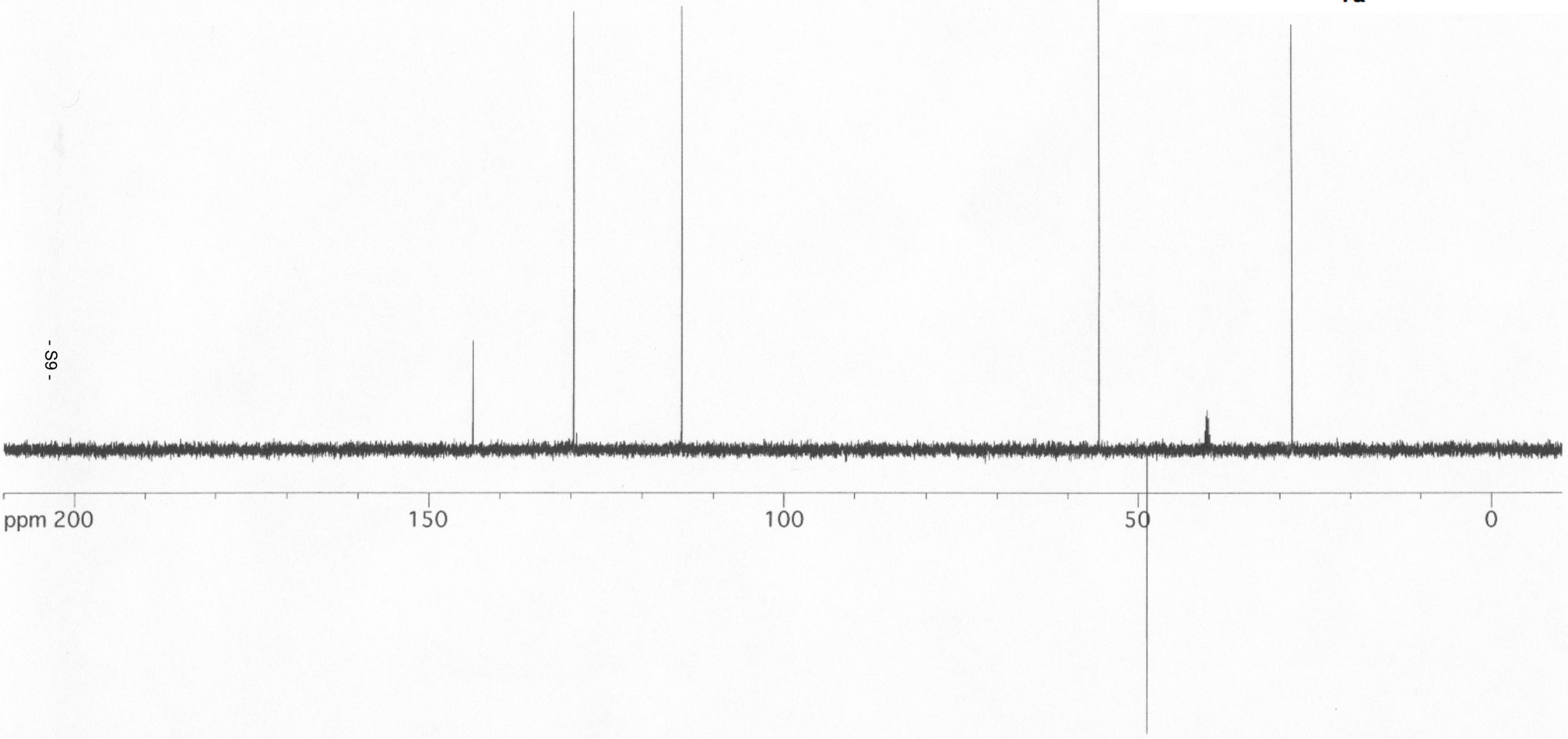
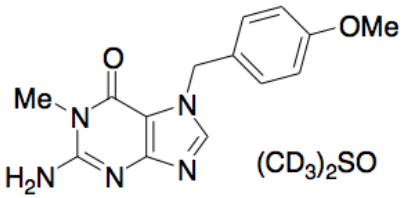


Fig. S8. DEPT-90 of **7a** ($(CD_3)_2SO$, 125 MHz)



7a

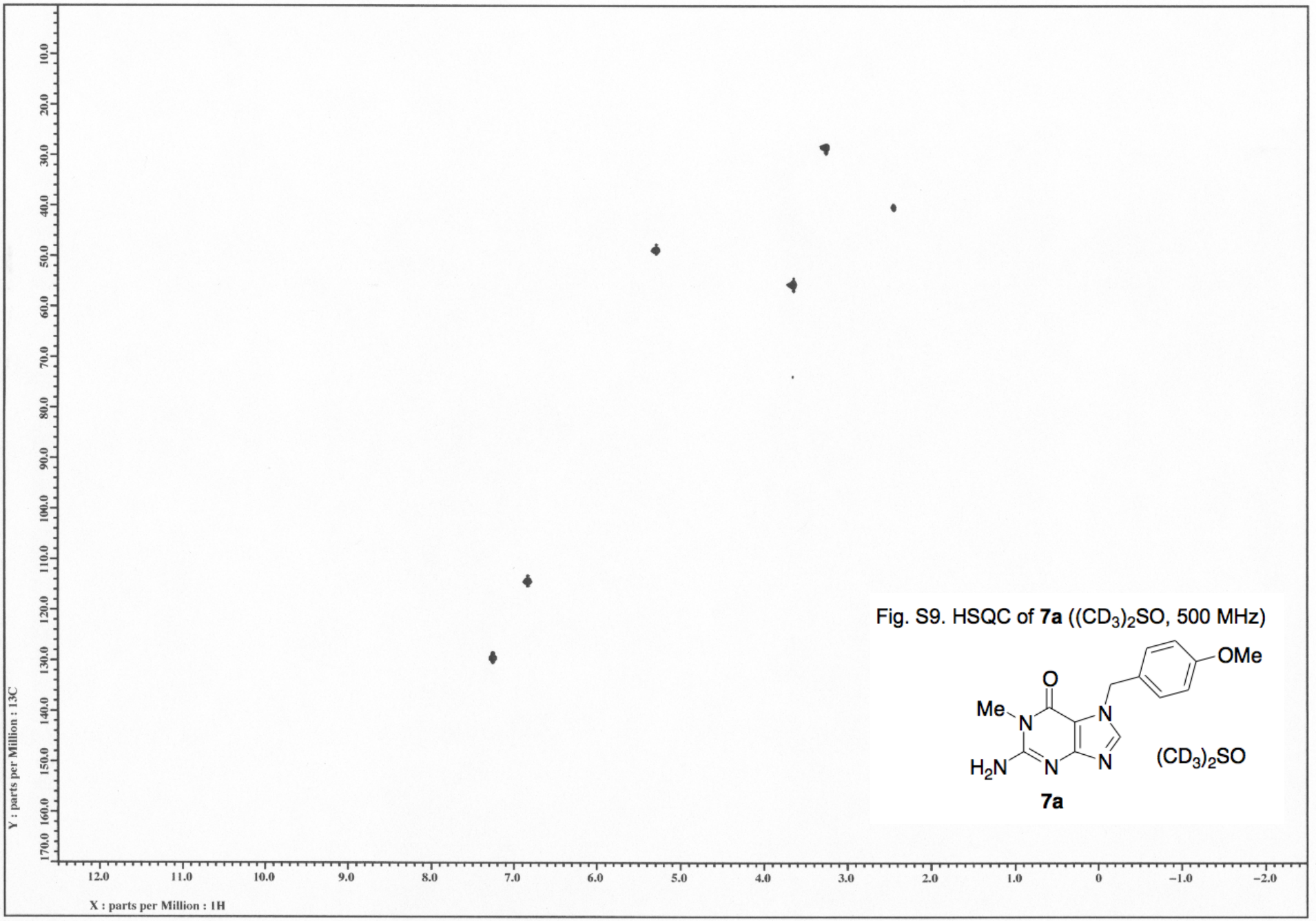
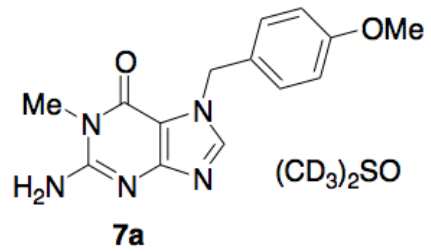


Fig. S9. HSQC of **7a** ($(CD_3)_2SO$, 500 MHz)

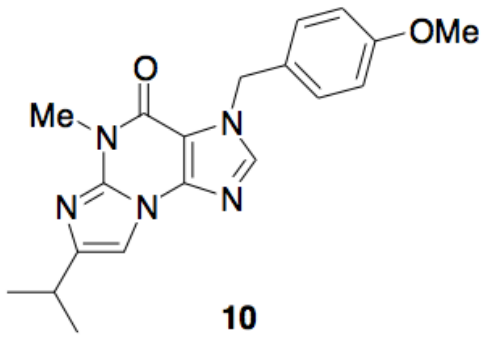
7a

Fig. S10. HMBC of **7a** ($(CD_3)_2SO$, 500 MHz)



7a

Fig. S11. ^{1}H NMR of **10** (CD_3OD, 500 MHz)



10

Fig. S12. ^{13}C NMR of **10** (CD_3OD, 125 MHz)

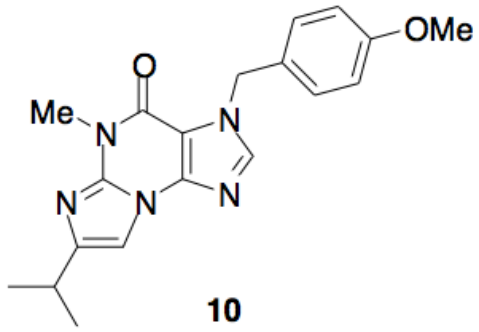


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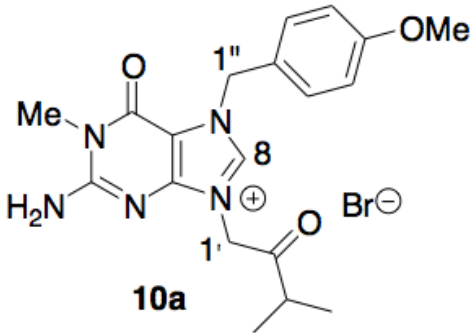
Fig. S13. ^{1}H NMR of **10a** ($(CD_3)_2SO$, 500 MHz)

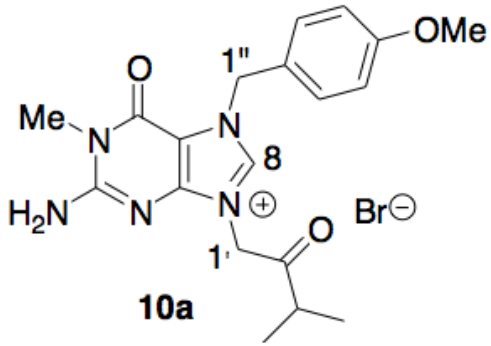


10a

X CH2Cl2

ppm 10 8 6 4 2 0

Fig. S14. ^{13}C NMR of **10a** ($(CD_3)_2SO$, 125 MHz)

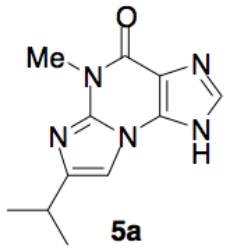


10a

X CH2Cl2

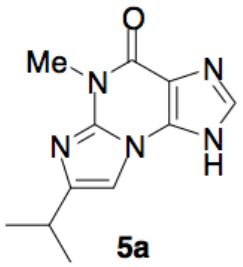
ppm 200 150 100 50 0

Fig. S15. ^{1}H NMR of **5a** ($(CD_3)_2SO$, 500 MHz)



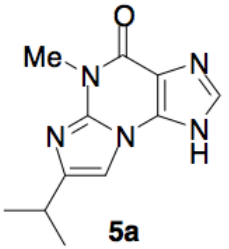
5a

Fig. S16. ^{13}C NMR of **5a** ($(CD_3)_2SO$, 125 MHz)



5a

Fig. S17. DEPT-135 of **5a** ($(CD_3)_2SO$, 125 MHz)



5a

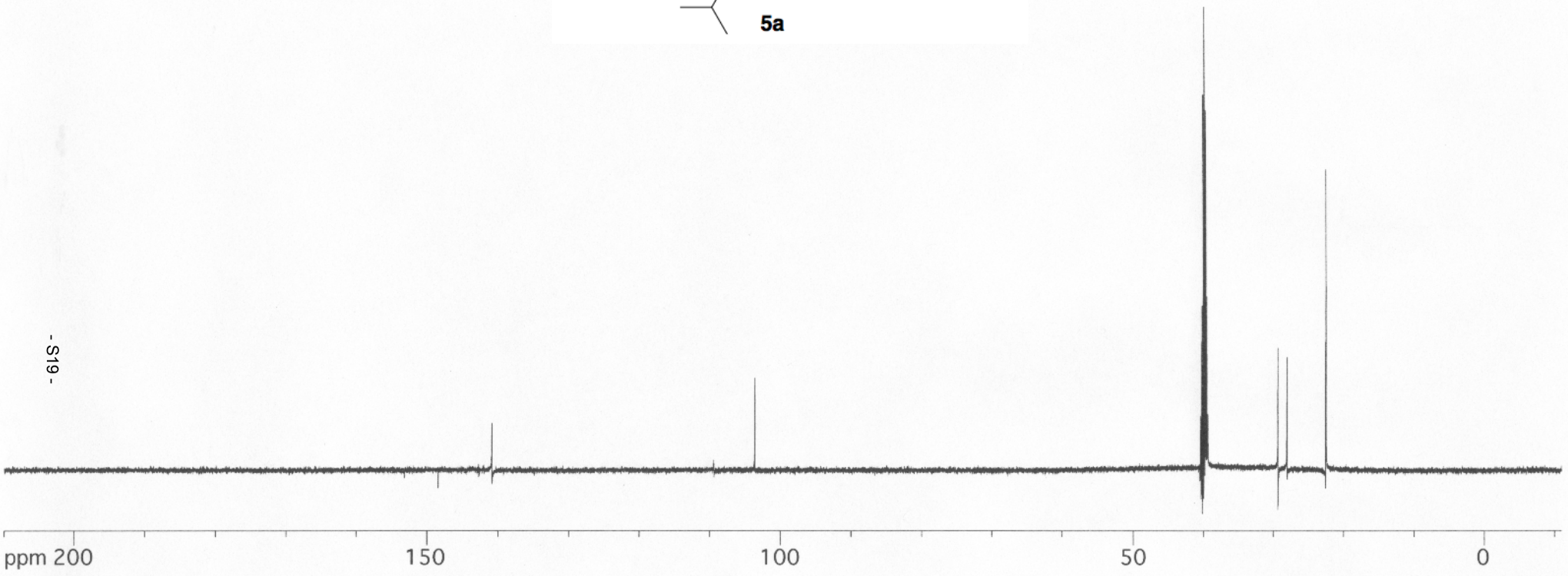
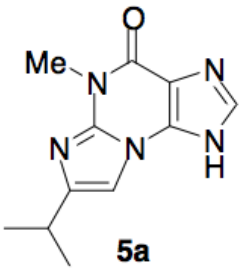
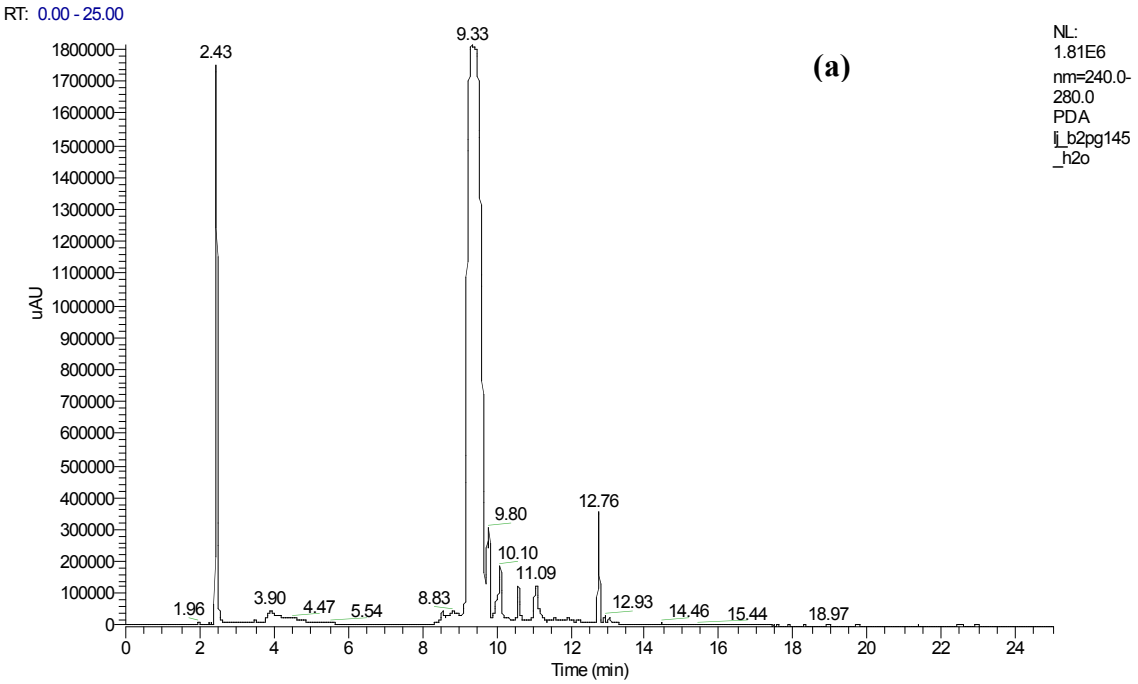
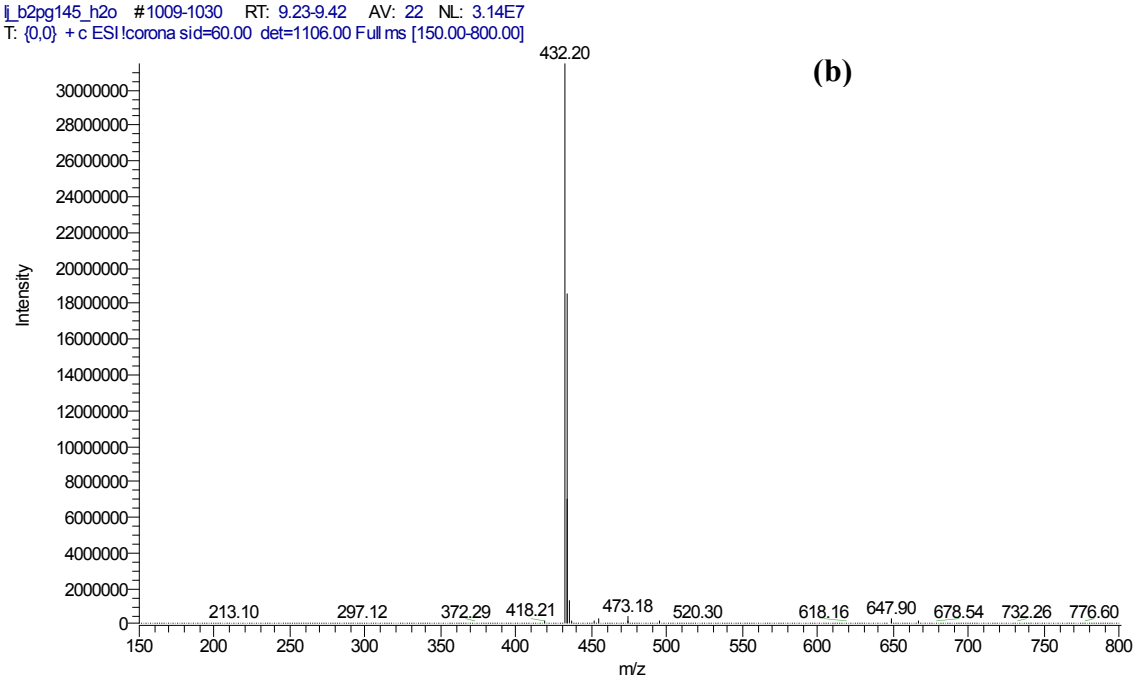


Fig. S18. HMBC of **5a** ($(CD_3)_2SO$, 500 MHz)



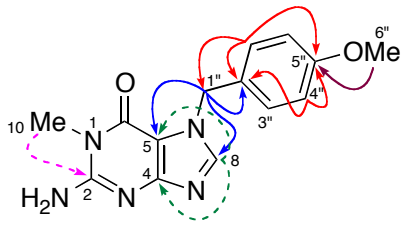
5a





Conditions: Reversed phase, Kinetex® C_{18} (2.6 µ, 4.6 x 150 mm), gradient, 0-100% CH_3CN in H_2O + 0.1% HCO_2H over 30 min, 0.7 $mL.min^{-1}$. Single quadrupole mass spectrometer (ThermoFisher, Surveyor MSQ): ESI ionization (positive mode).

Figure S13. LCMS of byproduct from debenzylation of **10** (workup, aqueous phase). (a) UV-vis chromatogram (λ 240-280 nm) (b) LR ESIMS of peak eluting at rt = 9.33 min, *m/z* 432.2 $[M+H]^+$.

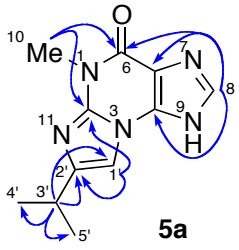


7a

Table S1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) and HMBC data of N^{7}-PMB-N^{1}-methylguanine (**7a**). HMBC optimized for $^{2,3}J_{HC}$ = 8.0 Hz

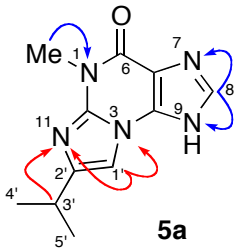
#	δ_C	δ_H (int, mult)	$J_{H\text{-}H}$ (Hz)	HMBC
2	154.3	-	-	-
4	153.5	-	-	-
5	107.0	-	-	-
6	157.9	-	-	-
8	143.4	8.08 (1H, s)	-	4, 5
10	27.8	3.36 (3H, s)	-	2
1"	48.3	5.35 (2H, s)	-	5, 8, 3"
2"	129.8	-	-	-
3"	129.2	7.29 (2H, d)	8.6	1", 2", 4"
4"	113.9	6.87 (2H, d)	8.6	2", 5"
5"	158.8	-	-	-
6"	55.1	3.70 (3H, s)	-	5"

^{1}H-^{13}C HMBC



5a

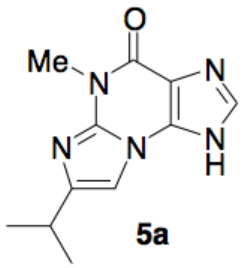
^{1}H-^{15}N HMBC



5a

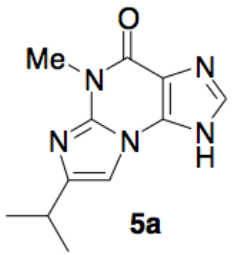
	δ_H[a]	δ_C[a]	δ_N[b]	J_{H-H} (Hz)	DEPT[a]	^{1}H–>^{13}C HMBC[c]	^{1}H–>^{15}N HMBC[c]
N-1	-	-	131.5	-	-	-	-
C-2	-	142.3	-	-	-	-	-
N-3	-	-	156.1	-	-	-	-
C-4	-	141.6	-	-	-	-	-
C-5	-	108.9	-	-	-	-	-
C-6	-	152.8	-	-	-	-	-
N-7	-	-	232.6	-	-	-	-
C-8	8.16 (1H, s)	140.5	-	-	CH	C-6, C-4, C-5	N-7, N-9
N-9	13.87 (1H, br, s)	-	166.1	-	-	-	-
C-10	3.57 (3H, s)	28.9	-	-	CH_3	C-6, C-2	N-1
N-11	-	-	221.9	-	-	-	-
C-1'	7.38 (1H, d)	103.2	-	1.0	CH	C-2', C-2	N-11, N-3
C-2'	-	148.0	-		-	-	-
C-3'	2.88 (1H, d sept)	27.7	-	6.9, 1.0	CH	C-2', C-1', C-4',5'	N-11
C-4',5'	1.25 (3H. d)	22.1	-	6.9	CH_3	C-2', C-3'	-

Table S2. ^{1}H, ^{13}C and ^{15}N NMR data of synthetic acremolin (**5a**, DMSO, 25 °C). HMBC long rage coupling = 8.0 Hz. [a] 125 MHz. [b] ^{15}N δ obtained by indirect detection from ^{1}H-^{15}N-HMBC crosspeaks. [c]600 MHz HMBC.



5a

Fig. S20. ^{1}H-^{15}N HMBC of **5a** ($(CD_3)_2SO$, 600 MHz)



5a

Fig. S21. ^{1}H-15N HMBC of **5a** ($(CD_3)_2SO$, 600 MHz) - expansion