Electronic Supplementary Information:

Synthesis of the Four Stereoisomers of 2,3-Epoxy-4-hydroxynonanal, Potential Genotoxic Products of Lipid Peroxidation, and Their Reactivity with Deoxyguanosine

Katya V. Petrova, Donald F. Stec, Markus W. Voehler, and Carmelo J. Rizzo*

Departments of Chemistry and Biochemistry, and Center in Molecular Toxicology, Vanderbilt University, VU Station B 351822, Nashville, Tennessee 37235-1822

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Procedures for the synthesis of (4*S*)- and (4*R*)-4-hydroxy-2*E*-nonenal

(*2S,3S*)-2,3-Epoxy-1-octanol. Prepared in 79% yield by Sharpless epoxidation of 2E-octen-1-ol as previously described.[1,2] $[\alpha]^{25}_{D}$ -40.7° (*c* 0.43, $CHCl_3$). lit:[2] $[\alpha]^{20}_{D}$ -40.4° (*c* 1.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$); δ 0.91 (t, 3H, *J*=7.0 Hz, CH_3), 1.31-1.36 (m, 4H, $2CH_2$), 1.42-1.49 (m, 2H, CH_2), 1.55-1.64 (m, 2H, CH_2), 1.73 (br. t, 1H, OH), 2.92-3.0 (m, 2H, CH_2), 3.62-3.65 (m, 1H), 3.88-3.90 (m, 1H).

(2*R*,3*R*)-2,3-Epoxy-1-octanol. Prepared in 87 % yield.[2] $[\alpha]^{24.5}_{D}$ +40.0° (*c* 1.11, $CHCl_3$). lit:[3] $[\alpha]^{27}_{D}$ +38.9° (*c* 1.11, $CHCl_3$ 99 % ee); lit:[2] $[\alpha]^{20}_{D}$ +40.0° (*c* 0.56, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$); δ 0.91 (t, 3H, *J*=6.9 Hz, CH_3), 1.31-1.35 (m, 4H, $2CH_2$), 1.44-1.46 (m, 2H, CH_2), 1.55-1.59 (m, 2H, CH_2), 1.73 (br. d, 1H, OH), 2.91-2.99 (m, 2H, CH_2), 3.68 (ddd, 1H, *J*=4.2, 7.2, 12.4 Hz), 3.93 (ddd, 1H, *J*=2.5, 5.3, 12.5 Hz).

(*2R,3S*)- Epoxyoctanal. (Diacetoxyiodo)benzene (4.61 g, 14.3 mmol) was added to a solution of (*2S,3S*)-epoxy-1-octanol (1.90 g, 13.0 mmol) and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) (0.20 g 1.3 mmol) in 25 mL dry methylene chloride. The reaction mixture was stirred at ambient temperature until the starting material was no longer detectable by GC/MS. The reaction mixture was diluted with methylene chloride (50 mL), then washed with a saturated aqueous solution of $Na_2S_2O_3$ (25 mL) and the aqueous layer was extracted with methylene chloride (5 x 25 mL). The combined organic layers were washed with 5 % $NaHCO_3$ (25 mL) and brine, dried over $MgSO_4$, filtrated and evaporated. Purification by flash chromatography on silica, eluting with 10 % ethyl acetate in hexanes afforded (*2R,3S*)-epoxyoctanal (1.51 g, 82 %). $[\alpha]^{24.7}_{D}$ +98.3° (*c* 0.48, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$); δ 0.91 (t, 3H, *J*=7.1 Hz, CH_3), 1.31-1.37 (m, 4H, $2CH_2$), 1.46-1.52 (m, 2H, CH_2), 1.63-1.71 (m, 2H, CH_2), 3.14 (dd, 1H, *J*=2.0, 6.3 Hz, H-2), 3.24 (ddd, 1H, *J*=2.0, 5.6, 11.0 Hz, H-3), 9.01 (d, 1H, *J*=6.3 Hz, CHO); *m/z* (GC-EI) 143 (0.5, M^++1), 71 (100), 55 (20), 41 (25).

(*2S,3R*)-Epoxyoctanal. Prepared from (2*R*,3*R*)-epoxy-1-octanol in 84 % yield following the procedure described above for (*2R,3S*)-epoxyoctanal. $[\alpha]_{D}^{25.7}$ -98.6° (*c* 0.35, $CHCl_3$).

(*4S*)-4-Hydroxy-2*E*-nonenal (1). (Methoxymethyl)triphenylphosphonium chloride (2.74 g, 7.98 mmol), which was dried before use, was placed in a flame-dried flask and dry THF (10 mL) was added. The suspension was cooled to -40 °C and potassium *tert*-butoxide (1.12 g, 9.98 mmol) dissolved in dry THF (10 mL) was added dropwise resulting in an orange solution. The mixture was stirred 15 min at -40 °C, then cooled to -78 °C. A solution of (*2R,3S*)-epoxyoctanal (0.85 g, 6.0 mmol) in dry THF (5 mL) was stirred over activated 4Å molecular sieves for 15 min, then added dropwise to the Wittig reagent at – 78 °C. The mixture was stirred for 15 min at – 78 °C, then

allowed to warm to room temperature over 1h. The reaction was quenched by the addition of saturated aqueous NH_4Cl (15 mL) to the reaction, and the mixture was stirred for 30 min. The mixture was extracted with methylene chloride (3 x 50 mL); the combined organics were washed with water, dried over $MgSO_4$, filtrated and concentrated. Purification by flash chromatography on silica, eluting at the beginning with 3:1 methylene chloride:hexanes (v/v) to remove trace of impurities, then followed by 65:35 hexanes:ether (v/v) to afford (4*S*)-HNE (**1**, 0.53 g, 56 %). $[\alpha]^{24.5}_D$ +44.7^o (*c* 0.36, $CHCl_3$) (lit:[4] $[\alpha]^{20}_D$= +48^o (*c* 0.69, $CHCl_3$)); ^{1}H NMR (400 MHz, CD_2Cl_2); δ 0.91 (t, 3H, *J*=6.9 Hz, CH_3), 1.29-1.51 (m, 6H, $3CH_2$), 1.54-1.69 (m, 2H, CH_2), 1.86 (br.s., 1H, OH), 4.43 (m, 1H, *J*=5.2 Hz, H-4), 6.27 (ddd, 1H, *J*=1.6, 7.9, J=15.7 Hz, H-2), 6.84 (dd, 1H, *J*=4.4, 15.6 Hz, H-3), 9.56 (d 1H, *J*=8.0 Hz, CHO); *m/z* (GC-EI) 157 (100, M^++1), 139 (25), 109 (40), 81 (30).

(*4R*)-Hydroxy-2*E*-nonenal (2). (*4R*)-HNE was prepared from (*2S,3R*)-epoxyoctanal in 68 % yield following the procedure described above for synthesis of **1**. $[\alpha]^{25}_D$ -46.7^o (*c* 0.73, $CHCl_3$) (lit:[4] $[\alpha]^{25}_D$ -46^o (*c* 0.45, $CHCl_3$));

(1) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; B., S. K. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(2) Wang, H.; Kozekov, I. D.; Harris, T. M.; Rizzo, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 5687-700.

(3) Richardson, T. I.; Rychnovsky, S. D. *Tetrahedron* **1999**, *55*, 8977-8996.

(4) de Montarby, L.; Mosset, P.; Gree, R. *Tetrahedron Lett.* **1988**, *29*, 3937-3940.

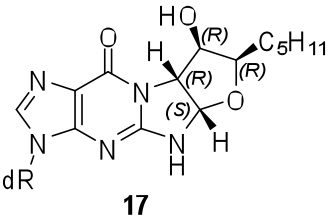

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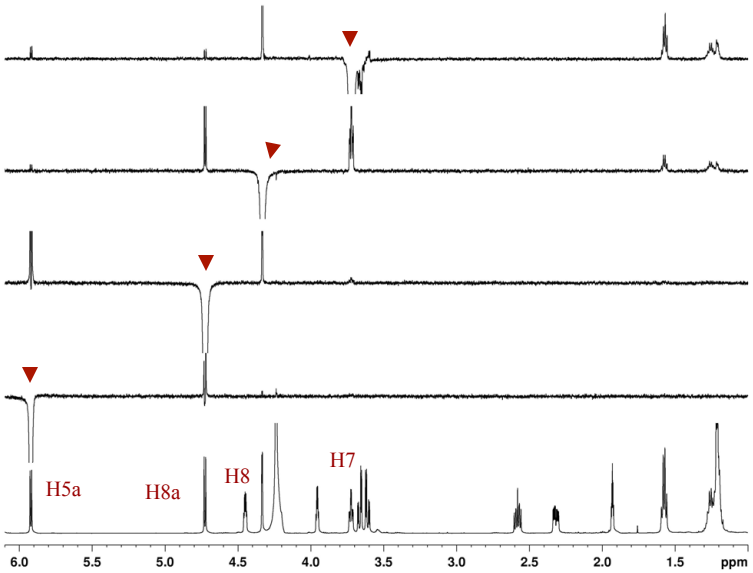
Figure S1. Selective NOE spectra of tetracyclic adduct **17**



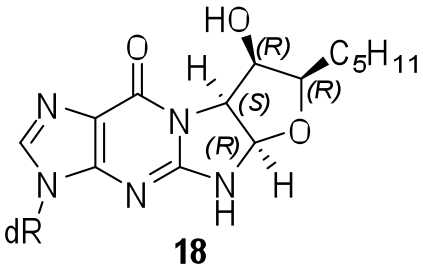


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Figure S2. Selective NOE spectra of tetracyclic adduct **18**

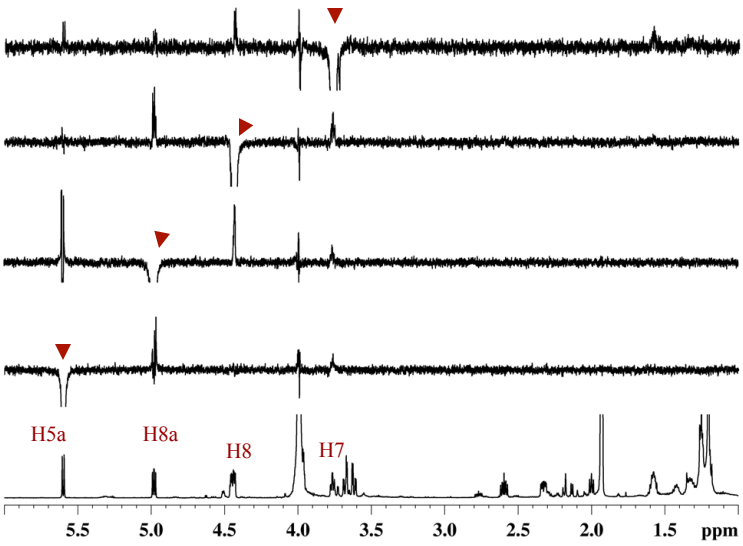
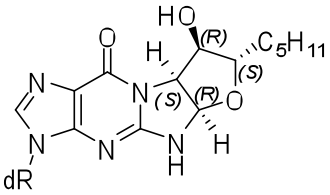


Figure S3. Selective NOE spectra of tetracyclic adduct **19**



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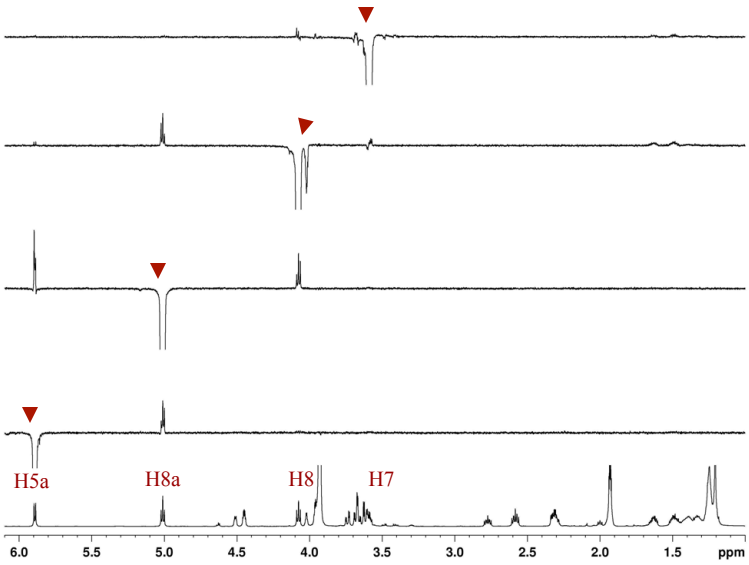
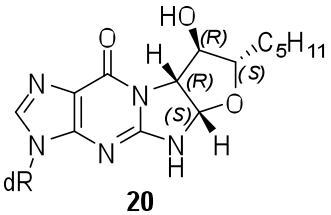


Figure S4. Selective NOE spectra of tetracyclic adduct **20**



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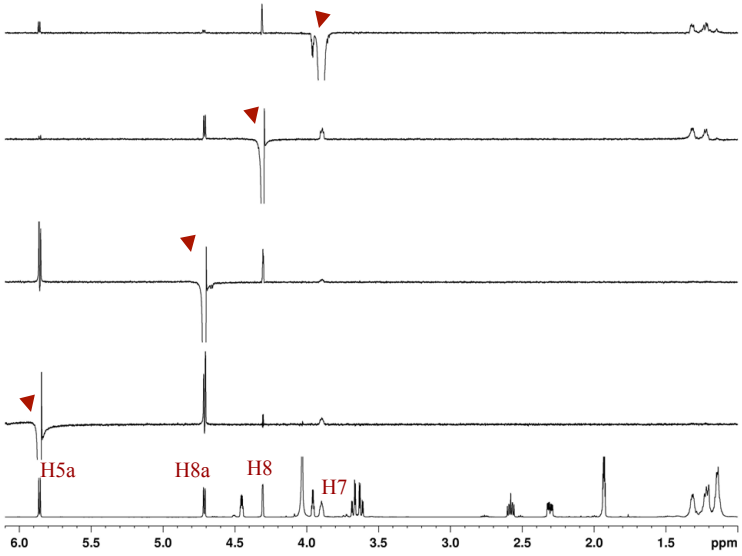
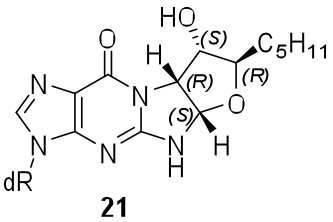


Figure S5. Selective NOE spectra of tetracyclic adduct **21**



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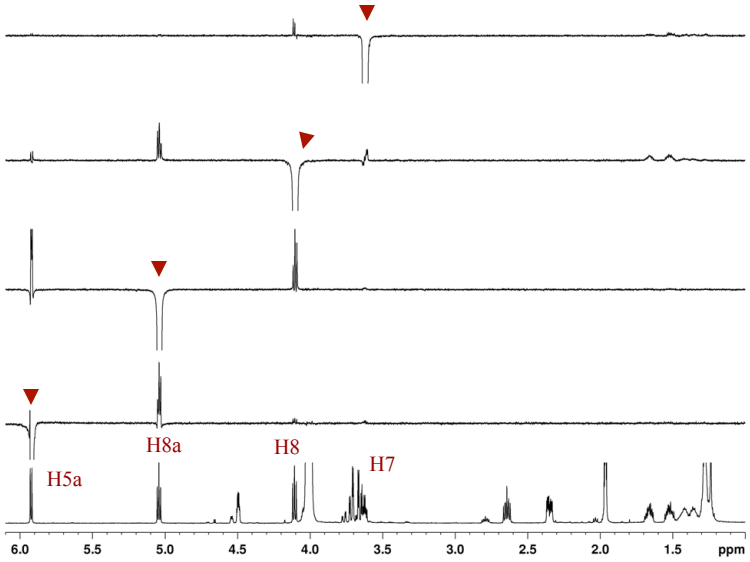
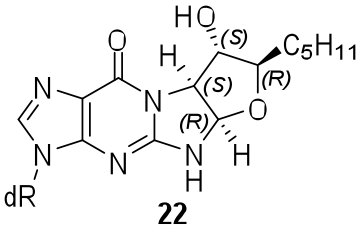


Figure S6. Selective NOE spectra of tetracyclic adduct **22**



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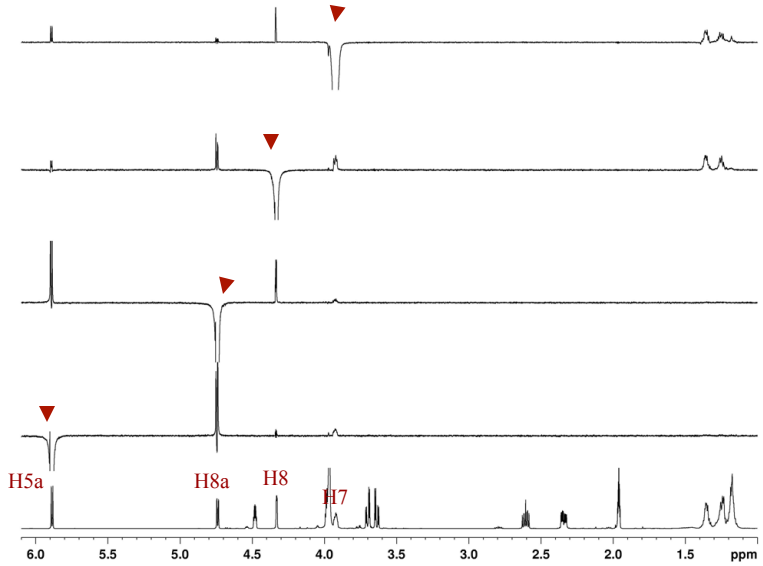
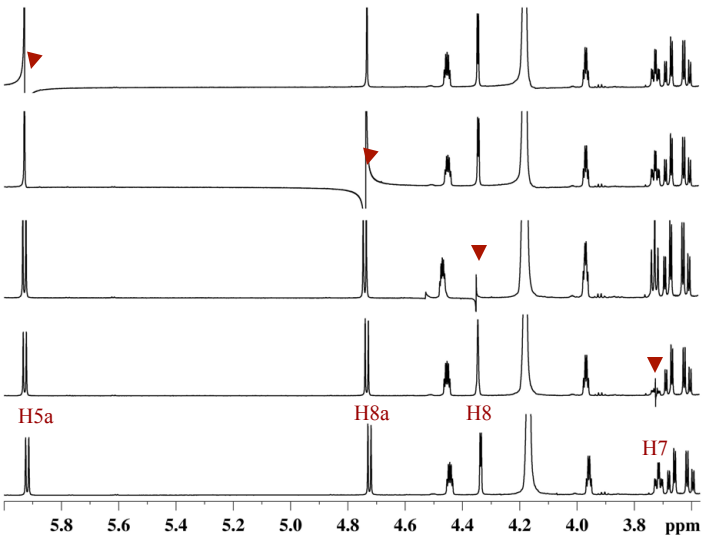


Figure S7. 1D 1H homonuclear decoupling experiments for tetracyclic adduct **15**



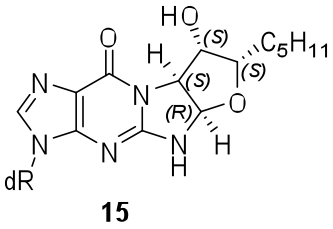


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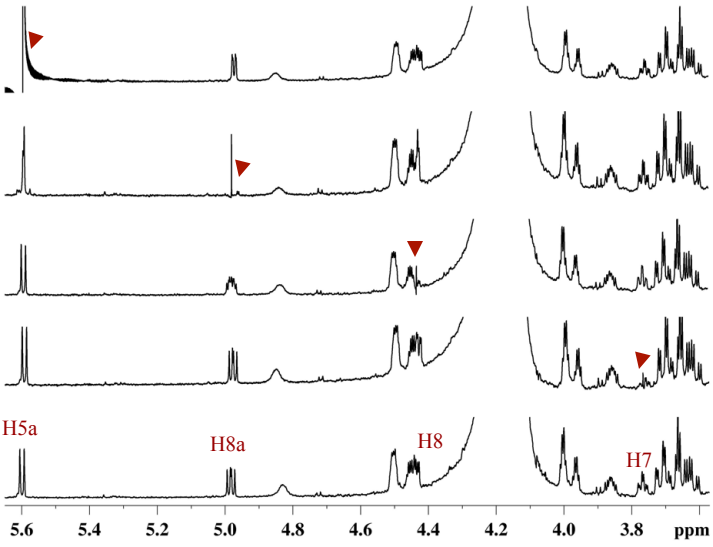
Figure S8. 1D 1H homonuclear decoupling experiments for tetracyclic adduct **16**



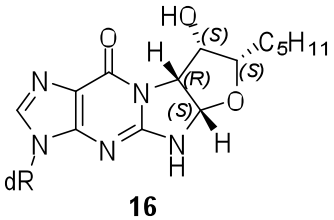


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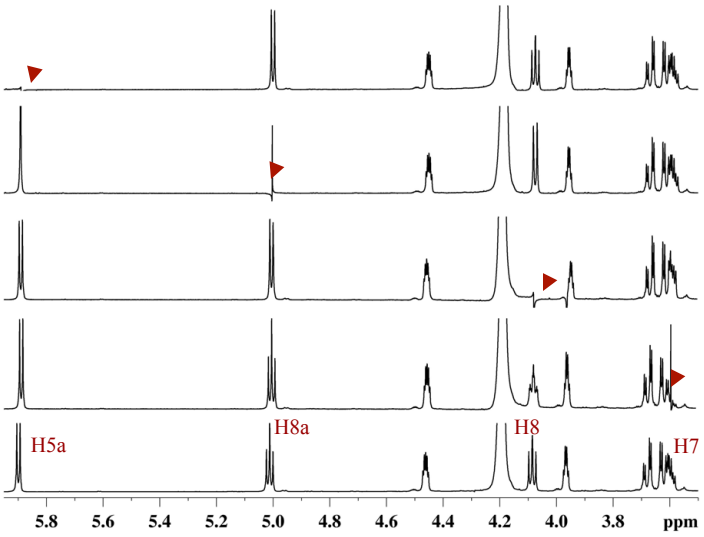
Figure S9. 1D 1H homonuclear decoupling experiments for tetracyclic adduct **21**



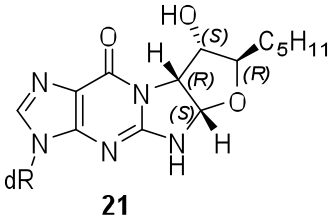


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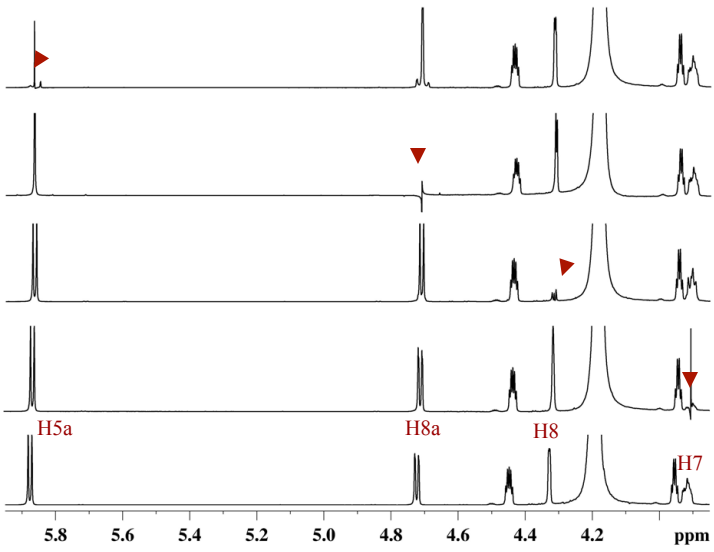
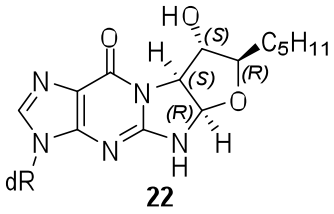
Figure S10. 1D 1H homonuclear decoupling experiments for tetracyclic adduct **22**





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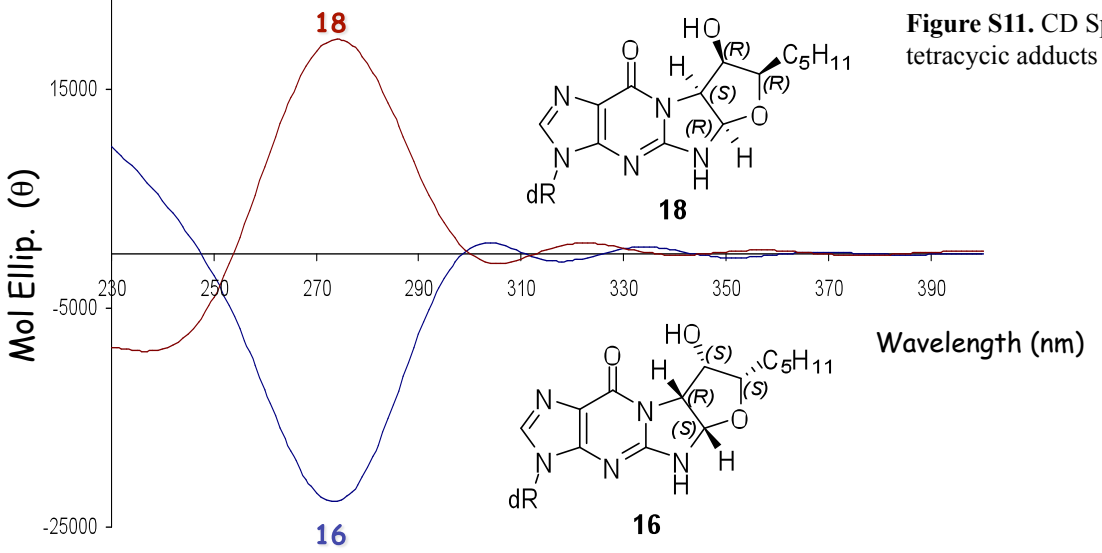


Figure S11. CD Spectra of tetracycic adducts **16** and **18**

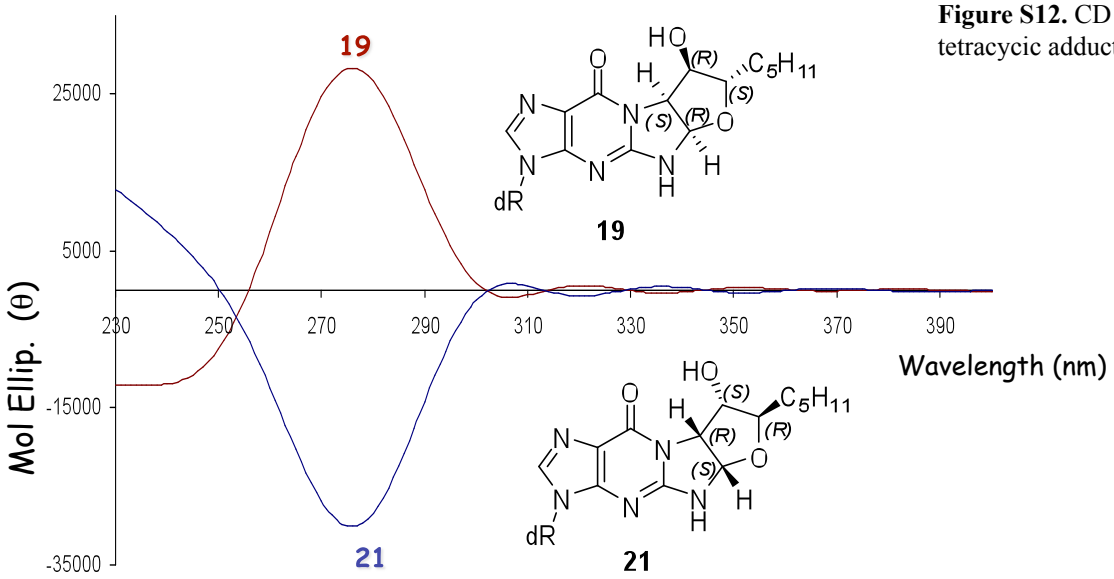


Figure S12. CD Spectra of tetracycic adducts **19** and **21**

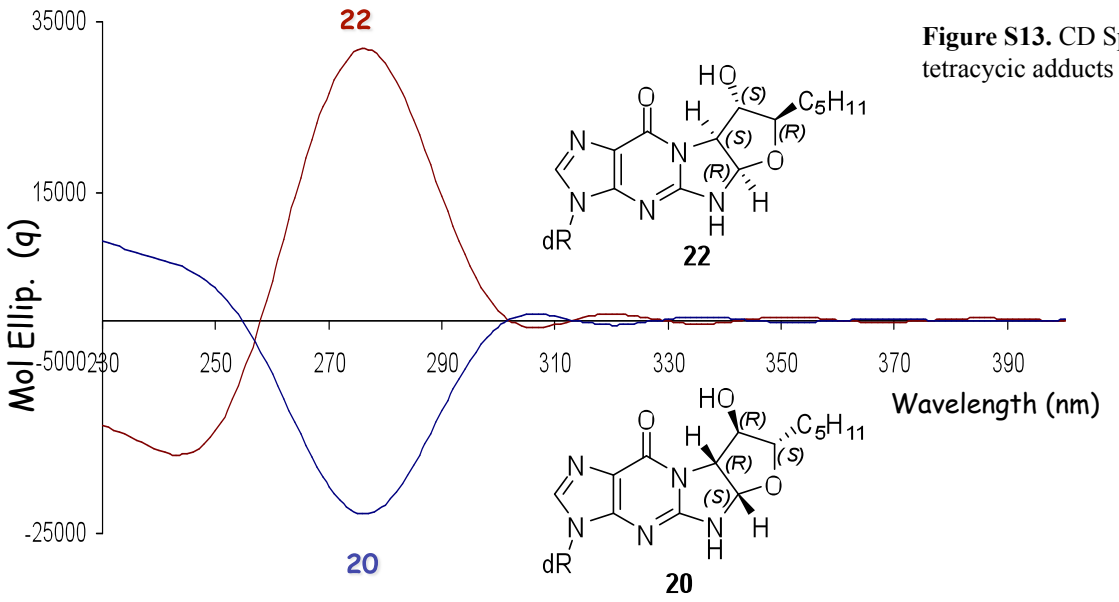


Figure S13. CD Spectra of tetracycic adducts **20** and **22**

Figure 14. Spectra of C7-(1,2-dihydroxyheptyl)-εdGuo adducts **11** and **12**

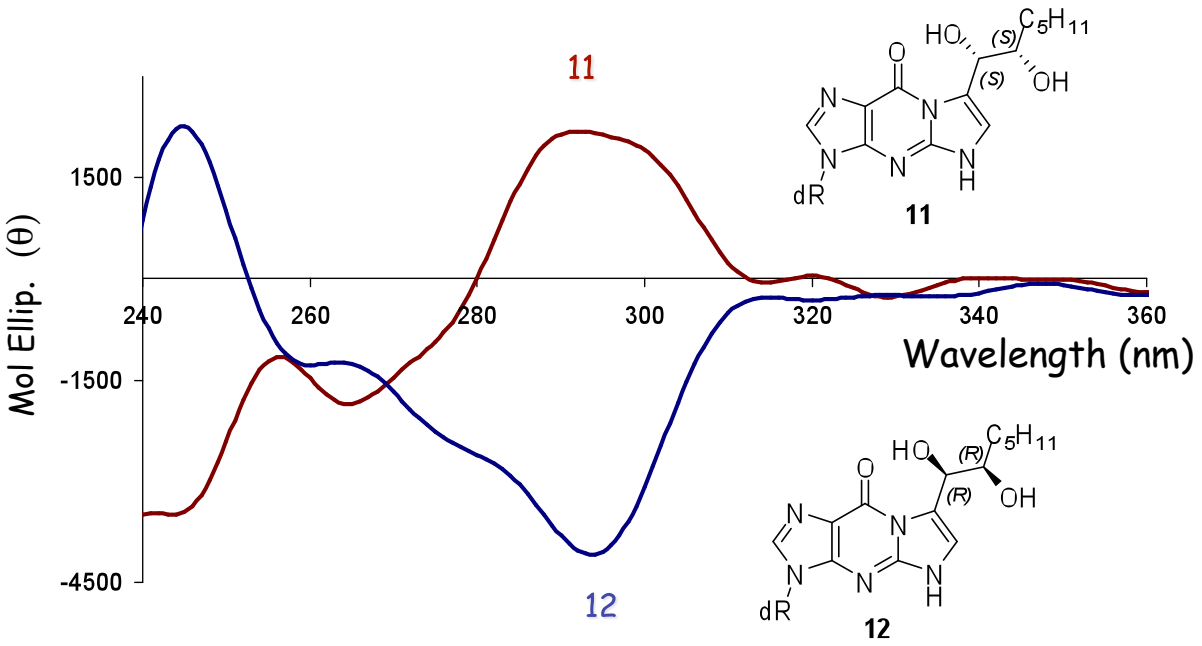


Figure 15. Spectra of C7-(1,2-dihydroxyheptyl)-εdGuo adducts **13** and **14**

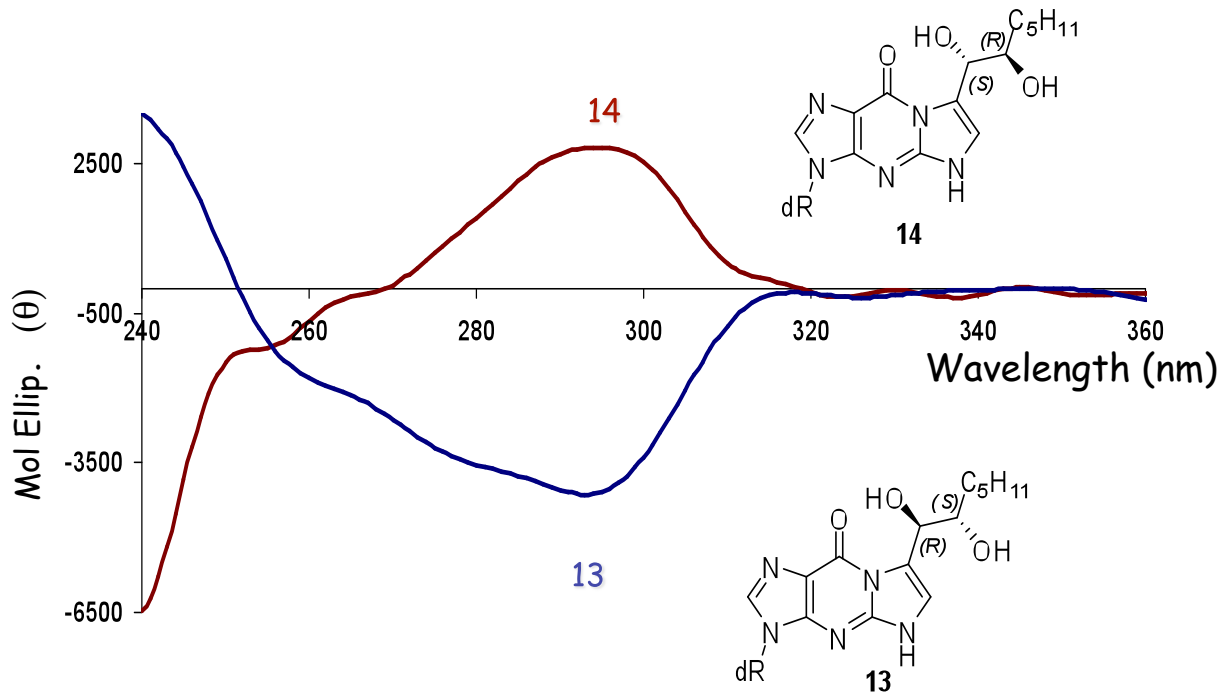
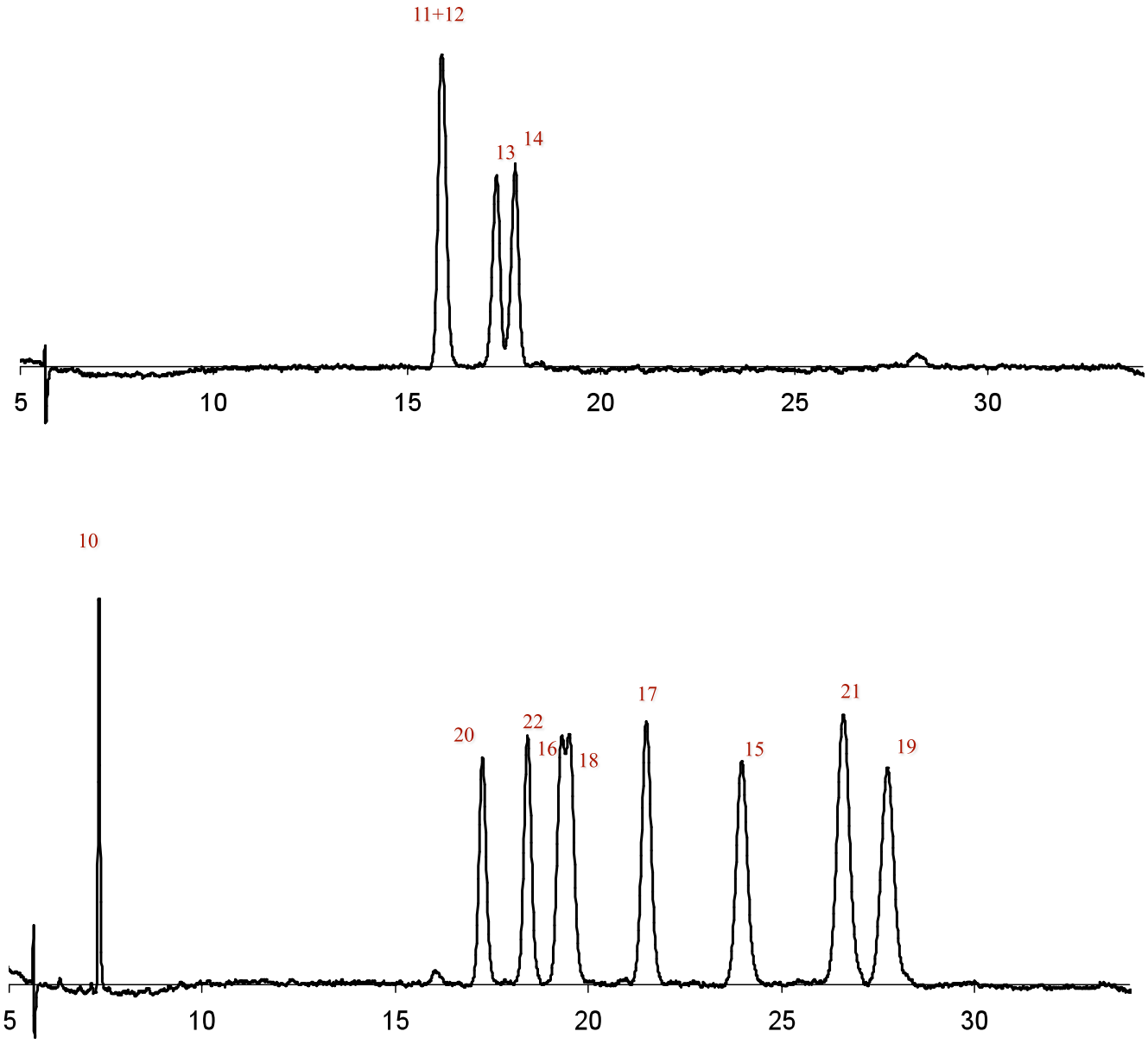


Figure S16. HPLC chromatograms of four C7-(1,2-dihydroxyheptyl)-εdGuo adducts **11-14** and eight tetracyclic adducts **15-22**



HPLC separation of EHN-dGuo adducts was conducted on a Beckman gradient HPLC system with a diode array UV detector monitoring at 260 nm using a Waters YMC ODS-AQ column (250 mm × 4.6 mm) at flow rate 1.0 mL/min. The mobile phase consisted of H_2O and CH_3CN using the following gradients: initially 90 % H_2O; a 3 min linear gradient to 78 % H_2O; isocratic at 78 % H_2O for 25 min, 4 min linear gradient to 20 % H_2O, followed by a 4 min linear gradient to 90 % H_2O and remained for 1 min at the initial conditions.

Figure S17. ^{1}H NMR spectrum and GC chromatogram of (*4*S)-4-hydroxy-2*E*-nonenal **1**

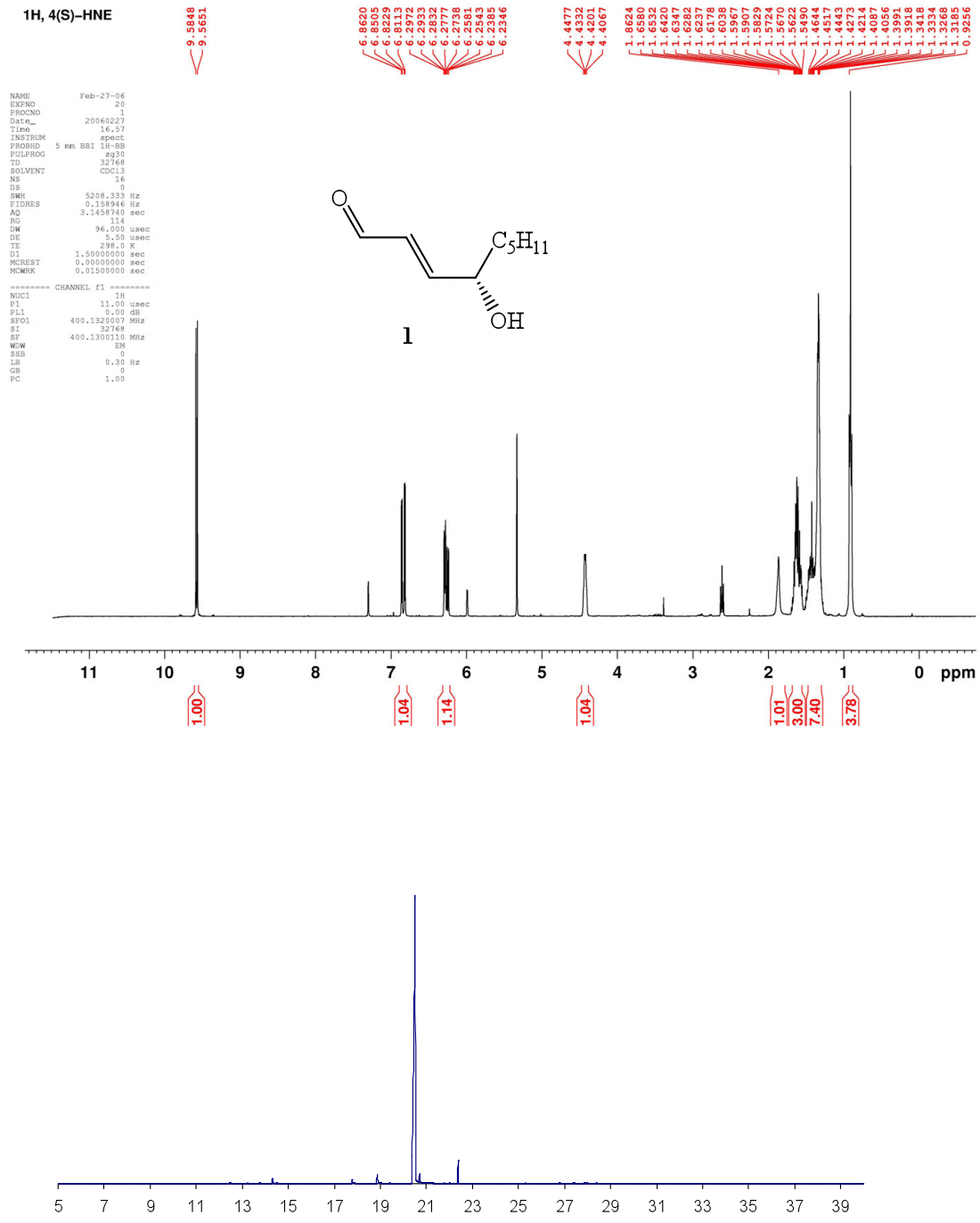
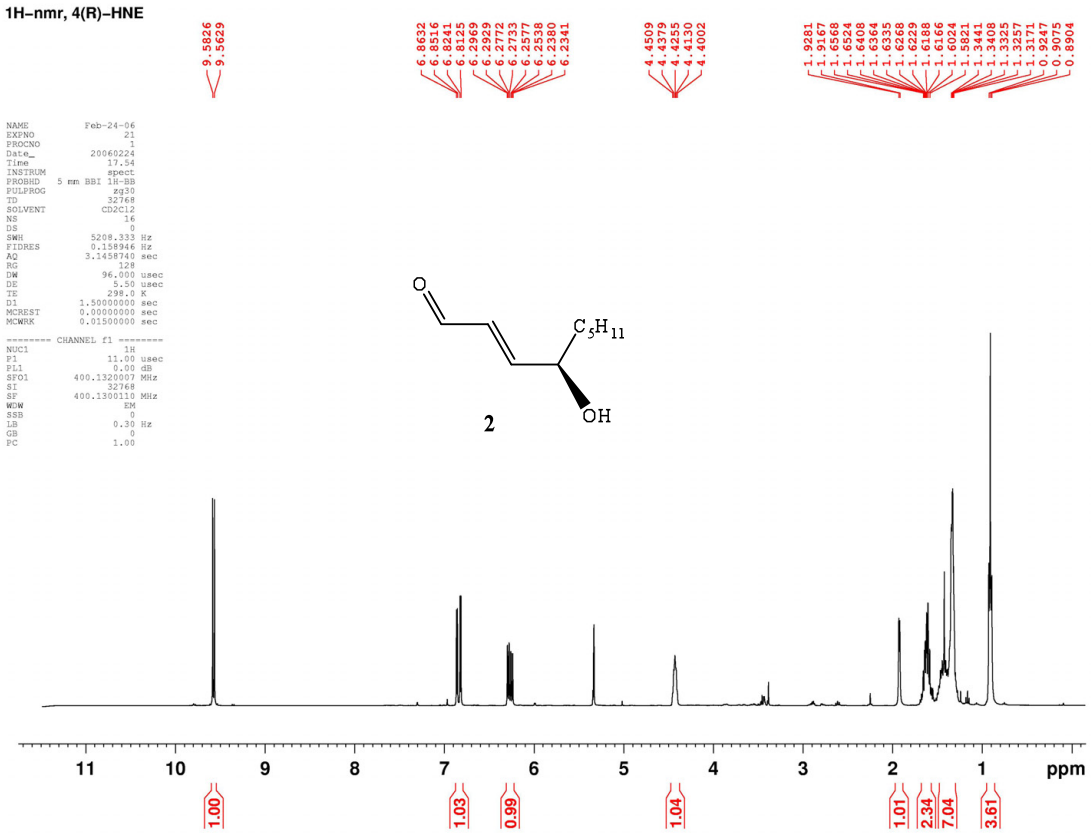
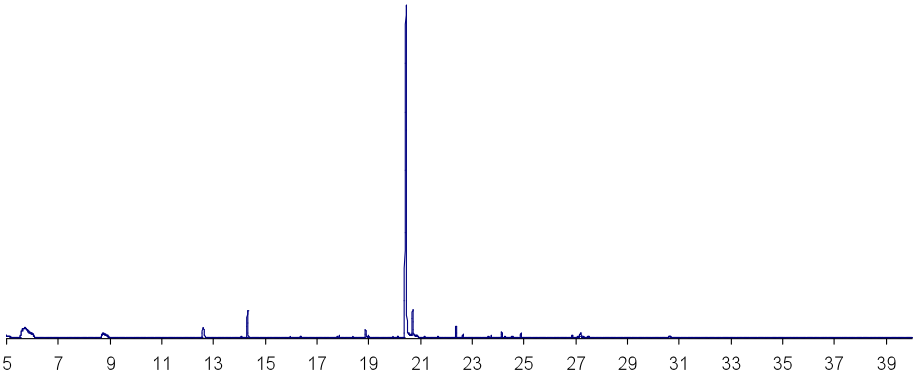


Figure S18. ^{1}H NMR spectrum and GC chromatogram of (*4R*)-4-hydroxy-2*E*-nonenal **2**





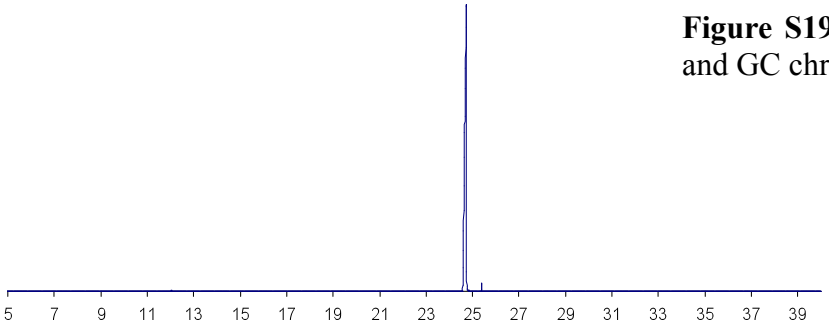
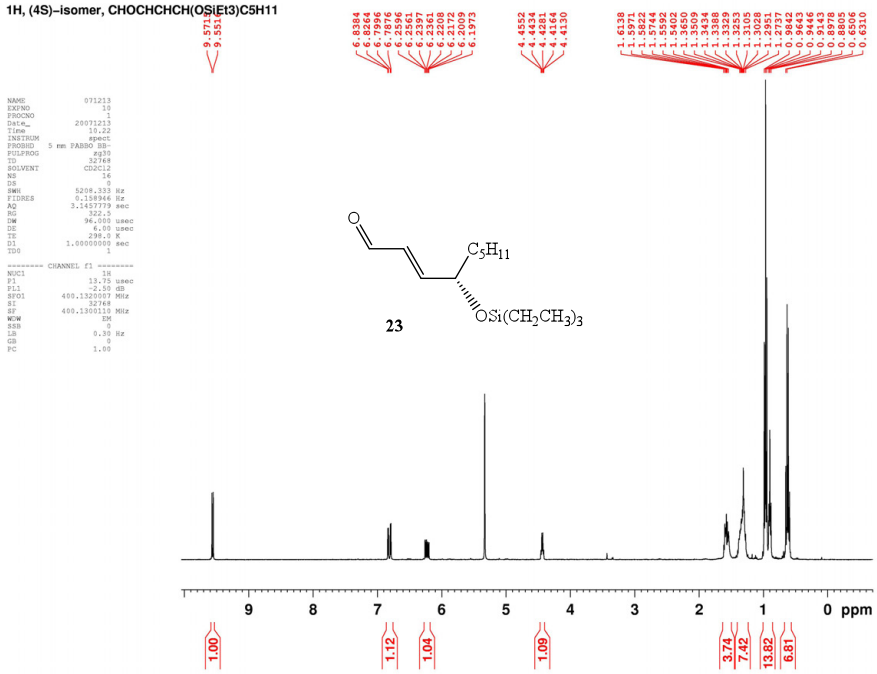


Figure S19. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **23**



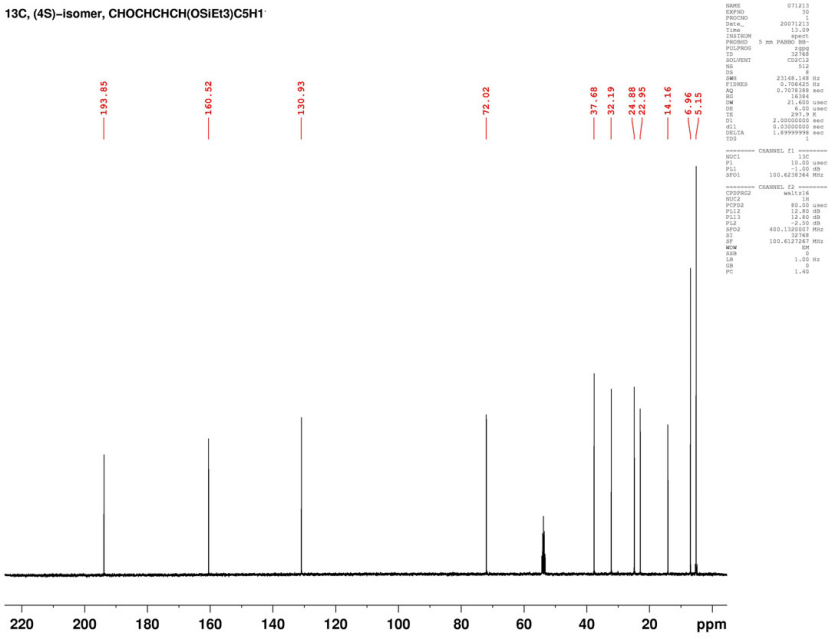
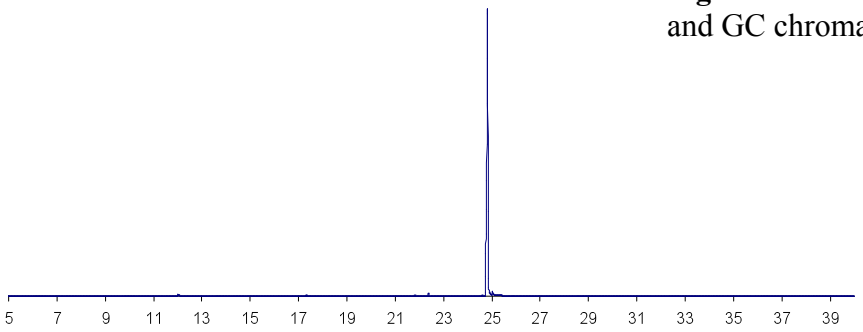
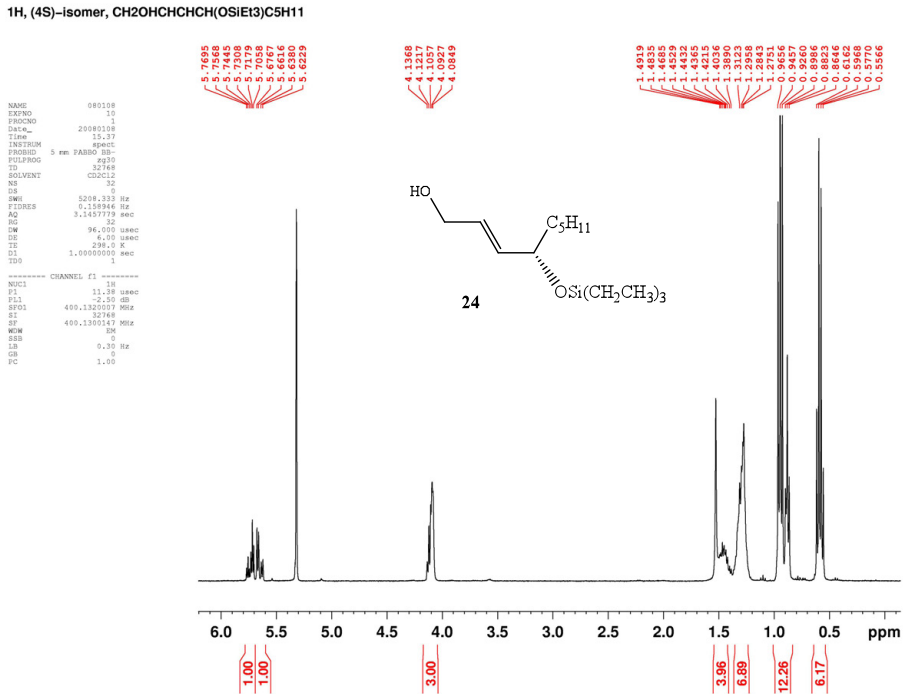
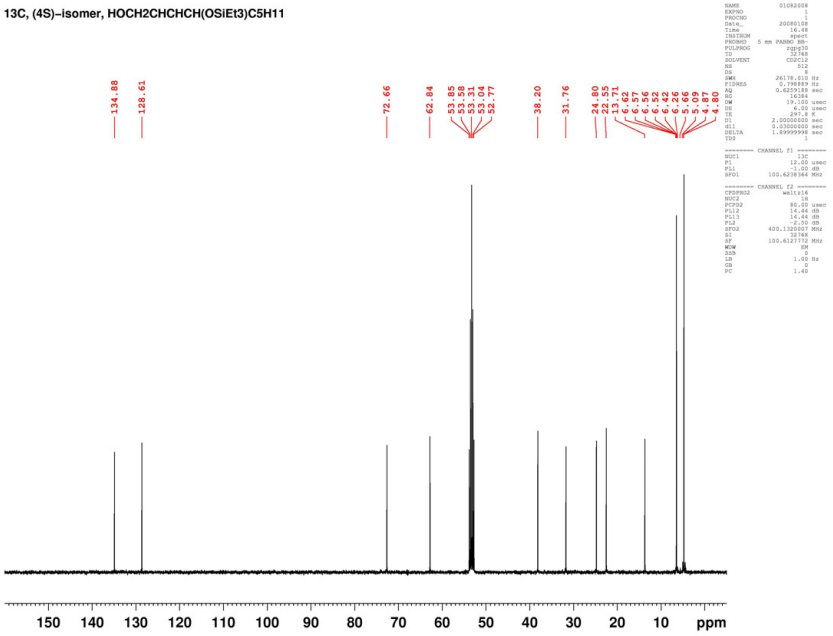


Figure S20. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **24**







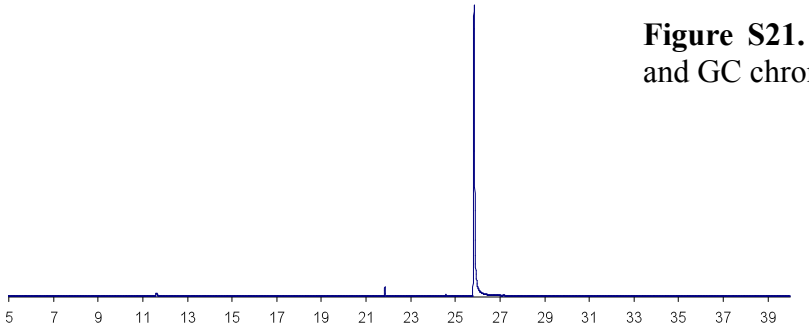
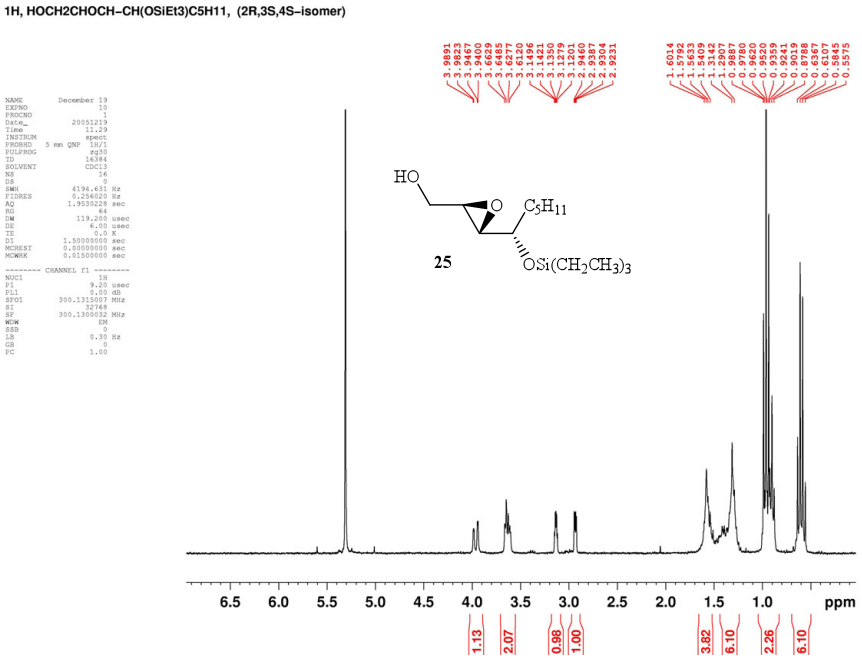
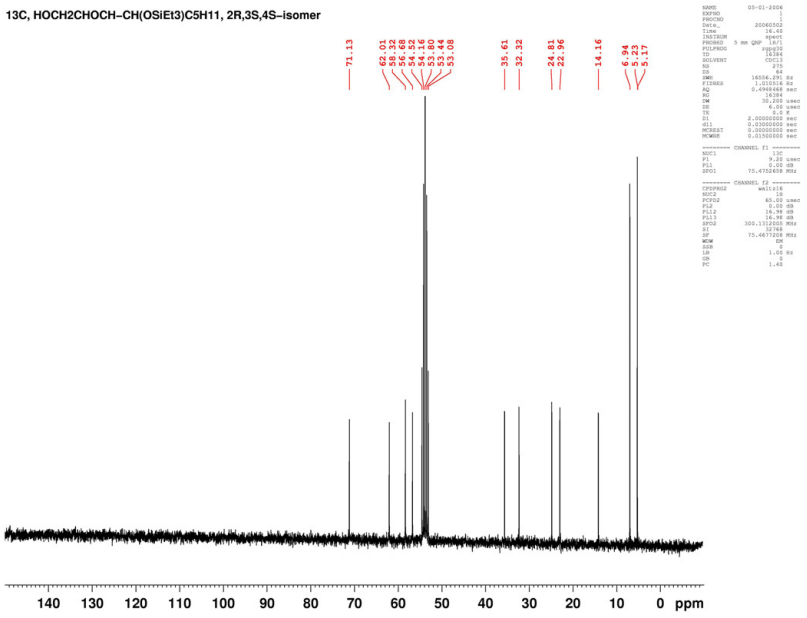


Figure S21. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **25**





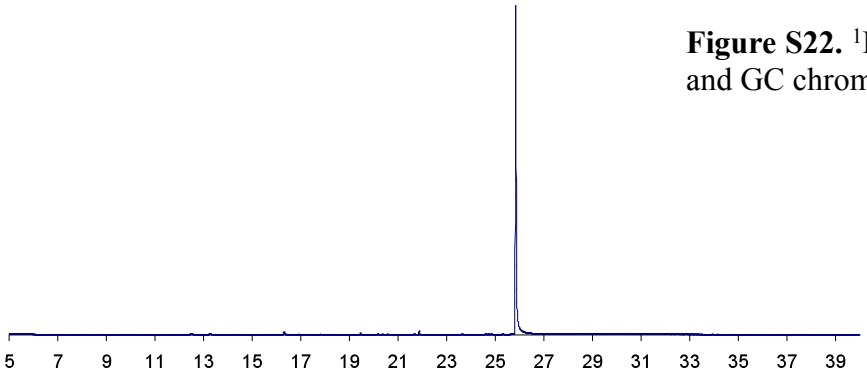
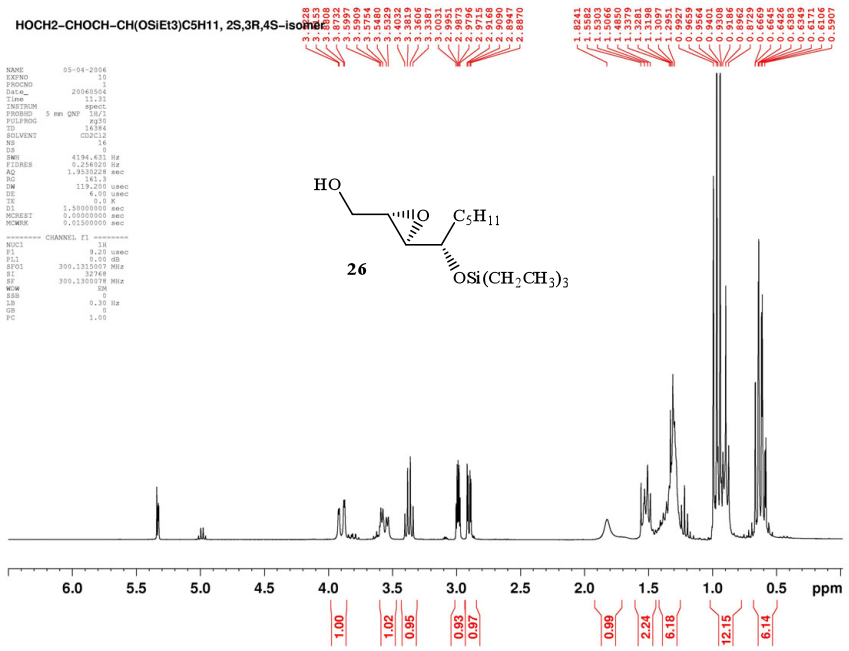
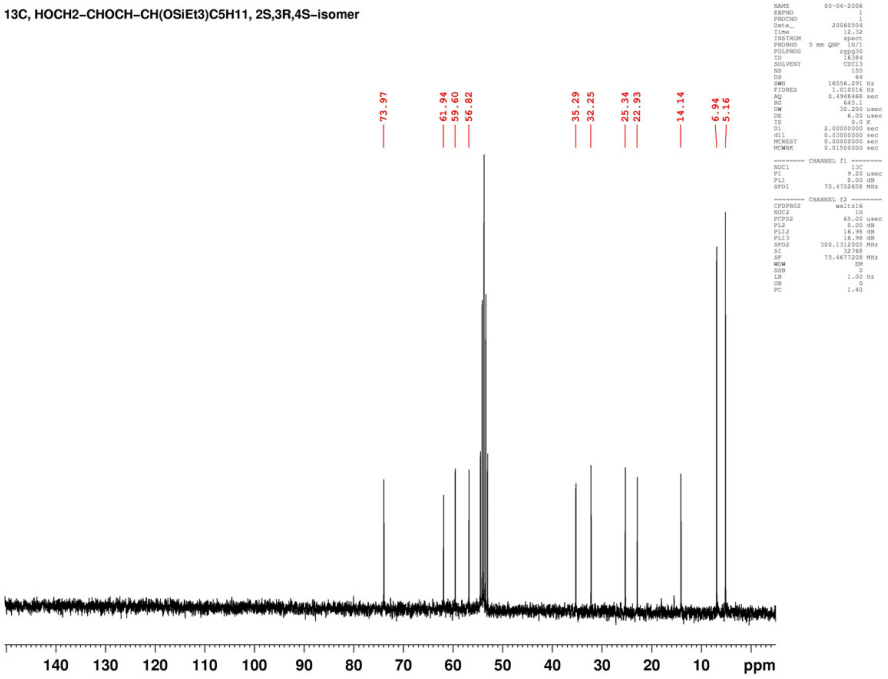


Figure S22. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **26**





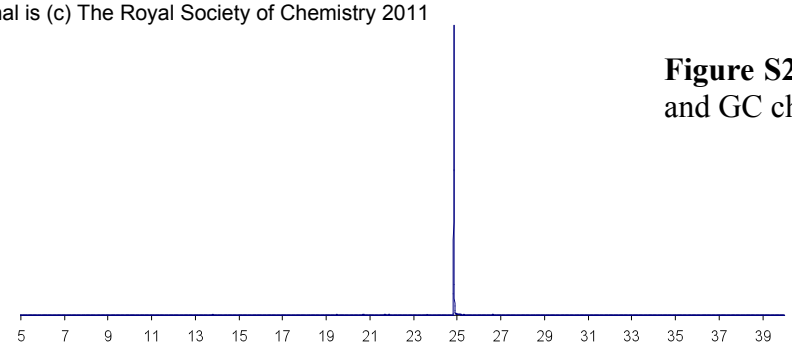
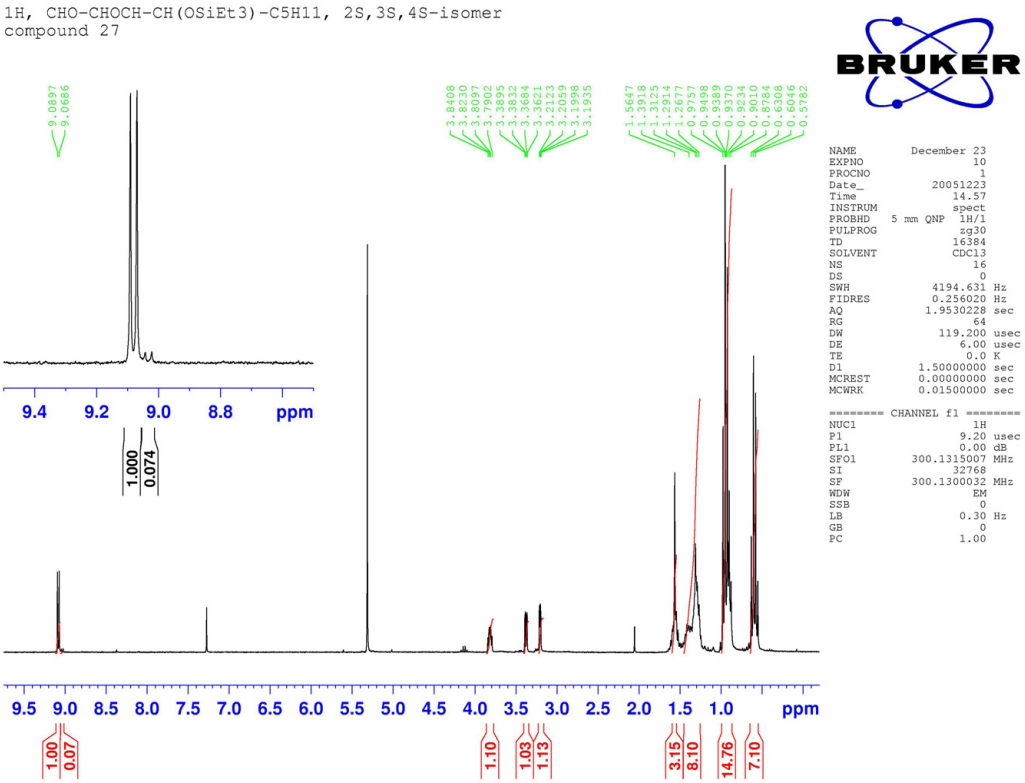
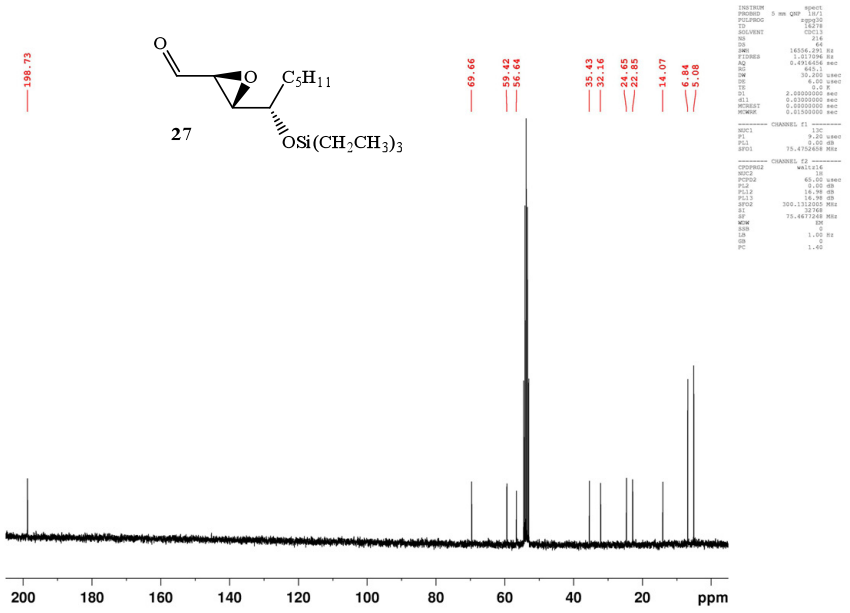


Figure S23. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **27**





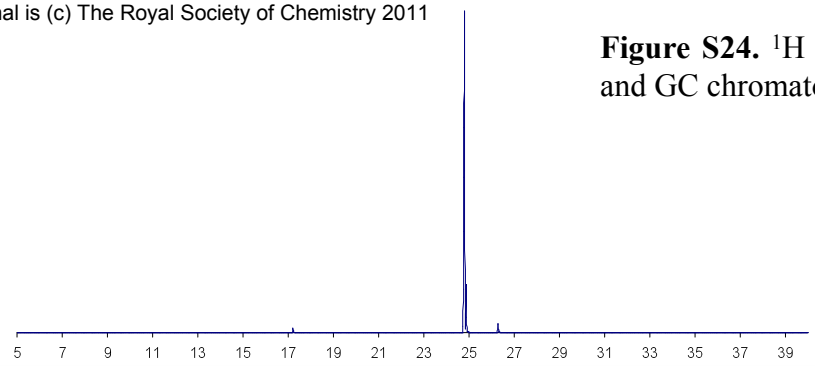
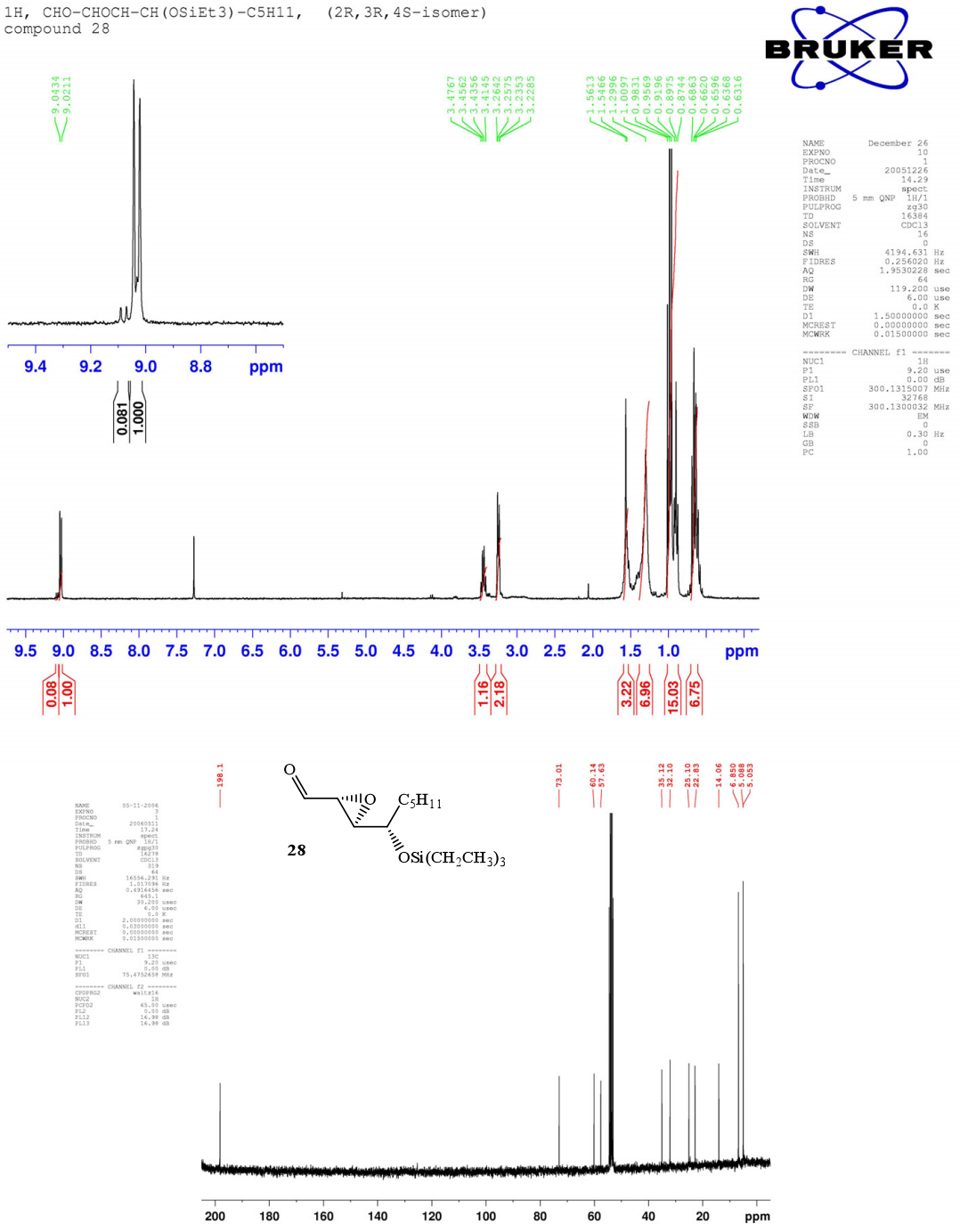


Figure S24. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **28**



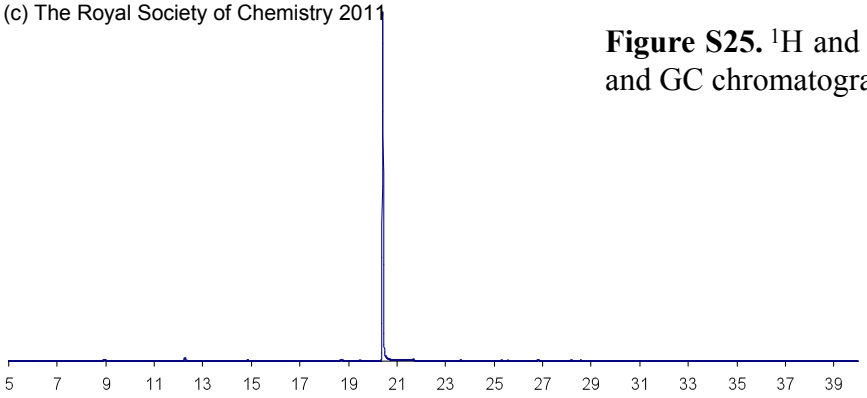
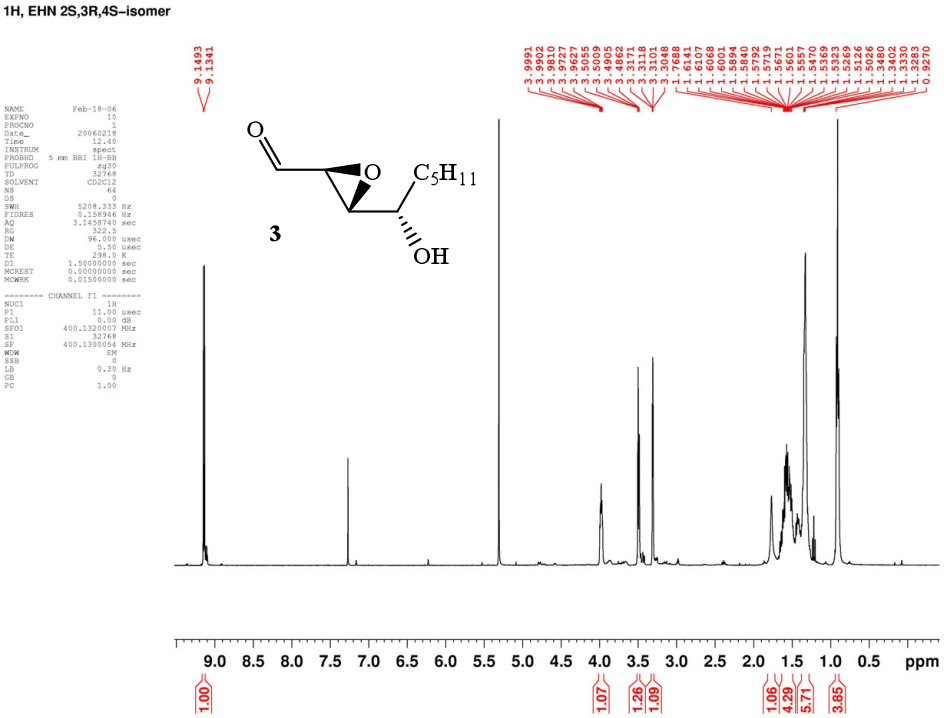


Figure S25. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **3**



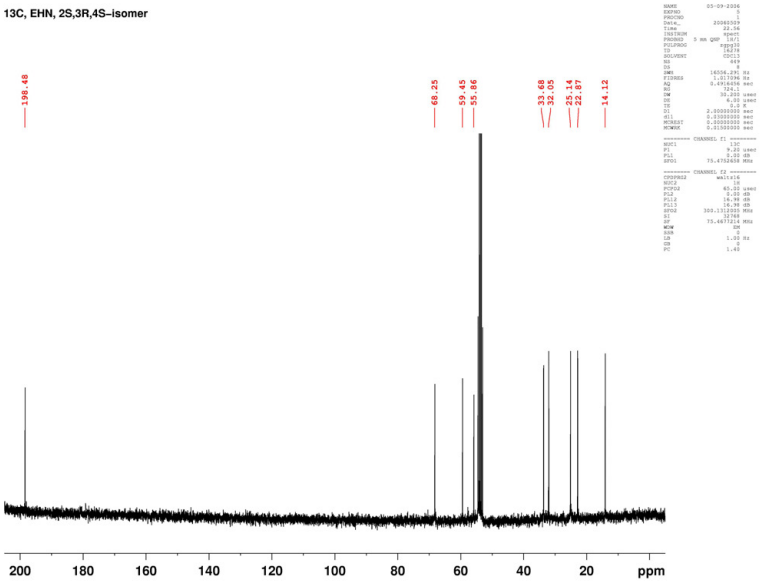
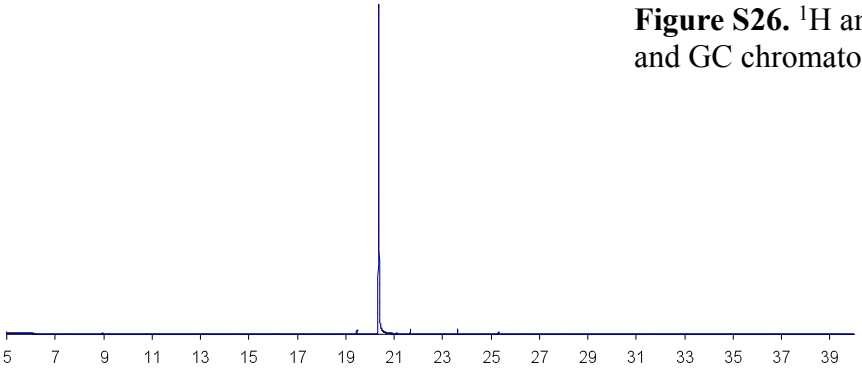
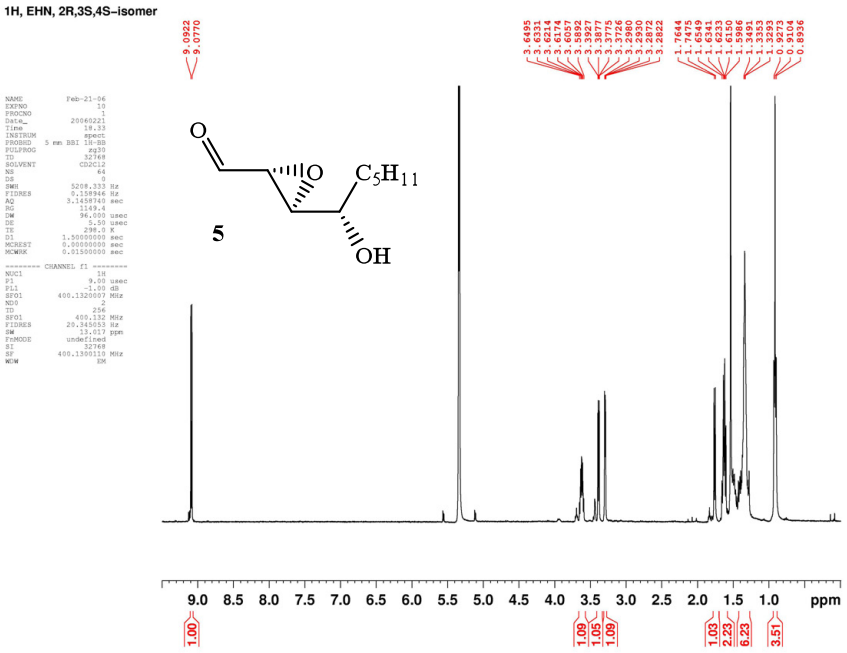
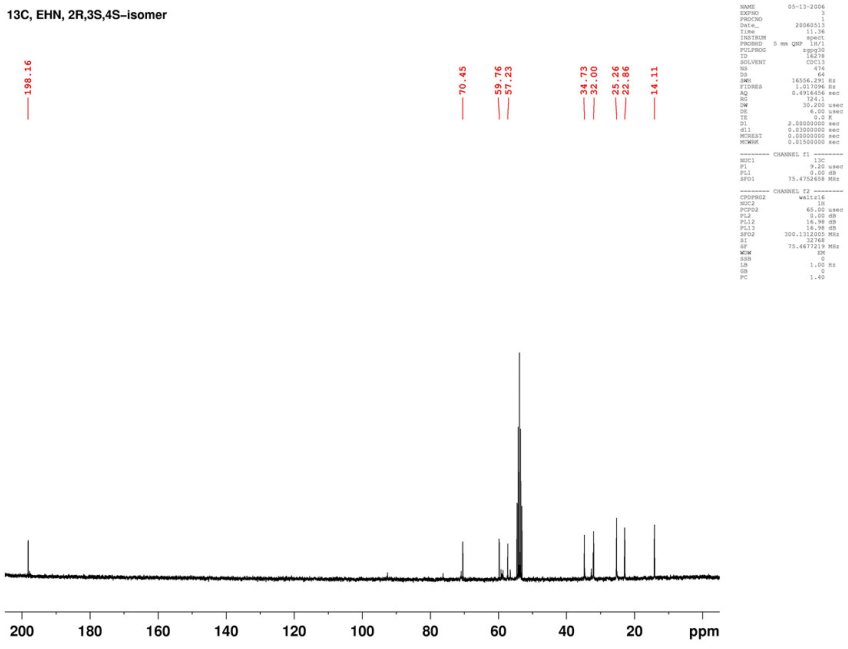


Figure S26. ^{1}H and ^{13}C NMR spectra and GC chromatogram of **5**







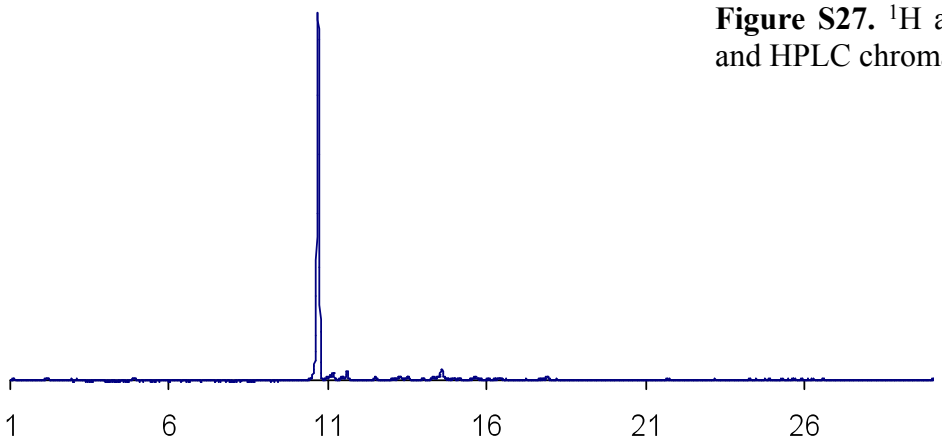
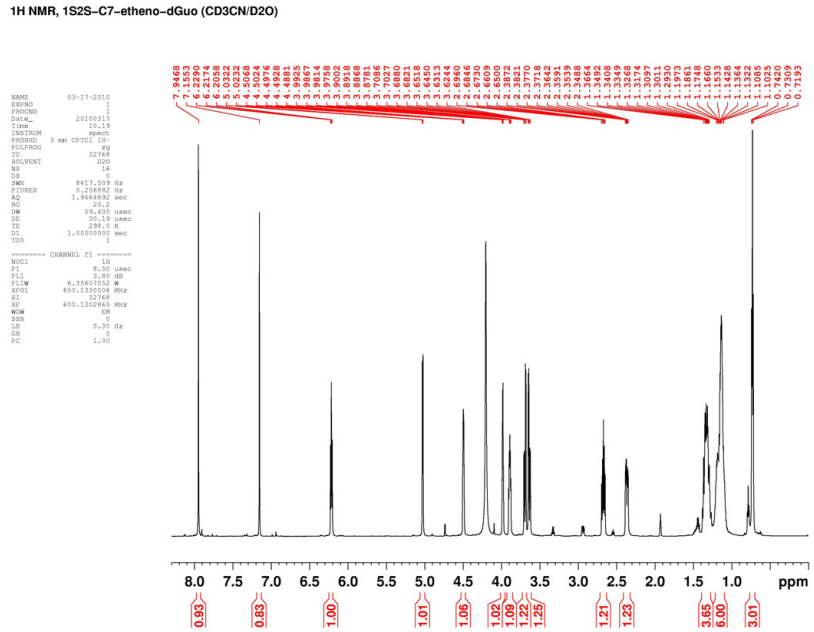
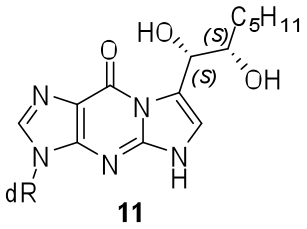


Figure S27. ^{1}H and ^{13}C NMR spectra and HPLC chromatogram of **11**





11

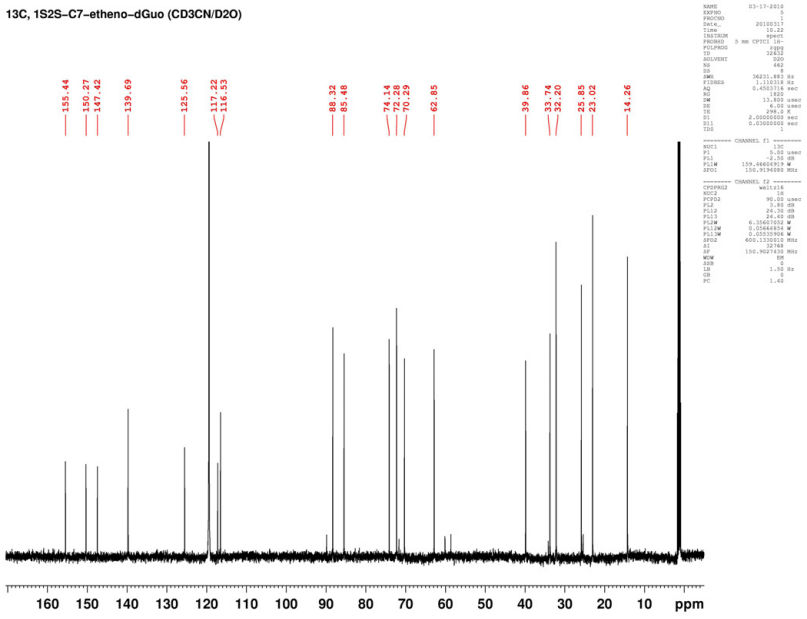
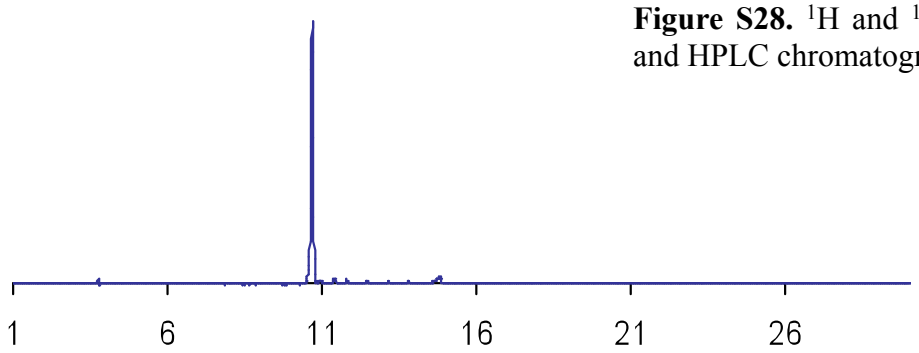
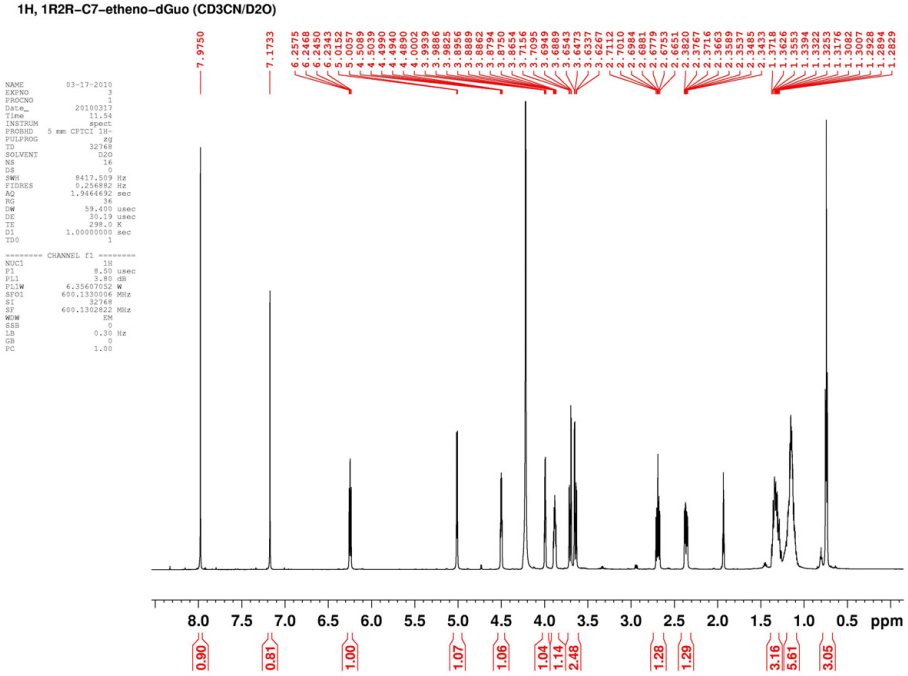
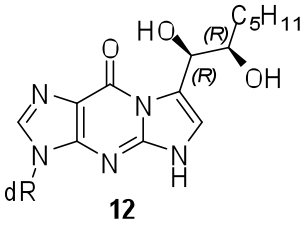


Figure S28. ^{1}H and ^{13}C NMR spectra and HPLC chromatogram of **12**







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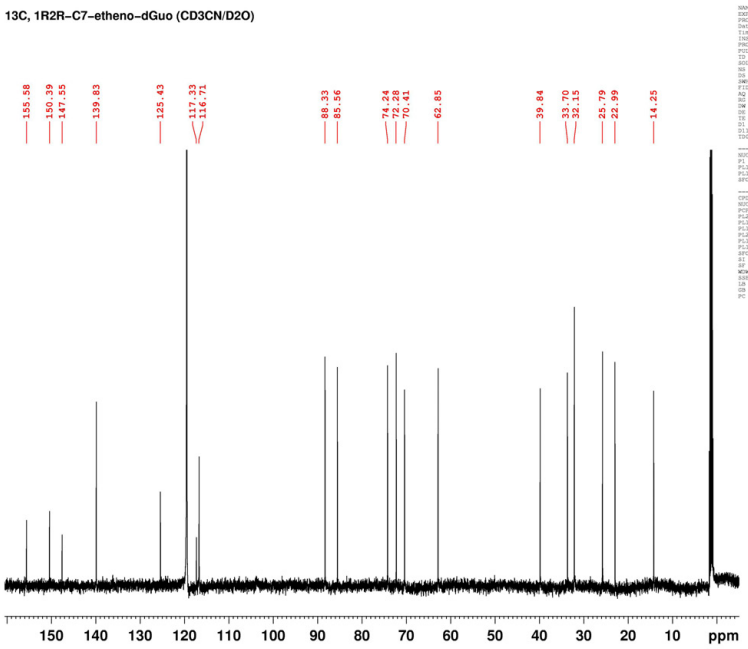
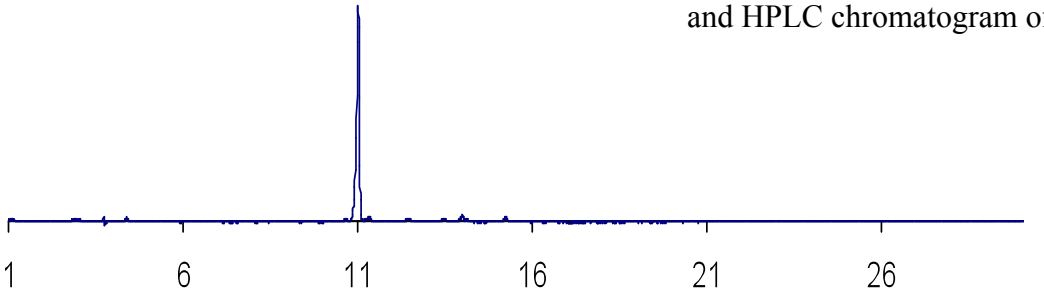
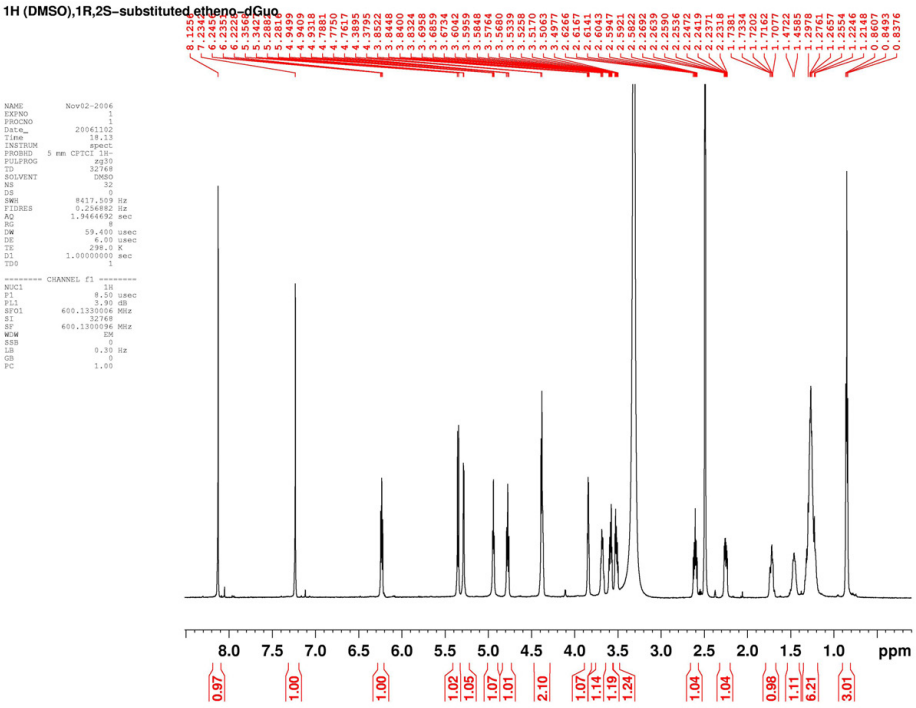
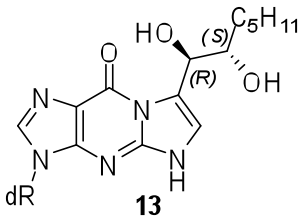


Figure S29. ^{1}H and ^{13}C NMR spectra and HPLC chromatogram of **13**







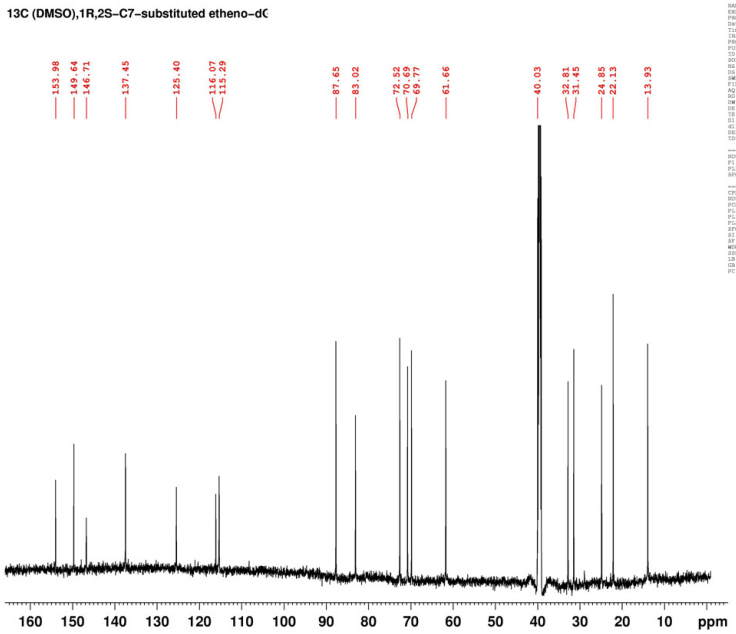
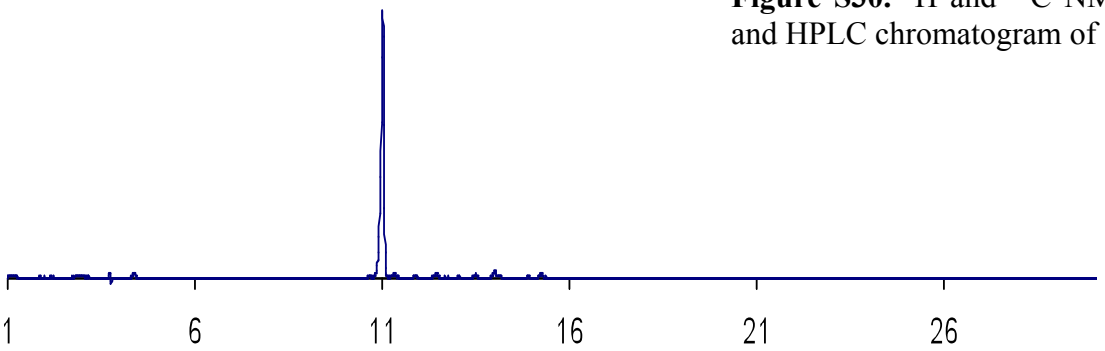
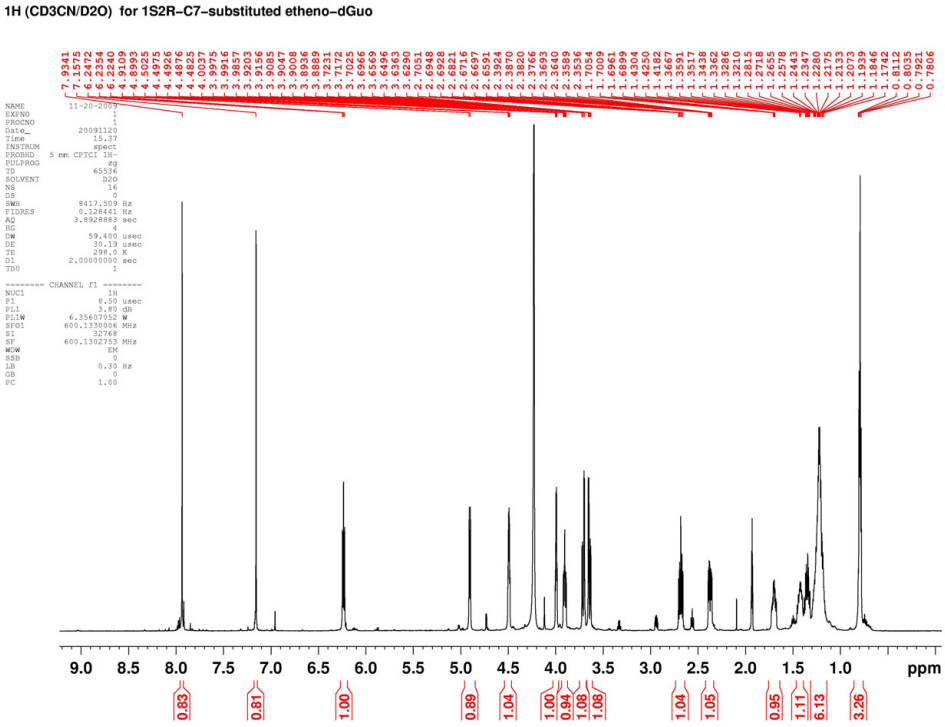
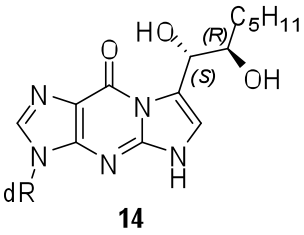


Figure S30. ^{1}H and ^{13}C NMR spectra and HPLC chromatogram of **14**







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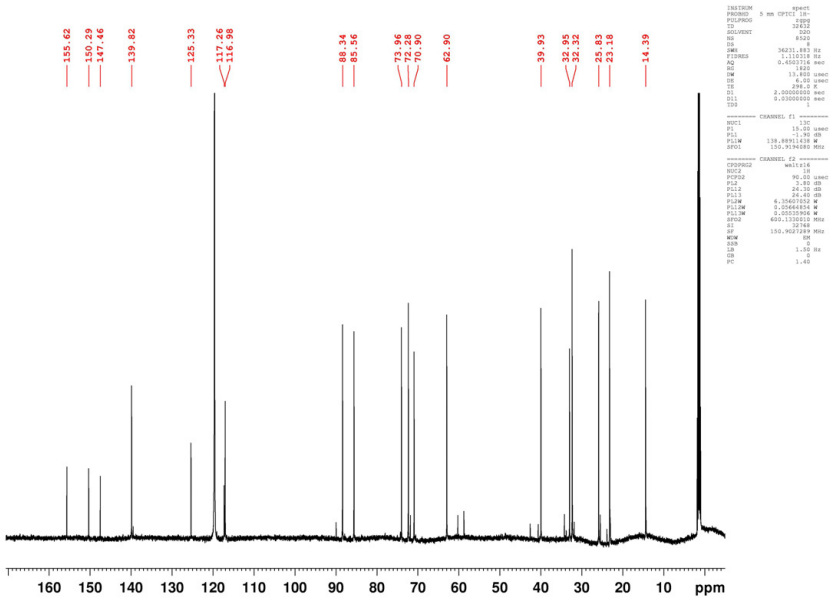
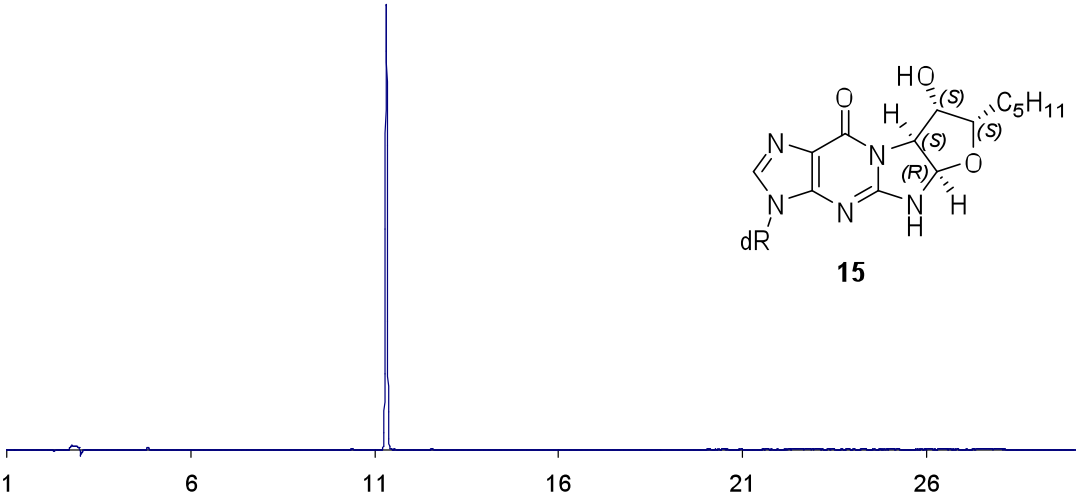


Figure S31. ^{1}H spectrum and HPLC chromatogram of **15**



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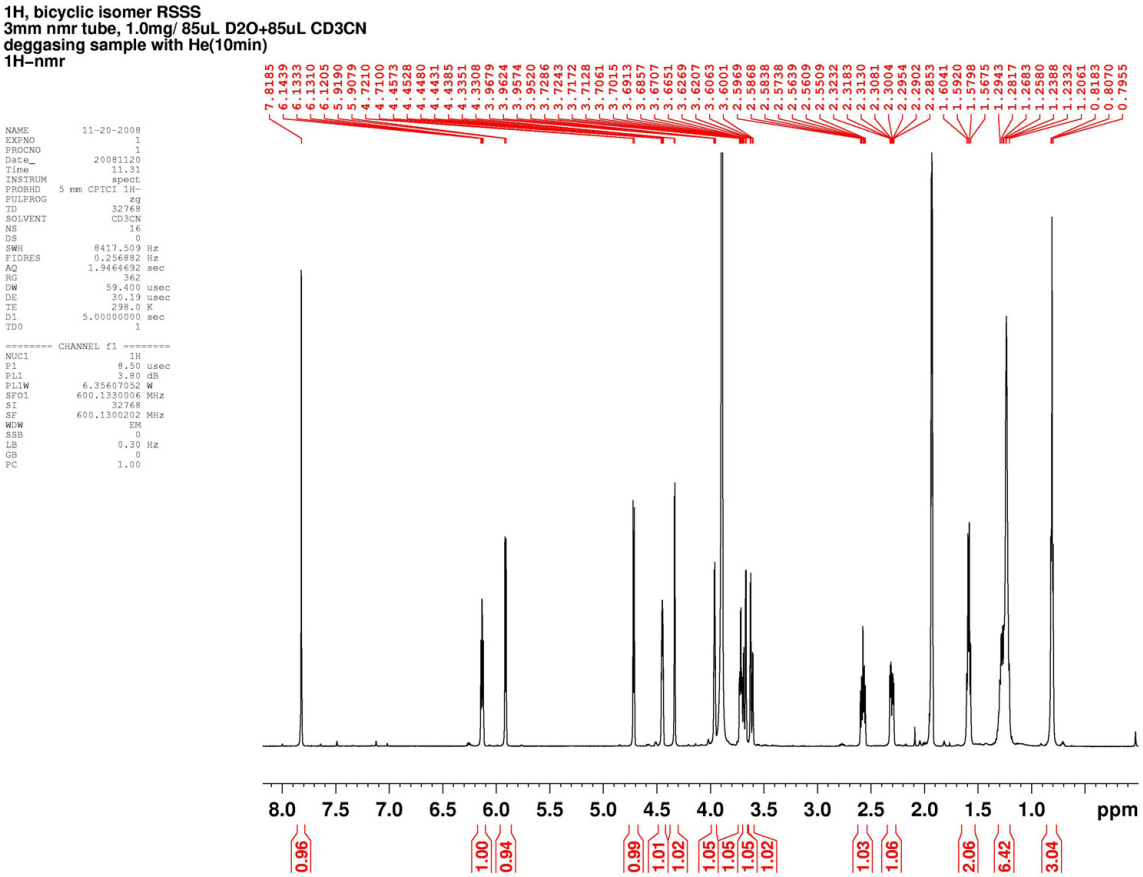
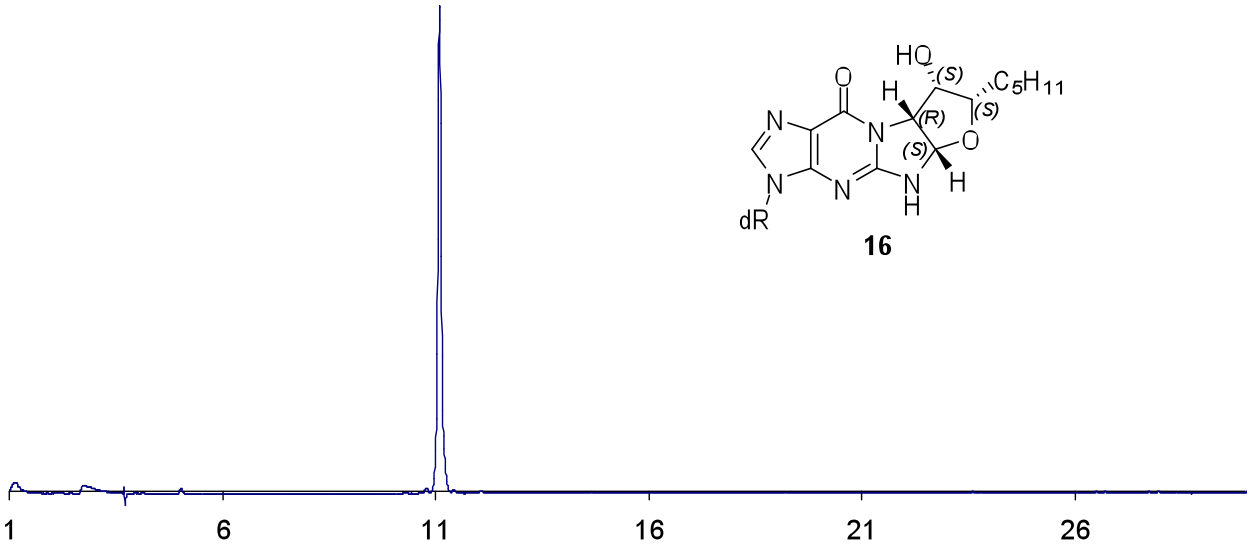


Figure S32. ^{1}H spectrum and HPLC chromatogram of **16**



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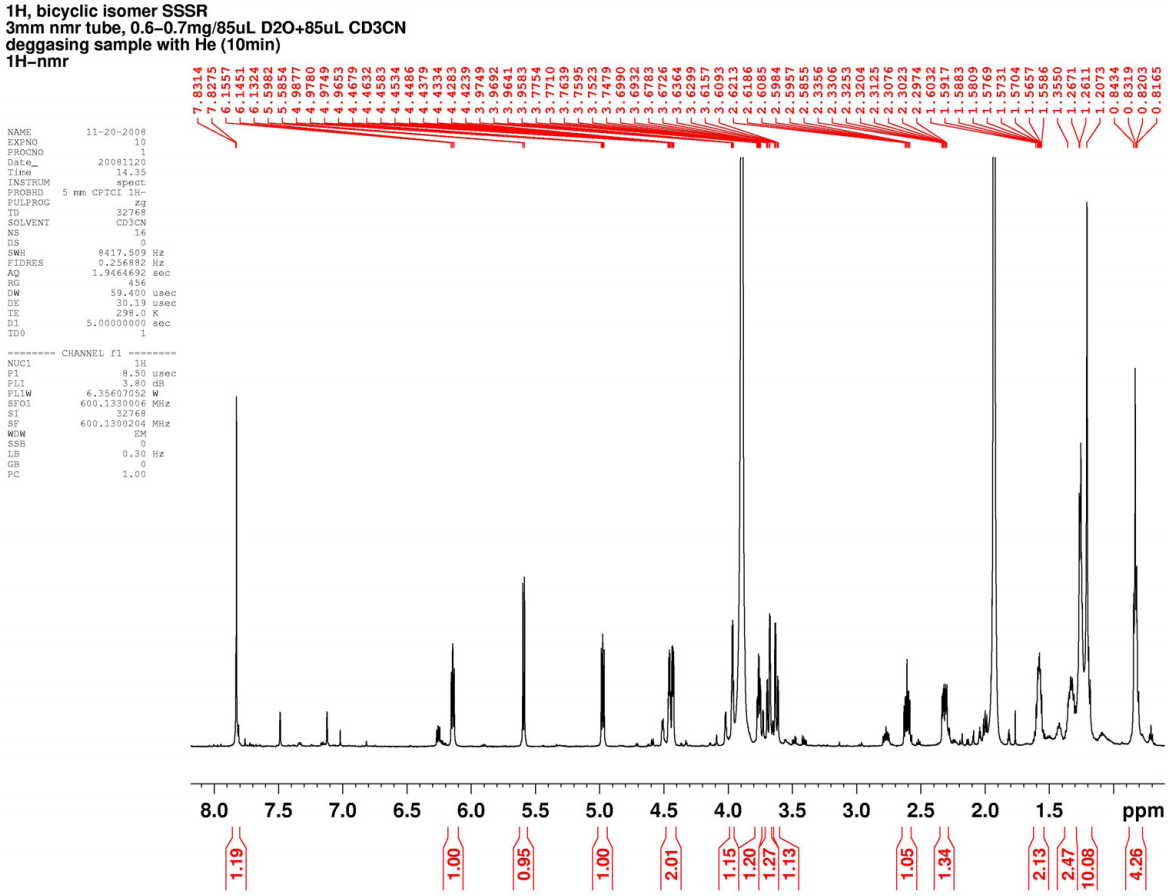
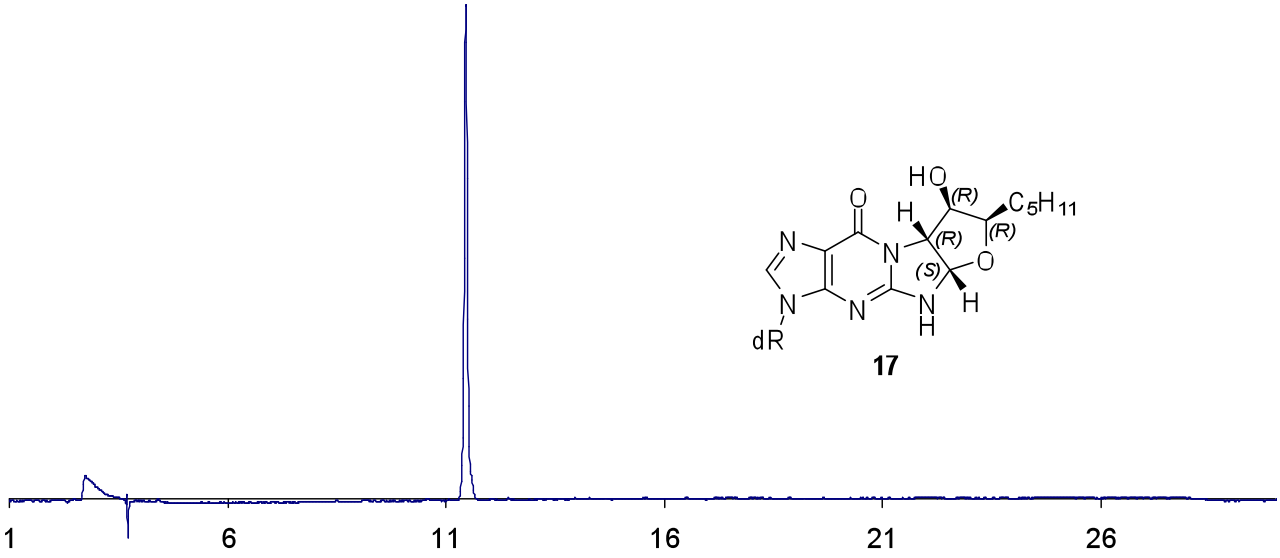


Figure S33. ^{1}H spectrum and HPLC chromatogram of **17**



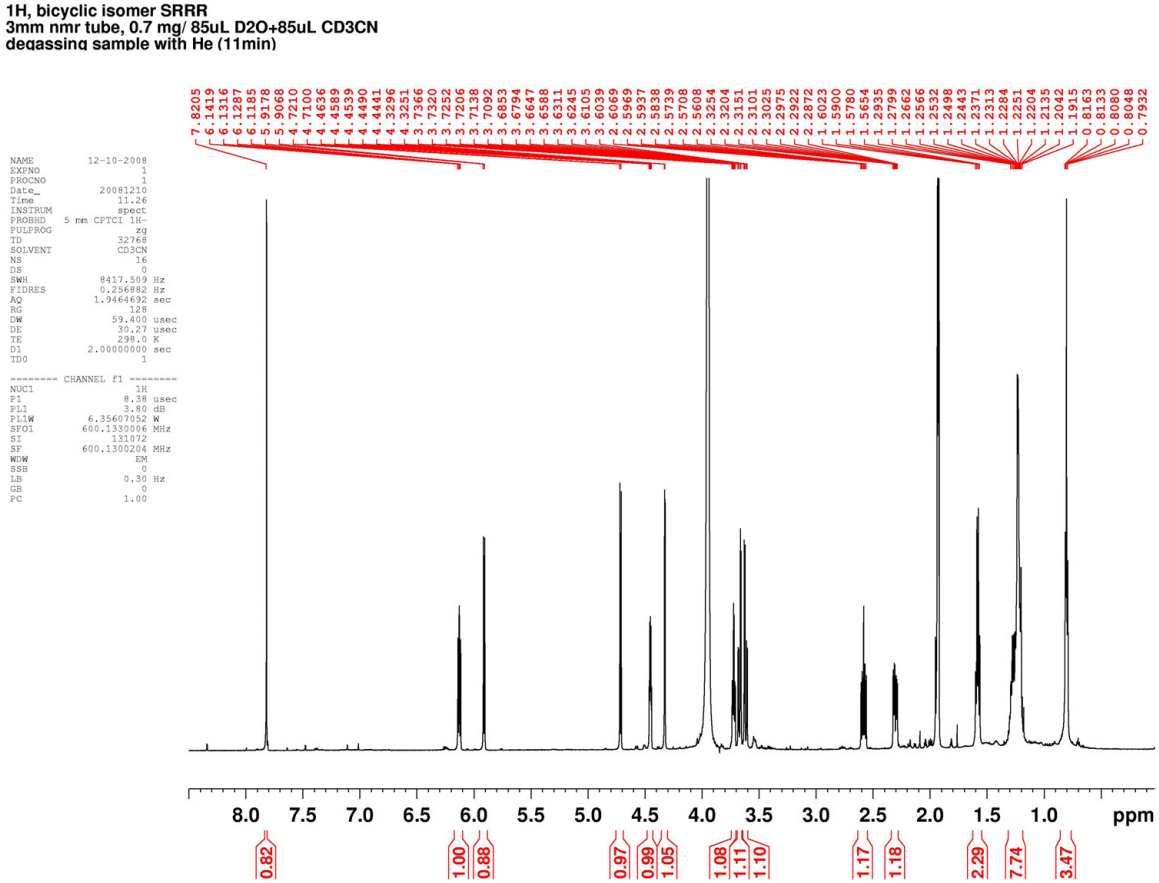
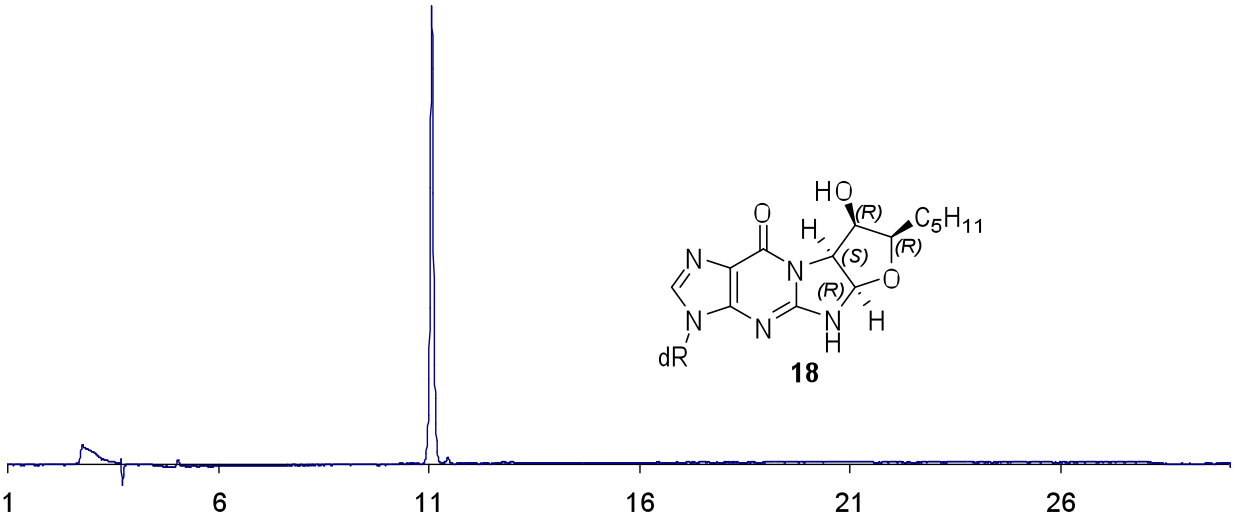


Figure S34. ^{1}H spectrum and HPLC chromatogram of **18**



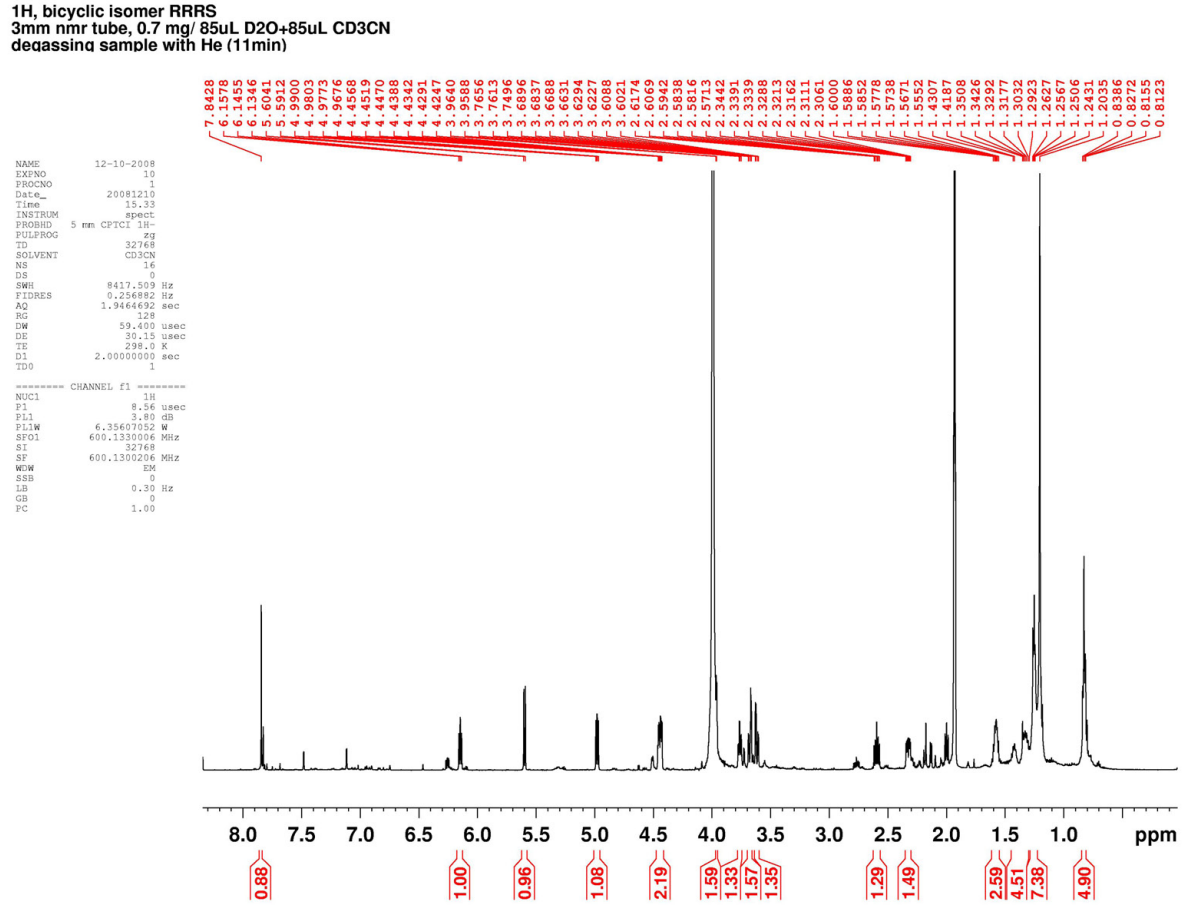
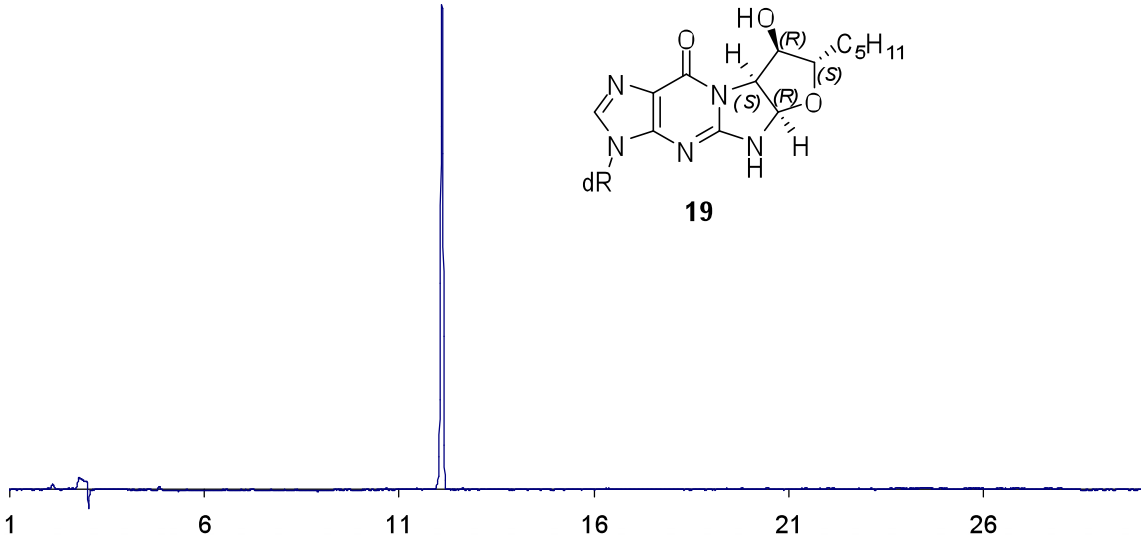


Figure S35. ^{1}H spectrum and HPLC chromatogram of **19**



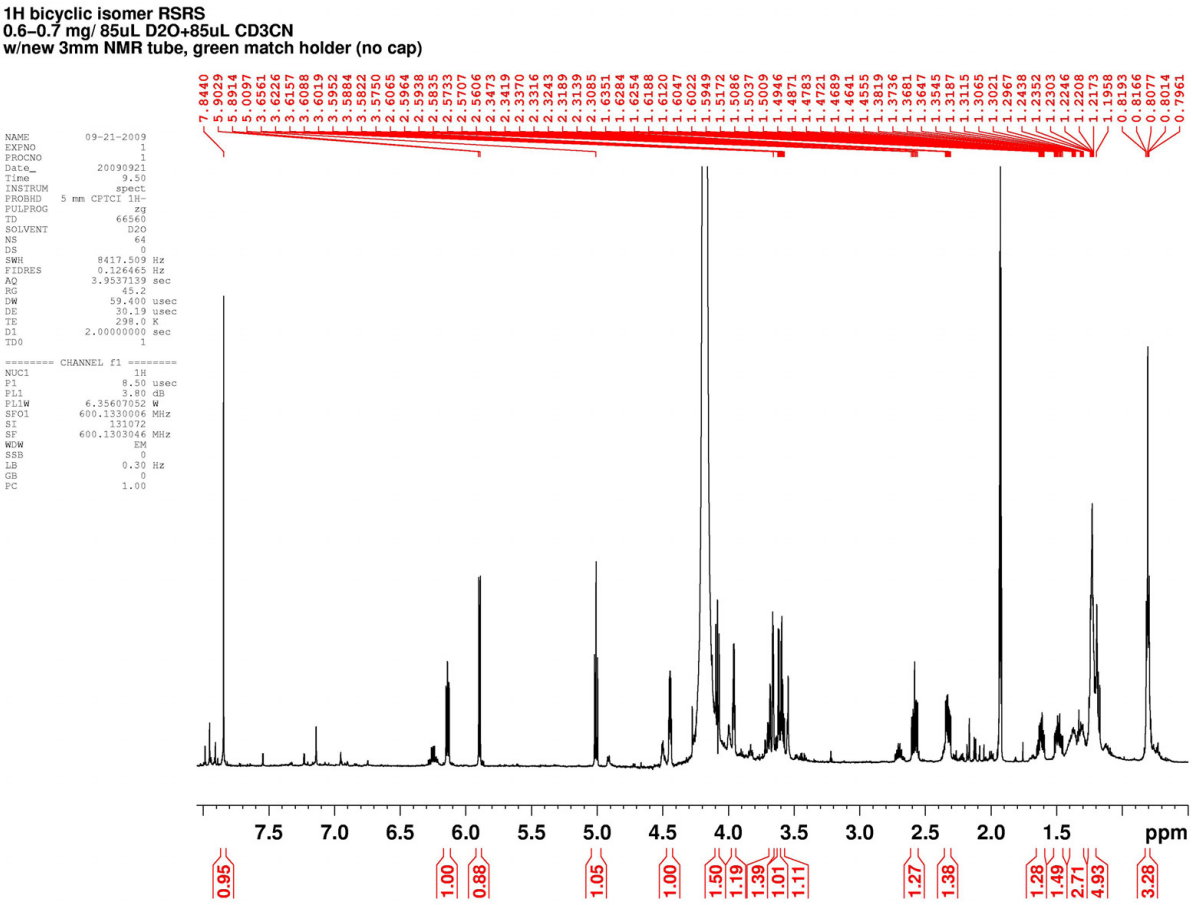
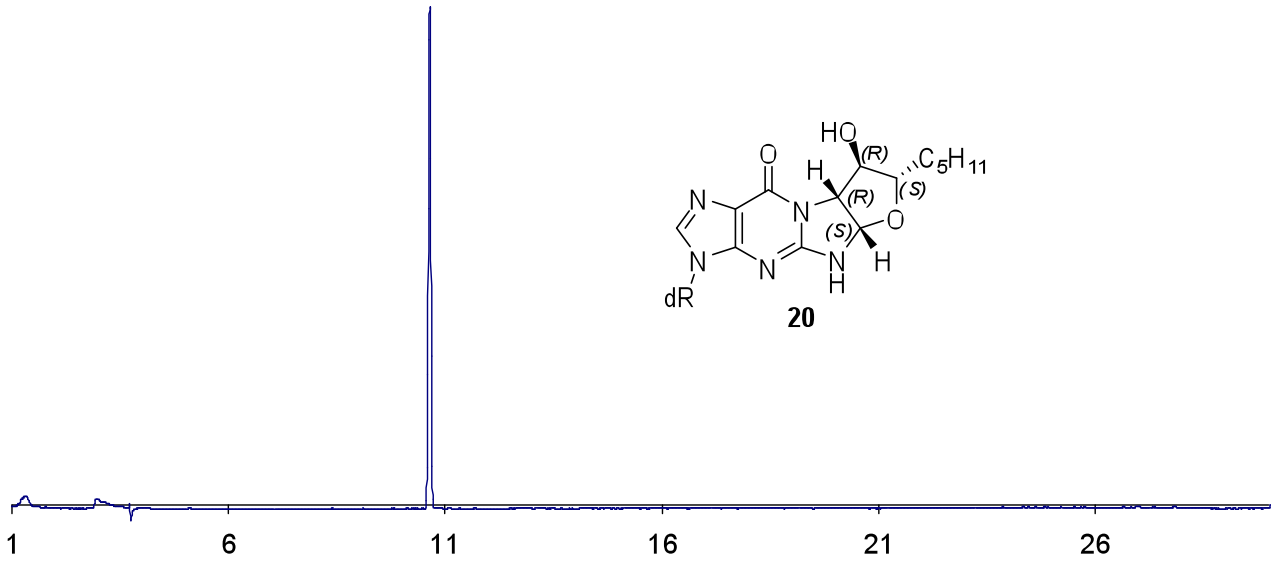


Figure S36. ^{1}H spectrum and HPLC chromatogram of **20**



1H, bicyclic isomer SSRR
3mm nmr tube, 0.9 mg/85uL D2O+85uL CD3CN
deggasing sample with He (11 min)
1H-nmr

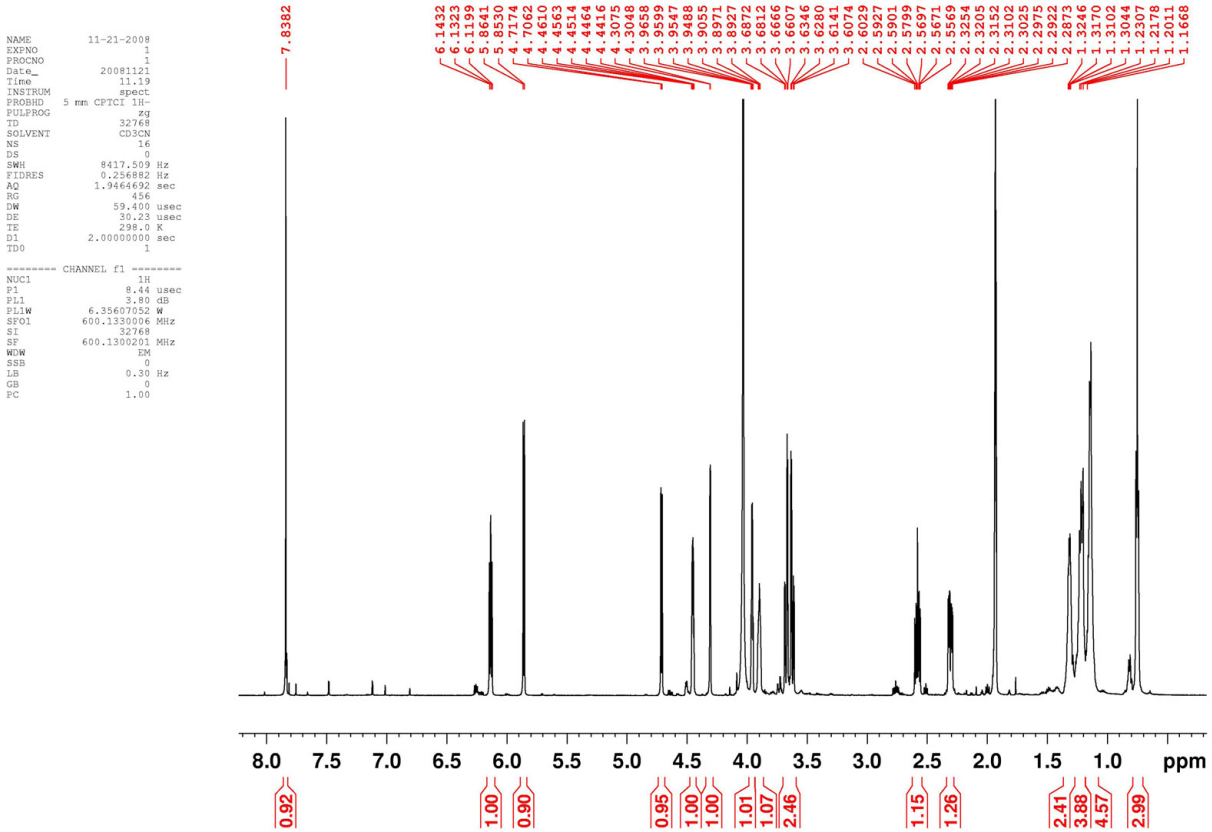
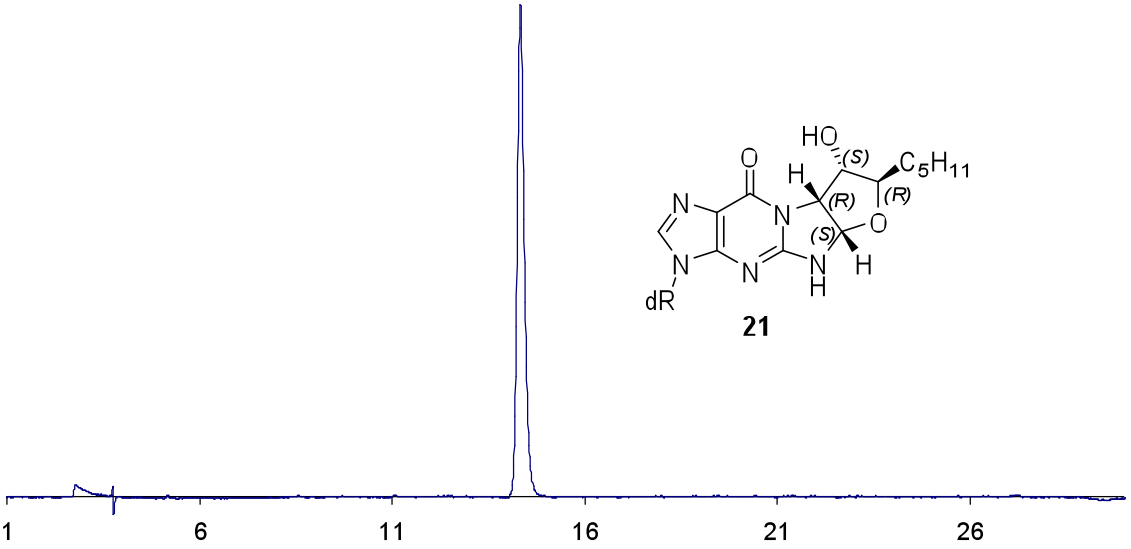


Figure S37. ^{1}H spectrum and HPLC chromatogram of **21**



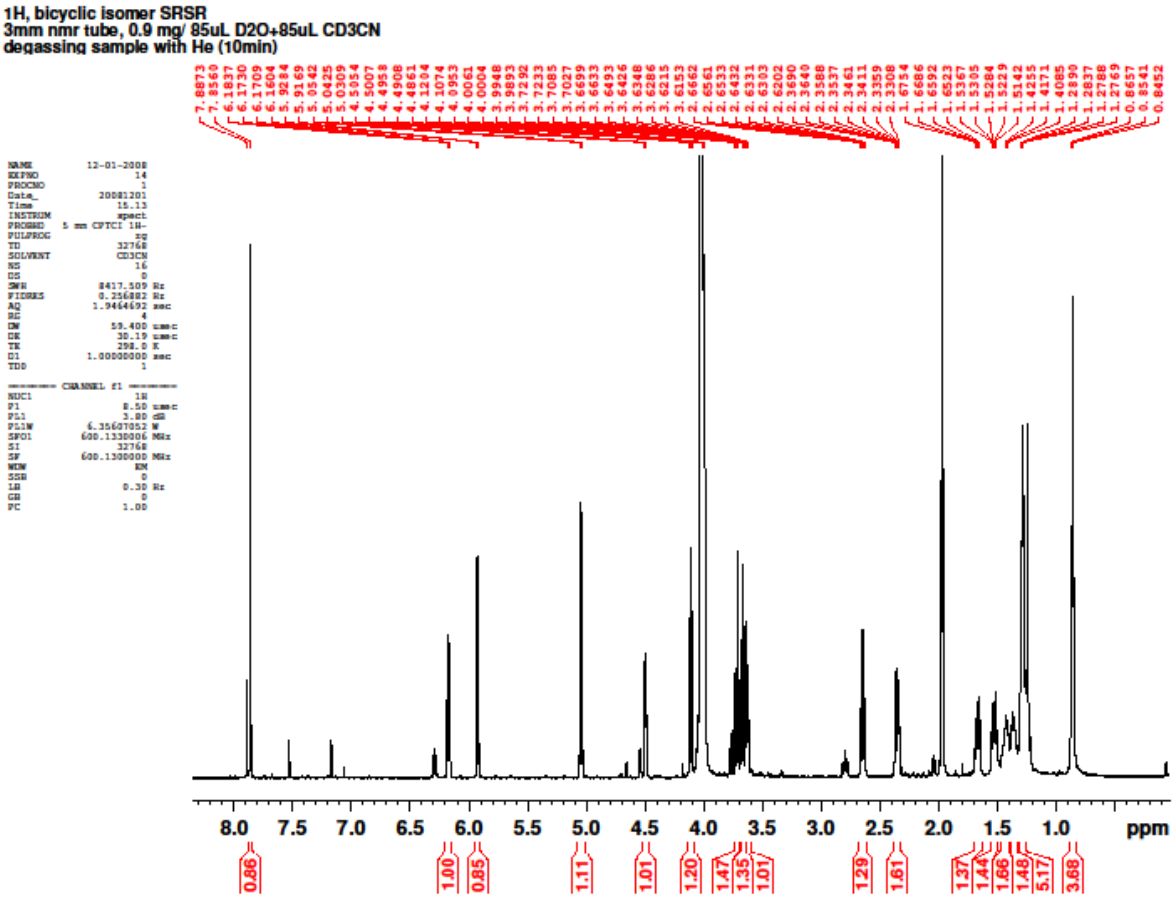
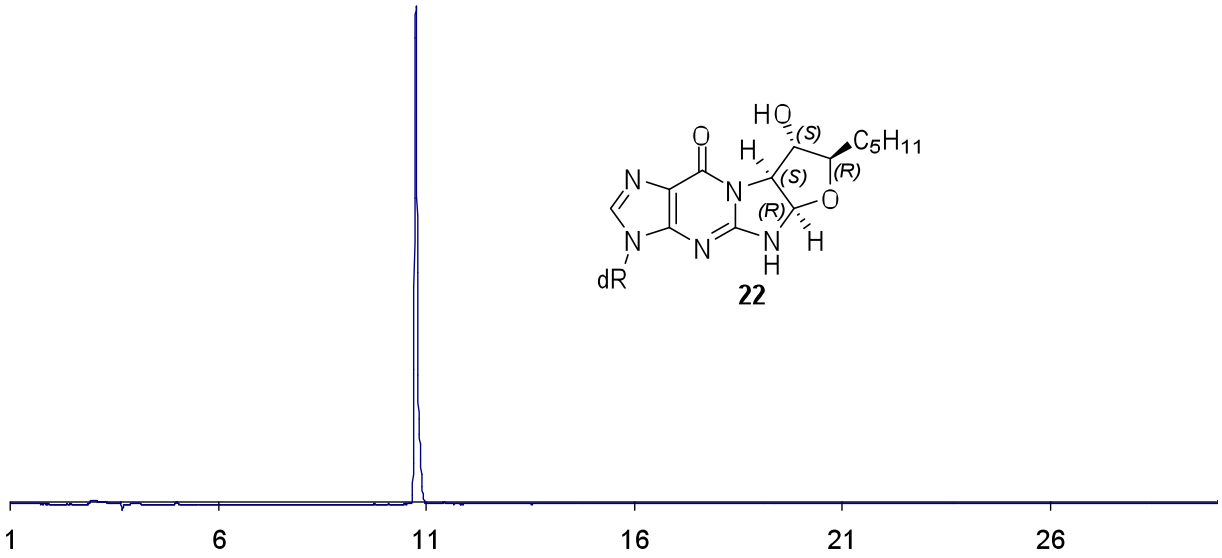


Figure S38. ^{1}H spectrum and HPLC chromatogram of **22**



1H, bicyclic isomer RRSS
3mm nmr tube, 1.4 mg/ 85uL D2O+85uL CD3CN
degassing sample with He (10min)