Double [3,3]-sigmatropic rearrangement in the enzymatic dioxygenation of benzyl azide : Preparation of novel synthetically valuable azido-diols.

Natalia Thevenet‡, Victoria de la Sovera‡, María Agustina Vila, Nicolás Veiga, David Gonzalez, Gustavo Seoane and Ignacio Carrera*.

Facultad de Química-Universidad de la República. General Flores 2124, CP 11800, Montevideo – Uruguay. E-mail: icarrera@fq.edu.uy

Electronic Supplementary Information

1. General Considerations

All solvents were distilled prior to use. Mass spectra (MS) were recorded on a Shimadzu GC–MS QP 1100 EX instrument using the electron impact mode (70 eV). High resolution mass spectra were obtained on a Bruker Daltonics Q-TOF spectrometer (ESI mode), at Polo Tecnólogico de Pando, Facultad de Química, Universidad de la República. Infrared spectra (IR) were recorded either on neat samples (KBr disks) or in solution on a Shimadzu FT-IR 8101A spectrophotometer. NMR spectra were obtained in $CDCl_3$ on a Bruker Avance DPX-400 instrument. Proton chemical shifts (δ) are reported in ppm downfield from TMS as an internal reference, and carbon chemical shifts are reported in ppm relative to the center line of the $CDCl_3$ triplet (77.0 ppm). Optical rotations were measured on a Zuzi 412 polarimeter using a 0.5 dm cell. $[\alpha]_D$ values are given in units of $deg \bullet cm^2 \bullet g^{-1}$ and concentration values are expressed in g/100 mL. Analytical TLC was performed on silica gel 60F-254 plates and visualized with UV light (254 nm) and/or p-anisaldehyde in acidic ethanolic solution. Flash column chromatography was performed using silica gel (Kieselgel 60, EM reagent, 230–400 mesh) Chemicals and reagents were purchased from Sigma-Aldrich and used as received. *E. coli* JM109 (pDTG601) was generously donated by Prof. David T. Gibson (1938-2014). All his strains collection is now managed by Prof. Rebecca Parales (University of Califormia, Davis). Shake-flask cultivation was carried out using a Thermo Forma orbital shaker. A Sartorious-Biostat A plus bioreactor fitted with a 5 liter baffled vessel was used as bioreactor.

2. Biotransformation procedures

Media composition. Luria-Bertani (LB) medium used for cell growth contained: Bacto-Tryptone (10 g/L), Bacto-Yeast Extract (5 g/L), and sodium chloride (10 g/L). Agar (15 g/L) was added for solid media. When needed the medium was supplemented with sterile ampicillin sodium salt (0.1 g/L).

Mineral Salts Broth (MSB) used for the precultures contained: K_2HPO_4 (16 g/L), KH_2PO_4 (14 g/L), $(NH_4)_2SO_4$ (5 g/L), Bacto-Yeast Extract (15 g/L); after sterilization the medium was supplemented with sterile glucose (30 g/L), $MgSO_4.7H_2O$ (2 g/L) and ampicillin sodium salt (0.1 g/L).

Defined mineral salt medium for the bioreactor consisted of: KH_2PO_4 (7.5 g/L), citric acid (2.0 g/L), $MgSO_4 \bullet 7H_2O$ (5.0 g/L), ferric ammonium citrate (0.3 g/L), 98% H_2SO_4 (1.4 mL/L) and trace metal solution (1.5 mL/L). After sterilization, pH is regulated to 6.8 by addition of conc. ammonium hydroxide, followed by supplementation with sterile thyamine hydrochloride (0.3 g/L) and ampicillin sodium salt (0.1 g/L). Trace metal solution contained: citric acid (40 g/L), $MnSO_4 \bullet 2H_2O$ (30 g/L), NaCl (10 g/L), $FeSO_4 \bullet 7H_2O$ (1 g/L), $CoCl_2 \bullet 6H_2O$ (1 g/L), $ZnSO_4 \bullet 7H_2O$ (1 g/L), $CuSO_4 \bullet 5H_2O$ (0.1 g/L), H_3BO_3 (0.1 g/L), $NaMoO_4 \bullet 2H_2O$ (0.1 g/L); pH was adjusted to 3.0 with ammonium hydroxide.

M9 minimal salt medium for biotransformations in shake-flask contained: Na_2HPO_4 (12.8 g/L), KH_2PO_4 (3 g/L), NaCl (0.5 g/L).

Plate preparation. *E.coli* JM109 (pDTG601) cells properly stored in cryovials, are streaked into LB-agar plates (supplemented with ampicillin sodium salt). The streaked plate is incubated at 37 ºC for 24 hours. Single-cell colonies are chosen for the precultures preparation.

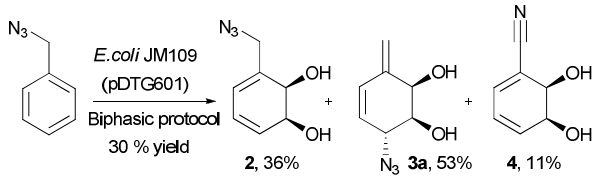
Bioreactor cultures and biotransformations. Growth and biotransformation in the bioreactor were carried out using a modification of our previously published procedure.[1] Thus, 5mL of LB medium supplemented with ampicillin sodium salt (0.1 g/L) and glucose (5 g/L) was inoculated with a single colony of *E. coli* JM109 (pDTG601), and grown overnight at 37 ºC and 150 rpm. Two 500 mL shake-flasks containing 150 mL of MSB medium were inoculated with 1mL of the grown culture. These preculture flasks were placed in an orbital shaker at 37 ºC and 150 rpm, for 12 hrs. Both entire cultures were used to inoculate the bioreactor (Sartorius Biostat A plus), charged with an initial volume of 2.5 L of defined mineral salt medium, and set to 500 rpm, 30 ºC, and air flow rate of 4L/min. The pH value was controlled automatically to 6.8 by addition of conc. ammonium hydroxide during the whole process. A pulse of antifoam agents (Aldrich's Antifoam Y: Silicone dispersion in water 1:1) was added at the beginning of the run. At 6 hours after inoculation the dissolved oxygen value sharply increased (indicating carbon deprivation), whereupon a glucose fed-batch was started by adding glucose (0.7 g/mL solution) from an initial rate of 0.08 mL/min to 0.54 mL/min in a 20 hours period. When the biomass concentration reached aprox 15 g/L cdw (OD_{600} = 15), IPTG was added to induce TDO expression (IPTG final concentration in bioreactor of 10 mg/L), and the stirrer speed was set to 900 rpm. After the culture reached the stationary phase (c.a. 26 hours, 50 g/L cdw aprox), glucose feeding was decreased to 0.25 mL/min and benzyl azide addition was started. A solution of benzyl azide in liquid paraffin (0.5 M, 160 mL) was added at a flow rate of 20 mL/min using a peristaltic pump.

Downstream process. After the biotransformation was completed (aprox. 4 hours), the pH of the medium in the bioreactor was adjusted to 7.5. The culture broth was centrifuged at 7000 rpm and 4 ºC for 30 minutes, the supernatant was collected and the cell pellet properly disposed. Centrifugation allows the separation of the liquid paraffin (which contains no detectable amounts of products) from the aqueous phase. In this stage, the clean culture broth (aprox. 3.0 L) is left at room temperature for one week to afford complete conversion of the rearrangement. The isolation of the diol from the aqueous supernatant was carried out using liquid-liquid extraction with ethyl acetate. The aqueous media was previously washed with hexanes to completely remove liquid paraffin traces. The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford a crude material that was purified by column chromatography (SiO_2, EtOAc: Hex 7:3) to obtain 3.0 -4.8 grams of **3a.**

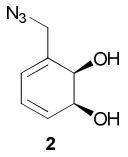
Biotransformation using Resting Cells. Once the culture described in the section "Bioreactor cultures and biotransformations" reached the stationary phase, 100mL of media were taken from the reactor and centrifuged at 5000rpm for 15 minutes. The supernatant was discarded and the pellets were suspended in 200mL of minimal media (M9) supplemented with glucose solution (0.7g/mL) to a final concentration of 30mM. The reaction was carried out in 250mL flasks containing 50mL of this culture and substrate was added as 0.5M solution in liquid paraffin to a final concentration of 30mM. The flasks were incubated in orbital shaker overnight at 28ºC, 150rpm.

3. Synthetic procedures and spectral data

a) Benzyl azide biotransformation with *E.coli* JM109 (pDTG601)

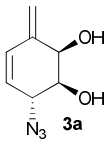


Biotransformation protocol in shake flasks and in the bioreactor is described in section 2.



(1*S*,2*R*)-3-azidomethyl-cyclohex-3,5-diene-1,2-diol (2)

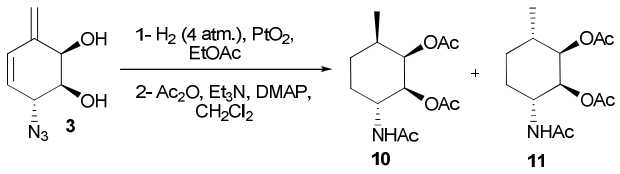
Since this compound is unstable to produce the rearranged azide **3** it was fully characterized as its stable isopropylidene derivative (compound 9, vide infra). White solid; Rf 0.3 (n-hexane/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.66 (brs, 1H), 2.78 (brs, 1H), 4.01 (s, 2H), 4.24 (m, 1H), 4.34 (m, 1H), 5.99 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.6, 68.2, 68.6, 123.0, 124.3, 129.8, 135.0; IR (cm^{-1}) ν 3294, 2924, 2098, 1600, 1269, 1076, 1053, 1010, 914 cm^{-1}



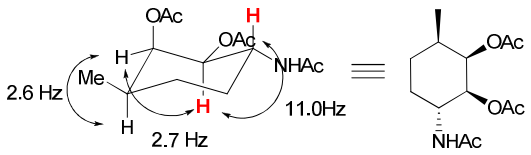
(1*S*,2*R*,6*R*)-6-azido-3-methylenecyclohex-4-ene-1,2-diol (3a)

White solid; Rf 0.4 (n-hexane/EtOAc = 4/6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (dd, *J* = 9.9, 1.9 Hz, 1H), 5.69 (d, *J* = 9.9 Hz, 1H), 5.35 (s, 3H), 5.26 (s, 3H), 4.20 (ddd, *J* = 7.1, 2.2, 2.2 Hz, 1H), 3.78 (ddd, *J* = 6.9, 6.9, 2.8 Hz, 1H), 2.55 (d, *J* = 6.9 Hz, 1H), 2.31 (d, *J* = 3.8 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ141.9, 130.0, 142.6, 118.7, 114.8, 73.5, 71.5,61; IR(cm^{-1}) ν 3278, 2098, 1286, 1271, 1252, 1053, 1010, 914; MS (EI, 70eV) m/z(%): 167 (11, M+), 149 (10), 124 (24); 110(30), 93 (69), 77 (66), 65 (100), 55 (86), 41 (47); HRMS (ESI+) calcd for $C_7H_9N_3NaO_2$ 190.0587 $[M+Na]^+$ found 190.0578; α_D (MeOH, 2,375g/100ml)= - 202,7.

Relative Stereochemistry Determination of C3: To confirm absolute configuration in C3 (N_3 substituent) compound **3** was totally reduced and acetyl protected as shown below.

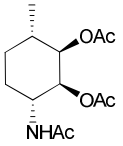


To a solution of compound **3** (0.03 g, 0.18 mmol) in 15 mL of ethyl acetate, 0.012 g of platinium dioxide and potassium bicarbonate (0.003 g) were added. This mixture was treated under hydrogen (4 atm) in a Parr hydrogenator for 48 hours at room temperature. When no starting material was detected by TLC, the catalyst was filtered, and the solvent removed to afford a crude material which was dissolved in CH_2Cl_2 (2 mL). The following reagents were added at room temperature and under nitrogen atmosphere: acetic anhydride (0.2 mL, 2.18 mmol), anhydrous triethylamine (0.4 mL, 2.96 mmol) and catalytic amount of 4-dimethylaminopyridine. After complete consumption of starting material, the reaction mixture was neutralized by the addition of a sodium bicarbonate saturated solution until no more bubbling was observed. The organic layer was washed with copper sulfate and sodium chloride saturated solution. and dried over anhydrous Na_2SO_4. Solvent was removed *in vacuo* and acetilated compounds were isolated by silica gel column chromatography (ethyl acetate: hexane, 15:85). Two compounds (**10** and **11**) were obtained (70:30 mixture) characterized as epimers in C4, as expected by previous reports.[2] J coupling analysis for both compounds showed an anti-relationship between the OAc in C2 and the NHAc group in C3.



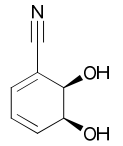
10

(1*R*,2*S*,3*R*,6*R*)-3-acetamine-6-methyl-1,2-cyclohexane-1,2-diyl diacetate (10): Colorless oil; Rf 0.3 (n-hexane/EtOAc = 3/7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, *J* = 8.6, 1H), 5.23 (dd, *J* = 2.7, 2.6 Hz, 1H), 4.76 (dd, *J* = 11.0, 2.7 Hz, 1H), 4.26 (dddd, *J* = 11.3, 8.5, 7.2, 4.2 Hz, 1H), 2.16 (m, 1H), 2.15 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.77 (m, 1H), 1.50 (m, 2H), 1.26 (m, 1H), 0.91 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 170.7, 169.9, 74.5, 72.9, 47.9, 34.4, 31.3, 26.9, 23.5, 21.0, 20.9, 17.1; HRMS (ESI+) calcd for $C_{13}H_{22}NO_5$ 272.1492 $[M+H]^+$ found 272.1505.



11

(1*R*,2*S*,3*R*,6*S*)-3-acetamine-6-methyl-1,2-cyclohexane-1,2-diyl diacetate (11): Colorless oil; Rf 0.3 (n-hexane/EtOAc = 3/7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 (d, *J* = 8.4, 1H), 5.04 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.98 (dd, *J* = 5.0, 2.7 Hz, 1H), 4.28 (dddd, *J* = 13.5, 9.0, 8.7, 4.5 Hz, 1H), 2.10 (s, 3H), 2.09 (m, 1H), 2.07 (s, 3H), 2.03 (m, 1H), 1.97 (s, 3H), 1.91 (m, 1H), 1.52 (m, 1H), 1.35 (m, 1H), 1.05 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.4, 169.7, 74.3, 70.4, 48.8, 32.2, 26.3, 25.6, 23.5, 21.1, 21.0, 16.2.

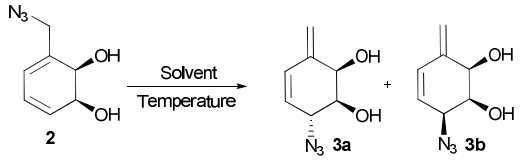


4

(5*R*,6*S*)-5,6-dihydroxycyclohexa-1,3-dienecarbonitrile[3]

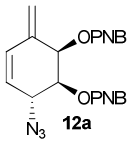
White solid; Rf 0.3 (n-hexane/EtOAc = 4/6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 5.6 Hz, 1H), 6.30 (ddd, J = 9.6, 3.8, 0.9 Hz, 1H), 6.13 (ddd, J = 9.7, 5.5, 1.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 137.0, 122.3, 118.3, 113.9, 66.7, 66.4. MS (EI, 70eV) m/z(%): 137 (0.2, M+), 119 (100), 91 (32); 64 (20).

b) Rearrangement Study:



2 3a 3b

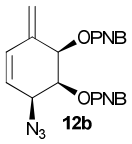
Compound **2** (0.02g, 0.12 mmol) freshly purified by column chromatography to avoid traces of rearranged products, was dissolved in 1.5 mL of the indicated solvent (see Table 1 in the manuscript) in a proper sealed vial. The system was heated at the indicated temperature until no more starting material was detected by TLC. Solvent was evaporated *in vacuo*, and the crude mixture analyzed by NMR. Epimers **3a** and **3b** were inseparable by column chromatography. To obtain pure compounds for spectral characterization the corresponding *p*-nitrobenzoates **12a** and **12b** were prepared and purified by column chromatography.



12a

(1*S*,2*R*,6*R*)-6-azido-3-methylenecyclohex-4-ene-1,2-diyl di-*p*-nitrobenzoate (12a)

Fully Characterized as free diol **3a**, Colorless oil; Rf 0.4 (n-hexane/EtOAc = 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (ddd, *J* = 9.0, 2.0, 2.0 Hz, 2H), 8.30 (ddd, *J* = 9.0, 2.0, 2.0 Hz, 2H), 8.15 (ddd, *J* = 9.0, 2.6, 2.2 Hz, 4H), 6.44 (dd, *J* = 9.9, 1.8 Hz, 1H), 6.21 (d, *J* = 2.7 Hz, 1H), 5.89 (d, *J* = 9.9 Hz, 1H), 5.53 (s, 1H), 5.48 (s, 1H), 5.47 (dd, 8.1, 2.8 Hz, 1H), 4.63 (ddd, *J* = 8.2, 2.0, 2.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 150.9, 136.4, 134.7, 134.7, 134.4, 134.4, 131.7, 131.0, 131.0, 130.9, 130.9, 123.8, 123.8, 123.8, 123.8, 123.6, 118.2, 71.5, 71.4, 57.2 ; IR (cm^{-1}) ν 2918, 2104, 1732, 1606, 1527; MS (EI, 70eV) m/z(%): 270 (19), 150 (100), 120 (16); 104 (25), 92 (15), 76 (16); α_D (MeCN, 1,08g/100ml)= - 143,2.



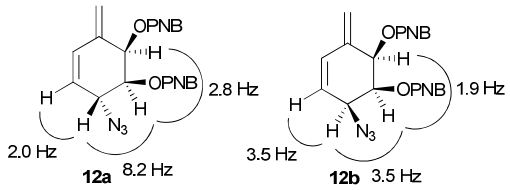
12b

(1*S*,2*R*,6*S*)-6-azido-3-methylenecyclohex-4-ene-1,2-diyl di-*p*-nitrobenzoate (12b)

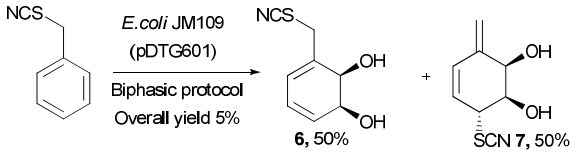
Colorless oil; Rf 0.5 (n-hexane/EtOAc = 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 8.9 Hz, 4H), 8.18 (ddd, *J* = 8.8, 1.9, 1.9 Hz, 2H), 8.15 (dd, *J* = 9.0, 2.1 Hz, 2H) 6.61 (dd, *J* = 10.0, 1.9 Hz, 1H), 6.04 (d, *J* = 1.9 Hz, 1H), 5.92 (m, 2H), 5.44 (s, 1H), 5.39 (s, 1H), 4.51 (ddd, *J* = 3.5, 3.5, 3.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (100

MHz, $CDCl_3$) δ 163.85, 150.98, 137.13, 134.93, 134.58, 131.02, 130.94, 130.44, 125.11, 123.93, 123.87, 121.93, 74.51, 72.25, 58.34; IR (cm^{-1}) ν 2957, 2924, 2854, 2104, 1732, 1607, 1531, 1464, 1410, 1348, 1321, 1278, 1264, 1170, 1099; MS (EI, 70eV) m/z(%): 465 (M+, 0.04), 270 (2), 256 (30), 150 (100), 134 (4), 120 (13), 104 (21), 92 (9), 76 (8); HRMS (ESI+) calcd for $C_{21}H_{15}N_5NaO_8$ 488.0818 $[M+Na]^+$ found 488.0884; α_D (MeCN, 1,53g/100ml)= + 168,3.

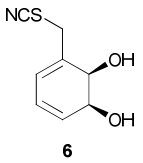
Comparison of J coupling values in compounds **12a** and **12b** allows for determination of the azide stereochemical relative configuration, and is in agreement with the previous assignment for the major compound.



c) Benzyl thiocyanate biotransformation with *E.coli* JM109 (pDTG601)

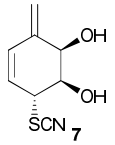


Biotransformation protocol is described in section 2.



(1*S*,2*R*)-3-(thiocyanatomethyl)cyclohexa-3,5-diene-1,2-diol (6)

Since this compound is unstable because of the SCN migration, it was fully characterized as the rearranged compound **7.** White solid; Rf 0.2 (n-hexane/EtOAc = 3/7); ^{1}H NMR (400 MHz, $CDCl_3$) 6.0(m, 3H), 4.40 (d, *J* = 6.2 Hz, 1H), 4.30 (dd, *J* = 6.2, 2.6 Hz, 1H), 3.78 (s, 2H).

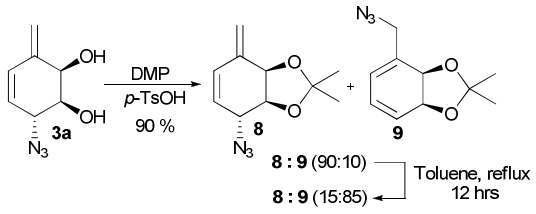


(1*S*,2*R*,6*R*)-6-thiocyanato-3-methylenecyclohex-4-ene-1,2-diol (7)

White solid; Rf 0.3 (n-hexane/EtOAc = 3/7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (d, *J* = 10.0 Hz, 1H), 5.72 (dd, *J* = 9.8, 1.2 Hz 2H), 5.44 (s, 1H), 5.32 (s, 1H), 4.57 (s, 1H), 4.03 (s, 2H), 3.40 (s, OH), 2.96 (s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.34, 132.11, 123.48, 119.49, 111.03, 73.13, 70.66, 50.09; IR (cm^{-1}) ν 3371, 2910, 2154, 1398, 1302,

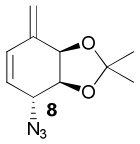
1237, 1099, 1070, 1055, 1005, 918; MS (EI, 70eV) m/z(%): 183 (M+,33), 140 (36), 139 (10); 122 (13), 107 (100), 95 (28); HRMS (ESI+) calcd for $C_8H_9NNaO_2S$=206.0246 $[M+Na]^+$ found 206.0256; α_D (MeOH, 2,5g/100ml)= - 336.

d) Isopropylidene protection of compound 3a.



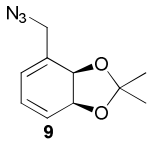
To a solution of compound **3a** (0.5 g, 2.99 mmol) in acetone (5 mL, 68.19mmol), 7.5 mL (61.10 mmol) of dimethoxypropane were added along with a catalytic amount of *p*- toluene sulfonic acid at room temperature. After complete consumption of starting material was observed, the reaction was quenched by the addition of solid sodium bicarbonate, filtered and concentrated under reduced pressure. The reaction crude was suspended in diethylether, washed with brine and dried over Na_2SO_4. Concentration of the filtered solution gave dark yellow oil. NMR analysis of this crude product revealed a 90:10 mixture of compounds **8** and **9**.

Compound **8** (0.4 g, 1.93 mmol), was dissolved in 10 mL of toluene and heated to reflux. After 3 hours, solvent was removed *in vacuo.* NMR analysis of this crude product revealed a 15:85 mixture of compounds **8** and **9**. Purification by silica gel column chromatography (ethyl acetate: hexane, 5:95) gave product **9** with 80% yield.



(1*S*,2*R*,6*R*)-6-azido-3-methylene-1,2-O-isopropylidenecyclohex-4-en-1,2-diol (8)

Since this compound is unstable to produce the endo-diene **9** it was fully characterized as its stable free diol (compound **3a**) Colorless oil; Rf 0.7 (n-hexane/EtOAc = 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (dd, *J* = 10.0, 2.2 Hz, 1H), 5.67 (d, *J* = 10.1 Hz, 1H), 5.47 (s, 1H), 5.46(s, 1H), 4.71 (d, *J* = 5.5 Hz, 1H), 4.17 (dd, *J* = 5.5, 5.5 Hz, 1H), 4.07 (m, 1H), 1.50 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 130.7, 124.3, 121.2, 109.5, 77.7, 73.5, 59.7, 28.3, 26.3



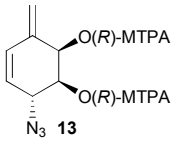
(1*S*,2*R*)-3-azidometyl-1,2-O-isopropylidenecyclohex-3,5-dien-1,2-diol (9)

Colorless oil; Rf 0.7 (n-hexane/EtOAc = 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.06 (dd, *J* = 9.6, 5.5 Hz, 1H), 5.98 (m, 2H), 4.74 (dd, *J* = 8.8, 3.5 Hz, 1H), 4.68 (d, *J* = 8.9 Hz, 1H), 4.00 (s, 2H), 1.43 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (100

MHz, $CDCl_3$) δ 132.0, 125.3, 123.7, 121.6, 105.9, 71.2, 70.8, 53.7, 26.8, 24.8; IR (cm-1) ν 2988, 2889, 2102, 1240, 1211, 1159, 1051, 1040, 864; MS (EI, 70eV) m/z(%): 207 (M+, 0.1), 149 (36), 137 (12); 120 (39), 107 (100), 95 (73), 94 (95), 77 (59), 66 (58), 65 (58), 43 (61); HRMS (ESI+) calcd for $C_{10}H_{13}N_3NaO_2$=230.0890 [M+Na]+ found 230.0900; α_D (MeOH, 2,0g/100ml)= 118,1.

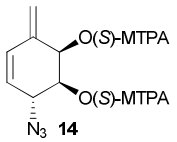
d) Enantiopurity determination for compound 3a. It is well known that dioxygenation by the Toluene Dioxygenase enzymatic complex gives enantiopure cis-cyclohexadienediols (>99% e.e.) of monosubstituted arenes.[4] In order to assure enantiomeric purity of **3a** Mosher derivatives **13 and 14** was prepared. A single di-mosher ester was found detected by NMR in both cases (see below). In agreement with the reports of Boyd (*JCS Perkin Trans* 1, 1998, 1935-1943 and *JACS* 1991, 113, 666-667) we could confirm that the dihydroxylation reaction gives a single enantiomer. This is so because the bis-MTPA esters prepared using both enantiomers of the reagent gave two different spectra with well differentiated signals for all the ring protons. *Since the preparation of each bis-MTPA esters gave a single compound the biotransformation was confirmed to be enantioespecific*.

To a solution of compound **3a** (0.015g, 0.09mmol) in anhydrous CH_2Cl_2 (0.15M) at 0 ºC (*S*) or (*R*)-(+)-α-methoxy-α-trifluoromethyl-α-phenylacetic acid chloride (0.037mL, 0.20 mmol) and 4-dimethylaminopyridine (0.024g, 0.20mmol) were added. After complete consumption of starting material CH_2Cl_2 was evaporated. The reaction crude was suspended in ethyl acetate and then washed with water, copper sulfate and sodium chloride saturated solutions. Organic layer was dried over anhydrous Na_2SO_4, and filtered.



13

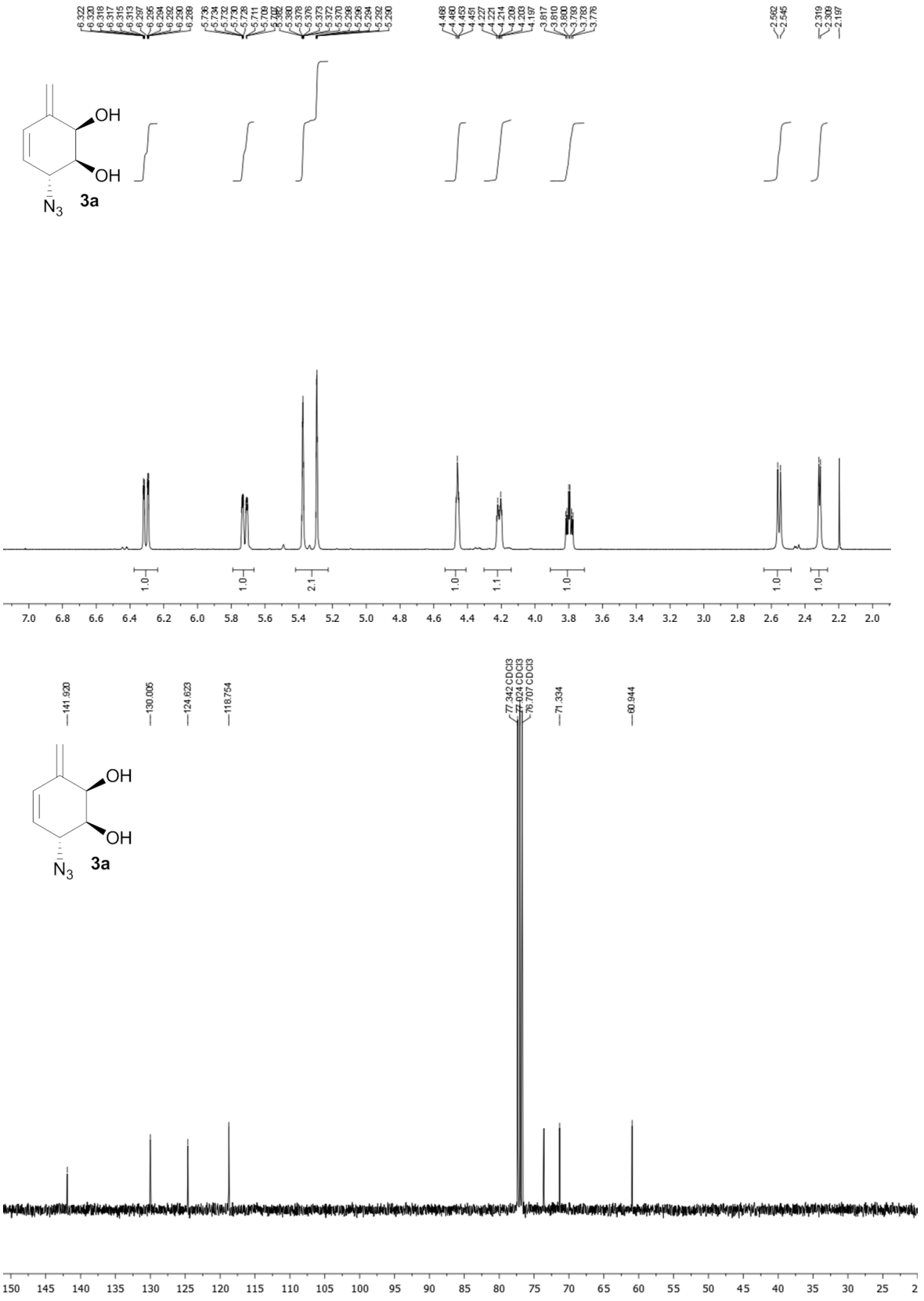
13 *(R)*-MTPA, Fully Characterized as free diol **3a**. Colorless oil; Rf 0.5 (n-hexane/EtOAc = 8/2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (m, 2H), 7.43-7.28 (m, 8H), 6.19 (d, *J* = 10.0 Hz, 1H), 5.91 (d, *J* = 1.9 Hz, 1H), 5.70 (ddd, *J* = 10.0, 1.6, 1.6 Hz, 1H), 5.52 (s, 1H), 5.38 (s, 1H), 5.30 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.23 (ddd, *J* = 9.0, 2.1, 2.1 Hz, 1H), 3.65 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.1, 136.5, 132.0, 131.5, 129.8, 129.8, 129.5, 129.0, 129.0, 129.0, 129.0, 128.3, 128.3, 128.3, 128.3, 127.2, 127.0, 124.4, 121.5, 84.9, 84.6, 73.7, 74.0, 58.1, 56.0, 55.1; IR (cm-1) ν 2106, 1757, 1271, 1242, 1170, 1122, 1030, 1018, 993;

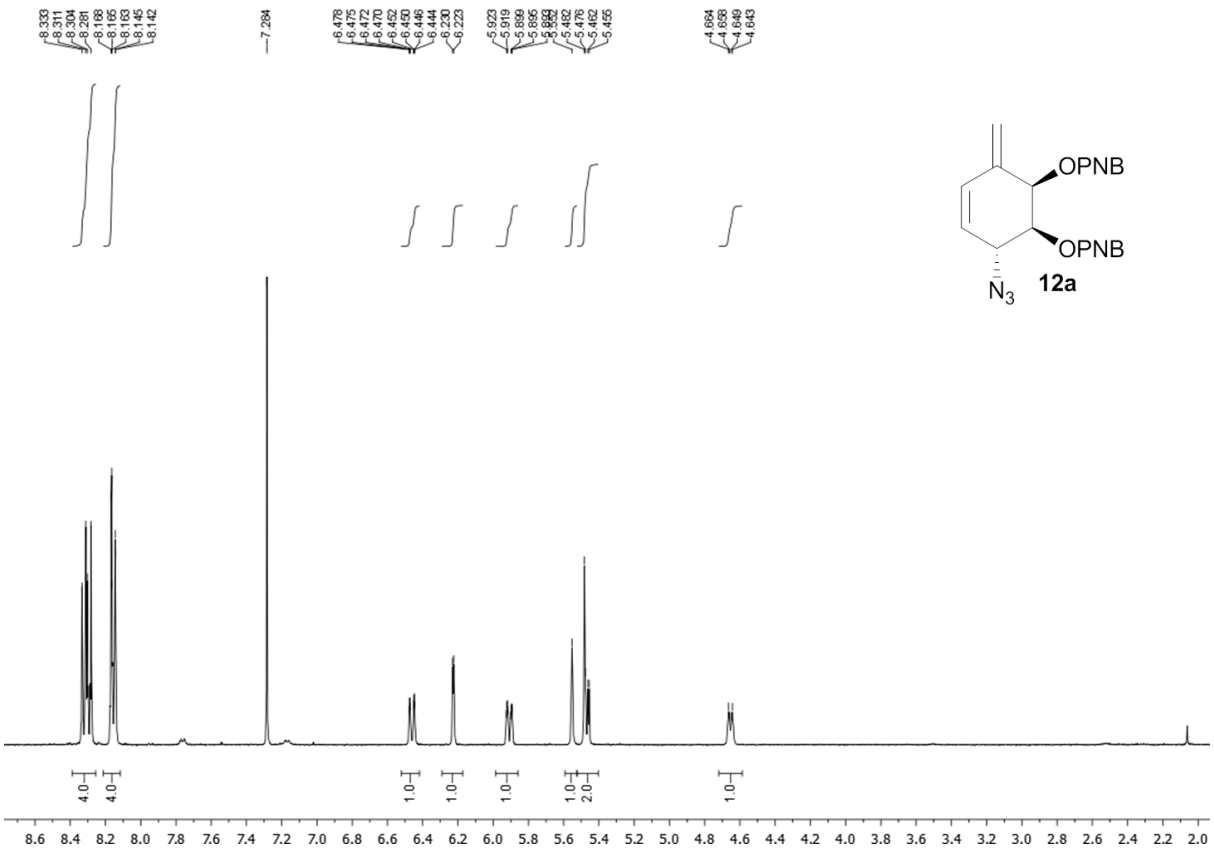


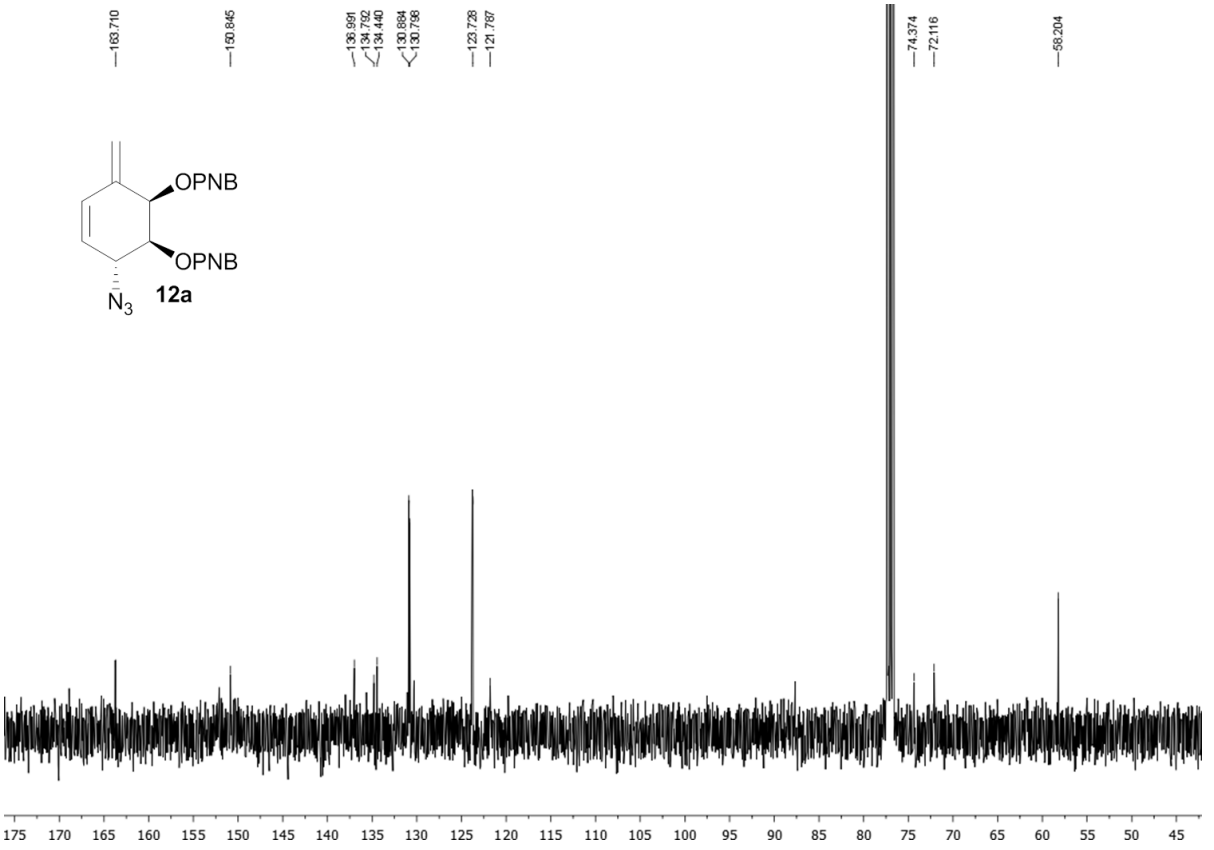
14

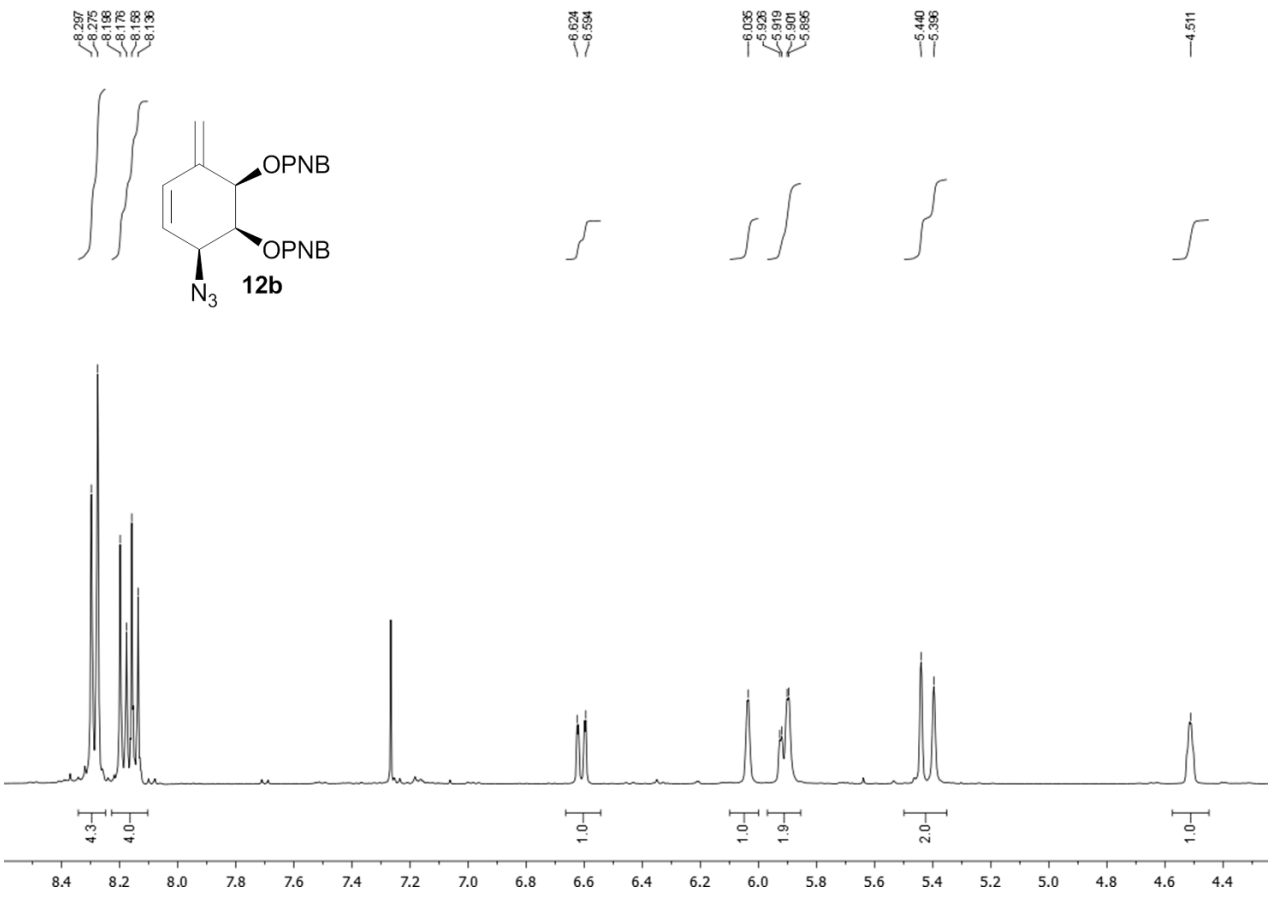
14 *(S)*-MTPA, Fully Characterized as free diol **3a.** Colorless oil; Rf 0.5 (n-hexane/EtOAc = 8/2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (m, 2H), 7.41 (m, 5H), 7.34 (m, 1H), 7.26 (m, 3H), 6.28 (d, *J* = 9.9 Hz, 0H), 6.02 (d, *J* = 2.3 Hz, 2H), 5.73 (dt, *J* = 9.9 Hz, J = 1.8 Hz, 1H), 5.51 (s, 1H), 5.40 (s, 1H), 5.30 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.04 (dt, *J* = 8.6, 2.4 Hz, 1H), 3.48 (s, 4H), 3.43 (s, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.4, 136.9, 131.9, 131.6, 129.8, 129.8, 129.7, 129.7, 129.1, 128.5, 128.5, 128.3, 128.3, 127.3, 127.3, 126.2, 124.5, 124.5, 121.6, 85.0, 84.7, 73.9, 73.1, 58.3, 55.6, 55.3.

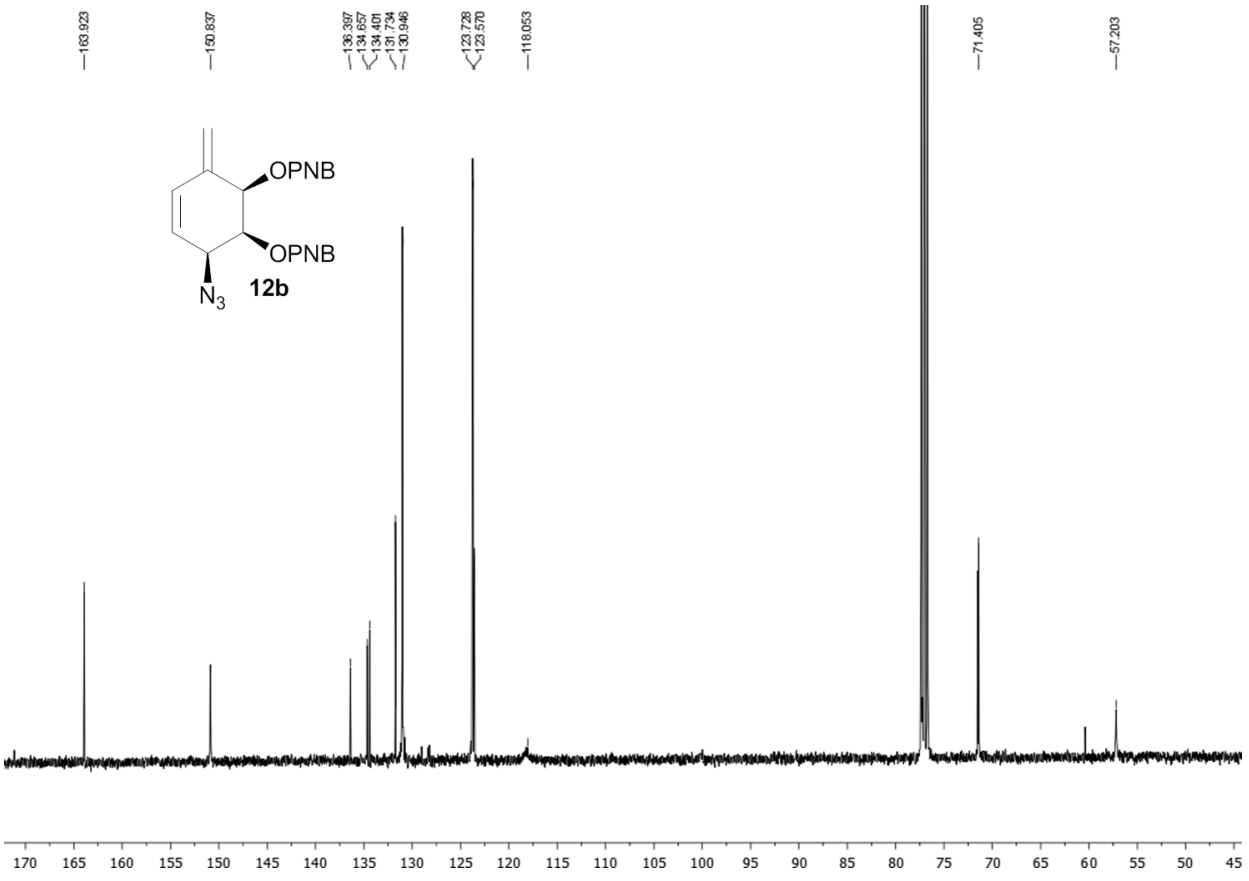
4. ^{1}H and ^{13}C NMRs (all cases measured in $CDCl_3$)

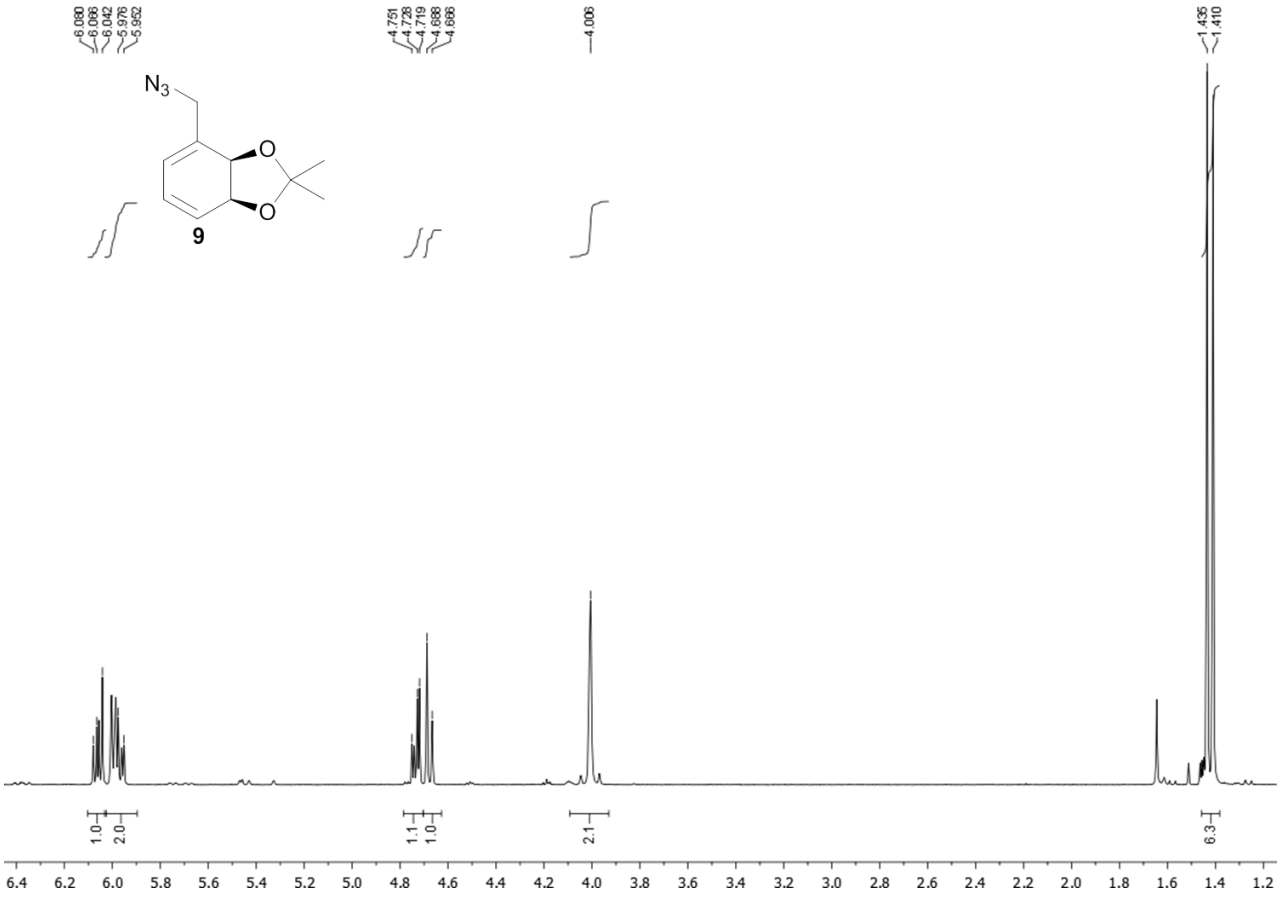


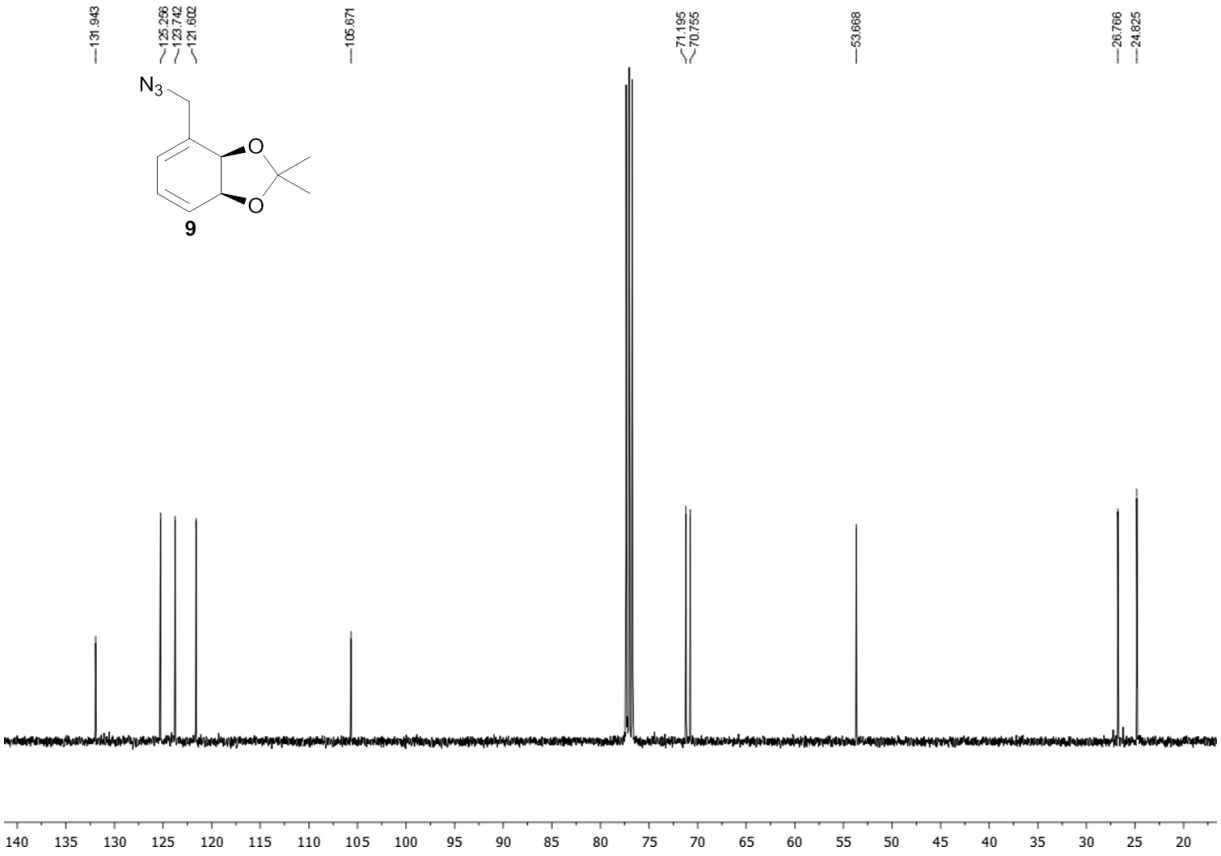

8.333
8.311
8.304
8.281
8.188
8.165
8.163
8.145
8.142
7.284
6.478
6.475
6.472
6.470
6.452
6.450
6.446
6.444
6.230
6.223
5.923
5.919
5.899
5.895
5.482
5.476
5.462
5.455
4.664
4.658
4.649
4.643
OPNB
OPNB
N3
12a
4.0
4.0
1.0
1.0
1.0
1.0
2.0
1.0
8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0

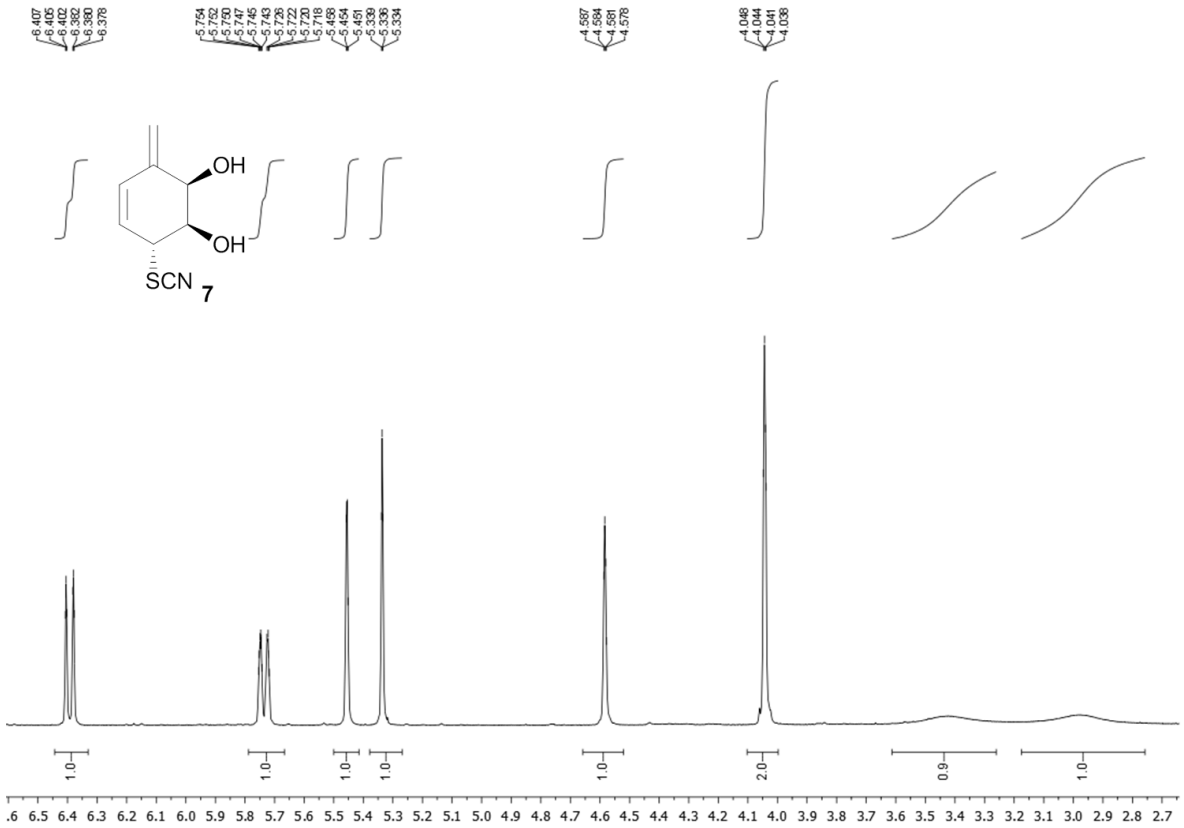

163.710
150.845
138.991
134.792
134.440
130.884
130.786
123.728
121.787
74.374
72.116
58.204
OPNB
OPNB
N3
12a
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45

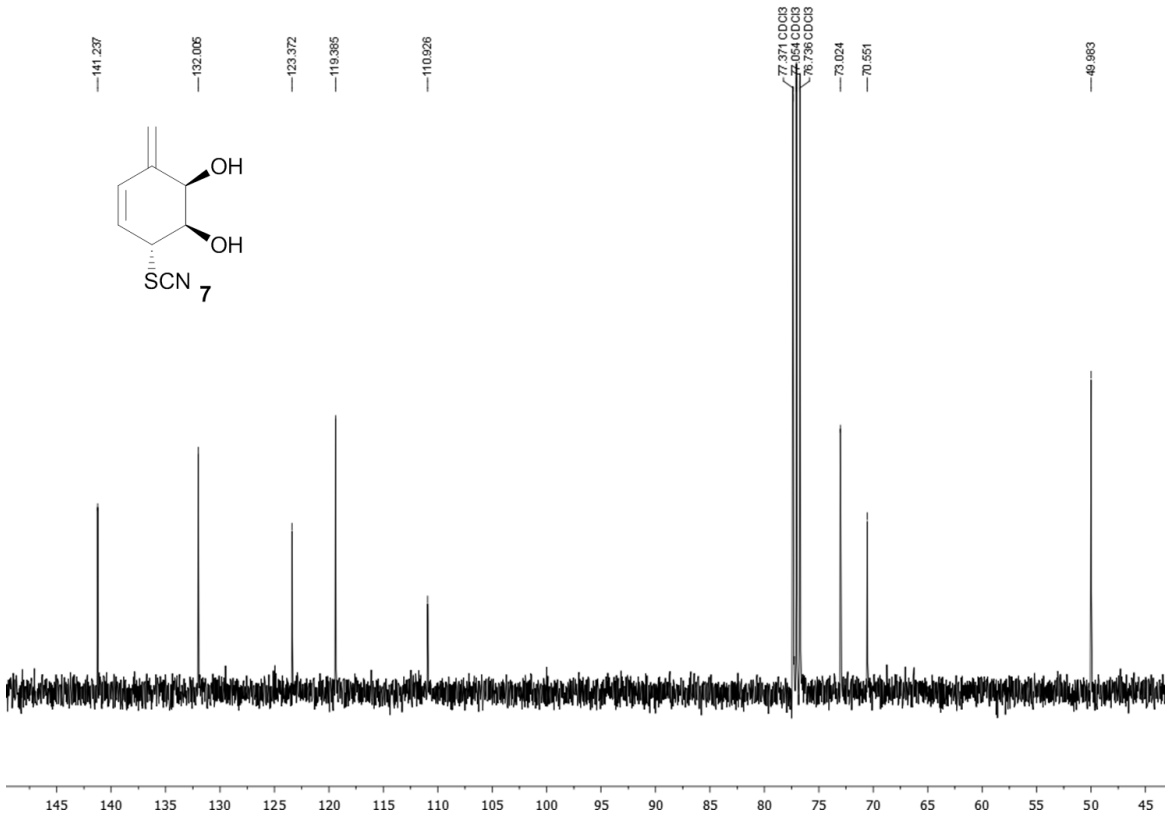

8.297
8.275
8.198
8.176
8.168
8.136
6.624
6.594
6.035
5.928
5.919
5.901
5.895
5.440
5.396
4.511
OPNB
OPNB
N3
12b
4.3
4.0
1.0
1.0
1.9
2.0
1.0
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4

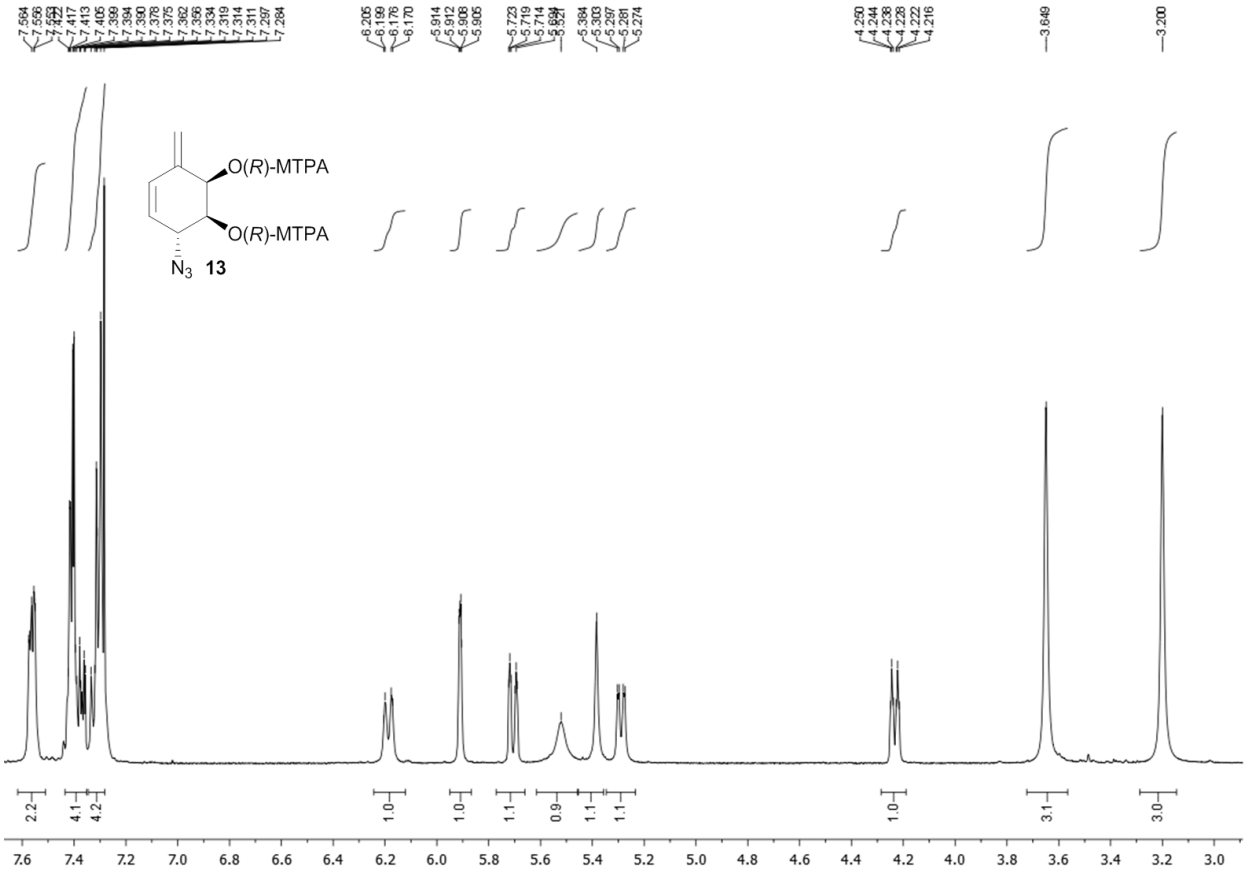

163.923
150.837
136.397
134.657
134.401
131.734
130.946
123.728
123.570
118.053
71.405
57.203
OPNB
OPNB
N3
12b
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45

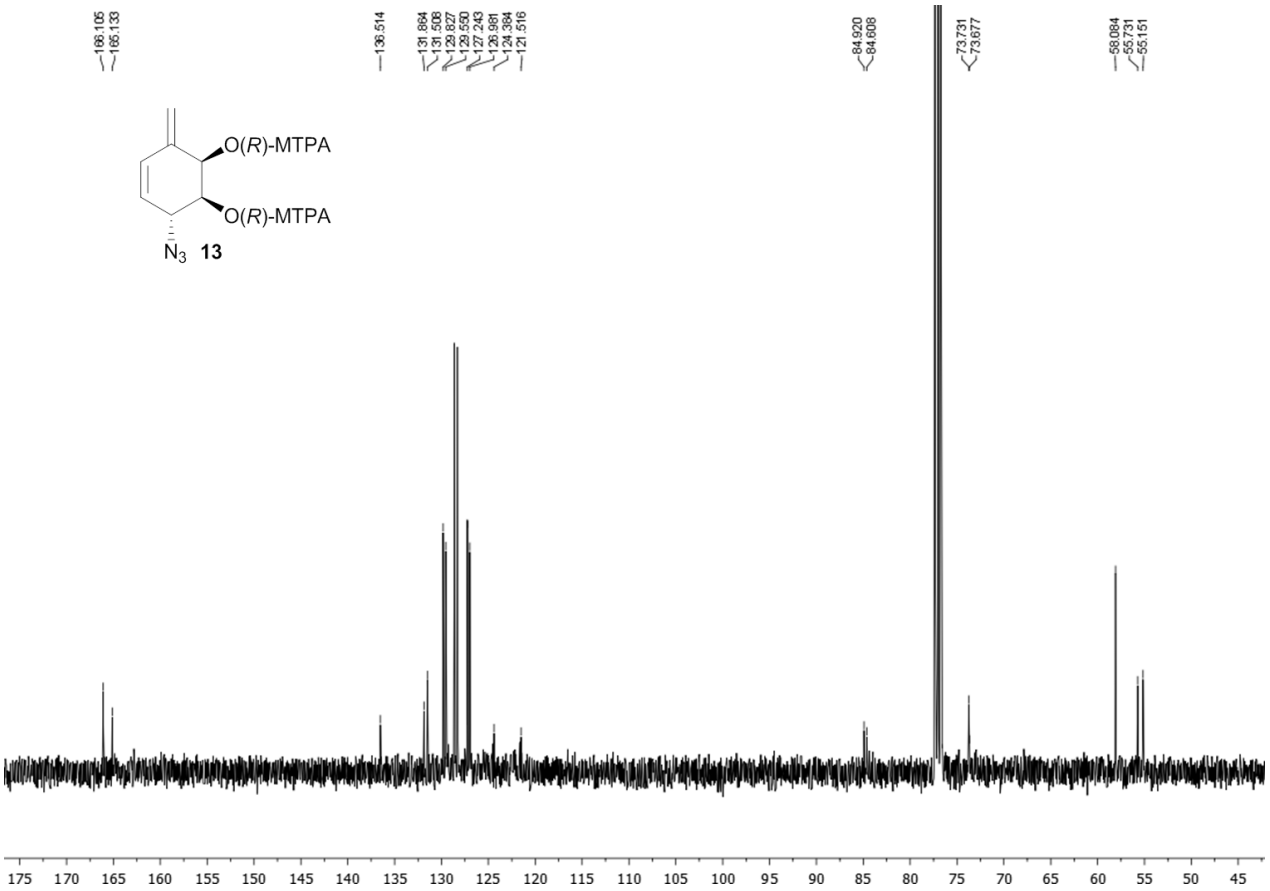

6.080
6.066
6.042
5.976
5.952
4.751
4.728
4.719
4.688
4.666
4.006
1.435
1.410
N3
O
O
9
1.0
2.0
1.1
1.0
2.1
6.3
6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2

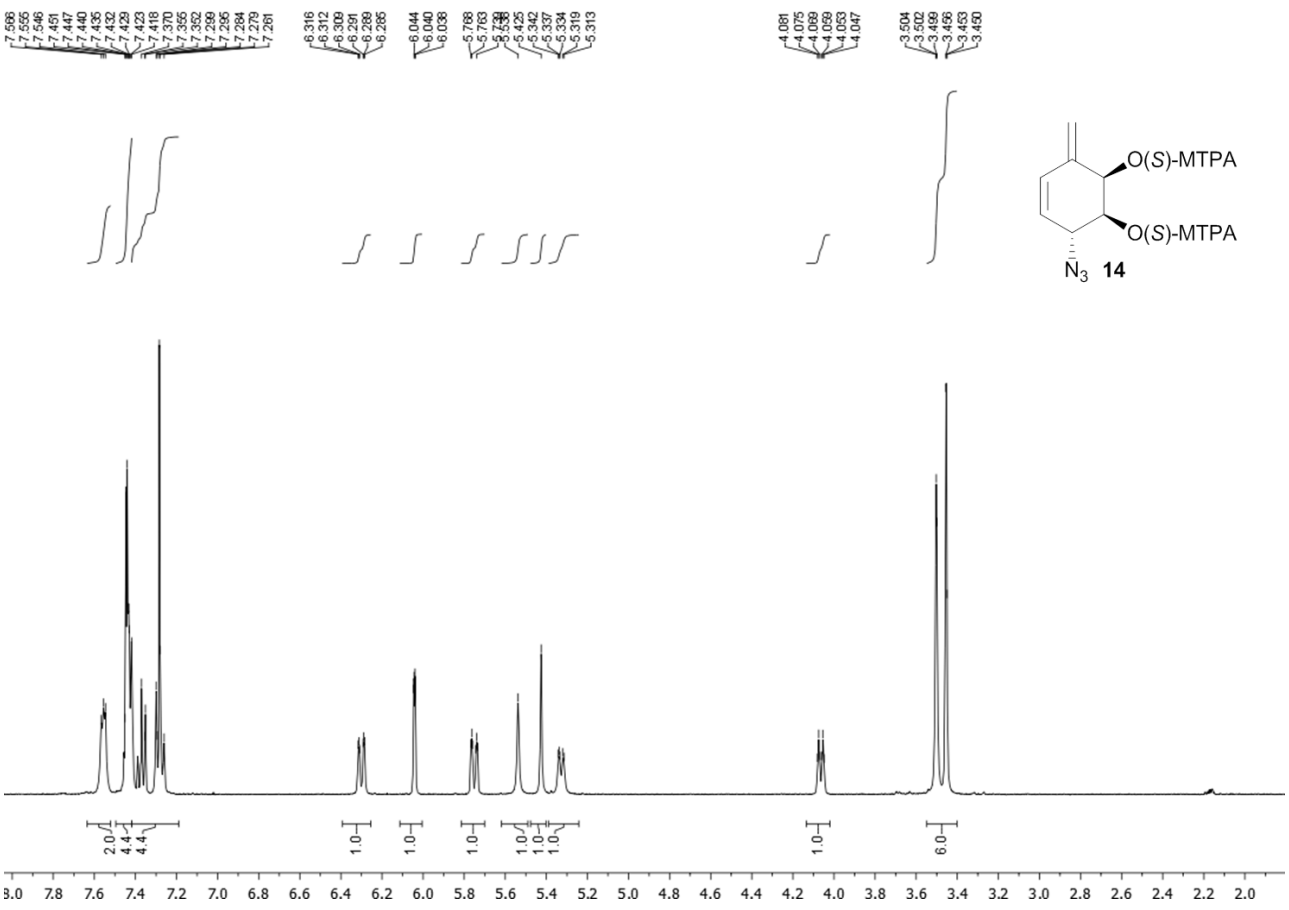

131.943
125.266
123.742
121.602
105.671
71.195
70.755
53.668
26.766
24.825
N3
O
O
9
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20

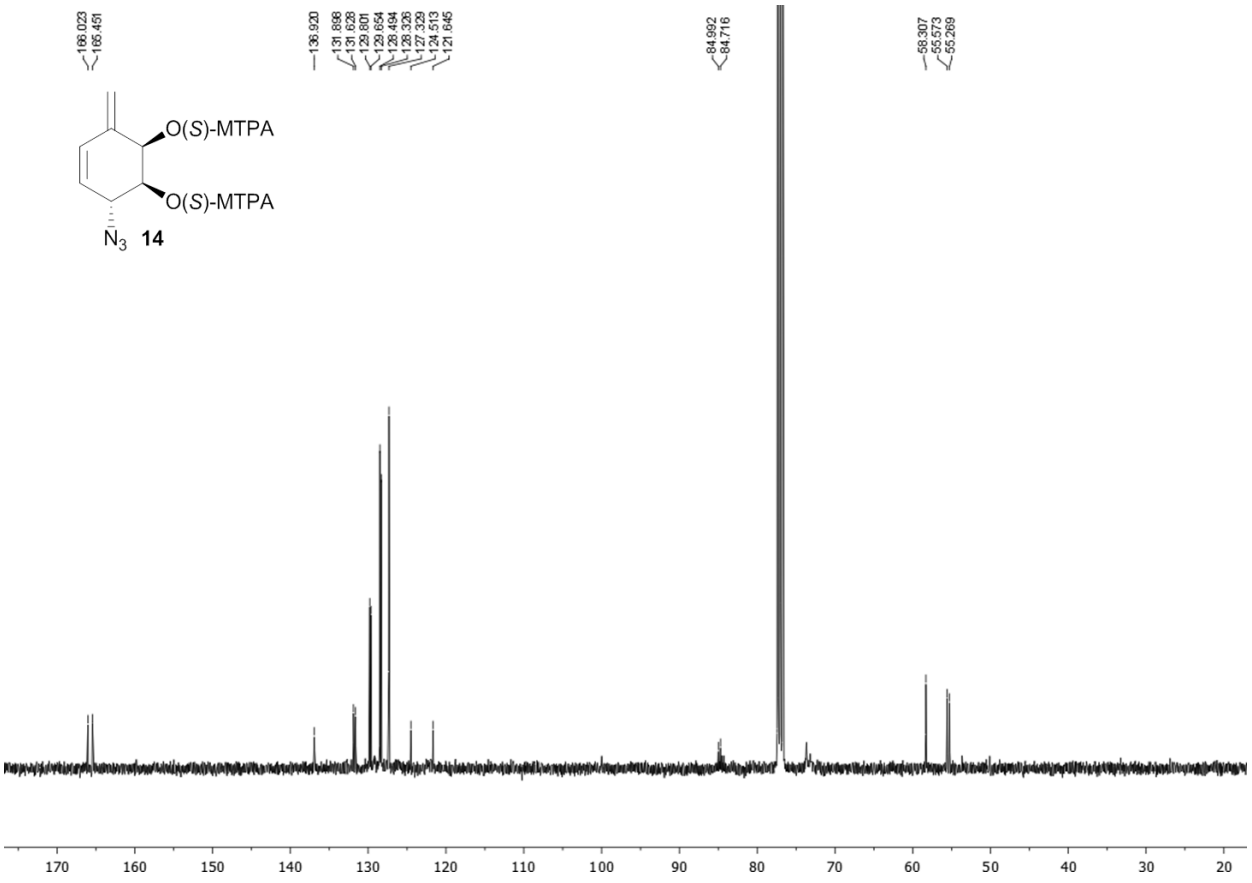

6.407
6.405
6.402
6.382
6.380
6.378
5.754
5.752
5.750
5.747
5.745
5.743
5.726
5.722
5.720
5.718
5.458
5.454
5.451
5.339
5.336
5.334
4.587
4.584
4.581
4.578
4.046
4.044
4.041
4.038
OH
OH
SCN
7
1.0
1.0
1.0
1.0
1.0
2.0
0.9
1.0
.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7

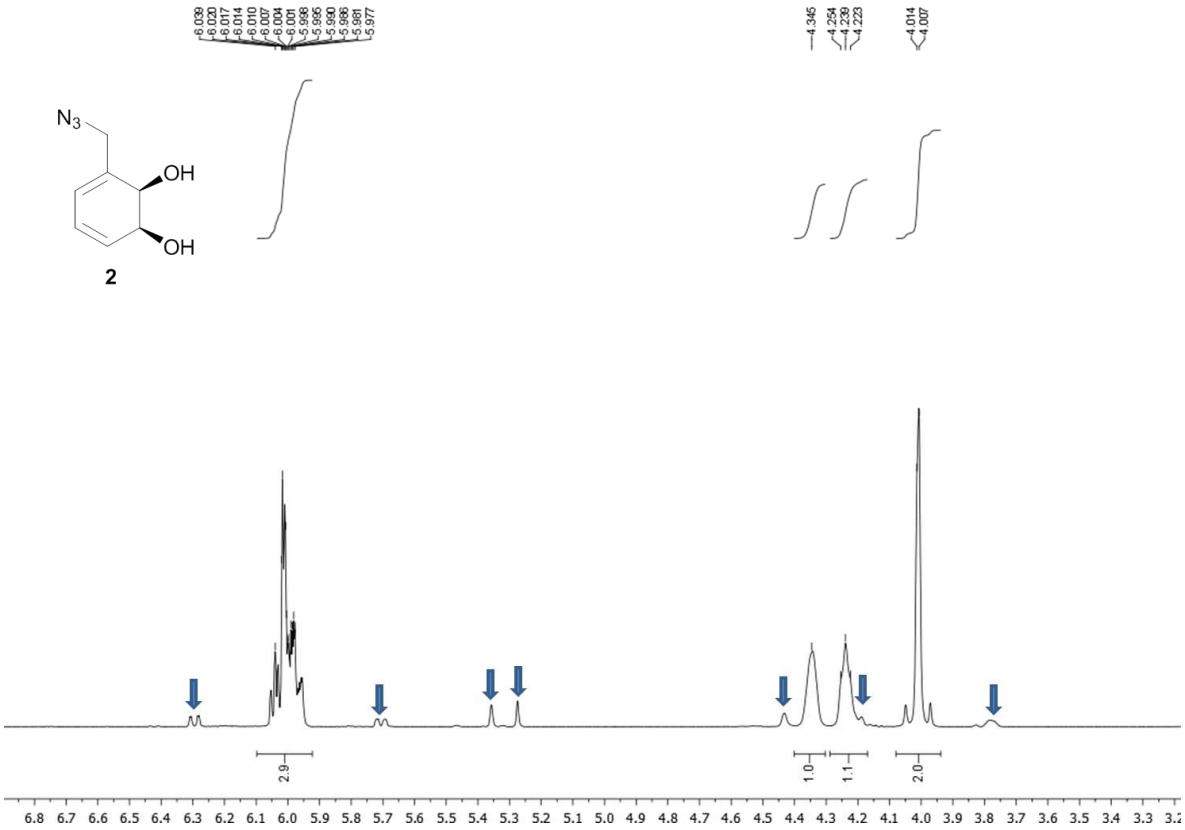

141.237
132.005
123.372
119.365
110.926
77.371 CDCl3
77.054 CDCl3
76.736 CDCl3
73.024
70.551
49.983
OH
OH
SCN
7
145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45


O(R)-MTPA
O(R)-MTPA
N3 13

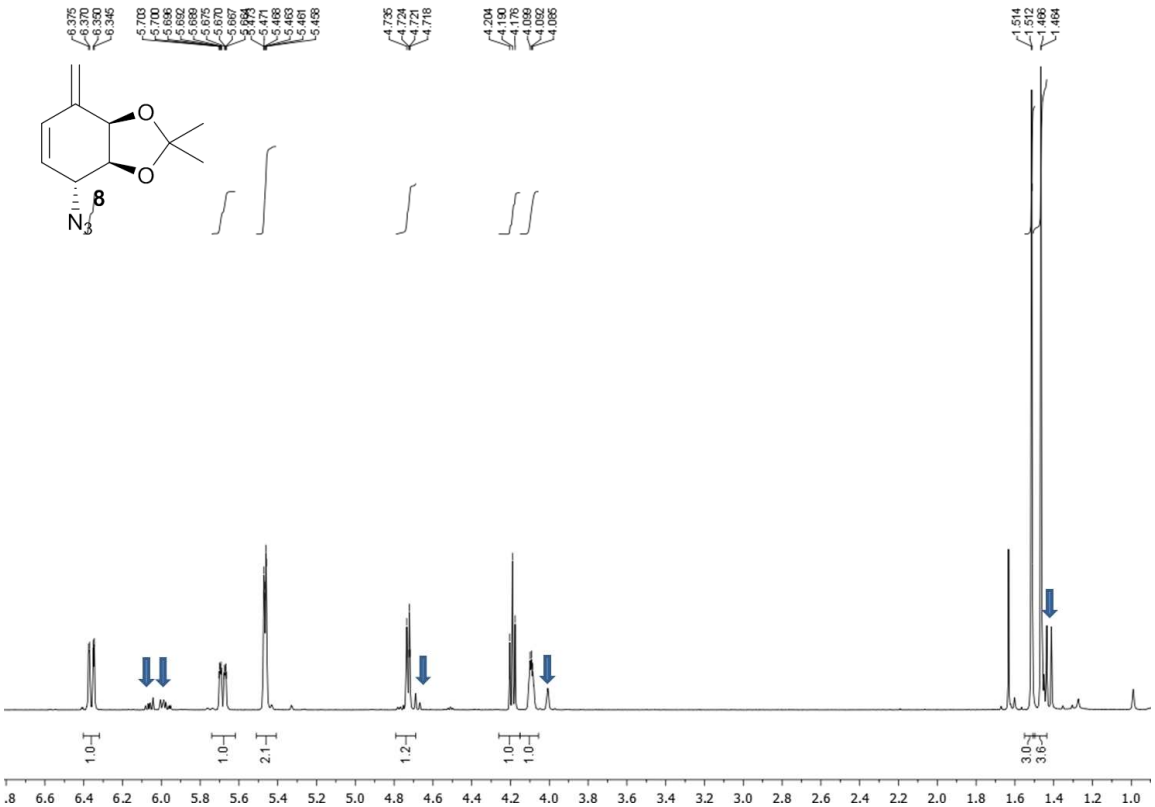

O(R)-MTPA
O(R)-MTPA
N3 13


O(S)-MTPA
O(S)-MTPA
N3 14

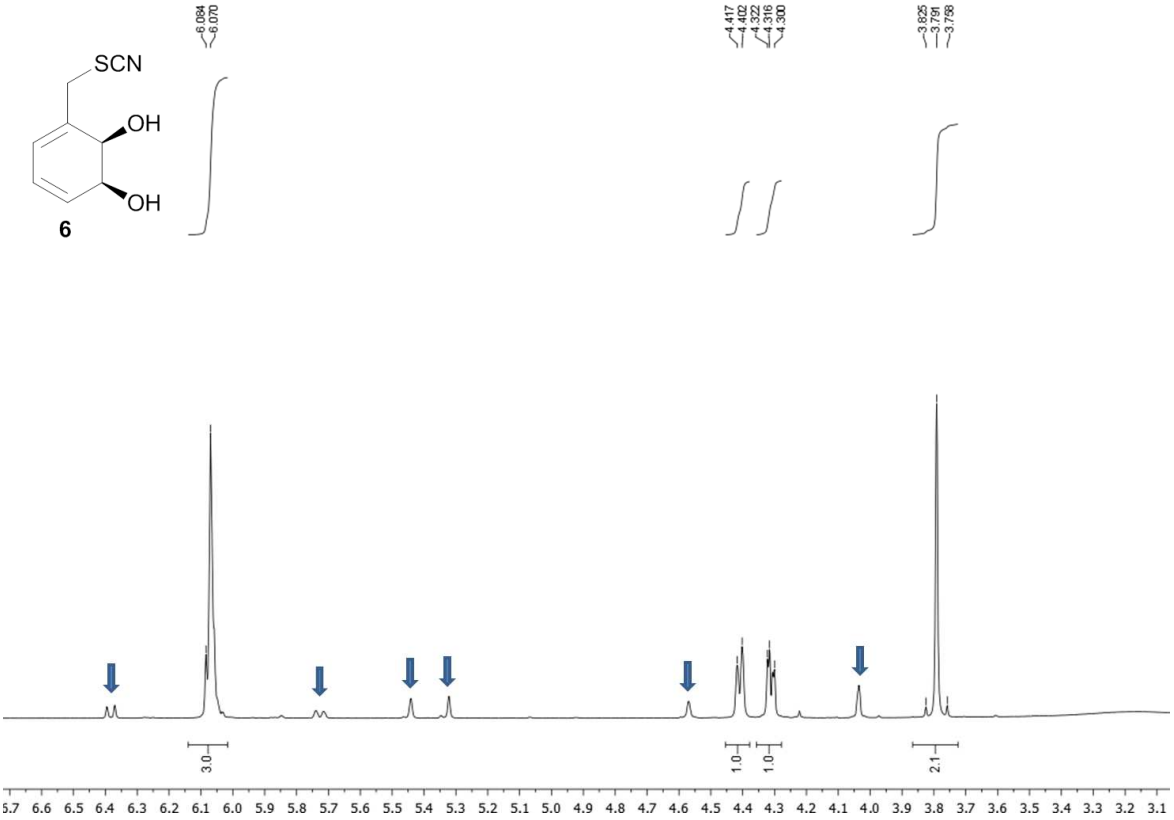

O(S)-MTPA
O(S)-MTPA
N3 14



Minor compound (blue arrows) corresponds to rearranged product **3a**



Minor compound (blue arrows) corresponds to rearranged product **9**



Minor compound (blue arrows) corresponds to rearranged product **7**

5. Computational calculations

All geometry optimizations were performed in gas phase and by means of the methods described hereinafter as implemented in Gaussian 09.[5] The final structures obtained were all minima in the potential energy surface, being the nature of the stationary points verified through vibrational analysis. For the molecular modelling approach to the [3,3] sigmatropic shift, different initial geometries of the compounds **2**, **3a**, **3b**, **8** and **9** were built, so as to explore the different features of the potential energy surface of each system brought about by the rotation of the rotatable bonds. These inputs were optimized by means of a PM3 semiempirical method. The minimum energy structure was selected for each compound, and restricted Density Functional Theory (DFT) geometry optimization runs were carried out using the B3LYP functional and the 6-31+G(d,p) basis set. Thermodynamic and structural parameters of the processes were determined from the output of the calculations.

In accordance with the experimental data (Figure S1), the computational results predict that the [3,3] sigmatropic shift for **2** is favoured from a thermodynamic point of view. The process is spontaneous at room temperature and at 100 °C, giving rise to negative ΔG^0 values no matter which face of the ring is considered for the azide rearrangement. Conversely, when the diol function is protected with the isopropylidene group, the thermodynamic tendency is reversed, and the transformation of compound **9** in compound **8** is not spontaneous, neither at room temperature (ΔG^0 = 2,01 kcal/mol) nor in toluene relfux (ΔG^0 = 2,24 kcal/mol).

In the light of the abovementioned results, the driving force of the sigmatropic shift seems to be related to the ability of the compounds to establish through-space attracting interactions between the azide and diol functions. Products **3a** and **3b** can set one N_3-HO hydrogen bond each, although this is not possible for **2**, since the azide group is positioned far away from the diol function owing to electrostatic repulsion (Figure S1). Moreover, **3a** undergoes an additional stabilization by means of a rapid conformational transition in which through-space N_3-OH electrostatic interactions are significant. Besides, when preventing the N_3-HO bond formation through the protection of the diol function in **8**, the sigmatropic rearrangement is inverted. Following steric and electrostatic repulsions, the azide group in **9** is placed as far as possible from the isopropylidene group.

We have carried out a complete computational study on these systems, whose results are in agreement with the experimental proposal about the mechanistic and energetic aspects of the sigmatropic shift. A more comprehensive kinetic and thermodynamic discussion on this matter will be provided in a future publication of our group.

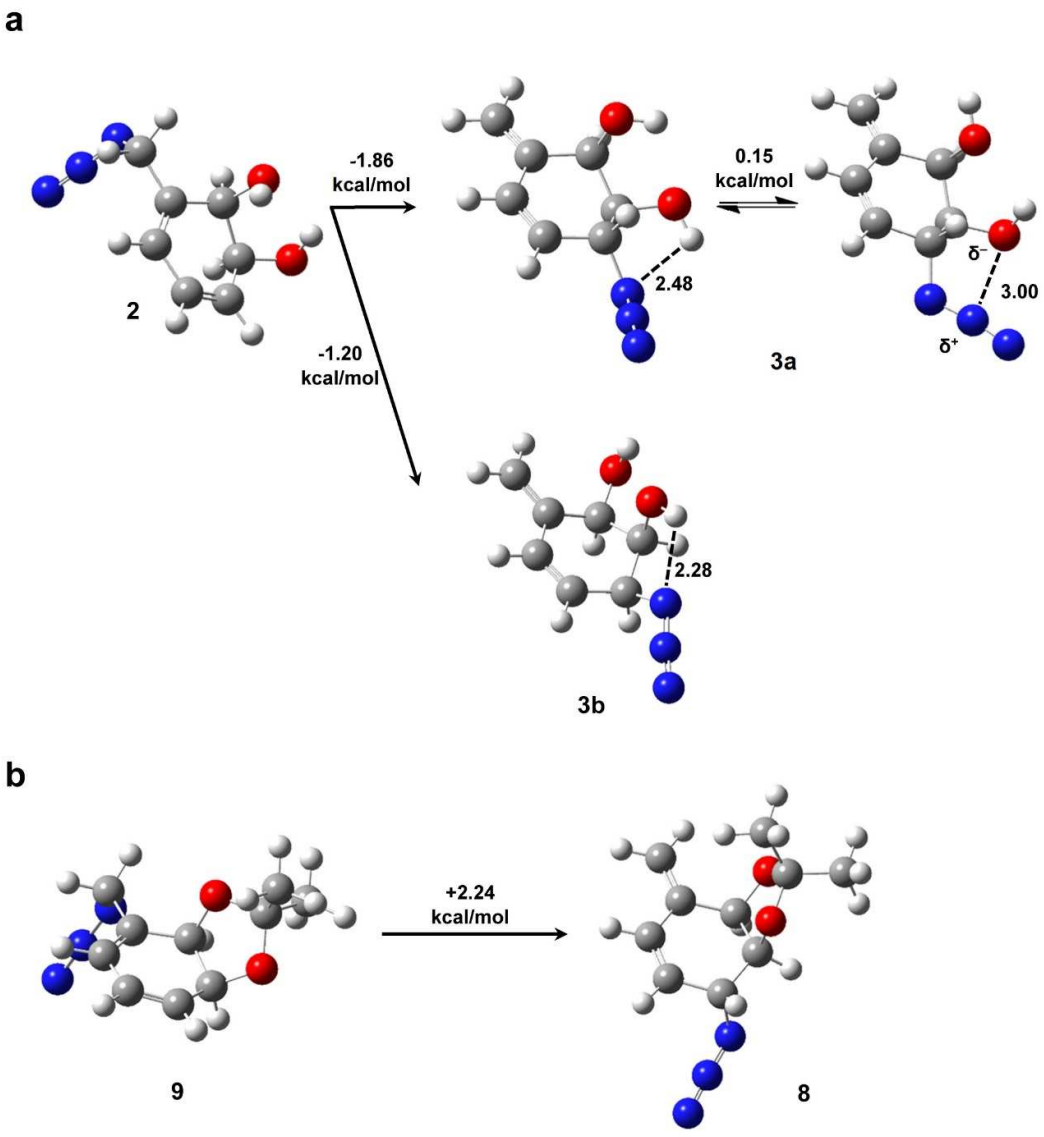


Figure S1. RB3LYP/6-31+G(d,p) optimized geometries for (a) compounds **2**, **3a** and **3b**, and (b) compounds **8** and **9**. The calculated ΔG^0 values at 25 °C (a) and 110,6 °C (toluene boiling point) (b) are shown for each reaction. The intramolecular hydrogen bonds and electrostatic interactions involving the azide group are depicted as dashed lines, with the corresponding distances in Å. Color code: C (grey), H (white), O (red), N (blue).

6. References

1. M. A. Vila, M. Brovetto, D. Gamenara, P. Bracco, G. Zinola, S. Rodríguez, G. Seoane and I. Carrera, *J. Mol. Catal. B: Enzym.*, 2013, **96**, 14-20.
2. M. E. Lagreca, I. Carrera, G. Seoane and M. Brovetto, *Tetrahedron Letters*, 2014, **55**, 853-856.
3. D. R. Boyd, N. D. Sharma, M. V. Berberian, K. S. Dunne, C. Hardacre, M. Kaik, B. Kelly, J. F. Malone, S. T. McGregor and P. J. Stevenson, *Advanced Synthesis & Catalysis*, 2010, **352**, 855-868.
4. D. Gonzalez, D. T. Gibson and T. Hudlicky, *Aldrichimica acta*, 1999, **32**, 35-62.
5. R. A. Gaussian 09, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.