

The catalytic versatility of non-toxic 1,4-dialkyltriazolium salts: *in situ* modification facilitates diametrically opposed catalysis modes in one pot

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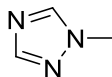
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1.0 General

Nuclear magnetic resonance (NMR) spectra were recorded on: Bruker Avance III 400 MHz, Bruker DPX400 400 MHz and Bruker Avance II 600 MHz spectrometers. ^1H NMR spectra were recorded at 400.23 MHz, 400.13 MHz and 600.13 MHz respectively. Chemical shifts are reported in ppm and coupling constants (J) are quoted in Hertz. ^{13}C NMR spectra were recorded on the previously mentioned instruments (100.64 MHz, 100.61 MHz & 150.9 MHz, respectively) with total proton decoupling. Spectra recorded in CDCl_3 were referenced to residual CHCl_3 at 7.26 ppm for ^1H and 77.0 ppm for ^{13}C . Spectra recorded in $\text{DMSO}-d_6$ were referenced to residual DMSO at 2.50 ppm for ^1H and 39.52 for ^{13}C . A Waters micromass LCT-TOF mass spectrometer was used in ESI positive and ESI negative modes for electrospray ionization mass spectrometry. Flash chromatography was carried out using silica gel, particle size 0.04-0.063 mm and using a stepwise solvent polarity gradient correlated with TLC mobility. TLC analysis was performed on precoated 60F₂₅₄ slides, and visualised by either UV irradiation or KMnO_4 staining. All liquid aldehydes were freshly distilled at reduced pressure prior to use, all solid aldehydes were recrystallised prior to use, unless otherwise stated, all chemicals were purchased from Aldrich and used as received. Anhydrous methanol was distilled over sodium before use. Anhydrous THF was distilled over sodium-benzophenone ketyl radical before use. All reactions were carried out under a protective argon atmosphere unless otherwise stated. Careful drying of all catalysts/ionic liquids is essential for best results in acetalisation reactions – a convenient procedure for this follows: the catalysts/ionic liquids were dissolved in dry toluene under argon. The solvent was removed *in vacuo* and the procedure was repeated twice, taking care that the compound was not exposed to air. The catalysts/ionic liquids were then dried under high vacuum for 2 h and used in the reaction. For all known compounds the spectral characteristics were in agreement with those reported in the literature.

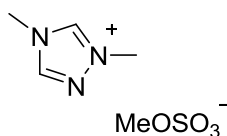
2.0 Catalyst synthesis and characterisation

2.1 1-Methyl-1*H*-1,2,4-triazole (**11**)



A 250 mL round bottomed flask containing a magnetic stirring bar, was charged with 1,2,4-triazole (2 g, 0.03 mol) in MeCN (148 mL). To this MeI (2.67 mL, 0.03 mol) was added and the reaction mixture was stirred at 45 °C for 2 days after which a white precipitate was obtained. The mixture was filtered by vacuum filtration using a Buchner funnel. The solid was washed with CH₂Cl₂ (30 mL) and the combined organic filtrate was concentrated *in vacuo* giving a pale yellow solid (2.03 g, 82%). The compound was used directly in the following reactions to furnish (**11c,d,f,g,h**) without further purification.

2.2 1,4-dimethyl-4*H*-1,2,4-triazol-1-ium methyl sulfate (**11c**)



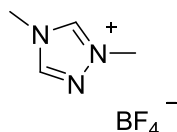
To a 100 mL round bottomed flask containing a magnetic stirring bar, triazole **11** (1g, 12 mmol) in MeCN (10 mL) was added. To this dimethyl sulfate (1.3 mL, 14 mmol) was added. The reaction flask was fitted with a condenser and heated under reflux for 2 d. Upon completion of reaction, the product was obtained by concentration of the solution *in vacuo* as a colourless oil (2.5 g, 98%). The salt catalyst was stored under argon.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.38, (s, 3H), 3.89 (s, 3H), 4.06 (s, 3H), 9.10 (s, 1H), 9.96 (s, 1H).

¹³C NMR (100 MHz, CDCl₃): δ = 33.9, 38.5, 52.9, 143.4, 145.3.

HRMS: calcd. for [*M*-MeOSO₃]⁺ C₄H₈N₃ requires 98.0718, found 98.0715.

2.3 1,4-dimethyl-4*H*-1,2,4-triazol-1-ium tetrafluoroborate (**11d**)



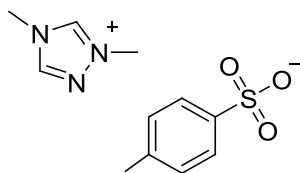
To a 50 mL round bottomed flask containing a magnetic stirring bar, **11** (500 mg, 6 mmol) in MeCN (8 mL) was added. Trimethyloxonium tetrafluoroborate (1.15g, 6 mmol) was added, the reaction flask was fitted with a condenser and placed under an argon atmosphere. The reaction mixture was stirred under reflux for 2 d. The solvent was removed *in vacuo*, the resulting residue was dissolved in CH₂Cl₂ and extracted using H₂O (2 x 20 mL). The combined organic layers were dried over MgSO₄, filtered and the solvent was removed under reduced pressure giving the pure catalyst **11d** (712 mg, 64%). *Note*: catalyst was used immediately due to rapid degradation.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.87 (s, 3H), 4.18 (s, 3H), 9.20 (s, 1H), 9.92 (s, 1H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 33.4, 38.6, 143.6, 145.4.

HRMS: calcd. for [*M*-BF₄]⁺ C₄H₈N requires 98.0718, found 98.0717.

2.4 1,4-dimethyl-4*H*-1,2,4-triazol-1-ium 4-methylbenzenesulfonate (**11f**)



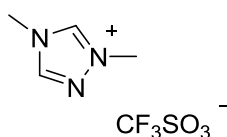
To a 100 mL round bottomed flask fitted with a magnetic stirring bar, **11** (1 g, 12 mmol) was added. This was dissolved in CH₂Cl₂ (4 mL), ethanol (8mL) and methyl tosylate (2.18g, 12 mmol) was added. The reaction flask was fitted with a condenser and the reaction was stirred at 80 °C for 2 d, after which the solvent was removed *in vacuo*. The resulting residue was dissolved in CH₂Cl₂ and extracted using H₂O (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. This produced the off-white powder **11f** (2.85 g, 82%). Mp 112-114 °C

^1H NMR (DMSO- d_6): δ = 2.29 (s, 3H), 3.88 (s, 3H), 4.05 (s, 3H), 7.12 (d, J = 7.8 Hz, 2H), 7.49 (d, J = 7.8 Hz, 2H), 9.11 (s, 1H), 9.99 (s, 1H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 20.8, 33.9, 38.5, 125.5, 128.1, 137.8, 143.4, 145.3, 145.9.

HRMS: calcd. for $[M-\text{OTf}]^+ \text{C}_4\text{H}_8\text{N}$ requires 98.0718, found 98.0718.

2.5 1,4-dimethyl-4H-1,2,4-triazol-1-ium trifluoromethanesulfonate (11g)



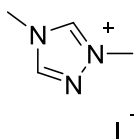
A 100 mL round bottomed flask fitted with a magnetic stirring bar was charged with triazole **11** (600 mg, 7.23 mmol) in MeCN (5 mL). To this distilled methyl triflate (1.6 mL, 14.5 mmol) was added and stirred at rt for 2 d. The solvent was removed under reduced pressure giving the pure compound **11g** as a pale yellow oil (1.4 g, 78% yield). *Note*: Catalyst was tested immediately due to rapid degradation.

^1H NMR (DMSO- d_6): δ = 3.78 (s, 3H), 3.93 (s, 3H), 8.97 (s, 1H), 9.79 (s, 1H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 34.2, 38.7, 143.4, 145.3.

HRMS: calcd. for $[M-\text{OTf}]^+ \text{C}_4\text{H}_8\text{N}$ requires 98.0718, found 98.0719.

2.6 1,4-Dimethyl-4H-1,2,4-triazol-1-ium iodide (11h)



To a 100 mL round bottomed flask containing a magnetic stirring bar, triazole **11** (2g, 24 mmol) in DCM (8 mL) and ethanol (12 mL) was added. To this methyl iodide (6 mL, 0.8 mol) was added. The reaction flask was fitted with a condenser and heated at 40 °C for 3 d. Upon completion of reaction, the product was obtained by concentration of the solution *in vacuo* as a yellow solid (4.43 g, 82%). The salt catalyst was stored under argon and kept under light free conditions. Mp 121-123 °C, lit 119-123 °C^{1a}

^1H NMR (400 MHz, DMSO- d_6): δ = 3.88 (s, 3H), 4.06 (s, 3H), 9.10 (s, 1H), 9.96 (s, 1H).

2.7 General procedure 1: counterion exchange (triazolium ions 11a, 11b and 11e):

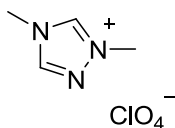
General procedure 1a: Preparation of anion exchange resin (AER):

A 1% acid in methanol solution was passed through a glass column packed with Amberlyst[®] A-26 (OH⁻-form) until the pH of eluates reached the same value as that of the original solution, and then the resin was washed with methanol until neutral pH. The process was carried out at room temperature, using gravity as a driving force.

General procedure 1b for anion exchange:

A methanolic solution of the triazolium salt **11h** (50-60 mM) was passed through a column packed with Amberlyst A-26, previously loaded with the selected anion, and then washed with 25 mL of methanol. The combined eluants were concentrated *in vacuo* removed and the oil obtained was dried under vacuum at 60 °C. The amount of halide contents in the exchanged ionic liquids was determined by a silver chromate test following a similar protocol described by Sheldon and co-workers.² An aqueous solution of potassium chromate (5 % p/v in Milli-Q water, 0.257 M) was added to the sample. A silver nitrate aqueous solution (0.24 % p/v in Milli-Q water, 0.014 M) was added dropwise to 1 mL of the solution and the end point was reached when a red persistent suspension of silver chromate was observed. This test was carried out on each sample twice. Volumes were measured with a 1 mL syringe, and 0.1 mL contains 9 drops of the silver nitrate aqueous solution, consequently 1 drop = 0.011 mL. (0.011 mL of AgNO₃ was enough to react with 200 ppm (mg/L) of iodide and 6 ppm (mg/L) Cl).

2.8 1,4-dimethyl-4*H*-1,2,4-triazol-1-ium perchlorate (**11a**)



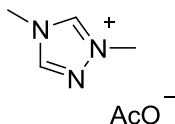
Catalyst **11a** was prepared as per general procedure 1a, where Amberlyst®A-26 (OH-form) was washed with a methanolic solution of perchloric acid until the pH of the eluate was the same as the pH of the acidic solution. The resin was washed with methanol until pH was neutral. The catalyst **11h** was then washed through following general procedure 1b producing compound **11a** (100%).

^1H NMR (400 MHz, DMSO- d_6): δ = 3.89 (s, 3H), 4.07 (s, 3H), 9.12 (s, 1H), 9.98 (s, 1H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 34.0, 38.6, 143.3, 145.3.

HRMS: calcd. for $[M-\text{ClO}_4]^+$ $\text{C}_4\text{H}_8\text{N}$ requires 98.0718, found 98.0719.

2.9 1,4-dimethyl-4*H*-1,2,4-triazol-1-ium acetate (**11b**)



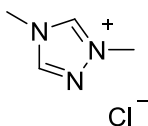
Catalyst **11b** was prepared as per general procedure 1a, where Amberlyst®A-26 (OH-form) was washed with a methanolic solution of acetic acid until the pH of the eluate was the same as the original solution. The resin was then neutralised by washing with methanol. The catalyst **11h** was then passed through the column following general procedure 1b producing the pure catalyst **11b** (100%).

^1H NMR (400 MHz, DMSO- d_6): δ = 3.89 (s, 3H), 4.07 (s, 3H), 9.11 (s, 1H), 9.98 (s, 1H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 34.1, 38.6, 143.3, 145.3.

HRMS: calcd. for $[M-\text{AcO}]^+$ $\text{C}_4\text{H}_8\text{N}$ requires 98.0718, found 98.0715.

2.10 1,4-dimethyl-4H-1,2,4-triazol-1-ium chloride (**11e**)



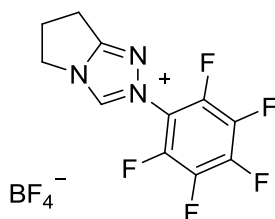
Catalyst **11e** was prepared as per general procedure 1a, where Amberlyst®A-26 (OH-form) was washed with a methanolic solution of hydrochloric acid until the pH of the eluate was the same as the pH of the acidic solution. The resin was washed with methanol until the pH of the eluates was neutral. The catalyst **11h** was then washed through following general procedure 1b producing the pure catalyst **11e** (100%).

^1H NMR (400 MHz, DMSO- d_6): δ = 3.90 (s, 3H), 4.07 (s, 3H), 9.15 (s, 1H), 10.06 (s, 1H).

^{13}C NMR (100 MHz, DMSO- d_6): δ = 34.0, 38.7, 143.4, 145.3.

HRMS: calcd. for $[M\text{-OTf}]^+$ $\text{C}_4\text{H}_8\text{N}$ requires 98.0718, found 98.0719.

2.11 2-(perfluorophenyl)-6,7-dihydro-5H-pyrrolo[2,1-c][1,2,4]triazol-2-ium tetrafluoroborate (**19**)



An oven dried round bottomed flask fitted with a magnetic stirring bar was charged with 2-pyrrolidinone (500 mg, 5.88 mmol) and CH_2Cl_2 (30 mL). Trimethyloxonium tetrafluoroborate (871 mg, 5.88 mmol) was added and the reaction was stirred at room temperature for 12 h. Pentafluorophenylhydrazine (1.16 g, 5.88 mmol) was added and allowed to stir for a further 2 h. The solvent was removed *in vacuo* and the resulting orange solid was heated under vacuum at 110 °C for 2 h. Triethyl orthoformate (4.83 mL, 29.4 mmol) was added and heating was continued at 110 °C for 1 h. Upon cooling, toluene (60 mL) was added and the white solid product was filtered, rinsed with toluene (3 x 5 mL) and heated under vacuum at 120 °C for 6 h to provide triazolium salt **19** (1.36g, 64 %). Mp 243-244 °C lit. 242-245 °C.^{1b}

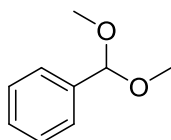
^1H NMR (400 MHz, acetone- d_6): δ = 3.00 (ddd, J = 15.4, 8.0, 8.0 Hz, 2H), 3.43 (t, J = 8.0 Hz, 2H), 4.76 (t, J = 8.0 Hz, 2H), 10.21 (s, 1H).

3.0 General procedure 2: the acetalisation of aldehydes

A 20 mL reaction vessel was fitted with a magnetic stirring bar, charged with catalyst (0.08 mmol), fitted with a septum and flushed with argon. Benzaldehyde (170 μL , 1.67 mmol) was added followed by dry methanol (3.4 mL) *via* syringe. The solution was then stirred under argon at room temperature for 24 h. When conversion was judged to be either complete or > 95% conversion (by ^1H NMR spectroscopic analysis) the reaction was quenched with PhNHNH₂ and solvent was removed *in vacuo*. The crude product was either purified by flash-chromatography or the yield was determined by ^1H NMR spectroscopy using an internal standard

4.0 Characterisation data for acetalisation of aldehydes

4.1 Benzaldehyde dimethyl acetal (**6**)



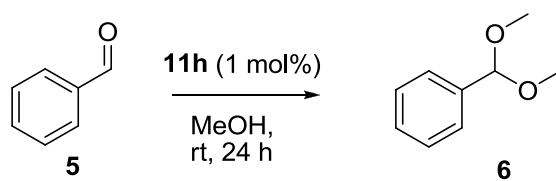
The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (6.8 mg, 0.03 mmol), methanol (6.7 mL) and benzaldehyde (340 μL , 3.34 mmol). After purification of the crude material by flash chromatography (5:1 hexane:EtOAc) the product **6** was obtained as a pale yellow liquid (496 mg, 98%).

The NMR spectra of **6** were consistent with those previously reported.³

^1H NMR (400 MHz, CDCl₃): δ = 3.36 (s, 6H), 5.42 (s, 1H), 7.34-7.39 (m, 3H), 7.47-7.49 (m, 2H).

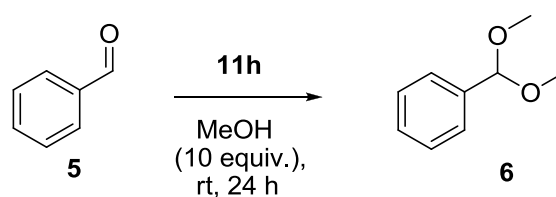
^{13}C NMR(100 MHz, CDCl₃): δ = 52.2, 102.7, 126.2, 127.7, 128.0, 137.5.

4.1.2 Optimisation of the acetalisation of benzaldehyde:



MeOH	Loading (mol %)	Yield (%) ^a
10 equiv.	1	56
6 equiv.	1	47
4 equiv.	1	42
2 equiv.	1	35

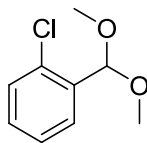
^aIsolated yield after chromatography



MeOH	Loading (mol %)	Yield (%) ^a
10 equiv.	20	69
10 equiv.	10	64
10 equiv.	5	60
10 equiv.	1	56
10 equiv.	0.5	35

^aIsolated yield after chromatography

4.2 2-Chlorobenzaldehyde dimethyl acetal (**14a**)



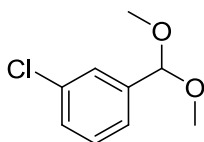
The dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (5.9 mg, 0.026 mmol), methanol (6.8 mL) and 2-chlorobenzaldehyde (400 μ L, 2.56 mmol). After purification of crude material by flash chromatography (5:1 hexane:EtOAc) the product **14a** was obtained as a pale yellow liquid (460 mg, 96 %).

The NMR spectra of **14a** were consistent with those previously reported.⁴

¹H NMR (400 MHz, CDCl₃): δ = 3.32 (s, 6H), 5.33 (s, 1H), 6.99-7.02 (m, 2H), 7.07-7.09 (m, 1H) 7.30-7.32 (m, 1H).

¹³C NMR (100 MHz, CDCl₃): δ = 52.2, 101.6, 124.4, 126.5, 128.1, 129.1, 133.8, 139.8.

4.3 3-Chlorobenzaldehyde dimethyl acetal (**14b**)



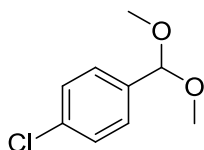
The dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (5.9 mg, 0.026 mmol), methanol (6.8 mL) and 3-chlorobenzaldehyde (400 μ L, 2.56 mmol). After purification of crude material by flash chromatography (5:1 hexane:EtOAc) the product **14b** was obtained as a pale yellow liquid (449 mg, 95 %).

The NMR spectra of **14b** were consistent with those previously reported.⁴

¹H NMR (400 MHz, CDCl₃): δ = 3.41 (s, 6H), 5.66 (s, 1H), 7.29-7.33 (m, 2H), 7.38-7.40 (m, 1H), 7.63-7.65 (m, 1H).

¹³C NMR (100 MHz, CDCl₃): δ = 53.4, 100.5, 126.1, 127.6, 129.1, 129.3, 132.7, 134.8.

4.4 4-Chlorobenzaldehyde dimethyl acetal (**14c**)



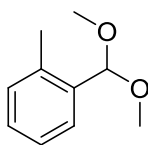
The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (8.1 mg, 0.036 mmol), methanol (7.1 mL) and 4-chlorobenzaldehyde (500 mg, 3.56 mmol). After purification of the crude material by flash chromatography (5:1 hexane:EtOAc) the product **14c** was obtained as a pale yellow liquid (653 mg, 98%).

The NMR spectra of **14c** were consistent with those previously reported.³

¹H NMR (400 MHz, CDCl₃): δ = 3.34 (s, 6H), 5.39 (s, 1H), 7.32 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H).

¹³C NMR (100 MHz, CDCl₃): δ = 52.2, 101.6, 124.6, 126.4, 128.4, 133.8.

4.5 2-Methylbenzaldehyde dimethyl acetal (**14d**)



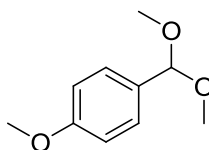
The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (3.8 mg, 0.017 mmol), methanol (2.3 mL) and 2-methylbenzaldehyde (100 μ L, 0.86 mmol). After purification of the crude material by flash chromatography (15:1 hexane:EtOAc) the product **14d** was obtained as a pale yellow liquid (131 mg, 91%).

The NMR spectra of **14d** were consistent with those previously reported.⁵

¹H NMR (400 MHz, CDCl₃): δ = 2.42 (s, 3H), 3.37 (s, 6H), 5.51 (s, 1H) 7.14-7.16 (m, 1H), 7.25-7.30 (m, 2H), 7.56 (dd, J = 6.5, 2.0 Hz, 1H).

¹³C NMR (100 MHz, CDCl₃): δ = 18.4, 52.5, 101.3, 124.9, 126.1, 127.9, 130.1, 135.2, 135.8.

4.6 4-Methoxybenzaldehyde dimethyl acetal (**14e**)



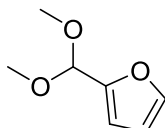
The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (3.8 mg, 0.017 mmol), methanol (2.2 mL) and 4-methoxybenzaldehyde (100 μ L, 0.82 mmol). The reaction mixture was then heated at 35 °C for 24 hours. **Note:** the use of PhNHNH₂ was not required. After purification of the crude material by flash chromatography (5:1 hexane:EtOAc) the product **14e** was obtained as a pale yellow liquid (134 mg, 91%).

The NMR spectra of **14e** were consistent with those previously reported.³

¹H NMR (400 MHz, CD₃OD): δ = 3.30 (s, 6H), 3.81 (s, 3H), 5.34 (s, 1H), 6.92 (d, J = 8.9 Hz, 2H), 7.32 (d, J = 8.9 Hz, 2H).

¹³C NMR (100 MHz, CD₃OD): δ = 51.2, 53.7, 102.7, 112.5, 127.1, 129.7, 160.0.

4.7 2-Dimethoxymethyl furan (**14f**)

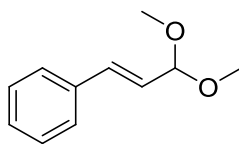


The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (3.6 mg, 0.016 mmol), methanol (2.1 mL) and furfural (77 μ L, 0.79 mmol). After purification of the crude material by flash chromatography (6:1 hexane:EtOAc) the product **14f** was obtained as a pale yellow liquid (103 mg, 92%).

¹H NMR (400 MHz, CDCl₃): δ = 3.30 (s, 6H), 5.37 (s, 1H), 6.31 (s, 1H), 6.36 (s, 1H), 7.39 (s, 1H).

¹³C NMR (100 MHz, CDCl₃): δ = 53.3, 107.9, 109.4, 112.1, 141.9, 149.9

4.8 3,3-Dimethoxypropenyl benzene (14g)



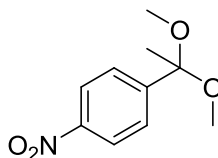
The desired dimethyl acetal was obtained following general procedure 2 using catalyst **11h** (3.6 mg, 0.016 mmol), methanol (2.1 mL) and cinnamaldehyde (100 μ L, 0.79 mmol). **Note:** the use of PhNHNH₂ was not required. After purification of the crude material by flash chromatography (8:1 hexane: EtOAc) the product **14g** was obtained as a pale yellow liquid (128 mg, 90%).

The NMR spectra of **14g** were consistent with those previously reported.⁶

¹H NMR (400 MHz, CDCl₃): δ = 3.41 (s, 6H), 5.00 (d, J = 5.1 Hz, 1H), 6.18 (dd, J = 16.4, 5.1 Hz, 1H), 6.69 (d, J = 16.4 Hz, 1H), 7.29-7.38 (m, 3H), 7.44-7.45 (m, 2H).

¹³C NMR (100 MHz, CDCl₃): δ = 52.3, 102.5, 125.2, 126.3, 127.6, 128.2, 133.2, 135.6.

4.9 4-nitroacetophenone dimethyl ketal (15)

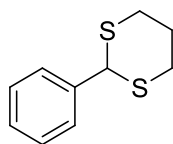


The desired dimethyl ketal was obtained following general procedure 2 using catalyst **11h** (22.5 mg, 0.1 mmol), methanol (2.6 mL) and 4-nitroacetophenone (165.2 mg, 1 mmol). The reaction mixture was then heated at rt for 24 hours. **Note:** the use of PhNHNH₂ was not required. After purification of the crude material by flash chromatography (15:1 hexane: EtOAc) the product **15** was obtained as a pale yellow solid (68 mg, 32%). M.p. 58-60 °C, lit. 60-62 °C.⁷

¹H NMR(400 MHz, CDCl₃): δ = 1.53 (s, 3H), 3.78 (s, 6H), 7.66 (d, J = 8.9 Hz, 2H), 8.17 (d, J = 8.9 Hz, 2H).

^{13}C NMR (100 MHz, CDCl_3): $\delta = 26.4, 48.6, 123.1, 123.9, 129.1, 146.7, 151.4$.

4.10 Phenyl-1,3-dithiane (16)

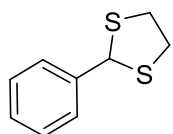


The desired dithiane was obtained following general procedure 2 using **11h** (11.2 mg, 0.05 mmol), 1,3-propanedithiol (300 μL , 2.98 mmol), THF (9.1 cm^3) and benzaldehyde (276 μL , 2.71 mmol). Upon completion of the reaction, the reaction mixture was added to a saturated solution of NaHCO_3 (5 mL) and the product was extracted with EtOAc (2 x 10 mL). The combined organic layers were then dried over MgSO_4 and the solvent was removed under reduced pressure. After purification by flash column chromatography the product **16** was obtained as a white solid (486 mg, 92%). M.p. 69-71 $^\circ\text{C}$, lit. 71-72 $^\circ\text{C}$.^{7b}

^1H NMR (400 MHz, CDCl_3): $\delta = 1.99\text{-}2.19$ (m, 2H), 2.96 (ddd, $J = 7.5, 4.3, 3.3$ Hz, 2H), 3.07 (ddd, $J = 14.5, 12.2, 2.5$ Hz, 2H), 5.16 (s, 1H), 7.28-7.36 (m, 3H), 7.46-7.48 (m, 2H).

^{13}C NMR (100 MHz, CDCl_3): $\delta = 24.9, 30.4, 51.7, 127.8, 128.5, 128.9, 139.1$.

4.11 Phenyl-1,3-dithiolane (17)



The desired dithiolane was obtained following general procedure 2 using catalyst **11h** (4.5 mg, 0.02 mmol), 1,2-ethanethiol (100 μL , 1.19 mmol), THF (200 μL) and benzaldehyde (110 μL , 1.08 mmol). After completion of the reaction, the reaction mixture was poured onto a saturated NaHCO_3 solution (5 mL) and the product was extracted with ethyl acetate (2 x 10 mL) 25 mL water. The organic layer was separated, dried over MgSO_4 and concentrated *in*

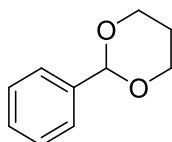
vacuo. After purification by flash chromatography (5:1 hexane:EtOAc) the product **17** was obtained as a colorless liquid (177 mg, 89%).

The NMR spectra of **17** were consistent with those previously reported.⁸

¹H NMR (400 MHz, CDCl₃): δ = 3.34-3.40 (m, 2H), 3.48-3.54 (m, 2H), 5.66 (s, 1H), 7.27-7.35 (m, 3H), 7.54-7.56 (m, 2H).

¹³C NMR (100 MHz, CDCl₃): δ = 39.8, 55.8, 127.5, 127.6, 128.1, 139.9.

4.12 Phenyl-1,3-dioxane (18)



The desired dioxane was obtained following general procedure 2 using catalyst **11h** (5.6 mg, 0.025 mmol), 1,3-propanediol (100 μ L, 1.38 mmol) and benzaldehyde (128 μ L, 1.26 mmol). After purification of the crude material by flash chromatography the product **18** was obtained as a pale yellow liquid (177 mg, 86 %).

¹H NMR (400 MHz, CDCl₃): δ = 1.40-1.51 (m, 1H), 2.15-2.28 (m, 1H), 4.04 (ddd, J = 14.0, 12.3, 1.7 Hz, 2H), 4.32 (ddd, J = 11.7, 5.3, 1.2 Hz, 2H), 5.51 (s, 1H), 7.32-7.39 (m, 3H), 7.48-7.50 (m, 2H).

¹³C NMR (100 MHz, CDCl₃): δ = 25.1, 67.5, 102.6, 125.3, 127.9, 128.1, 138.8.

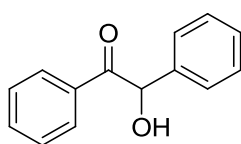
5.0 General procedure 3: the benzoin condensation

To a 5 mL round bottom flask, equipped with a magnetic stirring bar, Rb₂CO₃ (99.995 %, anhydrous, 0.044 mmol, 10.16 mg) that had been finely ground using a mortar and pestle, was added. The reaction vessel was put under vacuum and heated with a heat gun for one minute over two-minute intervals for a total of 4 minutes. Upon cooling, catalyst **11h** (10 mg, 0.044mmol) and (*E*)-stilbene (24.78 mg, 0.138 mmol) were added and the flask was fitted with a septum seal. The reaction was evacuated for 4 min and put under an atmosphere of Ar. The required aldehyde was distilled under vacuum and used directly. THF (1.1 M) was added

via syringe, followed by the aldehyde (1.100 mmol). The reaction was stirred at room temperature for 24 h after which CH₂Cl₂ (3.0 mL) and deionised H₂O (3.0 mL) were added. The organic layer was separated and the aqueous layer was washed with CH₂Cl₂ (4 x 3.0 mL). The organic extracts were combined, dried with MgSO₄, filtered and the solvent was removed *in vacuo*. The product was purified using flash column chromatography.

6.0 Characterisation data for benzoin condensation

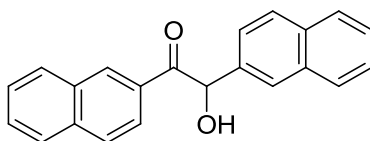
6.1 2-hydroxy-1,2-diphenylethanone (15a)



Prepared according to general procedure 3 using catalyst **11h**. Purified by column chromatography (6:4 CH₂Cl₂:hexane) to give pure **15a** (112 mg, 96 %) as a white solid. M.p. 131-132 °C, lit, 130-131 °C.⁹

¹H NMR (400 MHz, CDCl₃): δ = 4.56 (bs, 1H), 5.97 (s, 1H), 7.29-7.38 (m, 5H), 7.45 (app t, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 2H).

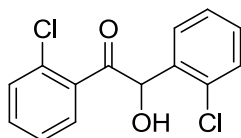
6.2 2-hydroxy-1,2-di(naphthalen-2-yl)ethanone (15b)



Prepared according to general procedure 3 using catalyst **11h**. Purified by column chromatography (6:4 CH₂Cl₂:hexane) gave pure **15b** (156 mg, 91%) as a white solid. M.p. 124-125 °C, lit, 124-126 °C.¹⁰

¹H NMR (400 MHz, CDCl₃): δ = 4.78 (bs, 1H), 6.31 (s, 1H), 7.49-7.65 (m, 5H), 7.79-7.84 (m, 5H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.98 (s, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 8.54 (s, 1H).

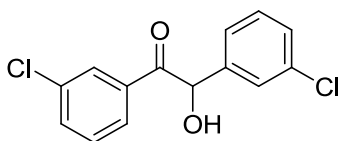
6.3 1,2-bis(2-chlorophenyl)-2-hydroxyethanone (15c)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (6:4, CH₂Cl₂:hexane) gave **15c** (42 mg, 27%) as an off white solid. M.p 64-65 °C, lit 64 °C.¹¹

¹H NMR (400 MHz, CDCl₃): δ = 4.46 (bs, 1H), 6.38 (s, 1H), 7.23-7.26 (m, 5H), 7.31-7.41(m, 3H).

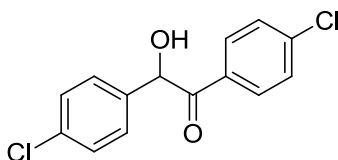
6.4 1,2-bis(3-chlorophenyl)-2-hydroxyethanone (15d)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (6:4 CH₂Cl₂:hexane) gave **15c** (142 mg, 92%) as an off white solid. M.p 76-77 °C, lit, 76-77 °C.¹¹

¹H NMR (400 MHz, CDCl₃): δ = 4.58 (bs, 1H), 5.91 (s, 1H), 7.23-7.29 (m, 3H), 7.35-7.39 (m, 2H), 7.51 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.92 (s, 1H).

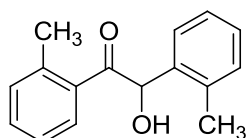
6.5 1,2-bis(4-chlorophenyl)-2-hydroxyethanone (15e)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (6:4 CH₂Cl₂:hexane) gave **15e** (144 mg, 93%) as an off white solid. M.p. 87-88 °C, lit, 86-87 °C.¹¹

¹H NMR (400 MHz, CDCl₃): δ = 4.49 (bs, 1H), 5.90 (s, 1H), 7.26-7.27 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 7.8 Hz, 2H).

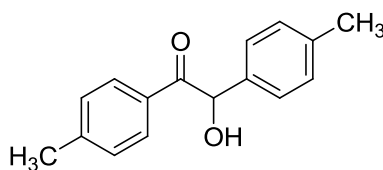
6.6 2-hydroxy-1,2-dio-tolyloethanone (15f)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (6:4 CH₂Cl₂:hexane) gave **15f** (24 mg, 18%) as an off white solid. M.p 76-77 °C, lit 76-78 °C.¹²

¹H NMR (400 MHz, CDCl₃): δ = 2.39 (s, 3H), 2.42 (s, 3H), 4.25 (s, 1H), 6.09 (s, 1H), 7.11-7.31 (m, 6H), 7.35 (t, J = 6.5 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H).

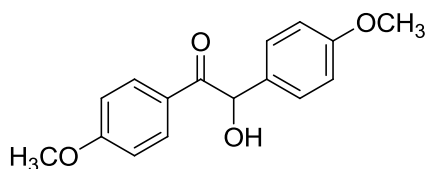
6.7 2-hydroxy-1,2-dip-tolyloethanone (15g)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (6:4 CH₂Cl₂:hexane) gave **15g** (117 mg, 89%) as an off white solid. M.p 88-89 °C, lit. 89-90 °C.¹¹

¹H NMR (400 MHz, CDCl₃): δ = 2.31 (s, 3H), 2.38 (s, 3H), 4.57 (s, 1H), 5.92 (s, 1H), 7.14-7.15 (d, J = 7.5 Hz, 2H), 7.20-7.28 (m, 4H), 7.84 (d, J = 8.5 Hz, 2H).

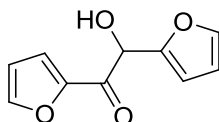
6.8 2-hydroxy-1,2-bis(4-methoxyphenyl)ethanone (**15h**)



Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (CH₂Cl₂) gave **15h** (53 mg, 35%) as an off white solid. M.p 110-111 °C, lit. 109-110 °C.¹³

¹H NMR (400 MHz, CDCl₃): δ = 3.77(s, 3H), 3.84 (s, 3H), 4.61 (s, 1H), 5.87 (s, 1H), 6.84-6.91 (m, 4H), 7.25-7.29 (m, 2H), 7.92 (d, J = 8.8 Hz, 2H).

6.9 1,2-di(furan-2-yl)-2-hydroxyethanone (**15i**)

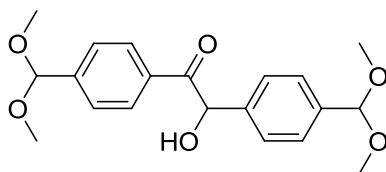


Prepared according to general procedure 3 using catalyst **11h**. Purification by column chromatography (CH₂Cl₂) gave **15i** (98 mg, 93%) as a white solid. M.p. 136-137 °C, lit. 136-137 °C.¹³

¹H NMR (400 MHz, CDCl₃): δ = 4.22 (bs, 1H), 5.83 (bs, 1H), 6.38 (dd, J = 3.0, 1.6 Hz, 1H), 6.45 (d, J = 3.0 Hz, 1H), 6.57 (dd, J = 3.0, 1.6 Hz, 1H), 7.27 (m, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.56 (s, 1H).

7.0 *In situ* catalyst modification: procedure

7.1 1,2-bis(4-(dimethoxymethyl)phenyl)-2-hydroxyethanone (**23**)



To a reaction vessel, containing a magnetic stirring bar, terephthalaldehyde (100 mg, 0.75 mmol) and catalyst **11h** (9 mg, 0.04 mmol) was added. The reaction vessel was placed under argon and anhydrous MeOH (67 μ L, 1.65 mmol) was added. The reaction mixture was stirred under argon for 24 h, after which time full conversion to the monoacetal **21a** occurred, this was then followed by the addition of DBU (6.6 μ L, 0.04 mmol) and dry THF (600 μ L) *via* syringe to the reaction vessel to generate basic conditions. The mixture was stirred under argon for a further 24 h, after which time the solvent was removed *in vacuo* and the resulting residue was dissolved in CH₂Cl₂ (3.0 mL). This was extracted using H₂O (3.0 mL x2) and the combined organic layers were concentrated under vacuum. The resulting crude compound was purified by flash column chromatography (6:4 CH₂Cl₂:hexane) giving the pure product **23** as a pale yellow solid (102mg, 75%). Mp 122-125 °C

Note: the use of Rb₂CO₃ in this reaction is not optimal, as it is a solid, and requires the flask to be opened to add it. In our experience this always resulted in lower product yields.

¹H NMR (400 MHz, CDCl₃): δ = 3.30 (s, 12H), 4.59 (bs, 1H), 5.43 (s, 2H), 5.95 (s, 1H), 6.82-6.84 (m, 2H), 7.27-7.29 (m, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.89 (d, J = 8.6 Hz, 2H).

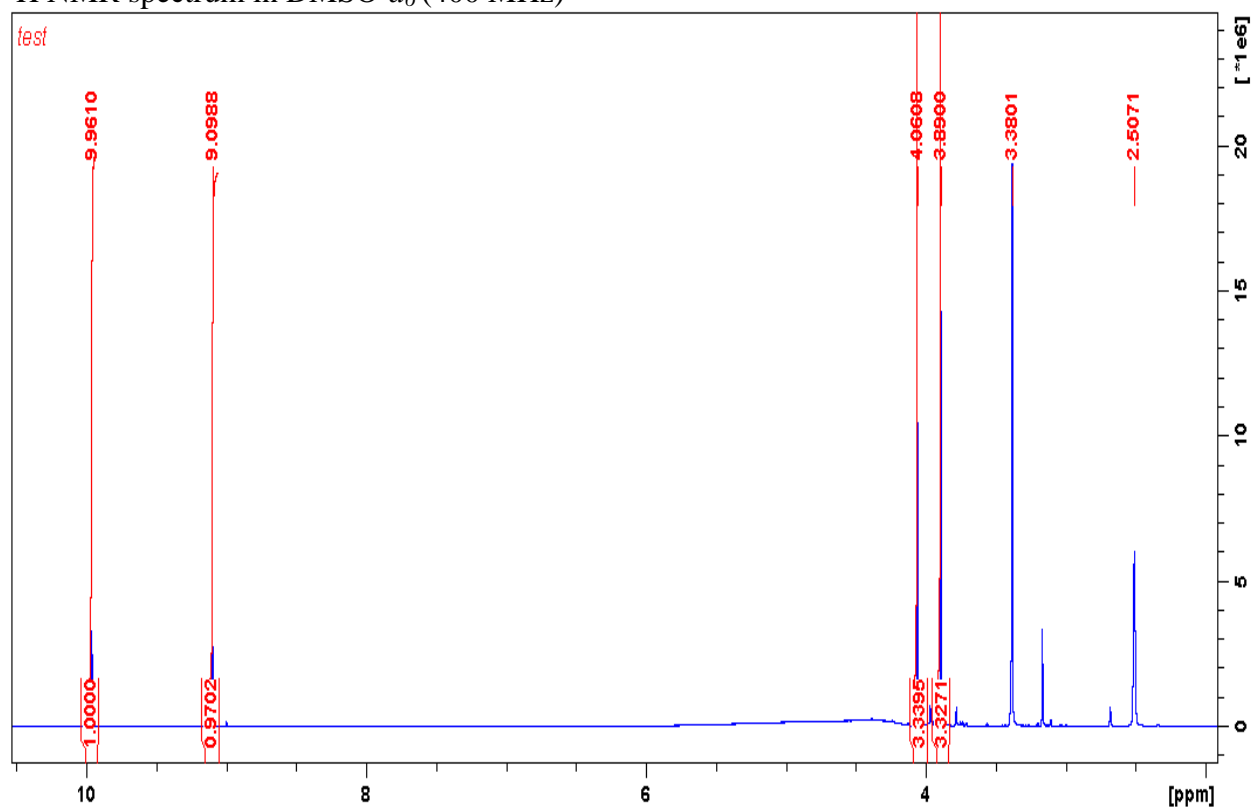
¹³C NMR (100 MHz, CDCl₃): δ = 52.4, 78.6, 103.5, 126.6, 127.3, 127.9, 128.9, 135.6, 135.8, 136.2, 145.1, 198.9.

HRMS: calcd. for [M]⁺ C₂₀H₂₄O₆ requires 360.1573, found 360.1575.

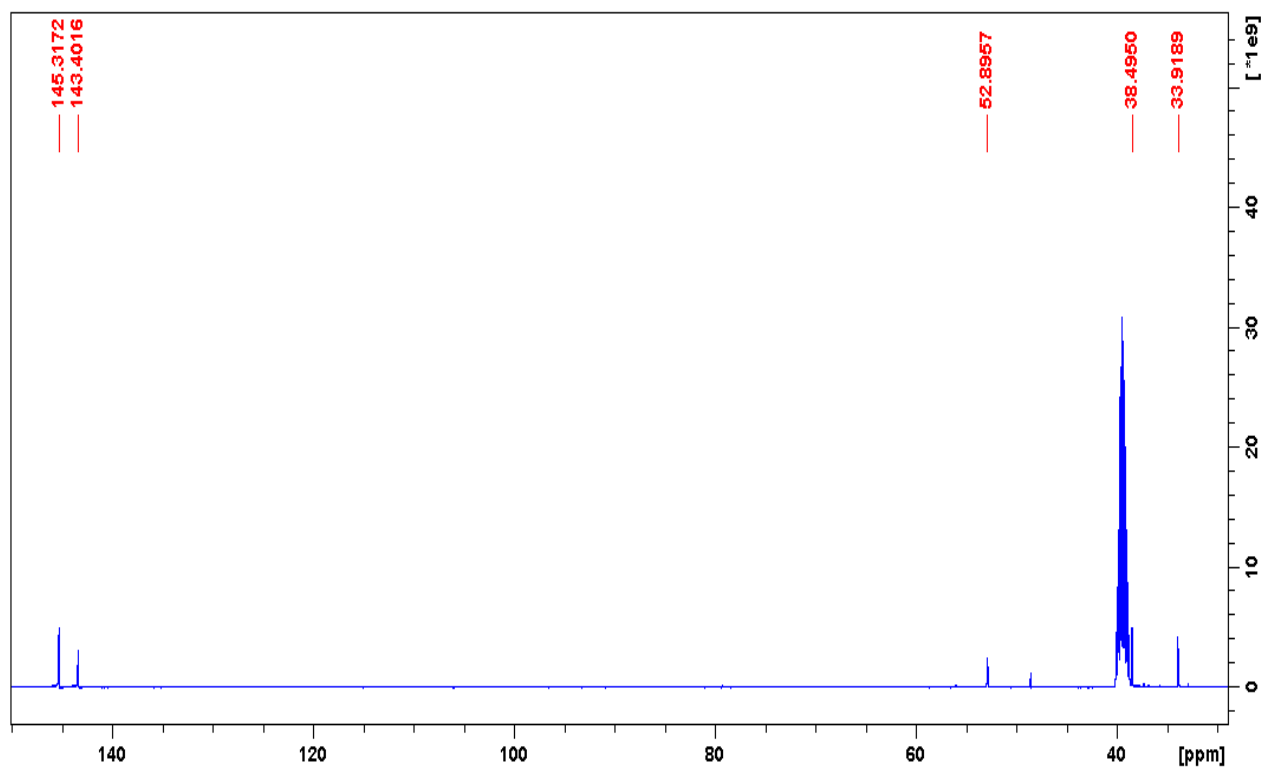
8.0 NMR spectra for catalysts and products

8.1 1,4-dimethyl-4H-1,2,4-triazol-1-ium methyl sulfate (11c)

^1H NMR spectrum in $\text{DMSO-}d_6$ (400 MHz)

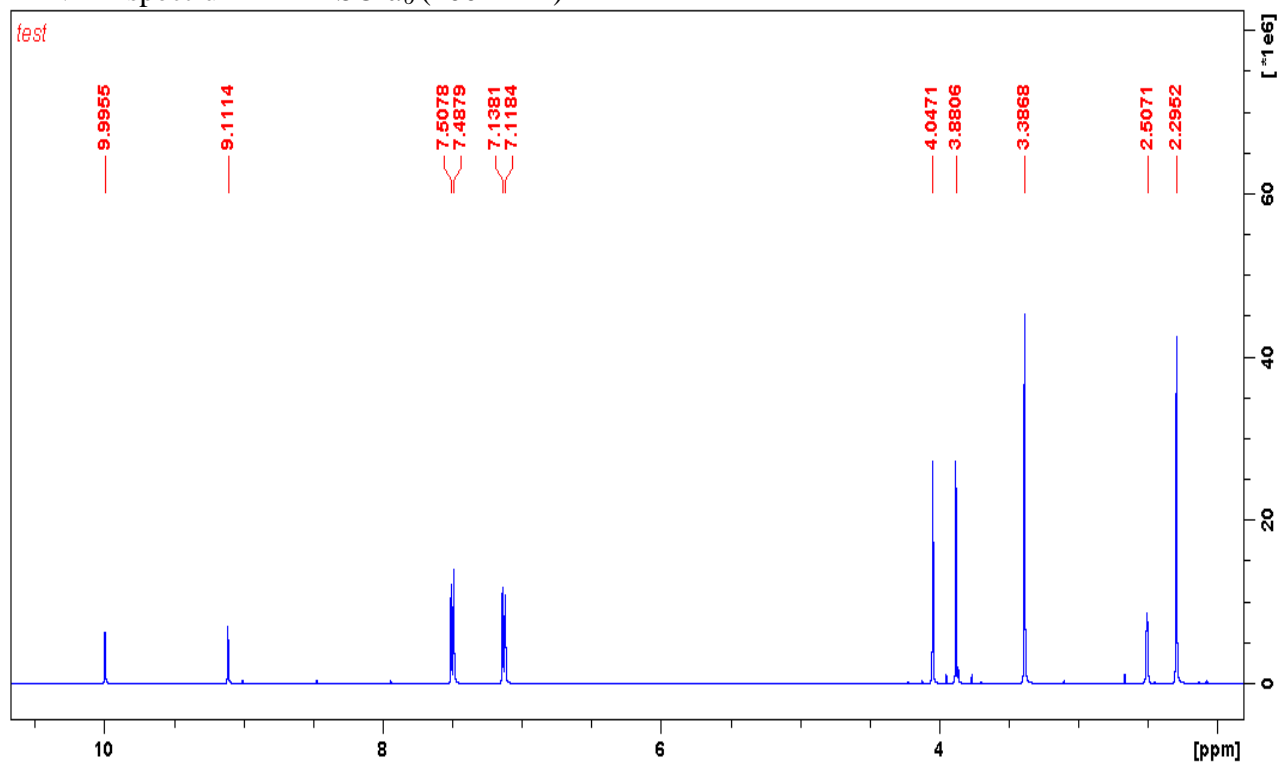


^{13}C NMR spectrum in $\text{DMSO-}d_6$ (100 MHz)

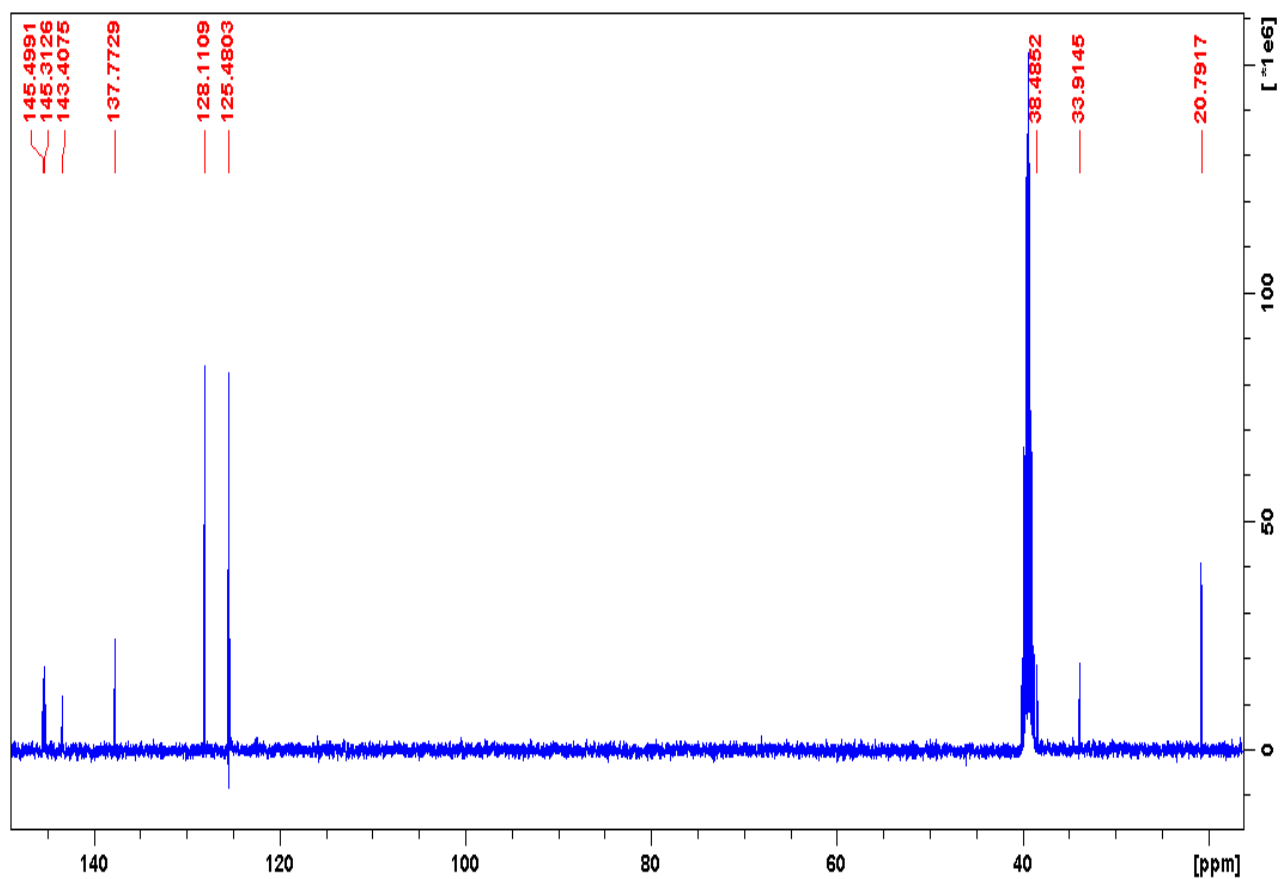


8.2 1,4-dimethyl-4H-1,2,4-triazol-1-ium 4-methylbenzenesulfonate (11f)

¹H NMR spectrum in DMSO-*d*₆ (400 MHz)

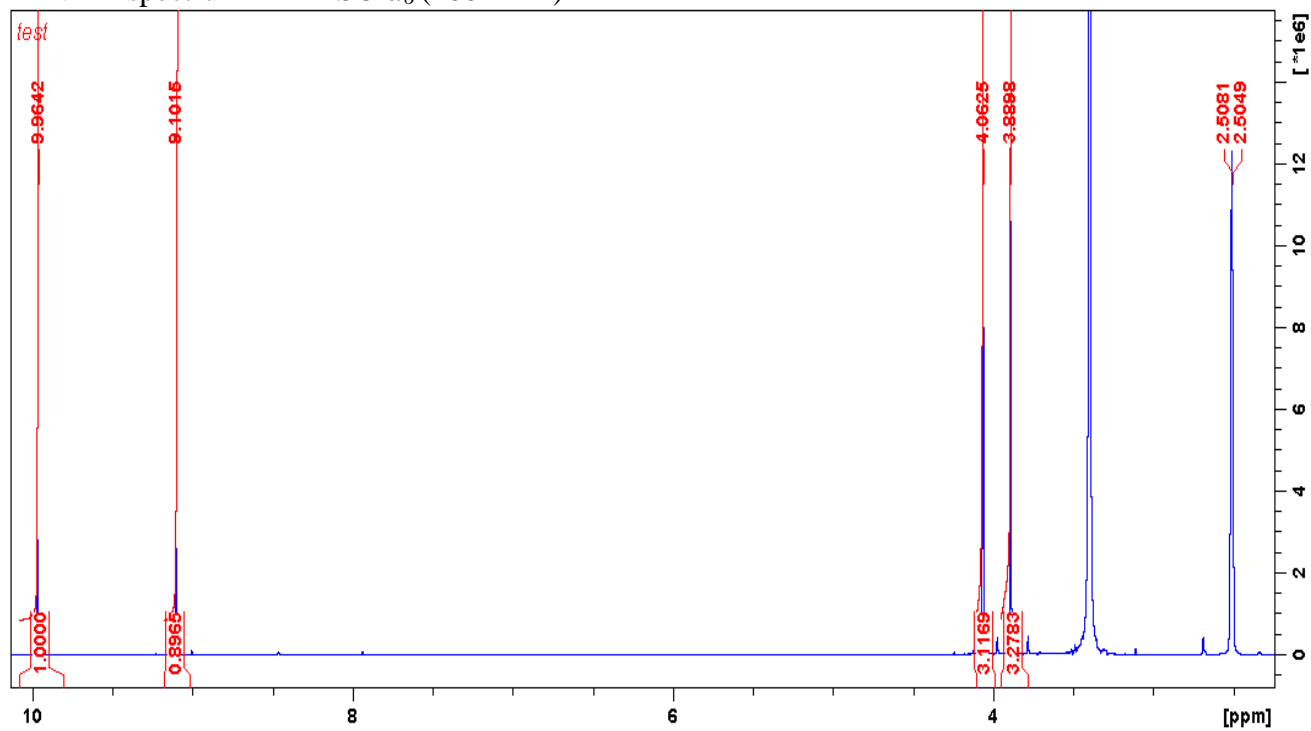


¹³C NMR spectrum in DMSO-*d*₆ (100 MHz)



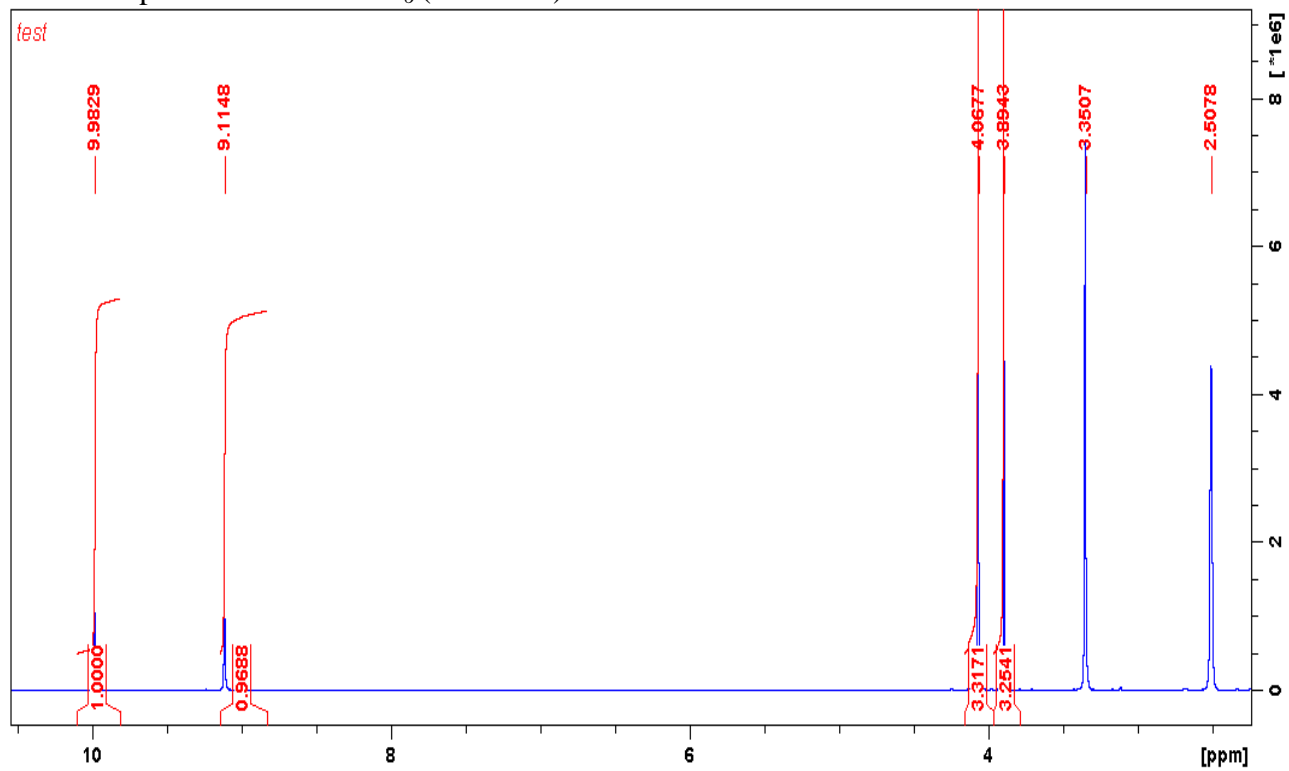
8.3 1,4-Dimethyl-4H-1,2,4-triazol-1-ium iodide (11h)

¹H NMR spectrum in DMSO-*d*₆ (400 MHz)

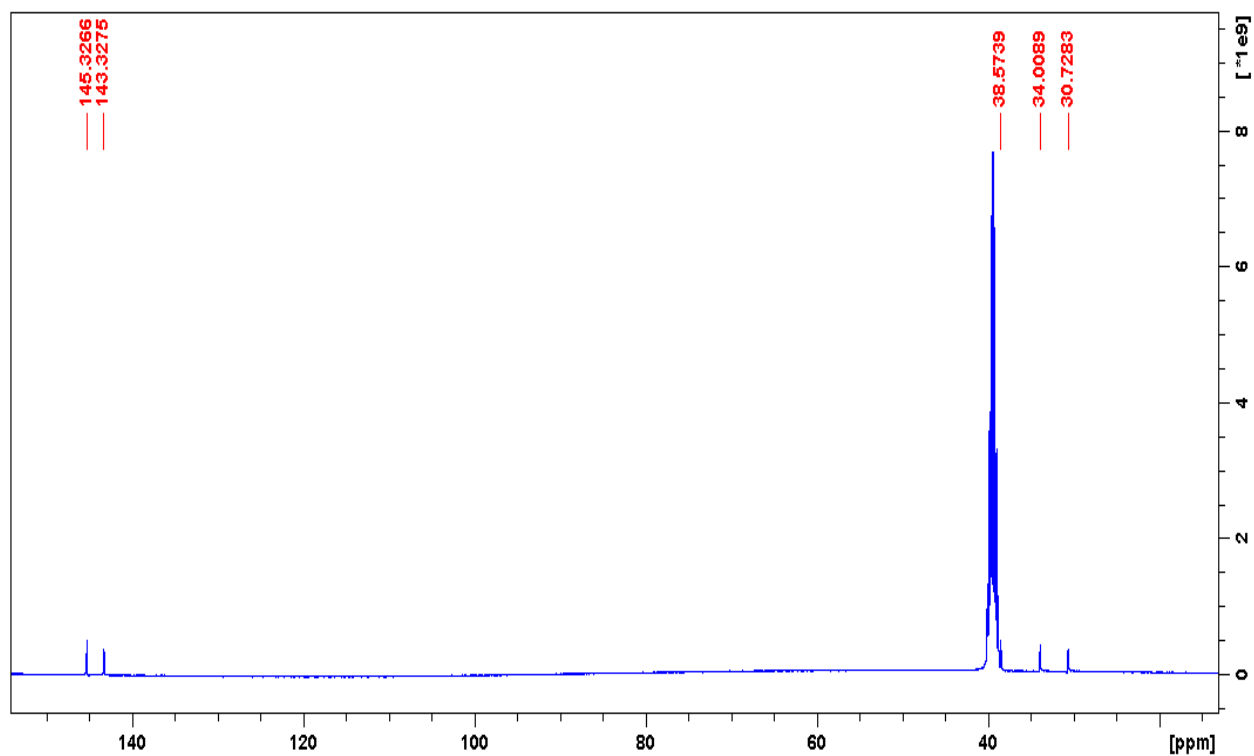


8.4 1, 4-dimethyl-4H-1,2,4-triazol-1-ium perchlorate (11a)

^1H NMR spectrum in $\text{DMSO-}d_6$ (400 MHz)

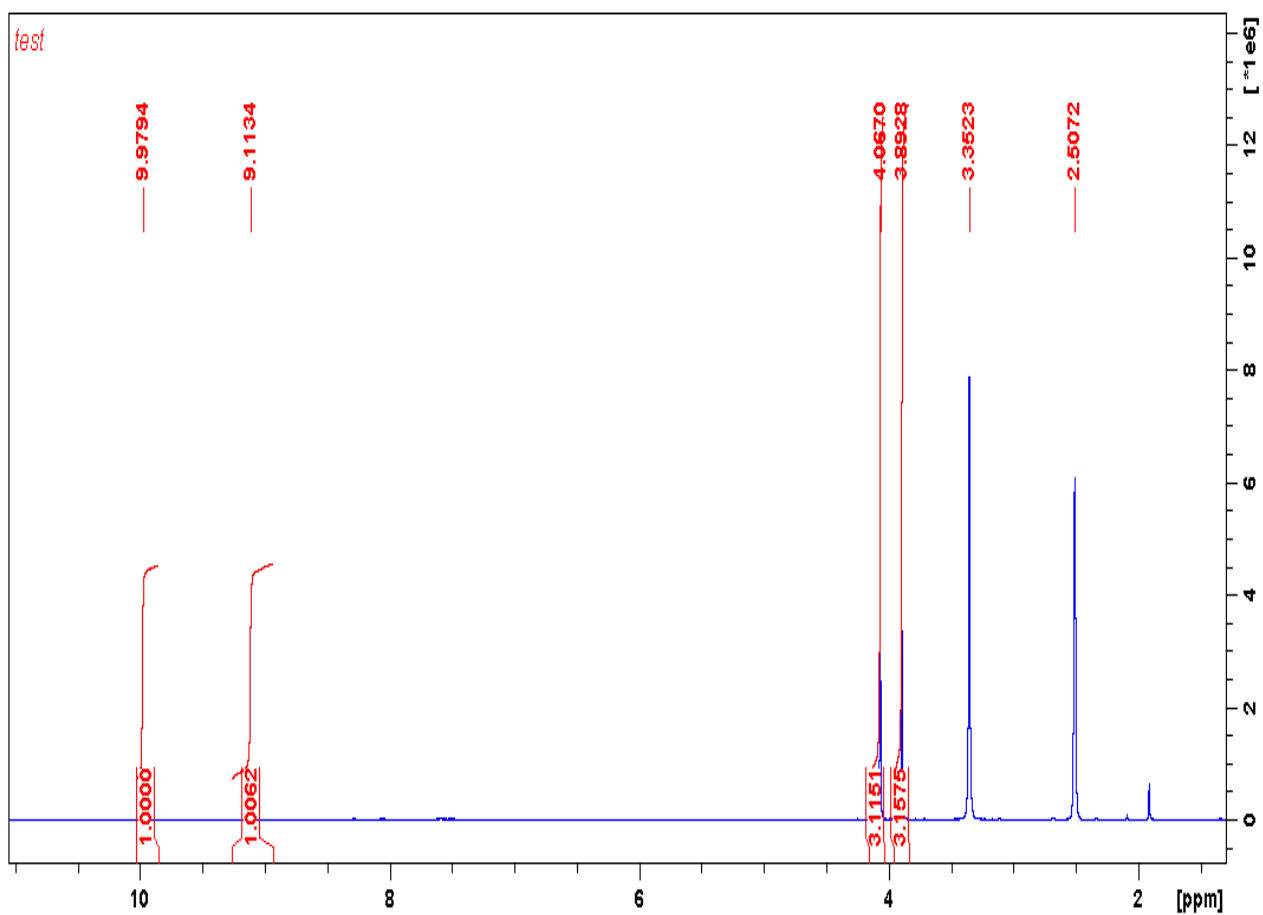


^{13}C NMR spectrum in $\text{DMSO-}d_6$ (100 MHz)

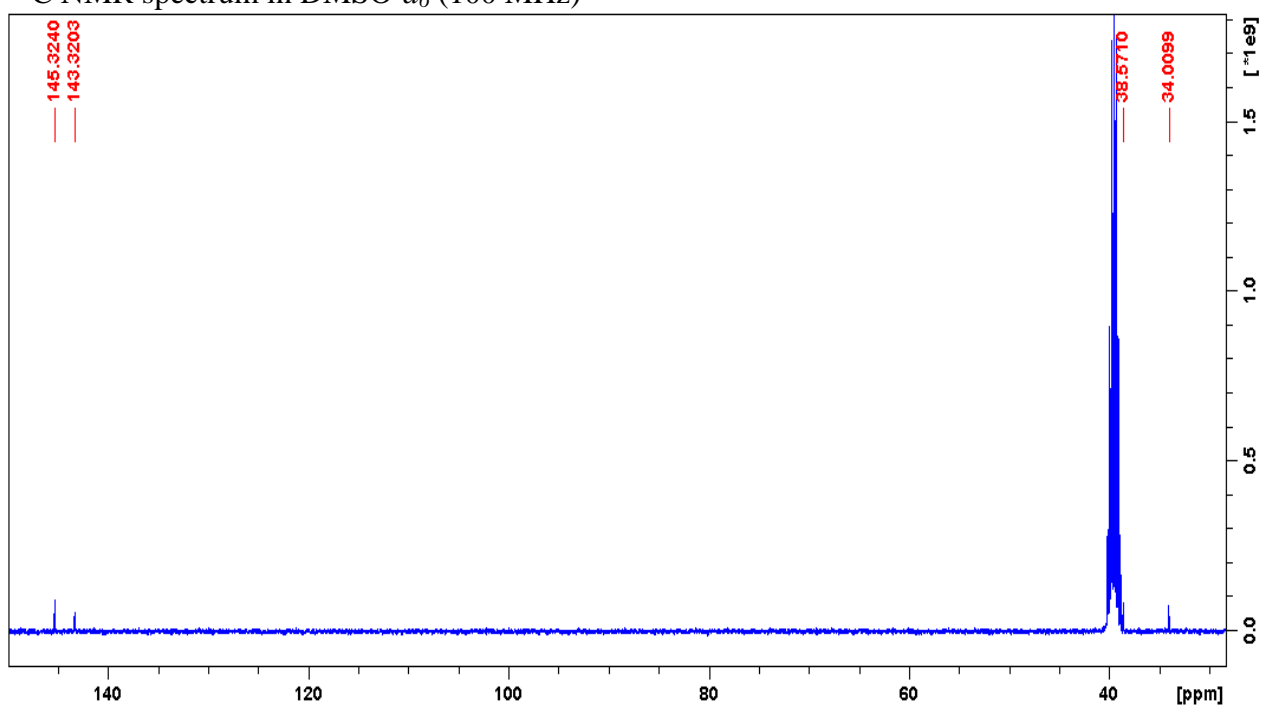


8.5 1,4-dimethyl-4H-1,2,4-triazol-1-ium acetate (11b)

¹H NMR spectrum in DMSO-*d*₆ (400 MHz)

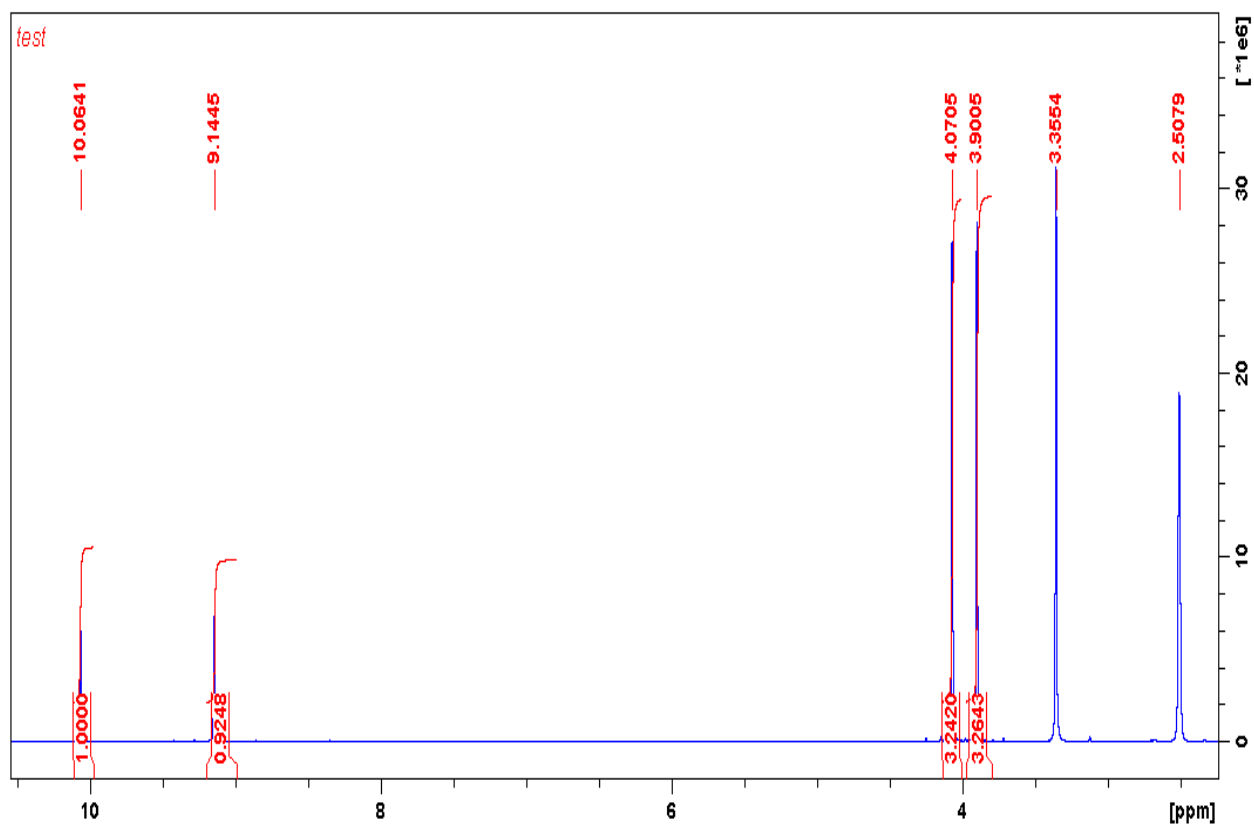


^{13}C NMR spectrum in $\text{DMSO-}d_6$ (100 MHz)

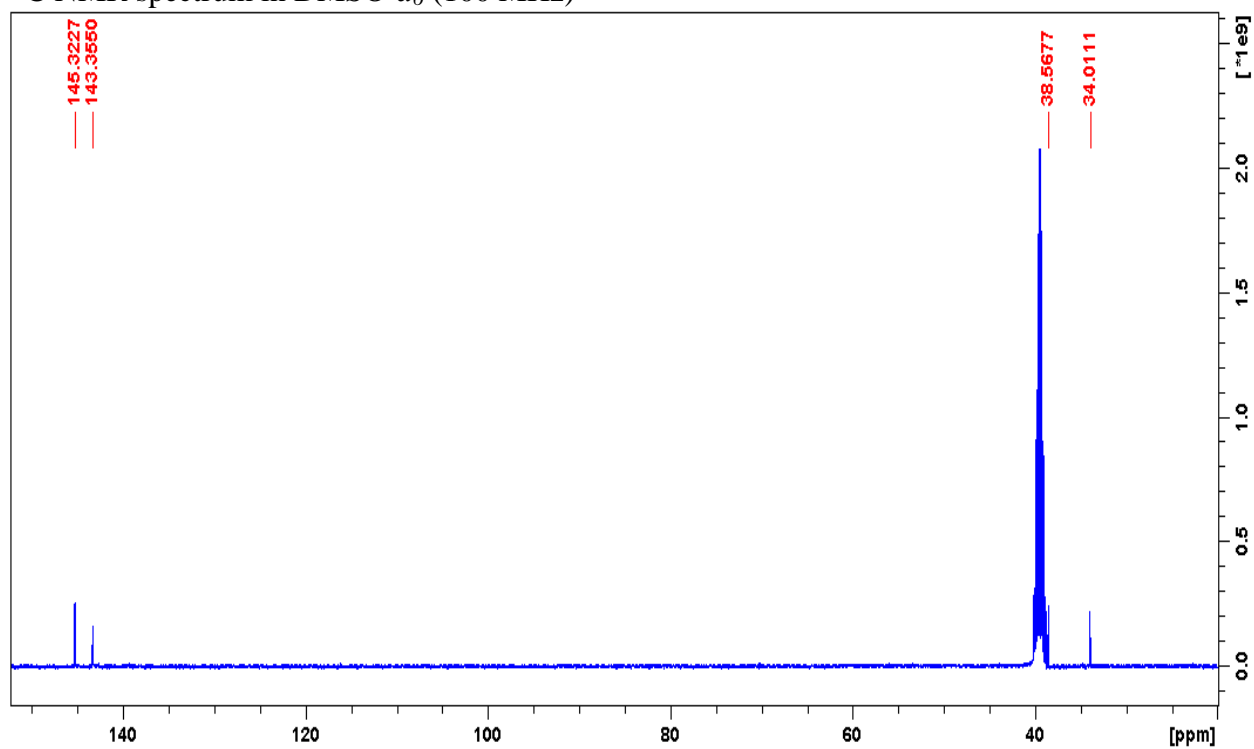


8.6 1, 4-dimethyl-4H-1,2,4-triazol-1-ium chloride (11e)

^1H NMR spectrum in $\text{DMSO-}d_6$ (400 MHz)

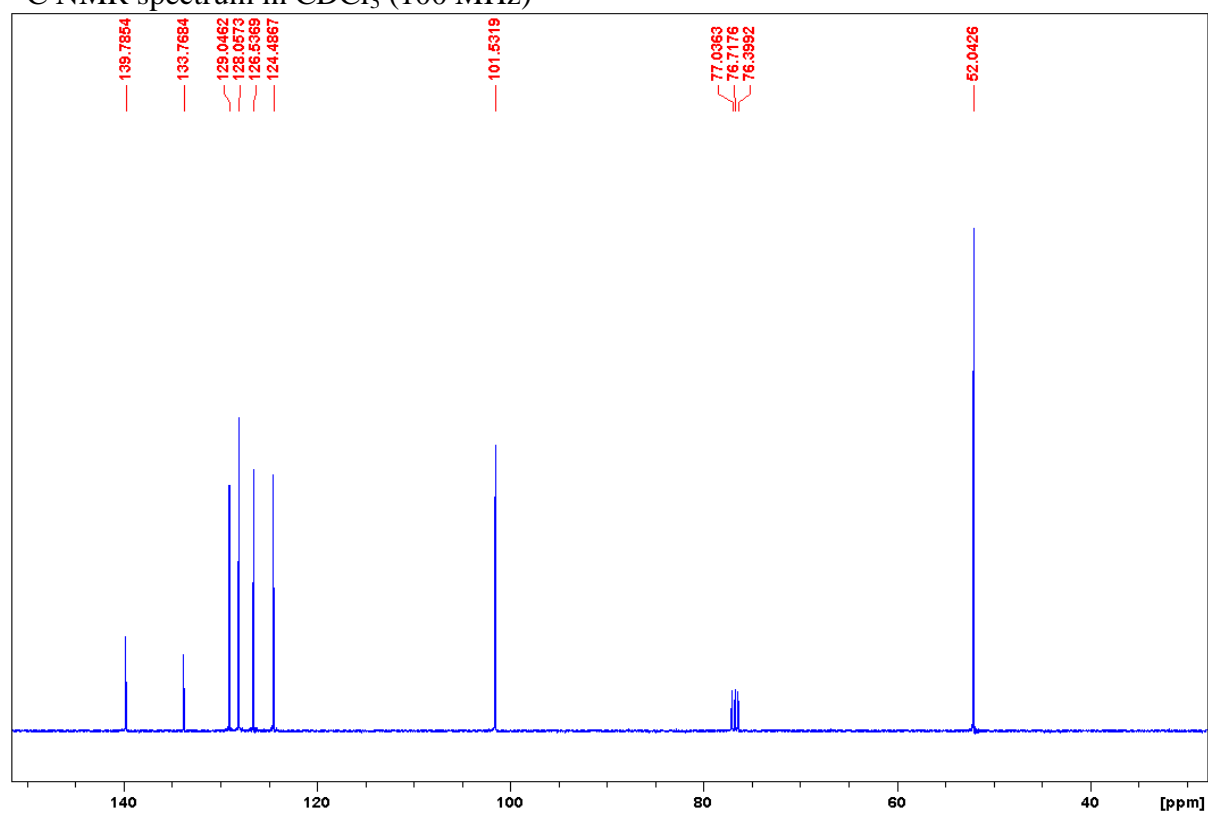
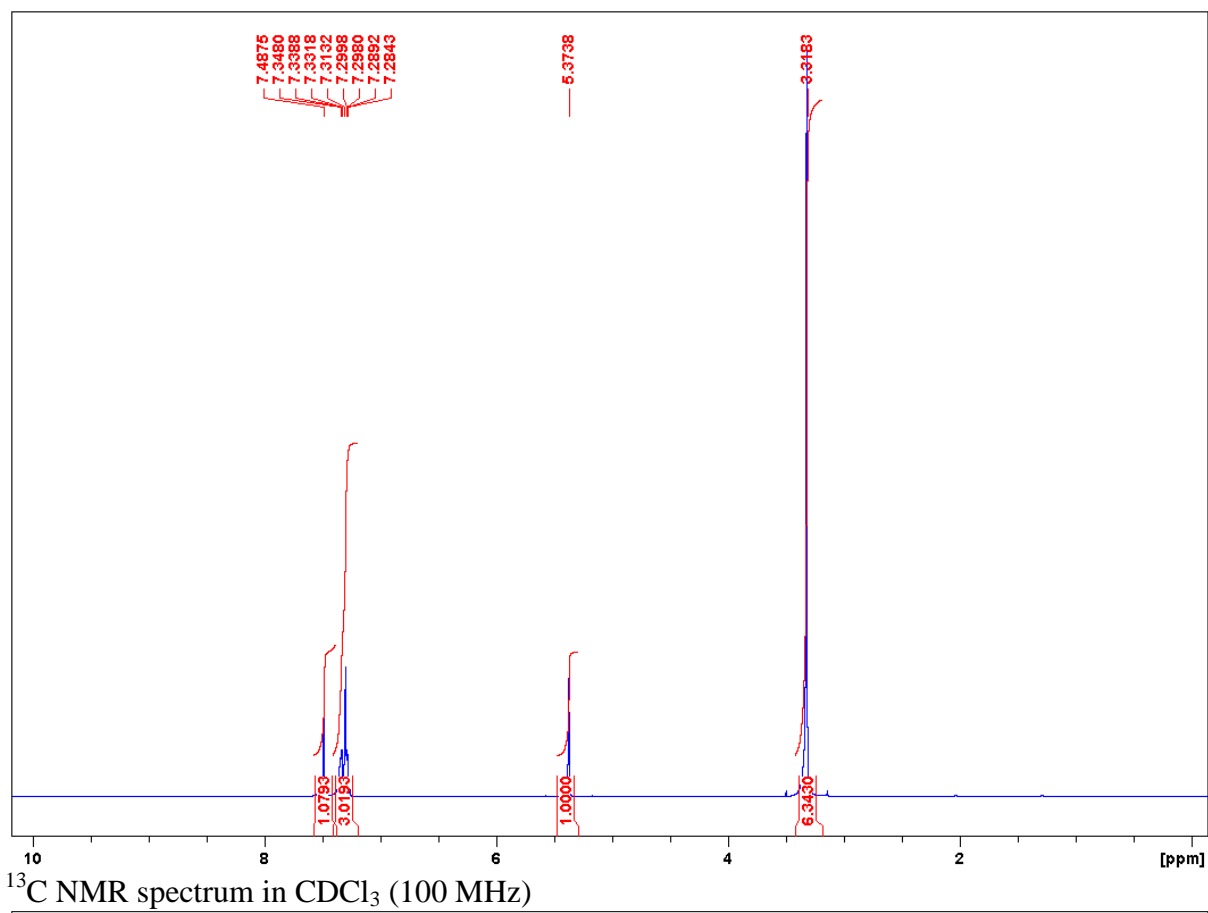


^{13}C NMR spectrum in $\text{DMSO-}d_6$ (100 MHz)



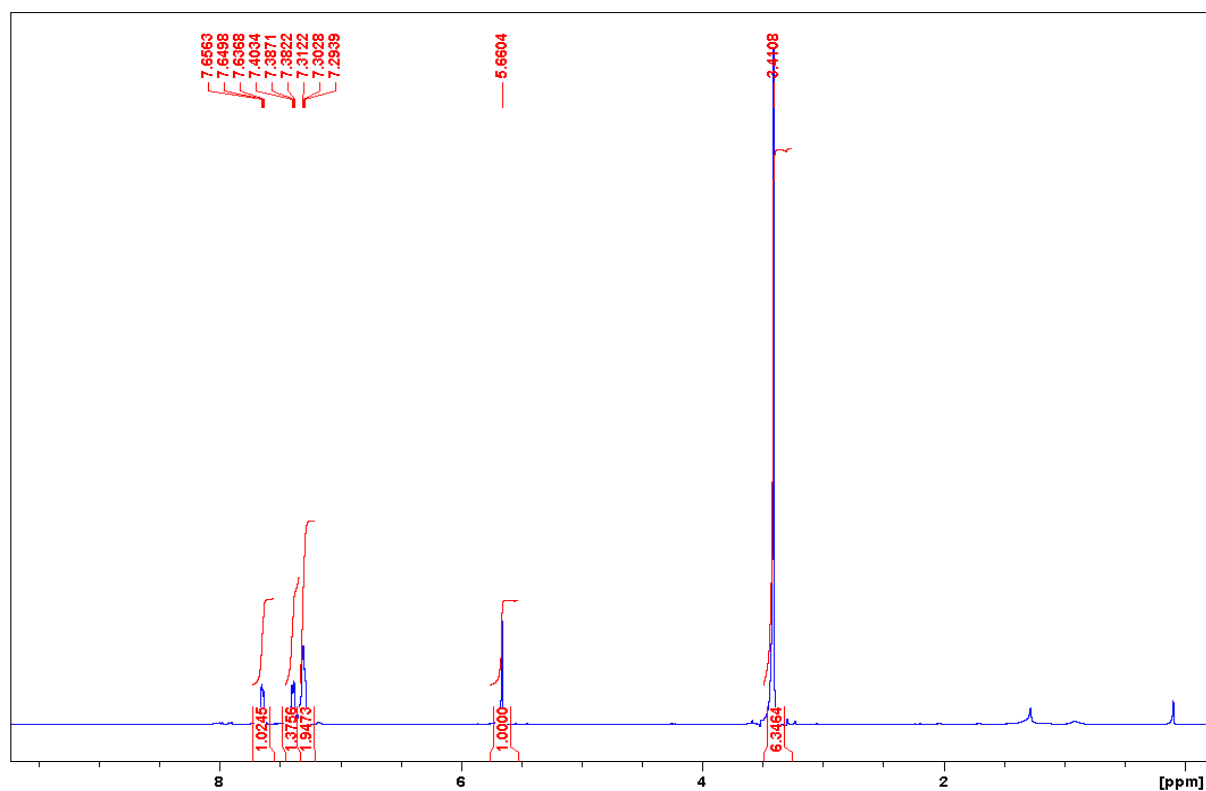
8.7 2-Chlorobenzaldehyde dimethyl acetal (14a)

^1H NMR spectrum in CDCl_3 (400 MHz)

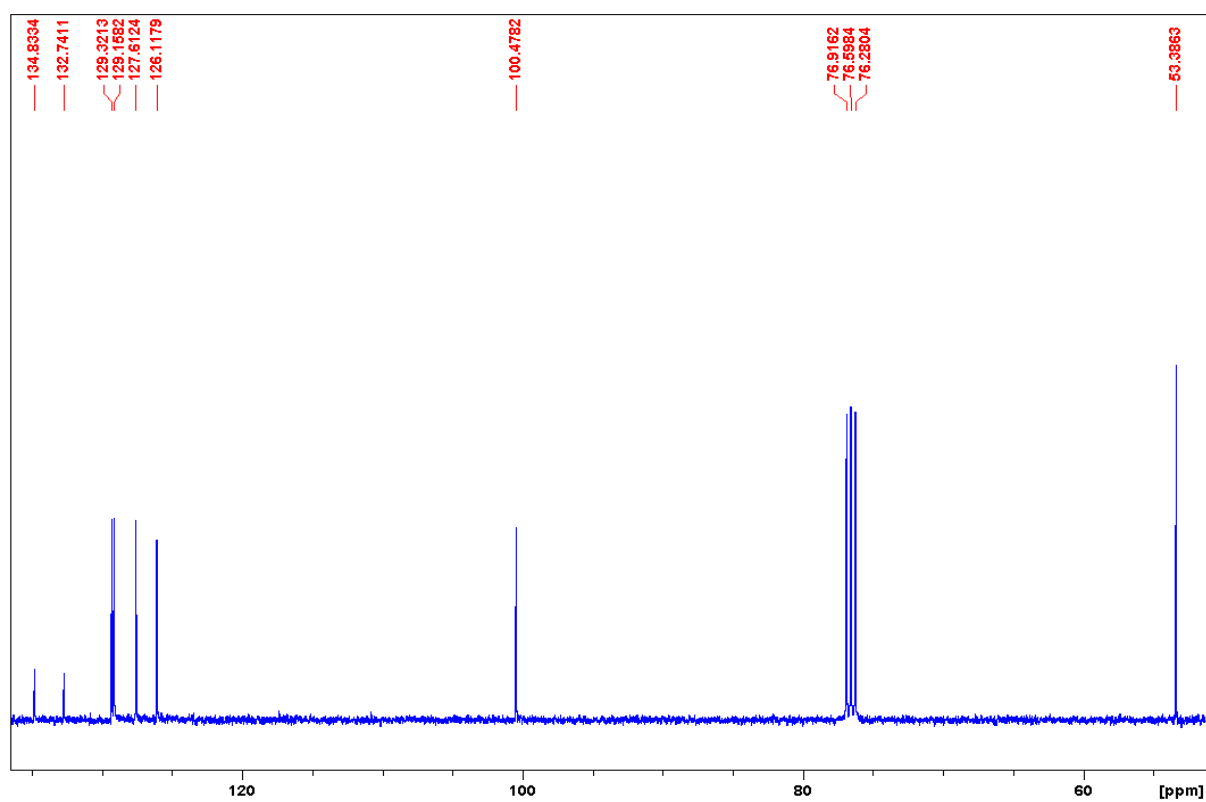


8.8 3-Chlorobenzaldehyde dimethyl acetal (14b)

^1H NMR Spectrum in CDCl_3 (400 MHz)

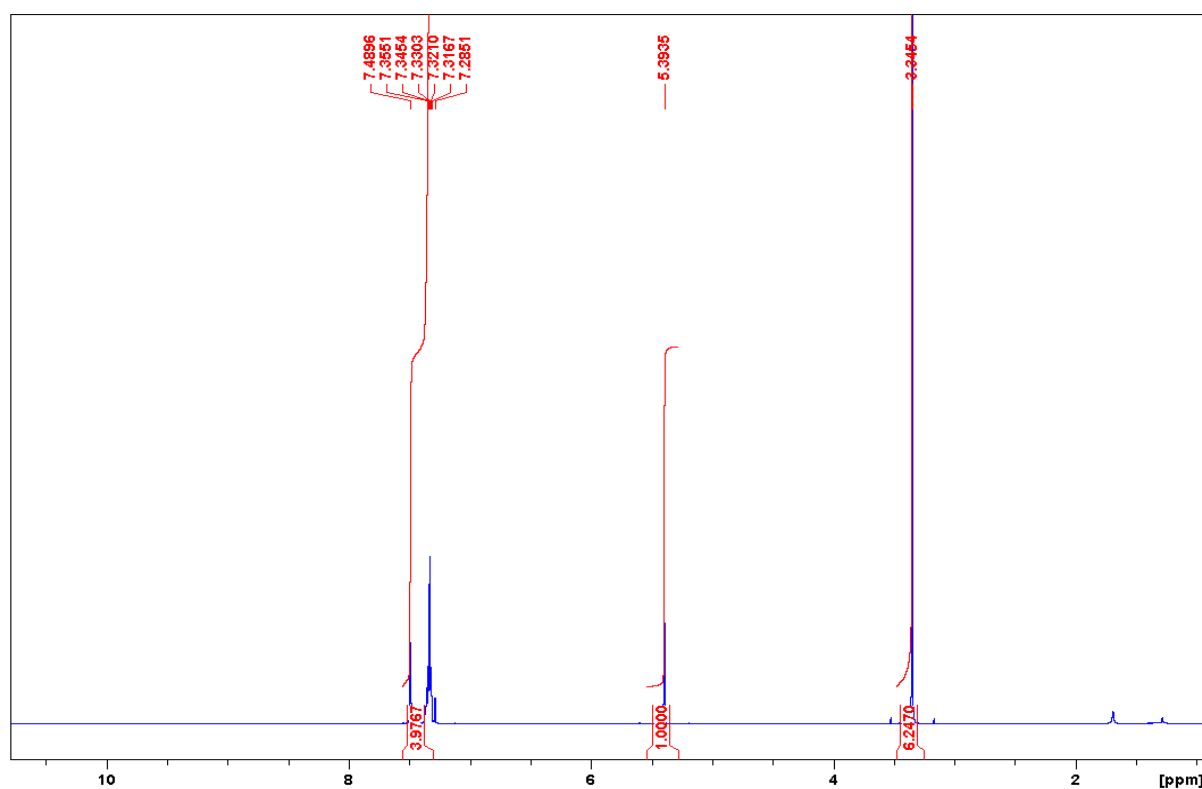


^{13}C NMR spectrum in CDCl_3 (100 MHz)

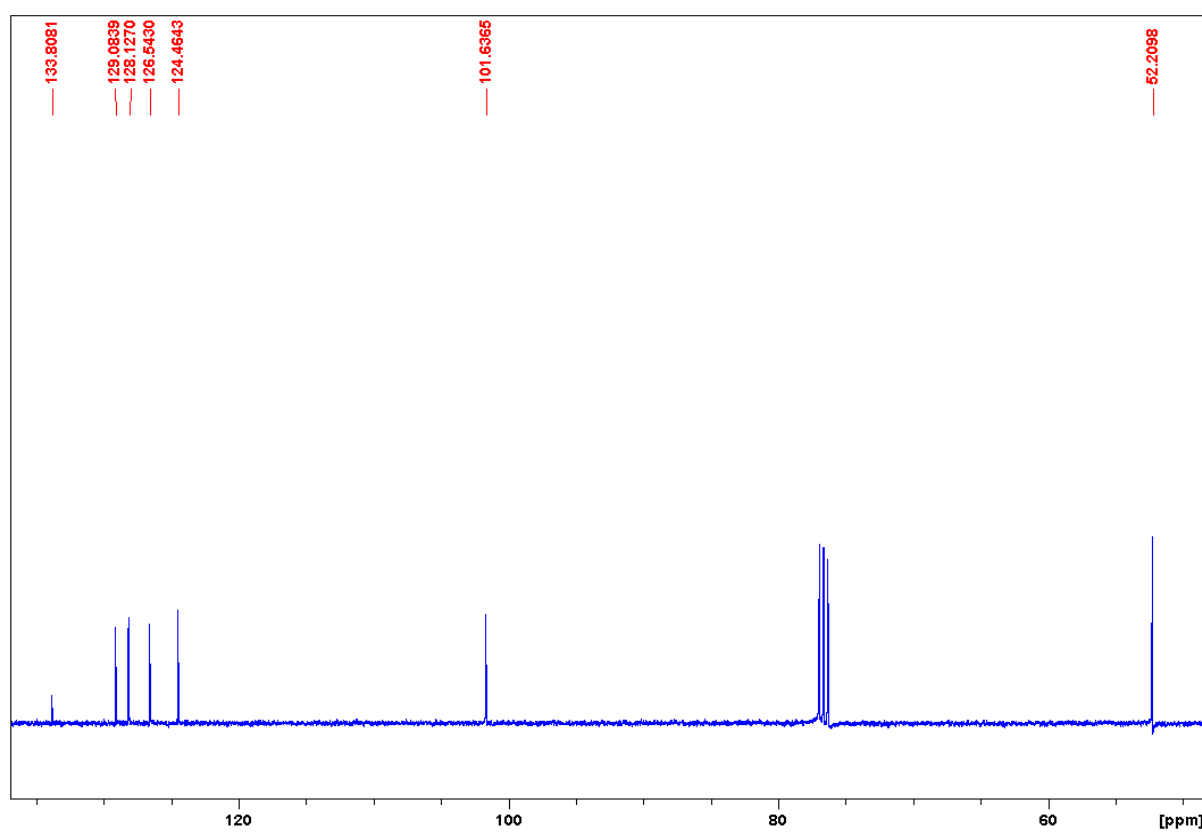


8.9 4-chlorobenzaldehyde dimethyl acetal (14c)

^1H NMR spectrum in CDCl_3 (400 MHz)

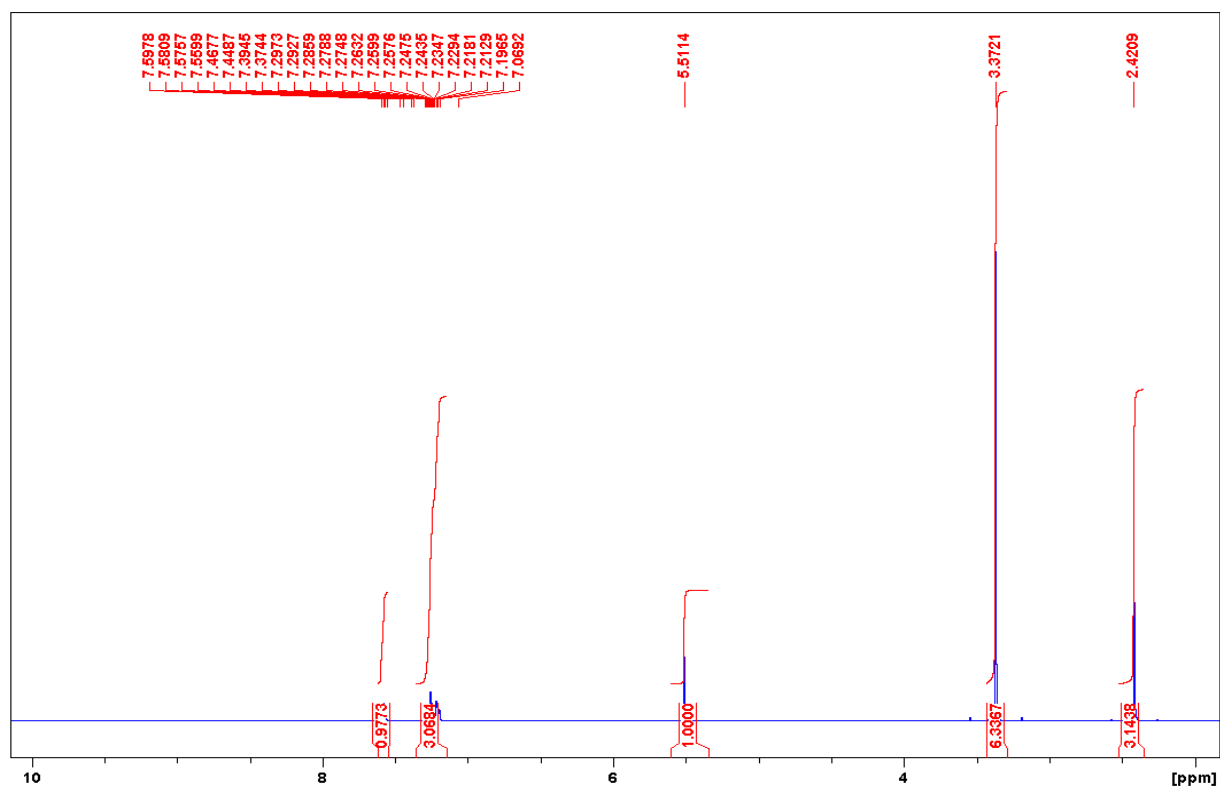


^{13}C NMR spectrum in CDCl_3 (100 MHz)

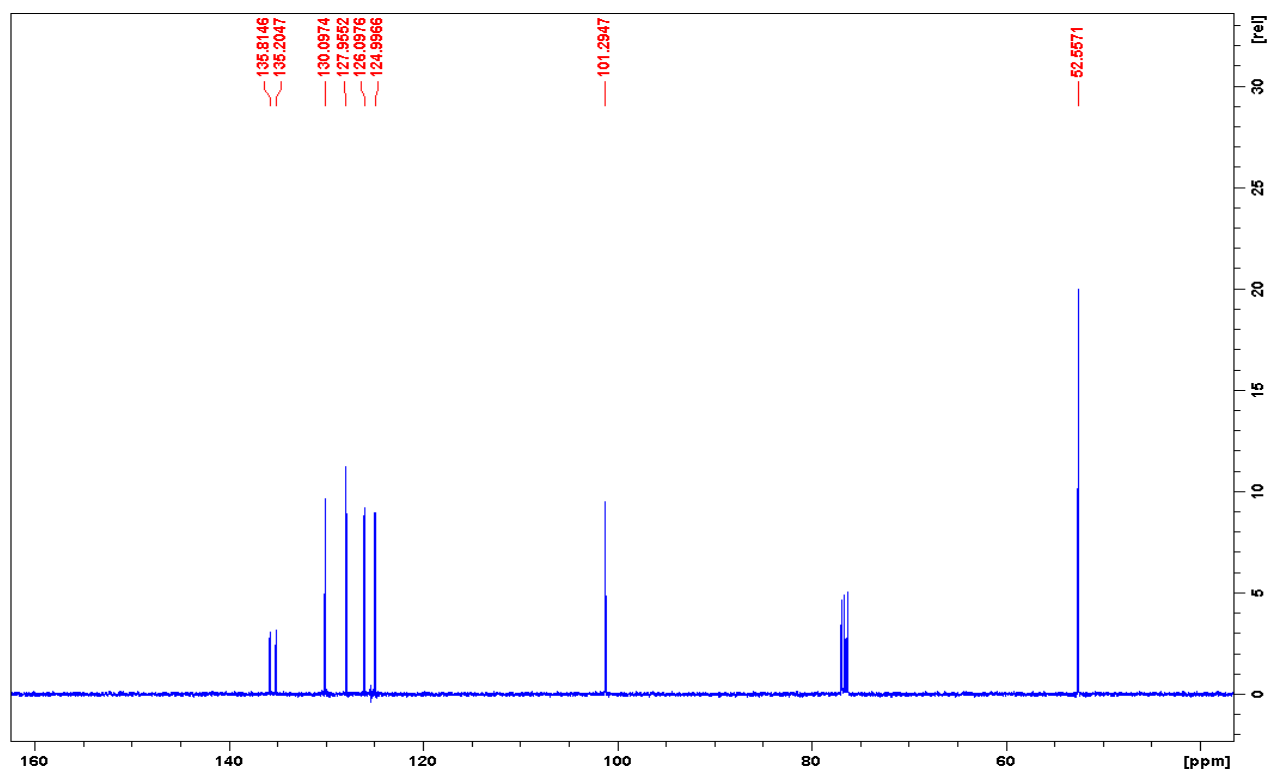


8.10 2-Methylbenzaldehyde dimethyl acetal (14d)

^1H NMR spectrum in CDCl_3 (400 MHz)

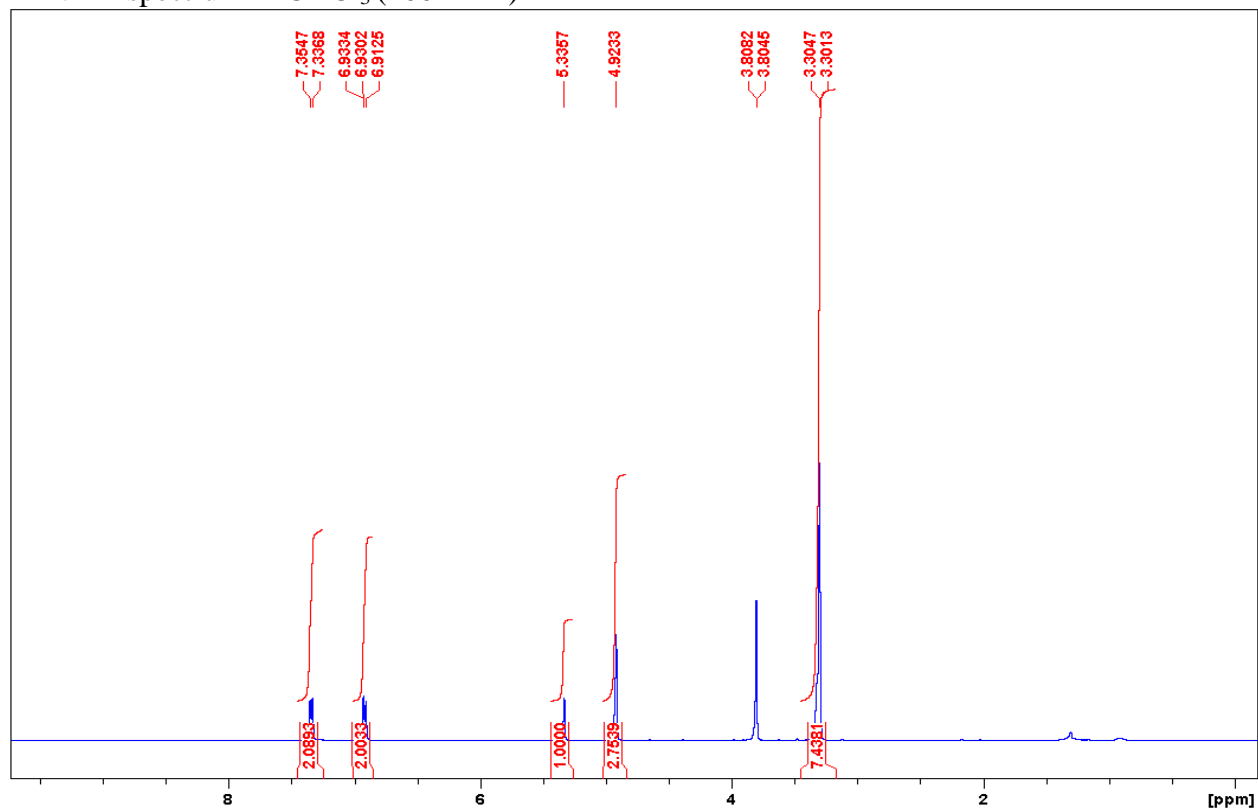


^{13}C NMR spectrum in CDCl_3 (100 MHz)

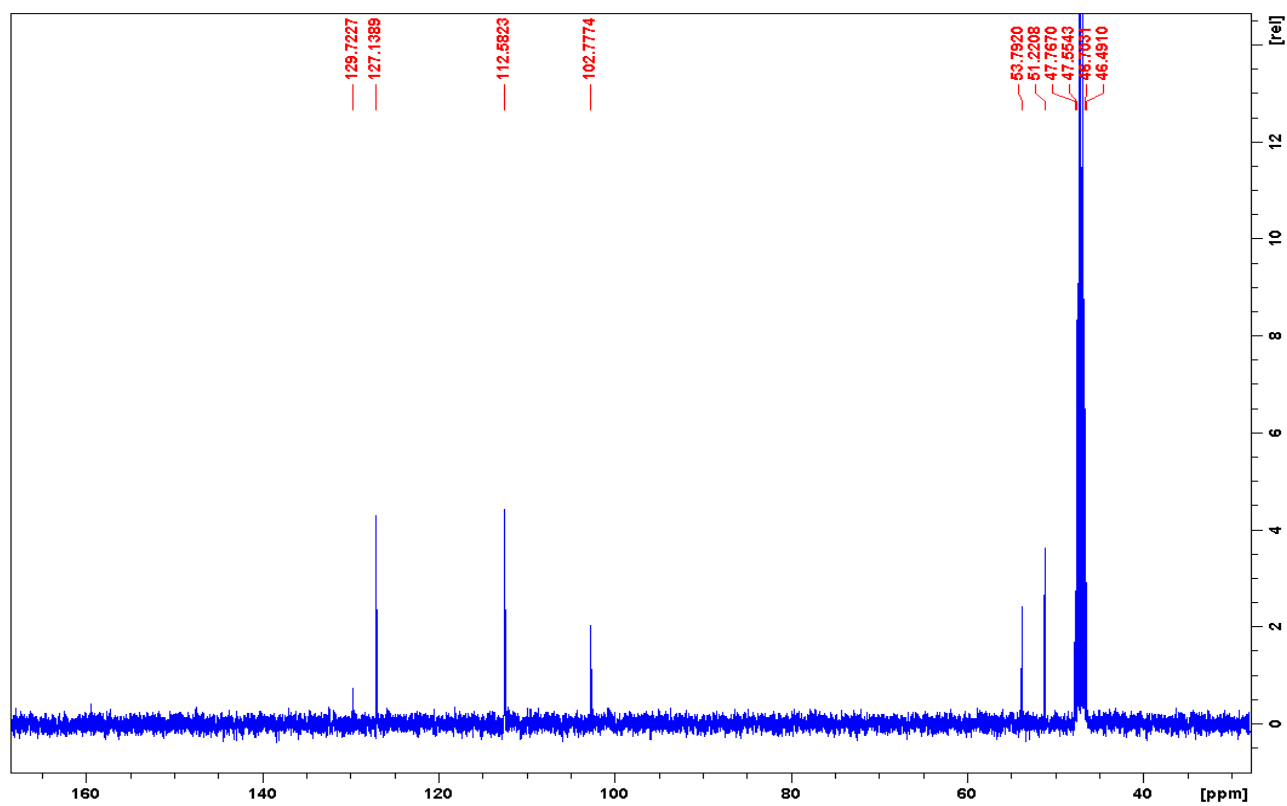


8.11 4-Methoxybenzaldehyde dimethyl acetal (14e)

^1H NMR spectrum in CDCl_3 (400 MHz)

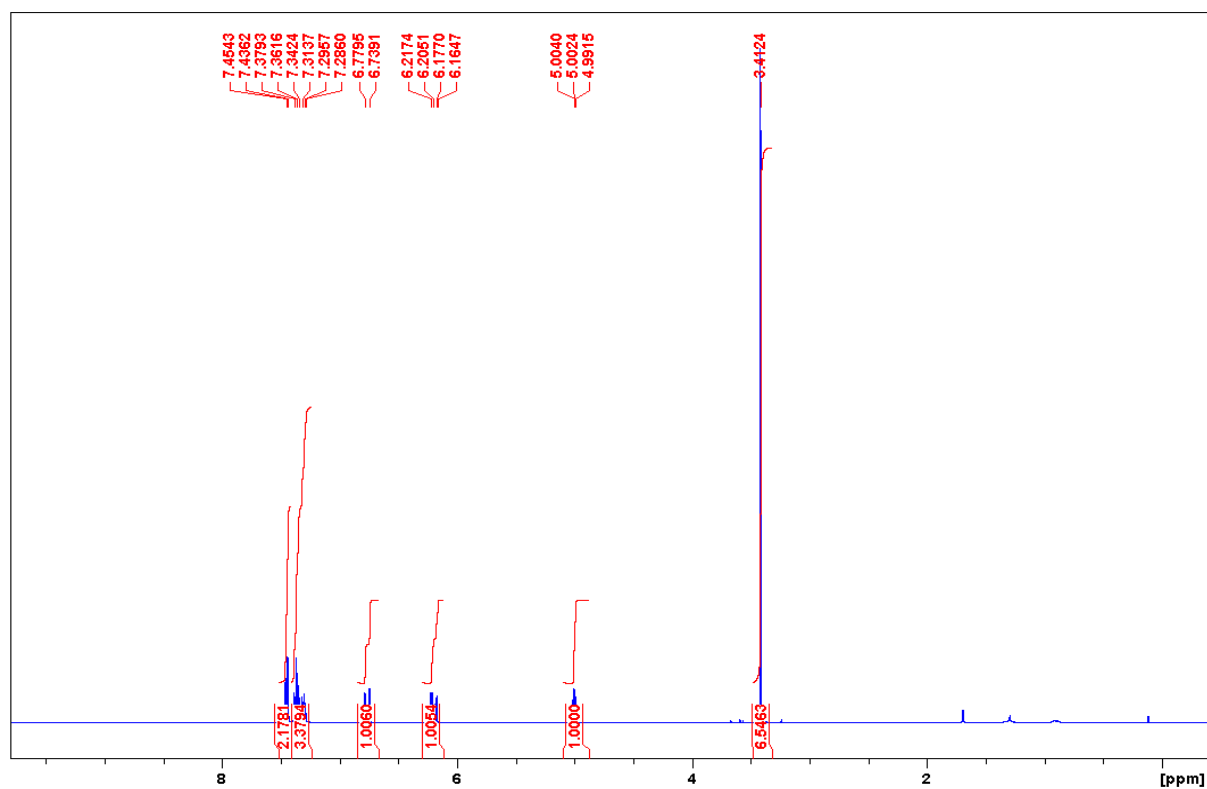


^{13}C NMR spectrum in CDCl_3 (100 MHz)

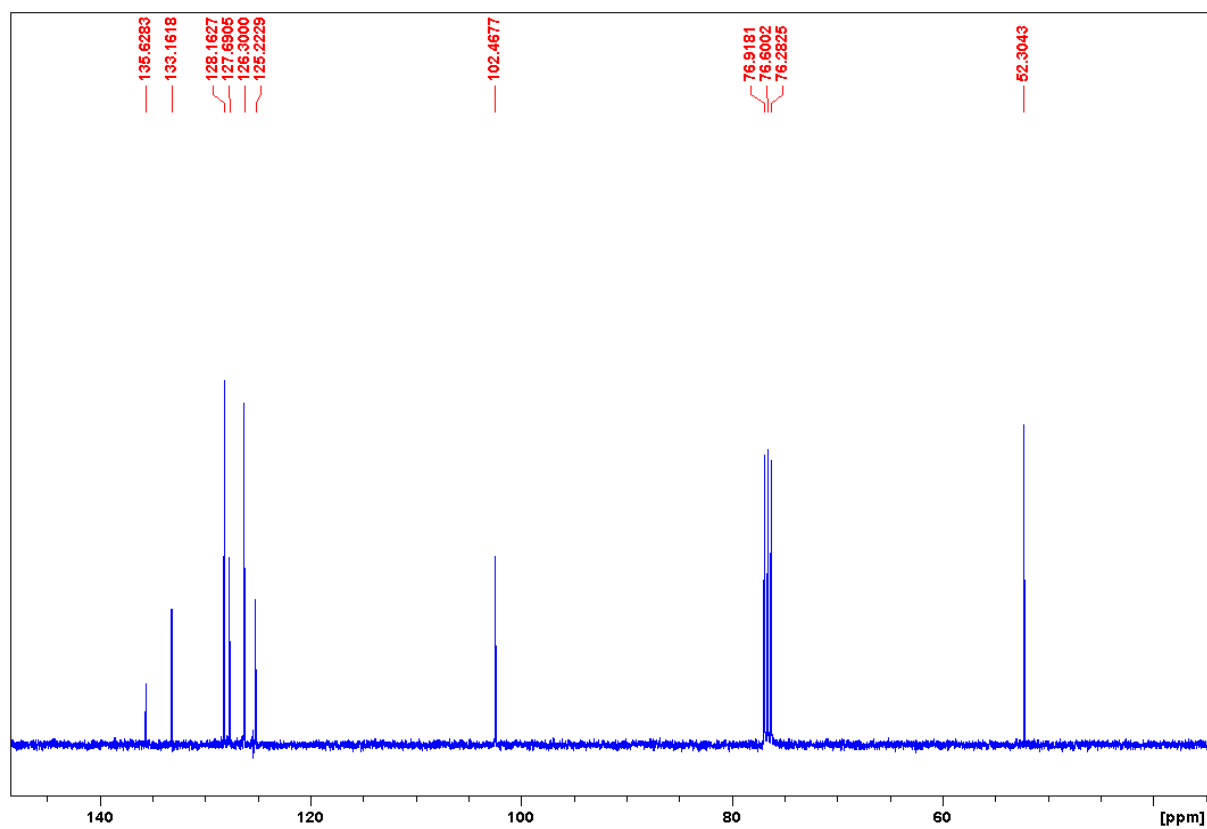


8.12 3,3-Dimethoxypropenyl benzene (14g)

^1H NMR spectrum in CDCl_3 (400 MHz)

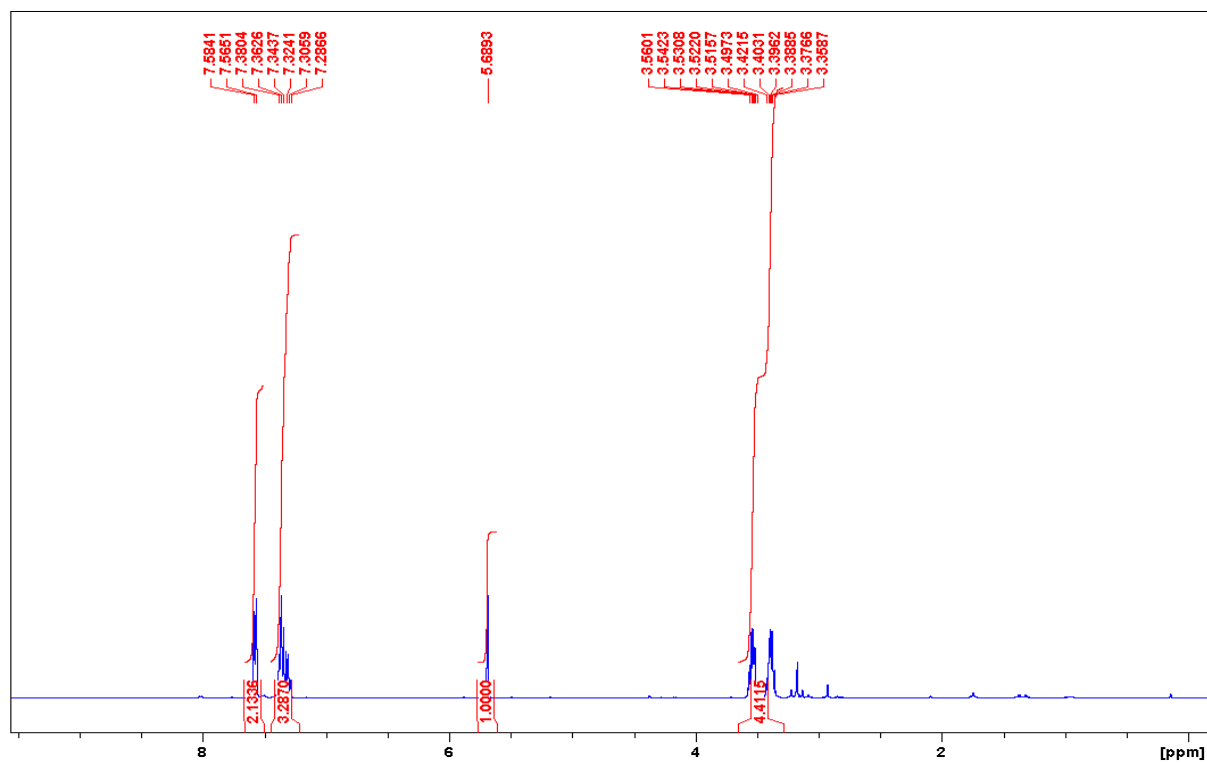


^{13}C NMR spectrum in CDCl_3 (100 MHz)

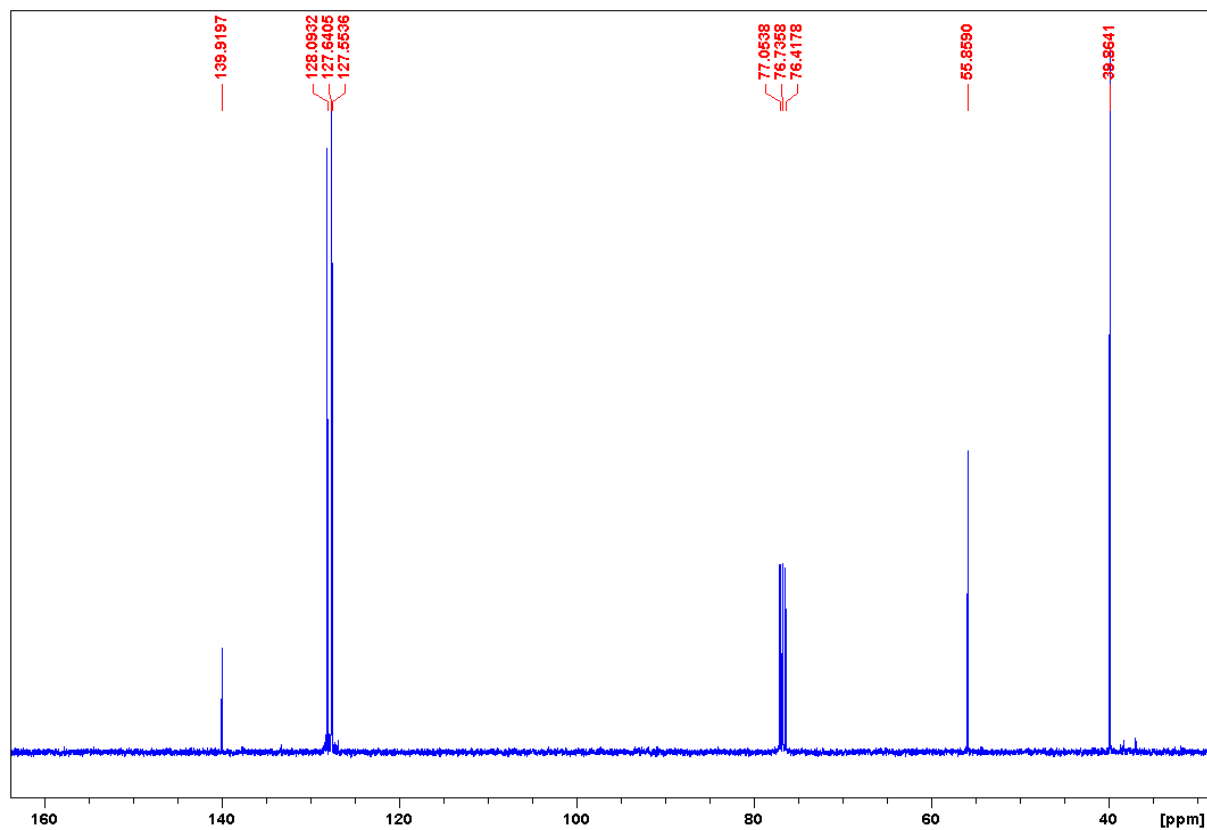


8.13 Phenyl-1,3-dithiolane (17)

^1H NMR spectrum in CDCl_3 (400 MHz)

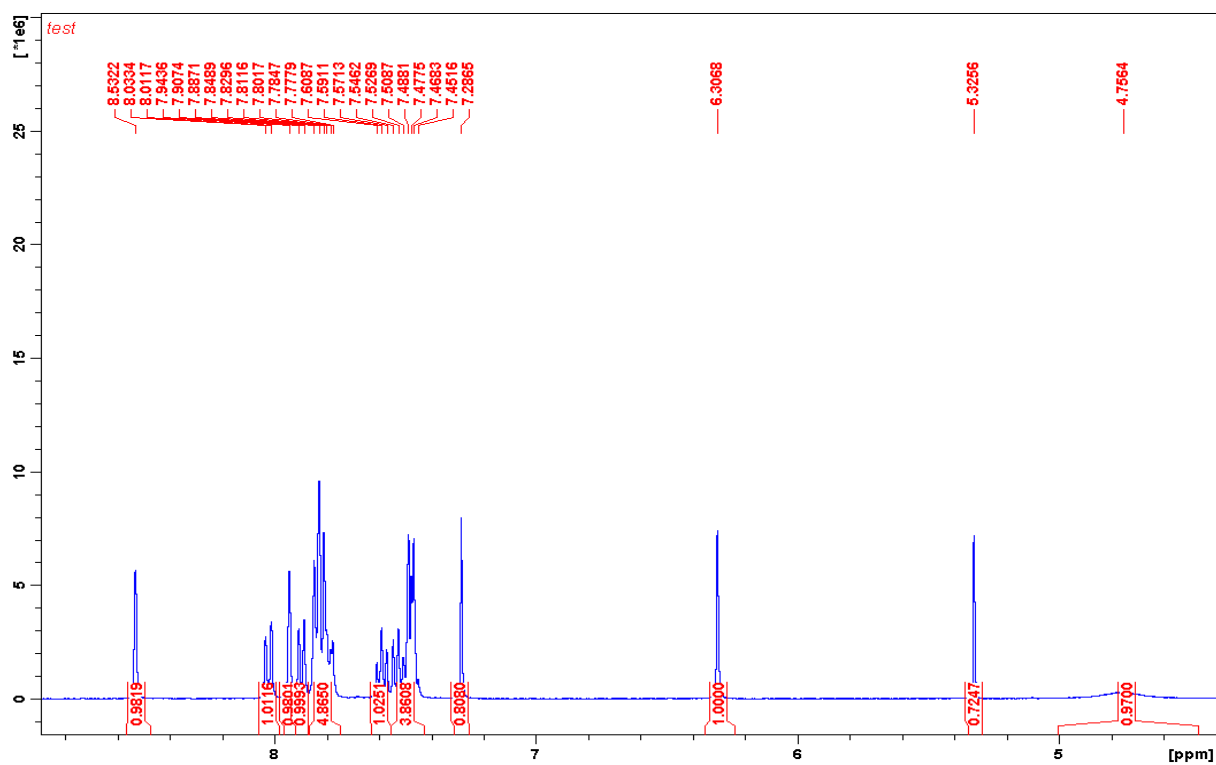


¹³C NMR spectrum in CDCl₃ (100 MHz)



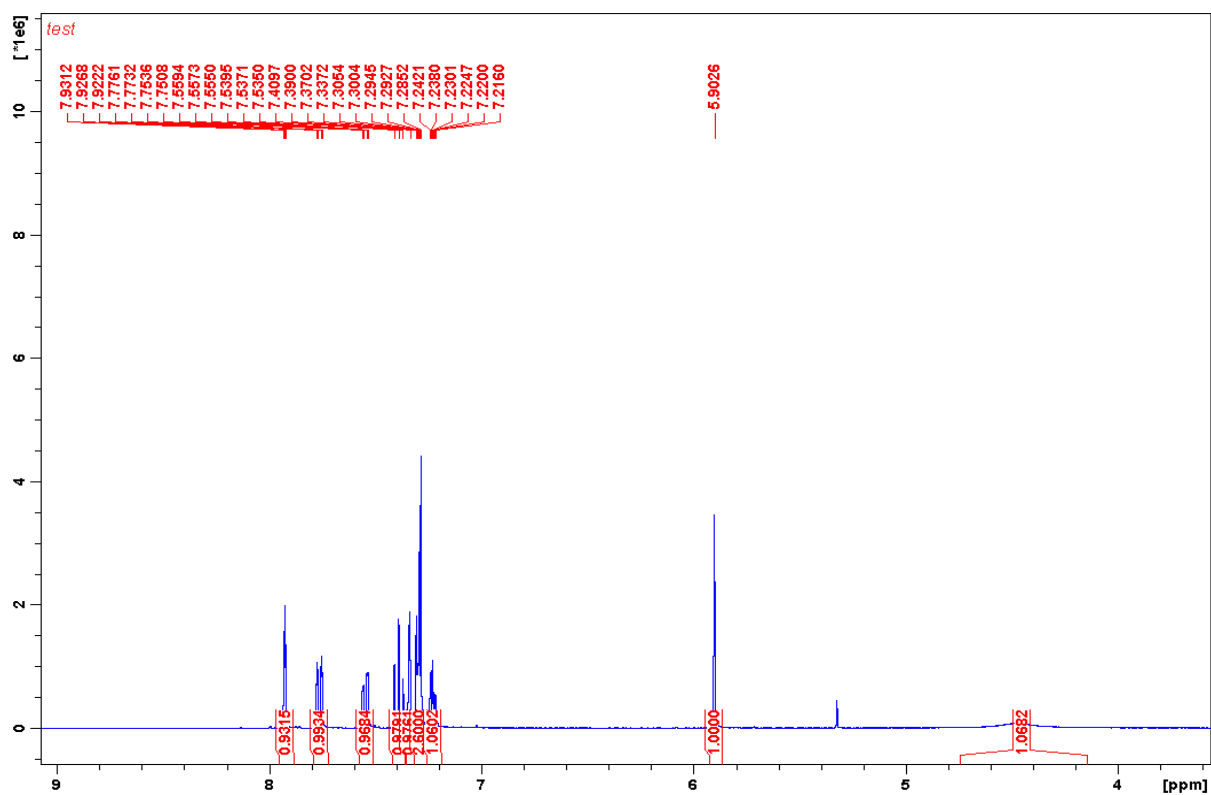
8.14 2-hydroxy-1,2-di(naphthalen-2-yl)ethanone (15b)

¹H NMR spectrum in CDCl₃ (400 MHz)



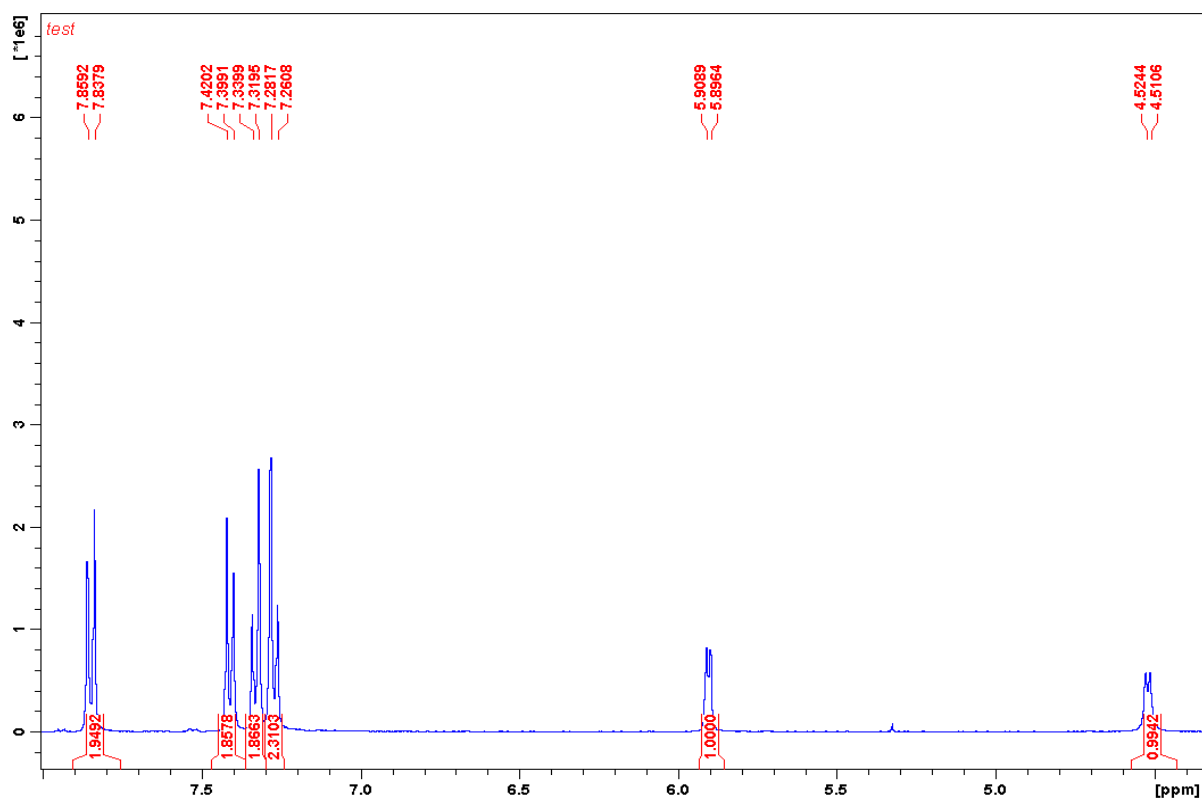
8.17 1,2-bis(3-chlorophenyl)-2-hydroxyethanone (15d)

¹H NMR spectrum in CDCl₃ (400 MHz)



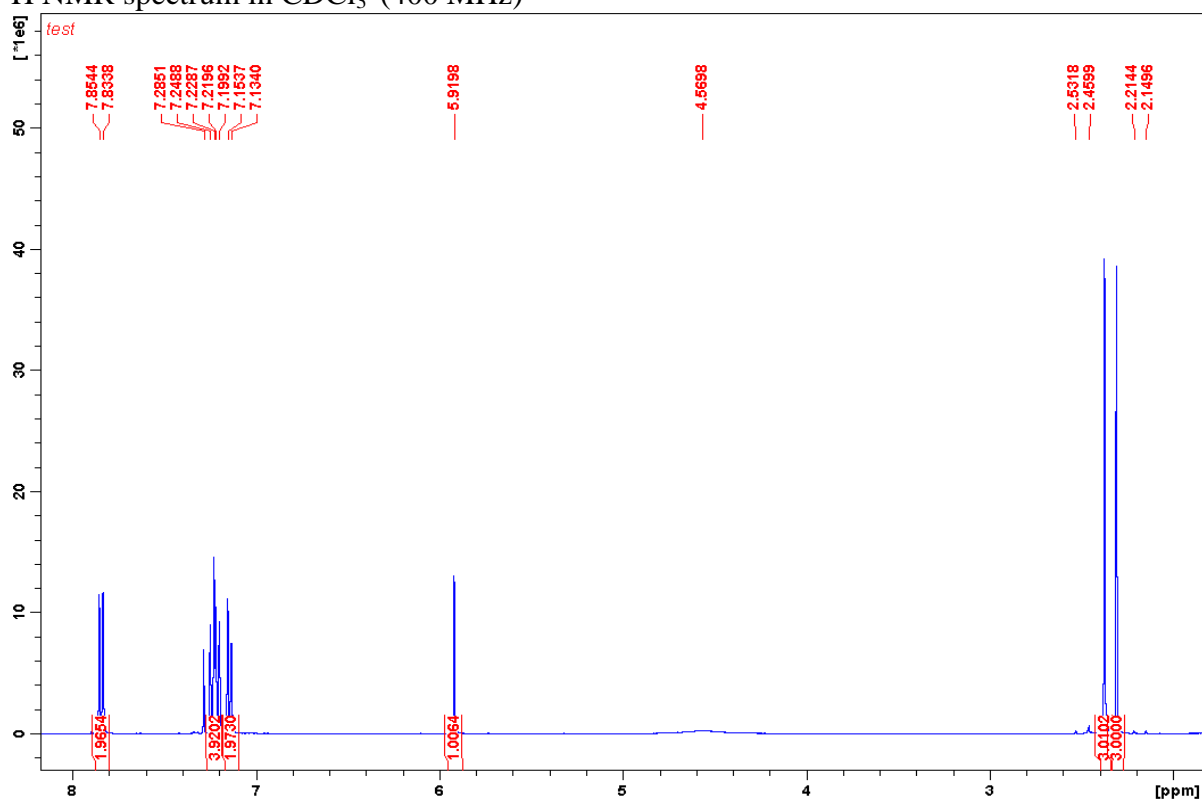
8.15 1,2-bis(4-chlorophenyl)-2-hydroxyethanone (15e)

¹H NMR spectrum in CDCl₃ (400 MHz)



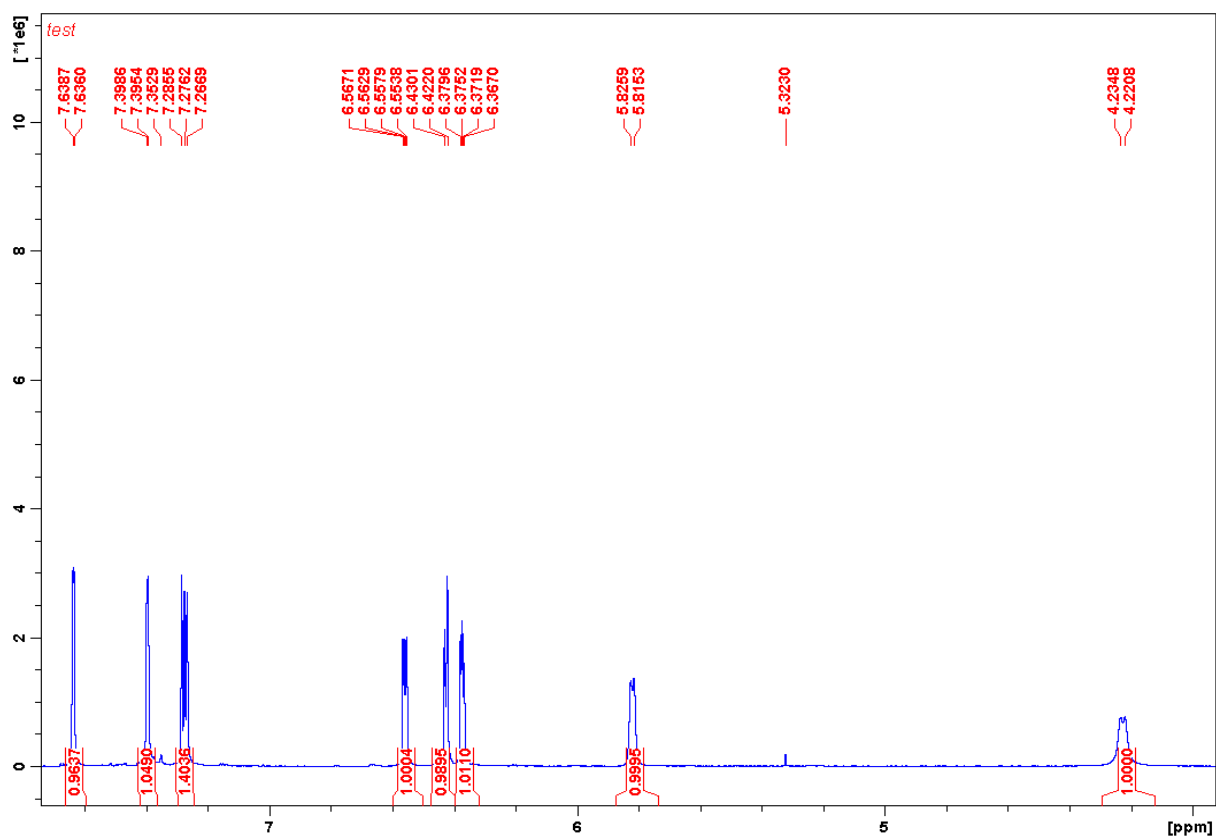
8.16 2-hydroxy-1,2-dip-tolyethanone (15g)

¹H NMR spectrum in CDCl₃ (400 MHz)



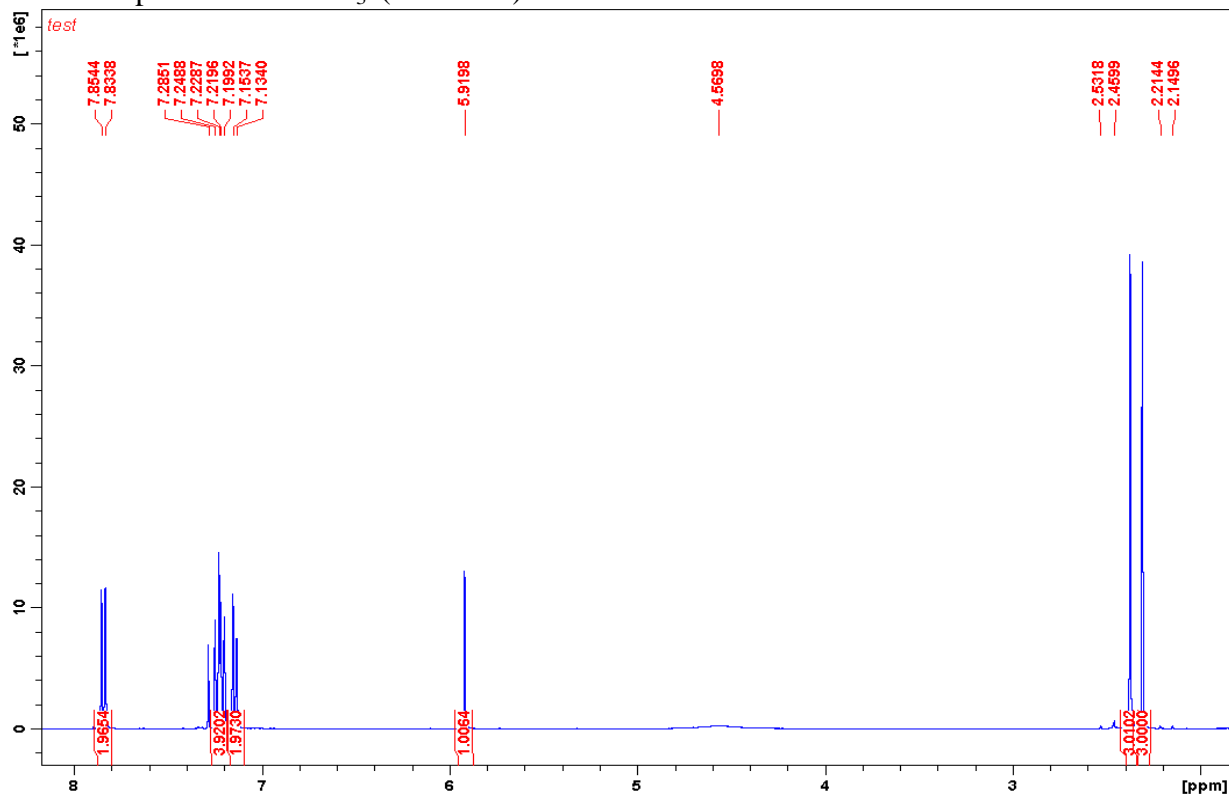
8.17 1,2-di(furan-2-yl)-2-hydroxyethanone (15i)

¹H NMR spectrum in CDCl₃ (400 MHz)

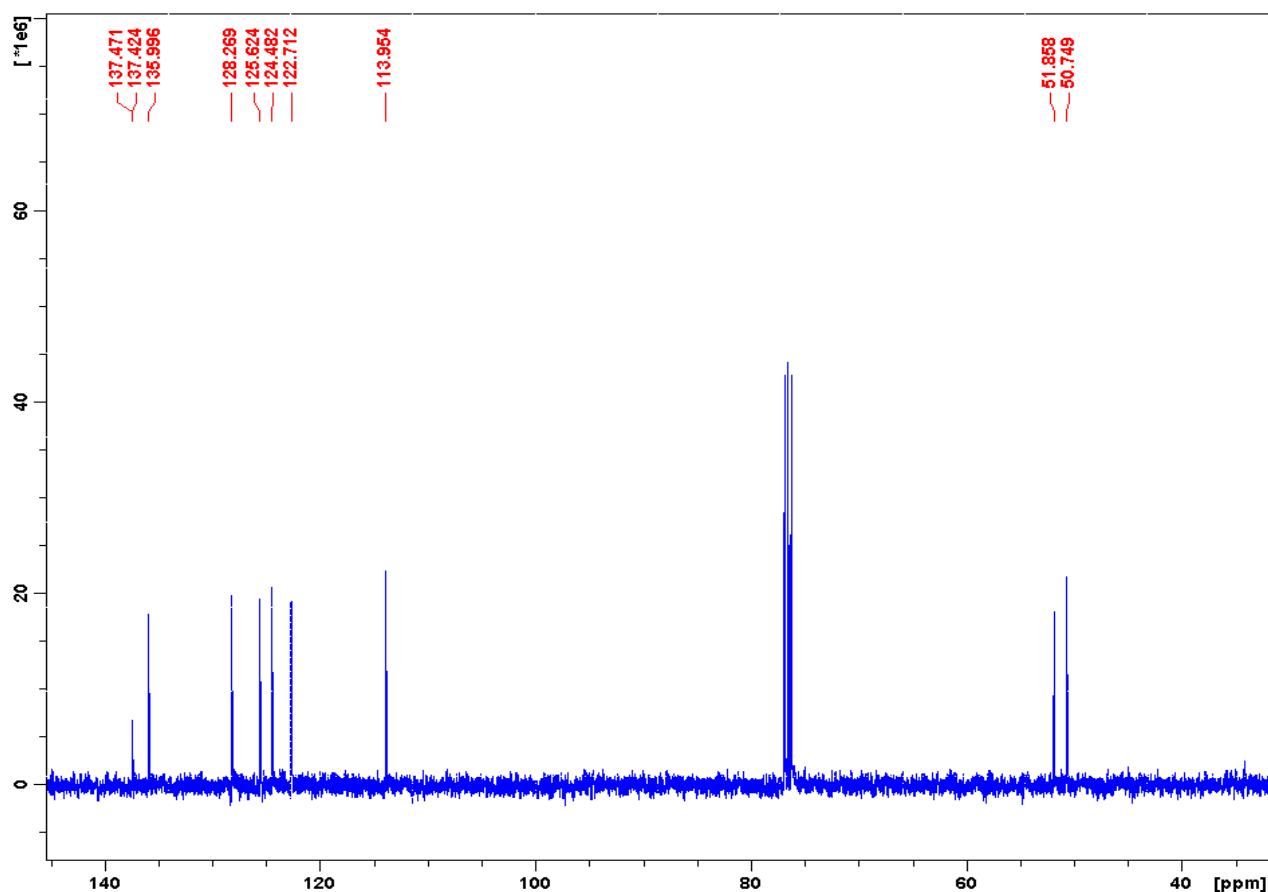


8.18 1,2-bis(4-(dimethoxymethyl)phenyl)-2-hydroxyethanone (23)

¹H NMR spectrum in CDCl₃ (400 MHz)



¹³C NMR spectrum in CDCl₃ (100 MHz)



9.0 Toxicity Screening

9.1 Antimicrobial toxicity screening study

For the antifungal studies, compounds were screened against the following yeast strains: *Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporan asahii* 1188 and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445). MIC values for antifungal study were defined as 80% inhibition (IC₈₀) of the control growth for yeast and 50% inhibition (IC₅₀) of the control growth for filamentous fungi. The MIC values for most fungi were recorded after 24 h and 48 h except for the dermatophytic strain (*T. mentagrophytes* 445) which was determined after 72 h and 120 h. Both triazolium salts were found to be non-toxic to all 12 fungi strains up to 2 mM concentration for dimethyl and 500 μM (solubility limit) for C₆F₅.

Next, triazolium salts were screened against four Gram positive organisms *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus epidermidis* HK6966/08, *Enterococcus sp.* HK14365/08 and four Gram negative organisms *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae*-ESBL positive HK14368/08 and *Pseudomonas aeruginosa* ATCC 9027. MIC values for the antibacterial study were defined as 95% inhibition (IC₉₅) of the control growth. Both triazolium salts were non-toxic to all 8 bacteria strains, up to 2 mM dimethyl and 500 μM (solubility limit) C₆F₅ concentration. Combined this screening data demonstrate that the triazolium salts do not exhibit high antimicrobial toxicity to a wide range (20 strains) of bacteria and fungi.

Finally, IC₅₀ values for the dimethyl salt were also determined after 18h against a screen of 5 bacteria (Table 1). This shows that the dimethyl salt has low antibacterial toxicity (high IC₅₀ values). The bacteria strains most sensitive to the dimethyl salt are the *putida* class. To complete the study C₆F₅ was also included in the antibacterial study and in agreement with the earlier study low toxicity was demonstrated against the 5 strains in Table 1.

	Compound	MIC - IC ₅₀ value (mM)				
		<i>E.coli</i>	<i>B. subtilis</i>	<i>P. fluorescens</i>	<i>P. putida</i> (CPI)	<i>P. putida</i> (KT2440)
1	dimethyl	>100	>100	>100	50-100	50-100
2	C ₆ F ₅	>0.50	>0.50	>0.50	>0.50	>0.50

Table 1 - IC₅₀ determination

9.2 Antifungal activity

In vitro antifungal activities of the compounds were evaluated on a panel of four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) and eight clinical yeast isolates (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitanae* 2446/I, *Trichosporon asahii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three ATCC strains were used as the

quality control strains. All of the isolates were maintained on Sabouraud dextrose agar prior to being tested.

Minimum inhibitory concentrations (MICs) were determined by modified CLSI standard of microdilution format of the M27-A3 and M38-A2 documents.^{14,15} Dimethyl sulfoxide (100 %) served as a diluent for all compounds; the final concentration did not exceed 2%. RPMI 1640 (Sevapharma, Prague) medium supplemented with L-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 M NaOH was used as the test medium. The wells of the microdilution tray contained 200 μL of the RPMI 1640 medium with 2- fold serial dilutions of the compounds (1000 to 0.244 $\mu\text{mol/L}$ for the new compounds) and 10 μL of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2 \text{ cfu.mL}^{-1}$. The trays were incubated at 35 °C and MICs were read visually after 24 h and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h. The MICs were defined as 80% inhibition (IC_{80}) of the control growth for yeasts and as 50% inhibition (IC_{50}) of the control growth for filamentous fungi. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

9.3 Antibacterial activity

In vitro antibacterial activities¹⁶ of the compounds were evaluated on a panel of three ATCC strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and five clinical isolates (*Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus epidermidis* HK6966/08, *Enterococcus* sp. HK14365/08, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae* ESBL HK14368/08) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. The above-mentioned ATCC strains also served as the quality control strains. All the isolates were maintained on Mueller-Hinton agar prior to being tested.

Dimethyl sulfoxide (100%) served as a diluent for all compounds; the final concentration did not exceed 2%. Mueller-Hinton agar (MH, HiMedia, Čadersky-Envitek, Czech Republic) buffered to pH 7.4 (± 0.2) was used as the test medium. The wells of the microdilution tray

contained 200 μL of the Mueller-Hinton medium with 2-fold serial dilutions of the compounds (1000 to 0.244 $\mu\text{mol/L}$) and 10 μL of inoculum suspension. Inoculum in MH medium was prepared to give a final concentration of 0.5 McFarland scale ($1.5 \times 10^8 \text{ cfu.mL}^{-1}$). The trays were incubated at 37 °C and MICs were read visually after 24 h and 48 h. The MICs were defined as 95% inhibition of the control growth. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

9.4 Toxicity Studies- Experimental

IC_{50} values for the compounds were determined at The School of Biotechnology, Dublin City University using a modification of the broth microdilution method described by Amsterdam.¹⁷ Strains were grown in nutrient broth overnight, washed with 0.01M sodium phosphate buffer and the cell number adjusted to give an optical density reading of 0.07 at 660 nm. The antimicrobial activity of the ILs were tested in 96 well microplates. 180 μL of Mueller-Hinton broth was pipetted into column 1 of the wells and 100 μL into the other wells. 20 μL of the chemical solution was transferred into column 1 giving a concentration of 200 mM. 100 μL of the solution from column 1 was then transferred to the next column and mixed. The procedure was repeated to give a series of two-fold dilutions. Each well was inoculated with 5 μL of bacterial culture. Wells containing medium only were used as blanks and wells containing medium and culture only were used as positive controls. The microplates were incubated overnight at 37 °C for *E.coli* and 30 °C for all other bacteria. The presence or absence of growth was determined by measuring the optical density of the wells at a wavelength of 405 nm using a plate reader. The IC_{50} values were determined as the concentration or range of concentrations that caused a 50% reduction in growth.

10.0 References

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