Supplementary Information

Inhibition of quorum sensing and biofilm formation in *Vibrio harveyi* by 4-Fluoro-DPD; a novel potent inhibitor of AI-2 signalling.

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‡ Equal contribution to the research; MK & FF completed the synthesis and S Forbes the microbiology

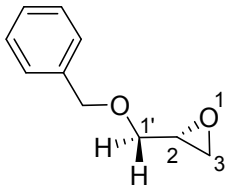
1. Chemical synthesis

1.1. Experimental

1.1.1 Materials and Methods

Chemicals were purchased from Aldrich Chemical Co., Gillingham, UK. Syntheses were monitored by thin layer chromatography on pre-coated 60 F_{254} silica gel aluminium backed plates (Merck, Darmstadt). Visualisation of spots for thin layer chromatography was performed using a 3% vanillin in 1% H_2SO_4/ethanol solution, 1% $KMnO_4$ in 7% K_2CO_3/10% NaOH solution and UV GL-58 Mineral-Light lamp. Flash column grade 40-63µm silica gel (Apollo scientific, Stockport, UK) was used in preparative scale column chromatography. NMR spectra were recorded using Bruker Avance spectrometers equipped with a 5 mm single-axis *Z*-gradient quattro nucleus probe, operating at 300 MHz and 400 MHz for ^{1}H and at 75 MHz and 100 MHz for ^{13}C. The spectrometer was running TOPSPIN NMR system software (Version 2.0). Chemical shifts (δ) are reported in parts per million (ppm), peak positions relative to Me_4Si (0.00 ppm) for ^{1}H and ^{13}C NMR spectra. ^{19}F NMR spectra were recorded on a Bruker Avance-400 spectrometer operating at 376 MHz and chemical shifts were referenced to hexafluorobenzene at 161.7 ppm. Abbreviations used for splitting patterns are: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; p, pentet; m, multiplet. Mass spectra were recorded at the School of Chemistry, University of Manchester using Micromass PLATFORM II (ES) and Thermo Finnigan MAT95XP (Accurate mass and GCMS) instruments.

(*R*)-2-((Benzyloxy)methyl)oxirane (4)

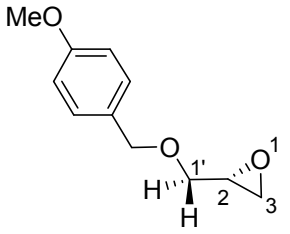


4

A suspension of 60% sodium hydride (1.60 g, 40.5 mmol) in hexane (15 ml) was stirred for 10 minutes. The excess hexane was removed and (*R*)-glycidol (2.0 g, 27.0 mmol) in dry DMF (20 ml) was added dropwise to the reaction mass at 0 °C. Benzyl bromide (4.2 ml, 35.1 mmol) was added to the mixture after 20 minutes. After 48 hours, the solution was diluted with ethyl acetate (100 ml) and washed with water (5x20 ml). The organic layer was dried

($MgSO_4$) and concentrated under reduced pressure. The resulting liquid was purified by column chromatography (hexane:ethyl acetate, 8:2) to give 3.0 g (70%) of (**4**) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.21 (m, 5H, Ar-H), 4.63 (d, 1H, J 12Hz, $\underline{CH_2}$-Ar), 4.53 (d, 1H, J 12 Hz, $\underline{CH_2}$-Ar), 3.76 (dd, 1H, $^2J_{1'a\text{-}1'b}$ 11.4 Hz, $^3J_{1'a\text{-}2}$ 3.0 Hz, H-1'a), 3.41 (dd, 1H, $^2J_{1'b\text{-}1'a}$ 11.4 Hz, $^3J_{1'b\text{-}2}$ 5.7 Hz, H-1'b), 3.22-3.13 (1H, m, H-2), 2.76 (t, 1H, $^3J_{3a\text{-}2}$= $^2J_{3a\text{-}3b}$= 4.5 Hz, H-3a), 2.61 (dd, 1H, $^3J_{3b\text{-}2}$ 3.0 Hz, $^2J_{3a\text{-}3b}$ 4.5 Hz, H-3b). [The product was confirmed by comparison of the spectral data with that reported in the literature].[1]

(*R*)-2-((4-Methoxybenzyloxy)methyl)oxirane (5)

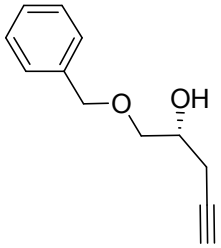


5

A suspension of 60% sodium hydride (0.84 g, 21 mmol) in hexane (25 ml) was stirred for 10 minutes. The excess hexane was removed and dry DMF (20 ml) was added. The reaction mass was cooled to 0 °C under nitrogen atmosphere. (*R*)-Glycidol (1.13 ml, 17.0 mmol) in dry DMF (10 ml) was added dropwise to the reaction mixture at 0 °C. The cooling bath was removed once the addition was completed and the reaction was stirred for 1 h at rt. The reaction was cooled to 0 °C and *p*-methoxybenzyl chloride (2.6 ml, 19 mmol) and tetrabutylammonium iodide (0.24 g, 0.87 mmol) was added. The reaction was stirred for 2 days at rt, quenched with saturated ammonium chloride solution (50 ml) and extracted with diethyl ether (3 × 75 ml). The combined organic layers were washed with water (5 × 50 ml), dried ($MgSO_4$) and filtered, and the solvent removed under reduced pressure. Purification of the residue by column chromatography eluting with diethyl ether–hexane (2:8) gave (**5**) as a colourless oil (2.1 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, 2H, J 8.7 Hz, Ar-H), 6.86 (d, 2H, J 8.7 Hz, Ar-H), 4.52 (d, 1H, J 11.4 Hz, $\underline{CH_2}$-Ar), 4.46 (d, 1H, J 11.4 Hz, $\underline{CH_2}$-Ar), 3.78 (s, 3H, OMe), 3.71 (dd, 1H, $^2J_{1'a\text{-}1'b}$ 11.4 Hz, $^3J_{1'a\text{-}2}$ 3.1 Hz, H-1'a), 3.38 (dd, 1H, $^2J_{1'b\text{-}1'a}$ 11.4 Hz, $^3J_{1'b\text{-}2}$ 5.8 Hz, H-1'b), 3.18-3.13 (1H, m, H-2), 2.76 (dd, 1H, $^3J_{3a\text{-}2}$ 4.1 Hz, $^2J_{3a\text{-}3b}$ 5.1 Hz, H-3a), 2.58 (dd, 1H, $^3J_{3b\text{-}2}$ 2.8 Hz, $^2J_{3a\text{-}3b}$ 5.1 Hz, H-3b). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-Ar), 130.1 (C-Ar), 129.6 (CH-Ar), 113.8 (CH-Ar), 72.9 ($\underline{CH_2}$-Ar), 70.5 (C-1'), 55.2

(OMe), 50.8 (C-2), 44.3 (C-3). [The product was confirmed by comparison of the spectral data with that reported in the literature].[2]

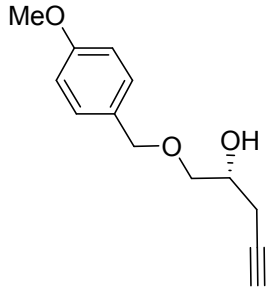
(*R*)-1-(Benzyloxy)-pent-4-yn-2-ol (6)



6

A solution of **(4)** (2.0 g, 12.2 mmol) in dry DMSO (16 ml) was added dropwise to a suspension of lithium acetylide ethylenediamine complex (90%, 1.84 g, 18.4 mmol) in dry DMSO (15 ml). The mixture was stirred at 0 °C under nitrogen atmosphere for 40 minutes. The reaction was then stirred at rt overnight. The reaction was quenched by addition of brine (30 ml), and then acidified with 10% hydrochloric acid. The mixture was extracted with ethyl acetate (3x100 ml) and washed with 5% sodium hydrogen carbonate solution (50 ml). The organic layer was dried ($MgSO_4$) and solvent was removed under vacuum. The residue was purified by column chromatography (ethyl acetate:hexane, 3:7) to give (**6**) as a colourless liquid (1.58 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.38-7.28 (m, 5H, Ar-H), 4.57 (s, 2H, $\underline{CH_2}$-Ar), 4.03-3.97 (m, 1H, H-2), 3.61 (dd, 1H, $^3J_{1a\text{-}2}$ 3.9 Hz, $^2J_{1a\text{-}1b}$ 9.3 Hz, H-1a), 3.50 (dd, 1H, $^3J_{1b\text{-}2}$ 6.6 Hz, $^2J_{1a\text{-}1b}$ 9.3 Hz, H-1b), 2.54 (d, 1H, $^3J_{2,OH}$ 4.8 Hz, OH), 2.45 (dd, 2H, $^3J_{2\text{-}3a}$=$^3J_{2\text{-}3b}$ 2.4 Hz, $^2J_{3a\text{-}3b}$ 6.3 Hz, H-3a, 3b), 2.03-2.01 (1H, m, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.7 (C-Ar), 128.8 (CH-Ar), 128.4 (CH-Ar), 127.5 (CH-Ar), 80.2 (C-4), 73.5 (C-1), 72.8 ($\underline{CH_2}$-Ar), 70.6 (C-5), 68.8 (C-2), 23.7 (C-3). [The product was confirmed by comparison of the spectral data with that reported in the literature].[3]

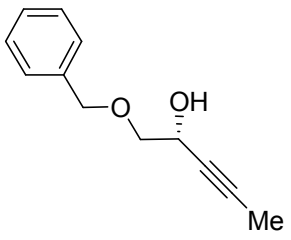
(*R*)-1-(4-Methoxybenzyloxy)-pent-4-yn-2-ol (7)



7

A solution of **(5)** (1.5 g, 7.7 mmol) in dry DMSO (10 ml) was added dropwise to a suspension of lithium acetylide ethylenediamine complex (90%, 1.1g, 11.6 mmol) in dry DMSO (10 ml). The mixture was stirred at 0°C under a nitrogen atmosphere for 40 minutes. The reaction mixture was then stirred at rt overnight. The reaction was quenched by addition of brine (20 ml), and then acidified by 10% hydrochloric acid. The mixture was extracted by ethyl acetate (3x75 ml) and was washed with 5% sodium hydrogen carbonate solution (25 ml). The organic layer was dried ($MgSO_4$) and solvent was removed under vacuum. The residue was purified by column chromatography (ethyl acetate:hexane, 3:7) to give (**7**) as a colourless liquid (1.0 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.24 (d, 2H, J 7.7 Hz, Ar-H), 6.86 (d, 2H, J 7.7 Hz, Ar-H), 4.47 (s, 2H, $\underline{CH_2}$-Ar), 3.98-3.89 (m, 1H, H-2), 3.76 (s, 3H, OMe), 3.56 (dd, 1H, $^3J_{1a\text{-}2}$ 3.7 Hz, $^2J_{1a\text{-}1b}$ 9.5 Hz, H-1a), 3.45 (dd, 1H, $^3J_{1b\text{-}2}$ 7.7 Hz, $^2J_{1a\text{-}1b}$ 9.4 Hz, H-1b), 2.59 (d, 1H, OH), 2.44-2.39 (m, 2H, H-3), 2.03-1.98 (1H, m, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-Ar), 129.8 (C-Ar), 129.5 (CH-Ar), 113.9 (CH-Ar), 80.3 (C-4), 73.1 (C-1), 72.5 ($\underline{CH_2}$-Ar), 70.5 (C-5), 68.8 (C-2), 55.2 (OMe), 23.5 (C-3). [The product was confirmed by comparison of the spectral data with that reported in the literature].[2]

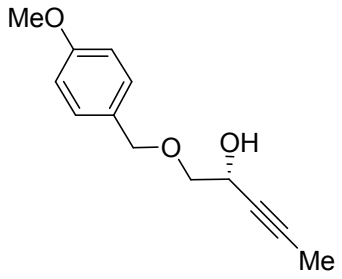
(*R*)-1-(Benzyloxy)pent-3-yn-2-ol (8)



8

A solution of **(6)** (1.4 g, 7.28 mmol) in dry DMSO (8 ml) was added dropwise to a solution of potassium *t*-butoxide (3.6 g, 29.51 mmol) in dry DMSO (20 ml) at room temperature under a nitrogen atmosphere. After stirring for 60 minutes, the reaction was quenched by the addition of brine (20 ml) and was acidified by the addition of 10% hydrochloric acid. The mixture was extracted with ethyl acetate (3x50 ml) and washed with water. After drying with magnesium sulfate, the organic solvent was removed under reduced pressure. The residue was purified by column chromatography (ethyl acetate:hexane, 3:7) to give 0.88 g (64%) of **(8)** as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.34-7.25 (m, 5H, Ar-H), 4.65-4.50 (m, 3H, $\underline{CH_2}$-Ar, H-2), 3.59 (dd, 1H, $^{3}J_{1a\text{-}2}$ 3.6 Hz, $^{2}J_{1a\text{-}1b}$ 9.9 Hz, H-1a), 3.53-3.47 (m, 1H, H-1b), 2.99 (s, 1H, OH), 1.82 (d, 3H, J 2.1 Hz, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.6 (C-Ar), 128.5 (CH-Ar), 127.8 (CH-Ar), 126.6 (CH-Ar), 82.0 (C-3), 77.0 (C-2), 74.0 (C-1), 73.4 ($\underline{CH_2}$-Ar), 61.8 (C-4), 3.6 (C-5). [The product was confirmed by comparison of the spectral data with that reported in the literature].[3]

(*R*)-1-(4-Methoxybenzyloxy)-pent-3-yn-2-ol (9)

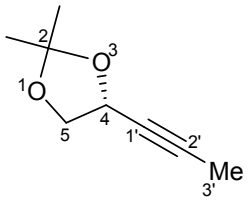


9

A solution of **(7)** (0.95 g, 7.28 mmol) in dry DMSO (5 ml) was added dropwise to a solution of potassium *t*-butoxide (2.2 g, 17.25 mmol) in dry DMSO (20 ml) at room temperature under nitrogen atmosphere. After stirring for 60 minutes, the reaction was quenched by addition of brine (20 ml) and was acidified by addition of 10% hydrochloric acid. The mixture was extracted with ethyl acetate (3x75 ml) and washed with water. After drying with magnesium sulfate, the organic solvent was removed under reduced pressure. The residue was purified by column chromatography (ethyl acetate:hexane, 3:7) to give (**9**) as a colourless liquid (0.62 g,

66%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.24 (d, 2H, J 8.4 Hz, Ar-H), 6.85 (d, 2H, J 8.4 Hz, Ar-H), 4.51-4.45 (m, 3H, $\underline{CH_2}$-Ar, H-2), 3.76 (s, 3H, OMe), 3.54 (dd, 1H, $^{3}J_{1a\text{-}2}$ 3.8 Hz, $^{2}J_{1a\text{-}1b}$ 9.7 Hz, H-1a), 3.46 (dd, 1H, $^{3}J_{1b\text{-}2}$ 7.7 Hz, $^{2}J_{1a\text{-}1b}$ 9.7 Hz, H-1b), 3.05 (s, 1H, OH), 1.80 (d, 3H, J 2.4 Hz, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (C-Ar), 129.8 (C-Ar), 129.5 (CH-Ar), 113.8 (CH-Ar), 81.8 (C-3), 77.4 (C-2), 73.7 (C-1), 72.9 ($\underline{CH_2}$-Ar), 61.7 (C-4), 55.2 (OMe), 3.6 (C-5). [The product was confirmed by comparison of the spectral data with that reported in the literature].[4]

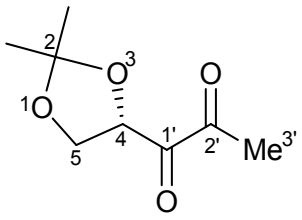
(*R*)-2,2-Dimethyl-4-(prop-1'-yn-1'yl)-1,3-dioxolane (10)



10

2,2-Dimethoxypropane (1.1 ml, 9.0 mmol) was added to a solution of (*R*)-Pent-3-yn-1,2-diol (**10**) (0.3 g, 3.0 mmol) in a dry DMF (3.0 ml) at room temperature. Two drops of concentrated H_2SO_4 were added and the reaction mixture was stirred overnight at room temperature. A saturated aqueous solution of $NaHCO_3$ (5 ml) was added. The mixture was extracted with diethyl ether (2x25 ml), the organic layers combined, and washed with water (3x10 ml). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (diethyl ether:hexane, 1:9) to give 0.32 g (75%) of (**10**) as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ 4.72–4.62 (m, 1H, H-4), 4.11 (1H, dd, $^{2}J_{5b\text{-}5a(gem)}$ 4.4, $^{3}J_{5a\text{-}4}$ 2.8 Hz, H-5a), 3.82 (dd, $^{2}J_{5b\text{-}5a(gem)}$ 7.8, $^{3}J_{5b\text{-}4}$ 7.1 Hz, 1H, H-5b), 1.86 (d, J 2.1, 3H, 3'-Me), 1.47 (s, 3H, 2-Me), 1.37 (s, 3H, 2-Me). [The product was confirmed by comparison of the spectral data with that reported in the literature][5]

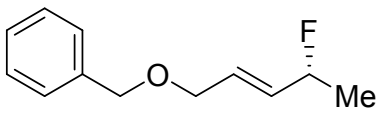
(*S*)-1-(2,2-Dimethyl-[1,3]-dioxolan-4-yl)-propane-1',2'-dione (11)



11

$NaIO_4$ (0.74 g, 3.47 mmol) was dissolved in H_2O (14 ml) was added to a solution of (**10**) (0.25 g, 1.78 mmol) in CCl_4 (9 ml) and MeCN (9 ml). The mixture was vigorously stirred, and RuO_2•H2O (6.0 mg) was added. The mixture was vigorously stirred for 15-30 min in air. The reaction mixture was filtered through silica using ethyl acetate as the eluant. The eluant was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate:hexane, 3:7) to give 0.16 g (52%) of (**11**) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14 (dd, 1H, $^3J_{4\text{-}5a}$ 7.9, $^3J_{4\text{-}5b}$ 5.5 Hz, H-4), 4.37 (t, 1H, $^2J_{5a\text{-}5b\ (gem)} = {}^3J_{5a\text{-}4}$ 8.2 Hz, H-5a), 4.00 (dd, 1H, $^2J_{5b\text{-}5a\ (gem)}$ 9.0, $^3J_{5b\text{-}4}$ 5.4 Hz, H-5b), 2.40 (3H, s, Me), 1.47 (3H, s, 2-Me), 1.42 (3H, s, 2-Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.1 (C=O), 194.4 (C=O), 73.8 (C-4), 66.7 (C-5), 26.8 (2-Me), 26.1 (2-Me), 24.2 (3'-Me).[The product was confirmed by comparison of the spectral data with that reported in the literature][5, 6]

(*E*)-(((4-Fluoropent-2-en-1-yl)oxy)methyl)benzene (15)



15

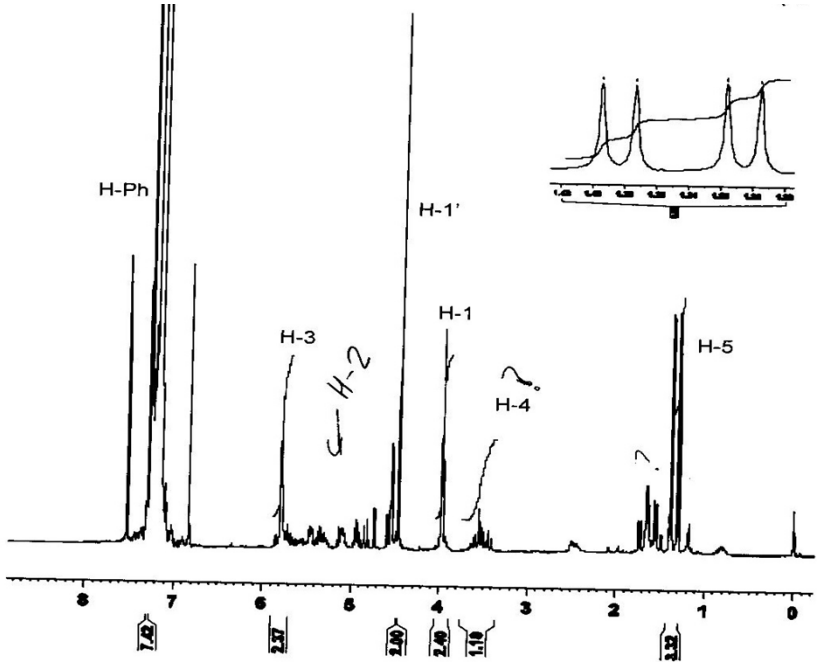
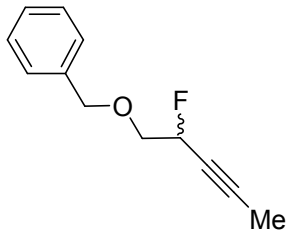


Figure SI 1: ^{1}H NMR spectrum of potentially (*E*)-(((4-Fluoropent-2-en-1-yl)oxy)methyl) benzene (**15**) in $CDCl_3$.

(±)-(((2-Fluoropent-3-yn-1-yl)oxy)methyl)benzene (16)



(±)-16

Xtal-Fluor-E (1.94 g, 8.47 mmol) was added to a stirred solution of triethylamine trihydrofluoride (1.5 ml, 9.29 mmol) and triethylamine (0.5 ml, 4.27 mmol) in dry dichloromethane (25 ml) at -72 °C. After stirring for 10 minutes, the solution of 1-(benzyloxy)-pent-3-yn-2-ol (**(±)-8**) (0.8 g, 4.2 mmol) in dry dichloromethane (10 ml) was added dropwise to the above mixture. The reaction was stirred at -72 °C for 1 hour and allowed to warm to room temperature. The reaction was complete in 3 hours. The reaction

mixture was quenched with 5% aqueous sodium hydrogen carbonate solution (10 ml), and extracted with diethyl ether (3x50 ml). After drying with magnesium sulfate, the solvent was removed *in vacuo*. A colourless liquid (0.59 g, 73%) of (**(±)-16**) was isolated after flash column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.33 (5H, m, H-Ar), 5.32-5.14 (1H, dm, $^{2}J_{H\text{-}F}$ 50.5 Hz, H-2), 4.65 (d, 1H, J 11.9 Hz, $\underline{CH_2}$-Ar), 4.59 (d, 1H, J 11.9 Hz, $\underline{CH_2}$-Ar), 3.78-3.61 (2H, m, H-1), 1.88 (3H, dd, $^{5}J_{H\text{-}F}$ 6.6, $^{5}J_{H\text{-}H}$ 2.1 Hz, Me). ^{19}F NMR (375 MHz, $CDCl_3$) δ -177.5.

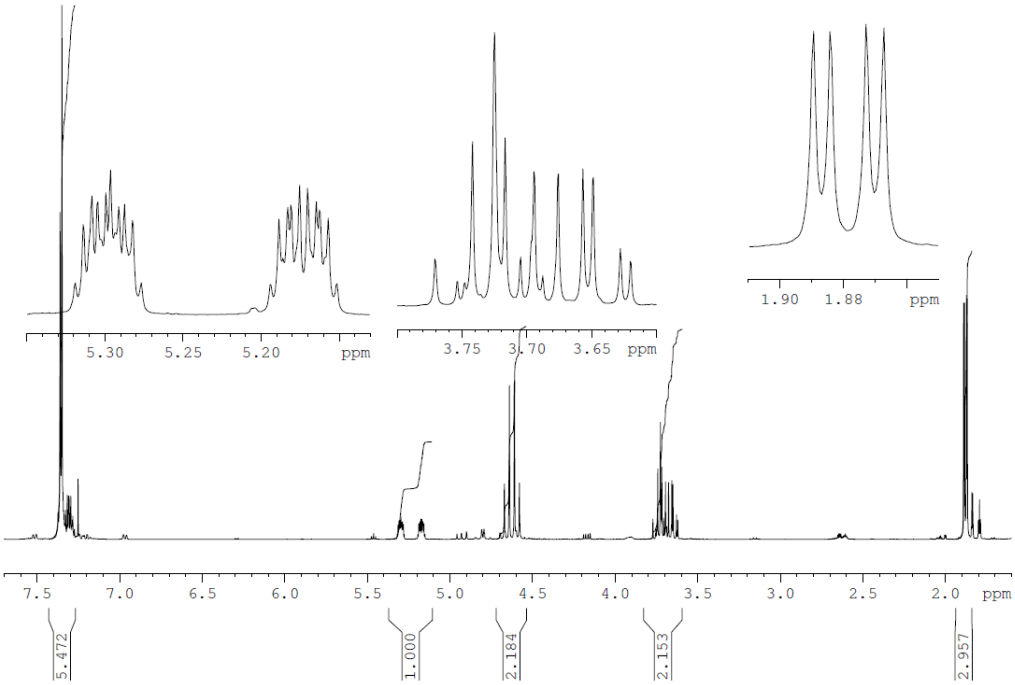


Figure SI 1: ^{1}H NMR spectrum of **(±)-**(((2-fluoropent-3-yn-1-yl)oxy)methyl)benzene (**16**) in $CDCl_3$.

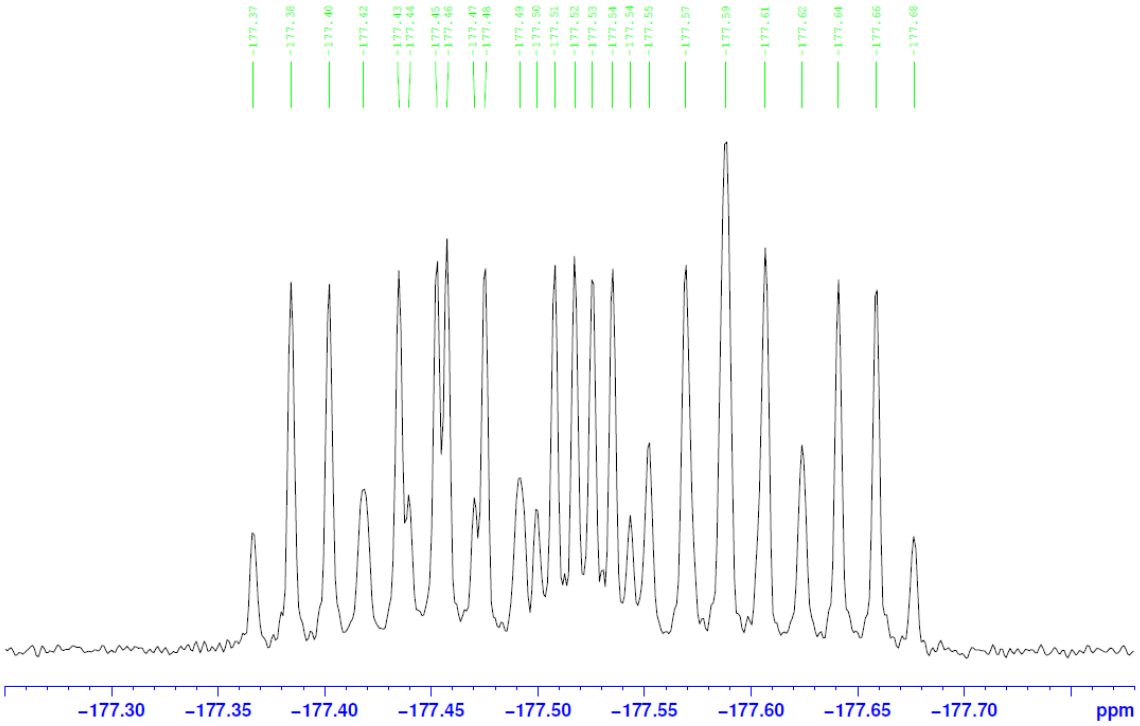
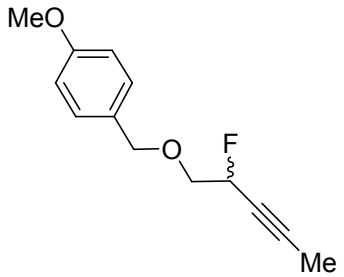


Figure SI 2: ^{19}F NMR spectrum of **(±)-**(((2-fluoropent-3-yn-1-yl)oxy)methyl)benzene (**16**) in $CDCl_3$.

(±)-(((2-Fluoropent-3-yn-1-yl)oxy)methyl)-4-methoxybenzene (17)



(±)-17

Xtal-Fluor-E (1.2 g, 5.24 mmol) was added to a stirred solution of triethylamine trihydrofluoride (0.88 ml, 5.47 mmol) and triethylamine (0.3 ml, 2.97 mmol) in dry dichloromethane (15 ml) at -72°C. After stirring for 5 minutes, the solution of 1-(4-

methoxybenzyloxy)-pent-3-yn-2-ol (**(±)-9**) (0.6 g, 2.72 mmol) in dry dichloromethane (5 ml) was added dropwise to the above mixture. The reaction was stirred at -72°C for 1 hour and allowed to warm to room temperature. The reaction was complete in 3 hours. The reaction mixture was quenched with 5% aqueous sodium hydrogen carbonate solution (5 ml), and extracted with diethyl ether (3x25 ml). After drying with magnesium sulfate, the solvent was removed *in vacuo.* The residue was purified using column chromatography (silica gel, diethyl ether:hexane (1:9 v/v)) to give 0.37 g (61%) of (**(±)-17**) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (2H, d, J 8.8 Hz, H-Ar), 6.91 (2H, d, J 8.8 Hz, H-Ar), 5.32-5.14 (1H, dm, $^{2}J_{H\text{-}F}$ 50.5 Hz, H-2), 4.60 (d, 1H, J 11.5 Hz, $\underline{CH_2}$-Ar), 4.54 (d, 1H, J 11.5 Hz, $\underline{CH_2}$-Ar), 3.82-3.60 (2H, m, H-1), 3.82 (3H, s, OMe), 1.89 (3H, dd, $^{5}J_{H\text{-}F}$ 6.5, $^{5}J_{H\text{-}H}$ 2.1 Hz, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3 (C-Ar), 129.6 (CH-Ar), 129.5 (C-Ar), 113.8 (CH-Ar), 82.5 (d, $^{1}J_{C\text{-}F}$ 166.2 Hz, C-2), 81.5 (C-3), 77.2 (C-4), 73.2 ($\underline{CH_2}$-Ar), 71.9 (d, $^{2}J_{C\text{-}F}$ 24.1 Hz, C-1), 55.3 (OMe), 3.6 (Me). ^{19}F NMR (375 MHz, $CDCl_3$) δ -177.5. MS (GCMS) m/e $[M]^{\cdot}$ 222.1.

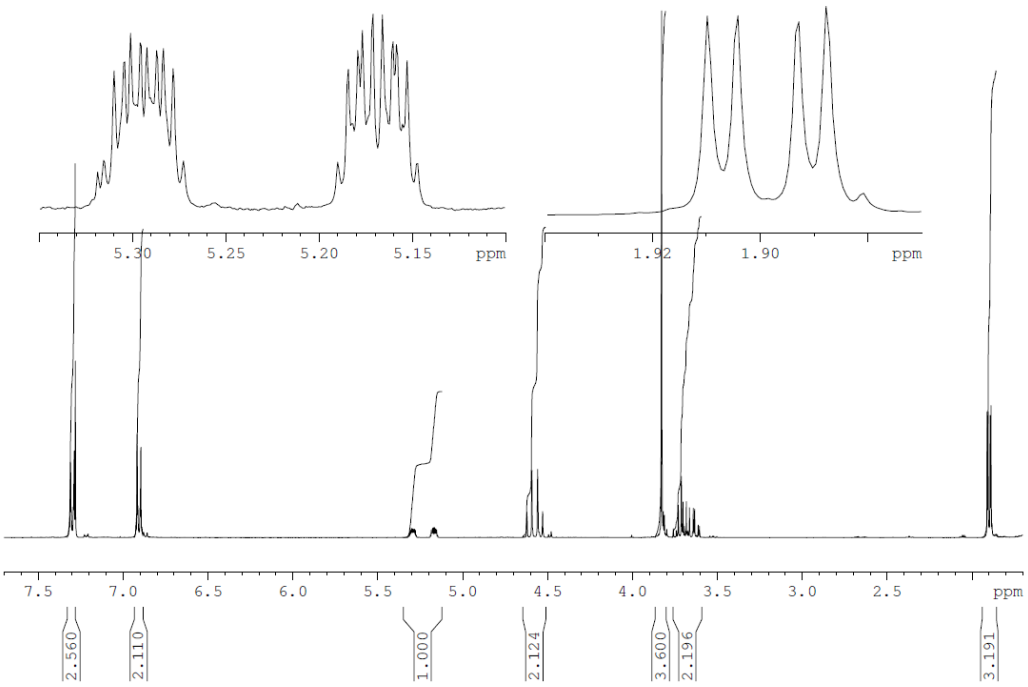


Figure SI 3: ^{1}H NMR spectrum of **(±)-**(((2-fluoropent-3-yn-1-yl)oxy)methyl)benzene (**17**) in $CDCl_3$.

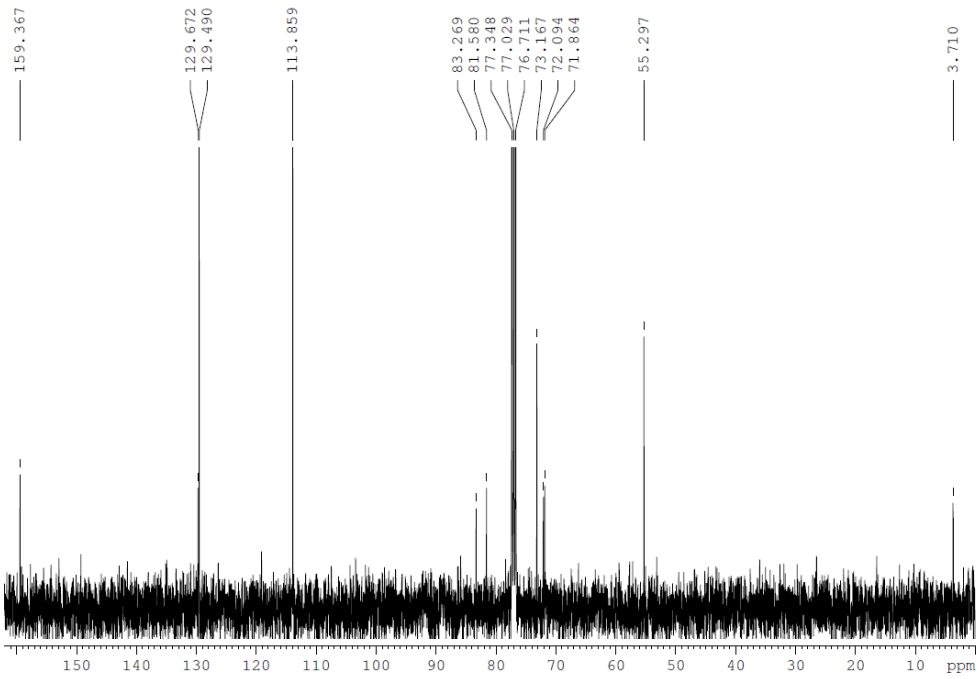


Figure SI 4: ^{13}C NMR spectrum of **(±)-**(((2-fluoropent-3-yn-1-yl)oxy)methyl)benzene (**17**) in $CDCl_3$.

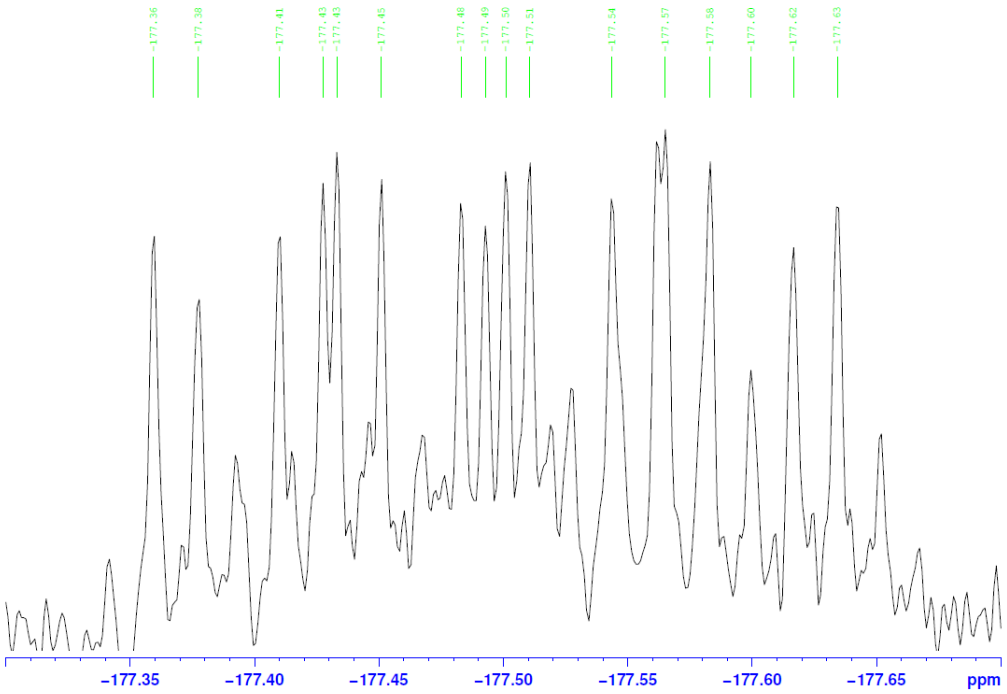
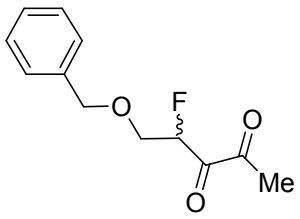


Figure SI 5: ^{19}F NMR spectrum of **(±)-**(((2-fluoropent-3-yn-1-yl)oxy)methyl)benzene (**17**) in $CDCl_3$.

(±)-5-(Benzyloxy)-4-fluoropentane-2,3-dione (18)



(±)-18

(((2-Fluoropent-3-yn-1-yl)oxy)methyl)benzene **((±)-16)** (350 mg, 1.8 mmol) in carbon tetrachloride (9 ml) and acetonitrile (9 ml) was added to a stirred solution of sodium periodate (890 mg, 4.16 mmol) in water (14 ml). The mixture was stirred vigorously and $RuO_2.H_2O$ (6.0 mg, 0.025 mmol) was added. The reaction mixture was stirred vigorously for 15 minutes, diluted with EtOAc (50 ml) and stirred well. The layers were separated and then the aqueous layer was extracted with EtOAc (2 x50 ml), the organic layer combined and dried ($MgSO_4$). After removing solvent under reduced pressure, the residue was purified by column chromatography (silica gel, ethyl acetate:hexane (1:9 to 3:7 v/v)) to give 0.31 g (71%) of (**(±)-18**) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.34-7.23 (5H, m, H-Ar), 5.84-5.59 (1H, dm, $^2J_{H-F}$ 48.3 Hz, H-4), 4.69 (d, 1H, J 11.7 Hz, $\underline{CH_2}$-Ar), 4.45 (d, 1H, J 11.7 Hz, $\underline{CH_2}$-Ar), 4.19 (1H, ddd, $^3J_{5a-F}$ 34.8, $^2J_{5a-5b}$ 11.7, $^3J_{5a-4}$ 4.1 Hz, H-5a), 3.91 (1H, ddd, $^3J_{5b-F}$ 21.2, $^2J_{5b-5a}$ 11.7, $^3J_{5b-4}$ 1.7 Hz, H-5b), 2.33 (3H, s, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0 (C-2), 191.8 (C-3), 136.9 (C-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 127.8 (CH-Ar), 92.4 (d, $^1J_{C-F}$ 188.9 Hz, C-4), 73.7 ($\underline{CH_2}$-Ar), 69.4 (d, $^2J_{C-F}$ 21.5 Hz, C-5), 24.1 (d, $^5J_{C-F}$ 1.8 Hz, C-1). MS (GCMS) m/e $[M]^{\cdot}$ 224.2.

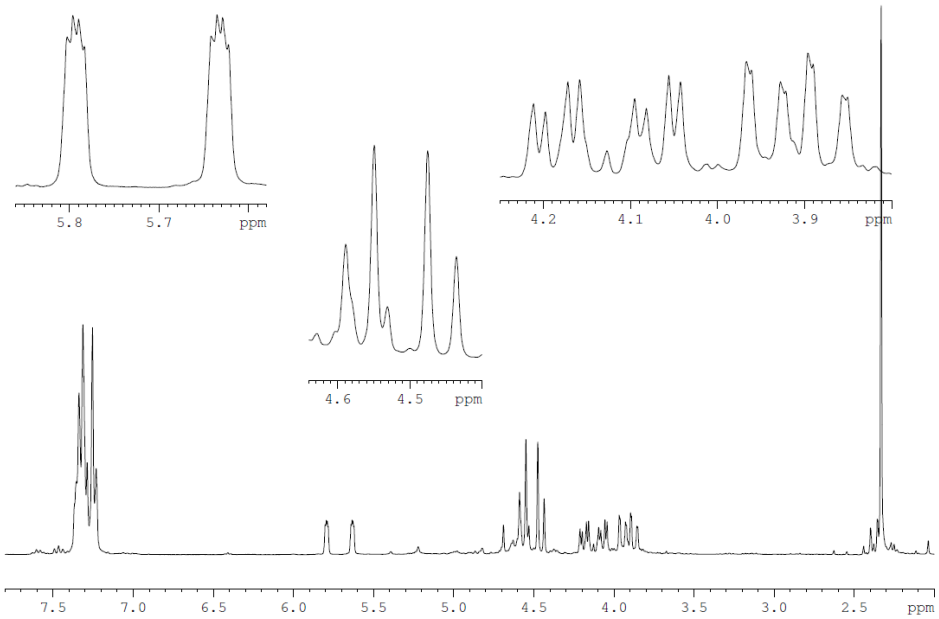


Figure SI 6: ^{1}H NMR spectrum of **(±)-**5-(benzyloxy)-4-fluoropentane-2,3-dione (**18**) in $CDCl_3$.

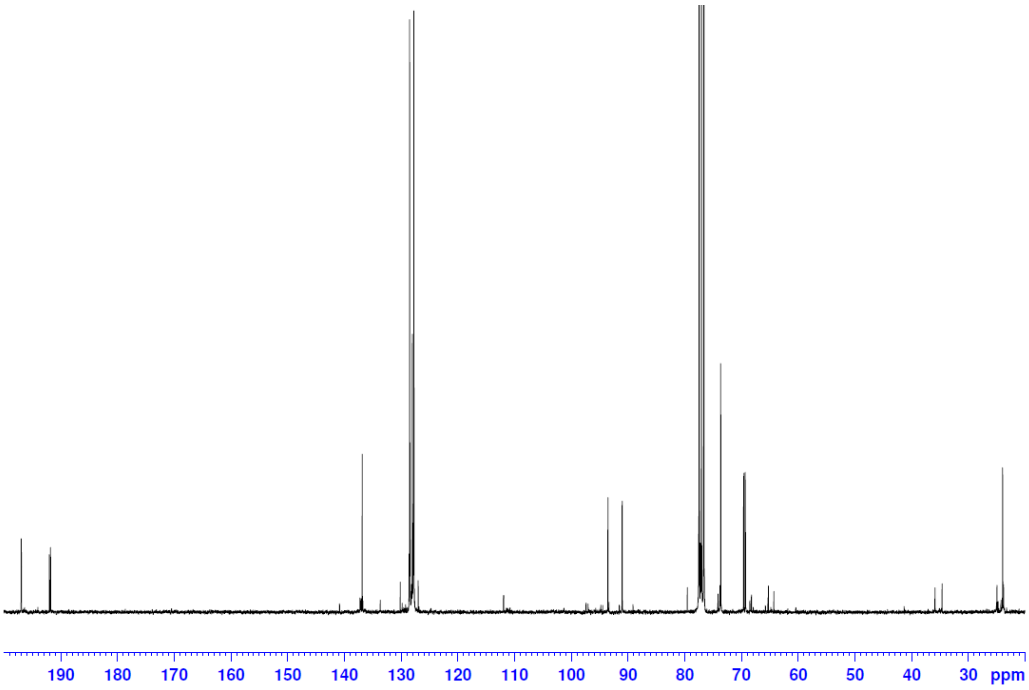
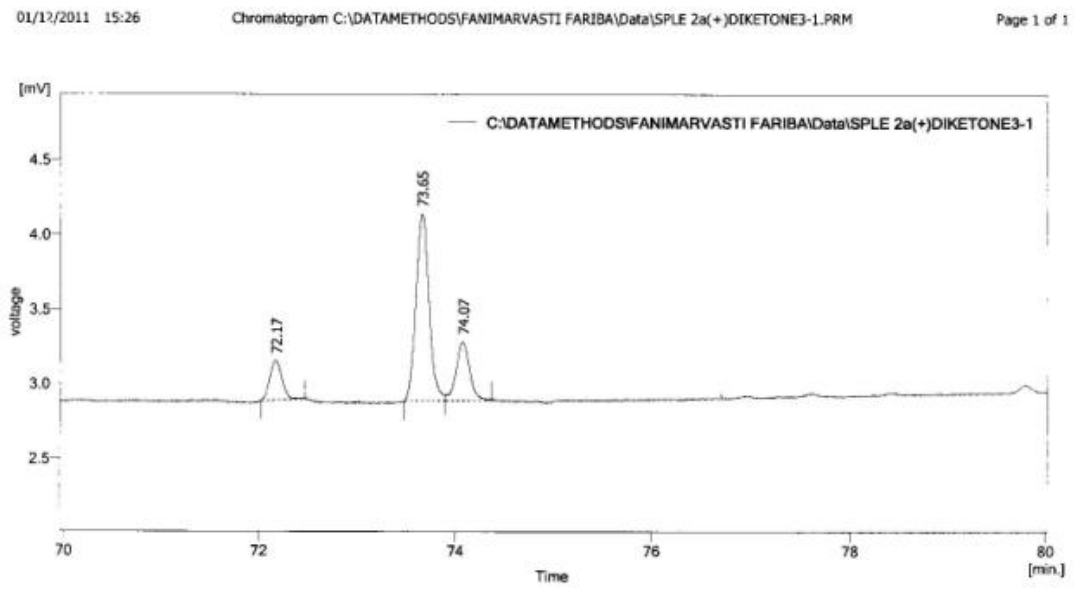


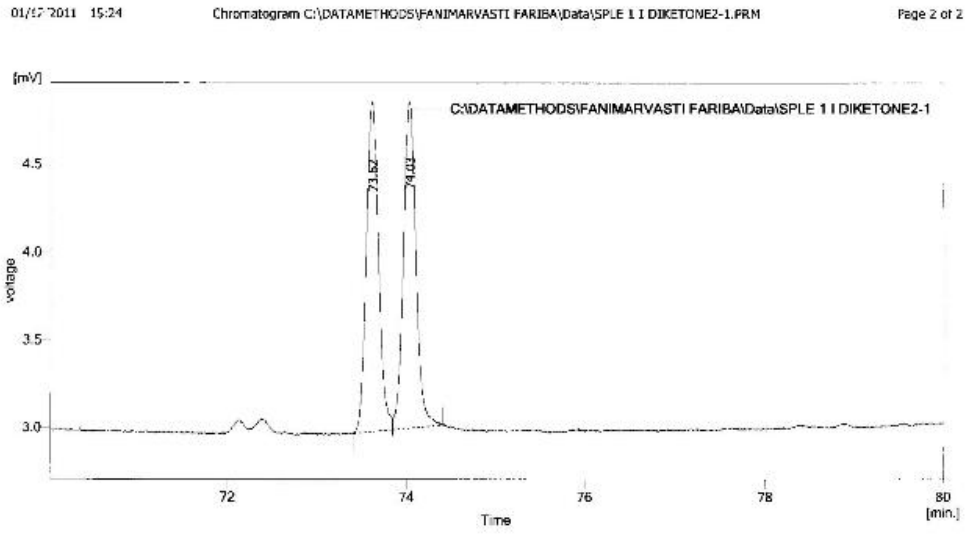
Figure SI 7: ^{13}C NMR spectrum of **(±)-**5-(benzyloxy)-4-fluoropentane-2,3-dione (**18**) in $CDCl_3$.



Result Table (Uncal - C:\DATAMETHODS\FANIMARVASTI FARIBA\Data\SPLE 2a(+)DIKETONE3-1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	72.173	2.295	0.262	12.9	13.7	0.14
2	73.653	11.674	1.255	65.7	65.6	0.14
3	74.073	3.812	0.395	21.4	20.7	0.15
	Total	17.782	1.912	100.0	100.0	

Figure SI 8: Chiral Gas chromatogram of (*S*)-5-(benzyloxy)-4-fluoropentane-2,3-dione (**18**).

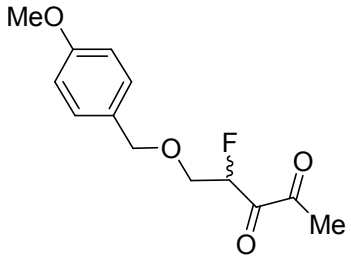


Result Table (Uncal - C:\DATAMETHODS\FANIMARVASTI FARIBA\Data\SPLE 1 I DIKETONE2-1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	73.617	17.785	1.871	49.4	50.1	0.15
2	74.030	18.238	1.862	50.6	49.9	0.15
	Total	36.023	3.733	100.0	100.0	

Figure SI 9: Chiral Gas chromatogram of (±)-5-(benzyloxy)-4-fluoropentane-2,3-dione (**18**).

(±)-5-(4'-Methoxybenzyloxy)-4-fluoropentane-2,3-dione (19)



19

The method of synthesis was similar to that described for (**(±)-18**), except that (((2-fluoropent-3-yn-1-yl)oxy)methyl)-4-methoxybenzene (**(±)-17**) was used. Diketone (**(±)-19**) (0.28 g, 82%) was isolated as a yellow liquid after flash column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (2H, d, J 8.8 Hz, H-Ar), 6.88 (2H, d, J 8.8 Hz, H-Ar), 5.72 (1H, ddd, $^{2}J_{4\text{-}F}$ 55.6, $^{3}J_{4\text{-}5a}$ 4.1, $^{3}J_{4\text{-}5b}$ 2.0 Hz, H-4), 4.52 (d, 1H, J 11.5 Hz, $\underline{CH_2}$-Ar), 4.40 (d, 1H, J 11.5 Hz, $\underline{CH_2}$-Ar), 4.11 (1H, ddd, $^{3}J_{5a\text{-}F}$ 34.9, $^{2}J_{5a\text{-}5b}$ 11.7, $^{3}J_{5a\text{-}4}$ 4.1 Hz, H-5a), 3.90 (1H, ddd, $^{3}J_{5b\text{-}F}$ 21.2, $^{2}J_{5b\text{-}5a}$ 11.7, $^{3}J_{5b\text{-}4}$ 2.0 Hz, H-5b), 3.81 (3H, s, OMe), 2.35 (3H, s, Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0 (C-2), 191.8 (C-3), 159.3 (C-Ar), 129.5 (CH-Ar), 128.8 (C-Ar), 113.8 (CH-Ar), 92.2 (d, $^{1}J_{C\text{-}F}$ 188.8 Hz, C-4), 73.4 ($\underline{CH_2}$-Ar), 69.2 (d, $^{2}J_{C\text{-}F}$ 22.0 Hz, C-5), 55.3 (OMe), 24.1 (Me, C-1). ^{19}F NMR (375 MHz, $CDCl_3$) δ -201.4 (ddd, $^{2}J_{F\text{-}H}$ 55.8 Hz, $^{3}J_{F\text{-}H}$ 34.8 Hz, $^{3}J_{F\text{-}H}$ 21.0 Hz). MS(GCMS) m/e $[M]^{\cdot}$ 254.1.

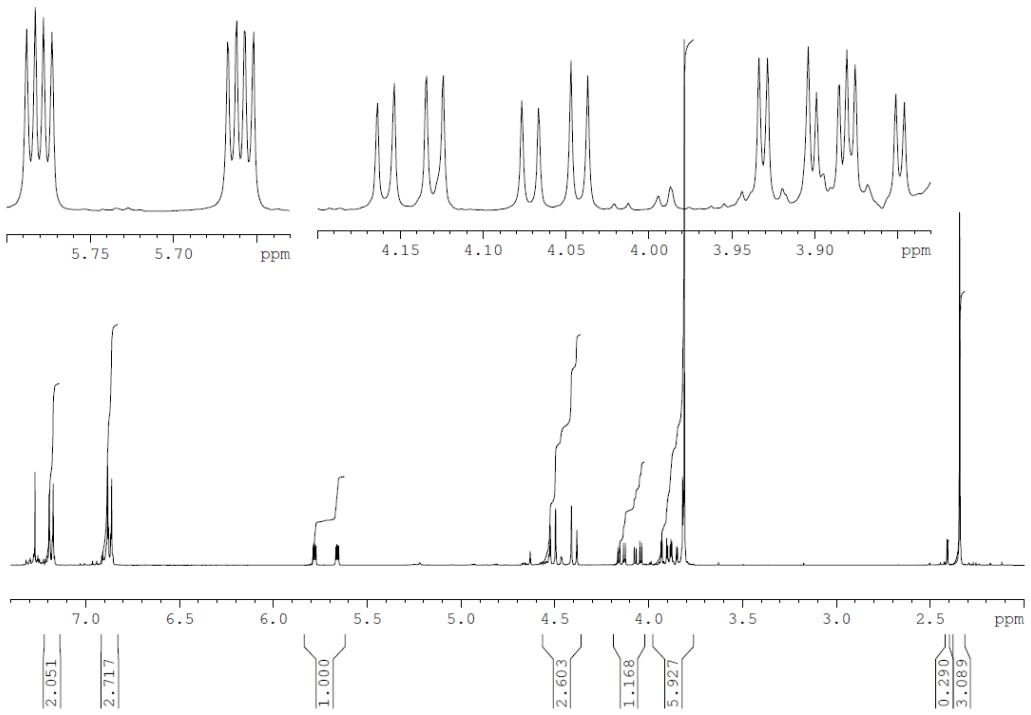


Figure SI 10: ^{1}H NMR spectrum of **(±)-**5-(4'-methoxybenzyloxy)-4-fluoropentane-2,3-dione (**19**) in $CDCl_3$.

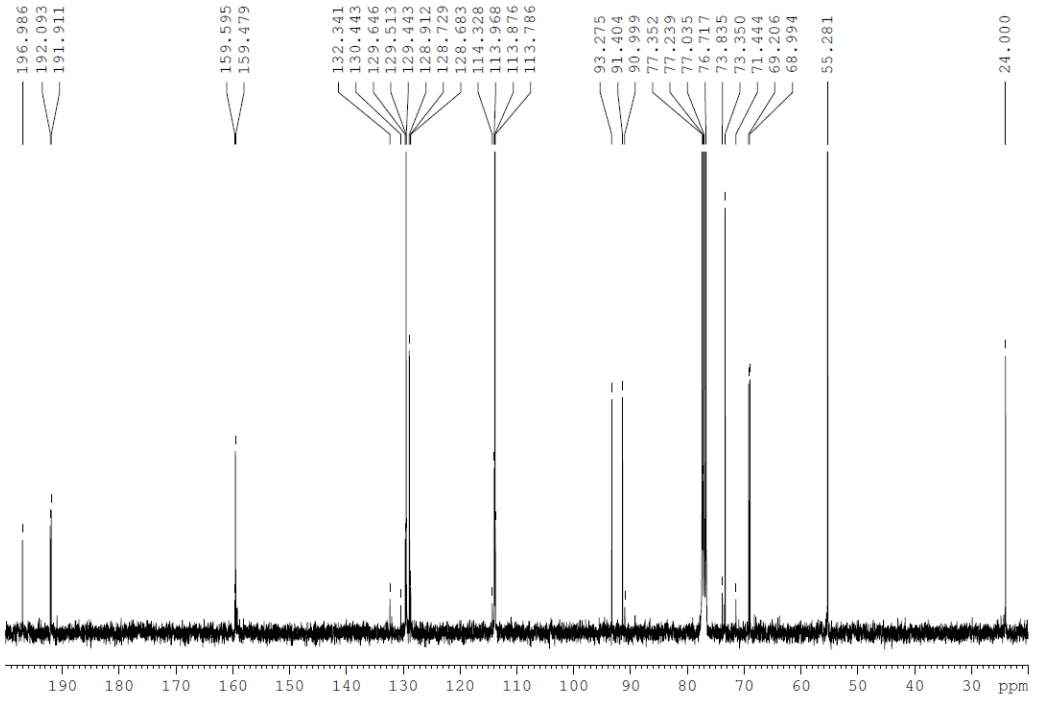


Figure SI 11: ^{13}C NMR spectrum of **(±)-**5-(4'-methoxybenzyloxy)-4-fluoropentane-2,3-dione (**19**) in $CDCl_3$.

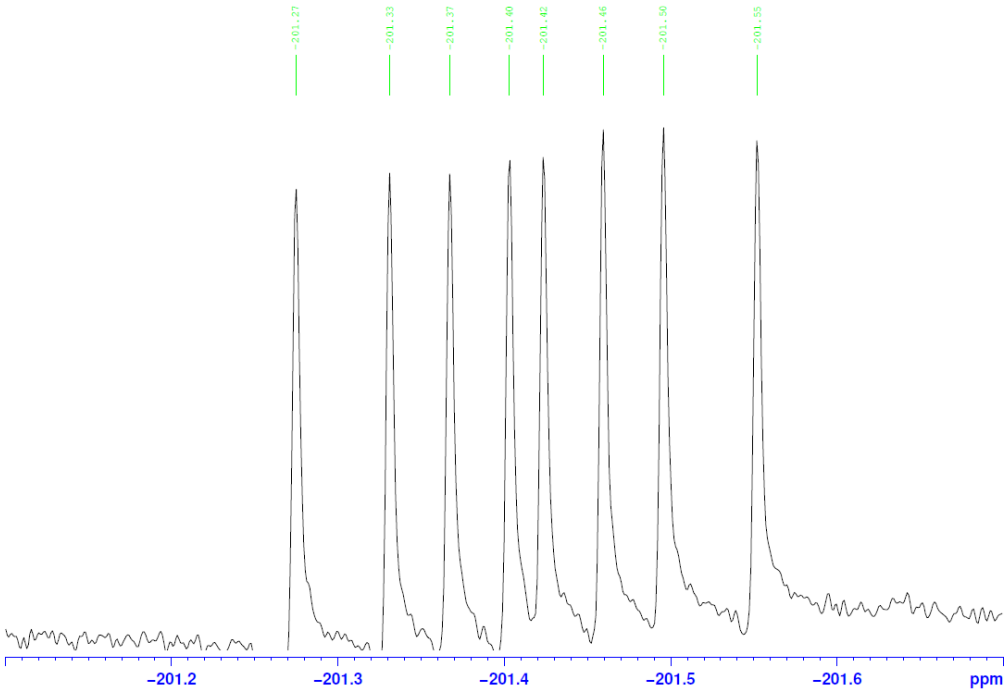
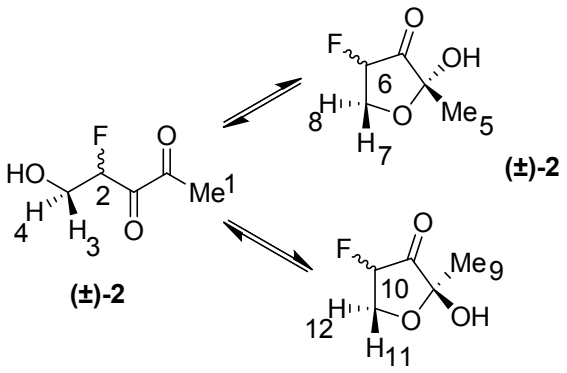


Figure SI 12: ^{19}F NMR spectrum of **(±)**-5-(4'-methoxybenzyloxy)-4-fluoropentane-2,3-dione (**19**) in $CDCl_3$.

(±)-5-Hydroxy-4-fluoropentane-2,3-dione (2)

To a solution of 5-(4'-methoxybenzyloxy)-4-fluoropentane-2,3-dione (**(±)-19**) (0.26 g, 1.02 mmol) in DCM (18 ml) and water (1.8 ml) was added DDQ (0.46 g, 2.04 mmol) at room temperature. The reaction mixture was stirred for 3 h. Saturated aqueous $NaHCO_3$ (10 ml) was added and the resulting dark red solution was stirred vigorously for 15 min. The layers were separated and the aqueous layer was extracted with ethyl acetate (10x30 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure**.** The residue was purified by flash column chromatography (ethyl acetate:hexane, 2:3) to give 5.4 mg (40 %) of (**(±)-2**) as a colourless oil as the non-hydrated form (2.0 mg, cyclic and acyclic) and the hydrated form (3.4 mg, cyclic) compounds. The hydrated and non-hydrated compounds existed in the ratio of 1:3 (acyclic:cyclic).



Non-cyclic (non-hydrated):

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.67 (1H, ddd, $^{2}J_{2\text{-}F}$ 55.6, $^{3}J_{2\text{-}3}$ 7.0, $^{3}J_{2\text{-}4}$ 4.0 Hz, H-2), 4.34 (1H, ddd, $^{3}J_{4\text{-}F}$ 28.4, $^{2}J_{4\text{-}3}$ 11.6, $^{3}J_{4\text{-}2}$ 5.2 Hz, H-4), 4.19 (1H, ddd, $^{3}J_{3\text{-}F}$ 25.4, $^{2}J_{3\text{-}4}$ 11.6, $^{3}J_{3\text{-}2}$ 2.4 Hz, H-3), 2.60 (d, J 2.2, Me H-1). ^{19}F NMR (375 MHz, $CDCl_3$) δ -202.7 (ddd, $^{2}J_{F\text{-}H}$ 55.6, $^{3}J_{F\text{-}H}$ 28.4, $^{3}J_{F\text{-}H}$ 25.1 Hz).

Cyclic (non-hydrated):

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (1H, ddd, $^{2}J_{6\text{-}F}$ 54.7, $^{3}J_{6\text{-}7}$ 5.3, $^{3}J_{6\text{-}8}$ 2.3 Hz, H-6), 4.08 (1H, dd, $^{3}J_{8\text{-}F}$ 20.9, $^{3}J_{8\text{-}6}$ 4.9 Hz, H-6), 3.97 (1H, ddd, $^{3}J_{7\text{-}F}$ 24.4, $^{2}J_{7\text{-}8}$ 10.3, $^{3}J_{7\text{-}6}$ 4.4 Hz, H-7), 1.66 (s, Me H-5). ^{19}F NMR (375 MHz, $CDCl_3$) δ -186.3 (ddd, $^{2}J_{F\text{-}H}$ 54.4, $^{3}J_{F\text{-}H}$ 24.4, $^{3}J_{F\text{-}H}$ 20.5 Hz).

Cyclic (non-hydrated):

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (1H, dt, $^{2}J_{10\text{-}F}$ 46.5, $^{3}J_{10\text{-}11}$ 5.1Hz, H-2), 4.38 (1H, ddd, $^{3}J_{11\text{-}F}$ 18.9, $^{2}J_{11\text{-}12}$ 10.7, $^{3}J_{11\text{-}10}$ 7.0 Hz, H-4), 4.19 (1H, ddd, $^{3}J_{3\text{-}F}$ 25.4, $^{2}J_{3\text{-}4}$ 11.6, $^{3}J_{3\text{-}2}$ 2.4 Hz, H-3), 1.65 (s, Me H-9). ^{19}F NMR (375 MHz, $CDCl_3$) δ -192.5 (ddd, $^{2}J_{F\text{-}H}$ 46.8, $^{3}J_{F\text{-}H}$ 23.4, $^{3}J_{F\text{-}H}$ 20.8 Hz).

^{13}C NMR (75 MHz, $CDCl_3$) δ 198.0 (C=O), 188.0 (C=O), 117.4, 113.8, 106.6, 102.6, 94.5 (d, $^{1}J_{C\text{-}F}$ 196.8 Hz), 91.7 (d, $^{1}J_{C\text{-}F}$ 197.2 Hz), 91.4 (d, $^{1}J_{C\text{-}F}$ 188.8 Hz), 69.5 (d, $^{2}J_{C\text{-}F}$ 24.5 Hz), 68.5 (d, $^{2}J_{C\text{-}F}$ 26.1 Hz), 60.9 (d, $^{2}J_{C\text{-}F}$ 23.9 Hz), 22.8 (Me, acyclic), 20.5 (Me, cyclic). ^{19}F NMR (375

GCMS m/z $[M]^{\cdot}$ 134.0. Accurate mass calcd for $C_5H_7O_3F$ (non-hydrated): 134.0374. Found: 134.0371.

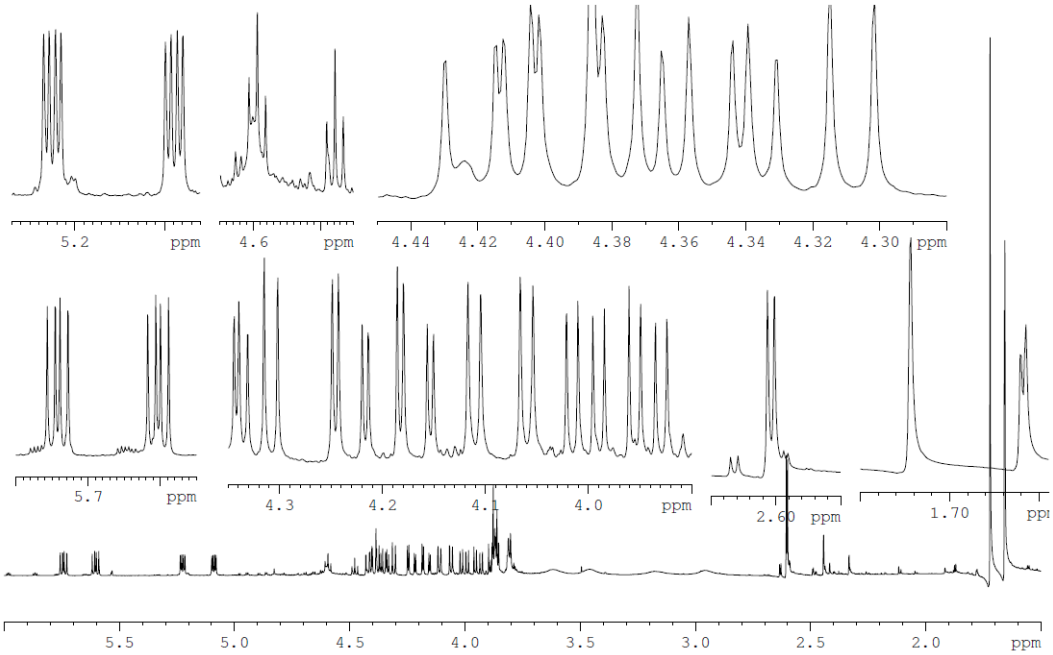


Figure SI 13: ^{1}H NMR spectrum of **(±)-**5-hydroxy-4-fluoropentane-2,3-dione (**2, non-hydrated**) in $CDCl_3$.

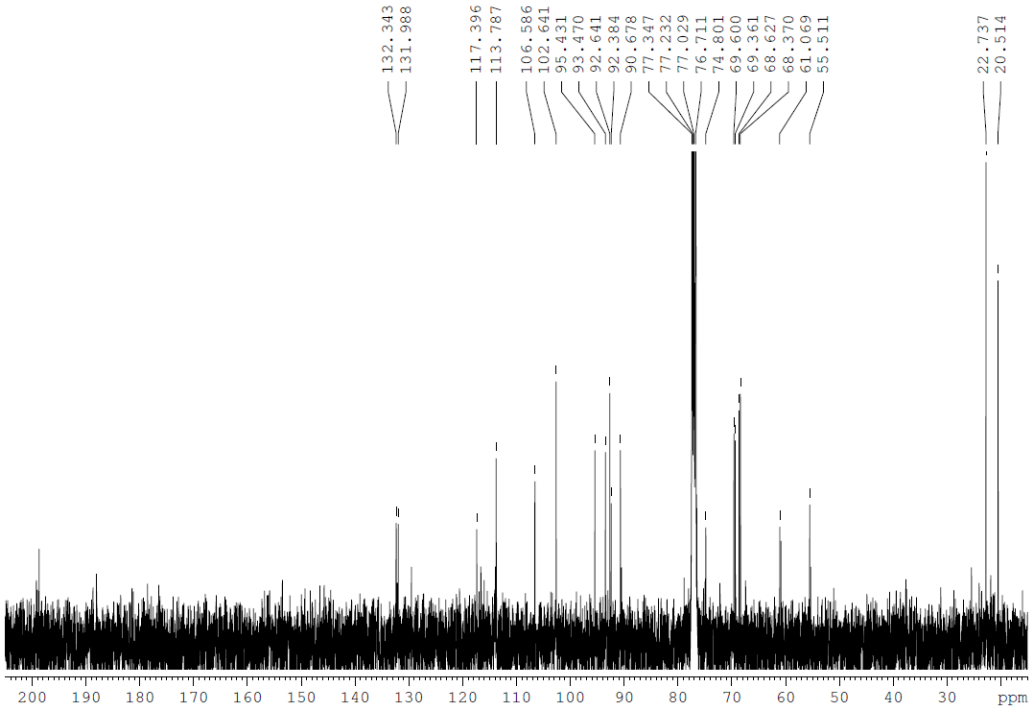


Figure SI 14: ^{13}C NMR spectrum of **(±)-**5-hydroxy-4-fluoropentane-2,3-dione (**2, non-hydrated**) in $CDCl_3$.

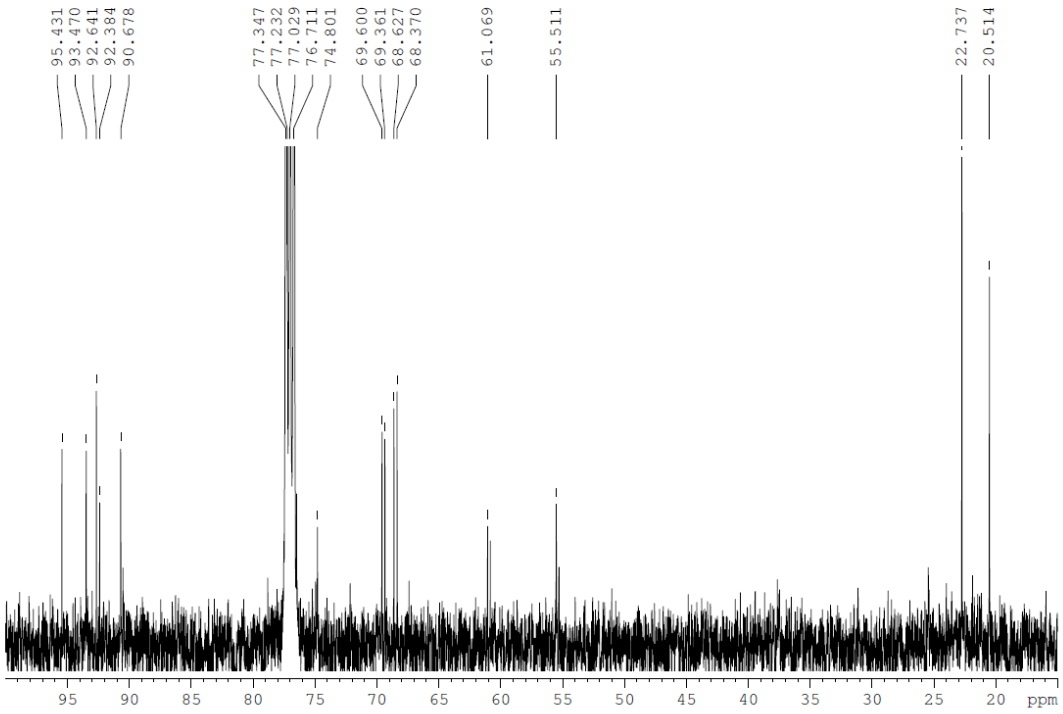


Figure SI 15: Section of ^{13}C NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, non-hydrated**) in $CDCl_3$.

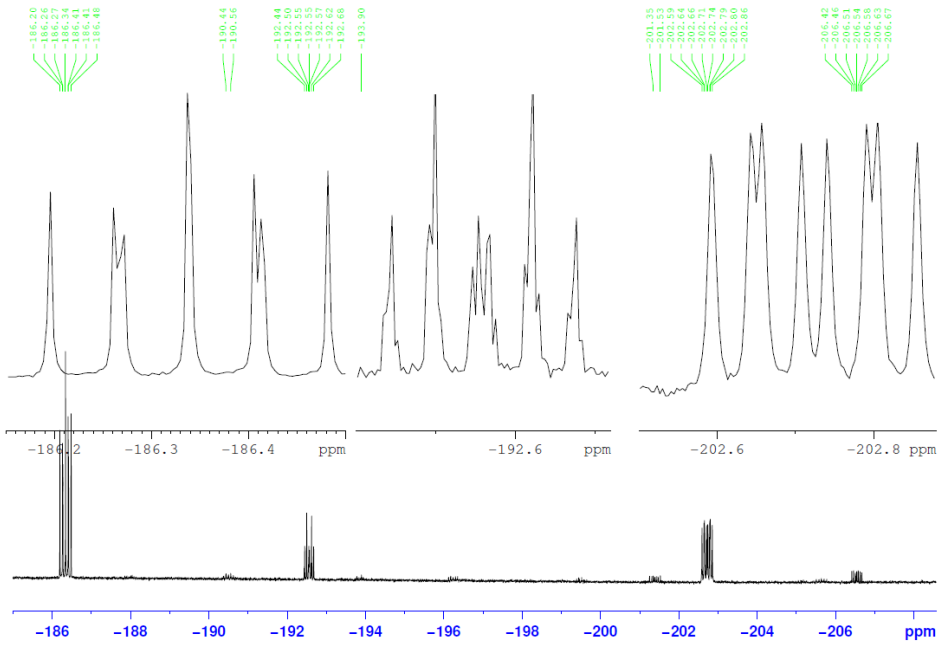
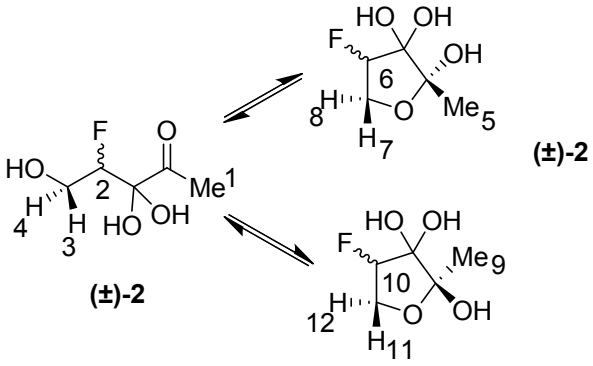


Figure SI 16: ^{19}F NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, non-hydrated**) in $CDCl_3$.

Hydrated isomer:



^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15-4.95 (1H, m), 4.35-4.04 (2H, m), 2.57 (d, J 1.2 Hz, Me non-cyclic). 1.78 (s, Me cyclic), 1.77 (s, Me cyclic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.0 (C=O), 115.8, 113.8, 106.2, 103.4, 95.9 (d, $^{1}J_{C\text{-}F}$ 190.1 Hz), 95.7 (d, $^{1}J_{C\text{-}F}$ 194.2 Hz), 92.6 (d, $^{1}J_{C\text{-}F}$ 189.1 Hz), 77.8 (d, $^{2}J_{C\text{-}F}$ 22.2 Hz), 75.2 (d, $^{2}J_{C\text{-}F}$ 29.3 Hz), 71.2 (d, $^{2}J_{C\text{-}F}$ 24.1 Hz), 70.2 (d, $^{2}J_{C\text{-}F}$ 22.5 Hz), 60.2 (d, $^{2}J_{C\text{-}F}$ 20.9 Hz), 25.0 (d, J 2.9 Hz, Me, acyclic), 20.3 (Me, cyclic). ^{19}F NMR (375 MHz, $CDCl_3$) δ -197.8 (ddd, $^{2}J_{F\text{-}H}$ 46.8, $^{3}J_{F\text{-}H}$ 27.4, $^{3}J_{F\text{-}H}$ 20.8 Hz), -183.2 (ddd, $^{2}J_{F\text{-}H}$ 57.2, $^{3}J_{F\text{-}H}$ 35.4, $^{3}J_{F\text{-}H}$ 21.8 Hz), -175.1(ddd, $^{2}J_{F\text{-}H}$ 58.5, $^{3}J_{F\text{-}H}$ 31.1, $^{3}J_{F\text{-}H}$ 27.1 Hz). GCMS m/z $[M]^{\cdot}$ 152.0. Accurate mass calcd for $C_5H_9O_4F$ (hydrated): 152.0479. Found: 152.0478.

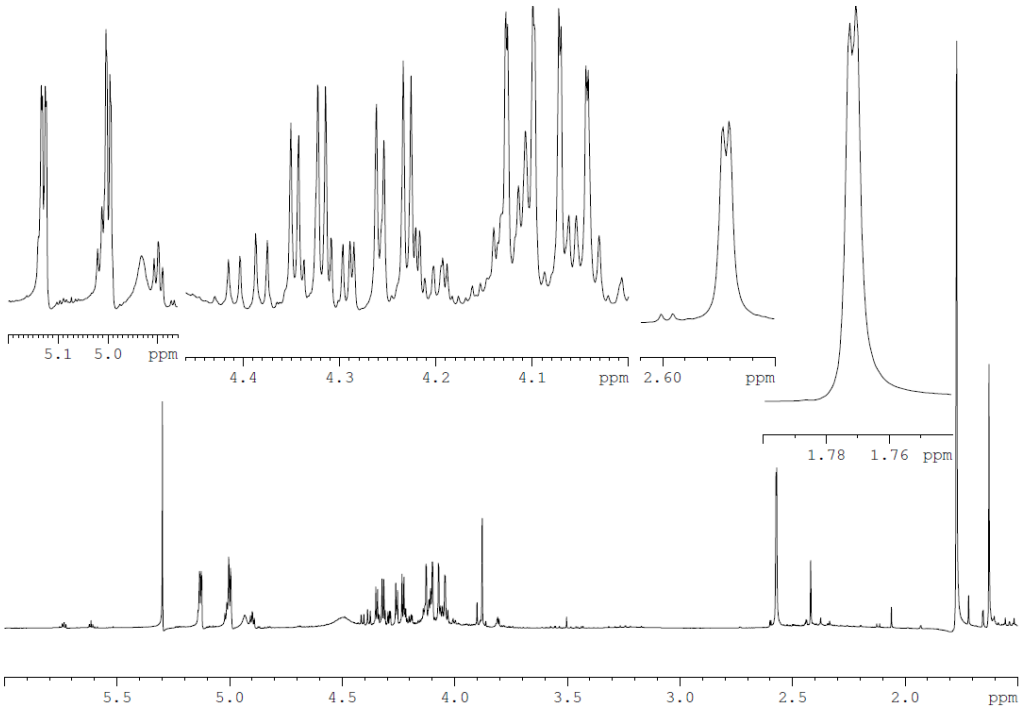


Figure SI 17: ^{1}H NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, hydrated**) in $CDCl_3$. The peak at ~1.56 (s) ppm is the residual water present in $CDCl_3$.

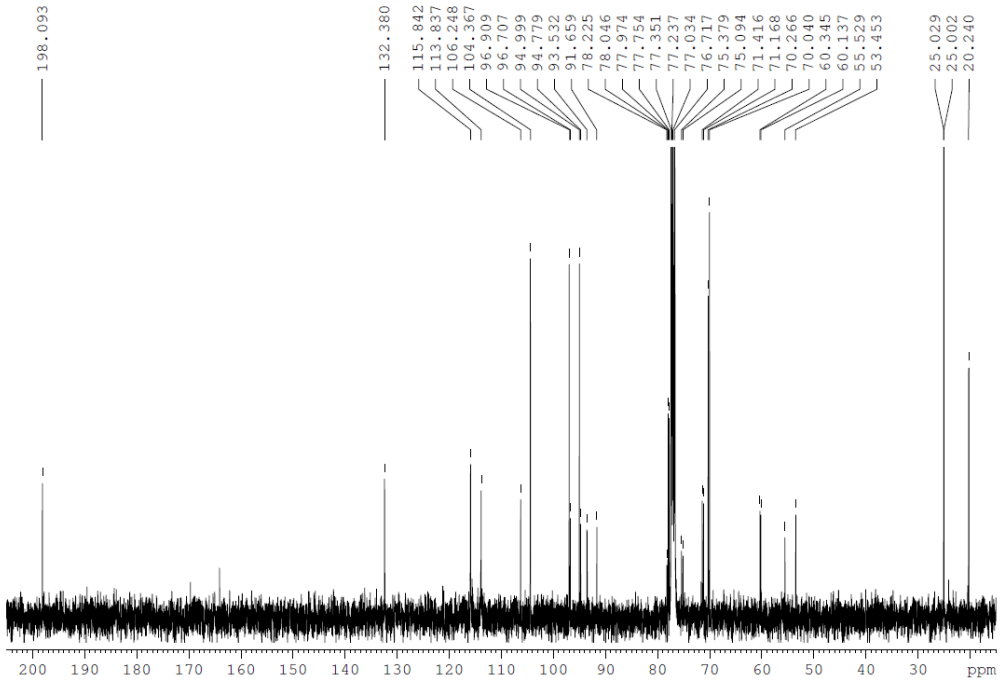


Figure SI 18: ^{13}C NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, hydrated**) in $CDCl_3$.

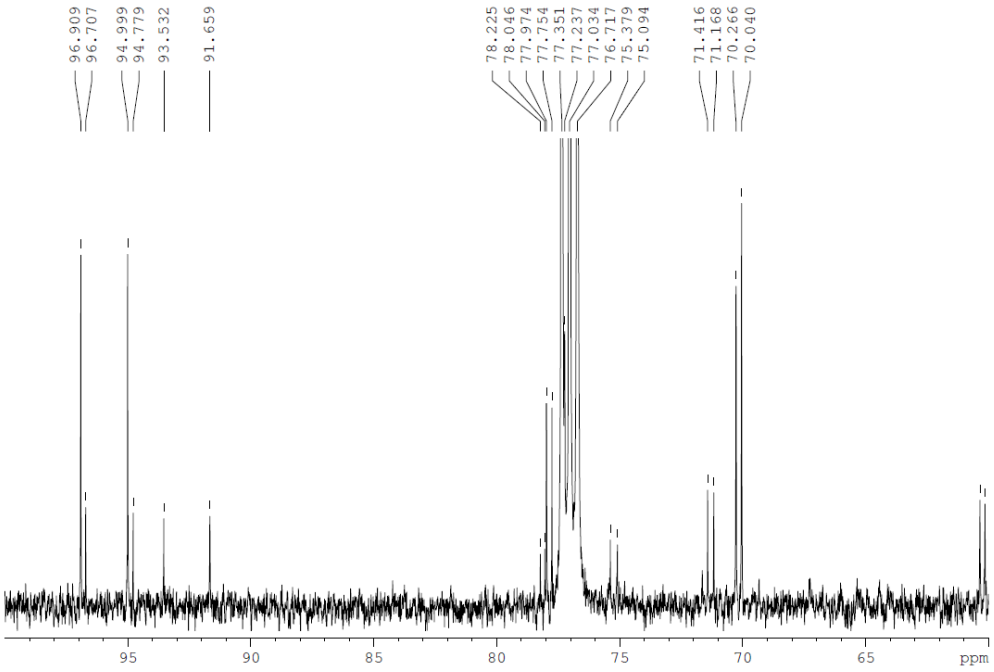


Figure SI 19: Section ^{13}C NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, hydrated**) in $CDCl_3$.

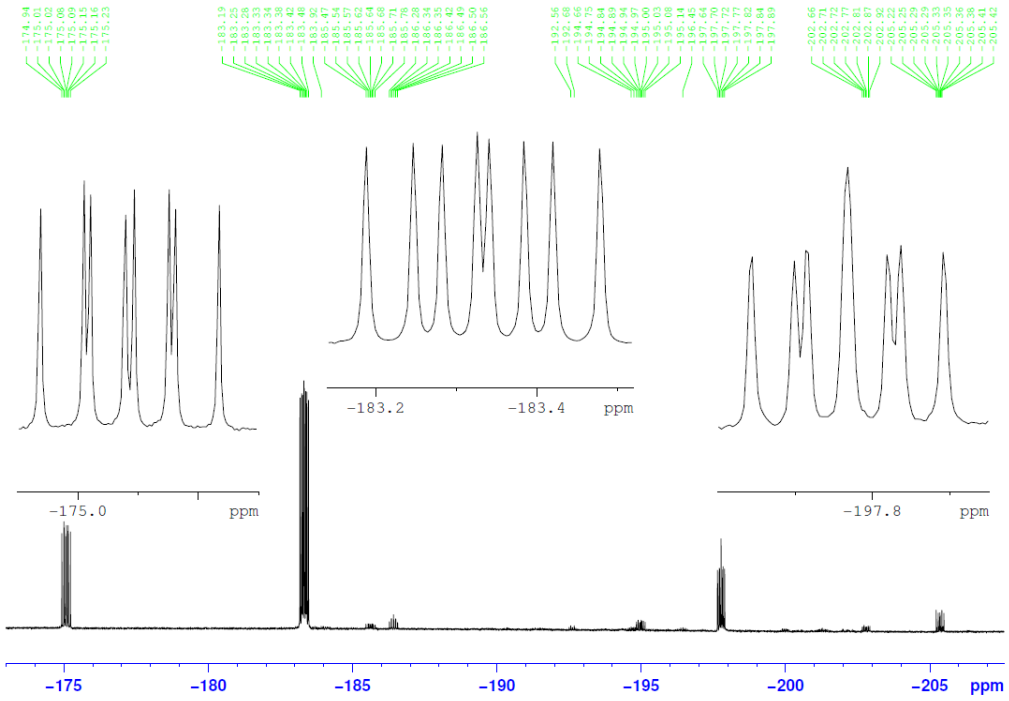
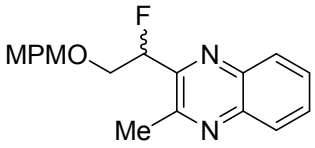


Figure SI 20: ^{19}F NMR spectrum of **(±)**-5-hydroxy-4-fluoropentane-2,3-dione (**2, hydrated**) in $CDCl_3$.

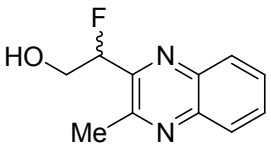
(±)-2-(1-Fluoro-2-(4-methoxybenzyloxy)ethyl)-3-methylquinoxaline (20)



(±)-20

To a solution of 5-(4'-methoxybenzyloxy)-4-fluoropentane-2,3-dione (**(±)-19**) (10 mg) in MeOH (5 ml) and a drop of conc HCl was added 1,2-phenylenediamine (5mg) at room temperature. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (25 ml) and the organic layer washed with water (10 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure**.** The residue was purified by flash column chromatography (ethyl acetate:hexane, 1:4) to give 11 mg (82 %) of (**(±)-20**) as an orange oil. The product was taken to next step for deprotection.

(±)-2-fluoro-2-(3-methylquinoxalin-2-yl)ethanol (21)



(±)-21

To a solution of 2-(1-fluoro-2-(4-methoxybenzyloxy)ethyl)-3-methylquinoxaline (**(±)-20**) (7mg, 0.21 mmol) in DCM (1.8 ml) and water (180 µl) was added DDQ (80 mg, 0.42 mmol) at room temperature. The reaction mixture was stirred for 3 h at room temperature and diluted with ethyl acetate (25 ml). Saturated aqueous $NaHCO_3$ (5 ml) was added and the resulting dark red solution was stirred vigorously for 15 min. The layers were separated and the organic layer was dried over anhydrous $MgSO_4$ and concentrated reduced pressure**.** The

residue was purified by flash column chromatography (ethyl acetate:hexane, 2:3) to give 2.4 mg (54 %) of (**(±)-21**) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (2H, dd, $^{2}J_{H-H}$ 8.4, $^{4}J_{H-H}$ 1.1Hz, Ar-H), 7.81-7.71 (2H, m, Ar-H), 5.84 (1H, dt, $^{2}J_{H-F}$ 46.5, $^{3}J_{H-H}$ 4.7Hz, H-2), 4.48-4.32 (m, 2H, H-1), 2.87 (3H, d, J 2.3 Hz, Ar-Me). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8 (C-Ar), 149.9 (C-Ar), 141.9 (C-Ar), 139.5 (C-Ar), 130.9 (CH-Ar), 129.6 (CH-Ar), 129.0 (CH-Ar), 128.5 (CH-Ar), 89.9 (d, $^{1}J_{C-F}$ 173.6, C-2), 62.9 (d, $^{2}J_{C-F}$ 24.1, C-1), 22.3 (d, $^{5}J_{C-F}$ 2.8, Ar-Me); ^{19}F NMR (375 MHz, $CDCl_3$) δ -188.1.

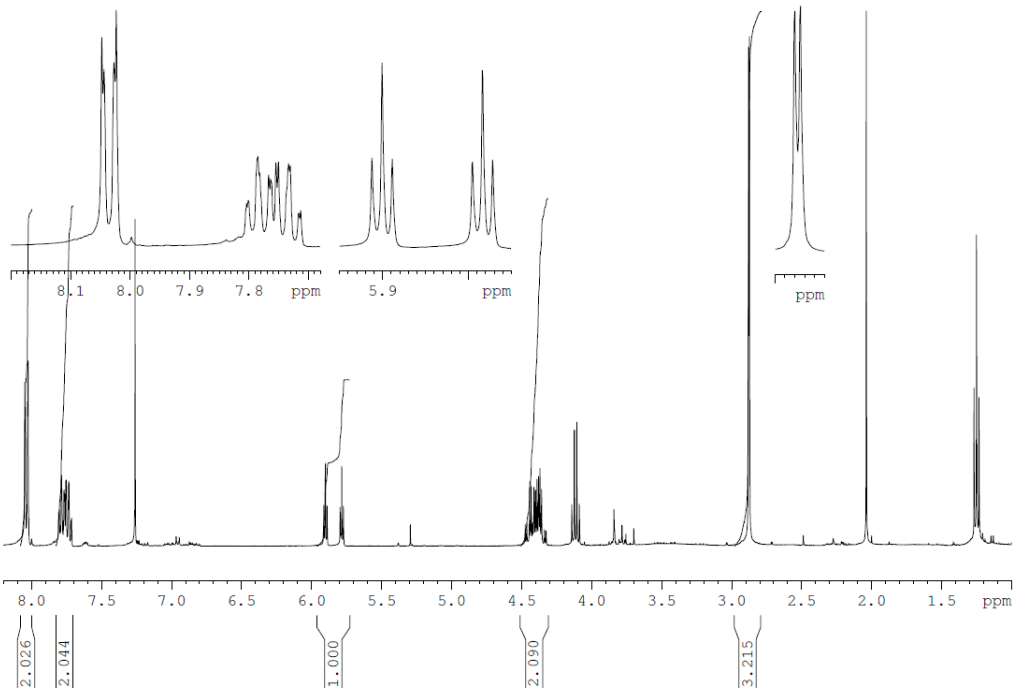


Figure SI 21: ^{1}H NMR spectrum of **(±)-**2-fluoro-2-(3-methylquinoxalin-2-yl)ethanol (**21**) in $CDCl_3$. The peaks at ~4.12 (q), 2.05 (s), 1.26 (t) ppm is the residual solvent ethyl acetate.

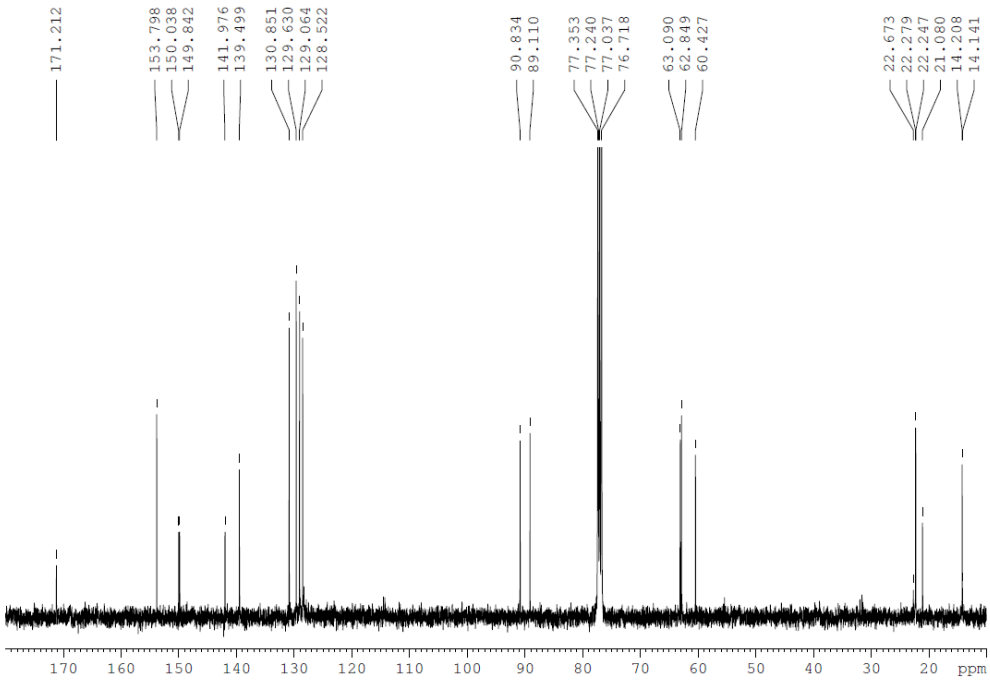


Figure SI 22: ^{13}C NMR spectrum of **(±)-**2-fluoro-2-(3-methylquinoxalin-2-yl)ethanol (**21**) in $CDCl_3$. The peaks at ~171.2, 60.4, 21.0, 14.1 ppm is the residual solvent ethyl acetate.

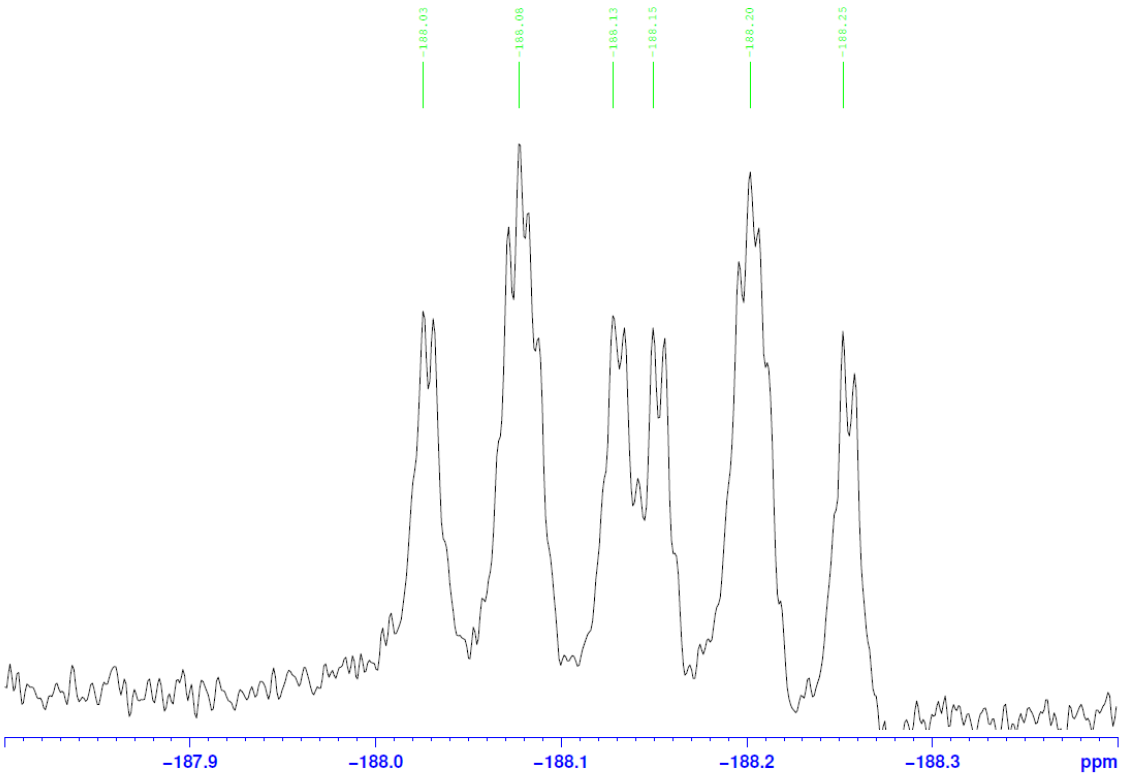


Figure SI 23: ^{19}F NMR spectrum of **(±)-**2-fluoro-2-(3-methylquinoxalin-2-yl)ethanol (**21**) in $CDCl_3$.

2. Microbiology:

2.1.1 Bioluminescence formation by *Vibrio harveyi* BB170

Vibrio harveyi BB170 is a mutant strain which responds only to AI-2.[7, 8] The AI-2 signalling molecule binds to receptor proteins on the bacterial cell surface leading to activation of the l*ux* operon. Expression of the *luxA* and *luxB* genes within this operon leads to the production of luciferase, an enzyme which catalyzes the oxidation of a long chain aliphatic aldehyde and a reduced flavin, with the liberation of excess free energy presenting as luminescence.[8] Thus, *V. harveyi* BB170 is a bacterial reporter strain frequently used to demonstrate the presence of AI-2 like molecules or inhibitors of AI-2 based quorum sensing through alterations in its bioluminescence production.[6-9]

2.1.2 Bioluminescence Assay

The biological activity of (S) DPD was assayed using Vibrio harveyi BB170 reporter strain that responds only to AI-2 like molecules.[6]

The bioluminescence assay was performed as described by Surette and Greenberg by testing different concentrations of synthetic DPD.

Vibrio harveyi BB170 ATCC number BAA-1117 was grown at 30 °C for 16 h with aeration in AB medium.

Bacterial culture was diluted to an OD600 of 0.2 and then was further diluted 1:5000 in fresh AB medium.

Aliquots (180 µl) of the culture were placed in a 96-well plate.

Doubling dilutions of synthetic DPD (20 µl) were prepared at 10 x in use concentration in sterile vibrio medium and were added to the 180 µl aliquots of inoculated AB medium.

Light production was measured using a luminescence reader (Biotek Synergy-2) every 30 min at 30 °C for up to 16 h.

(S)-DPD in sterile vibrio medium acted as a negative control

2.2. Inhibition of quorum sensing and biofilm formation in *Vibrio harveyi* BB170 by (±)F-DPD (2)

2.2.1. Bioluminescence inhibition assay

The biological activity of F-DPD (**2**) was investigated using *Vibrio harveyi* BB170, a reporter

strain that responds to AI-2 like molecules. Bioluminescence inhibition assays were performed as follows; *Vibrio harveyi* was grown for 16 h at 30°C with aeration in AB medium, diluted to an OD_{600} of 0.2 and then further diluted 1:5000 in sterile AB medium. Aliquots (180 µl) were delivered into a 96-well microtiter plate. F-DPD (**2**) was prepared in sterile water at 10 x in use concentrations and was diluted 1:10 (20 µl) into the bacterial inoculum. Plates were incubated for 40 h at 30 °C and luminescence was measured every 30 minutes using a BioTek Synergy-2 plate reader (Bio-Tek, UK). Fold decreases in luminescence at test F-DPD (**2**) concentrations were evaluated relative to peak luminescence of untreated control culture of *Vibrio harveyi* (positive control). Negative controls consisted of sterile AB growth medium containing F-DPD (**2**) at test concentrations. Ethanol controls were included.

2.2.2. Planktonic growth rate measurement

Diluted cultures of *Vibrio harveyi* containing graded concentrations of F-DPD (**2**) were prepared in a 96-well microtiter plate as described previously. Plates were incubated for 40 h at 30°C and optical density was measured every 30 minutes at 600 nm using a Titertek multiskan plate reader (Bio-Tek, UK). Positive controls (untreated *Vibrio harveyi*) negative controls (sterile AB growth medium containing F-DPD (**2**)) and ethanol controls were included.

2.2.3. Biofilm formation assay

Diluted cultures of *Vibrio harveyi* containing graded concentrations of F-DPD (**2**) were prepared in a 96-well microtiter plate as described previously. Aliquots (180 µl) were delivered into a 96-well microtiter plate. F-DPD (**2**) was prepared in sterile water at 10 x in use concentrations and was diluted 1:10 (20 µl) into the bacterial inoculum. Plates were incubated for 48 h at 30 °C, statically and aerobically in a humidified chamber to minimize evaporation. Excess media was decanted from the wells and the remaining biofilm was stained for 1 min with 1 % crystal violet solution before being washed 2 x with sterile PBS and left to air dry for 1 h. After drying bound crystal violet was solubilized in 200 µl of 70 % ethanol and $OD_{600\ was}$ determined relative to a sterile control.

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