Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary information

The Supplementary information contains the following figures:

The Supplementary information contains the following Tables:

Table S1. Effect of percentage agarose on exogenous VOC detection

Table S2. Effect of NaOH concentration on absorbance of aniline coupled with NQS

Table S3. Analytical data for 2-nitrophenol and aniline trapped in modified agarose gel

**S1. Optimisation of percentage agarose**

Percentage agarose had little effect on the absorbance readings when tested with 20 μg $mL^{-1}$ aniline in BHI (Table S1). However, 2 % agarose gave slightly higher and 0.5 % agarose slightly lower absorbance readings with 50 μg $mL^{-1}$ of 2-nitrophenol in BHI. In addition, gels containing 2 % agarose set more quickly than gels with a lower percentage of agarose. On that basis it was concluded that a 1% agarose gel was appropriate; this was applied to further work.

**S2. Optimisation of 2-nitrophenol detection method**

A yellow colour developed when agarose gel with added sodium hydroxide was exposed to 2-nitrophenol. Aliquots (100 μL, 150 μL and 200 μL) of 1% agarose with 2 mmol $L^{-1}$ NaOH were tested with 50 μg $mL^{-1}$ of 2-nitrophenol in BHI media. The smallest volume of agarose gel i.e. 100 μL was discarded due to downward curving of the surface of the gel. Absorption spectra of 2-nitrophenol trapped in agarose gel with both 150 μL and 200 μL of gel were generated (Figure S1). It was noted that an agarose gel volume of 150 μL produced the highest absorbance reading; on that basis 150 μL of agarose gel was used in all further work. In addition, the maximum absorption wavelength for 2-nitrophenol in 1 % agarose gel was 415 nm; all future 2-nitrophenol measurements were carried out at 415 nm.

The concentration range of sodium hydroxide added to agarose gel was assessed to determine the effect of increasing alkalinity on the yellow colour obtained after overnight incubation. Final NaOH concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mmol $L^{-1}$ in agarose

gel were tested with 50 μg $mL^{-1}$ of 2-nitrophenol in BHI media. The Absorbance signal, and visual yellow colour development, increased with increasing NaOH concentration. However, there was a limited increase in absorbance and colour development at a NaOH concentration greater than 2 mmol $L^{-1}$ (Figure S2); it was therefore concluded that the optimum NaOH in 1% agarose gel was 2 mmol $L^{-1}$.

**S3. Optimisation of aniline detection method**

An orange colour developed when NQS modified agarose gel was exposed to aniline. An absorption spectrum of aniline with 150 μL of 1% agarose gel modified with NQS was produced (Figure S3). The maximum absorption wavelength was 470 nm. All future measurements with aniline were carried out at 470 nm. An absorption spectrum of NQS against blank agarose gel indicated a maximum absorption wavelength of 355 nm and no overlap with the spectrum obtained for aniline coupled with NQS.

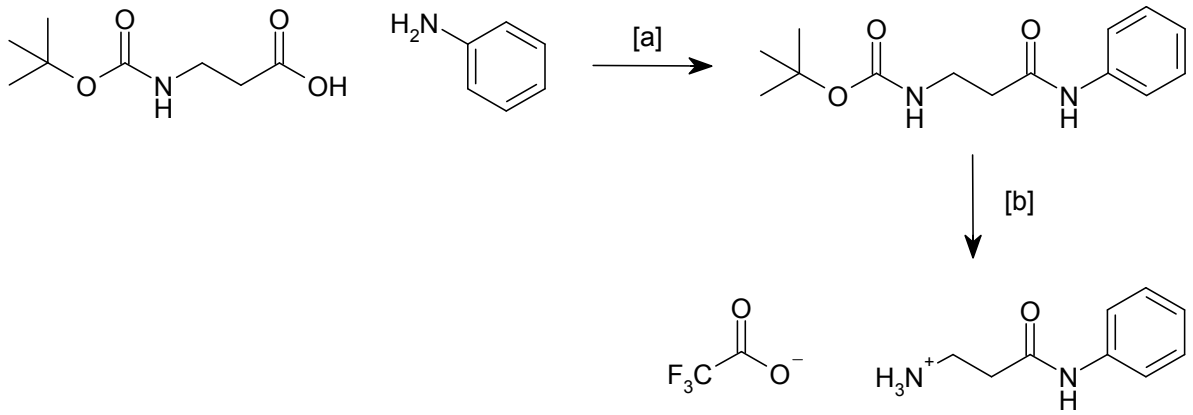
A range of NQS volumes added to agarose gel was tested (Figure S4); a 0.5 % NQS solution was added to 50 mL gel and the effect of amount of NQS in the gel was determined. NQS volumes of 0.05, 0.1, 0.125, 0.25, 0.5, 1.0 mL in 50 mL agarose gel were tested with 20 μg $mL^{-1}$ aniline in BHI media. The orange colour became more intense as NQS volume increased. However, at higher NQS volumes (1.0 and 0.5 mL), a red precipitate formed in the gel, therefore 0.25 mL NQS (0.5 %) was applied to further work.

The addition of NaOH to agarose gel and its effect on colour development was investigated. Final NaOH concentrations of 0, 0.01, 0.1, 0.5, 1.0 and 2.0 mmol $L^{-1}$ NaOH in 50 mL agarose gel with 0.25 mL NQS (0.5%) were tested with 20 µg $mL^{-1}$ aniline in BHI media (Table S2). The addition of NaOH had no effect on absorbance readings; therefore NaOH was omitted from all further work with aniline.

**S4 Analytical calibration data for 2-nitrophenol and aniline**

The linear range for 2-nitrophenol in BHI media, measured at 415 nm, was 10 - 50 µg $mL^{-1}$; whereas for aniline, measured at 470 nm, it was 5 - 20 µg $mL^{-1}$ in BHI media. All measurements were taken following 18 h of incubation at 37 °C. Analytical data for the quantification of 2-nitrophenol and aniline trapped in modified agarose gels are given in Table S3.

**S5. Synthesis of 3-amino-N-phenylpropanamide, TFA**




[a] DCCI, N-hydroxy succinimide [b] TFA

**S6. Analytical data for 3-amino-N-phenylpropanamide, TFA**

3-Amino-N-phenylpropanamide, TFA salt, obtained as an off white powder, m.p. 105-107 °C. LRMS (ESI) for $C_9H_{12}N_2O$. Calculated mass of molecular ion 165.21 $[M+H]^+$. Measured mass: 164.93. $^1$H-NMR (400 MHz; $d_6$-DMSO) $\delta_H$ 10.13 (1H, s, N*H*), 7.75 (3H, s, N$H_3^+$), 7.55 (2H, d, *J* = 7.6 Hz, Ar-*H*), 7.26 (2H, t, *J* = 8.0 Hz, Ar-*H*), 7.00 (1H, t, *J*= 7.4 Hz, Ar-*H*), 3.04 (2H, t, *J* = 7.0

Hz, C$H_2$), 2.65 (2H, t, *J* = 6.6 Hz, C$H_2$) (Figure S5); $^{13}$C-NMR (101 MHz; $d_6$-DMSO) $\delta_C$ 168.3 (*C*=O), 159.0 (q, *J* = 38 Hz, $CF_3CO_2^-$), 139.7 (Ar-*C*), 129.3 (Ar-*C*), 124.0 (Ar-*C*), 119.6 (Ar-*C*), 115.9 (q, J = 288 Hz, $CF_3CO_2^-$), 35.5 ($CH_2$), 33.9 ($CH_2$) (Figure S6).

**S7. Analytical data for 3-amino-N-phenylpropanamide**

3-Amino-N-phenylpropanamide was obtained as a white solid. $^1$H-NMR (400 MHz; $CDCl_3$) $\delta_H$ 9.98 (1H, broad s, N*H*), 7.51 (2H, d, J = 8 Hz, Ar-*H*), 7.26 (2H, t, J = 8 Hz, Ar-*H*), 7.02 (1H, t, J = 8 Hz, Ar-*H*), 3.05 (2H, broad m, C$H_2$), 2.43 (2H, t, J = 6 Hz, C$H_2$). (Figure S7). This data is consistent with that reported in the literature.[15]

$^{13}$C-NMR (101 MHz; $CDCl_3$) $\delta_C$ 171.2 (*C*=O), 138.5, (Ar-*C*), 129.0 (Ar-*C*), 123.9 (Ar-*C*), 119.9 (Ar-*C*), 38.8 ($CH_2$), 38.0 ($CH_2$). (Figure S8).

**Fig. S1. Absorption spectrum of 2-nitrophenol in NaOH modified - agarose gel (1%). Gel volume (◇) 150 µL, (■) 200 µL**

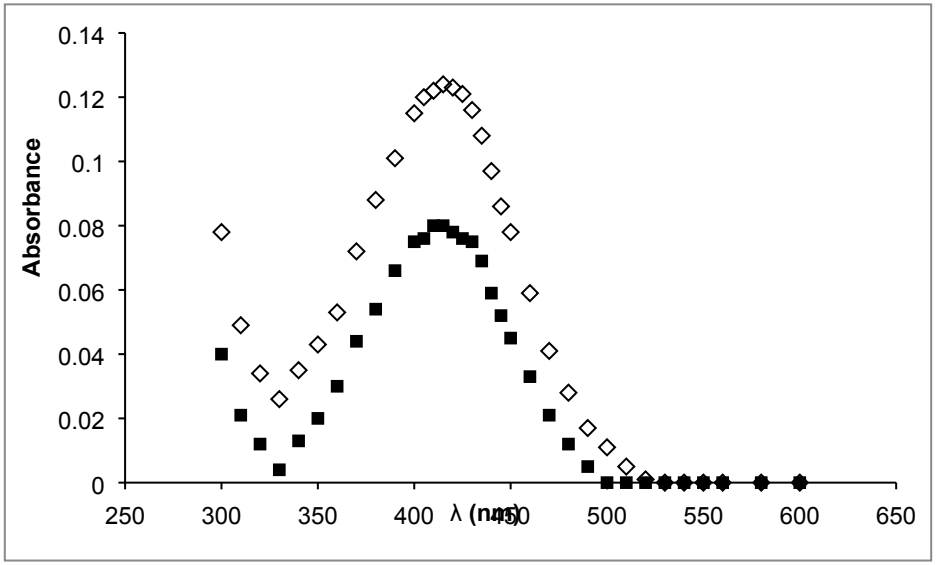
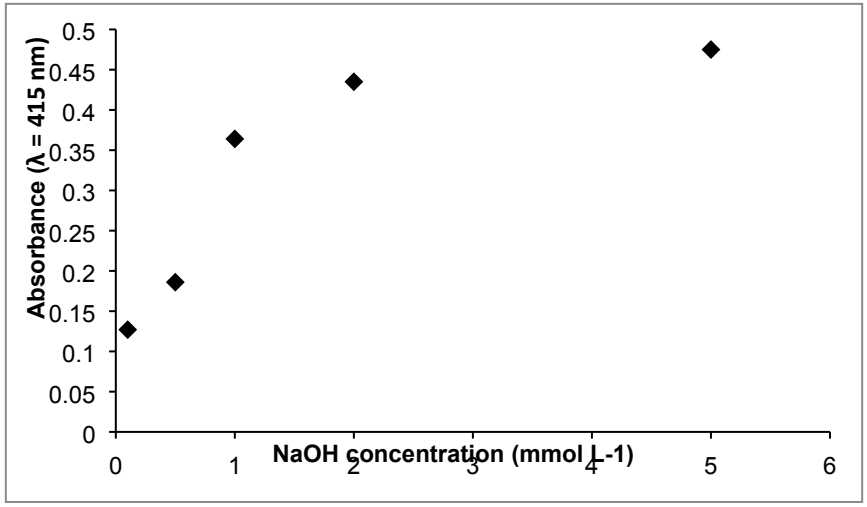


**Fig. S2. Effect of NaOH concentration on absorbance of 2-nitrophenol**




50 µg $mL^{-1}$ 2-nitrophenol in BHI; 150 µL gel; overnight incubation.

**Fig. S3. Absorption spectra in NQS modified - agarose gel (1%): (◇) aniline coupled with NQS in gel against reagent blank, (■) NQS against agarose gel reagent blank**

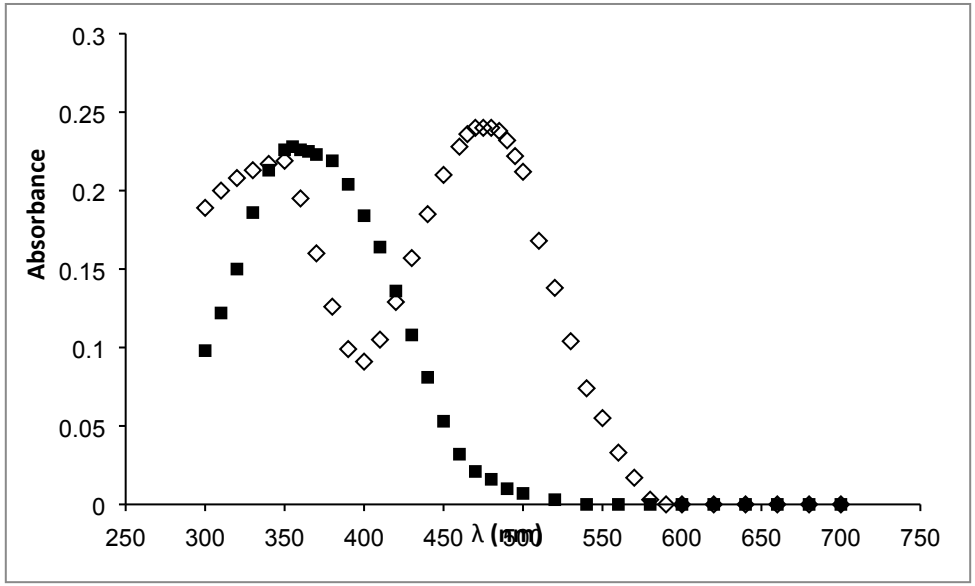
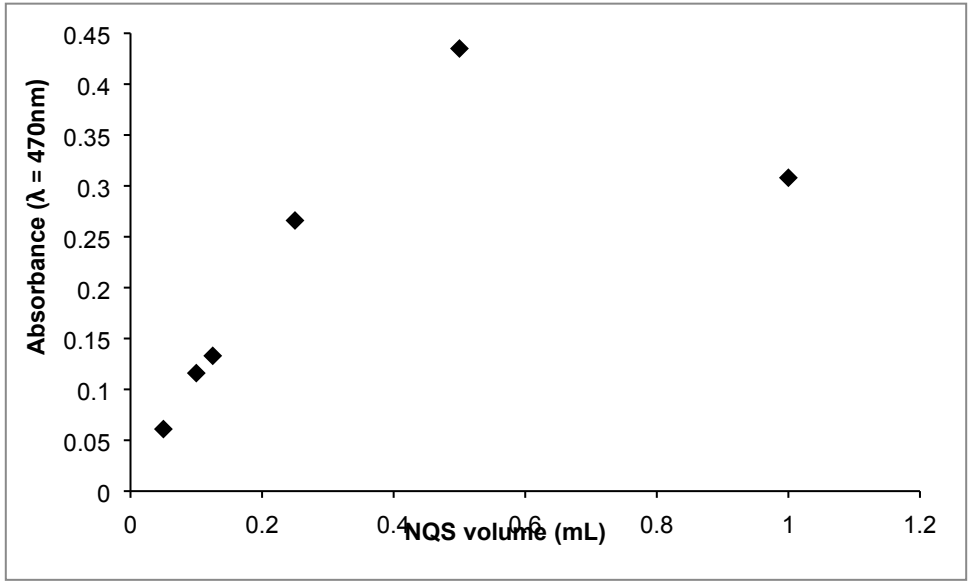


**Fig. S4. Effect of NQS volume on absorbance of aniline coupled with NQS**




20 µg $mL^{-1}$ aniline in BHI, 150 µL gel, overnight incubation.

**Fig. S5. $^{1}$H-NMR for 3-amino-N-phenylpropanamide, TFA**

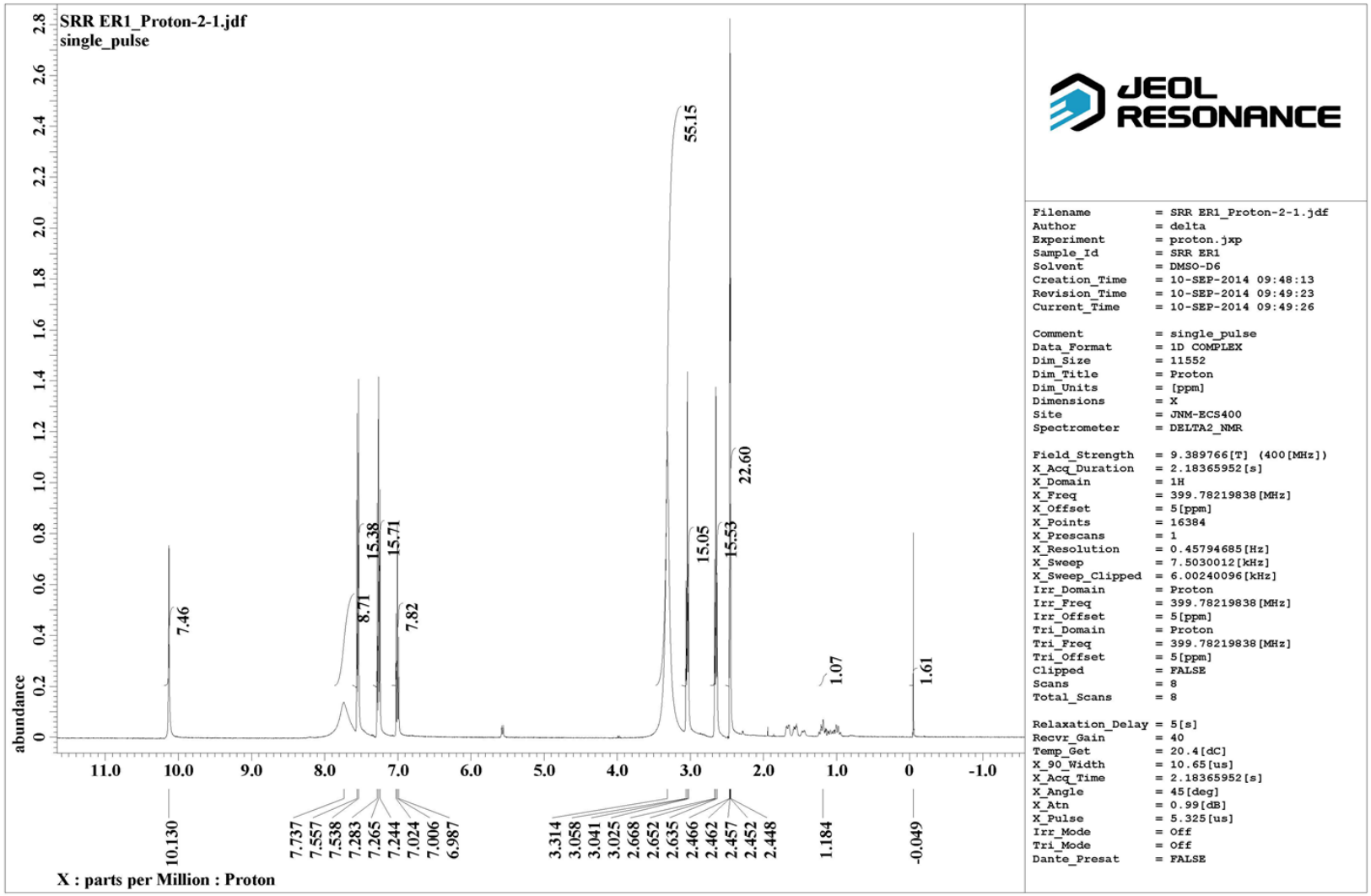


**Fig. S6. $^{13}$C-NMR for 3-amino-N-phenylpropanamide, TFA**

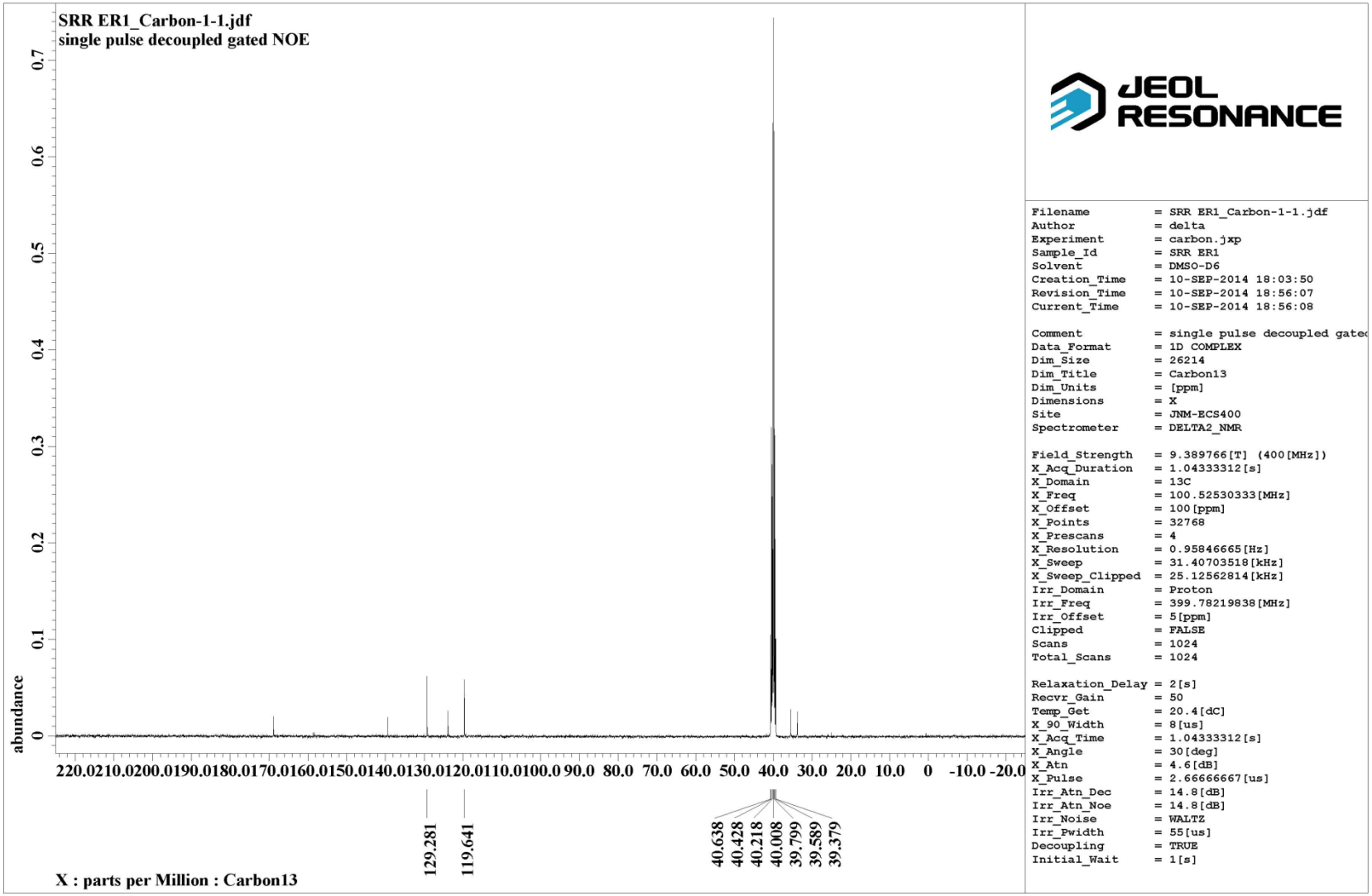


**Fig. S7. $^{1}$H-NMR for 3-amino-N-phenylpropanamide**

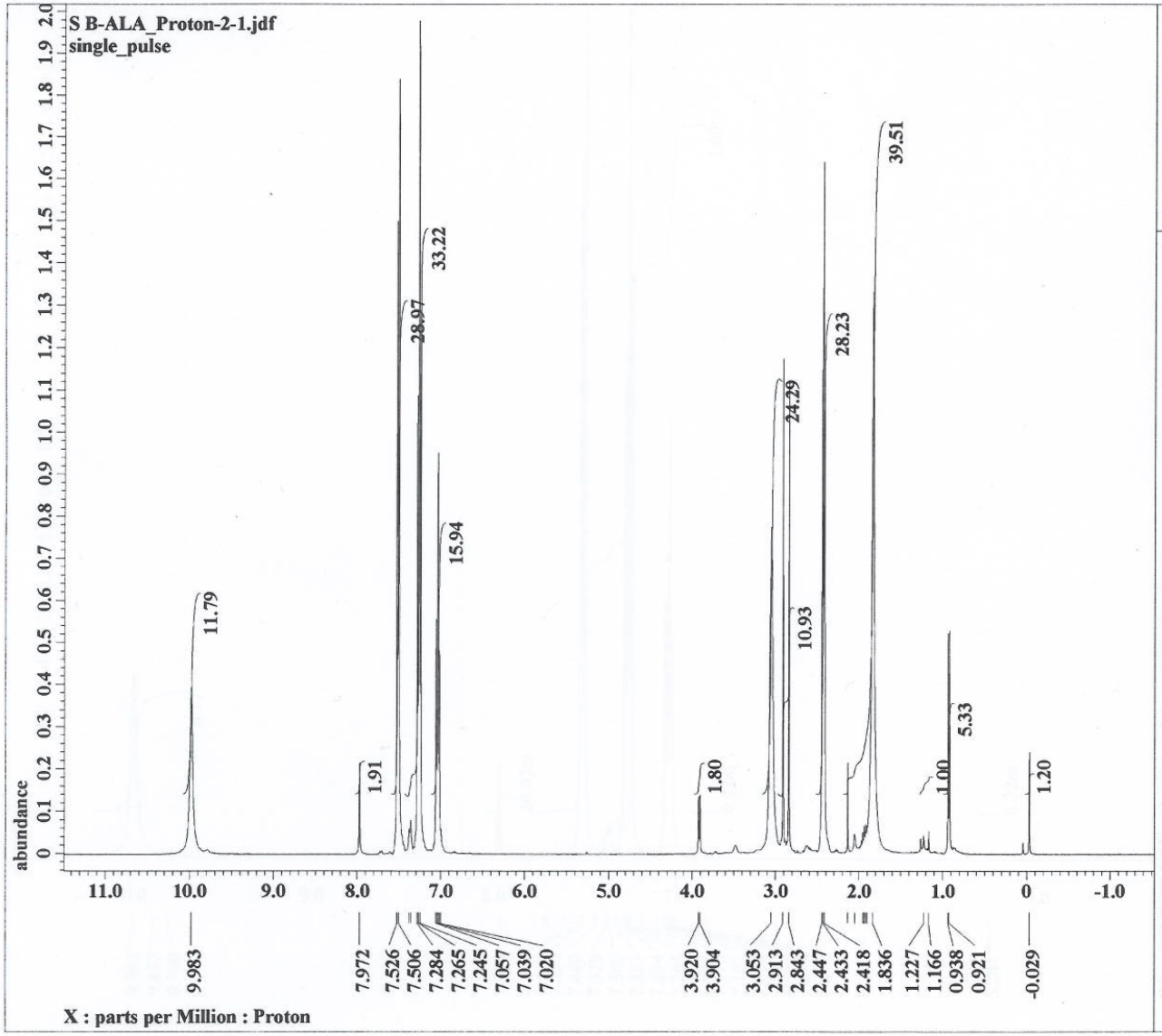



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**Fig. S8. $^{13}$C-NMR for 3-amino-N-phenylpropanamide**

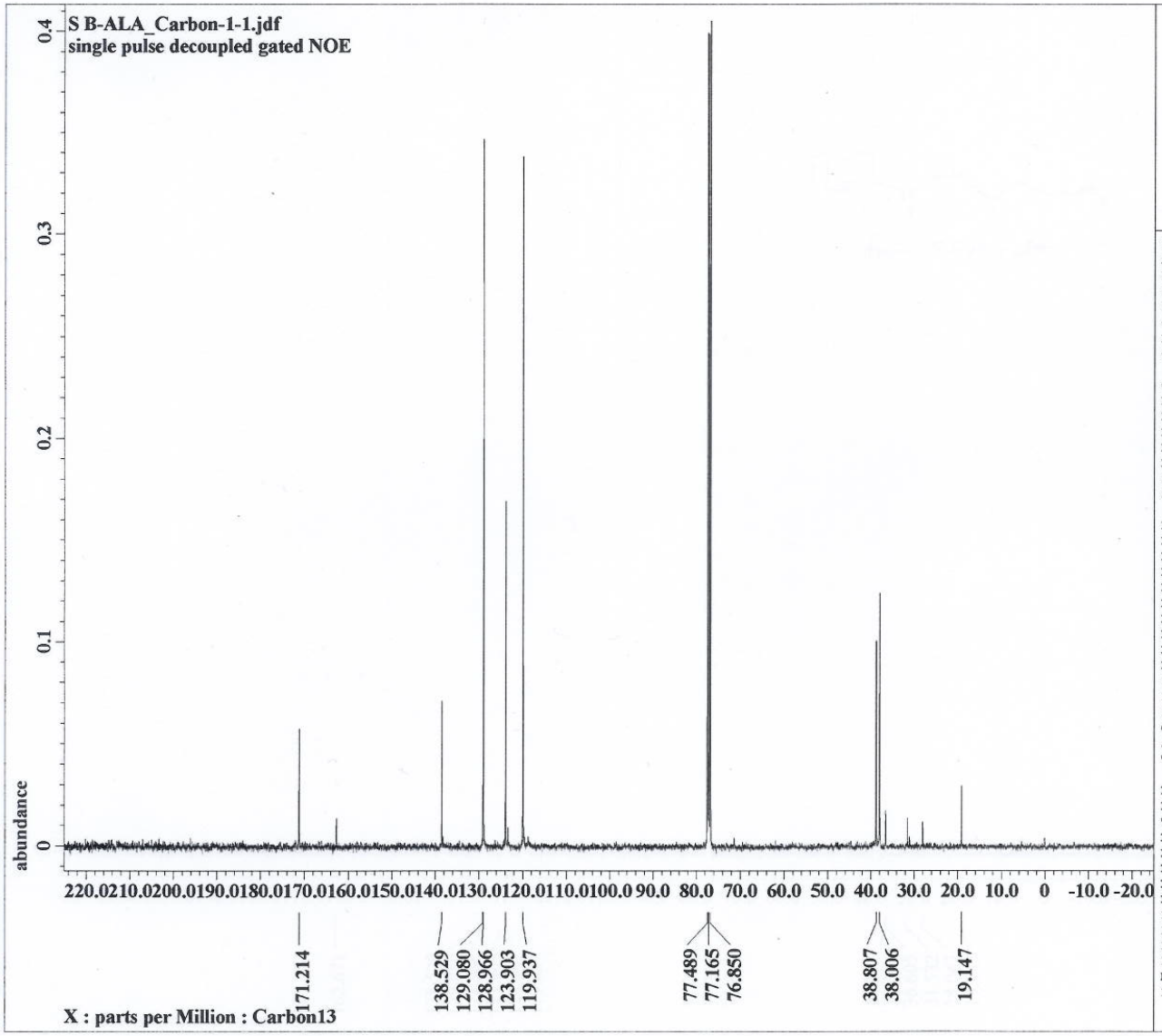


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**Table S1. Effect of percentage agarose on exogenous VOC detection**

| % Agarose* | 2-Nitrophenol (absorbance at 415 nm)† | Aniline (absorbance at 470 nm)‡ |
|---|---|---|
| 0.5 | 0.138 | 0.132 |
| 1 | 0.147 | 0.134 |
| 2 | 0.165 | 0.124 |

*150 µL volume; †50 µg $mL^{-1}$ 2-nitrophenol in BHI, overnight incubation; ‡20 µg $mL^{1}$ aniline in BHI, overnight incubation.

**Table S2. Effect of NaOH concentration on absorbance of aniline coupled with NQS**

| NaOH concentration (mmol $L^{-1}$)† | Absorbance at 470 nm* |
|---|---|
| 0 | 0.123 |
| 0.01 | 0.124 |
| 0.1 | 0.129 |
| 0.5 | 0.125 |
| 1 | 0.125 |
| 2 | 0.09 |

*20 µg $mL^{-1}$ aniline in BHI; †0.25 mL NQS (0.5 %) in 150 µL gel.

**Table S3. Analytical data for 2-nitrophenol and aniline trapped in modified agarose gel**

| VOC | Linear range ($\mu g\ mL^{-1}$) | y = mx + c | Correlation coefficient $r^2$ | n* | $\lambda_{max}$ (nm) |
|---|---|---|---|---|---|
| 2-Nitrophenol | 10 - 50 | y = 0.0024x + 0.0055 | 0.9998 | 4 | 415 |
| Aniline | 5 - 20 | y = 0.0095x + 0.0165 | 0.9901 | 4 | 470 |

*n, number of points on calibration curve.