Chemoselective detection and discrimination of carbonyl-containing compounds in metabolite mixtures by $^1$H-detected $^{15}$N NMR


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Experimental procedures for synthesis of $^{15}$N-QDA

$^{15}$N-hydroxphthalimide (1). A flask fitted with a Dean-Stark trap was charged with hydroxylamine hydrochloride (0.52 g, 7.4 mmol) and toluene (25 mL). To the suspension were added phthalic anhydride (1.15 g, 7.8 mmol) and pyridine (600 $\mu$L, 7.4 mmol). The reaction solution was stirred and heated to reflux. After 4h, the solution was allowed to cool to room temperature whereupon the product precipitated. The precipitate was collected by filtration and washed with Et$_2$O (15 mL) and then cold water (15 mL). The filtered solution was concentrated by rotary evaporation until more precipitation occurred. The second precipitated crop was collected and washed with Et$_2$O (10 mL) and cold water (15 mL). The washed solids were combined and dried in a vacuum desiccator to afford 1 (0.77 g, 63%) as a light yellow solid; mp 230-231 °C; $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 10.80 (s, 1 H), 7.82 (s, 4 H); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) $\delta$ 164.2, 164.0, 134.5, 128.8, 128.7, 123.0; $^{15}$N NMR (DMSO-d$_6$, 40.5 MHz) $\delta$ 155.9.

$N$-(2-hydroxyethyl)$-N,N$-dimethyl-1-dodecylammonium bromide (2). To a solution of $N,N$-dimethylethanolamine (1.0 g, 11.2 mmol) in acetone (50 mL) was added 1-bromododecane (3.0 g, 12.3 mmol). The reaction mixture was heated at reflux 40 h before cooling to 0 ºC. The precipitated solids were collected by filtration, washed with Et$_2$O (20 mL) and then dried in a vacuum desiccator. Bromide 2 (3.0 g, 80%) was obtained as a white solid; mp 195-199 °C (lit. $^1$ mp 193 °C); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 5.00 (bb, 1 H), 4.12 (t, $J$ = 4.8 Hz, 2 H), 3.74 (t, $J$ = 4.8 Hz, 2 H), 3.53 (m, 2 H), 3.36 (s, 6 H), 1.74 (m, 2 H), 1.33 (m, 4 H), 1.28-1.24 (m, 14 H), 0.86 (t, $J$ = 6.8 Hz, 3 H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 66.4, 65.8, 56.1, 52.3, 32.1, 29.8, 29.6, 29.6, 29.5, 29.4, 26.4, 23.1, 22.9, 14.3.

Scheme 1. Synthesis of $^{15}$N-QDA.

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N-(2-((1,3-dioxoisoindolin-2-yl)oxy)ethyl)-N,N-dimethyldecane-1-aminium bromide (3). To a solution of ammonium bromide 2 (0.68 g, 2.0 mmol) in a 2:1 mixture of THF:CH2Cl2 (15 mL) at room temperature were added successively phthalimide 1 (0.30 g, 1.8 mmol), triphenylphosphine (0.53 g, 2.0 mmol) and DIAD (400 μL, 2.0 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction solvents then were removed by rotary evaporation and EtOAc (20 mL) was added to the residue. The resultant suspension was briefly sonicated (bath sonicator, room temperature, 5 min). The undissolved solids were collected by filtration and then recrystallized from EtOAc to afford phthalimide 3 (465 mg, 53%) as a white solid; mp 128-131 °C; 1H NMR (CDCl3, 400 MHz) δ 7.81 (m, 2 H), 7.76 (m, 2 H), 4.73 (m, 2 H), 4.35 (m, 2 H), 3.74 (m, 2 H), 3.54 (s, 6 H), 1.80 (m, 2 H), 1.34 (m, 4 H), 1.26-1.19 (m, 14 H), 0.83 (t, J = 6.8 Hz, 3 H); 13C NMR (CDCl3, 175 MHz) δ 163.2, 128.6, 72.6, 66.5, 62.1, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 26.4, 23.0, 22.8, 14.3; 15N NMR (CDCl3, 40.5 MHz) δ 170.2. HRMS, calc’d C24H39N15NO3+ 404.29255, found 404.29254.

N-(2-15N-aminoxyethyl)-N,N-dimethyl-1-dodecylammonium bromide (15N-QDA). To a solution of phthalimide 3 (213 mg, 0.44 mmol) in CH2Cl2 (5 mL) at room temperature was added hydrazine monohydrate (85 μL, 1.75 mmol) in one portion. The reaction mixture was stirred at room temperature for 1 h and then filtered to remove any solids. The filtrate was concentrated to dryness by rotary evaporation. The residue was dissolved by addition of CH2Cl2 and the resultant suspension was filtered to remove the insoluble solids. The filtrate was then evaporated and the solid obtained was dried under high vacuum to afford 15N-QDA (147 mg, 95%); 1H NMR (CDCl3, 400 MHz) δ 6.00-5.75 (bb, 2 H), 4.12 (m, 2 H), 3.92 (m, 2 H), 3.54 (m, 2 H), 3.37 (s, 6 H), 1.68 (m, 2 H), 1.28 (m, 4 H), 1.26-1.19 (m, 14 H), 0.82 (t, J = 6.8 Hz, 3 H); 13C NMR (CDCl3, 100 MHz) δ 69.1, 65.6, 62.3, 51.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 26.4, 23.0, 22.8, 14.2; 15N NMR (CDCl3, 40.5 MHz) δ 96.6; HRMS, calc’d C16H37N15NO+ 274.2871, found 274.2872.
Figure S1: $^1$H, $^{13}$C and $^{15}$N NMR spectra of $^{15}$N-QDA

A. $^1$H NMR (CDCl$_3$, 400 MHz)
B. $^{13}$C NMR (CDCl$_3$, 100 MHz)

C. $^{15}$N NMR (CDCl$_3$, 40.5 MHz)
Figure S2. Modulation of intensity by INEPT delay for mixture of standards

1D HSQC spectra of the standard mixture of QDA* derivatives were recorded at 18.8 T, 20°C as described in the methods. The INEPT delay was systematically varied as shown to the right, in terms of the effective $J_{NH}$ value (in Hz). The optimum delay is close to 3 Hz.
Figure S3. NMR spectra of derivatized compounds in MeOD

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Spectra were recorded at 18.8 T as described in the text. A. HSQC of the standard mixture recorded with a $J_{NH}$ value set to 3 Hz. Assignments are shown on the Figure.

B. HSQC-TOCSY as A with a TOCSY mixing time of 60 ms. Red, green and cyan boxes denote aldehyde, sugars and ketone resonances, respectively. The horizontal red lines show TOCSY correlations from the aldoxime H1 to the H2 of the open chain sugar.
The standards mixture was prepared as described in the text. TOCSY spectra were recorded at 18.8 T with acquisition times of 0.512 s in $t_2$ and 31.25 ms in $t_1$, with a spin lock mixing time of 60 ms at a strength of 8 kHz. The data tables were zero filled once in $t_2$, linear predicted once in $t_1$ and zero filled to 2048 points. The free induction decays were processed using an unshifted Gaussian and a line broadening exponential of 2 Hz in both dimensions.

Upper: Unedited TOCSY showing all proton proton correlations
Lower: $^{15}$N HSQC edited TOCSY showing only compounds with scalar couplings of ca 2-3 Hz to $^{15}$N.