Supporting Information

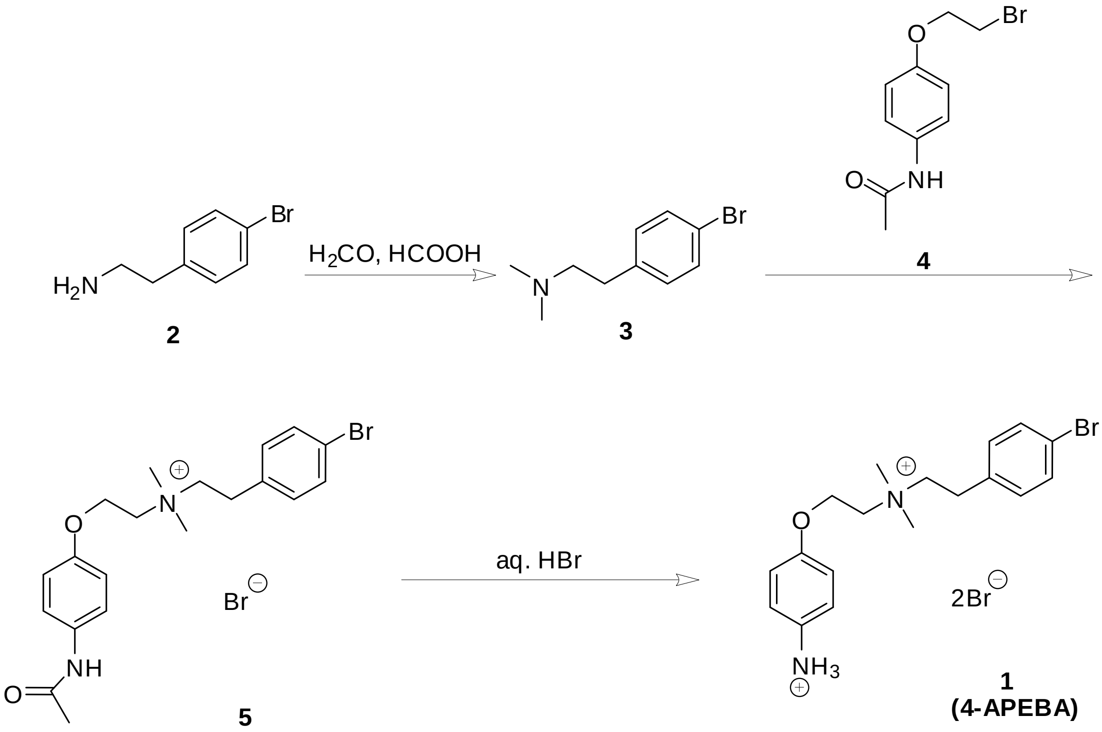
Targeted LC-MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA

Mark Eggink[a], Maikel Wijtmans[b], Ansgar Kretschmer[a], Jeroen Kool[a], Henk Lingeman[a], Iwan J. P. de Esch[b], Wilfried M. A. Niessen[a],*, Hubertus Irth[a]

VU University Amsterdam, Faculty of Science, [a]Section of BioMolecular Analysis, and [b]Pharmacochemistry, Section of Medicinal Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, the Netherlands.

Corresponding author: W.M.A. Niessen, mail@hyphenms.nl

Supporting Data: Synthesis route toward 4-APEBA (1) and chemical characterization of products



2-(4-Bromophenyl)-*N*,*N*-dimethylethanamine (3). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.28 (d, 2H, J=8.4 Hz), 6.96 (d, 2H, J=8.4 Hz), 2.70-2.58 (m, 2H), 2.42-2.34 (m, 2H), 2.17 (s, 6H).

2-(4-Acetamidophenoxy)-*N*-(4-bromophenethyl)-*N*,*N*-dimethylethanaminium bromide (5). ^{1}H-NMR (CD_3OD, 250 MHz) δ 7.47 (d, 2H, J=8.25 Hz), 7.46 (d, 2H, J=9.0 Hz), 7.26 (d, 2H, J=8.25 Hz), 6.93 (d, 2H, J=9.0 Hz), 4.51-4.43 (m, 2H), 3.94-3.87 (m, 2H), 3.72-3.63 (m, 2H), 3.28 (s, 6H), 3.20-3.12 (m, 2H), 2.08 (s, 3H). ^{13}C-NMR (CD_3OD, 50 MHz) δ 171.48, 155.46, 136.31, 134.20, 133.09, 132.21, 123.06, 122.20, 115.96, 66.95, 64.34, 63.39, 52.48, 29.40, 23.69.

4-(2-((4-Bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide (1, 4-APEBA). ^{1}H-NMR (D_2O, 250 MHz) δ 7.61 (d, 2H, J=8.25 Hz, H_a), 7.41 (d, 2H, J=9.0 Hz, H_i), 7.30 (d, 2H, J=8.25 Hz, H_b), 7.15 (d, 2H, J=9.0 Hz, H_h), 4.64-4.56 (m, 2H, H_g), 3.99-3.94 (m, 2H, H_f), 3.79-3.71 (m, 2H, H_d), 3.34 (s, 6H, H_e), 3.29-3.21 (m, 2H, H_c). For assignment codes of the protons, see Figure S6. ^{13}C-NMR (D_2O, 50 MHz) δ 158.58, 136.04, 133.23, 132.57, 126.00, 124.62, 122.12, 117.46, 66.71, 64.04, 63.62, 53.46, 29.54. 2D-NOESY (D_2O, 400 MHz): Diagnostic couplings: H_f x H_e; H_f x H_d; H_f x H_g; H_g x H_h; H_h x H_i; H_e x H_d; H_c x H_d; H_c x H_b (weak but present); H_a x H_b. M.p.=231.1-232.5 °C. LC-UV purity: >98 %. LC–MS purity: >98 %. IR (solid, cm^{-1}) ν 2780, 1657, 1507, 1250, 803, 520. HR-MS ($C_{18}H_{24}BrN_2O^+$): calc: 363.10665 (100 %), 365.10479 (99.48 %), found: 363.10684 (100 %), 365.10521 (99.17 %). Elem. Anal. ($C_{18}H_{25}Br_3N_2O$): calc (%) C 41.17, H 4.80, Br 45.65, N 5.33, O 3.05; found (%) C 41.25, H 4.89, Br 45.3, N 5.25, O 3.25.

Solution stability of 4-APEBA. 4-APEBA was left in aerated D_2O (30 mM, NMR tube) in room light at room temperature for 70 days. The solution does discolor a little bit and 4-APEBA starts to precipitate over time. However, ^{1}H-NMR analyses of the vortexed solution taken at regular intervals, including after 70 days, showed a spectrum identical to that of a freshly prepared solution. In a similar incubation experiment (23 mM in D_2O), the sample was tested after 30 days in a typical aldehyde derivatization (see main text) run alongside freshly dissolved 4-APEBA (23 mM, freshly prepared in D_2O). Here, no drop in derivatization activity was detected for the 30-day sample, i.e., both solutions worked equally well. Notwithstanding these excellent stability results, it is recommended to prepare fresh 4-APEBA solutions.

Supporting Figures

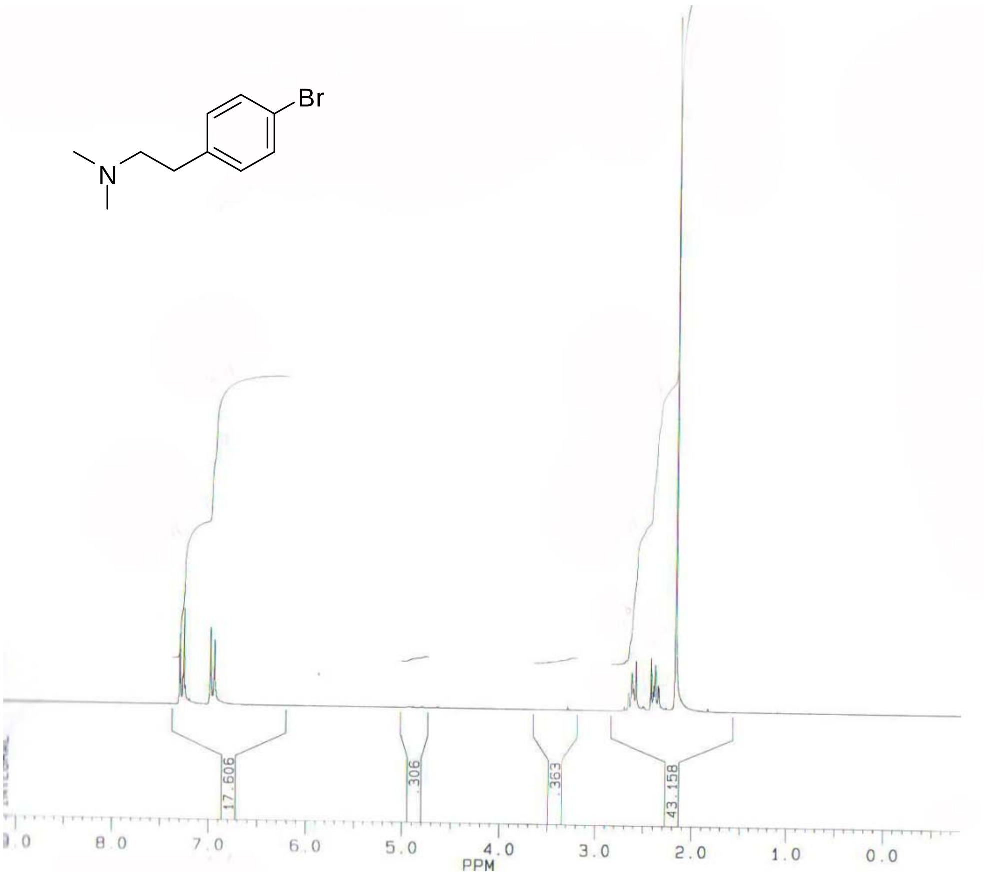


Figure S1. ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

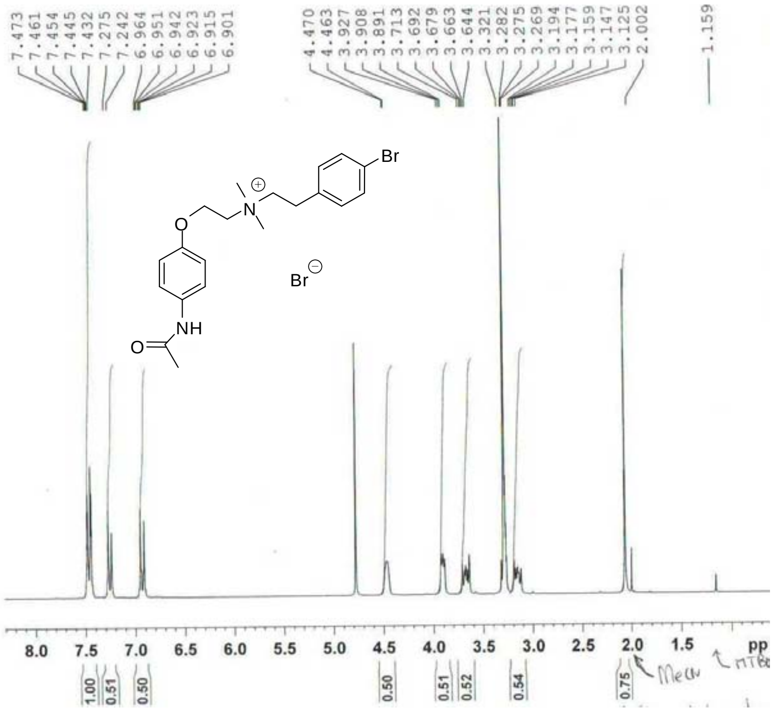


Figure S2. ^{1}H-NMR spectrum of recrystallized compound **5** in d_4-MeOD. Traces of crystallization solvents MTBE, MeCN and MeOH are visible.

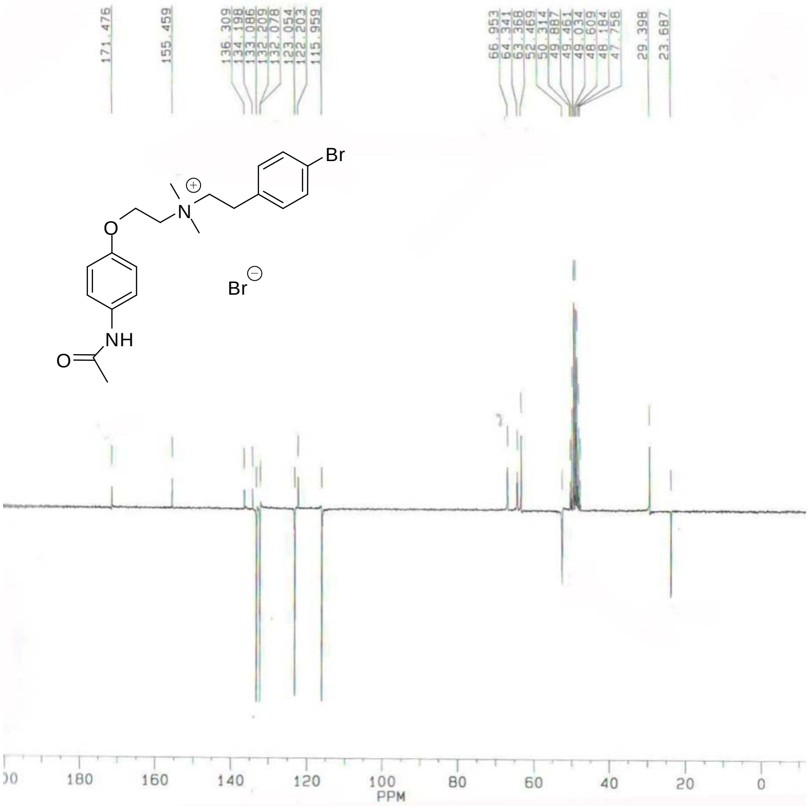


Figure S3. ^{13}C-APT spectrum of compound **5** in d_4-MeOD.

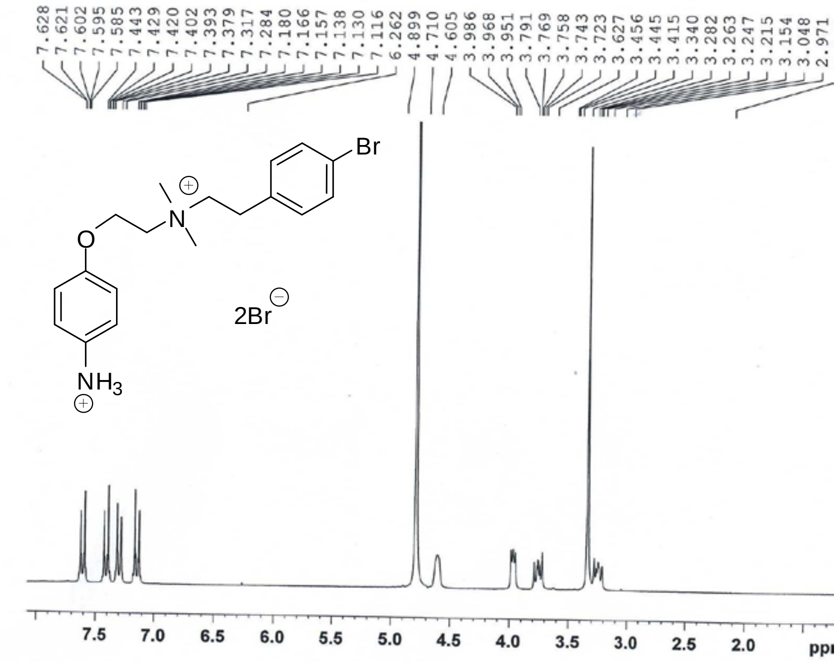


Figure S4. ^{1}H-NMR spectrum of compound **1** in D_2O.

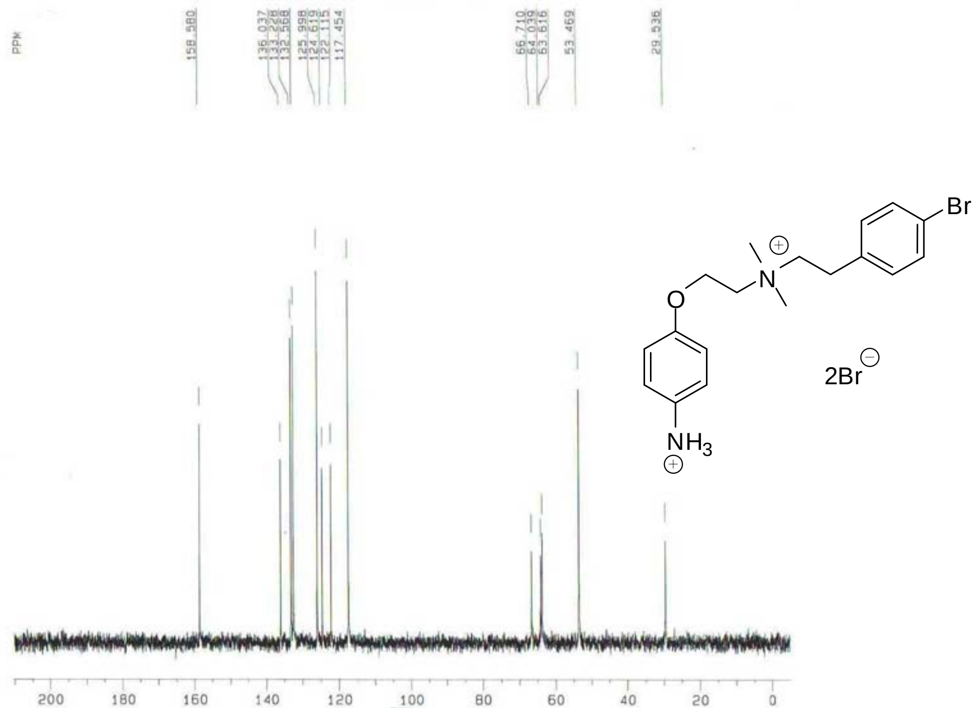
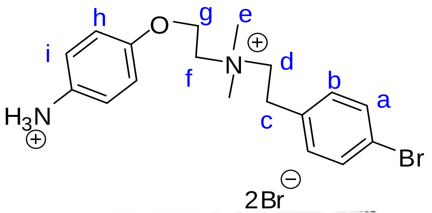


Figure S5. ^{13}C-spectrum of compound **1** in D_2O.



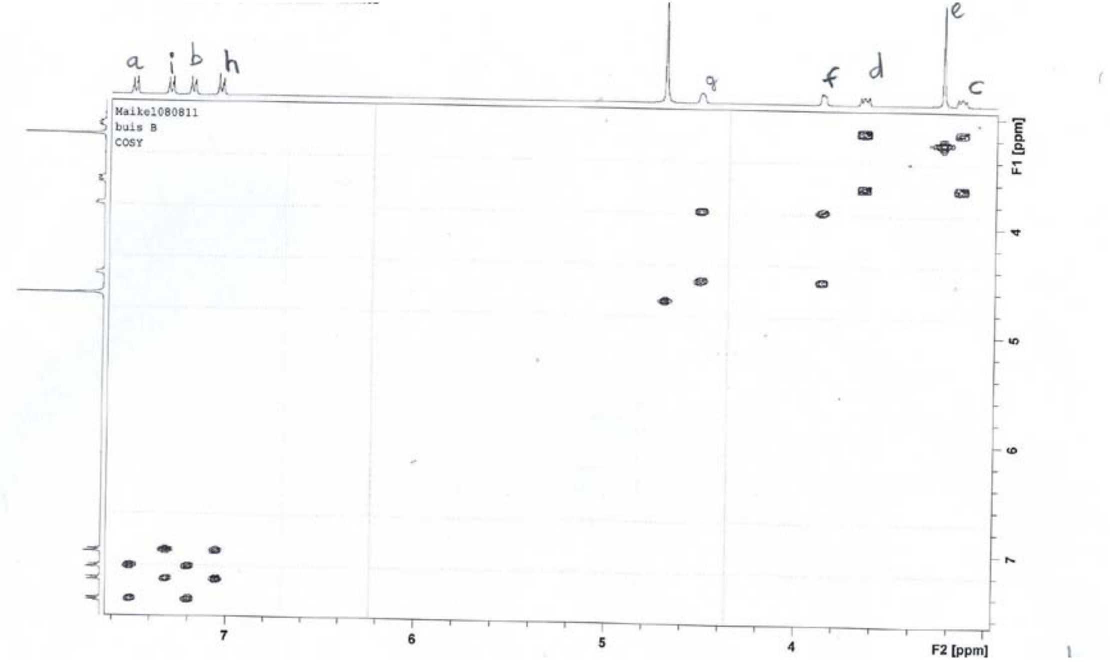


Figure S6. 2D-COSY of compound **1** in D_2O

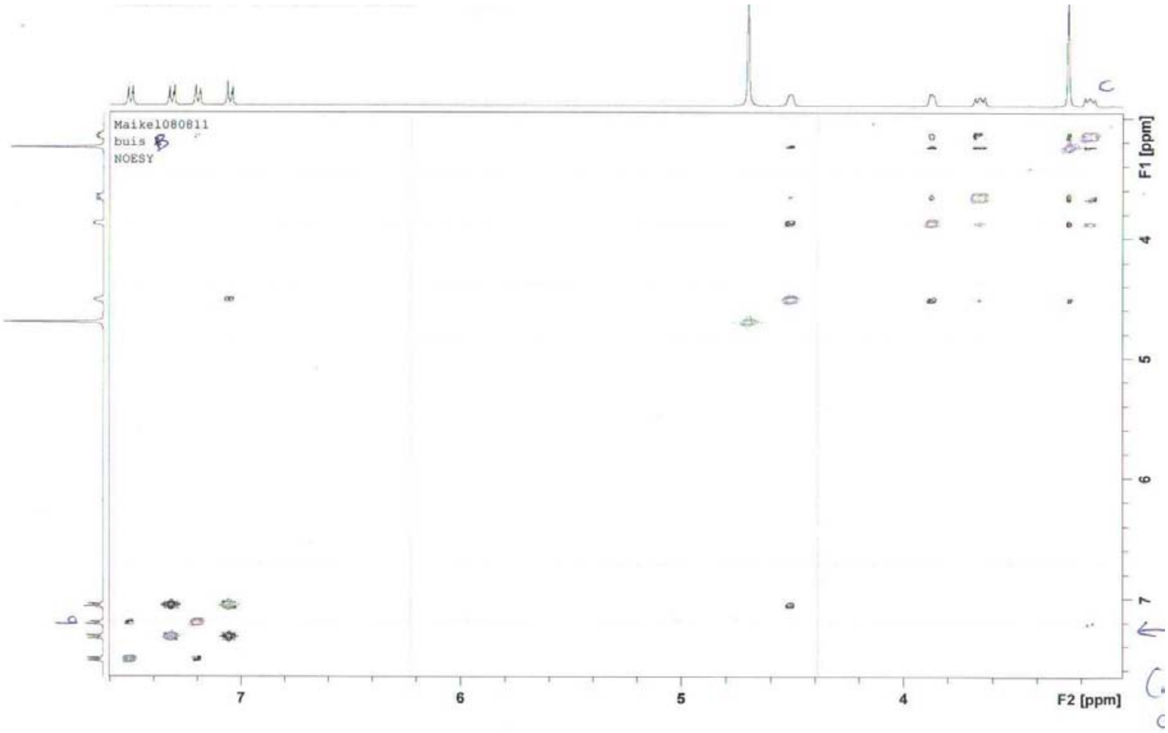


Figure S7. 2D-NOESY of compound **1** in D_2O.

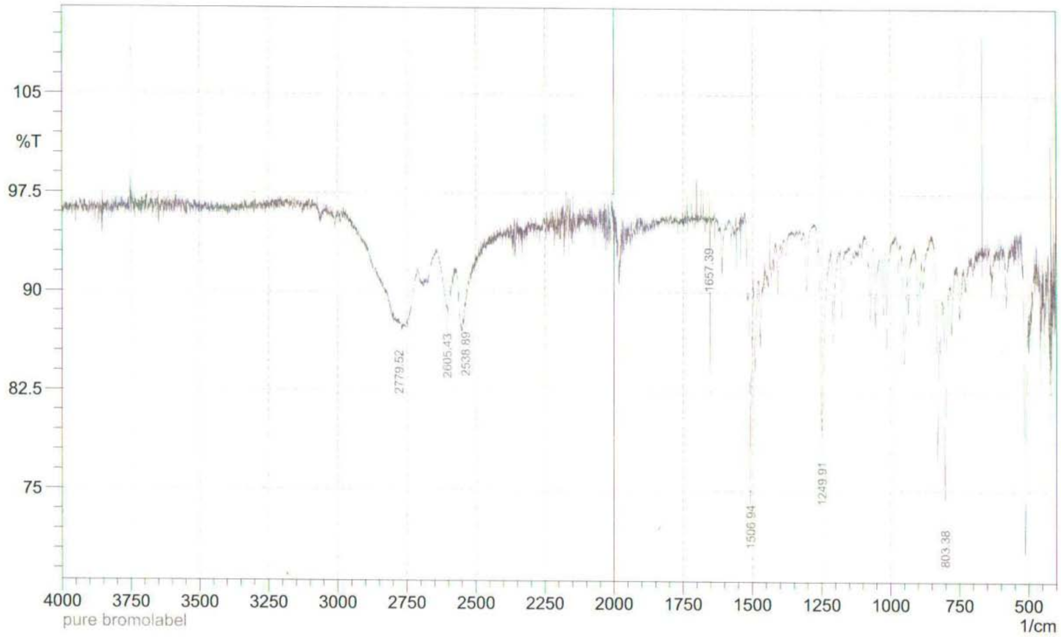


Figure S8. IR-spectrum of compound **1** as a solid.

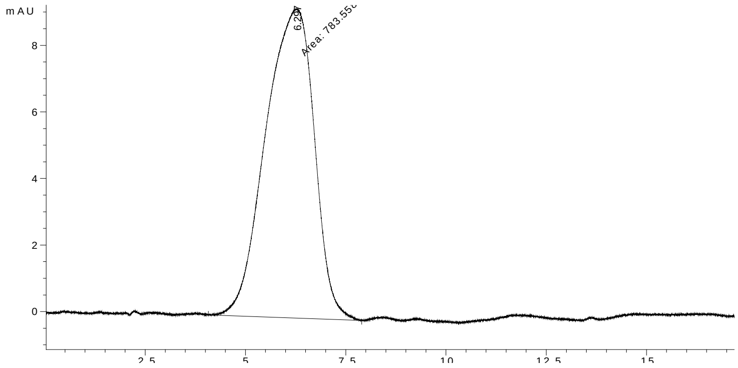


Figure S9. LC-UV chromatogram of compound **1** (detection at 277 nm).

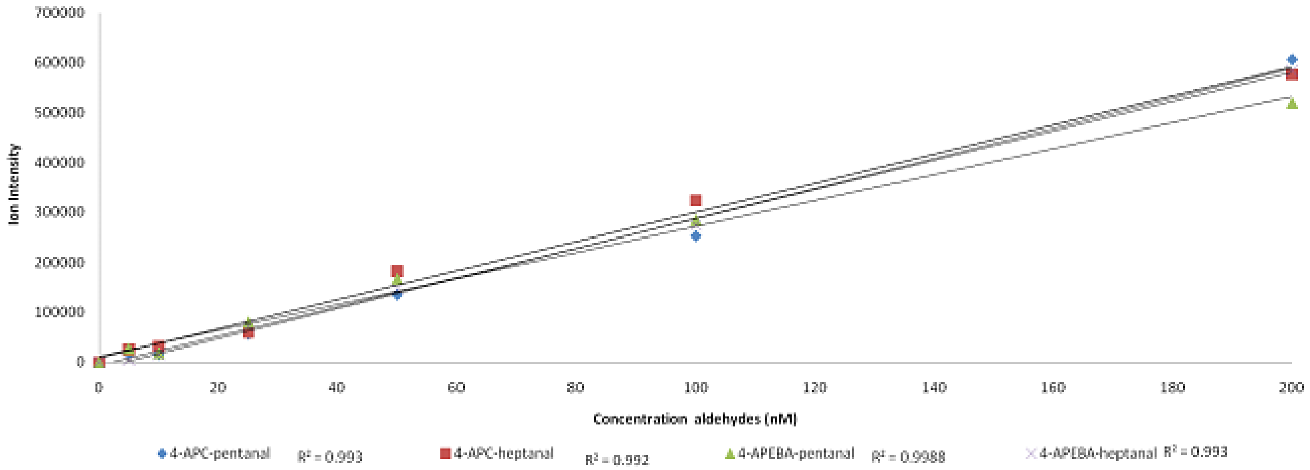


Figure S10: Signal intensity (EIC of a single m/z) comparison between 4-APC and 4-APEBA derivatives.

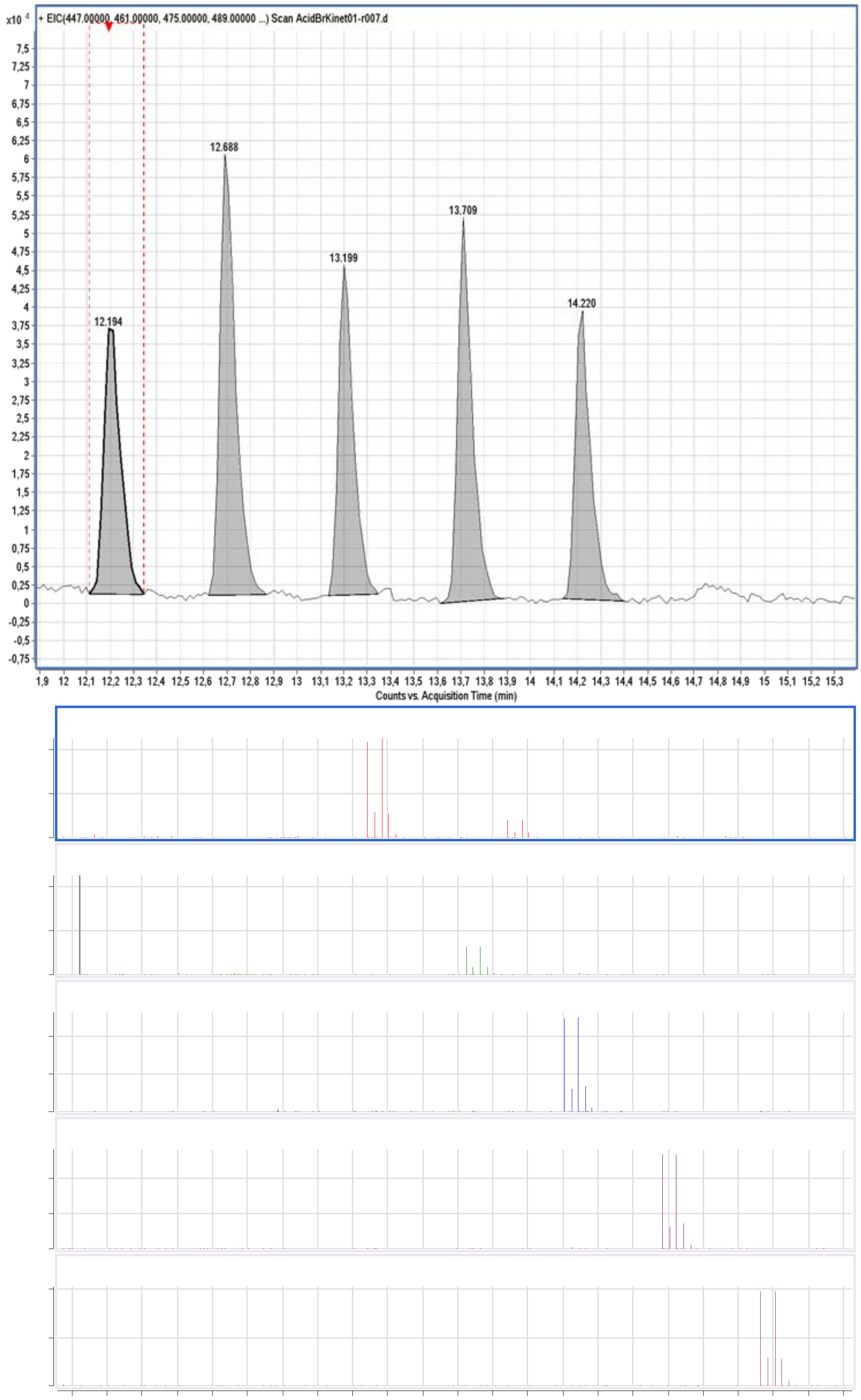


Figure S11: Combined extracted ion chromatogram of pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid with respectively the corresponding MS traces.

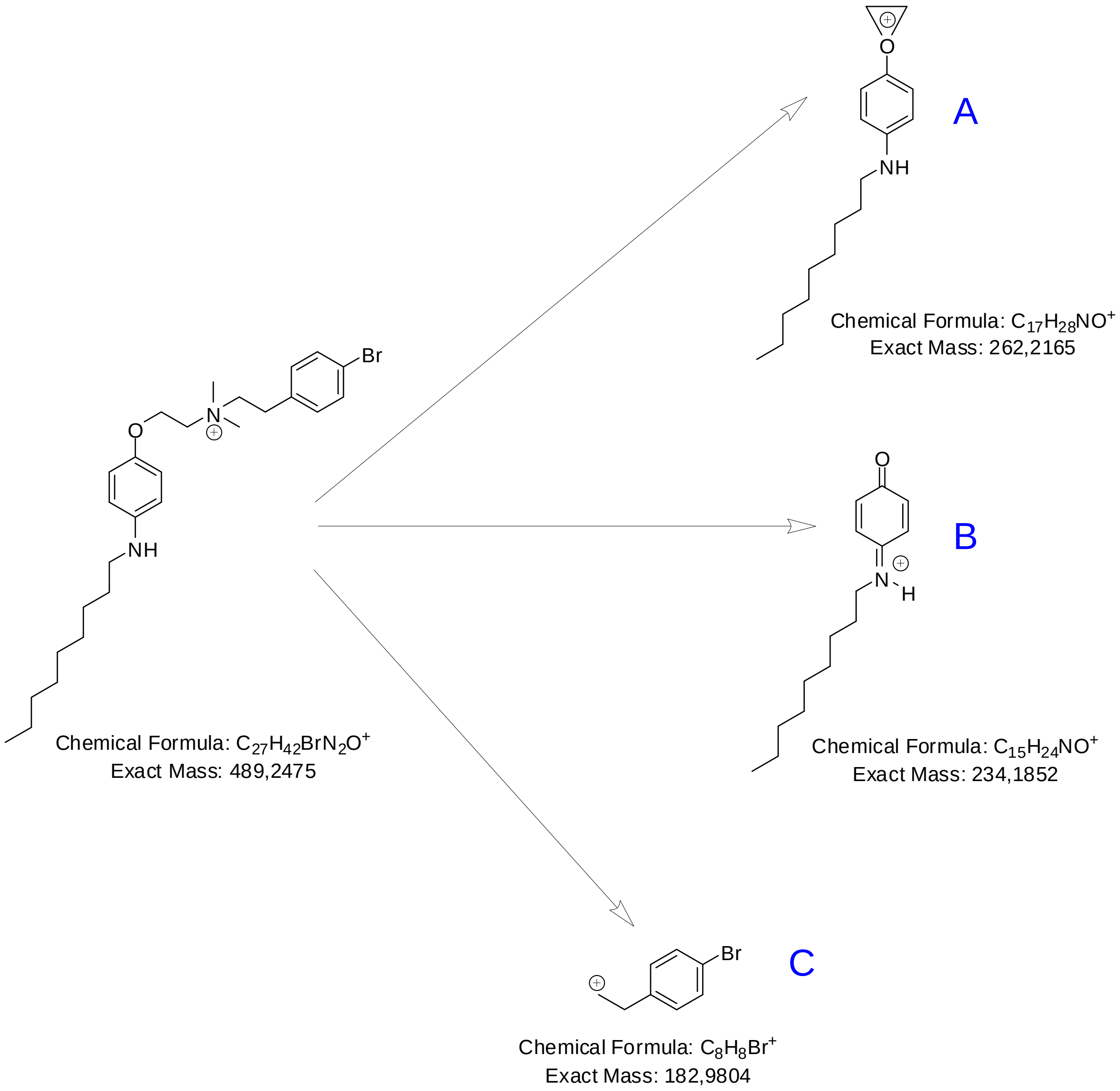


Figure S12: Major fragmentations of the adduct of 4-APEBA and nonanal

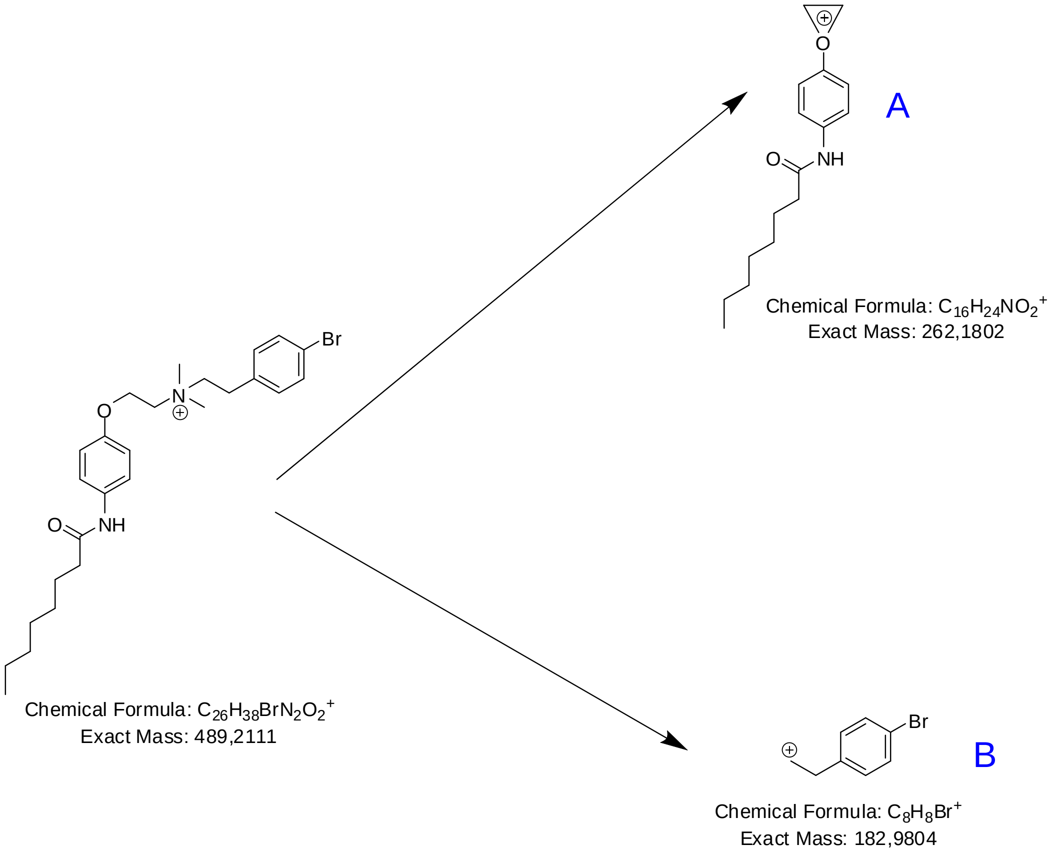


Figure S13: Major fragmentations of the adduct of 4-APEBA and octanoic acid.

Table S1: Chemical formulas generated from extract mass measurements.

Compounds	**t_R**	**Formula (M+)**	**Score**	**Mass**	**Calc Mass**	**Abs Diff (ppm)**
Octanal in human plasma	18.37	$C_{26}H_{40}BrN_2O$	100	475.2325	475.2324	0.15
Octanal human plasma +AAPH	18.45	$C_{26}H_{40}BrN_2O$	100	475.2317	475.2324	1.59
Oxidized aldehyde human plasma	16.91	$C_{24}H_{34}BrN_2O_3$	100	477.1757	477.1753	0.96
Oxidized aldehyde human plasma +AAPH	17.05	$C_{24}H_{34}BrN_2O_3$	100	477.1739	477.1753	2.81