Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting information for

A general solid phase method for the synthesis of sequence independent peptidyl-fluoromethyl ketones

by

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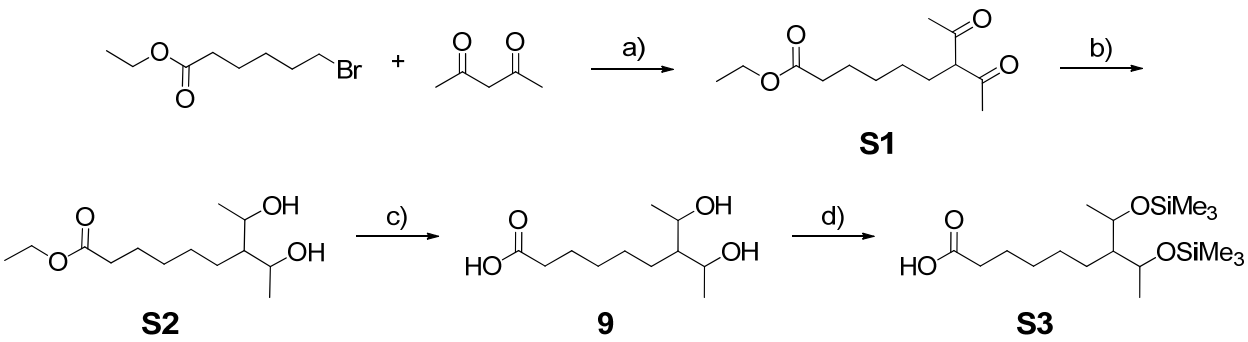

Scheme S1. Reagents and conditions: a) DBU, toluene, 38 h, *r.t.*, 21%; b) $NaBH_4$, methanol, *r.t.*, 1 h, 92%; c) aqueous NaOH (0.6 M), 15 min, reflux, 87%; d) Me_3SiCl, Et_3N, diethyl ether, *r.t.*

Ethyl 7-acetyl-8-oxononanoate (S1).[1] A solution of ethyl 6-bromohexanoate (3.44 mL, 19.40 mmol, 1 equiv) in toluene (10 mL) was added to a stirred solution containing acetyl acetone (2.00 mL, 19.40 mmol, 1 equiv) and DBU (2.90 mL, 19.40 mmol, 1 equiv) in toluene (50 mL). Reaction was stirred at room temperature for 38 hours, and then washed with water to remove the DBU salt. The organic layer was dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was loaded on a silica gel column and eluted first with dichloromethane/pentane=1:1 to remove the unreacted ethyl bromohexanoate and then with ethyl acetate/pentane=1:3 (R_f=0.75) to afford ethyl 7-acetyl-8-oxononanoate (**S1**) (0.98 g, 21%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.21-1.25 [overlapped peaks, 10H, C*H*$_2$(CH$_2$)$_2$COOEt, COOCH$_2$C*H*$_3$ keto and enol], 1.28–1.41 [overlapped peaks, 4H, C*H*$_2$(CH$_2$)$_3$COOEt enol and keto], 1.55–1.67 (overlapped peaks, 4H, C*H*$_2$CH$_2$COOEt enol and keto), 1.78–1.84 [overlapped peaks, 4H, C*H*$_2$(CH$_2$)$_4$COOEt enol and keto], 2.10 (s, 6H, COCH$_3$ keto or enol), 2.14 (s, 6H, COCH$_3$ keto or enol), 2.25 (t, ^3J=7.4 Hz, 2H, C*H*$_2$COOEt keto or enol), 2.28 (t, ^3J=7.4 Hz, 2H, C*H*$_2$COOEt keto or enol), 3.58 [t, ^3J=7.2 Hz, 1H, C*H*(COCH$_3$)$_2$], 4.09 (q, ^3J=7.1 Hz, 2H, COOC*H*$_2$CH$_3$ keto or enol), 4.10 (q, ^3J=7.1 Hz, 2H, COOC*H*$_2$CH$_3$ keto or enol); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 15.0, 23.6, 25.4, 25.6, 27.9, 28.8, 29.7, 29.8, 29.9, 31.2, 34.9, 35.0, 61.0, 69.6, 111.2, 174.3, 174.3, 191.7, 205.0; MS (ES$^+$) *m/z*: 243.2 [*M*+H]$^+$.

Ethyl-8-hydroxy-7-(1-hydroxyethyl)nonanoate (S2). A solution of 7-acetyl-8-oxononanoate **S1** (0.560 g, 2.31 mmol) in methanol (5 mL) was added dropwise to a stirred suspension of $NaBH_4$ (0.174 g, 4.62 mmol,) in methanol (25 mL). The mixture was stirred at room temperature until the TLC showed the reaction completion (approximately 1 hour). The solvent was removed and the residue was treated with an aqueous solution of sodium citrate 5% (30 mL). The mixture was extracted with ethyl acetate (3 x 30 mL), dried over anhydrous $MgSO_4$ and evaporated to afford ethyl 8-hydroxy-7-(1-hydroxyethyl)nonanoate (**S2**), as a mixture of diastereoisomers, as a colorless liquid (0.52 g, 92%). R_f=0.65 (ethyl acetate). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.22–1.50 (overlapped peaks, 17H, 2CH_3, CH_2CH_2CH_2CH_2CH_2COOCH_2CH_3), 1.61–1.70 (m, 1H–CH(CHOH)$_2$), 2.28–2.34 (m, 2H-CH_2COOEt), 3.93–3.97, 3.99–4.05, 4.12–4.23, 4.22–4.26 (m, -CHOH, from different diastereoisomers), 4.14 ppm (q, ^3J=7.1 Hz, -COOCH_2CH$_3$); ^{13}C-NMR (101 MHz, $CDCl_3$) δ ppm: 14.2, 19.2, 21.2, 21.6, 22.2, 22.3, 24.7, 24.77, 24.79, 25.92, 25.96, 27.7, 28.4, 29.4, 29.6, 29.7, 31.0, 33.7, 34.0, 34.22, 34.23, 34.29, 49.1, 49.4, 51.3, 60.17, 60.19, 68.0, 69.2, 71.3, 72.4, 173.75, 173.85, 173.87; MS (ES$^+$) *m/z*: 247.1 [M+H]$^+$.

8-Hydroxy-7-(1-hydroxyethyl)nonanoic acid (9). Ethyl-8-hydroxy-7-(1-hydroxyethyl) nonanoate (**S2**) (0.246 g, 1 mmol) was added over an aqueous solution of NaOH 0.6 M (10 mL) and the mixture was heated for 15 min to reflux. The reaction mixture was allowed to cool to room temperature and stirred for approximately 30 min. The aqueous solution was washed with ethyl acetate twice to remove the unreacted ethyl 8-hydroxy-7-(1-hydroxyethyl)nonanoate. The aqueous phase was then cooled on an ice bath, acidified to pH=4 and extracted with ethyl acetate (3 x 30 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and evaporated to afford 8-hydroxy-7-(1-hydroxyethyl)nonanoic acid (**9**) as a mixture of diastereoisomers, as a colorless oil (0.189 g, 87%). R_f=0.40 (ethyl acetate). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.21–1.47 (overlapped peaks, 12H, 2CH_3, CH_2CH_2CH_2CH_2 CH$_2$COOH), 1.65–1.71 (m, 1H, CH(CHOH)$_2$), 2.38 (m, 2H, CH_2COOH), 3.92–3.98, 3.98–4.03, 4.09–4.13, 4.21–4.25 (m, -CHOH, from different diastereoisomers); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 19.4, 21.4, 21.8, 22.4, 24.5, 24.6, 25.9, 26.1, 27.7, 28.5, 29.4, 29.7, 29.8, 31.0, 33.82, 33.87, 49.2, 49.5, 51.4, 68.2, 69.6, 71.5, 72.6, 178.3. MS (ES$^+$): *m/z*: 219.1 [M+H]$^+$, 459.6 [2M+Na]$^+$, 475.5 [2M+K]$^+$.

8-((Trimethylsilyl)oxy)-7-(1-((trimethylsilyl)oxy)ethyl)nonanoic acid (S3). A solution of trimethylchlorosilane (0.435 mL, 3.41 mmol, 3.1 equiv) in anhydrous diethyl ether (15 mL) was added very slowly over a period of 15 minutes to a solution of 8-hydroxy-7-(1-hydroxyethyl)nonanoic acid **9** (0.240 g, 1.10 mmol, 1 equiv) and triethylamine (0.435 mL, 3.41 mmol) in dry diethyl ether (30 mL). The reaction mixture was allowed to stir at room temperature overnight and the white salt formed was filtered. Dry pentane (60 mL) was added to the filtrate and an additional amount of salt was precipitate. The solution was filtered again and the solvent evaporated to dryness. Because the silylated acid (**S3)** is water (and heat) sensitive, solvent removal was performed under vacuum at 5°C to afford viscous colorless oil which was used immediately in the next step without any further purification.

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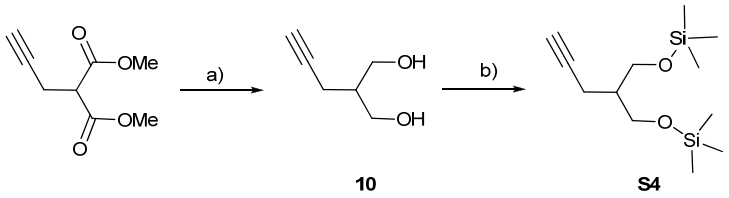


Scheme S2. Reagents and conditions: a) $LiAlH_4$, diethyl ether, *r.t.*, overnight, 80%; b) Me_3SiCl, Et_3N, diethyl ether, *r.t.*, overnight, 91%.

2-(Prop-2-yn-1-yl)propane-1,3-diol (10). A solution of dimethyl 2-(prop-2-yn-1-yl)malonate (1.00 mL, 6.99 mmol) was added dropwise during a period of 10 minutes to an ice cold suspension of $LiAlH_4$ (0.398 g, 10.49 mmol) in dry diethyl ether (50 mL), under argon. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with MeOH and water, washed three times with water, the organic phase was dried over anhydrous $MgSO_4$ and then evaporated to dryness. The residue was loaded on a chromatographic column and separated, using ethyl acetate as eluent, to afford 2-(prop-2-yn-1-yl)propane-1,3-diol (**10**) as colourless liquid (0.64 g, 80%). R_f=0.38 (silica gel, ethyl acetate). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.89–1.96 [m, 1H, C*H*$(CH_2OH)_2$], 2.00 (t, ^4J=2.7 Hz, 1H, CC*H*), 2.27 (dd, ^3J=6.9 Hz, ^4J=2.7 Hz, 2H, CH_2CCH), 3.25 (br s, CH_2OH), 3.72 [dd, ^2J=10.9 Hz, ^3J=6.3 Hz, 2H, C*H*H-OH ($H_{equatorial}$-six-membered ring (1,3-dioxan like structure) formed by hydrogen bonding)], 3.78 [dd, ^2J=10.9 Hz, ^3J=4.8 Hz, 2H, CH*H*-OH (H_{axial}-six-membered ring (1,3-dioxan like structure) formed by hydrogen bonding)]; ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 19.4, 43.4, 65.8, 71.8, 84.1; MS (ES^+): *m/z*: 115.0 $[M+H]^+$.

2,2,8,8-Tetramethyl-5-(prop-2-yn-1-yl)-3,7-dioxa-2,8-disilanonane (S4). Over a dry solution of 2-(prop-2-yn-1-yl)propane-1,3-diol (**10**) (0.356 g, 3.12 mmol, 1 equiv) and Et_3N (0.95 g, 9.36 mmol, 3 equiv) in anhydrous diethyl ether (40 mL), TMSCl (1.02 g, 9.36 mmol, 3 equiv) was added dropwise. The mixture was allowed to stir at room temperature overnight while a white salt precipitated. The precipitate was filtered and the filtrate was diluted with pentane, the mixture was cooled and the rest of the triethylamine salt precipitated. The filtrate was concentrated in *vacuum*, at low temperature, to afford the silylated alcohol **S4** as a colorless liquid which was immediately used in the next step (0.73 g, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 0.09 (s, 18H, OSi(CH_3)$_3$), 1.83–1.89 (m, 1H, CH(CH_2OTMS)$_2$), 1.92 (t, ^4J=2.7 Hz, 1H, CCH), 2.24 (dd, ^3J = 6.6 Hz, ^4J=2.7 Hz, 2H, CH_2CCH), 3.55–3.65 (m, 4H, CH(CH_2OTMS)$_2$).

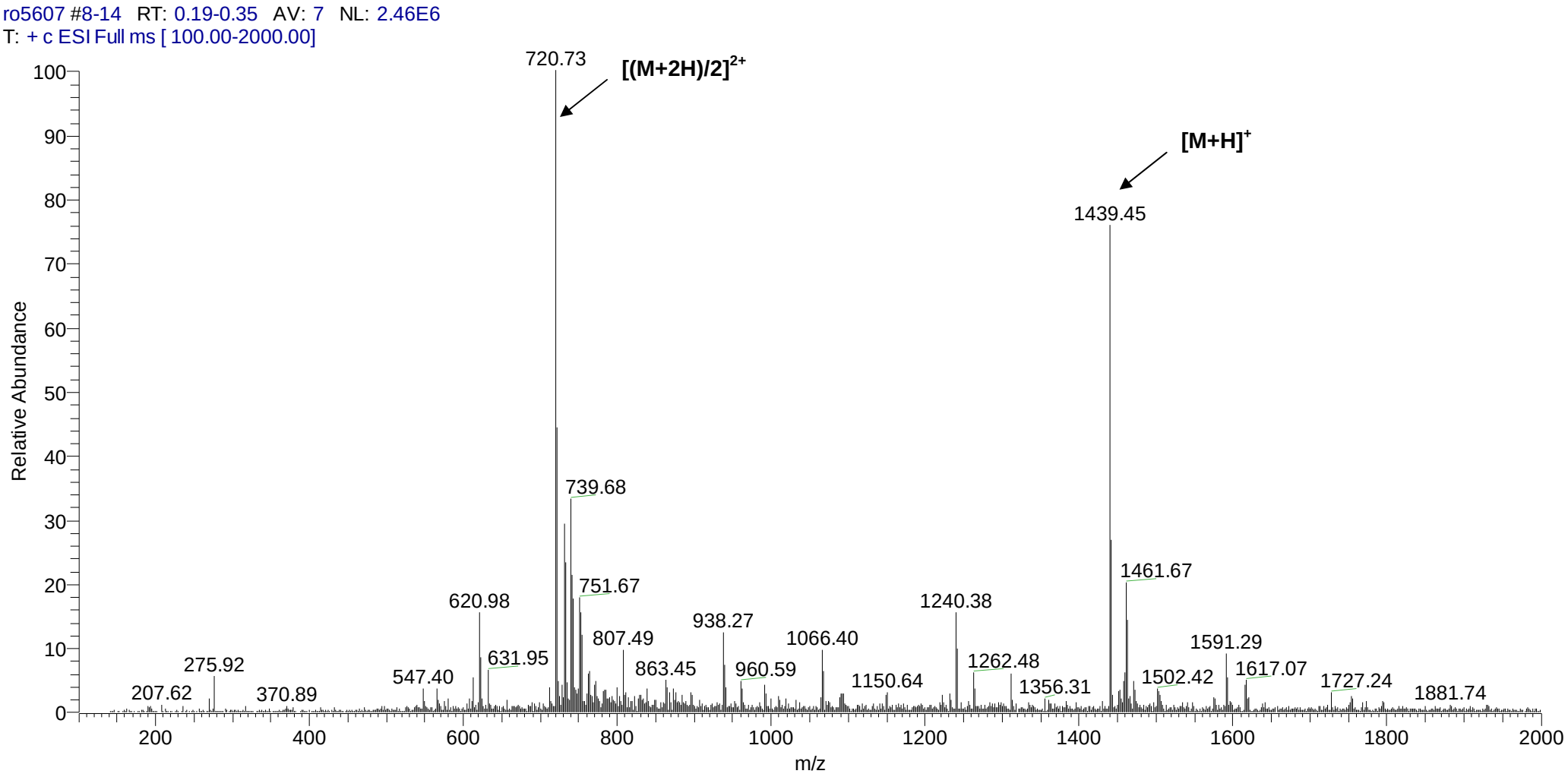
Synthesis of 2-azidoacetic acid

To a mixture of bromoacetic acid (3.00 g, 21.59 mmol, 1 equiv), NaN_3 (14.04 g, 215.91 mmol, 10 equiv) and NH_4Cl (13.86 g, 259.09 mmol, 12 equiv), water (60 mL) was added. The mixture was heated on an oil bath at 60^{o}C until all the solids dissolved and then the solution was allowed to stir at 60°C overnight. The mixture was acidified with concentrated HCl on an ice bath to pH=4 and extracted with ethyl acetate (8 x 30 mL). The organic phase was washed with water (2 x 20 mL), dried over anhydrous $MgSO_4$ and evaporated to give 2-azidoacetic acid as a colorless liquid (1.67 g, 76%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.96 (s, 2H, CH_2), 11.89 (s, 1H, COOH); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 52.87, 174.60; MS (ES$^+$) *m/z*: 101.9 [M+H]$^+$.

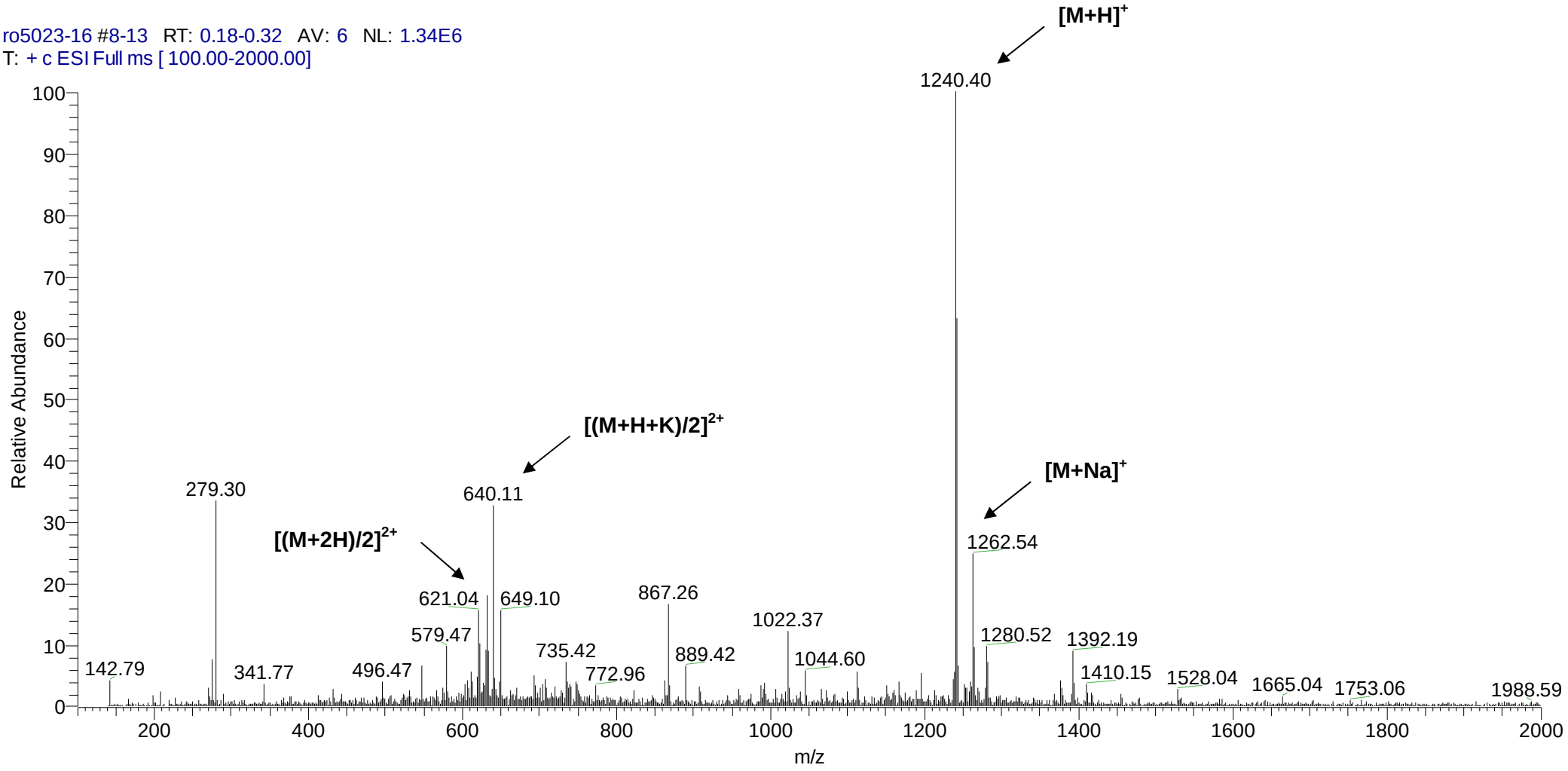
References

1. D. P. Shrout, D. A. Lightner, *Synthetic Commun.* 1990, **20**, 2075-2080.

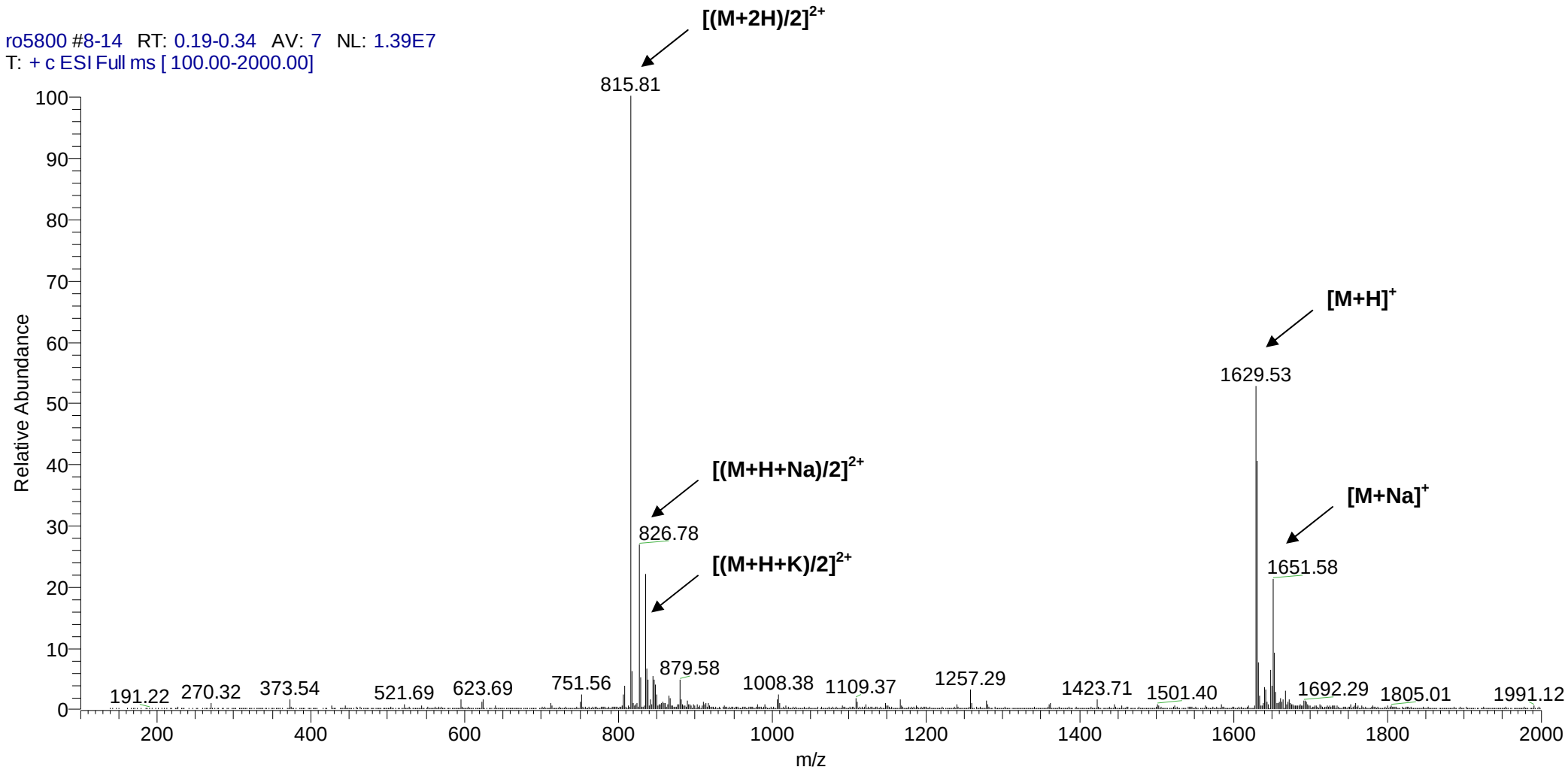
ES^+ MS spectrum of compound 15



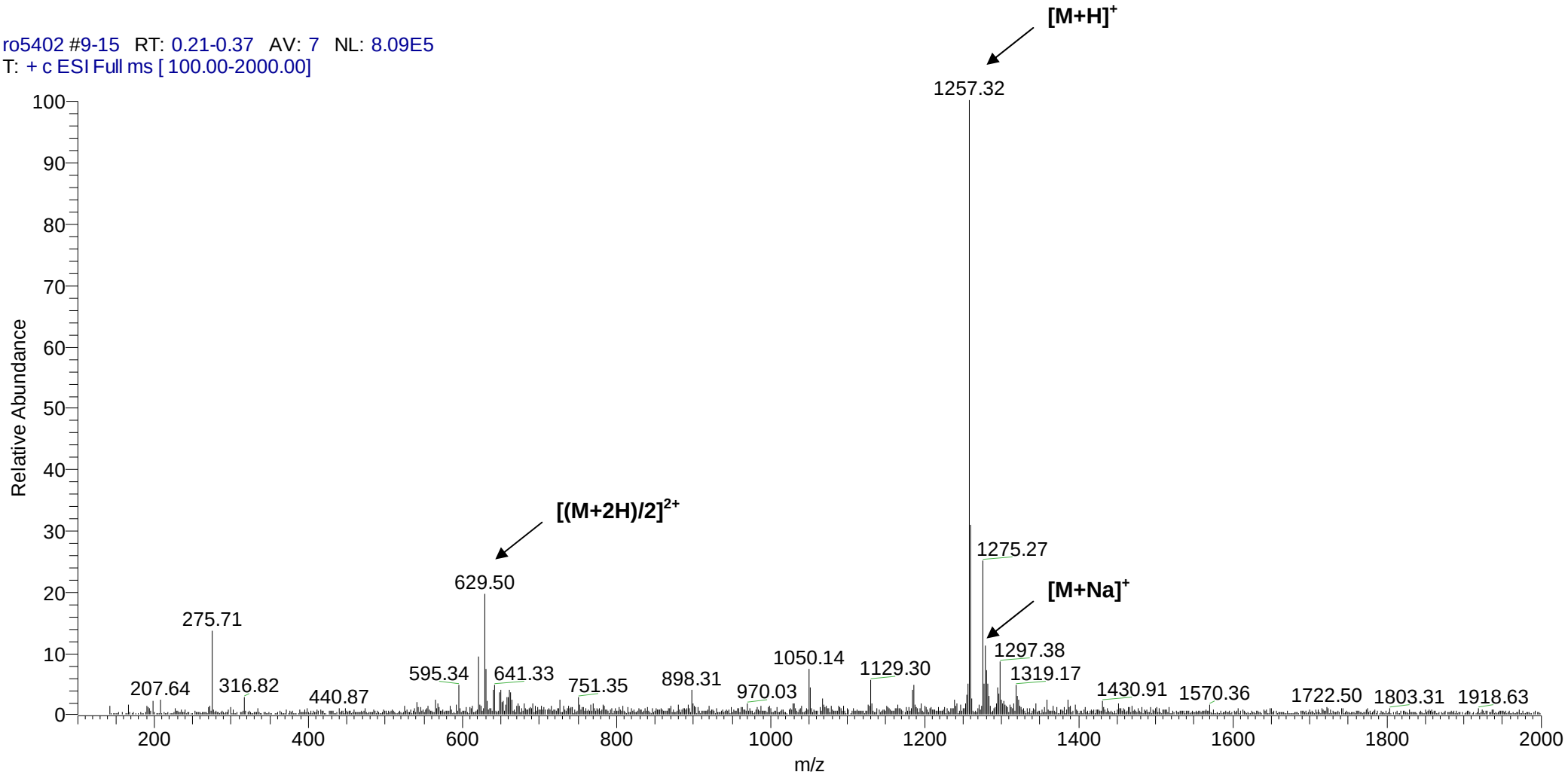
ES^+ MS spectrum of Biotin-Teg-FQQQTG-8 (16)



ES$^+$ MS spectrum of compound 19



ES^+ MS spectrum of Biotin-Teg-YQEQTG-8 (20)



ES^+ MS spectrum of compound 22