Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting Information

New Prodigiosin Derivatives – Chemoenzymatic Synthesis and Physiological Evaluation against Cisplatin-Resistant Cancer Cells

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1. Slopes and absorption spectra of prodiginines 16ba–bc for determination of molar extinction coefficients

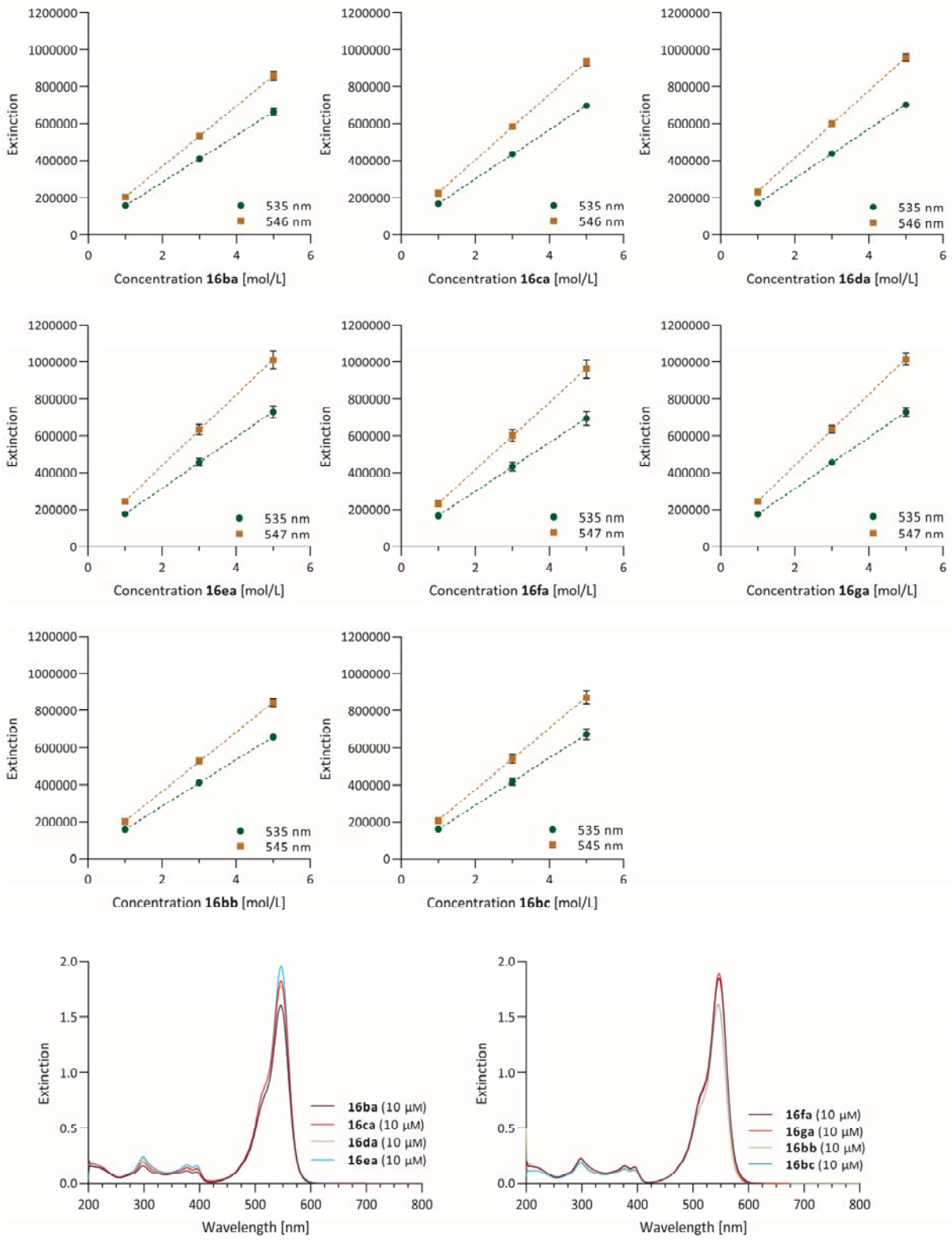


Figure S1: Extinction values for different molar concentrations of prodiginines **16ba–bc** in acidic ethanol. Measured extinctions at 535 nm and at the absorption maximum of 1 µM, 3 µM, and 5 µM prodiginine solutions were plotted against the concentrations and the molar extinction coefficients determined from the slope of linear regression in agreement with the Beer-Lambert law. Absorptions spectra of 10 µM prodiginine solutions in acidic ethanol were recorded from 200–800 nm.

Table S1: Fit parameters for determination of molar extinction coefficients of prodiginines **16ba–bc** from linear regression.

Prodiginine	λ_{max} [nm]	Replikates	535 nm			λ_{max}		
			Slope	Y-Intersect	R^2	Slope	Y-Intersect	R^2
16ba	546	3	126508	31353	0.9975	163533	42056	0.9976
16ca	546	3	132425	36969	0.9981	177092	50169	0.9980
16da	546	3	133375	37697	0.9984	181692	51814	0.9983
16ea	547	3	138350	39206	0.9929	191400	55522	0.9929
16fa	547	3	131608	36631	0.9902	182125	52236	0.9903
16ga	547	3	138200	38244	0.9966	192358	54403	0.9965
16bb	545	3	125450	32839	0.9979	160358	42714	0.9980
16bc	545	3	128500	32011	0.9939	166267	42189	0.9941

2. Influence of A-ring substitution on molar extinction coefficients of prodiginines 16ba–bc

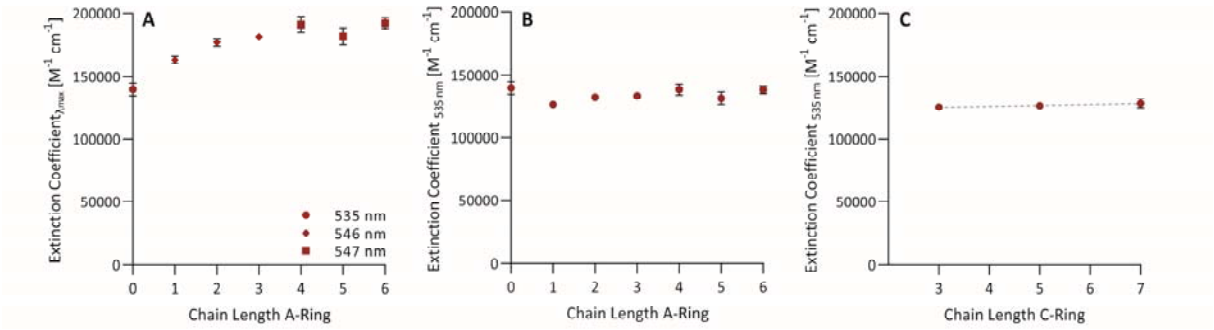


Figure S2: Molar extinction coefficients of chemically synthesised prodiginines **16ba–16bc** in acidic ethanol. All measurements were performed in triplicates and standard errors are given by error bars. (**A**) Molar extinction coefficients at the wavelength of maximum absorption in relation to the A-ring substitution with constant pentyl-substitution on the C-ring. The determined absorption maximum is indicated by varying symbols. (**B**) Molar extinction coefficients in relation to the A-ring substitution at 535 nm with constant pentyl-substitution on the C-ring. (**C**) Molar extinction coefficients of the methyl-substituted A-ring derivatives **16ba**, **16 bb**, and **16bc** at 535 nm in dependence of C-ring substitution. Linear regression was applied to determine the slope and allow approximation of extinction coefficients for prodiginines, which had not been synthesised by chemical means.

3. Molar extinction coefficients of synthesised prodiginines 16ba–bc and approximation of extinction coefficients for unknown derivatives

Table S2: Experimental molar extinction coefficients (ε) of chemically synthesised prodiginines **16ba–bc** at 535 nm and at the respective absorption maximum in acidic ethanol with standard errors.

Prodiginine	λ_{max} [nm]	ε_{535} [M^{-1} cm^{-1}]	$\varepsilon_{\lambda max}$ [M^{-1} cm^{-1}]
16ba	546	126508 ± 2380	163533 ± 3040
16ca	546	132425 ± 2211	177092 ± 2960
16da	546	133375 ± 1200	181692 ± 2797
16ea	547	138350 ± 4432	191400 ± 6105
16fa	547	131608 ± 4950	182125 ± 6805
16ga	547	138200 ± 3071	192358 ± 4337
16bb	545	125450 ± 2176	160358 ± 2707
16bc	545	128500 ± 3815	166267 ± 4848

The methyl-substituted prodiginine A-ring derivatives **16ba**, **16bb**, and **16c** show a linear correlation of their molar extinction coefficients at 535 nm (Figure S2C). Based on the equation for the linear regression fit, the experimentally determined extinction coefficients of prodiginines (Table S2) were used to approximate the extinction coefficients of derivatives that had not been accessed by chemical synthesis. As the C5-monopyrrole **4a** was fused with each MBC derivative **3a–g**, the extinction coefficients for shortened C3 or elongated C7 C-ring substituted prodiginines could be calculated. Therefore, the equation from the linear regression (Figure S2C) was employed (equation S1.1).

$$\varepsilon_{535}(n) = 762.5\ M^{-1}cm^{-1} \cdot n + 123007\ M^{-1}cm^{-1} \quad \text{(S1.1)}$$

ε: Molar extinction coefficient

n: A-ring chain length of prodiginines

Under the assumption that the slope of 762.5 M^{-1} cm^{-1} would behave similarly with increasing chain length on the A-ring, approximated extinction coefficients at 535 nm were calculated for prodiginines with C3 or C7 substitution on the C-ring (Table S3).

Table S3: Approximated* molar extinction coefficients at 535 nm in acidic ethanol of prodiginines, which had not been synthesised chemically.

Prodiginine	Substitution A-Ring	Substitution C-Ring	Approximated ε_{535} [M^{-1} cm^{-1}]
16ab	H	propyl	138275
16ac	H	heptyl	141325
16cb	ethyl	propyl	130900
16cc	ethyl	heptyl	133950
16db	propyl	propyl	131850
16dc	propyl	heptyl	134900
16eb	butyl	propyl	136825
16ec	butyl	heptyl	139875
16fb	pentyl	propyl	130083
16fc	pentyl	heptyl	133133
16gb	hexyl	propyl	136675
16gc	hexyl	heptyl	139725

*The calculation was based on the equation $\varepsilon_{535}(n)$ = 762.5 M^{-1} cm^{-1} · n + 123007 M^{-1} cm^{-1}.

4. Raw data of UV absorbance for prodiginines from biocatalysis for quantification of LC-MS extracts

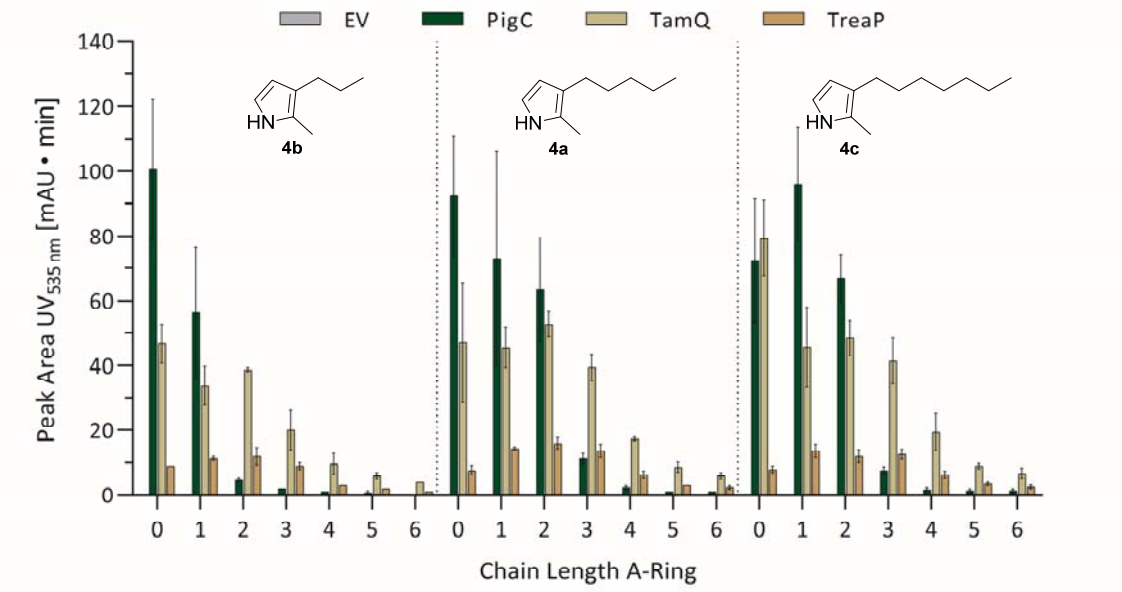


Figure S3: Raw data (integrated areas of UV absorbance at 535 nm) of prodiginines from *in vitro* reactions with the prodiginine ligases PigC, TamQ, TreaP and the empty vector control with varying chain lengths on the A- and C-ring. Methanolic extracts of biocatalytic reactions between monopyrroles **4a–c** and 5-alkyl MBC derivatives **3a–g** were subjected to coupled LC-MS measurements and the UV absorbance at 535 nm was integrated. The chain length of alkylated MBC is depicted on the x-axis of the bar diagram, the used monopyrrole is shown in the diagram. Each experiment was performed in triplicates. It should be noted that prodigiosin (**1**) and the prodiginines **16ab** and **16ac**, derived from unsubstituted MBC **3a**, exhibit an absorption maximum at 535 nm, while the absorption maximum for all other prodiginines was shifted to 545–547 nm. Thus, the true amount of all other A-ring substituted prodiginines is underestimated unless extinction coefficients are applied to quantify the exact amount of prodiginines in the extracts.

5. General information and instrumentation

High-resolution mass spectrometry (HRMS) data were recorded on a Bruker maXis instrument (at CeMSA in Düsseldorf) with a standard collision energy of 8 eV. However, for Boc-protected 1*H*-pyrroles a reduction to 2–4 eV was obligatory, as Boc-protecting groups showed fragmentation towards the carbamic acid [M–56.0626]. ^{1}H, ^{13}C, DEPT135, ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, and ^{1}H-^{13}C-HMBC NMR data were either collected on a Bruker Avance/DRX 600 or DRX 300 spectrometer. $CDCl_3$ or DMSO-d_6 were used as NMR solvents and their respective chemical shifts ($CDCl_3$: ^{1}H - 7.26 ppm, ^{13}C - 77.16 ppm; DMSO-d_6: ^{1}H - 2.50 ppm, ^{13}C - 39.52 ppm) used for referencing. Due to the significant amount of quaternary carbon atoms in MBC- and prodiginine derivatives, the default NMR scan numbers were significantly increased for ^{13}C (6000–8000 scans), COSY (10 scans), HSQC (10 scans), and HMBC (60–100 scans) pulse sequences to allow complete annotation of signals to corresponding ^{1}H and ^{13}C atoms. For melting point determination, the Büchi melting point apparatus B-540 was employed and the temperature increment was set to 2 °C/min. Thin layer chromatography was performed using Macherey-Nagel POLYGRAM SIL G/UV_{254} plates (40 x 80 x 0.2 mm). Visualisation of different compounds on TLC was achieved using various staining methods, including excitation under UV light, $KMnO_4$ (7.5 g/L $KMnO_4$, 50 g/L K_2CO_3, 625 mg/L NaOH in water) and *p*-anisaldehyde (300 mL glacial acetic acid, 6 mL conc. H_2SO_4, 3 mL *p*-anisaldehyde).

6. Quantitative NMR

Quantitative ^{1}H-NMR (qNMR) was used to determine the purity of chemically synthesised prodiginines. In a 1.5 mL reaction tube, triphenylmethane (used as secondary qNMR standard) and prodiginine were weighed and dissolved in 500 µL $CDCl_3$. For each prodiginine, four independent sets of ^{1}H-qNMR samples were prepared and spectra measured at 600 MHz to determine the purity, calculated by equation S2.1. For the calculation of purities, the methyl group of triphenylmethane (5.52 ppm) and the methoxy group of prodiginines (3.95 ppm) were integrated in a range of ± 50 Hz from the centre of the signal.

$$P_{Prod} = \frac{I_{Prod}}{I_{Std}} \cdot \frac{N_{Std}}{N_{Prod}} \cdot \frac{M_{Prod}}{M_{Std}} \cdot \frac{m_{Std}}{m_{Prod}} \cdot P_{Std} \qquad \text{(S2.1)}$$

Prod = Product
Std = Standard
m = Mass
N = Number of absorbing Protons
M = Molecular weight
I = Signal area/integral
P = Purity (in percent)

7. Photographic records

Photographs were recorded on a Canon EOS 6D digital reflex camera with the following instrument settings: ISO 250, shutter speed 1/13 s^{-1}, aperture F/13, focal distance 100 mm, manual white balance. For the purpose of image processing in Corel PHOTO-PAINT 2019, photographs were saved as .CR2 (Canon Raw 2) raw image files. By using Corel, all images (including the colour and grey control card) were edited to alter the light exposure from 0.0 to 1.0 and the brightness was increased from 0 to 25. Each reaction tube was saved individually as TIFF file with transparent background and 600 dpi resolution.

8. Chemical syntheses

(*Z*)-4'-Methoxy-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (1, prodigiosin)

Under N_2 atmosphere, 2-methyl-3-pentyl-1*H*-pyrrole (**4a**, 651 mg, 4.31 mmol, 2.50 eq.) and Boc-MBC (**3a**, 500 mg, 1.72 mmol, 1.00 eq.) were dissolved in MeOH (40 mL) and cooled to 0 °C. HCl in MeOH (1.25 M, 3.58 mL, 2.24 mmol, 1.30 eq.) was added dropwise and the solution stirred for 60 min at 21 °C. A second portion of HCl in MeOH (1.25 M, 3.58 mL, 2.24 mmol, 1.30 eq.) was added and the solution further stirred at 21 °C for 18 h. The reaction was quenched by the addition of 25% NH_3 (*aq*.) and the solvent evaporated. The residue was diluted with CH_2Cl_2 (100 mL) and washed with brine (2 x 100 mL). The organic phase was dried over $MgSO_4$, filtered over degreased cotton wool and the solvent evaporated. Chromatography on silica using CH_2Cl_2 + 0.7% 7 N NH_3 in MeOH, followed by chromatography on silica using *n*-pentane/CH_2Cl_2 (60:40) + 4% 7 N NH_3 in MeOH afforded the desired product (423 mg, 1.17 mmol, 68%) as deep purple amorphous solid.

δ^{1}H (300 MHz, $CDCl_3$) 0.88 (3 H, t, 11''-H), 1.22–1.39 (4 H, m, 9''-H, 10''-H), 1.52 (2 H, p, 8''-H), 2.38 (2 H, t, 7''-H), 2.54 (3 H, s, 6''-H), 3.99 (3 H, s, 7'-H), 6.07 (1 H, d, 3'-H), 6.34 (1 H, dt, 4-H), 6.67 (1 H, d, 3''-H), 6.91 (1 H, ddd, 3-H), 6.94 (1 H, s, 6'-H), 7.22 (1 H, td, 5-H), 12.55 (1 H, brs, 1-H), 12.69 (2 H, brs, 1'-H, 1''-NH).

δ^{13}C (76 MHz, $CDCl_3$) 12.6 (C-6''), 14.2 (C-11''), 22.6 (C-10''), 25.4 (C 7''), 29.9 (C-8''), 31.6 (C-9''), 58.8 (C-7'), 93.0 (C-3'), 111.9 (C 4), 116.1 (C-6'), 117.2 (C-3), 120.8 (C-5'), 122.4 (C-2), 125.3 (C-2''), 127.0 (C-5), 128.5 (C-3''), 128.6 (C-4''), 147.1 (C-5''), 147.8 (C-2'), 165.9 (C-4').

FT-IR (neat, cm^{-1}): 3215, 3154, 3098, 3070, 3011, 2954, 2924, 2853, 1633, 1604, 1575, 1544, 1509, 1469, 1148, 1412, 1385, 1351, 1278, 1264, 1250, 1198, 1135, 1065, 1042, 998, 988, 956, 888, 879, 836, 808, 785, 744, 716, 697, 650, 622, 594, 544, 502, 461.

T_m: 135.5–139.9 °C.

HRMS-ESI (m/z): $[M-Cl]^+$ calculated for $C_{20}H_{26}N_3O$ 324.2070, found 324.2077.

t_R (LC-MS Method): 7.67 min.

4-Methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3a, MBC)

In a 10 mL microwave vial, Boc-MBC **S1** (500 mg, 1.72 mmol, 1.00 eq.) was suspended in 2,2,2-trifluoroethanol (4.96 mL, 68.9 mmol, 40.0 eq.) and heated at constant power of 50 W up to 120 °C in a CEM Discover laboratory microwave. During subsequent cooling, the product crystallised from a dark purple to black solution. The product was recovered by filtration and repeatedly washed with EtOAc, to remove residual purple impurities. MBC **3a** (267 mg, 1.40 mmol, 81%) was finally obtained as metallic silver powder.

δ^1H (600 MHz, DMSO) 11.43 (1 H, s, 1-H), 11.24 (1 H, s, 1'-H), 9.30 (1 H, s, 7-H), 6.91 (1 H, m, 5'-H), 6.75 (1 H, s, 3'-H), 6.27 (1 H, m, 3-H), 6.12 (1 H, m, 4'-H), 3.83 (3 H, s, 10-H).

δ^{13}C (151 MHz, DMSO) 171.6 (C-6), 158.6 (C-4), 133.1 (C-2), 123.3 (C-2'), 120.3 (C-5'), 117.4 (C-5), 109.3 (C-4'), 108.3 (C-3'), 90.9 (C-3), 57.7 (C-10).

FT-IR (neat, cm^{-1}): 3245, 3192, 3125, 3039, 2952, 2840, 1593, 1546, 1517, 1504, 1422, 1358, 1346, 1302, 1276, 1234, 1187, 1160, 1116, 1044, 1013, 974, 829, 810, 770, 740, 694, 667, 625, 612, 604, 587, 463.

T_m: 250–265 °C (Decomposition).

HRMS-ESI (m/z): $[M+H]^+$ calculated for $C_{10}H_{11}N_2O_2$ 191.0815, found 191.0818.
t_R (LC-MS Method): 7.01 min.

(*Z*)-*N*-[(5-Bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl]-*N*-ethylethanamine (10)

Bromide **10** was synthesised according to a published procedure from Chawrai *et al*.(1)
In a 500 mL Schlenk flask under N_2 atmosphere, *N*,*N*-diethylformamide (10.7 g, 106.2 mmol, 3.00 eq.) was dissolved in dry CH_2Cl_2 (40 mL) and cooled to 0 °C. Phosphoryl bromide (25.4 g, 88.5 mmol, 2.50 eq.) was dissolved in dry CH_2Cl_2 and slowly added to the diethylformamide solution at 0 °C via transfer cannula. The solution was stirred for 1 h at 0 °C, during which time the solution had turned yellow and a white solid precipitated. A solution of 4-methoxy-3-pyrrolin-2-one (4.00 g, 35.4 mmol, 1.00 eq.) in dry CH_2Cl_2 (60 mL) was slowly added to the Vilsmeyer reagent at 0 °C. After complete addition, the reaction was allowed to warm to 21 °C, stirred for 30 min at 21 °C and then heated for 3 h at 40°C. Finally, the reaction was refluxed for 2 h at 50 °C. The reaction was quenched with ice water (400 mL), phases were separated and the aqueous phase adjusted to pH 7–8 with 1 M NaOH (*aq*.). The aqueous phase was then extracted with CH_2Cl_2 (5 x 100 mL) and merged organic phases washed with brine (2 x 150 mL). Drying over $MgSO_4$ and filtration provided the crude product as brown oil. Chromatography on silica with PE/EtOAc (80:20) + 1% TEA yielded the desired product (5.55 g, 21.4 mmol, 61%) as orange oil, which crystallised at –20 °C.

δ^1H (600 MHz, $CDCl_3$) 6.99 (1 H, s, 6-H), 5.59 (1 H, s, 4-H), 4.12 (2 H, q, *J* = 7.1 Hz, 8-H), 3.76 (3 H, s, 13-H), 3.39 (2 H, q, *J* = 7.2 Hz, 10-H), 1.29 (6 H, q, *J* = 7.1 Hz, 9-H, 11-H).
δ^{13}C (151 MHz, $CDCl_3$) 165.5 (C-3), 138.7 (C-6), 133.8 (C-5), 120.9 (C-2), 96.6 (C-4), 58.1 (C-13), 51.2 (C-10), 44.7 (C-8), 14.7 (C-11), 12.6 (C-9).

FT-IR (neat, cm^{-1}): 2974, 2938, 2876, 2866, 1630, 1525, 1486, 1459, 1448, 1406, 1382, 1325, 1275, 1185, 1106, 1076, 1058, 992, 944, 908, 858, 813, 801, 775, 719, 683, 676, 642, 565, 525, 478.

T_m: 43.3–45.0 °C

HRMS-ESI (m/z): $[M+H]^+$ calculated for $C_{10}H_{16}BrN_2O$ 259.0441, found 259.0443.

***tert*-Butyl 5'-formyl-4'-methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-1-carboxylate (Boc-MBC, S1)**

Boc-protected MBC was synthesised according to an original procedure from Aldrich *et al*. and modified by Klein *et al*.(2, 3) In a 500 mL Schlenk flask under N_2 atmosphere, tetrakis(triphenylphosphine)palladium(0) (1.18 g, 1.02 mmol, 0.05 eq.) was dissolved in degassed toluene (11 mL). A solution of bromide **10** (5.30 g, 20.5 mmol, 1.00 eq.) and *N*-Boc-pyrrole-2-boronic acid MIDA-Ester (9.88 g, 30.7 mmol, 1.50 eq.) in degassed 1,4-dioxane/water (9:1, 180 mL) were added to the Pd catalyst. After addition of Na_2CO_3 (6.51 g, 61.4 mmol, 3.00 eq.) the reaction was heated to 90 °C for 1 h. Anhydrous K_3PO_4 (8.68 g, 40.9 mmol, 2.00 eq.) was supplemented to the reaction and after additional 90 min of reflux, a second batch of Pd catalyst (1.18 g, 1.02 mmol, 0.05 eq.) was added. After 5 h of reflux, the solution was cooled to ambient temperature and ice water (400 mL) was added. The pH was adjusted with 30 mL 2 M HCl, followed by extraction with CH_2Cl_2 (3 x 150 mL). Merged organic phases were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification on silica with PE/EtOAc (80:20) provided the product (2.57 g, 8.86 mmol, 43%) as beige solid.

δ^1H (600 MHz, $CDCl_3$) 10.70 (1 H, brs, 1'-H), 9.54 (1 H, brs, 9'-H), 7.33 (1 H, dd, *J* = 3.4 Hz, *J* = 1.8 Hz, 5-H), 6.67 (1 H, dd, J = 3.6 Hz, J = 1.8 Hz, 3-H), 6.25 (1 H, t, *J* = 3.5 Hz, 4-H), 6.08 (1 H, d, *J* = 2.7 Hz, 3'-H), 3.89 (3 H, s, 7'-H), 1.62 (9 H, s, 10-H, 11-H, 12-H).

δ^{13}C (151 MHz, $CDCl_3$) 174.5 (C-8'), 157.8 (C-4'), 149.9 (C-6), 130.3 (C-2'), 126.2 (C-2), 124.7 (C-5), 118.4 (C-5'), 117.0 (C-3), 111.6 (C-4), 94.9 (C-3'), 85.9 (C-9), 58.1 (C-7'), 28.1 (C-10, C-11, C-12).

FT-IR (neat, cm^{-1}): 3207, 3156, 3111, 3013, 2993, 2977, 2936, 2857, 2831, 1744, 1597, 1546, 1504, 1455, 1445, 1389, 1366, 1304, 1284, 1255, 1230, 1189, 1170, 1160, 1138, 1082, 1067, 1041, 1015, 918, 878, 843, 821, 768, 732, 692, 639, 585, 571, 497.

T_m: 145–150 °C (Decomposition)

HRMS-ESI (m/z): $[M+H]^+$ calculated for $C_{16}H_{21}N_2O_4$ 291.1339, found 291.1344.

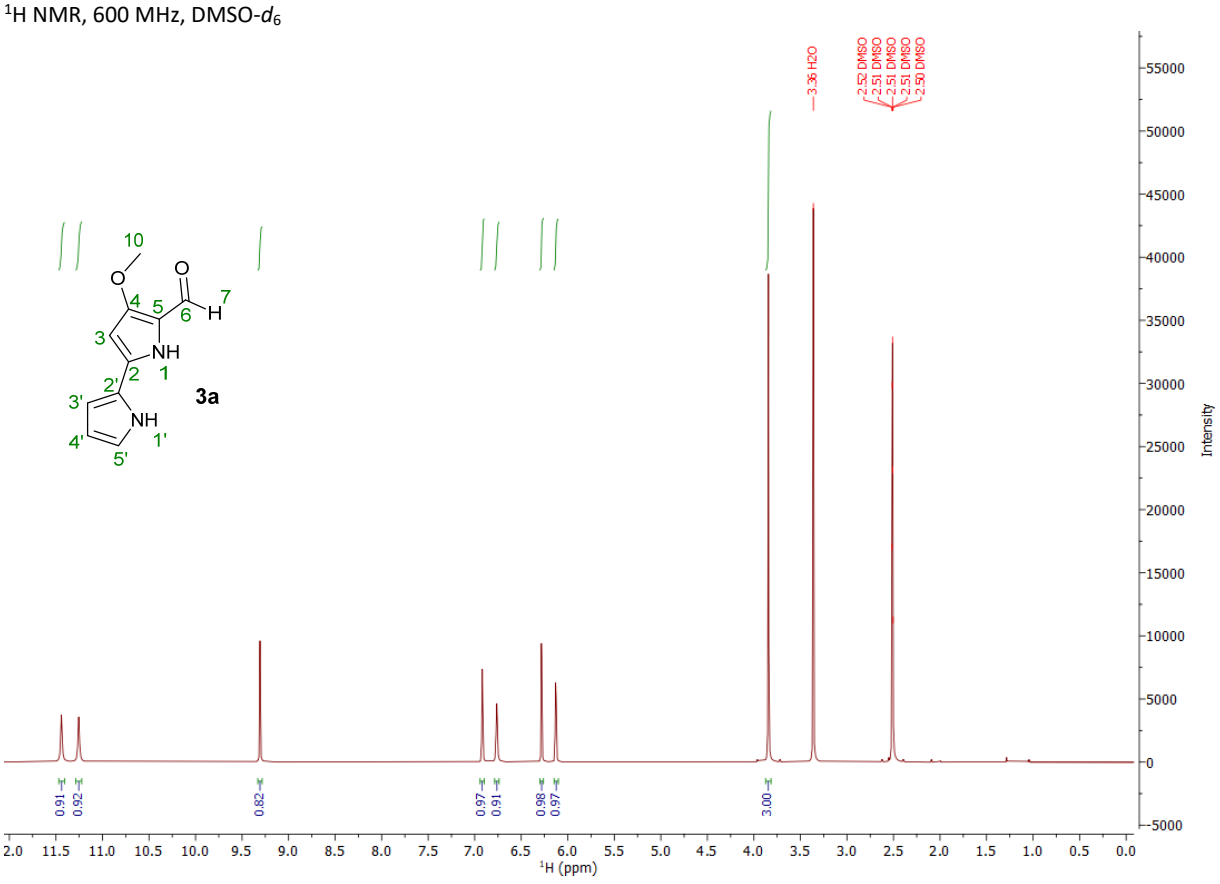
9. References

(1) S. R. Chawrai, N. R. Williamson, G. P. Salmond and F. J. Leeper, *Chem. Commun.*, 2008, (*16*), 1862-1864.
(2) L. N. Aldrich, E. S. Dawson and C. W. Lindsley, *Org. Lett.*, 2010, **12**(*5*), 1048-1051.
(3) A. Domröse, A. S. Klein, J. Hage-Hülsmann, S. Thies, V. Svensson, T. Classen, J. Pietruszka, K.-E. Jaeger, T. Drepper and A. Loeschcke, *Front. Microbiol.*, 2015, **6**:972.

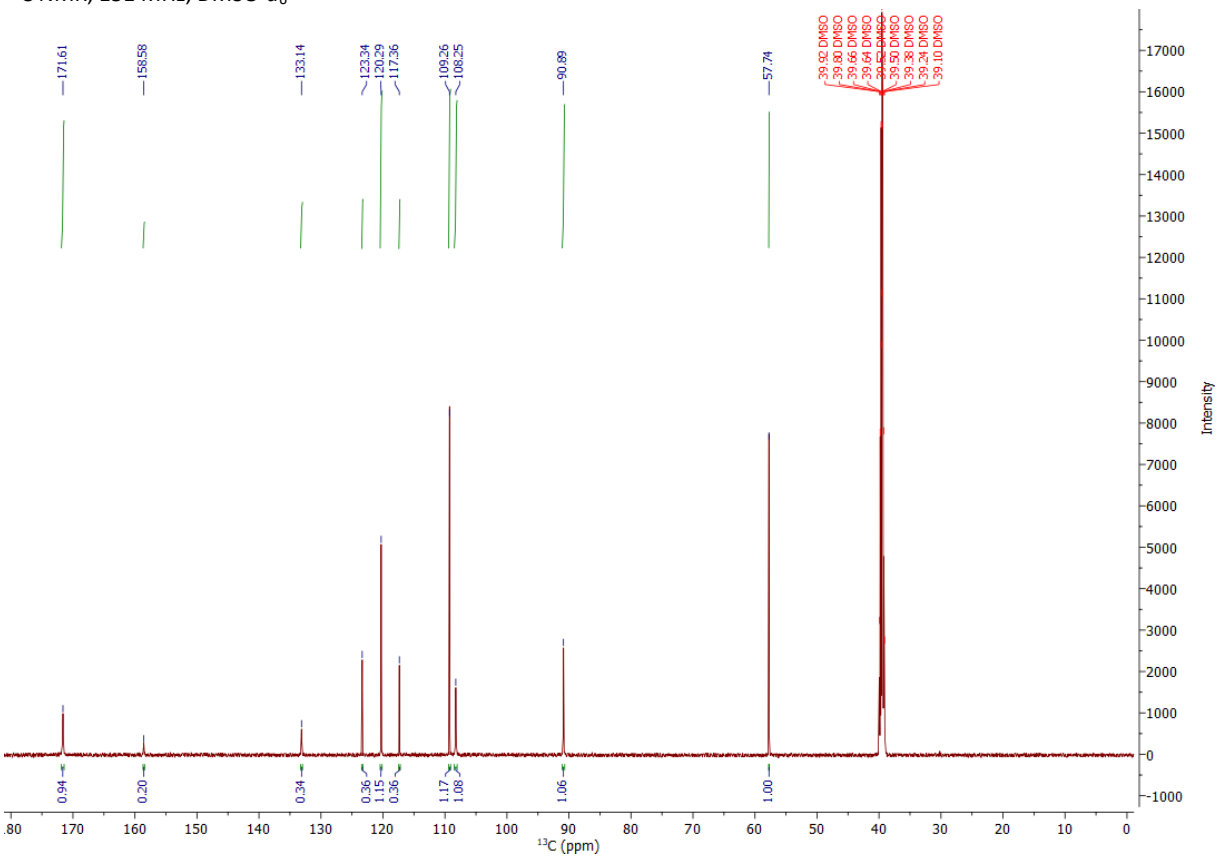
10. NMR Spectra

4-Methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3a)

^{1}H NMR, 600 MHz, DMSO-d_6

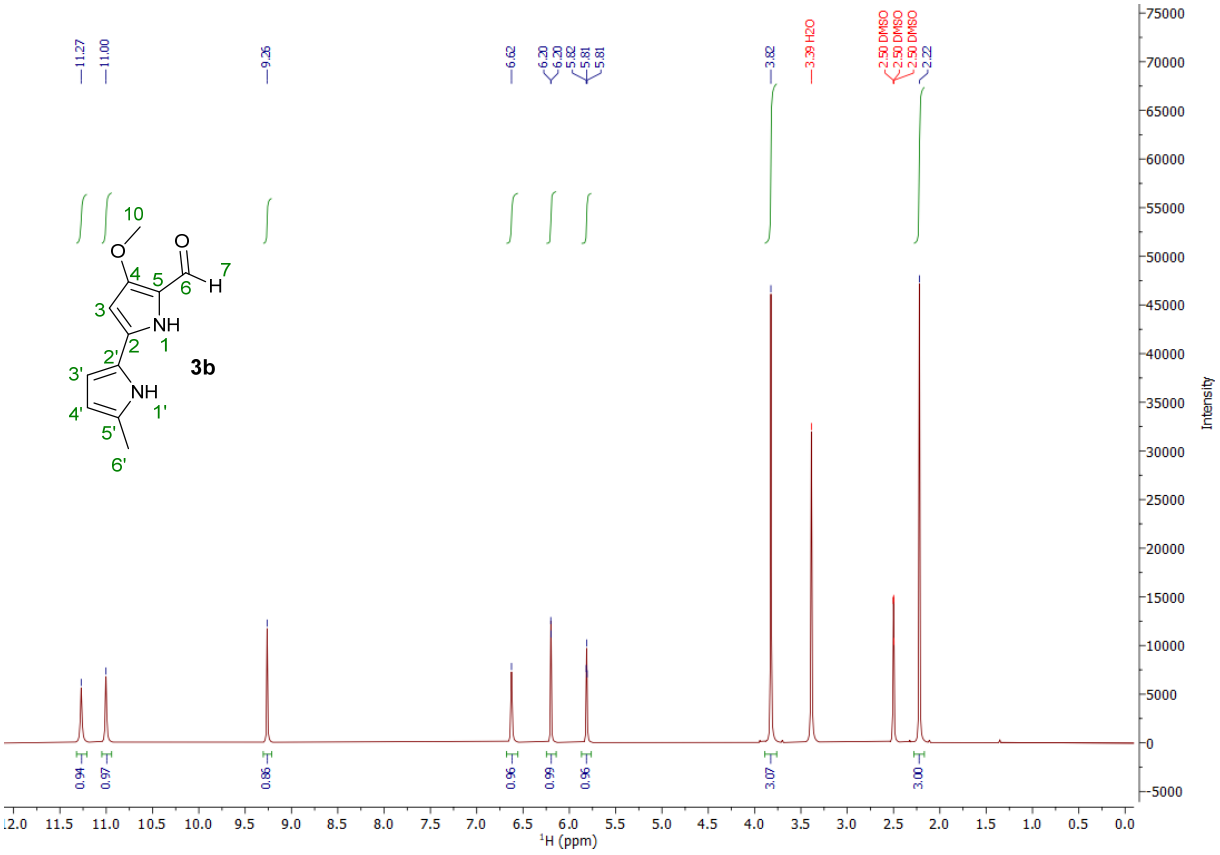


^{13}C NMR, 151 MHz, DMSO-d_6

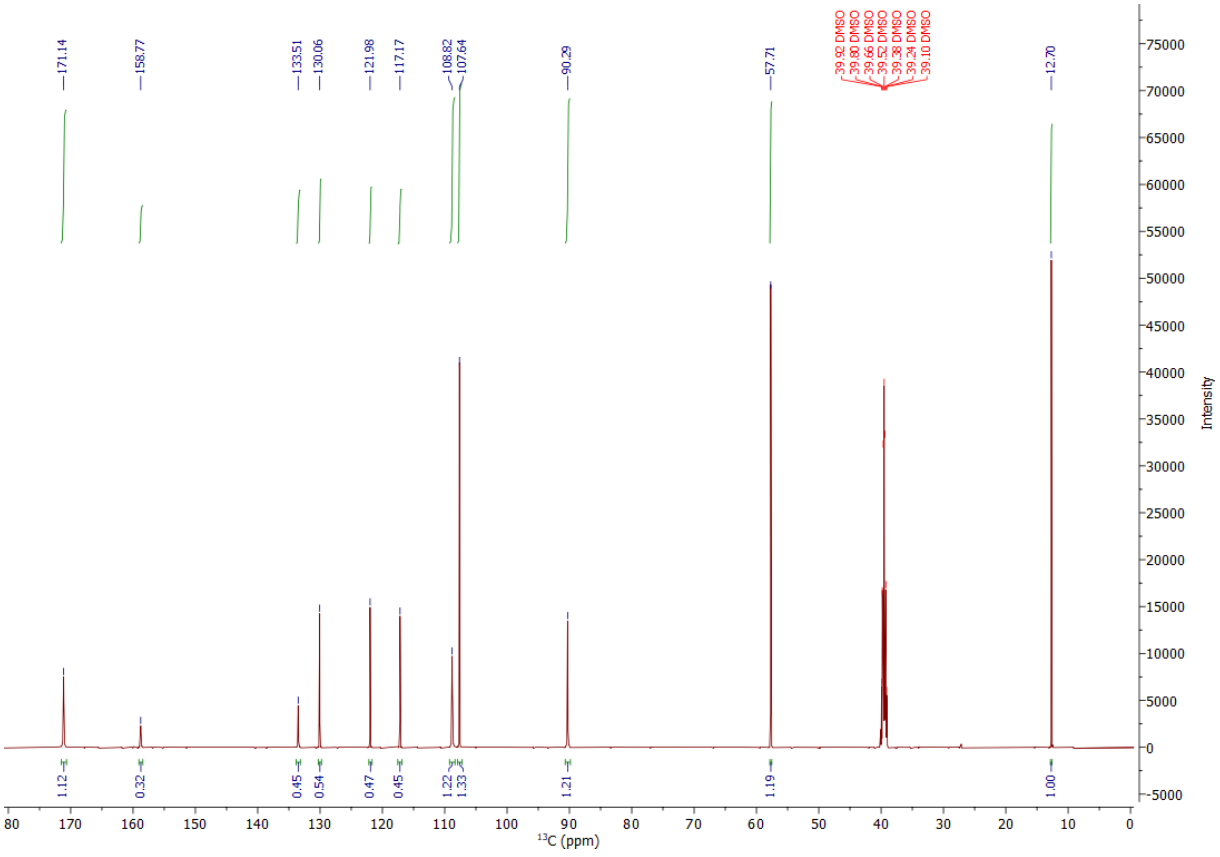


4-Methoxy-5'-methyl-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3b)

^{1}H NMR, 600 MHz, DMSO-d_6

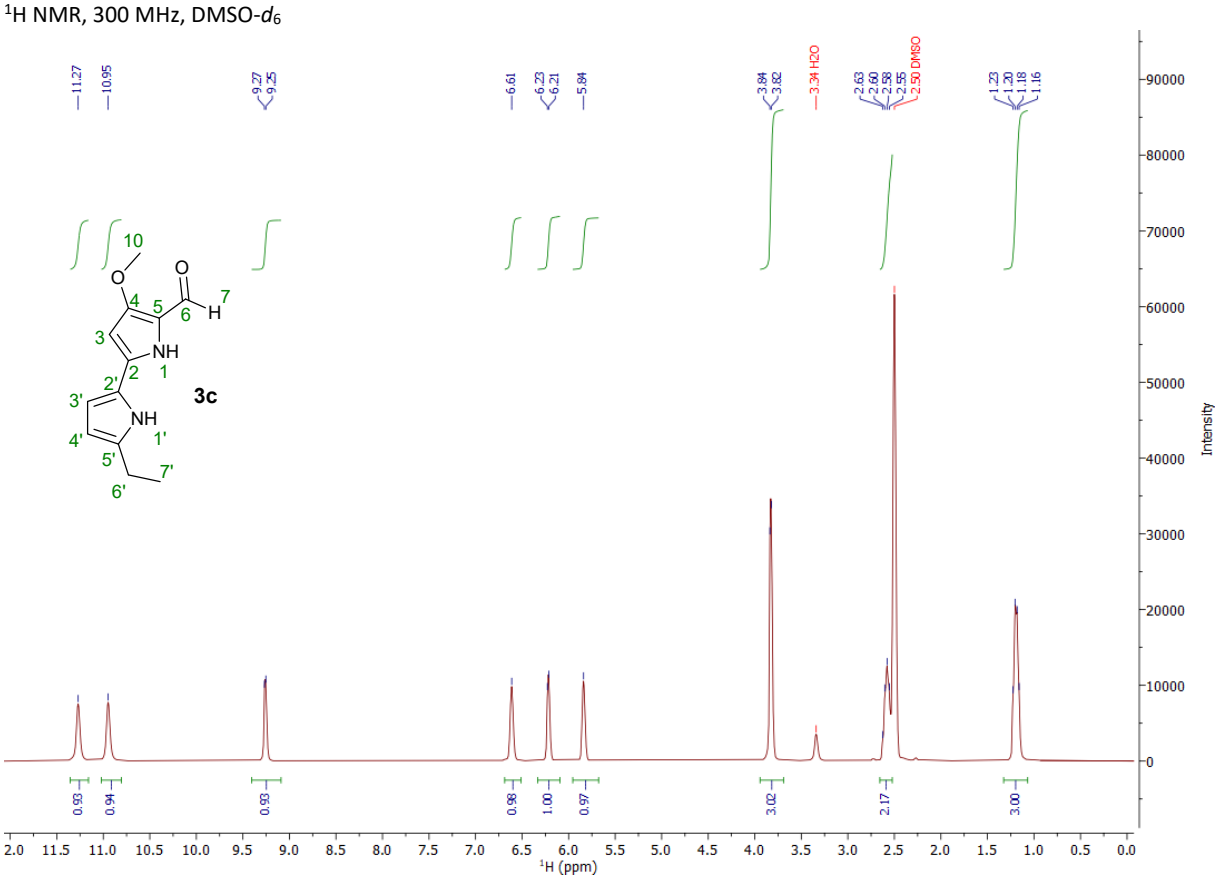


^{13}C NMR, 151 MHz, DMSO-d_6

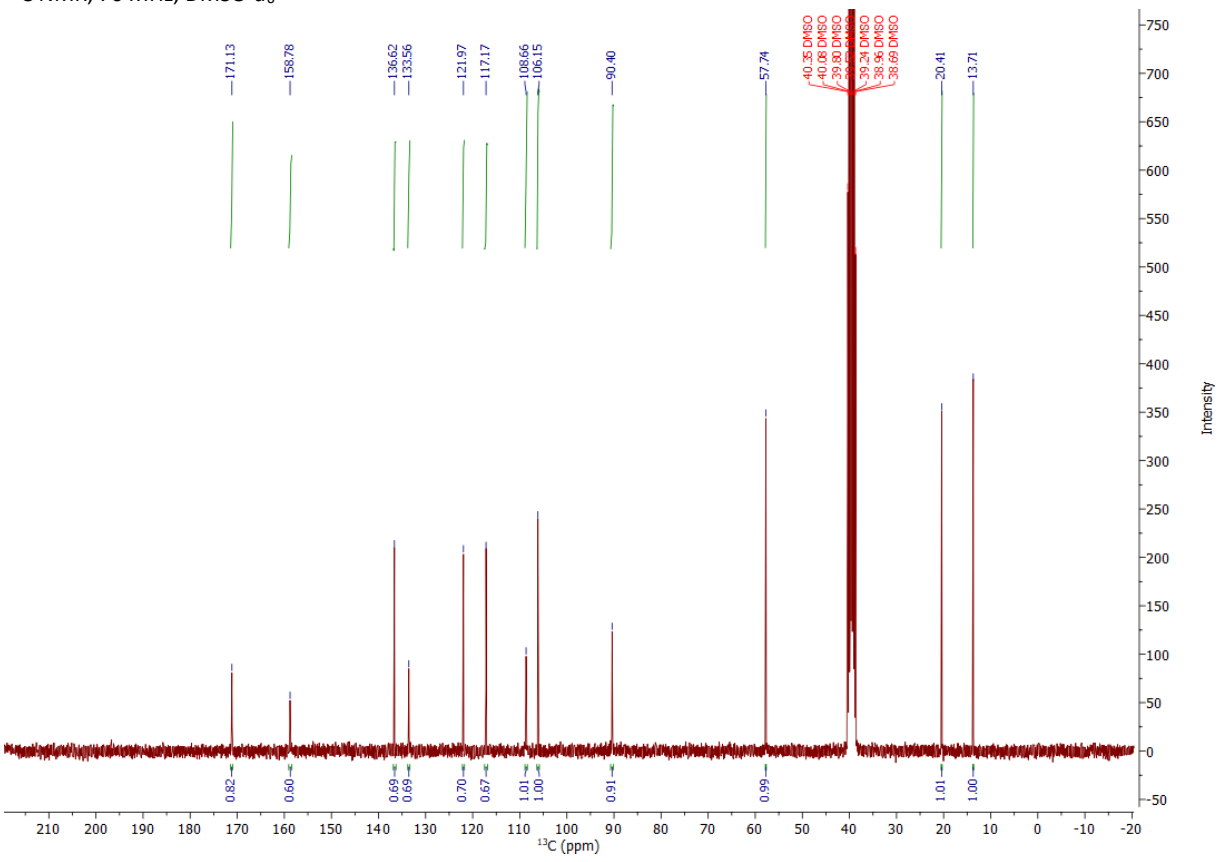


5'-Ethyl-4-methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3c)

^{1}H NMR, 300 MHz, DMSO-d_6

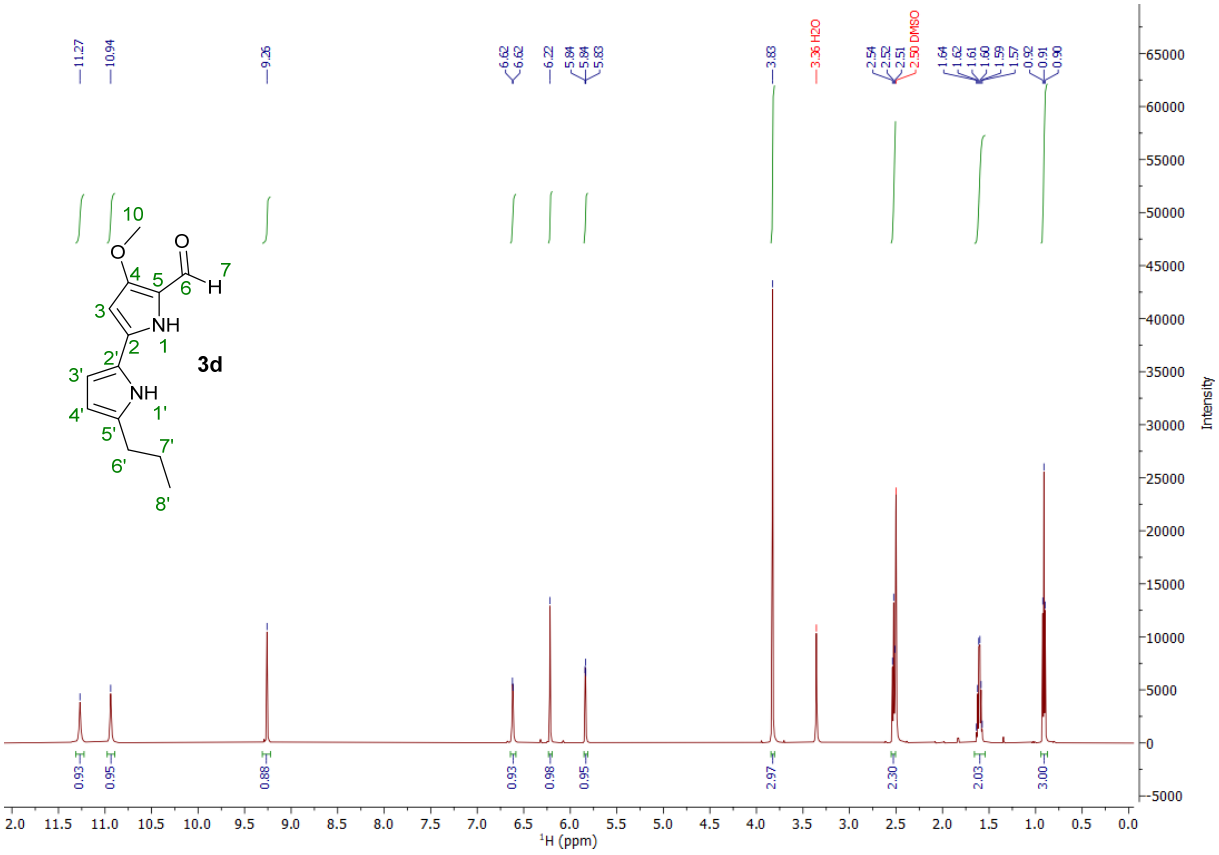


^{13}C NMR, 76 MHz, DMSO-d_6

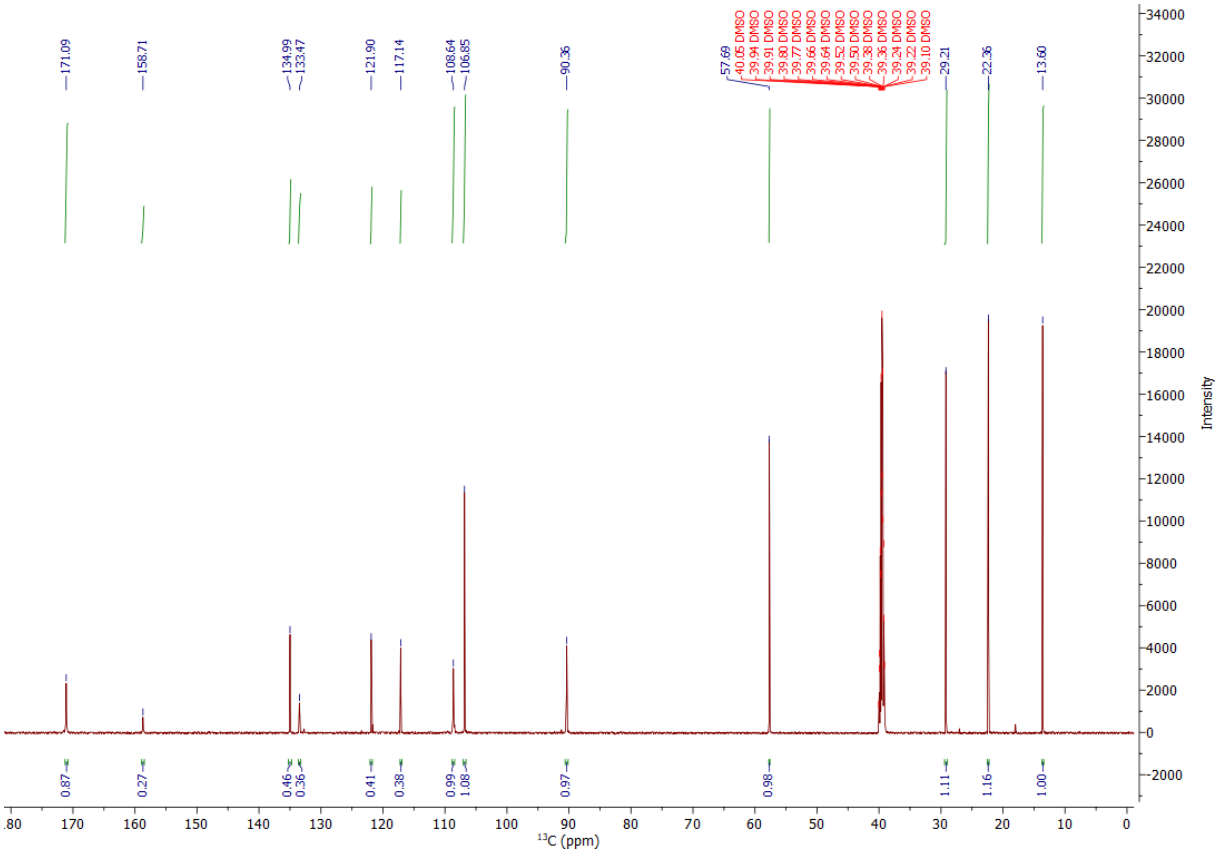


4-Methoxy-5'-propyl-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3d)

^{1}H NMR, 600 MHz, DMSO-d_6

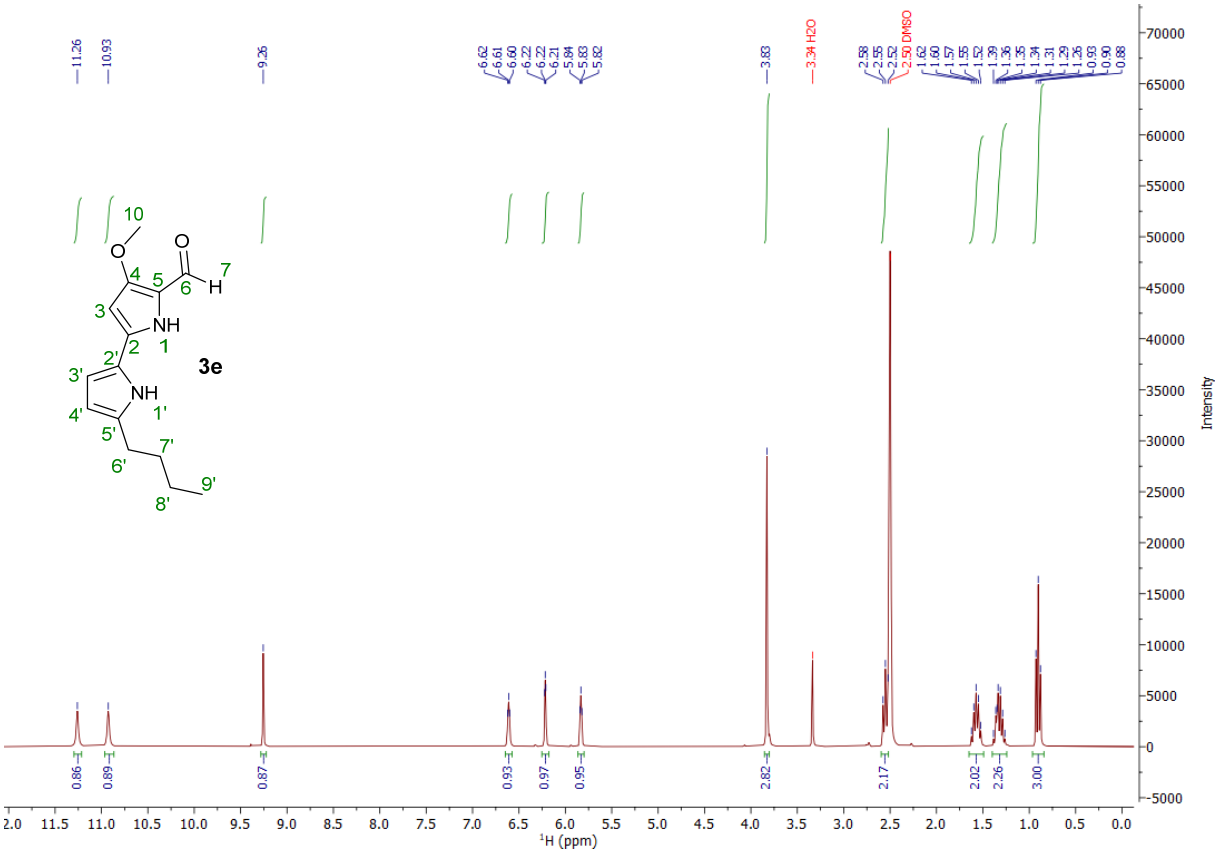


^{13}C NMR, 151 MHz, DMSO-d_6

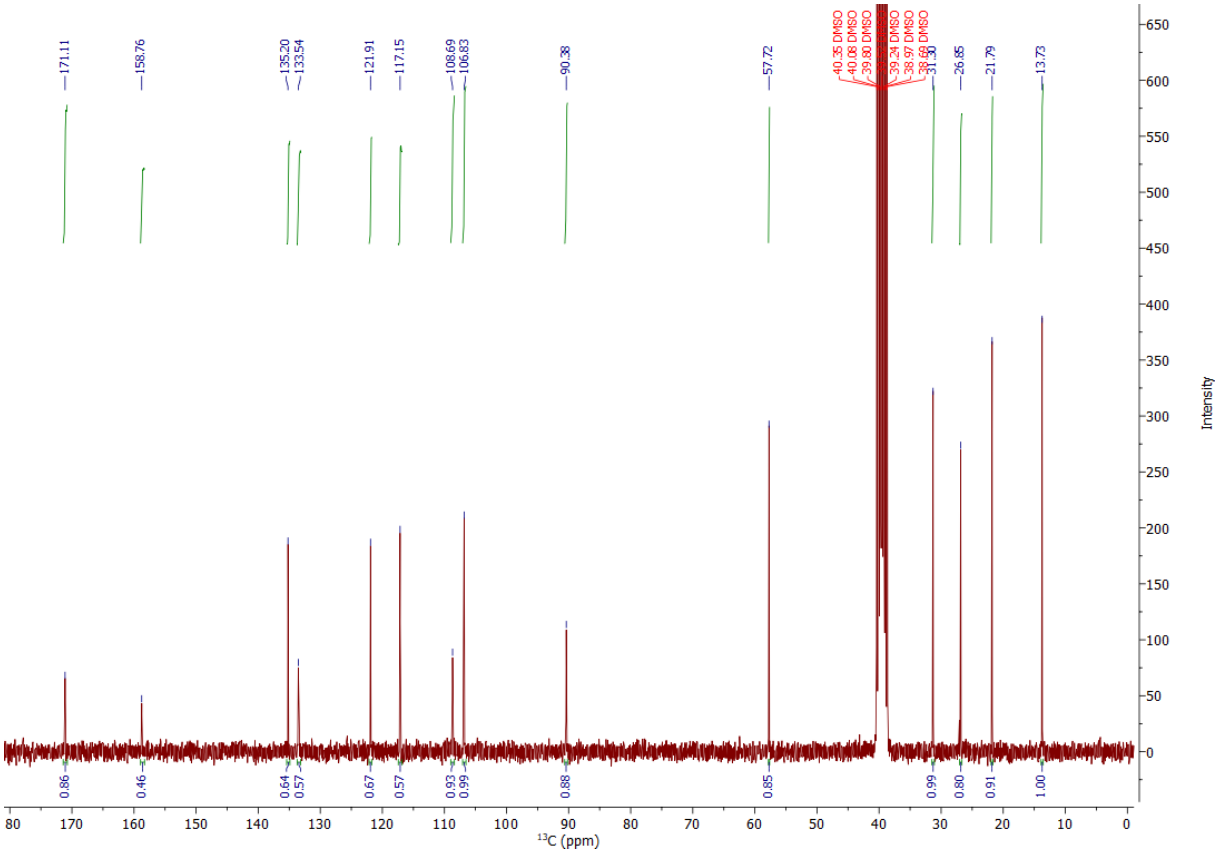


5'-Butyl-4-methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3e)

^{1}H NMR, 300 MHz, DMSO-d_6

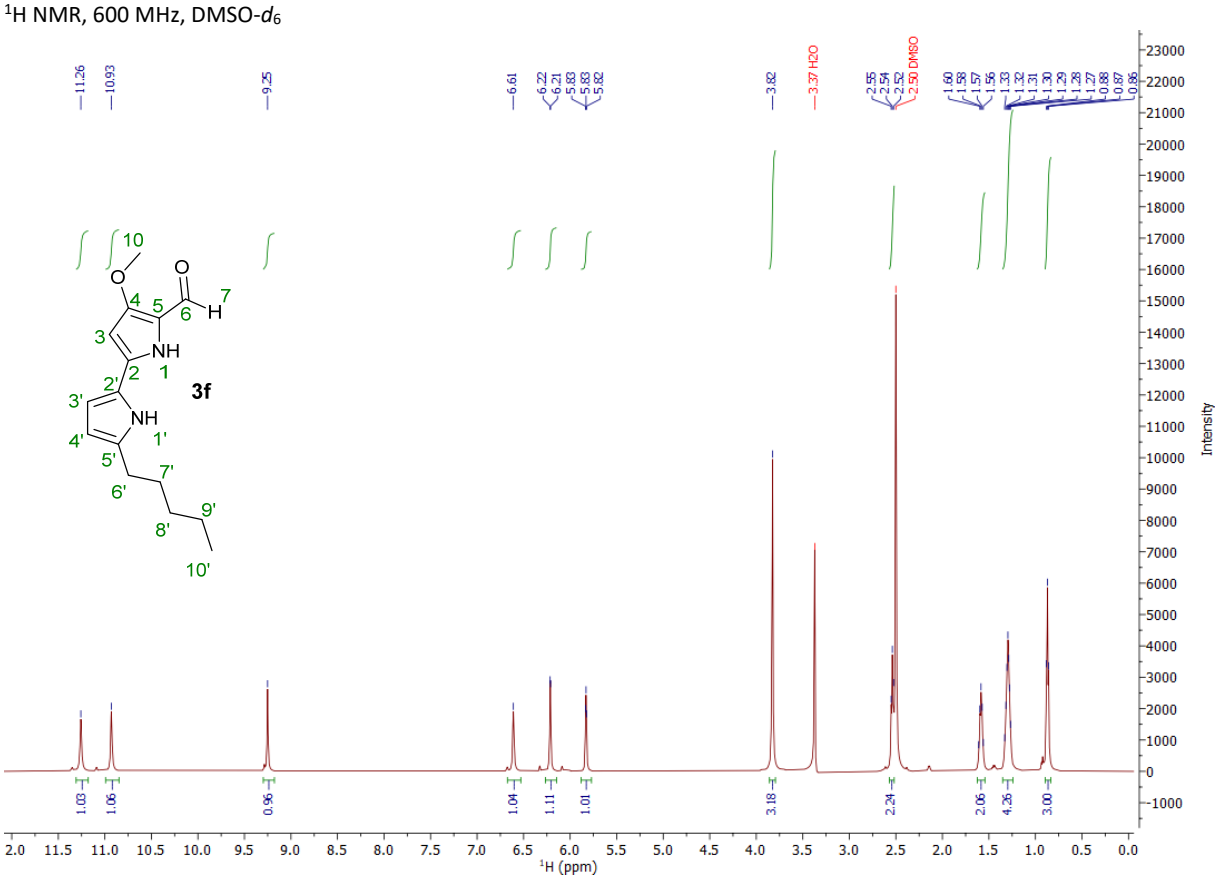


^{13}C NMR, 76 MHz, DMSO-d_6

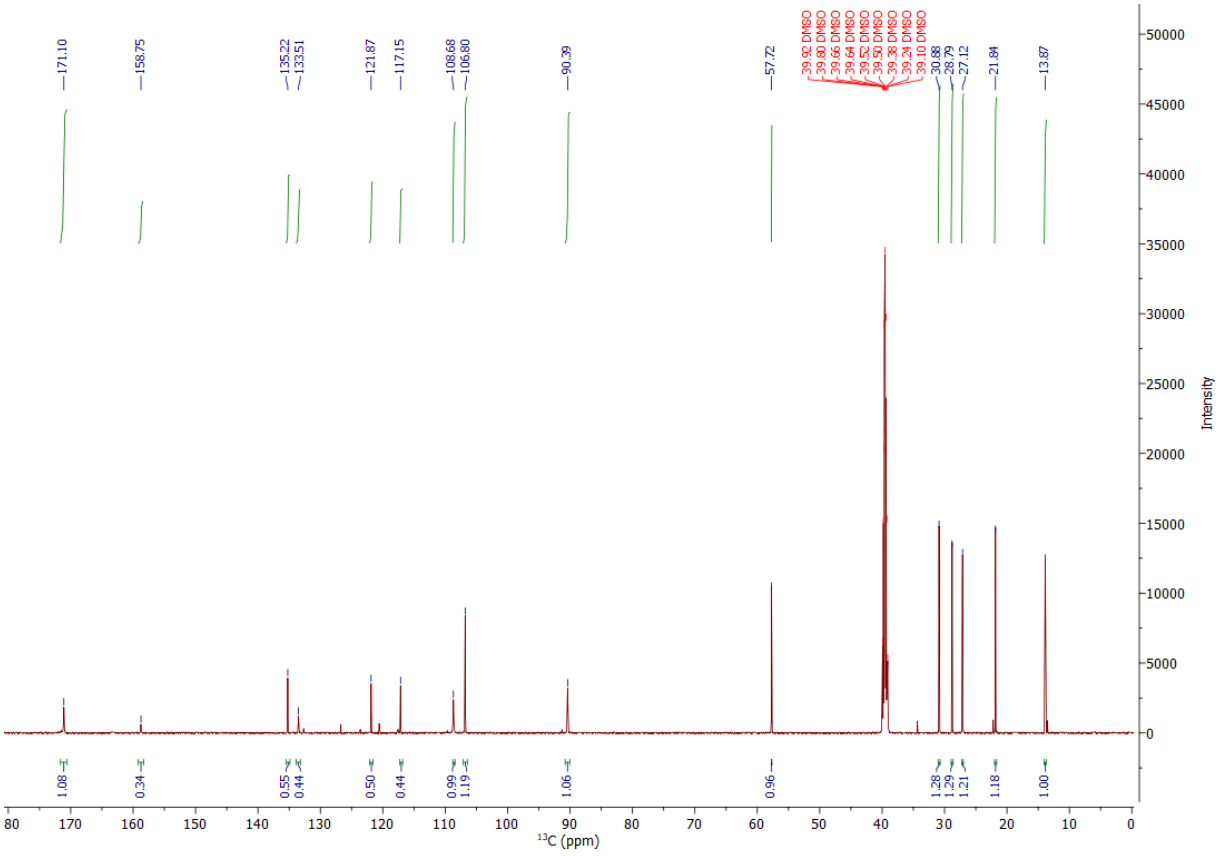


4-Methoxy-5'-pentyl-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3f)

^{1}H NMR, 600 MHz, DMSO-d_6

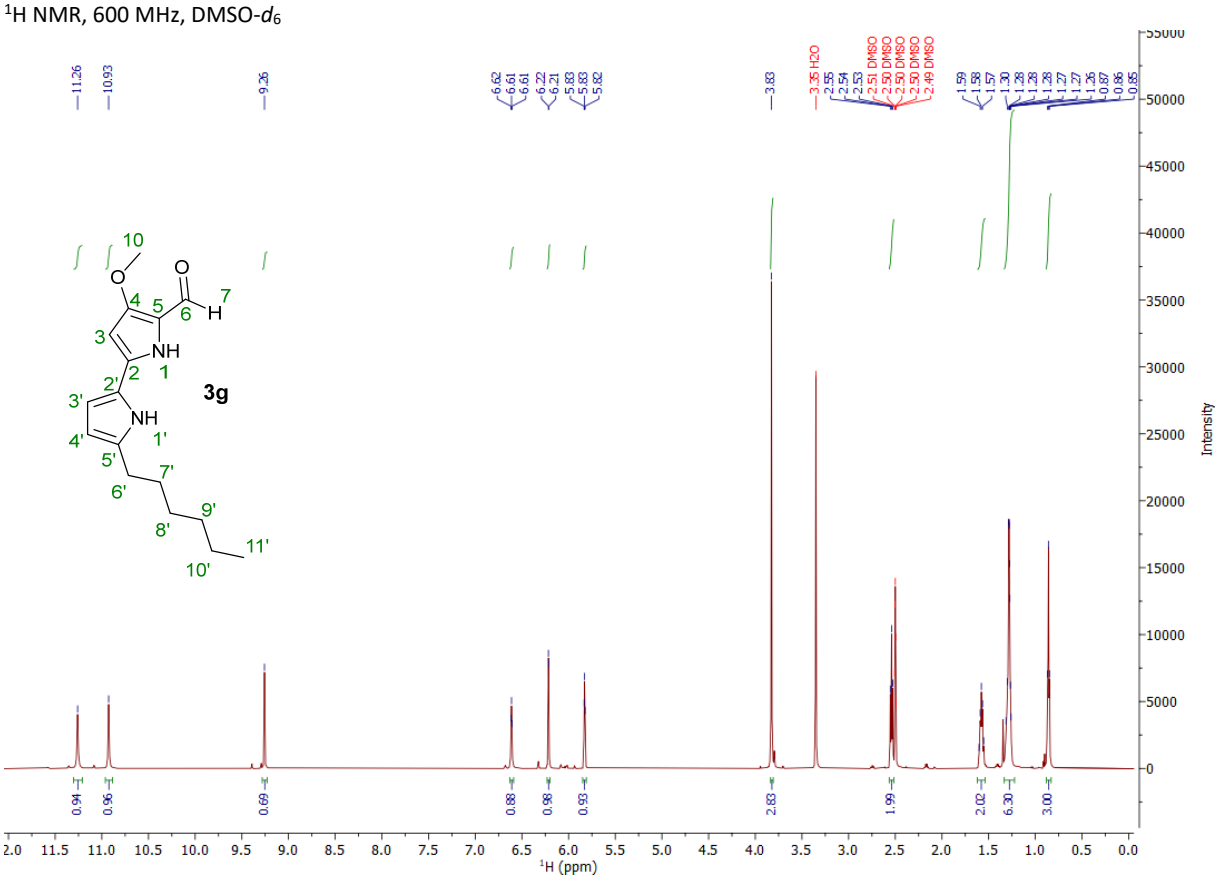


^{13}C NMR, 151 MHz, DMSO-d_6

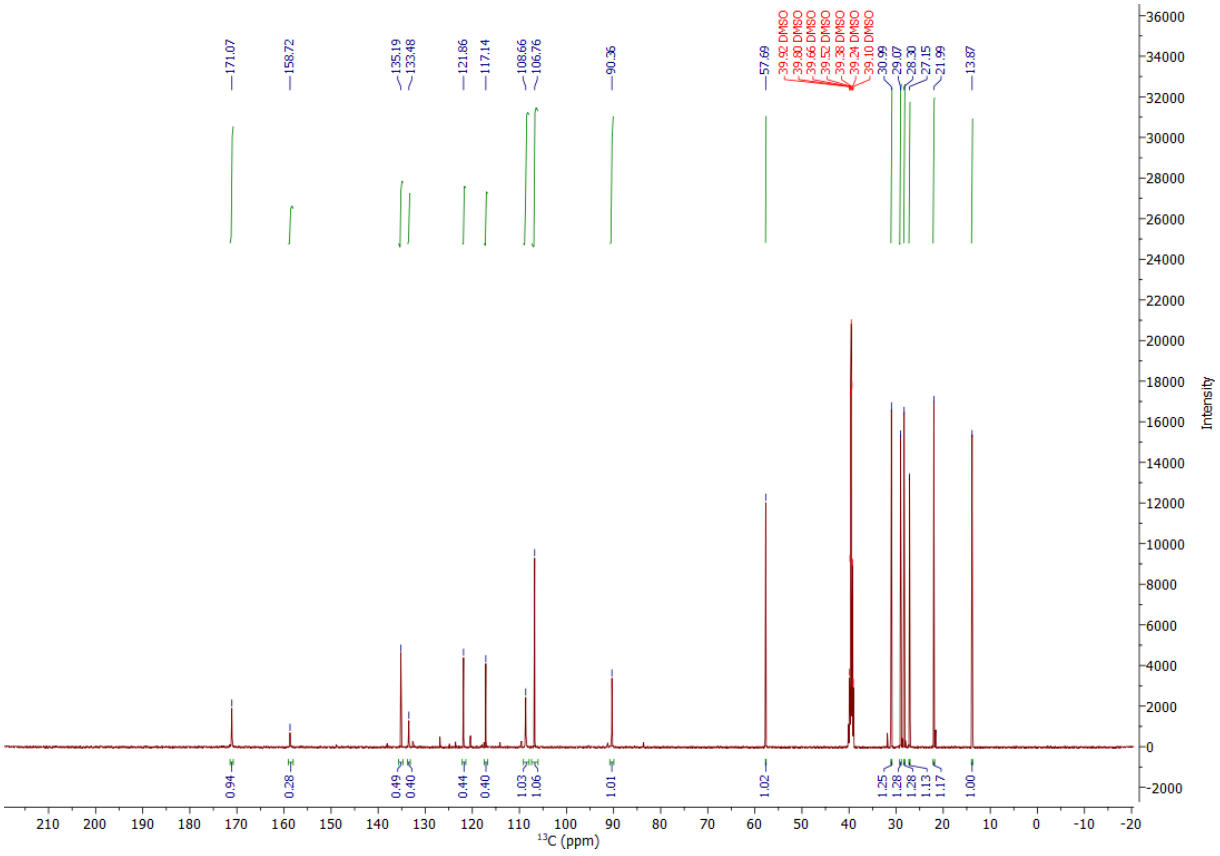


5'-Hexyl-4-methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-5-carbaldehyde (3g)

^{1}H NMR, 600 MHz, DMSO-d_6

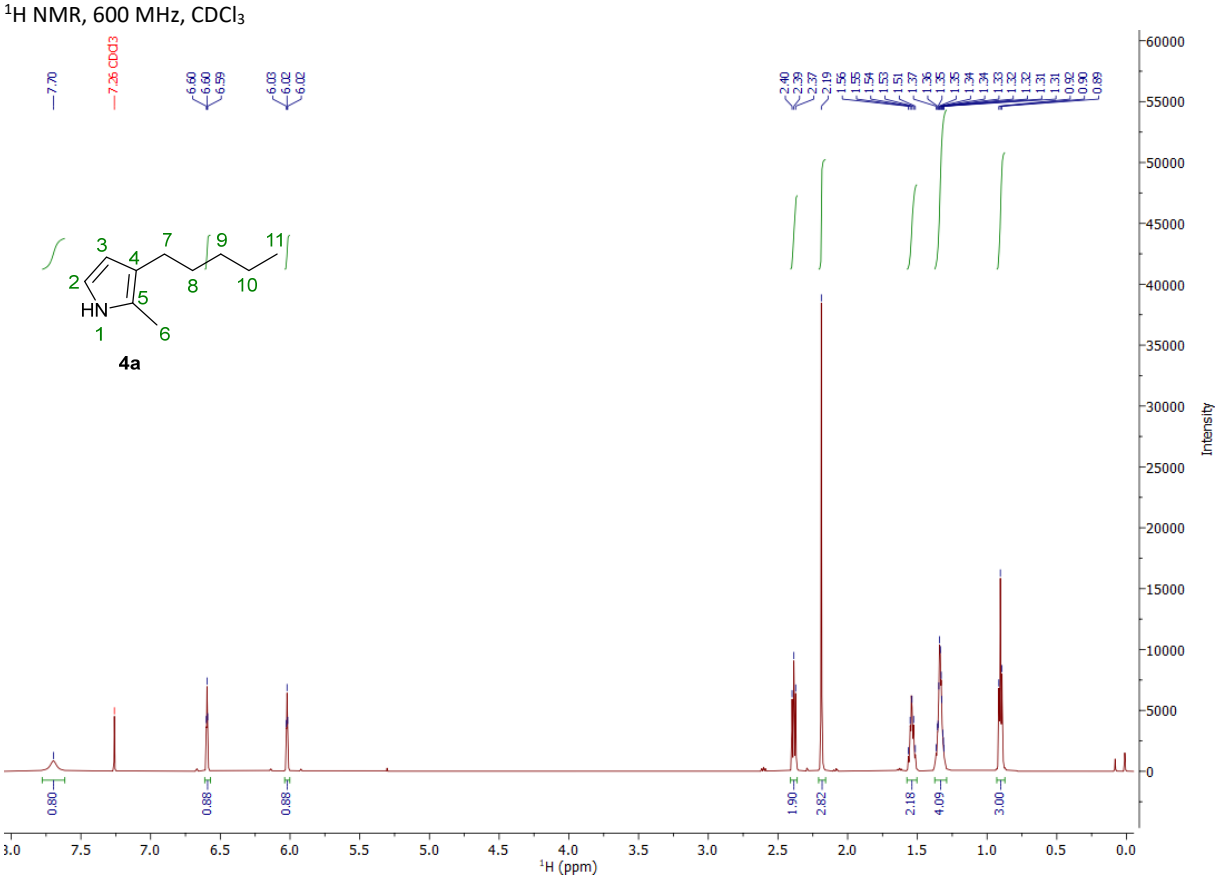


^{13}C NMR, 151 MHz, DMSO-d_6

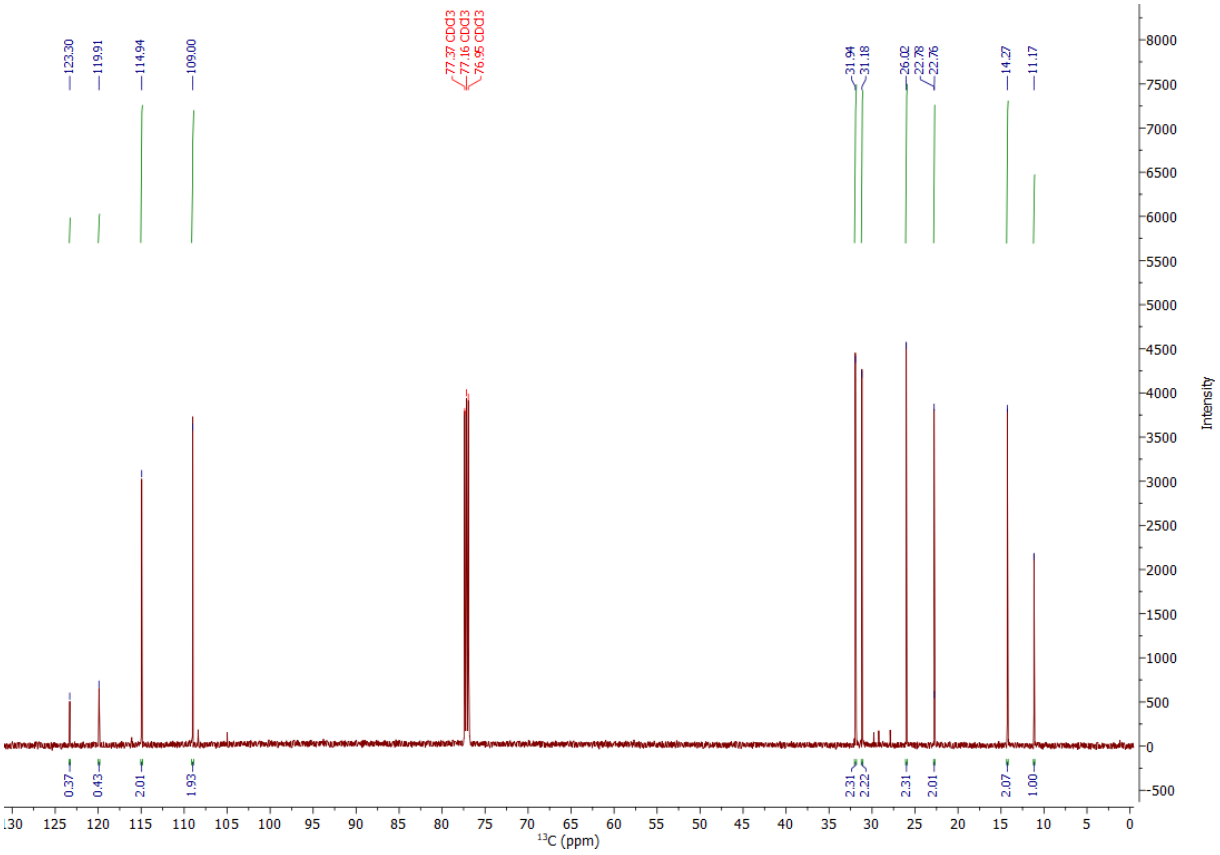


2-Methyl-3-pentyl-1*H*-pyrrole (4a)

^{1}H NMR, 600 MHz, $CDCl_3$

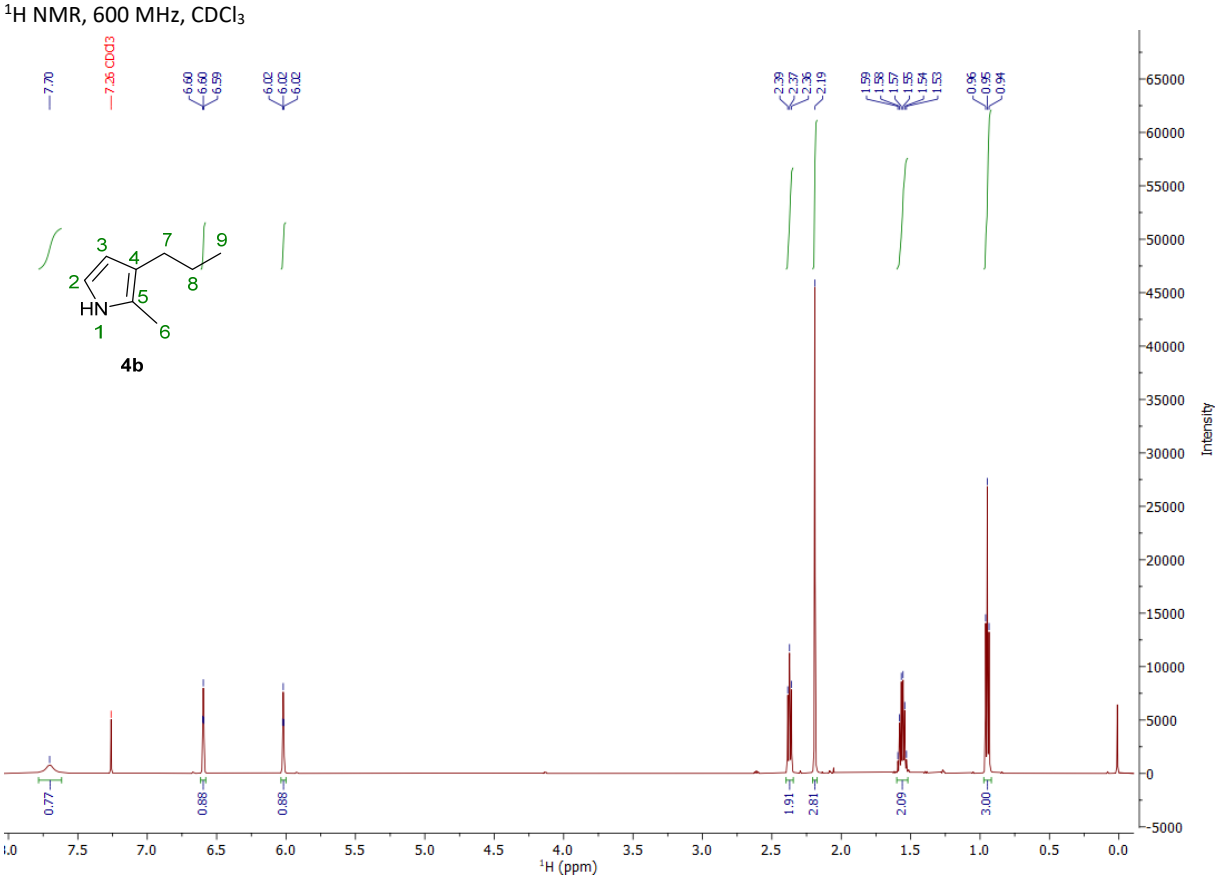


^{13}C NMR, 151 MHz, $CDCl_3$

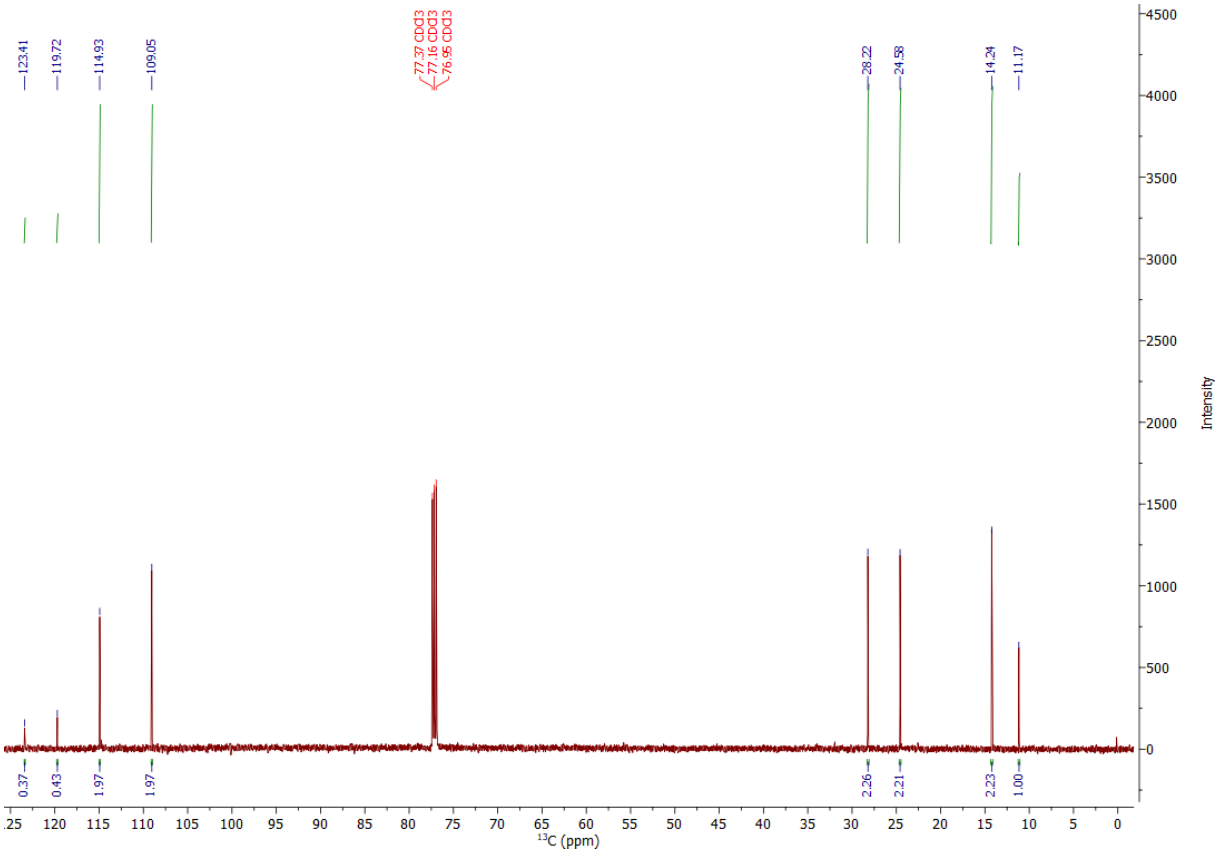


2-Methyl-3-propyl-1*H*-pyrrole (4b)

^{1}H NMR, 600 MHz, $CDCl_3$

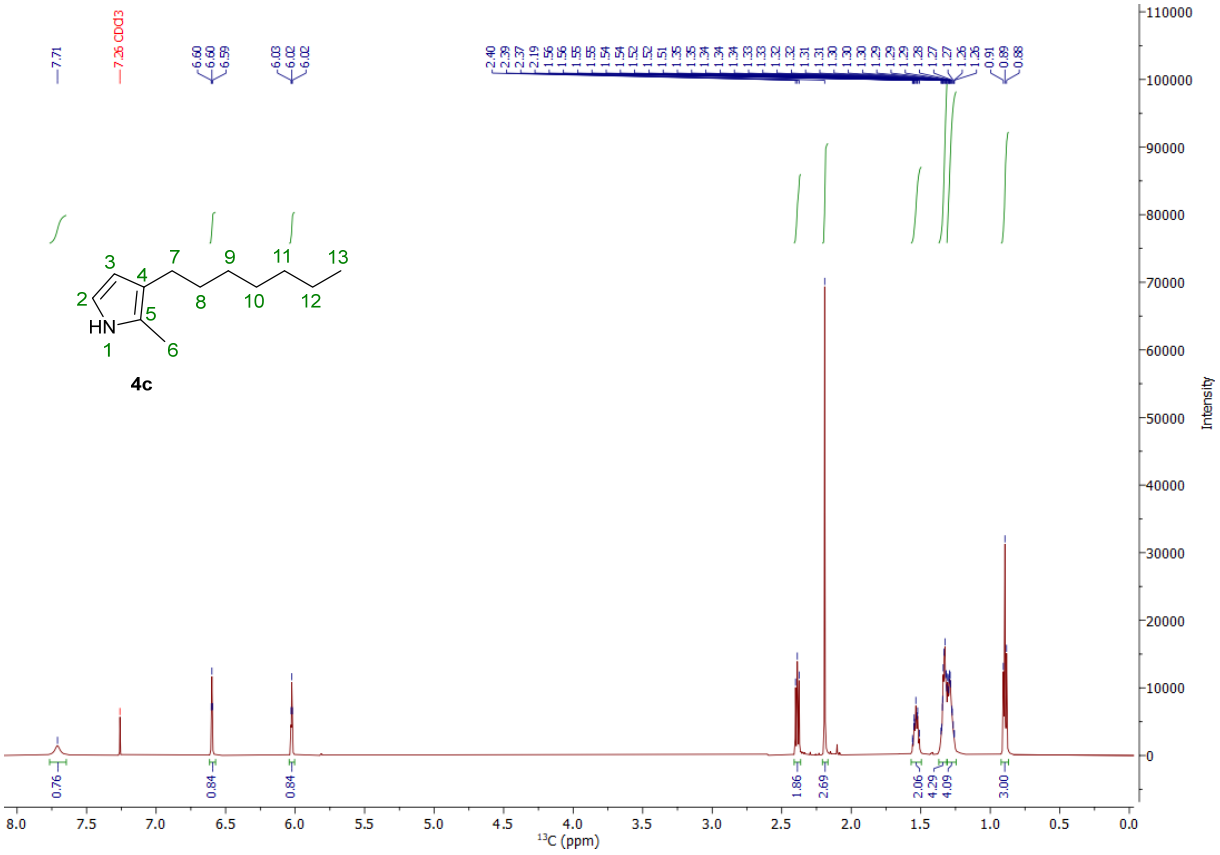


^{13}C NMR, 151 MHz, $CDCl_3$

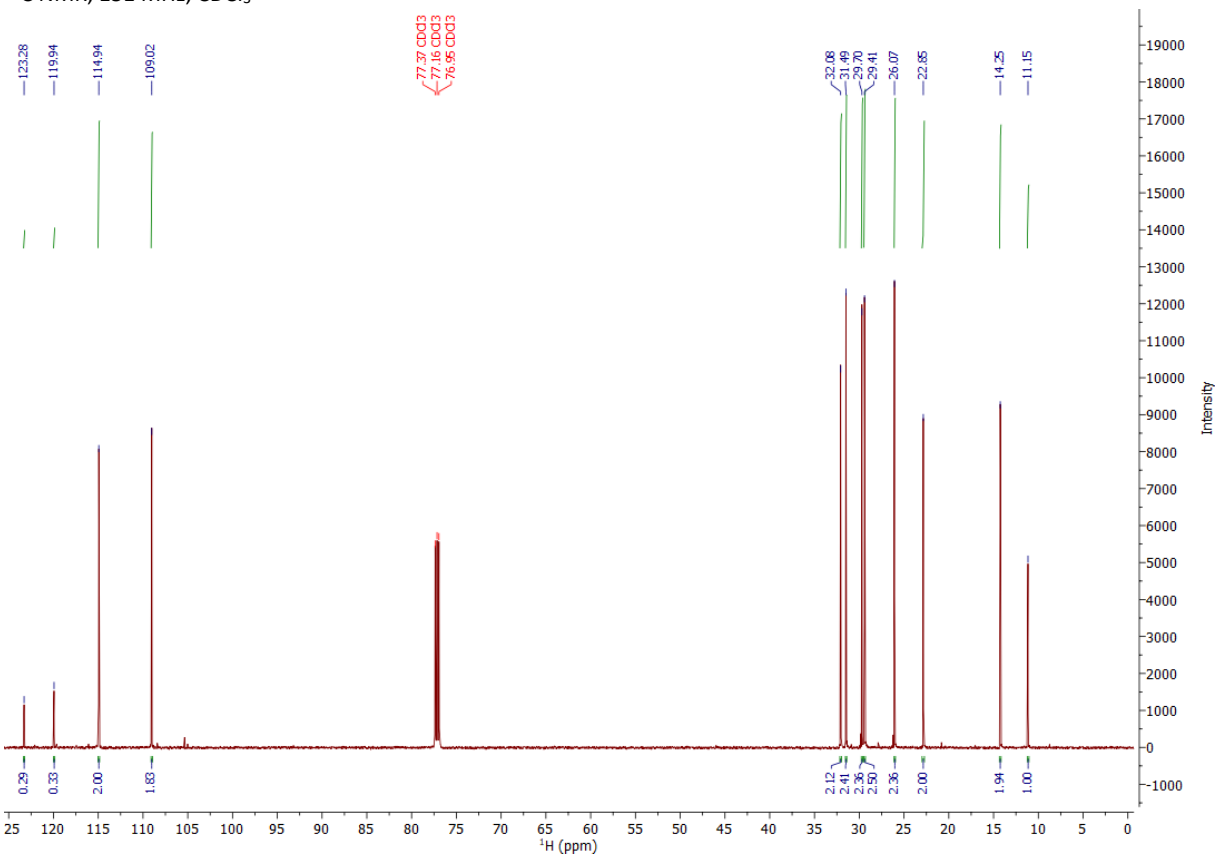


3-Heptyl-2-methyl-1*H*-pyrrole (4c)

^{1}H NMR, 600 MHz, $CDCl_3$

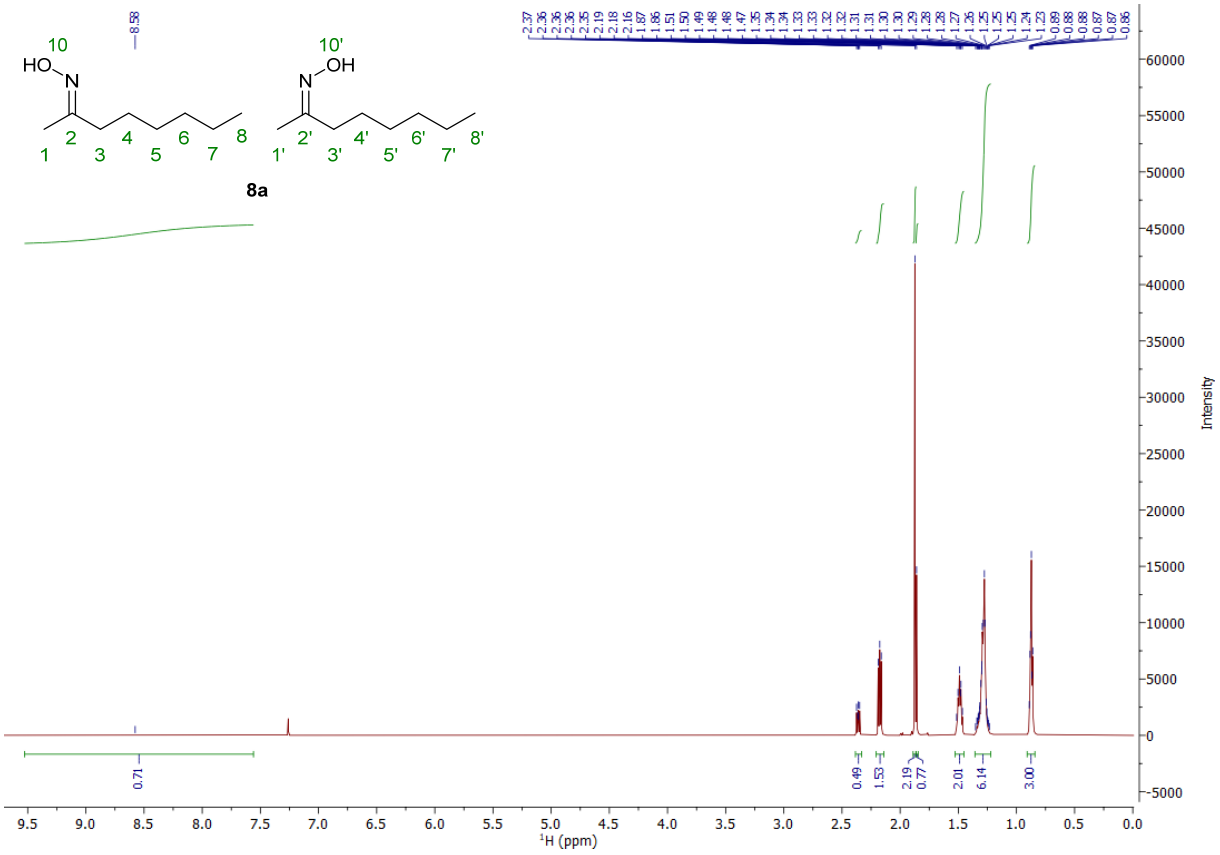


^{13}C NMR, 151 MHz, $CDCl_3$

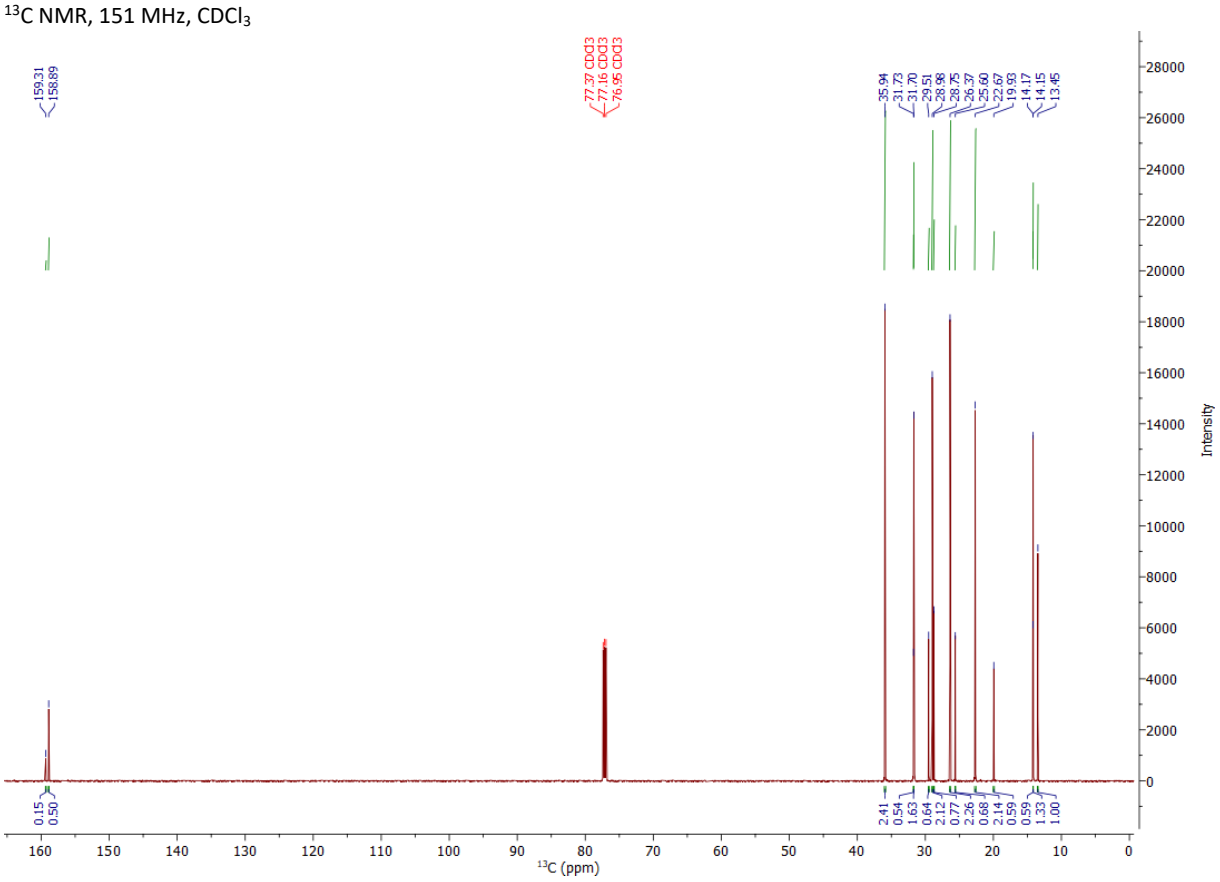


(*E*/*Z*)-Octan-2-one oxime (8a)

^{1}H NMR, 600 MHz, $CDCl_3$

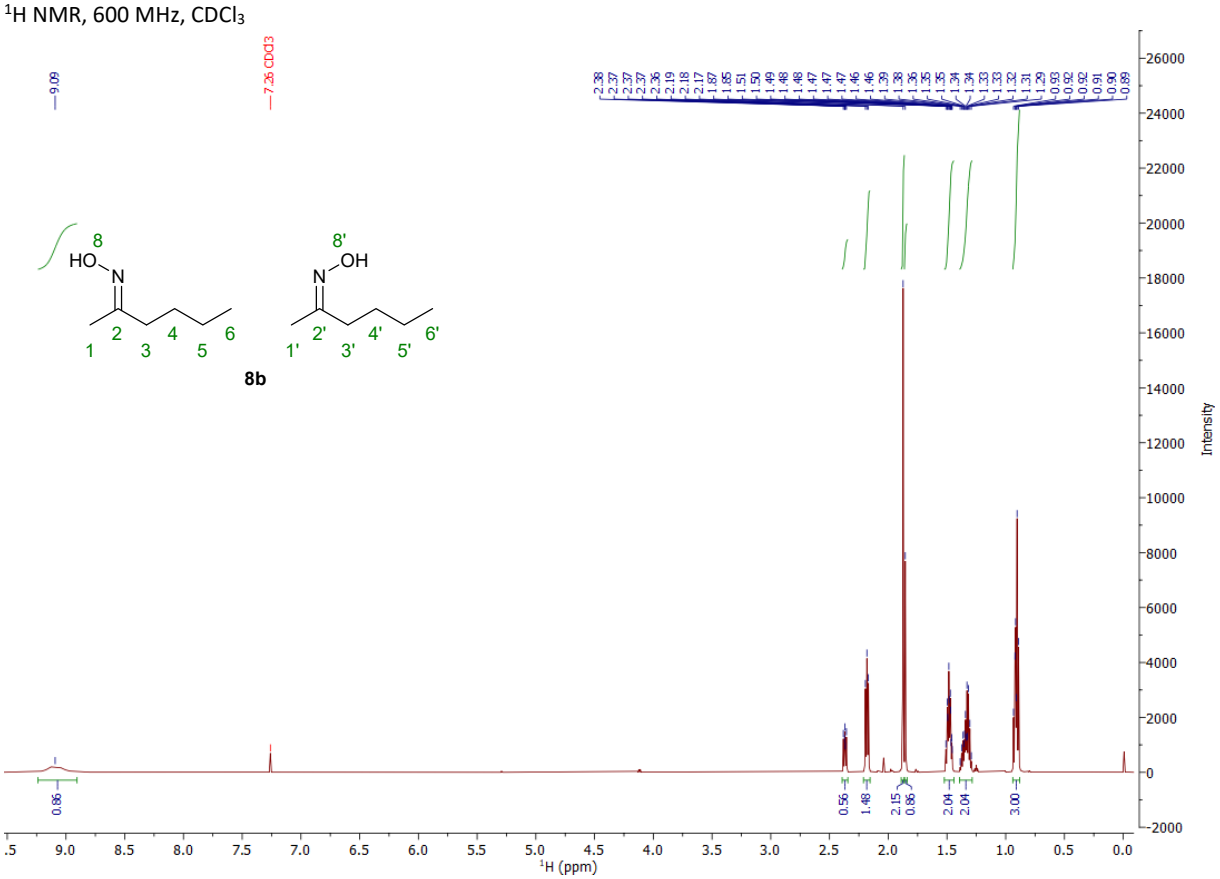


^{13}C NMR, 151 MHz, $CDCl_3$

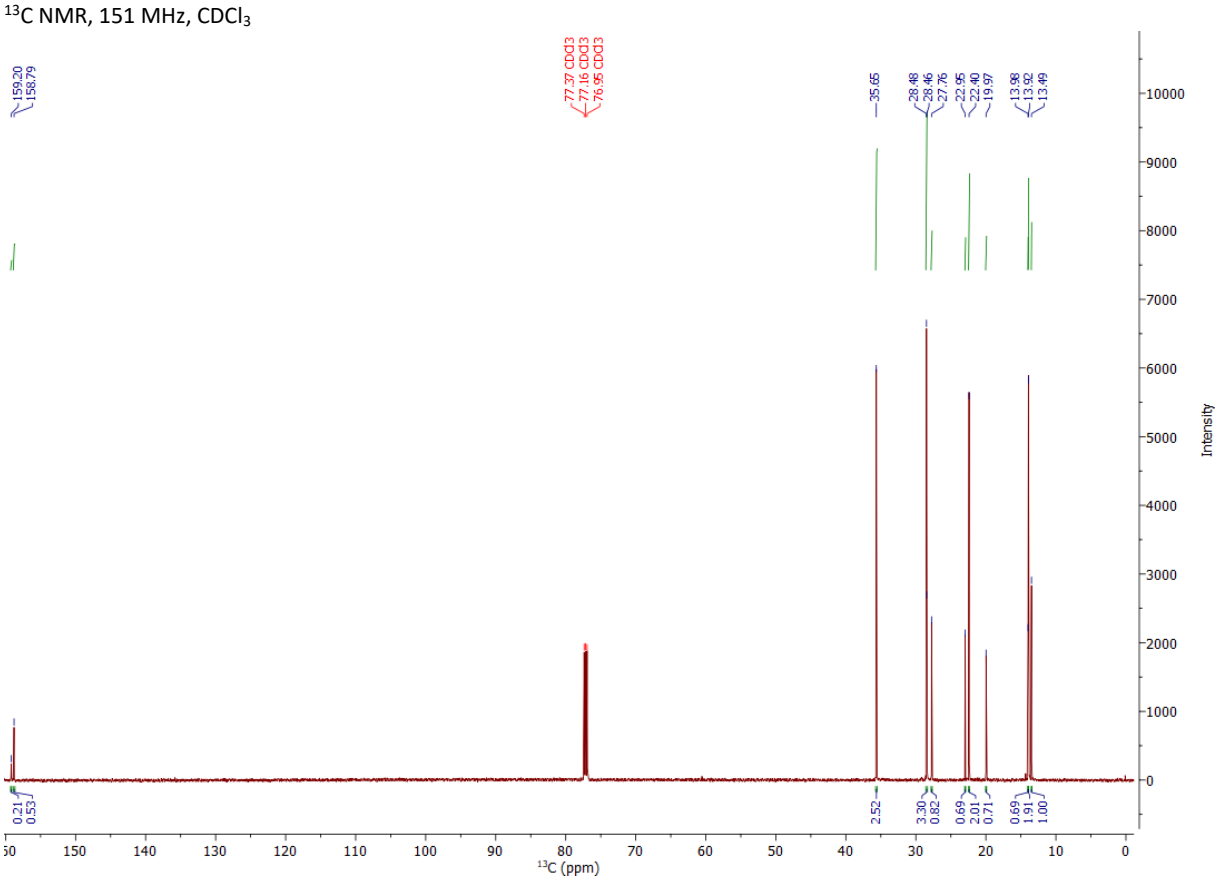


(*E*/*Z*)-Hexan-2-one oxime (8b)

^{1}H NMR, 600 MHz, $CDCl_3$

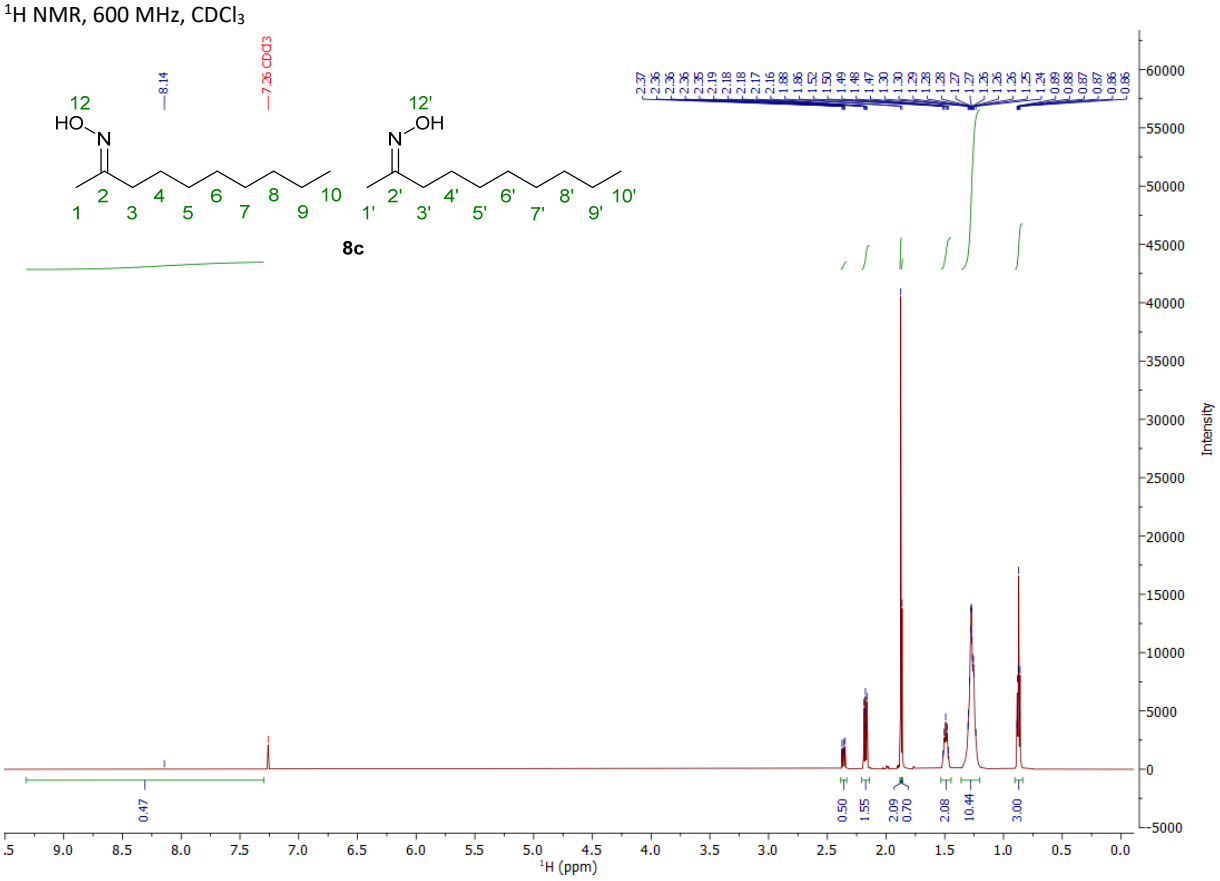


^{13}C NMR, 151 MHz, $CDCl_3$

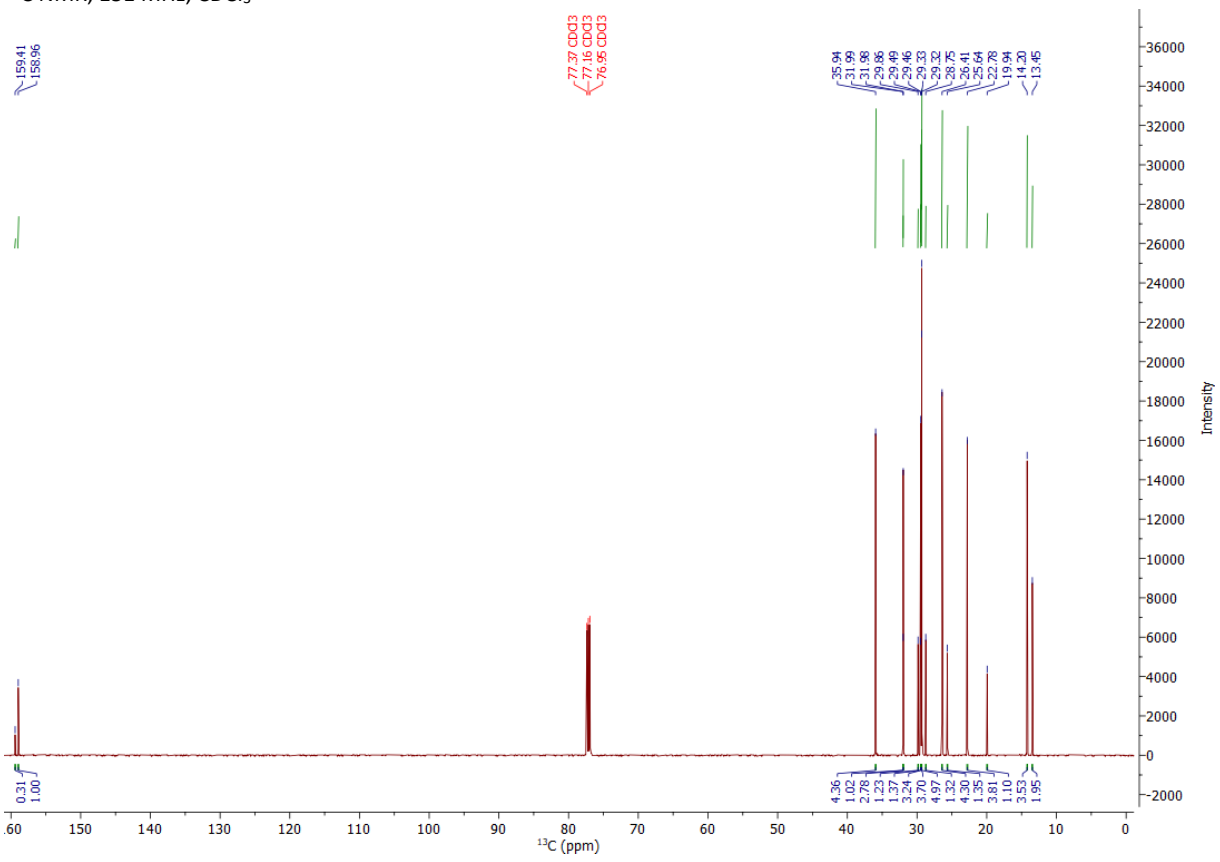


(*E*/*Z*)-Decan-2-one oxime (8c)

^{1}H NMR, 600 MHz, $CDCl_3$

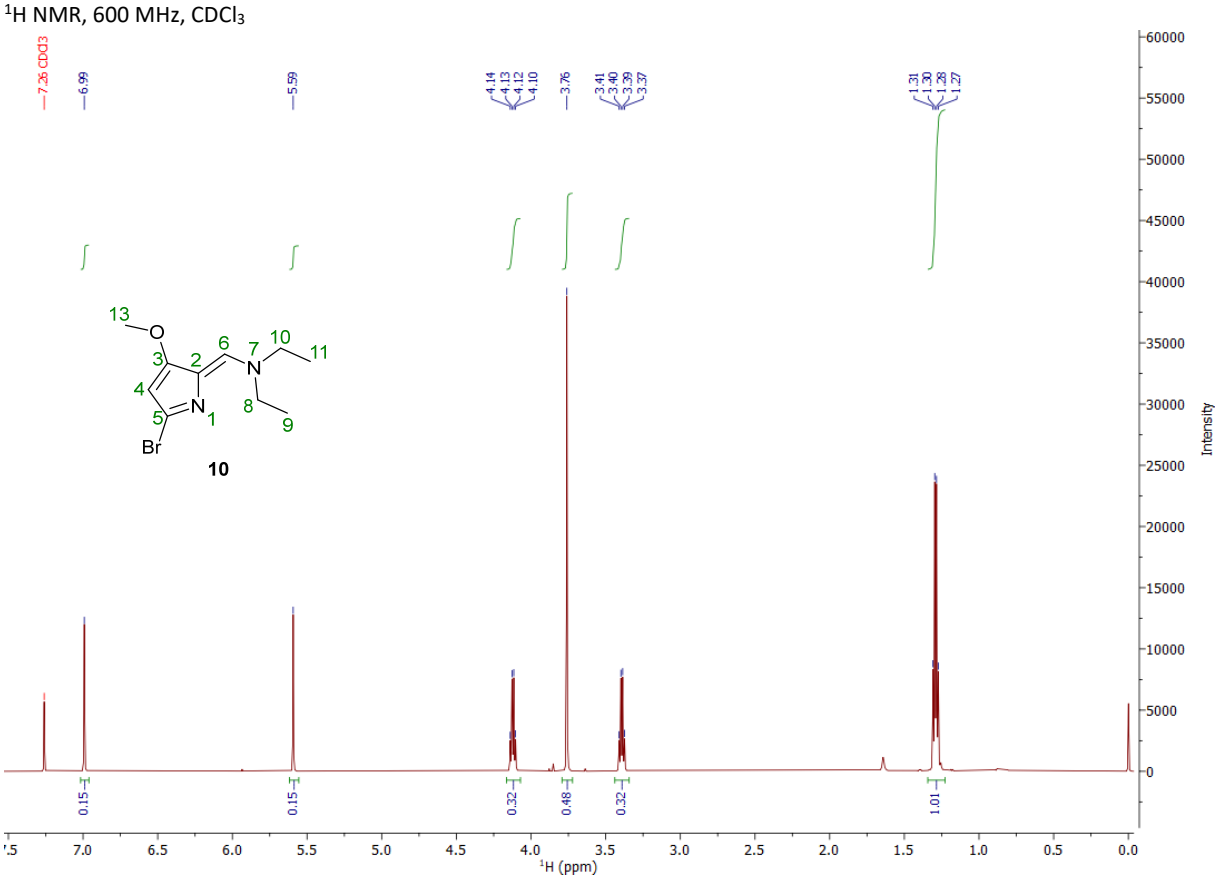


^{13}C NMR, 151 MHz, $CDCl_3$

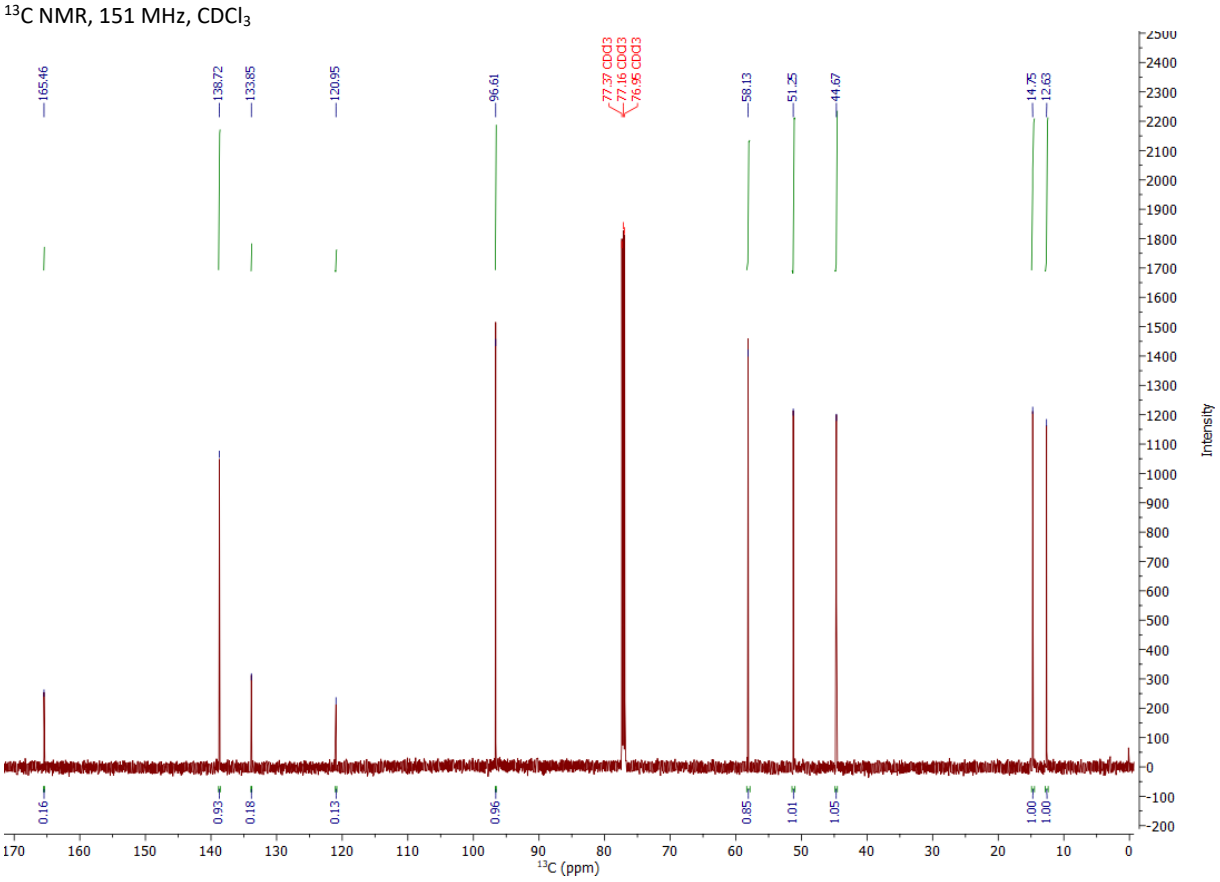


(*Z*)-*N*-[(5-Bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl]-*N*-ethylethanamine (10)

^{1}H NMR, 600 MHz, $CDCl_3$

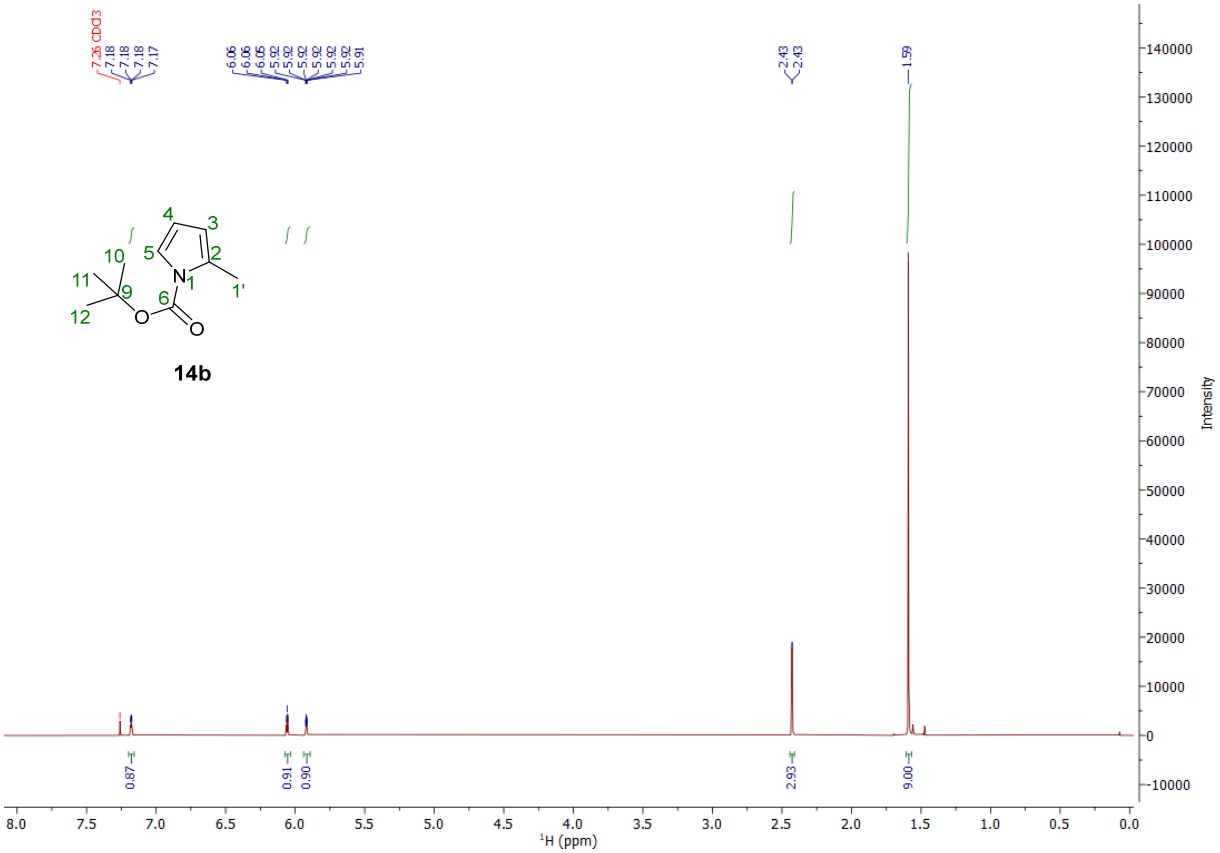


^{13}C NMR, 151 MHz, $CDCl_3$

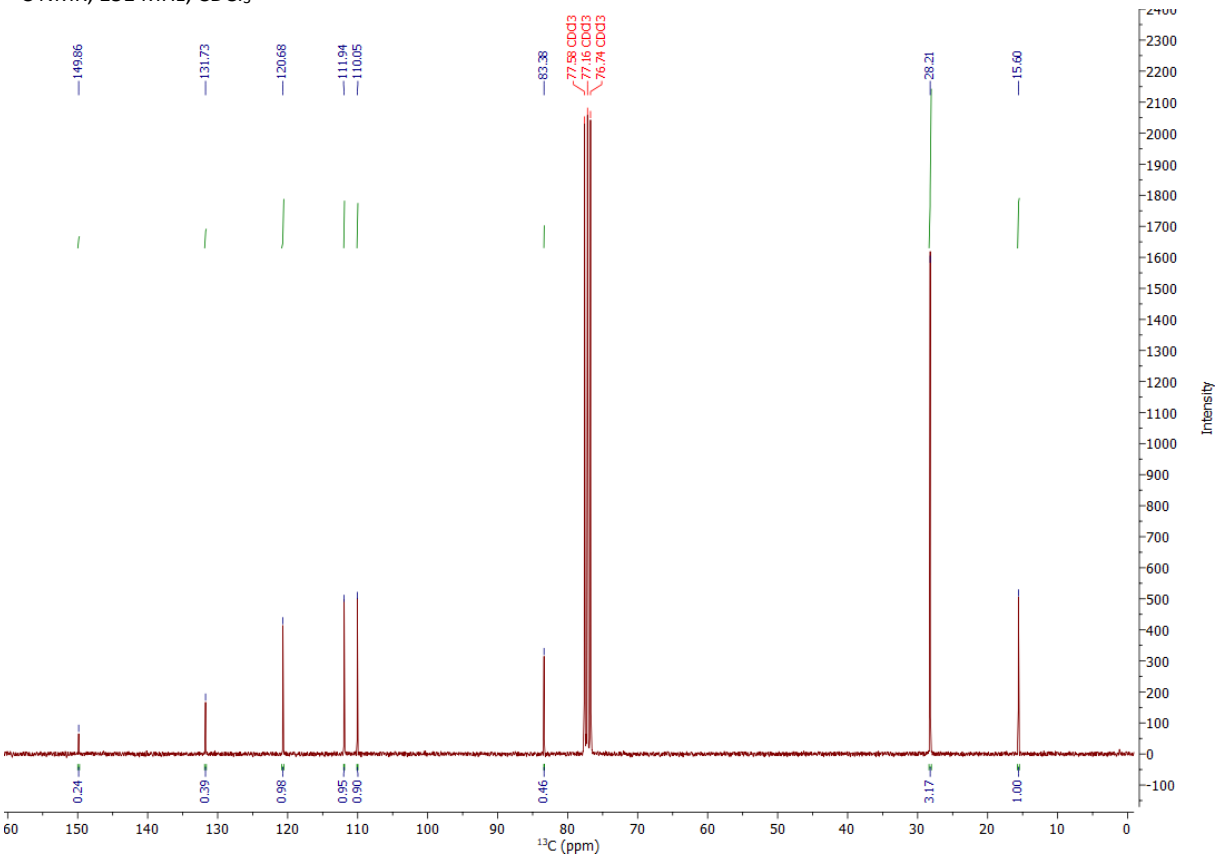


1-*tert*-Butyloxycarbonyl 2-methyl-1*H*-pyrrole (14b)

^{1}H NMR, 600 MHz, $CDCl_3$

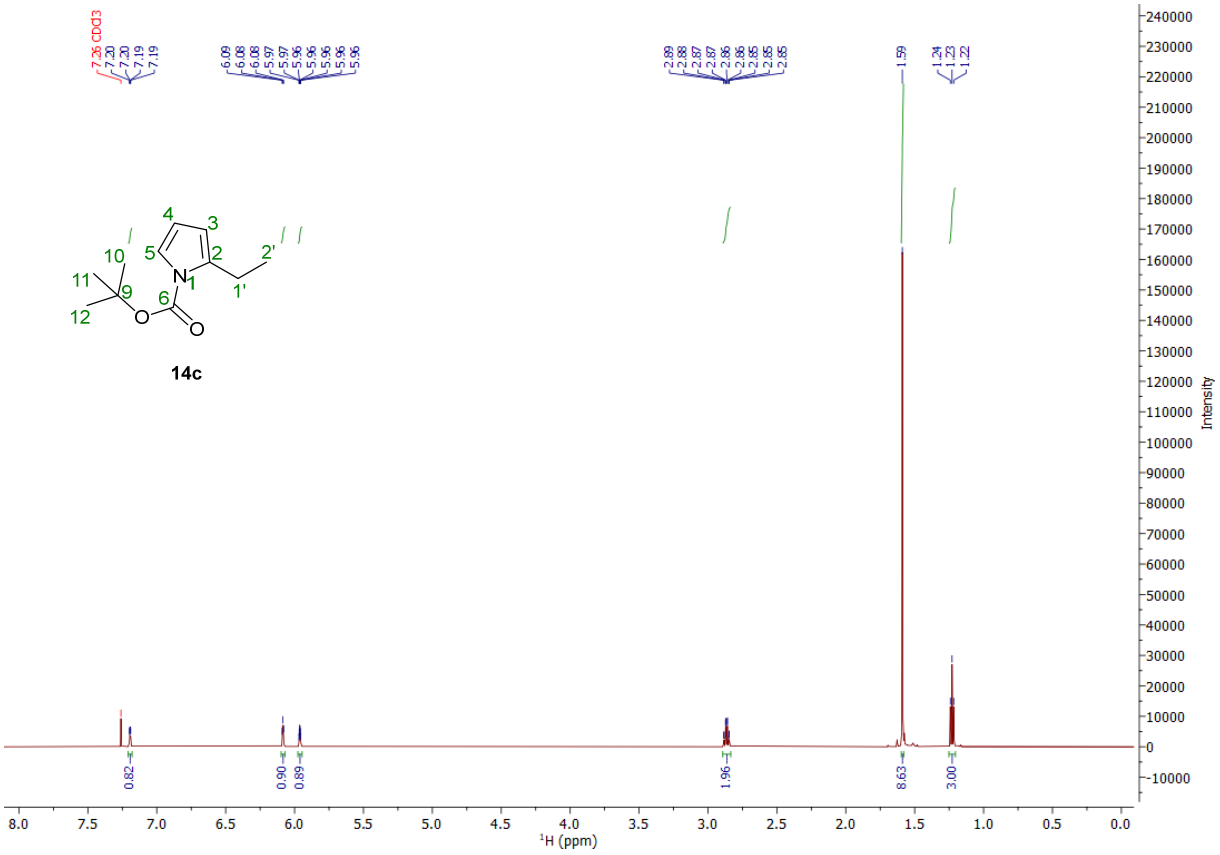


^{13}C NMR, 151 MHz, $CDCl_3$

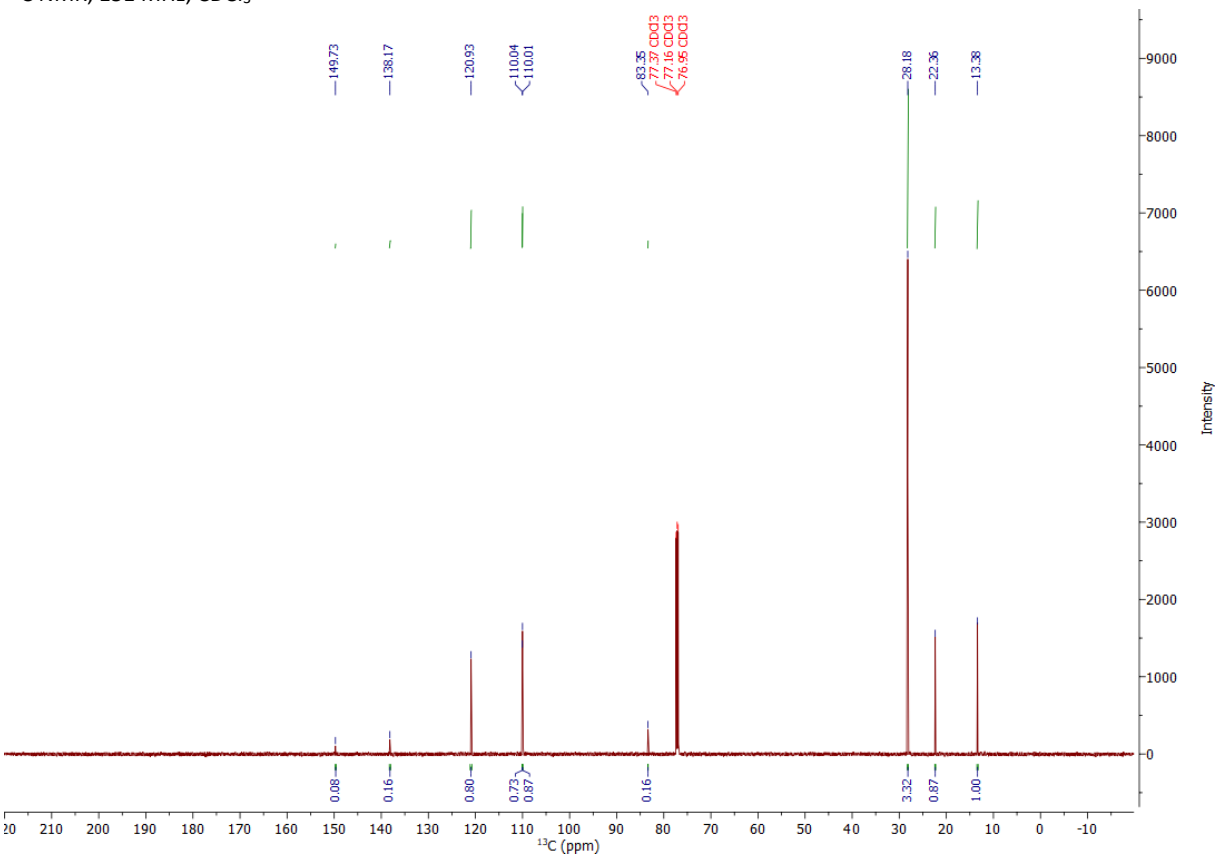


1-*tert*-Butyloxycarbonyl 2-ethyl-1*H*-pyrrole (14c)

^{1}H NMR, 600 MHz, $CDCl_3$

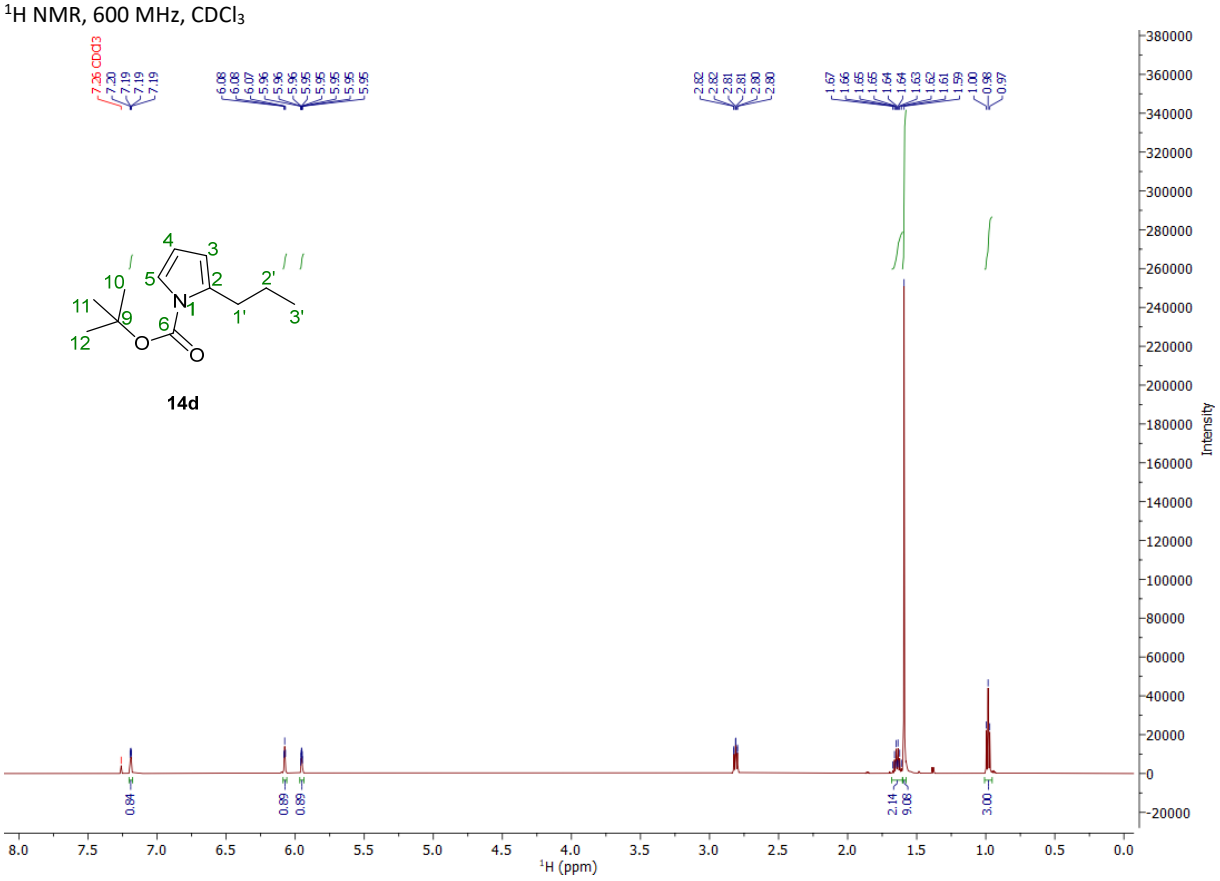


^{13}C NMR, 151 MHz, $CDCl_3$

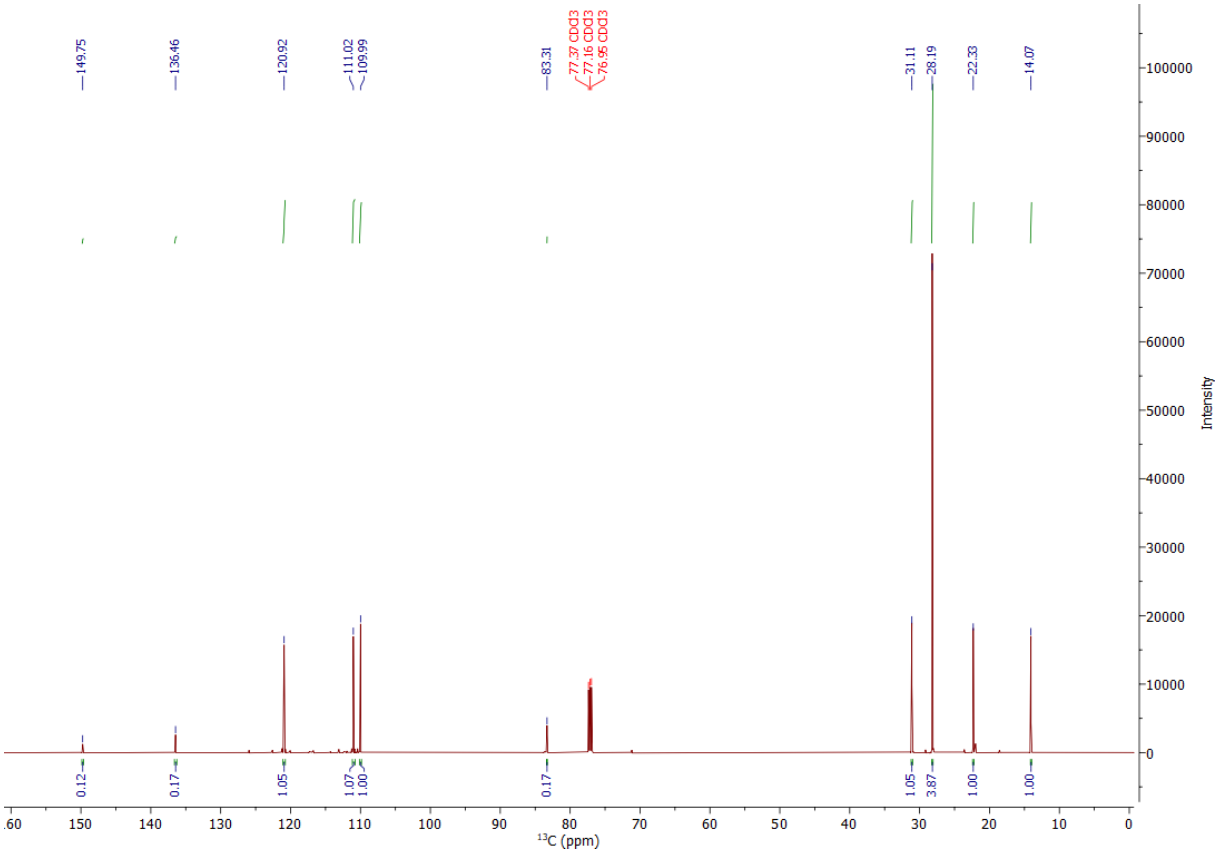


1-*tert*-Butyloxycarbonyl 2-propyl-1*H*-pyrrole (14d)

^{1}H NMR, 600 MHz, $CDCl_3$

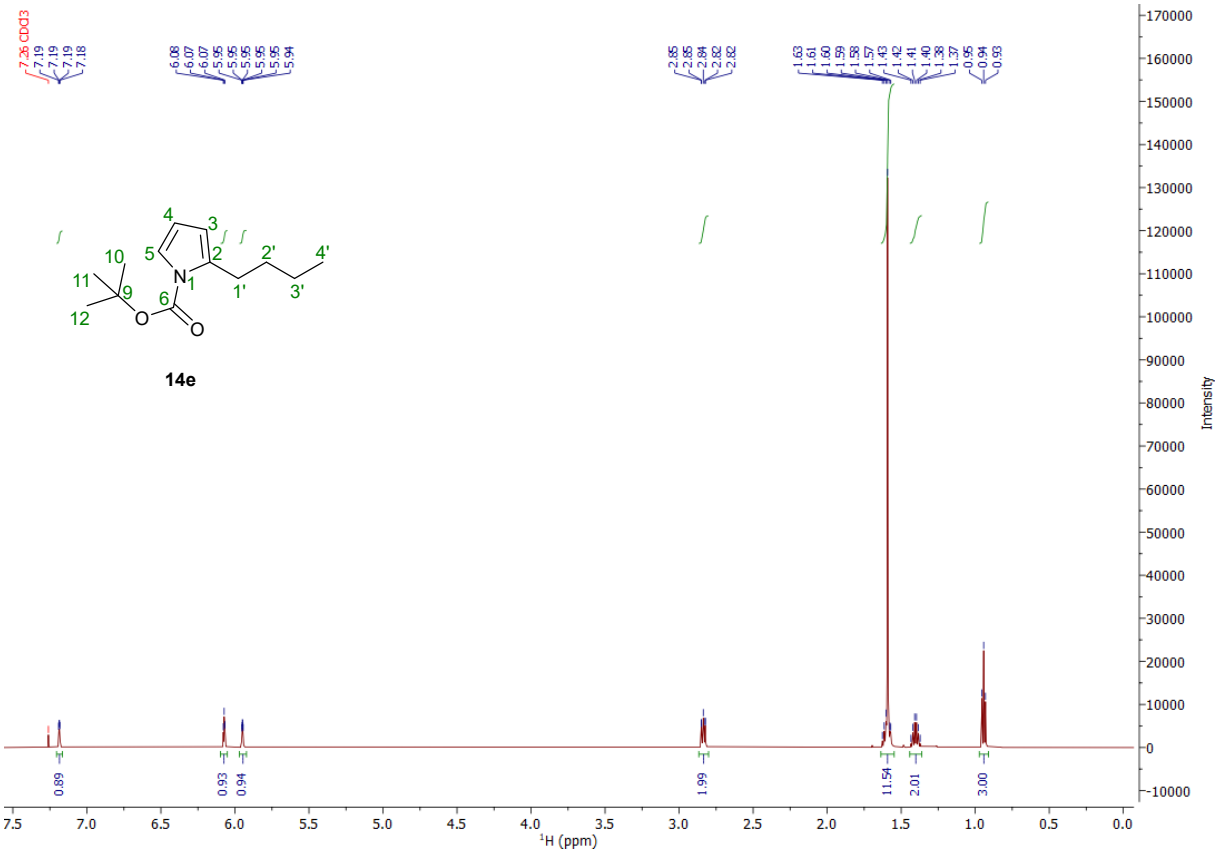


^{13}C NMR, 151 MHz, $CDCl_3$

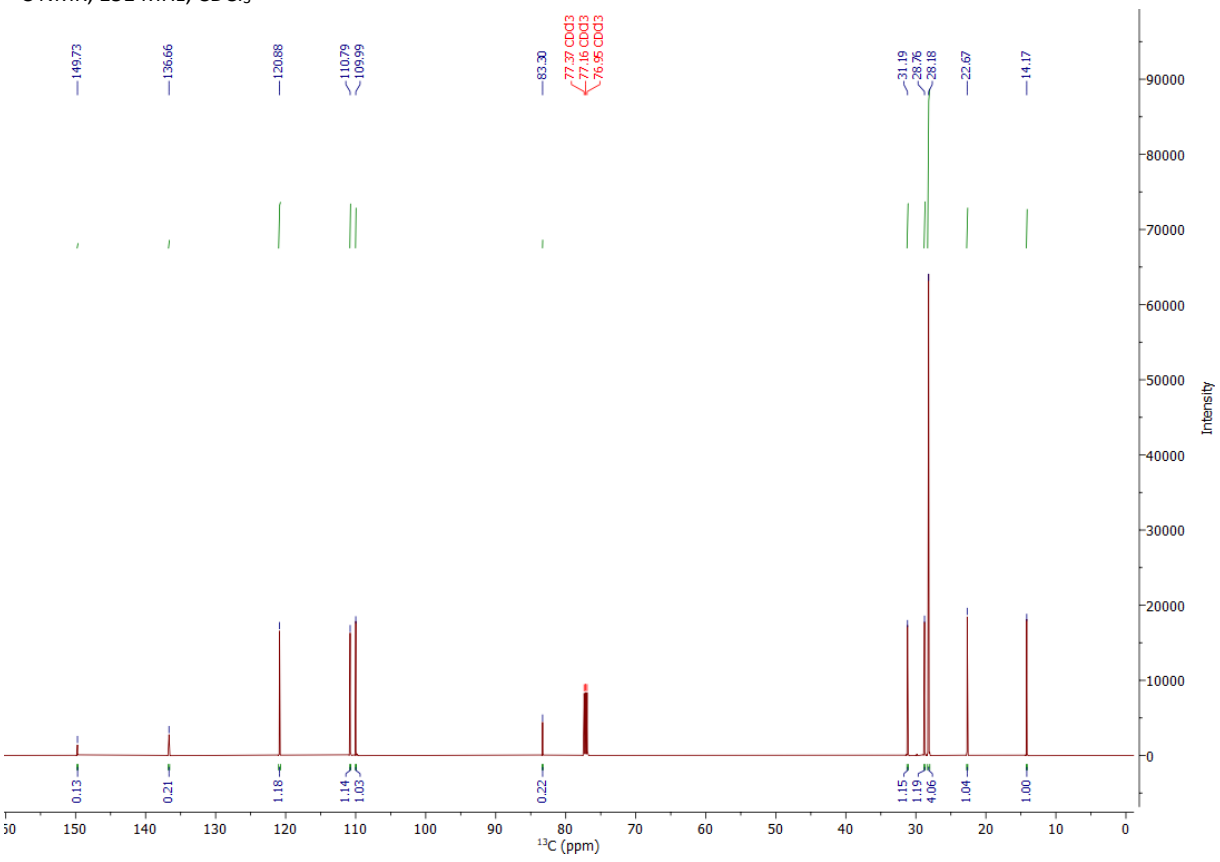


1-*tert*-Butyloxycarbonyl 2-butyl-1*H*-pyrrole (14e)

^{1}H NMR, 600 MHz, $CDCl_3$

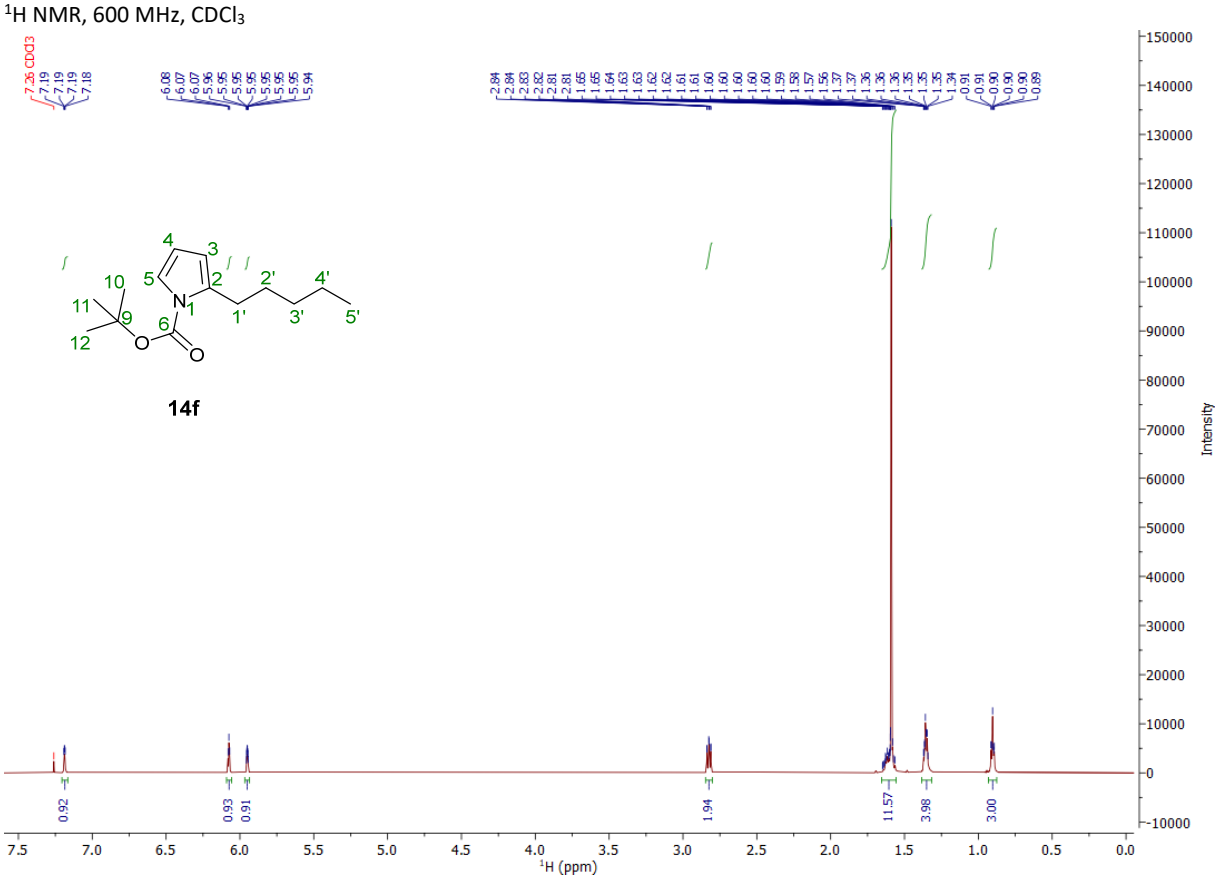


^{13}C NMR, 151 MHz, $CDCl_3$

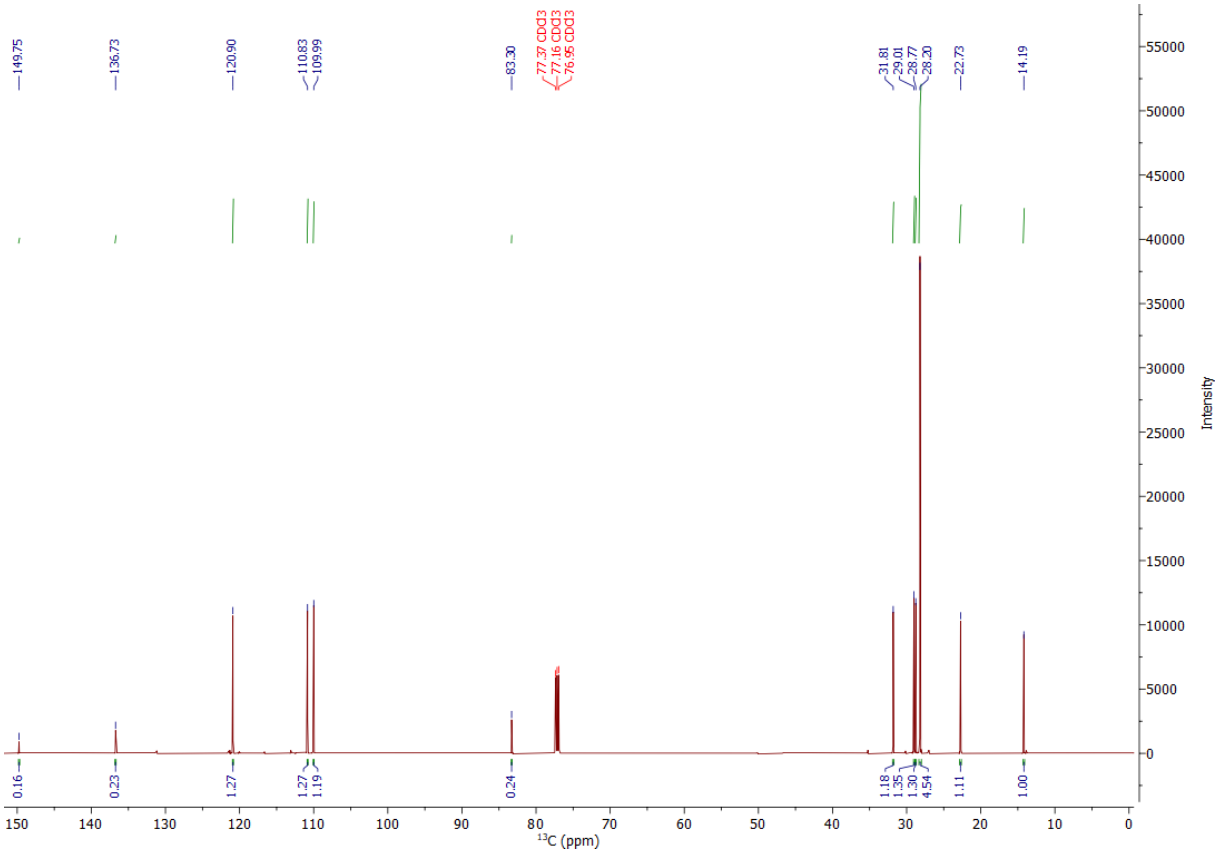


1-*tert*-Butyloxycarbonyl 2-pentyl-1*H*-pyrrole (14f)

^{1}H NMR, 600 MHz, $CDCl_3$

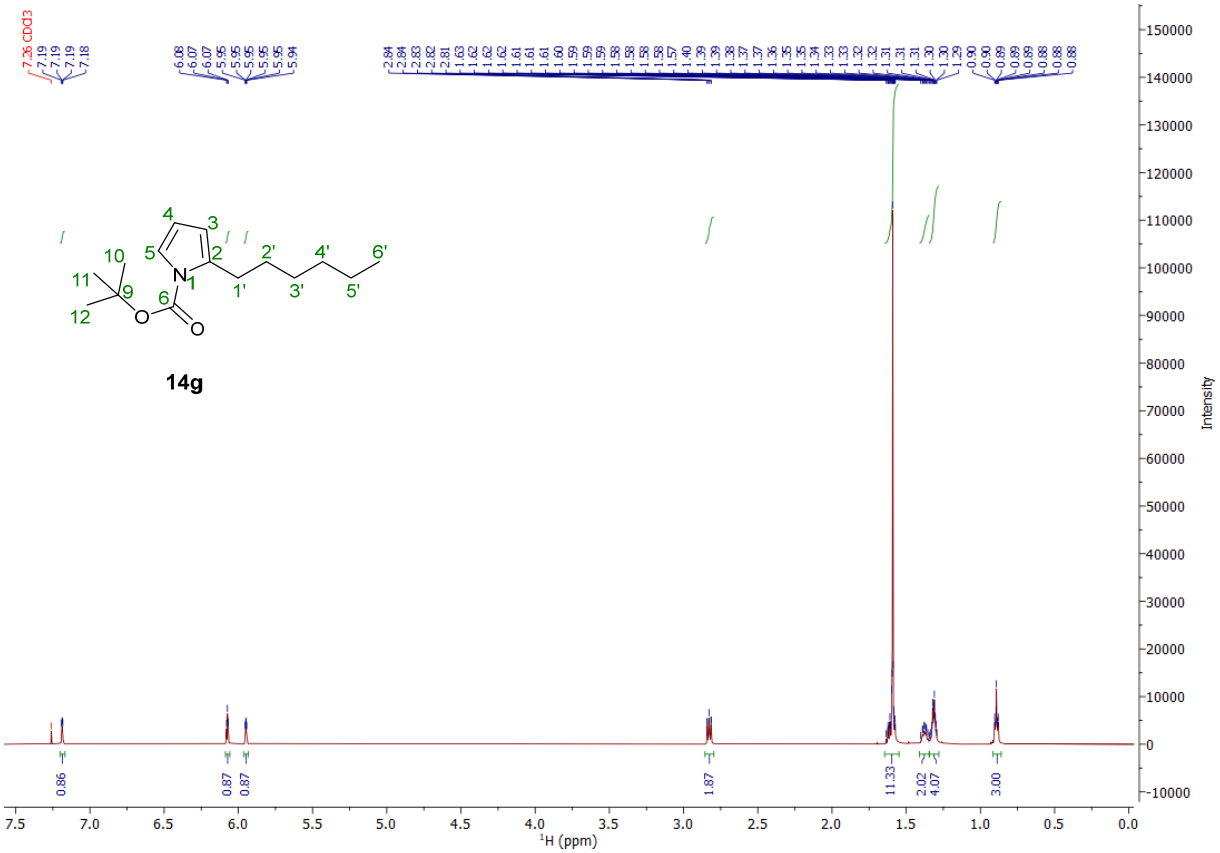


^{13}C NMR, 151 MHz, $CDCl_3$

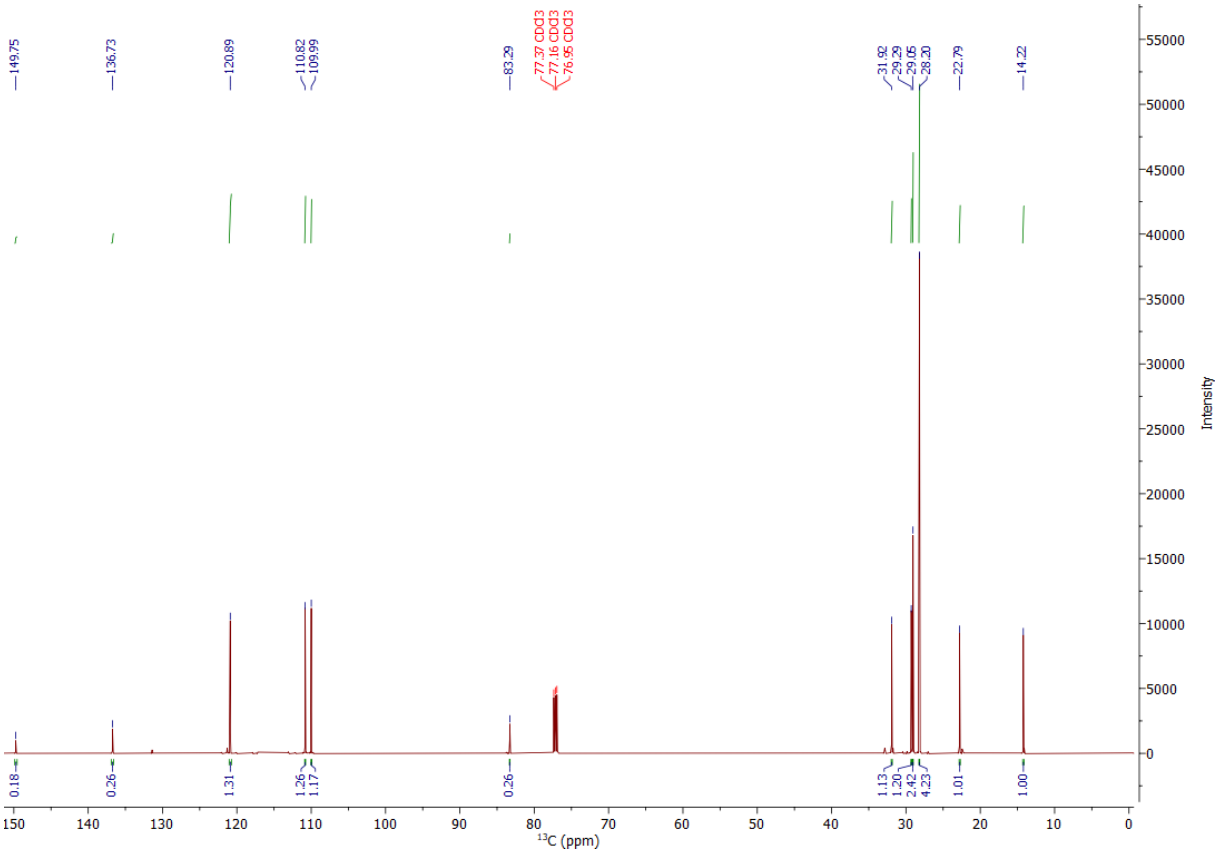


1-*tert*-Butyloxycarbonyl 2-hexyl-1*H*-pyrrole (14g)

^{1}H NMR, 600 MHz, $CDCl_3$

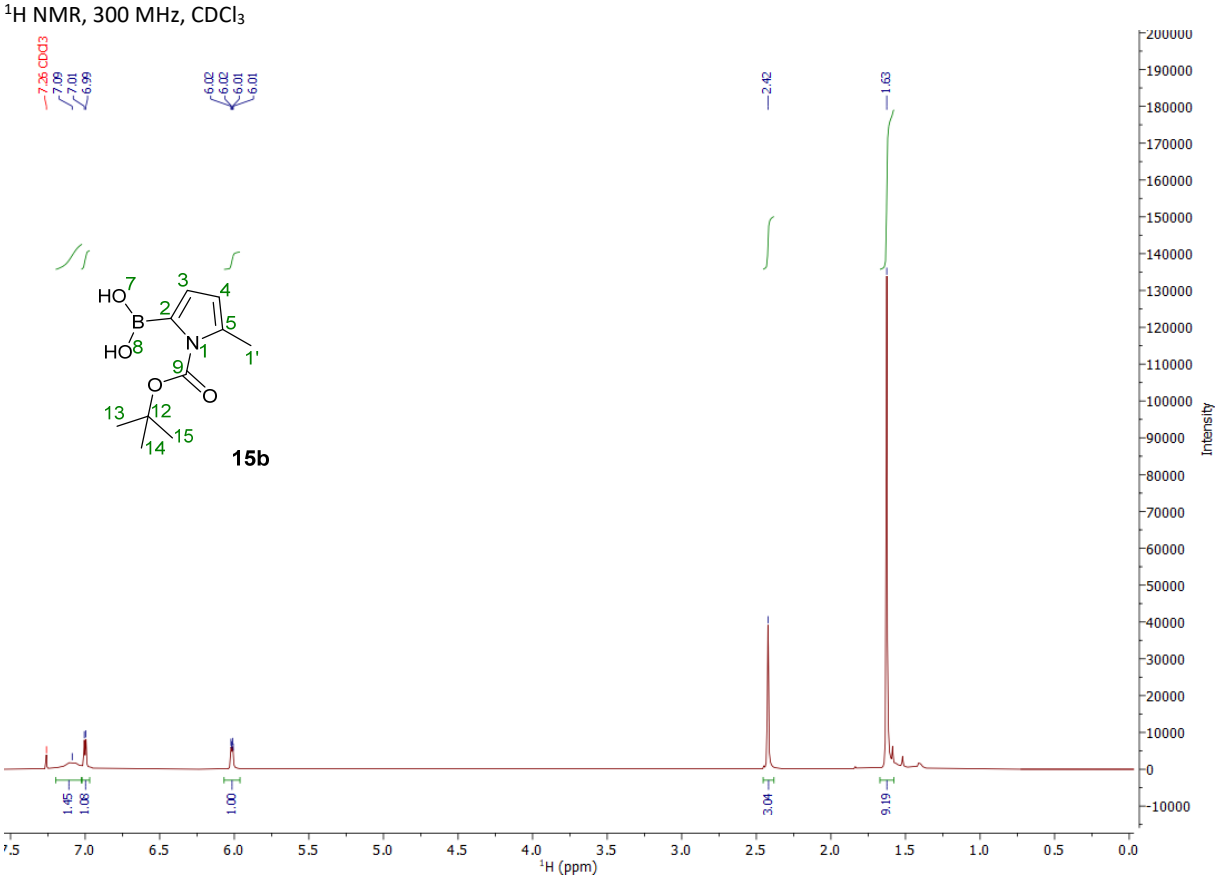


^{13}C NMR, 151 MHz, $CDCl_3$

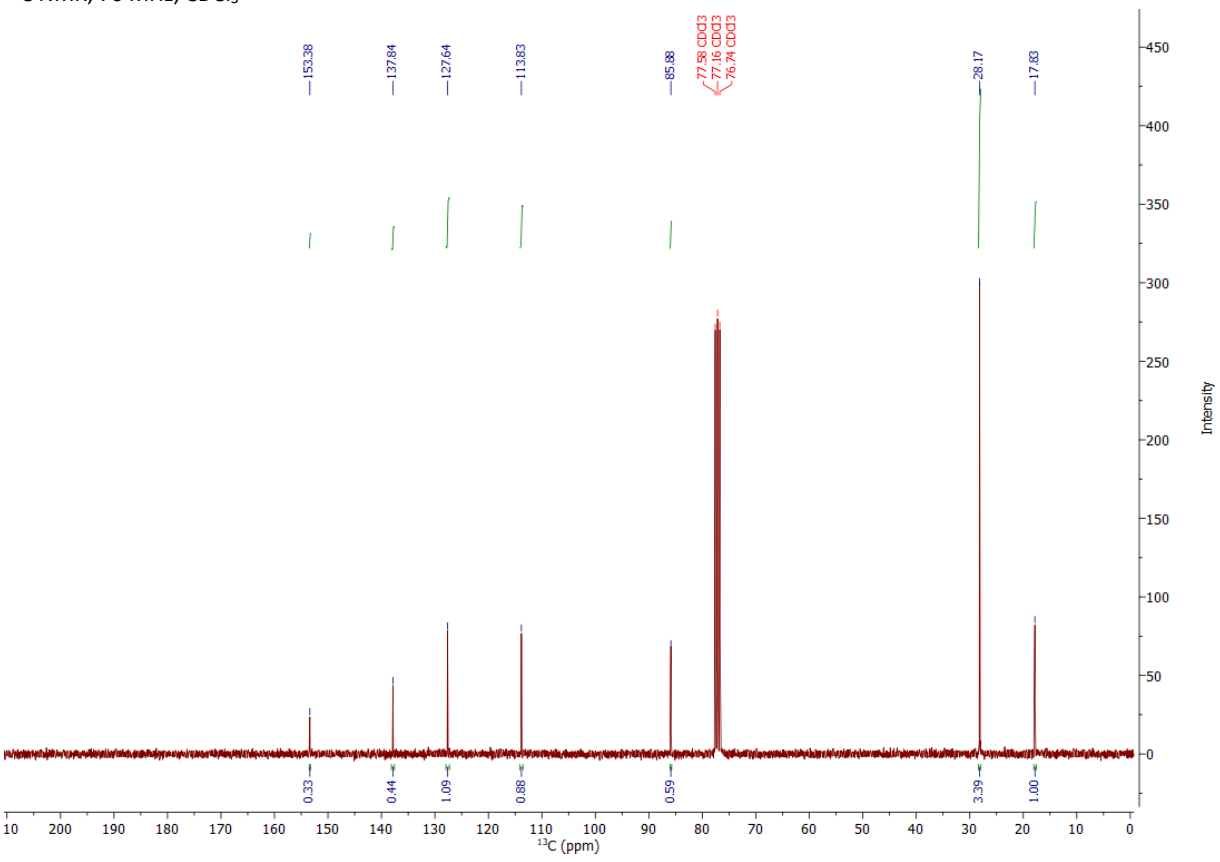


(1-(*tert*-Butoxycarbonyl)-5-methyl-1*H*-pyrrol-2-yl)boronic acid (15b)

^{1}H NMR, 300 MHz, $CDCl_3$

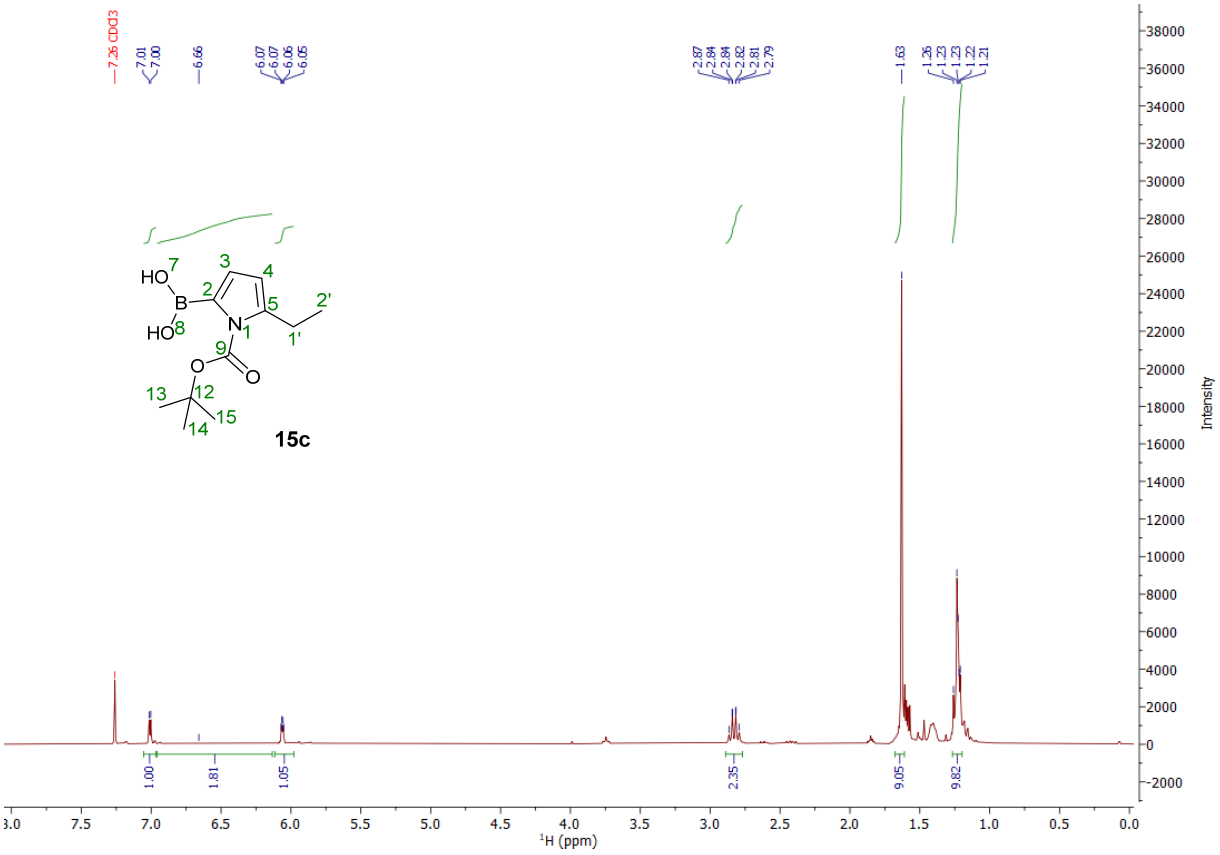


^{13}C NMR, 76 MHz, $CDCl_3$

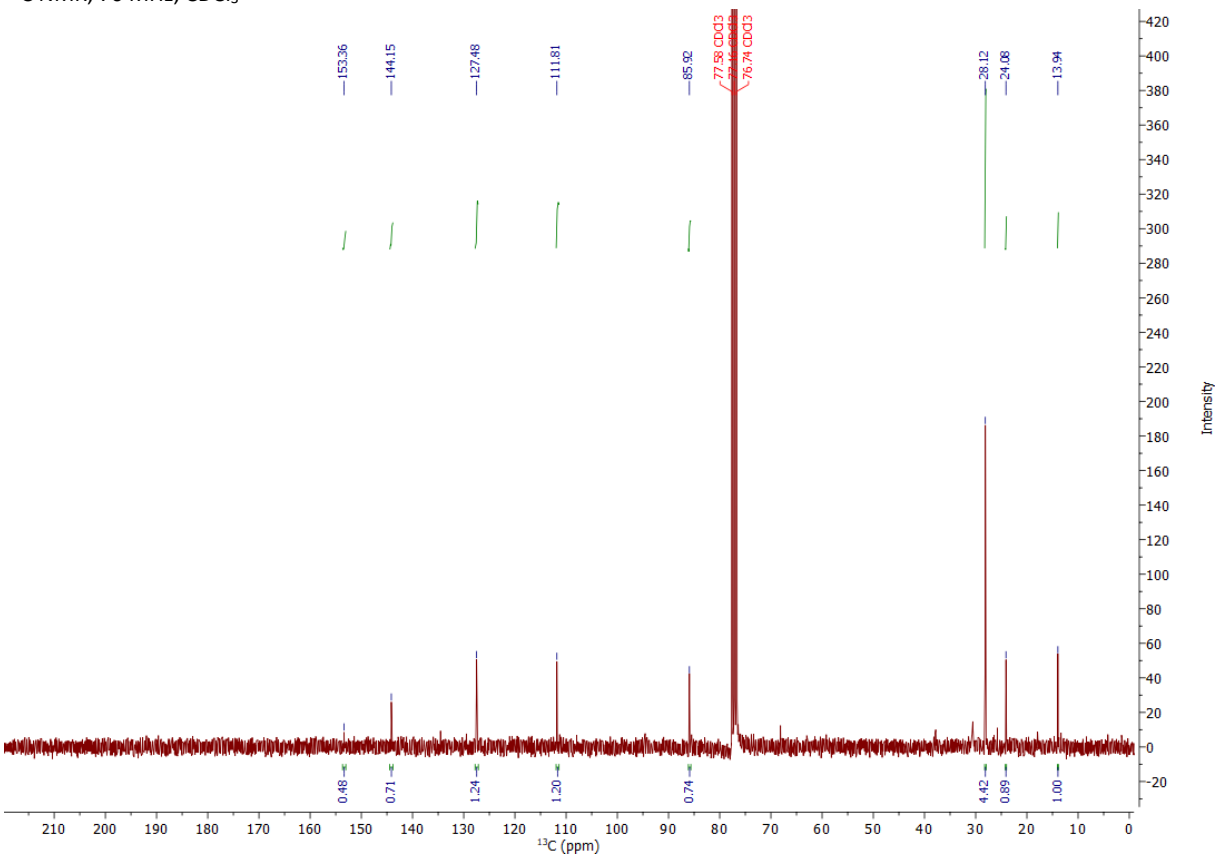


(1-(*tert*-Butoxycarbonyl)-5-ethyl-1*H*-pyrrol-2-yl)boronic acid (15c)

^{1}H NMR, 300 MHz, $CDCl_3$

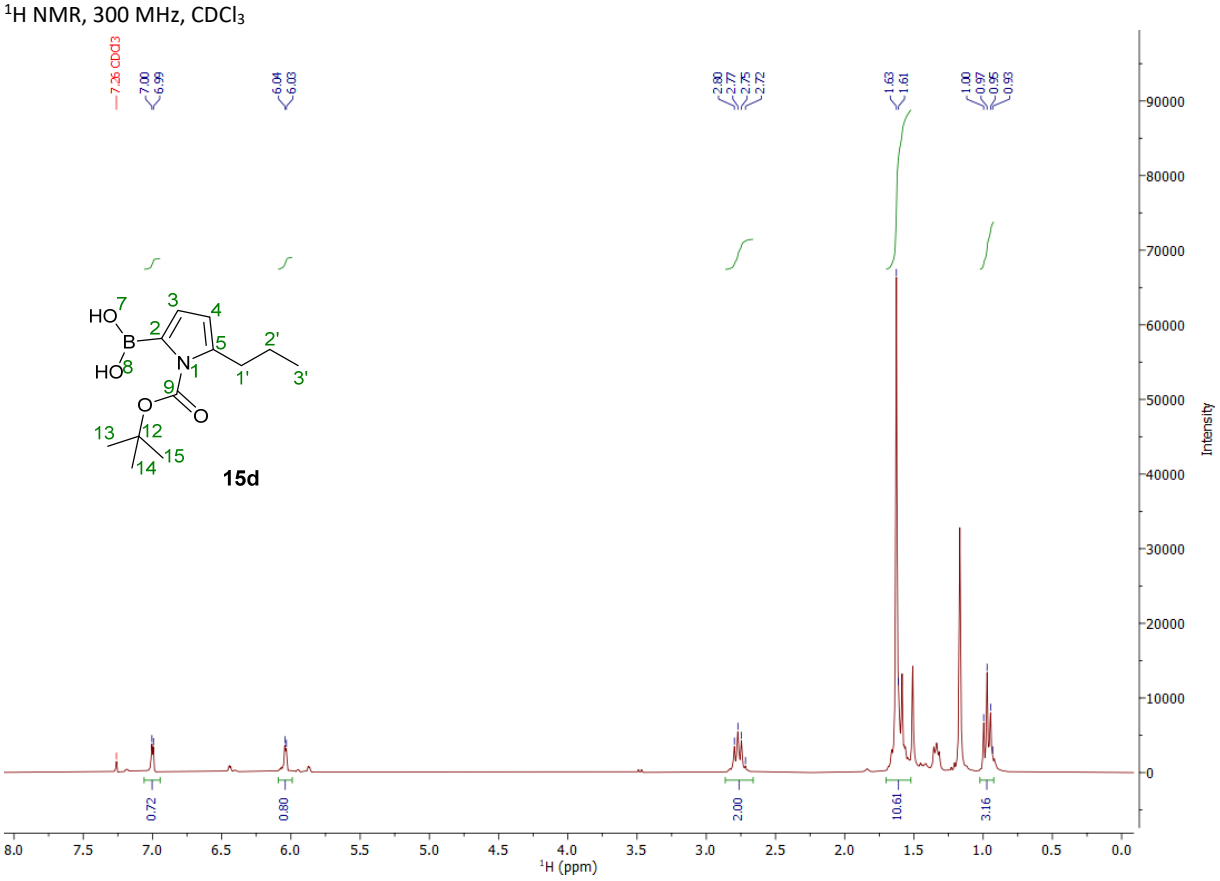


^{13}C NMR, 76 MHz, $CDCl_3$

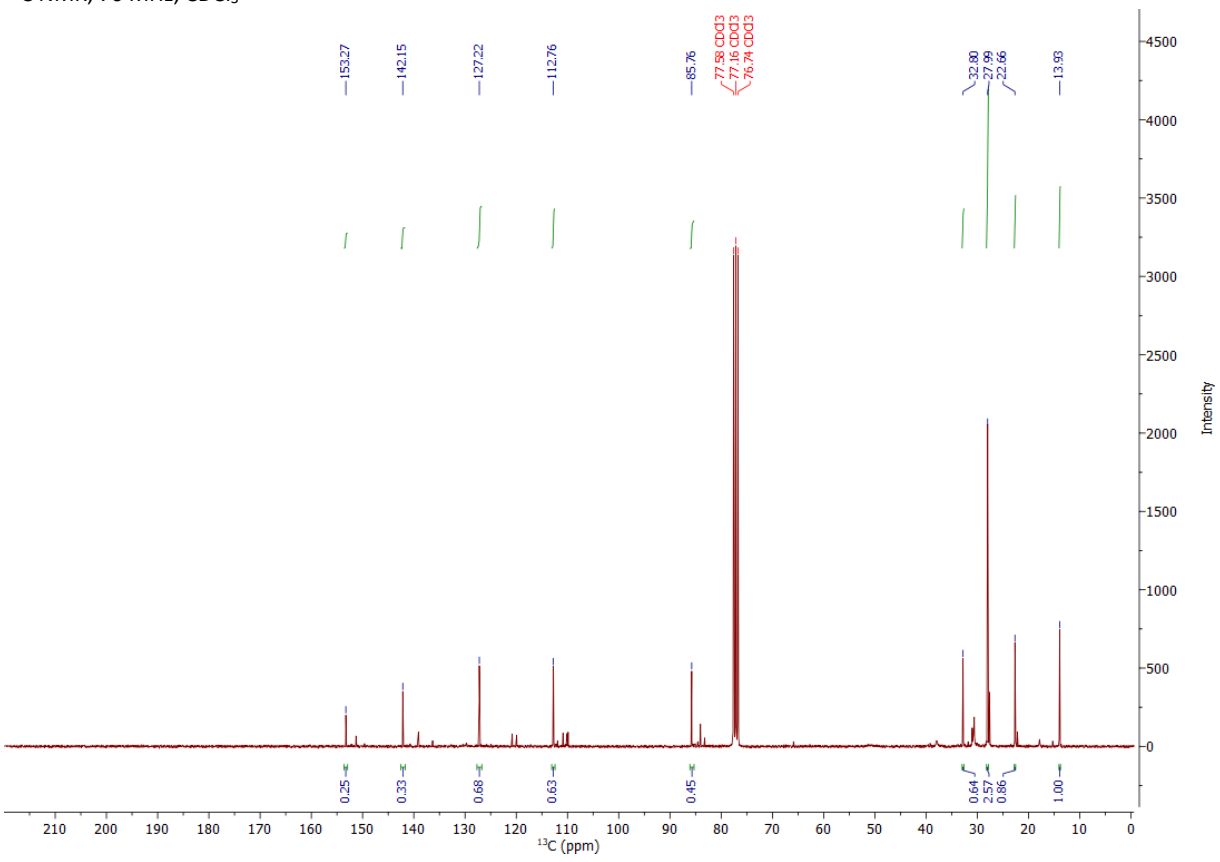


(1-(*tert*-Butoxycarbonyl)-5-propyl-1*H*-pyrrol-2-yl)boronic acid (15d)

^{1}H NMR, 300 MHz, $CDCl_3$

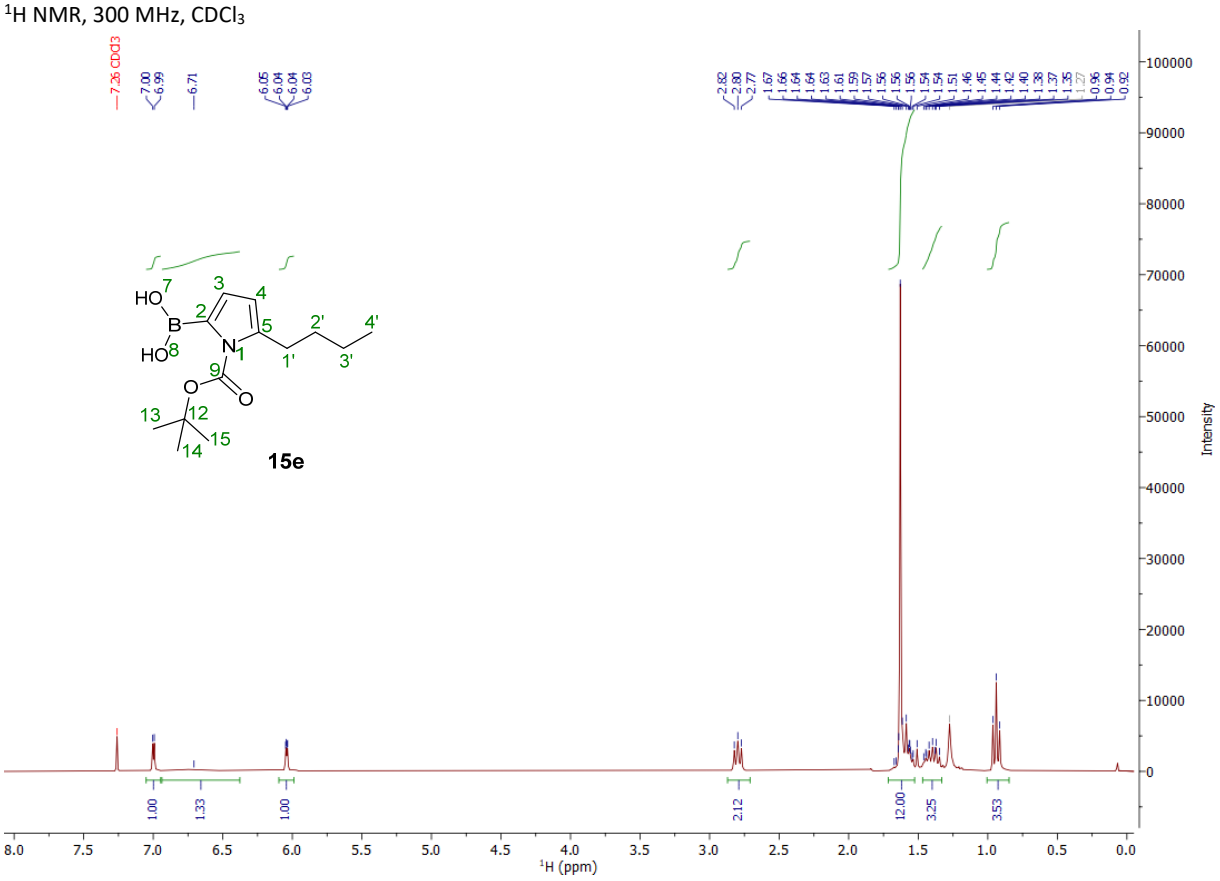


^{13}C NMR, 76 MHz, $CDCl_3$

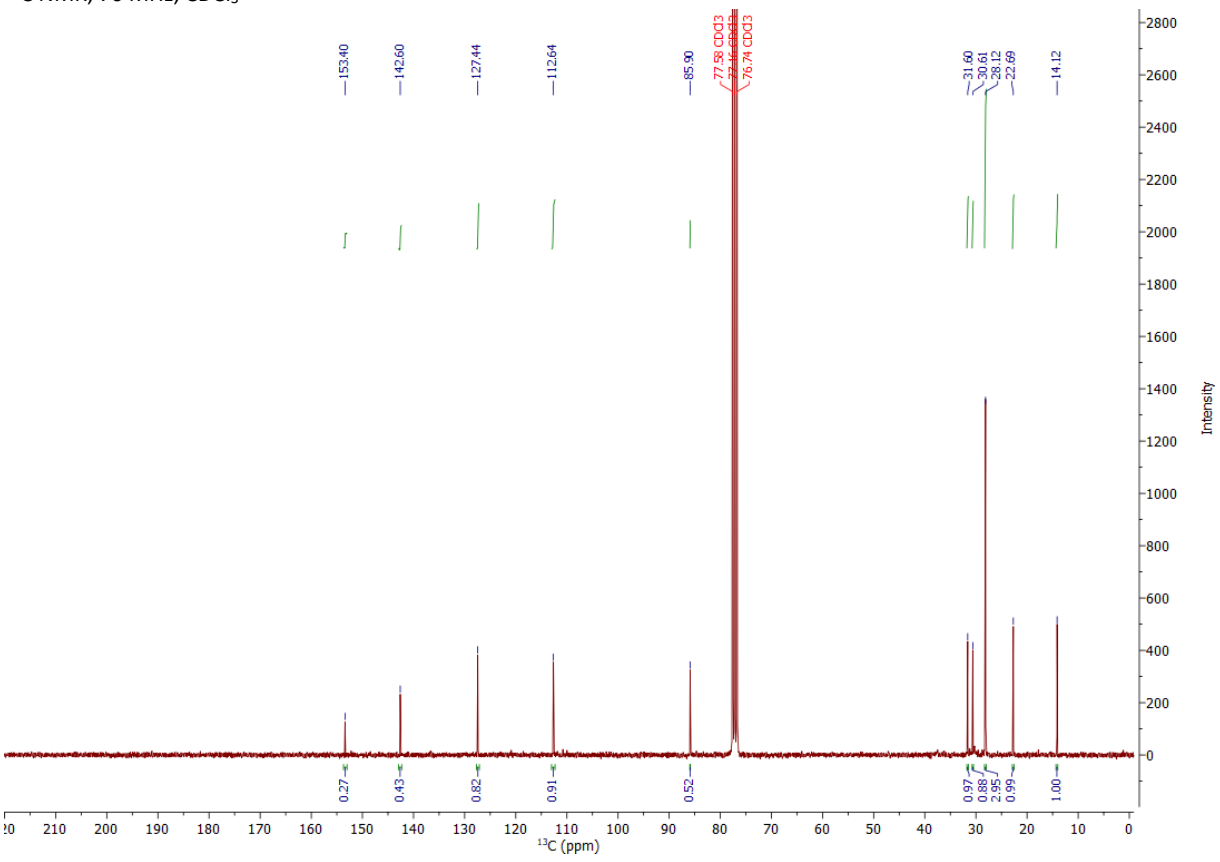


(1-(*tert*-Butoxycarbonyl)-5-butyl-1*H*-pyrrol-2-yl)boronic acid (15e)

^{1}H NMR, 300 MHz, $CDCl_3$

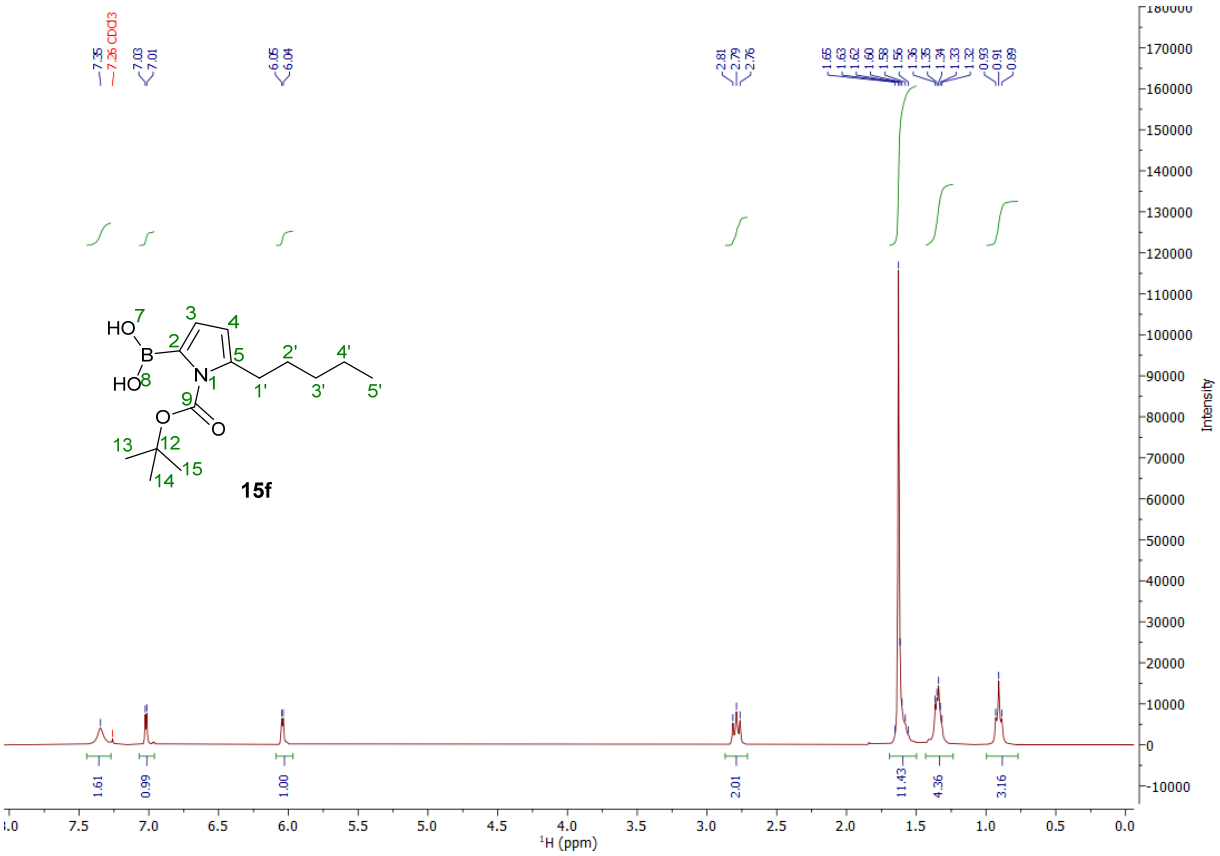


^{13}C NMR, 76 MHz, $CDCl_3$

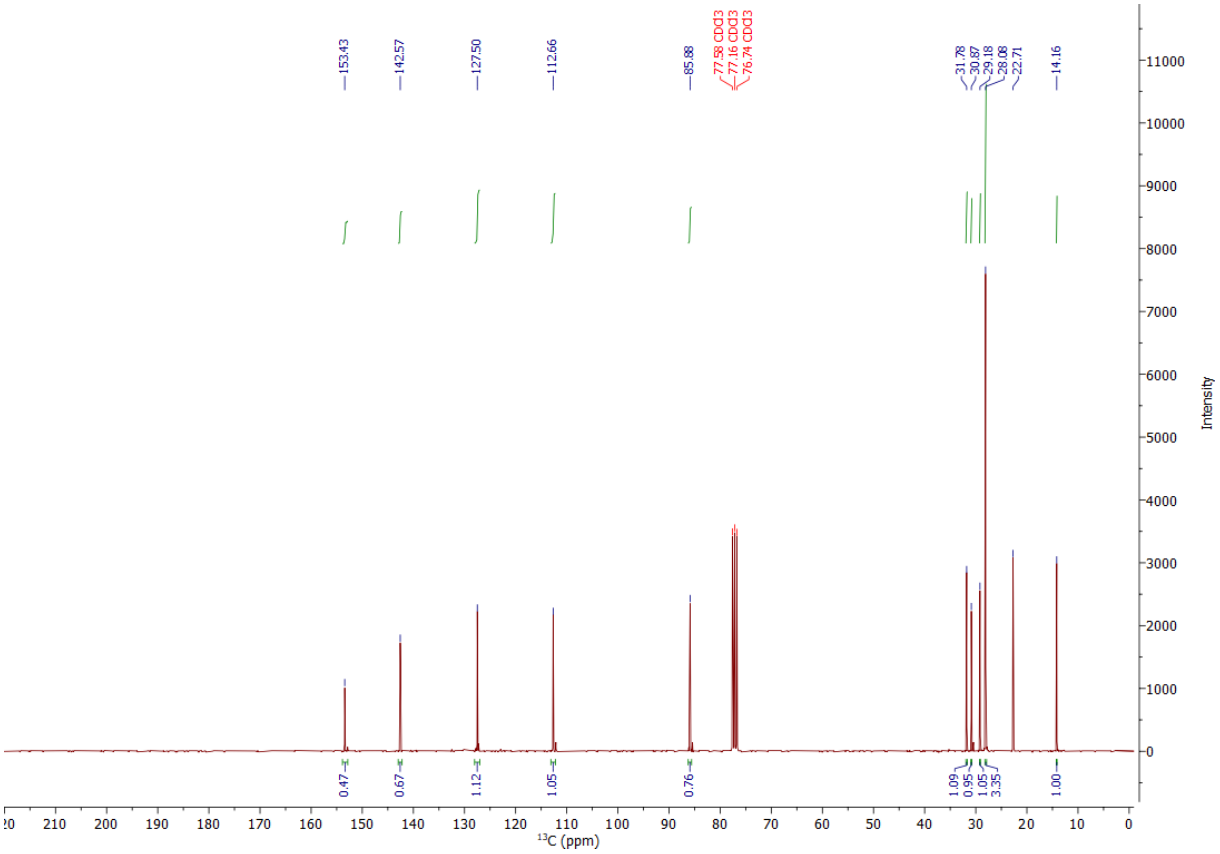


(1-(*tert*-Butoxycarbonyl)-5-pentyl-1*H*-pyrrol-2-yl)boronic acid (15f)

^{1}H NMR, 300 MHz, $CDCl_3$

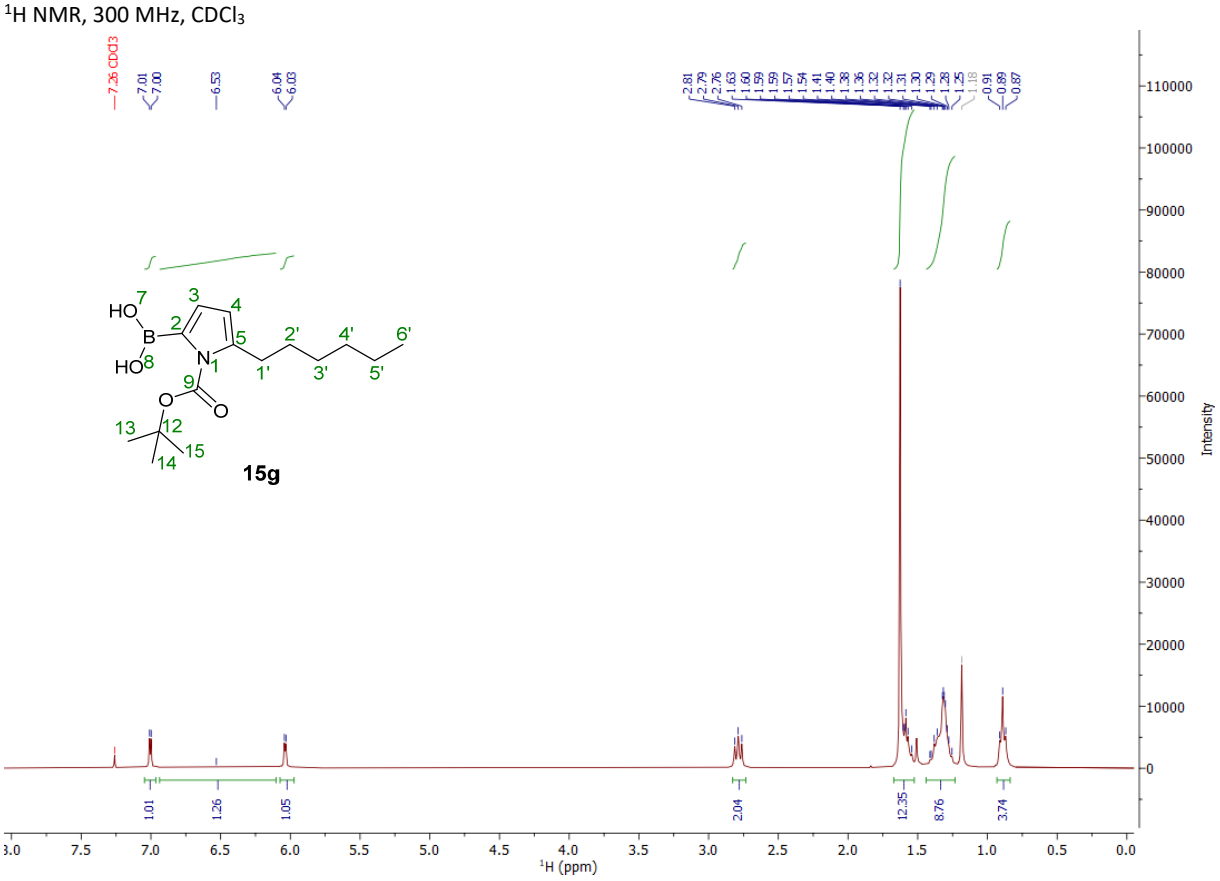


^{13}C NMR, 76 MHz, $CDCl_3$

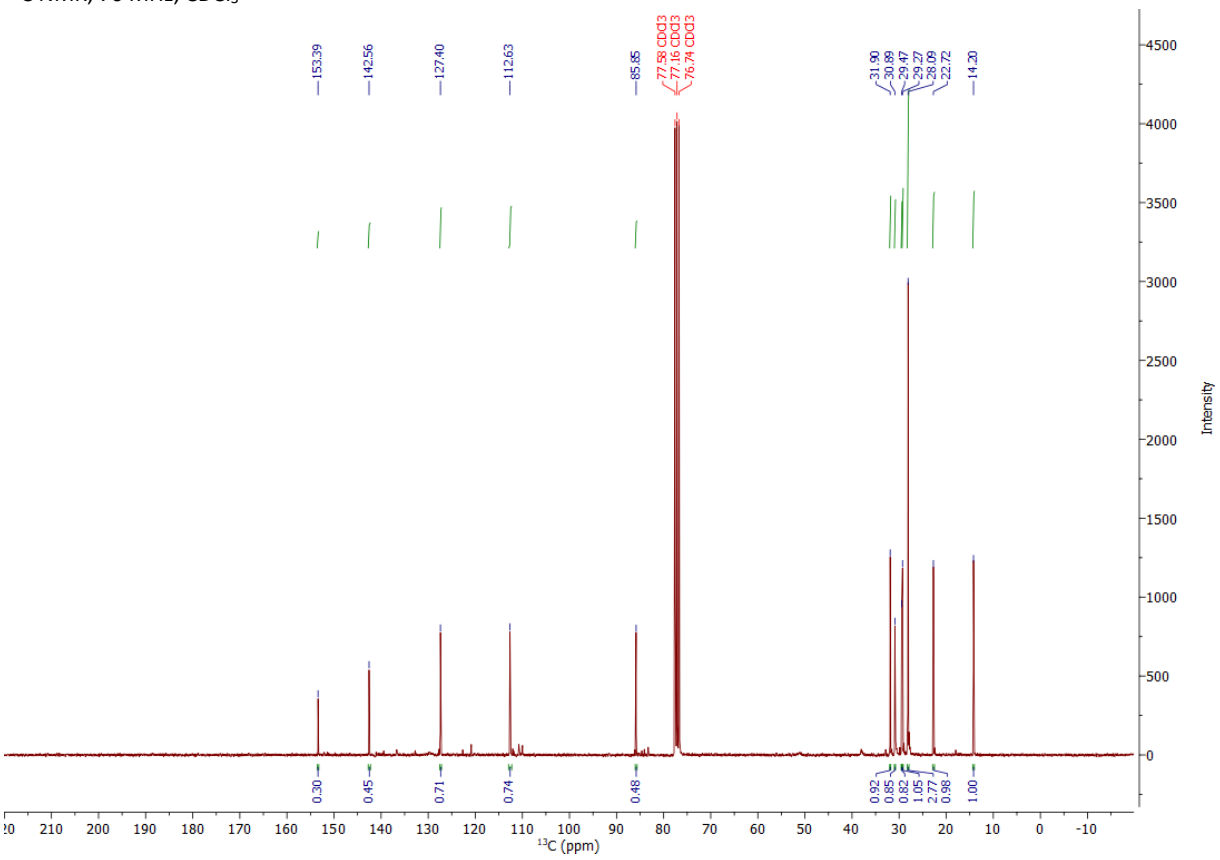


(1-(*tert*-Butoxycarbonyl)-5-hexyl-1*H*-pyrrol-2-yl)boronic acid (15g)

^{1}H NMR, 300 MHz, $CDCl_3$

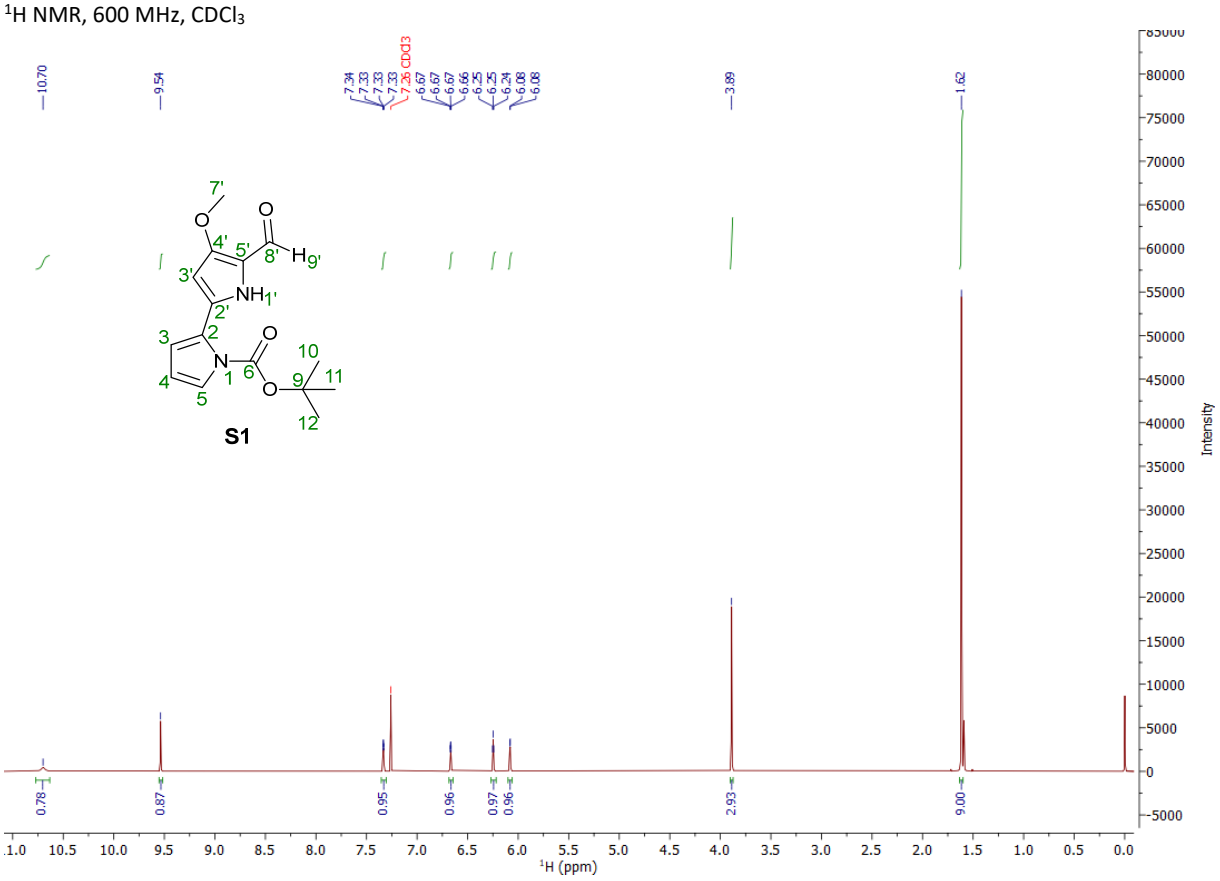


^{13}C NMR, 76 MHz, $CDCl_3$

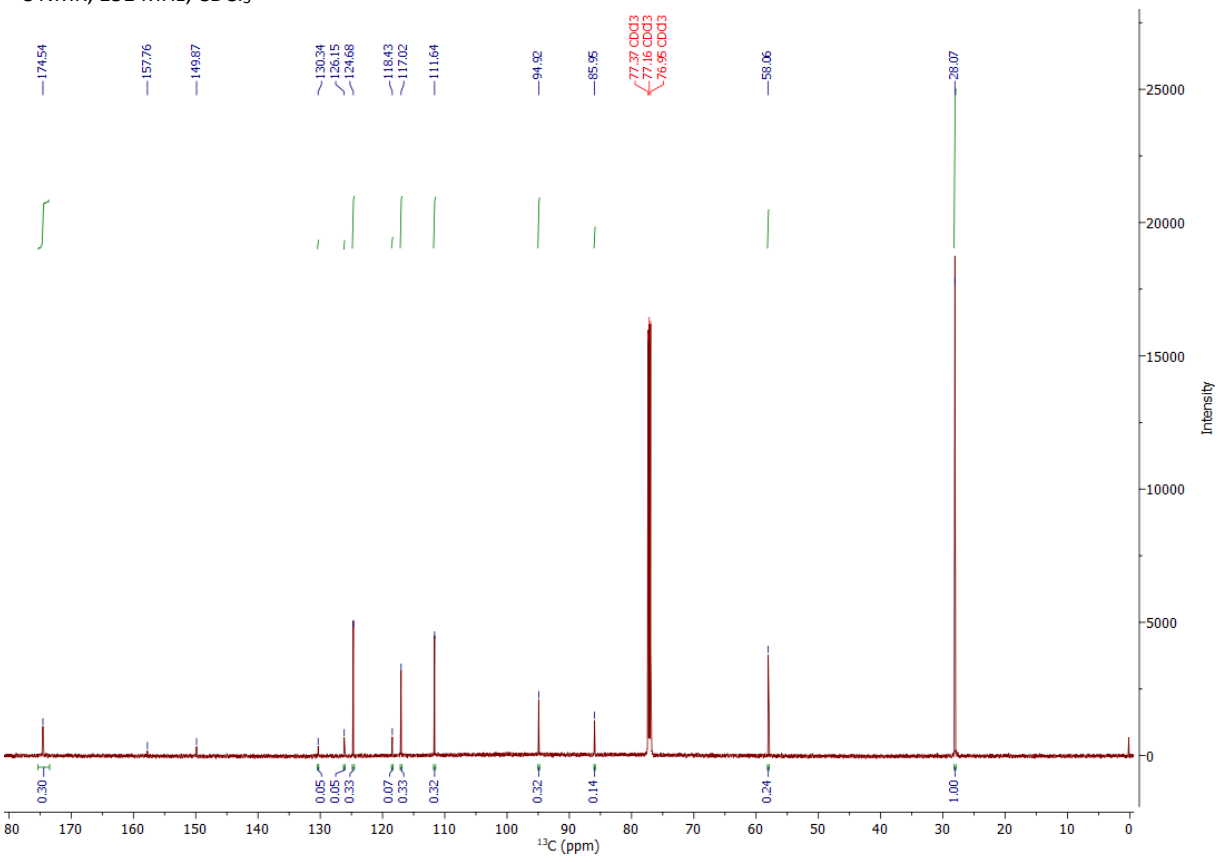


***tert*-Butyl 5'-formyl-4'-methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-1-carboxylate (S1)**

^{1}H NMR, 600 MHz, $CDCl_3$

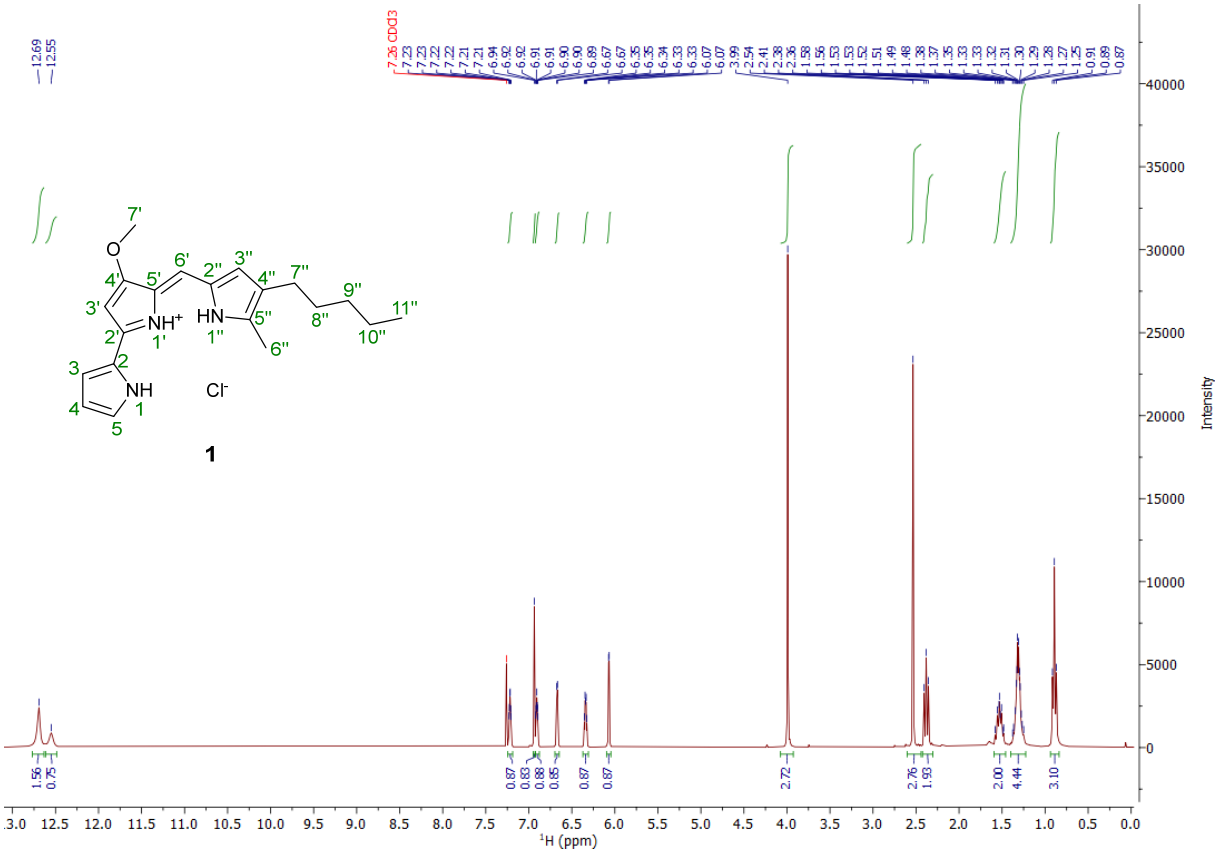


^{13}C NMR, 151 MHz, $CDCl_3$

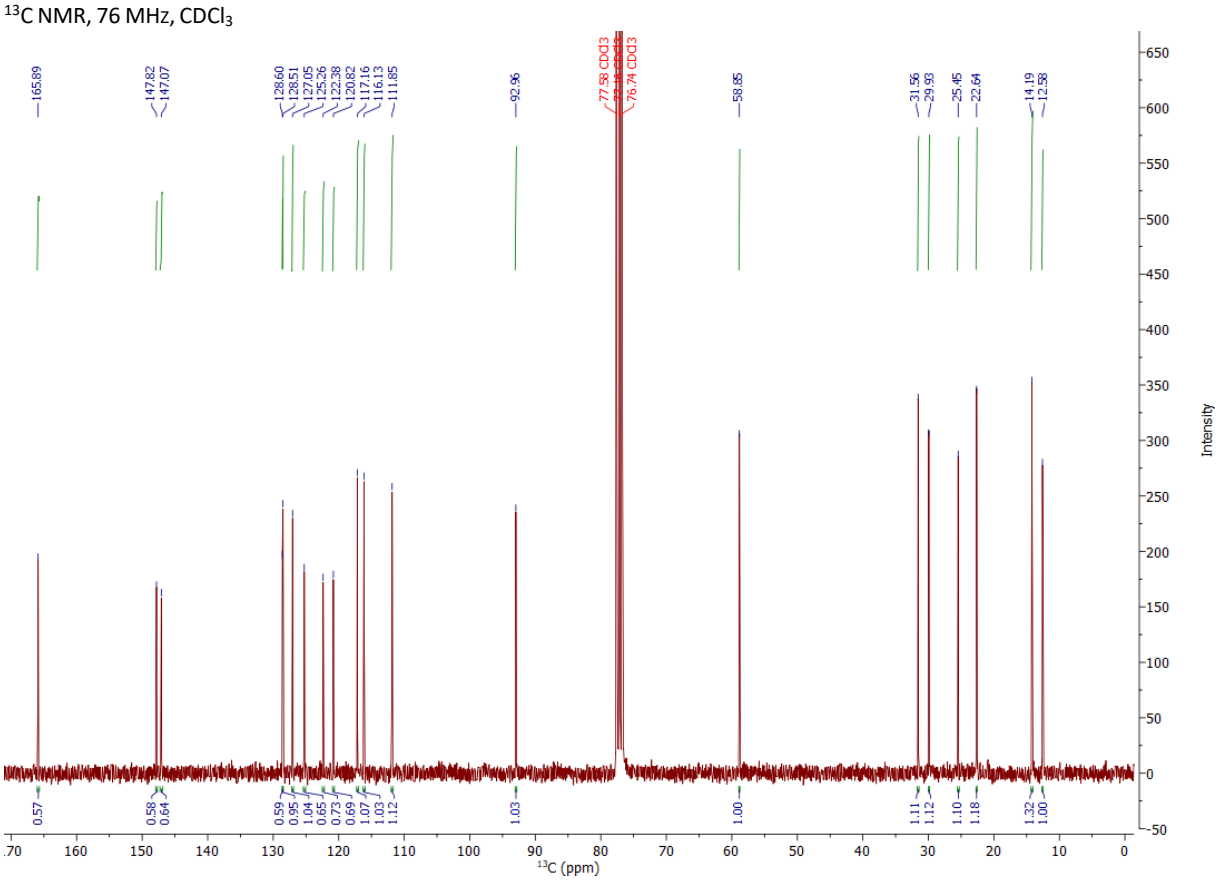


(*Z*)-4'-Methoxy-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (1, prodigiosin)

^{1}H NMR, 300 MHz, $CDCl_3$

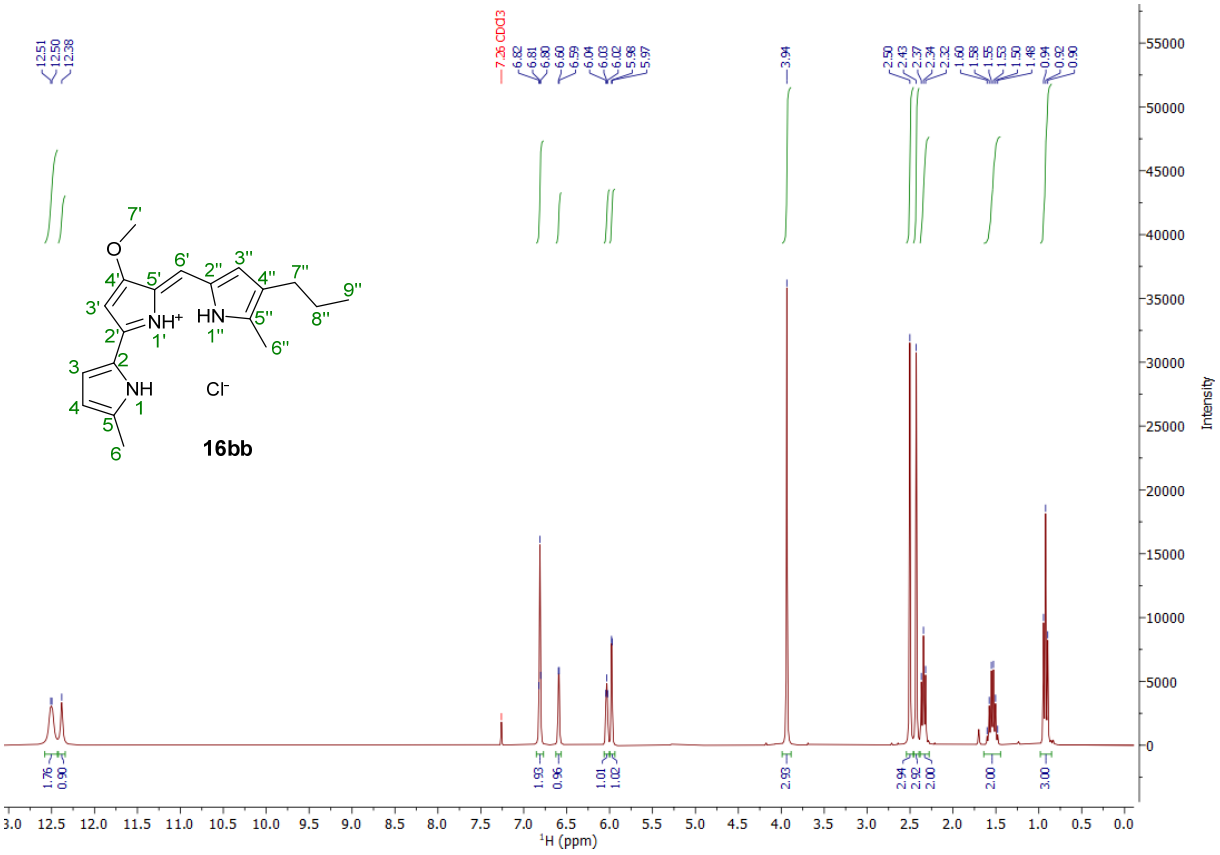


^{13}C NMR, 76 MHz, $CDCl_3$

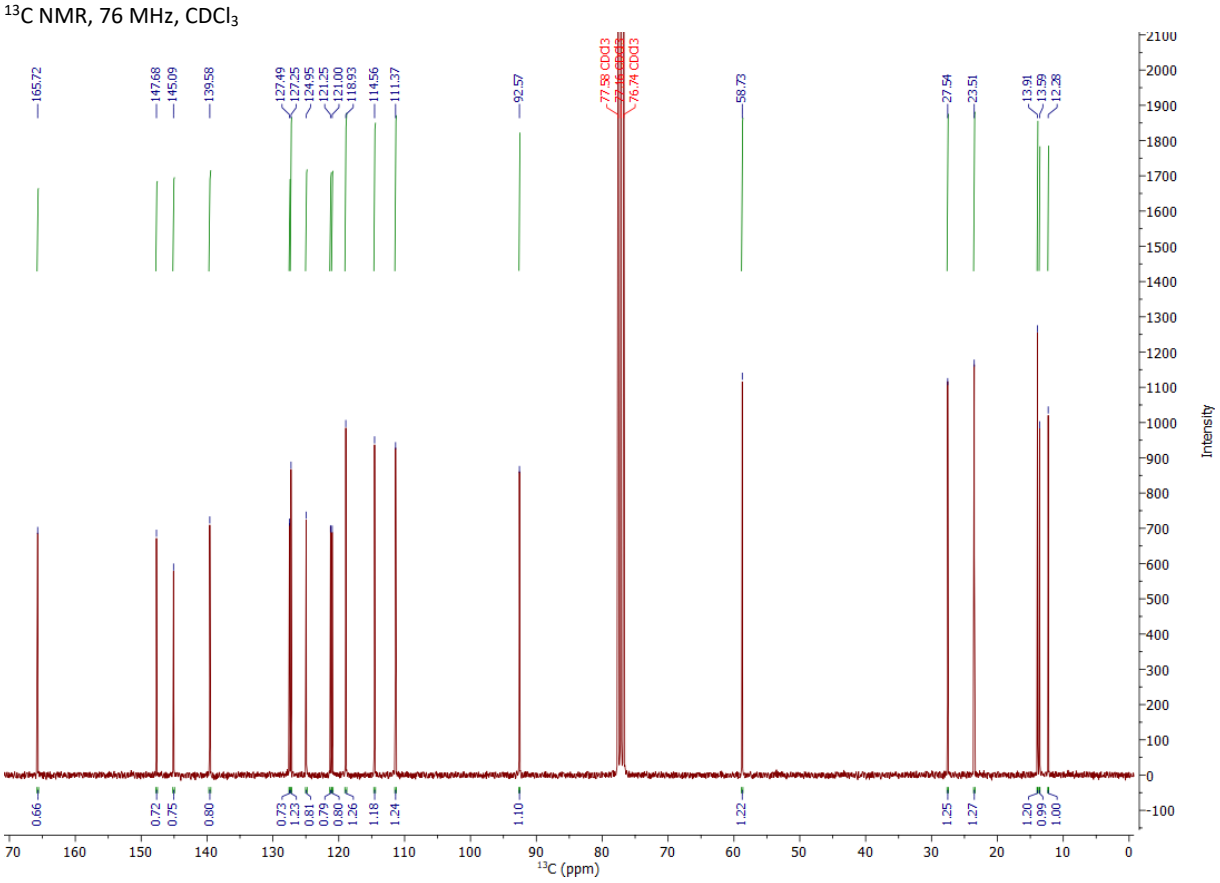


(*Z*)-4'-Methoxy-5-methyl-5'-((5-methyl-4-propyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16bb)

^{1}H NMR, 300 MHz, $CDCl_3$

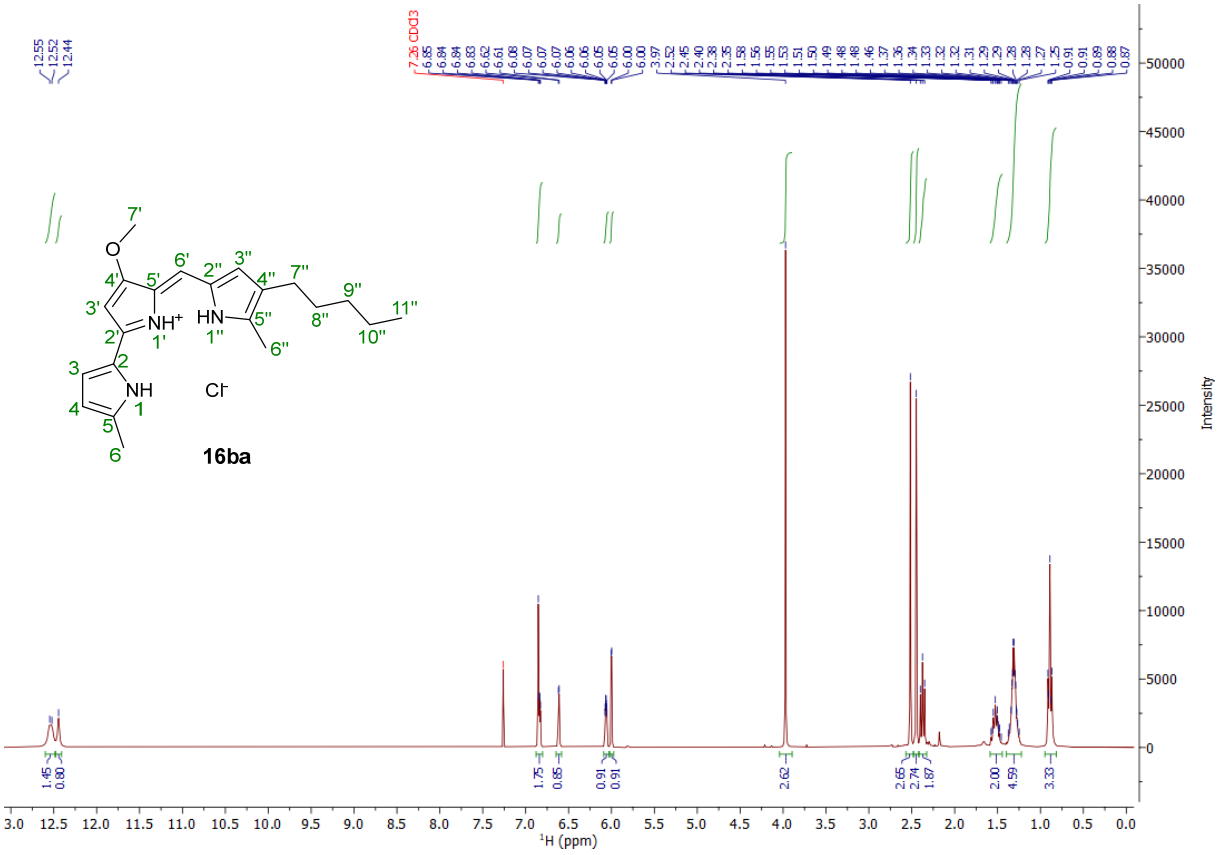


^{13}C NMR, 76 MHz, $CDCl_3$

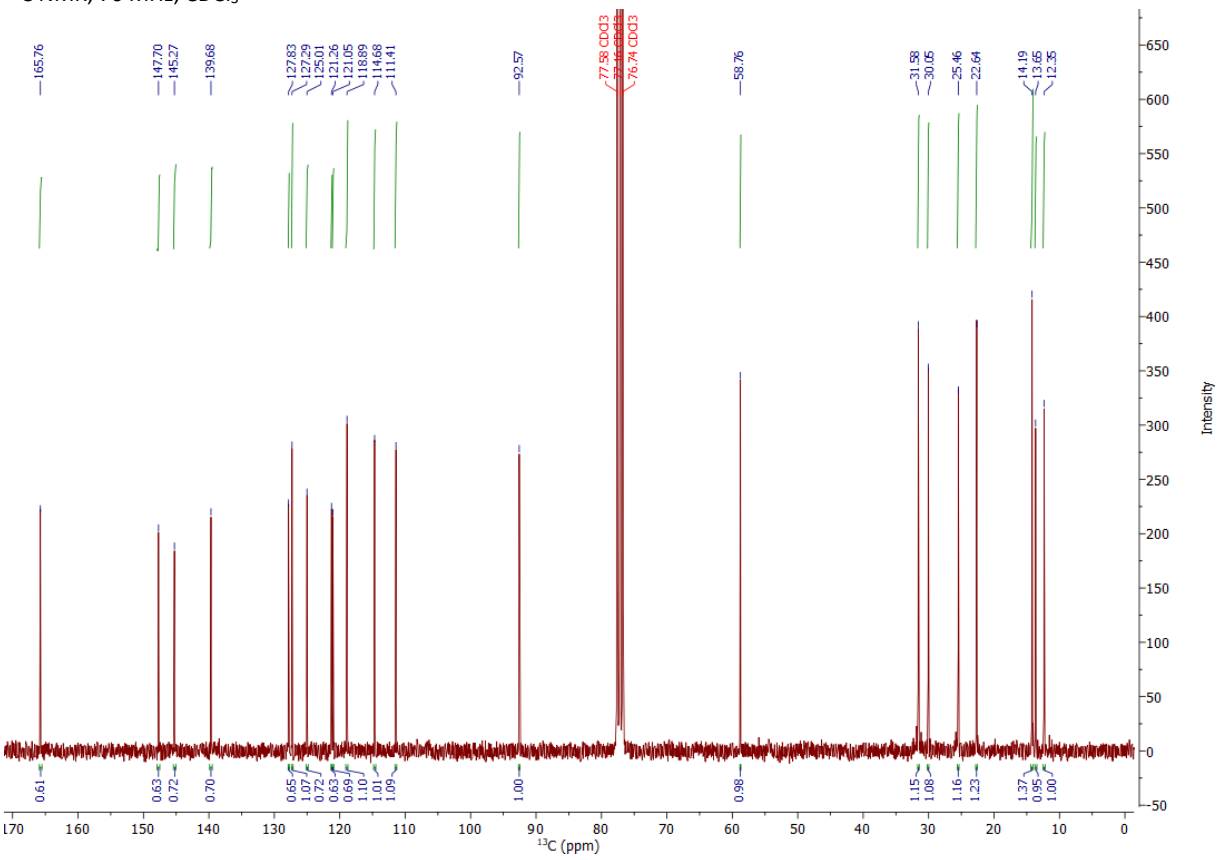


(*Z*)-4'-Methoxy-5-methyl-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16ba)

^{1}H NMR, 300 MHz, $CDCl_3$

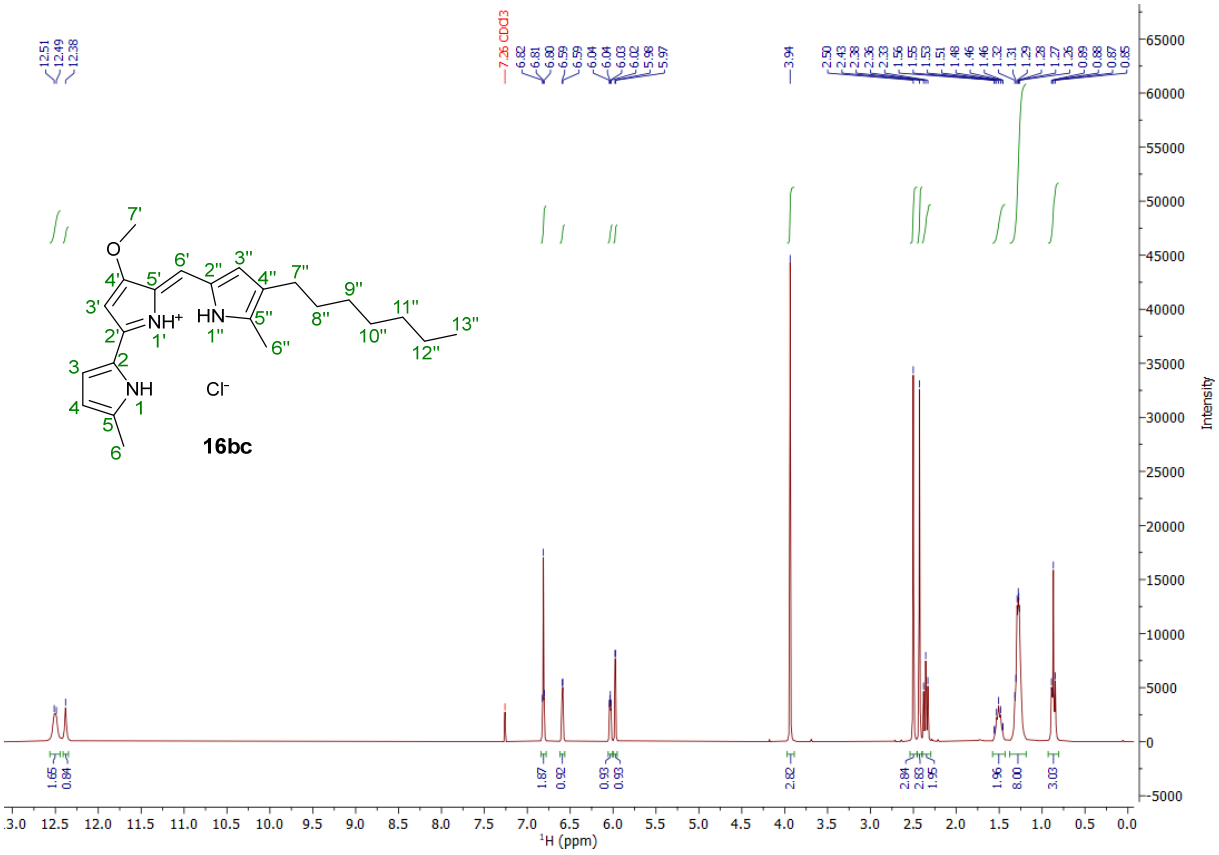


^{13}C NMR, 76 MHz, $CDCl_3$

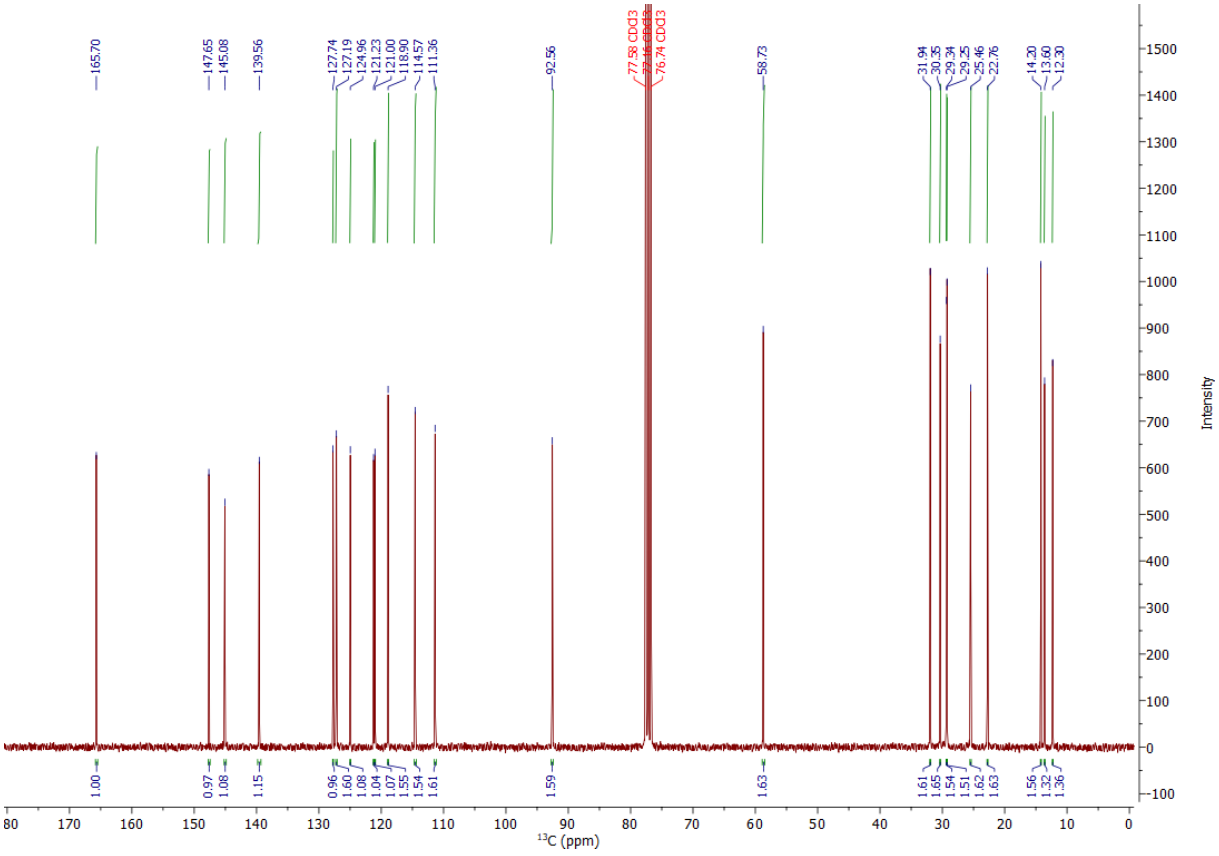


(*Z*)-4'-Methoxy-5-methyl-5'-((5-methyl-4-heptyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16bc)

^{1}H NMR, 300 MHz, $CDCl_3$

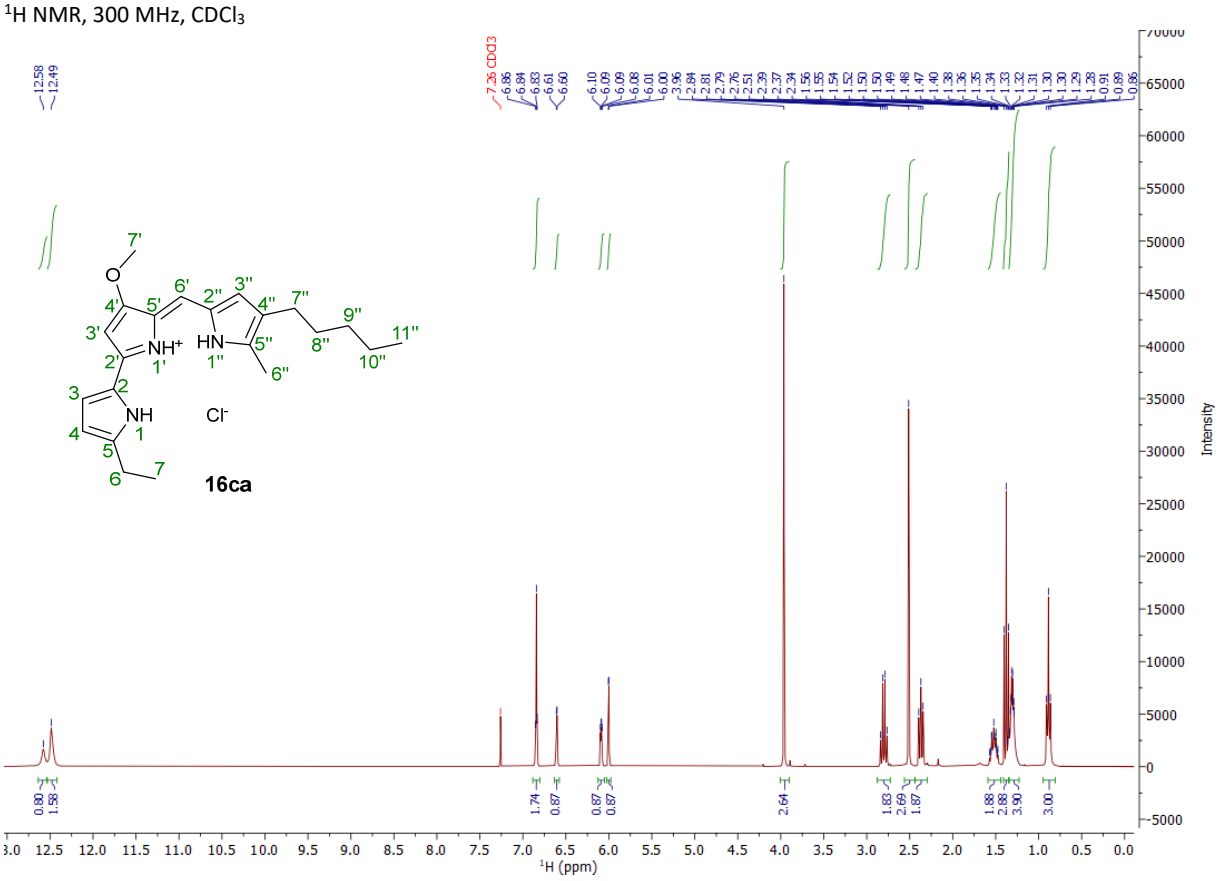


^{13}C NMR, 76 MHz, $CDCl_3$

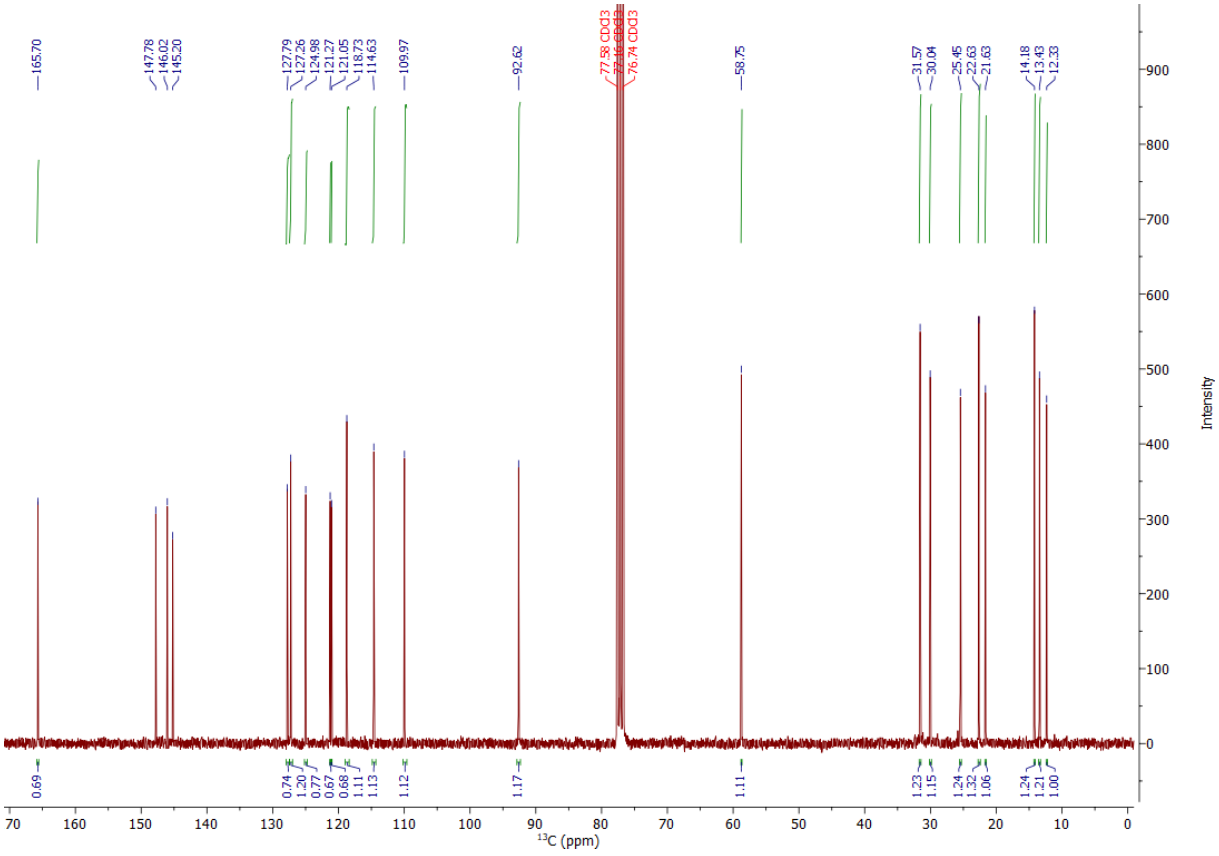


(*Z*)-5-Ethyl-4'-methoxy-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16ca)

^{1}H NMR, 300 MHz, $CDCl_3$

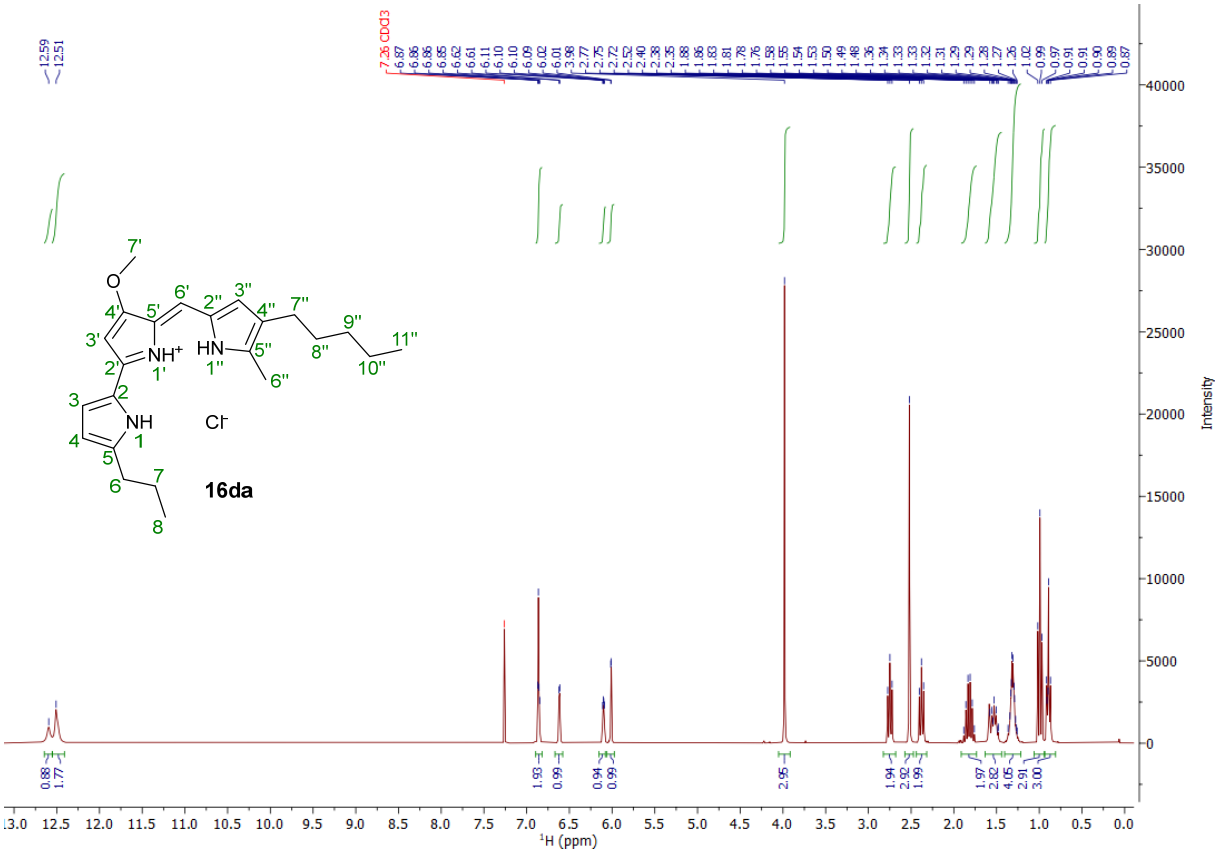


^{13}C NMR, 76 MHz, $CDCl_3$

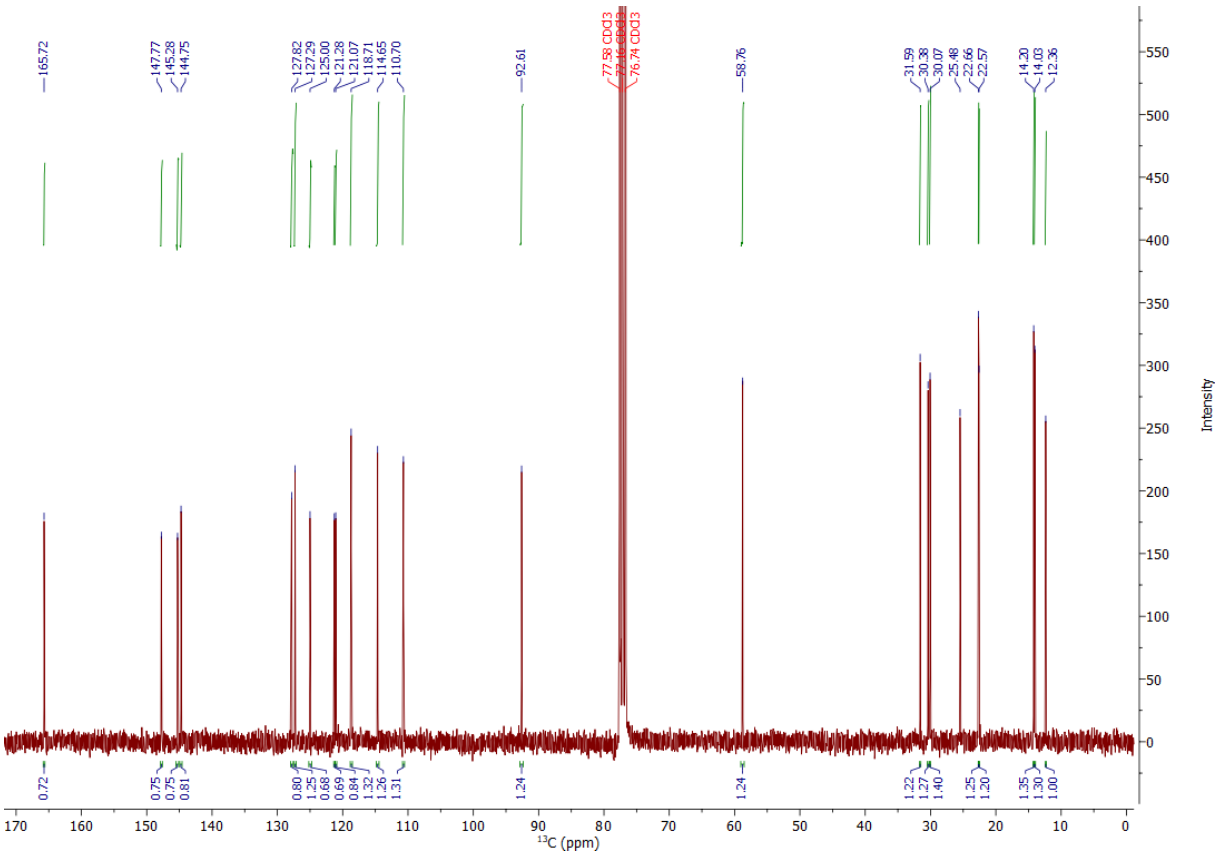


(*Z*)-4'-Methoxy-5-propyl-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16da)

^{1}H NMR, 300 MHz, $CDCl_3$

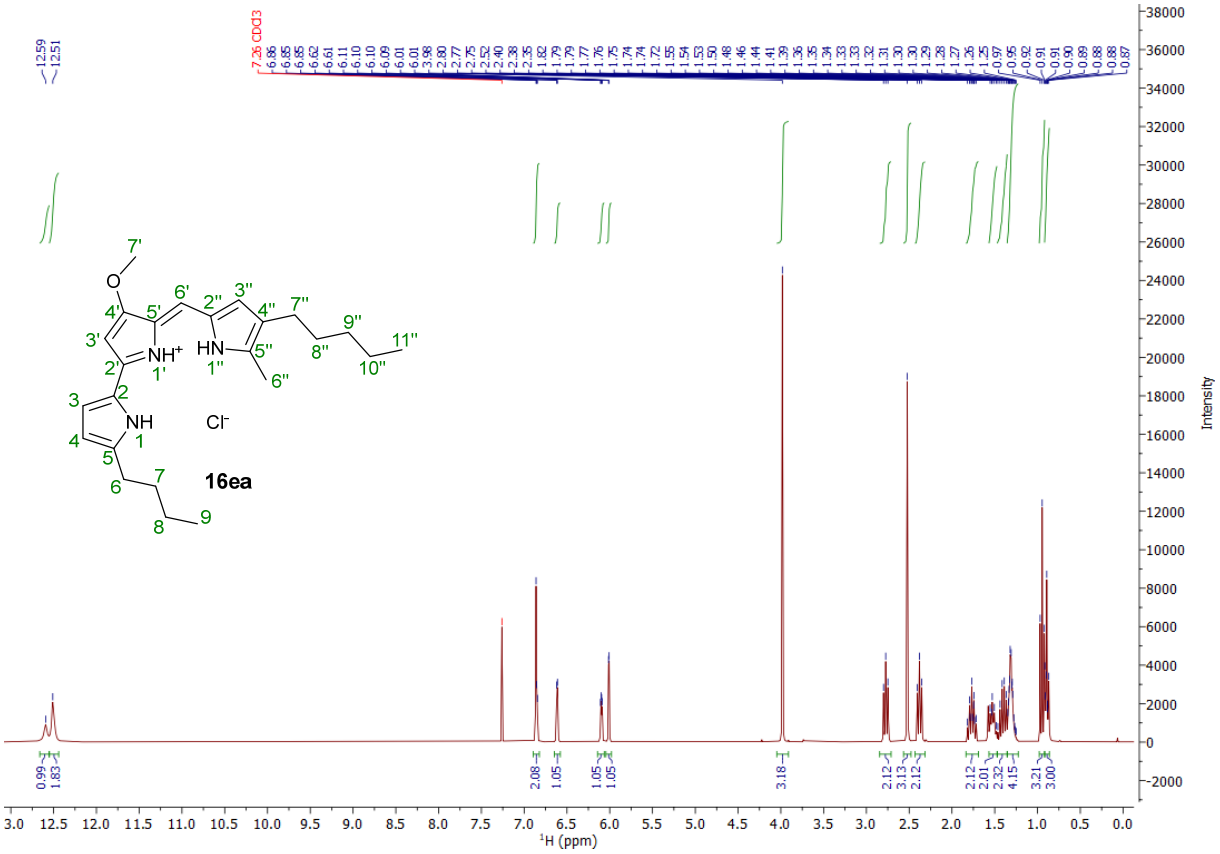


^{13}C NMR, 76 MHz, $CDCl_3$

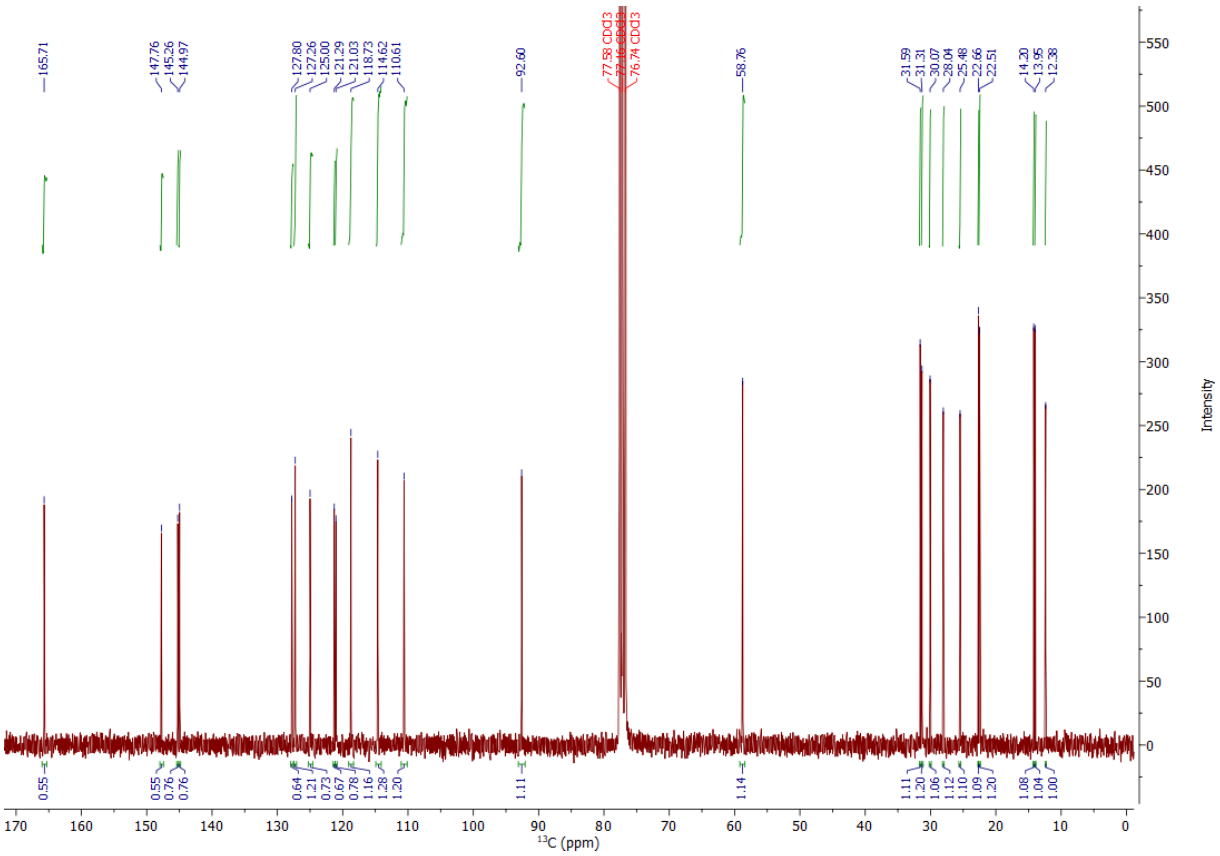


(*Z*)-5-Butyl-4'-methoxy-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16ea)

^{1}H NMR, 300 MHz, $CDCl_3$

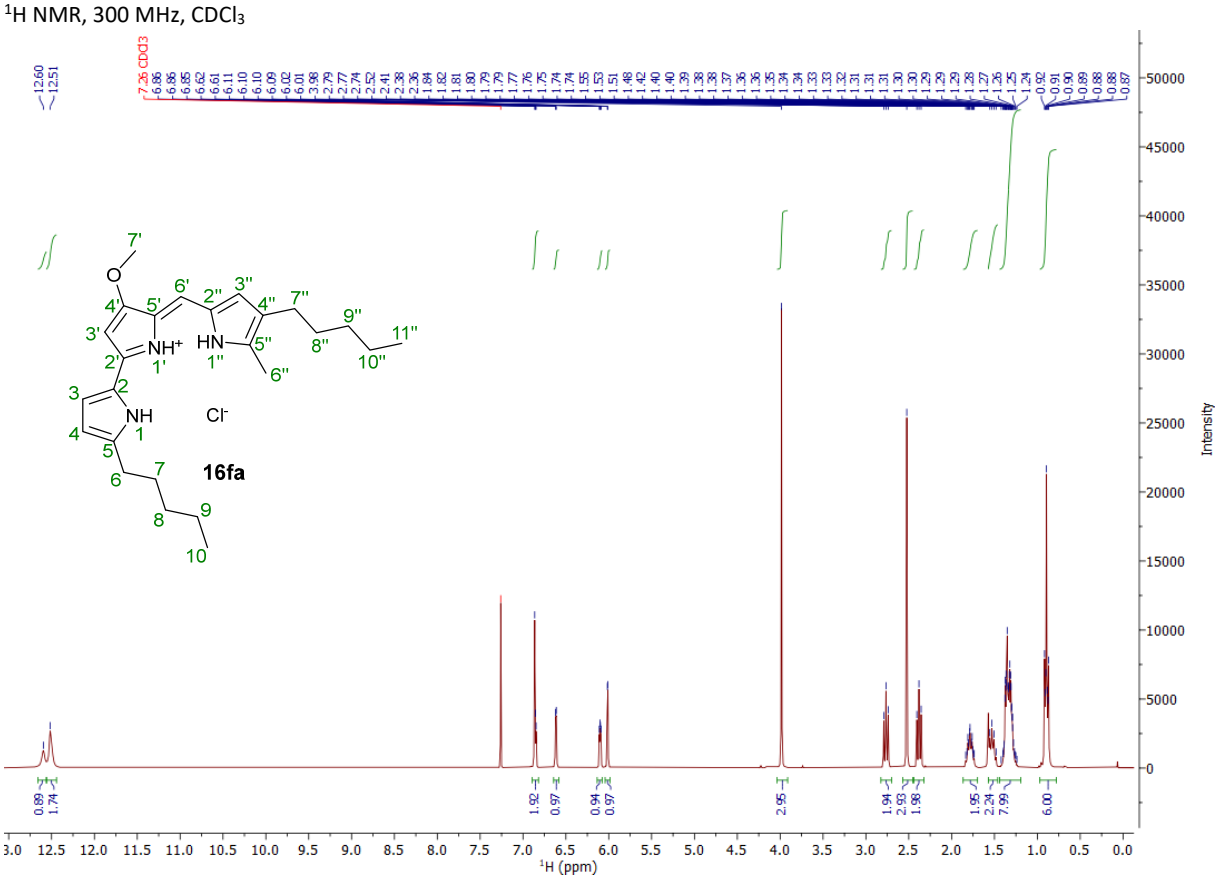


^{13}C NMR, 76 MHz, $CDCl_3$

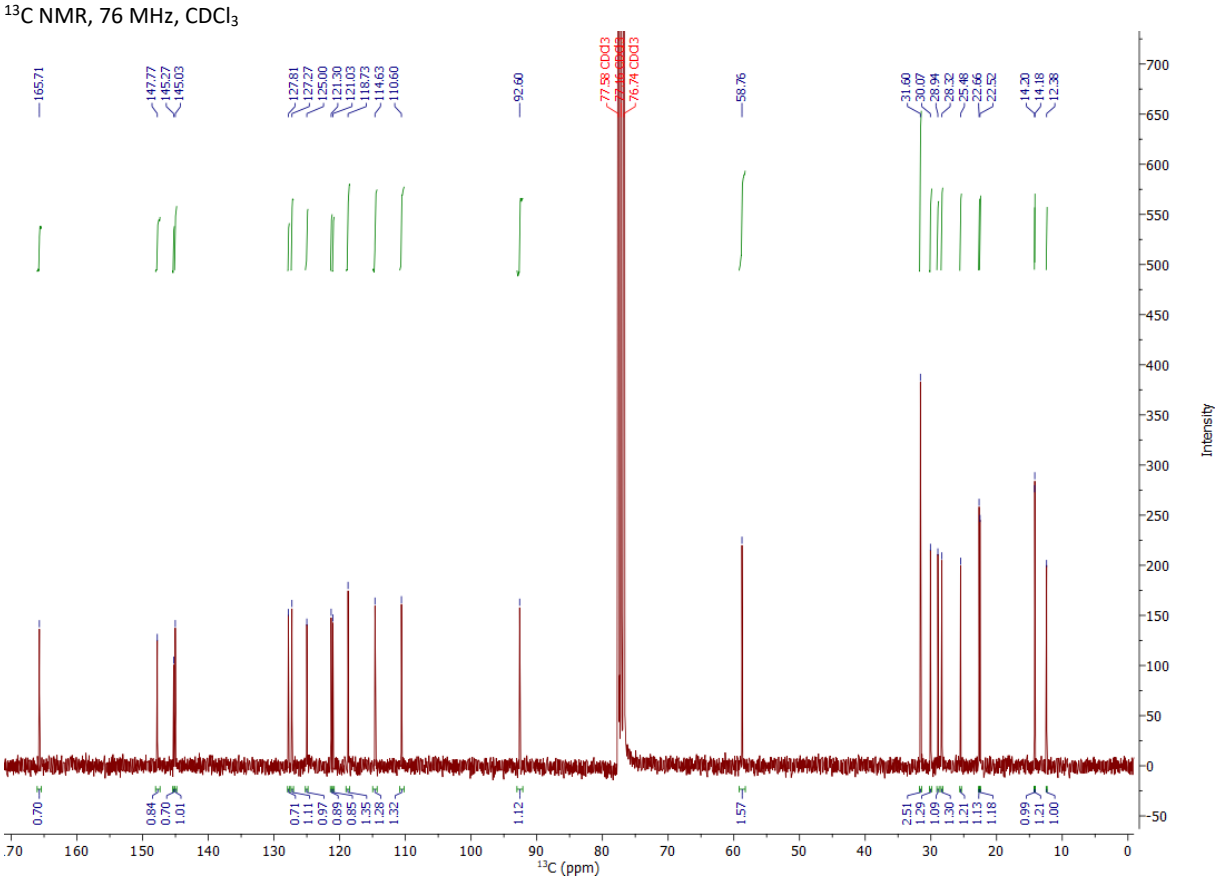


(*Z*)-4'-Methoxy-5-pentyl-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16fa)

^{1}H NMR, 300 MHz, $CDCl_3$

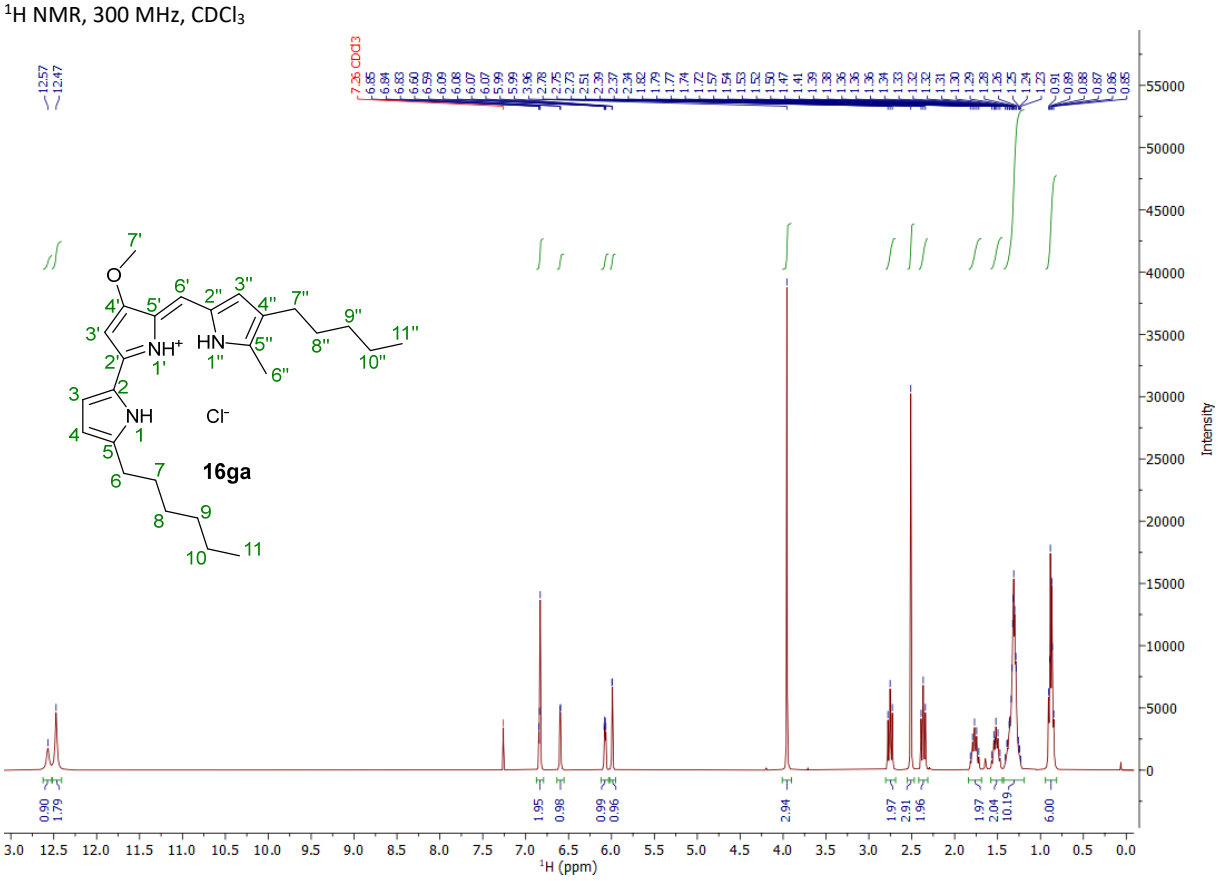


^{13}C NMR, 76 MHz, $CDCl_3$

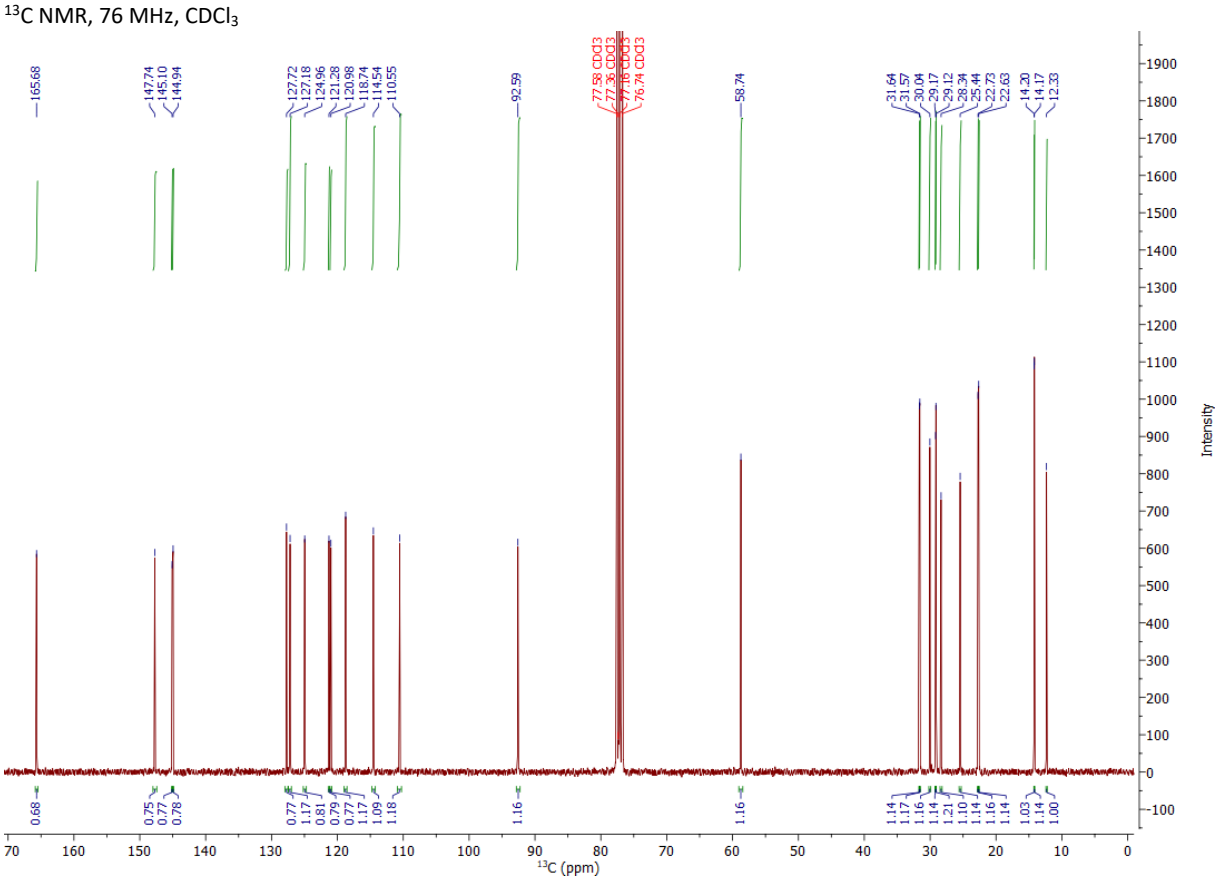


(*Z*)-5-Hexyl-4'-methoxy-5'-((5-methyl-4-pentyl-1*H*-pyrrol-2-yl)methylene)-1*H*,5'*H*-[2,2'-bipyrrol]-1'-ium chloride (16ga)

^{1}H NMR, 300 MHz, $CDCl_3$



^{13}C NMR, 76 MHz, $CDCl_3$



11. LC-MS Chromatograms – Synthetic References

MBC Derivatives

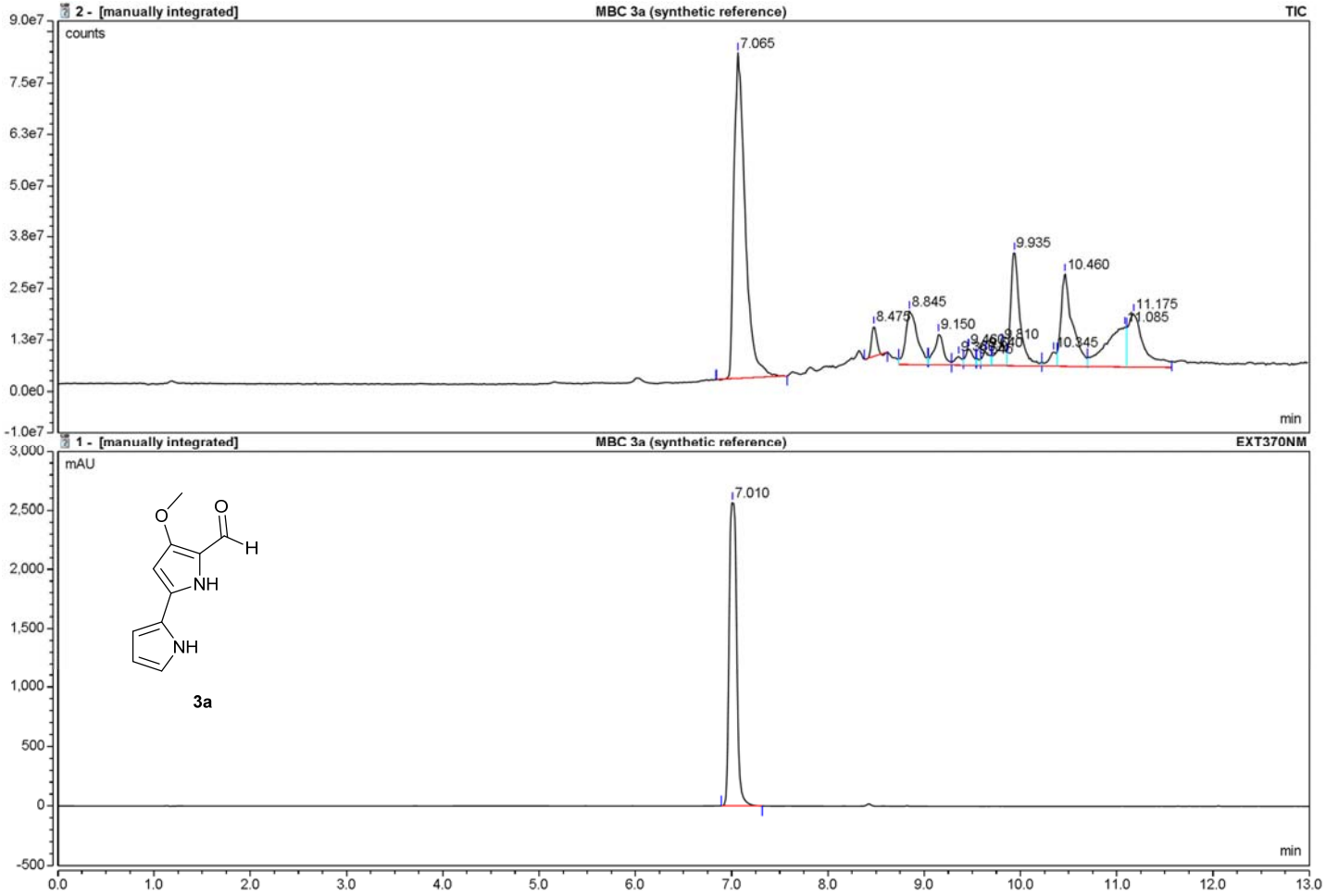


Figure S4: LC-MS data [total ion chromatogram (TIC, top) and UV_{370} trace (bottom)] of chemically synthesised MBC **3a**. Retention time t_R 7.01 min.

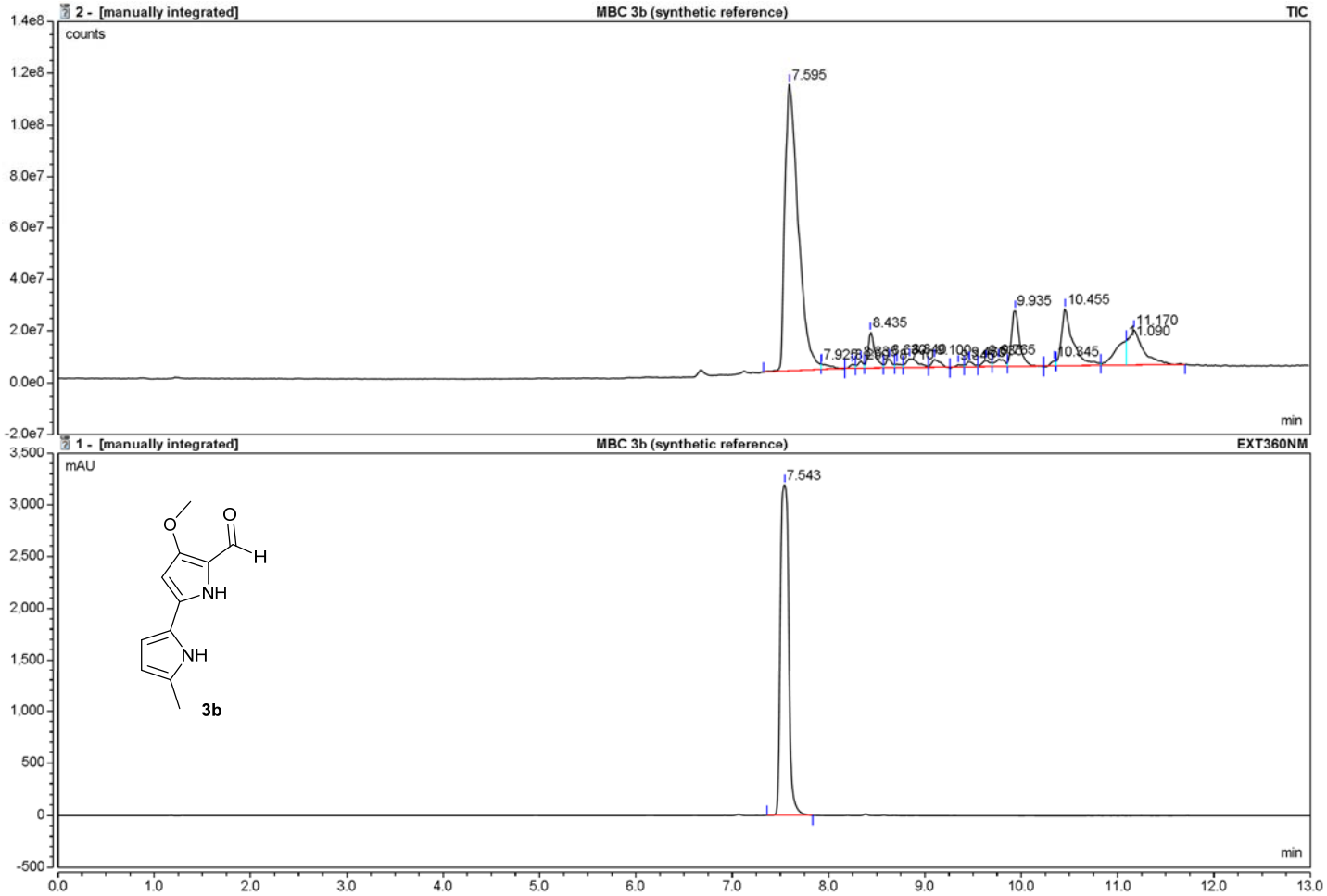


Figure S5: LC-MS data [TIC (top) and UV_{360} trace (bottom)] of chemically synthesised 5'-methyl-MBC **3b**. Retention time t_R 7.54 min.

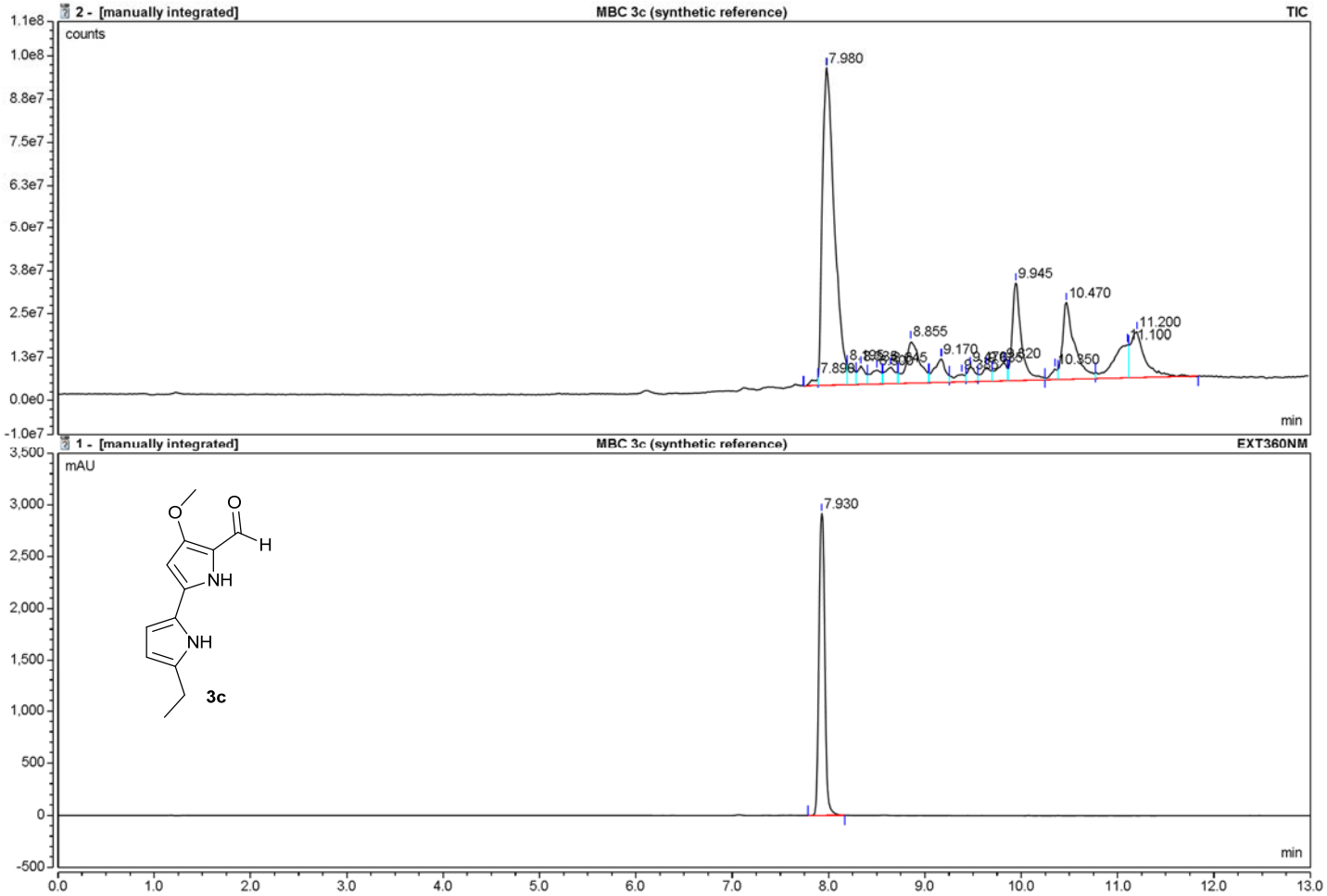


Figure S6: LC-MS data [TIC (top) and UV_{360} trace (bottom)] of chemically synthesised 5'-ethyl-MBC **3c**. Retention time t_R 7.93 min.

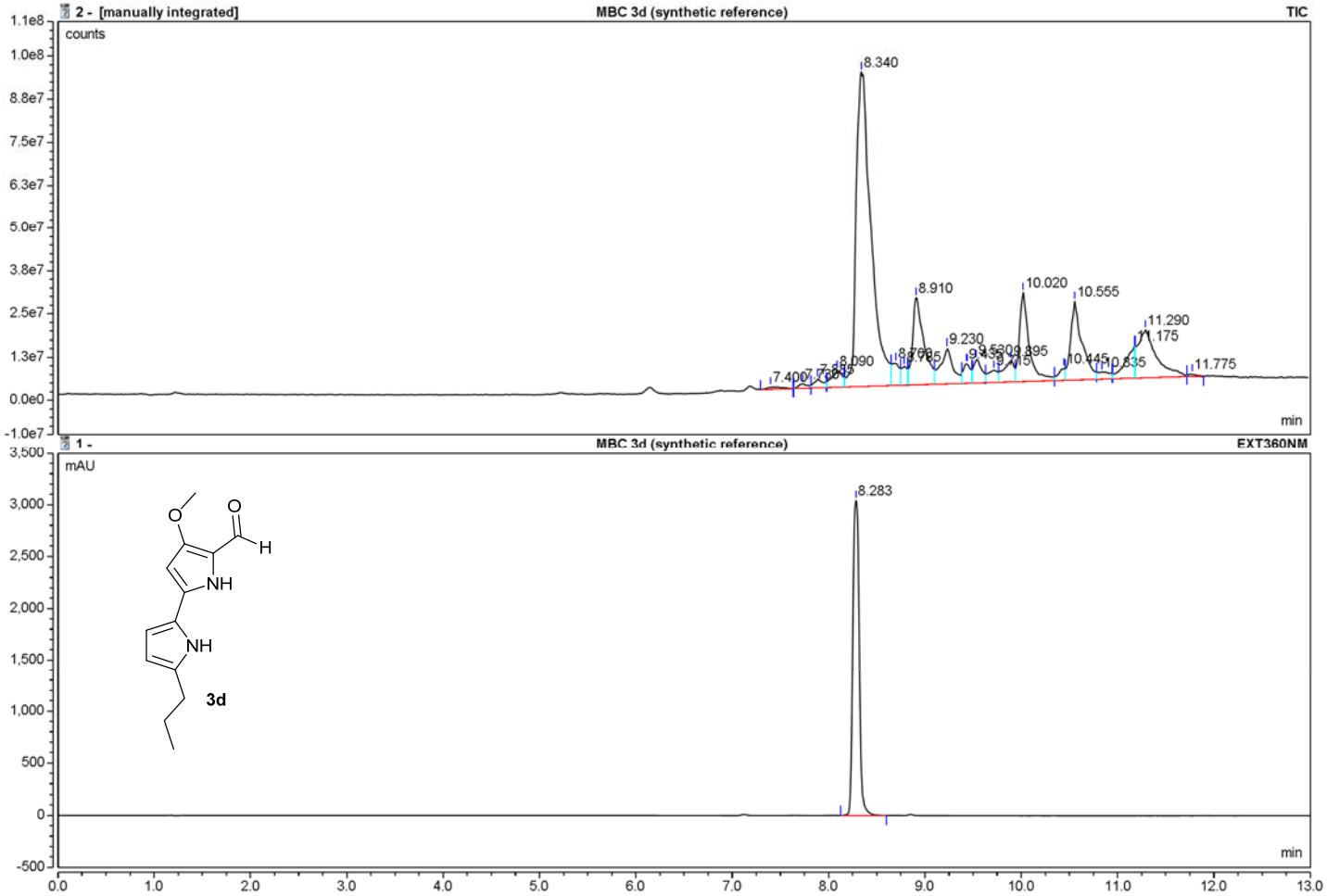


Figure S7: LC-MS data [TIC (top) and UV_{360} trace (bottom)] of chemically synthesised 5'-propyl-MBC **3d**. Retention time t_R 8.28 min.

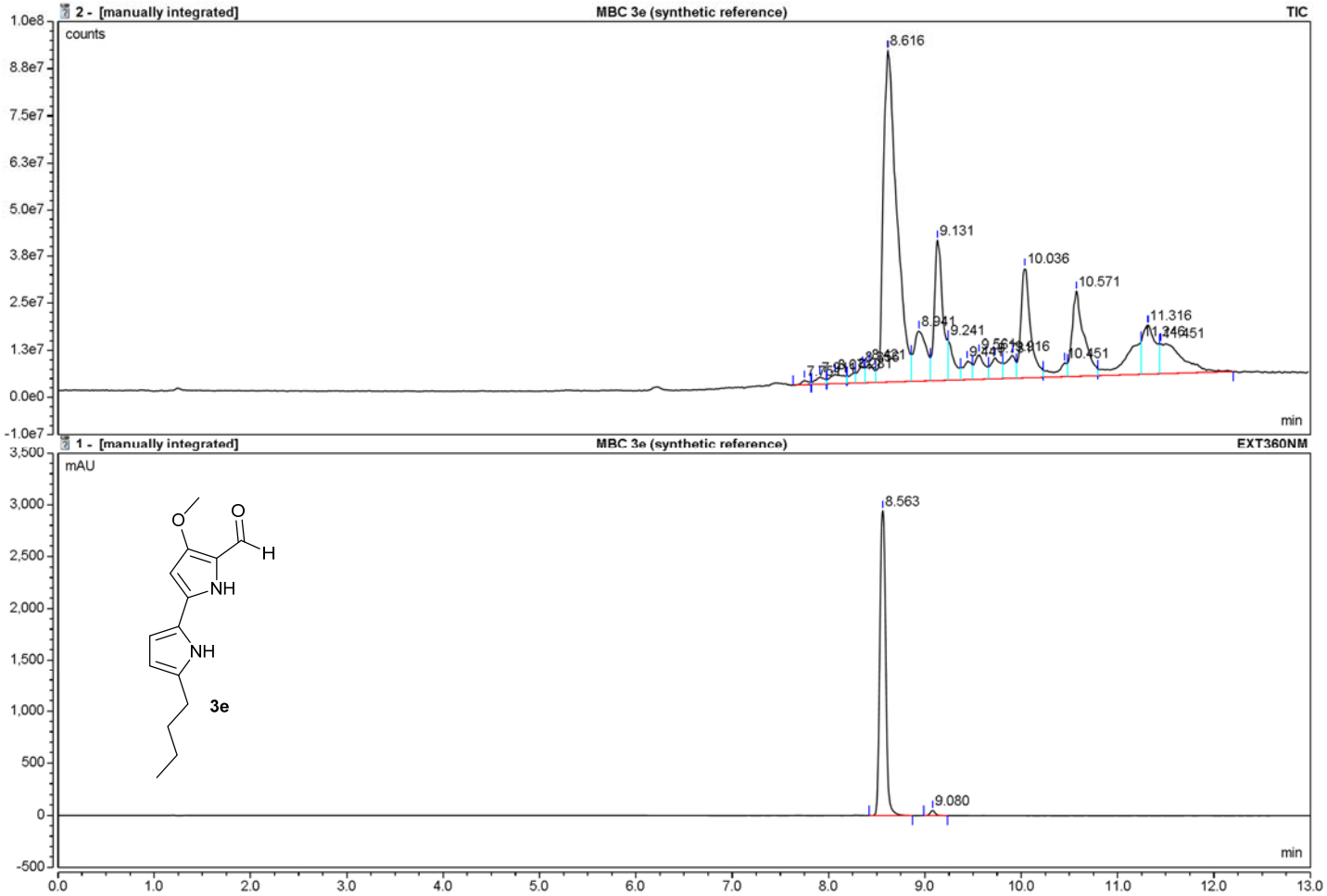


Figure S8: LC-MS data [TIC (top) and UV_{360} trace (bottom)] of chemically synthesised 5'-butyl-MBC **3e**. Retention time t_R 8.56 min.

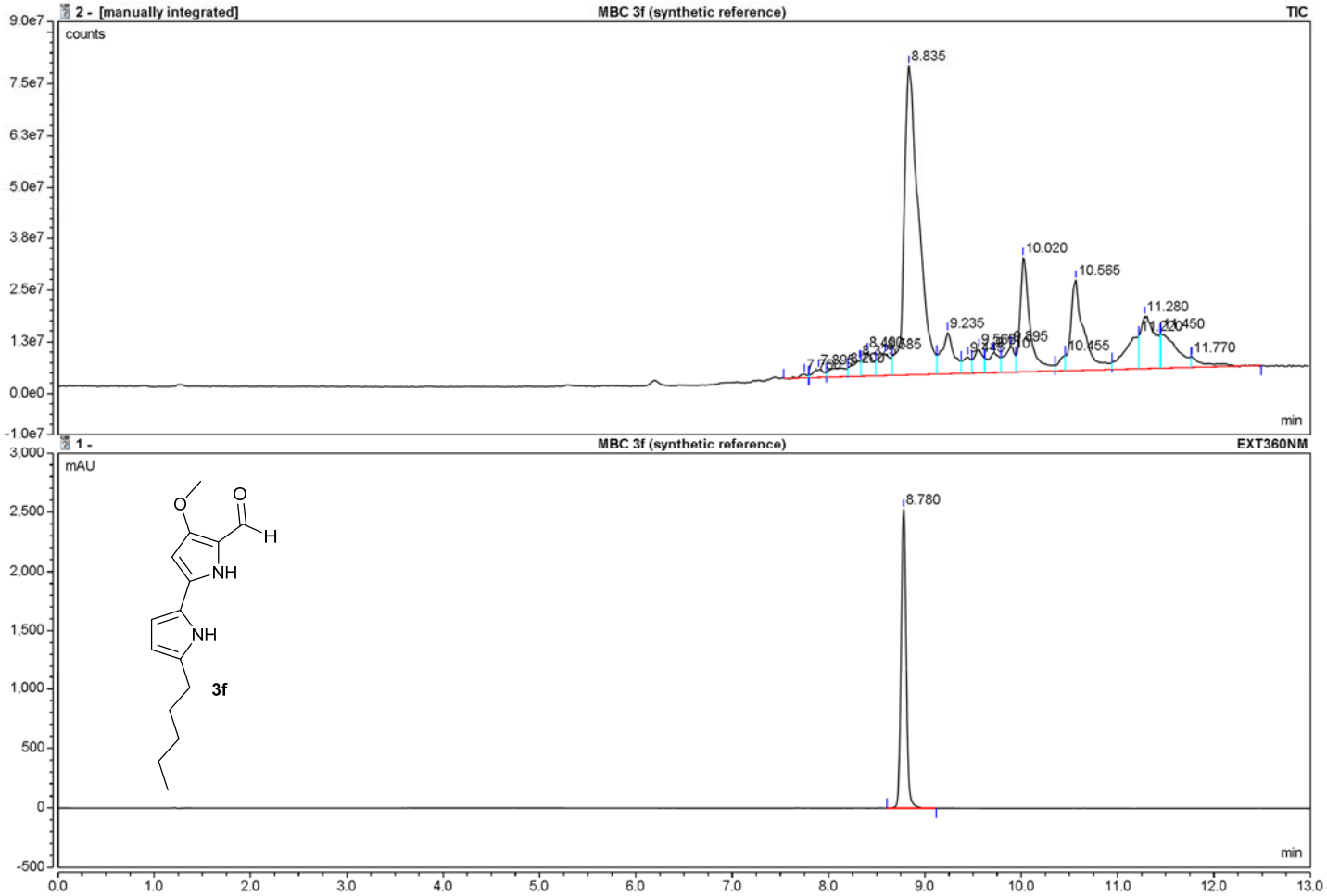


Figure S9: LC-MS data [TIC (top) and UV_{360} trace (bottom)] of chemically synthesised 5'-pentyl-MBC **3f**. Retention time t_R 8.78 min.

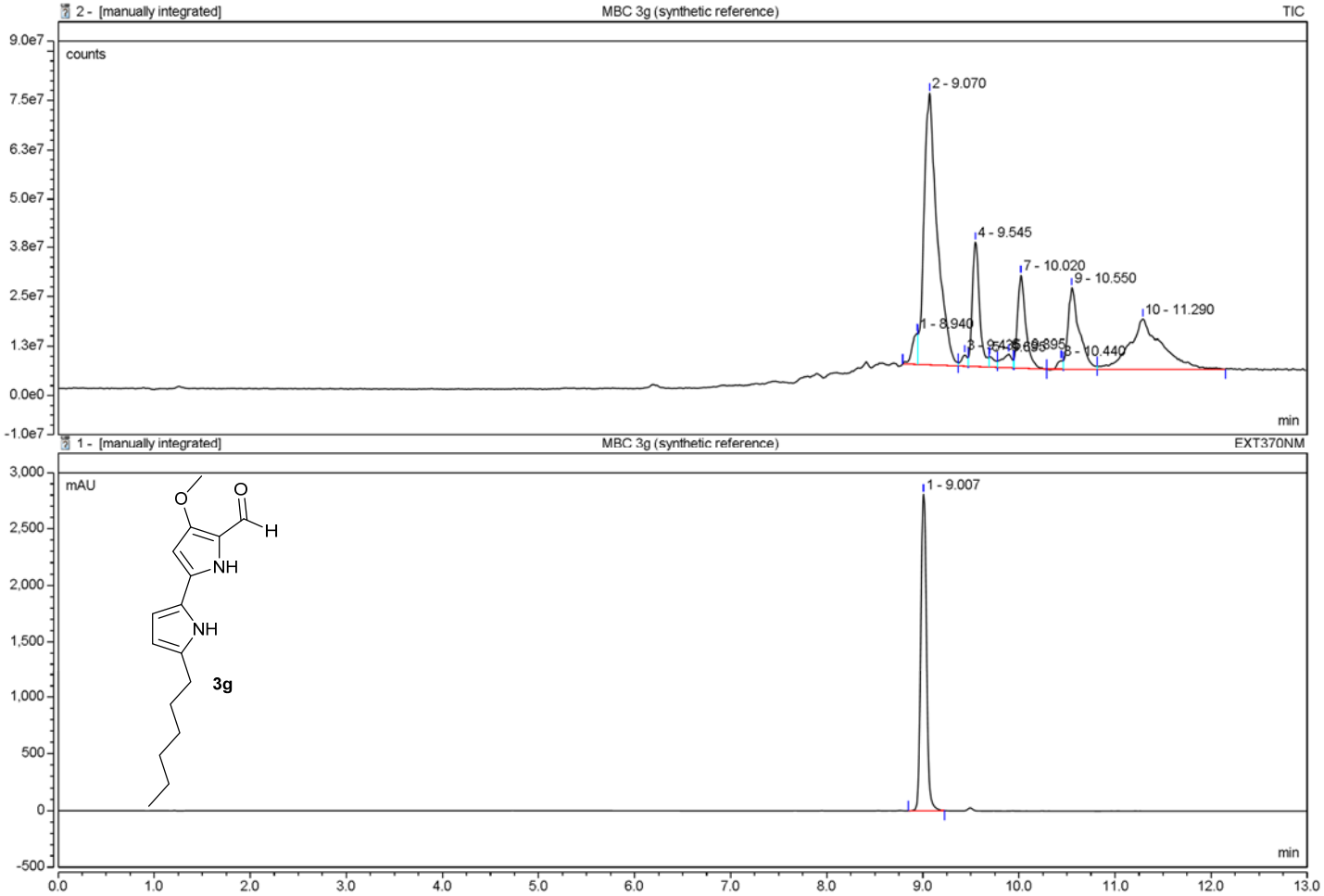


Figure S10: LC-MS data [TIC (top) and UV_{370} trace (bottom)] of chemically synthesised 5'-hexyl-MBC **3g**. Retention time t_R 9.07 min.

Pyrroles

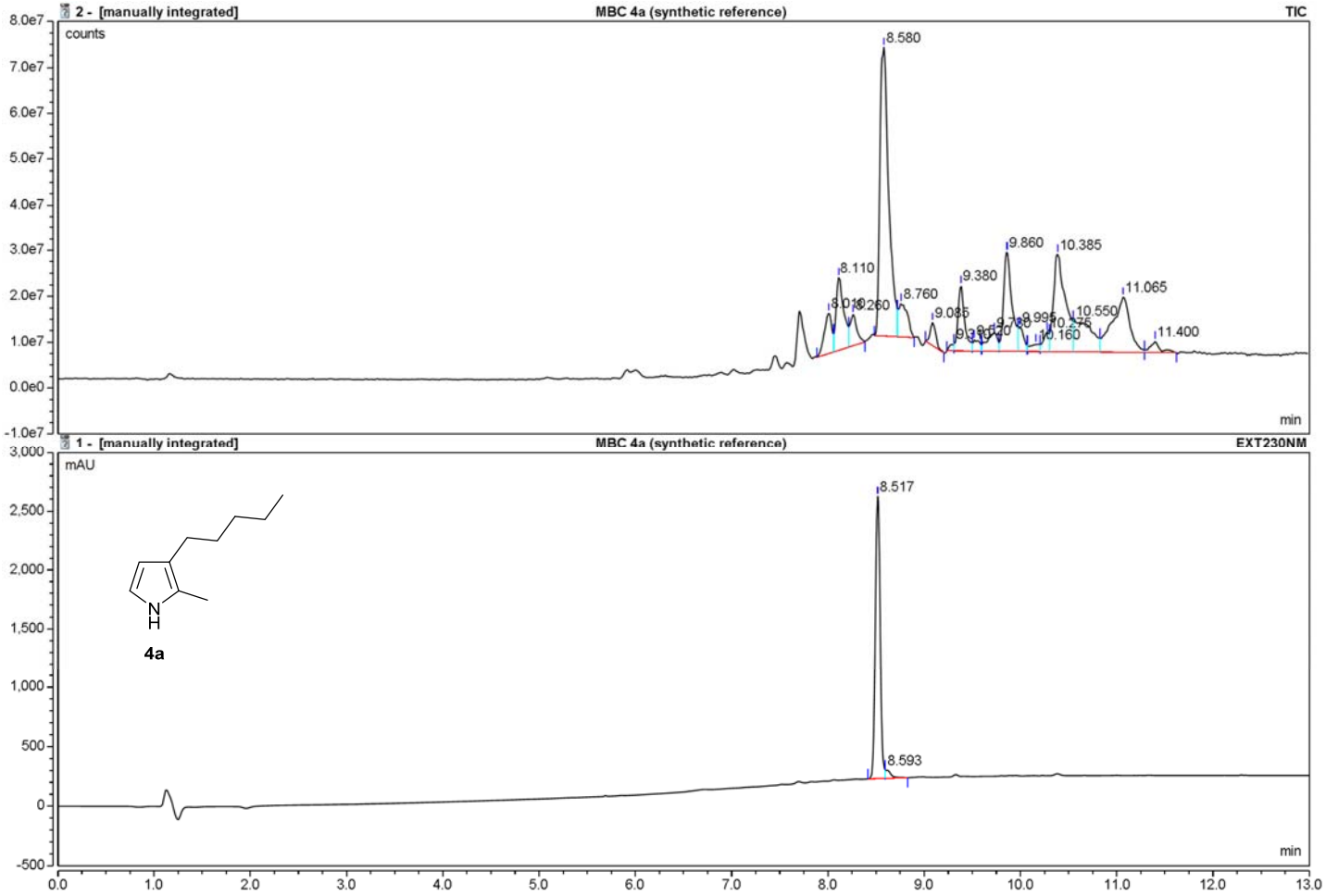


Figure S11: LC-MS data [TIC (top) and UV_{230} trace (bottom)] of chemically synthesised monopyrrole **4a**. Retention time t_R 8.52 min.

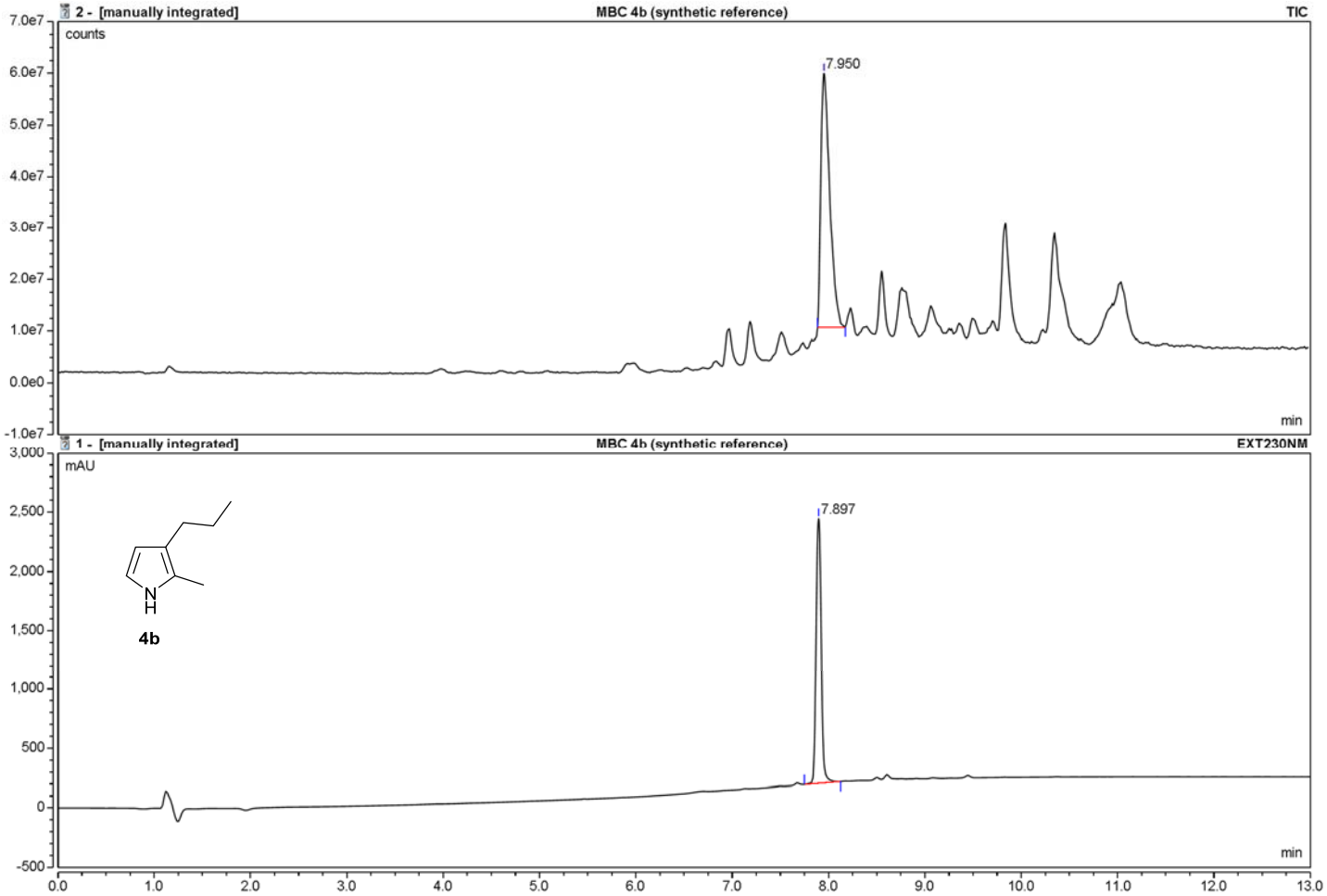


Figure S12: LC-MS data [TIC (top) and UV_{230} trace (bottom)] of chemically synthesised monopyrrole **4b**. Retention time t_R 7.90 min.

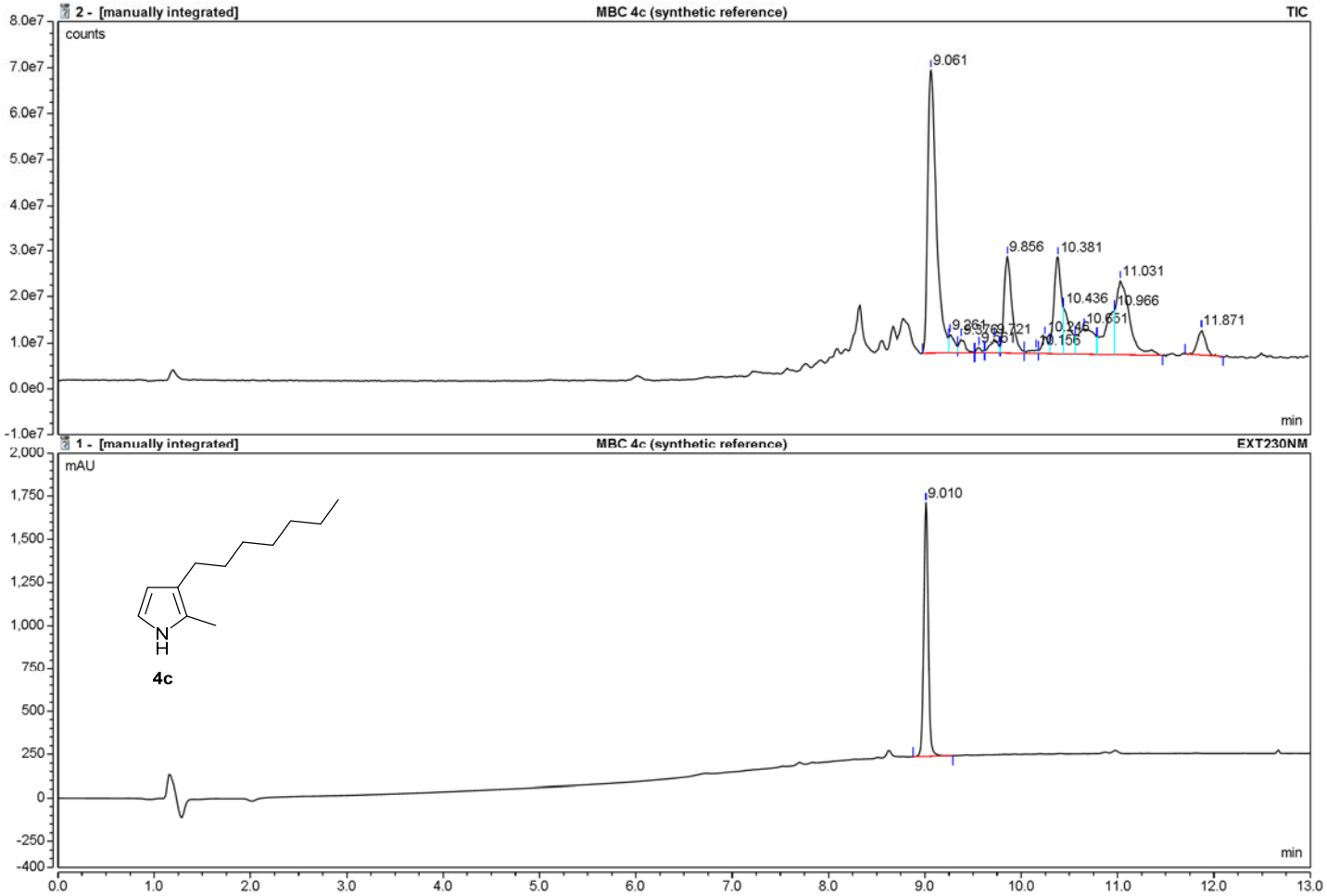


Figure S13: LC-MS data [TIC (top) and UV_{230} trace (bottom)] of chemically synthesised monopyrrole **4c**. Retention time t_R 9.01 min.

Prodiginines

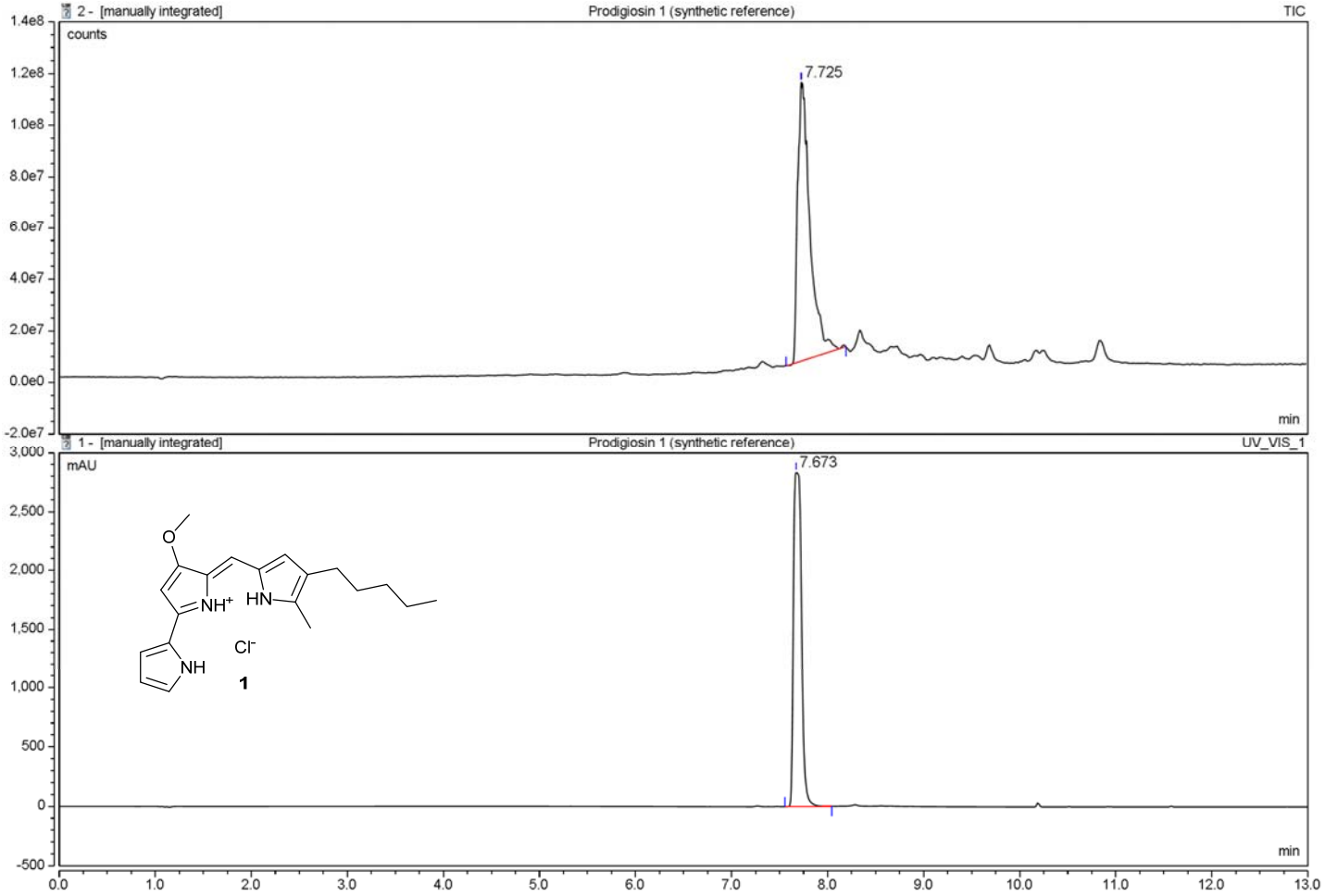


Figure S14: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodigiosin (**1**). Retention time t_R 7.67 min.

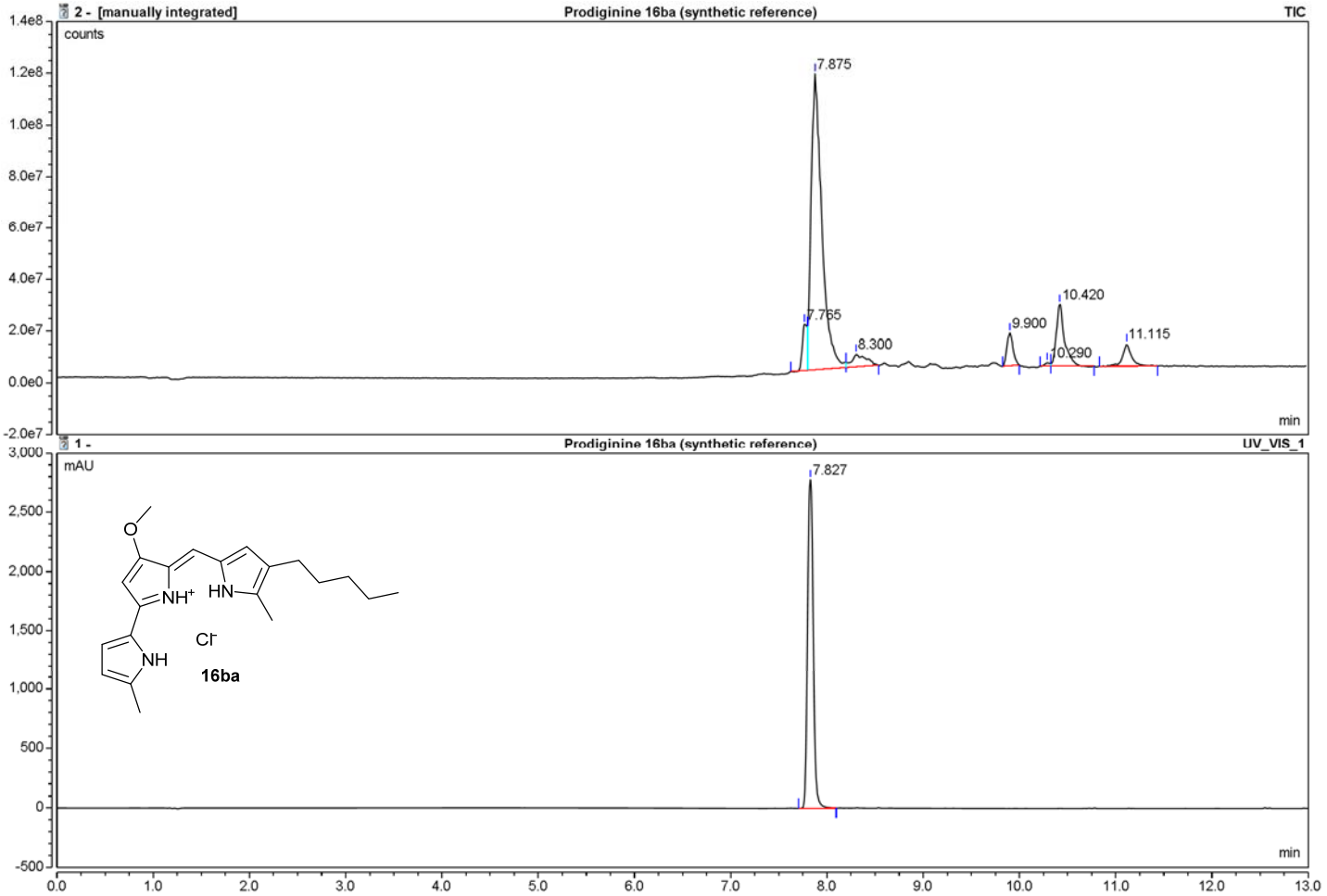


Figure S15: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16ba**. Retention time t_R 7.83 min.

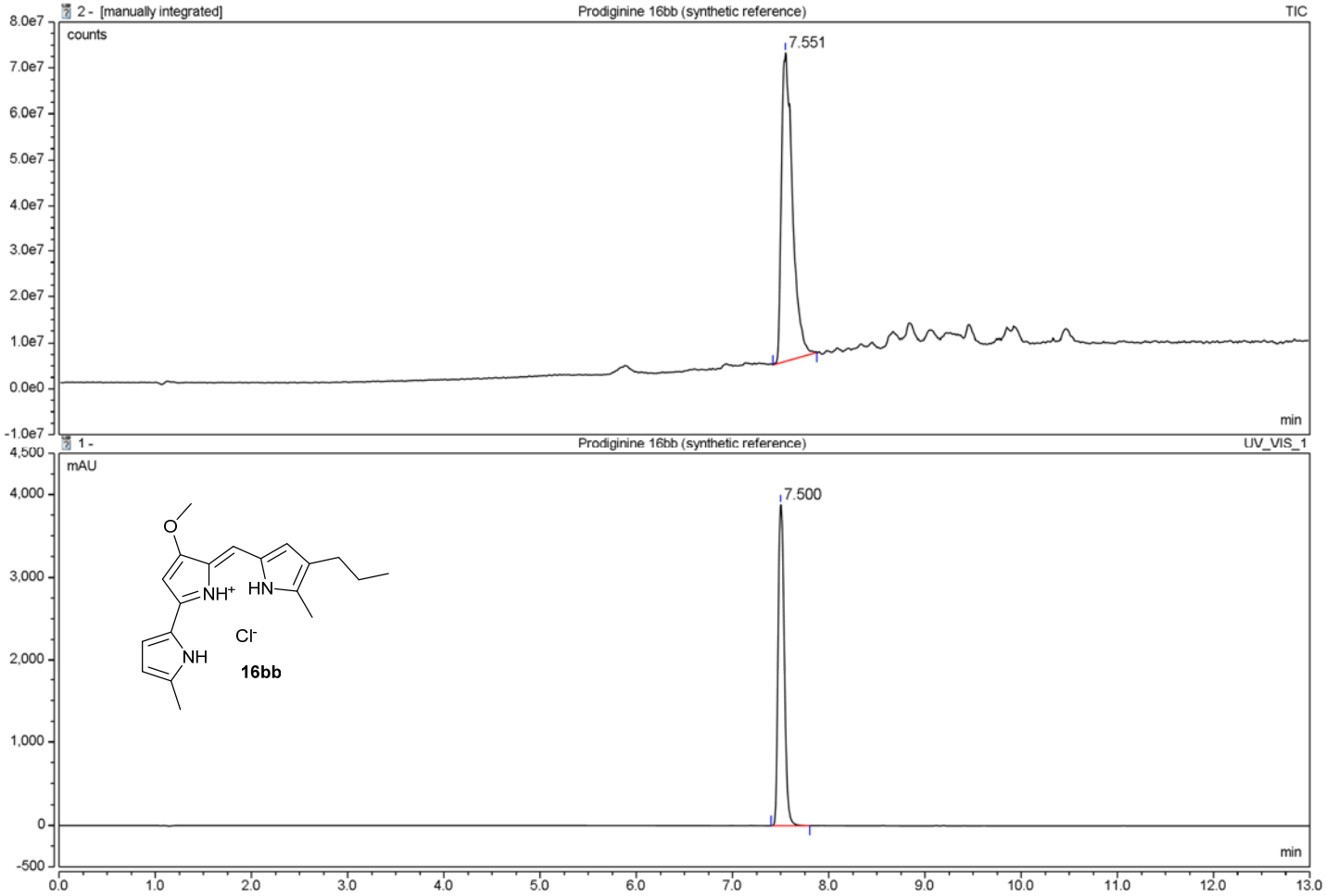


Figure S16: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16bb**. Retention time t_R 7.50 min.

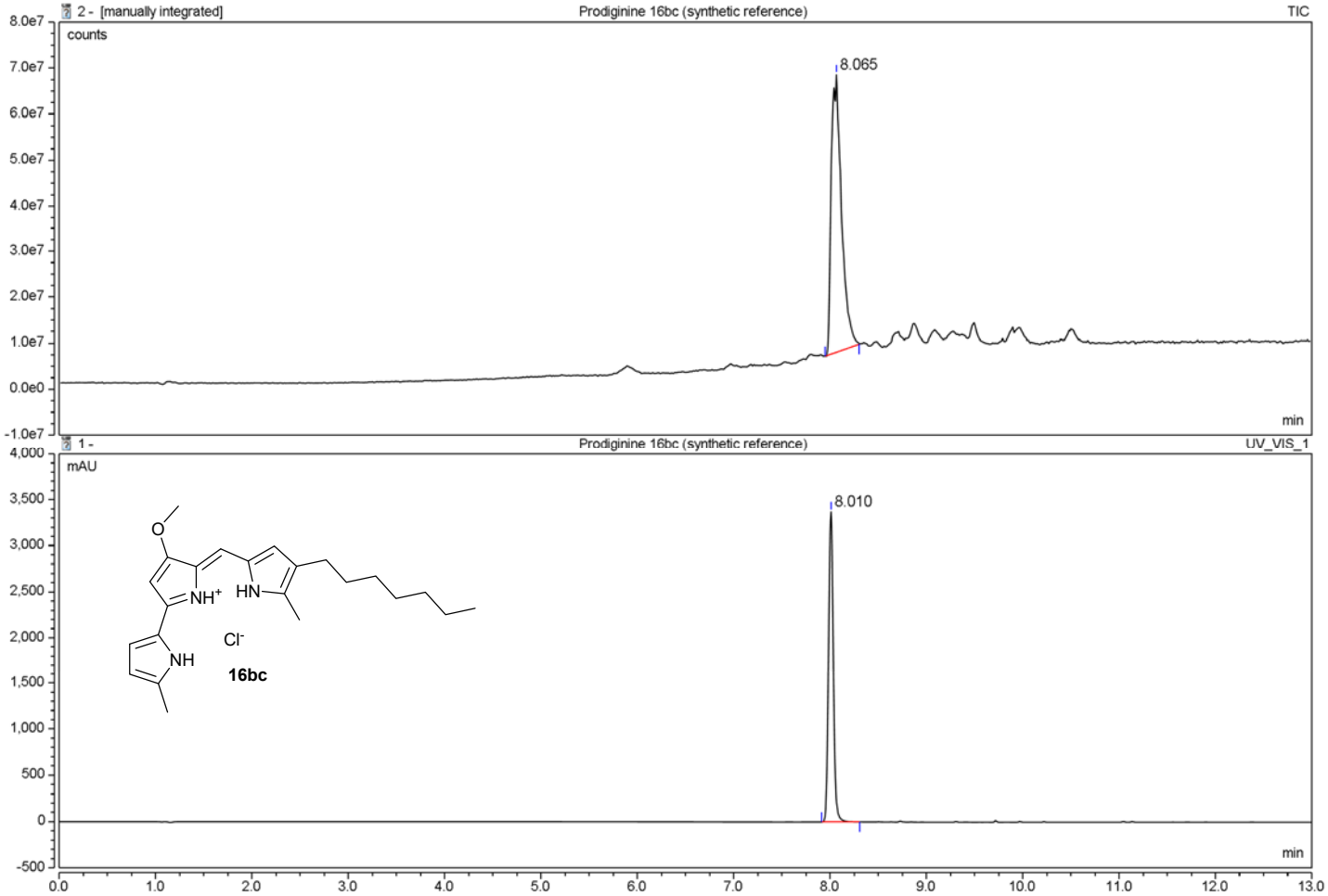


Figure S17: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16bc**. Retention time t_R 8.01 min.

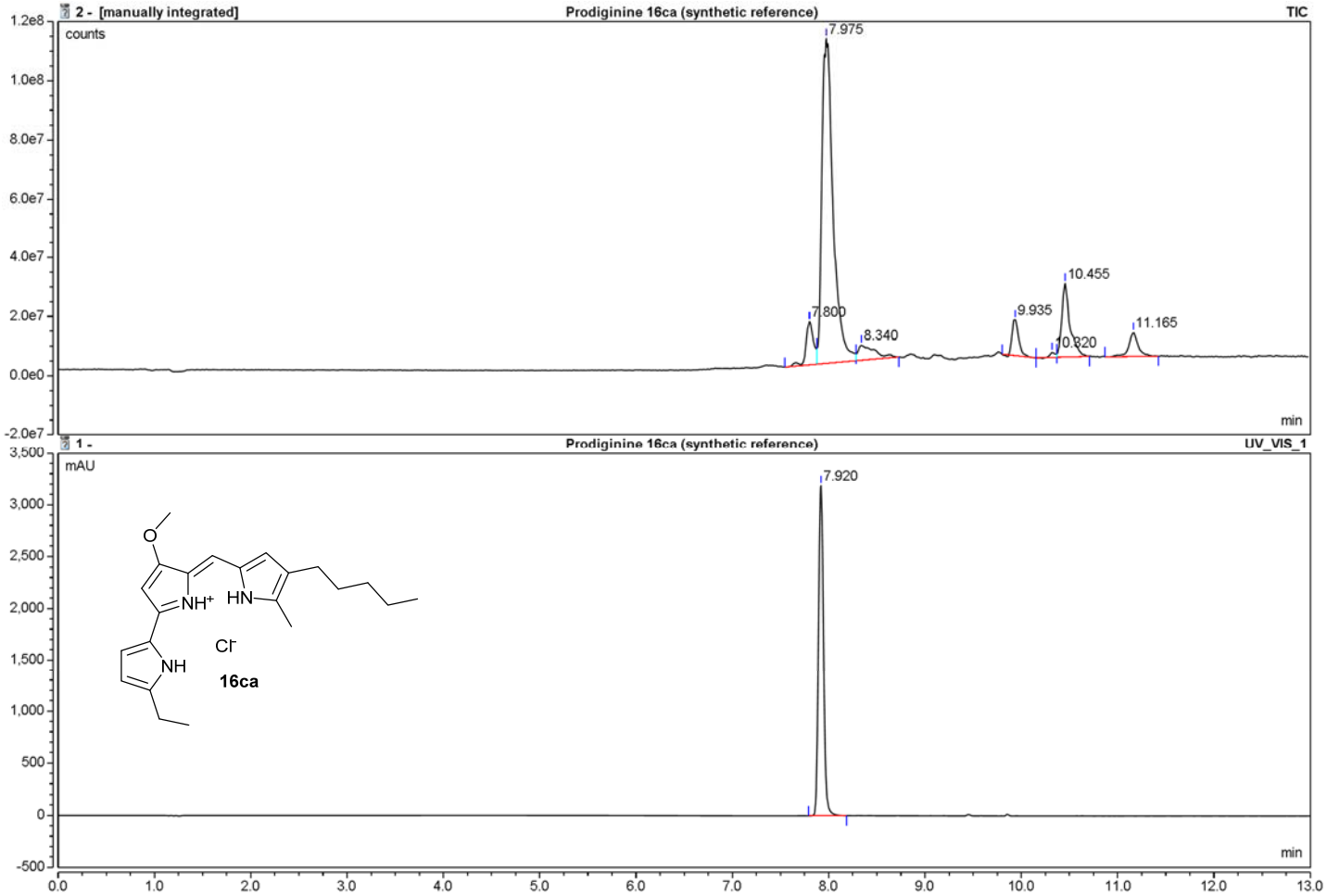


Figure S18: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16ca**. Retention time t_R 7.92 min.

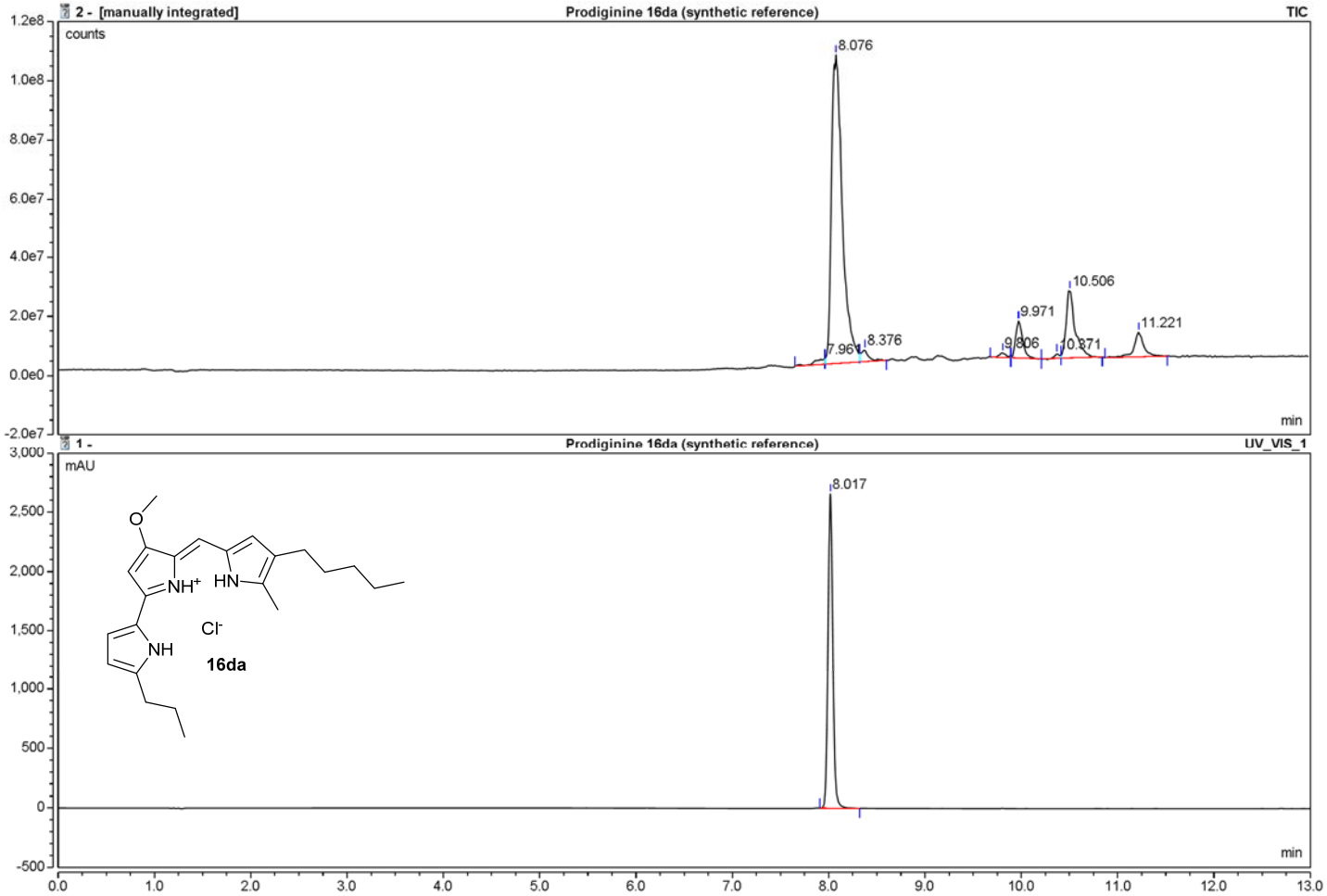


Figure S19: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16da**. Retention time t_R 8.02 min.

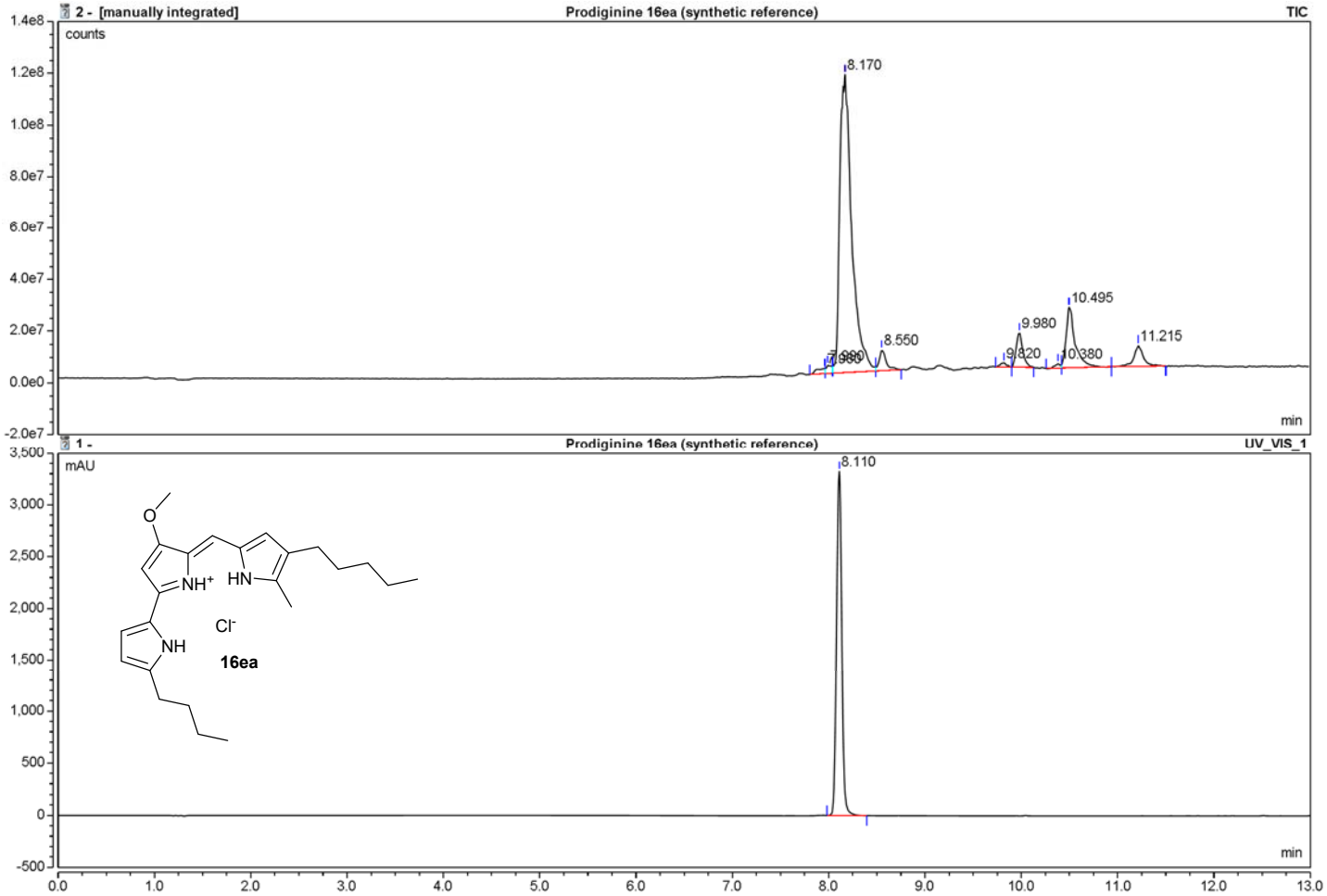


Figure S20: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16ea**. Retention time t_R 8.11 min.

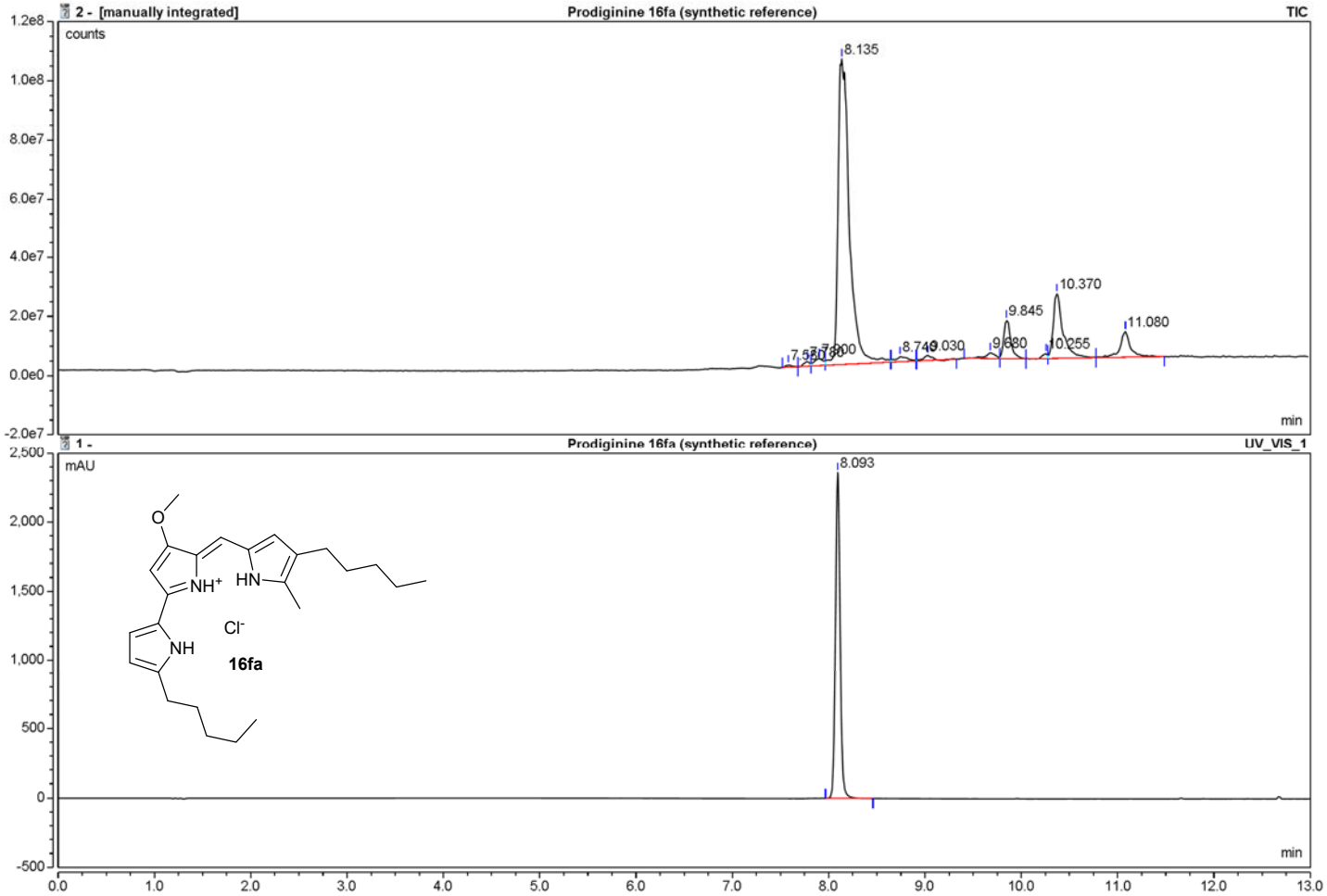


Figure S21: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16fa**. Retention time t_R 8.09 min.

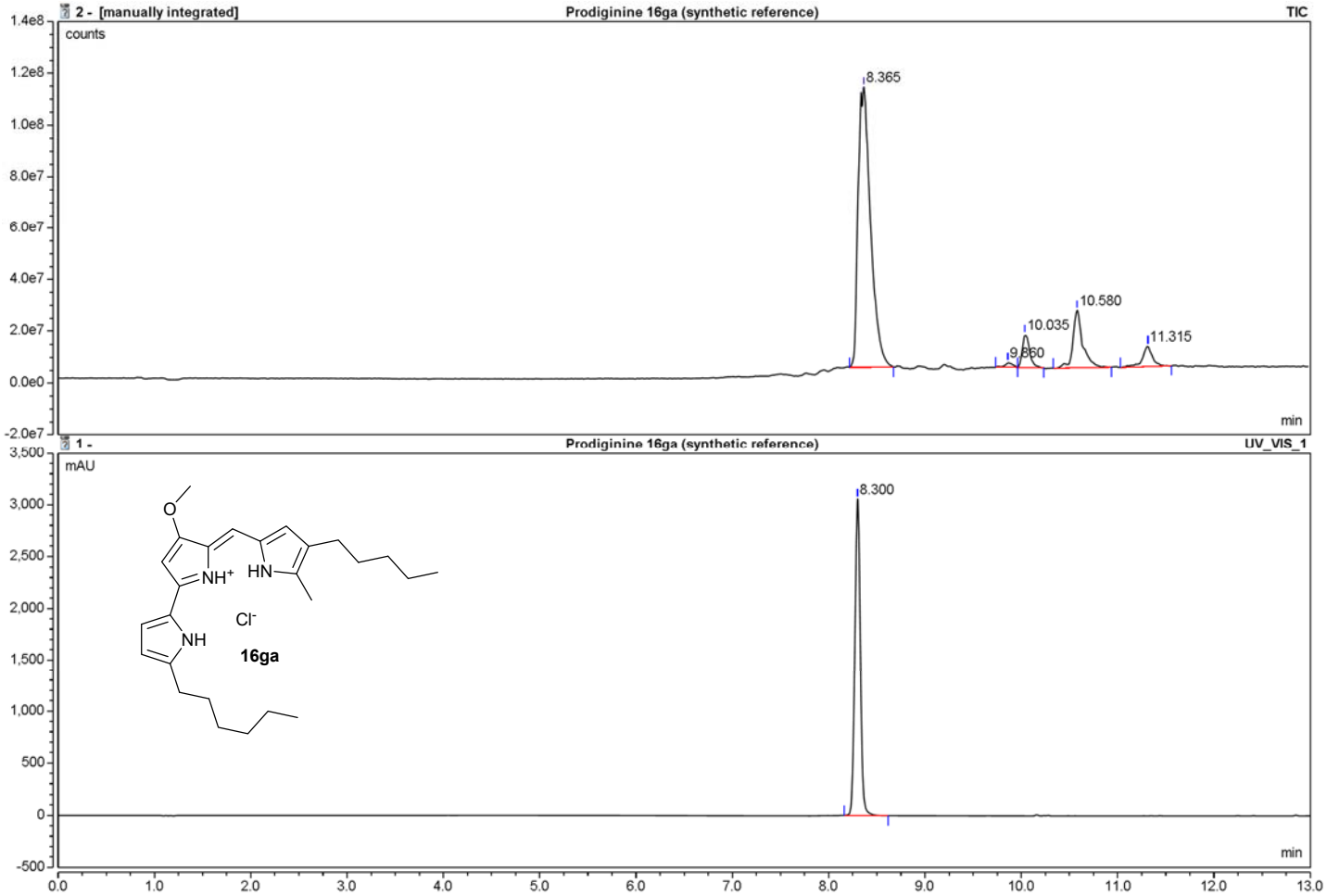


Figure S22: LC-MS data [TIC (top) and UV_{535} trace (bottom)] of chemically synthesised prodiginine **16ga**. Retention time t_R 8.30 min.

12. LC-MS Chromatograms – Biocatalytic *In Vitro* Experiments

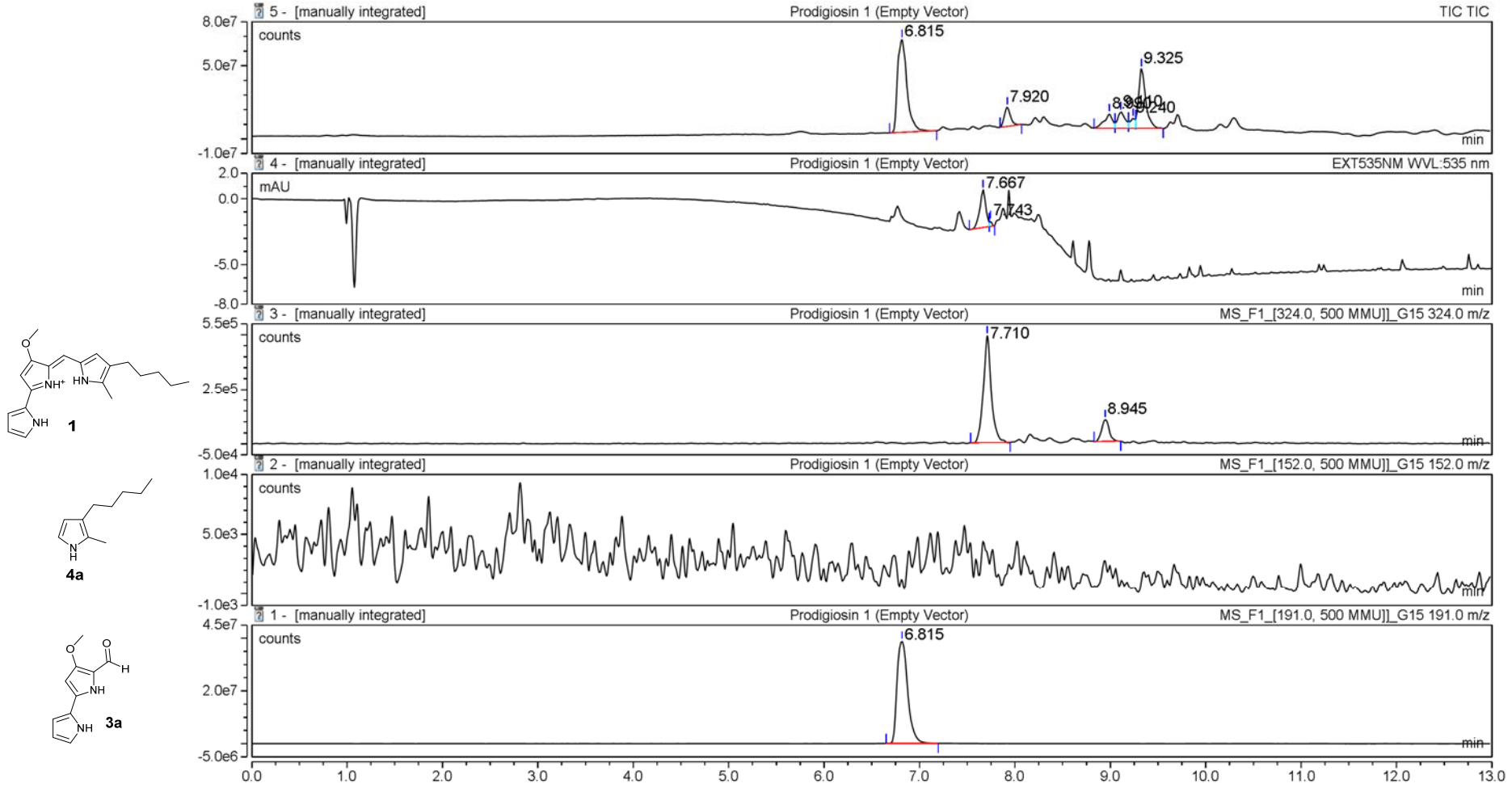


Figure S23: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **1** (m/z 324.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **1** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

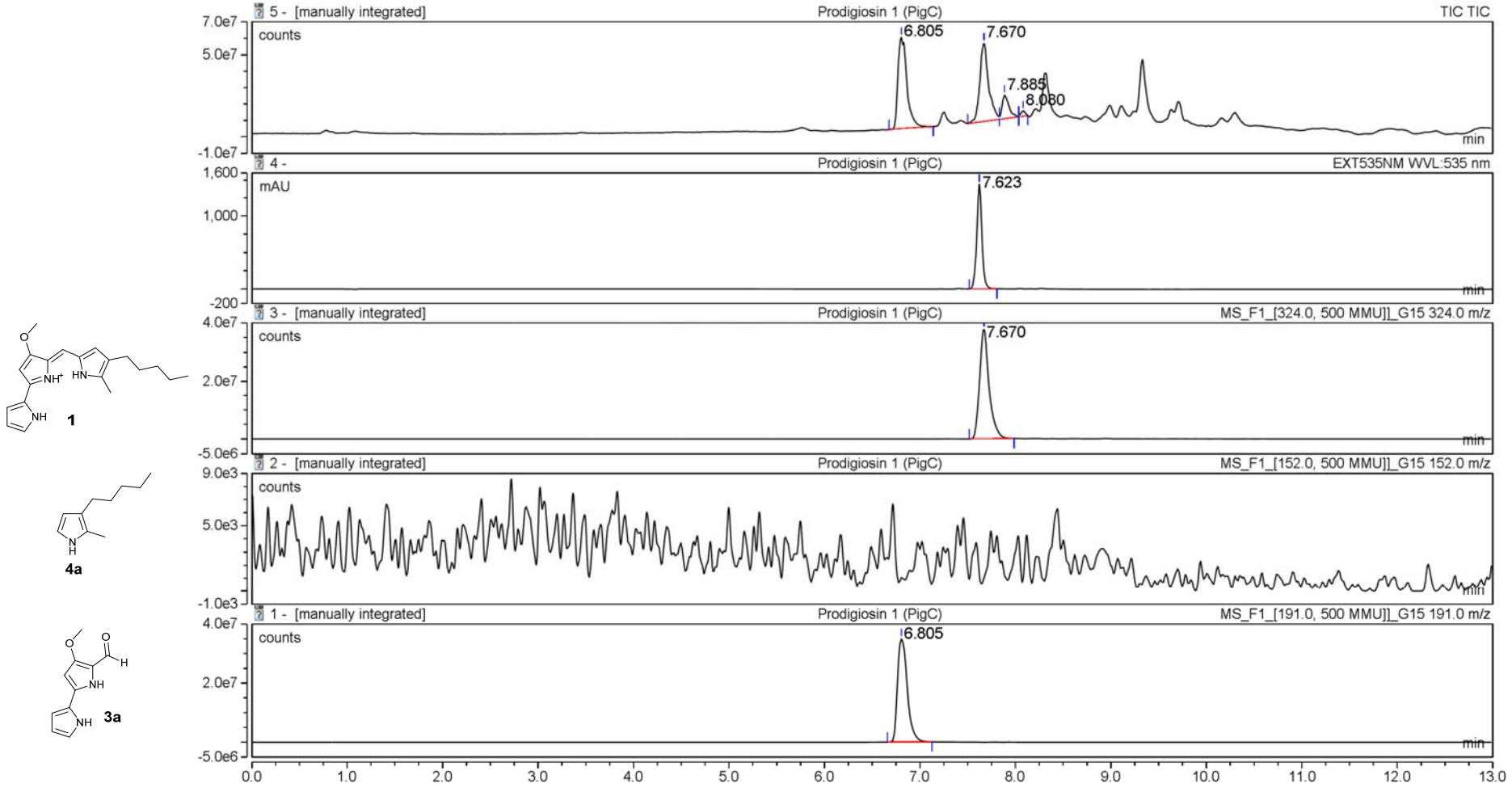


Figure S24: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodigiosin **1** (m/z 324.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodigiosin **1** t_R 7.62 min.

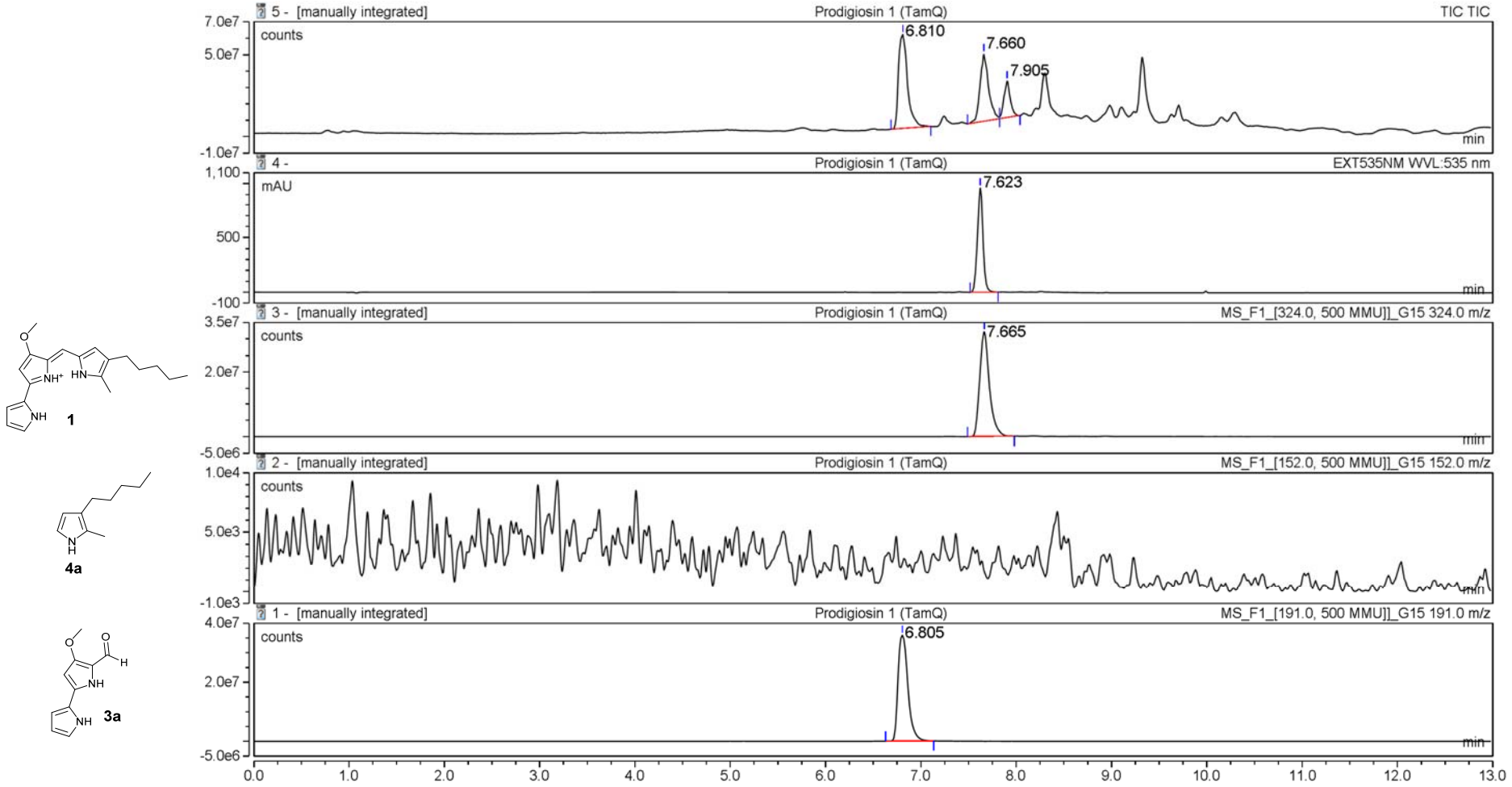


Figure S25: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodigiosin **1** (m/z 324.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodigiosin **1** t_R 7.62 min. Retention time of prodigiosin **1** t_R 7.62 min.

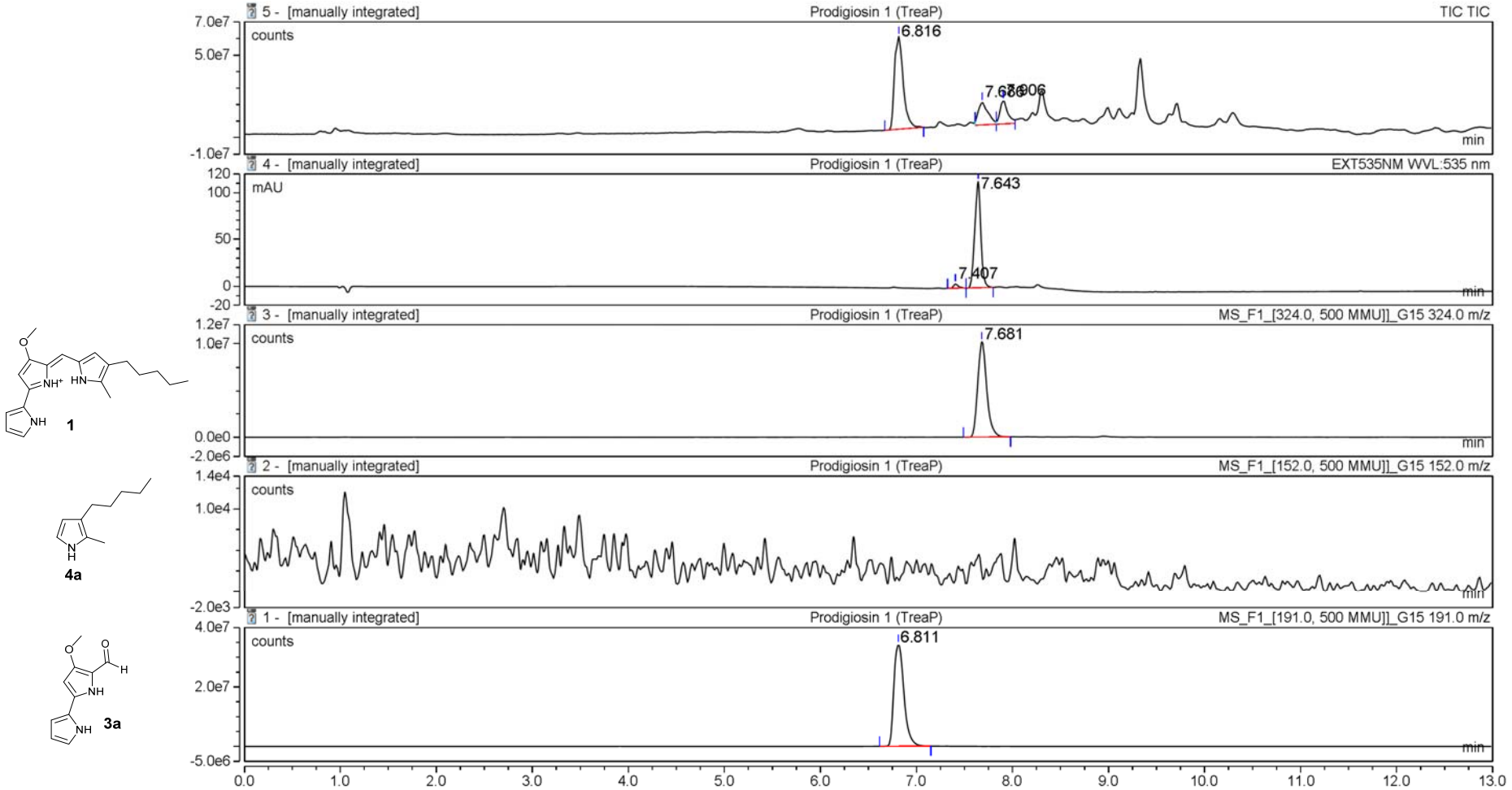


Figure S26: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodigiosin **1** (m/z 324.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodigiosin **1** t_R 7.62 min. Retention time of prodigiosin **1** t_R 7.64 min.

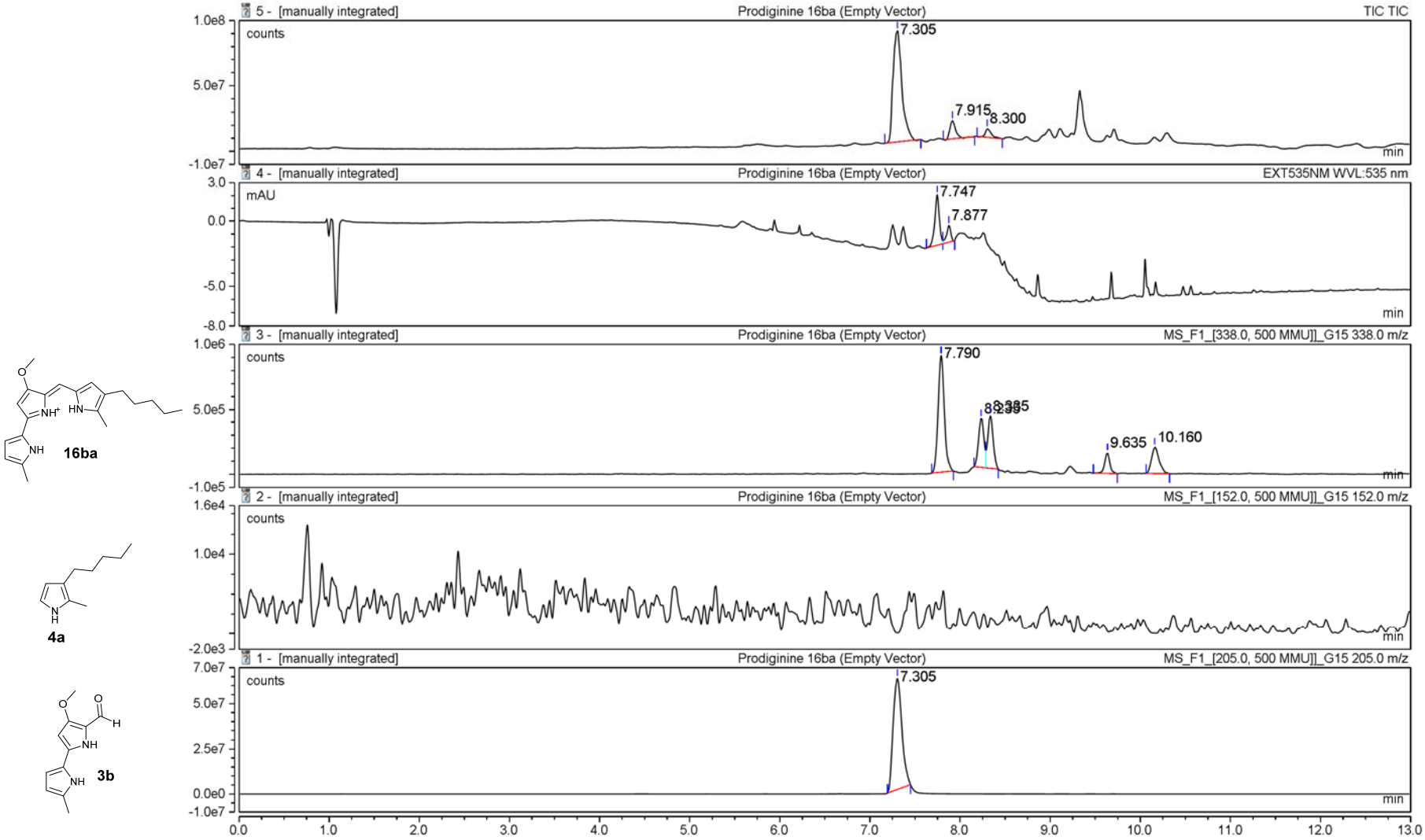


Figure S27: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ba** (m/z 338.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **1** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

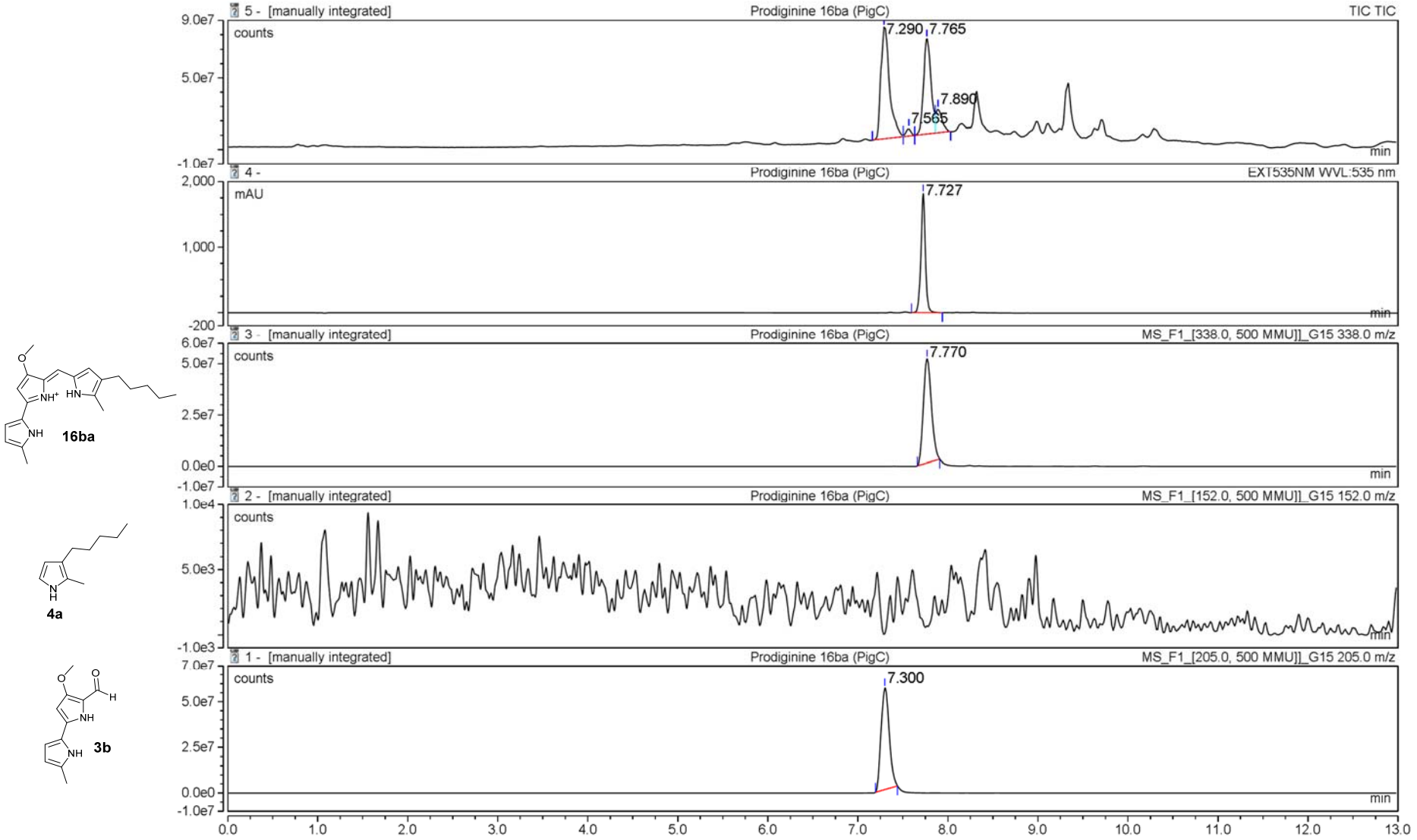


Figure S28: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ba** (m/z 338.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ba** t_R 7.73 min.

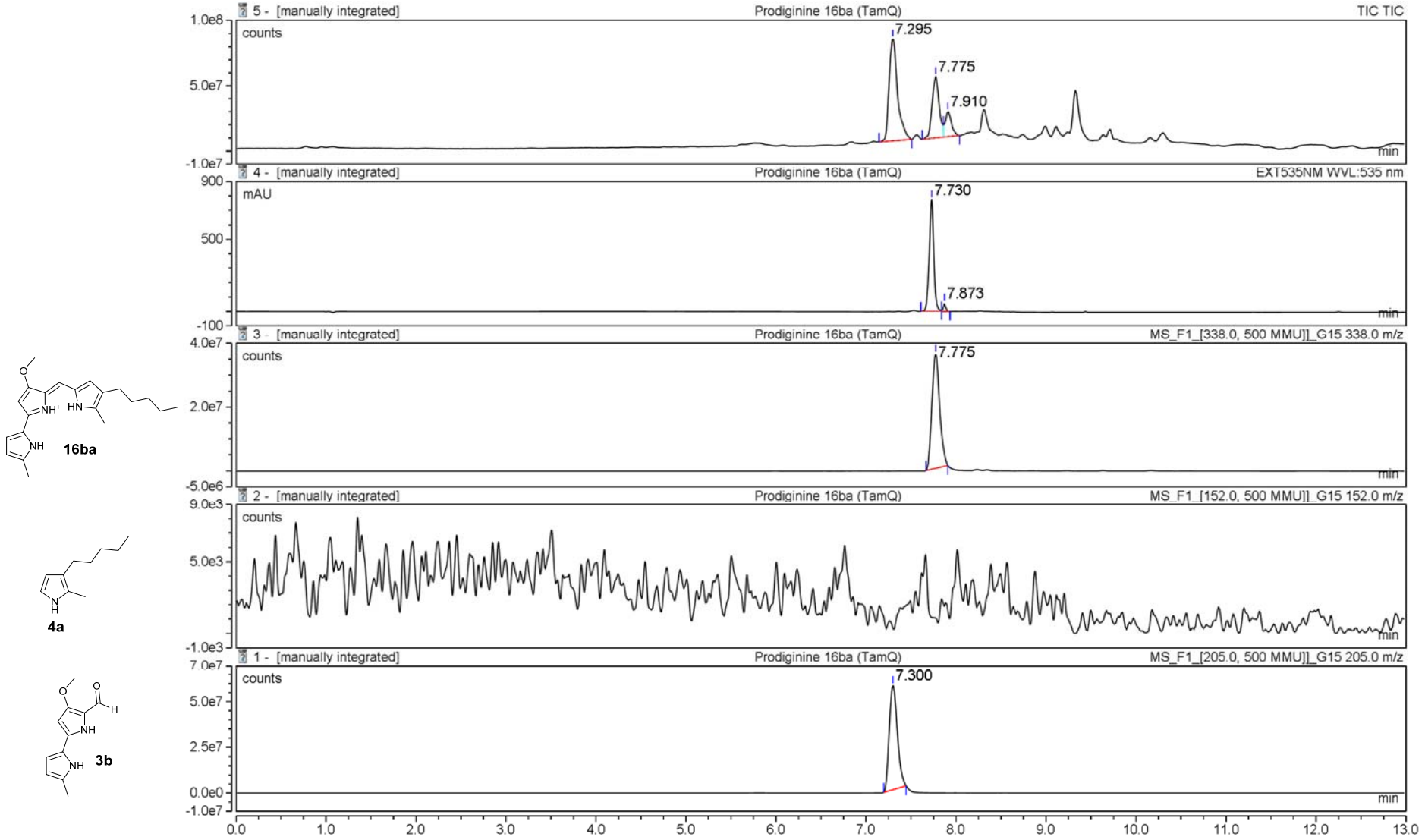


Figure S29: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ba** (m/z 338.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ba** t_R 7.73 min.

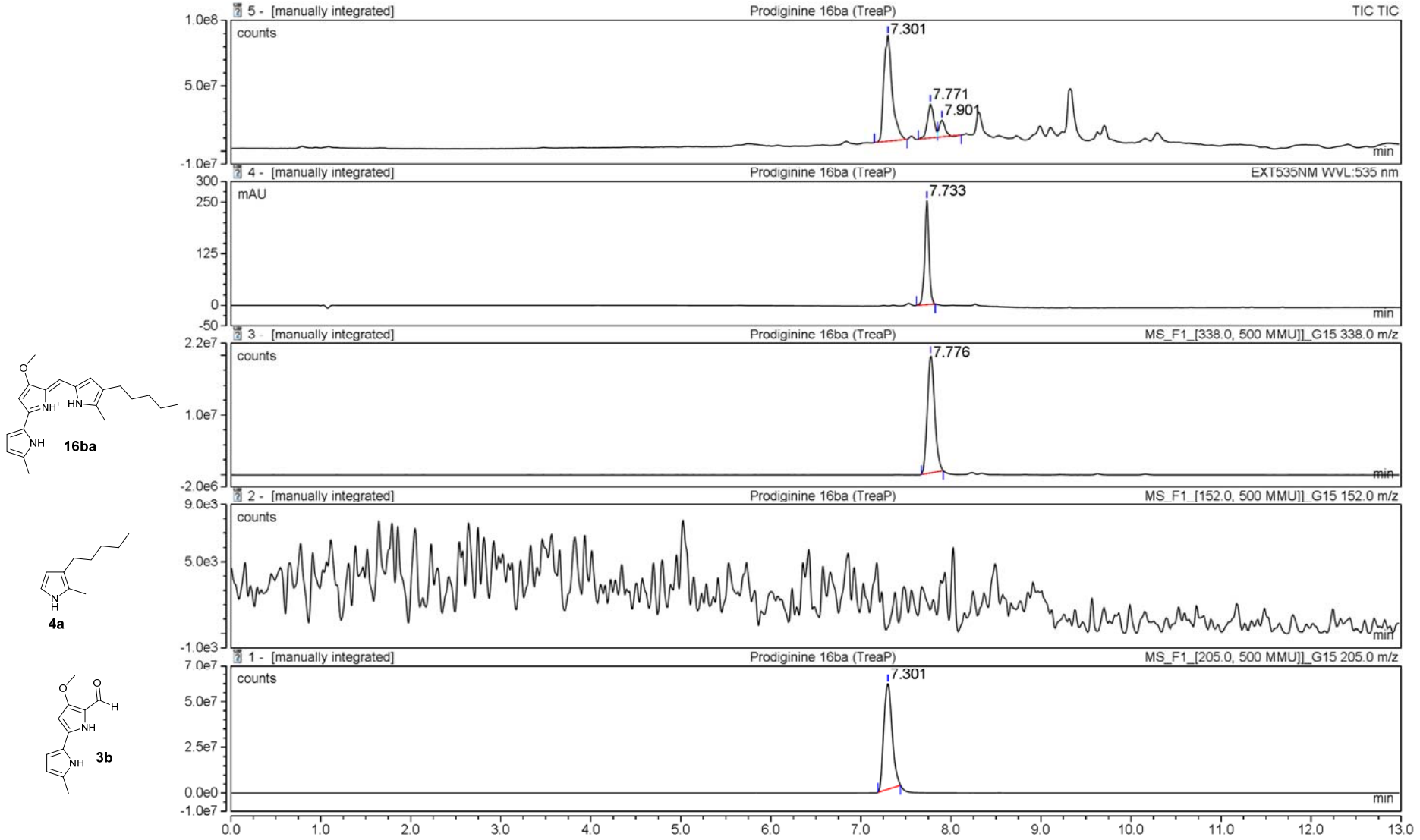


Figure S30: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ba** (m/z 338.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ba** t_R 7.73 min.

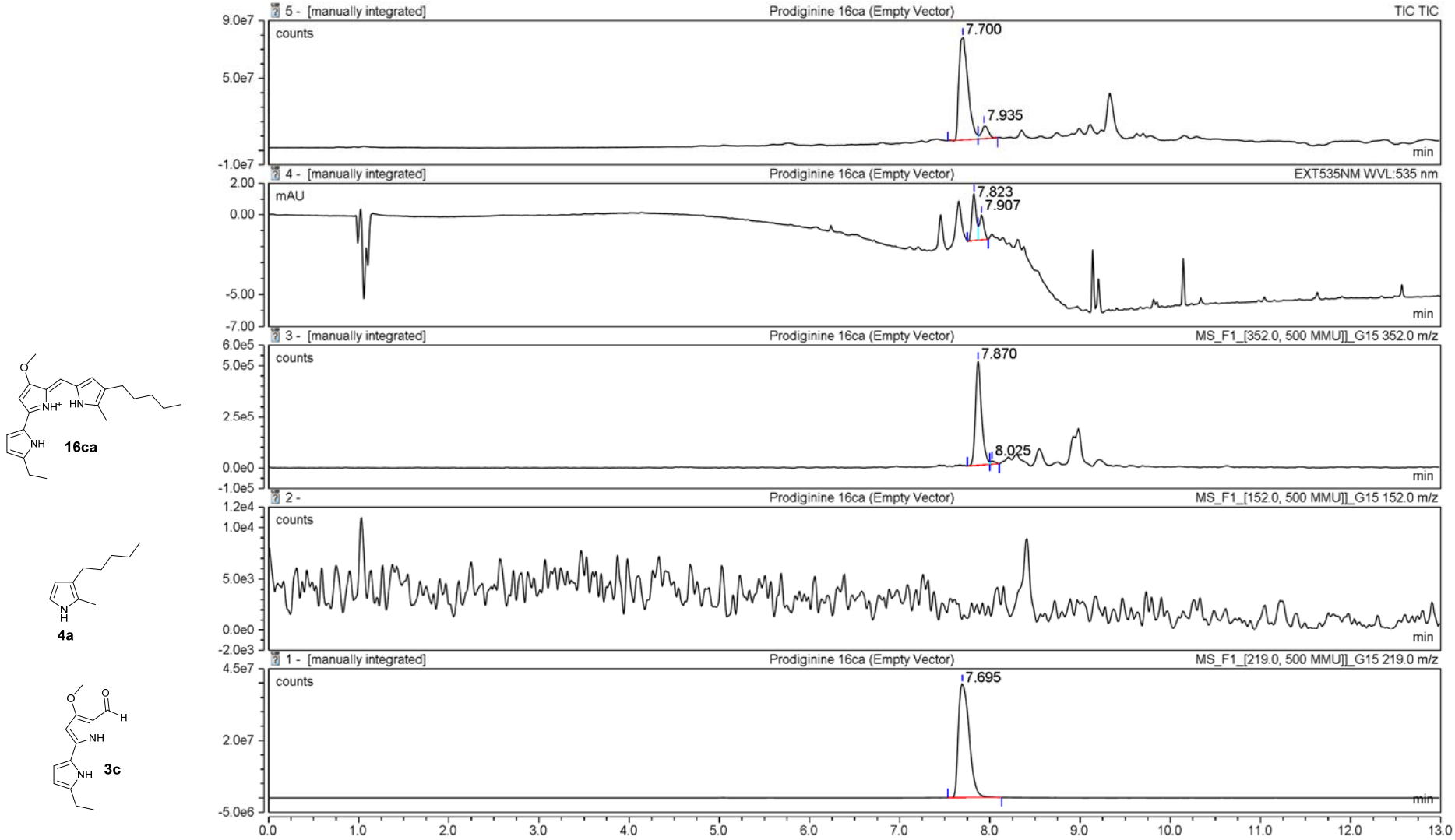


Figure S31: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ca** (m/z 352.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ca** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

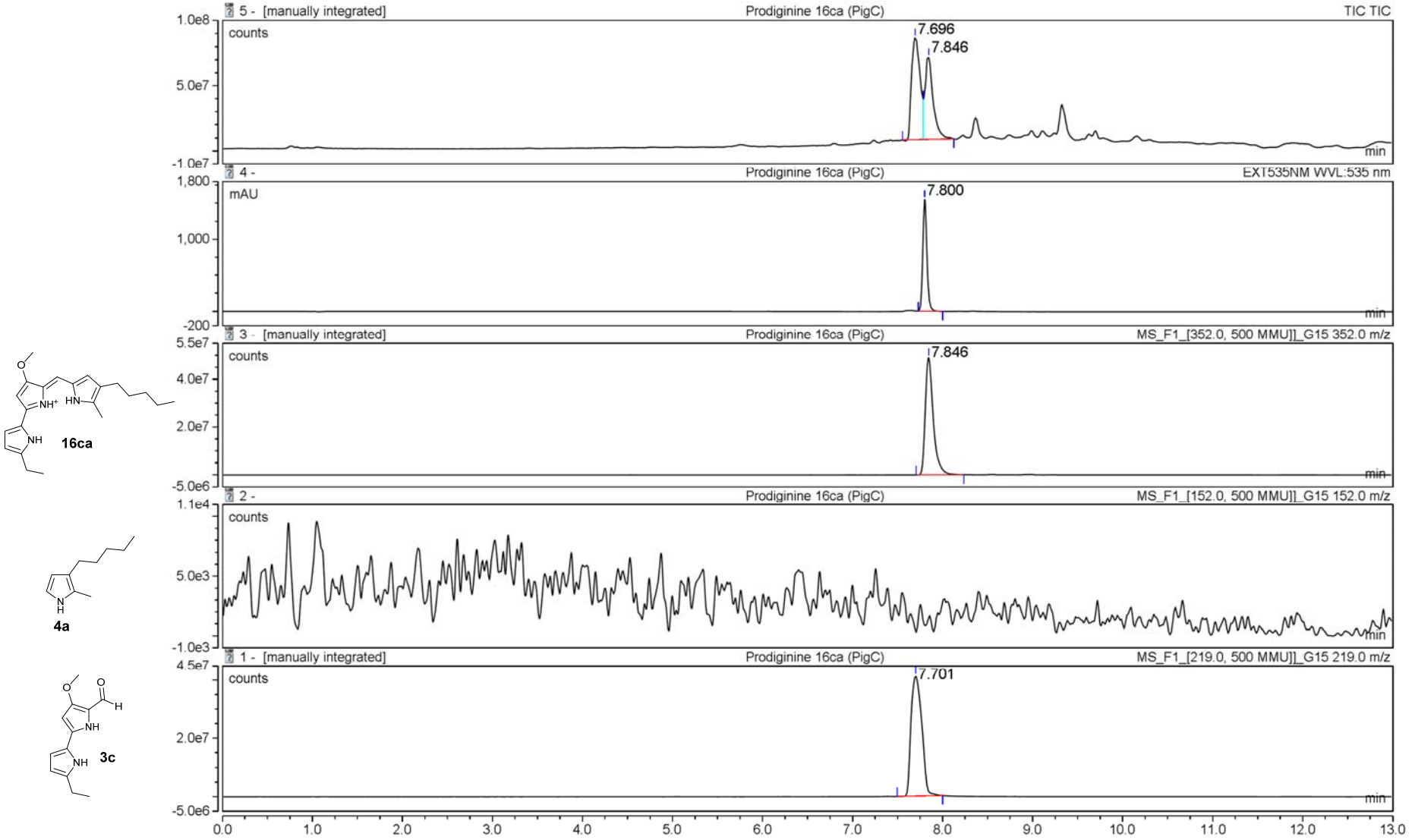


Figure S32: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ca** (m/z 352.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ca** t_R 7.80 min.

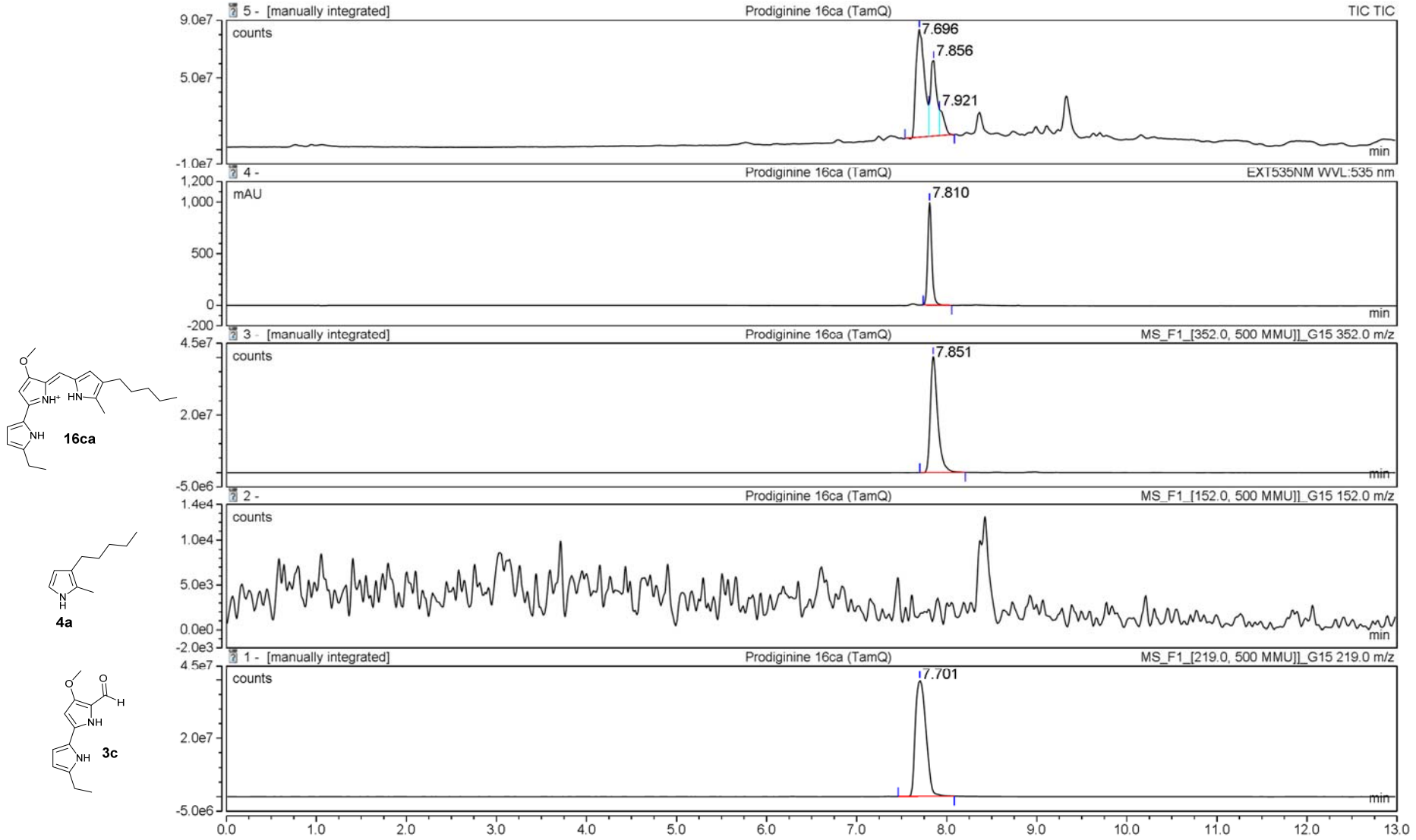


Figure S33: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ca** (m/z 352.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ca** t_R 7.81 min.

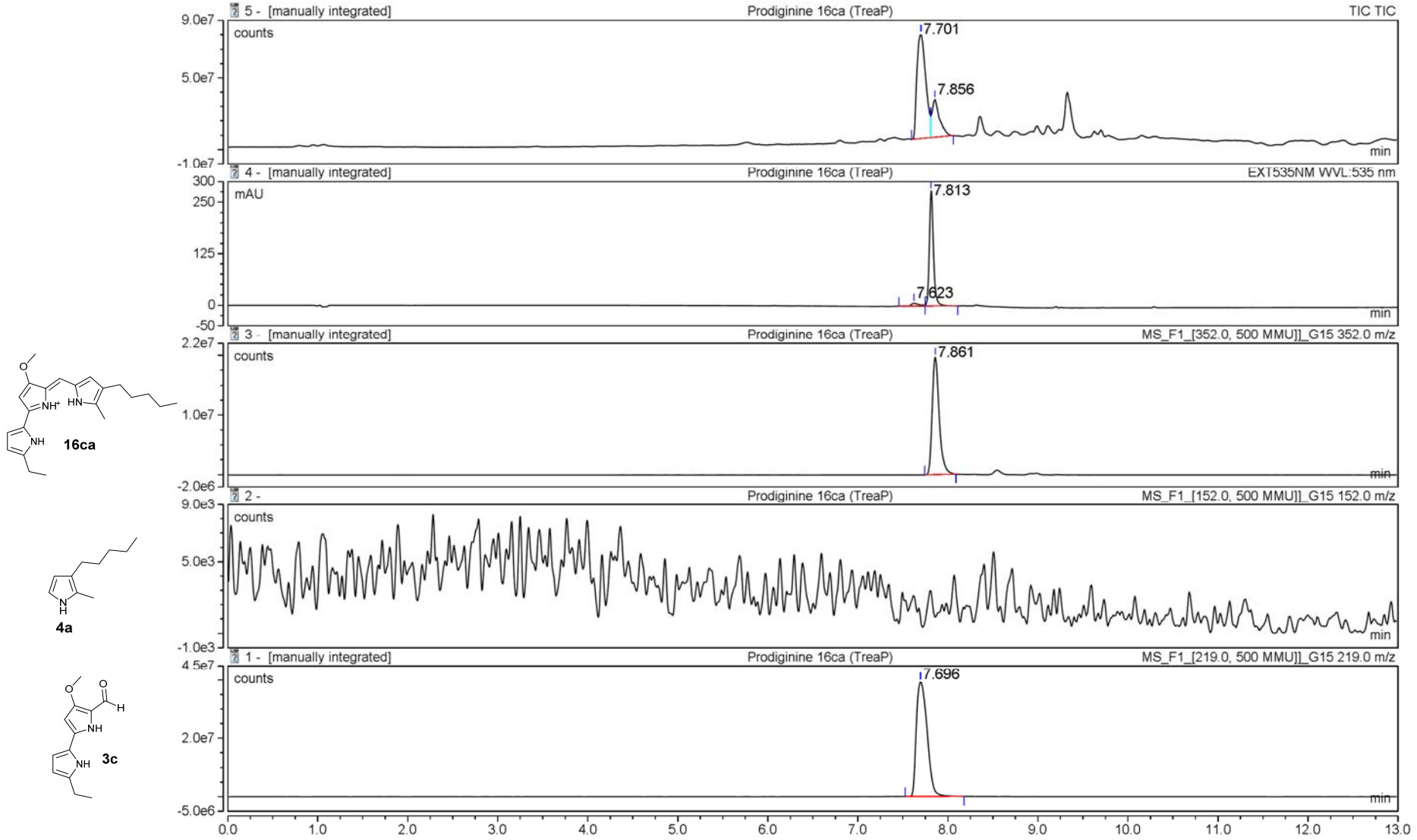


Figure S34: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ca** (m/z 352.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ca** t_R 7.81 min.

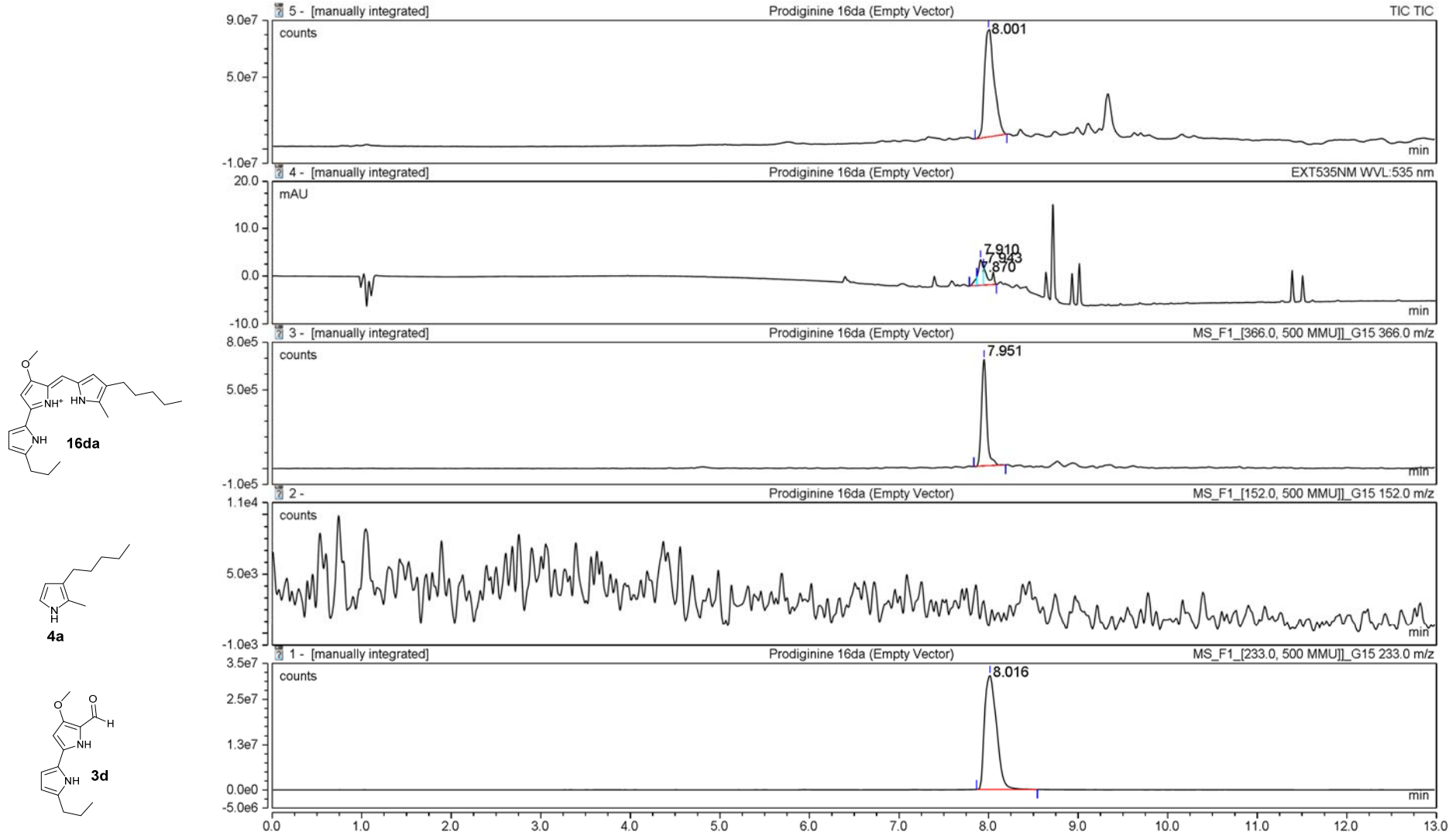


Figure S35: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16da** (m/z 366.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16da** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

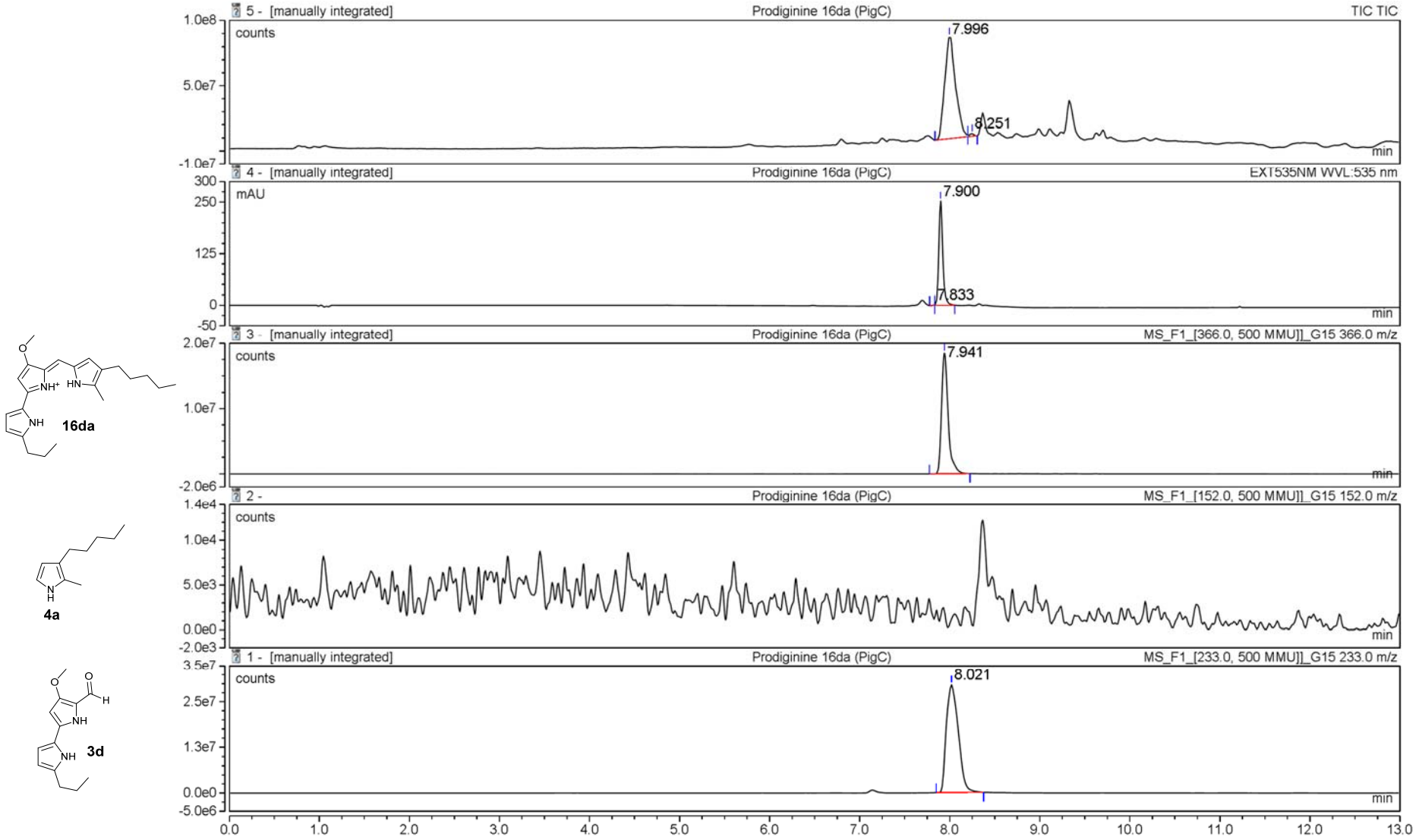


Figure S36: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16da** (m/z 366.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16da** t_R 7.90 min.

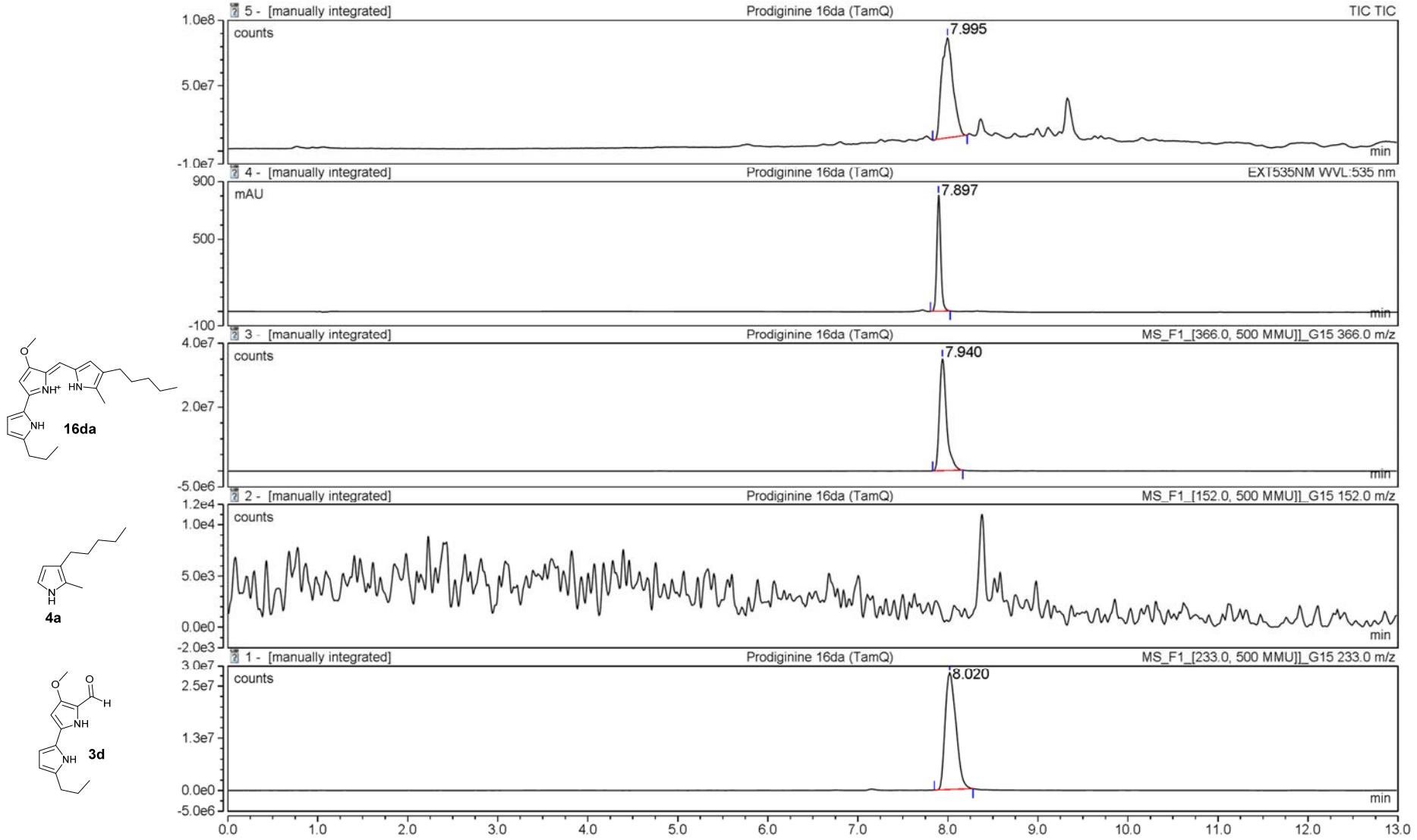


Figure S37: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16da** (m/z 366.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16da** t_R 7.90 min.

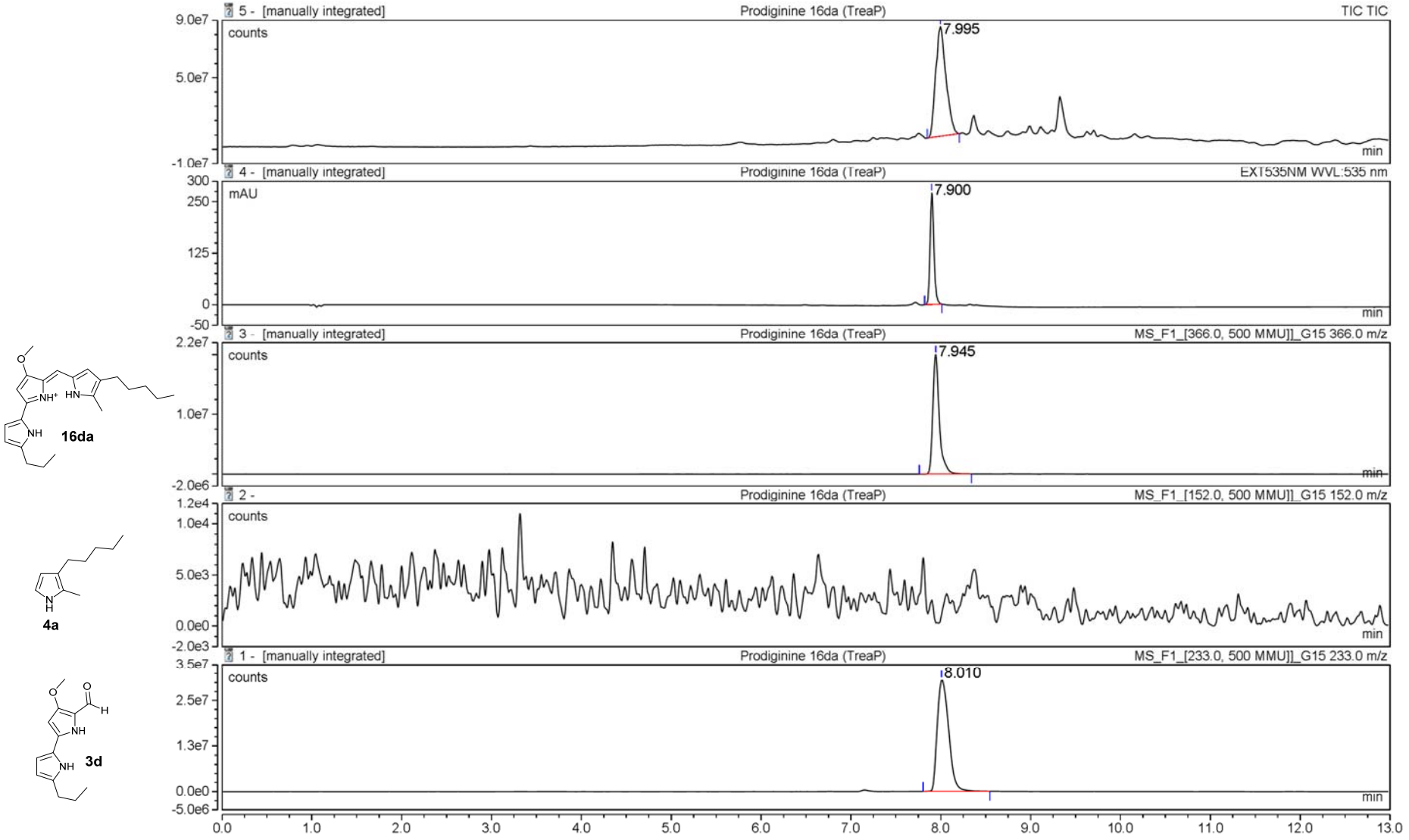


Figure S38: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16da** (m/z 366.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16da** t_R 7.90 min.

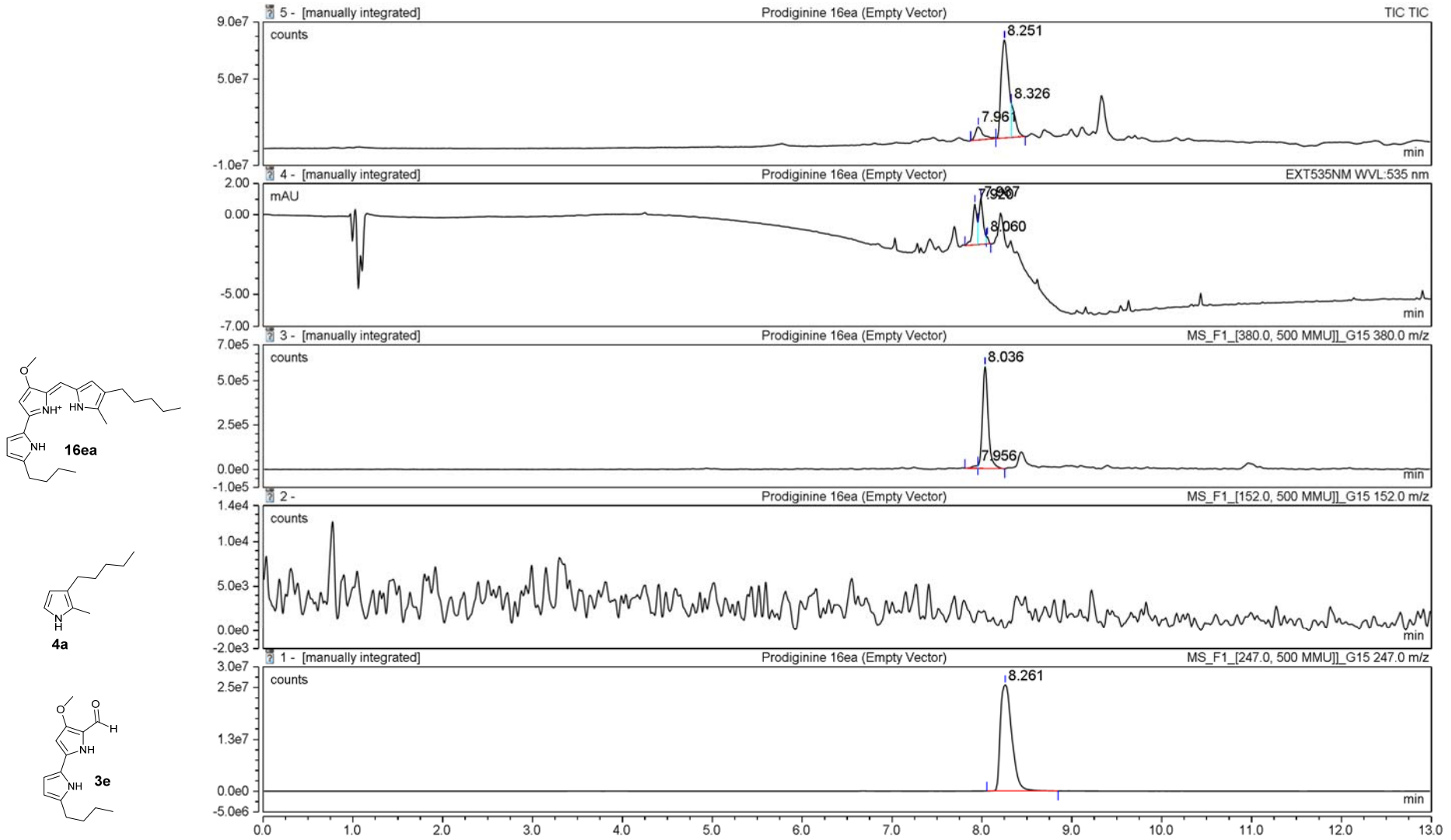


Figure S39: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ea** (m/z 380.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ea** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

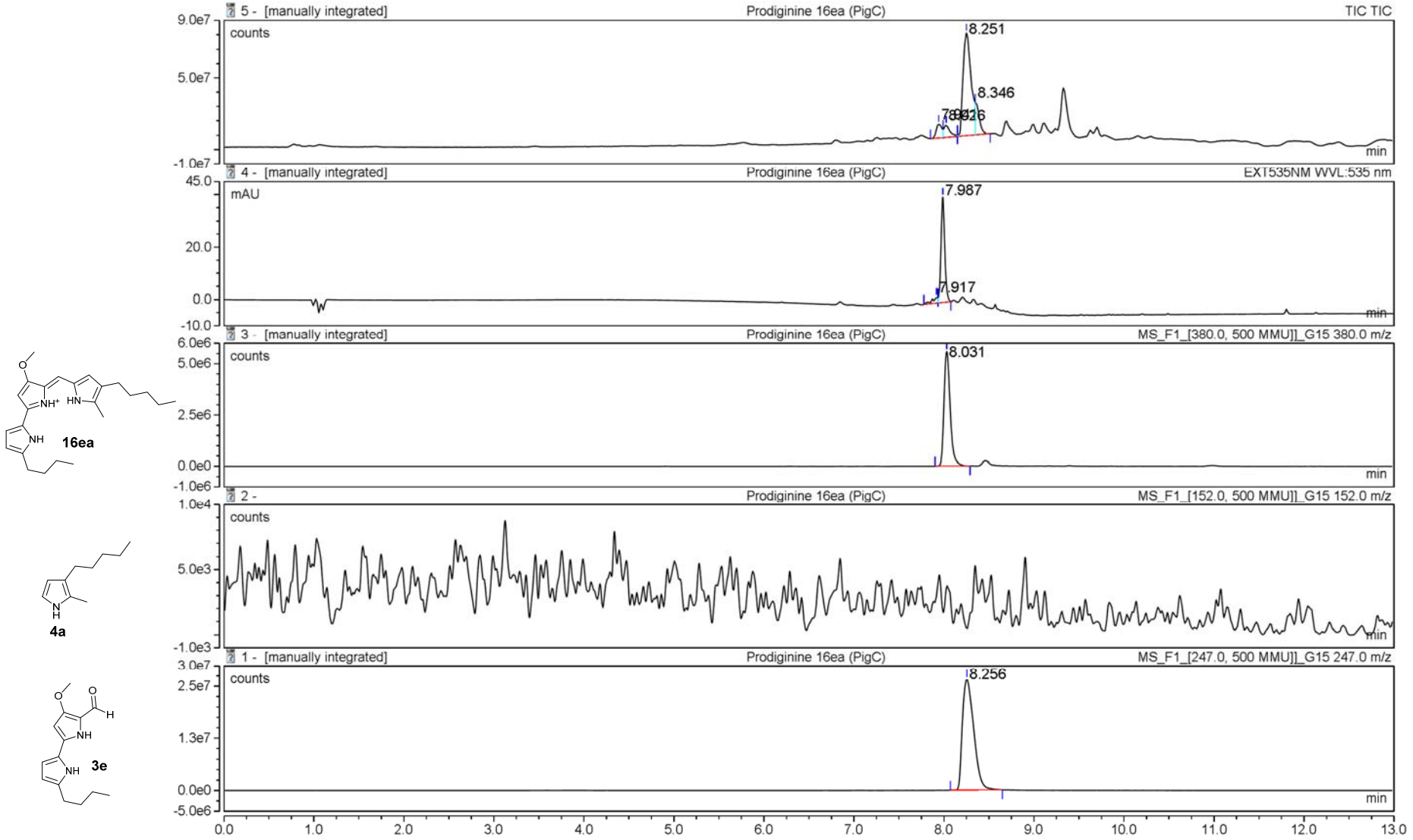


Figure S40: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ea** (m/z 380.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ea** t_R 7.99 min.

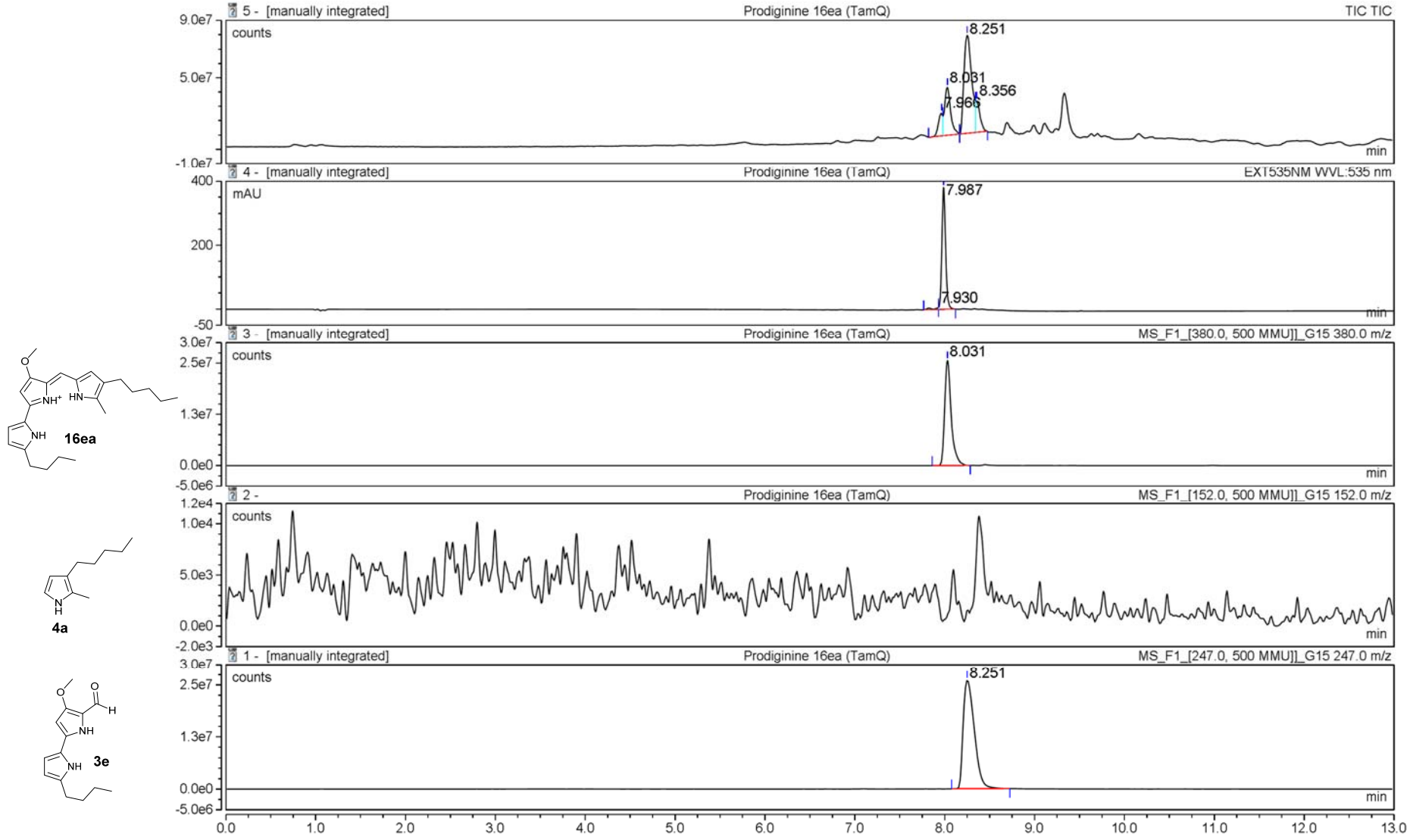


Figure S41: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ea** (m/z 380.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ea** t_R 7.99 min.

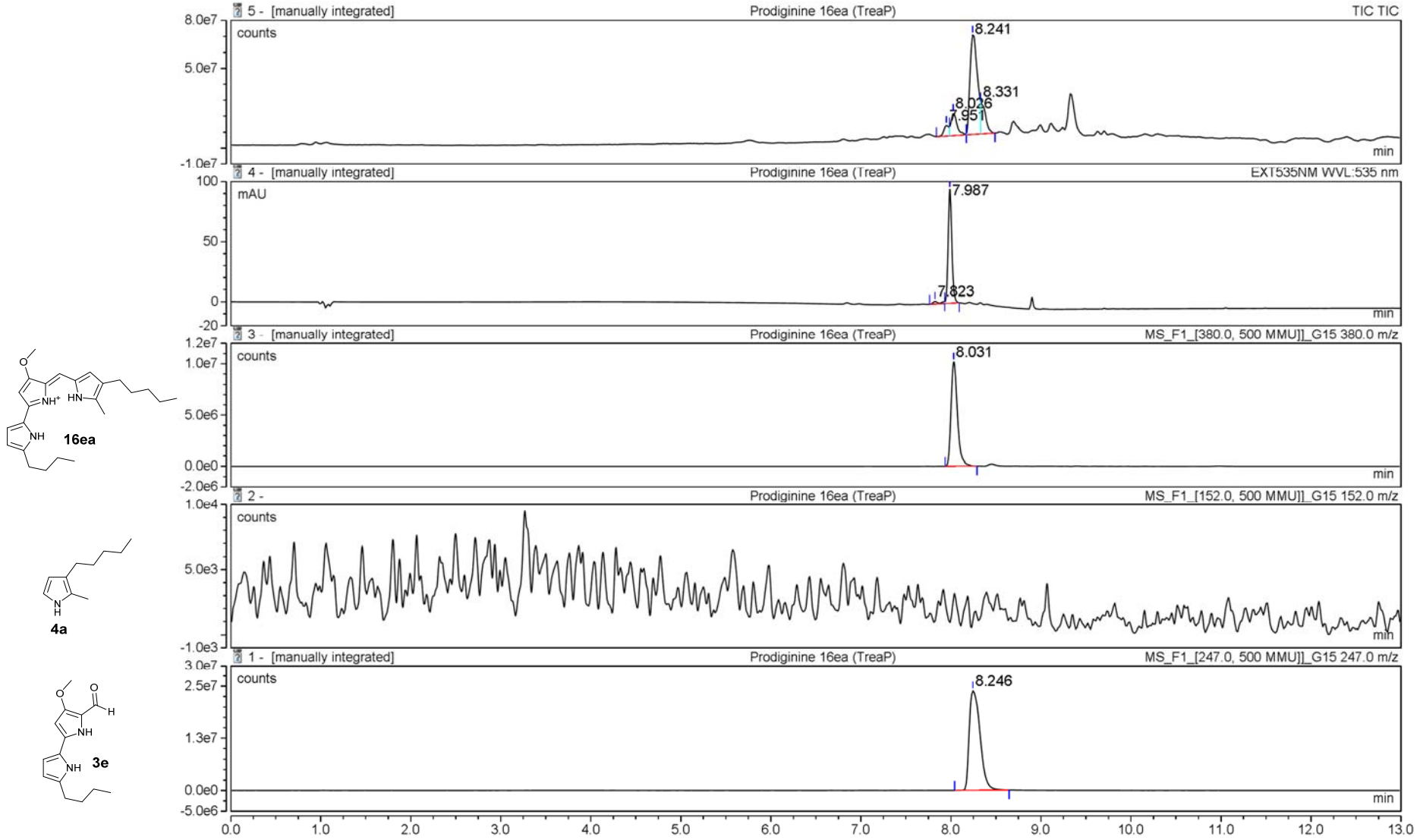


Figure S42: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ea** (m/z 380.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ea** t_R 7.99 min.

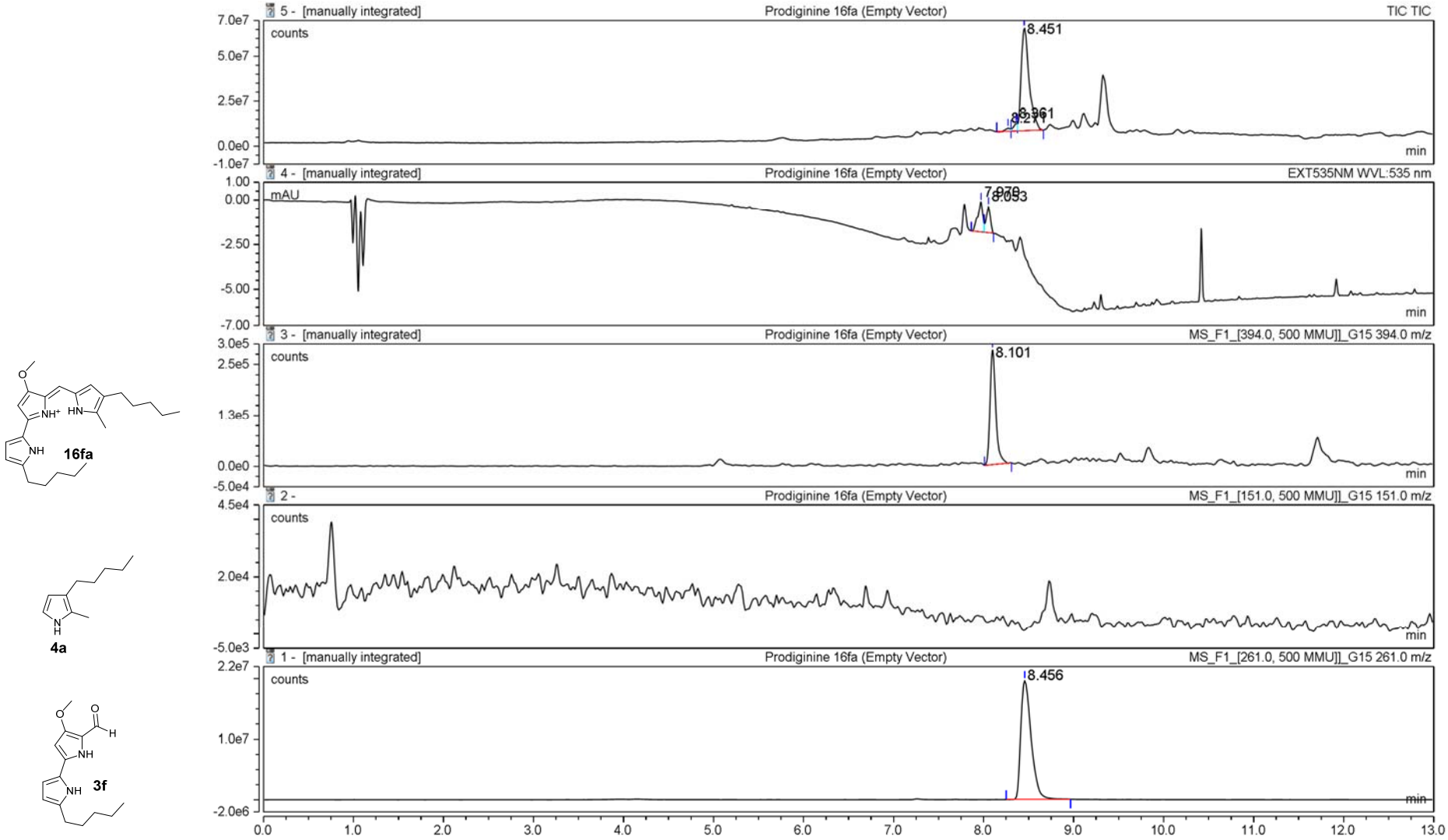


Figure S43: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16fa** (m/z 394.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16fa** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

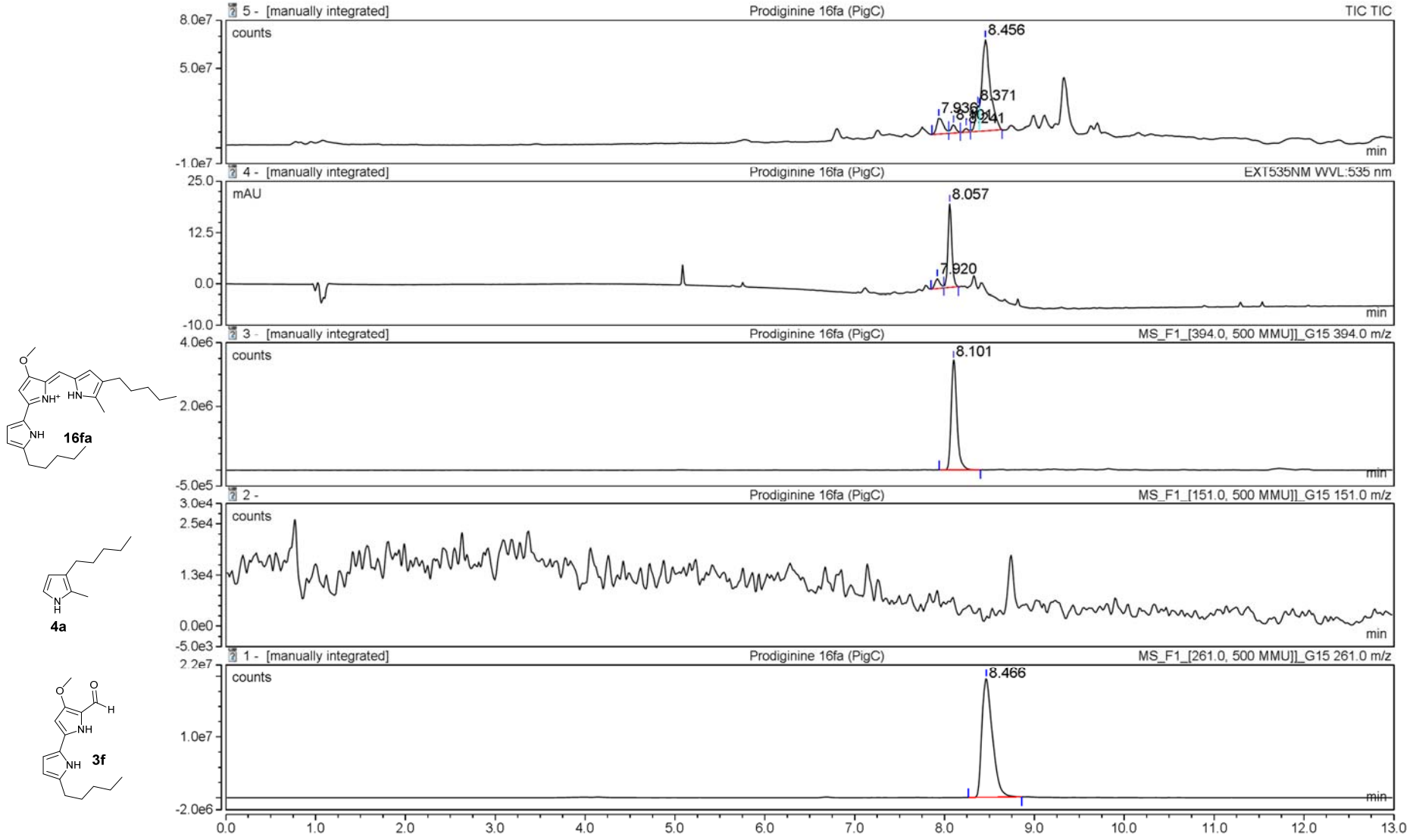


Figure S44: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ef** (m/z 394.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fa** t_R 8.06 min.

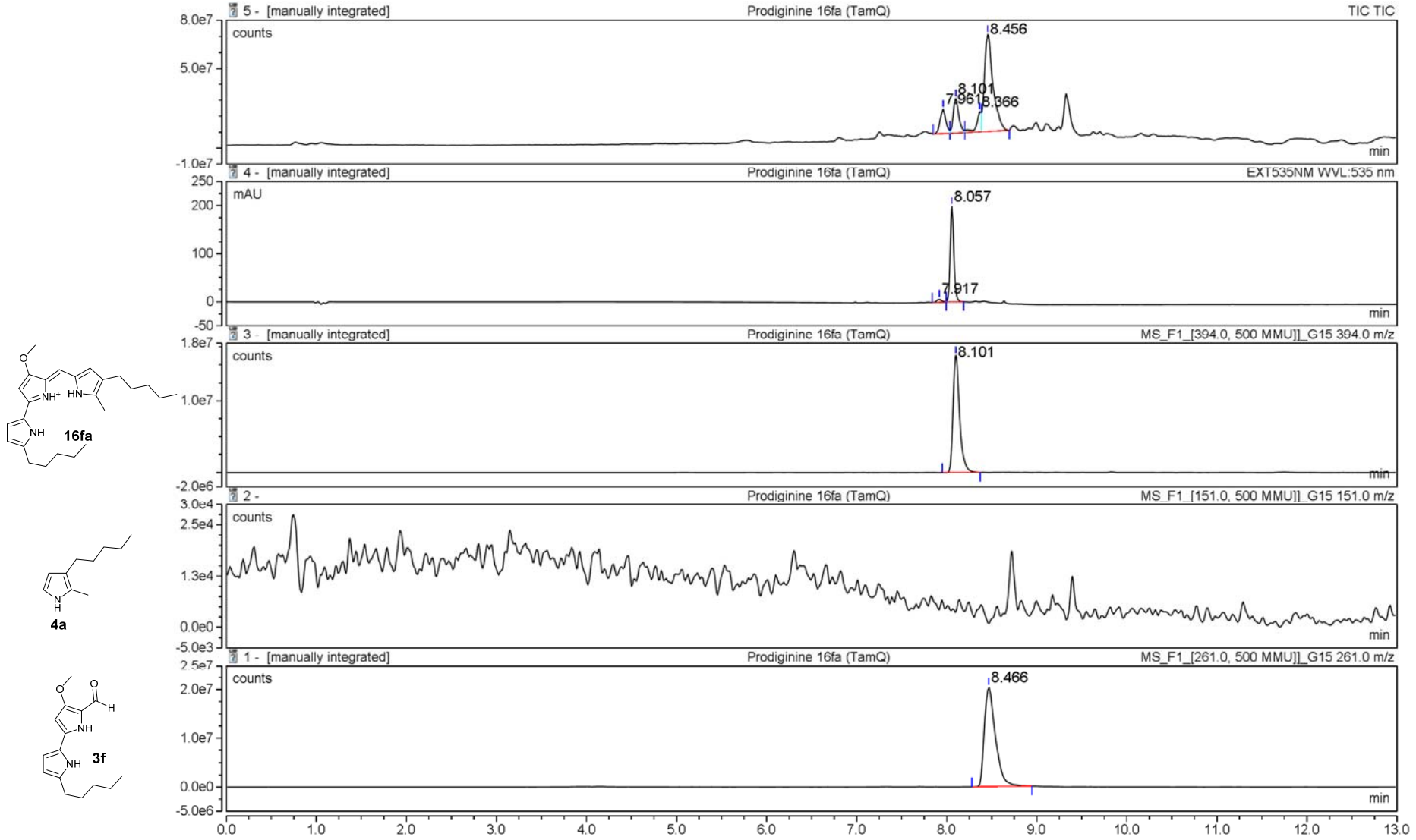


Figure S45: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ef** (m/z 394.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fa** t_R 8.06 min.

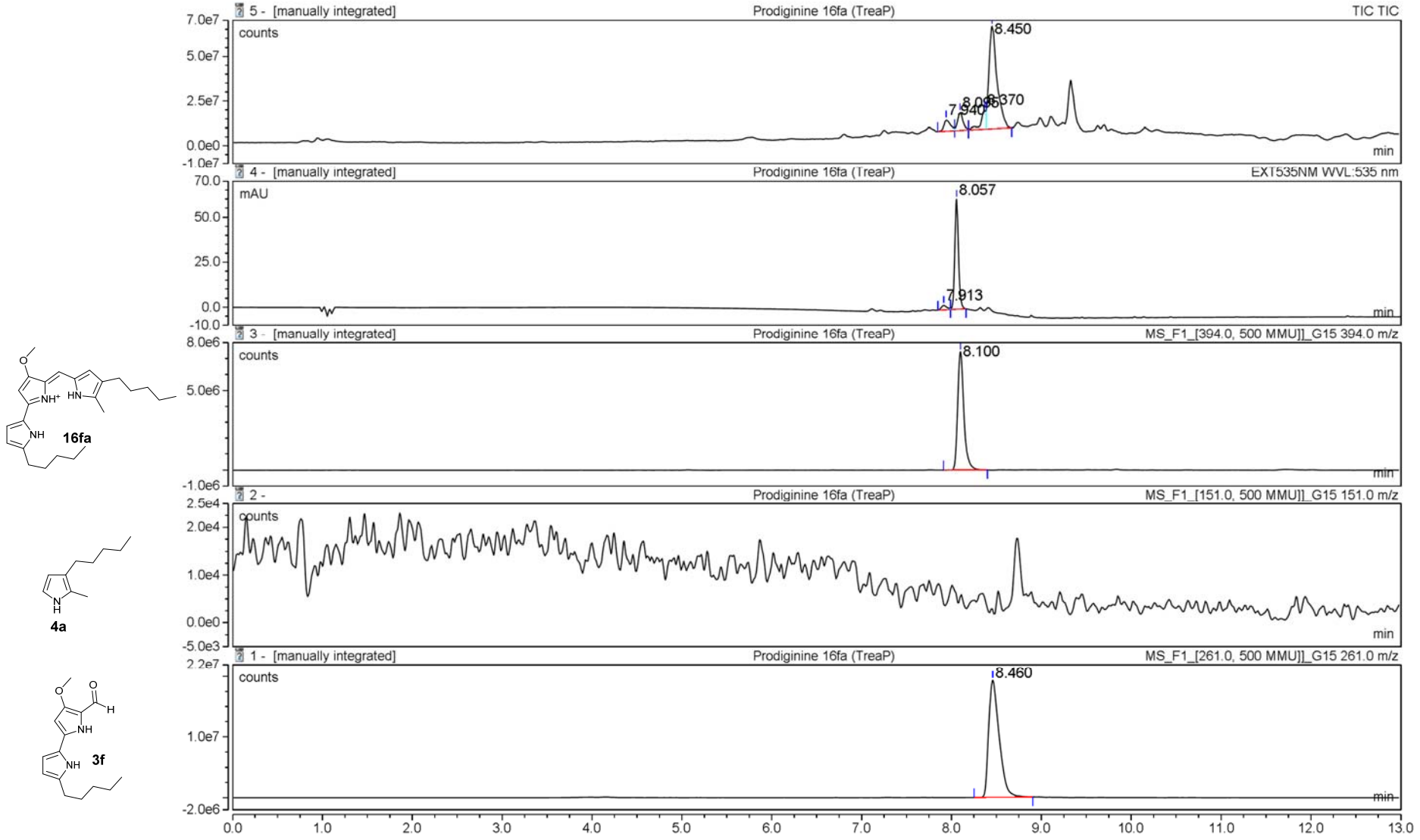


Figure S46: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ef** (m/z 394.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fa** t_R 8.05 min.

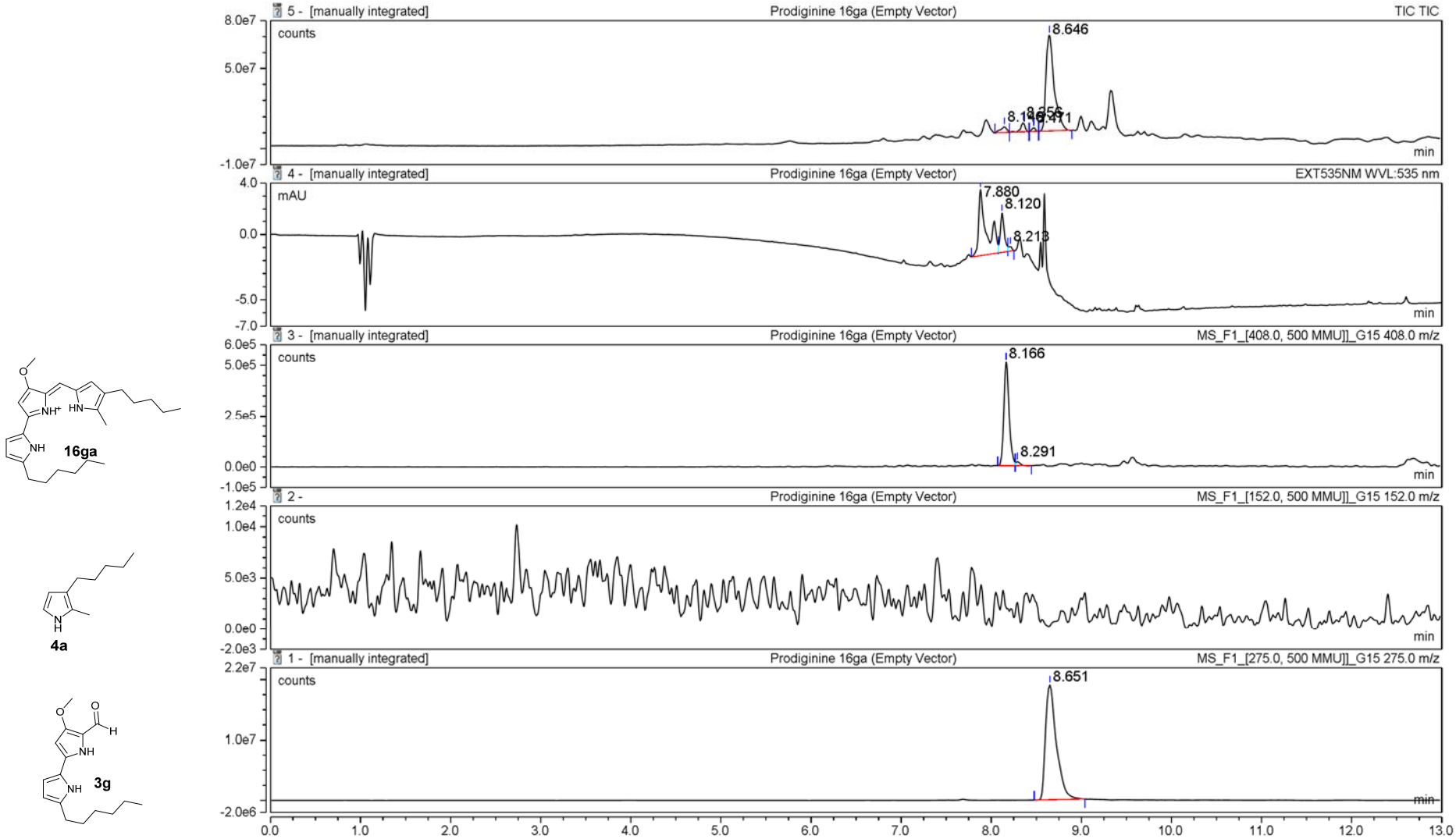


Figure S47: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ga** (m/z 408.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3a** (m/z 275.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4a** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ga** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

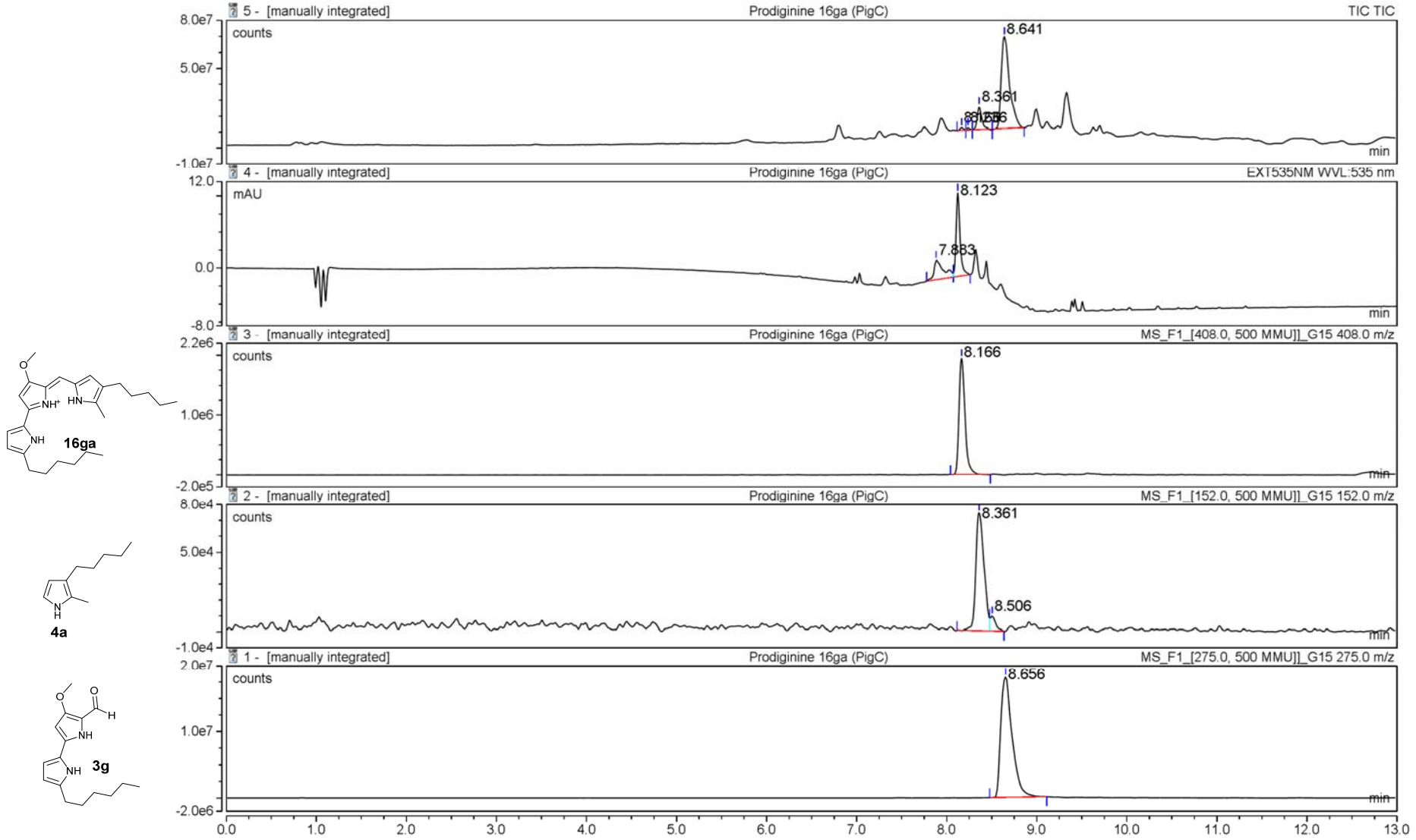


Figure S48: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eg** (m/z 408.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4a** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ga** t_R 8.12 min.

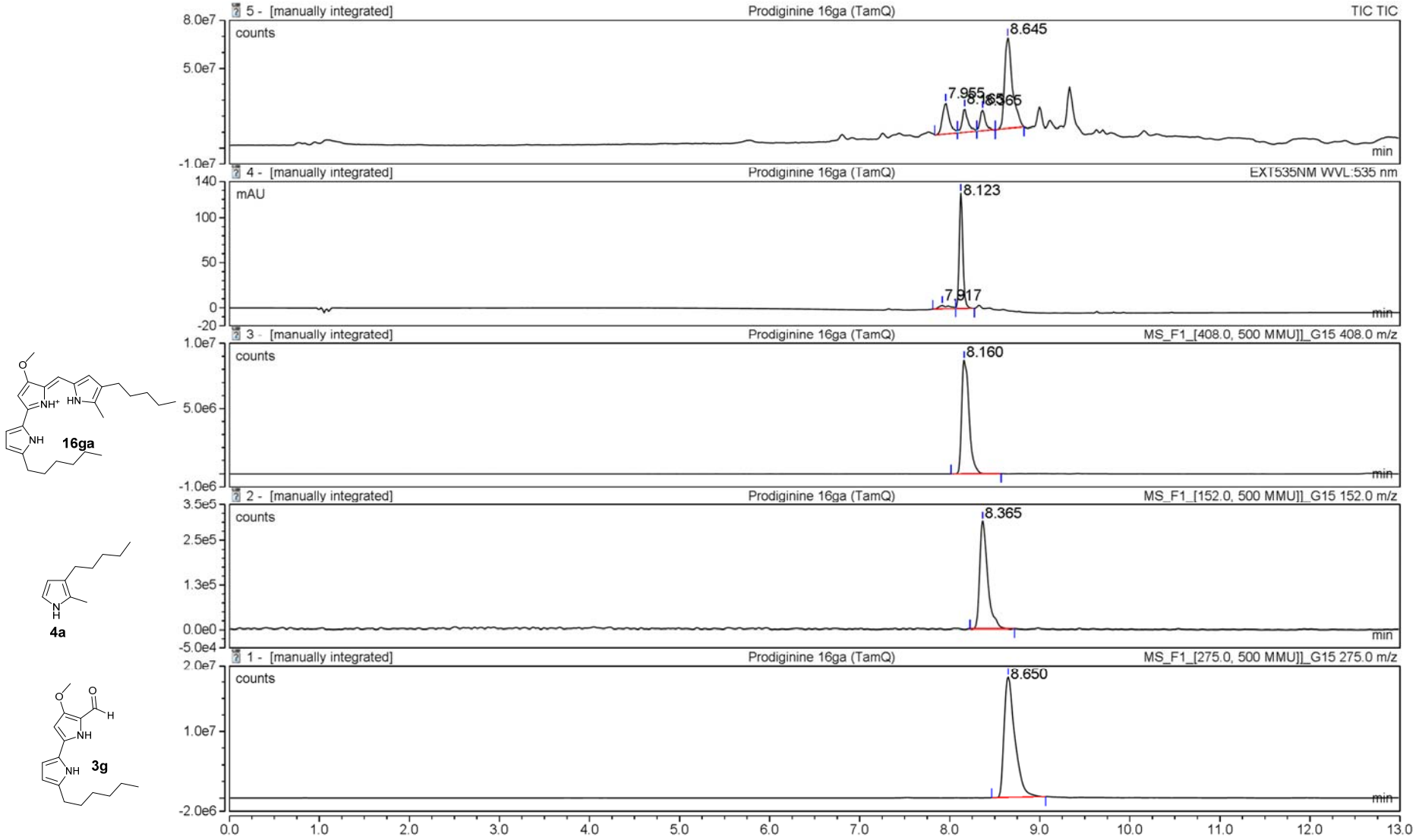


Figure S49: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eg** (m/z 408.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4a** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ga** t_R 8.12 min.

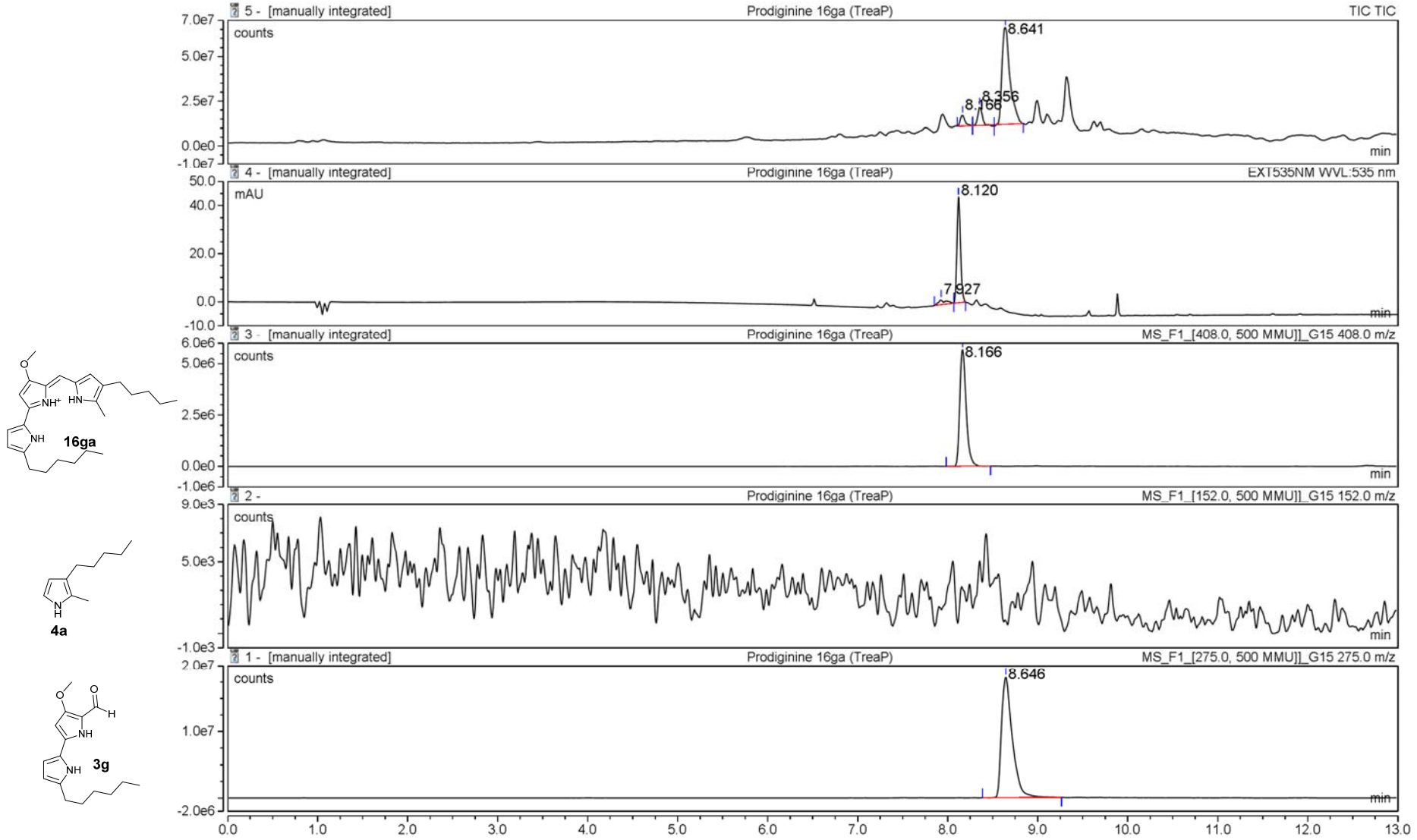


Figure S50: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eg** (m/z 408.0) and the educts pyrrole **4a** (m/z 152.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4a** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ga** t_R 8.12 min.

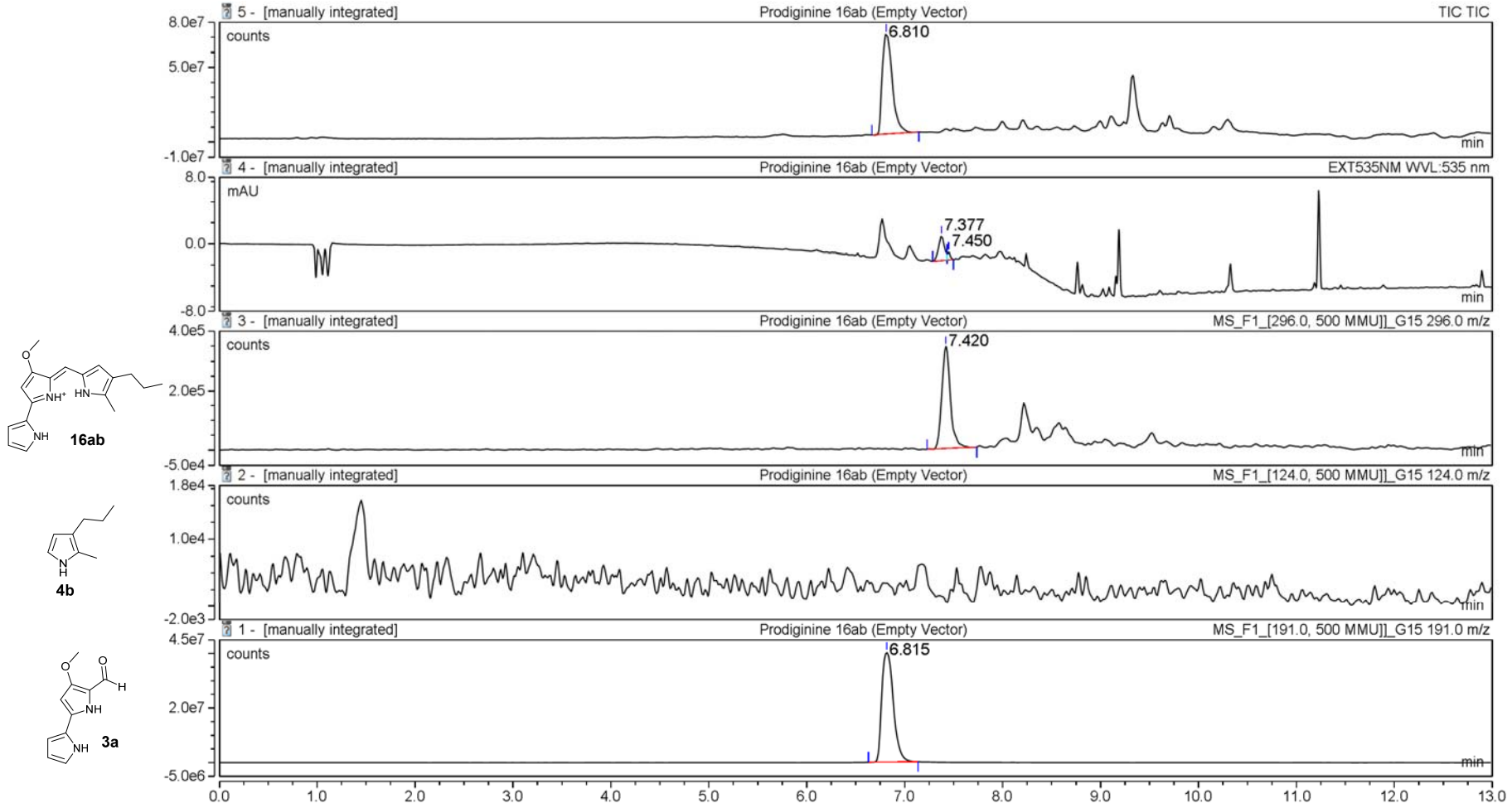


Figure S51: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ab** (m/z 296.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ab** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

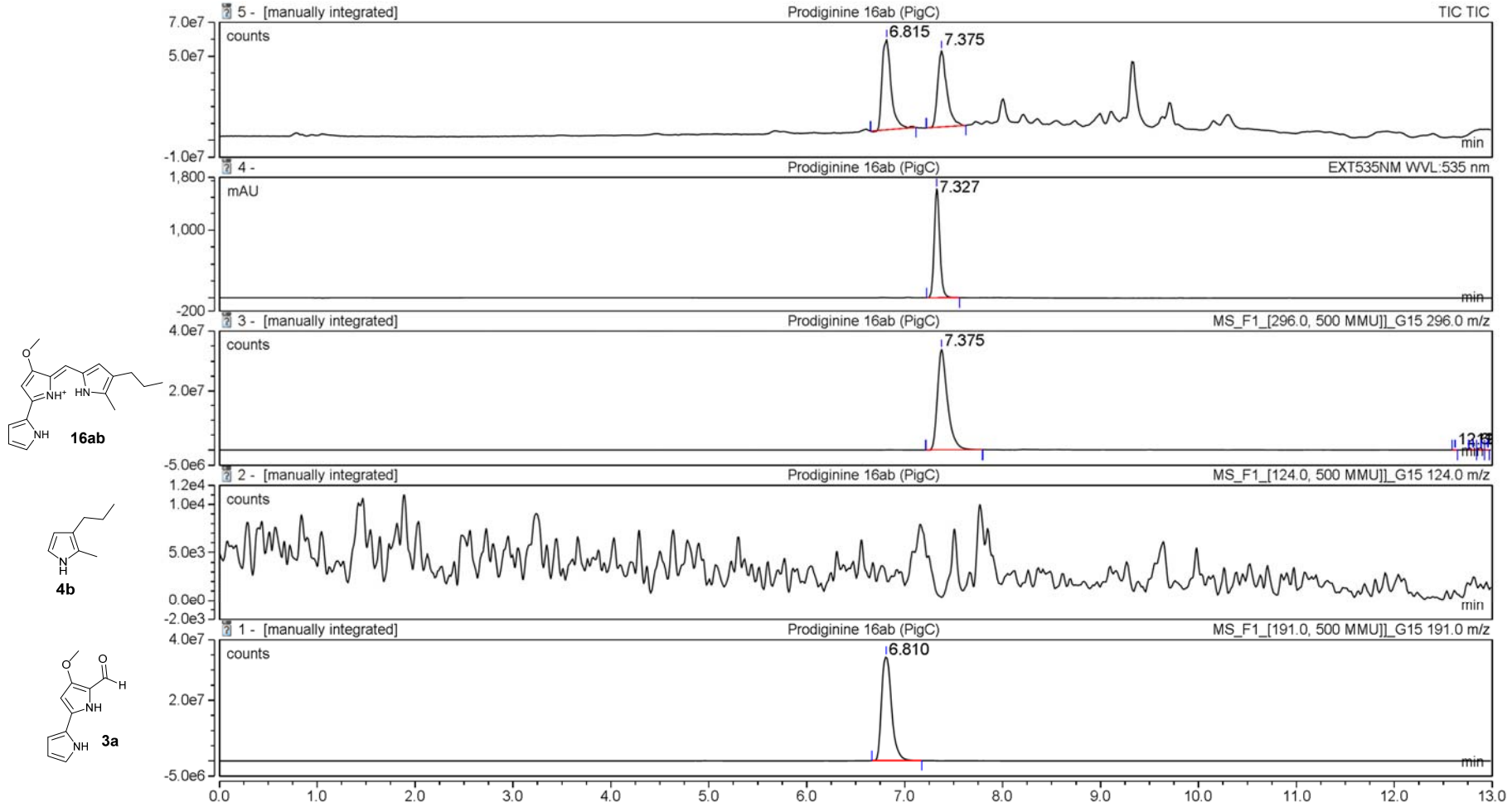


Figure S52: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ab** (m/z 296.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ab** t_R 7.33 min.

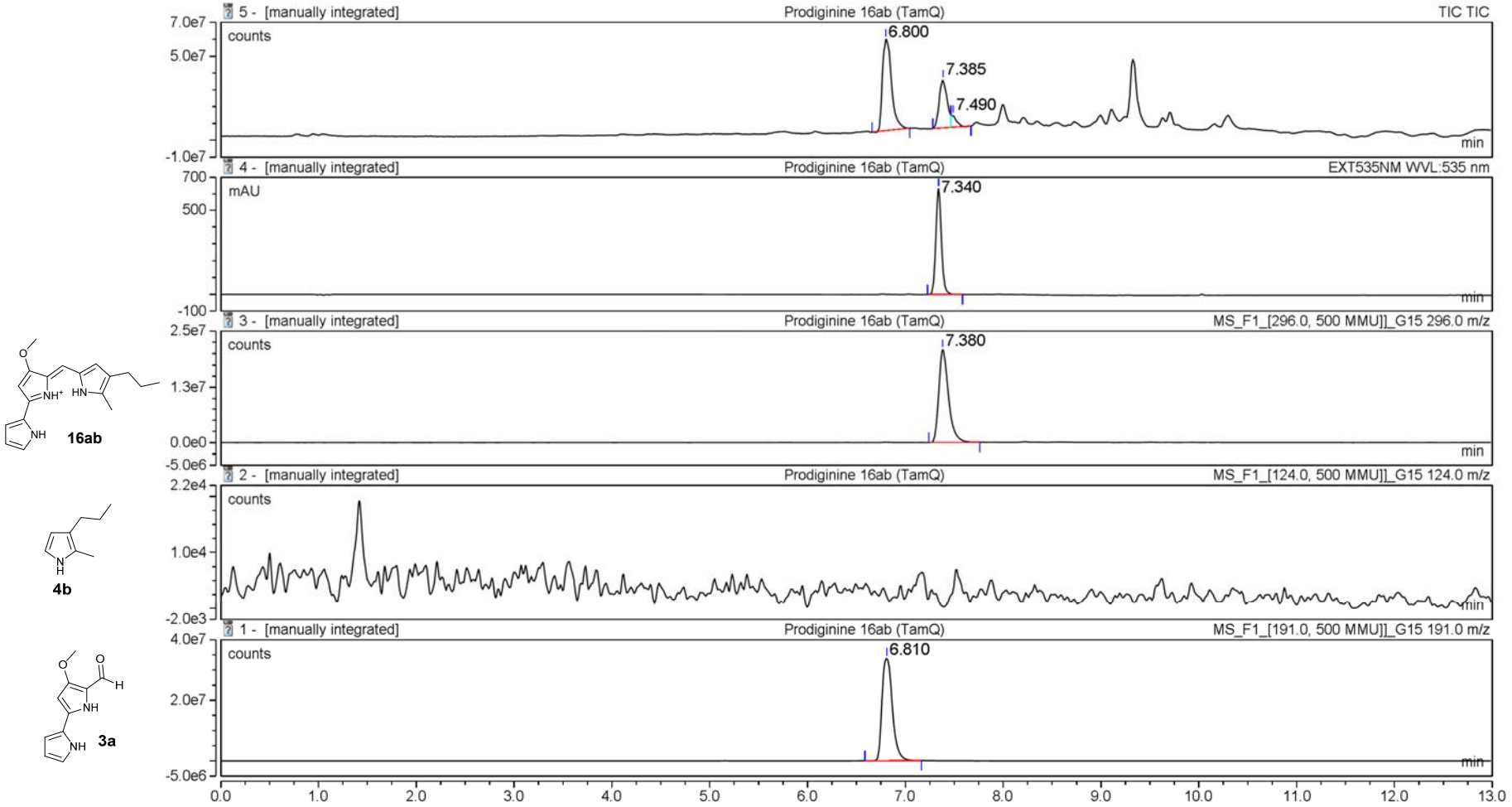


Figure S53: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ab** (m/z 296.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ab** t_R 7.34 min.

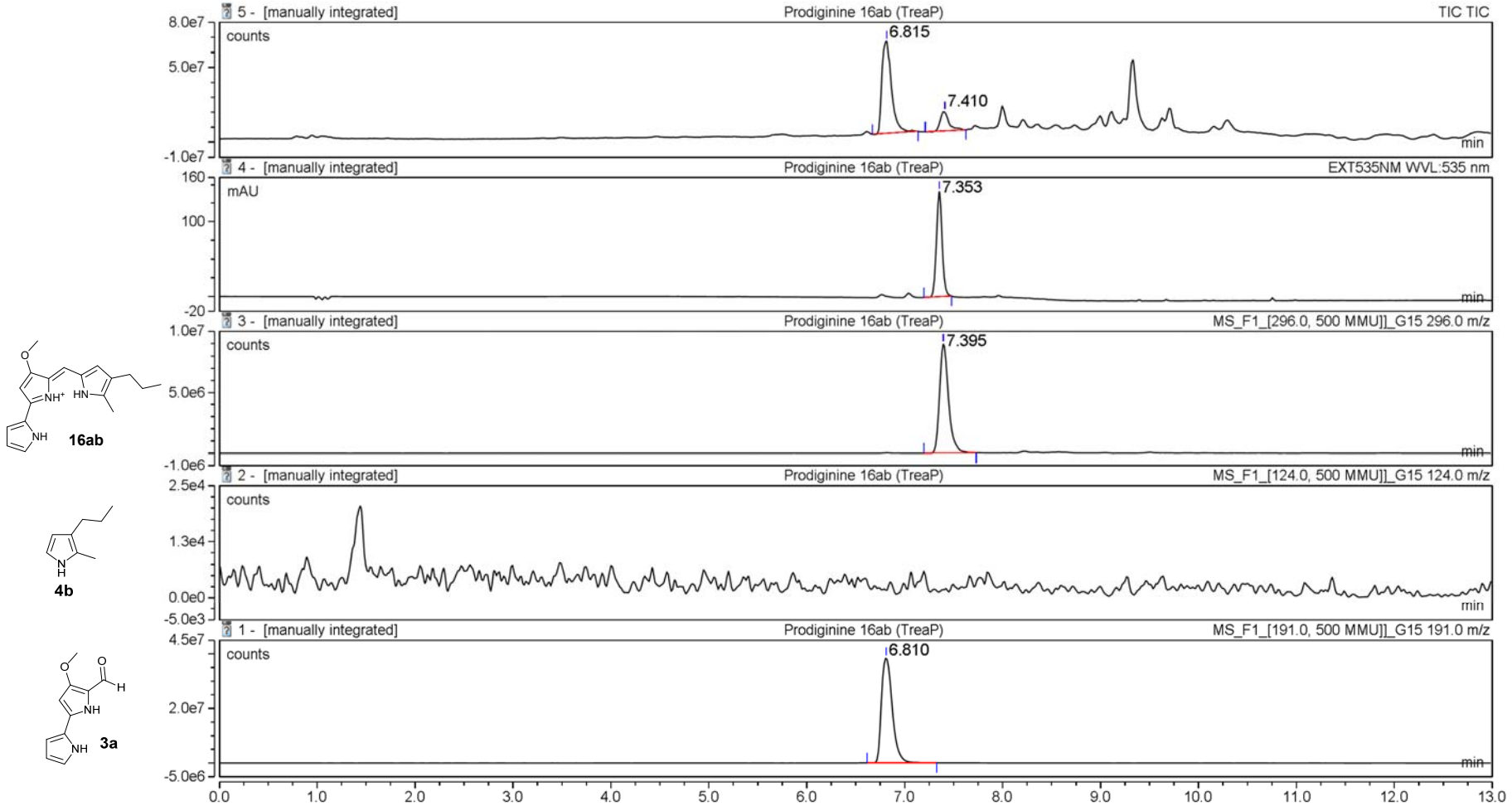


Figure S54: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ab** (m/z 296.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ab** t_R 7.35 min.

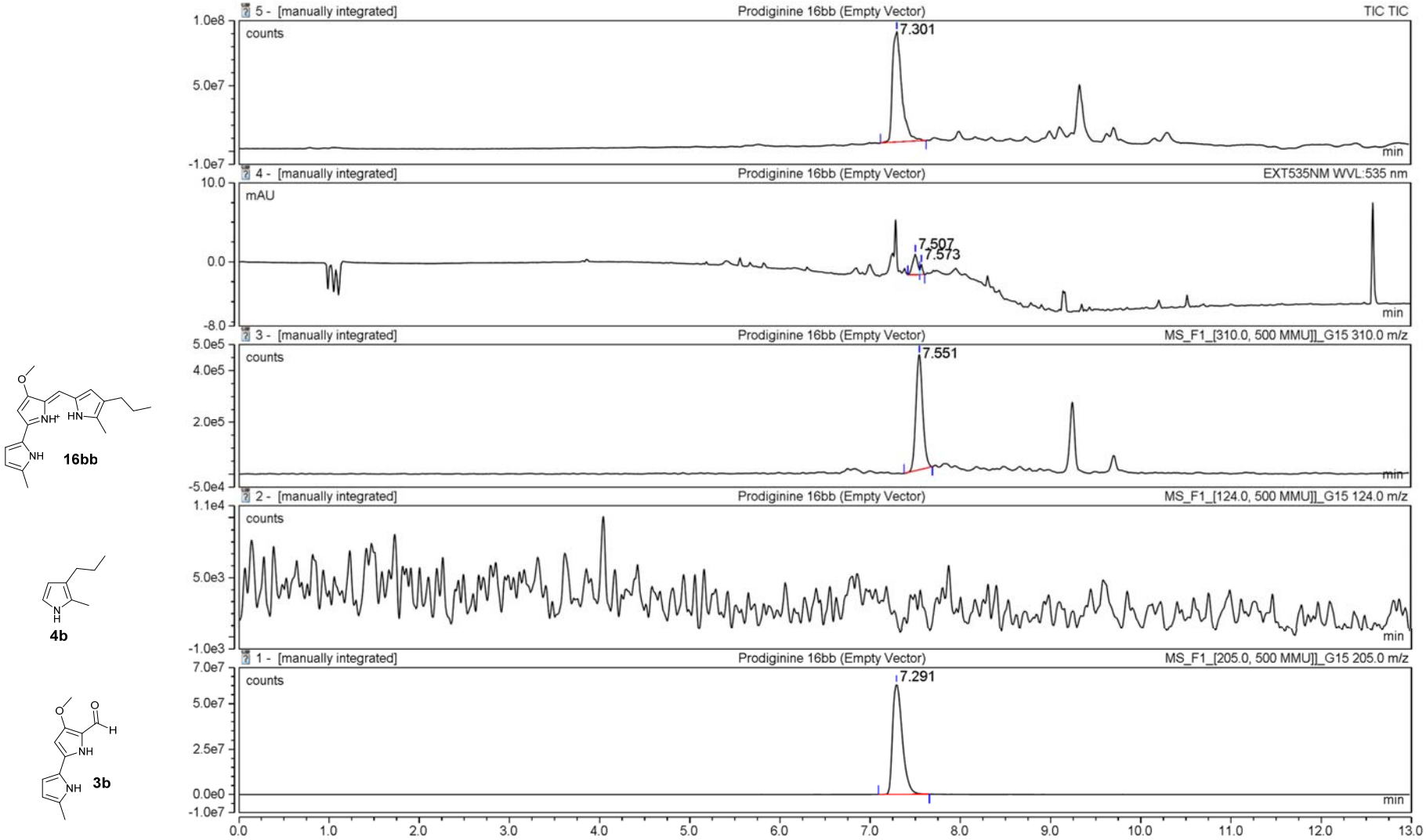


Figure S55: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16bb** (m/z 310.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16bb** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

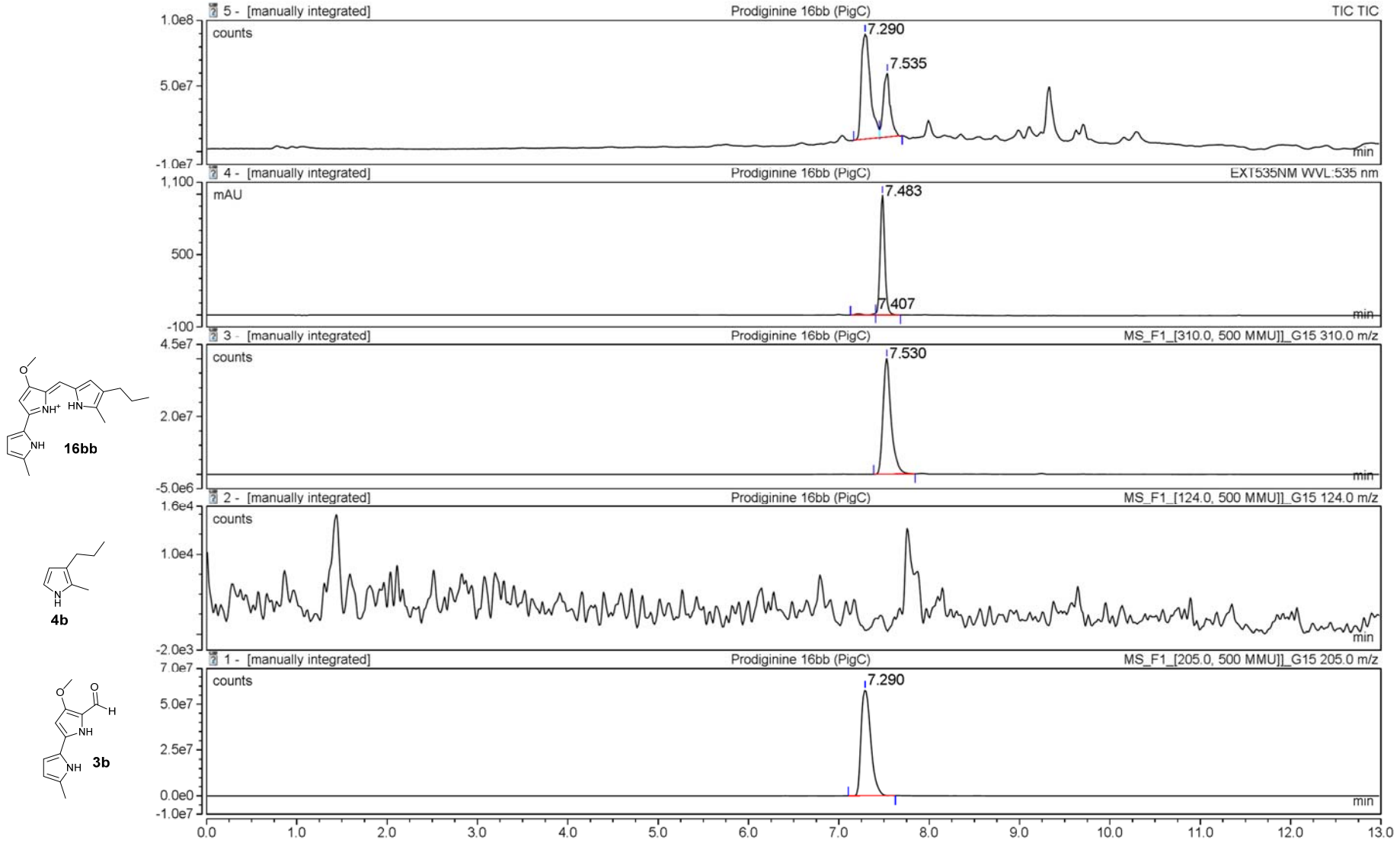


Figure S56: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bb** (m/z 310.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bb** t_R 7.48 min.

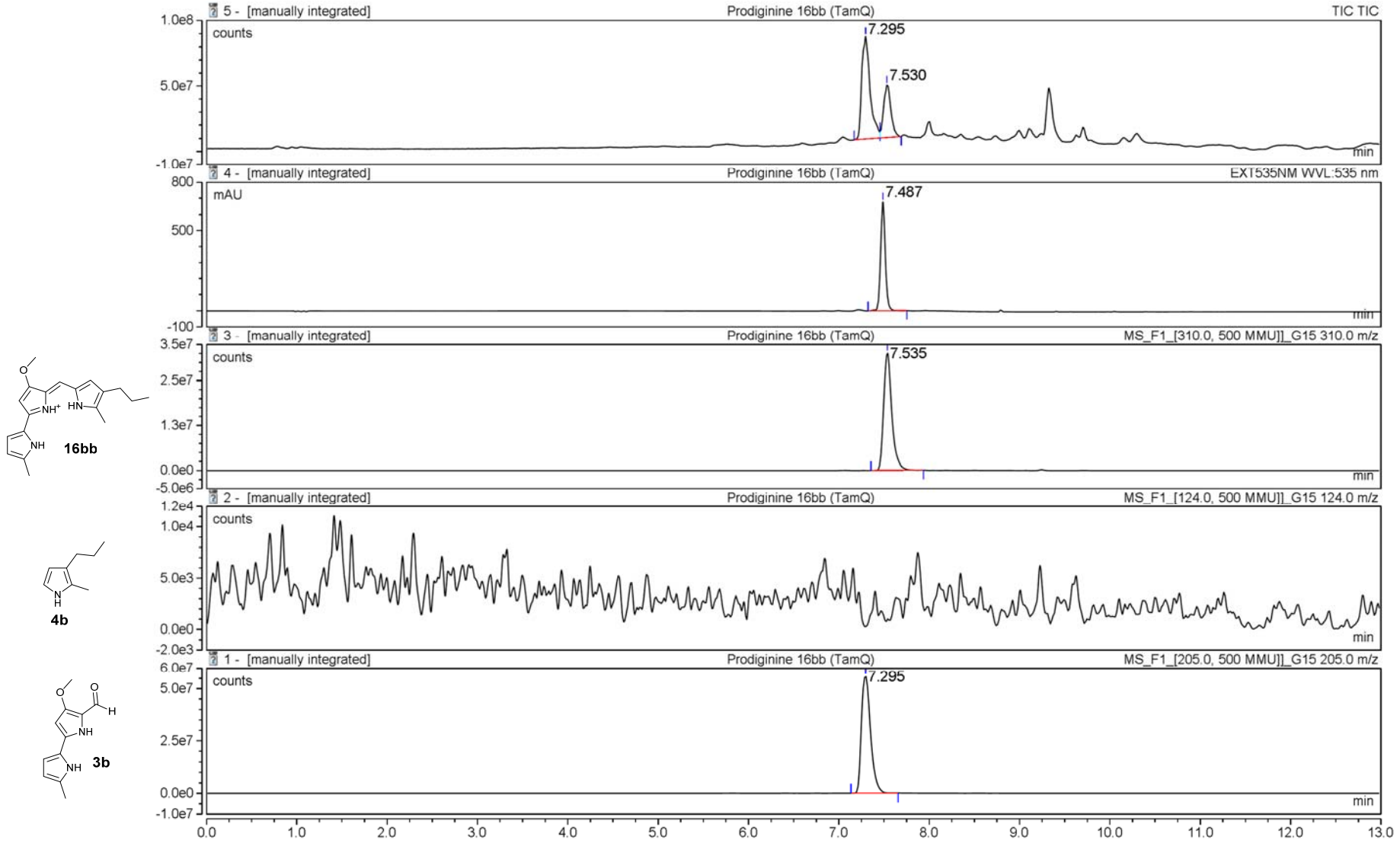


Figure S57: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bb** (m/z 310.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bb** t_R 7.49 min.

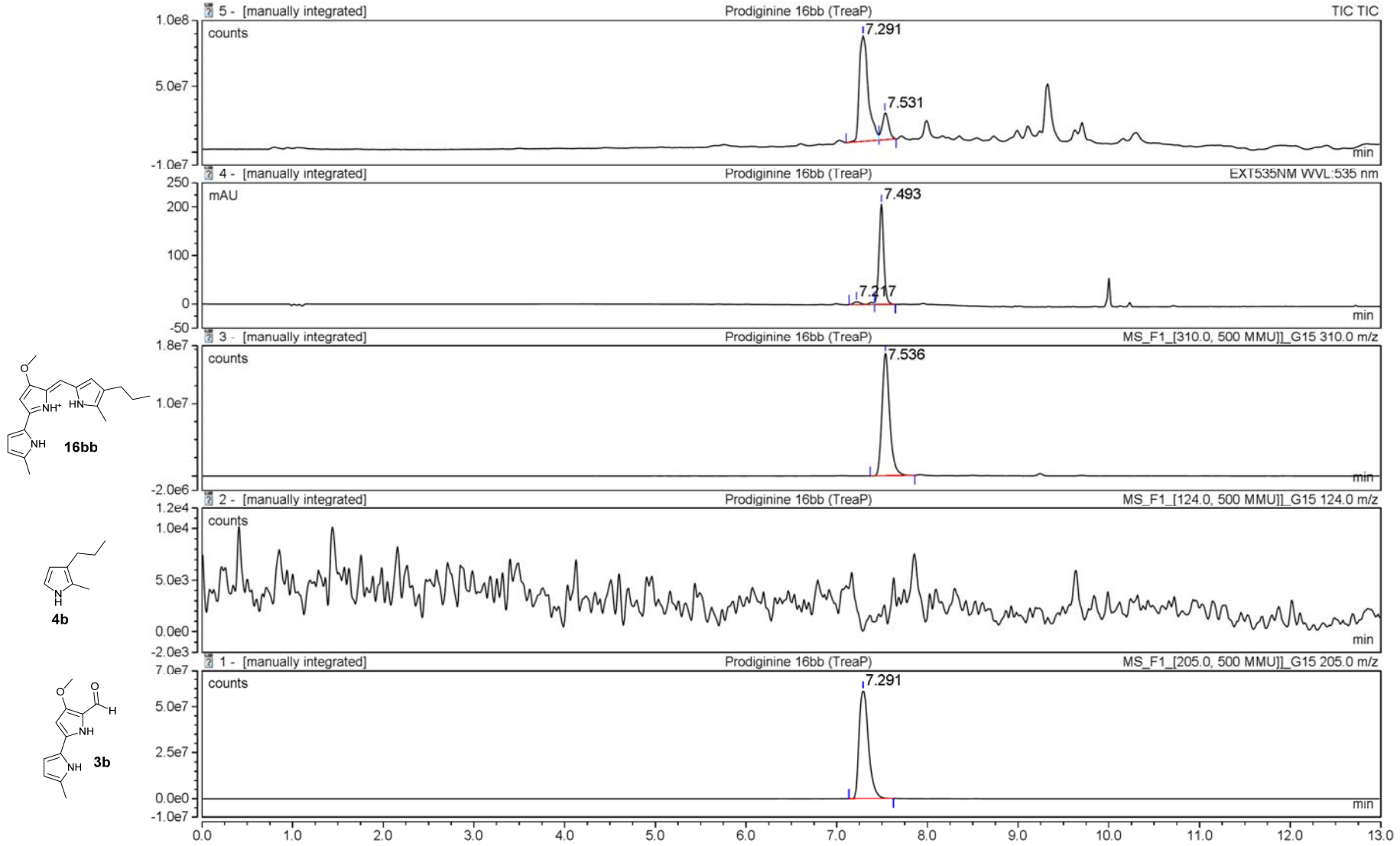


Figure S58: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bb** (m/z 310.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bb** t_R 7.49 min.

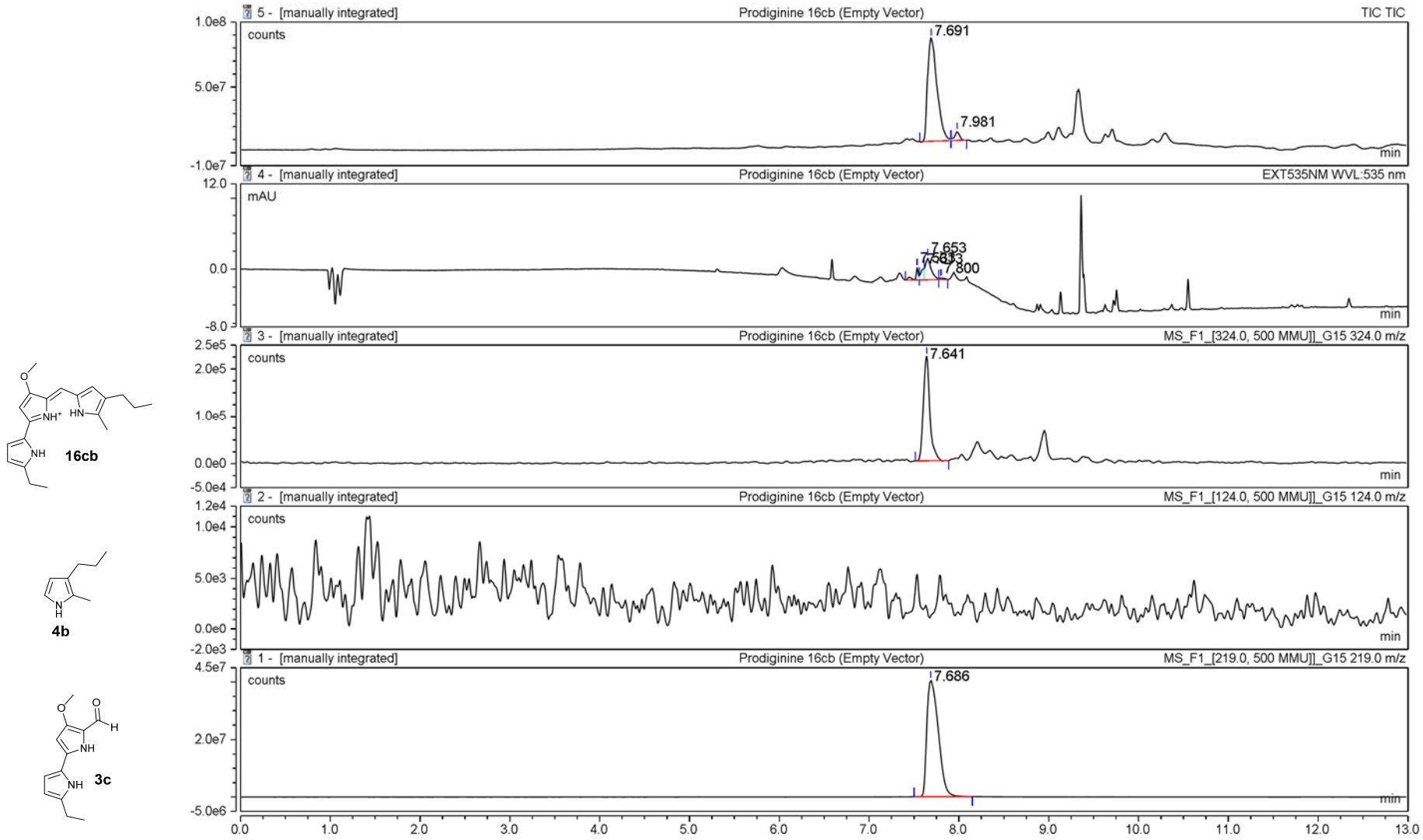


Figure S59: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16bc** (m/z 324.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16bc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

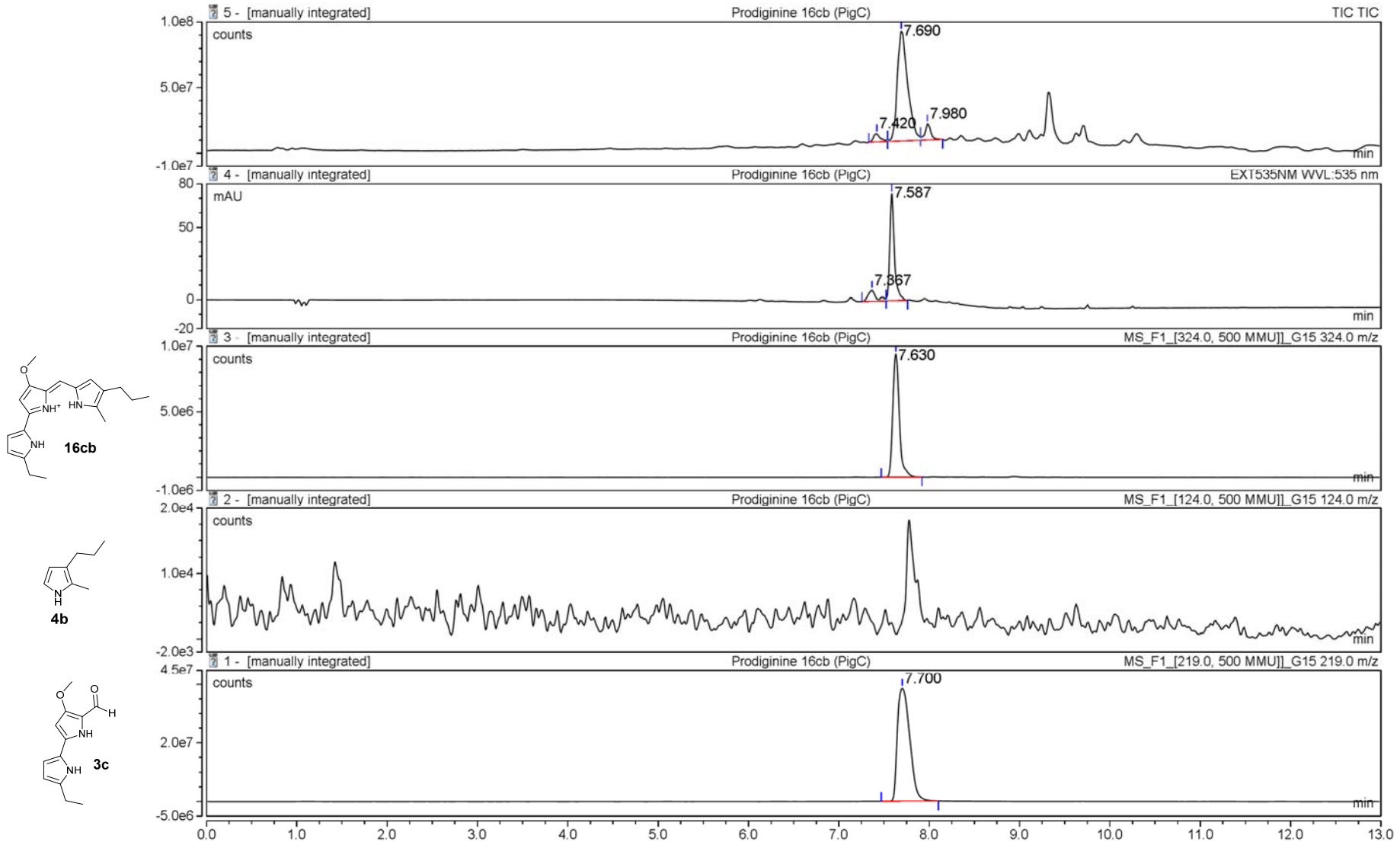


Figure S60: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cb** (m/z 324.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cb** t_R 7.59 min.

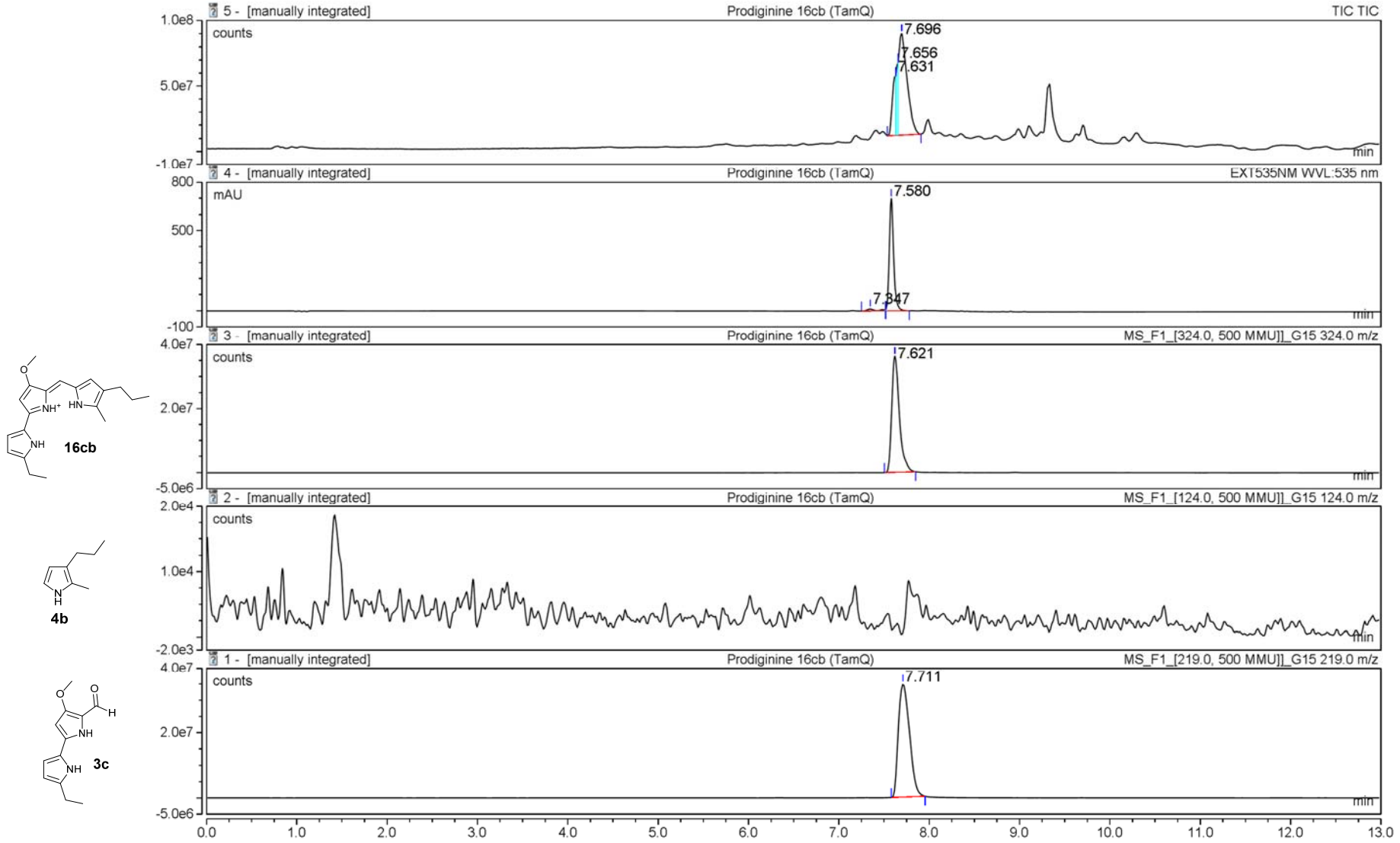


Figure S61: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cb** (m/z 324.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cb** t_R 7.58 min.

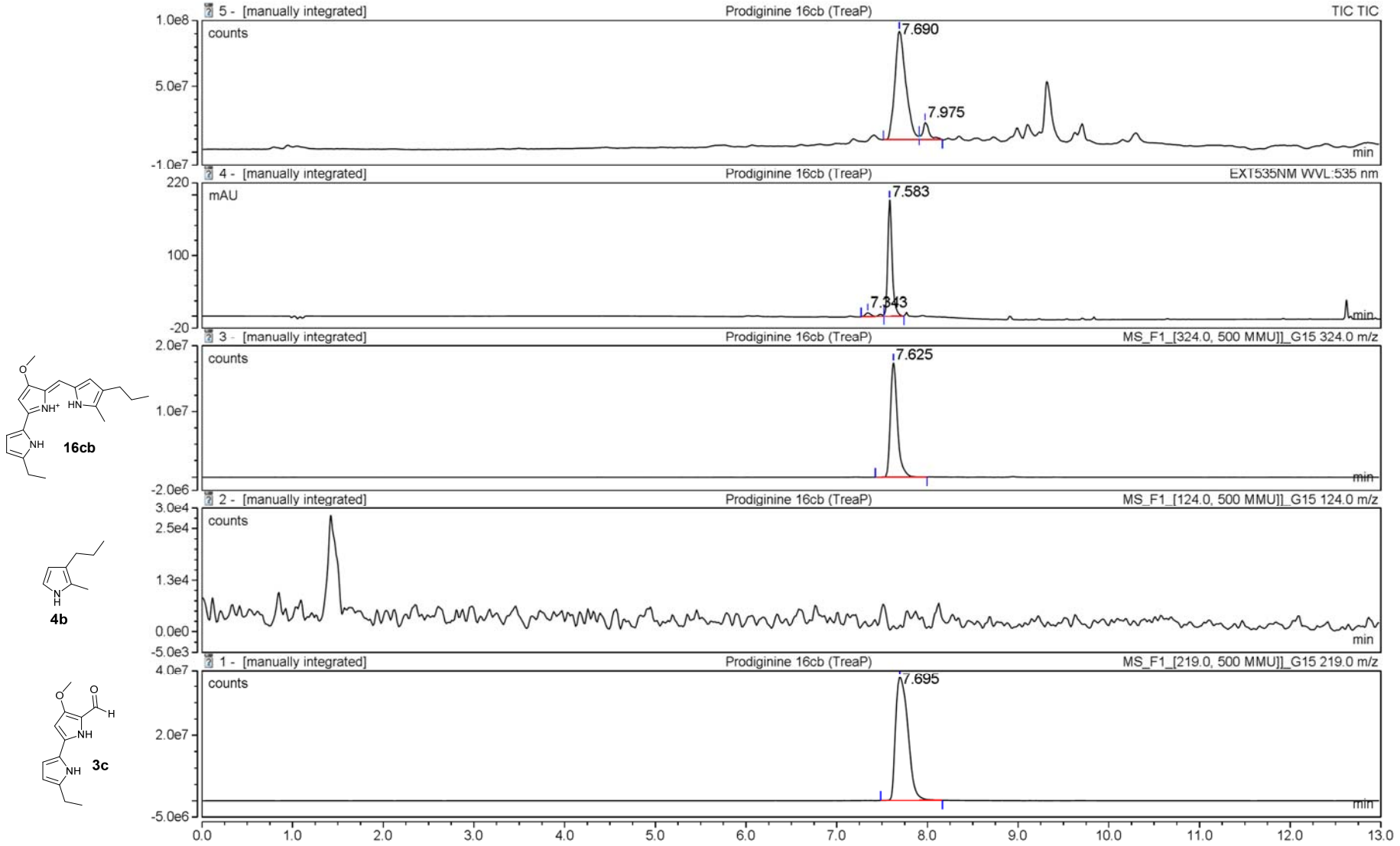


Figure S62: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cb** (m/z 324.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cb** t_R 7.58 min.

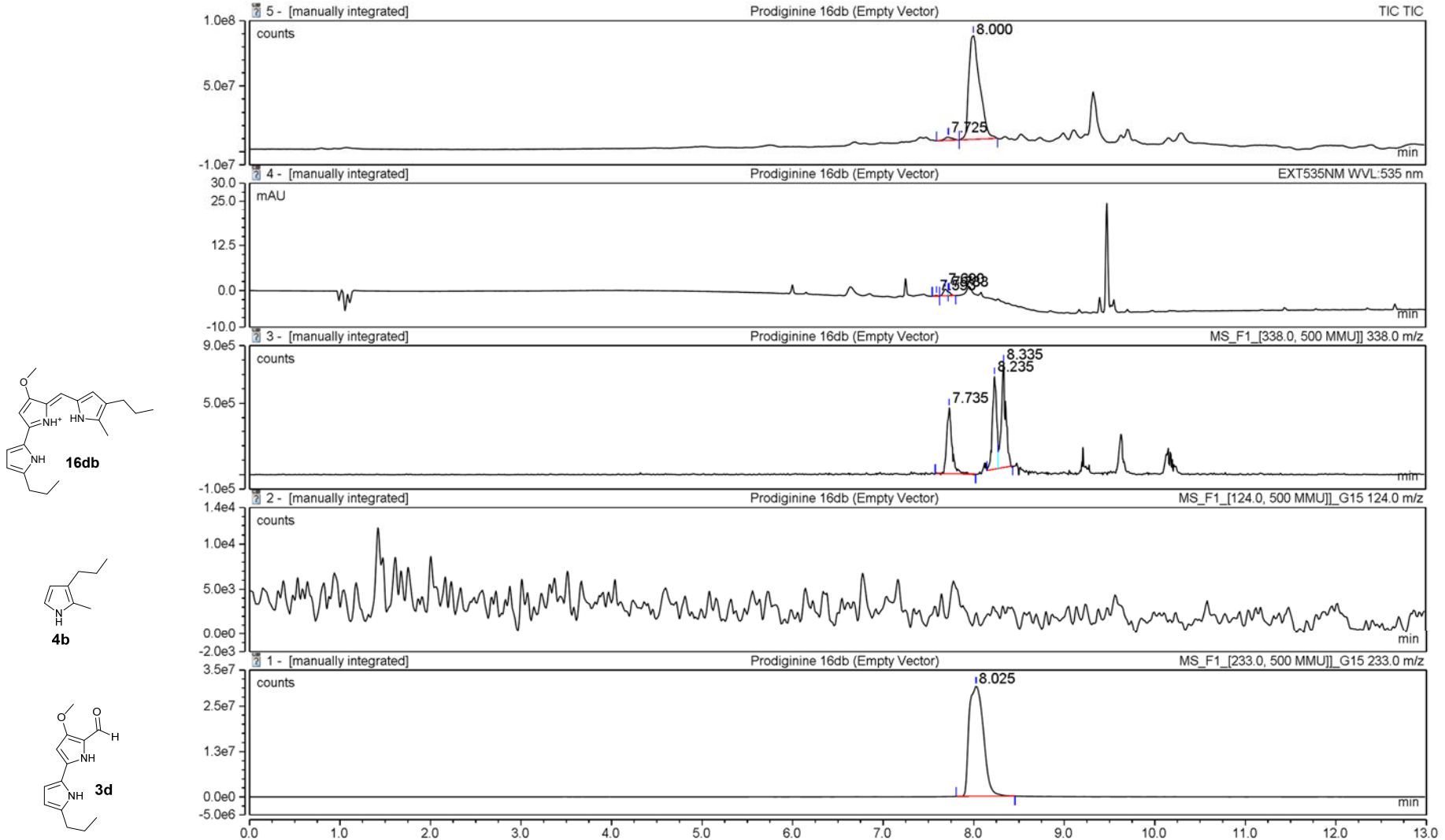


Figure S63: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16db** (m/z 338.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16db** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

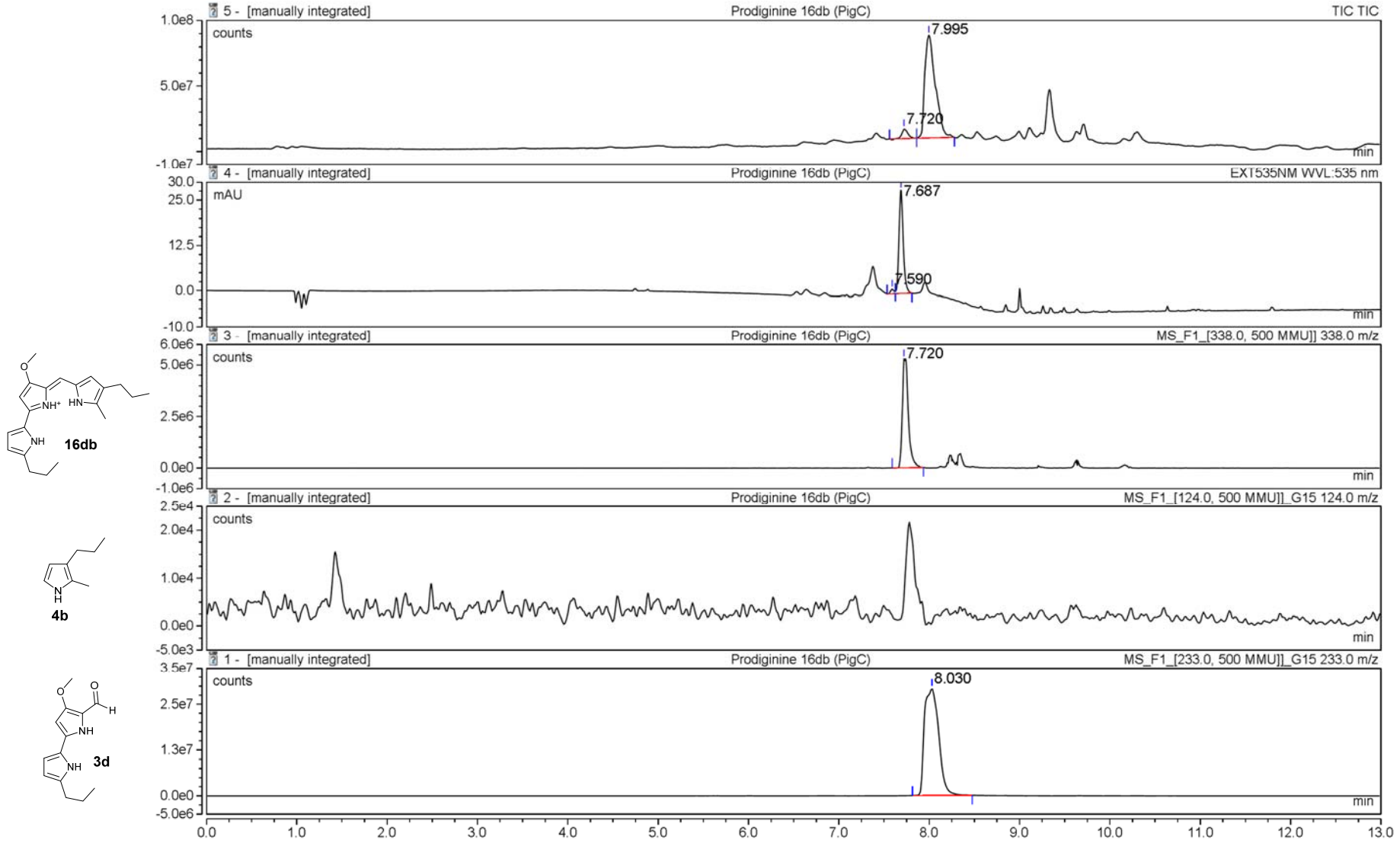


Figure S64: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16db** (m/z 338.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16db** t_R 7.69 min.

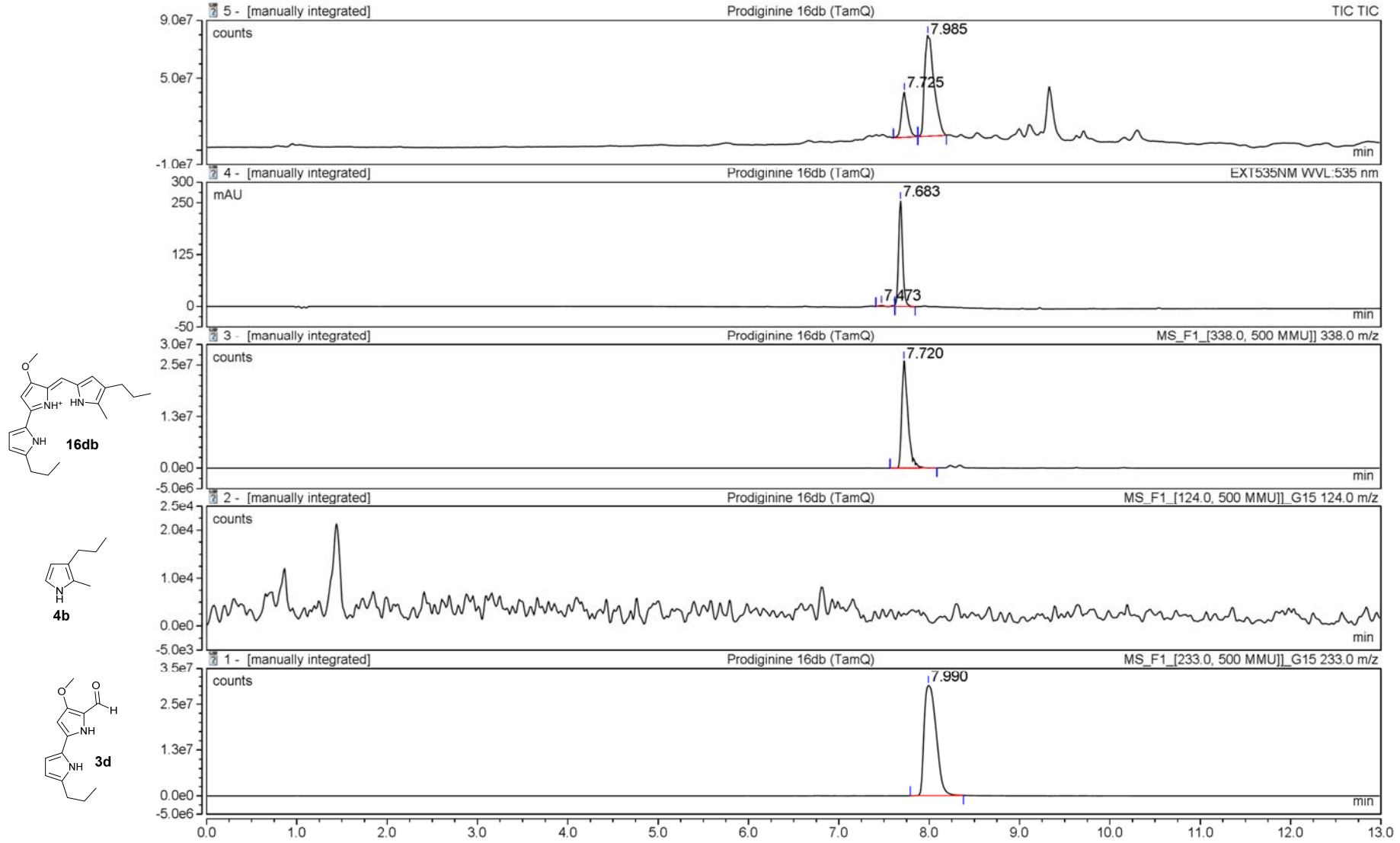


Figure S65: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16db** (m/z 338.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16db** t_R 7.68 min.

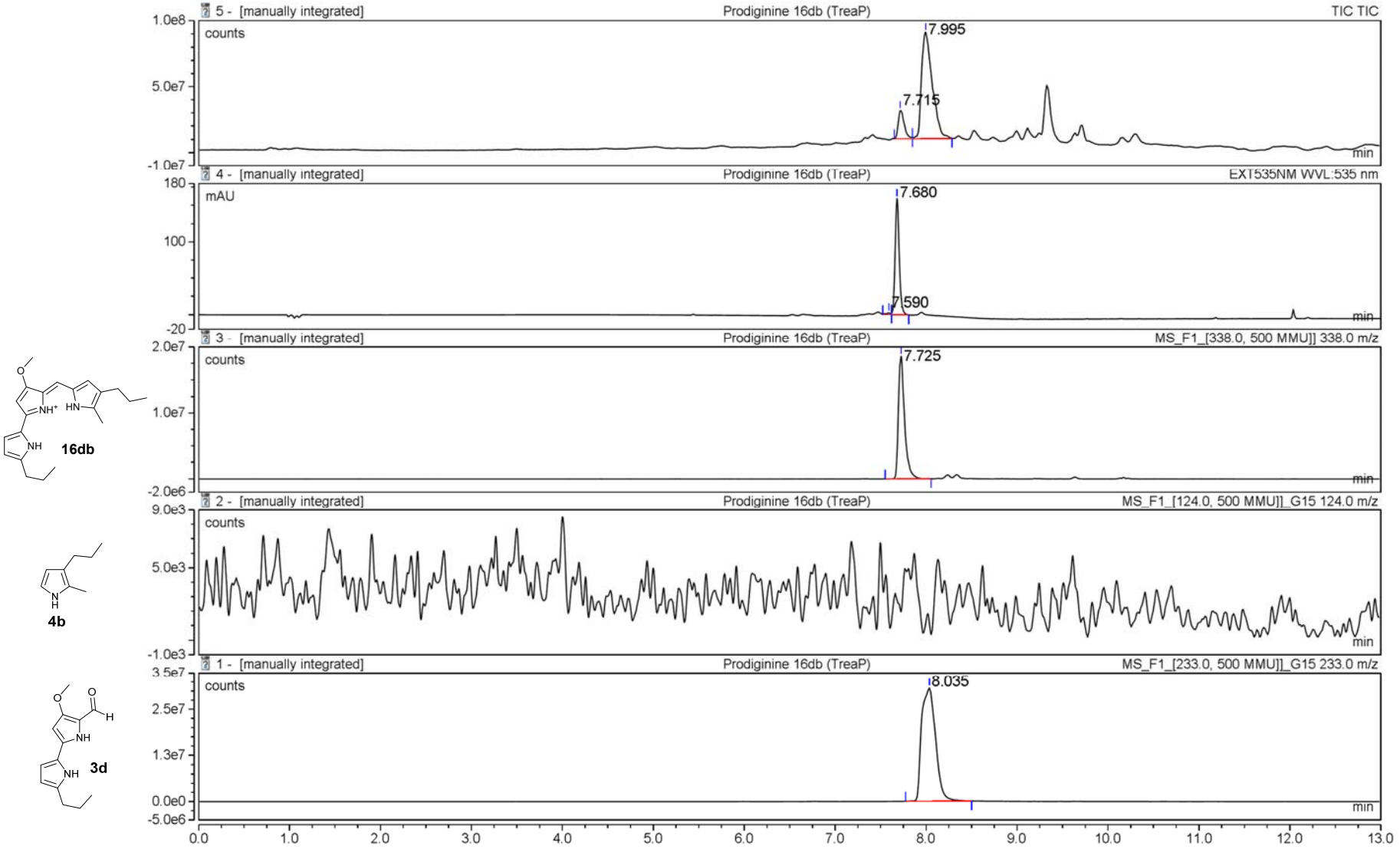


Figure S66: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16db** (m/z 338.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16db** t_R 7.68 min.

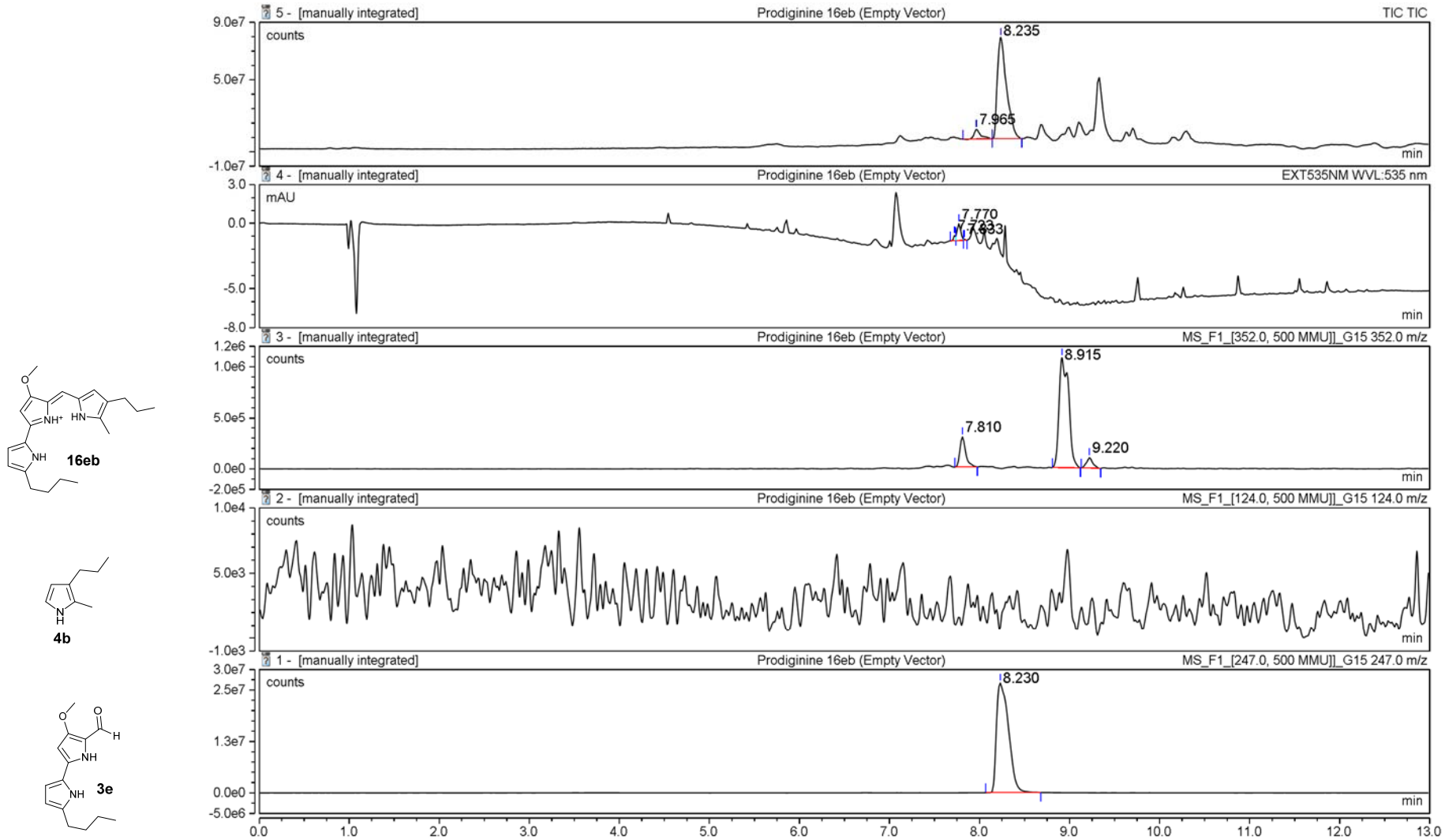


Figure S67: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16eb** (m/z 352.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16eb** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

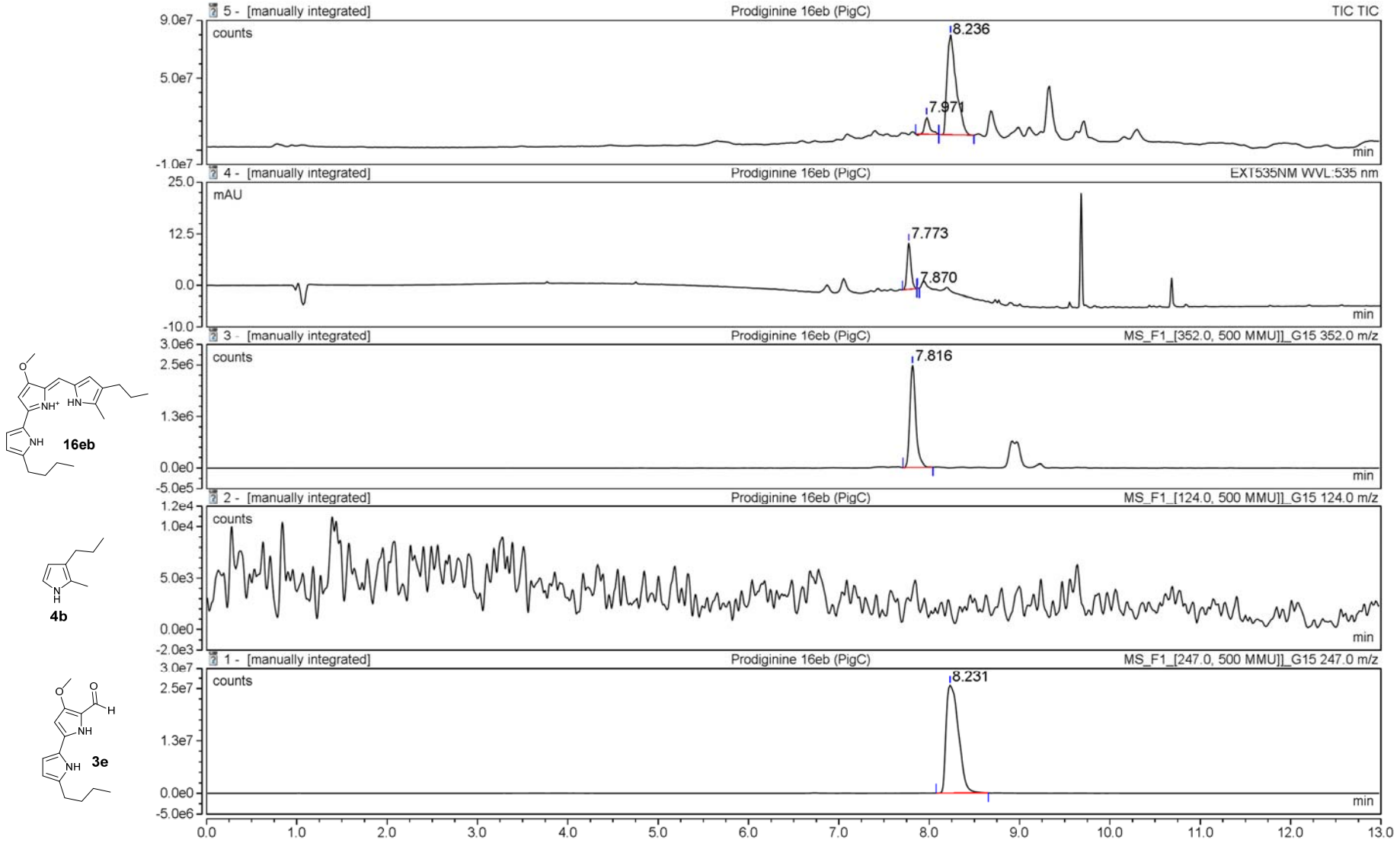


Figure S68: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eb** (m/z 352.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16eb** t_R 7.77 min.

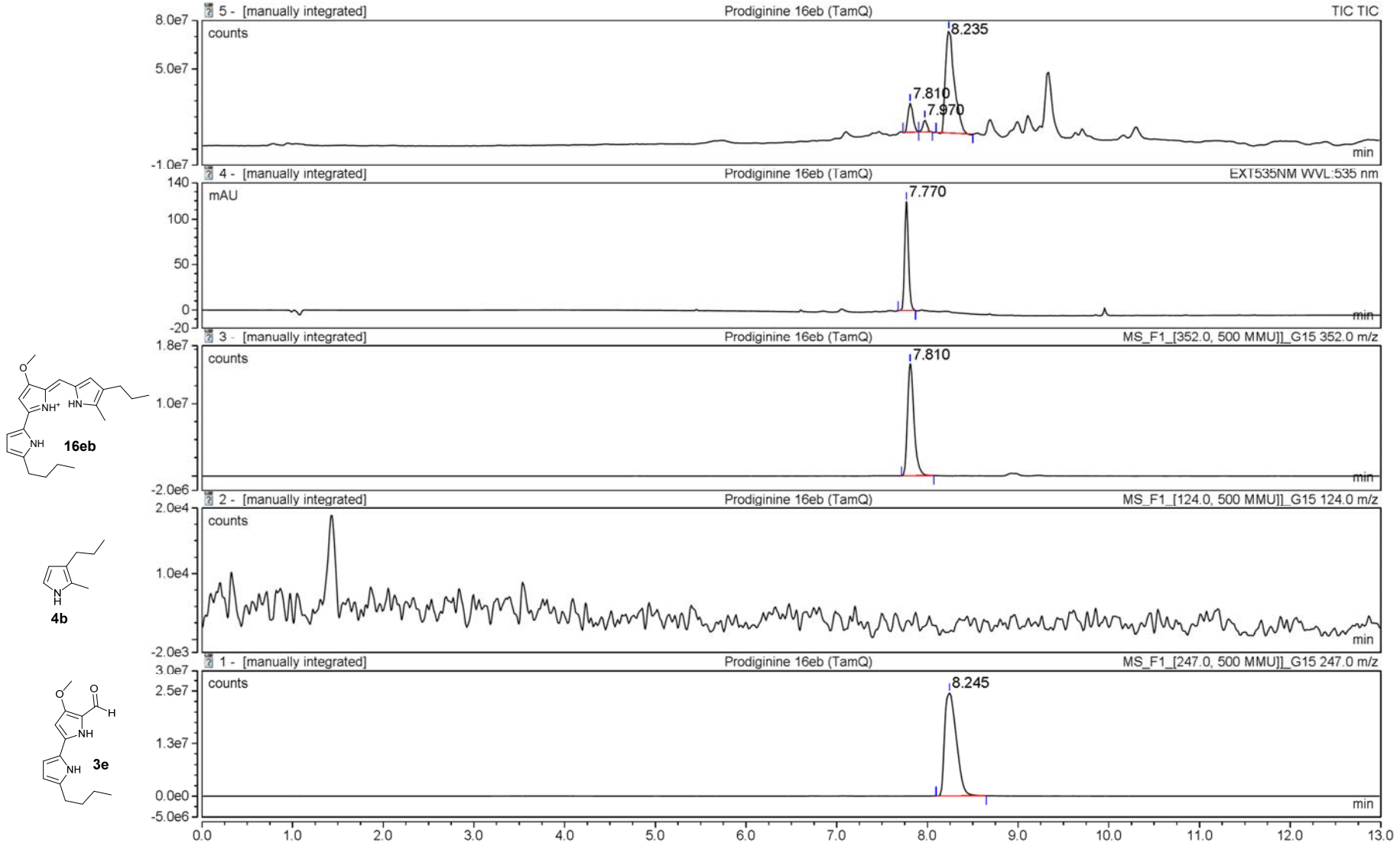


Figure S69: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eb** (m/z 352.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16eb** t_R 7.77 min.

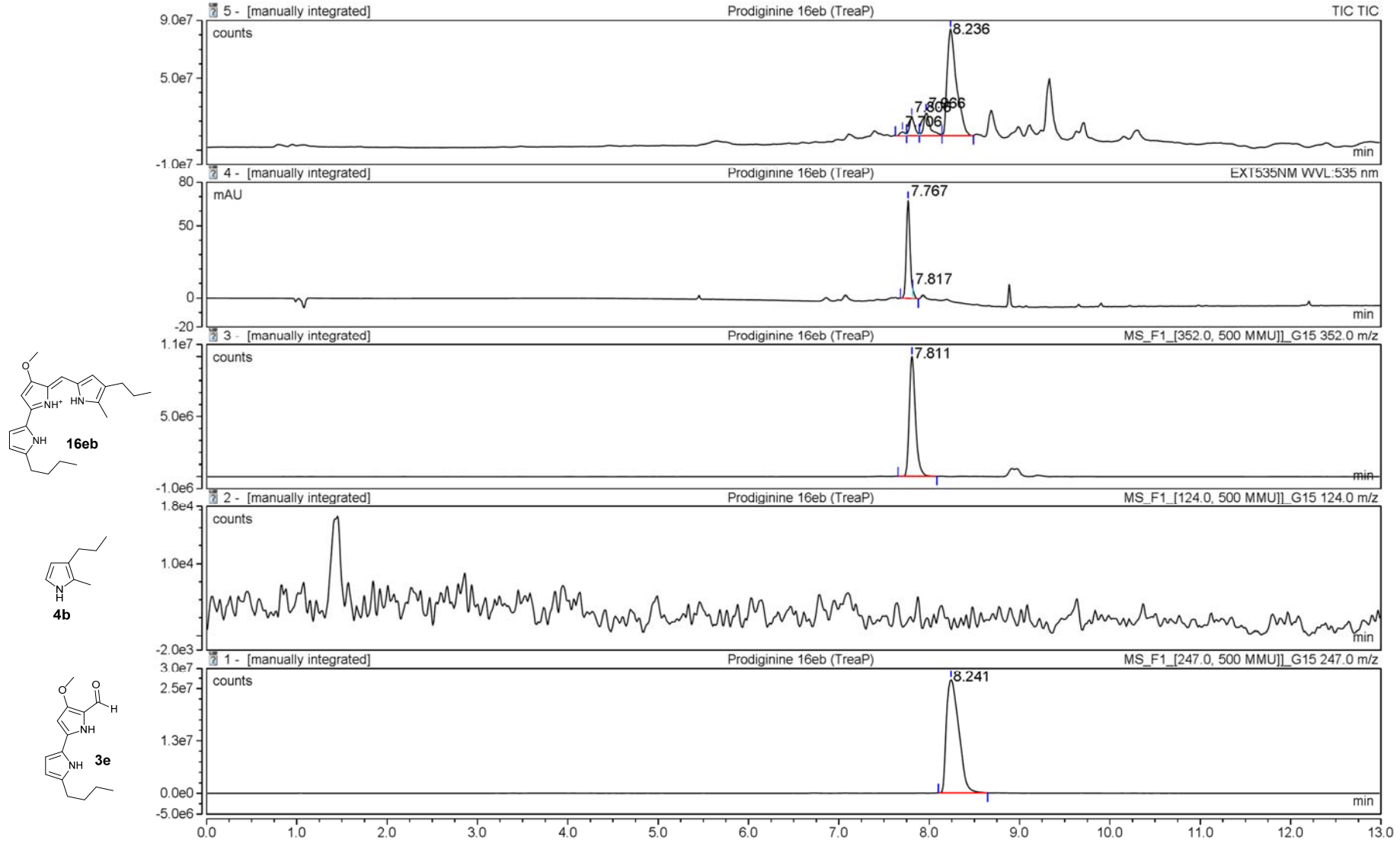


Figure S70: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16eb** (m/z 352.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16eb** t_R 7.77 min.

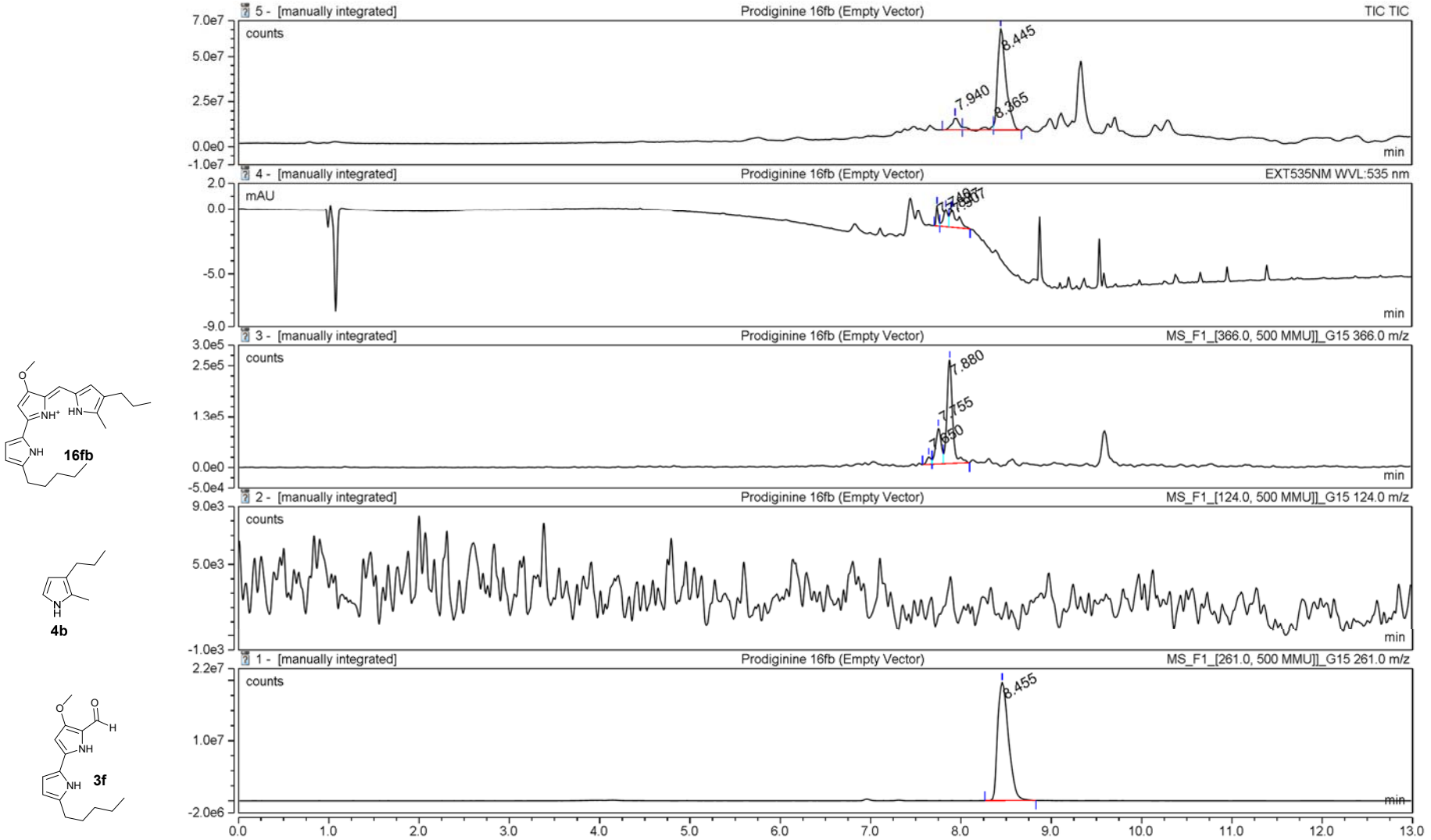


Figure S71: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16fb** (m/z 366.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16fb** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

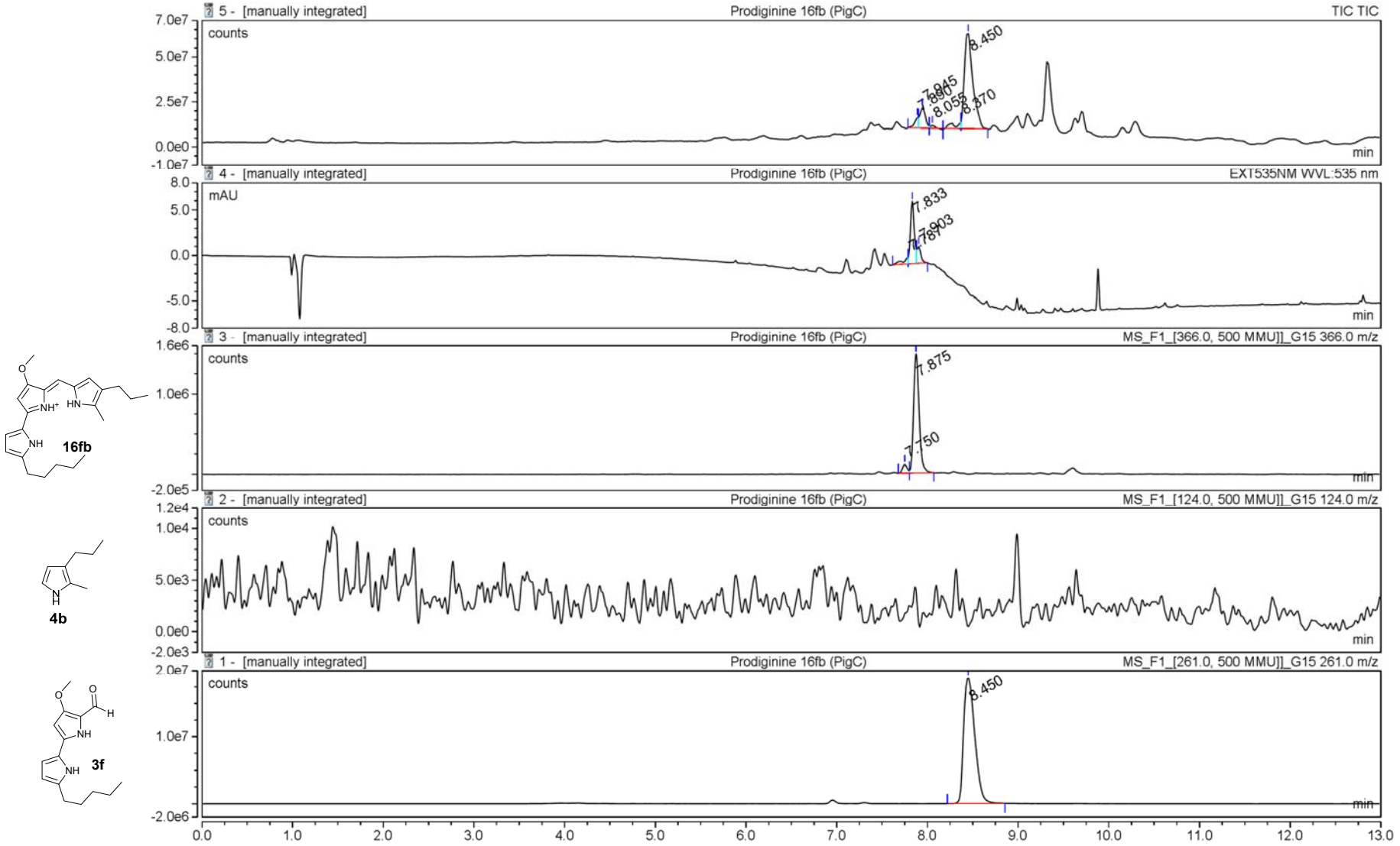


Figure S72: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fb** (m/z 366.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fb** t_R 7.83 min.

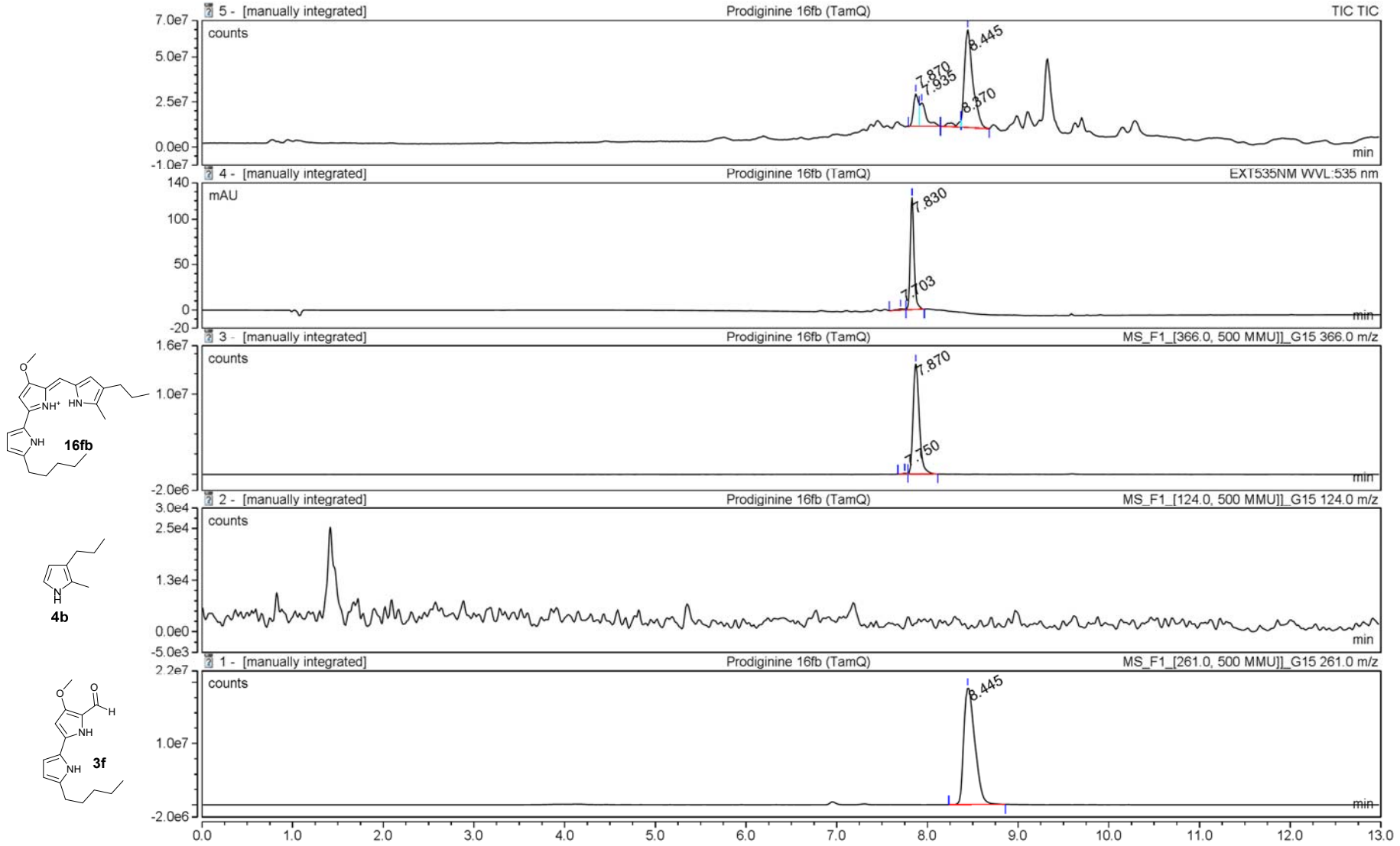


Figure S73: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fb** (m/z 366.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fb** t_R 7.83 min.

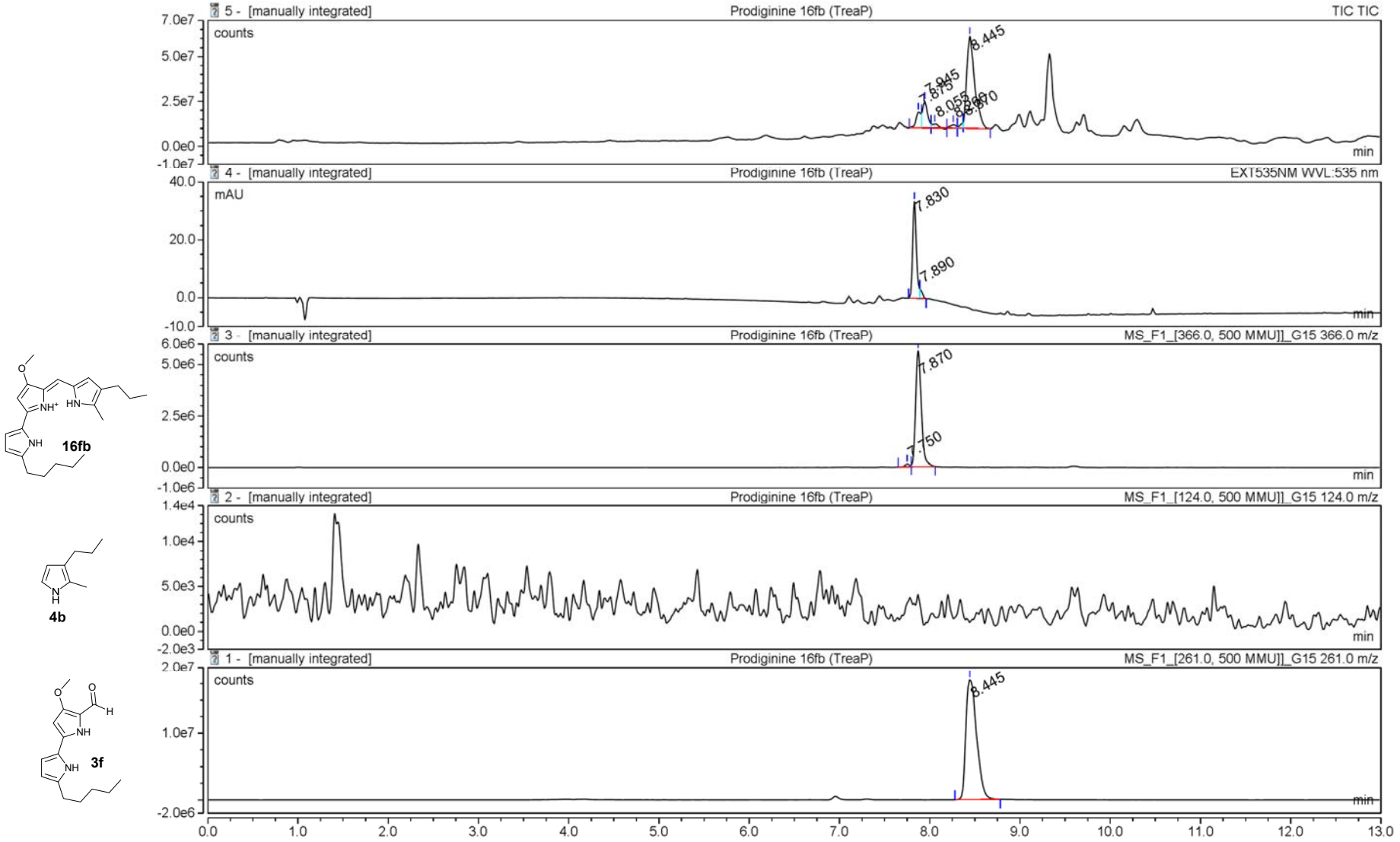


Figure S74: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fb** (m/z 366.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fb** t_R 7.83 min.

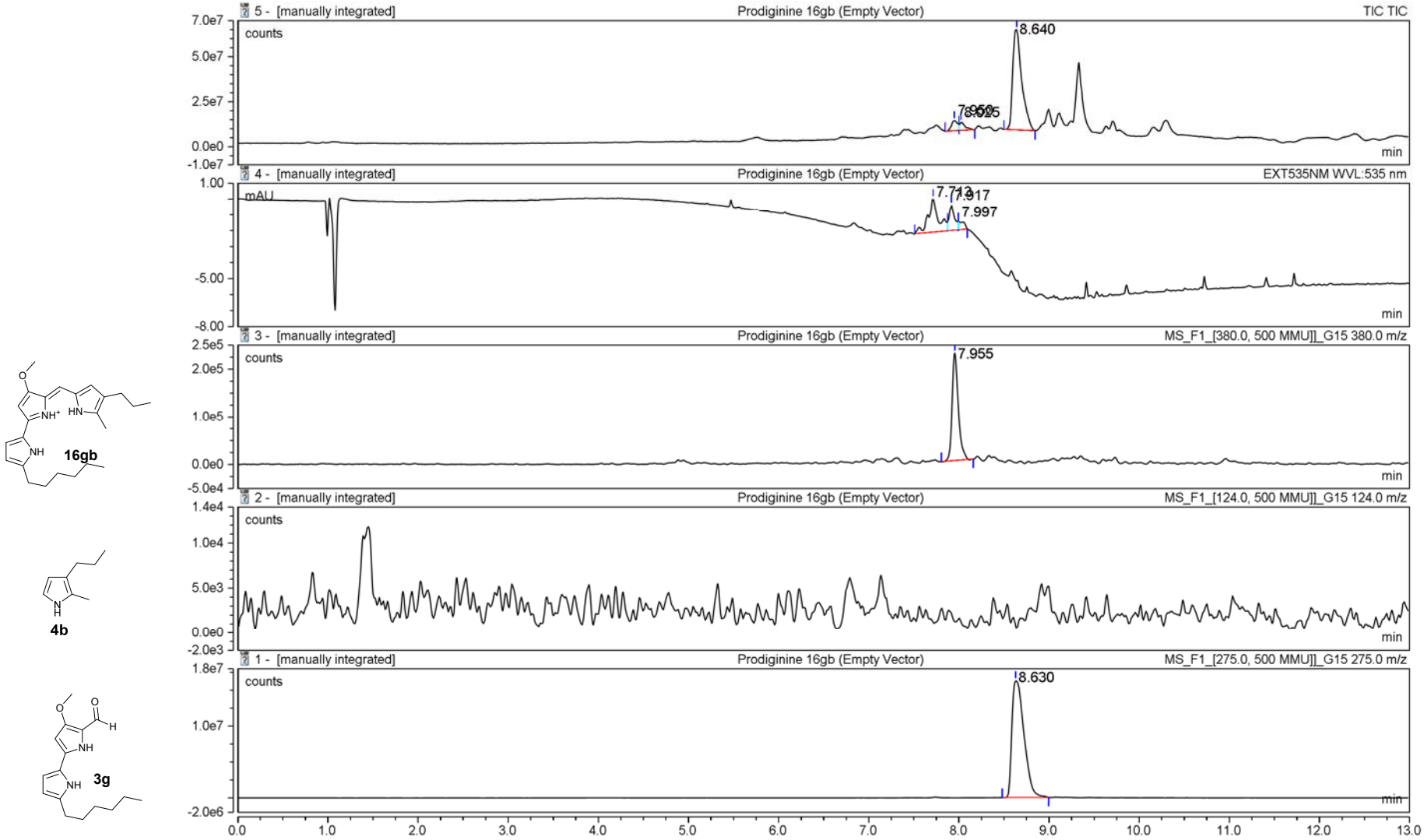


Figure S75: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16gb** (m/z 380.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4b** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16gb** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

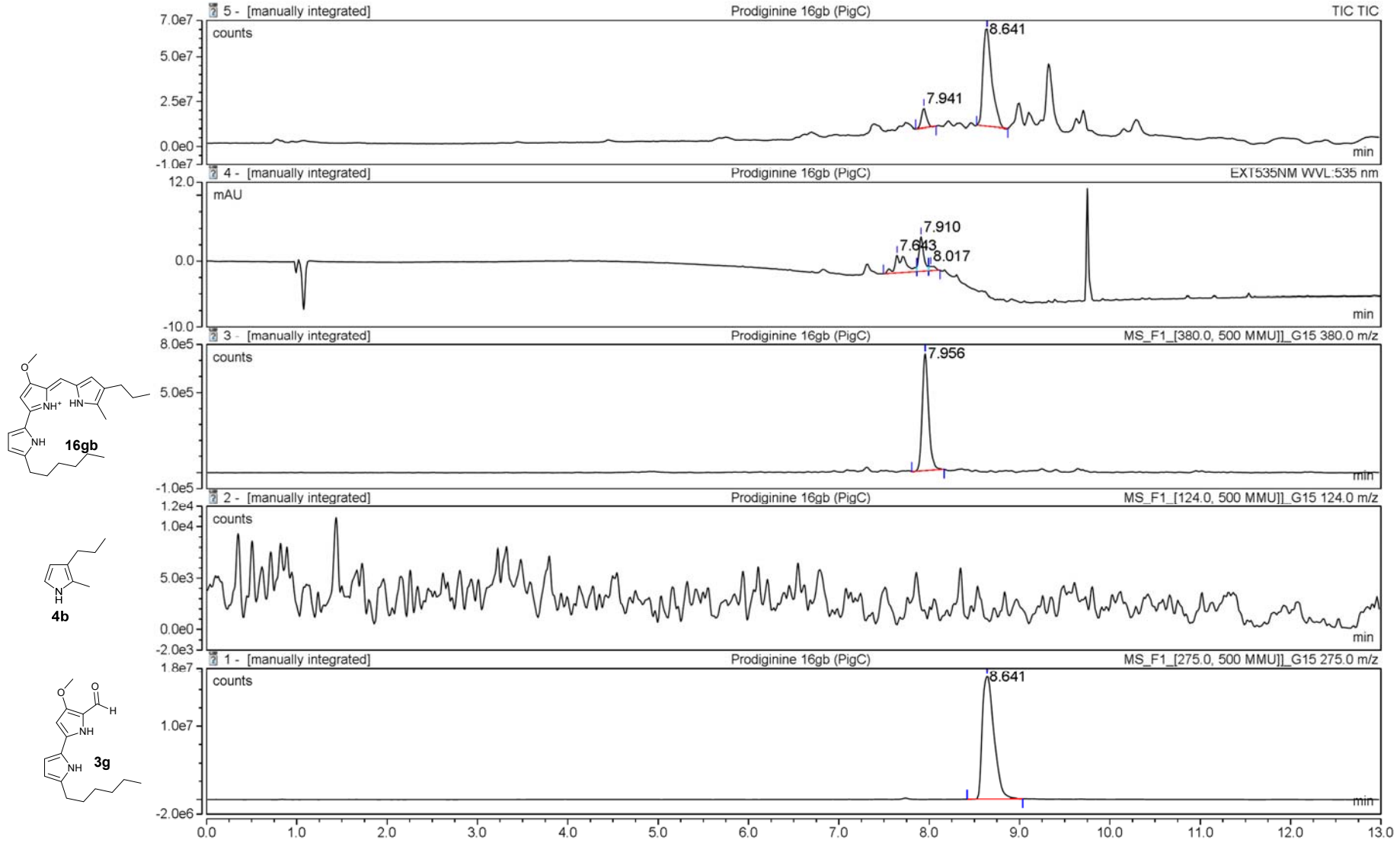


Figure S76: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gb** (m/z 380.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4b** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gb** t_R 7.91 min.

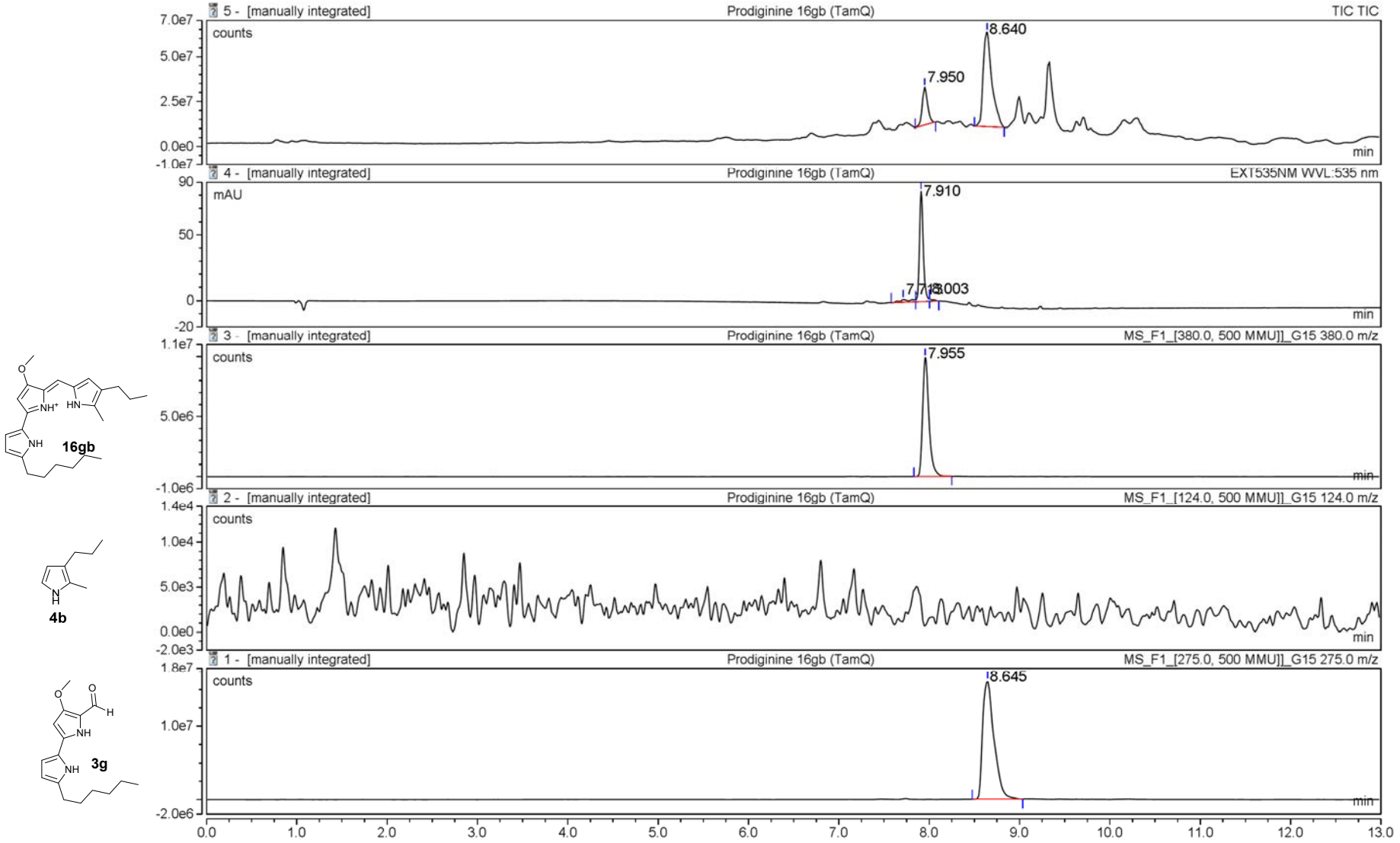


Figure S77: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gb** (m/z 380.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4b** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gb** t_R 7.91 min.

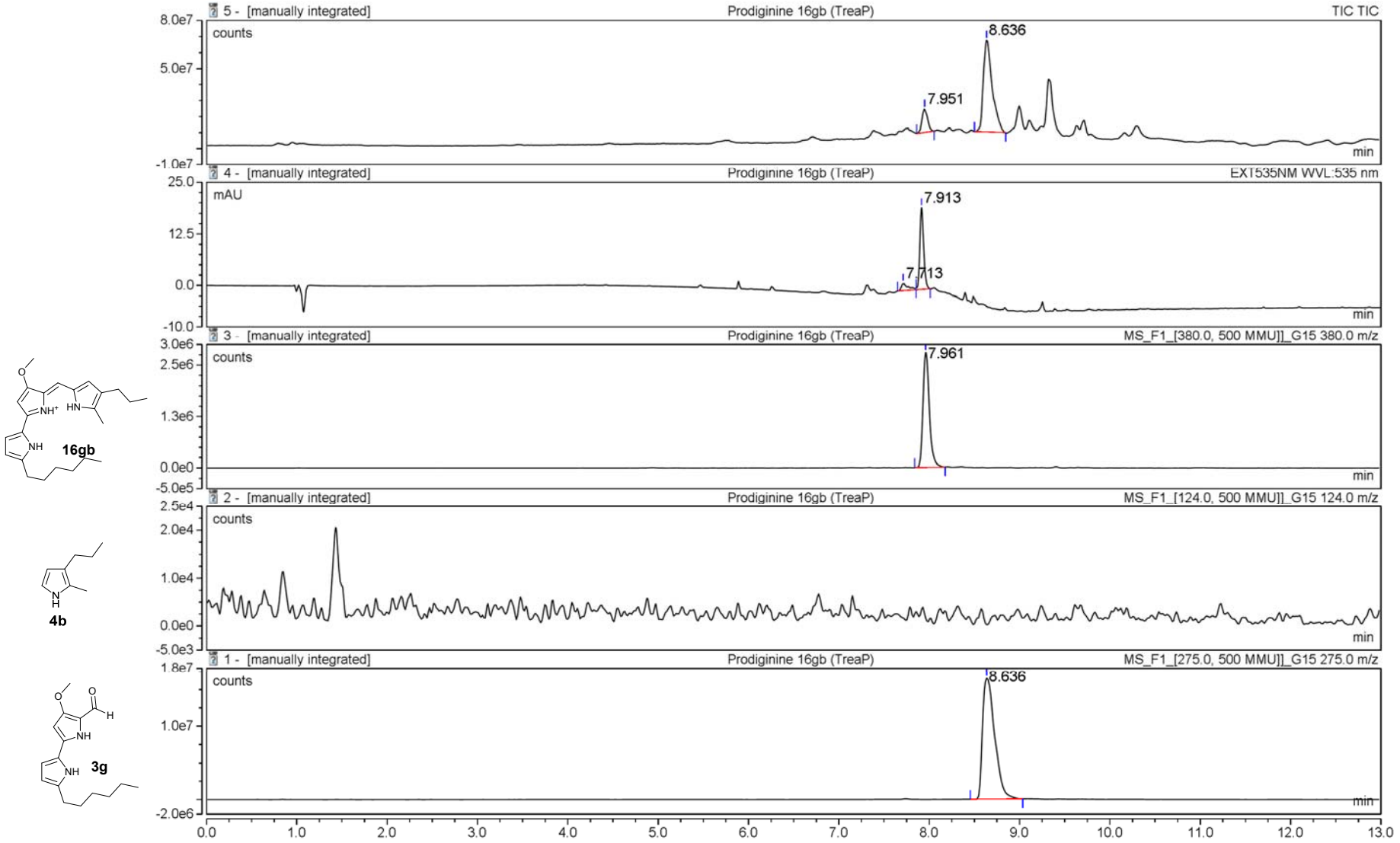


Figure S78: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gb** (m/z 380.0) and the educts pyrrole **4b** (m/z 124.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4b** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gb** t_R 7.91 min.

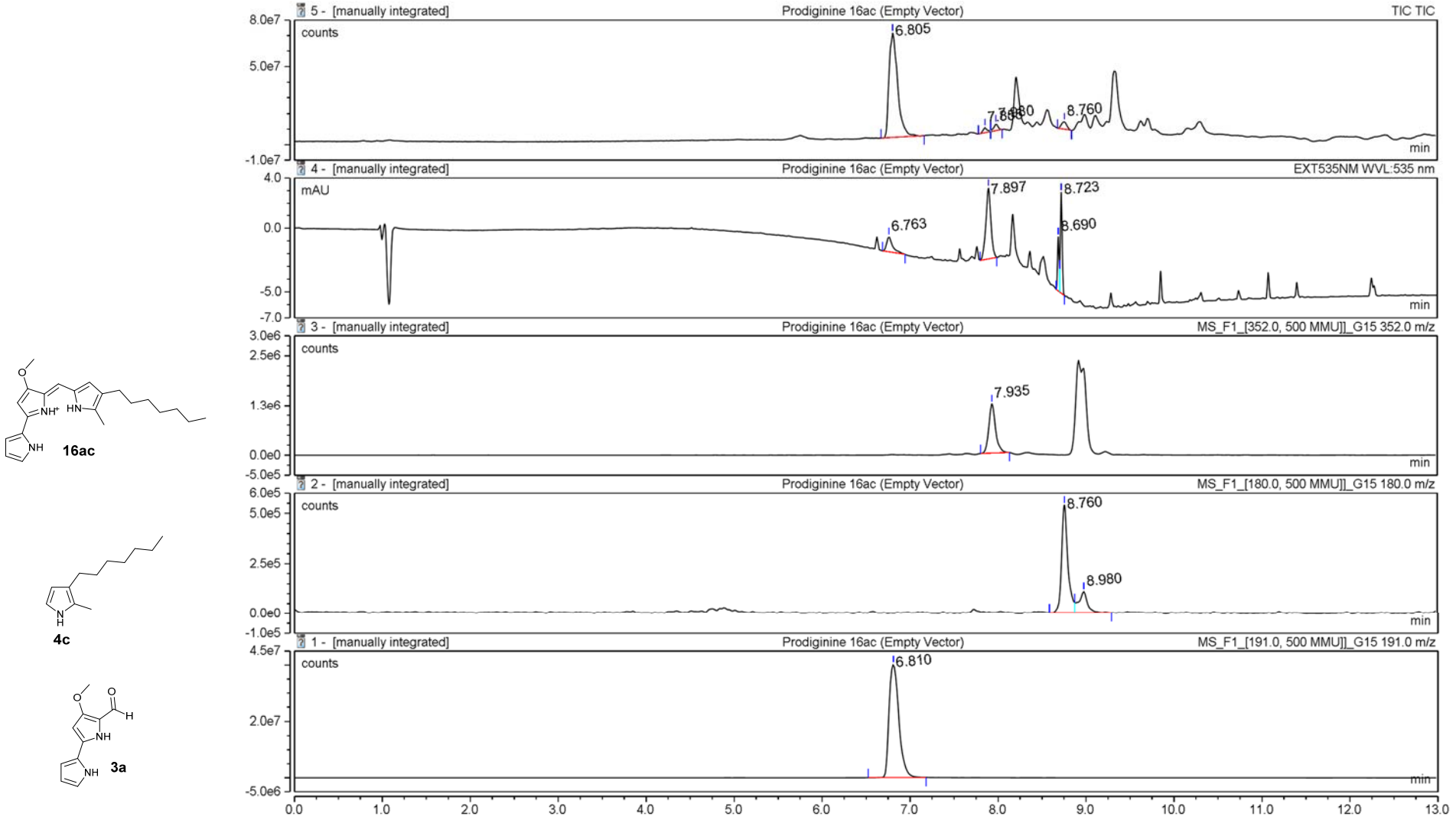


Figure S79: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ac** (m/z 352.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ac** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

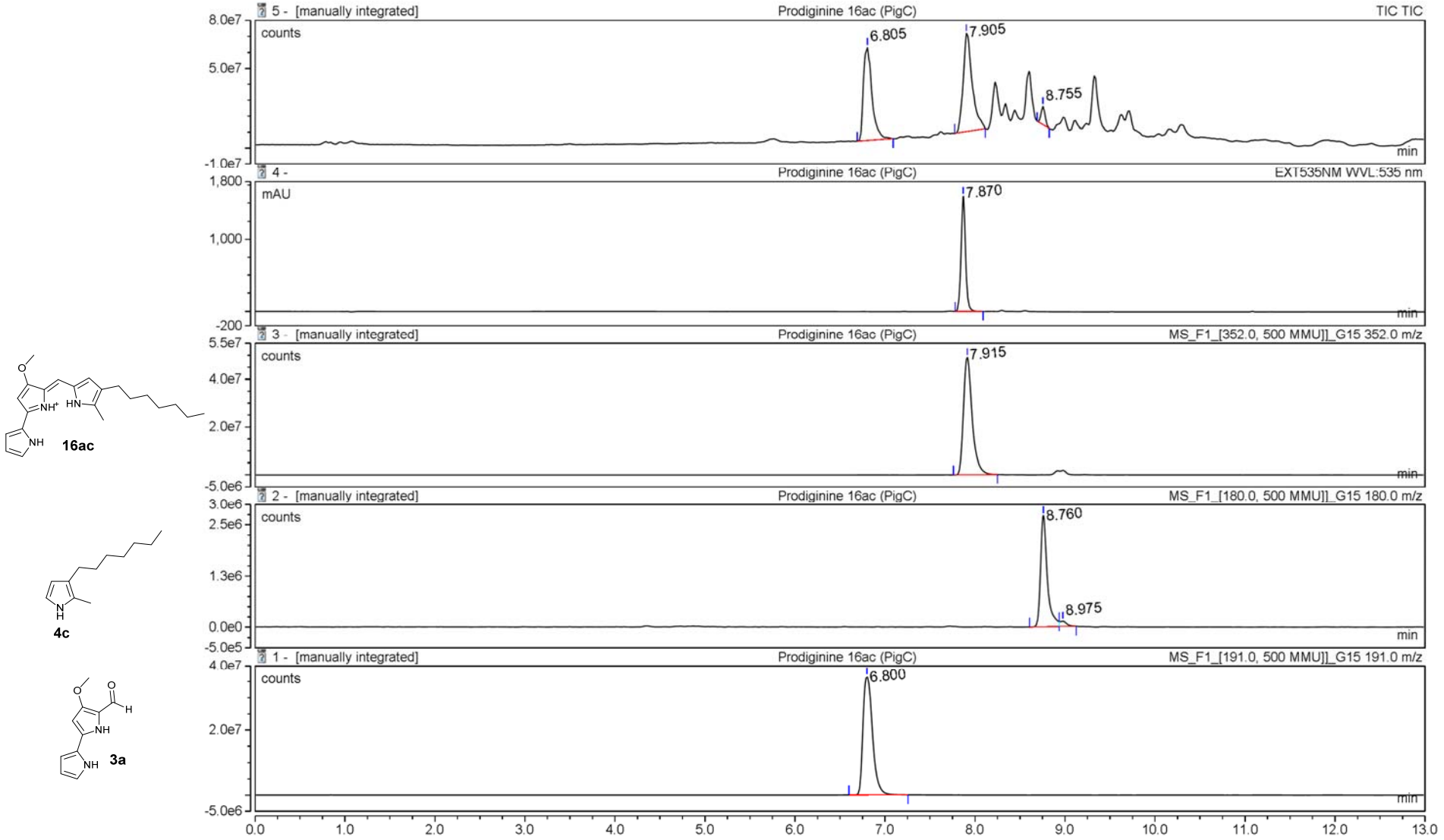


Figure S80: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ac** (m/z 352.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ac** t_R 7.87 min.

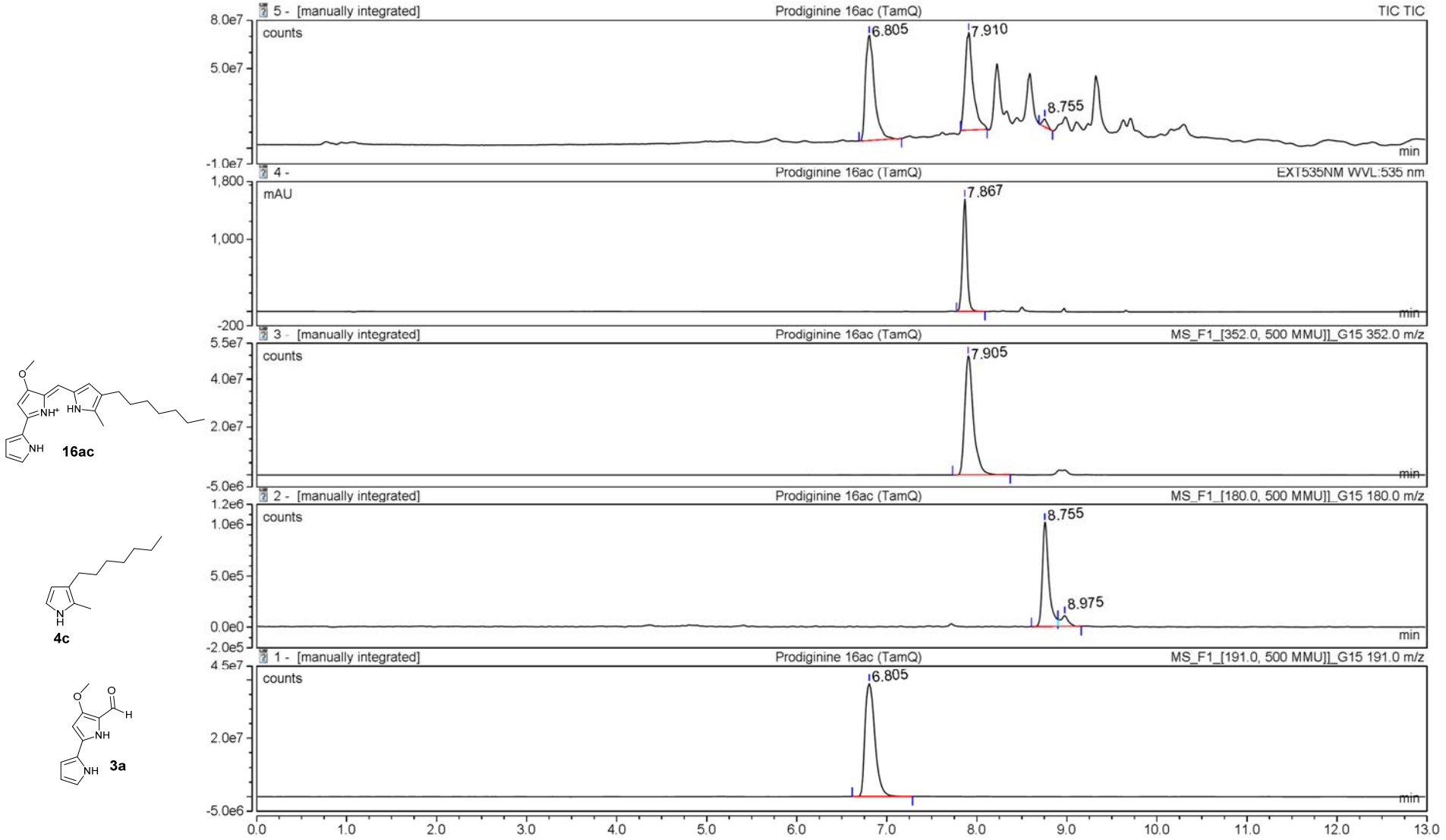


Figure S81: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ac** (m/z 352.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ac** t_R 7.87 min.

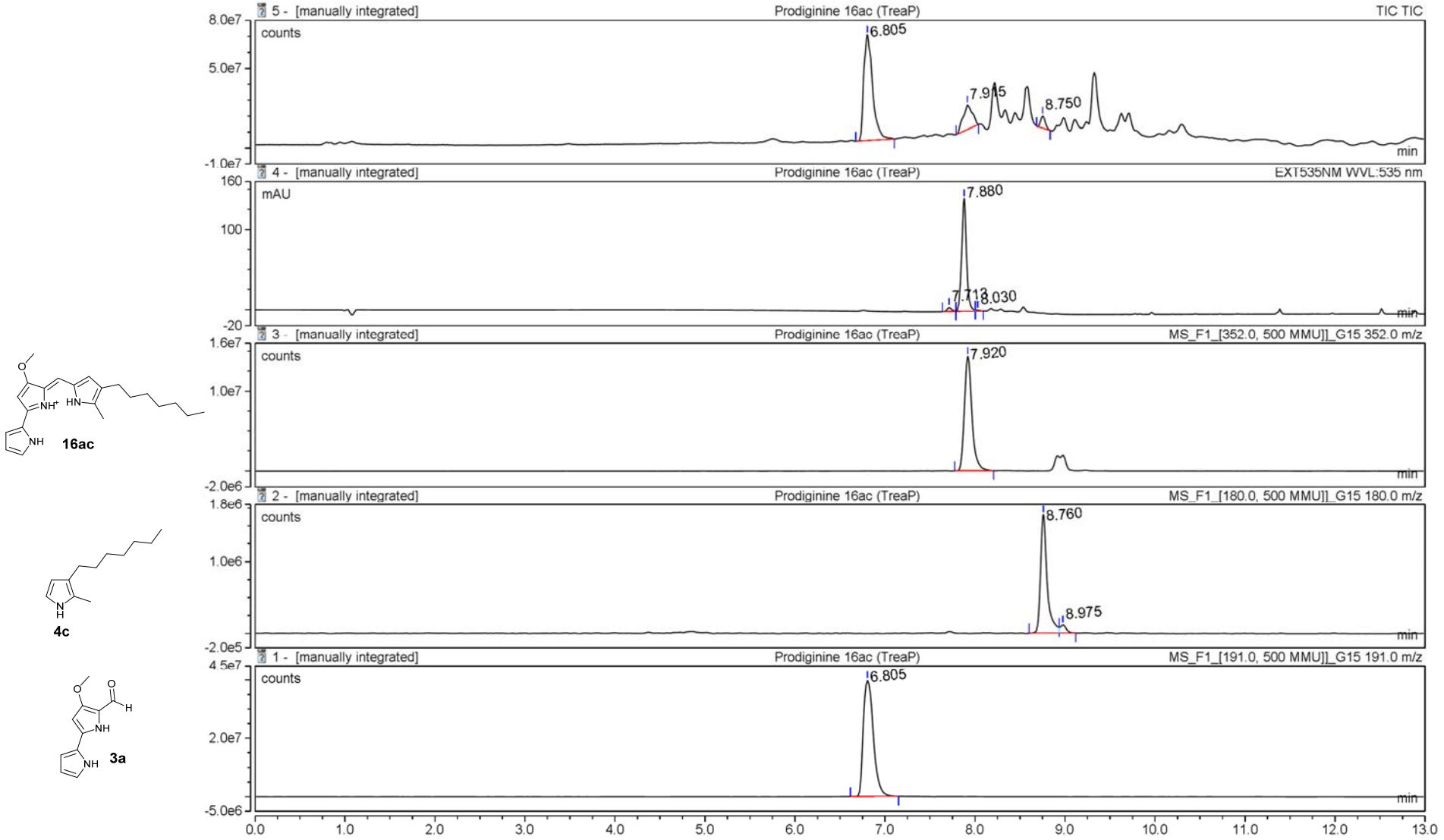


Figure S82: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ac** (m/z 352.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3a** (m/z 191.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3a**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ac** t_R 7.88 min.

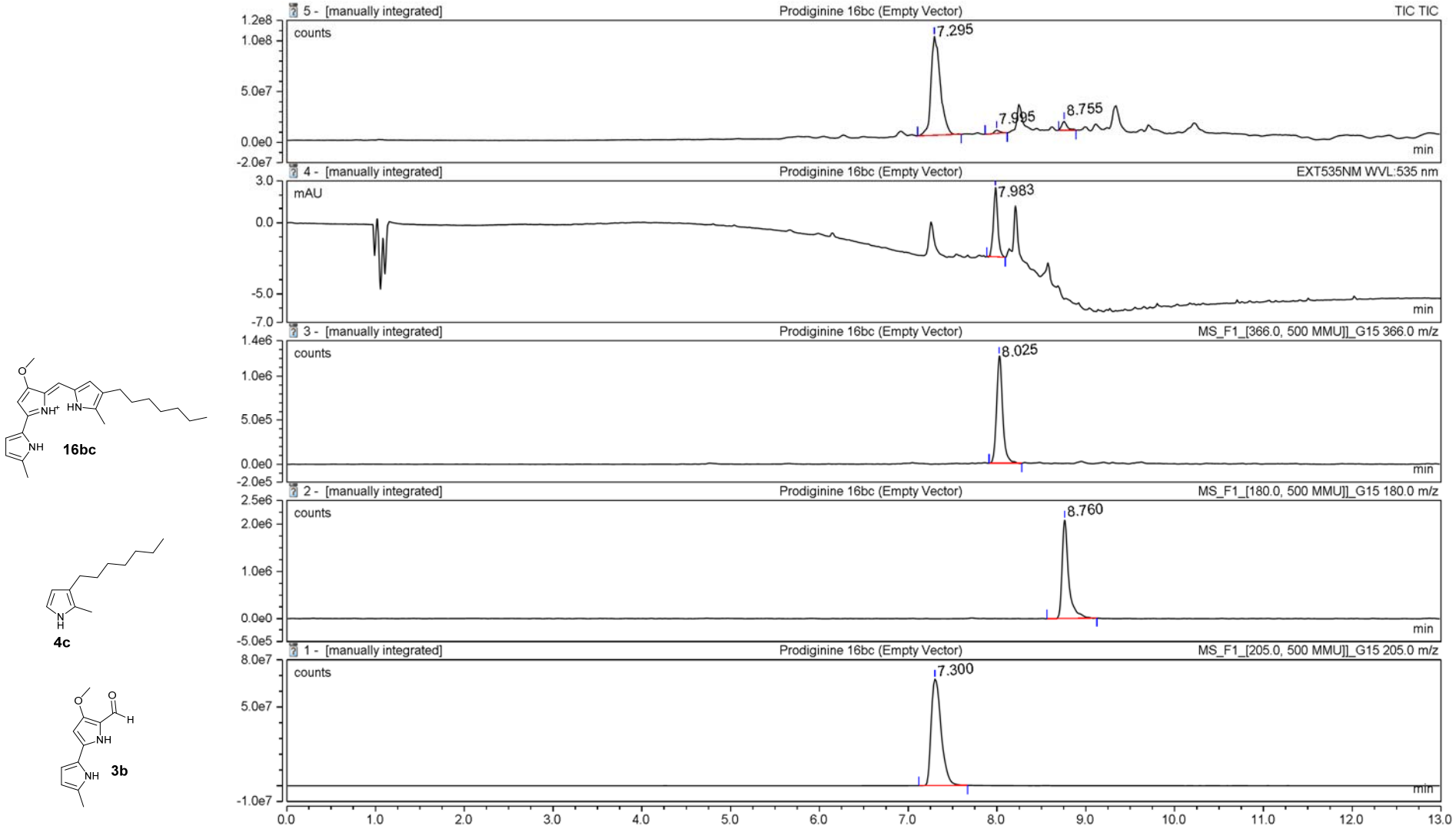


Figure S83: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16bc** (m/z 366.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16bc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

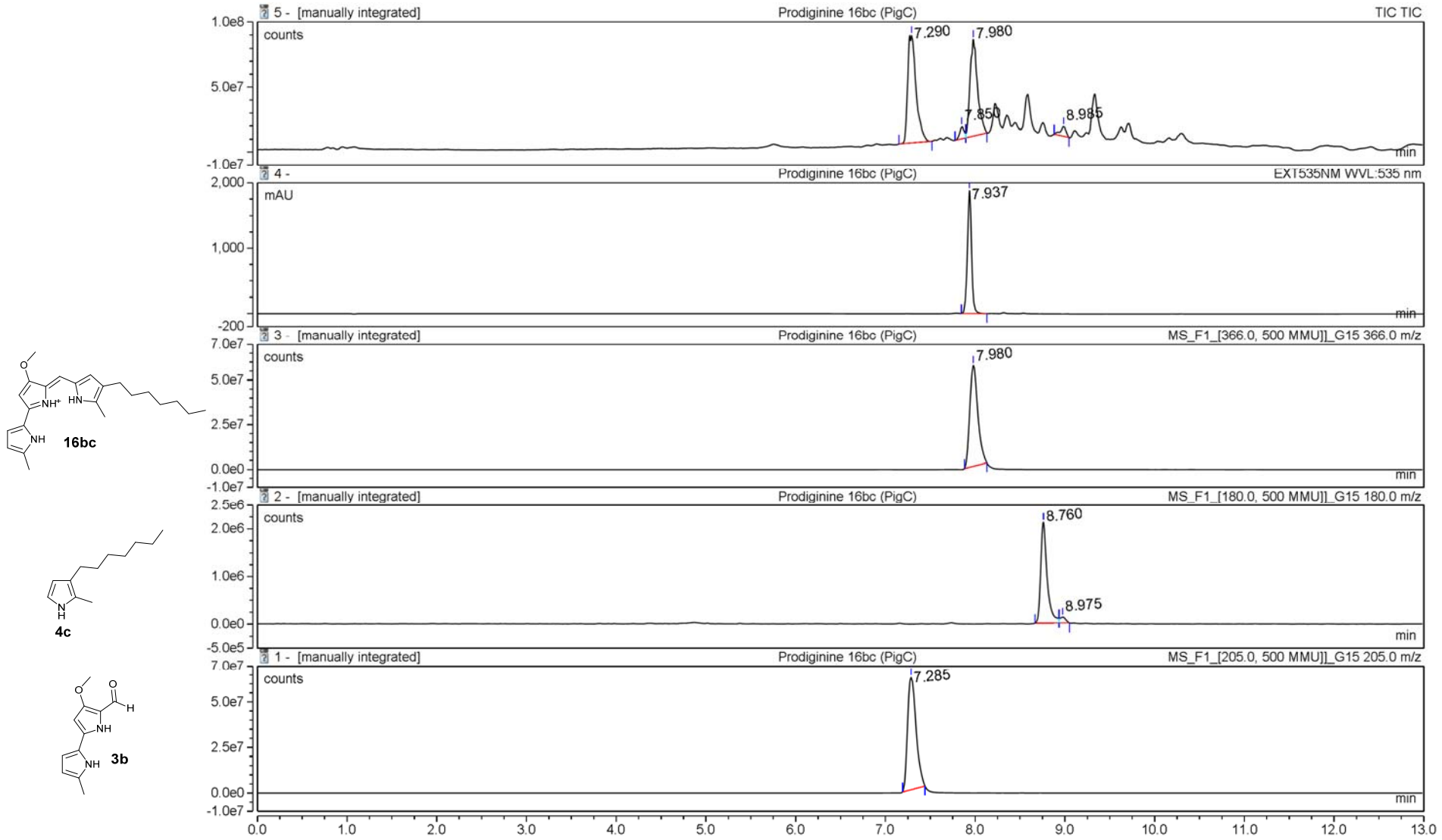


Figure S84: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bc** (m/z 366.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bc** t_R 7.94 min.

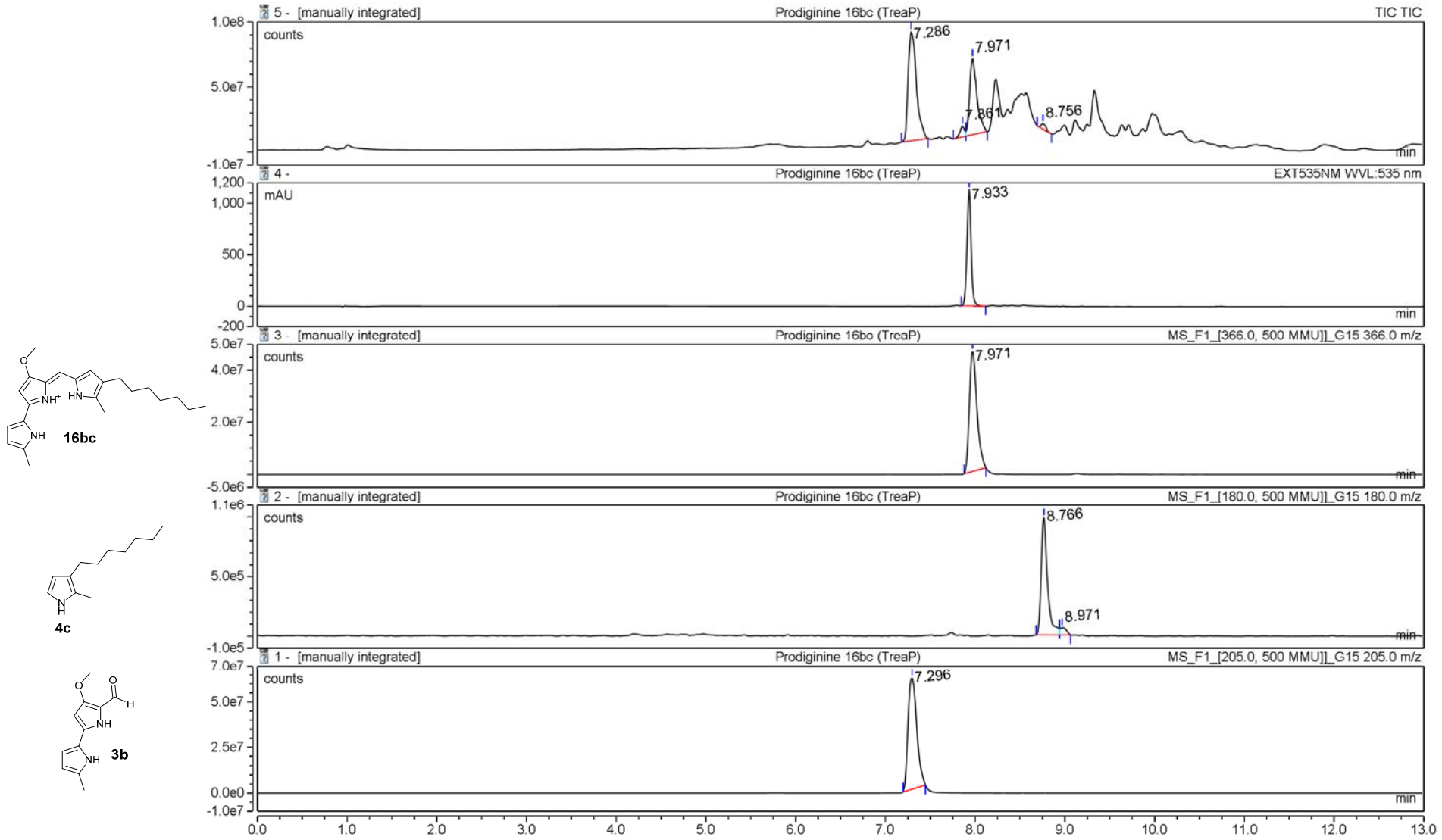


Figure S85: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bc** (m/z 366.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bc** t_R 7.93 min.

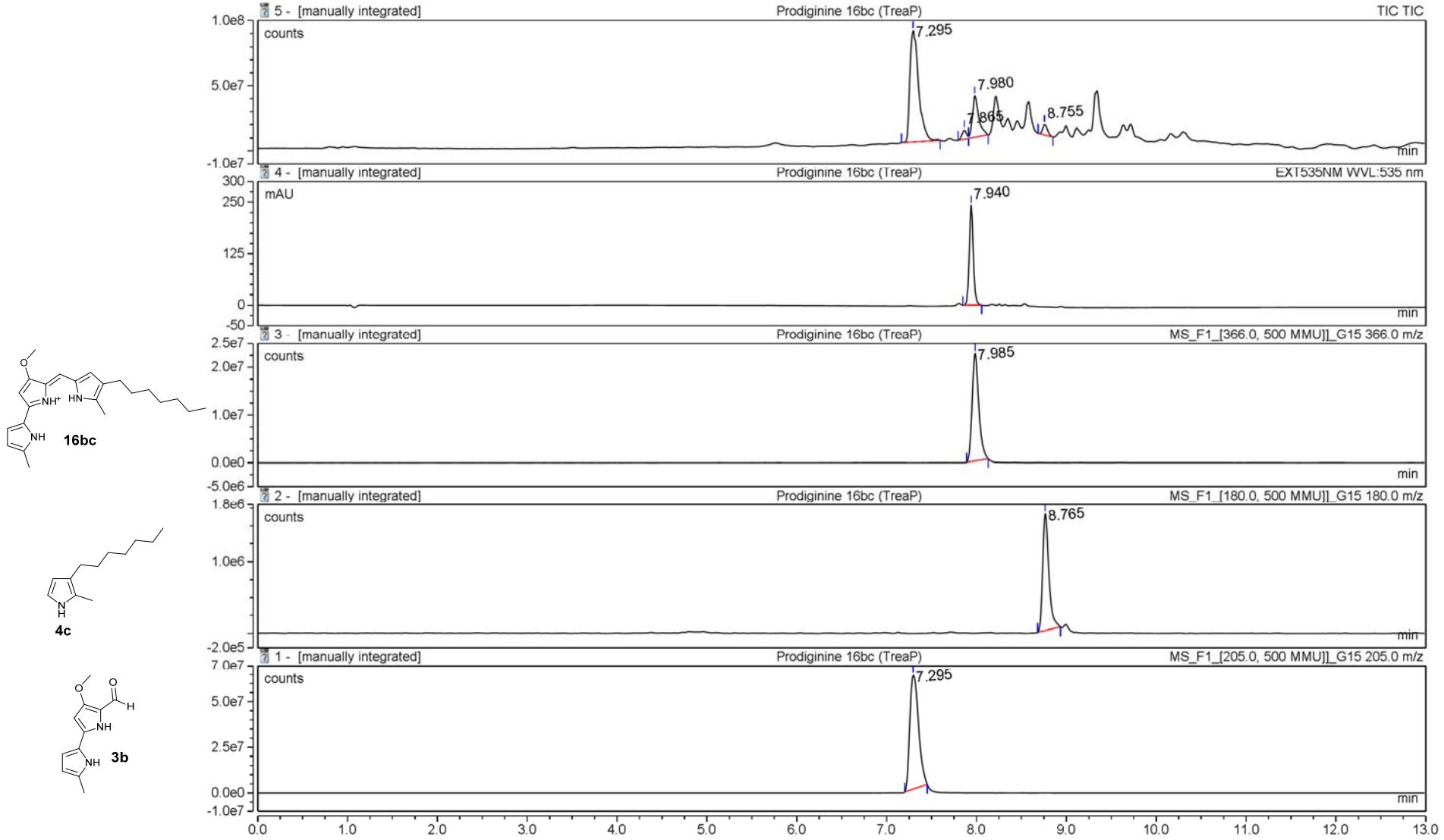


Figure S86: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16bc** (m/z 366.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3b** (m/z 205.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3b**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16bc** t_R 7.94 min.

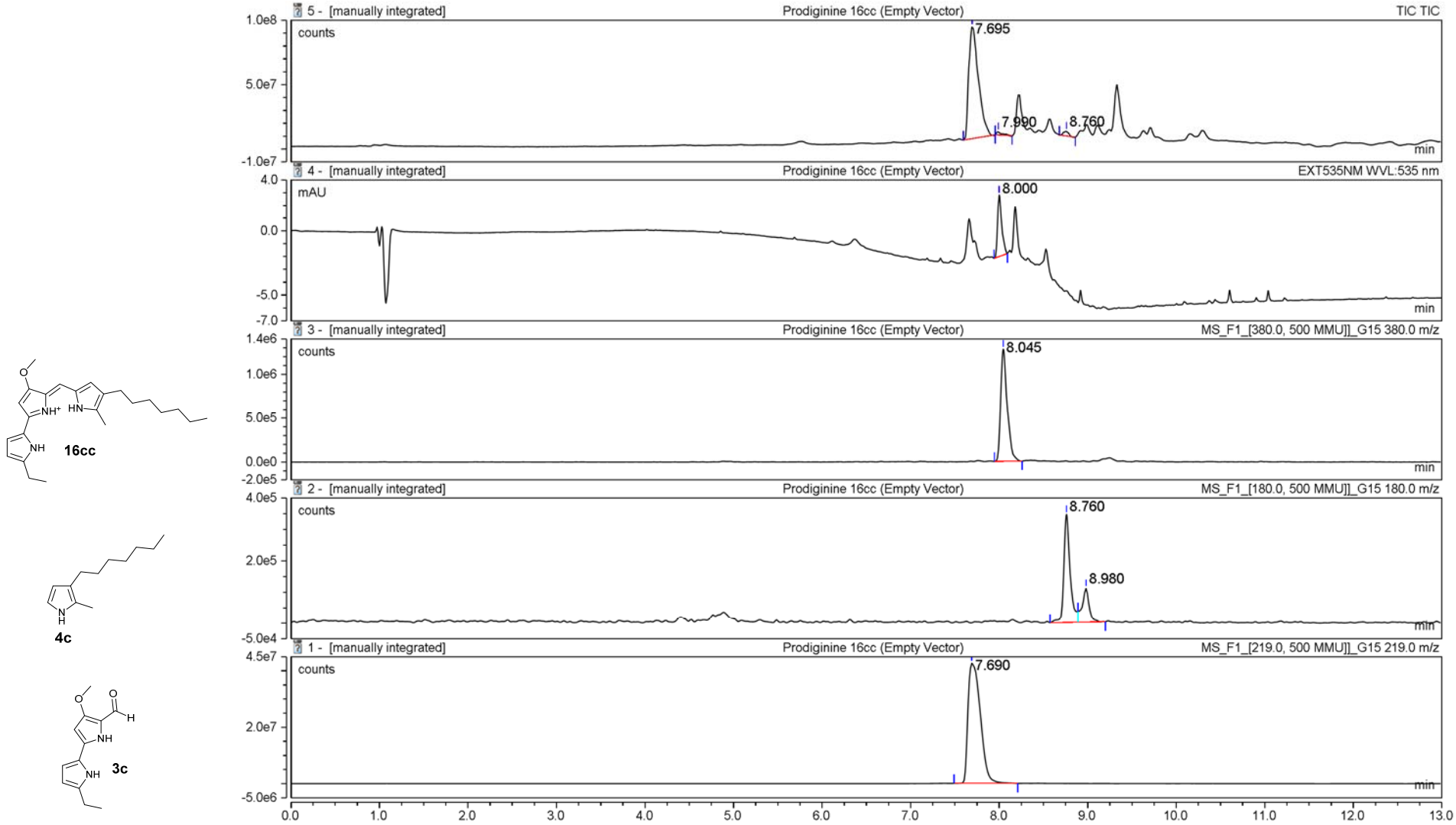


Figure S87: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16cc** (m/z 380.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16cc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

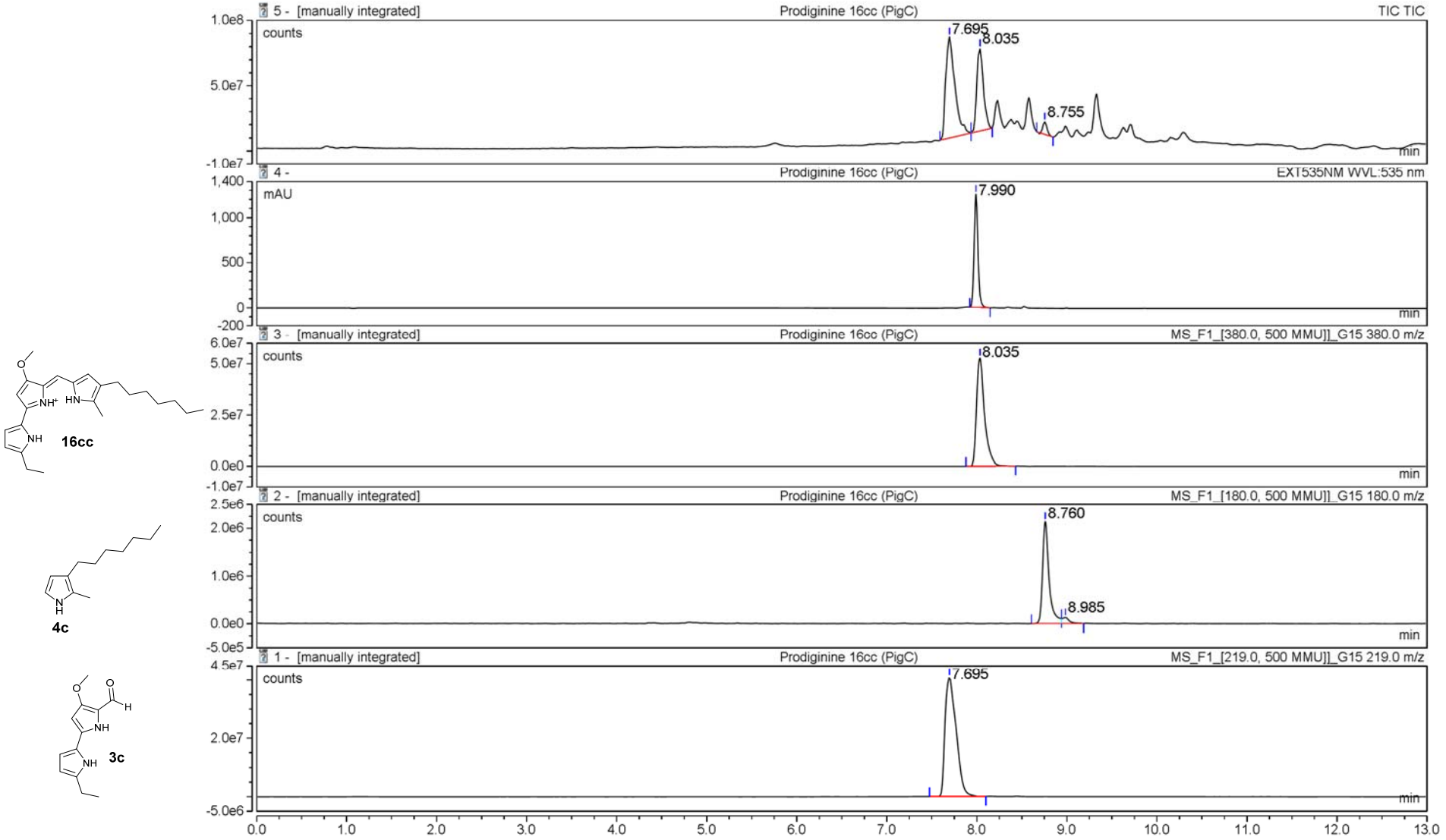


Figure S88: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cc** (m/z 380.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cc** t_R 7.99 min.

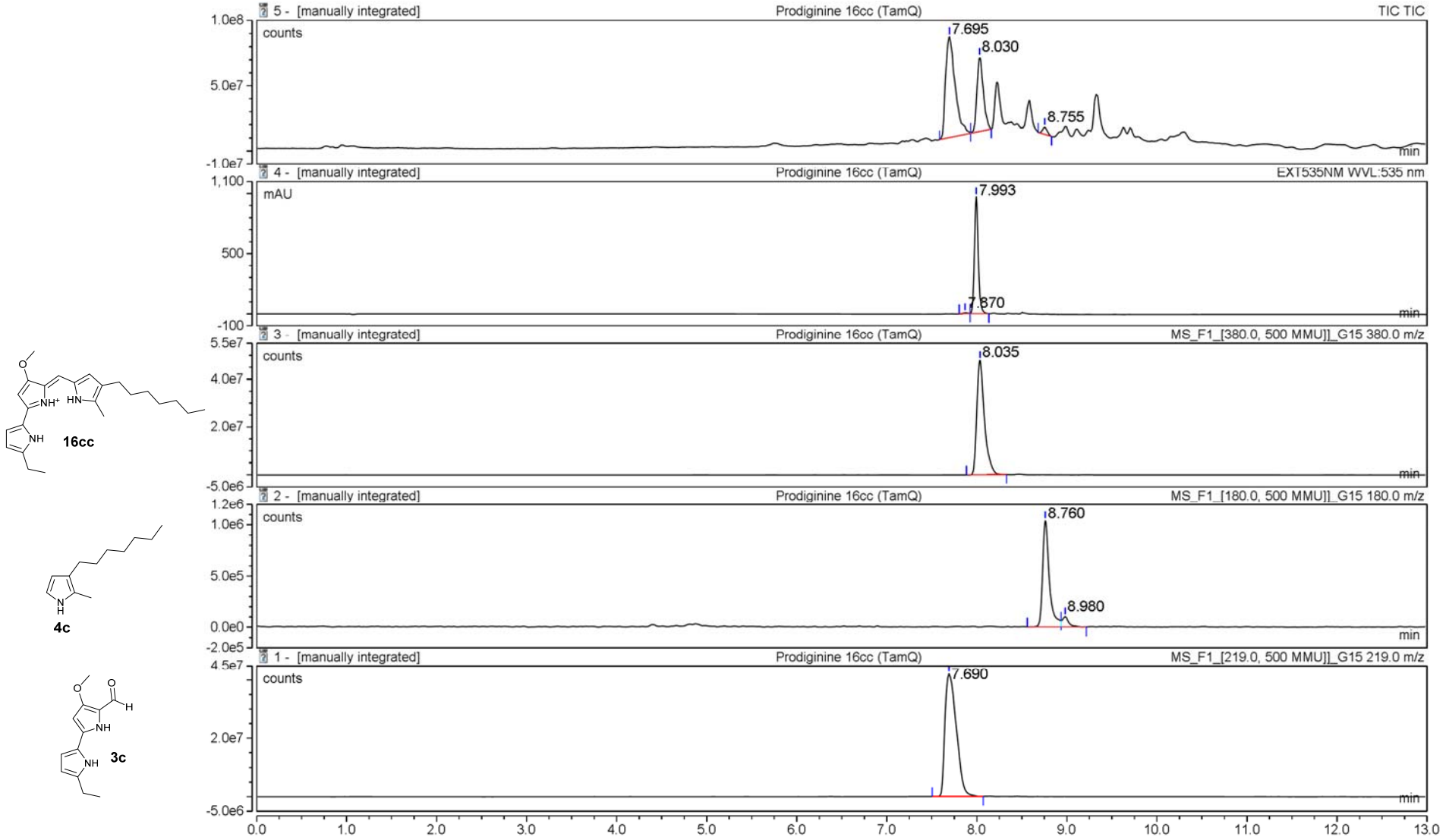


Figure S89: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cc** (m/z 380.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cc** t_R 7.99 min.

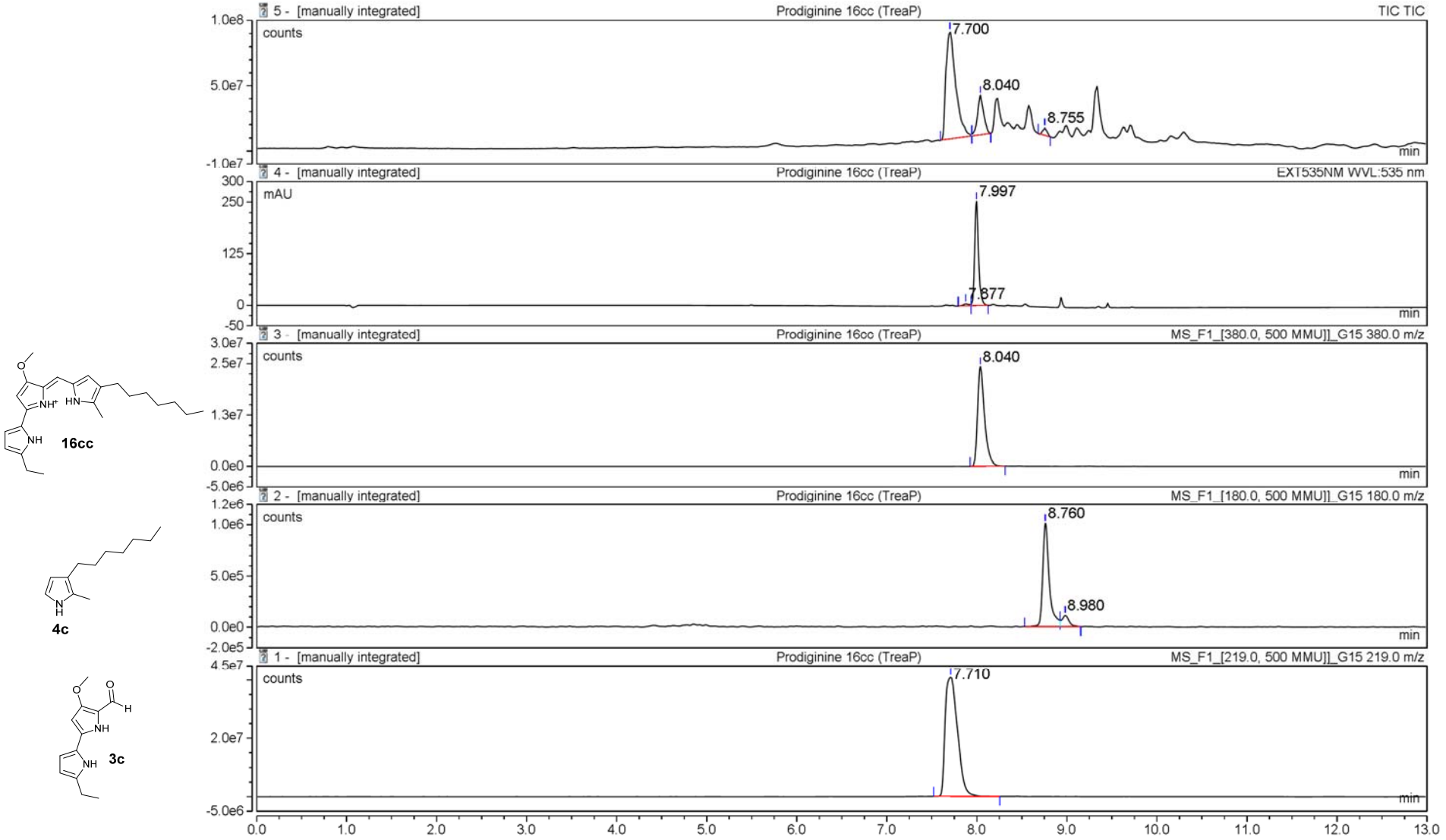


Figure S90: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16cc** (m/z 380.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3c** (m/z 219.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3c**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16cc** t_R 8.00 min.

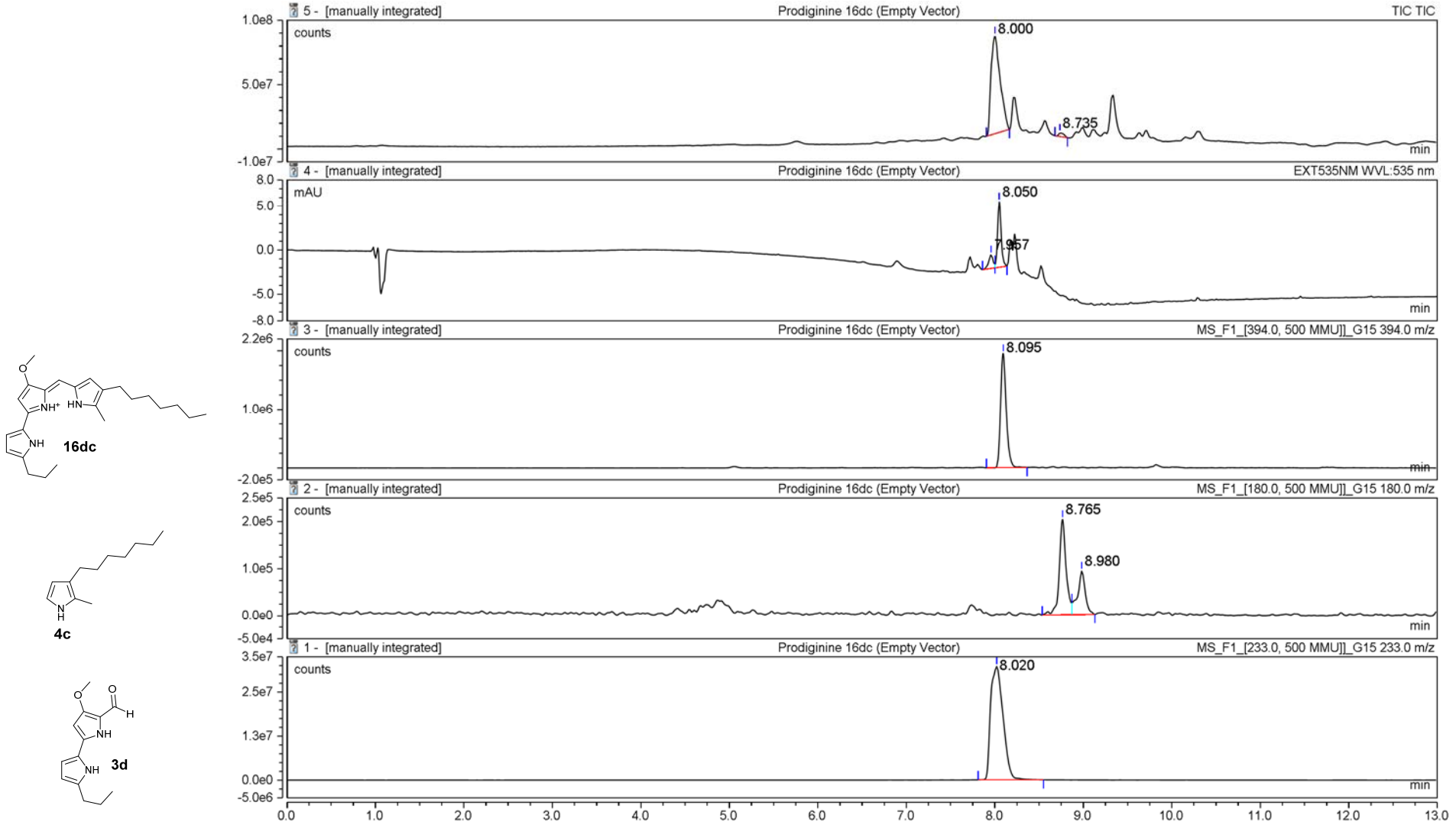


Figure S91: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16dc** (m/z 394.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16dc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

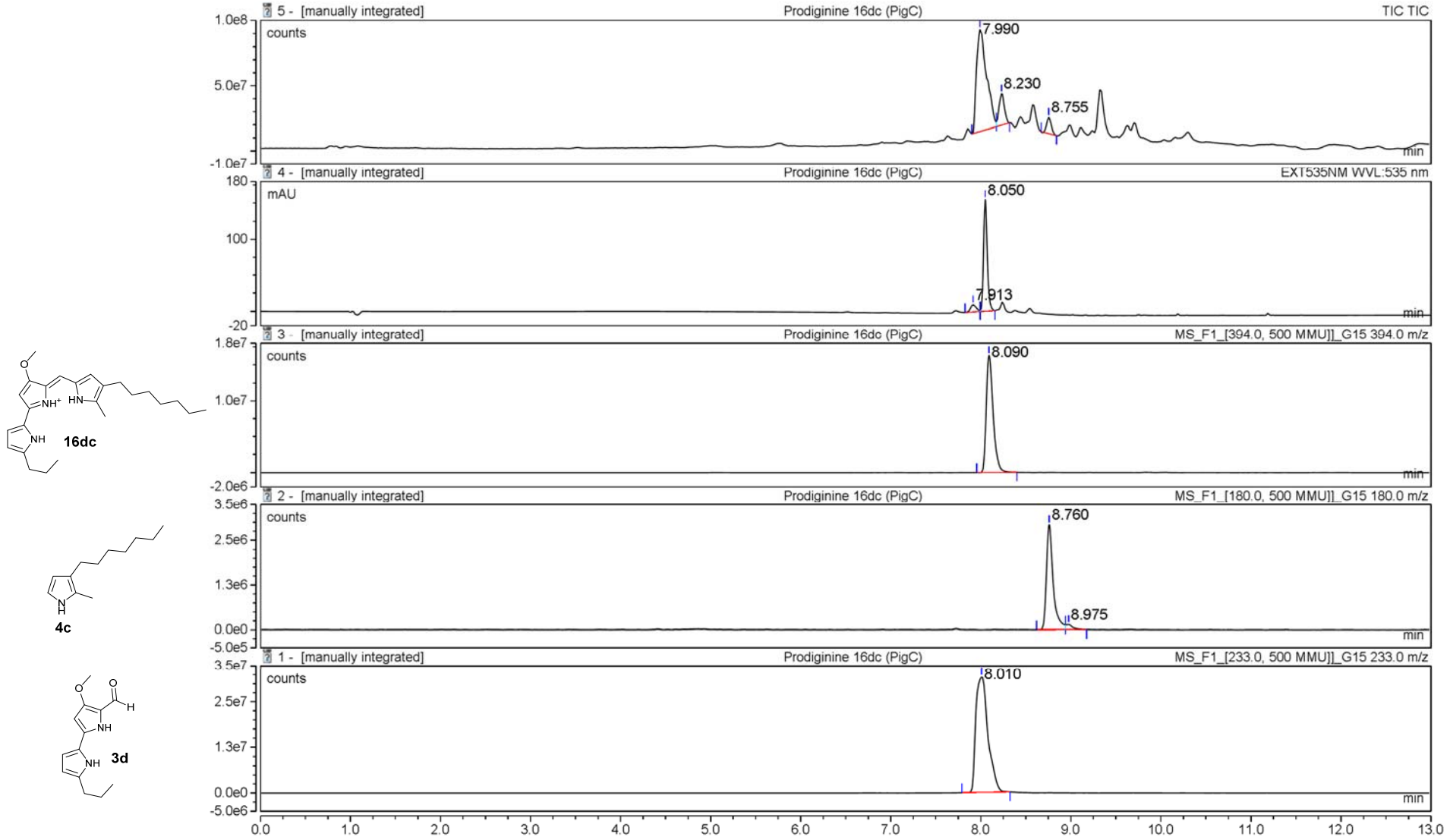


Figure S92: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16dc** (m/z 394.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16dc** t_R 8.05 min.

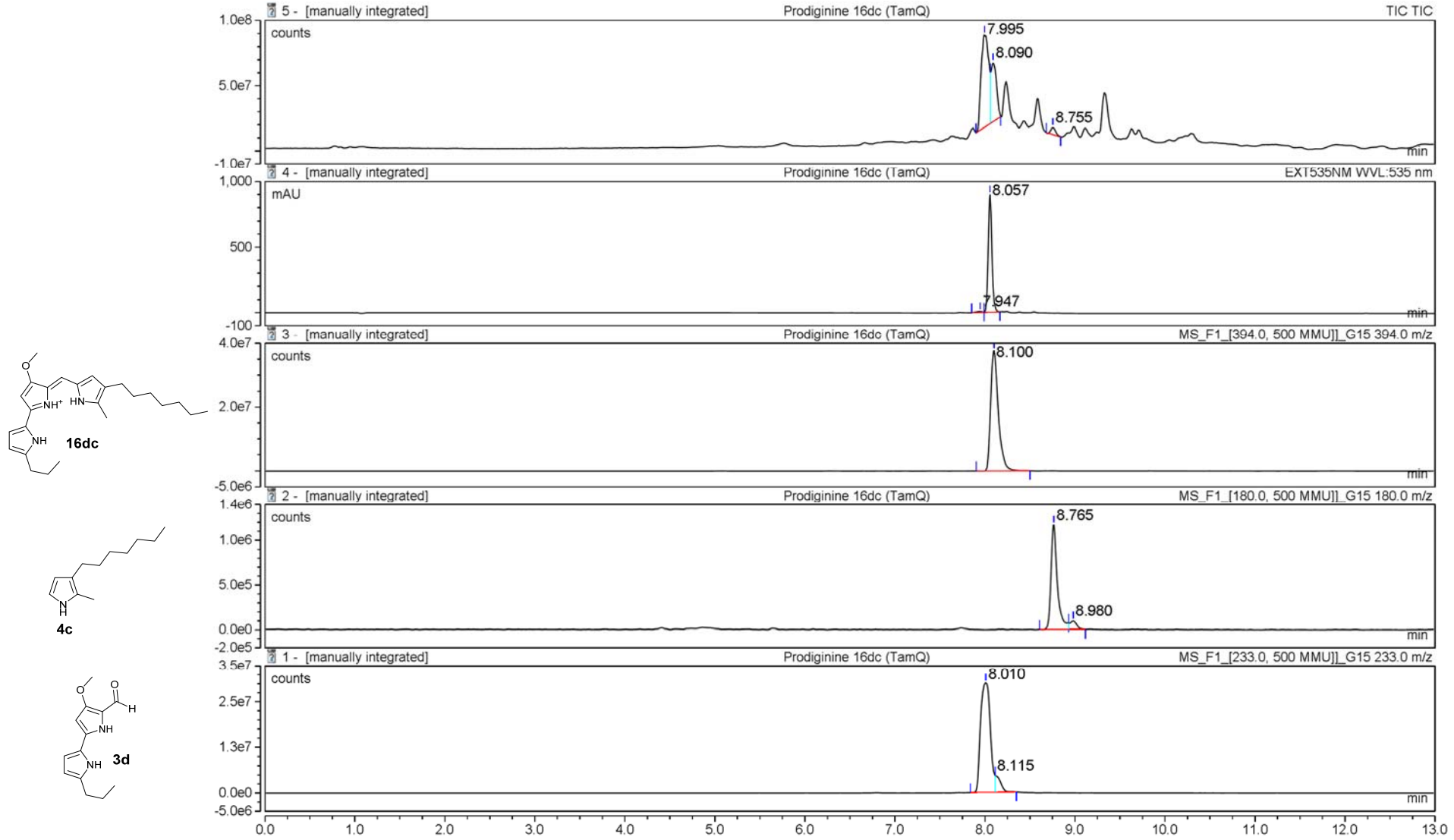


Figure S93: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16dc** (m/z 394.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16dc** t_R 8.06 min.

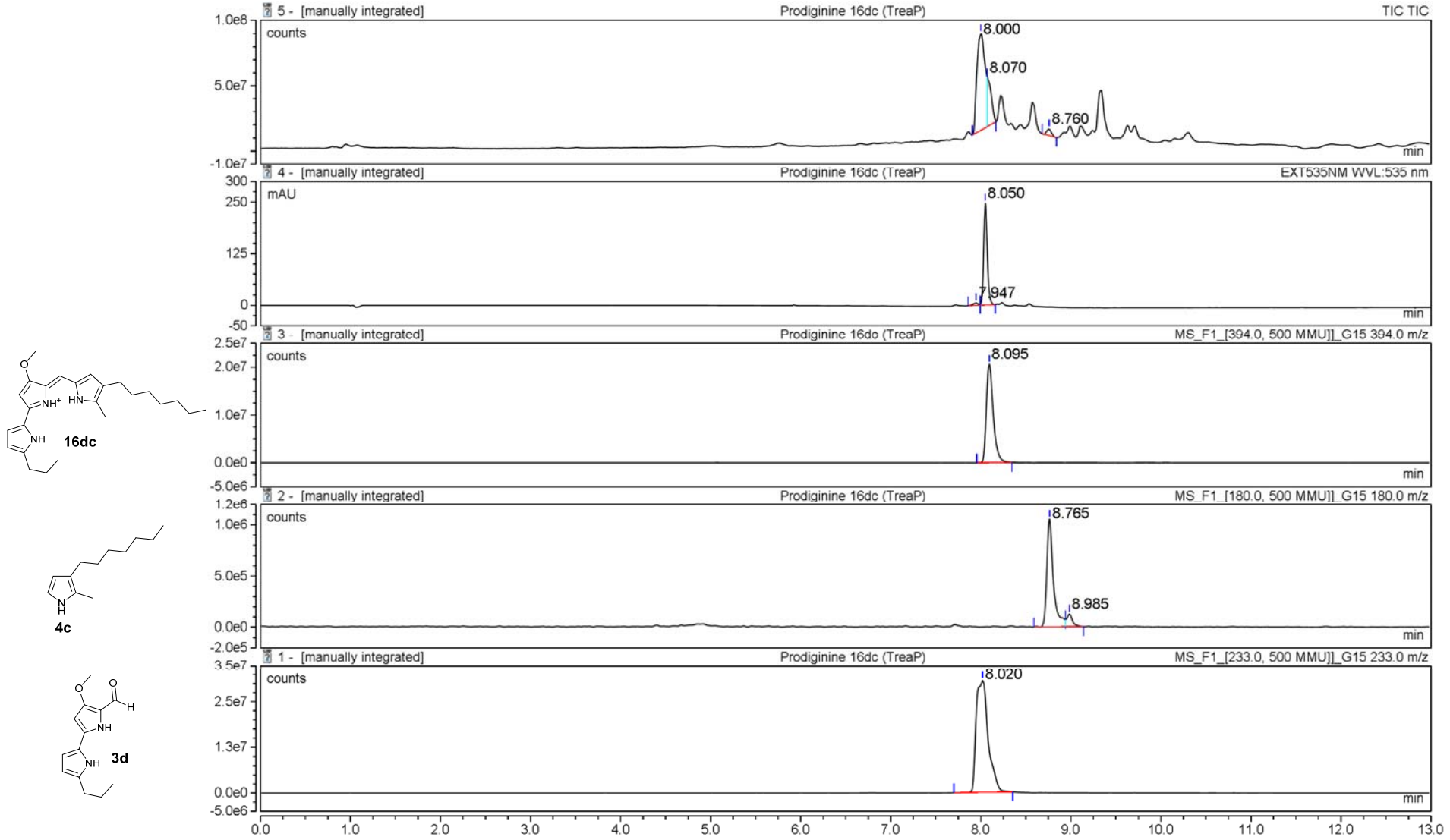


Figure S94: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16dc** (m/z 394.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3d** (m/z 233.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3d**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16dc** t_R 8.05 min.

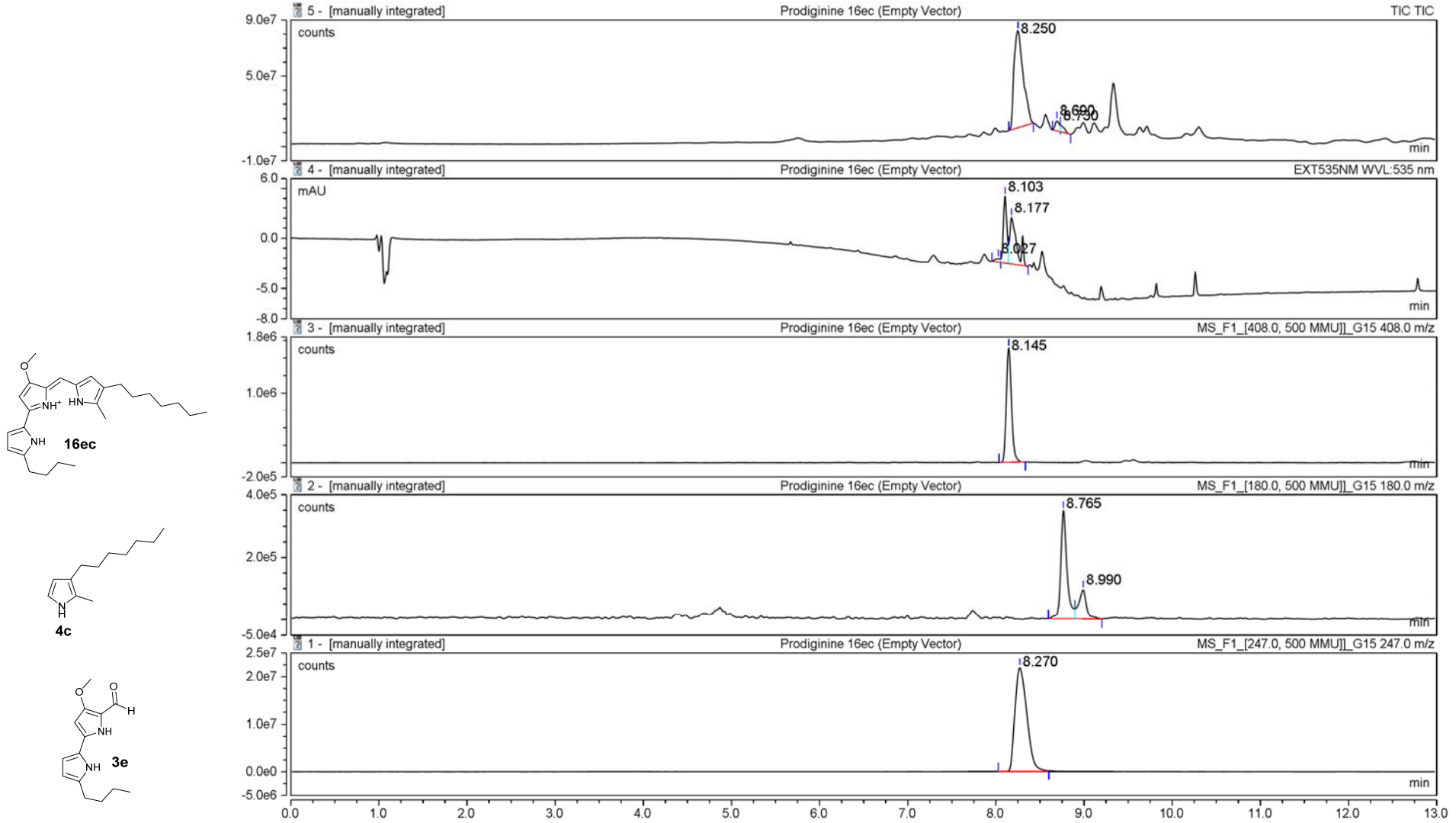


Figure S95: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16ec** (m/z 408.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16ec** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

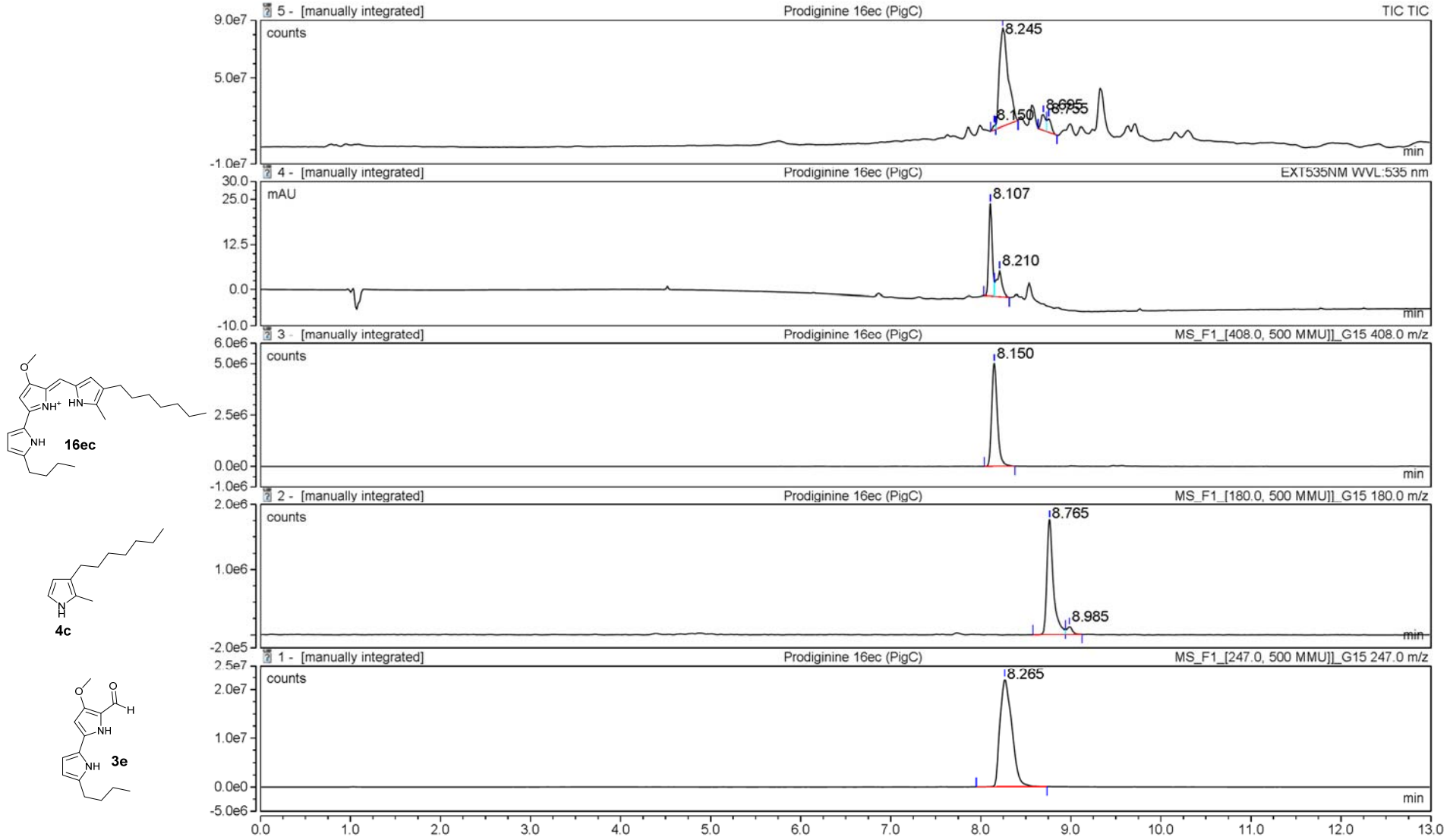


Figure S96: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ec** (m/z 408.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ec** t_R 8.11 min.

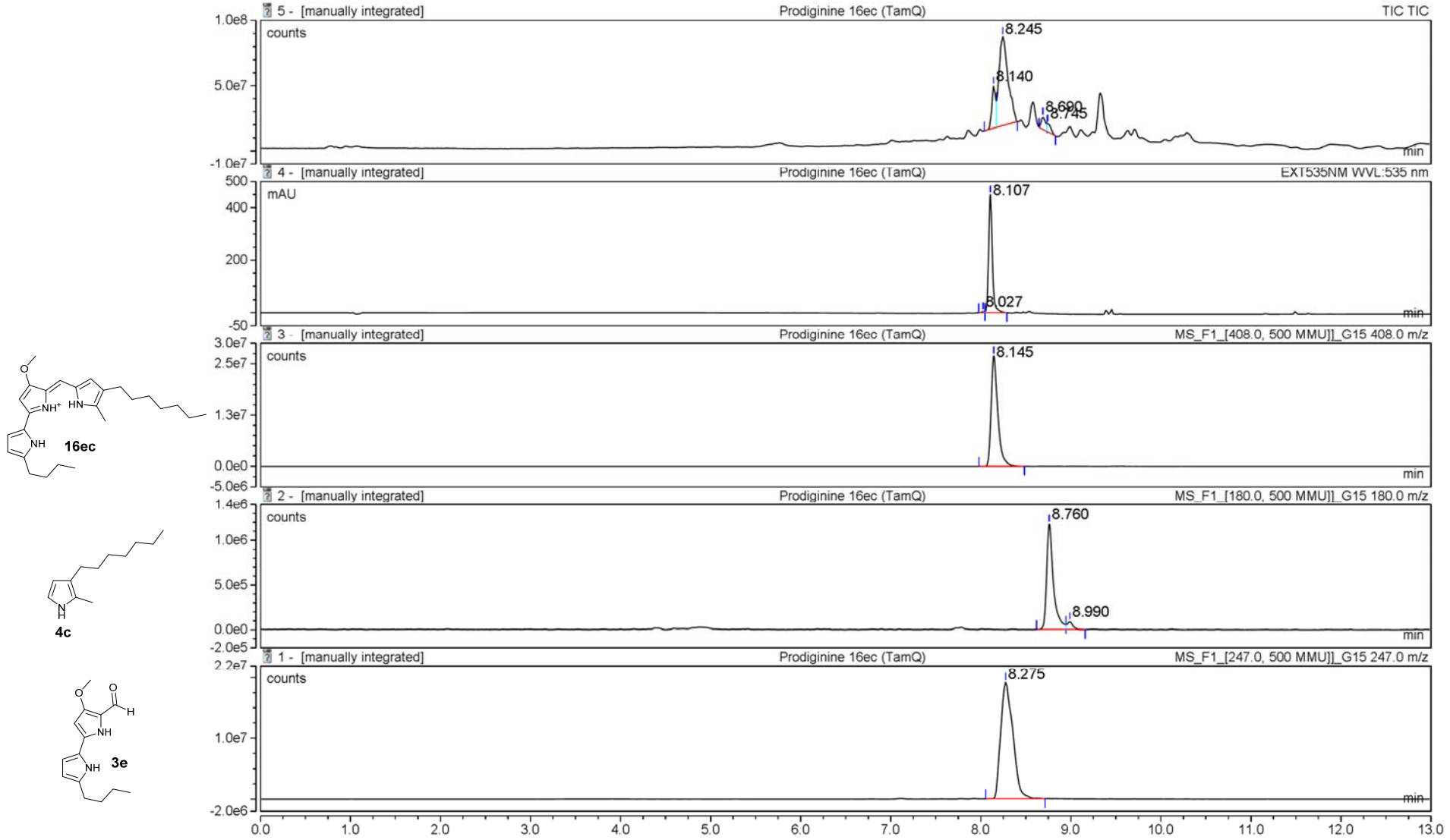


Figure S97: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ec** (m/z 408.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ec** t_R 8.11 min.

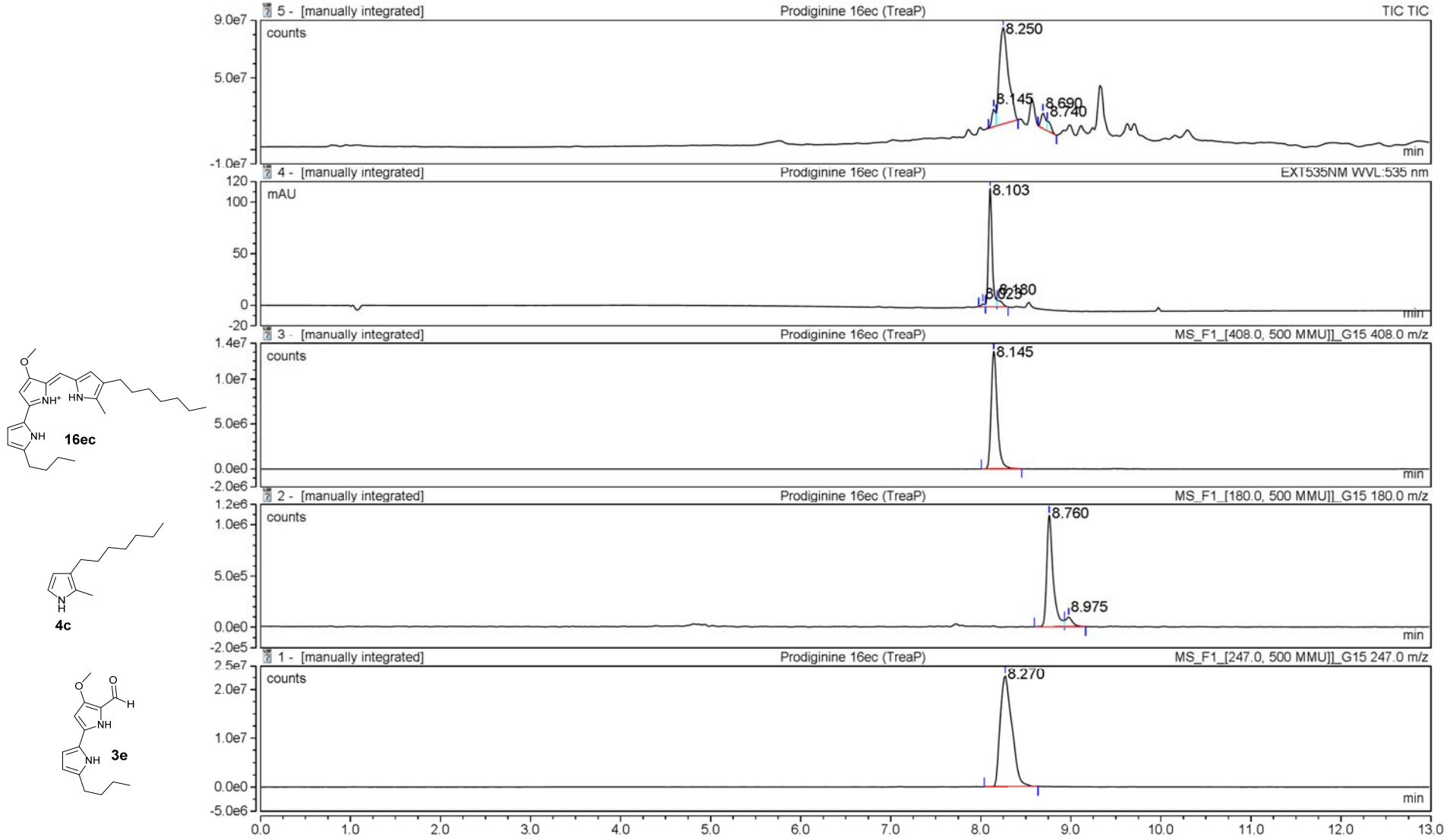


Figure S98: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16ec** (m/z 408.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3e** (m/z 247.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3e**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16ec** t_R 8.10 min.

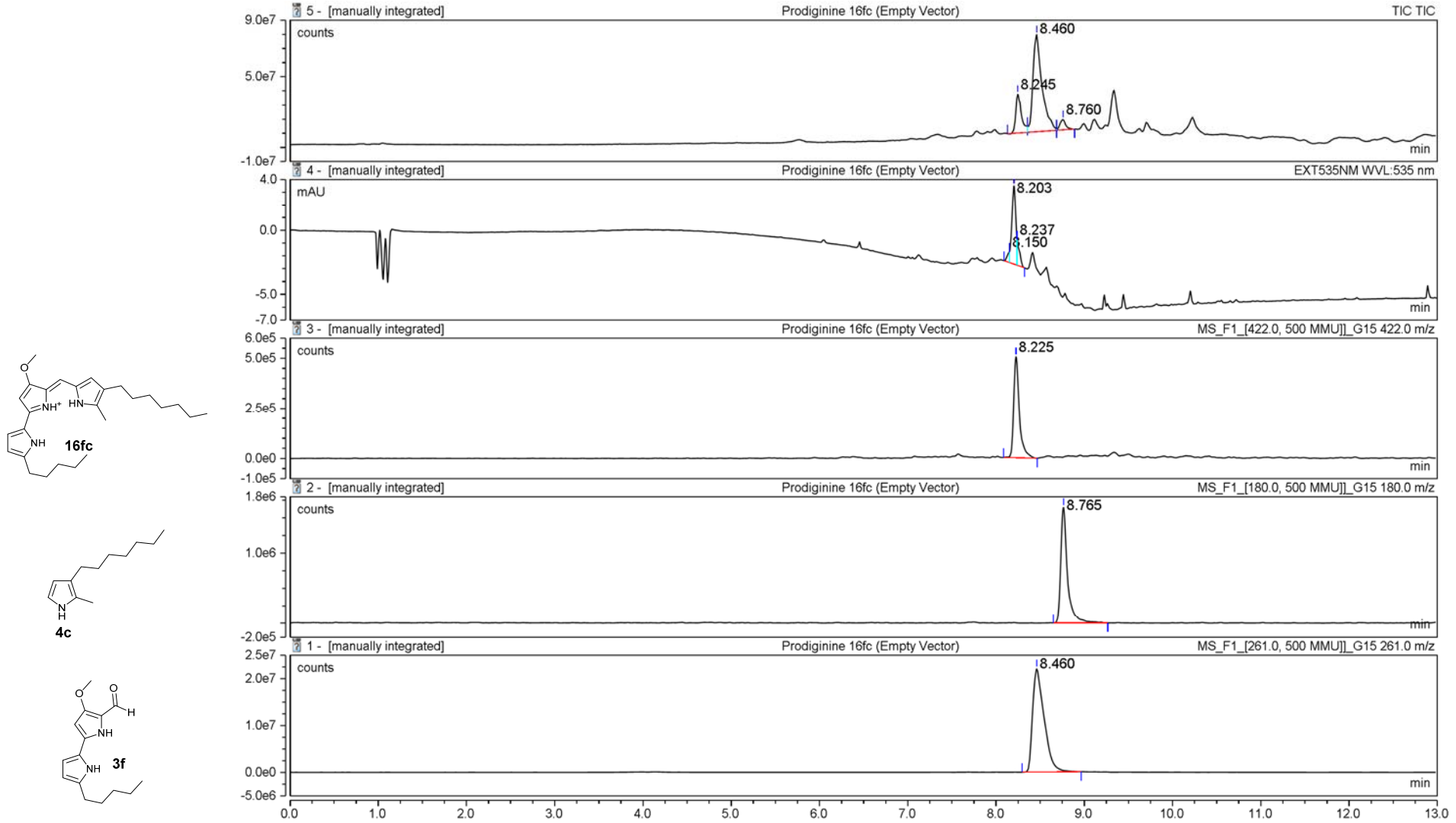


Figure S99: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16fc** (m/z 422.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16fc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

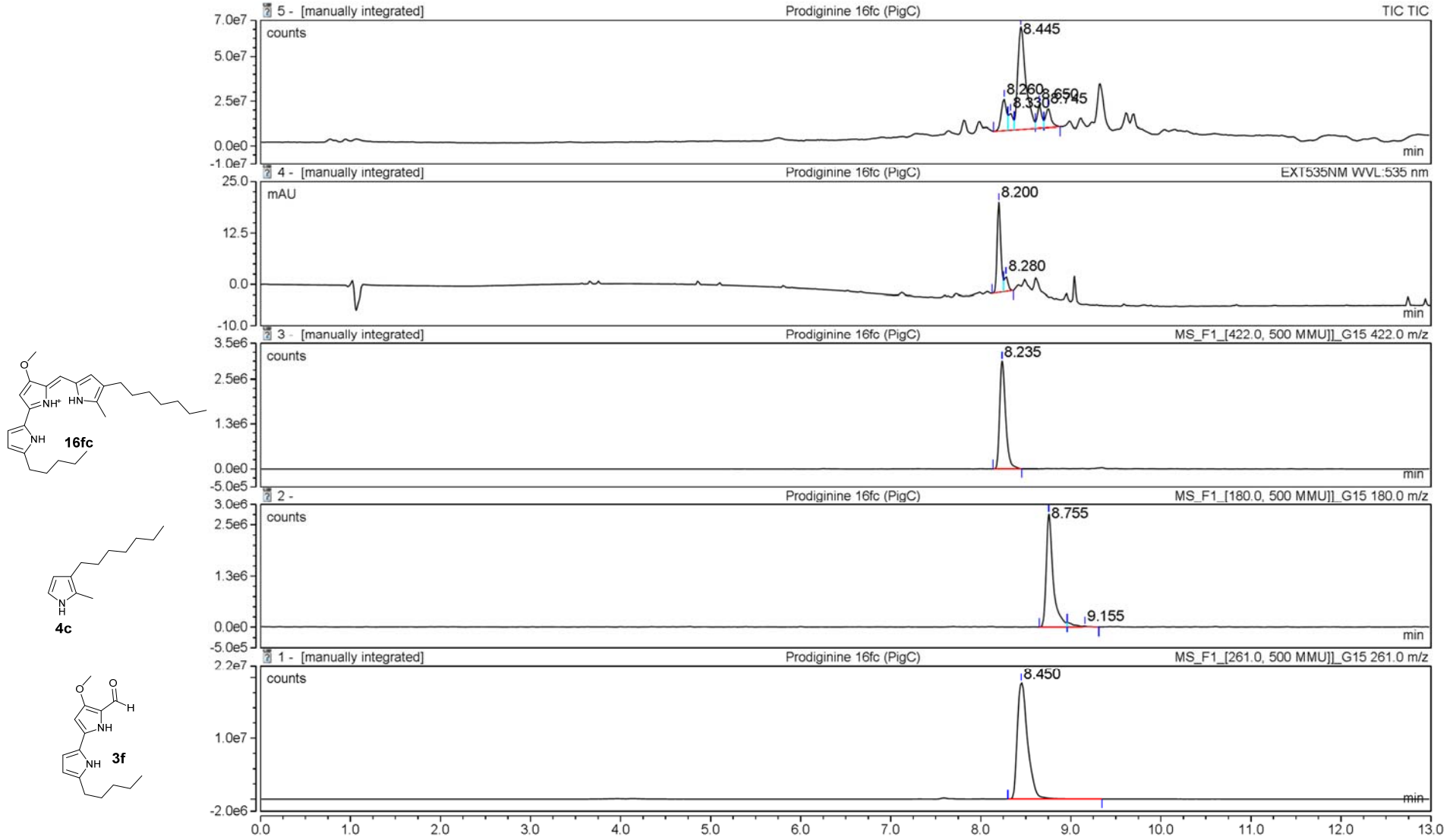


Figure S100: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fc** (m/z 422.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fc** t_R 8.20 min.

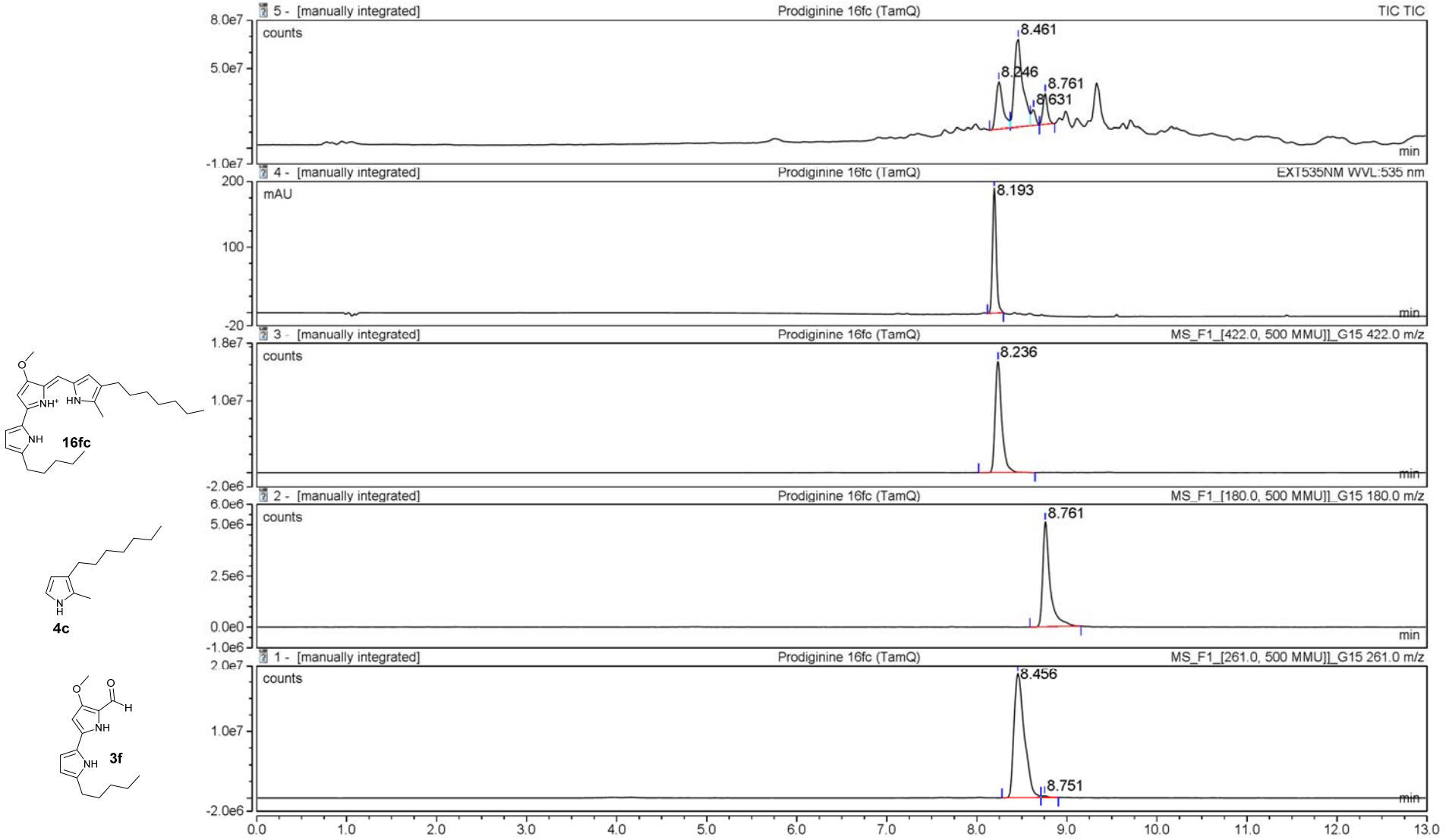


Figure S101: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fc** (m/z 422.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fc** t_R 8.19 min.

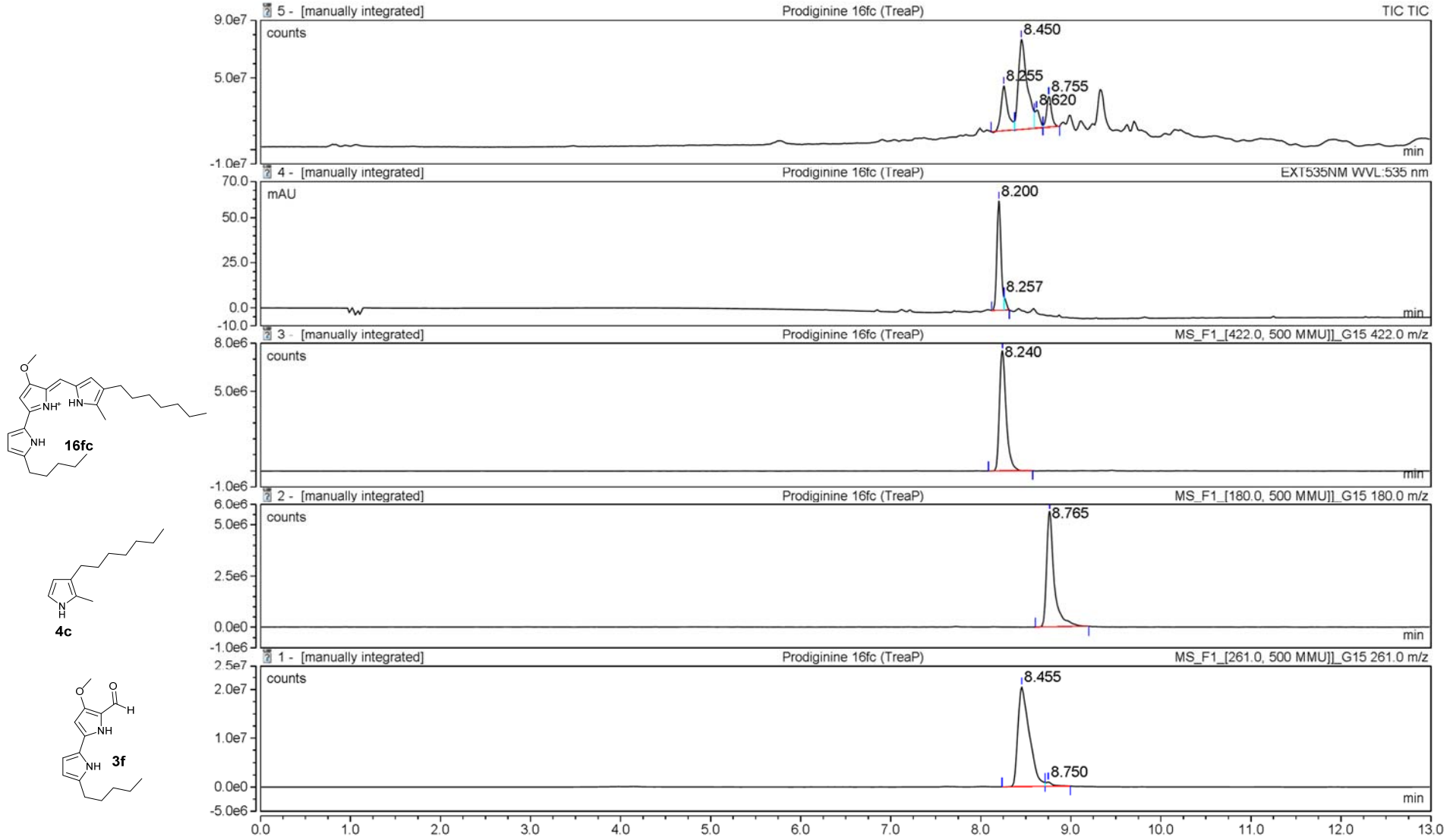


Figure S102: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16fc** (m/z 422.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3f** (m/z 261.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3f**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16fc** t_R 8.20 min.

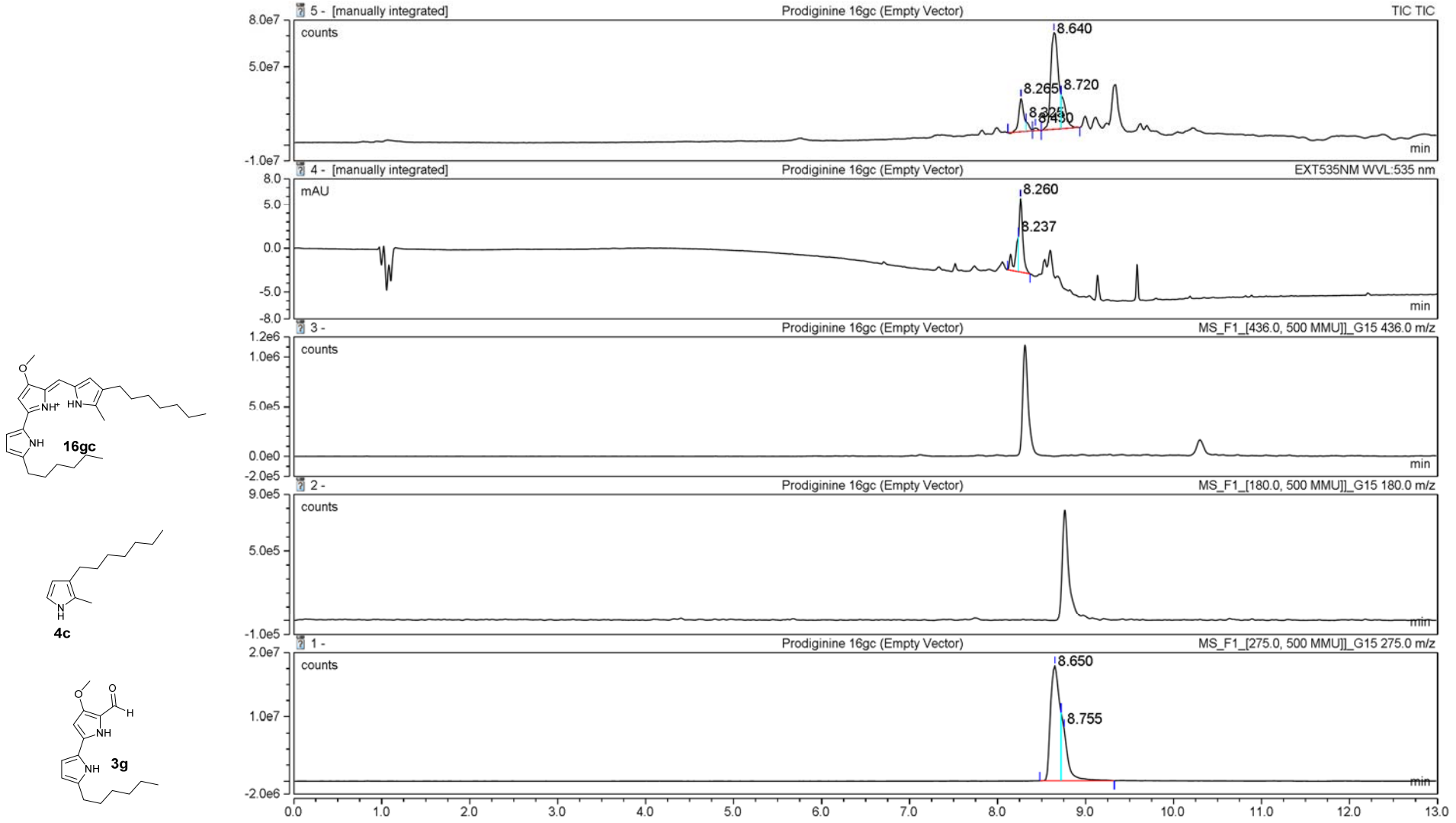


Figure S103: LC-MS data [from top to bottom: TIC, UV_{535} trace, and extracted ion chromatograms (EIC) of the potential product prodigiosin **16gc** (m/z 436.0) and the educts pyrrole **4c** (m/z 180.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the biocatalytic empty vector control reaction between pyrrole **4c** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. The small amount of prodigiosin **16gc** found in the EIC (middle chromatogramm) can be traced back to acid-catalysed condensation between pyrrole and MBC during chromatography with 0.1% formic acid. Integration of the corresponding signal from the UV_{535} trace gives an integral of 0 mAU·min.

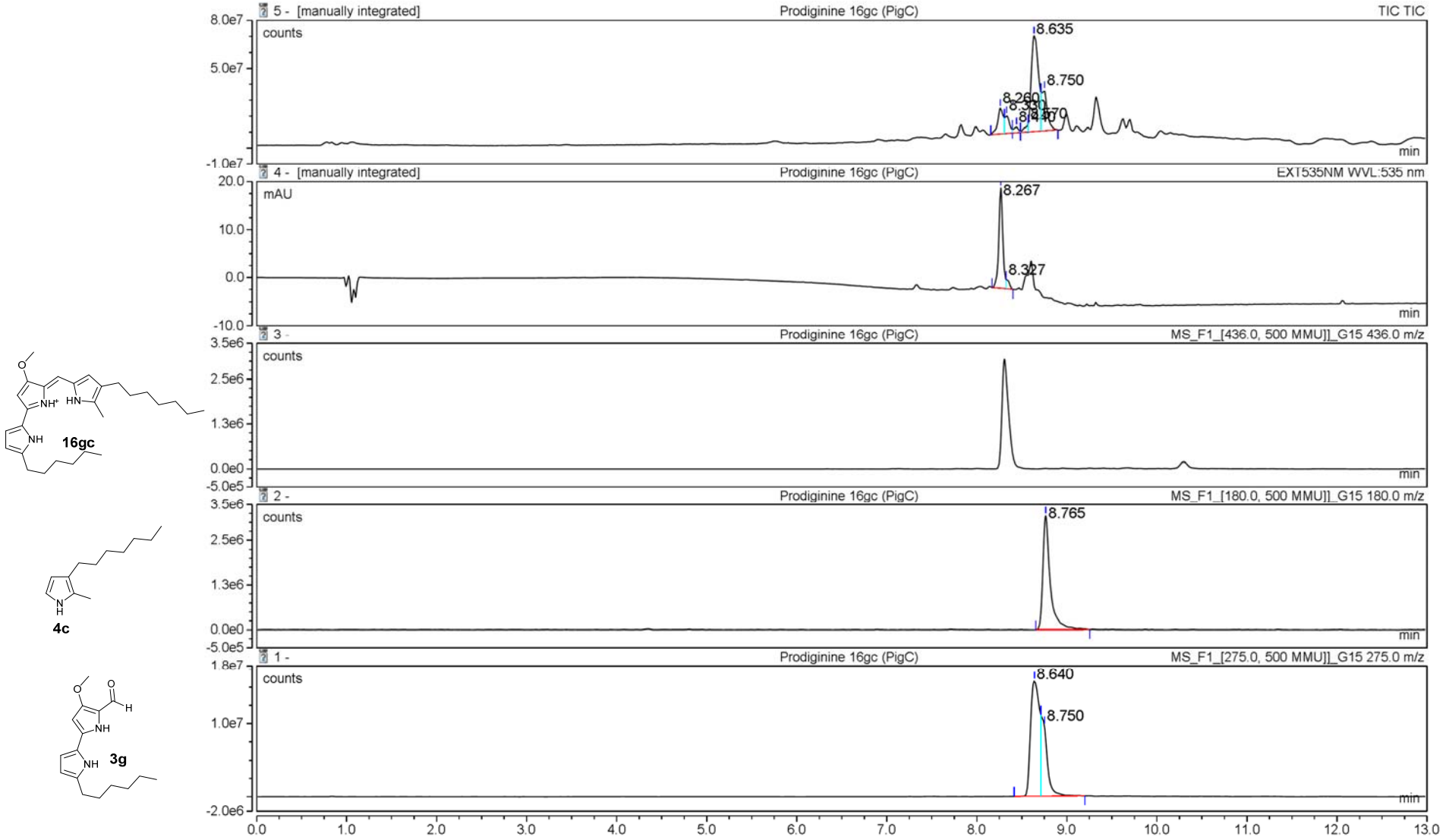


Figure S104: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gc** (m/z 436.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the PigC-catalysed condensation reaction between pyrrole **4c** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gc** t_R 8.27 min.

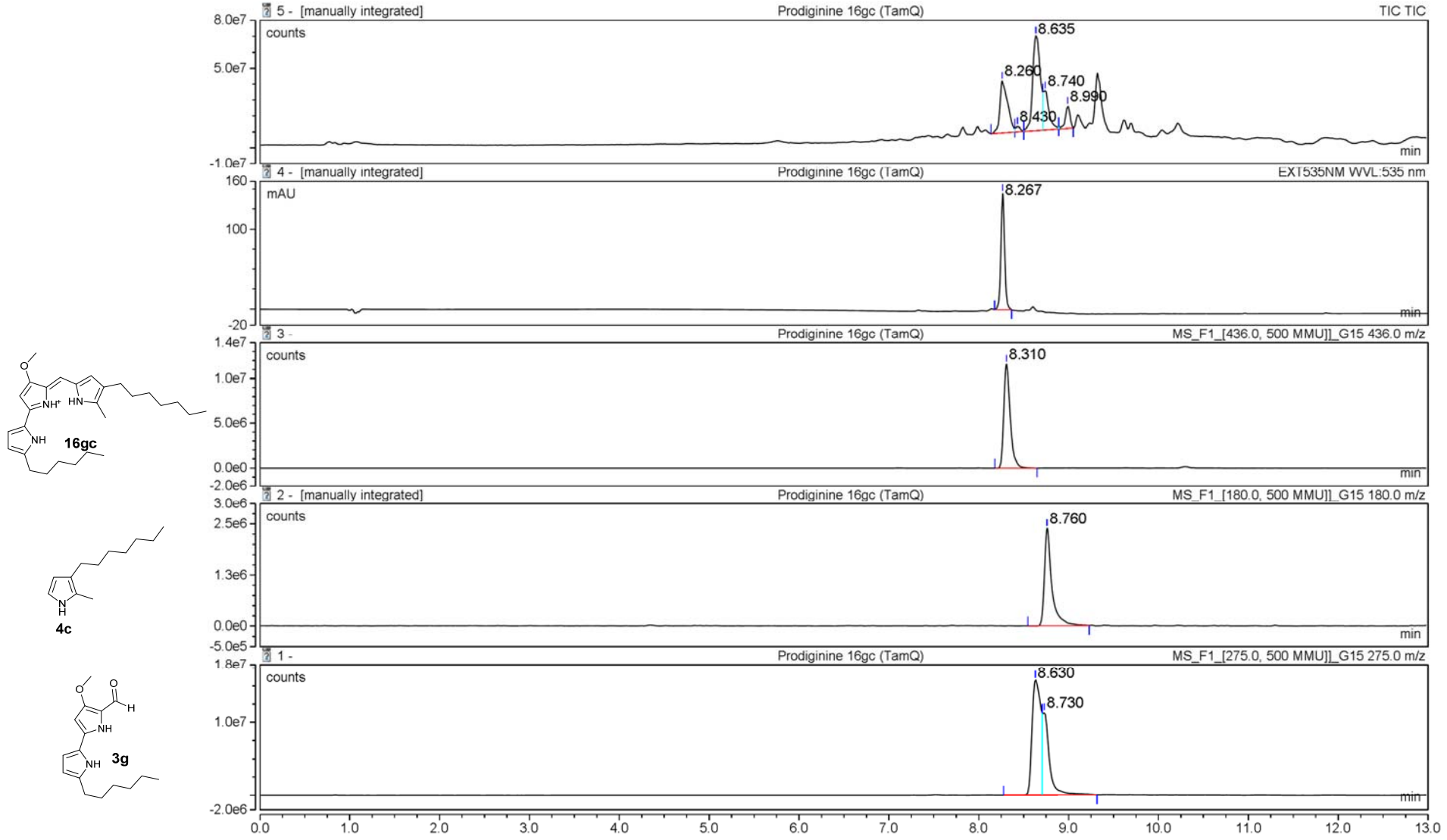


Figure S105: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gc** (m/z 436.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TamQ-catalysed condensation reaction between pyrrole **4c** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gc** t_R 8.27 min.

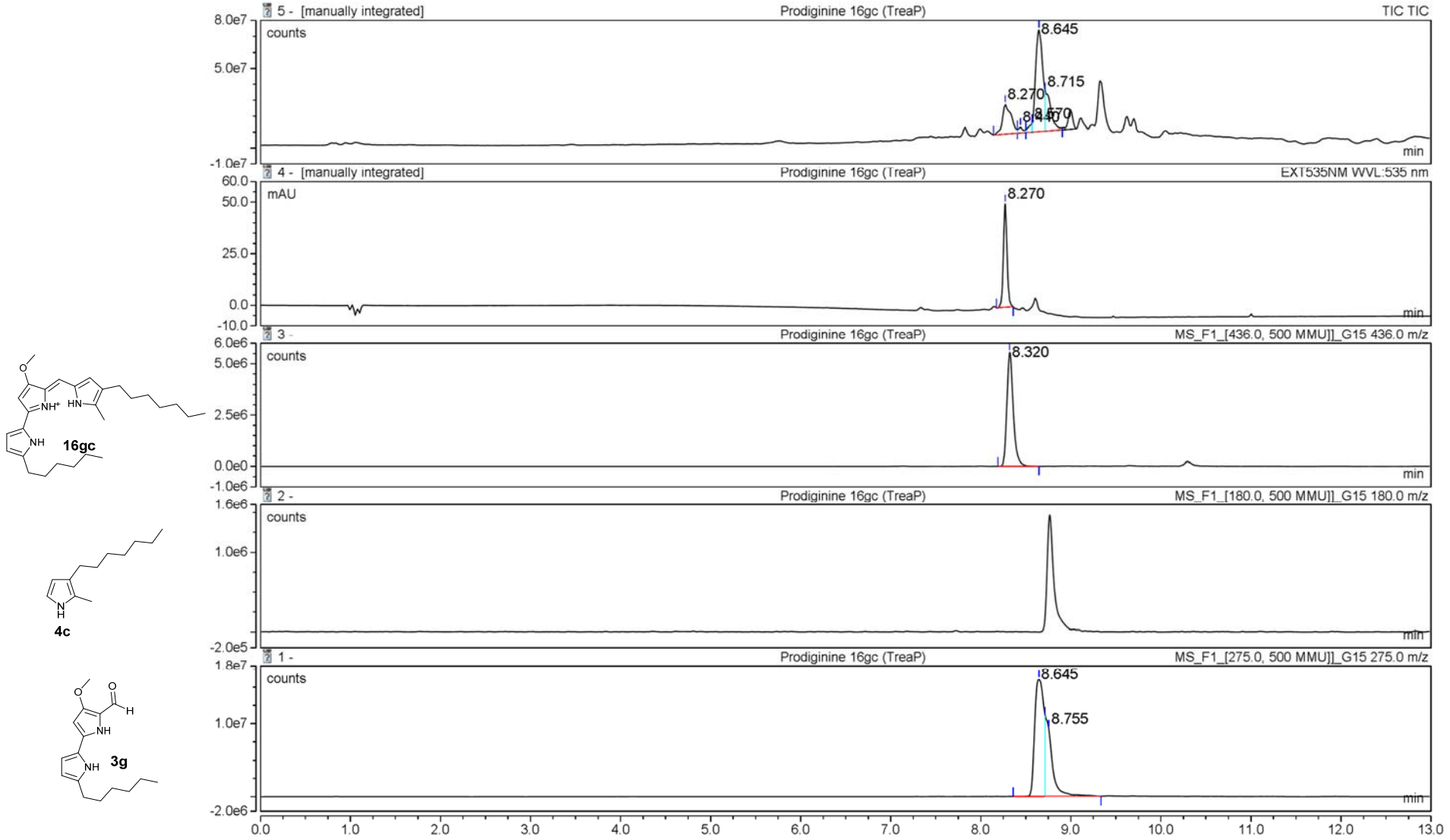


Figure S106: LC-MS data [from top to bottom: TIC, UV_{535} trace, and EIC of the potential product prodiginine **16gc** (m/z 436.0) and the educts pyrrole **4c** (m/z 191.0) and MBC **3g** (m/z 275.0)] from a representative methanolic reaction extract of the TreaP-catalysed condensation reaction between pyrrole **4c** and MBC **3g**. The structures of starting materials and the expected condensation product are shown on the left-hand site besides the corresponding EIC. Retention time of prodiginine **16gc** t_R 8.27 min.

13. LC-MS Chromatograms – Negative Controls

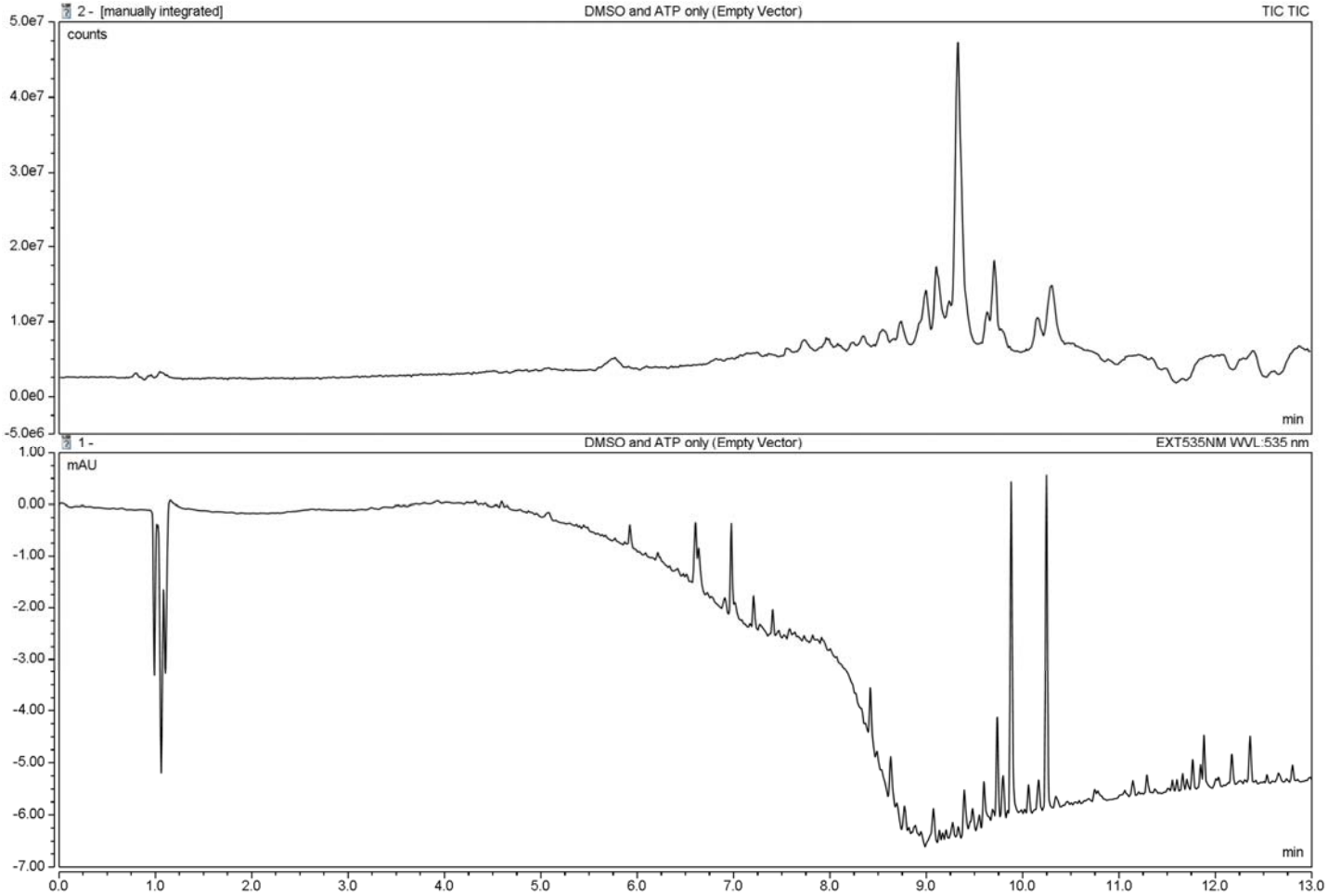


Figure S107: LC-MS data (TIC and UV_{535} trace) of the methanolic reaction extract from the empty vector negative control reaction, containing ATP and DMSO only, but no pyrroles or MBC derivatives.

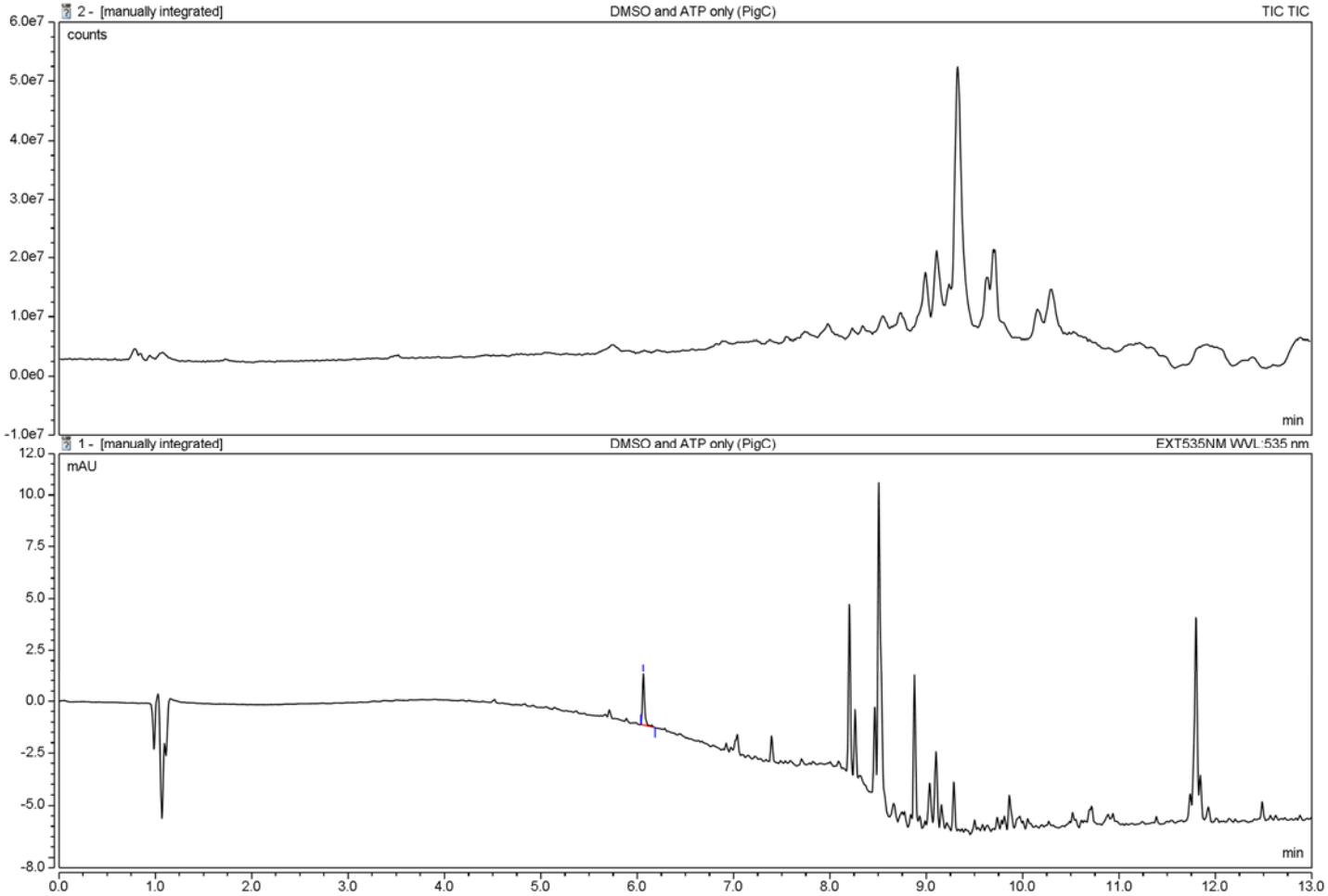


Figure S108: LC-MS data (TIC and UV_{535} trace) of the methanolic reaction extract from the PigC negative control reaction, containing ATP and DMSO only, but no pyrroles or MBC derivatives.

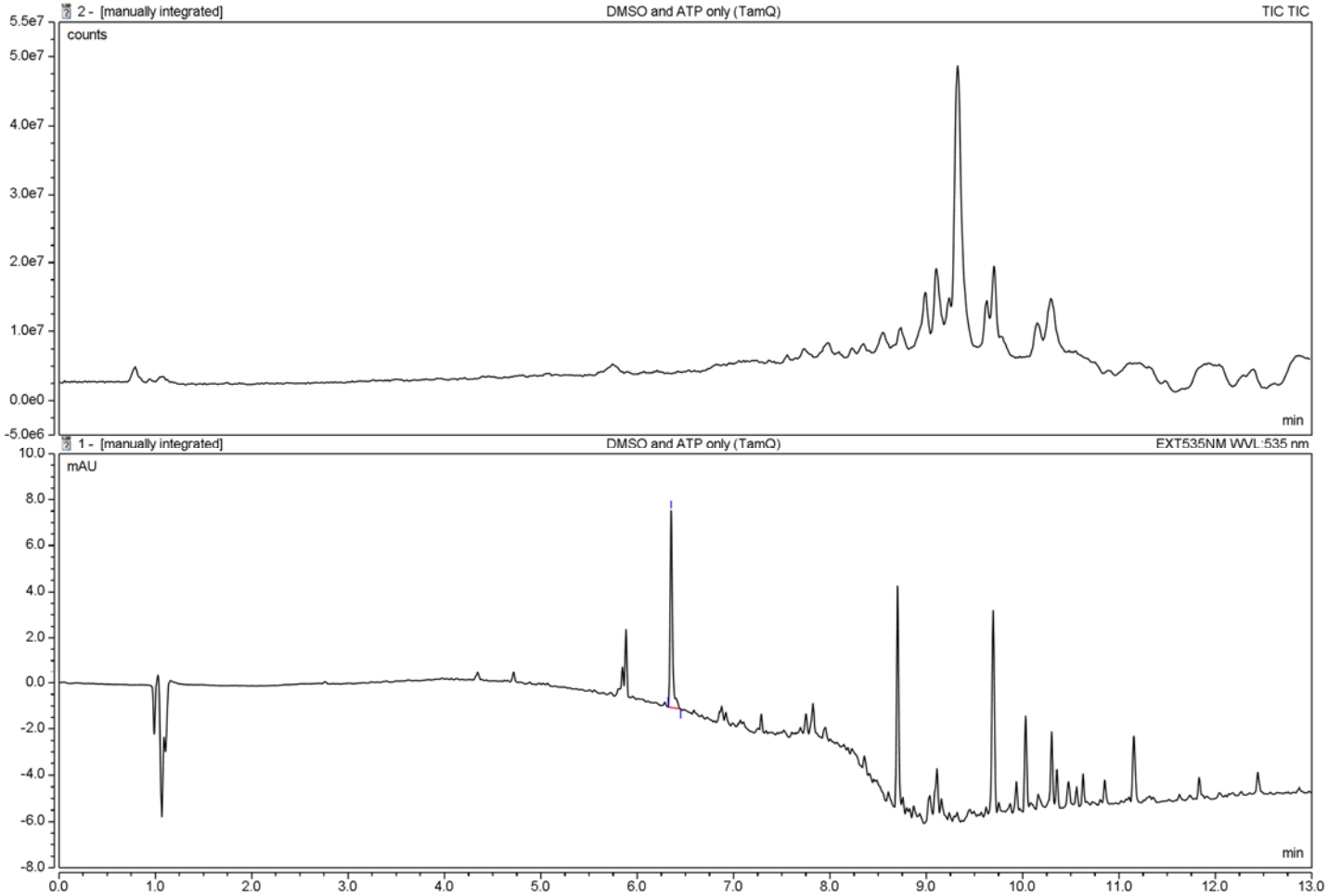


Figure S109: LC-MS data (TIC and UV_{535} trace) of the methanolic reaction extract from the TamQ negative control reaction, containing ATP and DMSO only, but no pyrroles or MBC derivatives.

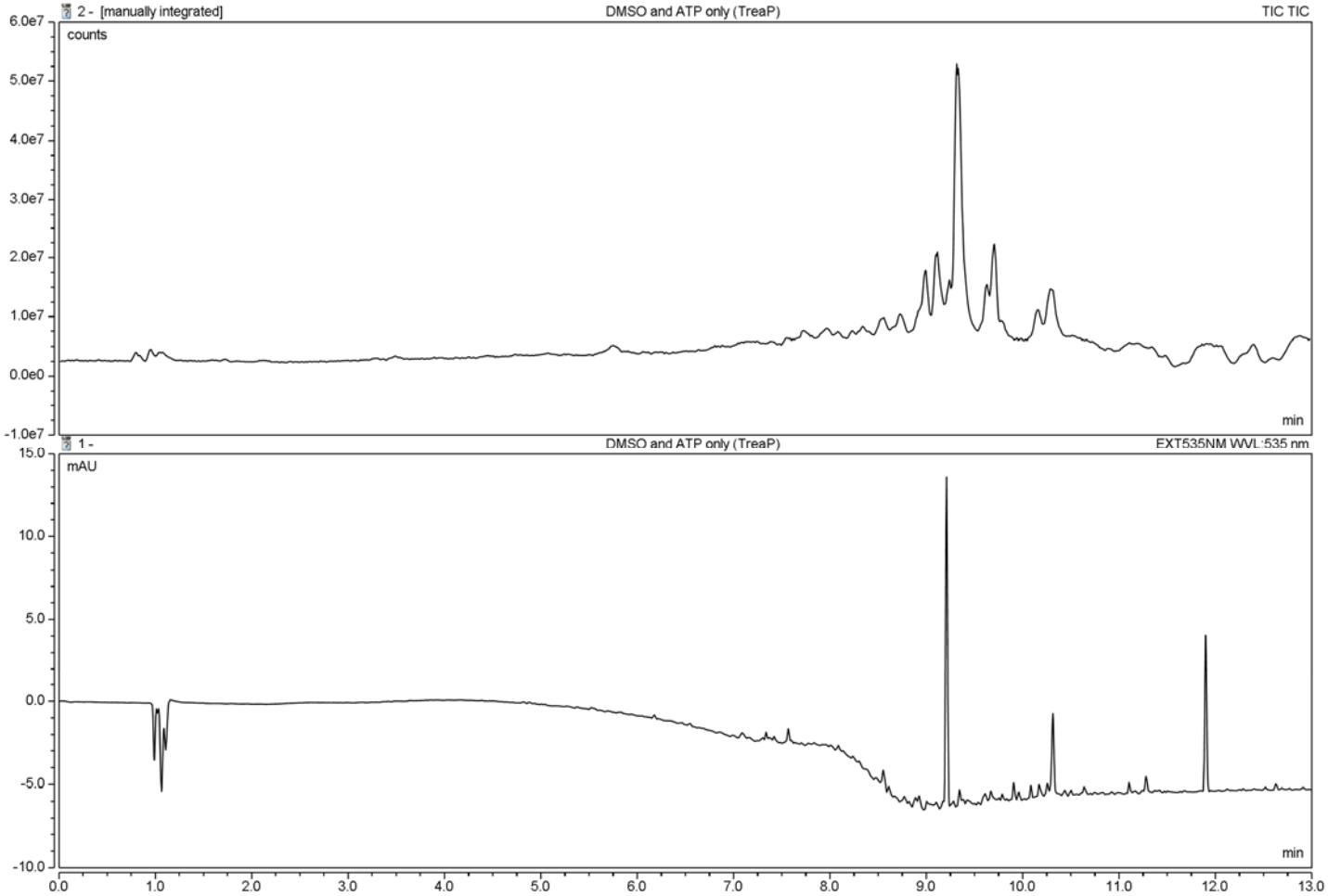


Figure S110: LC-MS data (TIC and UV_{535} trace) of the methanolic reaction extract from the TreaP negative control reaction, containing ATP and DMSO only, but no pyrroles or MBC derivatives.

14. LC-MS Chromatograms – Control Reactions

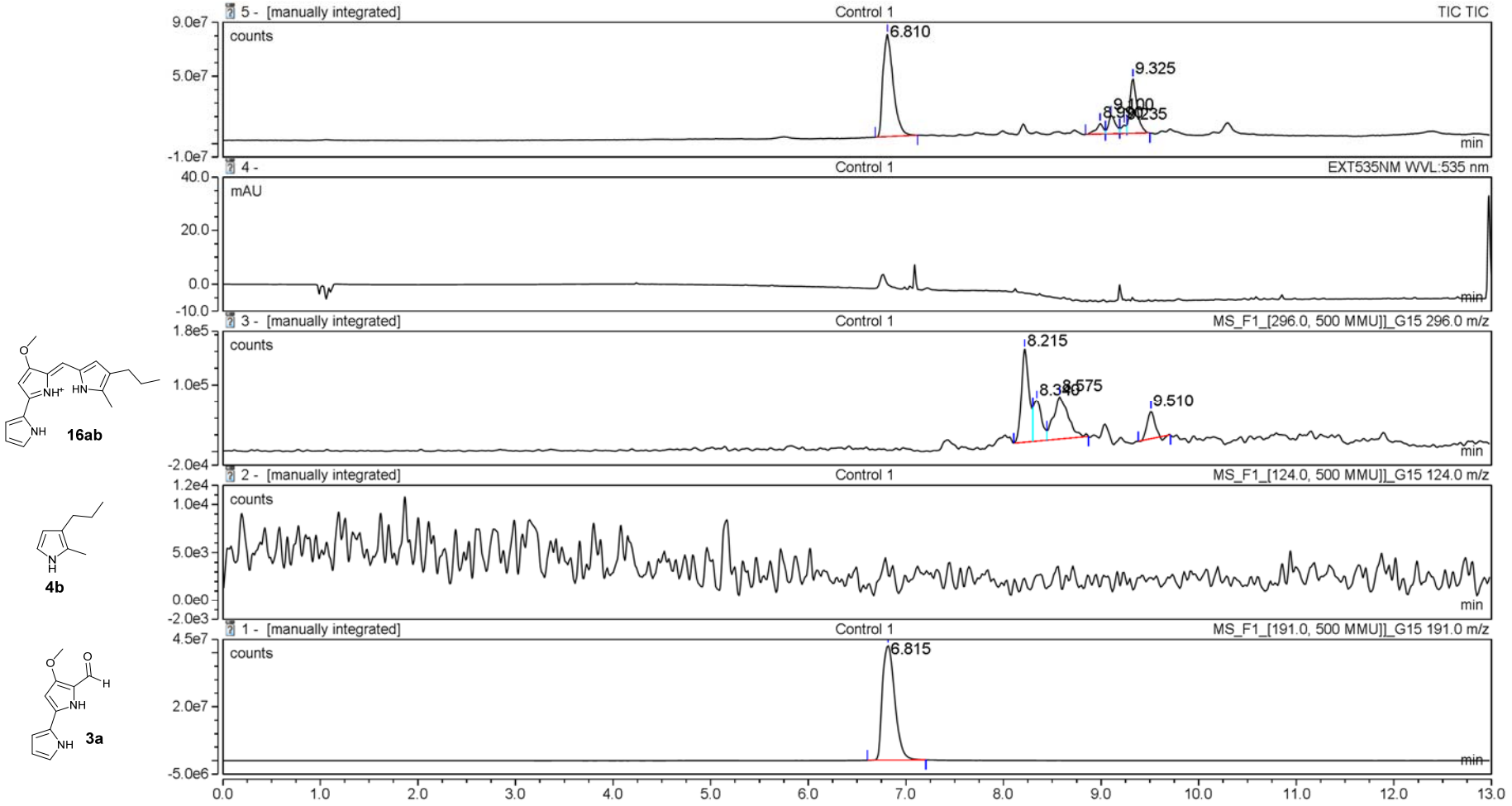


Figure S111: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 1, containing MBC **3a** (m/z 191.0) and pyrrole **4b** (m/z 124.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodiginine **16ab** (m/z 296.0) with a retention time t_R of 7.33 min is not found in the UV trace at 535 nm.

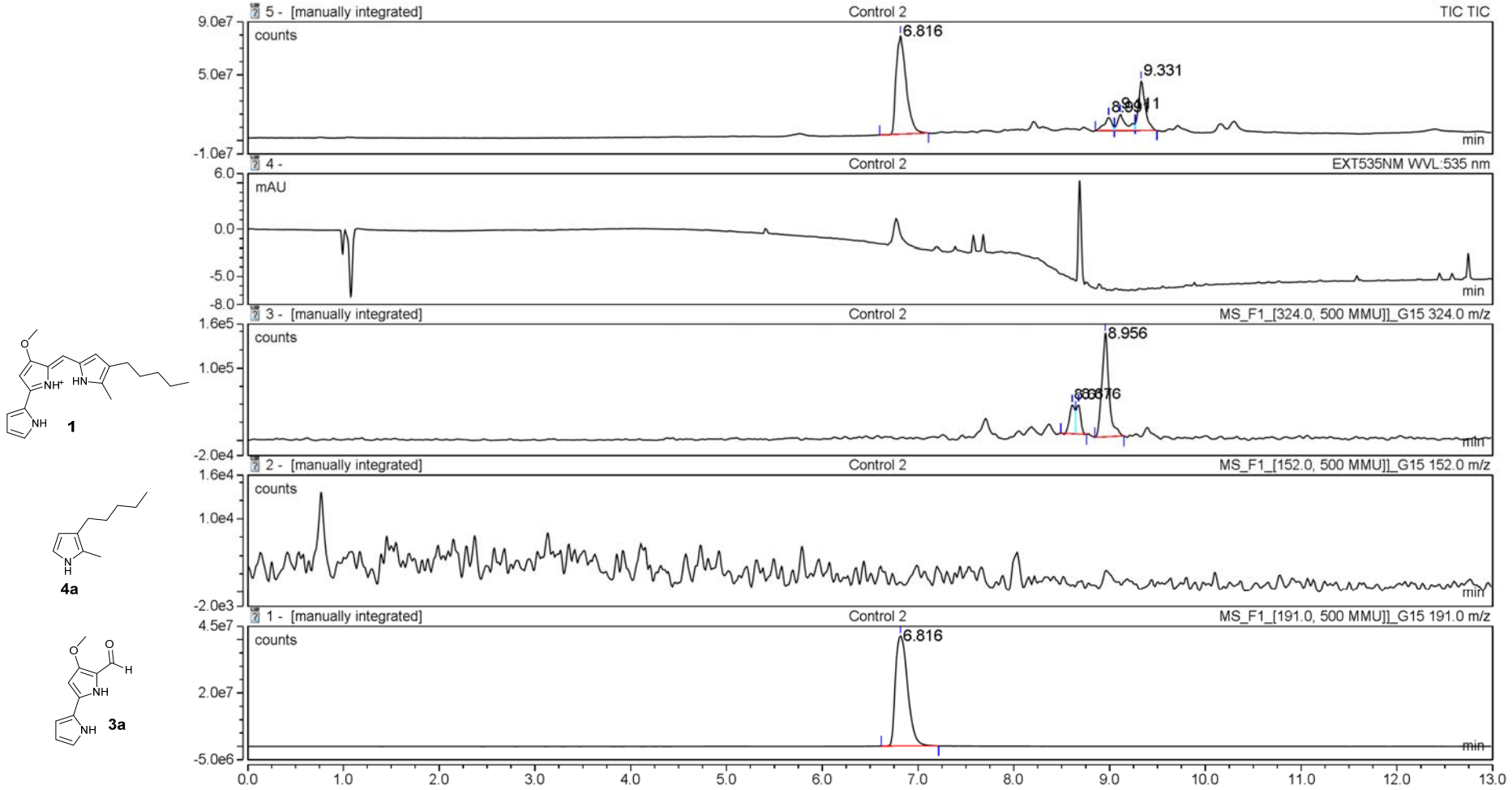


Figure S112: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 2, containing MBC **3a** (m/z 191.0) and pyrrole **4a** (m/z 152.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodigiosin **1** (m/z 324.0) with a retention time t_R of 7.62 min is not found in the UV trace at 535 nm.

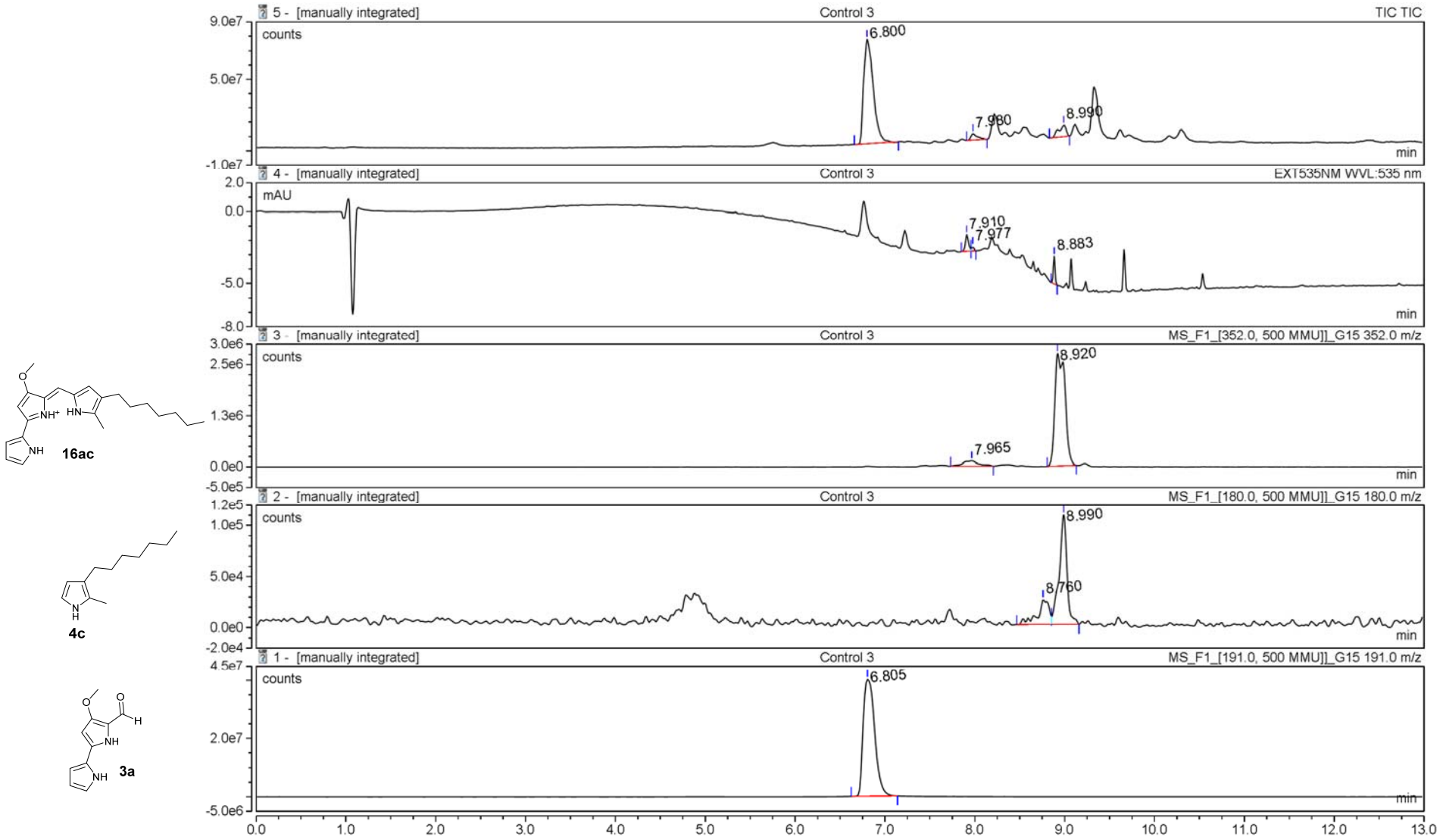


Figure S113: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 3, containing MBC **3a** (m/z 191.0) and pyrrole **4c** (m/z 180.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodiginine **16ac** (m/z 352.0) with a retention time t_R of 7.87 min is not found in the UV trace at 535 nm.

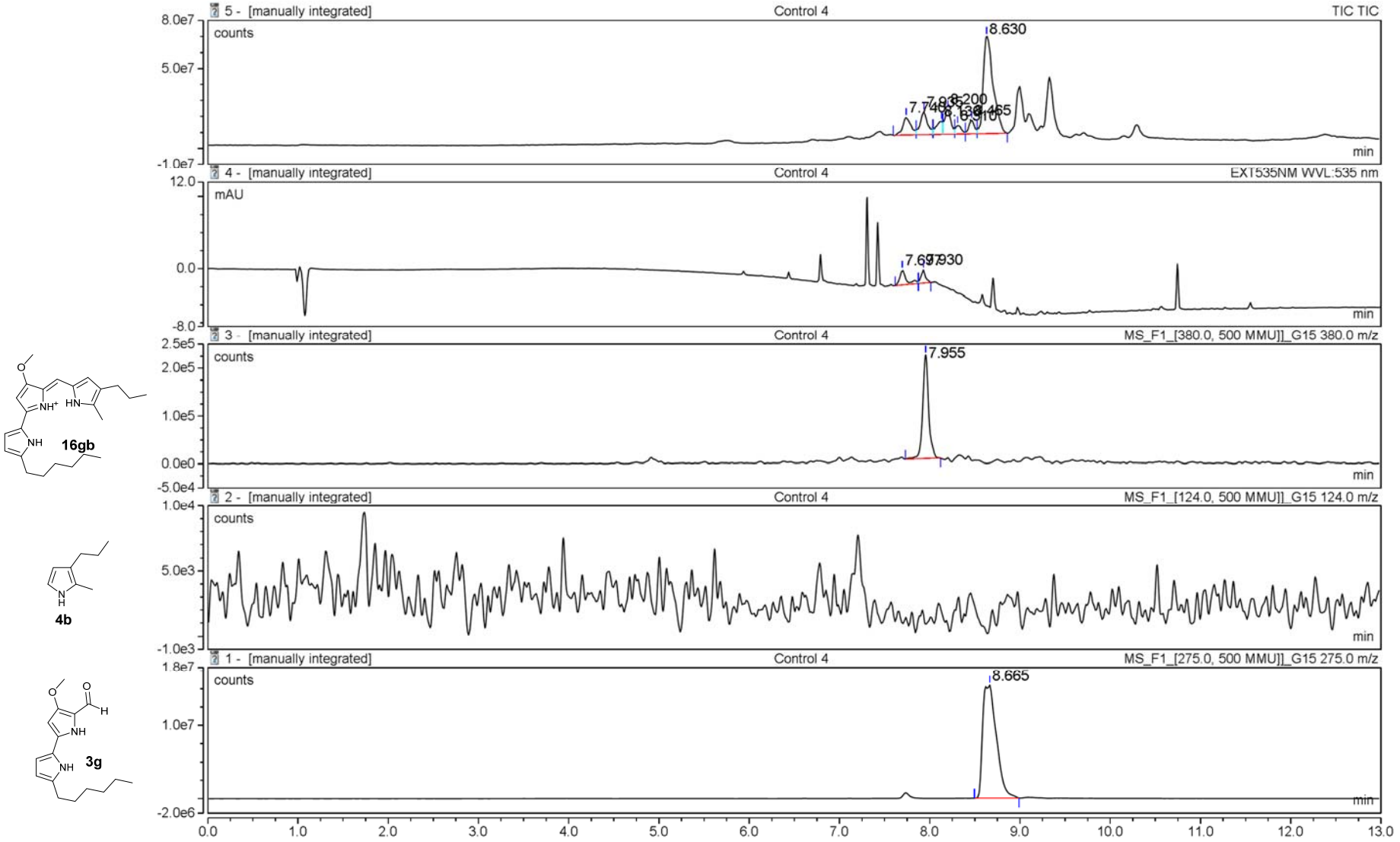


Figure S114: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 4, containing MBC **3g** (m/z 275.0) and pyrrole **4b** (m/z 124.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodiginine **16gb** (m/z 380.0) with a retention time t_R of 7.91 min is not found in the UV trace at 535 nm.

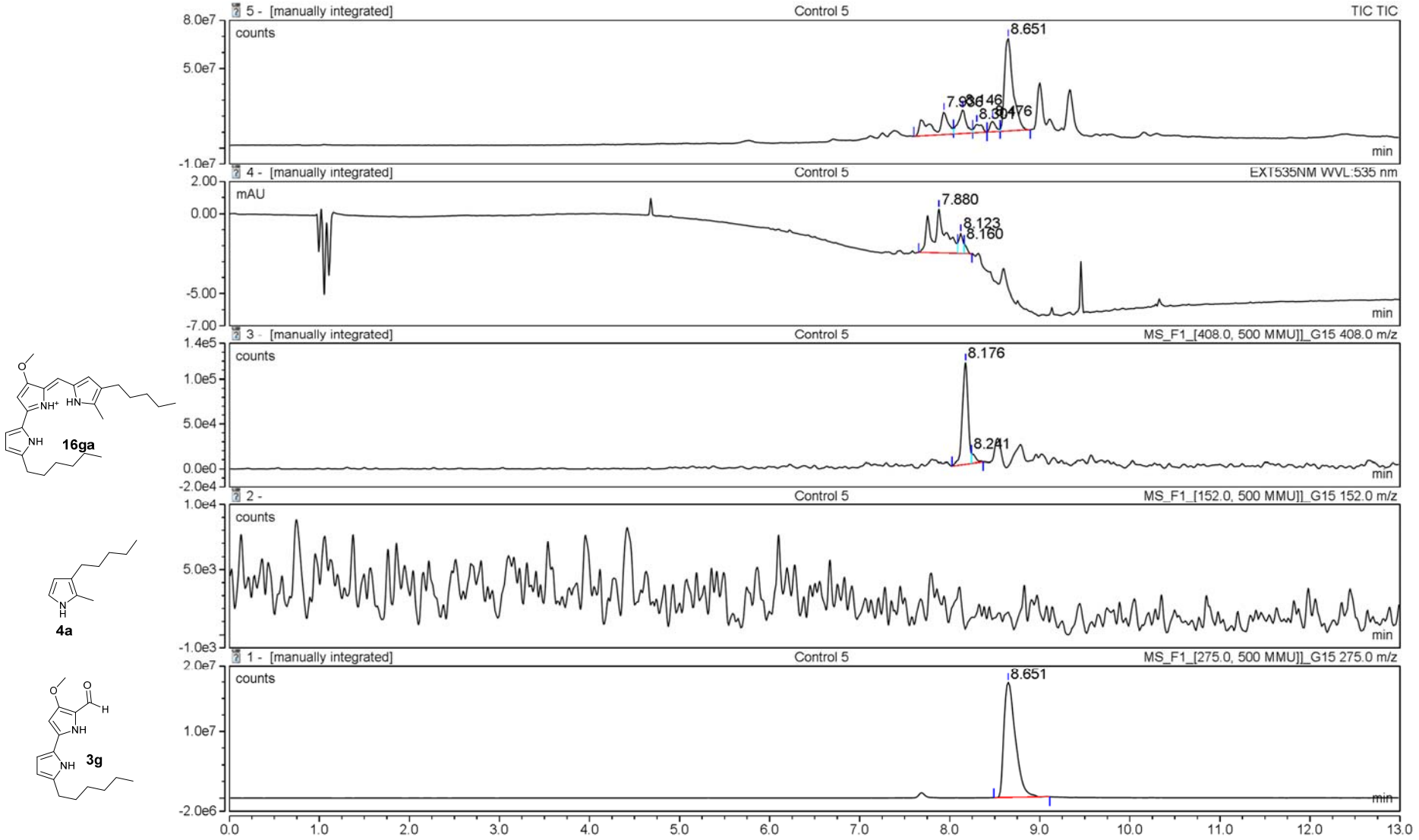


Figure S115: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 5, containing MBC **3g** (m/z 275.0) and pyrrole **4a** (m/z 152.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodiginine **16ga** (m/z 408.0) with a retention time t_R of 8.12 min is not found in the UV trace at 535 nm.

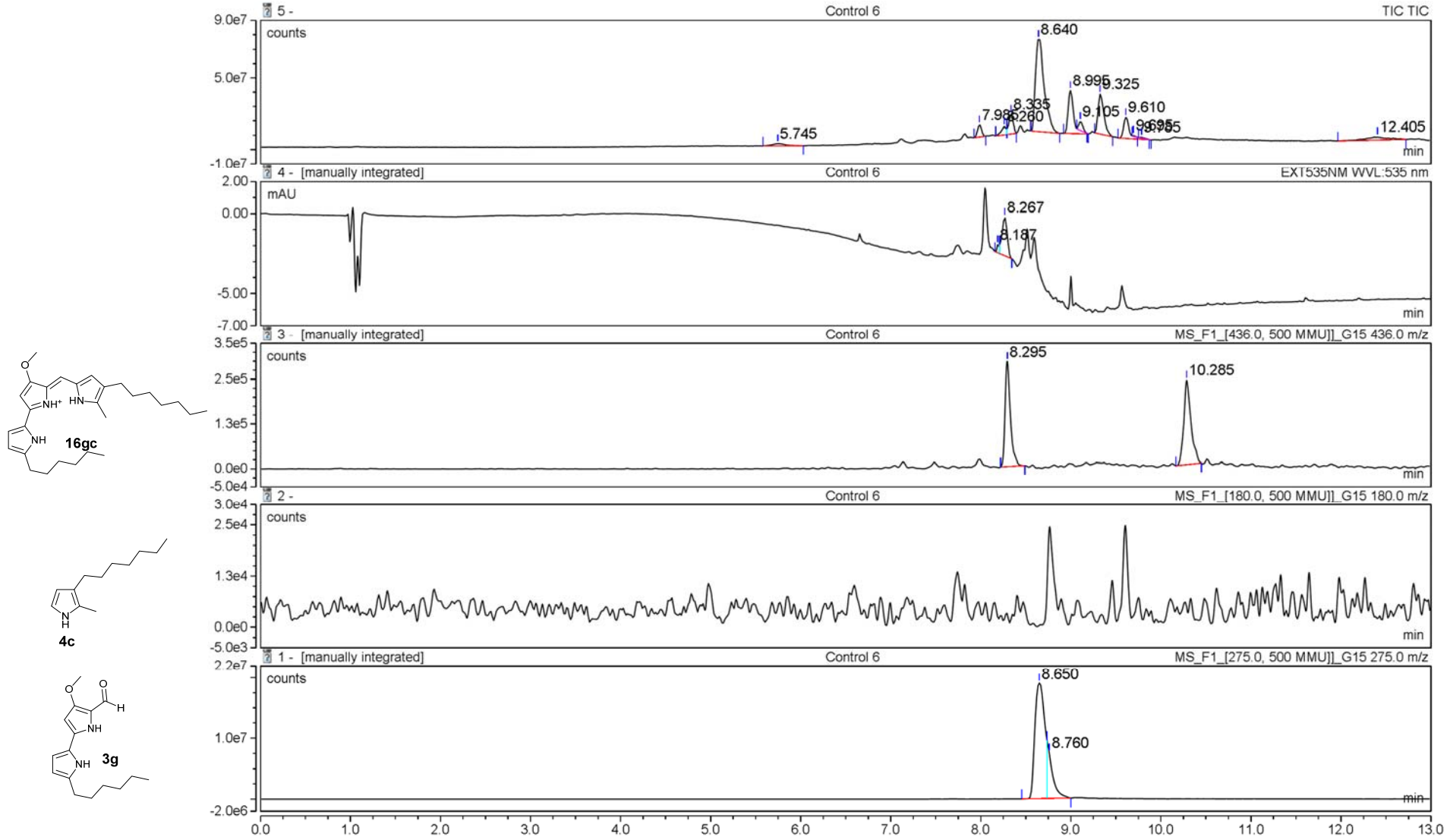


Figure S116: LC-MS data (TIC, UV_{535}, and extracted ion chromatograms) of control reaction 6, containing MBC **3g** (m/z 275.0) and pyrrole **4c** (m/z 180.0), ATP and KP_i buffer, but no enzyme containing cell lysates. The potential condensation product prodiginine **16gc** (m/z 436.0) with a retention time t_R of 8.27 min is not found in the UV trace at 535 nm.

15. Dose-response plots for cell viability screening of urothelial carcinoma cell lines RT-112 and RT-112res with chemically synthesised prodiginines 16ba–gb, prodigiosin (1), and obatoclax mesylate

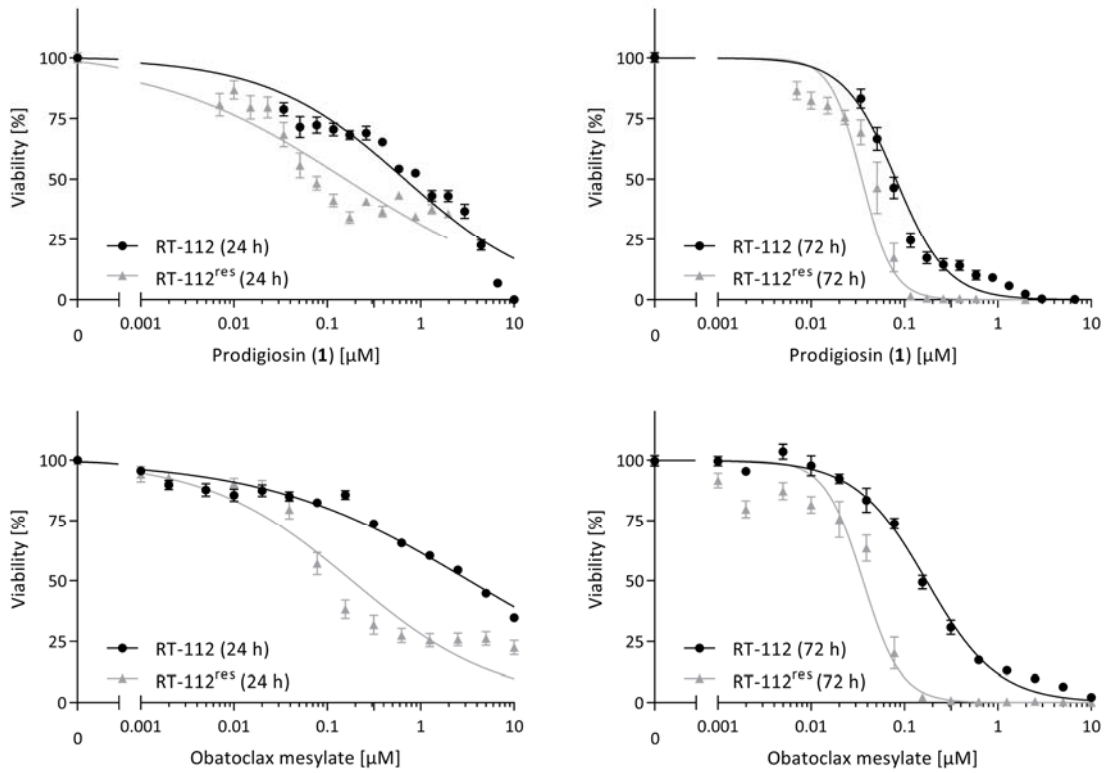


Figure S117: Dose-response curves of urothelial cancer cell lines RT-112 (black circles) and the cisplatin-resistant progeny RT-112res (grey triangles) for prodigiosin (**1**) and obatoclax mesylate. Cell viability was determined via MTT assay and plotted against the prodiginine concentration (µM). The left column contains the viability read out after 24 h of incubation, the right column contains the viability read out after 72 h of incubation. Error bars in the corresponding colour represent standard deviations. Three biological replicates were prepared for each experiment and each biological replicate comprised data of three technical replicates.

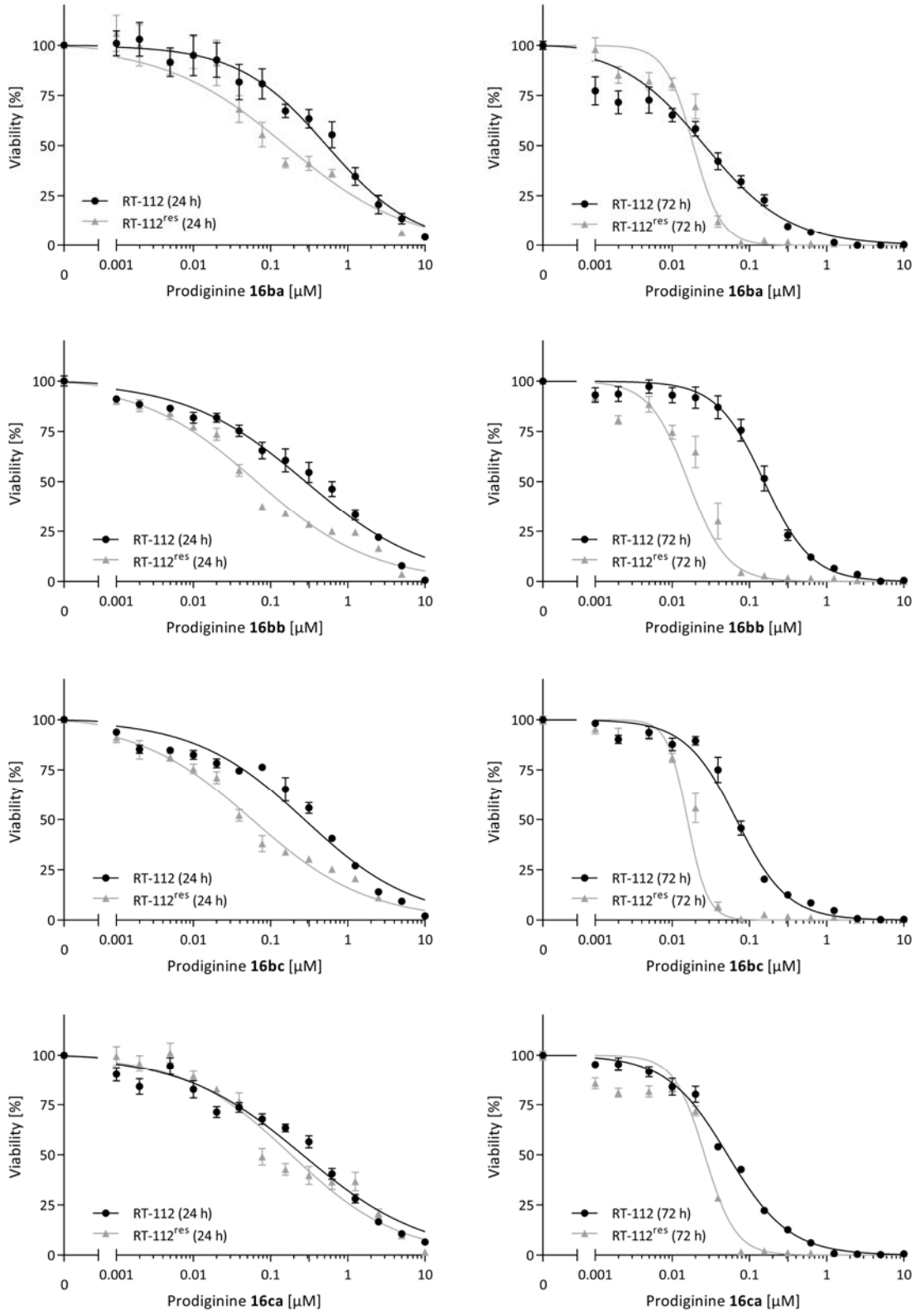


Figure S118: Dose-response curves of urothelial cancer cell lines RT-112 (black circles) and the cisplatin-resistant progeny RT-112res (grey triangles) for chemically synthesised prodiginines **16ba**, **16bb**, **16bc**, and **16ca**. Cell viability was determined via MTT assay and plotted against the prodiginine concentration (µM). The left column contains the viability read out after 24 h of incubation, the right column contains the viability read out after 72 h of incubation. Error bars in the corresponding colour represent standard deviations. Three biological replicates were prepared for each experiment and each biological replicate comprised data of three technical replicates.

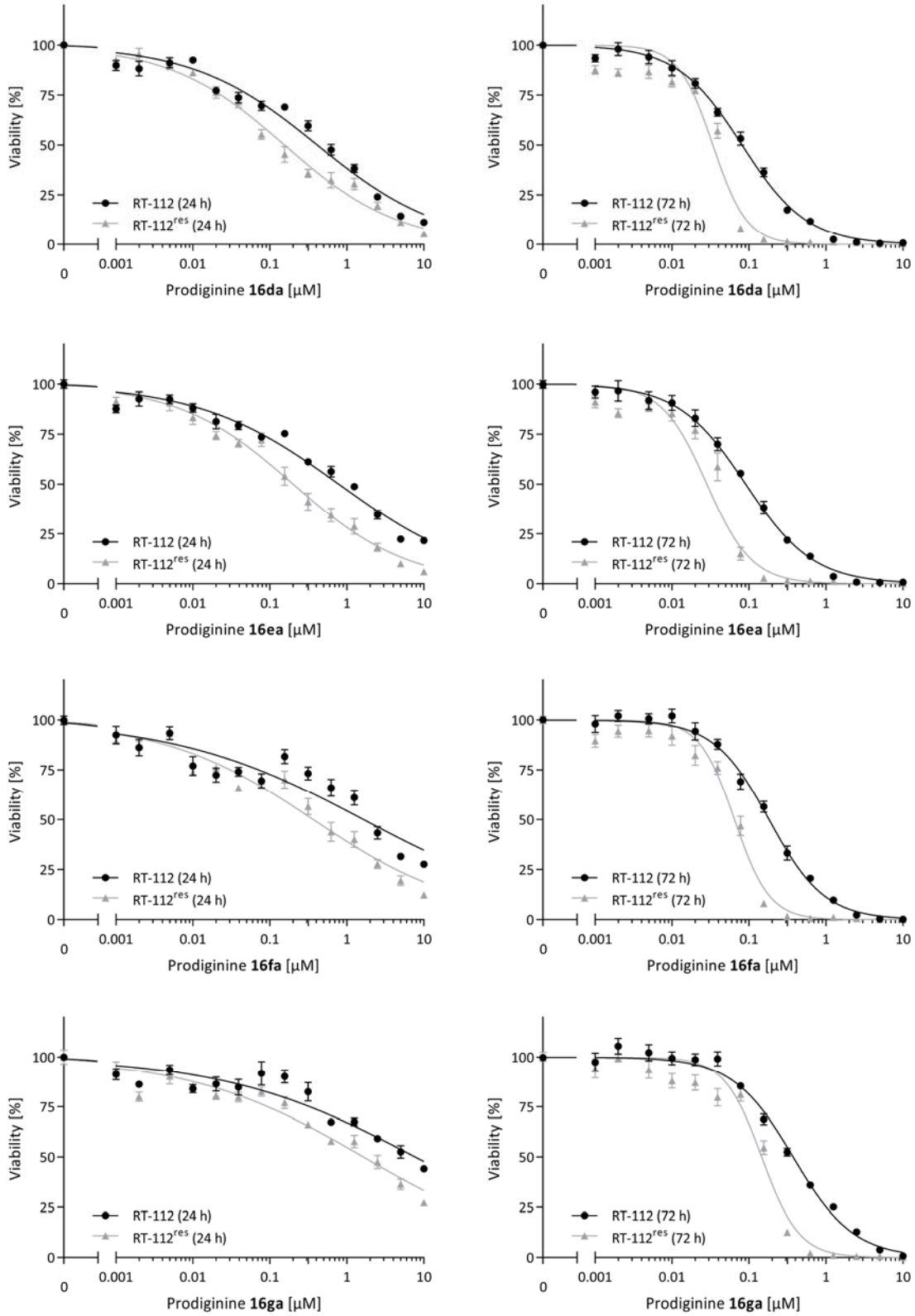


Figure S119: Dose-response curves of urothelial cancer cell lines RT-112 (black circles) and the cisplatin-resistant progeny RT-112res (grey triangles) for chemically synthesised prodiginines **16da**, **16ea**, **16fa**, and **16ga**. Cell viability was determined via MTT assay and plotted against the prodiginine concentration (µM). The left column contains the viability read out after 24 h of incubation, the right column contains the viability read out after 72 h of incubation. Error bars in the corresponding colour represent standard deviations. Three biological replicates were prepared for each experiment and each biological replicate comprised data of three technical replicates.

16. Inhibitory Concentrations (IC_{50}) of prodiginines against urothelial cancer cell lines RT-112 and RT-112res

Table S4: Inhibitory concentrations of prodigiosin (**1**), obatoclax mesylate, and the prodiginines **16ba–16ga** against urothelial carcinoma cell lines RT-112 (cisplatin sensitive) and RT-112res (cisplatin resistant) after 24 h and 72 h of incubation. The presented IC_{50} (nM) are visualised as bar diagram in figure 6B in the main article and summarise the data from the dose-response curves (Figure S117-S119). The displayed errors represent the 95% confidence interval.

	24 h		72 h	
Prodiginine	**RT-112**	**RT-112res**	**RT-112**	**RT-112res**
Prodigiosin (1)	675 ± 92	157 ± 31.4	73.8 ± 4.90	41.1 ± 3.80
Obatoclax mesylate	3327 ± 548	184 ± 36.3	172 ± 13.8	36.0 ± 2.99
16ba	535 ± 178	1586 ± 44.8	26.3 ± 3.12	18.8 ± 1.25
16bb	285 ± 60	66.3 ± 10.2	155 ± 20.1	16.1 ± 1.02
16bc	274 ± 48	57.4 ± 9.65	67.7 ± 5.96	15.7 ± 0.77
16ca	260 ± 51	185 ± 43.6	52.1 ± 3.29	26.3 ± 1.83
16da	414 ± 64	155 ± 24.0	81.3 ± 7.24	34.0 ± 2.92
16ea	789 ± 132	201 ± 34.8	90.8 ± 10.2	28.4 ± 2.57
16fa	1650 ± 758	367 ± 80.0	182 ± 19.0	64.9 ± 6.20
16ga	7752 ± 4588	1648 ± 489	375 ± 41.5	146 ± 1.66