Investigations Regarding the Utility of Prodigiosenes to Treat Leukemia

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Supporting Information

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General Experimental

All chemicals and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Ethyl acetate, hexanes and dichloromethane were obtained crude and purified *via* distillation, under air and at 1 atm pressure, before use. HPLC grade methanol, THF and chloroform were employed in reactions where stated. Anhydrous dichloromethane and THF were purchased from EMD Chemicals. Column chromatography was performed using 230-400 mesh Silicycle Ultra Pure Silica Gel, 150 mesh Brockmann III, activated, neutral aluminium oxide or 150 mesh Brockmann III, activated, basic aluminium oxide, as indicated. TLC was performed on silica gel or neutral aluminium oxide plates and visualized using UV light (254 and/or 365 nm) and/or developed with Vanillin stain. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. NMR spectra were recorded using a Bruker AVANCE 500 or a Bruker AC-250 spectrometer. All ^{1}H and ^{13}C NMR chemical shifts are expressed in parts per million (ppm) using the solvent signal [$CDCl_3$ (^{1}H 7.26 ppm; ^{13}C 77.16 ppm); DMSO (^{1}H 2.50 ppm; ^{13}C 39.52 ppm)] as the internal reference. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; as, apparent singlet; at, apparent triplet; q, quartet; m, multiplet. All coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded using ion trap (ESI TOF) instruments. UV analysis was carried out using a Varian CARY 100 BIO UV-Visible spectrophotometer; extinction coefficients (ε) are reported at the stated λ_{max} (nm) in units of $M^{-1}cm^{-1}$ in DMSO solvent, with the baseline manually corrected for the solvent. HPLC analysis was performed on a Varian ProStar 410 HPLC autosampler on a Regis® Pirkle covalent (*R*,*R*) Whelk-01 10/100 FEC, 25 cm x 4.6 mm column.

Zebrafish Husbandry, Embryo Collection & Embryo Staging

Use of zebrafish in this study was approved by the Dalhousie University Animal Care Committee (protocol no. 11-129). Zebrafish were maintained according to standard protocol. Embryos were collected and grown in E3 embryo media (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, and 0.16 mM $MgSO_4$, pH 7.5, supplemented with 1 x 10^{-5}% Methylene Blue [v/v]) at 28.5ºC. Embryos completely lacking melanocytes, xanthophores, and iridophores were obtained

through use of *casper* (*roy orbison*$^{-/-}$; *nacre*$^{-/-}$) double mutant zebrafish line.[1] Embryos were developmentally staged according to standard protocol.[2]

Xenotransplantation Of Human Leukemia Cells Into Zebrafish Embryos, Dissociation, & Immunofluorescence

Mutant *casper* zebrafish embryos at 48 hours post fertilization (hpf) were anaesthetized with 0.090 mg/mL Tricaine (Sigma-Aldrich) and used for cell transplantation using a protocol adapted from Haldi et al., (2006)l.[3,4] Briefly, CM-DiI-labeled K562 cells were loaded into a pulled glass micropipette and 25-50 cells were delivered, as a single injection, into the yolk sac of each embryo (Injection conditions: 40ms pulse time, 4.6 psi positive pressure) using a PLI-100 Pressure Injector (Harvard Apparatus, Holliston, MA) while under observation using a Leica MZ16F modular stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany). Following injection embryos were allowed to recover at 28 °C for 1 hour before transfer to 35 °C where they remained for the experiment. At 24 hours post injection (hpi), only embryos with a uniform fluorescent cell mass at the site of injection were used for proliferation studies. Embryo xenotransplanted with human cancer cells were then maintained in groups of 15-20 within individual Petri dishes prior to drug treatment. At 24 hpi, prodigiosenes were added directly to the fish water, at indicated concentrations, and embryos were incubated for 72 hours until 96 hpi. Embryos treated with 0.3% (v/v) DMSO served as a negative control for drug efficacy. Embryos treated with 20 μM imatinib mesylate (a targeted inhibitor for the BCR-ABL1 oncoprotein in K562 cells) served as a positive control for drug efficacy.[4] For the fluorescence imaging, a filter with excitation/emission wavelengths of 550/605 nm was used and all embryos were photographed under the same settings. Photographs reported are Z-stack projections: Images were taken at 0.5 micrometer intervals through the entire depth of each embryo and a composite photograph was produced using Zeiss AxioVision computer program (release 4.7.1), using a pre-programmed algorithm.

Experimental Procedures and Data

Synthesis of 2-Formyl Pyrroles (5)

4-[2-(Methoxycarbonyl)ethyl]-3,5-dimethyl pyrrole-2-carboxaldehyde (5a).[5] Following the general procedure for the synthesis of 2-formyl pyrroles (**5**), the title compound was obtained as a yellow solid (2.80 g, 80% yield from **4a**[6]). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.56 (brs, 1H, NH), 9.46 (s, 1H, CHO), 3.67 (s, 3H, OCH_3), 2.71, (t, 2H, J = 7.8 Hz, CH_2CH_2CO), 2.45 (t, 2H, J = 7.8 Hz, CH_2CH_2CO), 2.28 (s, 3H, CH_3), 2.27 (s, 3H, CH_3) ppm. ^{1}H NMR matches reported data.[5]

4-[(Methoxycarbonyl)methyl]-3,5-dimethyl pyrrole-2-carboxaldehyde (5b).[7] Following the general procedure for the synthesis of 2-formyl pyrroles (**5**), the title compound was obtained as a yellow solid (3.70 g, 95% yield from **4b**[6]). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.69 (brs, 1H, NH), 9.49 (s, 1H, CHO), 3.69 (s, 3H, OCH_3), 3.40, (s, 2H, CH_2CO), 2.29 (s, 6H, 2 x CH_3) ppm. ^{1}H NMR matches reported data.[7]

3-[2-(Methoxycarbonyl)ethyl]-4,5-dimethyl pyrrole-2-carboxaldehyde (5c).[5] A solution of dibenzyl oximinomalonate[5] (4.9 g, 15.6 mmol) in glacial acetic acid (8 mL) was added slowly, over 5 minutes, to a solution of **13a** (3.0 g, 16.1 mmol) in glacial acetic acid (10 mL), with stirring at 70 °C. A mixture of zinc dust (4.09 g, 62.6 mmol) and sodium acetate (1.92 g, 23.5 mmol) were added gradually, so as to maintain a temperature of around 90 °C. After the addition was complete, the reaction mixture was heated at 90 °C, with stirring, for one hour before being allowed to cool, followed by pouring into ice-water (400 mL) and thoroughly extracting with diethyl ether (4 x 200 mL). The combined organic extracts were washed with water (250 mL) and brine (250 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give benzyl 3-(3-methoxy-3-oxopropyl)-4,5-dimethyl-1H-pyrrole-2-carboxylate (**4c**), which was used without further purification. Following the general procedure for the synthesis of 2-formyl pyrroles (**5**), the title compound was obtained as a yellow solid (845 mg, 29% yield from **13a**). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.03 (br s, 1H, NH), 9.45 (s, 1H, CHO), 3.66 (s, 3H, OCH_3), 3.00 (t, 2H, J = 7.9 Hz, $CH_2CH_2CO_2Me$), 2.55 (t, 2H, J = 7.9 Hz, $CH_2CH_2CO_2Me$), 2.23 (s, 3H, CH_3), 1.94 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1, 173.1, 136.3, 134.9, 127.6,

117.8, 51.9, 35.9, 19.6, 11.8, 8.7 ppm; LRMS: 232.1 (M+Na); HRMS: 232.0933 Found, 232.0944 Calculated for $C_{11}H_{15}NO_3Na$. ^{1}H NMR matches reported data.[5]

4-[(Ethoxycarbonyl)methyl]-3-[2-(methoxycarbonyl)ethyl]-5-methylpyrrole-2-carboxaldehyde (5d). A solution of dibenzyl oximinomalonate[5] (4.12 g, 13.16 mmol) in glacial acetic acid (7 mL) was added slowly, over 5 minutes, to a solution of **13b** (3.5 g, 13.55 mmol) in glacial acetic acid (8.5 mL), with stirring at 70 °C. A mixture of zinc dust (3.54 g, 54.2 mmol) and sodium acetate (1.67 g, 20.33 mmol) were added gradually, so as to maintain a temperature of around 90 °C. After the addition was complete, the reaction mixture was heated at 90 °C, with stirring, for one hour before being allowed to cool, followed by pouring into ice-water (400 mL) and thoroughly extracting with diethyl ether (4 x 200 mL). The combined organic extracts were washed with water (250 mL) and brine (250 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give benzyl 4-(2-ethoxy-2-oxoethyl)-3-(3-methoxy-3-oxopropyl)-5-methyl-1H-pyrrole-2-carboxylate (**4d**), which was used without further purification. Following the general procedure for the synthesis of 2-formyl pyrroles (**5**), the title compound was obtained as a yellow solid (917 mg, 26% yield from **13b**). M.p. 62-65 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.75 (br s, 1H, NH), 9.51 (s, 1H, CHO), 4.13 (q, 2H, J = 7.2 Hz, O*CH*$_2$CH$_3$), 3.66 (s, 3H, OCH_3), 3.40 (s, 2H, *CH*$_2$CO$_2$Et), 3.03 (t, 2H, J = 7.8 Hz, *CH*$_2$CH$_2$CO$_2$Me), 2.60 (t, 2H, J = 7.8 Hz, CH$_2$*CH*$_2$CO$_2$Me), 2.28 (s, 3H, CH_3), 1.25 (t, 3H, J = 7.2 Hz, OCH$_2$*CH*$_3$) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.6, 173.1, 171.6, 137.3, 135.2, 127.8, 115.3, 61.2, 51.9, 36.3, 30.1, 19.3, 14.4, 12.0 ppm; LRMS: 304.1 (M+Na); HRMS: 304.1163 Found, 304.1155 Calculated for $C_{14}H_{19}NO_5Na$.

Synthesis of Dipyrrinones (6)

(*Z*)-Methyl 3-(5-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (6a).[8] Following the general procedure for the synthesis of dipyrrinones (**6**), the title compound was obtained as a brown solid (383 mg, 69% yield from **5a**). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.92 (brs, 1H, NH), 10.22 (brs, 1H, NH), 6.35 (s, 1H, *meso*-H), 5.09 (s, 1H), 3.90 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 2.73 (t, 2H, J = 7.8 Hz, *CH*$_2$CH$_2$CO), 2.44 (t, 2H, J = 7.8 Hz, CH$_2$*CH*$_2$CO), 2.36 (s, 3H, CH_3), 2.12 (s, 3H, CH_3) ppm. ^{1}H NMR matches reported data.[8]

(*Z*)-Methyl 2-(5-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)acetate (6b).[8] Following the general procedure for the synthesis of dipyrrinones (**6**), the title compound was obtained as a brown solid (1.83 g, 82% yield from **5b**). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.91 (brs, 1H, NH), 10.31 (brs, 1H, NH), 6.35 (s, 1H, *meso*-H), 5.09 (s, 1H), 3.89 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.40 (s, 2H, CH_2CO), 2.37 (s, 3H, CH_3), 2.13 (s, 3H, CH_3) ppm. ^{1}H NMR matches reported data.[8]

(*Z*)-Methyl 3-(2-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-4,5-dimethyl-1H-pyrrol-3-yl) propanoate (6c). Following the general procedure for the synthesis of dipyrrinones (**6**), the title compound was obtained as a brown solid (367 mg, 33% yield from **5c**). M.p. 225-230 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.86 (brs, 1H, NH), 10.12 (brs, 1H, NH), 6.34 (s, 1H, *meso*-H), 5.08 (s, 1H, CH), 3.89 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 2.86 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.47 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.32 (s, 3H, CH_3), 1.95 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 173.4, 168.1, 132.5, 128.6, 122.2, 121.5, 115.9, 100.4, 89.9, 58.3, 51.8, 35.9, 20.4, 11.6, 9.1 ppm; LRMS: 305.1 (M+H); HRMS: 305.1479 Found, 305.1496 Calculated for $C_{16}H_{21}N_2O_4$.

(*Z*)-Methyl 3-(4-(2-methoxy-2-oxoethyl)-2-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene) methyl)-5-methyl-1H-pyrrol-3-yl)propanoate (6d). Following the general procedure for the synthesis of dipyrrinones (**6**), the title compound was obtained as a brown solid (204 mg, 20% yield from **5d**). M.p. 200-204 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.88 (brs, 1H, NH), 10.33 (brs, 1H, NH), 6.32 (s, 1H, *meso*-H), 5.09 (s, 1H, CH), 3.90 (s, 3H, OCH_3), 3.67 (s, 6H, 2 x OCH_3), 3.42 (s, 2H, CH_2), 2.88 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.51 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.37 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 173.5, 172.6, 168.2, 133.6, 128.4, 122.8, 121.9, 113.2, 100.1, 90.1, 58.4, 52.2, 51.8, 36.1, 30.3, 20.1, 11.7 ppm; LRMS: 385.1 (M+Na); HRMS: 385.1357 Found, 385.1370 Calculated for $C_{18}H_{22}N_2O_6Na$.

Synthesis of 2-Bromodipyrrins (7)

(*Z*)-Methyl 3-(5-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (7a). Following the general procedure for the synthesis of 2-bromodipyrrins (**7**), the title compound was obtained as a yellow solid (343 mg, 52% yield from **6a**). M.p. 118-122 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.2-11.3 (br s, 1H, NH), 6.87 (s, 1H,

meso-H), 5.58 (s, 1H, CH), 3.83 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 2.71 (t, 2H, J = 7.8 Hz, $CH_2CH_2CO_2Me$), 2.43 (t, 2H, J = 7.8 Hz, $CH_2CH_2CO_2Me$), 2.30 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 166.8, 144.0, 137.5, 136.9, 131.4, 126.4, 121.5, 116.3, 99.3, 58.5, 51.7, 34.8, 19.7, 12.3, 9.6 ppm; LRMS: 367.1 (M+H); HRMS: 367.0653 Found, 367.0652 Calculated for $C_{16}H_{20}N_2O_3Br$.

(*Z*)-Methyl 2-(5-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)acetate (7b). Following the general procedure for the synthesis of 2-bromodipyrrins (**7**), the title compound was obtained as a yellow solid (414 mg, 72% yield from **6b**). M.p. 129-133 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ 6.88 (s, 1H, *meso*-H), 5.58 (s, 1H, CH), 3.83 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.38 (s, 2H, CH_2CO), 2.31 (s, 3H, CH_3), 2.15 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1, 167.0, 144.7, 138.1, 137.4, 131.1, 126.4, 116.5, 115.8, 99.5, 58.6, 52.2, 30.3, 12.5, 9.8 ppm; LRMS: 353.0 (M+H); HRMS: 353.0489 Found, 353.0495 Calculated for $C_{15}H_{18}N_2O_3$.

(*Z*)-Methyl 3-(2-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-4,5-dimethyl-1H-pyrrol-3-yl) propanoate (7c). Following the general procedure for the synthesis of 2-bromodipyrrins (**7**), the title compound was obtained as an orange solid (198 mg, 48% yield from **6c**). M.p. 100-104 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ 6.87 (s, 1H, *meso*-H), 5.58 (s, 1H, CH), 3.83 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 2.87 (t, 2H, J = 7.9 Hz, $CH_2CH_2CO_2Me$), 2.49 (t, 2H, J = 7.9 Hz, $CH_2CH_2CO_2Me$), 2.27 (s, 3H, CH_3), 1.95 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 167.1, 144.3, 137.8, 137.2, 134.0, 125.9, 118.3, 116.3, 99.4, 58.6, 51.9, 35.7, 20.3, 12.5, 9.0 ppm; LRMS: 367.1 (M+H); HRMS: 367.0635 Found, 367.0652 Calculated for $C_{16}H_{20}N_2O_3Br$.

(*Z*)-Methyl 3-(2-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-4-(2-methoxy-2-oxoethyl)-5-methyl-1H-pyrrol-3-yl) propanoate (7d). Following the general procedure for the synthesis of 2-bromodipyrrins (**7**), the title compound was obtained as a dark yellow oil (153 mg, 69% yield from **6d**). ^1H NMR ($CDCl_3$, 500 MHz) δ 6.85 (s, 1H, *meso*-H), 5.55 (s, 1H, CH), 3.82 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.39 (s, 2H, CH_2), 2.88 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.88 (t, 2H, J = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.29 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.0, 167.2, 145.5, 137.9, 137.9, 133.7, 126.0, 116.0, 115.2,

99.7, 58.6, 52.2, 51.8, 36.0, 30.1, 20.0, 12.5 ppm; LRMS: 426.1 (M+H); HRMS: 425.0688 Found, 425.0707 Calculated for $C_{18}H_{22}N_2O_5Br$.

Synthesis of Prodigiosenes (8)

(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)propanoate (8a). Following the general procedure for the synthesis of prodigiosenes (**8**), the title compound was obtained as a deep red solid (79 mg, 55% yield from **7a**). M.p. 78-83 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.89 (s, 1H, *meso*-H), 6.65 (d, 1H, *J* = 2.5 Hz, PyH), 6.60 (as, 1H, PyH), 6.11 (t, 1H, *J* = 2.5 Hz, PyH), 6.08 (s, 1H, PyH), 3.97 (s, 3H, OCH_3), 3.56 (s, 3H, OCH_3), 2.56 (t, 2H, *J* = 7.8 Hz, $CH_2CH_2CO_2Me$), 2.32 (t, 2H, *J* = 7.8 Hz, $CH_2CH_2CO_2Me$), 2.11 (s, 3H, CH_3), 1.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 168.9, 159.1, 137.0, 129.7, 128.7, 125.7, 122.8, 120.7, 113.4, 112.43, 112.36, 110.0, 95.5, 58.6, 51.7, 35.0, 19.9, 10.3, 9.8 ppm; LRMS: 354.2 (M+H); HRMS: 354.1789 Found, 354.1812 Calculated for $C_{20}H_{24}N_3O_3$; ε_{476nm} = 33,300; HPLC analysis: 96%.

(*Z*)-Methyl 2-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)acetate (8b). Following the general procedure for the synthesis of prodigiosenes (**8**), the title compound was obtained as a deep red solid (124 mg, 65% yield from **7b**). M.p. 68-72 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.87 (s, 1H, *meso*-H), 6.70 (as, 1H, PyH), 6.66 (d, 1H, *J* = 4.0 Hz, PyH), 6.17 (t, 1H, *J* = 3.0 Hz, PyH), 6.03 (s, 1H, PyH), 3.95 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.28 (s, 2H, CH_2CO), 2.14 (s, 3H, CH_3), 1.89 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.3, 168.9, 159.3, 137.8, 137.2, 130.0, 128.7, 125.7, 122.7, 114.8, 113.4, 112.4, 110.0, 95.5, 58.5, 51.99, 30.3, 10.5, 9.8 ppm; LRMS: 340.2 (M+H); HRMS: 340.1667 Found, 340.1656 Calculated for $C_{19}H_{22}N_3O_3$; ε_{472nm} = 41,700; HPLC analysis: 99%.

(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4,5-dimethyl-2H-pyrrol-3-yl)propanoate (8c). Following the general procedure for the synthesis of prodigiosenes (**8**), the title compound was obtained as a deep red solid (52 mg, 54% yield from **7c**). M.p. 51-56 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.88 (s, 1H, *meso*-H), 6.66-6.67 (m, 2H, 2 x PyH), 6.14 (ad, 1H, *J* = 2.5 Hz, PyH), 6.06 (s, 1H, PyH), 3.97 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 2.86 (t, 2H, *J* = 7.9 Hz, $CH_2CH_2CO_2Me$), 2.45 (t, 2H, *J* = 7.9 Hz, $CH_2CH_2CO_2Me$), 1.82 (s, 3H, CH_3), 1.75 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 168.6, 158.1, 137.3, 131.5, 128.4, 125.0, 123.0,

117.6, 112.9, 112.63, 112.56, 110.1, 95.3, 58.5, 51.7, 35.7, 20.3, 10.5, 8.9 ppm; LRMS: 354.2 (M+H); HRMS: 354.1793 Found, 354.1812 Calculated for $C_{20}H_{24}N_3O_3$; ε_{478nm} = 40,200; HPLC analysis: 99%.

(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4-(2-methoxy-2-oxoethyl)-5-methyl-2H-pyrrol-3-yl)propanoate (8d). Following the general procedure for the synthesis of prodigiosenes (**8**), the title compound was obtained as a deep red solid (34 mg, 37% yield from **7d**). M.p. 40-45 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.85 (s, 1H, *meso*-H), 6.67-6.80 (m, 2H, PyH), 6.15 (at, 1H, *J* = 3.0 Hz, PyH), 6.05 (s, 1H, PyH), 3.96 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 3.29 (s, 2H, CH_2CO_2Me), 2.89 (t, 2H, *J* = 8.0 Hz, $CH_2CH_2CO_2Me$), 2.51 (t, 2H, *J* = 8.0 Hz, $CH_2CH_2CO_2Me$), 1.80 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 172.3, 169.0, 139.2, 137.3, 132.1, 128.4, 125.5, 123.2, 114.5, 113.11, 113.09, 112.8, 110.3, 95.7, 58.6, 52.2, 51.8, 36.1, 30.2, 20.1, 10.8 ppm; LRMS: 412.2 (M+H); HRMS: 412.1870 Found, 412.1867 Calculated for $C_{22}H_{26}N_3O_5$; ε_{470nm} = 30,000; HPLC analysis: 99%.

Synthesis of Diketones 13a and 13b

Methyl-4,6-Dioxoheptanoate (12).[5] a) Carbon tetrachloride (0.2 mL, ~2 mmol) was added drop-wise to a suspension of magnesium turnings (4.0 g, 164.4 mmol) in HPLC grade methanol (15 mL), with stirring under N_2, and the mixture was warmed until evolution of H_2 gas commenced (~30 °C). Additional methanol (50 mL) was added slowly and heating was continued, increasing to 50 °C, until the magnesium had completely dissolved (~2 hours). ^{t}Butylacetoacetate (**9**, 26.7 mL, 161.2 mmol) was then added over 15 minutes and the resulting reaction mixture was heated to reflux temperature for 1 hour, after which time the reaction mixture was cooled to r.t. and filtered, washing with methanol, and then dried under vacuum. b) 3-Methoxycarbonyl propionyl chloride was added over 15 minutes to a stirred suspension of the product (**10**) from part a), above, in diethyl ether (75 mL) and the reaction mixture was heated to reflux temperature. Precipitation of magnesium during the reaction hindered stirring, therefore a couple of boiling chips were added. After 30 minutes the reaction was cooled to 0 °C and acidified slowly with 2 M aqueous H_2SO_4 (~40 mL), until the residue had completely dissolved. The two layers were then separated and the aqueous phase was extracted with ether (3 x 60 mL). The organic extracts were combined and washed with water (3 x 100 mL), dried over anhydrous

magnesium sulfate, filtered and concentrated to give a pale yellow oil, which was used in part c), below, without further purification. c) *p*-Tosic acid (150 mg, 0.8 mmol) was added to the crude product (**11**) from part b), above, and the neat mixture was heated to reflux temperature (~160 °C) for 1 hour, until gas evolution had ceased. The reaction mixture was then cooled to r.t., dissolved in diethyl ether (100 mL) and extracted quickly with ice-cold NaOH solution (2 M, 35 mL then 3 x 20 mL). The aqueous extracts were run directly into ice-cold sulphuric acid solution (2 M, 80 mL), which was, in turn, extracted with dichloromethane (3 x 80 mL). The organic extracts were combined and washed with brine (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give the crude product, which was purified by distillation under reduced pressure (aspirator, head temp. ~120 °C) to give *methyl-4,6-dioxoheptanoate* **12** (10.83 g, 39% yield) as a clear colourless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ (enol tautomer) 15.11 (brs, 1H, OH), 5.48 (s, 1H, CH), 3.64 (s, 3H, OCH_3), 2.61-2.58 (m, 4H, CH_2CH_2), 1.98 (s, 3H, CH3C=C); (keto tautomer) 3.62 (s, 3H, OCH_3), 3.59 (s, 2H, CH_2), 2.77 (t, 2H, J = 6.5 Hz, CH_2), 2.61-2.58 (m, 2H, CH_2), 2.20 (s, 3H, CH_3CO) ppm. The keto:enol ratio was estimated to be 1:3.

Methyl-5-Methyl-4,6-Dioxoheptanoate (13a).[5] Iodomethane (2.4 mL, 38.3 mmol) was added drop-wise to a vigorously stirred suspension of methyl-4,6-dioxoheptanoate (**12**, 5.5 g, 31.9 mmol) and potassium carbonate (4.41 g, 31.9 mmol) in acetone (75 mL). The flask was then fitted with a large condenser and the reaction heated to 80 °C, with vigorous stirring overnight. After cooling slightly the reaction mixture was filtered whilst still hot, washing with acetone, and then concentrated. The residue was then dissolved in chloroform (50 mL) and washed with water (2 x 30 mL) and brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give the crude product, which was purified via distillation under reduced pressure (vacuum pump, head temp. ~84 °C) to give *methyl-5-methyl-4,6-dioxoheptanoate* (**13a**, 5.16 g, 87% yield) as a colourless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ (enol tautomer) 3.67 (s, 3H, OCH_3), 2.70-2.80 (m, 2H, $CH_2CH_2CO_2Me$), 2.55-2.65 (m, 2H, $CH_2CH_2CO_2Me$), 2.12 (s, 3H, CH_3CO), 1.35 (s, 3H, CH_3C=C); (keto tautomer) 3.71 (q, 1H, J = 7.0 Hz, $CHCH_3$), 3.64 (s, 3H, OCH_3), 2.70-2.80 (m, 2H, $CH_2CH_2CO_2Me$), 2.55-2.65 (m, 2H, $CH_2CH_2CO_2Me$), 2.18 (s, 3H, CH_3CO), 1.32 (d, 3H, J = 7.0 Hz, $CHCH_3$) ppm. The keto:enol ratio was estimated to be 3:1.

Methyl-5-[(Ethoxycarbonyl)Methyl]-4,6-Dioxoheptanoate (13b).[5] Methyl-4,6-Dioxoheptanoate (**12**, 5.0 g, 29.0 mmol) was added dropwise to a vigorously stirred suspension of potassium carbonate (4.41 g, 31.9 mmol) in acetone (70 mL) and the resulting mixture was

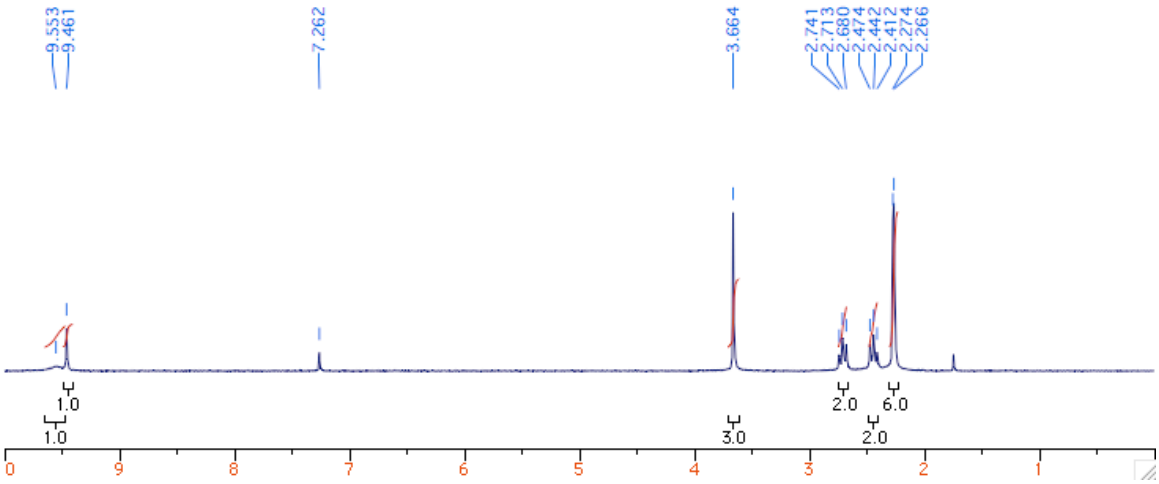
heated to gentle reflux (~55 °C), during which time ethyl bromoacetate (4.5 mL, 40.7 mmol) was added dropwise. Following the addition, the reaction mixture was heated to 75 °C with vigorous stirring for 5 hours. After cooling slightly the reaction mixture was filtered whilst still hot, washing with acetone, and then concentrated. The residue was then dissolved in chloroform (50 mL) and washed with water (2 x 30 mL) and brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give the crude product, which was purified via distillation under reduced pressure (vacuum pump, head temp. ~140 °C) to give *methyl-5-[(ethoxycarbonyl)methyl]-4,6-dioxoheptanoate* (**13b**) (5.67 g, 76% yield) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ (enol tautomer) 4.04 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.59 (s, 3H, OCH_3), 3.19 (s, 2H, $CH_2C{=}C$), 2.80-2.90 (m, 2H, $CH_2CH_2CO_2Me$), 2.55-2.65 (m, 2H, $CH_2CH_2CO_2Me$), 2.12 (s, 3H, CH_3CO), 1.17 (t, 3H, J = 7.0 Hz, CH_2CH_3); (keto tautomer) 4.09-4.18 (m, 1H, $CHCH_2$), 4.04 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.57 (s, 3H, OCH_3), 2.80-2.90 (m, 2H, $CH_2CH_2CO_2Me$), 2.80-2.84 (m, 2H, $CHCH_2$), 2.50-2.60 (m, 2H, $CH_2CH_2CO_2Me$), 2.21 (s, 3H, CH_3CO), 1.17 (t, 3H, J = 7.0 Hz, CH_2CH_3) ppm. The keto:enol ratio was estimated to be 1:1.

NMR Spectra

2-Formyl Pyrroles (5)

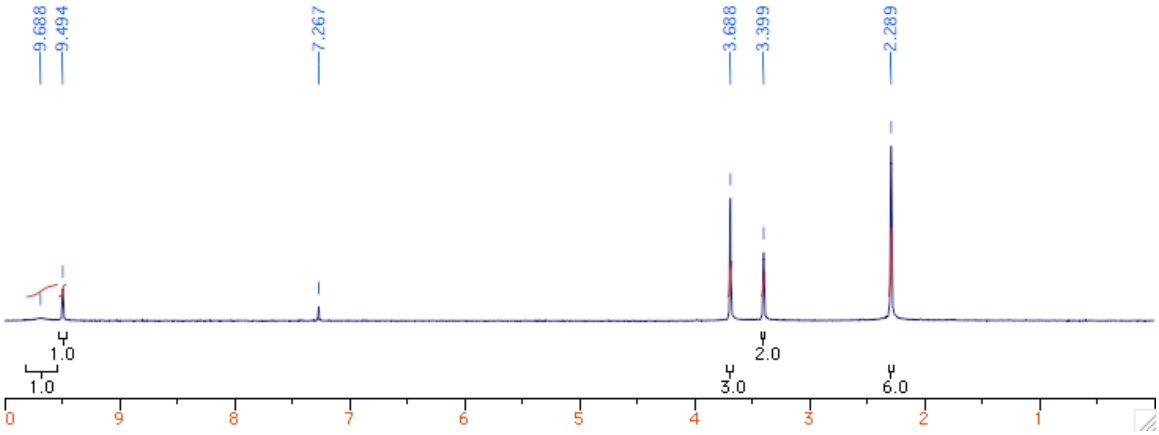
4-[2-(Methoxycarbonyl)ethyl]-3,5-dimethyl pyrrole-2-carboxaldehyde (5a).

^{1}H NMR ($CDCl_3$, 250 MHz):



4-[(Methoxycarbonyl)methyl]-3,5-dimethyl pyrrole-2-carboxaldehyde (5b).

^{1}H NMR ($CDCl_3$, 250 MHz):



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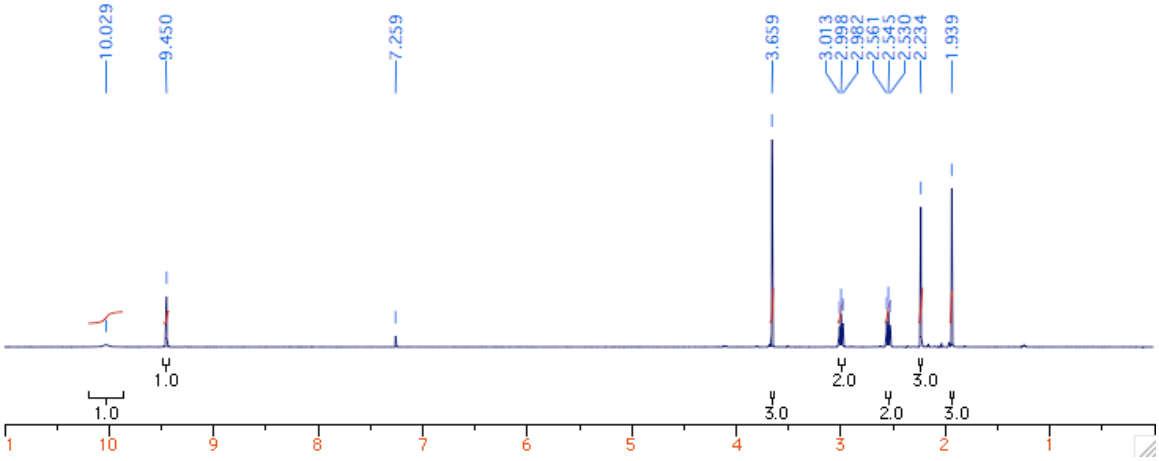
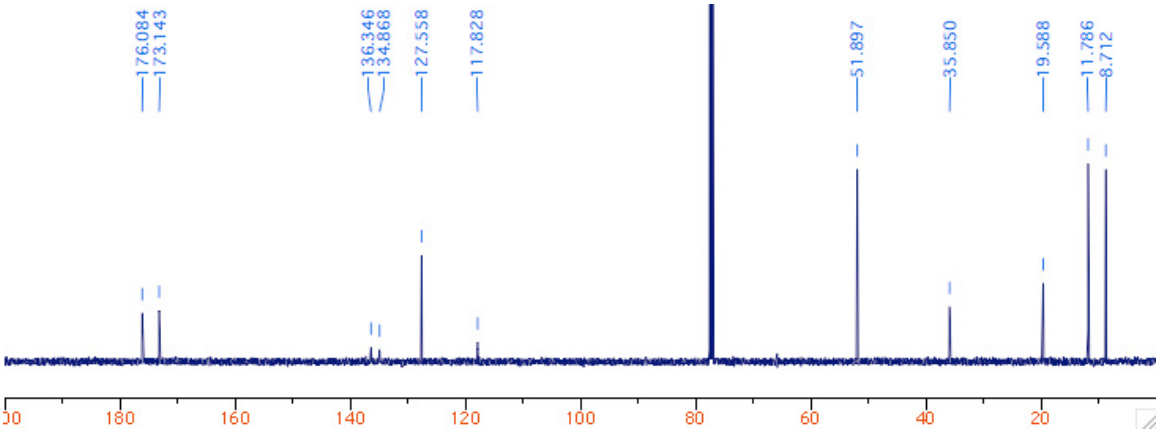
3-[2-(Methoxycarbonyl)ethyl]-4,5-dimethyl pyrrole-2-carboxaldehyde (5c).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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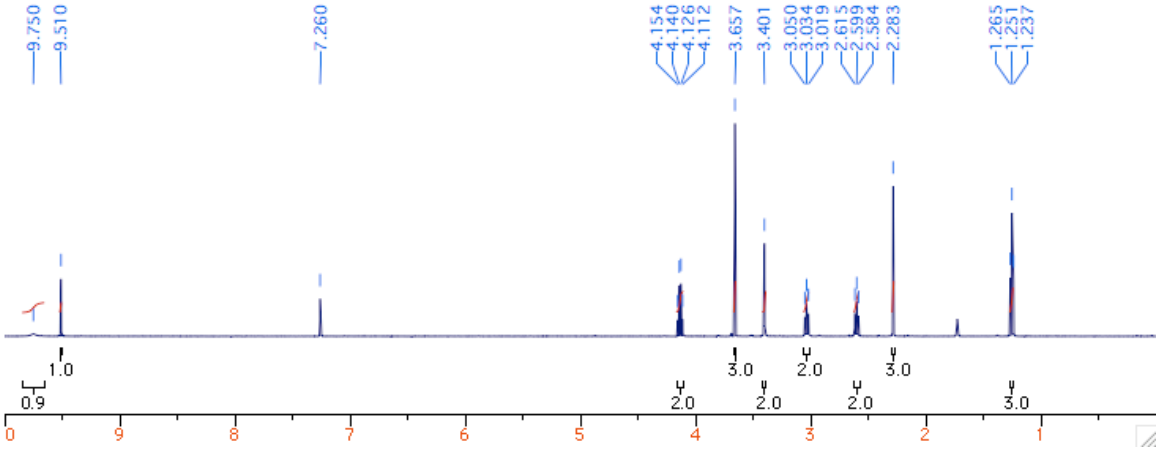
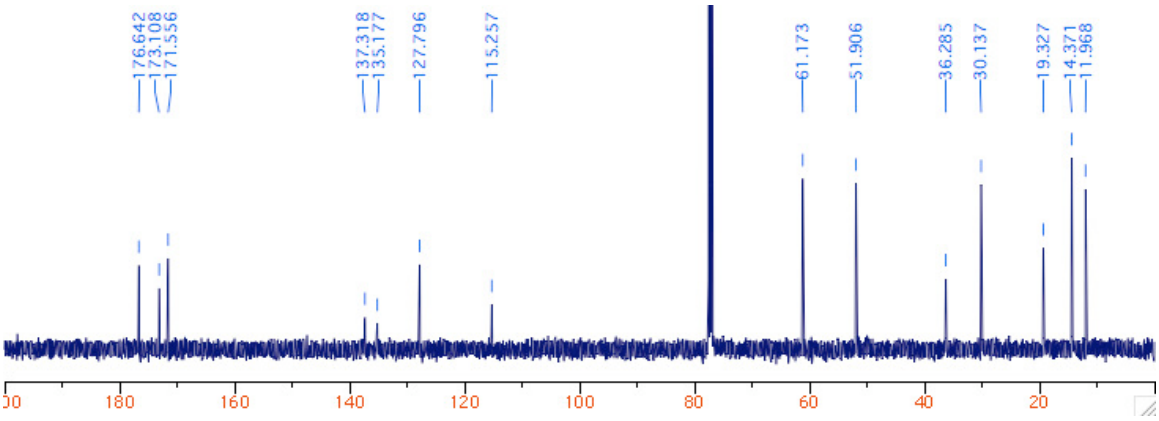
4-[(Ethoxycarbonyl)methyl]-3-[2-(methoxycarbonyl)ethyl]-5-methylpyrrole-2-carboxaldehyde (5d).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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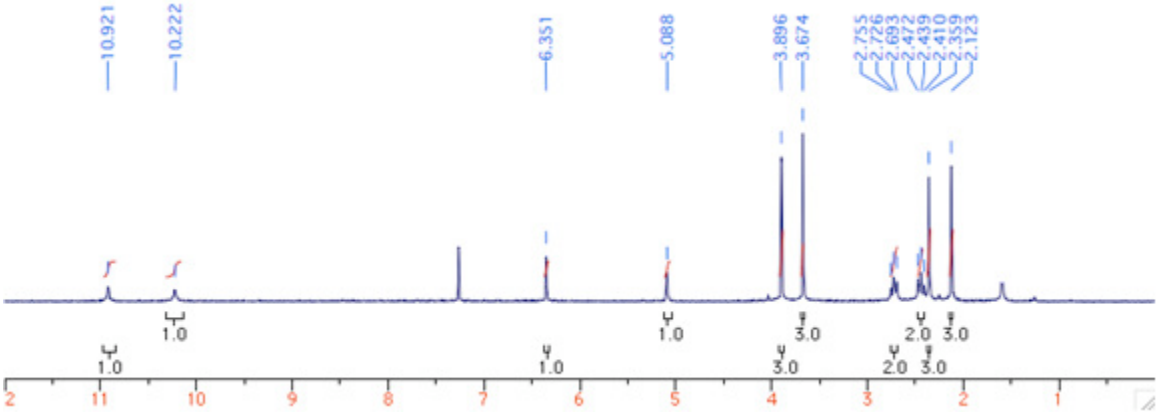
Dipyrrinones (6)

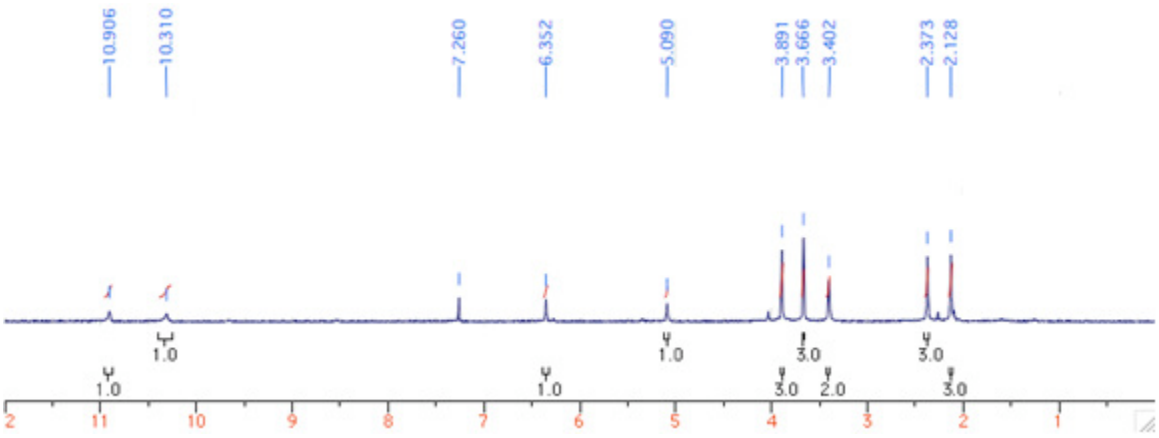
(*Z*)-Methyl 3-(5-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (6a).

^{1}H NMR ($CDCl_3$, 250 MHz):



(*Z*)-Methyl 2-(5-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)acetate (6b).

^{1}H NMR ($CDCl_3$, 250 MHz):



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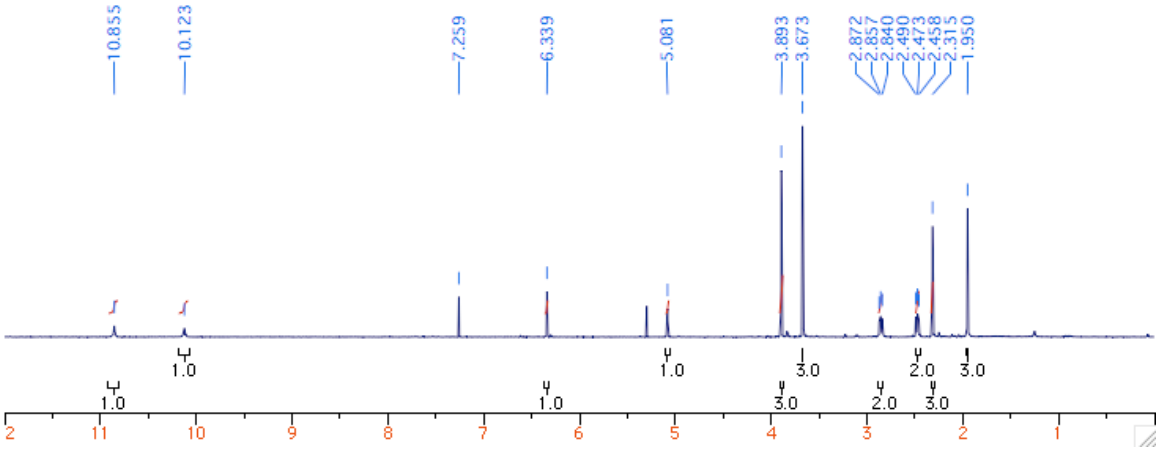
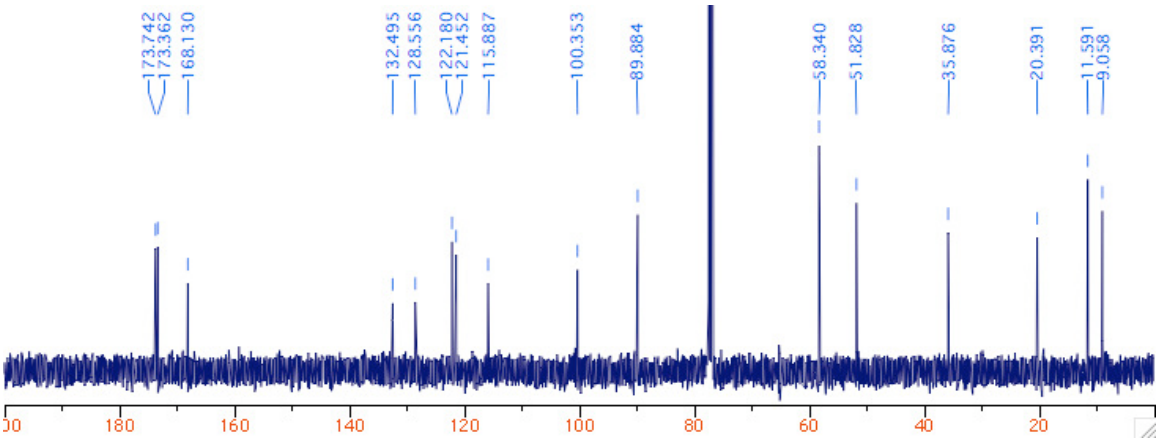
(*Z*)-Methyl 3-(2-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene)methyl)-4,5-dimethyl-1H-pyrrol-3-yl) propanoate (6c).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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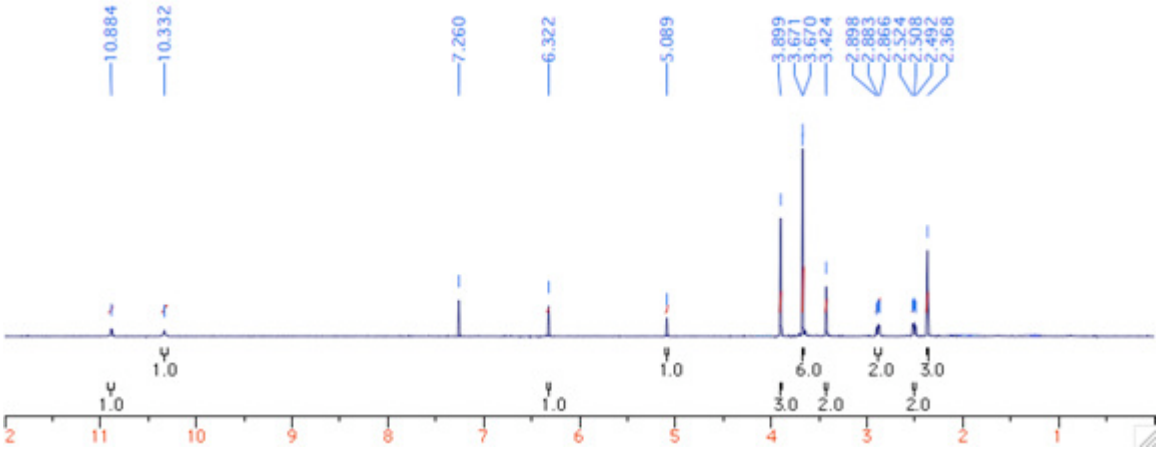
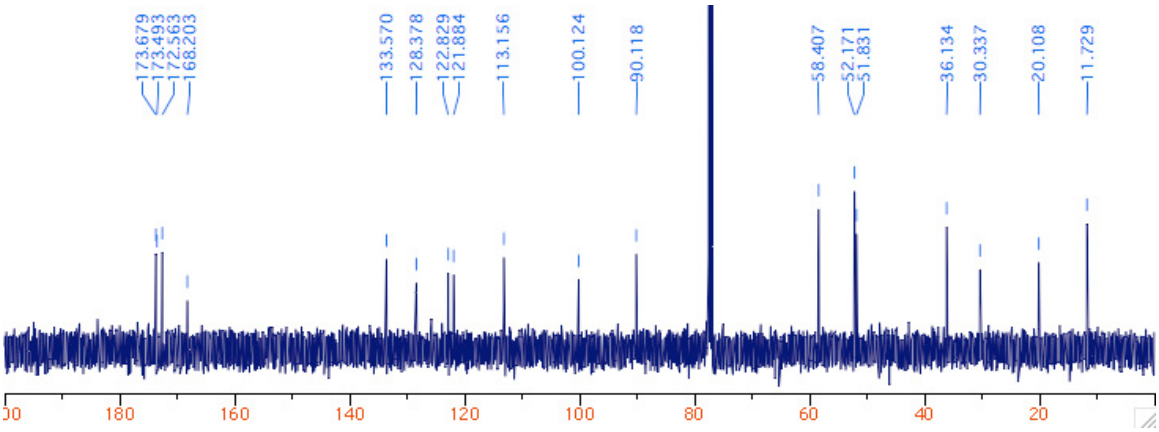
(*Z*)-Methyl 3-(4-(2-methoxy-2-oxoethyl)-2-((3-methoxy-5-oxo-1H-pyrrol-2(5H)-ylidene) methyl)-5-methyl-1H-pyrrol-3-yl)propanoate (6d).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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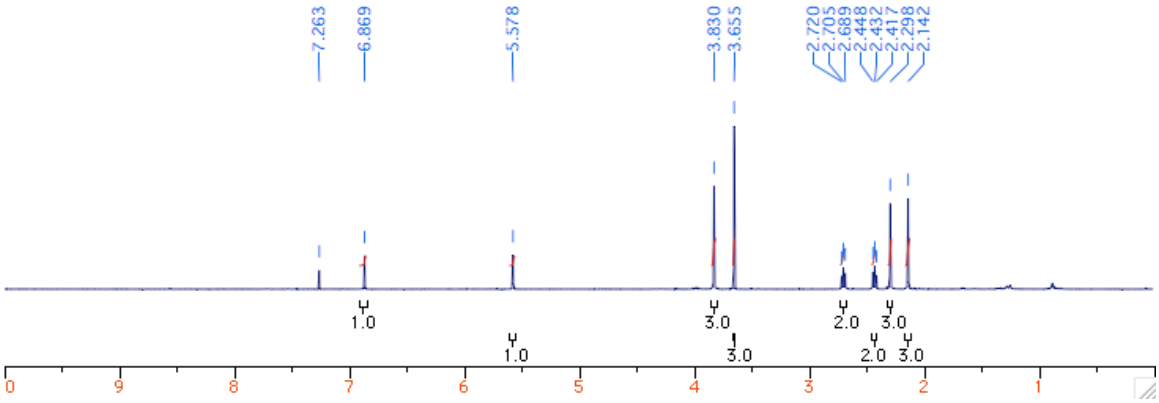
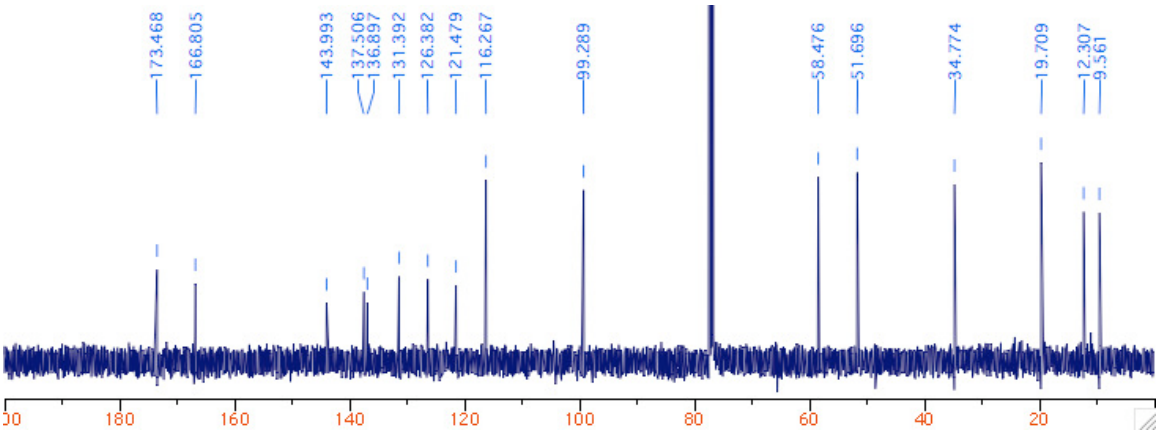
2-Bromodipyrrins (7)

(*Z*)-Methyl 3-(5-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (7a).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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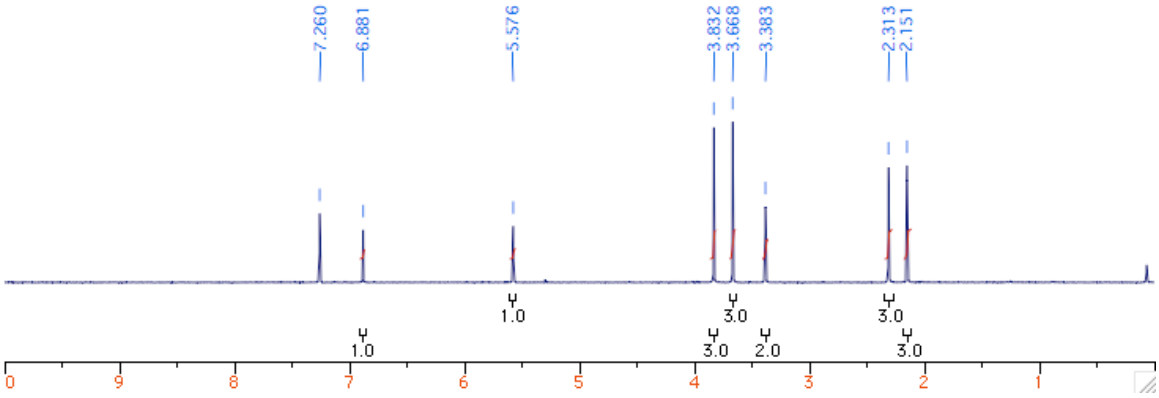
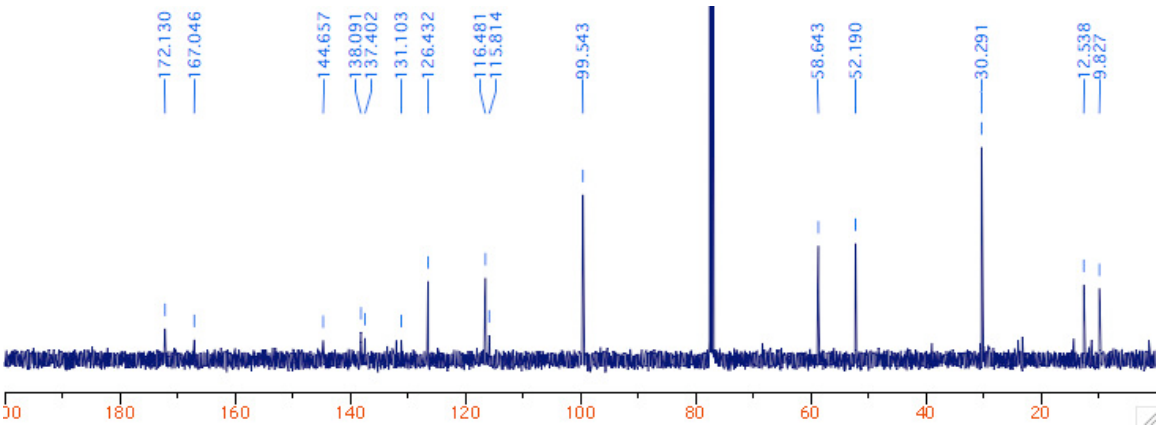
(*Z*)-Methyl 2-(5-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)acetate (7b).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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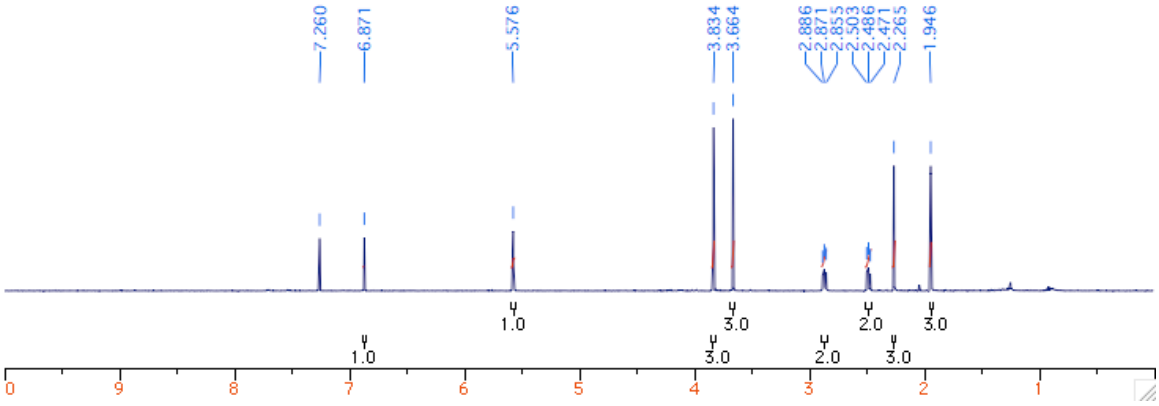
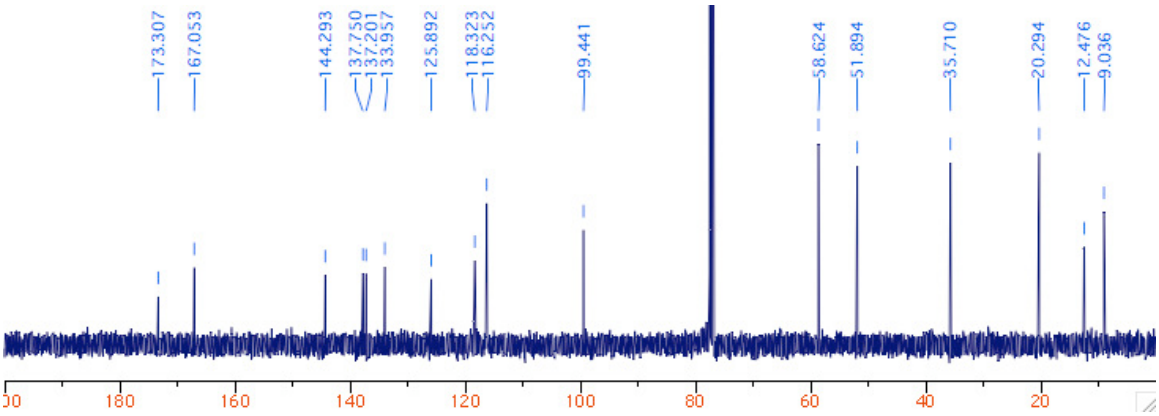
(*Z*)-Methyl 3-(2-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-4,5-dimethyl-1H-pyrrol-3-yl) propanoate (7c).

^{1}H NMR ($CDCl_3$, 500 MHz):

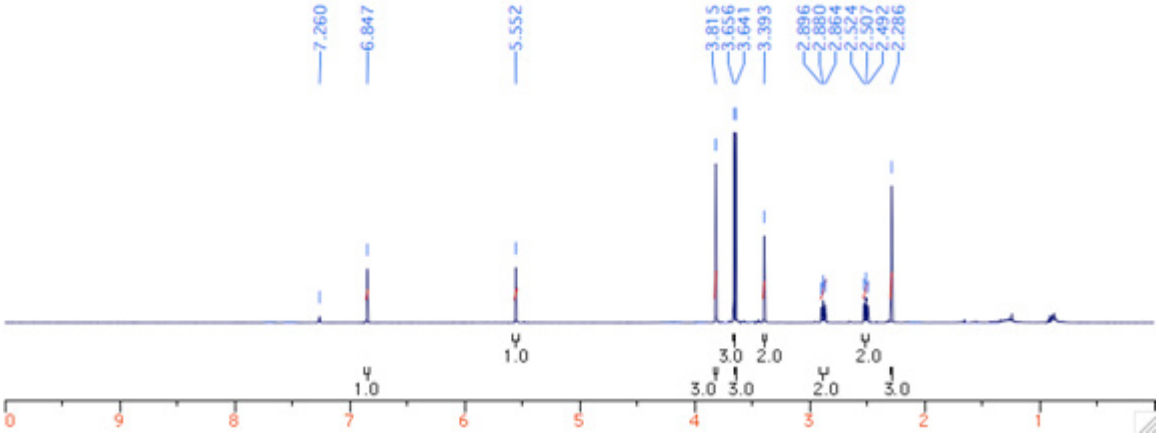


^{13}C NMR ($CDCl_3$, 125 MHz):

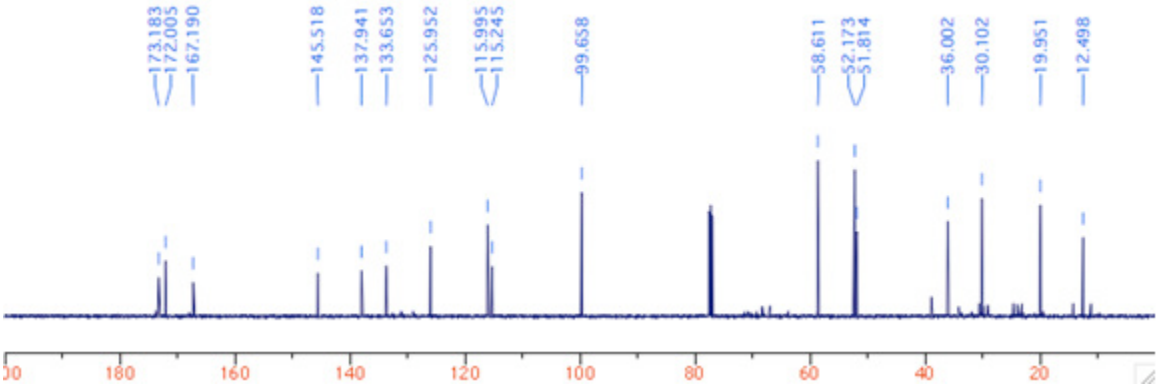


(*Z*)-Methyl 3-(2-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-4-(2-methoxy-2-oxoethyl)-5-methyl-1H-pyrrol-3-yl) propanoate (7d).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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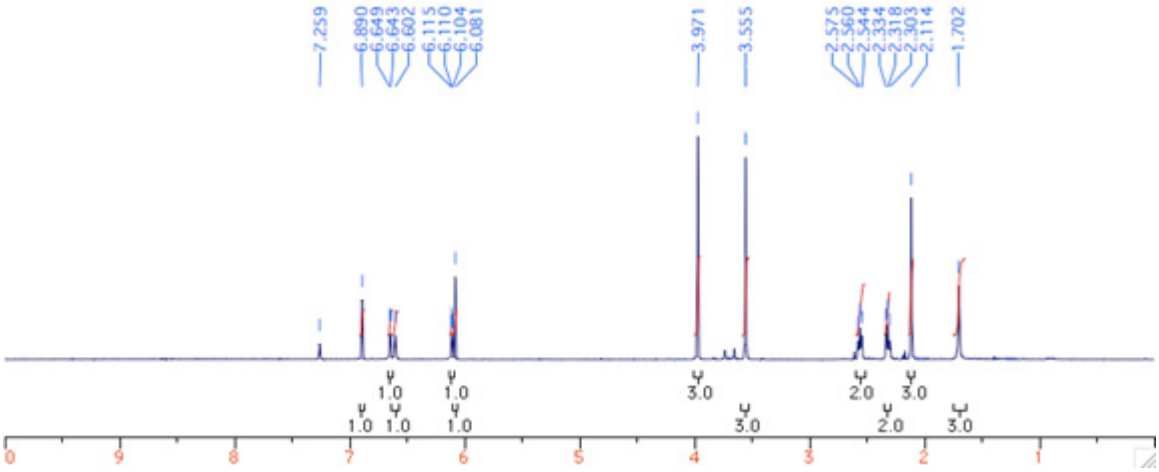
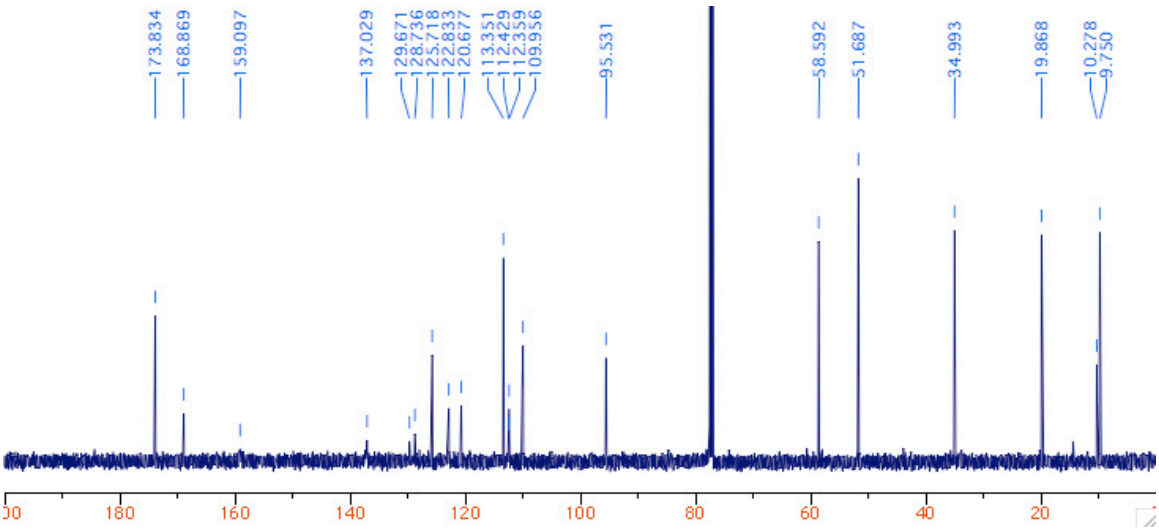
Prodigiosenes (8)

(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)propanoate (8a).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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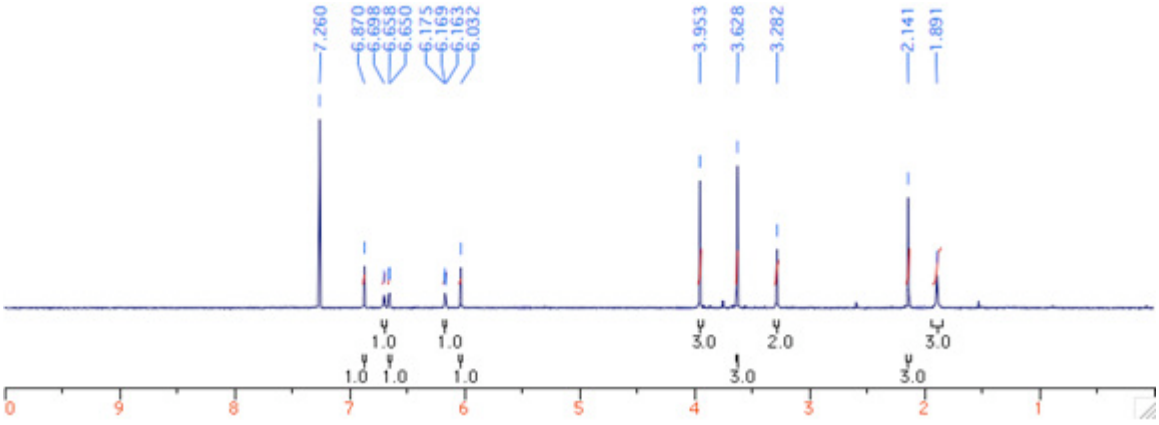
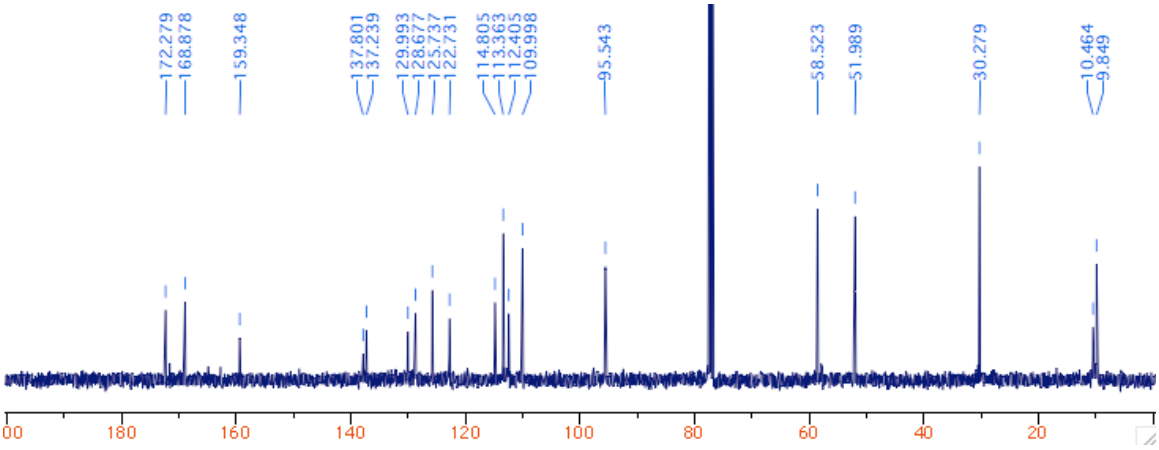
(*Z*)-Methyl 2-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)acetate (8b).

^{1}H NMR ($CDCl_3$, 500 MHz):

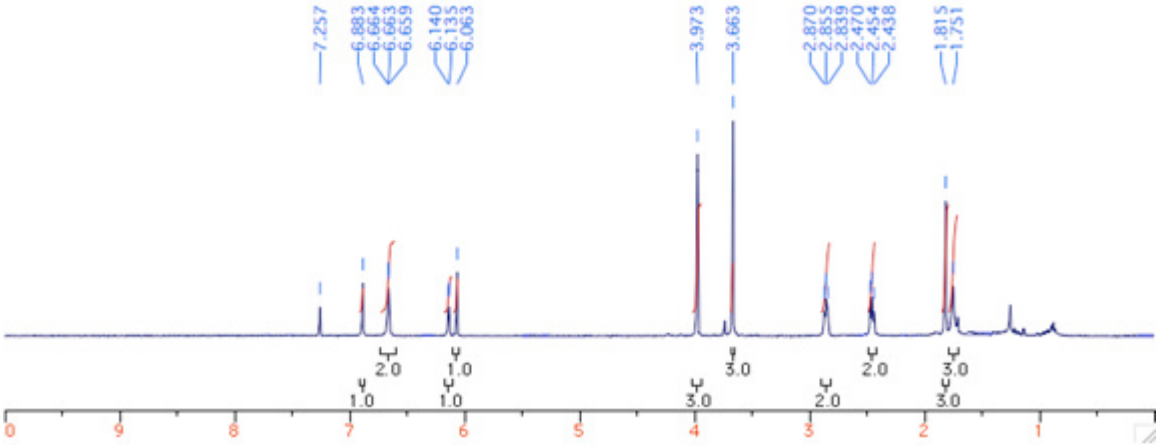


^{13}C udeft NMR ($CDCl_3$, 125 MHz):

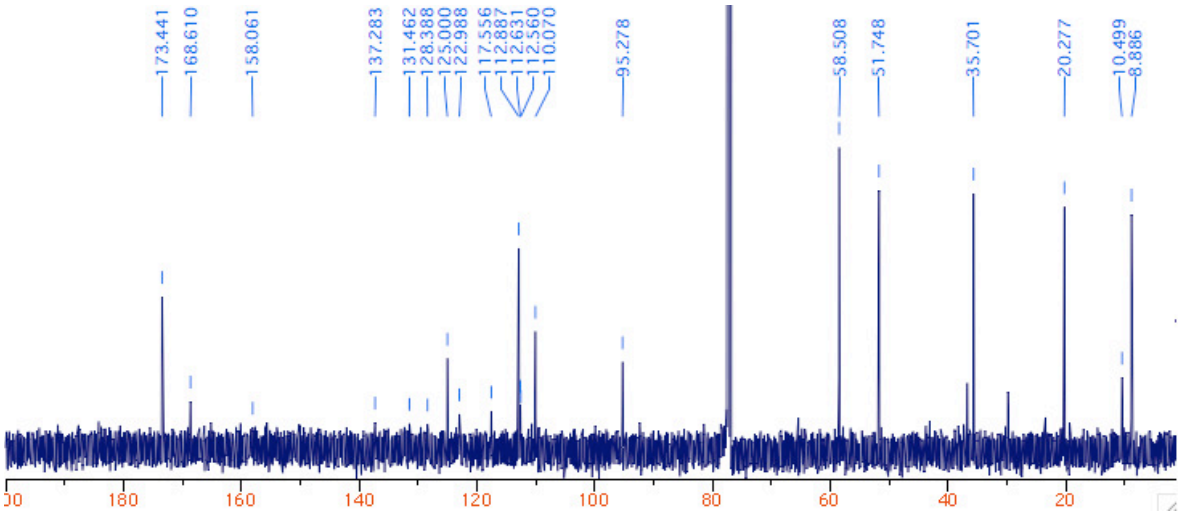


(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4,5-dimethyl-2H-pyrrol-3-yl)propanoate (8c).

^{1}H NMR ($CDCl_3$, 500 MHz):

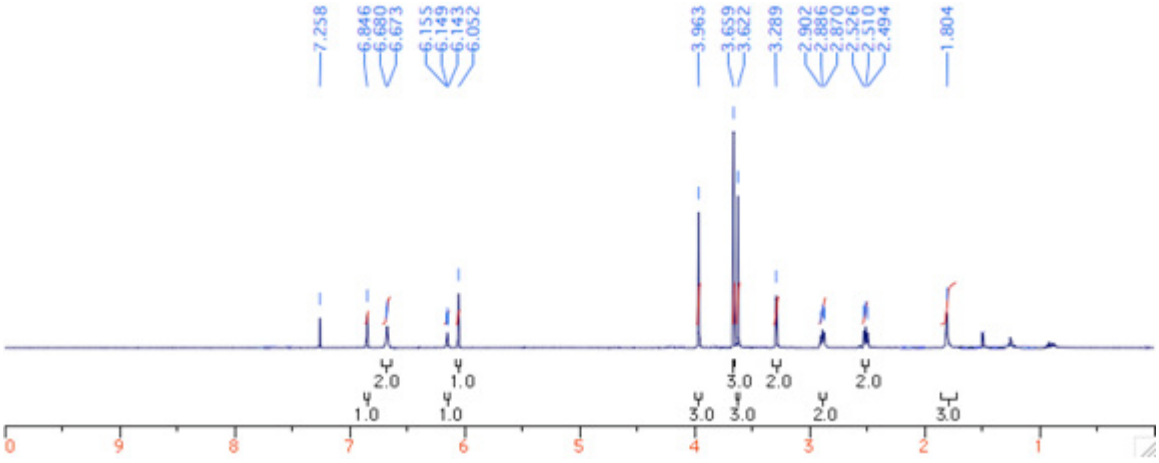


^{13}C NMR ($CDCl_3$, 125 MHz):

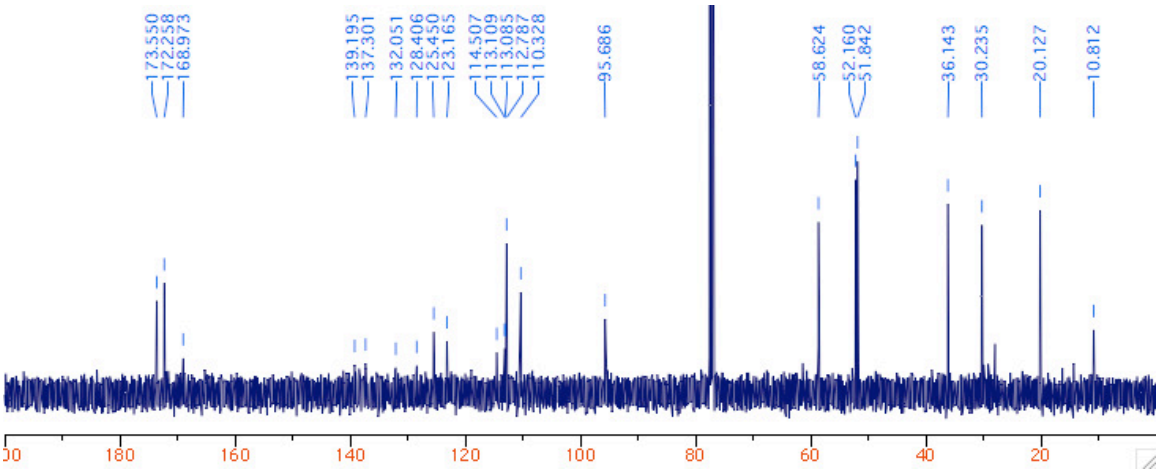


(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4-(2-methoxy-2-oxoethyl)-5-methyl-2H-pyrrol-3-yl)propanoate (8d).

^{1}H NMR ($CDCl_3$, 500 MHz):



^{13}C NMR ($CDCl_3$, 125 MHz):



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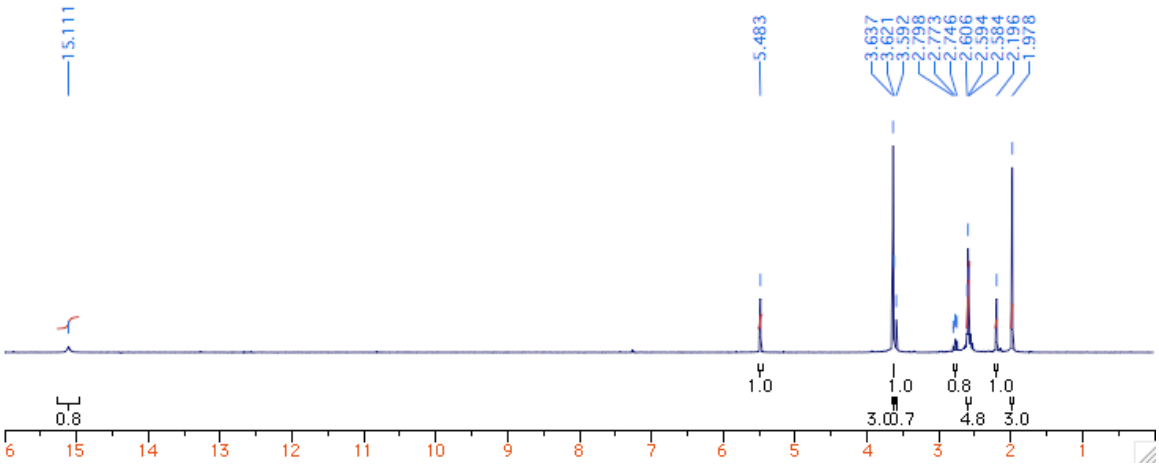
Diketones

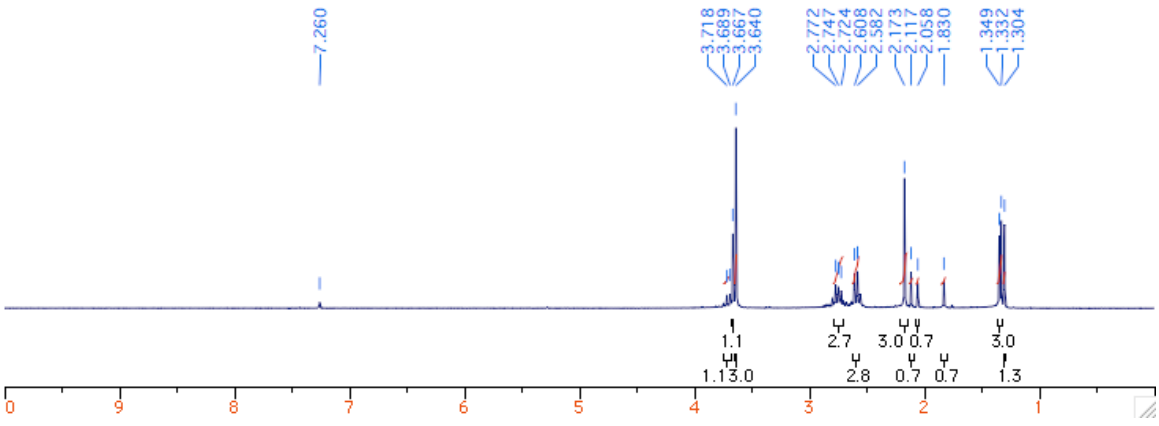
Methyl-4,6-Dioxoheptanoate (12).

^{1}H NMR ($CDCl_3$, 250 MHz):



Methyl-5-Methyl-4,6-Dioxoheptanoate (13a).

^{1}H NMR ($CDCl_3$, 250 MHz):



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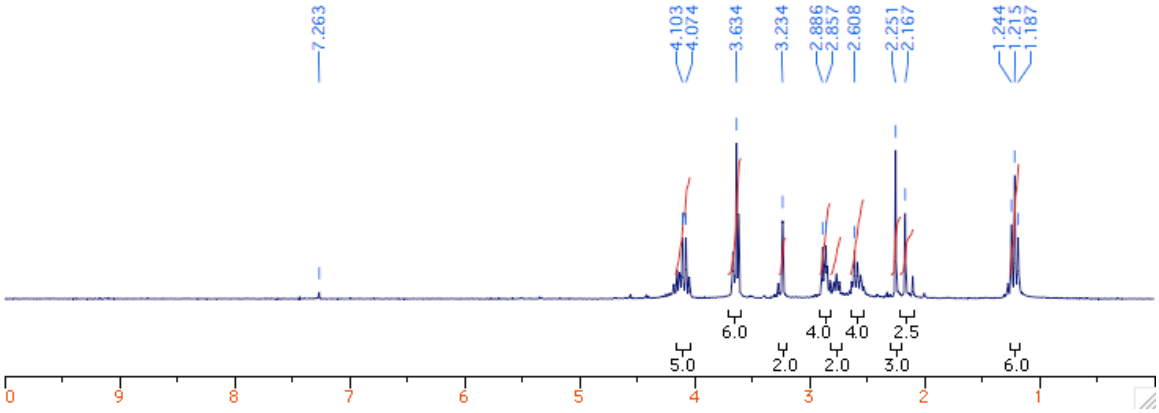
Methyl-5-[(Ethoxycarbonyl)Methyl]-4,6-Dioxoheptanoate (13b).

^{1}H NMR ($CDCl_3$, 250 MHz):



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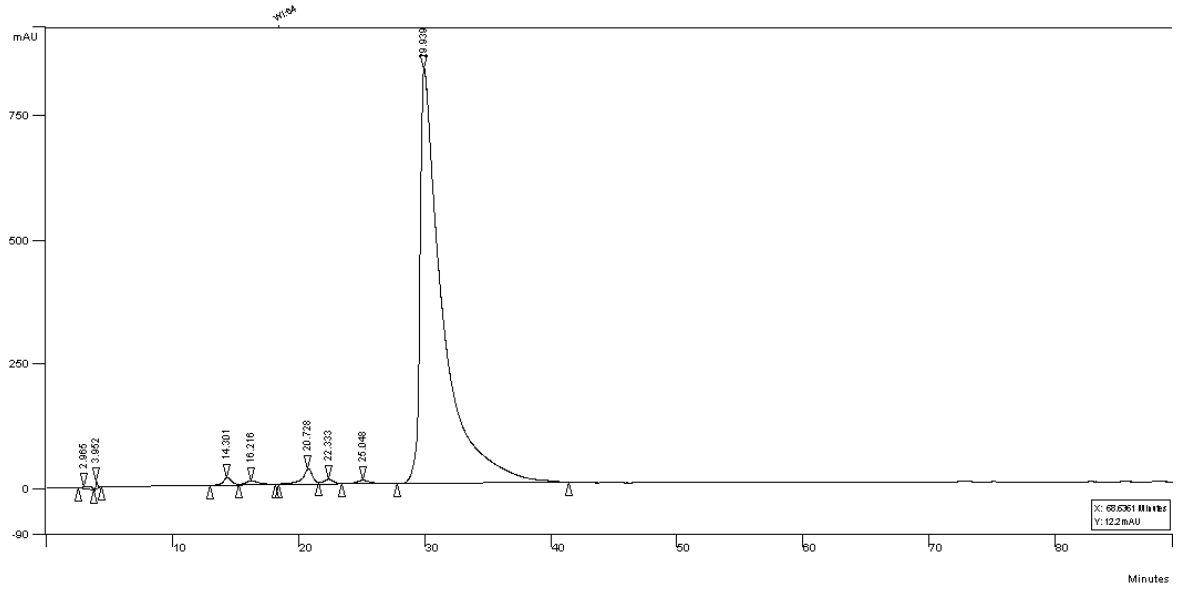
HPLC Traces

Prodigiosenes (8)

(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)propanoate (8a).



Solvent: Methanol:Water:NH_4OH ratio 90:9.8:0.2
15 µL injection
Flow rate: 0.75 mL/min
Detector: 451 nm
Retention time: 29.9 minutes
Result: 96%

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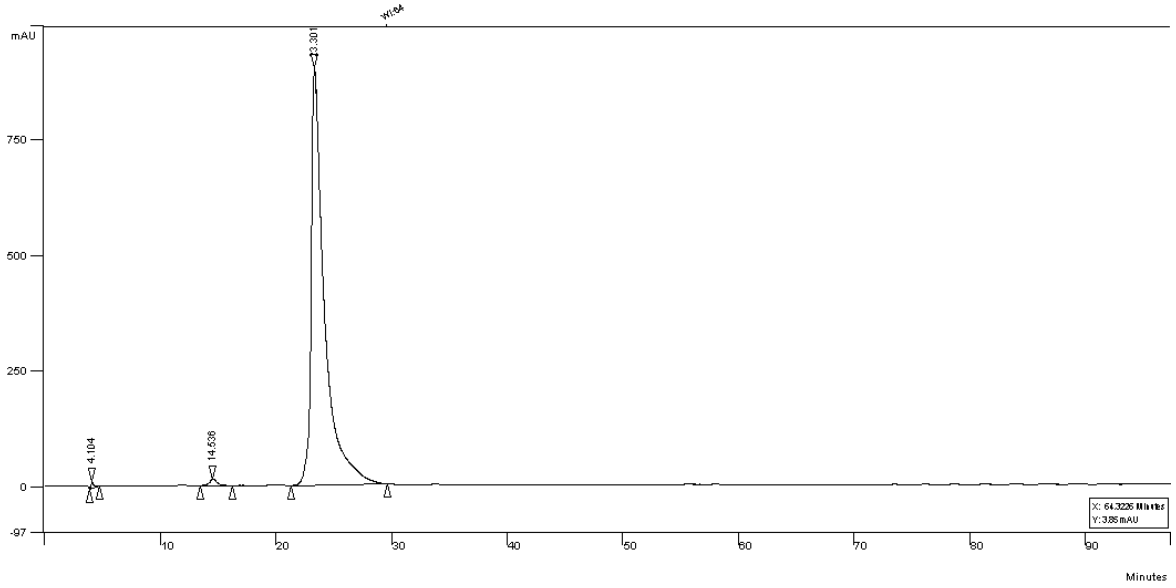
(*Z*)-Methyl 2-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)acetate (8b).



Solvent: Methanol:Water:NH_4OH ratio 90:9.8:0.2
15 µL injection
Flow rate: 0.75 mL/min
Detector: 451 nm
Retention time: 23.3 minutes
Result: 99%

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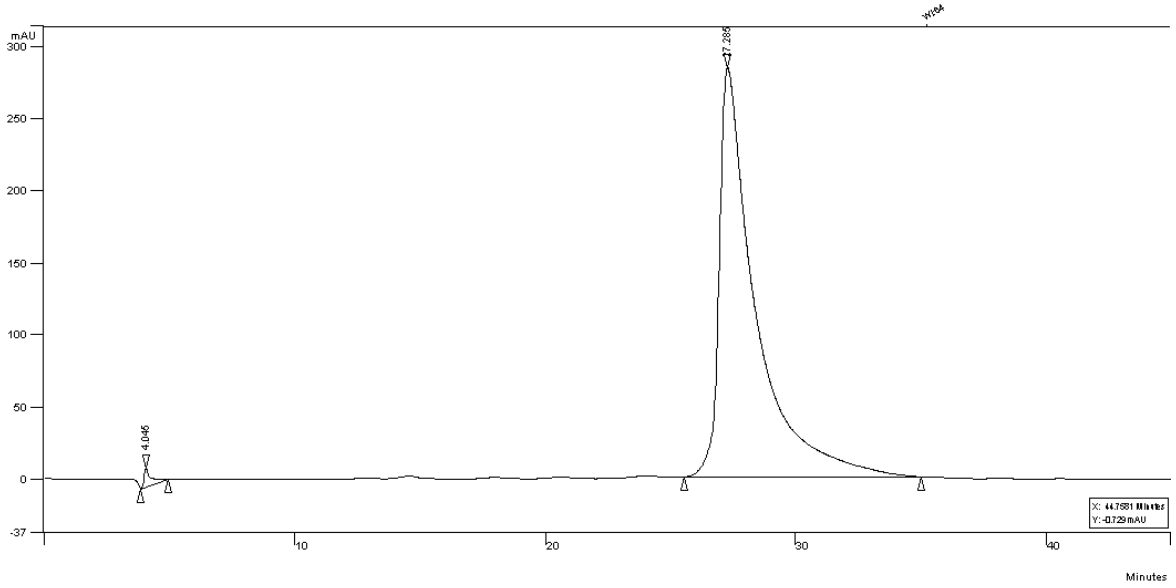
(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4,5-dimethyl-2H-pyrrol-3-yl)propanoate (8c).



Solvent: Methanol:Water:NH_4OH ratio 90:9.8:0.2
15 µL injection
Flow rate: 0.75 mL/min
Detector: 451 nm
Retention time: 27.3 minutes
Result: 99%

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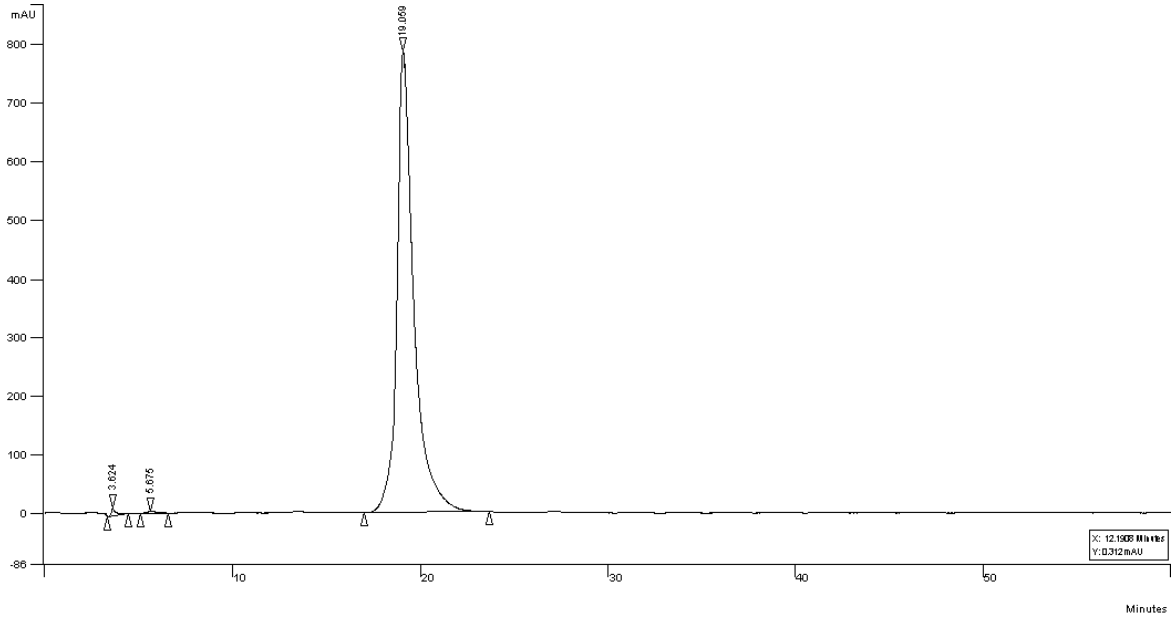
(*Z*)-Methyl 3-(2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-4-(2-methoxy-2-oxoethyl)-5-methyl-2H-pyrrol-3-yl)propanoate (8d).



Solvent: Methanol:Water:NH_4OH ratio 90:9.8:0.2
15 µL injection
Flow rate: 0.75 mL/min
Detector: 451 nm
Retention time: 19.1 minutes
Result: 99%

References

(1) White, R. M.; Sessa, A.; Burke, C.; Bowman, T.; LeBlanc, J.; Ceol, C.; Bourque, C.; Dovey, M.; Goessling, W.; Burns, C. E.; Zon, L. I. *Cell Stem Cell* 2008, *2*, 183.

(2) Kimmel, C. B.; Ballard, W. W.; Kimmel, S. R.; Ullmann, B.; Schilling, T. F. *Dev Dyn* 1995, *203*, 253.

(3) Haldi, M.; Ton, C.; Seng, W. L.; McGrath, P. *Angiogenesis* 2006, *9*, 139.

(4) Corkery, D. P.; Dellaire, G.; Berman, J. N. *Br. J. Haematol.* 2011, *153*, 786.

(5) Lash, T. D.; Mani, U. N.; Lyons, E. A.; Thientanavanich, P.; Jones, M. A. *J. Org. Chem.* 1999, *64*, 478.

(6) Paine III, J. B.; Dolphin, D. *J. Org. Chem.* 1985, *50*, 5598.

(7) Smith, K. M.; Eivazi, F.; Martynenko, Z. *J. Org. Chem.* 1981, *46*, 2189.

(8) Regourd, J.; Al-Sheikh Ali, A.; Thompson, A. *J. Med. Chem.* 2007, *50*, 1528.