# Supporting Information

## Synthesis and Chemical Characterisation of Target Identification Reagents Based on an Inhibitor of Human Cell Invasion by the Parasite *Toxoplasma gondii*

K. M. Evans, J. D. Haraldsen, R. J. Pearson, A. M. Z. Slawin, G. E. Ward and N. J. Westwood*

Centre for Biomolecular Sciences, School of Chemistry, University of St Andrews, North Haugh, St Andrews KY16 9ST, UK.
*njw3@st-andrews.ac.uk*

## Contents

## Experimental Section

Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Dichloromethane (DCM) was dried by heating under reflux over calcium hydride and distilled under an atmosphere of nitrogen. *m*CPBA was purified by dissolving in DCM and washing with an aqueous solution of buffered $KH_2PO_4$ (1.0 M) at pH 7.4 – 7.5. The DCM phase was then treated as for a separation. PE 40-60 refers to the fraction of light PE 40-60 boiling in the range 40-60 ˚C. Melting points were recorded using an Electrothermal 9100 capillary melting point apparatus. Values are quoted to the nearest 0.5 ˚C. IR spectra were measured on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. $^1$H NMR spectra were recorded at 300 and 500 MHz. $^{13}$C NMR spectra were recorded at either 75 or 125 MHz. *J* values are quoted in Hz. Assignment of spectra was carried out using PENDANT, COSY, HSQC and HMBC experiments. Low and high-resolution mass spectral analysis were recorded in either EI, CI or ES operating in positive ion mode.

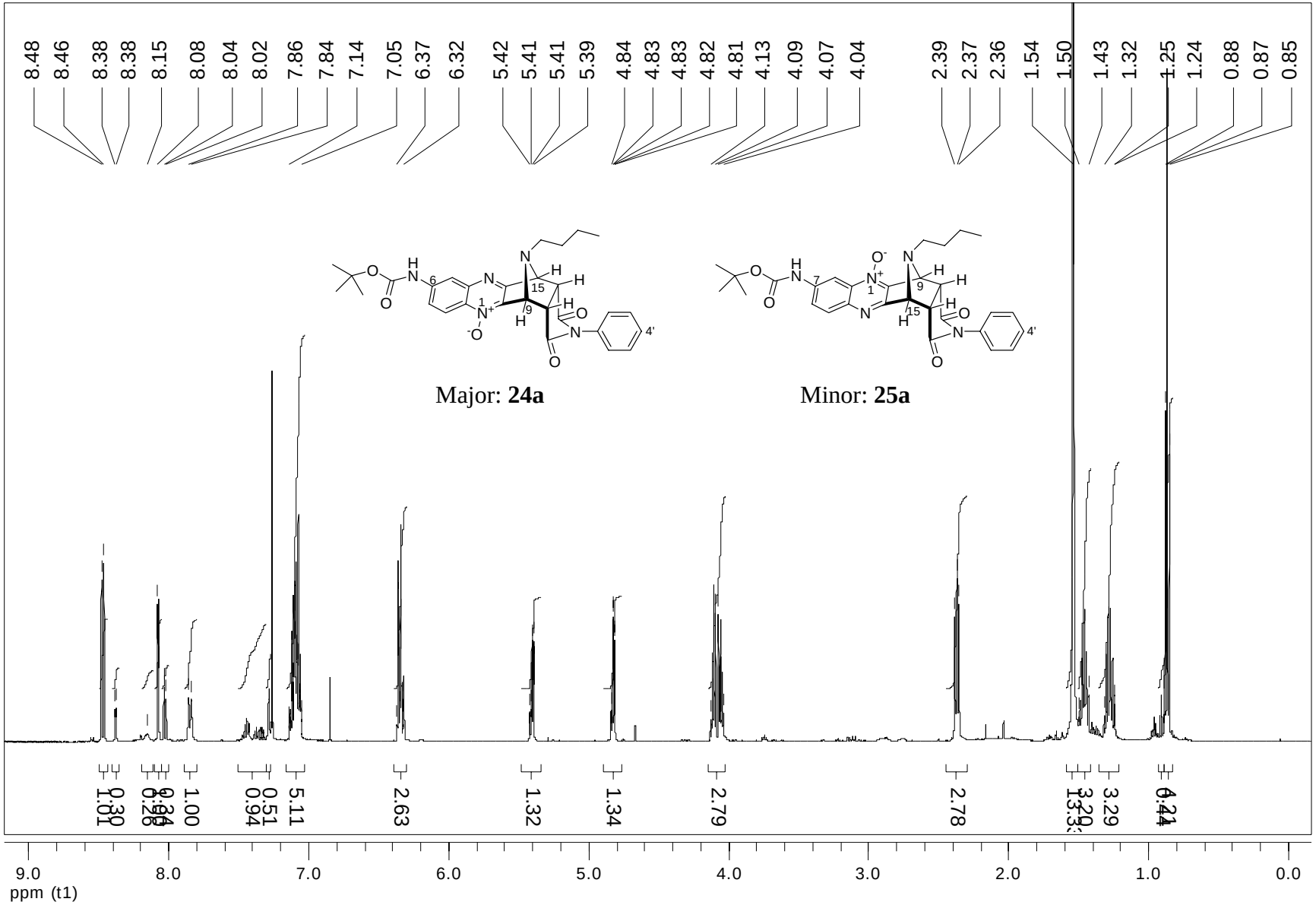
**Ethyl 2,3-dimethyl-5-bromo-6-quinoxalinecarbamate, 10.** To a solution of ethyl-2,3-dimethyl-6-quinozaline carbamate **(8)** (418 mg, 1.70 mmol) in dry DCM (6 mL) was added bromine (280.5 mg, 90 μL, 1.75 mmol, 1.03 equiv.) at room temperature. The reaction was heated at 55 $^o$C for 2 h. After cooling, the crude reaction mixture was diluted with DCM (50 mL), washed with 10% sodium bicarbonate solution (50 mL), 10% sodium thiosulfate solution (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography eluting with EtOAc:hexane 1:2 gave one major product **10** as a white solid (400 mg, 0.99 mmol, 58%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.30 (3H, t, $^3J$ = 7.5 Hz, $OCH_2C\underline{H}_3$), 2.67 (3H, s, $C\underline{H}_3$), 2.71 (3H, s, $C\underline{H}_3$), 4.22 (2H, q, $^3J$ = 7.5, $OC\underline{H}_2CH_3$), 7.52 (1H, br s, N<u>H</u>), 7.87 (1H, d, $^3J$ = 8.0, 1 × Ar-<u>H</u>), 8.58 (1H, d, $^3J$ = 8.0, 1 × Ar-<u>H</u>); MS-ES+ (*m/z*) 348 ($[M + Na]^+$, $^{81}$Br, 88%), 346 ($[M + Na]^+$, $^{79}$Br,100). A number of other minor products were isolated that had mass spectra consistent with the incorporation of more than one bromine atom. No further characterisation was carried out on these compounds.

**Ethyl 2,3-bis(bromomethyl)quinoxalin-6-ylcarbamate 1,4-dioxide, 12.** 1,4-Dibromo-2,3-butanedione (541 mg, 2.2 mmol) was added to a stirred solution of diamine **11** (433 mg, 2.22 mmol) in acetic acid (20 mL). The reaction was heated at 100 $^o$C for 45 min. before being poured onto a 10% sodium hydrogencarbonate solution (1L) containing ice. The reaction was then extracted with DCM (3

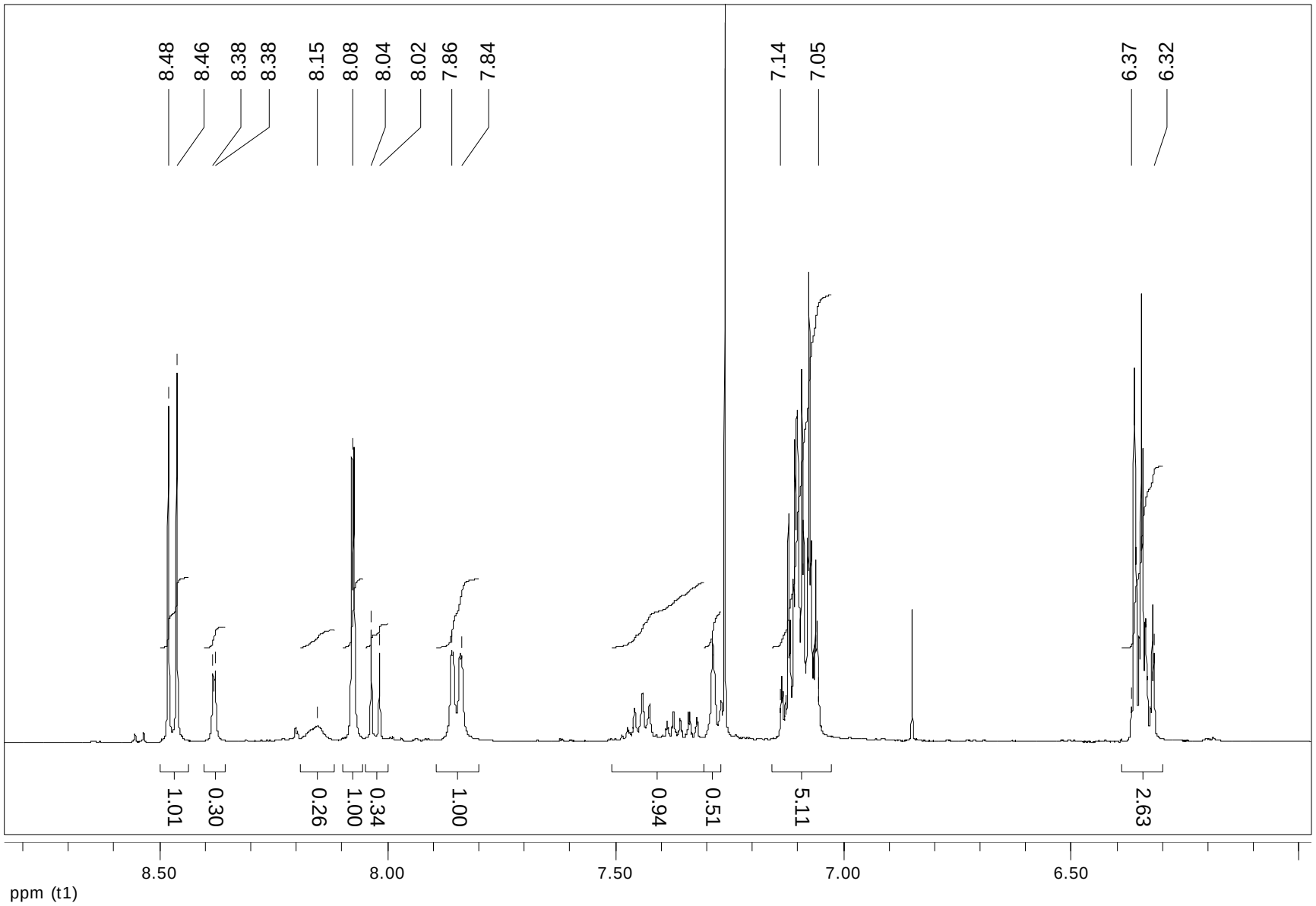
× 300ml) and the combined organic extracts dried ($MgSO_4$) and concentrated *in vacuo* to give a crude sample of **ethyl 2,3-bis(bromomethyl)quinoxalin-6-ylcarbamate (9)** (457 mg, 1.13 mmol, 51%) as a brown foam. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.30 (3H, t, $^3J$ = 7.5, $OCH_2C\underline{H}_3$), 4.21 (2H, q, $^3J$ = 7.5, $OC\underline{H}_2CH_3$), 4.81-4.83 (6H, 2 × s, 2 × $C\underline{H}_3$), 6.90 (1H, br s, N<u>H</u>), 7.79 (1H, dd, $^3J$ = 8.0, $^3J$ = 2.0, 1 × Ar-<u>H</u>), 7.92 (1H, d, $^3J$ = 8.0, 1 × Ar-<u>H</u>), 8.03 (1H, d, $^3J$ = 2.0, 1 × Ar-<u>H</u>). A sample of crude **9** (200 mg, 0.50 mmol) was reacted with purified *m*CPBA (428 mg, 1.24 mmol, 2.5 equiv.) in dry THF (5 mL) at room temperature for 5 h. A further aliquot of *m*CPBA (428 mg, 1.24 mmol, 2.5 equiv.) was then added and the reaction stirred overnight. 10% Sodium bicarbonate solution (50 mL) was added and the reaction extracted with DCM (3 × 30ml). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The crude reaction mixture was then purified by column chromatography eluting with EtOAc:hexane 1:2 to give the desired compound **12** (17mg, 0.039mmol, 8%) as a pale yellow solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.31 (3H, t, $^3J$ = 7.5 Hz, $OCH_2C\underline{H}_3$), 4.28 (2H, q, $^3J$ = 7.5 Hz, $OC\underline{H}_2CH_3$), 4.82-4.87 (6H, 2 × s, 2 × $C\underline{H}_3$), 7.34 (1H, br s, N<u>H</u>), 8.20 (1H, dd, $^3J$ = 8.0, $^3J$ = 2.0 Hz, 1 × Ar-<u>H</u>), 8.42 (1H, d, $^3J$ = 2.0 Hz, 1 × Ar-<u>H</u>), 8.52 (1H, d, $^3J$ = 8.0 Hz, 1 × Ar-<u>H</u>).

## Representative $^{1}H$ and $^{13}C$ spectra

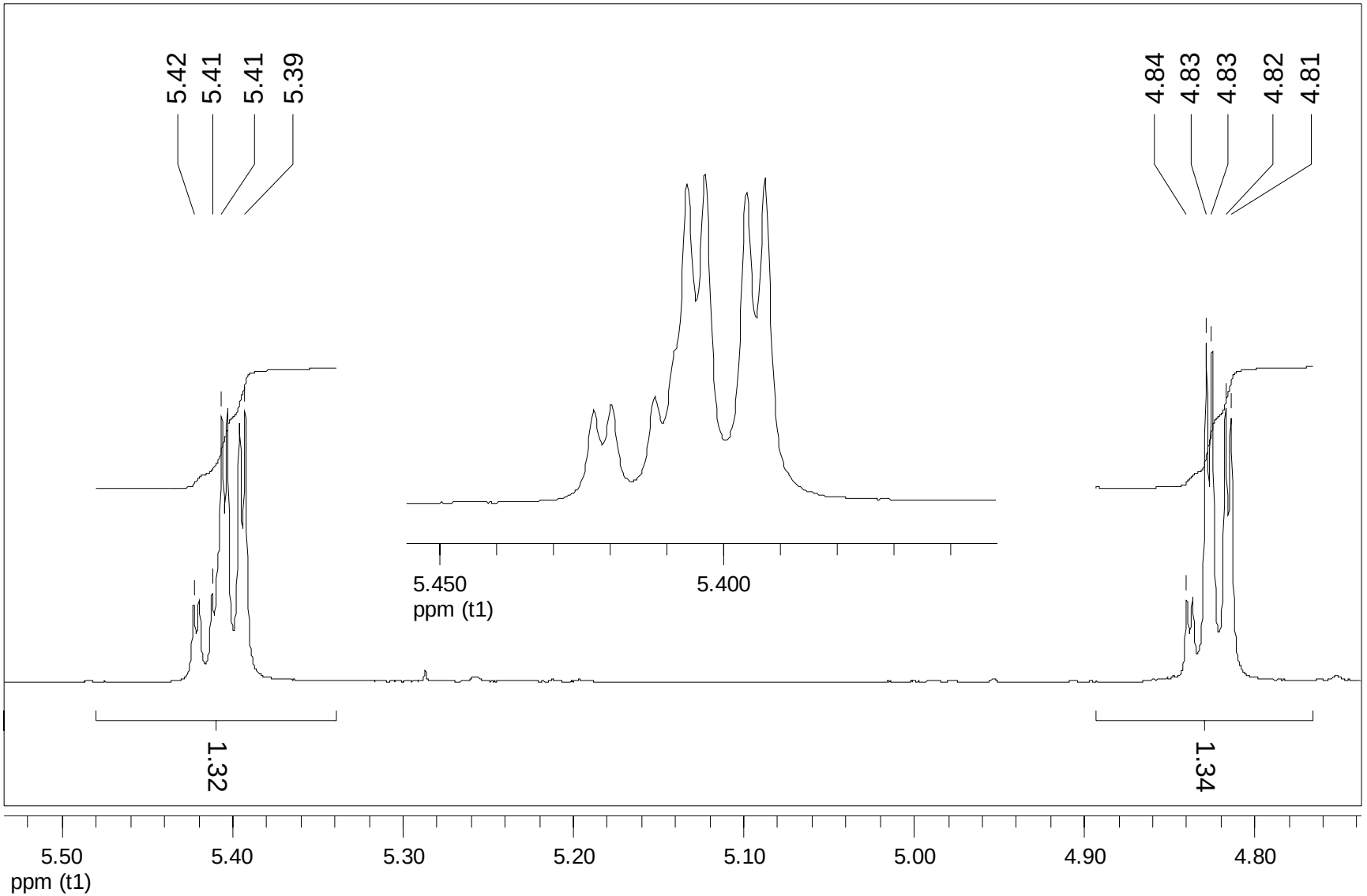
300 MHz $^{1}H$ NMR in $CDCl_3$ *Endo* regioisomers, **24a**/**25a**



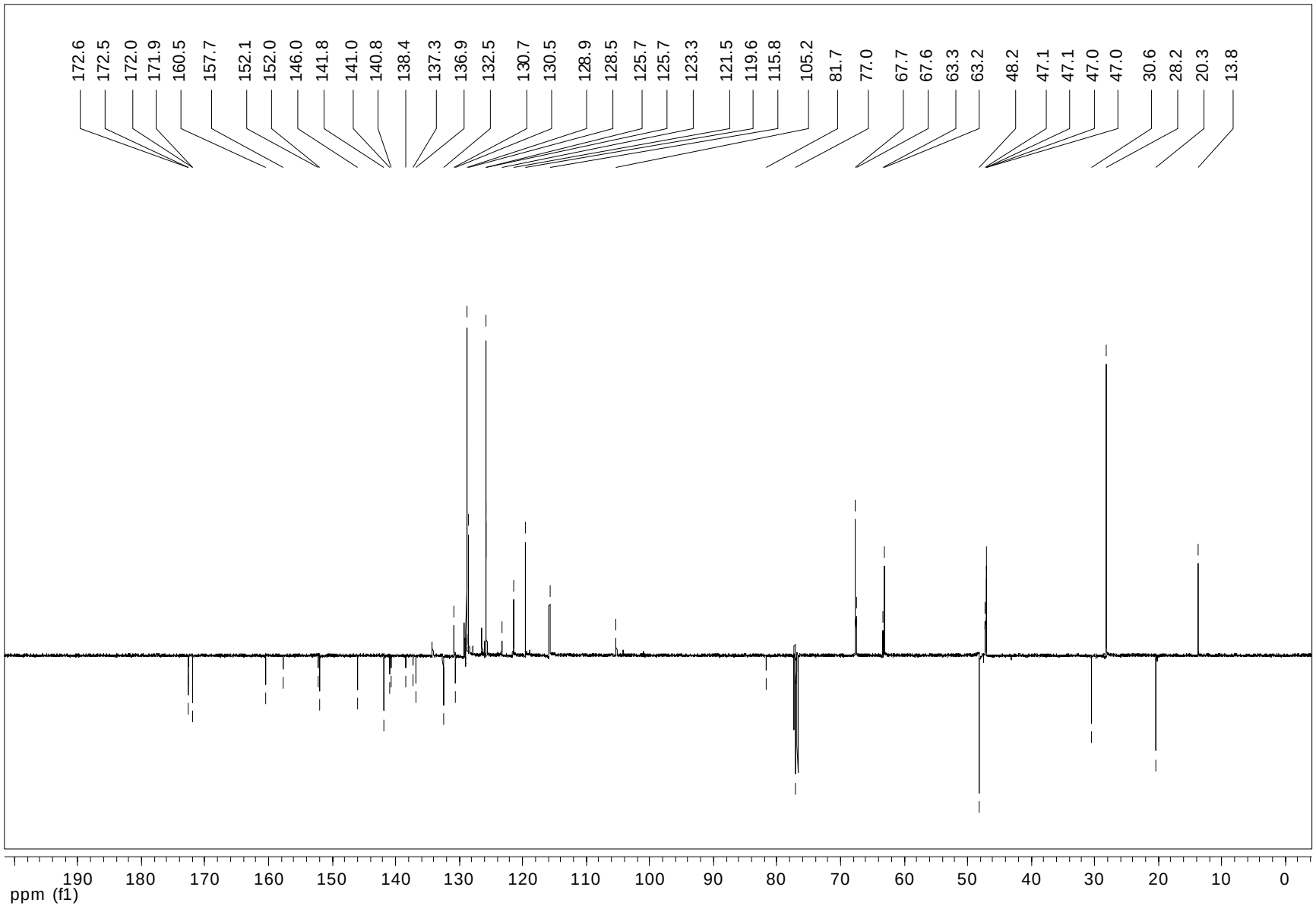
300 MHz $^{1}H$ NMR in $CDCl_3$ *Endo* regioisomers, **24a**/**25a** Expansion of the aromatic region



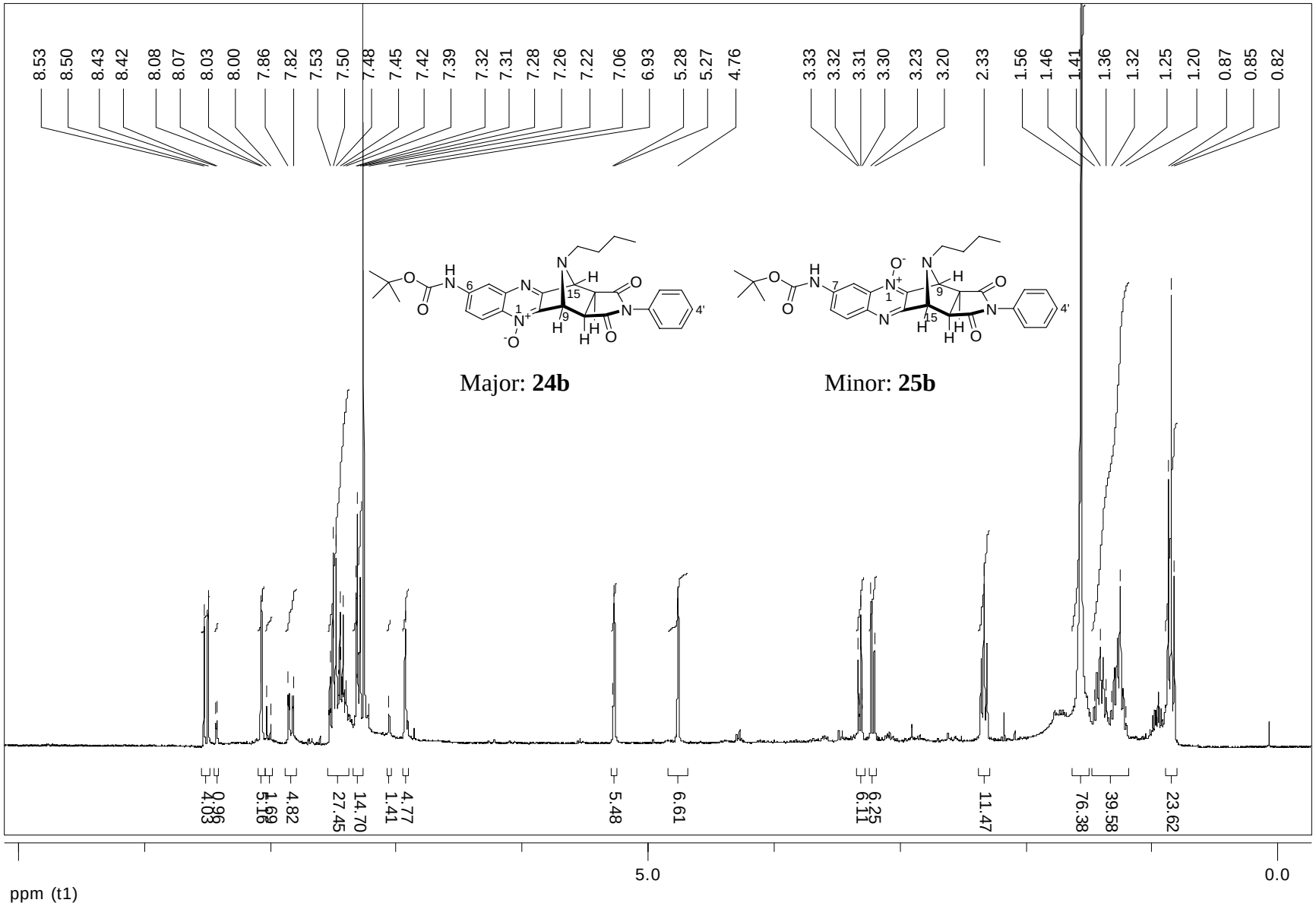
300 MHz $^{1}$H NMR in $CDCl_3$ *Endo* regioisomers, **24a**/**25a** *Endo* topology expressed by the $^{1}$H NMR signals corresponding to H9 of **24a** ($\delta$ 5.40 ppm) and **25a** ($\delta$ 5.42 ppm) and H15 of **24a** ($\delta$ 4.82 ppm) and **25a** ($\delta$ 4.83 ppm).



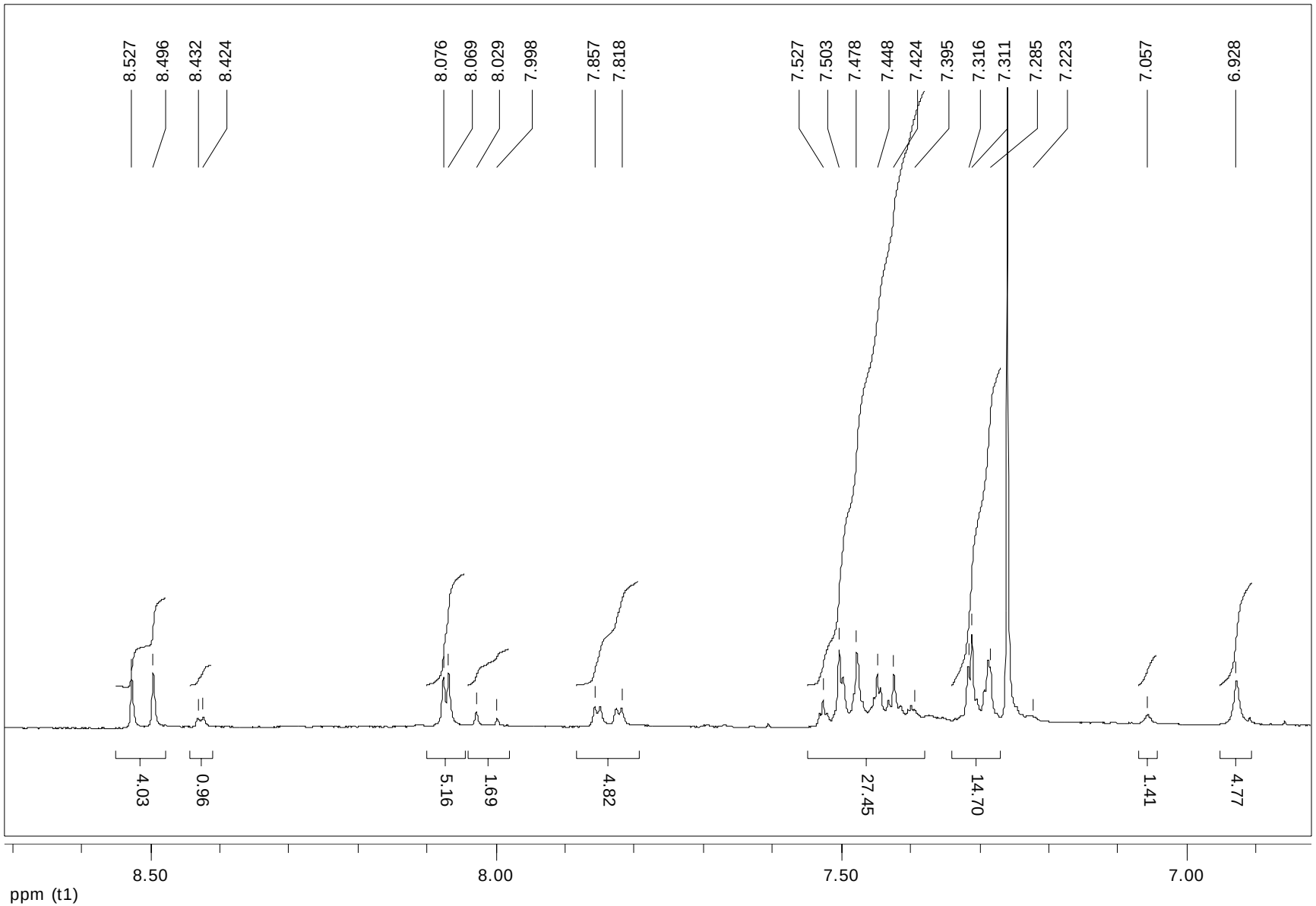
300 MHz $^{13}C$ NMR in $CDCl_3$ *Endo* regioisomers, **24a**/**25a**



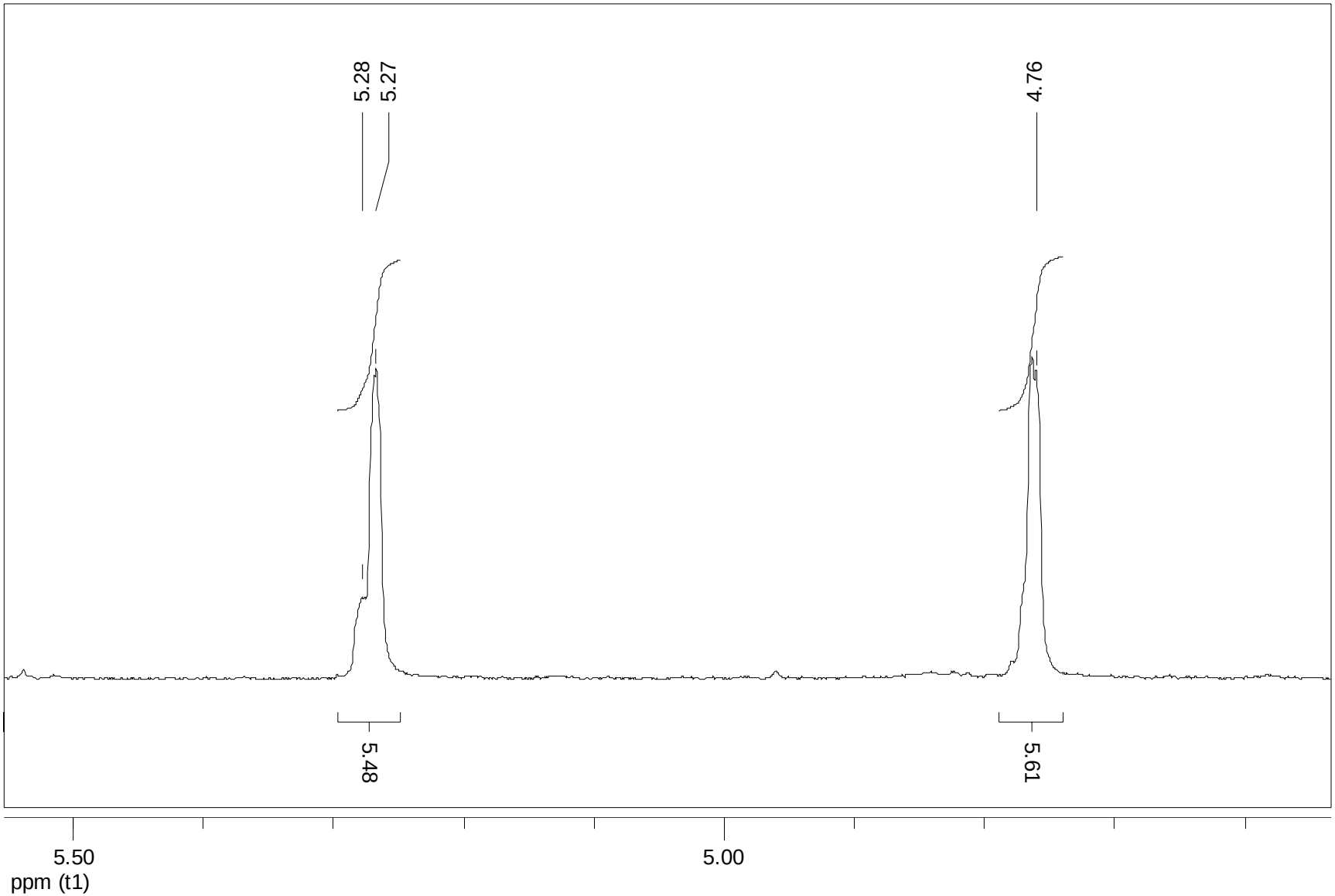
300 MHz $^{1}$H NMR in $CDCl_3$ *Exo* regioisomers, **24b**/**25b**



300 MHz $^{1}H$ NMR in $CDCl_3$ *Exo* regioisomers, **24b**/**25b** Expansion of the aromatic region



300 MHz $^{1}$H NMR in $CDCl_3$ *Exo* regioisomers, **24b**/**25b** *Exo* topology expressed by the $^{1}$H NMR signals corresponding to H9 of **24b** ($\delta$ 5.27 ppm) and **25b** ($\delta$ 5.28 ppm) and H15 of **24b** ($\delta$ 4.76 ppm) and **25b** ($\delta$ 4.76 ppm).



300 MHz $^{13}C$ NMR in $CDCl_3$ *Exo* regioisomer, Major: **24b**