Solution Structures of the Prototypical 18 kDa Translocator protein ligand, PK 11195, Elucidated with $^{1}H/^{13}C$-NMR Spectroscopy and Quantum Chemistry

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Supporting information

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***In Vitro* Binding Assay.** The binding affinities (IC_{50} value) of **1a** and **1b** for TSPO were determined in rat brain mitochondrial membranes by competition experiments against [^{3}H]**1a**. Crude mitochondrial membranes were prepared as described previously.[1] Crude preparation (0.8 mL; 0.5 mg protein per/mL) was incubated with [^{3}H]**1a** (0.58 nM; 100 µL) and the test compound (added in 100 µL) for 90 min at 4 ºC. The incubation was ended by rapid filtration through a glass filter paper (Whatman GF/B) that had been pre-soaked in poly(ethyleneimine) (0.3 %), after which the filters were washed three times with ice-cold HEPES buffer (50 mM; 3 mL), using a multi-cell harvester, M-48R. Aquasol-2 scintillator (5 mL) was added and the filter bound radioactivity was counted in a liquid scintillation counter (Beckman Coulter). Non-specific binding was determined in the presence of **1a** (10 µM). IC_{50} values were calculated by non-linear regression (one site competition) on Prism software (Graph-Pad).

Determination of Energy Barrier with Dynamic ^{1}H-NMR. Energy barriers to amide bond rotation in **1a** were calculated according to the method of Shanan-Atidi and Bar-Ali[2] by making use of the relationship:

$$P_A - P_B = \Delta P = [(X^2 - 2)/3]^{3/2}.\ 1/X$$

where P_A and P_B are the population fractions of species A and B and $X = 2\pi\delta\nu\tau$, and $\delta\nu$ is the chemical shift difference between the signals at very slow exchange and τ is defined by the relation $1/\tau = (1/\tau_A) = (1/\tau_B)$ where τ_A and τ_B are the lifetimes of species A and B, respectively.

The rates of exchange are k_A and k_B which obey:

$k_A = (1/2\tau)(1 - \Delta P)$ and $k_B = (1/2\tau)(1 + \Delta P)$

The free energy of activation can be deduced using Eyring's equation *i.e.*

$\Delta G_A^{\neq} = RT_c ln[(k/h\pi)(T_c/d\nu)[X/(1 - \Delta P)]$ and $\Delta G_B^{\neq} = RT_c ln[(k/h\pi)(T_c/d\nu)[X/(1 + \Delta P)]$

The difference between these two is given by:

$\Delta G = RT_c ln(P_A/P_B) = RT_c\ [(1 + \Delta P)/(1 - \Delta P)]$

When the values of the constants are introduced, the free energies of activation may be calculated in calories per mole as

$\Delta G_A^{\neq} = 4.575T_c[10.62 + log(X/(2\pi(1 - \Delta P)) + log(T_c/\delta\nu)]$ and

$\Delta G_B^{\neq} = 4.575 T_c[10.62 + log(X/(2\pi(1 + \Delta P)) + log(T_c/\delta\nu)]$

Values of $log(X/(2\pi(1 \pm \Delta P))$ were obtained for particular values of ΔP from the published plot of Shanan-Atidi and Bar-Ali.[2]

Tables

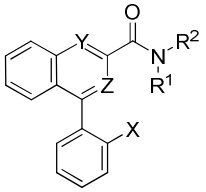
Table S1. Assignment of ^{13}C-NMR Chemical Shifts for the *N*-Me, *s*-Bu and Carbonyl Carbons of 1a from Theory [(B3LYP/6-311+G(2d,p) in $CHCl_3$] and Experiment ($CDCl_3$).

Signal	Chemical shift (δ ppm)	
	Theory	Experimental
$CH_2\mathbf{C}H_3$ (Z_1)	13.32	11.12
$CH_2\mathbf{C}H_3$ (Z_2)	13.32	11.04
$CH_2\mathbf{C}H_3$ (E_1)	13.14	11.05
$CH_2\mathbf{C}H_3$ (E_2)	13.23	10.87
$CH\mathbf{C}H_3$ (Z_1)	19.01	17.23
$CH\mathbf{C}H_3$ (Z_2)	19.22	17.31
$CH\mathbf{C}H_3$ (E_1)	20.34	18.58
$CH\mathbf{C}H_3$ (E_2)	20.43	18.45
$\mathbf{C}H_2CH_3$ (Z_1)	31.48	26.30
$\mathbf{C}H_2CH_3$ (Z_2)	31.51	26.30
$\mathbf{C}H_2CH_3$ (E_1)	32.39	27.38
$\mathbf{C}H_2CH_3$ (E_2)	32.44	27.41
$N\mathbf{C}H_3$ (Z_1)	32.97	30.50
$N\mathbf{C}H_3$ (Z_2)	32.87	30.39
$N\mathbf{C}H_3$ (E_1)	29.20	26.65
$N\mathbf{C}H_3$ (E_2)	29.29	26.65
CH (Z_1)	57.11	50.38
CH (Z_2)	57.14	50.58
CH (E_1)	65.28	55.57
CH (E_2)	64.30	55.75
CO (Z_1)	179.54	168.12
CO (Z_2)	179.18	168.12
CO (E_1)	180.68	168.38
CO (E_2)	180.56	168.38

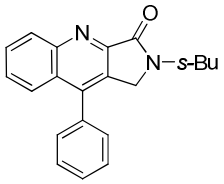
Table S2. Assignment of ^{13}C-NMR Chemical Shifts of the *s*-Bu Carbons of 1b from Theory [(B3LYP/6-311+G(2d,p) in $CHCl_3$] and Experiment ($CDCl_3$).

	Chemical shift (δ)	
	Theory	Experimental
$CH_2\mathbf{C}H_3$ (Z)	13.54	8.65, 8.73
$CH_2\mathbf{C}H_3$ (*E*)	13.27	
$CH\mathbf{C}H_3$ (Z)	24.52	18.66, 18.71
$CH\mathbf{C}H_3$ (*E*)	24.85	
$\mathbf{C}H_2CH_3$ (Z)	35.21	27.98, 27.93
$\mathbf{C}H_2CH_3$ (*E*)	36.39	
CH (*Z)*	52.52	44.92
CH (*E*)	59.20	

Table S3. Binding Affinities (IC_{50} values) for TSPO of *N*-Methyl Tertiary Amido Ligands, their *N-Desmethyl*-secondary Amido Analogs, and of a Conformationally Restrained Analog (8).



1



2

Ligand	X	Y	Z	R^1	R^2	IC_{50} (nM)
1a	Cl	CH	N	Me	*s*.Bu	0.5
1b[a]	Cl	CH	N	H	*s*.Bu	1,570
1c	H	N	CMe	Me	*s*.Bu	2.1[3]
1d	H	N	CMe	H	*s*.Bu	230[b]
1[e]	Me	N	CMe	Me	Bn	4.6[4]
1f	Me	N	CMe	H	Bn	10,270[c]
1g	H	CH	CH	Me	Bn	64[4]
1g	H	CH	CH	H	Bn	2,700[4]
8						10,000[3]

[a]*R*-enantiomer.

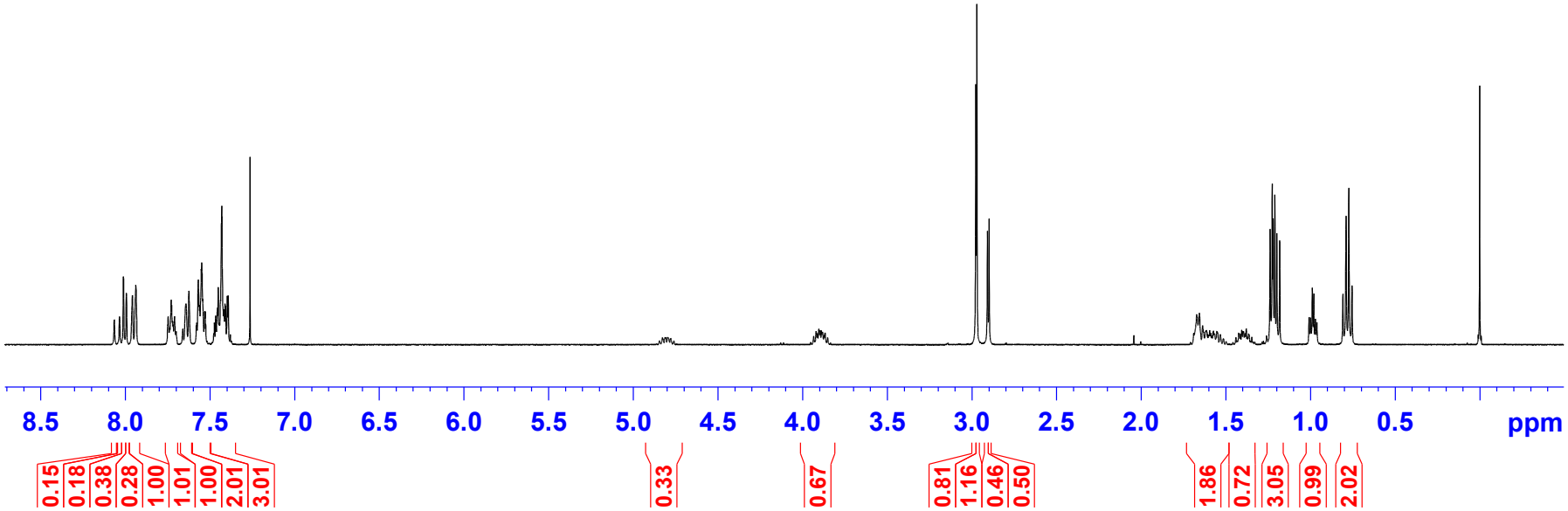


Figure S1A. Full ^{1}H-NMR spectrum of **1a** in $CDCl_3$ at 24 °C.

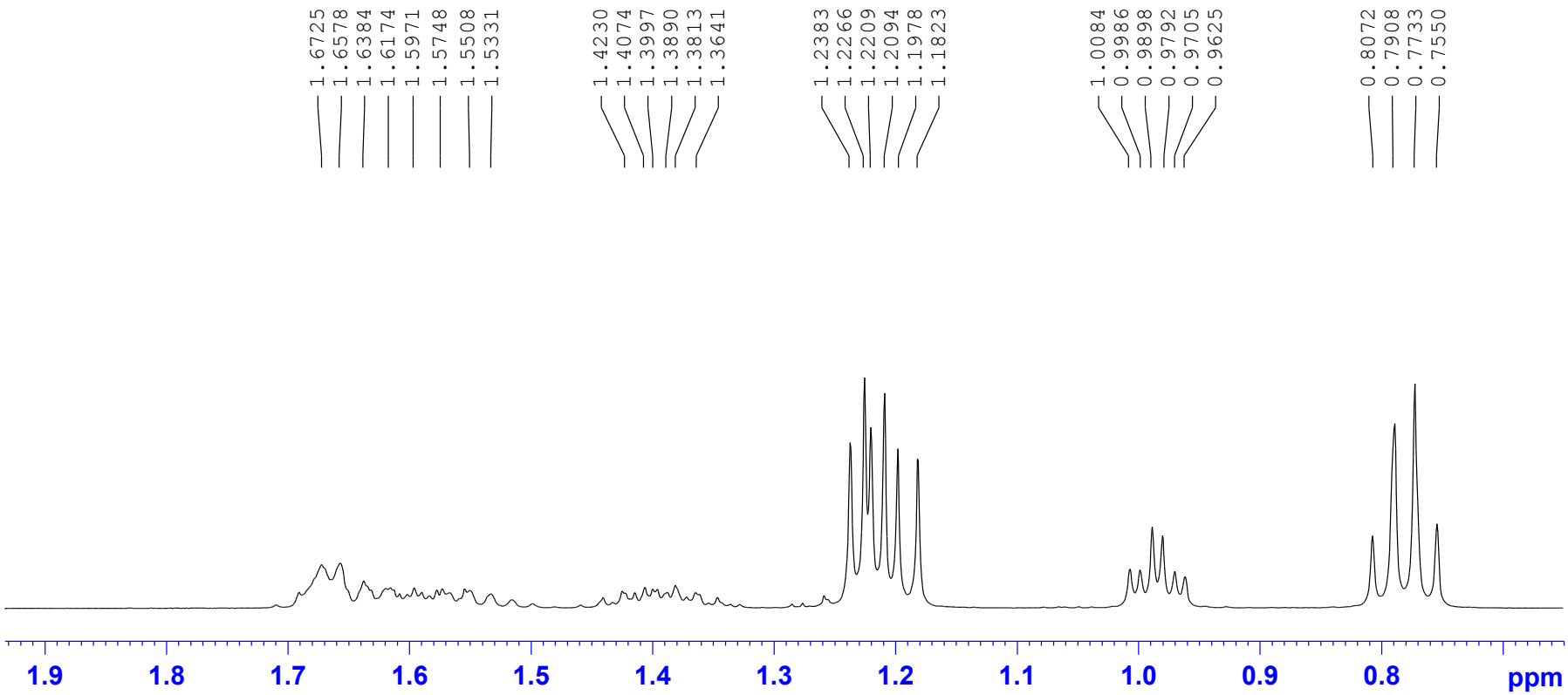


Figure S1B. Expanded ^{1}H-NMR spectrum of **1a** in $CDCl_3$ at room temperature at high field.

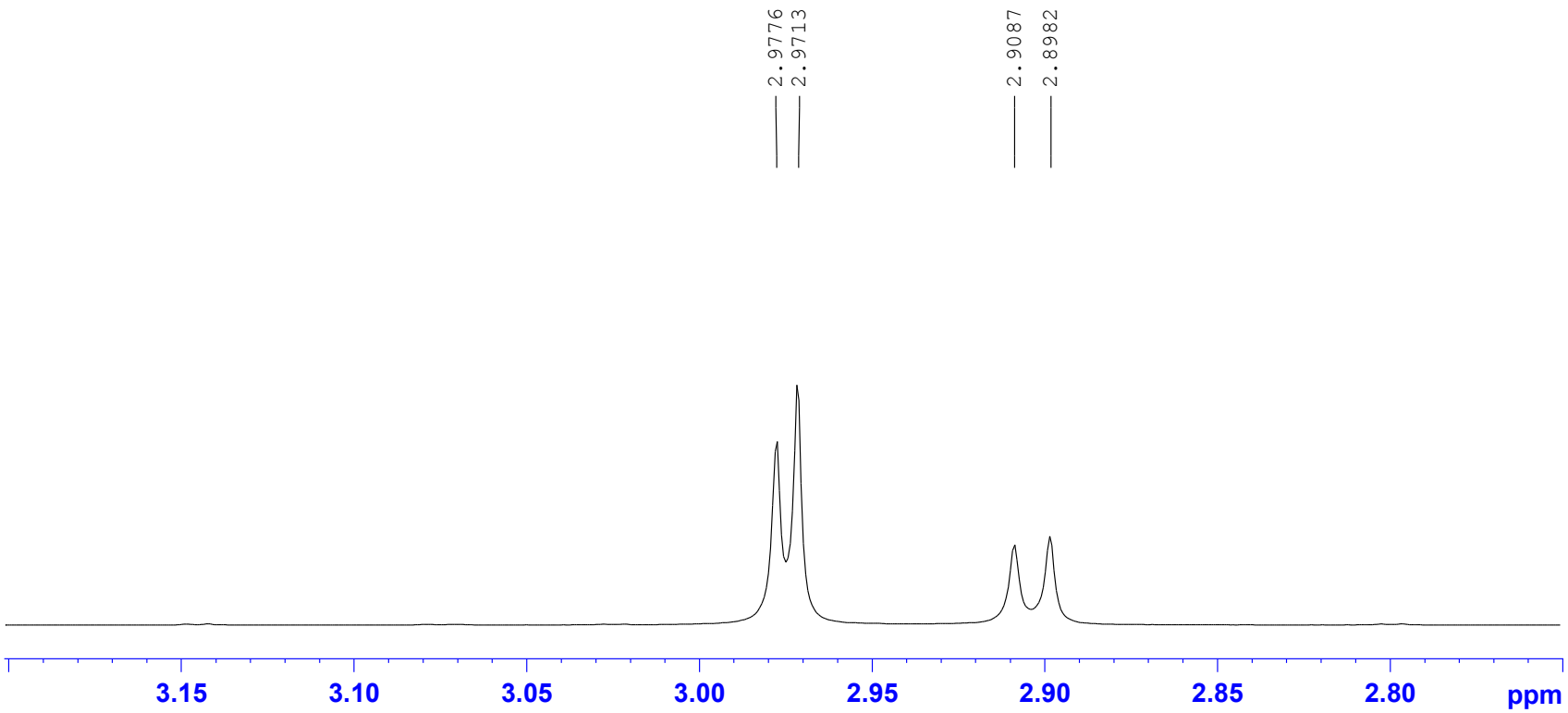


Figure S1C. Expanded ^{1}H-NMR spectrum of **1a** in $CDCl_3$ at room temperature at 2.7−3.2 ppm.

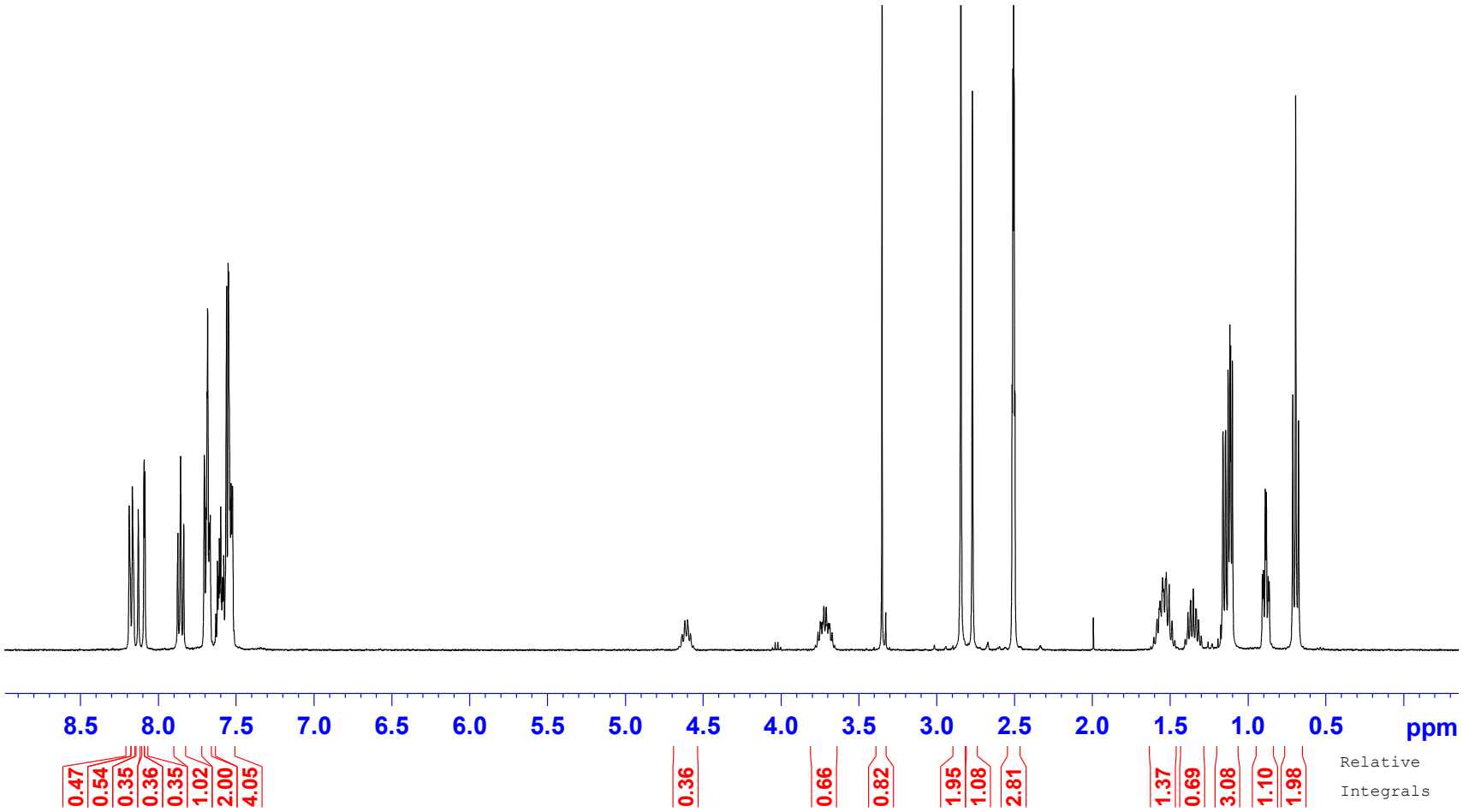


Figure S2. [1]H-NMR spectrum of **1a** in d_6–DMSO at 24 °C.

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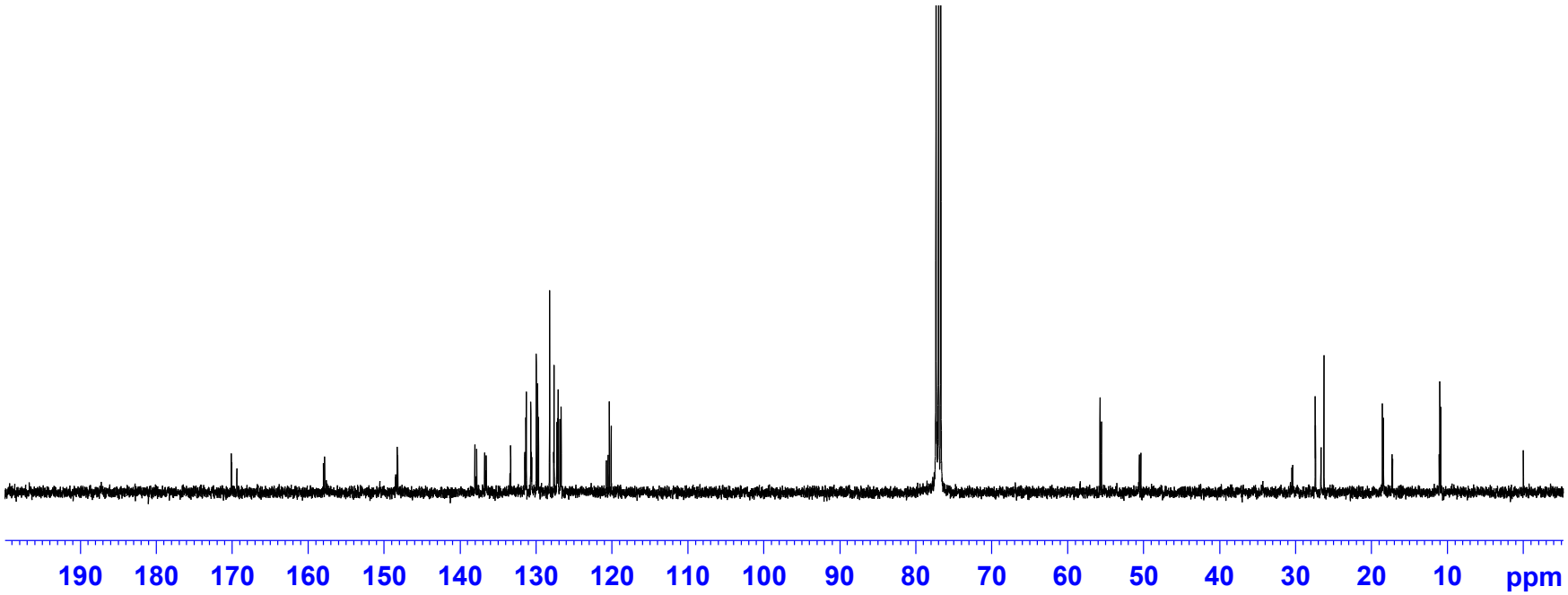


Figure S3A. Full ^{13}C-NMR spectrum of **1a** in $CDCl_3$ at room temperature.

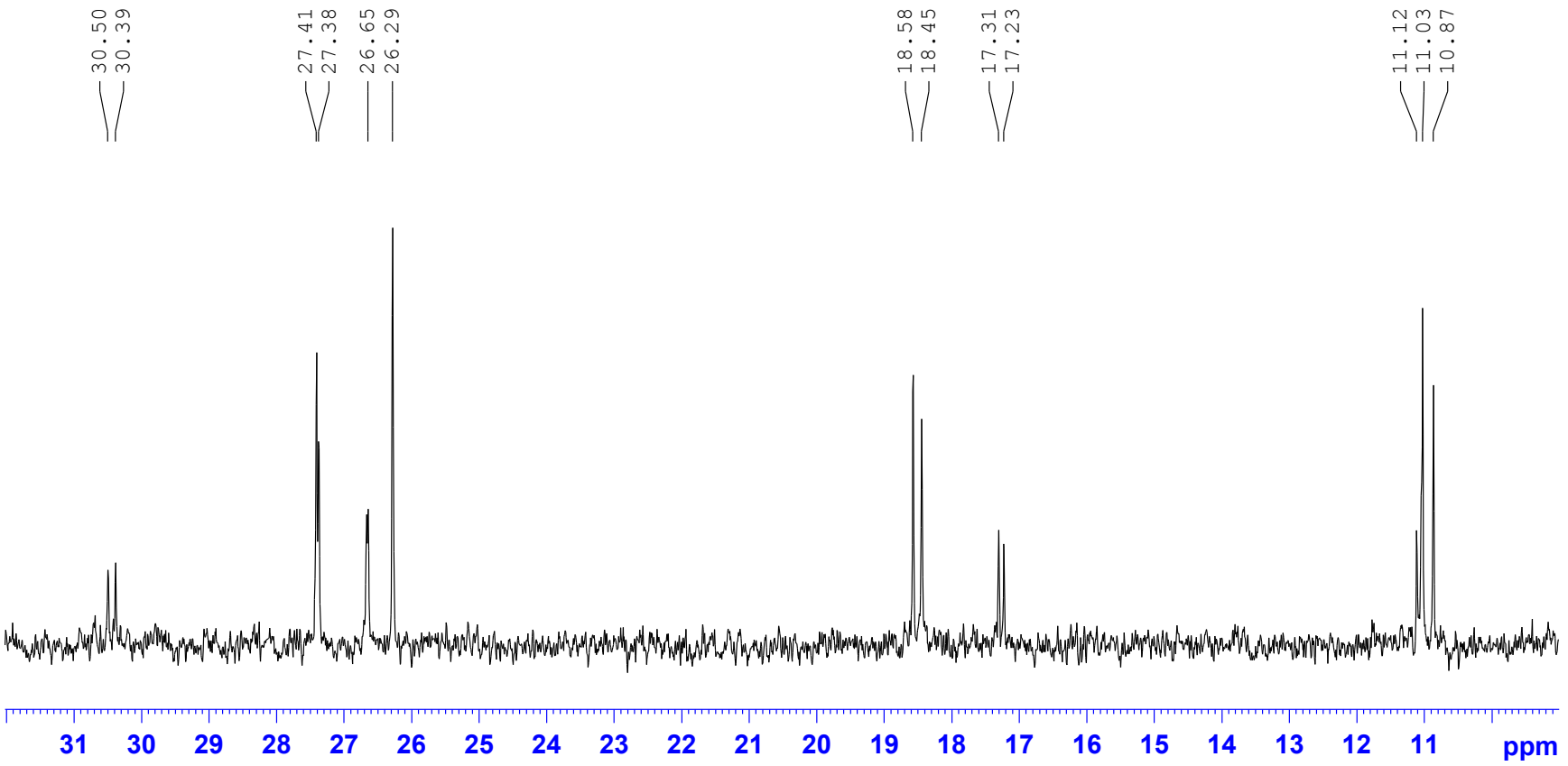


Figure S3B. ^{13}C-NMR spectrum of **1a** in $CDCl_3$ at room temperature (9−32 ppm)

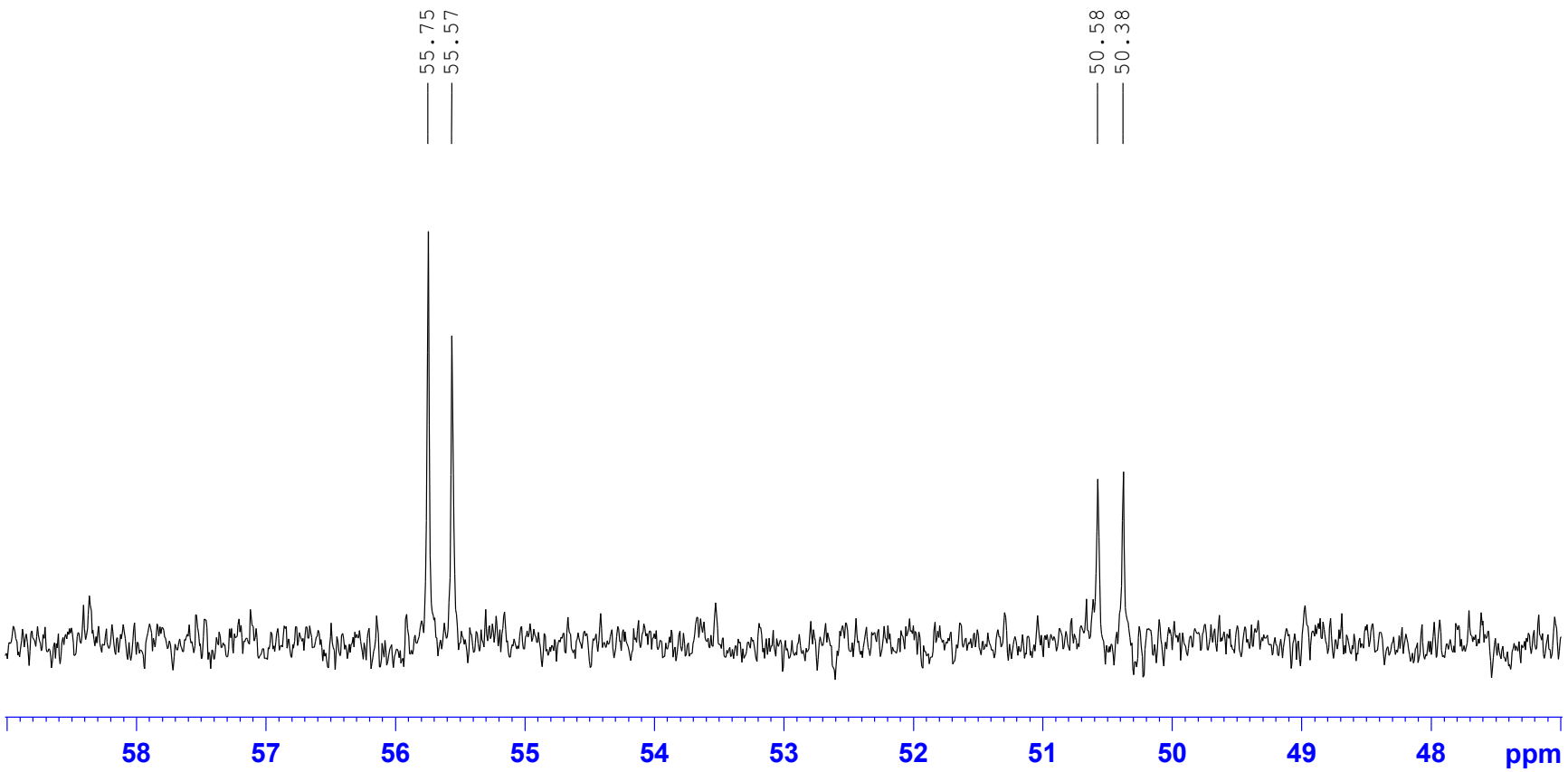


Figure S3C. ^{13}C-NMR spectrum of **1a** in $CDCl_3$ at room temperature (45−57 ppm).

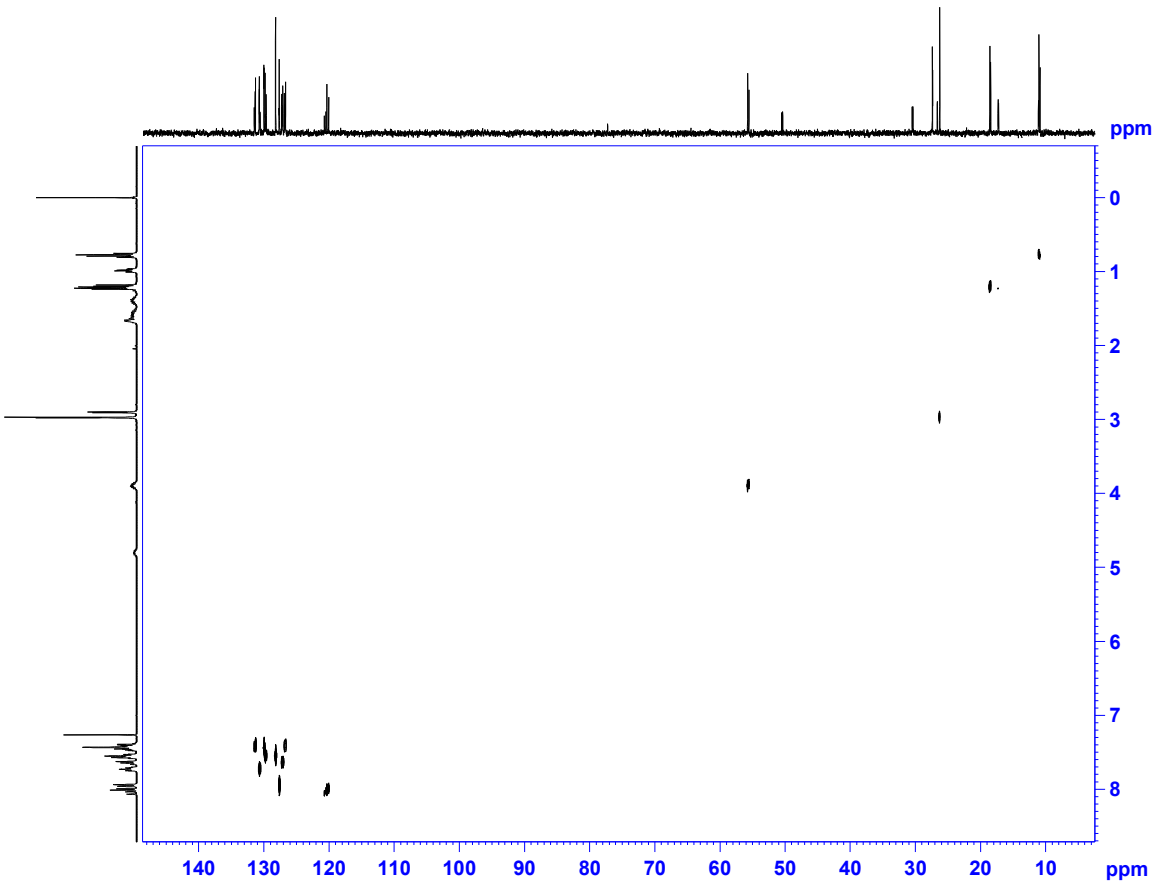


Figure S4. $^{1}H/^{13}C$-COSY NMR spectrum of **1a** in $CDCl_3$ at room temperature.

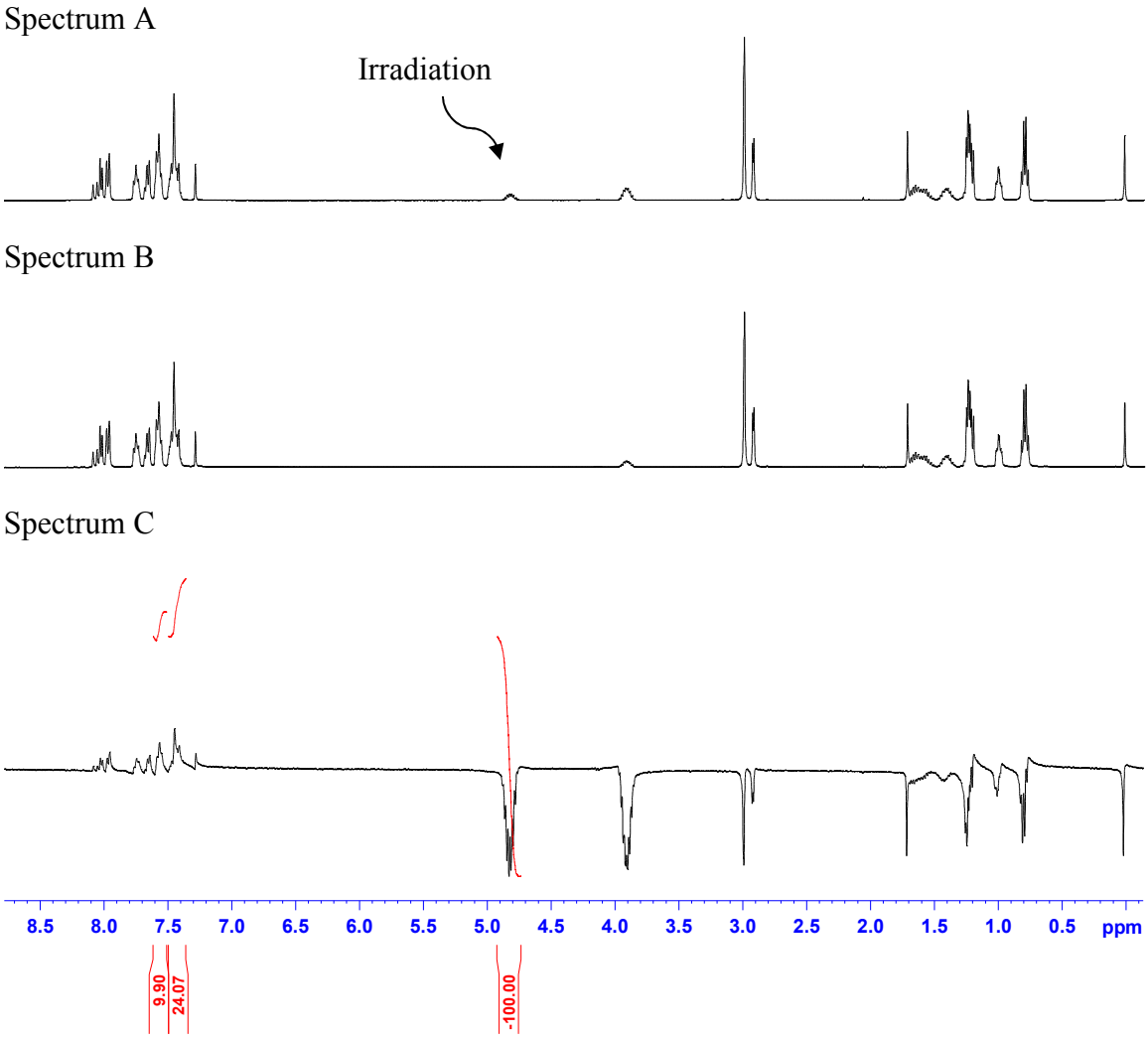


Figure S5. NOE spectroscopy of **1a.** Spectrum A: ^{1}H-NMR of **1a** in $CDCl_3$. Spectrum B: ^{1}H-NMR of **1a** in $CDCl_3$ after irradiation of *Z* rotamer *s*-butyl C-**H** signal. Spectrum C: NOE difference spectrum for A and B, showing increase of signals for chlorophenyl ring protons.

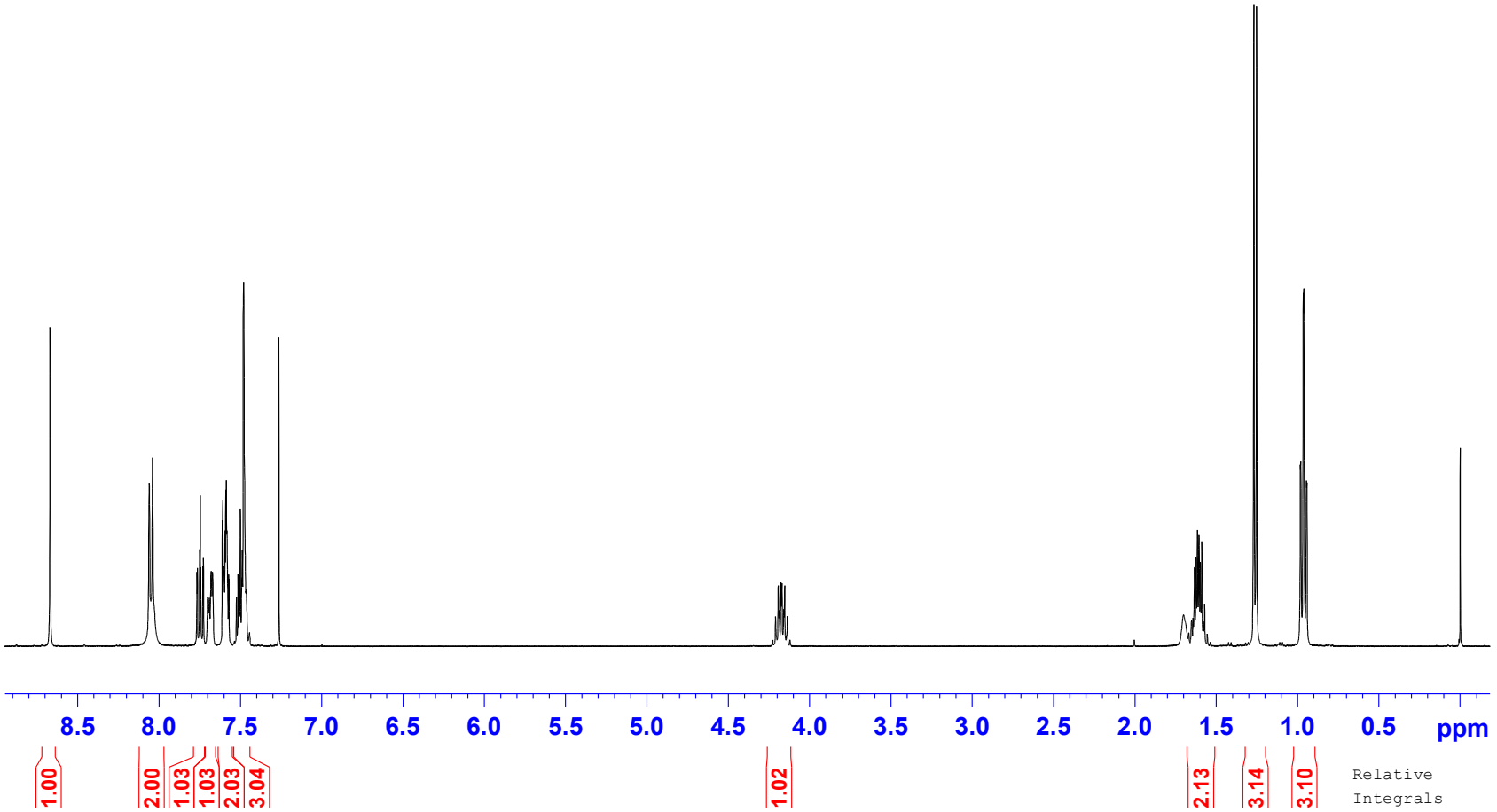


Figure S6. Full ^{1}H-NMR spectrum of **1b** in $CDCl_3$ at room temperature.

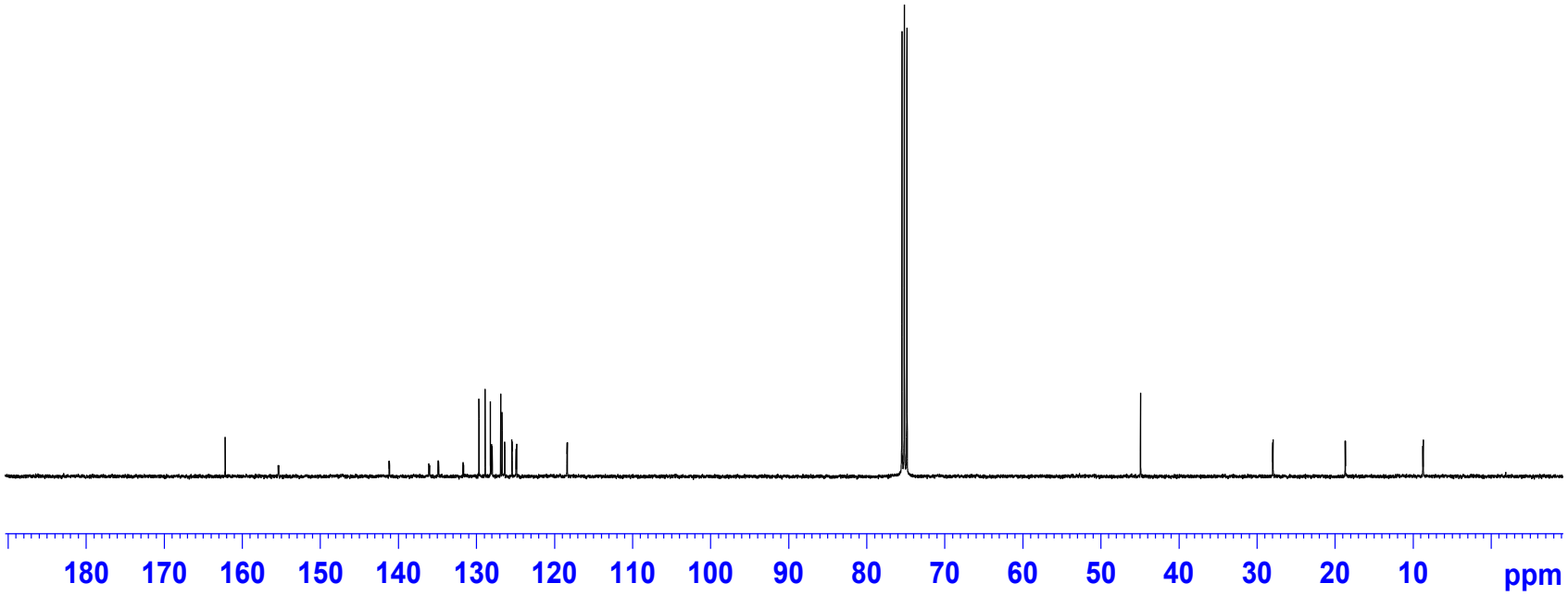


Figure S7A. Full ^{13}C-NMR spectrum of **1b** in $CDCl_3$ at room temperature.

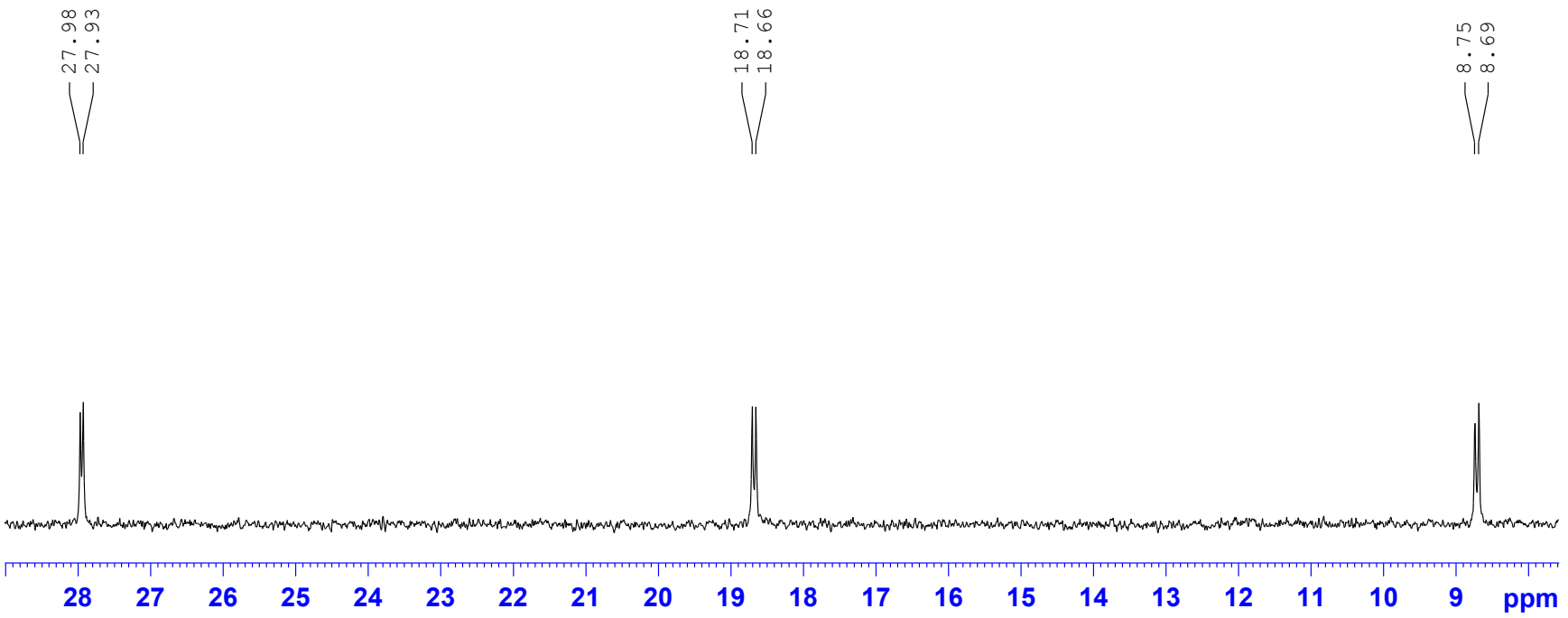


Figure S7B. ^{13}C-NMR spectrum of **1b** in $CDCl_3$ at room temperature, in the range 8−29 ppm.

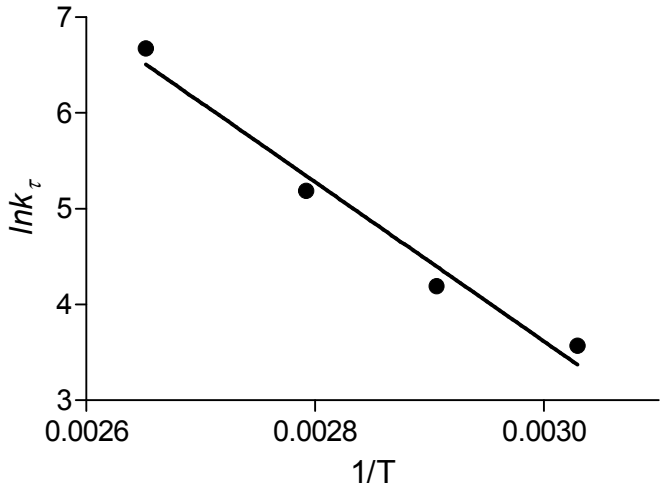
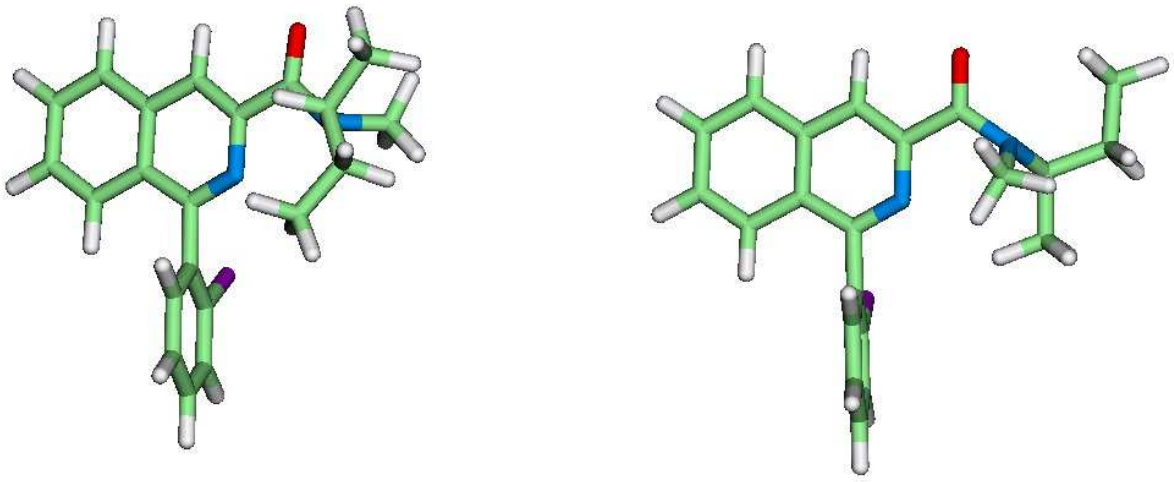
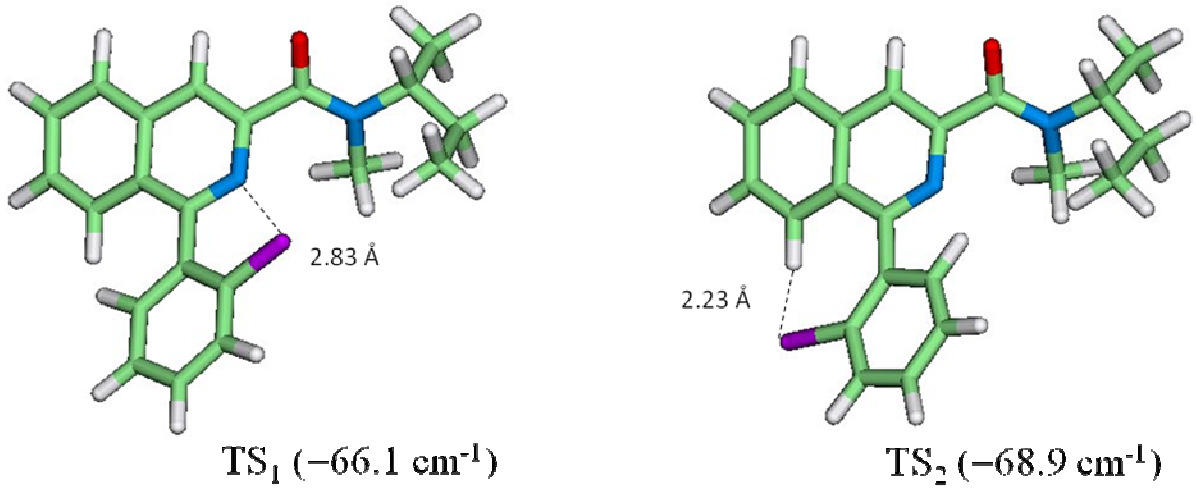


Figure S8. Ln rate of amide bond rotation (k_r; *Hz*) in **1a** versus inverse of absolute temperature (*K*).



TS_1 (−92.5 cm^{-1}) TS_2 (−101.1 cm^{-1})

Figures S9. Transition states for the amide bond isomerization of the Z_1 form of **1a**. Geometry was optimized at the B3LYP/6-31G* level in the solvent reaction field of chloroform. Values in parenthesis represent the imaginary vibrational frequency. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen; violet, chlorine.



Figures S10. Transition states for the chlorophenyl group rotation of the *Z1* isomer of **1a**. Geometry was optimized at the B3LYP/6-31G* level in the solvent reaction field of chloroform. The steric clash between the Cl and the isoquinoline nitrogen in TS_1 and the Cl and the C8-H atom of the isoqunolinyl moiety in TS_2 are indicated by the dashed lines. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen; violet, chlorine.

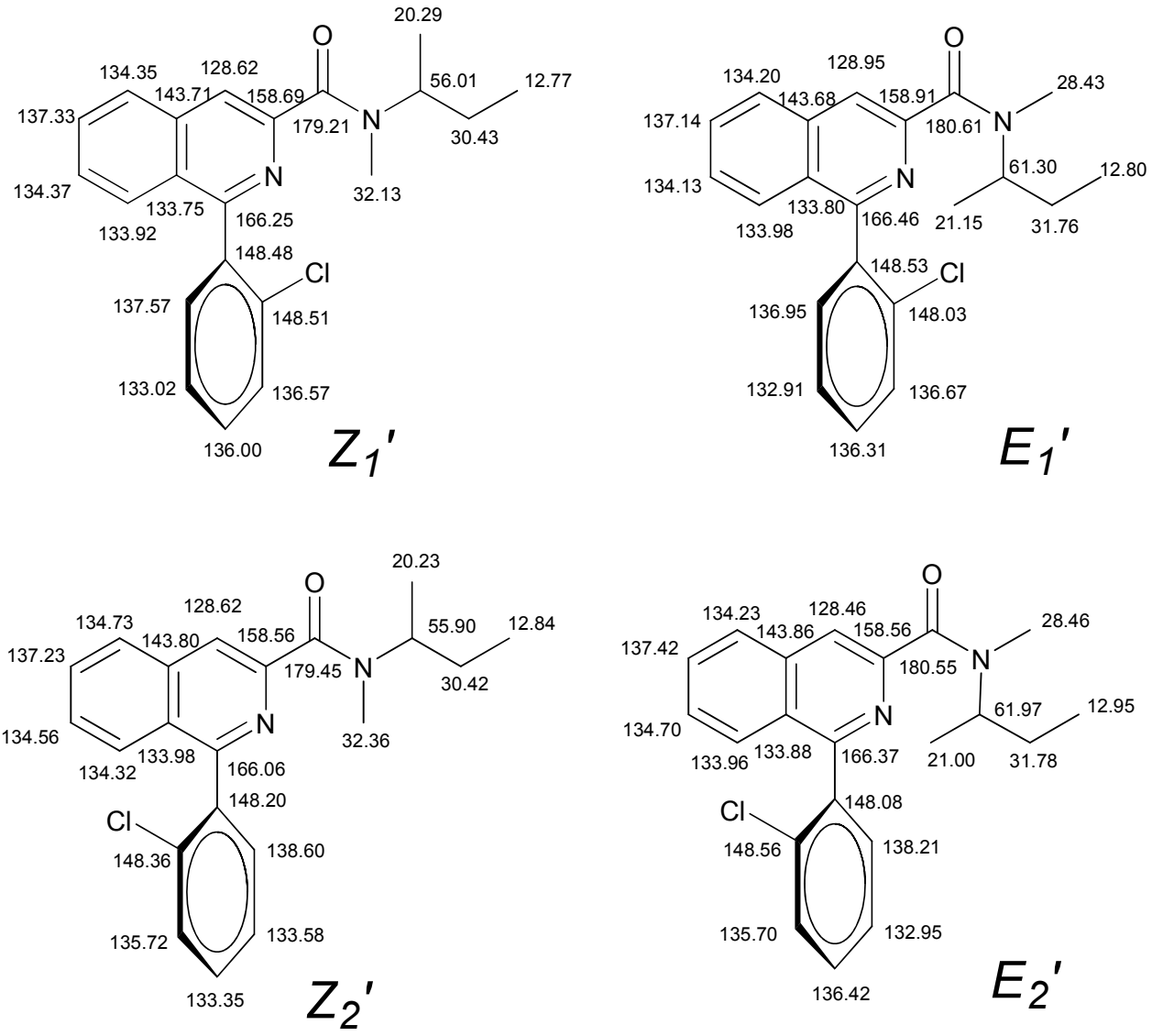


Figure S11. Calculated ^{13}C chemical shifts for the additional isomers of **1a** (Z_1', Z_2', E_1', and E_2') at the level of B3LYP/6-311+G(2d,p) in the solvent reaction field of chloroform. These were obtained by rotating ϕ_3 in the respective Z_1, Z_2, E_1, and E_2 rotamers.

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(2) Shanan-Atidi, H., and Bar-Eli, K. H. (1970) A convenient method for obtaining free energies of activation by the coalescence temperature of an unequal doublet. *J. Phys. Chem. 74,* 961−963.

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