Novel synthesis and structural characterization of a high affinity paramagnetic kinase probe for the identification of non-ATP site binders by NMR

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Purity of Final Compounds

Compound Number	Condition 1 HPLC % purity	Condition 2 HPLC % purity
17	100	100
18	100	100
18b	100	100
19	97.0	99.0
20	100	100
21	100	100
22	98.5	99.2
23	98.5	99.0
1	98.8	100
3	98.7	98.7

Purity in two solvent systems (H_2O-CH_3CN and H_2O-MeOH) was determined using a HPLC (Agilent 1100, Agilent, Waldbronn, Germany) under the following conditions: UV-DAD; Agilent Zorbax SB-C18 column 3 μm, 2.1 x 30 mm ; Condition 1: solvent A, H_2O with 0.1% formic acid; solvent B, acetonitrile with 0.1% formic acid; gradient, 0-7 min, from 95% A and 5% B to 0% A and 100% B; 7-9 min 0% A and 100% B; Condition 2: solvent A, H_2O with 0.1% formic acid; solvent B, methanol with 0.1% formic acid; gradient, 0-7 min, from 95% A and 5% B to 0% A and 100% B; 7-9 min 0% A and 100%; B flow rate, 0.8 mL/min, temperature, 40 ˚C, pressure 18.0 MPa ± 0.1.

Simulation of nuclear relaxation rates vs distance away from the nitroxide

$$R_{1M} = \frac{2}{15}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[\frac{7\tau_c}{1+\omega_S^2\tau_c^2} + \frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right] + \frac{2}{5}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\omega_I^2 g_e^4 \mu_B^4 S^2(S+1)^2}{(3kT)^2 r^6}\left[\frac{3\tau_{Curie}}{1+\omega_I^2\tau_{Curie}^2}\right] \quad (1)$$

$$R_{2M} = \frac{1}{15}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[4\tau_c + \frac{13\tau_c}{1+\omega_S^2\tau_c^2} + \frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right] + \frac{1}{5}\left(\frac{\mu_o}{4\pi}\right)^2 \frac{\omega_I^2 g_e^4 \mu_B^4 S^2(S+1)^2}{(3kT)^2 r^6}\left[4\tau_{curie} + \frac{3\tau_{Curie}}{1+\omega_I^2\tau_{Curie}^2}\right] \quad (2)$$

$$\tau_c^{-1} = \tau_s^{-1} + \tau_r^{-1} + \tau_M^{-1} \quad (3)$$

$$\tau_{Curie}^{-1} = \tau_r^{-1} + \tau_M^{-1} \quad (4)$$

Physical constants:

$\mu_o = 4\pi \times 10^{-7}$ kg m s^{-2} A^{-2}; $\gamma_I = 2.6752 \times 10^8$ rad s^{-1} T^{-1}; $g_e = 2.0023$;

$\mu_B = 9.2740 \times 10^{-24}$ J T^{-1}; $k = 1.3807 \times 10^{-23}$ J K^{-1};

Parameters used in simulation:

$S = ½$; $\tau_s = 1 \times 10^{-7}$ s; $\tau_r = 15 \times 10^{-9}$ s for kinase catalytic domain;

$\tau_r = 25 \times 10^{-9}$s for full length kinase, $\tau_s = 1 \times 10^{-7}$ s; $\tau_r << \tau_M$; $\tau_s << \tau_M$;

$\omega_I = 2\pi \times 600 \times 10^6$ Hz; $\omega_S = 658 \times \omega_I$, T = 298 kelvin;

	τr=15ns	for kinase catalytic		
		domain		
r (meters)	R1M dipolar (Hz)	R2M dipolar (Hz)	R1M curie (Hz)	R2M curie (Hz)
5.00E-10	25.49	41113.00	0.02	47.68
6.00E-10	8.54	13768.00	0.01	15.97
7.00E-10	3.39	5460.00	0.00	6.33
8.00E-10	1.52	2450.00	0.00	2.84
1.00E-09	0.40	642.00	0.00	0.74
1.50E-09	0.03	56.40	0.00	0.07
2.00E-09	0.01	10.04	0.00	0.01
2.50E-09	0.00	2.63	0.00	0.00
	τr=25ns	for full length kinase		
r (meters)	R1M dipolar (Hz)	R2M dipolar (Hz)	R1M curie (Hz)	R2M curie (Hz)
5.00E-10	16.63	63029.00	0.01	79.45
6.00E-10	5.57	21108.00	0.00	26.61
7.00E-10	2.21	8370.00	0.00	10.55
8.00E-10	0.99	3756.00	0.00	4.74
1.00E-09	0.26	985.00	0.00	1.24
1.50E-09	0.02	86.46	0.00	0.11
2.00E-09	0.00	15.39	0.00	0.02
2.50E-09	0.00	4.03	0.00	0.01

Equations (1) and (2) are the relaxation rates R_{1M} and R_{2M} contributed by the unpaired electron from the nitroxide.[1] The first and second terms of equations (1) and (2) are the dipolar and Curie contributions, respectively. Equations (3) and (4) are the correlation times for the dipolar and Curie relaxation contributions, respectively. The parameters used in the simulation are S =1/2 (electron spin quantum number), $\tau_s = 1 \times 10^{-7}$ sec (electron relaxation time),[2] ω_I = angular frequency on 600 MHz spectrometer and T = 298 kelvin. The dipolar and Curie part of the relaxation rates R_{1M} and R_{2M} were simulated for two τ_r (rotational correlation time) times, [3] 15 ns (for kinase catalytic domain) and 25 ns (for full length kinase) with τ_M (chemical exchange) larger than both τ_s and τ_r. With these parameters, only R_{2M} dipolar measurements will give quantitative distance information. Data here shows that from 5 to 15 Å away the R_{2M} dipolar measurements are greater than 56 Hz and 86 Hz for 15 ns and 25 ns τ_r, respectively. With a ligand in fast exchange and assuming a fraction bound of at least 10%, the PRE at 15 Å will contribute 5.6 Hz and 8.6 Hz (for 15 ns and 25 ns τ_r) to the R_{2obs} (R_2 observe), which may give quantitative distance measurements. Above 15 Å, the quantitative distance measurements become difficult due to smaller PRE, accuracy in measurements, and the $1/r^6$ dependence with relaxation rates. 25 Å (for a τ_r of 25 ns) away is about the limit of detection of ligand binding with a PRE of 0.4 Hz contribution to R_{2obs} at 10% bound.

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