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Supporting Information

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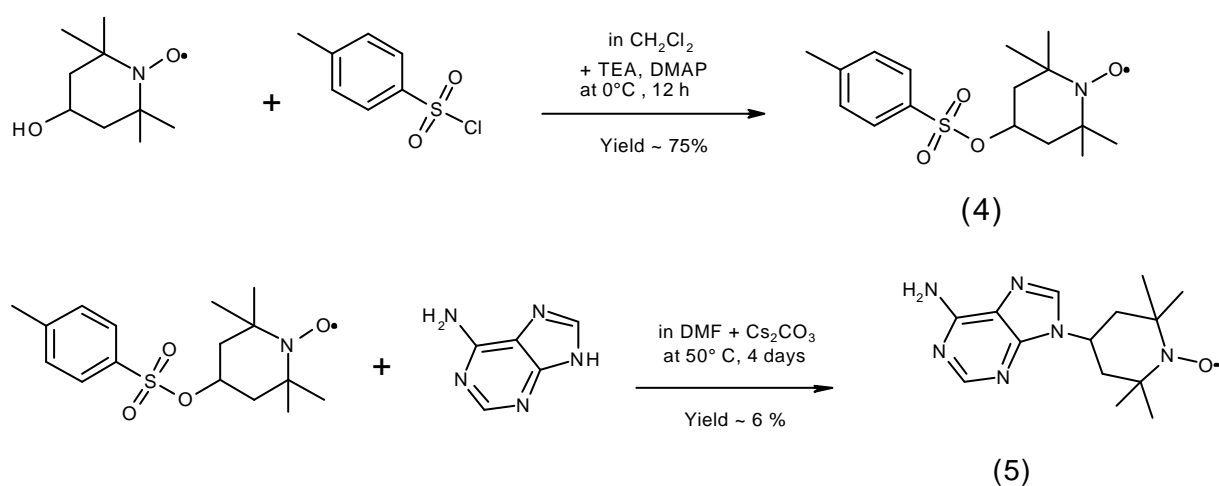
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Supporting Information

for

Strategies for the NMR-Based Identification and Optimization of Allosteric Protein Kinase Inhibitors

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Scheme S1. Synthesis of spin-labeled adenine analogue **3**

4-Tosyloxy-2,2,6,6-tetramethyl-piperidine-N-oxyl (Tempo-tosylate) (4): A solution of 755 mg (4 mM) Tosylchlorid in 5 mL Dichlormethan (DCM) was dropped during 10 min into a solution of 500 mg (2.9 mM) 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl [4-hydroxy-Tempo], 1.1 mL (7.9 mM) triethylamin and 100 mg DMAP (0.81 mM) in 20 mL of DCM at 0°C. The solution was stirred overnight at 0°C, and the DCM phase was washed with NaHCO₃ (saturated solution), water and brine, dried over Na₂SO₄, filtered and concentrated to dryness. The solid was dried at high vacuum and room temperature during 2 hours, and purified through silica column chromatography with hexane and ether (1:1). Yield: 710 mg orange crystals (75 %). TLC pure (silica in hexane/ether 1:1) (R_f = 0.4), MS: MH⁺ 327, AP-ES, pos scan; M⁺=326=20%, 124=100 % EI; NMR (Bruker: 360 MHz in CDCl₃): OK

3-[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)] adenine (Adenine-Tempo) (5): 50 mg (0.76 mM) **4** was added to a slurry of 200 mg (1.48 mM) adenine in 10 mL DMF and 500 mg (2.12 mM) Cs₂CO₃ at room temperature. The mixture was stirred during 6 h at 50°C. Additional 50 mg (0.76 mM) **4** was added and the mixture was stirred during 12 hr at 50°C. Additional 3 times 50 mg (0.76 mM) **4** was added after roughly every 12 hours. After 4 days at 50°C the mixture was filtered, washed with DMF, and evaporated to dryness. The remaining 1.2 g orange crystals were dissolved in DCM + 5% CH₃OH, filtered and evaporated again to yield 0.8 g orange crystals which were purified through gradient silica column chromatography with DCM/CH₃OH (5 to 30 %) (UV detection at 250 nm). Yield = 13 mg orange crystals (6%) **5**. TLC (silica, 15% ethanol + acetic acid ester, R_f = 0.25), MS = MH⁺ 290.3 (AP-ES, pos. scan); ¹H NMR (Bruker: 500 MHz, TOCSY and ROESY analysis for the regio chemistry in CDCl₃) = OK.