Synthesis of zapalog \(TMP-DANB-SLF)

Thomas Schwarz (thomas.schwarz@childrens.harvard.edu)
Thomas L. Schwarz Lab

Amos Gutnick
Thomas L. Schwarz Lab

Matthew Banghart
University of California San Diego

Emma West
HMS

Method Article

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Abstract

Protocol for synthesis of the photocleavable hetero-dimerizer zapalog (TMP-DANB-SLF). Adapted from: Gutnick A., Banghart M. R., West E. R., Schwartz T. L., "The light-sensitive dimerizer zapalog reveals distinct modes of immobilization for axonal mitochondria.", Nature Cell Biology (2019) https://doi.org/10.1038/s41556-019-0317-2

Procedure

1. Preparation of A - Trimethoprim \(500 mg, 1.72 mmol) was suspended in 40% HBr \(7 mL) at room temperature. The mixture was then stirred at 100 oC for 30 min. LCMS detected the desired product, the reaction mixture was adjusted to pH 7 with 1N NaOH. The precipitate was collected by filtration and dried to give 180 mg compound A. The reaction was repeated at 5 g scale to give A 1.5 g. 1H NMR \(400 MHz, d-DMSO): δ 8.11 \(s, 1H), 7.46\(s, 1H), 6.49\(s, 2H), 6.03\(s, 2H), 5.67\(s, 2H), 3.70\(s, 6H), 3.47\(s, 2H). 2. Preparation of B-2 - To a solution of B-1 \(500 mg, 3.0 mmol) in DMF \(2 ml) added K_2CO_3 \(632 mg, 4.6 mmol) and ethyl 4-bromobutyrate \(585 mg, 3.0 mmol). The reaction mixture was stirred at room temperature overnight, and then heated for 3 h at 50 oC. The solution was extracted with ethyl acetate, washed with H_2O and dried over Na_2SO_4 .The solvent was removed to give 800 mg of B-2 as a white solid in 95% yield. The reaction was repeated at 10 g scale to give B-2 16.2 g. 3. Preparation of B-3 - A solution of B-2 \(500 mg, 1.78 mmol) in 1.5 ml acetic acid was slowly added to a solution of 65% HNO_3 \(10 ml) and acetic anhydride \(2 ml) at 0 oC. The reaction was stirred for 3 h, poured into ice-cold water. The precipitate was immediately collected by filtration, washed extensively with water, dried under vacuum to give 350 mg of B-3 as a pale yellow solid in 61% yield. The reaction was repeated at 16 g scale to give B-3 11 g. 4. Preparation of B-4 - To a solution of B-3 \(350 mg, 1.08 mmol) in 26 ml MeOH at 0 oC was slowly added $NaBH_4$ \(105 mg, 2.78 mmol) in portions. The reaction was stirred for 3 h, quenched by addition of 20 ml NH4Cl \(aq.). The reaction mixture was extracted with ethyl acetate, washed with brine and dried over Na2SO4. After removal of the solvents in vacuo, the crude product was purified by flash chromatography \(petroleum ether: ethyl acetate = 2:1) to give 280 mg of B-4 as a white solid in 79% yield. The reaction was repeated at 11 g scale to give B-4 9.6 g. 5. Preparation of B-5 - To a solution of B-4 \(100 mg, 0.305 mmol) and KI \(51 mg, 0.305 mmol) in 2 ml DMF at 0 oC was slowly added NaH \(14 mg, 0.336 mmol), the mixture was stirred at r.t. for 10min, allybromide \(46 mg, 0.367 mmol) was added. The reaction was stirred at 70 oC overnight, water and EtOAc were added, the organic layer was washed with water and brine, dried by Na2SO4, concentrated and purified by prep-TLC. 1H NMR showed the structure was correct. The reaction was repeated at 1 g scale to give B-5 916 mg. 6. Preparation of B-6 - To a solution of B-5 \(60 mg, 0.163 mmol) and osmium tetraoxide \(1 mg,0.004 mmol) in 1 ml THF and 1 ml water was added Sodium periodate \(140 mg, 0.653 mmol) at r.t. under Ar. Then the reaction was stirred at 50 oC for 2h, TLC showed the reaction was consumed completely. The mixture was purified by prep-TLC \(1H NMR showed the product is not pure). The reaction was repeated at 910 mg scale to give B-6 700 mg. 7. Preparation of B-7 - To a solution of B-6 \(30 mg, 0.081 mmol) in MeOH \(3 mL) was added NaBH4 \(3 mg, 0.081 mmol) at 0 oC and the reaction was stirred at r.t. for 2h, TLC showed the

reaction was consumed completely. The mixture was purified by prep-TLC. 1H NMR showed the structure was correct. The reaction was repeated at 700 mg scale to give B-7 370 mg. 8. Preparation of B - To a solution of B-7 \(100 mg, 0.291 mmol) and CBr4 \(116 mg, 0.350 mmol) in DCM was added PPh3 \(92 mg, 0.350 mmol) 0 oC under Ar. Then the reaction was stirred at r.t. overnight. The mixture was purified by prep-TLC. 1H NMR showed the structure was correct. The reaction was repeated at 700 mg scale to give B 630 mg. 1H NMR \(400 MHz, CDCl3): δ 7.59 \(s, 1H), 7.32\(s, 1H), 5.23\(d, J = 6.0 Hz, 1H), 4.17-4.10\(m, 4H), 3.99\(s, 3H), 3.70-3.47\(m, 4H), 2.54\(t, J = 7.2Hz, 2H), 2.19\(m, 2H), 1.54\(m, 3H), 1.27\(t, J = 7.2 Hz, 3H). 9. Preparation of 5 - To a mixture of A \(10 mg, 0.036 mmol) in DMF \(0.5 mL) was added K2CO3 \(6 mg, 0.040 mmol), and the mixture was stirred at r.t. for 10min. Then B \(16.5 mg, 0.040 mmol) was added to this mixture, the mixture was stirred at 40 oC for 3h under Ar, LCMS showed the desired mW ion peak. The mixture was purified by prep-TLC. LCMS and 1H NMR confirmed the structure. The reaction was repeated at 260 mg scale to give compound 5 140 mg. 1H NMR \(400 MHz, CDCl3): δ 7.68 \(s, 1H), 7.58 \(s, 1H), 7.38 \(s, 1H), 6.35 \(s, 2H), 5.51\(br s, 2H, 5.23\(m, 1H), 5.00\(s, 2H), 4.17-4.10\(m, 4H), 3.91\(s, 3H), 3.77\(s, 6H), 3.59\(s, 3H), 3.58\(m, 1H), 2.52\(t, J = 7.2Hz, 2H), 2.18\(m, 2H), 1.51\(t, J = 6.0 Hz, 3H), 1.26\(t, J = 7.2 Hz, 3H). 10. Preparation of 6 - To a solution of 5 \(20 mg, 0.032 mmol) in 0.5 ml THF and 0.5 ml water was added LiOH.H2O \(5 mg, 0.119 mmol) at 0oC under Ar. Then the reaction was stirred at r.t. overnight, LCMS showed that the SM was consumed completely. The mixture was purified by prep-TLC to give 15 mg compound 6. The reaction was repeated at 120 mg scale to give compound 6 70 mg. 11. Preparation of TMP-DANB-SLF - To a solution of 6 \(15 mg, 0.025 mmol) in 0.5 ml DMF was added DIEA \(6 mg, 0.045 mmol) and HATU \(12 mg, 0.030 mmol) at 0oC under Ar. The mixture was stirred for 10min. Then SLF-NH2 \(16 mg, 0.030 mmol) was added to the reaction solution, the mixture was stirred at r.t. overnight. LCMS showed the reaction was difficult to purify. The reaction was repeated at 40 mg scale, and the mixture was purified by prep-TLC to give 30 mg crude product \(purity: 82.3%), then the crude was purified by HPLC to give 11.7 mg product \(purity: 93.6%). Further purifications by prep-TLC gave the target compound. The reaction was repeated at 30 mg scale, and two batches of product were combined and twice purified via prep-TLC to give 12.0 mg target compound. MS: 1108\[M+1]+. HPLC Purity @ 254 nm: 98.2%

Supplementary Files

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