Research Square

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Tristriazole ligand for use in the Cu-Catalyzed [3+2] azide-alkyne cycloaddition on protein surfaces

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Method Article

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Abstract

Introduction

Polytriazoles have been shown recently to be useful ligands in the Cu\(I)-catalyzed \[3+2] cycloaddition of azides and alkynes.[1] These ligands stabilize the Cu\(I) species allowing efficient catalysis in aerobic, aqueous conditions. Since such ligands are prepared by the reaction they are used to facilitate, an autocatalytic effect is observed in their synthesis. Tristriazole 1 was prepared in the manner prescribed in reference 1 and used as a ligand in the \[3+2] cycloaddition on protein surfaces \(Scheme 1).[2,3] See figure in Figures section.

Reagents

• Sodium azide, 99.5% \(Sigma-Aldrich, cat. no. S2002) • N,N-Dimethylformamide \(DMF) \(Rathburn, cat. no. RG2014) • Ethyl chloroacetate, 99% \(Sigma-Aldrich, cat. no. E16856) • Diethyl ether \(Rathburn, cat. no. RG2013) • Sodium sulfate \(anhydrous) \(Acros Organics, cat. no. 196640010) • Tripropargylamine, 98% \(Sigma-Aldrich, cat. no. T84964) • Acetonitrile \(Rathburn, cat. no. RH1015) • 2,6-lutidine, 99% \(Sigma-Aldrich, cat. no. L3900) • Copper\(I) iodide, 99.999% \(Sigma-Aldrich, cat. no. 215554) • Methanol \(Rathburn, cat. no. RH1019)

Equipment

• Magnetic hotplate stirrer \(e.g. IKA® RCT Basic) • 50 mL one-neck round bottom flask • 100 mL two-neck round bottom flask • Rubber septa \(to fit neck of flask) • Disposable syringes • Disposable needles • Teflon-coated magnetic stir bar • 250 mL separatory funnel • 250 mL Erlenmyer flasks • Glass sinter funnel for filtration • Balloon fitted to disposable 2.5 mL syringe barrel • Rotary evaporator \(Büchi) • Pyrex chromatographic column \(approx. diameter 3 cm)

Procedure

1) Weigh 2.31 g sodium azide \(35.5 mmol) and transfer to a 50 mL 1 neck round bottom flask equipped with a Teflon coated magnetic stir bar. CAUTION Sodium azide is highly toxic and care should be taken to avoid contact during transfer and workup. 2) Suspend the solid in 5 mL of DMF. Add 3.0 mL of ethylchloroacetate dropwise to the stirred mixture by syringe. These steps are carried out in open air and no effort was made to exclude water or oxygen. 3) Cap the reaction vessel with a rubber septum and stir at room temperature for 24 hours. 4) After 24 hours of reaction time, dilute the reaction mixture with diethyl ether \(~10 mL) and transfer to a 250 mL seperatory funnel. Add an additional 140 mL of diethyl ether. Wash the mixture with deionized water \(2 × 50 mL). 5) Dry the organic layer over Na_2SO_4 in a 250 Erlenmyer with such an amount of Na_2SO_4 that the solid is free flowing when the flask is swirled. At this point filter off the drying agent and rinse with 25 mL diethyl ether. 6) Concentrate the product by rotary evaporation until the volume is approximately 10 mL. This solution is to be used directly in the next step

7) Seal a 100 mL 2-neck round bottom flask equipped with a Teflon coated magnetic stir bar with two rubber septa. Flush the flask with argon for 10 minutes using an argon filled balloon equipped with an 19 gauge disposable needle for both entry and another 19 gauge needle for an exit. Remove the exit needle after flushing with argon. 8) Add 0.65 mL of tripropargylamine \(4.75 mmol) and 5.0 mL of acetonitrile by syringe. Cool the solution to 0 ºC using an ice/water bath and add 0.60 mL of 2,6-lutidine by syringe to the stirred solution. Add copper\(I) iodide as a solid; carefully but quickly removing and replacing one of the septa during the addition. 9) After all reagents have been added, flush the reaction with argon for 10 minutes. Remove the ice bath after one hour of stirring and stir at room temperature for 48 hours. 9) After the prescribed reaction time, remove solvent by rotary evaporation and purify the residue by column chromatography by loading the residue onto a silica column with ethyl acetate and eluting with the same solvent. TLC analysis should be used to locate the fractions with the desired product. 10) Combine the fractions containing the product and remove the solvent by rotary evaporation. The product should be a white solid and can be used without additional purification. 11) The solid can be crystallized if further purification is desired. Dissolve the solid in a minimal amount of boiling methanol. Cool slowly to room temperature and add diethyl ether dropwise until the solution is cloudy. Warm gently to redissolve all material and the let stand. The compound crystallizes as small white needles that can be isolated by filtration. Drying under vacuum is recommended.

Timing

76 hours \(including purification)

Critical Steps

Use of fresh copper\(I) salt \(CuI or CuBr) in the cycloaddition.

Troubleshooting

Low yield/No reaction: It is essential to use a copper\(I) salt \(CuI and CuBr gave similar results). Oxidized reagent \(e.g. copper\(II) species) will not catalyze the cycloaddition therefore fresh copper\(I) is strongly recommended. Rigorous exclusion of oxygen is also recommended, though no effort was made to degas solvents in this procedure.

Anticipated Results

Typical yields before crystallization are 70-80% over two steps. After recrystalization the overall yield is typically 60-65%. m.p. = 114-116 ºC; ^{1}H NMR \(400 MHz, $CDCl_3$): δ = 7.86 \(3H, s, CHtriazole), 5.16 \(6H, s, N-CH_2), 4.25 \(6H, q, J = 7.1, OCH_2CH_3), 3.80 \(6H, s, NCH_2CO_2Et), 1.28 \(9H, t, J = 7.1, OCH_2CH_3). ^{13}C NMR \(100 MHz, $CDCl_3$): δ = 14.1 \(OCH_2CH_3), 47.2 \(N-CH_2), 50.9 \(NCH_2CO_2Et), 62.3 \(OCH_2CH_3), 125.3 \(CHtriazole), 144.4 \(C4º, triazole), 166.4 \(COEt).

References

1. Chan, T.R.; Hilgraf, R.; Sharpless, K.B.; Fokin, V.V. _Org. Lett._ 2004, **6**, 2853-2855. 2. Kramer, H.B. Ph.D. Thesis. 2006. University of Oxford. 3. van Kasteren, S.I; Kramer, H.B.; Jensen, H.H.; Campbell, S.J.; Kirkpatrick, J.; Oldham, N.J.; Anthony, D.C.; Davis, B.G. _Nature_, 2007, **446**, 1105-1109.

Figures

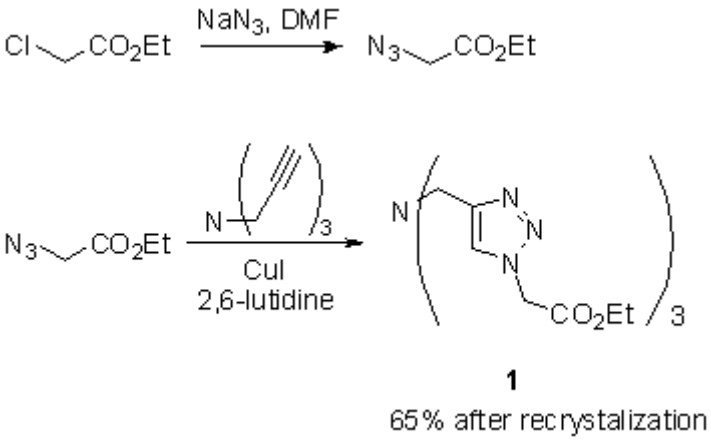

Figure 1

The scheme