One pot preparation of adenosine building blocks for click chemistry

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Method Article

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Abstract

Introduction

Reactions in which the chemical partners react selectively without interference by biological functionalities have become important tools in construction of new materials and labelled biomolecules.\[1] Among them the cross-linking chemistry based on metal ion \(most commonly Cu\(I)) catalysed Huisgen \[3 + 2] cycloaddition to form a connection in the form of a stable triazole ring seems to be ideal for use in biological systems since it can be performed also under aqueous conditions.\[2],\[3],\[4] Appropriately modified building blocks can be used in automated solid-phase oligonucleotide synthesis and later conjugated \("clicked") into various derivatives that can be used in antisense/antigene/siRNA therapeutic approaches, diagnostics or research \(e.g., through fluorophore labelling). There is thus certainly quite some incentive to develop methods for efficient and simple incorporation of groups at the nucleoside level groups that are armed for 1,3-dipolar cycloadditions \("Click Chemistry")

Reagents

Reagents • Allyl alcohol \(3) was purchased from Lancaster \(4678) • Methanol \(VWR 52542-25) was dried with I2 and Mg, and stored over molecular sieves • Bromoacetyl bromide \(4) was purchased from Aldrich \(B56412) • THF was purchased from Merck \(1.08107) and freshly distilled from CaH2 • Diethyl ether was purchased from VWR \(23811.292) • Dichloromethane \(DCM) was purchased from Sigma-Aldrich \(32222) • Triethylamine was purchased from Merck \(27468-1) and kept over CaH2 • Acetonitrile \(HPLC grade) was purchased from Merck \(21555-25) • 2-azidoethylamine was prepared according to a know procedure.\[5] • 5'-\(4-methoxytrityl)adenosine \(1) was prepared according to a know procedure.\[6] • Propargylamine was purchased from Fluka \(81825) • Conc. ammonia 25% was purchased from Merck \(35432-25) • Pyridine was purchased from Merck \(1.07463) and dried by distillation from CaH2 • Potassium tertbutoxide was purchased from Lancaster \(6164) • Merck Silica gel 60 \(0.040-0-063mm) was purchased from VWR \(1.09385.9025) CRITICAL THF should be freshly distilled just before the reaction. All glassware used during the reaction stages should be oven dried prior to use. Glassware should be allowed to cool to room temperature by storage in a dessicator containing self-indicating silica gel.

Procedure

Preparation of reagent 2\[7] 1] Add 40 mL diethyl ether to a 250 mL round bottomed flask containing a Teflon-coated magnetic stirrer bar. 2] Add 0.05 mL pyridine to the flask. 3] Add 3.8 mL bromoacetyl bromide 4 to the flask, close the flask with a glass stopper. 4] Put the flask on the ice-bath and turn on stirring. 5] Make a solution of 16 mL of diethyl ether, 2.9 mL of allyl alcohol \(3) and 6.4 mL of triethyl amine. 6] Equip the flask with a dropping funnel and add the mixture from 5] dropwise to the cooled solution of bromoacetyl bromide. 6] Stir for 30 min. 7] Add an additional 20 mL of diethyl ether to the flask. 8] Stir for an additional 3 h. 9] Filter off the precipitate on a glass filter funnel. 10] Evaporate the

filtrate under reduced pressure on a rotary evaporator and purify further using vacuum distillation \(Bp = 80 ˚C at 15mm Hg). Synthesis of alkyne and azide functionalized nucleosides 5 and 6 1] Add 269 mg of compound 1 to a 100 mL round bottomed flask 2] Add 10 mL THF, and evaporate the solvent under reduced pressure on a rotary evaporator, repeat the procedure. 3] Add a magnetic stirrer bar and leave the compound for drying under vacuum for 30 min. 4] Add 20 mL THF to the flask, close the flask with a glass tube \(containing a stopcock) equipped with a balloon containing Ar \(g), open the stopcock to flush the compound with argon. 5] Add 73 mg of potassium t-butoxide while stirring, continue stirring for 30 min. 6] Add 116 mg of allyl bromoacetate \(2) and continue stirring for an additional 2 h. 7] Remove the solvent by evaporation under reduced pressure on a rotary evaporator. 8] Dissolve the residue in 10 mL dry methanol and add 275 mg of propargyl amine \(10eq), or alternatively 2-azidoethylamine, close the flask with a stopper and continue stirring at room temperature over night. 9] Evaporate the solvent under reduced pressure on a rotary evaporator. 10] Dissolve the the residue in 1 mL of DCM and load it on the column \(5g of Merck Silica gel 60, packed with DCM on a glass column). First use 200 mL DCM and then a stepwise gradient of methanol in DCM \(from 1 to 10% methanol) to elute the compund. Collect fractions containing product and remove solvent under reduced pressure.

Timing

For 2: Count ca 6-7 h including distillation For 5 or 6: 1.5 days including over night reaction and purification

Troubleshooting

Low yield: Repeat reaction with fresh reagents and dry glassware, ensuring that all are anhydrous. The amount of 2 \(1.3 eq) in synthesis of 5 and 6 is critical and optimized for high 2'-selectivity and yield. Compound 2 is a strong irritant to eyes, wear protection shield.

Anticipated Results

Typical yields of 5 and 6 over two steps are 50-65% after purification by chromatography. Compound 5, Analytical data: 1H NMR \(400 MHz, CDCl3) δH 8.31 \(1 H, s, H8), 8.04 \(1 H, s, H2), 7.28 \(12 H, m, MMtr), 6.83 \(2 H, m, MMtr), 6.17 \(1 H, d, H1' J\(H,H)=4.1 Hz), 5.84 \(1 H, s, NH), 4.54 \(1 H, t, H2' J\(H,H)=4.6 Hz), 4.45 \(1 H, t, H3' J\(H,H)=4.8 Hz), 4.19 \(3 H, m, H4'+CH2, 4.03 \(2 H, m, CH2), 3.77 \(3 H, s, OMe), 3.55 \(2 H, dd, CH25' J\(H,H)=3.5 Hz, J\(H,H)=10.7 Hz), 2.18 \(1 H, t, J\(H,H)=2.5 Hz); 13C NMR \(100 MHz, CDCl3) δC 28.9 \(CH2), 55.5 \(OMe), 65.5 \(CH2), 71.0 \(CH2), 72.2 \(C3'), 74.1, 85.1 \(C4'), 86.2 \(C2'), 88.2 \(C1'), 113.5, 127.4, 128.2, 128.5, 130.6, 133.2, 142.1, 152.2, 162.2, 163.0, 170.1 \(C=O), MS \(ESI) m/z calcd for C35H34N6O6: 634.25; found 635.25 \[M+ + H]. Compound 6, Analytical data: 1H NMR \(400 MHz, CDCl3) δH 8.30 \(1 H, s, H8), 8.03 \(1 H, s, H2), 7.30 \(12 H, m, MMtr), 6.84 \(2 H, m, MMtr), 6.81 \(1 H, d, H1' J\(H,H)=3.8 Hz), 5.81 \(1 H, s, NH), 4.55 \(1 H, t, H2' J\(H,H)=4.8 Hz), 4.47 \(1 H, t, H3' J\(H,H)=5.2 Hz), 4.29 \(3 H, m, H4'+CH2, 4.03 \(2 H, m, CH2), 3.79 \(3 H, s, OMe), 3.46 \(6 H, m, CH25' + CH2x2), 2.88 \(1 H, bs); 13C NMR \(100 MHz, CDCl3) δC 38.8, 41.5, 50.8, 54.8, 55.4 \(OMe), 63.2 \

(CH25'), 71.0 \(CH2), 70.2 \(CH2), 70.6 \(C2'), 83.9 \(C4'), 84.2 \(C3'), 87.3 \(C1'), 87.8, 100.2, 113.5, 120.4, 127.4, 128.0, 128.2, 128.5, 130.5, 131.3, 135.2, 138.9, 144.0, 144.1, 149.6, 153.4, 155.7, 158.9, 169.6\(C=O), MS \(ESI) m/z calcd for C34H35N9O6: 665.27; found 666.19 \[M+ + H].

References

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Figures

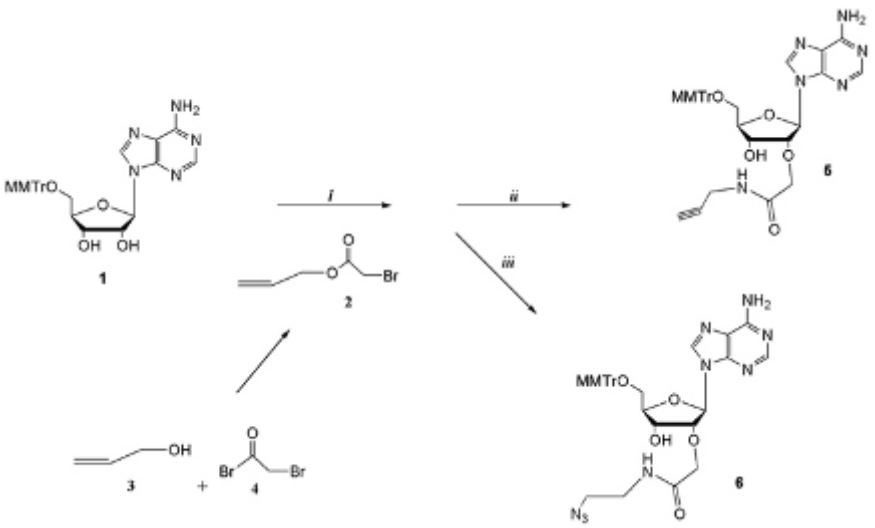

Figure 1

Scheme Synthesis of nucleosides with "clickable" linkers (i) tBuOK, THF, r.t., (ii) propargylamine (for compound 5) or 2-azidoethylamine (for compound 6) MMTr = 4-methoxytrityl, tBuOK = potassium tertbutoxide, THF = tetrahydrofurane

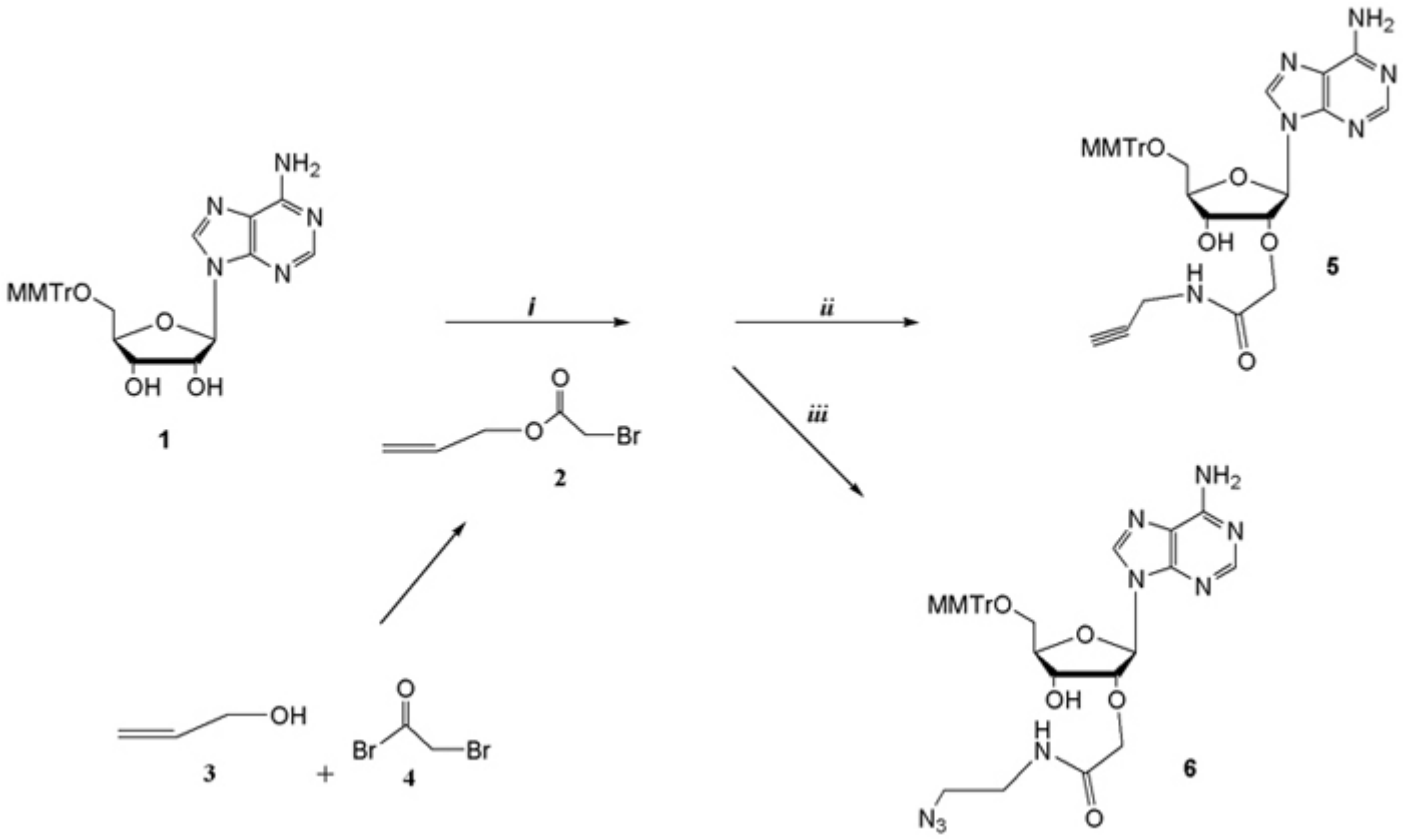

Figure 2