Synthesis of 1-methyl-7-nitroisatoic anhydride (1M7)

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Method Article

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Abstract

SHAPE \(selective 2'-hydroxyl acylation analyzed by primer extension) chemistry is a powerful approach for single-nucleotide resolution analysis of RNA secondary and tertiary structure. SHAPE uses hydroxyl-selective electrophiles to react with and form covalent adducts at the ribose 2'-hydroxyl group. The most useful and robust reagent for routine SHAPE experiments is 1-methyl-7-nitroisatoic anhydride \(1M7). This protocol describes a straightforward synthesis of 1M7 from commercially available precursors. The synthesis of 1-methyl-7-nitroisatoic anhydride was originally described by Mortimer _et al._ . The reaction scheme is:  Reaction Scheme

Reagents	MW \(g/mol)	Amount \(g)	Amount \(mL)	Moles \(mmol)	ratio
4NIA	208.13	5.000		24.023	1
NaH	24	0.990		24.744	1.03
MeI	141.94		1.499	24.023	1

Amounts of each starting material can be varied using the above Table.

Reagents

• 4-nitroisatoic anhydride \(4NIA) \(AstaTech, cat. no. 69441) • Sodium hydride \(NaH) – 60% dispersion in mineral oil \(Sigma-Aldrich, cat. no. 452912) • Methyl iodide \(MeI) \(Sigma-Aldrich, cat. no. 289566) • Anhydrous dimethylfluoride \(DMF) \(Sigma-Aldrich, cat. no. 227056) • HCl \(Fisher Scientific, cat. no. A144) • H_2O • Ether \(Fisher Scientific, cat. no. E138) **REAGENT SETUP** • H_2O and HCl should be ice cold before use.

Equipment

• Round bottom flasks \(250 and 500 mL) • Bunsen burner • N_2 tank and gas line • Teflon-coated stirring bar • Rubber septa • Disposable syringes \(1 mL, 20 mL, 60 mL) • Disposable needles \(18 gauge) • Stirring plate • Buchner funnel • Vacuum rotary evaporator • Watch glass • Oven maintained at 90 °C **EQUIPMENT SETUP** • All glassware used in the described reactions are washed with soap and water, rinsed with water, rinsed with acetone, and flame-dried over Bunsen burner. Rubber septa are immediately placed over flasks, flasks are flushed with N_2, and allowed to cool.

Procedure

1. Dissolve 4NIA in 60 mL DMF in a 250 mL flame-dried round bottom flask under N_2. 2. In a separate 500 mL flame-dried round bottom flask under N_2, make a slurry of NaH in 15 mL DMF and stir. 3. Slowly add 4NIA solution dropwise to slurry of NaH in DMF. Stir for 5 minutes. 4. Slowly add MeI dropwise and then stir at room temperature for 4 hours. 5. Pour reaction into 100 mL _ice cold_ 1N HCl. 6. Filter resulting

bright orange precipitate by vacuum filtration in a Buchner funnel. 7. Rinse precipitate twice with _ice cold_ water, followed by 3 rinses with ether. 8. Dry overnight in oven on watch glass.

Anticipated Results

Analytical data Yield ~80%; bright orange powdery solid. ^{1}H NMR \[400 MHz, CO\(CD_3)$_2$]: σ 3.69 \(s, 3H, -NCH3-), 8.12 \(dd, J=8.8 Hz, 2 Hz, 1 H, ArH), 8.2 \(d, J=2hz, 1h, ArH), 8.34 \(d, J=8.4 Hz, 1 H, ArH).

Figures

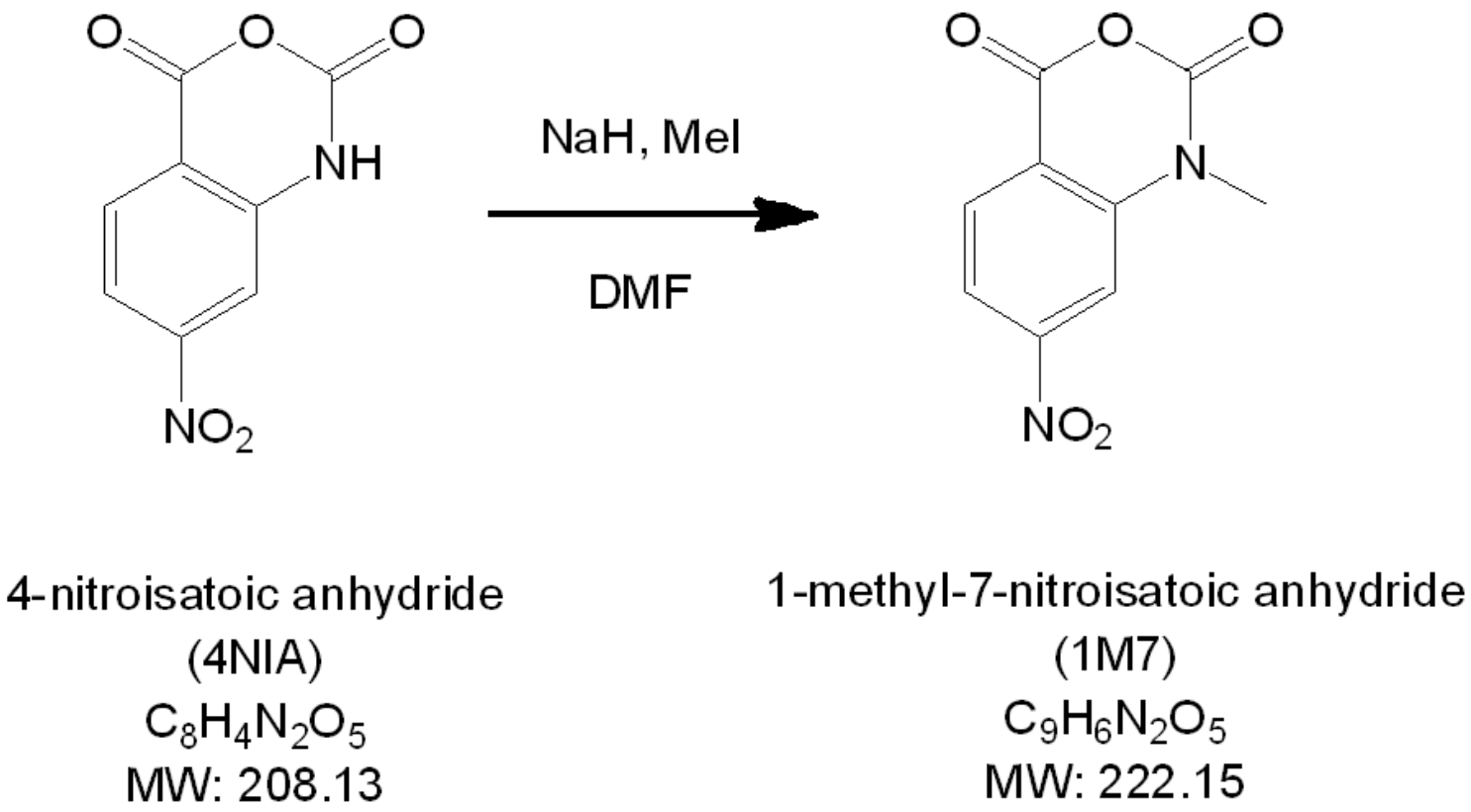

Figure 1

Reaction scheme Reaction Scheme