Supporting Protocol: Synthesis of 1-methyl-7-nitroisatoic anhydride (1M7)

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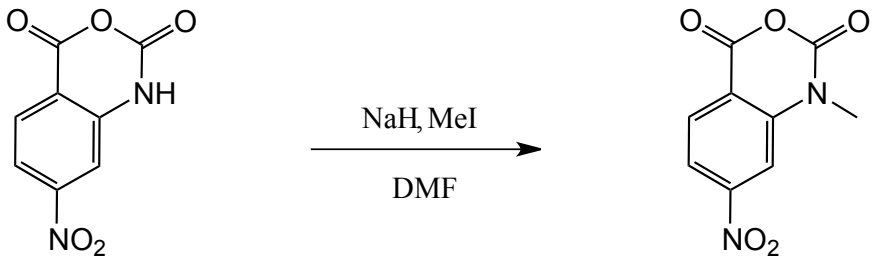
Synthesis of 1-methyl-7-nitroisatoic anhydride was originally described by Mortimer *et al.*[1].



4-nitroisatoic anhydride
(4NIA)

$C_8H_4N_2O_5$

MW: 208.13

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

$C_9H_6N_2O_5$

MW: 222.15

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 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).

REFERENCE

1. Mortimer, S.A. & Weeks, K.M. A fast-acting reagent for accurate analysis of RNA secondary and tertiary structure by SHAPE chemistry. *J Am Chem Soc* **129**, 4144-5 (2007).