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of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

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September 2014: The paragraphs above replace the section "Handling and Disposal of Hazardous Chemicals" in the originally published version of this article. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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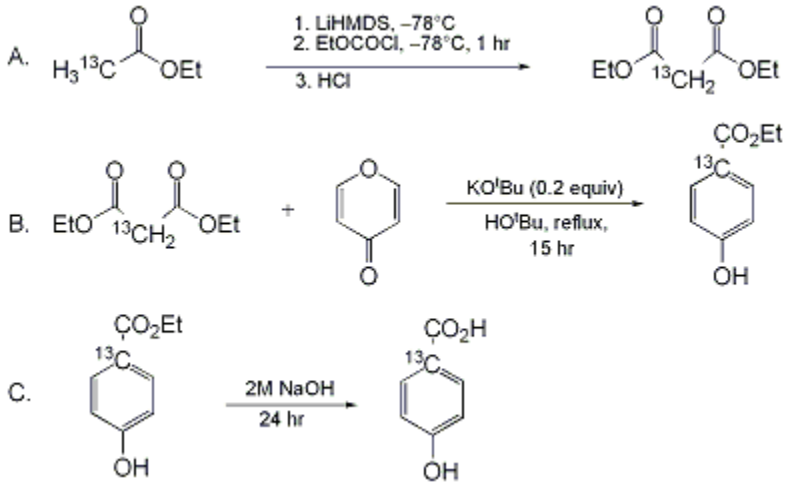
4-HYDROXY[1-^{13}C]BENZOIC ACID

[Benzoic-1-^{13}C acid, 4-hydroxy-]



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1. Procedure

A. Diethyl [2-^{13}C]malonate (Note 1). A flame-dried, 100-mL, round-bottomed Schlenk flask equipped with a rubber septum and a magnetic stirring bar is purged with argon. The flask is charged with 15 mL of anhydrous tetrahydrofuran (THF) (Note 2) and 5.8 mL (28 mmol, 2.5 equiv) of hexamethyldisilazane (Note 3). After the solution is cooled to 0°C in an ice-water bath, 9.4 mL (23.5 mmol, 2.1 equiv) of a solution of butyllithium (2.5 M in hexanes) (Note 4) is added slowly via a syringe to the stirred solution. The ice bath is removed and the mixture is allowed to warm to room temperature. After the solution is stirred for 30 min, it is cooled to −78°C using an acetone-dry ice bath and equilibrated for 5 min at the same temperature. Then 1.00 g (11.2 mmol, 1 equiv) of ethyl [2-^{13}C]acetate (Note 5) is added within 5 min via a syringe, and the acetate-containing flask is rinsed with 0.5 mL of anhydrous THF. Stirring is continued at −78°C for 20 min, and 1.07 mL (11.2 mmol, 1 equiv) of ethyl chloroformate (Note 6) is added within 5 min via a syringe. The mixture is stirred for 1 hr at −78°C, and 5 mL of 6 M hydrochloric acid (HCl) is added in one portion (Note 7). The mixture is allowed to warm to room temperature, and after the addition of 20 mL of water, the pH of the solution is adjusted to 1-2 with 2 M HCl. The mixture is extracted with diethyl ether (3 × 50 mL), and the combined organic phases are washed successively with 2 M HCl, water, and brine (30 mL each). The HCl and water phases are combined and reextracted with ether (50 mL). The organic layer is washed with brine (20 mL) and added to the combined organic phases. The combined extracts are dried over anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated under reduced pressure with a rotary evaporator (200 mbar/35°C, 150 mm/35°C). The crude product is distilled in a microdistillation apparatus at 90 mbar (67.5 mm) to give 1.66 g(10.3 mmol, 92%) of diethyl [2-^{13}C]malonate as a colorless liquid (Notes 8 and 9).

B. Ethyl 4-hydroxy[1-^{13}C]benzoate . A 100-mL, single-necked, round-bottomed flask equipped with a magnetic stirring bar, pressure-equalizing addition funnel and a reflux condenser beyond the dropping funnel (Note 10) is charged with 20 mL of tert-butyl alcohol (t-BuOH) (Note 11), 1.01 g (10.5 mmol, 1.05 equiv) of 4H-pyran-4-one (Note 12), and 1.61 g (10.0 mmol, 1 equiv) of diethyl [2-^{13}C]malonate . The condenser is sealed with a silica gel drying tube, and the stirred solution is put in an oil bath at 105° C. A solution of 0.22 g (2.0 mmol, 0.2 equiv) of potassium tert-butoxide (Note 13) in 20 mL of tert-butyl alcohol (t-BuOH) is added dropwise via the funnel during 20 min; the mixture turns red and turbid. After the mixture is heated under reflux for 15 hr, it is allowed to cool to room temperature.

Water (30 mL) is added, followed by 5 mL of 2 M HCl. After removal of most of the solvent with a rotary evaporator, the aqueous mixture is extracted with diethyl ether (3 × 50 mL). The combined organic phases are washed with water and brine (each 30 mL). Drying over Na_2SO_4, filtration, and removal of the solvent under reduced pressure with a rotary evaporator affords the crude product. Flash chromatography on silica gel (Note 14) with ethyl acetate/petroleum ether (4:1) as eluent affords 1.34 g (8.01 mmol, 80%) of ethyl 4-hydroxy[1-^{13}C]benzoate , mp 112-113°C (Note 15).

C. 4-Hydroxy[1-^{13}C]benzoic acid . A 25-mL, one-necked, round-bottomed flask equipped with a magnetic stirring bar is charged with 1.25 g (7.50 mmol) of ethyl 4-hydroxy[1-^{13}C]benzoate and 11.3 mL (22.5 mmol, 3 equiv) of 2 M sodium hydroxide (NaOH). After the solution is stirred for 24 hr at room temperature, 17 mL of 2 M HCl is added slowly, whereby the product precipitates. Water is added (10 mL), and the mixture is extracted with diethyl ether (3 × 50 mL). The combined organic phases are washed with 1 M HCl (2 × 30 mL). Removal of the solvent under reduced pressure on a rotary evaporator and drying under reduced pressure affords 1.02 g (7.34 mmol, 98%) of 4-hydroxy[1-^{13}C] benzoic acid (mp 212-213°C) (Note 16), which can be used for feeding experiments without further purification.

2. Notes

1. The procedure follows closely that of Mueller and Leete[2] with slight improvements by the submitters.
2. Tetrahydrofuran was distilled from potassium and benzophenone under an argon atmosphere immediately before use. The checkers used anhydrous THF purchased from Aldrich Chemical Company, Inc.
3. Hexamethyldisilazane (98%), purchased from Lancaster Synthesis Inc. or Aldrich Chemical Company, Inc. , was used as received.
4. Butyllithium (2.5 M in hexanes) was purchased from Aldrich Chemical Company, Inc. The actual concentration was determined by titration with diphenylacetic acid or 4-biphenylmethanol.[3]
5. Ethyl [2-^{13}C]acetate is commercially available (Aldrich Chemical Company, Inc.), but expensive. The compound can be prepared by O-ethylation[4,5,6] of the cheaper sodium [2-^{13}C]acetate (Aldrich Chemical Company, Inc.) or via [2-^{13}C]acetyl chloride.[7]
6. Ethyl chloroformate (97%), purchased from Aldrich Chemical Company, Inc. , was used as received.
7. The solution should be quenched prior to warming to room temperature. Solutions of lithiated ethyl acetate decompose rapidly at 0°C.[8]
8. The receiver is cooled to −10°C. Cooling to −78°C is not advisable because obstruction may occur. The product (bp 123-125°C/90 mbar, 67.5 mm) can be separated from the hydrolysis products trimethylsilanol and traces of hexamethyldisiloxane.[9] At the end, the apparatus is rinsed with diethyl ether to obtain all the product.
9. The spectral data are as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27 (t, 6 H, J = 7.2), 3.34 (d, 2 H, J = 132), 4.19 (q, 4 H, J = 7.2) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.1, 41.7 (^{13}C), 61.5, 166.7 (d, J = 59) ; MS (EI): 161 [M+] (3), 134 (40), 116 (100), 106 (7), 89 (52), 61 (31) ; IR (KBr) cm^{-1}: 3465 (br), 2986, 2942, 1754, 1733, 1467, 1448, 1410, 1369, 1319, 1267, 1189, 1151, 1097, 1035, 949, 866, 844, 787, 666, 603 .
10. Because of the relatively high melting point of the solvent (23-26°C), it is not advisable to put the condenser directly on the flask. With the addition funnel between the flask and the condenser, most of the tert-butyl alcohol is condensed as a liquid and does not collect as a solid on the cold condenser.
11. tert-Butyl alcohol (=99.7%), purchased from Fluka Chemical Corp. or Fisher Scientific , was used as received.
12. 4H-Pyran-4-one is commercially available (98+%, Aldrich Chemical Company, Inc.), but the substance is expensive. It can be synthesized by decarboxylation of chelidonic acid monohydrate (Lancaster Synthesis Inc.) following the procedure of De Souza and co-workers.[10]
13. Potassium tert-butoxide (99%), purchased from Fluka Chemical Corp. or Aldrich Chemical Company, Inc. , was used as received.
14. Flash chromatography was performed on E. Merck silica gel 230-400 mesh: 150 g of silica gel was loaded on a 7- × 2-in size column using a minimum amount of ethyl acetate as loading solvent. The checkers used a 90-g silica column purchased from Biotage.
15. The spectral data are as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39 (t, 3 H, J = 7.2), 4.36 (q, 2 H, J = 7.2), 6.50 (s, 1 H), 6.85-6.93 (m, 2 H), 7.93-7.99 (m, 2 H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.4, 61.1,

115.3 (d, J = 1.5), 122.7 (^{13}C), 132.0 (d, J = 60), 160.4 (d, J = 9.1), 167.2 (d, J = 77) ; MS (EI): 167 [M+] (30), 139 (23), 122 (100), 94 (11), 83 (10) ; IR (KBr) cm^{-1}: 3218 (br), 1672, 1602, 1583, 1441, 1370, 1306, 1286, 1239, 1169, 1104, 1018, 847, 768, 723, 697, 618 .
16. The spectral data are as follows: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.77-6.85 (m, 2 H), 7.75-7.81 (m, 2 H), 10.2 (s, br, ≈0.8 H), 12.4 (s, br, ≈0.8 H) ; (The coupling pattern of the aromatic protons is even at 600 MHz not clearly resolved.) ^{13}C NMR (75 MHz, DMSO-d_6) δ: 115.3, 121.6 (^{13}C), 131.7 (d, J = 59), 161.8 (d, J = 8.5), 167.3 (d, J = 74) ; MS (EI) 139 [M+] (100), 122 (94), 94 (20) ; IR (KBr) cm^{-1}: 3394 (br), 2966, 2831, 2660, 2562, 1677, 1602, 1588, 1504, 1440, 1421, 1309, 1282, 1243, 1169, 1100, 933, 852, 766, 617, 548 .

Waste Disposal Information

All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

3. Discussion

4-Hydroxybenzoic acid acts as biosynthetic precursor for several secondary metabolites.[11] Since in many cases decarboxylation takes place on route to the metabolites,[12] ring-^{13}C-labeled 4-hydroxybenzoic acids are in demand for biosynthetic studies.

Previous syntheses of ring-labeled 4-hydroxybenzoic acid use many steps and show low overall yields. 4-Hydroxy[3-^{13}C]benzoic acid was synthesized in six steps from ethyl [1-^{13}C]acetate with 2.8% overall yield.[13] 4-Hydroxy[3,5-$^{13}C_2$]benzoic acid was prepared in five steps from [1,3-$^{13}C_2$]acetone with an overall yield of less than 4.5%.[12b] 4-Hydroxy[2,6-$^{13}C_2$]benzoic acid was generated microbiologically from [1-^{13}C]glucose by using a mutant strain of *Klebsiella pneumoniae*.[14] For 120 mg of product, 18 g of labeled glucose was necessary. Methyl 4-methoxy[3,5-$^{13}C_2$]benzoate was obtained from [1,3-$^{13}C_2$] acetone in five steps with 32% overall yield.[15] Baldwin and co-workers[16] synthesized methyl 4-methoxy [3,4,5-$^{13}C_3$]benzoate in four steps from [1,2,3-$^{13}C_3$]acetone without indicating the overall yield.

The present procedure affords 4-hydroxy[1-^{13}C]benzoic acid from ethyl [2-^{13}C]acetate in three steps with 72% overall yield. It is based on an observation of Woodward[17] that ethyl 4-hydroxybenzoate is formed by base-catalyzed condensation of 4H-pyran-4-one with diethyl malonate . The submitters studied several solvent-base combinations for this reaction and found that tert-butyl alcohol/potassium tert-butoxide gave the highest yields. When a stoichiometric amount of the base is used, an excess of 4H-pyran-4-one has to be used.[4b] This can be avoided by the use of substoichiometric amounts as given in the procedure.

The use of ethyl [2-^{13}C]acetoacetate instead of diethyl [2-^{13}C]malonate in the condensation reaction with 4H-pyran-4-one afforded ethyl 4-hydroxy[1-^{13}C]benzoate in 87% yield. In this case, 1.1 equiv of 4H-pyran-4-one and 1.1 equiv of potassium tert-butoxide were optimal. The addition of catalytic amounts of the base was not satisfactory. Ethyl [2-^{13}C]acetoacetate was prepared from ethyl [2-^{13}C] acetate as described for diethyl [2-^{13}C]malonate.[18] The maximum yield for this reaction on a 10-mmol scale was only 70% after distillation. 4H-Pyran-4-one reacted with nitromethane and potassium tert-butoxide (each 1.1 equiv) to afford 4-nitrophenol in 75% yield after purification by flash chromatography. This gives easy access to 4-nitro[4-^{13}C]phenol. With 2,4-pentanedione , the condensation with 4H-pyran-4-one under the same reaction conditions gave 4-hydroxyacetophenone in 45-50% yield after purification.

Ethyl 4-hydroxy[1-^{13}C]benzoate can be converted into other ring-^{13}C-labeled compounds like 3,4-dihydroxy[1-^{13}C]benzoic acid, 1,3,4-trihydroxy[1-^{13}C]benzene, and D,L-[1'-^{13}C]tyrosine.[4b]

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Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

4-Hydroxybenzoic-1-^{13}C acid:
Benzoic-1-^{13}C acid, 4-hydroxy- (14); (211519-30-1)

Diethyl malonate-2-^{13}C:
Propanedioic-2-^{13}C acid, diethyl ester (10); (67035-94-3)

1,1,1,3,3,3-Hexamethyldisilazane:
Disilazane, 1,1,1,3,3,3-hexamethyl- (8);
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)- (9); (999-97-3)

Butyllithium:
Lithium, butyl- (8,9); (109-72-8)

Ethyl acetate-2-^{13}C:
Acetic-2-^{13}C acid, ethyl ester (9); (58735-82-3)

Ethyl chloroformate:
Formic acid, chloro-, ethyl ester (8);
Carbonochloridic acid, ethyl ester (9); (541-41-3)

Ethyl 4-hydroxybenzoate-1-^{13}C:
Benzoic-1-^{13}C acid, 4-hydroxy-, ethyl ester (14); (211519-29-8)

tert-Butyl alcohol (8);
2-Propanol, 2-methyl- (9); (75-65-0)

4H-Pyran-4-one (8,9); (108-97-4)

Potassium tert-butoxide:
tert-Butyl alcohol, potassium salt (8);
2-Propanol, 2-methyl-, potassium salt (9); (865-47-4)

Diphenylacetic acid:
Acetic acid, diphenyl- (8);
Benzeneacetic acid, α-phenyl- (9); (117-34-0)

4-Biphenylmethanol (9); (3597-91-9)

Sodium acetate-2-^{13}C:
Acetic-2-^{13}C acid, sodium salt (9); (13291-89-9)

Acetyl-2-^{13}C chloride:
Acetyl-2-^{13}C chloride (8,9); (14770-40-2)

Trimethylsilanol:
Silanol, trimethyl- (8,9); (1066-40-6)

Hexamethyldisiloxane:
Disiloxane, hexamethyl- (8,9); (107-46-0)