Note

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Efficient syntheses of benzyl and *n*-octyl [1,3-$^{13}C_2$]acetoacetates, and their application to syntheses of ^{13}C-labelled pyrrole and ^{13}C-labelled hymecromone†

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Benzyl [1-^{13}C]acetate (2a) was prepared via esterification of sodium [1-^{13}C]acetate (1) with benzyl bromide in the presence of 18-crown-6-ether in 97% yield. *n*-Octyl [1-^{13}C]acetate (2b) was rapidly obtained by microwave irradiation of 1-bromooctane and potassium [1-^{13}C]acetate (obtained by salt exchange of 1) absorbed on Al_2O_3 in 82% yield. Solvent-free Claisen condensation of benzyl or *n*-octyl [1-^{13}C]acetate (2a or 2b) in the presence of potassium *tert*-butoxide efficiently gave benzyl or *n*-octyl [1,3-$^{13}C_2$]acetoacetate (3a or 3b) in 51 or 68% yield, respectively. Dibenzyl 2,4-dimethyl[2,4-$^{13}C_2$]pyrrole-3,5-di[^{13}C]carboxylate (4) was synthesized from benzyl [1,3-$^{13}C_2$]acetoacetate (3a) in 54% yield. [2,4-$^{13}C_2$]Hymecromone (6) (7-hydroxy-4-methyl[2,4-$^{13}C_2$]coumarin) was obtained from *n*-octyl [1,3-$^{13}C_2$]acetoacetate (3b) and 1,3-benzenediol (5) in 73% yield.

Keywords: ^{13}C-labelling synthesis; ^{13}C-labelled synthetic unit; ^{13}C-labelled acetoacetate; ^{13}C-labelled pyrrole; ^{13}C-labelled hymecromone

Introduction

^{13}C-Labelled compounds are useful for tracer studies in life science research. For example, we have previously investigated vitamin B_{12} biosynthesis and phenacetin metabolism by using δ-aminolevulinic acid (a biosynthetic intermediate of tetrapyrrole), L-aspartic acid or phenacetin regioselectively labelled with ^{13}C.[1–3] Therefore, efficient ^{13}C-labelling synthetic methods are required. We have obtained various ^{13}C-labelled compounds by using [^{13}C]urea as the ^{13}C-source,[4] and we considered it important to develop efficient syntheses of a range of ^{13}C-labelled units that could be used to prepare various ^{13}C-labelled compounds. One such unit would be ^{13}C-labelled acetoacetate (*β*-keto ester), whose active methylene can readily be utilized in further reactions. Here, we describe efficient ^{13}C-labelling syntheses of benzyl and *n*-octyl acetoacetates, labelled with ^{13}C at the carbonyl carbons of acetoacetate from sodium ^{13}C-labelled acetate. Further, we applied these compounds to the syntheses of a ^{13}C-labelled pyrrole derivative and ^{13}C-labelled hymecromone, which we require for studies on vitamin B_{12} biosynthesis and on hymecromone metabolism, respectively.

Results and discussion

Syntheses of ^{13}C-labelled acetoacetates

Suitable conditions for the Claisen condensation of ethyl acetate to afford ethyl acetoacetate have been reported.[5,6] However, the rather low yield from ethyl acetate, or the large quantity of ethyl acetate used and the large recovery of unchanged ethyl acetate mean that these methods are unsuitable for synthesizing ^{13}C-labelled acetoacetates. Recently, Yoshizawa *et al*. reported that solvent-free Claisen condensation of ethyl acetate in the presence of potassium *tert*-butoxide by heating at 80°C for 20 min efficiently afforded ethyl acetoacetate in 73% yield (calculated from ethyl acetate).[7] In addition, this method could transform benzyl acetate, having a bulky ester moiety, to benzyl acetoacetate by heating at 100°C for 30 min in 75% yield (calculated from benzyl acetate), even though the traditional Claisen condensation of benzyl acetate in toluene and *tert*-butanol by heating at reflux for 16 h was unsuccessful. Benzyl acetate is easy to handle because of its high boiling point (206°C) compared with that (76.5–77.5°C) of ethyl acetate. Therefore, we attempted to synthesize benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) via benzyl [1-^{13}C]acetate (**2a**) derived from sodium [1-^{13}C]acetate (**1**). As shown in Scheme 1, the esterification of sodium [1-^{13}C]acetate (**1**) with benzyl bromide in the presence of 18-crown-6-ether in dry CH_3CN by heating at reflux under an N_2

†*This work is dedicated to the late Prof. J. R. Jones.*

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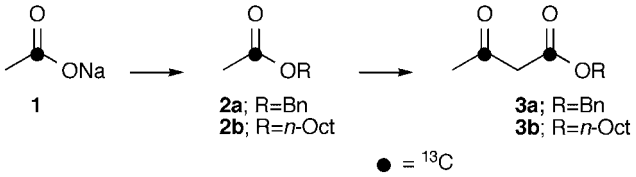


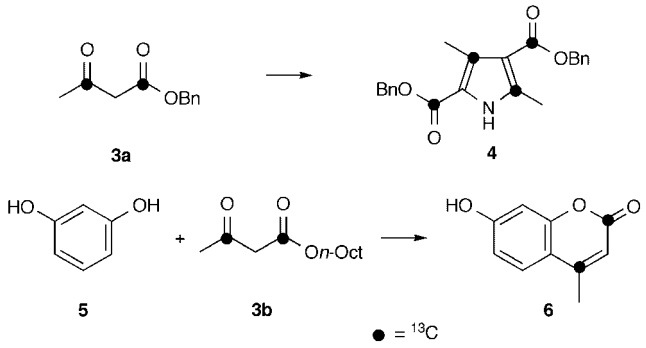
Scheme 1

atmosphere for 15 h gave benzyl [1-^{13}C]acetate (**2a**) in 97% yield, after modifying the previous methods.[8,9] The following solvent-free Claisen condensation of benzyl [1-^{13}C]acetate (**2a**) in the presence of potassium *tert*-butoxide by heating at 100°C under an N_2 atmosphere for 30 min gave benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) in 51% yield. Moreover, the synthesis of *n*-octyl [1,3-$^{13}C_2$]acetoacetate (**3b**) was carried out via *n*-octyl [1-^{13}C]acetate (**2b**), which also has a bulky ester, derived from sodium [1-^{13}C]acetate (**1**). Acetyl bromide was prepared by heating sodium acetate in the presence of benzoic acid and benzyl bromide at 120°C under an N_2 atmosphere for 1 day, then esterified with *n*-octanol in the presence of 4-dimethylaminopyridine at room temperature for 1.5 days to give *n*-octyl acetate in 58–66% yield (after distillation) from sodium acetate. However, these routes involve a number of manipulations. Therefore, we examined the microwave irradiation of 1-bromooctane and potassium [1-^{13}C]acetate (derived from the salt exchange of **1**) absorbed on Al_2O_3 for 5 min; chromatography of the combined reaction mixture absorbed on Al_2O_3 on silica gel gave *n*-octyl [1-^{13}C]acetate (**2b**) in 82% yield. Although the microwave irradiation time was 5 min for the scale of approximate 1 g of sodium [1-^{13}C]acetate (**1**), this esterification procedure is very rapid and offers a high yield compared with the above-mentioned process. Then, solvent-free Claisen condensation of *n*-octyl [1-^{13}C]acetate (**2b**) at 100°C for 1.5 h afforded *n*-octyl [1,3-$^{13}C_2$]acetoacetate (**3b**) in 68% yield.

Syntheses of ^{13}C-labelled pyrrole and ^{13}C-labelled hymecromone by utilization of the ^{13}C-labelled acetoacetates

As shown in Scheme 2, 0.5 equiv. of benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) was treated with $NaNO_2$ in acetic acid at room temperature for 2 h to give the α-nitroso-β-keto ester. After completion of this reaction, the remaining benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) and zinc powder were added, and heating at reflux for 1 h afforded dibenzyl 2,4-dimethyl[2,4-$^{13}C_2$]pyrrole-3,5-di[^{13}C]carboxylate (**4**) in 54% yield; this is a modification of the Knorr pyrrole synthesis method described by Fischer.[10]

[2,4-$^{13}C_2$]Hymecromone (**6**) (7-hydroxy-4-methyl[2,4-$^{13}C_2$]coumarin), which is a cholagogue, was obtained from the reaction of *n*-octyl [1,3-$^{13}C_2$]acetoacetate (**3b**) and 1,3-benzenediol (**5**) in the presence of an acidic catalyst under an N_2 atmosphere at room temperature for 12 h in 73% yield by modifying the synthetic methods reported by Russell and Frye.[11]



Scheme 2

Experimental

Materials and instruments

Sodium [1-^{13}C]acetate (99 atom% ^{13}C) was purchased from Cambridge Isotope Laboratories. All other chemicals were of analytical grade. Melting points were measured on a Yanako micro-melting point apparatus and are uncorrected. All ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Varian Gemini-300 spectrometer. IR spectra were recorded on a Jasco VALORA-III FT-IR spectrometer. MS spectra were obtained with a JEOL JMS-DX302 spectrometer. Microwave irradiation was carried out with a Mitsubishi Electric Corp. RO-F6 (2450 MHz, 500 W).

Benzyl [1-^{13}C]acetate (2a)

To sodium [1-^{13}C]acetate (**1**) (1.94 g, 23.4 mmol) and 18-crown-6-ether (0.26 g, 1.0 mmol) was added dry CH_3CN (10 ml), and the mixture was stirred at room temperature for 10 min under an N_2 atmosphere. To this suspension was added dropwise benzyl bromide (3.0 ml, 25.2 mmol) for 10 min at 0°C, then the mixture was heated under reflux for 15 h. The reaction was quenched with water and the mixture was extracted with Et_2O. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$ and evaporated. Distillation of the crude product gave benzyl [1-^{13}C]acetate (**2a**) (3.42 g, 97%), b.p. 96–100°C (18 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 2.12 (d, 3H, $^2J_{1H13C}$ = 6.9 Hz, $C\underline{H}_3{}^{13}CO$–), 5.12 (d, 2H, $^3J_{1H13C}$ = 3.3 Hz, –$^{13}COOC\underline{H}_2$–), 7.36 (m, 5H, phenyl-H); ^{13}C-NMR ($CDCl_3$) δ: 170.8 (C-1); IR (neat) cm^{-1}: 3035, 2927, 1694, 1498, 1456, 1377, 1363, 1211, 1027, 962, 750, 697; EI-MS *m/z* (rel. int.%): 151 (M^+, 41), 108 (100), 107 (17), 91 (98), 90 (42), 89 (12), 79 (22), 77 (16), 65 (16), 51 (11), 44 (30).

Benzyl [1,3-$^{13}C_2$]acetoacetate (3a)

Benzyl [1-^{13}C]acetate (**2a**) (3.02 g, 20.0 mmol) was added dropwise to potassium *tert*-butoxide (1.59 g, 14.2 mmol) at 0°C under an N_2 atmosphere, and the reaction mixture was heated at 100°C for 30 min. The reaction was quenched with 10% HCl at 0°C. The organic layer was separated and the water layer was extracted with Et_2O. The combined extract was washed with sat. $NaHCO_3$ aq. and brine, dried over anhydrous $MgSO_4$ and evaporated. Chromatography of the crude product on silica gel with hexane:ethyl acetate (4:1) gave benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) (0.99 g, 51%), ^{1}H-NMR ($CDCl_3$) δ: 2.25 (d, 3H, $^2J_{1H13C}$ = 6.0 Hz, $C\underline{H}_3{}^{13}CO$–), 3.50 (t, 2H, $^2J_{1H13C}$ = 6.9 Hz, –$^{13}COC\underline{H}_2{}^{13}COO$–), 5.17 (d, 2H, $^3J_{1H13C}$ = 3.0 Hz, –$^{13}COOC\underline{H}_2$–), 7.36 (m, 5H, phenyl-H); ^{13}C-NMR ($CDCl_3$) δ: 166.8 (C-1), 200.1 (C-3); IR (neat) cm^{-1}: 3035, 2927, 1701, 1676, 1613, 1359, 1309, 1132, 1024, 750, 698; EI-MS *m/z* (rel. int.%): 194 (M^+, 12), 165 (19), 108 (15), 107 (78), 91 (100), 79 (11), 65 (10), 59 (13), 44 (16).

n-Octyl [1-^{13}C]acetate (2b)

A solution of sodium [1-^{13}C]acetate (**1**) (5.0 g, 60.2 mmol) in water was adjusted to pH 1 with 3 M HCl and extracted with Et_2O. The combined extract was re-extracted with a solution of KOH (3.38 g) in

water. Aluminum oxide (20 g) was added to the extract, and the mixture was evaporated. The residue was divided into six portions, and to each was added 1-bromooctane (2.1 ml, 12.2 mmol; total 12.6 ml, total 73.2 mmol), followed by microwave irradiation for 5 min. Chromatography of the combined six reaction mixtures absorbed on aluminum oxide on silica gel with hexane:ethyl acetate (40:1–20:1) gave *n*-octyl [1-^{13}C]acetate (**2b**) (6.91 g, 82%), ^{1}H-NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 6.8$ Hz, $-(CH_2)_7\underline{CH_3}$), 1.23 (br, 12H, $-CH_2(\underline{CH_2})_6CH_3$), 2.05 (d, 3H, $^{2}J_{1H13C} = 6.9$ Hz, $\underline{CH_3}^{13}CO-$), 4.05 (dt, 2H, $J = 3.0$ Hz, $^{3}J_{1H13C} = 3.3$ Hz, $-^{13}COO\underline{CH_2}-$); ^{13}C-NMR ($CDCl_3$) δ: 171.1 (C-1); IR (neat) cm^{-1}: 2957, 2929, 2858, 1701, 1468, 1365, 1211, 1039.

n-Octyl [1,3-$^{13}C_2$]acetoacetate (3b)

n-Octyl [1-^{13}C]acetate (**2b**) (6.91 g, 39.9 mmol) was added dropwise to potassium *tert*-butoxide (3.15 g, 28.1 mmol) at 0°C under an N_2 atmosphere, and the reaction mixture was heated at 100°C for 1.5 h. The reaction was quenched with 10% HCl at 0°C. The organic layer was separated and the water layer was extracted with Et_2O. The combined extract was washed with sat. $NaHCO_3$ aq. and brine, dried over anhydrous $MgSO_4$ and evaporated. Chromatography of the crude product on silica gel with hexane:ethyl acetate (15:1) gave *n*-octyl [1,3-$^{13}C_2$] acetoacetate (**3b**) (2.93 g, 68%), ^{1}H-NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 6.3$ Hz, $-(CH_2)_7\underline{CH_3}$), 1.23 (br, 12H, $-CH_2(\underline{CH_2})_6CH_3$), 2.27 (d, 3H, $^{2}J_{1H13C} = 6.0$ Hz, $\underline{CH_3}^{13}CO-$), 3.45 (t, 2H, $^{2}J_{1H13C} = 6.6$ Hz, $-^{13}CO\underline{CH_2}^{13}COO-$), 4.14 (dt, 2H, $J = 3.0$ Hz, $^{3}J_{1H13C} = 3.8$ Hz, $-^{13}COO\underline{CH_2}-$); ^{13}C-NMR ($CDCl_3$) δ: 167.1 (C-1), 200.3 (C-3); IR (neat) cm^{-1}: 2957, 2928, 2858, 1700, 1679, 1617, 1457, 1406, 1359, 1309, 1221, 1134, 1029, 788; EI-MS *m/z* (rel. int.%): 216 (M^+, 0.8), 105 (100), 87 (39), 70 (19), 57 (20), 44 (34), 43 (16).

Dibenzyl 2,4-dimethyl[2,4-$^{13}C_2$]pyrrole-3,5-di[^{13}C]carboxylate (4)

A solution of sodium nitrite (0.58 g, 8.41 mmol) in water (0.8 ml) was added dropwise to a solution of benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) (1.56 g, 8.03 mmol) in acetic acid (4.7 ml) at 0°C for 10 min, and the mixture was stirred at room temperature. The reaction was complete after 2 h, and at that time, benzyl [1,3-$^{13}C_2$]acetoacetate (**3a**) (1.56 g, 8.03 mmol) and zinc powder (1.27 g, 19.42 mmol) were added, and the mixture was heated under reflux for 1 h. The reaction mixture was added to water (20 ml) at 0°C, and the mixture was left to stand for 6 h and then filtered. The residue was dried and dissolved in $CHCl_3$. The solution was filtered, and the filtrate was evaporated to give dibenzyl 2,4-dimethyl[2,4-$^{13}C_2$]pyrrole-3,5-di[^{13}C]carboxylate (**4**) (1.59 g, 54%), m.p. 136.0°C; ^{1}H-NMR ($CDCl_3$) δ: 2.48 (d, 3H, $^{2}J_{1H13C} = 6.9$ Hz, C-4-$\underline{CH_3}$), 2.58 (d, 3H, $^{2}J_{1H13C} = 6.9$ Hz, C-2-$\underline{CH_3}$), 5.29 (d, 2H, $^{3}J_{1H13C} = 3.0$ Hz, C-3-$^{13}COO\underline{CH_2}-$), 5.31 (d, 2H, $^{3}J_{1H13C} = 3.3$ Hz, C-5-$^{13}COO\underline{CH_2}-$), 7.40 (10H, m, phenyl-H), 8.94 (1H, brs, –NH–); ^{13}C-NMR ($CDCl_3$) δ: 131.6 (C-4), 139.2 (C-2), 161.0 (C-3-$^{13}\underline{C}O-$), 165.0 (C-5-$^{13}\underline{C}O-$); IR (KBr) cm^{-1}: 3309, 1660, 1625, 1416, 1259, 1178, 1074; EI-MS *m/z* (rel. int.%): 367 (M^+, 53), 276 (36), 260 (15), 231 (7), 152 (5), 141 (4), 91 (100), 65 (4).

[2,4-$^{13}C_2$]Hymecromone (6) (7-Hydroxy-4-methyl[2,4-$^{13}C_2$]coumarin)

To a mixture of *n*-octyl [1,3-$^{13}C_2$]acetoacetate (**3b**) (2.93 g, 13.5 mmol) and 1,3-benzenediol (**5**) (1.49 g, 13.5 mmol) was added dropwise conc. H_2SO_4 (12 ml) at 0°C under an N_2 atmosphere, and the mixture was stirred at room temperature for 12 h. The reaction was quenched with ice–water at 0°C. The resultant solid was collected by filtration and dissolved in EtOH (100 ml). To this solution was added silica gel (10 g) and the mixture was evaporated. Chromatography of the residue on silica gel with hexane:ethyl acetate (3:2–1:1) gave [2,4-$^{13}C_2$]hymecromone (**6**) (1.75 g, 73%), ^{1}H-NMR (DMSO-d_6) δ: 2.37 (d, 3H, $^{2}J_{1H13C} = 6.0$ Hz, C-4-$\underline{CH_3}$), 6.13 (d, 1H, $^{2}J_{1H13C} = 4.9$ Hz, C-3-H), 6.71 (d, 1H, $J = 2.3$ Hz, C-8-H), 6.81 (dd, 1H, $J = 2.3$, 8.7 Hz, C-6-H), 7.60 (dd, 1H, $J = 8.7$ Hz, $^{3}J_{1H13C} = 3.8$ Hz, C-5-H); ^{13}C-NMR (DMSO-d_6) δ: 153.7 (C-4), 160.4 (C-2); IR (neat) cm^{-1}: 3147, 1655, 1593, 1556, 1451, 1379, 1359, 1319, 1267, 1244, 1200, 1132, 1063, 1016, 981, 896, 845, 805, 762, 740, 693, 640, 581, 533; EI-MS *m/z* (rel. int.%): 178 (M^+, 100), 149 (100), 148 (56), 121 (13), 92 (13), 78 (4), 66 (5).

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