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Preparation of mono-labelled aliphatic polyacids via isoxazole derivatives

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ABSTRACT

A method allowing the selective oxygen labelling of aliphatic polyacids, for example, succinic and glutaric acids, is reported. This process is based on the hydrolysis of a 3-alkyl-4-nitroisoxazol-5-yl group by Na^{18}OH and affords the products in high yields. The same procedure was applied for the preparation of labelled aliphatic carboxylic acids.

3-Methyl-4-nitro-5-styrylisoxazoles **1** (Fig. 1) are valuable synthons that can be prepared on multigram scale from commercially available isoxazole **2** and an aromatic aldehyde. For example, we have shown that **1** could be used for the preparation of spiroisoxazolines,[1] heteroarylpropionic acids,[2–4] 3-indolepropionic acids[5] and 3-arylglutaric acids.[6] In these syntheses the 4-nitroisoxazolyl core is used as an activator of the exocyclic alkene and is converted into a carboxylate by reaction with excess sodium hydroxide.[7]

During previous studies we showed that alkaline hydrolysis of 3-methyl-4-nitro-5-styrylisoxazole **1** gave the corresponding cinnamic acid **6** (Scheme 1).[8] In particular, when Na^{18}OH was used, the ^{18}O-bilabelled carboxylic group in the above compound was obtained.[9] This was demonstrated by the presence of a signal at *m/z* 152 in the mass spectrum which was in agreement with the proposed bilabelled cinnamic acid.[9] Similarly, 3-methyl-4-nitro-5-styrylisoxazoles of type **1** were successfully employed to prepare 3-[$^{2}H_5$]-phenyl-2-propenoic-[^{18}O]-bilabelled acids.[9] Bromination of compound **1** gave the corresponding dibromo derivative **4** which in turn was converted into arylpropionic acid. Hydrolysis of **4** using Na^{18}OH gave the corresponding ^{18}O-bilabelled acids (Scheme 1).

Methods affording labelled carboxylates are of significant interest for investigating the metabolism of fatty acids and for proteomics studies.[10] Current literature describes:[11,12] (i) generation of an alkyl-metal reagent and its reaction with $C^{18}O_2$, (ii) hydrolysis of an acid derivative, for example, an ester or an acyl chloride and (iii) exchange procedures using $H_2{}^{18}O$ run on a preformed carboxylate.

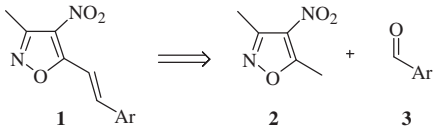

Figure 1. The polyfunctional 5-styryl-4-nitroisoxazole scaffold **1**.

These methods worked well for the preparation of simple labelled carboxylates, however, they could not be applied to the selective labelling of molecules possessing two or more carboxylates. Conversely, our strategy, in which a carboxylate is introduced as a 4-nitroisoxazole, could be employed for the preparation of such compounds. In order to demonstrate this point, we applied this procedure to the preparation of mono-labelled succinic acids **9a–e** and glutaric acids **12a–e** (Scheme 2, Table 1). These syntheses were based on previous studies demonstrating the reactivity of isoxazoles **1a–e** as Michael acceptors toward classic donors, such as nitromethane[3] and diethyl malonate.[4] Hence, compounds **1a–e** were reacted with nitromethane to afford adducts **7a–e**, which in turn were converted into acids **8a–e** using the Victor Meyer procedure. Compounds **8a–e** were subsequently reacted with 5 equiv of Na^{18}OH, readily generated by the addition of Na^0 to commercially available enriched $^{18}OH_2$. Delightfully, this reaction furnished 2-arylsuccinic acids **9a–e** in high isolated yields (Table 1). Importantly the mass spectra of compounds **9a–e** and **12a–e** displayed peaks demonstrating the double addition of ^{18}O to C-5 of the isoxazole. For example, the mass spectrum of compound **9a** showed a peak at *m/z* = 198 only, clearly indicating the presence of two atoms of ^{18}O in the mass ion M^+. The fragmentation showed two main peaks at *m/z* = 150 and *m/z* = 154 which were attributed

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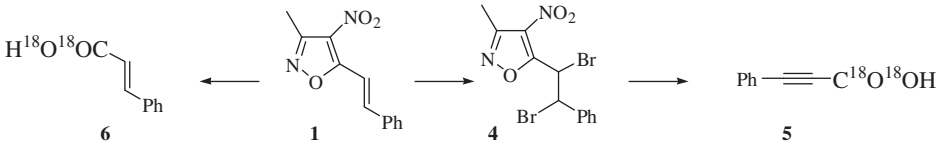
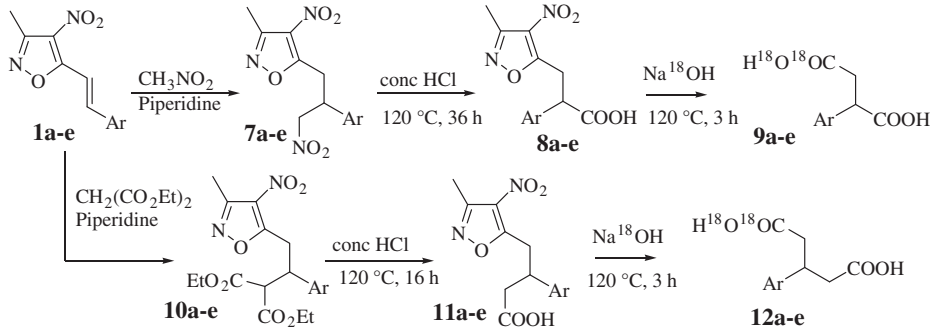


Scheme 1. Preparation of [^{18}O]-bilabelled cinnamic **6** and phenylpropionic acid **5**.



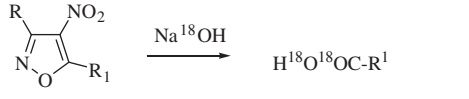
Scheme 2. Preparation of [^{18}O]-mono-labelled phenylsuccinic acids **9a–e** and phenylglutaric acids **12a–e**.

Table 1
Yields and significant *m/z* data of phenylsuccinic acids **9a–e** and phenylglutaric acids **12a–e**

Entry	Ar	Reactant	Product	Yield[a] (%)	*m/z*		
					[M$^+$]	[M$^+$–C^{16}O$_2$]	[M$^+$–C^{18}O$_2$]
1	C_6H_5	**8a**	**9a**	83	198 (38%)	154 (30%)	150 (15%)
2	*p*-Cl–C_6H_4	**8b**	**9b**	76	232 (21%)	188 (25%)	184 (63%)
3	*p*-CH_3O–C_6H_4	**8c**	**9c**	69	228 (21%)	184 (16%)	180 (47%)
4	*p*-CH_3–C_6H_4	**8d**	**9d**	75	212 (27%)	168 (28%)	164 (33%)
5	*p*-NO_2–C_6H_4	**8e**	**9e**	61	243 (17%)	199 (12%)	195 (57%)
6	C_6H_5	**11a**	**12a**	79	212 (15%)	168 (10%)	164 (24%)
7	*p*-Cl–C_6H_4	**11b**	**12b**	76	246 (18%)	202 (16%)	198 (32%)
8	*p*-CH_3O–C_6H_4	**11c**	**12c**	65	242 (10%)	198 (8%)	194 (39%)
9	*p*-CH_3–C_6H_4	**11d**	**12d**	77	226 (23%)	182 (13%)	182 (25%)
10	*p*-NO_2–C_6H_4	**11e**	**12e**	62	257 (11%)	213 (6%)	209 (14%)

[a] Isolated yield after crystallisation.

to [M$^+$–C^{18}O$_2$] and [M$^+$–C^{16}O$_2$]. The absence of a signal at *m/z* = 152 attributed to [M$^+$–C^{18}O^{16}O] ruled out the presence of mono-labelled carboxylic acids which were not formed under the adopted conditions. Similarly, isoxazoles **1a–e** were reacted with diethyl malonate to afford adducts **10a–e** which in turn were hydrolysed to give compounds **11a–e**. Reaction of compounds **11a–e** with 5 equiv of Na^{18}OH afforded the expected glutaric acids 12a–**e** which showed mass spectra consistent with double addition of ^{18}O to C-5 of the isoxazole (Table 1). As a final extension of this study, we have shown that 4-nitroisoxazoles could be used as starting materials for the preparation of simple aliphatic ^{18}O-bilabelled acids (Scheme 3). Therefore, 3,5-dimethyl-4-nitroisoxazole (**2**), 3,5-diethyl-4-nitroisoxazole (**14**) and 3-methyl-5-butyl-4-nitroisoxazole (**15**) were prepared according to literature procedures.[13]



2 R = R^1 = Me; **16** R = R^1 = Me;
14 R = R^1 = Et; **17** R = R^1 = Et;
15 R = Me , R^1 = *n*-Bu; **18** R = Me , R^1 = *n*-Bu;

Scheme 3. Preparation of [^{18}O]-labelled aliphatic acids **16–18**.

Alkaline hydrolysis of compounds **2**, **14** and **15** with Na^{18}OH led to labelled acetic, propionic and pentanoic acids, respectively. The mass spectra of those acids showed peaks at *m/z* values in agreement with the presence of a C^{18}O^{18}OH group.

In conclusion, we have shown that 4-nitroisoxazoles could be employed for introduction of labelled carboxylates at specific positions of carbon frameworks. The desired labelled carboxylates were obtained in high isolated yields. This study adds to the numerous applications of 4-nitroisoxazoles in synthesis and furnishes examples involved in the study of fatty acid metabolism and represents an efficient means to prepare labelled carboxylic acids.

1. General procedure for the preparation of compounds 9a–e and 12a–e

4-Nitroisoxazoles **8a–e** or **11a–e** (0.5 mmol), in a round-bottomed flask, were suspended in 1 N Na^{18}OH solution (2.5 mL). The reaction mixture was refluxed for 3 h, then allowed to cool to room temperature and the pH was adjusted to 1 by addition of 6 N HCl. The solvent was evaporated to yield a solid which was purified by crystallisation.

1.1. 2-Phenylsuccinic acid (9a):[14]

Colourless solid (82 mg, yield 83%) mp 164–166 °C; ν_{max} (KBr)/cm^{-1}: 3600–3200 broad, 1694, 726, 700; δ_H (200 MHz, $CDCl_3$)

11.20 (2H, br s), 7.22–6.99 (5H, m), 3.95–3.78 (1H, dd, J = 9.5, J = 5.5 Hz), 3.06–2.57 (2H, m); δ_C (50.3 MHz, $CDCl_3$) 175.2, 173.9, 139.0, 129.1, 128.3, 127.7, 47.7, 38.2; m/z 198 (M^+, 38%). Elemental Anal. Calcd for $C_{10}H_{10}O_2{}^{18}O_2$: C, 60.6; H, 5.1. Found: C, 60.2; H, 5.2.

1.2. 2-(4-Chlorophenyl)succinic acid (9b):[15]

Colourless solid (88 mg, yield 76%) mp 201–203 °C; ν_{max} (KBr)/cm^{-1}: 3620–3190 broad, 1708; δ_H (200 MHz, DMSO-d_6) 12.5 (2H, br s), 7.37–7.21 (4H, m), 3.93–3.71 (1H, m), 2.91–2.42 (2H, m); δ_C (50.3 MHz, DMSO-d_6) 178.9, 173.1, 133.9, 132.1, 129.6, 128.8, 46.9, 37.9; m/z 232 (M^+, 21%). Elemental Anal. Calcd for $C_{10}H_9ClO_2{}^{18}O_2$: C, 51.63; H, 3.90. Found: C, 51.2; H, 3.7.

1.3. 2-(4-Methoxyphenyl)succinic acid (9c):[16]

Colourless solid (79 mg, yield 69%) mp 205–206 °C; ν_{max} (KBr)/cm^{-1}: 3620–3190 broad, 1704s; δ_H (200 MHz, acetone-d_6) 11.1 (2H, br s), 7.28–7.26 (2H, m), 6.91–6.87 (2H, m), 3.99 (q, J = 4.0 Hz, 1H), 3.76 (s, 3H), 3.10 (dd, J = 8.0 Hz, J = 4.0 Hz, 1H), 2.61 (d, J = 12.0 Hz, 1H); δ_C (50.3 MHz, acetone-d_6) 174.6, 172.9, 159.9, 131.5, 129.7, 114.8, 55.5; m/z 228 (M^+, 21%). Elemental Anal. Calcd for $C_{11}H_{12}O_3{}^{18}O_2$: C, 57.9; H, 5.3. Found: C, 57.5; H, 5.4.

1.4. 3-Phenylpentanedioic acid (12a):[6]

Colourless solid (84 mg, 79% yield), mp 103–107 °C; ν_{max} (KBr)/cm^{-1}: 3665, 1743, 1270; δ_H (400 MHz, $CDCl_3$) 11.90 (2H, br s), 7.20–7.16 (5H, s), 3.35–3.31 (1H, m), 2.66–2.63 (2H, m), 2.47–2.45 (2H, m); δ_C (100.6 MHz, $CDCl_3$) 175.1, 141.6, 135.5, 128.0, 126.6, 36.6, 20.2; m/z 212 (M^+, 15%). Elemental Anal. Calcd for $C_{11}H_{12}O_2{}^{18}O_2$: C, 62.3; H, 5.7. Found: C, 62.5; H, 6.0.

1.5. 3-(4-Chlorophenyl)pentanedioic acid (12b):[6]

Colourless solid (94 mg, 76% yield), mp 131–136 °C; ν_{max} (KBr)/cm^{-1}: 3480, 1745, 1286; δ_H (400 MHz, $CDCl_3$) 12.25 (2H, br s), 7.32 (2H, d, J = 8.0), 7.26 (2H, J = 8.0), 3.41–3.35 (1H, m), 2.66–2.64 (2H, m), 2.54–2.47 (2H, m); δ_C (100.6 MHz, $CDCl_3$) 172.3, 142.9, 130.5, 129.1, 128.4, 37.5, 20.3; m/z 246 (M^+, 18%). Elemental Anal. Calcd for $C_{11}H_{11}ClO_2{}^{18}O_2$: C, 53.6; H, 4.5. Found: C, 53.3; H, 4.7.

1.6. 3-(4-Methoxyphenyl)pentanedioic acid (12c):[6]

Colourless solid (78 mg, 65% yield), mp 99–103 °C; ν_{max} (KBr)/cm^{-1}: 3560, 1736, 1289; δ_H (400 MHz, $CDCl_3$) 12.15 (2H, br s), 7.71 (2H, d, J = 8.0), 7.39 (2H, J = 8.0), 3.81 (3H, s), 3.54–3.47 (1H, m), 2.66–2.62 (2H, m), 2.58–2.54 (2H, m); δ_C (100.6 MHz, $CDCl_3$) 174.9, 158.8, 141.6, 128.9, 121.1, 61.5, 35.4, 20.2; m/z 242 (M^+, 10%). Elemental Anal. Calcd for $C_{12}H_{14}O_3{}^{18}O_2$: C, 59.5; H, 5.8. Found: C, 59.1; H, 6.0.

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