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Synthesis and characterisation of ^{13}C and ^{15}N isotopomers of a 1-acyl-7-nitroindoline

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Summary

Efficient methods are described for synthesis of isotopomers of the water-soluble, photolabile 1-acyl-7-nitroindoline **5** with either ^{13}C in the carbonyl of the acyl substituent or ^{15}N in the nitro group. The isotopic incorporations were verified by IR difference spectroscopy coupled with flash photolysis.

Key Words: nitration; protecting group; infrared spectroscopy; photolysis; FTIR

Introduction

We have previously reported[1,2] that photolysis of 1-acyl-7-nitroindolines **1** and **2** in neutral aqueous solution cleanly yields a carboxylate anion and a 7-nitrosoindole **3** or **4** (Scheme 1). Release of the carboxylate anion upon flash irradiation is rapid and the reagents are, for example, suitable for study of synaptic transmission by L-glutamate in mammalian brain tissue, where sub-millisecond release of the neurotransmitter is needed to mimic normal physiology.[3,4]

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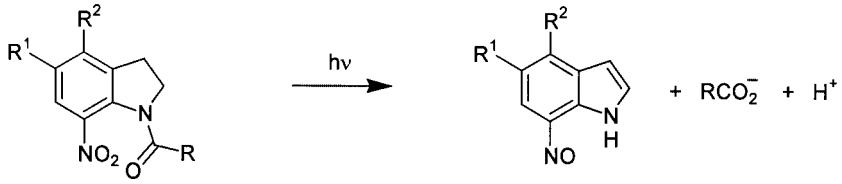
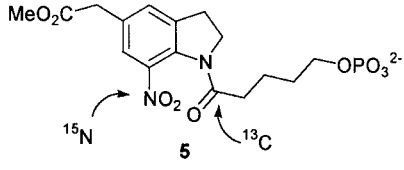


1 $R^1 = CH_2CO_2Me$, $R^2 = H$
2 $R^1 = H$, $R^2 = OMe$

3 $R^1 = CH_2CO_2Me$, $R^2 = H$
4 $R^1 = H$, $R^2 = OMe$

Scheme 1.

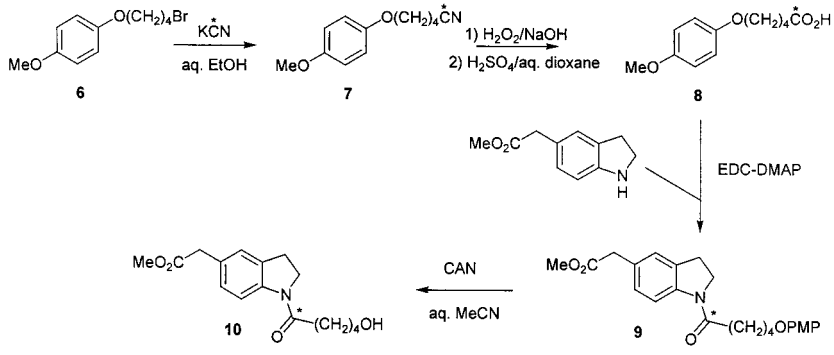
As part of a programme to examine the mechanism of this novel photocleavage reaction, we planned to use time-resolved infrared spectroscopy, as we had done previously for 1-(2-nitrophenyl)ethyl phosphates.[5,6] To facilitate band assignment in this approach, we required particular ^{15}N and ^{13}C isotopomers of a 1-acyl-7-nitroindoline with high water solubility and chose **5**, previously made in a non-isotopic form, as a suitable model. We describe here the synthesis and IR characterisation of two isotopomers carrying either ^{13}C in the carbonyl of the 1-acyl group or ^{15}N in the 7-nitro group.



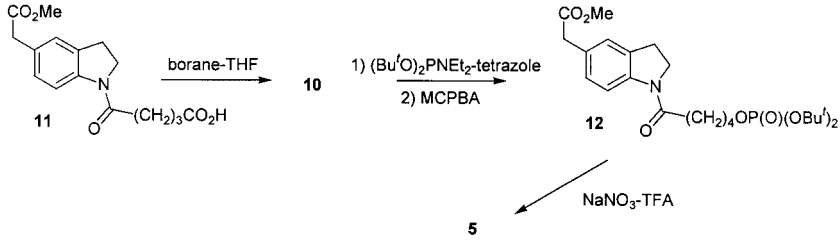
5

In our previous synthesis,[1] the *N*-acyl substituent was derived from glutaric anhydride but a different approach was necessary for the ^{13}C isotopomer to ensure labelling only at the required position. Scheme 2 shows the initial stages of the successful route, leading to the key alcohol **10**. The *p*-methoxyphenyl protecting group[7] was chosen in preference to the more commonly used *p*-methoxybenzyl to ensure its survival under the severe conditions necessary for hydrolysis of the nitrile **7**. Blocking the hydroxyl group also avoids problems of intramolecular participation and facilitates isolation and purification of the early precursors **7** and **8**. The methods described here may be of wider use for synthesis of labelled hydroxycarboxylic acids.

For synthesis of the [^{15}N]isotopomer, our previous route was also somewhat unsatisfactory, as the isotopic nitrogen would have been introduced at an early stage. We therefore altered the sequence of synthetic steps, enabling the isotope to be introduced in the final step.



Scheme 2.



Scheme 3.

The alternate synthesis of alcohol **10** (unlabelled) that facilitates this procedure is shown in Scheme 3, together with the final two steps of the preparation of **5** that followed a common route for either isotopomer, namely phosphorylation of **10** to give the phosphotriester **12**. Treatment of [^{13}C] **12** with non-isotopic $NaNO_3$–TFA, or non-isotopic **12** with [^{15}N]$NaNO_3$–TFA then effected the required nitration and concurrently cleaved the *t*-butyl protecting groups to release the appropriate isotopomer of **5**.

The isotopic incorporations were verified by difference IR spectroscopy of **5** before and after photolysis. Figure 1 shows the difference spectrum for the non-isotopic compound, where negative bands represent vibrations present prior to photolysis and positive bands are from species formed by photolysis.[5,6] The time frame of the spectrum shown is averaged over the interval from 70 ms to 29 s after the light flash and contains no time-resolved information, i.e. individual spectra at 70 ms and at 29 s were identical, which confirms in this time frame the absence of reactions after photolysis.

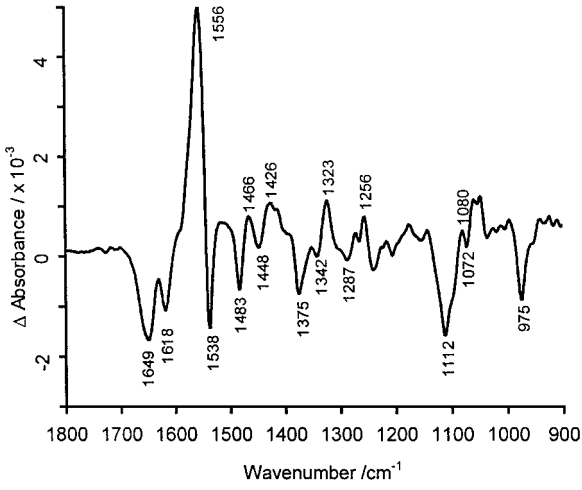


Figure 1. IR difference spectrum of photolysis of compound 5 in aqueous solution

The most prominent change in the difference spectrum for [^{13}C]**5** was for the strong antisymmetric carboxylate vibration of the photoreleased product, that shifted as expected from 1556 to 1512 cm^{-1}, while in the spectrum of [^{15}N]**5**, the antisymmetric and symmetric nitro stretches of the starting compound shifted from 1538 to 1510 cm^{-1} and 1375 to 1362 cm^{-1}, respectively. The magnitudes of these isotopic shifts for the nitro bands are consistent with previous results.[6] These characteristic primary isotope shifts confirm that the isotopic substitutions are in the expected positions. Other effects of the isotopic substitutions were more complex. For example, the two negative bands at 1649 and 1618 cm^{-1}, unaffected by ^{15}N substitution appeared as three negative bands at 1635, 1603 and 1577 cm^{-1} upon ^{13}C substitution. The amide band present in the starting compound is expected near 1650 cm^{-1} and a shift to 1603 cm^{-1} with ^{13}C substitution is consistent with this. Full assignment of the remaining bands in this region and elsewhere in the spectrum, where there is substantial overlap of positive and negative bands, would require further experimentation. Notably there was no evidence for a nitroso monomer band at $\sim$1500 cm^{-1} in the photoproducts. This is consistent with data for the pure nitrosoindole **3** [spectrum in $CHCl_3$].[1] Evidently, the nitroso group of this compound undergoes rapid dimerisation. A detailed analysis of the IR spectra was beyond the scope of the present work, particularly as the time scale of the photocleavage was beyond the time resolution of equipment available to us.

Experimental

General details are as previously described.[2] [^{13}C]KCN was from Cambridge Isotope Laboratories and [^{15}N]$NaNO_3$ was from Aldrich. Details given are for non-isotopic syntheses and the isotopic materials were prepared using identical methods.

5-*(4-Methoxyphenoxy)pentanonitrile* 7

A solution of potassium cyanide (1.43 g, 22 mmol) and 4-(4-methoxy phenoxy)butyl bromide (5.18 g, 20 mmol), prepared as described,[8] in EtOH (50 ml) and water (10 ml) was heated under reflux for 18 h. The solvent was evaporated and the residue was dissolved in Et_2O and washed with water. The organic phase was washed with brine, dried and evaporated to give **7** as white crystals (3.52 g, 87%), mp 36.5–37.5°C (EtOAc–petroleum ether) (Found: C, 67.31; H, 7.31; N, 6.49. $C_{12}H_{15}NO_2 \cdot 0.5\ H_2O$ requires C, 67.27; H, 7.53; N, 6.54%); δ_H (90 MHz) 6.81 (4 H, s, ArH), 3.84–4.04 (2 H, m, ArOCH_2), 3.76 (1 H, s, OMe), 2.32–2.52 (2 H, m, CH_2CN) and 1.72–1.96 (4 H, m, CH_2CH_2).

5-*(4-Methoxyphenoxy)pentanoic acid* 8

A solution of the nitrile **7** (2.05 g, 10 mmol) in a mixture of acetone (60 ml) and methanol (30 ml) was treated with 2 M aqueous NaOH (10 ml) and H_2O_2 (27.5 wt% aqueous solution, 10 ml). The solution was stirred at room temperature for 1 h and treated with further H_2O_2 (10 ml). After 2 h the solution was concentrated and the residue was dissolved in water and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give 5-(4-methoxyphenoxy)pentanamide as a white solid (2.21 g, 99%). The crude amide was dissolved in dioxane (20 ml), treated with 0.5 M aqueous H_2SO_4 (100 ml) and heated under reflux for 2.5 h. After cooling to room temperature the solution was washed with EtOAc and the combined organic phases were washed with brine, dried and evaporated to give **8** as white plates (1.57 g, 70%), mp 95–96°C (EtOAc–petroleum ether) (Found: C, 64.23; H, 7.10. $C_{12}H_{16}O_4$ requires C, 64.27; H, 7.19%); δ_H (400 MHz) 6.82 (4 H, s, ArH), 3.91–3.94 (2 H, m, ArOCH_2), 3.76 (1 H, s, OMe), 2.42–2.46 (2 H, m, CH_2CO_2H) and 1.81–1.84 (4 H, m, CH_2CH_2).

Methyl 1-[5-(4-methoxyphenoxy)pentanoyl]indoline-5-acetate **9**

Methyl indoline-5-acetate (0.28 g, 1.5 mmol), prepared as previously described,[1] was dissolved in dry MeCN (30 ml), cooled under nitrogen to 0°C and treated with DMAP (0.49 g, 4 mmol) and 5-(4-methoxyphenoxy)pentanoic acid **8** (0.23 g, 1.2 mmol), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.38 g, 2 mmol). The solution was stirred at room temperature for 18 h, concentrated under reduced pressure and a solution of the residue in EtOAc was washed successively with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–petroleum ether (2:3)] gave **9** as white crystals (0.44 g, 95%), mp 90–91°C (EtOAc–petroleum ether) (Found: C, 69.59; H, 6.81; N, 3.46. $C_{23}H_{27}NO_5$ requires C, 69.50; H, 6.85; N, 3.52%); δ_H (600 MHz) 8.17 (1 H, d, *J* 8.3, 7-H), 7.07–7.11 (2 H, m, 4, 6-H), 6.82 (4 H, s, ArH), 4.05 (2 H, t, *J* 8.5, 2-H), 3.96 (2 H, t, *J* 6.0, $ArOCH_2$), 3.76 (3 H, s, OMe), 3.68 (3 H, s, CO_2Me), 3.57 (2 H, s, 4-CH_2), 3.17 (2 H, t, 3-H), 2.49 (2 H, t, *J* 6.9, $NCOCH_2$) and 1.86–1.94 (4 H, m, CH_2CH_2).

Methyl 1-(5-hydroxypentanoyl)indoline-5-acetate **10**

A solution of **9** (596 mg, 1.5 mmol) in acetonitrile (240 ml) was cooled to −15°C and treated with an ice-cold solution of ceric ammonium nitrate (2.47 g, 4.5 mmol) in water (60 ml). The mixture was stirred at −15°C for 5 min and quenched by the addition of saturated aqueous $NaHSO_3$ (10 ml). The solvent was removed under reduced pressure and a solution of the residue in EtOAc was washed successively with saturated aqueous $NaHSO_3$, saturated aqueous $NaHCO_3$ and brine, dried and evaporated. Flash chromatography (EtOAc) gave **10** as white needles (197 mg, 45%), mp 68–69°C (EtOAc–petroleum ether) (Found: C, 65.91; H, 7.23; N, 4.66. $C_{16}H_{21}NO_4$ requires C, 65.96; H, 7.26; N, 4.81%); δ_H (600 MHz) 8.17 (1 H, d, *J* 8.3, 7-H), 7.07–7.11 (2 H, m, 4,6-H), 4.05 (2 H, t, *J* 8.5, 2-H), 3.65–3.69 (3 H, m, CH_2OH), 3.68 (3 H, s, CO_2Me), 3.57 (2 H, s, 4-CH_2), 3.18 (2 H, t, 3-H), 2.46 (2 H, t, *J* 6.9, $NCOCH_2$) and 1.82–1.89 (2 H, m, CH_2CH_2) and 1.64–1.69 (2 H, m, CH_2CH_2).

In an alternate synthesis, not suitable for preparation of the [^{13}C]labelled isotopomer, the acid **11** (1.22 g, 4 mmol), prepared as previously described,[1] was dissolved in dry THF (80 ml), cooled to −10°C and treated dropwise with a solution of 1 M borane in THF

(8 ml). The mixture was stirred at −10°C for 2.5 h and quenched with water. The solution was saturated with $NaHCO_3$ and extracted thoroughly with EtOAc. The organic extract was washed with brine, dried and evaporated and the reside was flash chromatographed (EtOAc) to give **10** as white needless (1.06 g, 91%), identical to the material described above.

Methyl 1-{5-[di(tert-butoxy)phosphoryloxy]pentanoyl}indoline-5-acetate 12

A solution of **10** (437 mg, 1.5 mmol) in dry THF (20 ml) was treated under nitrogen with 1H-tetrazole (631 mg, 9 mmol) and di-*t*-butyl-*N*, *N*-diethylphosphoramidite (93% purity; 804 mg, 3 mmol) and the mixture was stirred at room temperature for 3 h. The solution was cooled to 0°C and treated dropwise with a solution of *m*-chloroperbenzoic acid (55% peracid; 1.29 g, 4.5 mmol) in CH_2Cl_2 (5 ml). The solution was stirred at 4°C for 1 h then diluted with ether (20 ml) and washed with 10% aqueous $Na_2S_2O_5$. The organic phase was separated and the aqueous phase was re-extracted with ether. The combined organic phases were washed successively with 10% aqueous $Na_2S_2O_5$, saturated aqueous $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–petroleum ether (4:1)] followed by trituration with ether gave **12** as white plates (701 g, 97%), mp 55–56°C (EtOAc–petroleum ether) (Found: C, 59.58; H, 7.76; N, 2.85. $C_{24}H_{38}NO_7P$ requires C, 59.62; H, 7.92; N, 2.90%); δ_H (400 MHz) 8.16 (1 H, d, *J* 8.2, 7-H), 7.07–7.11 (2 H, m, 4,6-H), 4.05 (2 H, t, *J* 8.3, 2-H), 4.00 (2 H, dt, $J_{H,P}$ 6.3, *J* 7.0, CH_2OP), 3.68 (3 H, s, CO_2Me), 3.57 (2 H, s, 4-CH_2), 3.18 (2 H, t, 3-H), 2.46 (2 H, t, *J* 6.9, $NCOCH_2$) and 1.74–1.90 (4 H, m, CH_2CH_2) and 1.48 (18 H, s, 2 × CMe_3).

1-*[5-(Dihydroxyphosphoryloxy)pentanoyl]indoline-5-acetate disodium salt 5*

A solution of **12** (242 mg, 0.5 mmol) in TFA (5 ml) was treated with $NaNO_3$ (47 mg, 0.55 mmol) and the mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in water (30 ml) and adjusted to pH 6.4 with NaOH. The solution was washed with ether and lyophilised, then redissolved in a small volume of 25 mM Na phosphate, pH 6.0 and

purified by preparative HPLC as previously described.[1] The recovered material (0.36 mmol, 72%) was concentrated and stored in frozen aqueous solution (98 mM). Analytical reverse-phase HPLC, UV and NMR spectroscopy showed that the product was identical to that previously described. [1]

IR spectroscopy

Details of the FT-IR spectrometer and flash lamp have been described previously.[5] Solutions of **5** (100 mM) were prepared in 200 mM Na MOPS buffer, pH 7.0 in D_2O-based solvent and difference spectra were recorded at 20°C. The path length of the sample cell was 15 μm.

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