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SYNTHESIS OF [^{15}N] AND [SIDE-CHAIN 1-^{13}C] ISOTOPOMERS OF 1-(2-NITROPHENYL)ETHYL PHOSPHATES

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SUMMARY

Nitration of 1-phenylethyl acetate with NH_4NO_3-trifluoroacetic anhydride gave a mixture of ortho- *and* para-*nitro products, which were separable by chromatography after saponification of the acetate. The* ortho-*isomer [1-(2-nitrophenyl)ethanol] was converted to the 1-(2-nitrophenyl)ethyl esters of monomethyl phosphate and of the γ-phosphate of ATP. The title isotopomers were prepared using either ammonium [^{15}N]nitrate or [side-chain 1-^{13}C]1-phenylethyl acetate as starting materials. A correction is made to the reported ^{1}H NMR spectrum of caged ATP and the effects of isotopic substitution on the mass spectral fragmentation of 1-(2-nitrophenyl)ethanol are tabulated.*

We recently described the preparation of caged ATP **1**, the photolabile P^3-1-(2-nitrophenyl)ethyl ester of adenosine triphosphate, as its deuterated and [$^{18}O_1$] isotopomers **2** and **3** respectively [1], and the latter of these compounds has been used to aid assignment of infrared absorption bands in a flash photolysis–fast FTIR study of the photolysis of caged ATP [2]. In continuance of this spectroscopic study we required further isotopomers of caged ATP and now

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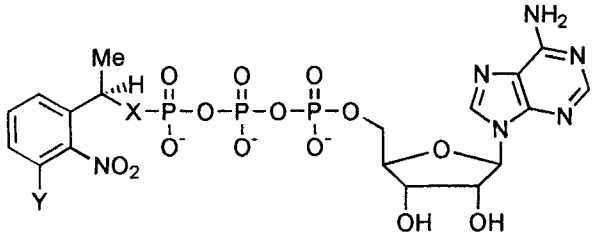
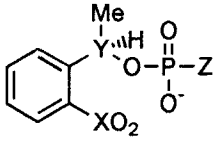
describe the synthesis of the [^{15}N] and [^{13}C] compounds **5a** and **6a** respectively, together with the related isotopomers **5b** and **6b** of methyl 1-(2-nitrophenyl)ethyl phosphate **4b**.



1 X = ^{16}O, Y = H
2 X = ^{16}O, Y = D
3 X = ^{18}O, Y = H



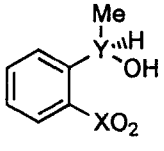
4 X = ^{14}N, Y = ^{12}C
5 X = ^{15}N, Y = ^{12}C
6 X = ^{14}N, Y = ^{13}C

a Z = ADP
b Z = OMe

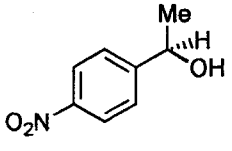
For all compounds prepared it was desired to attain high isotopic enrichment in order to observe clear isotope-related shifts in the IR absorption bands. For the [^{15}N] compounds **5a,b** this was achieved with the nitration method described by Crivello [3], which uses ammonium or potassium nitrate and trifluoroacetic anhydride (TFAA). The method has been applied extensively for nitration of carbo- and heterocyclic aromatics, and of a range of other substrates, but only in one example, [^{15}N]nitration of hexadeuteriobenzene [4], has it been used to prepare a [^{15}N]nitro compound. Since the reactants are used in equimolar stoichiometry and yields are generally high, the method may deserve more attention for isotopic synthesis, as it offers excellent utilisation of the isotopic nitrogen without any dilution of the enrichment level. An alternative method with similar advantages uses boron trifluoride monohydrate in place of TFAA [5].

Nitration of toluene was reported by Crivello to give *ortho*- and *para*-nitrotoluenes in 53 and 35% yields respectively, together with 1% of the *meta*-isomer [3]. In exploratory non-isotopic experiments 1-phenylethyl acetate was nitrated smoothly and the ^{1}H NMR spectrum of the crude reaction product, after acetate hydrolysis, showed two components in a ratio of 40:60. The

acetates were not separable by chromatography but after hydrolysis the *ortho*- and *para*-isomeric alcohols **7a** and **8** respectively were readily separated, with the *ortho*-isomer being the less abundant and earlier eluted component. The identity of the *ortho*-isomer **7a** was confirmed by comparison with the previously reported ^{1}H NMR spectrum of an authentic sample [1]. In separate experiments with authentic compounds (prepared by $NaBH_4$ reduction of the commercially-available nitro-acetophenones) it was shown that the *meta*- and *para*-1-(nitrophenyl)ethanols could not be separated under the conditions used here. However, the *meta*-isomer is expected to be a very minor component and could not be detected by NMR spectroscopy of the isolated *para*-isomer **8**. The ratio of *ortho*-isomer formation was less favourable than that reported for nitration of toluene, presumably because the larger 1-acetoxyethyl substituent has a greater steric demand, but an adequate amount of material could nevertheless readily be obtained.



7a X = ^{14}N, Y = ^{12}C
7b X = ^{15}N, Y = ^{12}C
7c X = ^{14}N, Y = ^{13}C



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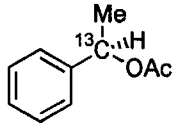

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The alcohol **7a** was cleanly oxidised with Jones reagent [6] to 2-nitroacetophenone, which could be converted to caged ATP **1** as previously described [1,7]. For preparation of the caged methyl phosphate **4b**, the alcohol **7a** was first converted to 1-(2-nitrophenyl)ethyl phosphate as previously described [1,8]. This compound in weakly acidic aqueous solution was stirred vigorously with excess ethereal diazomethane [9], which resulted in clean conversion to the required diester **4b**.

In considering the ^{1}H NMR spectra of the various compounds prepared here, it became apparent that in our previous report [1] of the signals from the nitrophenyl ring of caged ATP **1**, the assignments for H-6 and H-4 had inadvertently been transposed. Thus the text should have

read that the signals centred at δ 7.85, 7.77, 7.61 and 7.34 (D_2O solution) correspond respectively to H-3, H-4, H-5 and H-6. The opportunity to correct this error is welcomed.

The methods described above were readily applied to synthesise the required ^{15}N and ^{13}C isotopomers. For the ^{15}N series, 1-phenylethyl acetate was nitrated as described using ammonium [^{15}N]nitrate, while for the ^{13}C series, [side-chain 1-^{13}C]1-phenylethyl acetate **9** was prepared by $NaBH_4$ reduction and subsequent acetylation of [carbonyl-^{13}C]acetophenone.

The labelled 1-(2-nitrophenyl)ethanol isotopomers **7b** and **7c** were analysed by mass spectroscopy as previously descsribed [1] and as expected retained the full isotopic enrichment (> 98%) of the starting materials. Table 1 shows the effects of the ^{15}N and ^{13}C isotopic substitutions on the EI-mass spectrum of 1-(2-nitrophenyl)ethanol. Taken together with the previous data [1] for its deuterated and ^{18}O isotopomers, the results indicate that complex rearrangements and multiple pathways are involved in fragmentation of this compound. For example, although the compound shows no molecular ion and the fragment ion of highest mass (m/z 152) corresponds to

Table 1. Effects of isotopic substitution on the principal fragment ions in the EI-mass spectrum of 1-(2-nitrophenyl)ethanol **7a**.

Fragment ions of unlabelled alcohol **7a** m/z	Labelled Compound	
	[^{15}N] **(6b)**	[^{13}C] **(6c)**
152	+1	+1
149	+1	+1
134	+1	+1
121	n.c.	+1
105	n.c.	+1
104	n.c.	+1
93	+1	a
91	n.c.	b
77	n.c.	c
65	n.c.	n.c.
51	n.c.	n.c.
43	n.c.	+1

n.c, no change; a, position unchanged but intensity *ca.* 20% greater than in unlabelled compound; b, *ca.* 20% reduced intensity at m/z 91 but 5-fold intensity increase at m/z 92; c, intensity unchanged but 2.5-fold intensity increase at m/z 78.

loss of $CH_3^{\bullet}$ from the molecular ion, the abundant ion at m/z 43 corresponds to CH_3CO^+, i.e. an alternate fragmentation in which the methyl side chain remains attached to the charge-carrying species. Further discussion is unwarranted in the absence of more extensive investigation.

Results of flash photolysis–FTIR experiments with the labelled compounds prepared here will be described elsewhere.

EXPERIMENTAL

General details have been given previously [1]. Ammonium [^{15}N]nitrate and [carbonyl-^{13}C]acetophenone were purchased from Aldrich, Gillingham, Dorset, U.K. The negative ion FAB mass spectrum was determined on a VG 70-250SE instrument with the sample in a glycerol matrix. As in the Discussion section, descriptions are given in full for preparation of unlabelled compounds. Isotopomers were prepared by identical methods from appropriate starting materials.

Nitration of 1-phenylethyl acetate. – Ammonium nitrate (0.80 g, 10 mmol) and 1-phenylethyl acetate (1.64 g, 10 mmol) were placed in a round-bottomed flask equipped with a magnetic stirrer and a condenser fitted with a drying tube. Trifluoroacetic anhydride (10 ml) was added and the mixture was stirred at room temp. for 1.5 h. For part of this time the reaction generated sufficient heat to cause reflux of the TFAA. The reaction mixture was poured into water (50 ml) and extracted with ether, and the ether extract was washed with aq. $NaHCO_3$ and brine, dried and evaporated. A solution of the residue in MeOH (50 ml) and 2 M aq. KOH (7.5 ml) was heated under reflux for 0.5 h, then cooled and neutralised with 2 M aq. HCl. The solution was concentrated under reduced pressure, diluted with water and extracted with ether. The ether extract was washed with water and brine, dried and evaporated to leave a pale yellow oil (1.46 g) which showed two spots on TLC [EtOAc-petroleum ether (3:7)] with R_f values 0.19 and 0.30. Flash chromatography [EtOAc-petroleum ether (1:3)] gave 1-(2-nitrophenyl)ethanol **7a** as the less polar fraction (0.54 g). The more polar fraction was 1-(4-nitrophenyl)ethanol **8** (0.65 g), δ_H 8.13 (2 H, d, *J* 8.6, 3,5-H), 7.51 (2 H, d, 2,6-H), 4.99 (1 H, q, *J* 6.3, ArC*H*), 3.08 (1 H, s, OH) and 1.49 (3 H, d, Me).

[side-chain 1-^{13}C]1-Phenylethyl acetate **9**. – A solution of [carbonyl-^{13}C]acetophenone (2 g) in EtOH (30 ml) was cooled to 4 °C and $NaBH_4$ (0.63 g) was added. The mixture was stirred at 4 °C for 1 h, then neutralised by careful addition of glacial acetic acid and concentrated under reduced pressure. The residue was partitioned between ether and water, and the ether extract was washed with water and brine, dried and evaporated. The residue was dissolved in a mixture of dry pyridine (10 ml) and acetic anhydride (6 ml) and kept at room temp. overnight, then diluted with water and extracted with ether. The ether extract was washed with aq. $NaHCO_3$, 1 M aq. HCl, water and brine, dried and evaporated at leave the acetate **9** as a colourless oil (2.59 g, 95%), δ_H 7.33-7.45 (5 H, m, Ar-H), 5.88 (1 H, dq, J_{CH} 148.3, J_{HH} 6.4, ArC*H*), 2.06 (3 H, s, $COCH_3$) and 1.53 (3 H, dd, J_{CCH} 4.0). This material was used for nitration as described above without further purification.

2-Nitroacetophenone. – A solution of 1-(2-nitrophenyl)ethanol (0.50 g, 3 mmol) in acetone (5 ml) was cooled to 10 °C and an aliquot (0.8 ml) of a solution of CrO_3 (2.67 M in 4 M aq. sulphuric acid) was added dropwise with stirring. After 10 min, the mixture was diluted with water and extracted with ether. The ether extract was washed with water and brine, dried and evaporated to leave a yellow oil (0.44 g) which contained 2-nitroacetophenone in approx. 90% purity (^{1}H NMR) which was used without purification.

P^3-*1-(2-Nitrophenyl)ethyl adenosine triphosphate* **1**. – The [^{15}N] and [^{13}C] isotopomers **5a** and **6a** of this compound were prepared from the appropriate labelled 2-nitroacetophenone as previously described [1] for the deuterated isotopomer **2**.

1-(2-Nitrophenyl)ethyl methyl phosphate **4b**. – The alcohol **7a** was converted to 1-(2-nitrophenyl)ethyl phosphate as previously described [8], except that instead of precipitation as its barium salt, it was isolated from the phosphorylation reaction by anion exchange chromatography on DEAE-cellulose, using a linear gradient of triethylammonium bicarbonate (TEAB; 10-250 mM). Fractions containing the product were evaporated as previously described [7] to give the

monophosphate as its triethylammonium salt. An aqueous solution of this salt (20 mM, 15 ml) was adjusted to pH 4.5 with 1 M aq. HCl, cooled in ice and stirred vigorously with an ethereal solution of diazomethane (15 ml, approx. 300 mM). After 0.5 h the diazomethane had been completely consumed and the mixture was diluted with water (approx. 20 ml). The aqueous layer was washed with ether, filtered, adjusted to pH 5.5 and concentrated (approx. 1 ml). This solution was injected onto a preparative reverse phase HPLC column (2 x 30 cm; Waters C_{18} packing material, Cat. No. 20594) equilibrated in 10 mM Na phosphate, pH 5.5, and the column was eluted at 2.5 $ml.min^{-1}$ with the same buffer for *ca.* 2.5 h. The mobile phase was changed to water-acetonitrile (60:40) which eluted the phosphodiester **4b**. The eluted fractions were diluted 4-fold to reduce the acetonitrile concentration, then applied to a column of DEAE-cellulose (2 x 40 cm) and eluted with a linear gradient of TEAB (10-150 mM, total volume 2000 ml). Final processing of the fractions to remove excess TEAB was as previously described [7] and gave the diester **4b** as its triethylammonium salt (0.22 mmol, 73%) [Found: M^- (FAB) 260. $C_9H_{11}NO_6P$ requires m/z 260]; δ_H (90 MHz; Na salt; D_2O; acetone ref.) 7.41-8.09 (4 H, m, Ar-H), 5.78 (1 H, dq, $J_{HP,HH}$ 7, ArC*H*), 3.37 (3 H, d, J_{HP} 8.5, OMe) and 1.62 (3 H, d, CHCH_3).

HPLC analyses. – Anion exchange HPLC was performed on a Whatman Partisphere SAX column (Cat. No. 4621-0505). For the caged ATP compounds **1**, **5a** and **6a**, the mobile phase was 0.2 M ammonium phosphate, pH 5.85 plus 5% MeOH (v/v) at 1.5 $ml.min^{-1}$. Retention times were 3.2 and 15.3 min for caged and free ATP respectively. For the caged methyl phosphates **4b-6b**, the mobile phase was 15 mM sodium phosphate, pH 6.5 plus 3% MeOH (v/v) at 1.5 $ml.min^{-1}$. Retention times were 1.5 and 5.7 min for caged methyl phosphate and caged phosphate respectively. Both mobile phase buffers were prepared from solutions of the respective monobasic salts adjusted to the specified pH values with sodium hydroxide.

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