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Synthesis of [carbonyl-^{14}C]-4-benzoylbenzoic acid, a photolabelling reagent.

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SUMMARY

[carbonyl-^{14}C]-4-Benzoylbenzoic acid, a photolabelling reagent, was synthesised with high specific activity in 57 % radioactive yield from [carboxyl-^{14}C]-benzoic acid. Radioactive synthesis allowed elucidation of the mechanism of a previous unlabelled synthesis using benzoic acid, methyl ester. An improvement in the experimental conditions led to a new synthesis of radioactive benzoylbenzoic derivatives with good yield.

Key words: photolabelling, [carbonyl-^{14}C]-4-benzoyl-benzoic acid, lithiation, mechanism.

INTRODUCTION

Radioactive photolabelling reagents are commonly used to characterise the ligand binding sites of an enzyme. Several reagents have been reported (1). Among them, benzophenone derivatives offer certain advantages: they offer excellent chemical stability and are able to react with C-H bonds of the enzyme to form covalent bonds. (2)

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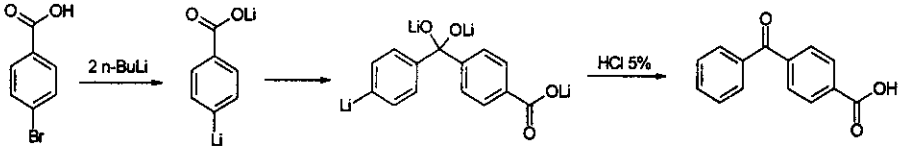
Our purpose was the synthesis of [carbonyl-^{14}C]-4-benzoyl-benzoic acid. This compound represents an interesting platform to obtain photoactivable analogues of a large panel of toxins, enzyme substrates and proteins.

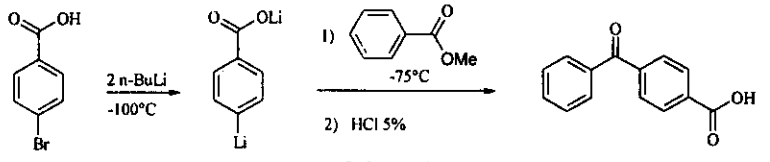
The synthesis of the [carboxyl-^{14}C]-4-benzoyl-benzoic acid has already been achieved by carbonatation with $^{14}CO_2$ of the Grignard reagent of 4-bromobenzophenone dimethylketal, which was obtained in two steps from 4-bromobenzophenone (**3**). In order to avoid the synthesis of unlabelled precursors, and to decrease the number of steps, we studied other synthesic routes.

4-Benzoylbenzoic acid (CAS Registry Number: 611-95-0) has been synthesised by dilithiation of 4-bromobenzoic acid and self-condensation of the lithium dianion at -20 °C (**4**) (*Scheme 1*). In the case we are dealing with, i.e. the synthesis of a labelled compound, the precursor would be the [^{14}C-carboxyl]-4-bromobenzoic acid and the final product would be labelled on both carbonyl and carboxyl positions. Thus this route did not satisfactorily answer the question we first addressed, i.e. the synthesis of the monolabelled [^{14}C-carbonyl]-4-benzoylbenzoic acid.



Scheme 1

Unlabelled 4-benzoyl-benzoic acid has also been obtained by condensation of the dilithio salt of 4-bromobenzoic acid on benzoic acid, methyl ester (**5**) (*Scheme 2*). This route was chosen due to the facile synthesis of [^{14}C-carboxyl]-benzoic acid, methyl ester and to the unique labelling position on the carbonyl group.

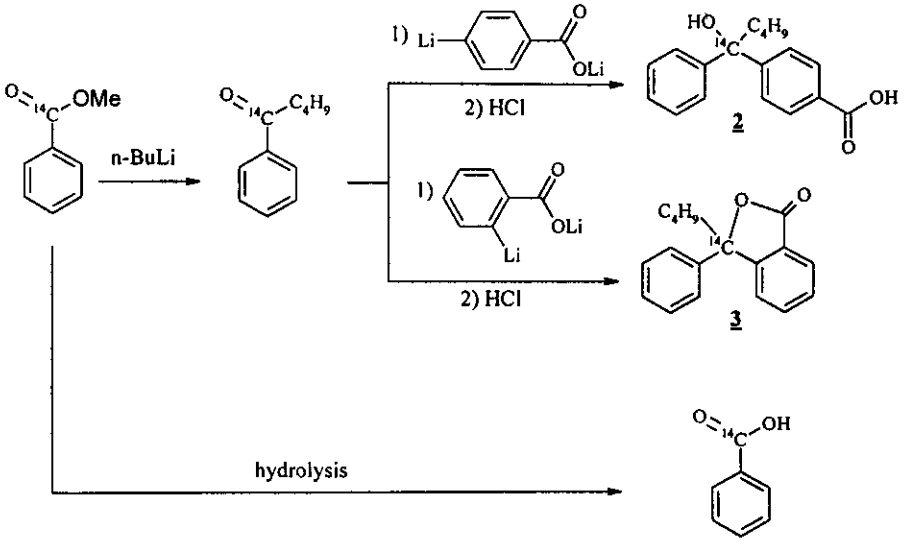


Scheme 2

RESULTS & DISCUSSION

Starting from [^{14}C-carboxyl]-benzoic acid, methyl ester, radioactive synthesis of 4-benzoyl-benzoic acid, according to **(5)**, gave an unexpected result: a 22 % chemical yield after purification but a zero radiochemical yield.

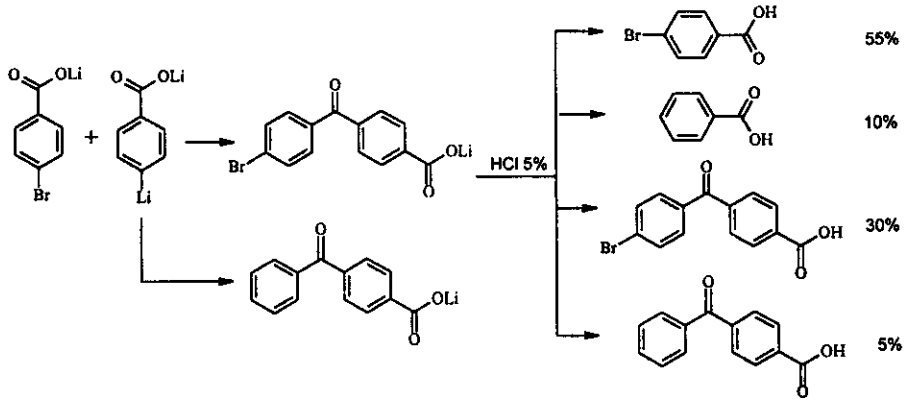
This led us to think that the 4-benzoylbenzoic acid, according to this procedure, did not arise as previously proposed **(5)** from the condensation of the dilithio salt of 4-bromobenzoic acid on [^{14}C-carboxyl]-benzoic acid, methyl ester (Scheme 2) but was produced by the self-condensation of the lithium derivatives (Scheme 1). The only labelled products obtained were the [carboxyl-^{14}C]-benzoic acid and two impurities: 4-[[1-^{14}C]-1-butyl-1-hydroxy-1-phenyl]-benzoic acid **2** and [3-^{14}C]-3-butyl-3-phenyl-3*H*-isobenzofuran-1-one **3** which may have arisen from the addition of excess n-butyl lithium on the introduced [^{14}C-carboxyl]-benzoic acid, methyl ester and from the condensation of both potential forms of the dilithio salt of bromobenzoic acid on the *resulting product* (*Scheme 3*).



Scheme 3

Therefore, in order to check this hypothesis, we examined the nature of the lithio derivatives in the reaction mixture before the addition of the ester. According to the experimental protocol used by **(5)**, the hydrolysis of the lithio derivatives after 2 hours at –78°C (table 1) gave 10% of benzoic acid, 30% of 4-bromobenzoylbenzoic acid and 5% of 4-benzoylbenzoic acid. This

experiment clearly indicates that two major problems have occurred : the remaining 4-bromobenzoic acid (55%) and the presence of an important amount of 4-bromobenzoylbenzoic acid (30%), formed according to *Scheme 4*. In fact the majority of the reactant has not been dilithiated and a part of this dilithio derivative (30 %) had already reacted before the addition of the ester. In these reaction conditions, only 10 % of the initial reactant (which is hydrolysed in benzoic acid) appeared available to react with the ester.



Scheme 4

In order to prevent the self-condensation of the dilithio compound, the reaction solution is kept below –100°C. In order to totally convert the 4-bromobenzoic acid into its dilithiated anion, the n-butyl lithium ratio was increased to 2.2 eq. and its reactivity was enhanced by the use of 0.1 eq. of N,N,N',N'-tetramethylethylenediamine (TMEDA). In these conditions, hydrolysis after 1 hour led to 70% of benzoic acid (Table 1). With an increase of TMEDA to 0.2 eq., 99% of 4-bromobenzoic acid was transformed into benzoic acid after hydrolysis (Table 1).

Eq n-BuLi	Eq TMEDA	Reaction time at -100°C	Reaction time at -78°C	% of 4-bromobenzoic acid (a)	% benzoic acid (a)	% 4-bromobenzoyl benzoic acid (a)	% 4-benzoyl benzoic acid (a)
2	-	(b)	2h	55	10	30	5
2.2	0.1	1h	-	20	70	7	3
2.2	0.2	1h	-	<1	99	-	-

a) HPLC measurement after hydroiysis, U.V. detection: 280 nm
b) Addition time 15 min

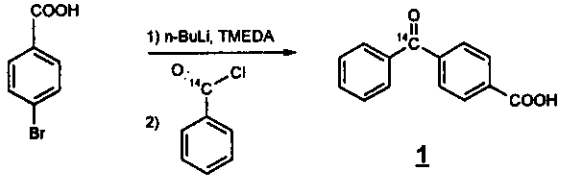
Table 1

In these optimised conditions, for which the 4-bromobenzoic acid is totally converted into its dilithio reactive form, the addition of [carboxyl-^{14}C]-benzoic acid, methyl ester gave rise to [carbonyl-^{14}C]-4-benzoylbenzoic acid 1 in only 10% radiochemical yield and with an important isotopic dilution (6 times). In fact benzoic acid, methyl ester seems to be insufficiently reactive even at –78°C, whereas at this temperature the unlabelled dilithio compound self-condensed.

In order to overcome this difficulty, we used an electrophile presumed more reactive than the ester, *i.e.* the acylchloride. [carboxyl-^{14}C]- Benzoylchloride was synthesised in 82% yield from [carboxyl-^{14}C]-benzoic acid with thionyl chloride. It did indeed react at -78°C, but is also consumed by the excess of n-butyl lithium (Scheme 3).

To prevent the formation of impurities of type (2) and (3) (Scheme 3) a stoichiometric amount n-butyl lithium was used. The resultant incomplete dilithiation of 4-bromobenzoic acid was enhanced by the use of sub-stoichiometric amount of [carboxyl-^{14}C]-benzoylchloride.

Since some self-condensation of the dilithio compound can not be totally avoided, the formation of unlabelled benzoylbenzoic acid, and thus the isotopic dilution, was prevented by carbonation of the dilithio 4-benzoylbenzoic acid, leading after hydrolysis to the formation of the related diacid, which was further easily eliminated. Following these conditions, [carbonyl-^{14}C]-4-benzoyl-benzoic acid was obtained in 57% radiochemical yield without isotopic dilution (Scheme 5).



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Scheme 5

EXPERIMENTAL

General

[^{14}C] Barium carbonate used for the classical synthesis of [carboxyl-^{14}C]-benzoic acid was obtained from Slavia. Reagents were purchased from Aldrich. Solvents were distilled with appropriate desiccant reagents and kept over molecular sieves. HPLC analyses were carried out on a Merck L 6200 system. Radioactive monitors were models LB 505 and LB

503 from Berthold. The UV detector was a Merck system, model L 4250. Radioactive TLC were recorded on Berthold system, model LB 2821. Specific activities were determined on a Finnigan mass spectrometer, model 4600.

4-Benzoylbenzoic acid from [^{14}C-carboxyl]-benzoic acid, methyl ester.

To 102 mg (0.5 mmol) of 4-bromobenzoic acid dissolved in 4 mL of dry THF and cooled to –100°C were added, 0.58 mL (1 mmol) of a 1.74 M n-butyl lithium hexane solution, over 15 min, under nitrogen atmosphere. The reaction mixture was kept below –95°C during the addition, then was allowed to warm to –78°C and was stirred for 2 h. at this temperature. A solution of [^{14}C-carboxyl]-benzoic acid, methyl ester (0.184 mCi, 0.5 mmol) in 0.5 mL of THF was added slowly in order to keep the temperature below –75°C. After 2 h. the reaction mixture was hydrolysed with a 5% HCl solution.

The crude was analysed by TLC (silica gel-hexane, ethyl acetate, acetic acid (70-30-0.5)-UV visualisation and radioactive detection). 4-Bromobenzoylbenzoic acid (non-radioactive product) Rf: 0.02; 4-benzoylbenzoic acid Rf: 0.32 (non-radioactive product); 2 Rf: 0.43 (10%); 4-bromobenzoic acid Rf: 0.47 (non-radioactive product); benzoic acid Rf: 0.52 (70%); 3 Rf: 0.83 (18%); benzoic acid, methyl ester Rf: 0.86 (2%).

The reaction solution was also analysed by HPLC (silicagel column -hexane, ethyl acetate, acetic acid (80-15-0.25); benzoic acid, methyl ester Rt. : 2.84 min ; 3 Rt. : 3.24 min ; benzoic acid Rt. : 5.18 min ; 4-bromobenzoylbenzoic acid Rt. : 5.40 min ; 2 Rt. : 8.12 min ; 4-benzoylbenzoic acid Rt. : 9.06 min ; 4-bromobenzoic acid Rt. : 21 min.

The solution was extracted with diethylether. The organic layers were extracted with a saturated solution of sodium bicarbonate, washed to neutral pH and the diethylether was evaporated. The dried residue has been identified by mass spectrometry as the lactone 3 (CI/NH_3) (m+1/z 267; m+18/z 284). The aqueous layer was acidified with HCl and extracted with diethylether. The purification was achieved on a silica gel column with hexane, ethyl acetate, acetic acid (70-30-0.5) giving rise to a 22% chemical yield (50 mg of unlabelled 4-benzoylbenzoic acid were obtained) and a 0% radiochemical yield. The second impurity has been identified by mass spectrometry as 2 (CI/NH_3) 267 (M-OH); 284 (+ NH_3); 302 (NH_3+NH_4^+).

Nature of the lithium anions of the reaction solution.

Method A:

To a solution of 4-bromobenzoic acid (203 mg, 1 mmol) in 4 mL of THF and cooled to –100°C were added slowly 1.2 mL (2.09 mmol) of a 1.74 M n-butyl lithium solution in hexane. The temperature was kept below –95°C over the addition and the solution was stirred at –75°C for 2 hours. The reaction mixture was hydrolysed with a 5% HCl aqueous solution.

The products were analysed by HPLC measurement. See results Table 1.

Method B:

To a solution of 4-bromobenzoic acid (203 mg, 1 mmol) and 16 µL (0.1 mmol) of TMEDA in 4 mL of THF and cooled to –100°C were added slowly 0.65 mL (1.13 mmol) of a 1.74 M n-butyl lithium solution in hexane. The reaction mixture was stirred at –100°C for 1 hour and hydrolysed with 5% HCl aqueous solution.

The products were analysed by HPLC measurement. See results Table 1.

Method C:

Same experimental protocol as method B except that the volume of TMEDA was 32µL (0.2 mmol)

The products were analysed by HPLC measurement. See results Table 1.

[carbonyl-^{14}C]-4-Benzoylbenzoic acid 1 from [carboxyl-^{14}C]-benzoic acid, methyl ester with TMEDA.

4-Bromobenzoic acid (101 mg, 0. 5 mmol) was dilithiated with 0.65 mL (1.13 mmol) of a 1.74 M n-butyl lithium hexane solution and 37 µL (0.23 mmol) of TMEDA (20% of n-butyl lithium). The reaction solution was kept at –100°C for 1 hour. [Carboxyl-C^{14}]-methyl benzoate (0.5 mmol, 0.184 mCi) was added dropwise to keep the temperature below –95°C. After 2 hours at –78°C, the solution was hydrolysed with a 5% HCl solution. The reaction solution was analysed as previously described by TLC and HPLC. The radioactive quantification of the different products gave 4-benzoylbenzoic acid 9%, benzoic acid 26%, 3 24%, 2 37%, unknown impurities 2%. The measurement of 4-benzoylbenzoic acid by HPLC with an authentic sample gave a 52% yield and a 9% radiochemical yield.

[carbonyl-^{14}C]-4-Benzoylbenzoic acid 1 from [carboxyl-^{14}C]-benzoyl chloride

To 22.8 mCi (0.42 mmol, SA 54.3 mCi/mmol) of dry [carboxyl-^{14}C]-benzoic acid was added 1 mL of distilled thionyl chloride and the mixture was refluxed under nitrogen atmosphere for 1 h. The solution was evaporated to dryness, the residue was dissolved in anhydrous benzene and evaporated again to dryness (twice).

To a solution of 102 mg (0.5 mmol) of 4-bromobenzoic acid in 4 mL of dry THF were added 16 µL (0.1mmol) of TMEDA, under nitrogen atmosphere, and the solution was kept below -100 °C.

0.64 ML of n-butyl lithium (1.56 mmol/mL-1 mmol) was added slowly with a syringe to avoid the increase of the temperature. The THF solution, which became yellow, was stirred for 1 h at -100 °C. The previous [carboxyl-^{14}C]-benzoyl chloride dissolved in 0.5 mL of dry THF was introduced and the solution was stirred over 1 h at -78°C. The solution was carbonated with CO_2 and then hydrolysed with 2N HCl. The product was extracted with diethyl ether and controlled by TLC on silica gel; hexane, ethyl acetate, acetic acid (70-30-0.5) Rf 0.2 and HPLC (R_t 9.15 min on silica gel column; mobile phase: hexane, ethyl acetate, acetic acid (85-15-0.25). The product was purified on silica gel column with hexane, ethyl acetate, acetic acid (70-30-0.5).

Results: 13 mCi of pure [carbonyl-^{14}C]-4-benzoyl-benzoic acid were obtained (radioactive yield 57 %). TLC analysis on silica gel hexane, ethyl acetate, acetic acid (70-30-0.5) Rf 0.2 showed one spot. The mass spectrometry (CI/NH_3) of [carbonyl-^{14}C]-4-benzoylbenzoic acid 1 (m+1/z= 229, m+18/z= 246), (SA 54,3 mCi/mmol) was in agreement with the mass spectrometry analysis of an authentic sample of the non-labelled acid.

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