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N.C.A [^{11}C]CO_2 AS A SAFE SUBSTITUTE FOR PHOSGENE IN THE CARBONYLATION OF PRIMARY AMINES

A. Schirbel, M.H. Holschbach*, H.H. Coenen

Institut für Nuklearchemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

SUMMARY

An efficient one-pot synthesis of [^{11}C]ureas and [^{11}C]isocyanates via dehydration of intermediately formed carbamate salts is described as a general alternative to their formation via [^{11}C]phosgene. After optimization of the reaction parameters, in-target produced n.c.a. [^{11}C]CO_2 can be used for labelling in a one pot reaction within a very short reaction time of 10 minutes resulting in good radiochemical yields. The developed method has been applied to the ^{11}C-carbonylation of aniline, benzyl- and phenethylamine and 1,2-diaminobenzene yielding the appropriate n.c.a. [^{11}C]ureas in about 65, 85, 25 and 70% radiochemical yield (RCY), respectively. The presented reaction sequence can be handled easily and safely and lends itself to simple automation.

Key words: ^{11}C-labelling, [^{11}C]carbon dioxide, [^{11}C]carbonylation, [^{11}C]ureas, [^{11}C]isocyanates

**Author for correspondence*
Fax (00)49-(0)2461- 612535, e-mail m.holschbach@fz-juelich.de

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INTRODUCTION

The urea group is often found as a structural element in biomolecules and pharmaceuticals. Labelling this moiety with the short-lived, positron emitting nuclide carbon-11 ($t_{½}$ = 20.4 min; β^+ = 99.8 %) is useful for positron emission tomography investigations. So far, ^{11}C-labelling of ureas in the carbonyl function requires the radiosynthesis of the labelled secondary precursor [^{11}C]phosgene, which can be prepared via different pathways. One possibility is the zinc catalyzed reduction of [^{11}C]CO_2 to [^{11}C]CO at 400°C followed either by catalytic chlorination using $PtCl_4$ at elevated temperature (1) or by a photochemical reaction with elemental chlorine (2). Alternatively, [^{11}C]methane can be used as labelled primary precursor and then be converted to [^{11}C]CCl_4 with elemental chlorine followed by catalytic oxidation to [^{11}C]phosgene (3). In all these multistep syntheses [^{11}C]phosgene is obtained with low radiochemical yields (RCY < 50 %) and specific activities (26-29 GBq/µmole) within relatively long reaction times (ca. 20 min) (1-3). For a reliable production of [^{11}C]phosgene both synthetic procedures require a sophisticated technical set up and experienced experimentalists.

Due to the drawbacks of the [^{11}C]phosgene synthesis the direct carbonylation of amines with n.c.a. [^{11}C]CO_2 represents an attractive alternative for the synthesis of carbon-11-labelled ureas. The first attempt (4) using silylated or diphenylphosphite-preactivated amines resulted in low radiochemical yields (RCY 2-5 %). Recently, the strong base lithium bis(trimethylsilyl)amide was carbonylated using n.c.a. [^{11}C]CO_2 yielding unsubstituted [^{11}C]urea with a radiochemical yield of about 60 % (5).

In this paper an efficient one-pot synthesis of [^{11}C]ureas and [^{11}C]isocyanates via dehydration of intermediately formed carbamate salts is described as a general alternative to their formation via [^{11}C]phosgene.

EXPERIMENTAL

General

All chemicals were purchased from commercial sources in the highest purity available. Solvents and reagents used in the labelling reactions were purified by standard laboratory procedures before use.

Melting point determinations employed a Mettler FP 61 and are rounded to the nearest degree. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 200 spectrometer in ≈ 5% solution at 25°C. All shifts are given in δ ppm using the signals of the appropriate solvent residues as a reference.

Analytical radio-HPLC was performed on a system consisting of a Sykam pump (S 1000) and a Sykam UV/vis detector (S 3300) with a wavelength of 254 nm. Sample injection was accomplished by a Rheodyne-Injector-block (7125). For measurement of radioactivity the outlet of the UV detector was connected to a NaI(Tl) well type scintillation detector and the recorded data was processed by a software system (Nuclear Interface GmbH). All given radiochemical yields are corrected for decay. The specific activities of the products were determined by HPLC assay of aliquots of the labelled products and comparison with standard curves of UV absorption recorded from solutions of known concentrations. HPLC was performed using a Nucleosil 100 - 5C18 (250x4 mm) column and a flow of 1 ml/min of the appropiate eluent.

Precursors and standards

2-Phenyl-ethylisocyanate

2-Phenylethylamine (4 g, 33 mmole) was dissolved in diethylether (10 mL) and HCl (4.4 mL of a 7.5 M solution in diethylether, 33 mmole) was added dropwise under ice cooling. 2-Phenylethylammonium hydrochloride precipitated as white needles and was isolated by filtration. Recrystallization from ethylacetate yielded 2.9 g (56 %) of the analytically pure product. The

hydrochloride (1.89 g, 12 mmole) was transformed into 2-phenylethyl isocyanate using bis(trichloromethyl)carbonate (triphosgene) according to Majer et al. (7). The isocyanate was obtained with 19.8 % yield as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$): δ 7.30-7.50 (m, 5H), 3.6 (t, 2H), 3.00 (t, 2H). ^{13}C-NMR ($CDCl_3$): δ 138, 129, 128, 126, 44, 38.

N,N´-Dibenzylurea

N,N´-Dibenzylurea was prepared according to Wolman et al. (8). Carbohydrazide (900 mg, 10 mmole) and benzylamine (10.7 g, 100 mmole) were dissolved in dimethylacetamide (20 mL) and the mixture was cooled in ice. Under vigorous stirring iodine (10 g, 40 mmole) was added to the solution. After 5 min excess of iodine was removed with aqueous 1N sodium thiosulfate (5 mL). The product was precipitated by the addition of water (100 mL), filtered off and air-dried giving 150 mg (6 % yield) of product as white needles. M.p.: 170.7°C,(lit (8) 170°C) ^{1}H-NMR ($CDCl_3$): δ 7.25-7.40 (m, 10H), 4.65 (s, 2H), 4.45 (d, 4H). ^{13}C-NMR (DMSO-d_6): δ 159, 142, 128, 127, 126, 43.

N,N´-Di(2-phenylethyl)urea

N,N´-Di(2-phenylethyl)urea was prepared from 2-phenylethylamine in the same way as described for N,N´-dibenzylurea. The product was obtained as a white solid with 37 % yield. M.p.: 132.0°C (lit (8) 135-137°C) ^{1}H-NMR ($CDCl_3$): δ 7.20-7.40 (m, 10H), 4.25 (s, 2H), 3.45 (t, 4H), 2.85 (t, 4H).

N,N´-Di(2-aminophenyl)urea

1,2-Diaminobenzene (1 g, 9.2 mmole) and urea (276 mg, 4.6 mmole) were dissolved in glacial acetic acid (20 mL) and the solvent was distilled off (9). The resulting residue was dissolved in 1 N NaOH (10 mL), extracted with diethylether (3 x 25 mL) and the combined organic layers were dried over magnesium sulfate. Evaporation of the solvent afforded a white solid which was recrystallized from ethanol, yielding 226 mg (21 %) of white needles, m.p.: 244°C. ^{1}H-NMR (DMSO-d_6): δ 9.50 (s, 2H), 6.95 - 7.05 (m, 8H) 4.55 (s, 4H).

N,N´-Diphenylcarbodiimide

Triphenylphospine (13.1 g, 50 mmole) was dissolved in benzene (100 mL) and bromine (8 g, 50 mmole) was added dropwise at 0 - 5°C. To the resulting suspension triethylamine (10.1 g, 100 mmole) was added. After stirring for 5 min at room temperature N,N´-diphenylurea (10.6 g, 50 mmole) was added and the mixture was refluxed for 90 min. The precipitate formed was filtered off, the filtrate was evaporated to dryness and the residue taken up in diethylether (50 mL). After filtration of the mixture the filtrate was evaporated to dryness and the residue distilled under reduced pressure to give N,N´-diphenylcarbodiimide as a colourless liquid with 5.2 % yield. $Bp_{1.1}$: 135°C. ^{1}H-NMR ($CDCl_3$): δ 7.10-7.40 (m, 10H).

Radiosyntheses

Production of [^{11}C]CO_2

[^{11}C]Carbon dioxide was produced using 17 MeV protons from a BC 1710 Baby Cyclotron at the Research Center Juelich GmbH via the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen. The [^{11}C]carbon dioxide was trapped in a capillary steel coil cooled with liquid nitrogen before being transferred into the reaction vessel with a stream of argon (25 mL/min). ^{11}C-labelling was performed using a remotely controlled apparatus placed in a lead shielded box.

N,N´-Diphenyl[^{11}C]urea **3**

[^{11}C]CO_2 was trapped at -90°C in a vial containing aniline (0.12 mmol) and triethylamine (0.59 mmol) in dichloromethane (1 mL). The solution was kept for 5 min at -20°C and phosphorylchloride (0.13 mmol) in dichloromethane (250 µL) was added via syringe. The solution was heated to 20°C for another 5 min. and after removing the solvent at reduced pressure under argon flow, HPLC solvent (2 mL) was added. Aliquots of the solution were analyzed by HPLC using methanol/water 50/50 (v/v) as the mobile phase; the water phase contained 1 % triethylamine and was adjusted to pH 3.0 with phosphoric acid.

The k´-values for [^{11}C]N,N´-diphenylurea **3** and [^{11}C]phenylisocyanate **2** were 10.4 and 5.1, respectively. [^{11}C]N,N´-diphenylcarbodiimide **4** eluted with acetonitrile/water 80/20 (v/v).

N,N´-Dibenzyl[^{11}C]urea **7**

This product was prepared as described for N,N´-diphenyl[^{11}C]urea **3**, starting from benzylamine. Acetonitrile/water 40/60 (v/v) was used as the mobile phase; the water phase contained 1 % triethylamine and was adjusted to pH 3.0 with phosphoric acid. The k´-values were 3.8 for N,N´-dibenzyl[^{11}C]urea **7** and 8.2 for [^{11}C]benzylisocyanate **5**.

N,N´-Di(2-phenylethyl)[^{11}C]urea **8**

It was prepared as described for N,N´-diphenyl[^{11}C]urea **3**, starting from 2-phenylethylamine. Acetonitrile/water 40/60 (v/v) was used as the mobile phase; the water phase contained 1 % triethylamine and was adjusted to pH 3.0 with phosphoric acid. The k´-values were 7.4 for N,N´-di(2-phenylethyl) [^{11}C]urea **8** and 11.4 for 2-[^{11}C]phenylethylisocyanate **6**.

[^{11}C]Benzimidazolone **9**

Compound **9** was prepared as described for N,N´-diphenyl[^{11}C]urea **3**, starting from *o*-diaminobenzene. Acetonitrile/0.1 M NaH_2PO_4 15/85 (v/v) was used as the mobile phase. The k´-value for [^{11}C]benzimidazolone **9** was 5.6. N,N´-di(2-aminophenyl)[^{11}C]urea **10** eluted with acetonitrile/water 80/20 (v/v).

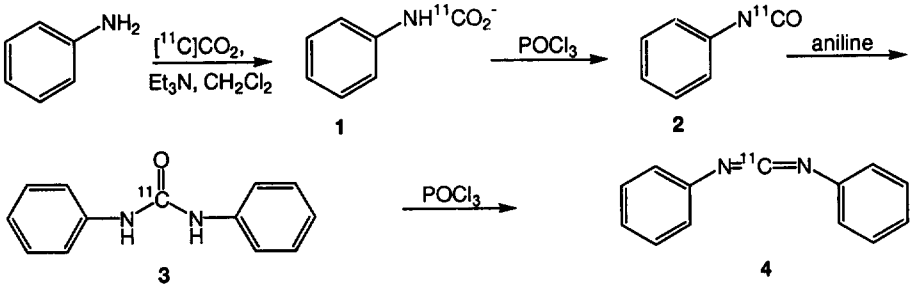
RESULTS AND DISCUSSION

The direct carbonylation of primary amines using both [^{12}C]CO_2 and [^{14}C]CO_2, thus avoiding the use of phosgene, has recently been described by Dean (6, 10). It has been shown, that in both cases the amines, after conversion into their carbamate salts, were dehydrated to isocyanates which were obtained in high yields as the final products. The aim of the study presented in this paper was to

modify this synthetic approach for a possible use on the no-carrier-added level using n.c.a.[^{11}C]CO_2 as the carbonyl synthon.

Carbonylation of aniline

Using the aromatic amine aniline as a model compound we investigated its reaction with n.c.a. [^{11}C]CO_2 under a variety of different reaction conditions (Scheme 1). The reaction was performed in dichloromethane as recommended solvent (6).



Scheme 1. ^{11}C-Carbonylation of aniline

In the presence of a tertiary amine, aniline can efficiently be converted with n.c.a.[^{11}C]CO_2 into the corresponding carbamate salt **1** and subsequently be dehydrated with phosphorylchloride to give [^{11}C]phenylisocyanate **2**. In contrast to reactions under stoichiometric conditions, carbon-11 labelled **2** was isolated in relatively low RCY because it reacts in situ with the excess of aniline to the corresponding [^{11}C]urea **3**. Correspondingly, the excess of the dehydrating reagent phosphorylchloride causes a consecutive reaction generating N,N´-diphenyl[^{11}C]carbodiimide **4** as side product, although in low RCY. Therefore, the isocyanate **2** and the urea **3** cannot be prevented from further reaction under n.c.a. reaction conditions. Also, attempts to isolate and analyze the carbamate formed in the first reaction step failed due to its high reactivity and water sensitivity. Hence, optimization of the individual reaction parameters of the consecutive steps was performed by determining the radiochemical yields of the isocyanate, the urea and the carbodiimide.

As a first step the concentration of aniline was optimized, keeping all conditions except the one examined as constant (Fig. 1). A ≥ 125 mM solution of the amine proved to be useful for obtaining high radiochemical yields (65 ± 4 %) of N,N′-diphenyl[^{11}C]urea **3** (Fig. 1), while higher concentrations did not lead to a significant increase in the RCY. The required substrate-concentration is relatively high and reflects the low reactivity of carbon dioxide. On the other hand, the optimal concentration found is typical for reactions of n.c.a. [^{11}C]CO_2 with other substrates, e.g. Grignard compounds. The curve depicted in Fig. 1 for isocyanate formation shows a maximum, because higher aniline concentrations favor the formation of the corresponding urea. The sigmoid curve forms of the two other compounds (**3**, **4**) are typical for product formation via consecutive reactions.

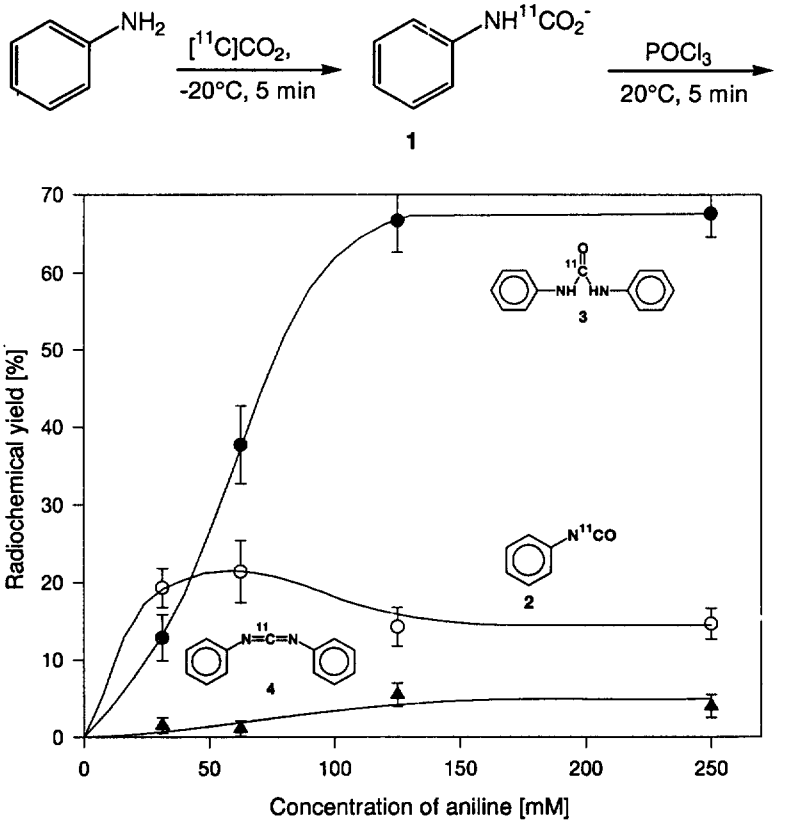


Fig. 1. Dependence of the RCY of **2**, **3**, and **4** on the concentration of aniline

In order to examine the influence of the reaction temperature on the [^{11}C]carbamate formation step, only the temperature was varied from -40 to +20°C. Fig. 2 shows that the temperature of the first reaction step, the formation of **1**, is the crucial factor for obtaining a high RCY of the urea **3**. In agreement with the literature [6] optimal yields of about 65 % were obtained at -20°C. Surprisingly an increase or decrease of only 10°C resulted in significantly lower yields of **3**. The remarkable temperature dependence of this reaction could be explained by a low reaction rate at low temperature and decomposition reactions ocurring at higher temperature; a strict control of the reaction temperature seems to be indispensable.

The effect of the reaction time on the formation of carbamate **1** is shown in Fig. 3. Under otherwise stable conditions a saturation yield was obtained for all

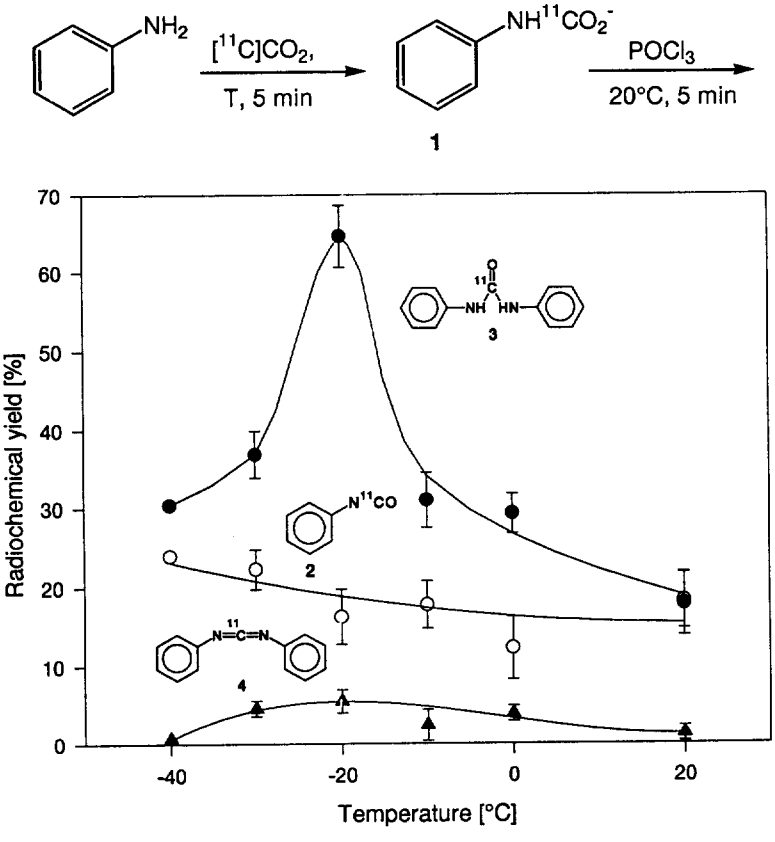


Fig. 2. Effect of temperature on the [^{11}C]carbamate-formation step

three compounds (**2**, **3**, **4**) after a very short reaction time of only 5 minutes, whereas longer reaction times did not increase the RCY, a fact which is favourable in view of the short half-life of carbon-11.

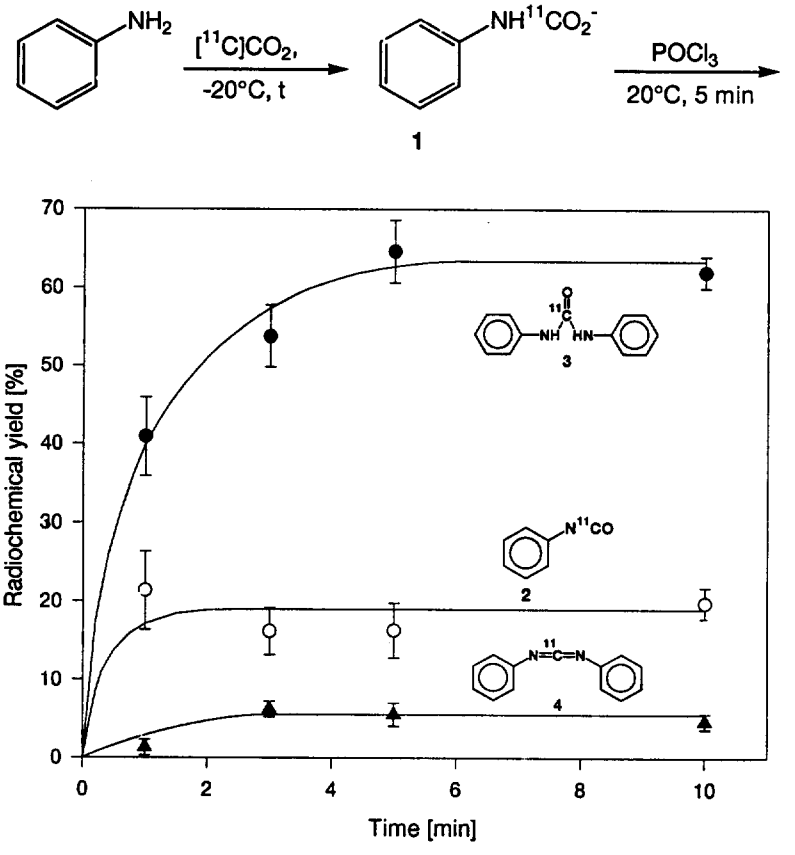


Fig. 3. Effect of reaction time on the [^{11}C]carbamate-formation step

After optimization of the carbamate-formation, the second reaction step, dehydration of **1** to the isocyanate **2**, was optimized with respect to reaction time and temperature; carbamate synthesis was performed using constant optimized reaction parameters.

As can be seen in Fig. 4, the time dependence of isocyanate-formation is very similar to that during the optimization of the first reaction step. A radiochemical

saturation yield of N,N′-diphenyl[^{11}C]urea was again obtained within a reaction time of only 5 minutes after addition of $POCl_3$ so that the total reaction time required is about 10 minutes.

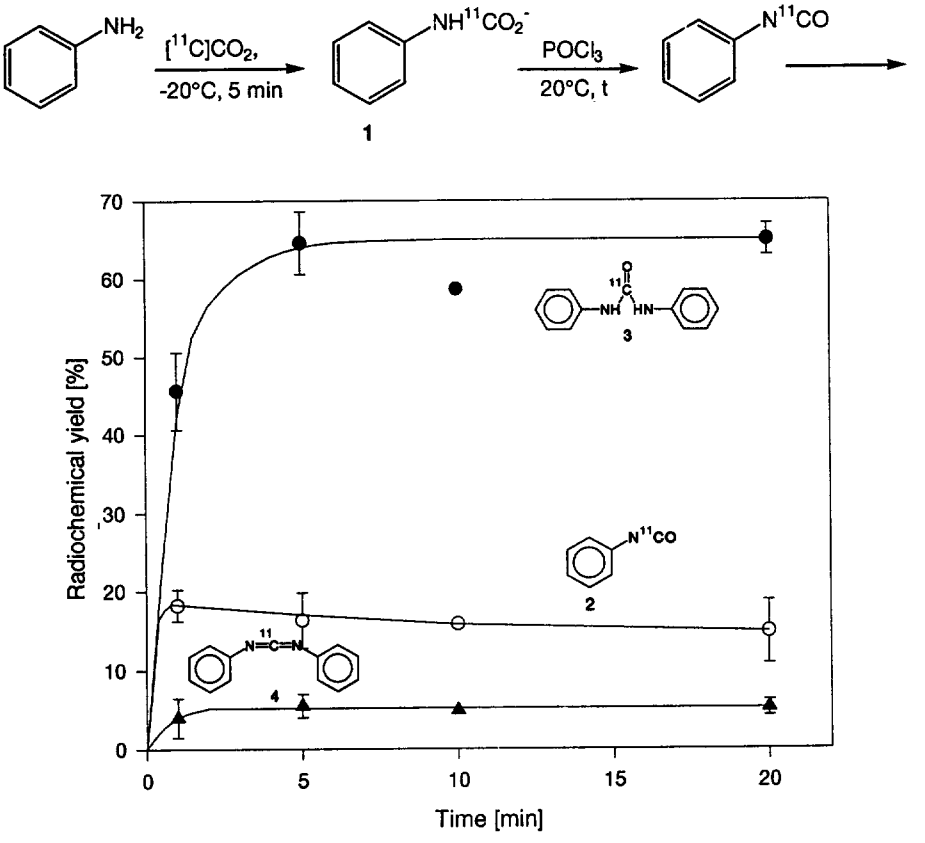
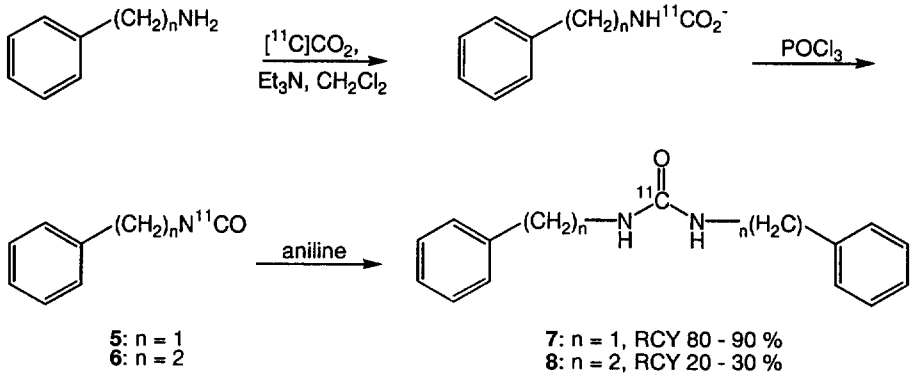


Fig. 4. Effect of reaction time on the [^{11}C]isocyanate-formation step

In contrast to the first reaction step, no significant effect of the reaction temperature between -20 and +40°C was observed on the reaction temperature in the [^{11}C]isocyanate-formation step. Consequently, it is possible to perform the second reaction step at room temperature without the necessity of cooling or heating.

Carbonylation of aliphatic amines

In order to demonstrate the versatility of the above described reaction sequence other model compounds were chosen for the carbonylation with n.c.a.[^{11}C]CO_2 using the described optimal reaction conditions. Benzylamine and 2-phenylethylamine served as model compounds. Labelling of these compounds resulted in RCYs of 85 ± 5 and 20 ± 5 %, respectively, for the corresponding [^{11}C]ureas **7** and **8** (Scheme 2).

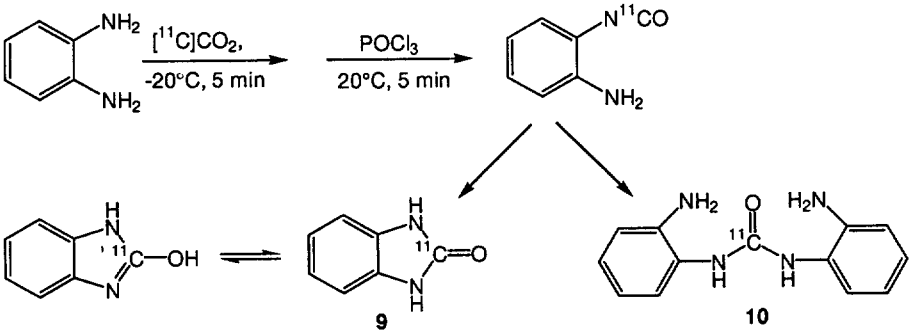


Scheme 2. Reaction scheme for the synthesis of alkyl substituted [^{11}C]ureas **7**, **8**

According to literature findings (6), the relatively low RCY for N,N´-di(2-phenylethyl)[^{11}C]urea **8** is not atypical for carbonylation reactions of aliphatic amines. It is assumed, that this problem could partly be overcome by the use of strong pentaalkylguanidine bases instead of triethylamine (6). In the present work, both carbon-11 labelled isocyanates **5** or **6** could not be isolated or detected although these compounds must have been formed as intermediates during the labelling reaction. Obviously, all the labelled isocyanates were immediately transformed to the corresponding [^{11}C]ureas due to the high nucleophilicity of the amines.

Carbonylation of 1,2-diaminobenzene

There are several pharmaceuticals containing a benzimidazolone structure which can potentially be labelled by the presented labelling procedure. Therefore carbon-11 carbonylation reactions were performed with *o*-diaminobenzene as a model compound using the same optimized reaction conditions. The desired cyclized product [^{11}C]2(3H)-benzimidazolone ([^{11}C]2-hydroxybenzimidazolone) **9** was obtained in 20 - 30 % RCY. The main problem in this reaction was the competitive formation of the corresponding open chain urea **10** in a RCY of about 40 % (Scheme 3).



Scheme 3. Reaction scheme for the synthesis of [^{11}C]benzimidazolone **9**

Several attempts were made to increase the RCY of [^{11}C]benzimidazolone **9** by driving the reaction into the desired direction, e.g. variation of the amine concentration or of the reaction temperature. These attempts led to a better ratio of **9**/**10**, but also to a decrease in absolute RCY. The use of other bases instead of triethylamine, e.g. 2,6 di-*tert*-butylpyridine, *n*-butyllithium and Hünig's base (N-ethyldiisopropylamine) resulted in a drastic decrease of the radiochemical yield (< 2 %). Changing the solvent from dichloromethane to acetonitrile, toluene, diethylether or DMF also led to very low radiochemical yields. Finally, the crucial factor affecting the RCY proved to be the amount of phosphorylchloride (Fig. 5). The best results were achieved using a 14-fold excess of *o*-diaminobenzene giving rise to the desired product **9** in 65 - 75 % RCY.

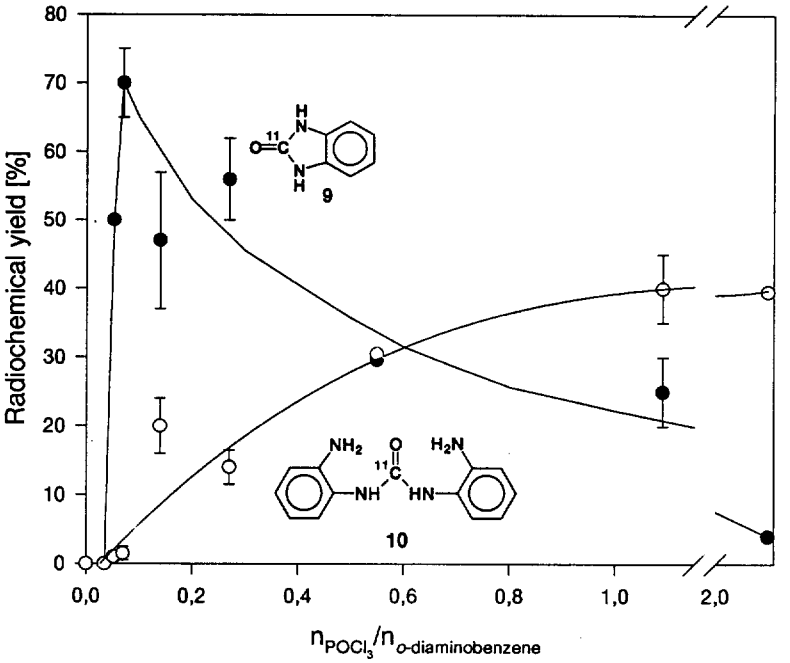


Fig. 5. Effect of $POCl_3$ concentration on the RCY of **9** and **10**

CONCLUSION

In conclusion, several [^{11}C]ureas and [^{11}C]isocyanates can be prepared in a one-pot synthesis by direct carbonylation of primary amines using n.c.a. [^{11}C]CO_2. The labelling reactions can be performed within a very short reaction time of 10 minutes with radiochemical yields up to 90 %. Compared to labelling conditions with [^{11}C]phosgene it is possible to obtain higher radiochemical yields within shorter reaction times. As with [^{11}C]phosgene it is still problematic to obtain high specific activities due to the high affinity of the required amines to CO_2. The observed specific activities are also in the range of 25 GBq/µmole and work is in progress to overcome this problem. However, the presented reaction sequence can be performed as a one-pot synthesis, can therefore be handled easily and safely, and lends itself to simple automation, thus avoiding the drawbacks of the potentially hazardous [^{11}C]phosgene synthesis.

CGP-12177, a β-adrenoreceptor agonist, pimozide and benperidol, antagonists for the D_2 dopamine receptor are interesting target molecules for the new method developed here. CGP-12177 and pimozide have already been radiolabelled with carbon-11 in the urea-carbonyl position using [^{11}C]phosgene (11,12) whereas labelling of benperidol in the carbonyl position has not yet been performed.

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