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Research Article

Synthesis of NCA [*carbonyl*-^{11}C]amides by direct reaction of *in situ* generated [^{11}C]carboxymagnesium halides with amines under microwave-enhanced conditions

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Summary

No-carrier-added (NCA) aromatic and aliphatic [*carbonyl*-^{11}C]amides were rapidly (< 5 min) synthesized in one pot in useful radiochemical yields (20–65%, decay-corrected) by directly coupling amines with NCA [^{11}C]carboxymagnesium halides generated *in situ* from Grignard reagents and cyclotron-produced [^{11}C]carbon dioxide. In this system cyclohexylcarboxymagnesium chloride (**1b**) is more reactive than 4-fluorophenylcarboxymagnesium bromide (**2b**) and primary amines (e.g. aniline, aminopyridines) far more reactive than secondary amines (e.g. 2-(methylamino)pyridine). The scope of the reaction was widened considerably by the application of microwaves, which allowed reactions to be carried out at much higher temperature than the boiling point of the solvent (i.e. tetrahydrofuran, b.p. 67°C).

Key Words: carbon-11; amide; grignard reagent; microwave; [^{11}C]carbon dioxide

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Introduction

Positron emission tomography (PET) imaging of animal or human organs requires the development of new radiotracers labeled with positron-emitters, such as carbon-11 ($t_{1/2} = 20.4$ min) and fluorine-18 ($t_{1/2} = 109.8$ min). Several methods have been developed to introduce carbon-11 label into organic compounds from primary cyclotron-produced radioactive precursors, such as no-carrier-added (NCA) [^{11}C]carbon dioxide and [^{11}C]methane.[1–3] Most of these involve multiple steps. The fact that carbon-11 has a very short half-life besets radiochemists with the considerable challenge of developing procedures that are preferably single-step, rapid, isotope-efficient and easily automated to produce radiotracers in high radiochemical purity and high radiochemical yield.

Amides constitute a major functionality in a large proportion of radiopharmaceuticals.[4,5] Methods are known for introducing carbon-11 into the carbonyl position. Traditionally, NCA [*carbonyl*-^{11}C]amides are prepared by reacting cyclotron-produced [^{11}C]carbon dioxide with organometallic reagents, such as Grignard reagents, to obtain either the [^{11}C]carboxymagnesium halides or [^{11}C]carboxylic acid followed by their conversion into a more reactive species, such as a [^{11}C]acid chloride,[6–11] and reaction with the amine. Other strategies to activate [^{11}C]acids have also been reported.[12–17] Nevertheless, the final step or reaction with amine is not only time consuming, but also a part of the process that is not always reliable. A labeling procedure that uses direct reaction of amine with [^{11}C]carboxymagnesium halide or [^{11}C]carboxylic acid generated *in situ* would provide a considerable reduction in time and efforts and would therefore become highly attractive.

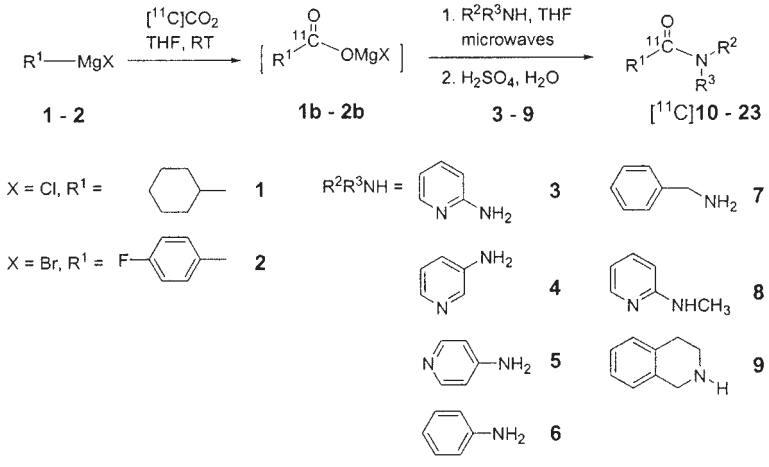
Lasne *et al.*[18,19] reported the synthesis of [^{11}C]aliphatic amides in moderate decay-corrected radiochemical yields (RCYs) by treatment of magnesium halide carboxylates with amines in tetrahydrofuran (THF) in the presence of 2.5 equivalents of alkylmagnesium halides. The RCYs were 15–60%, decay-corrected to the end of synthesis (EOS), and in most cases reactions were complete in 5 min. However, success was limited to the most reactive aliphatic Grignard reagents and amines. If this single-pot procedure is to be generally useful its scope must be expanded.

Microwave-enhanced radiochemistry[20–22] can provide a faster, cleaner, more selective and more highly atom-efficient approach than

conventional radiochemistry. The shorter reaction time coupled with other benefits, such as higher product purity due to less reaction mixture decomposition and the promotion of otherwise sluggish reactions, make it an ideal tool to be explored in radiochemistry with short-lived positron-emitters. Here we wish to report the synthesis of NCA [*carbonyl*-^{11}C]amides, both aliphatic and aromatic, by direct reaction of amines with [^{11}C]carboxymagnesium halides generated *in situ* from Grignard reagents and [^{11}C]carbon dioxide under microwave-enhanced conditions.

Results and discussion

The two Grignard reagents used in this study, namely cyclohexylmagnesium chloride (**1**) and 4-fluorophenylmagnesium bromide (**2**), were chosen not merely for their commercial availability, but more importantly because they each represent the aliphatic and aromatic moieties found in a large number of amide receptor ligands, especially the 5-HT$_{1A}$ receptor ligands, WAY-100635[23] and *p*-MPPF.[24] A range of commercially available primary and secondary aromatic amines of interest to our radioligand development program was selected for this study. However, the choice of Grignard reagent and amine need not be limited to those listed in Scheme 1.



Scheme 1. Synthesis of NCA [*carbonyl*-^{11}C]amides

Both Grignard reagents needed to be filtered through a polypropylene filter (0.45 μ) before use. Initial mixing of the Grignard reagent with THF in the standard Pyrex glass reaction vessel was carried out in an argon-protected glove box to minimize ingress of atmospheric carbon dioxide, a potential source of carrier. Reactions were performed under NCA conditions. Most of the amines have good solubility in THF, except 4-aminopyridine, which has moderate solubility. After the hydrolysis with sulfuric acid in THF, additional water was needed to dissolve the salts. The reaction mixture separated into aqueous and organic layers. The product [^{11}C]amide and unreacted [^{11}C]acid were retained in the organic layer whilst the salts were kept in the aqueous layer and easily removed. A representative chromatogram from the analysis of crude organic phase containing [^{11}C]**13**, showing absorbance (254 nm) and radioactivity detector responses is illustrated in Figure 1. The results for the synthesis of [*carbonyl*-^{11}C]amides are summarized in Table 1. Non-labeled compounds were also prepared and characterized using LC-MS or GC-MS. Reaction conditions and properties are summarized in Table 2. Authentic compounds were separated and used for co-injection with the radioactive samples.

Both Grignard reagents **1** and **2** react with NCA [^{11}C]carbon dioxide at room temperature (RT) in THF. In the absence of amines, the [^{11}C]carboxymagnesium halides proceed to react with excess Grignard reagent to form $R^1\,{}^{11}COR'$ and $R^1_3\,{}^{11}COH$ at elevated temperature (100°C). In the presence of amine, even non-reactive ones such as

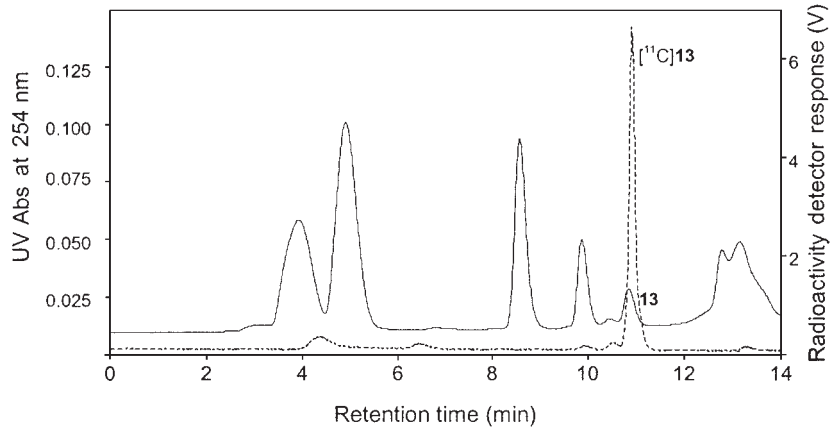


Figure 1. HPLC analysis of the crude organic phase containing [^{11}C]13, showing absorbance at 254 nm (—) and radioactivity detector (- - -) channel outputs

Table 1. NCA [*carbonyl*-^{11}C]amides from the direct coupling of NCA [^{11}C]carboxymagnesium halides with amines under microwave-enhanced conditions*

Entry	Grignard reagent	Amine	Reaction conditions			[^{11}C]amide	RCY(%)
			(°C)	(min)	(W)		
1	**1**	**3**	70	10	0	**10**	37
2	**1**	**3**	70	10	200	**10**	55
3	**1**	**3**	100	2	200	**10**	54
4	**1**	**4**	120	2	200	**11**	38
5	**1**	**5**	100	2	200	**12**	65
6	**1**	**6**	130	7	300	**13**	55
7	**1**	**7**	100	5	200	**14**	34
8	**1**	**8**	130	10	300	**15**	14
9	**1**	**9**	130	7	300	**16**	<5
10	**2**	**3**	100	10	0	**17**	22
11	**2**	**3**	100	10	200	**17**	45
12	**2**	**4**	120	2	200	**18**	14
13	**2**	**5**	120	3	200	**19**	32
14	**2**	**6**	130	7	300	**20**	22
15	**2**	**7**	100	5	200	**21**	11
16	**2**	**8**	130	10	300	**22**	0
17	**2**	**9**	130	10	300	**23**	0

*Grignard reagent, 0.2 mmol from 2 M solution in ether (100 µl) diluted in THF (400 µl); amine, 0.04 mmol in THF (100 µl); sulfuric acid [0.2 mmol in THF (200 µl)]; water (200 µl).

Table 2. Synthesis and identification of amides from direct coupling of carboxylic acids and amines in the presence of excess Grignard reagent*

Entry	Grignard reagent	Acid	Amine	Amide	Retention time in HPLC (min)	Mass by LC-MS or GC-MS#
1	**1**	**24**	**3**	**10**	9.4	205 $[M+1]^+$
2	**1**	**24**	**4**	**11**	6.7	205 $[M+1]^+$
3	**1**	**24**	**5**	**12**	4.9	205 $[M+1]^+$
4	**1**	**24**	**6**	**13**	10.8	203 $[M]^{+\#}$
5	**1**	**24**	**7**	**14**	9.8	217 $[M]^{+\#}$
6	**2**	**25**	**3**	**17**	8.6	217 $[M+1]^+$
7	**2**	**25**	**4**	**18**	6.1	217 $[M+1]^+$
8	**2**	**25**	**5**	**19**	4.7	217 $[M+1]^+$
9	**2**	**25**	**6**	**20**	10.4	215 $[M]^{+\#}$
10	**2**	**25**	**7**	**21**	9.6	229 $[M]^{+\#}$

*Grignard reagent, [1 mmol from 2 M solution in ether (500 µl) diluted in THF (500 µl)]; acid [0.33 mmol in THF (200 µl)]; amine [0.33 mmol in THF (200 µl)]; sulfuric acid [0.7 mmol in THF (700 µl)]; water (700 µl).

pyridine, the formation of ketone and alcohol from **1** can be inhibited, even at 120°C. It was noted that the intermediate, $C_6H_{11}{}^{11}COOMgCl$ (**1b**), from the reaction of cyclohexylmagnesium chloride with [^{11}C]

carbon dioxide, is much more reactive towards amine than the excess Grignard reagent. The reverse is true for reactions involving 4-fluorophenylmagnesium bromide; 4-F-$C_6H_4{}^{11}$COOMgBr (**2b**) is much more reactive towards excess Grignard reagent than amine which leads to the formation of ketone and tertiary alcohol. The addition of pyridine did not prevent the formation of [^{11}C]triarylmethanol in this case, even at 100°C. This may be a reflection of the relative C–Mg bond strength in these two Grignard reagents.[25,26]

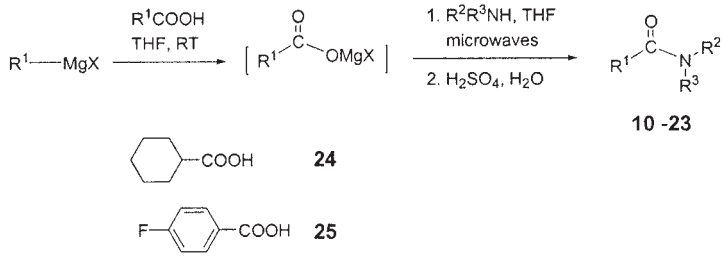
Cyclohexylmagnesium chloride was more reactive and gave much higher [^{11}C]amide RCYs than 4-fluorophenylmagnesium bromide (Table 1). Reactions starting with 4-flurophenylmagnesium bromide require higher temperature. For example, reaction of **1b** with 2-aminopyridine (Table 1, entry 2) took place at 70°C but no [^{11}C]amide was detected from the reaction of **2b** with the same amine at 70°C. When the temperature was elevated to 100°C, the corresponding amide was made in high RCY (Table 1, entry 11). However, further increase of reaction temperature does not necessarily increase the yields; instead more side reactions and decomposition take place.

Control of the reaction temperature is pivotal to the success of the reaction. Microwave technology provides a fast, precise way to control temperature. In this work a single mode microwave reactor equipped with temperature and pressure sensors was used. At an early stage, reactions using microwave reactor and heating block were compared. The use of microwaves improved the reaction significantly; for example RCYs increased 30–50% (e.g. from 37 to 55% and from 22 to 45%) for the two compared reactions (Table 1, entries 1 and 2, entries 10 and 11). It is also to our advantage that the microwave instrument enables the reaction to be carried out at much higher temperature than the boiling point of the solvent (here THF, b.p. 67°C). With crimp sealing of the septum no radioactivity was lost during the heating process even when the reaction mixture in THF was heated to 150°C and 80 p.s.i.

The carboxymagnesium halides are more reactive towards primary amines than secondary amines. There are insufficient data to elucidate the mechanism, but it appears that the reactivity of the amines is a function of both their electronic and steric properties. Only 14% RCY was achieved in the reaction of **1b** with 2-(methylamino)pyridine (Table 1, entry 8). Variation of temperature, reaction time and concentration did not improve the yield. The RCY for the reaction of **1b** with tetrahydroisoquinoline was even lower (Table 1, entry 9). Not

surprisingly, no amide was obtained from the aromatic Grignard reagent **2** with secondary amines.

Non-labeled compounds were prepared using the reactions outlined in Scheme 2. No optimization of yields was attempted. The compounds served as standards for radio-HPLC analysis and were characterized by LC-MS(ES) or GC-MS (EI) (Table 2).



Scheme 2. Synthesis of amides by direct coupling of carboxylic acids and amines in the presence of excess Grignard reagent

In conclusion, both aromatic and aliphatic NCA [^{11}C]amides were successfully synthesized by the direct coupling of NCA [^{11}C]carboxymagnesium halides, generated *in situ*, with primary amines. The procedure is less successful with secondary amines. The scope of the reaction has been widened considerably by the application of microwaves. Control of the reaction temperature at the right level is pivotal to the success of the reaction; without the use of a microwave reactor this would be difficult to achieve.

Experimental

Materials

Cyclohexylmagnesium chloride (2.0 M solution in diethyl ether packaged under nitrogen in Sure/Seal^{TM} bottle, Aldrich), 4-fluorophenylmagnesium bromide (2.0 M solution in diethyl ether packaged under nitrogen in Sure/Seal^{TM} bottle, Aldrich) were used as received. 2-Aminopyridine (99 + %, Aldrich), 3-aminopyridine (99 + %, Aldrich), 4-aminopyridine (99 + %, Aldrich), aniline (99%, Aldrich), benzylamine (99%, Aldrich), 2-(methylamino)pyridine (98%, Aldrich), 1,2,3,4-tetrahydroisoquinoline (98%, Aldrich) were used as received. THF

(99.9 + %, inhibitor-free, Aldrich), sulfuric acid (95–98%, Aldrich), phosphoric acid (85% in water, ACS grade, EM Science), ammonium formate (96%, J.T. Baker), acetonitrile (high purity solvent, Burdick & Jackson) were also used without further treatment.

General methods

LC-MS analysis was performed on a Thermo Finnigan Surveyor LC system equipped with a Finnigan Quest LC Q_{DECA} ESI probe. GC-MS analysis was performed on a Thermo Finnigan Trace GC equipped with Polaris Q system. Microwave irradiation was performed with a CEM Discover^{TM} microwave reactor. HPLC analysis was performed on a system comprising a Beckman Coulter Gold HPLC module [System Gold 126 solvent module coupled with a 166 UV absorbance detector (single wavelength)] plus a Bioscan Flow Count radioactivity detector (diode or PMT). Radioactivity was measured using a calibrated Biodex Medical Systems Atomlab^{TM} 300 dose calibrator.

NCA [^{11}C]Carbon dioxide was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on a cyclotron (PETtrace; GE) using a nitrogen gas target (1% oxygen, pressure 150 p.s.i.) bombarded for 5 min with a 5 μA beam of 16 MeV protons. NCA[^{11}C]carbon dioxide was first collected in a 5-loop stainless steel tube cryogenic trap, equipped with a cartridge of mixtures of chromium(III) oxide and copper(II) sulfate on silica gel to remove nitrogen oxides and another cartridge of phosphorous(V) oxide to remove moisture,[27] before delivery to the reaction vessel by helium at 5 ml/min. Generally, this process gives [^{11}C]carbon dioxide with a specific radioactivity in the range 40–80 GBq/μmol within 5 min of the end of irradiation.

Labeled compounds were identified by co-injection of the non-labeled standards and co-elution from the column (Phenomenex Luna C18, 10 μ, 100 Å, 250 × 4.6 mm i.d.) with acetonitrile-0.1 M ammonium formate solution containing 15.6 mM phosphoric acid, (pH = 3.5) as mobile phase at 2 ml/min.

General procedure for the synthesis of NCA [*carbonyl-*^{11}*C*]*amides*, [^{11}*C*] ***10–23***

Within an argon-protected glove box, Grignard reagent (RMgX, 2 M solution in ether; 100 μl, filtered through a 0.45 μ filter) and THF (400 μl) were added to a standard Pyrex glass microwave reaction vessel

(10 ml; CEM). The vessel was crimp-sealed with a PTFE-coated septum. [^{11}C]Carbon dioxide was bubbled into the vial at 5 ml/min at RT for 6 min *via* PEEK tubing (1/16 in) pierced through the septum. Amine (0.04 mmol) in THF (100 µl) was added to the reaction mixture using an epidermal needle also at RT. The mixture was then irradiated in the microwave reactor at the pre-set temperature and for a set time. At the end of microwave irradiation, stock sulfuric acid (1M in THF; 200 µl) and water (200 µl) were added successively through epidermal needles. The aqueous layer (~250 µl) was removed and the organic layer (~700 µl) containing the ^{11}C-labeled products analyzed by radio-HPLC with UV absorbance (254 nm) and radioactivity detection.

General procedure for the synthesis of unlabeled amides, ***10–23***

Within an argon-protected glove box, Grignard reagent (RMgX; 2 M solution in ether, 500 µl; filtered through 0.45 µ filter) and THF (500 µl) were added to a standard Pyrex glass microwave reaction vessel (10 ml; CEM). The vessel was crimp-sealed with a PTFE-coated septum. Acid (RCOOH; 0.3 mmol) in THF (200 µl) was added to the mixture at 0°C, followed 5 min later by amine (1.2 mmol) in THF (200 µl), again at 0°C. The mixture was then irradiated in the microwave reactor at a pre-set temperature and for a set time. At the end of microwave irradiation, stock sulfuric acid (1 M in THF; 700 µl), and water (700 µl) were added successively to destroy excess Grignard reagent and to dissolve the salt formed again using epidermal needles. The aqueous layer (0.6 ml) was removed and the organic layer (2.1 ml) containing the unlabeled products was analyzed by HPLC as the crude reaction mixture. The identity of compounds was confirmed by HPLC-MS or GC-MS analysis.

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