Direct fixation of [^{11}C]-CO_2 by amines: formation of [^{11}C-*carbonyl*]-methylcarbamates†

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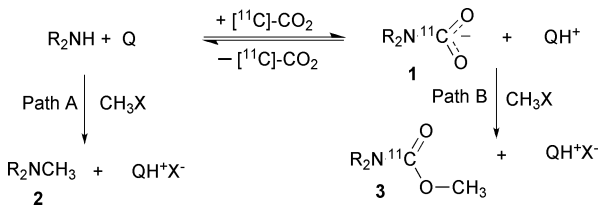
[^{11}C-*Carbonyl*]-methylcarbamates can be synthesised directly from [^{11}C]-CO_2 and primary or secondary amines in a one-pot, two-step reaction. The use of either diazabicyclo[5.4.0]undec-7-ene (DBU) or 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) enables efficient trapping of [^{11}C]-CO_2 in small volumes of DMF as [^{11}C]-carbamate salts. Subsequent reaction with a variety of methylating agents rapidly generates the desired [^{11}C-*carbonyl*]-methylcarbamates in high radiochemical yields. The usefulness of the method is illustrated by the efficient radiosynthesis of a kappa opioid receptor imaging radiotracer, useful in positron emission tomography (PET).

Introduction

Positron emission tomography (PET) is a powerful biomedical imaging technique, which continues to develop as a useful tool for cancer imaging, drug development and basic research.[1] PET relies upon the supply of radiotracers, labelled with positron emitting radionuclides; most commonly carbon-11 (half-life 20.4 min) or fluorine-18 (half-life of 109.7 min).[2] The short half-life of carbon-11 severely restricts the type and number of chemical steps that can be employed in the production of a target molecule.[3] While cyclotron-produced [^{11}C]-CO_2 is the starting material for the radiosynthesis of the vast majority of [^{11}C]-labelled radiotracers used for PET, it is commonly transformed into other, more versatile synthons such as [^{11}C]-iodomethane[4] or [^{11}C]-methyl triflate.[5] These are then reacted with more complex substrates (precursors) to generate the desired radiotracer.

Methods of direct reaction of [^{11}C]-CO_2 with amines have been limited to a few examples of mainly esoteric interest. Pre-formed *N*-silyl amines have been labelled with [^{11}C]-CO_2 and reduced to [^{11}C]-*N*-methylamines.[6] However, the conditions were complex, yields low, and products unisolated. Simple symmetrical [^{11}C]-labelled ureas have been reported by the low-temperature trapping of [^{11}C]-CO_2 with amines followed by treatment with $POCl_3$.[7] Unsymmetrical [^{11}C]-labelled ureas have been synthesised by the sequential reaction of [^{11}C]CO_2 with phosphinimines followed by treatment with amines, but only phenylureas were prepared by this method.[8] Recent interest in "green" chemistry has resulted in significant advances in the direct fixation of carbon dioxide into organic molecules.[9] Specifically, the synthesis of carbamates from amines, CO_2 and an alkylating agent has been the subject of much scrutiny, and reaction conditions have been developed which allow efficient CO_2 fixation.[10] Catalysts studied for this particular transformation include alkali carbonates,[11] zeolites[12] and guanidines.[13] 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) in particular has been the subject of much study in this regard,[14,15] and while its mechanism of action is still debatable,[16] this amidine seems particularly efficient as a CO_2 fixation and transfer agent. Indeed, during this work, an elegant study showed the utility of DBU as an agent to prepare [^{11}C]-benzylcarbamates directly from [^{11}C]-CO_2 and amines.[17]

We report here the use of DBU and an even more efficient fixation agent, 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP), to directly trap [^{11}C]-CO_2 at ambient temperature and facilitate (a) the fixation of [^{11}C]-CO_2 into solution from a dilute N_2 gas stream as carbamic acid salts **1** and (b) the rapid and efficient synthesis of [^{11}C]-methylcarbamates **3** from a variety of primary and secondary amines (Scheme 1). The practical application of this [^{11}C-*carbonyl*]-carboxymethylation radiolabelling method to complex functionalised molecules is demonstrated by the efficient radiosynthesis of [^{11}C-*carbonyl*]-GR103545, a positron-emitting radiopharmaceutical developed for the PET imaging of kappa opioid neuroreceptors.[18]



Scheme 1 Q = fixation agent, either DBU or BEMP.

Results and discussion

Efficiency of [^{11}C]-CO_2 trapping

The abilities of DBU and BEMP to trap [^{11}C]-CO_2 in solution were determined by measuring the equilibrium distribution of [^{11}C]-CO_2 between the gas and liquid phase of solutions of fixation agent in DMF at various concentrations in a sealed container.[19] In pure DMF, the partition ratio [^{11}C]-CO_2 liquid–gas (PR) was 7.5, in

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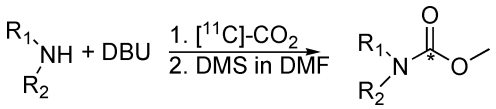
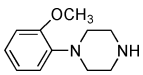
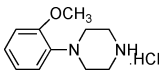
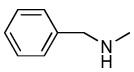
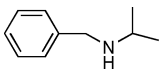
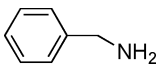
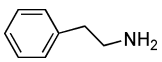
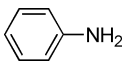
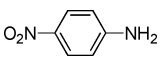
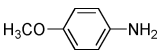
† Electronic supplementary information (ESI) available: Full experimental details on the radiosynthesis of [^{11}C-*carbonyl*]-GR103545, [^{11}C]-CO_2 gas–liquid partition data, and ^{1}H and ^{13}C NMR spectra of 4-(2-methoxyphenyl)-1-piperazinecarboxylic acid methyl ester. See DOI: 10.1039/b916419g

Table 1 Synthesis of [^{11}C-*carbonyl*]-methylcarbamates from [^{11}C]-CO_2 and primary or secondary amines using DBU[a]



Entry	Amine substrate	Radiochemical yield (%)[b]
1		65–75 ($n = 6$)
2		80, 79
3		90, 95
4		76, 69
5		96, 91
6		85–90 ($n = 3$)
7		11, 23, 32, 40, 28
8		3, 8, 20[c]
9		22, 40, 55[c]

[a] Standard conditions: amine (4.16 μmols) + DBU (42.4 μmols) in DMF (80 μL) treated with [^{11}C]-CO_2 in N_2 (10 mL min^{-1}). Reaction at r.t. for 1 min then treated with solution of DMS (52.8 μmols) in DMF (400 μL). Reaction quenched with aq. NH_3 after 10 s. [b] See experimental for definition. [c] 8.32 μmols of amine used.

good agreement with literature values.[20] PRs increased in a linear manner with increasing concentrations for both fixation agents, but the effect was much more dramatic for BEMP than DBU, *e.g.* at 100 mM, the PR for DBU in DMF was 25, while for BEMP in DMF it was over 250. Thus, while DBU is effective at trapping CO_2 in solution as previously reported, BEMP is even more so.

[^{11}C]-Carboxymethylation of model amines

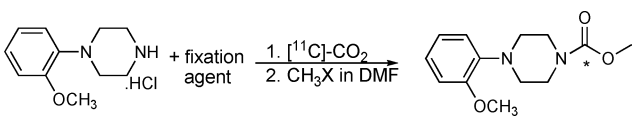
Initial experiments were carried out using model primary and secondary aliphatic and aromatic amine substrates (Table 1). Small volume (80 μL) solutions of amine in DMF containing DBU were used to trap cyclotron-produced [^{11}C]-CO_2 from a stream of N_2 as such a scale is practical for the synthesis of PET radiotracers for imaging studies.

Trapping of radioactivity was essentially quantitative when the [^{11}C]-CO_2 was bubbled through the amine solution at room temperature at 10 mL min^{-1}. Increasing the flow to 70 mL min^{-1} resulted in significant breakthrough (60%) of [^{11}C]-CO_2. A solution of dimethylsulfate (DMS, 10 μL) in DMF (400 μL) was then added and the reaction monitored by radio-HPLC. Control experiments showed that (a) radiochemical yields were not increased by increasing the reaction time of [^{11}C]-CO_2 with amine past 1 min and (b) yields were not improved by increasing the reaction time of the methylating agent to more than 10 s. That both reaction steps are rapid bodes well for radiosyntheses with the short-lived isotope carbon-11.

Radiochemical yields of [^{11}C]-methylcarbamates were good to excellent for both primary and secondary aliphatic amines (Table 1, entries 1–6), even for the sterically hindered *N*-benzyl-*N*-isopropylamine (Table 1, entry 4). However, aromatic amines were more problematic. Aniline gave variable results with yields varying between 11–40% (Table 1, entry 7), while the even less nucleophilic 4-nitroaniline gave only 3–8% radiochemical yield (Table 1, entry 8, first 2 runs). Yields from aromatic amines could be improved somewhat by doubling the amine concentration (Table 1, entries 8 and 9, last runs). Using the hydrochloride salts of amines as opposed to the free base had little effect on the reaction (compare Table 1, entries 1 and 2).

With 2-methoxyphenylpiperazine hydrochloride (MPP) as a model amine, the effects of amine concentration, fixation agent, and methylating agent on radiochemical yields were examined (Table 2). It became immediately apparent that BEMP was superior to DBU in this reaction (compare Table 2, entries 1 *vs.* 2 and 3 *vs.* 4). Reducing the concentration of MPP had only a modest effect on radiochemical yields; even at a concentration of only 0.5 mg mL^{-1}, radiochemcial yields were still moderate (Table 2, entry 12). There was a more complex interplay between the stoichiometry of the amine and the alkylating agent, which suggests that at lower amine concentrations, too much methylating agent is deleterious to the reaction (compare Table 2, entries 10 and 11). This, perhaps,

Table 2 A study of amine concentration, fixation agent, and methylating agent[a]



Entry	[MPP]/mg mL^{-1}	Fixation agent/μL	Methylating agent/μL	Radiochemical yield (%)[b]
1	10	DBU (6)	DMS (5)	79
2	10	BEMP (6)	DMS (5)	93
3	10	DBU (6)	DMS (1)	39
4	10	BEMP (6)	DMS (1)	75
5	10	DBU (1)	DMS (5)	10
6	10	BEMP (1)	DMS (5)	48
7	5	BEMP (6)	DMS (5)	87
8	5	BEMP (3)	DMS (5)	94
9	5	BEMP (6)	DMS (1)	96
10	1	BEMP (6)	DMS (5)	60
11	1	BEMP (1)	DMS (1)	90
12	0.5	BEMP (6)	DMS (2)	45
13	0.5	BEMP (6)	MeI (2)	79
14	0.5	BEMP (6)	MeOTs (2)	78
15	0.5	BEMP (6)	TMOF (2)	0.7
16	0.5	BEMP (6)	DMS (2)	33[c]

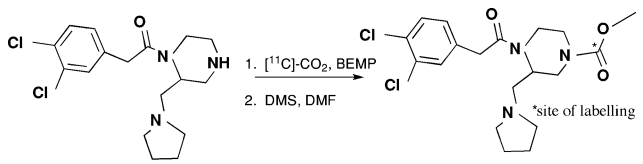
[a] A solution of MPP hydrochloride + fixation agent in DMF (80 μL) treated with [^{11}C]-CO_2 in N_2 (10 mL min^{-1}). Reaction at r.t. for 1 min then treated with solution of alkylating agent in DMF (400 μL). Reaction quenched with aq. NH_3 after 10 s. [b] See experimental for definition - average of two runs. [c] 2 μL of water added to amine solution.

is due to competing methylation of amine (Scheme 1, Path A) at low amine concentrations.

Both methyl tosylate (MeOTs) and iodomethane (MeI) were efficacious as methylating agents (Table 2, entries 13 and 14). However, solutions of the former had to be freshly prepared, while the latter might not be useful in the radiosynthesis of high specific activity radiopharmaceuticals.[21] Trimethylorthoformate (TMOF) was ineffective as a methylation agent, reflecting its much lower methylating ability (Table 2, entry 15). Anhydrous reagents were used throughout here but the reaction proved quite tolerable to the presence of water as addition of 2 μL (1.4 M) only reduced radiochemical yields moderately (compare Table 2, entries 12 and 16).

Application of the method to the radiosynthesis of a PET radiopharmaceutical

GR103545, a potent and selective agonist for the kappa opiod receptor,[22] is currently being used as PET imaging agent in non-human primates.[23] It has previously been labelled with carbon-11 by a multi-step radiosynthesis involving the production and coupling of [^{11}C]-methylchloroformate to its norcarboxymethyl amine precursor[18] or by reacting [^{11}C]MeI with CO_2-saturated solutions of said amine.[24] Our [^{11}C]-CO_2 fixation approach was applied to this radiosynthesis (Scheme 2) using a "loop" method,[25] whereby the [^{11}C]-CO_2 is trapped in a loop of narrow-bore steel tubing pre-coated with a solution the norcarboxymethylamine precursor and BEMP in DMF. This technique provides a higher surface area for interaction of [^{11}C]-CO_2 with the precursor solution, promotes easy automation of the process, and enables us to use only 0.1 mg of the norcarbomethoxy precursor while producing high specific activity product in practical yields.



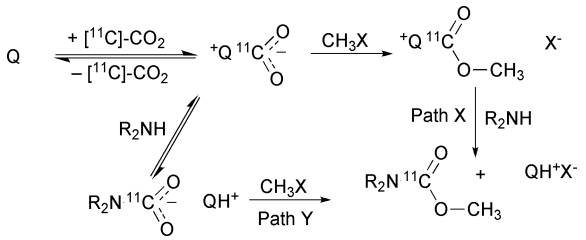
norcarbomethoxy GR103545 [^{11}C-*carbonyl*]-GR103545

Scheme 2

Cyclotron-produced [^{11}C]-CO_2 (24 GBq) was converted into purified, formulated, sterile and pyrogen-free [^{11}C-*carbonyl*]-GR103545 (2.6–3.8 GBq) in only 23 min with radiochemical purities >98% and specific activities of 108–162 GBq μmol^{-1} (all values at end-of-synthesis).[26]

Mechanism

The role of the CO_2-fixation agent, DBU or BEMP, in the reaction is still open to speculation. It is obvious that fixing the CO_2 in solution is essential but other aspects, such as providing a highly polarisable soft counter-ion,[13,15–17] could also play a significant part in their effectiveness. The observation that the phosphazene, BEMP, is superior to the amidine, DBU, does not allow a distinction between the two plausible reaction pathways outlined in Scheme 3. However, if Path X is significant then it ought to be possible to reverse the order of addition of reagents *i.e.* trap [^{11}C]-CO_2 in a solution of fixation agent containing CH_3X, followed



Scheme 3 Q = fixating agent, either DBU or BEMP.

by addition of amine. Preliminary experiments of this type have produced very low radiochemical yields (data not shown).

The reactivity of the carbonic acid salt to the methylating agent is exceedingly high. This is apparent, not only from the very short reaction time required, but also from the successful radiosynthesis of [^{11}C]-GR103545. This molecule contains a tertiary amine, yet carboxymethylation competes well with methylation at this site.

Experimental

A Scanditronix MC 17 cyclotron was used for radionuclide production. [^{11}C]-CO_2, produced by the ^{14}N(p,α)^{11}C nuclear reaction, was concentrated from the gas target in a stainless steel coil cooled to −178 °C. Upon warming, the [^{11}C]-CO_2 in a stream of N_2 gas was passed through a NO_x trapping column[27] and a drying column of P_2O_5 prior to use. Purifications and analyses of radioactive mixtures were performed by high performance liquid chromatography (HPLC) with an in-line UV (254 nm) detector in series with a NaI crystal radioactivity detector (purifications) or a Bioscan Flowcount coincidence radioactivity detector (analyses). Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-712M). Proton and carbon-13 NMR spectra were recorded at 25 °C on a Varian Mercury 400 MHz spectrometer. Chemicals were obtained from Aldrich, Tocris, or Fisher. Dimethylformamide (DMF) was distilled from barium oxide. DBU, BEMP, and DMF were stored over 4 Å molecular sieves prior to use. Automated radiosyntheses were controlled by Labview™ software. Unless stated otherwise, all radioactivity measurements were normalised for radioactive decay.

Synthesis of reference methylcarbamates

A solution of amine (see Table 1, 0.5 g) and triethylamine (1 mL) in EtOAc (10 mL) was treated with methylchloroformate (0.5 mL) and the mixture stirred until reaction was complete by HPLC analysis (5 min to 16 h). Saturated aq. K_2CO_3 was added (10 mL) and the mixture stirred for 15 min. The aqueous layer was extracted with EtOAc, and the combined organic fractions washed with water, aq. HCl (1 N) and brine, then dried (Na_2SO_4) and filtered through a short plug of silica. Evaporation gave the desired carbamates. With the exception of 4-(2-methoxyphenyl)-1-piperazinecarboxylic acid methyl ester, all carbamates are known compounds and had ^{1}H NMR spectra in accord with their structure.

4-(2-Methoxyphenyl)-1-piperazinecarboxylic acid, methyl ester. Yield 67%. White crystals from diisopropyl ether; mp 66–69 °C.

[^{11}C]-CO_2 partition experiments

5 mL (nominal volume) conical vials were used. Each vial volume was measured (by filling with water and weighing) and exactly half-filled with solutions of carbon dioxide fixation agent (DBU or BEMP) in DMF (*ca.* 2.5 mL). [^{11}C]-CO_2, was bubbled into the vials (30–300 MBq), which were then septum-sealed and left to stand at ambient temperature for 10 min. Control experiments showed that equilibrium had been reached by this time. For each vial, the total radioactivity was measured and 1 mL of the gas phase above the solution removed with a gas-tight syringe, then measured for radioactivity. The partition ratios (PR) of [^{11}C]-CO_2 between the liquid and gas phases were calculated as:

PR = [Activity in vial – (F × Activity in syringe)]/[F × Activity in syringe]; where F = volume of gas phase (in mL).

[^{11}C-carbonyl]-carboxymethylation reactions

[^{11}C]-CO_2 was bubbled (using a 2" × 21G needle at 10 mL min^{-1}) into a solution of test amine and carbon dioxide fixation agent (DBU or BEMP) in DMF (80 μL) in a septum-sealed 1 mL conical vial. The vial was vented directly to a 3 mL syringe barrel filled with silica-coated NaOH (Ascarite™) to trap any escaping [^{11}C]-CO_2. Trapping of [^{11}C]-CO_2 was >95% in all cases. After 1 min, a solution of methylating agent in DMF (400 μL) was added followed, after 10 s, by aq. ammonia (0.01 N, 0.5 mL). Radioactivity in the vial and NaOH trap were measured and aliquots of the reaction mixture analysed by HPLC and compared to standards of methylcarbamates to determine conversion yields to [^{11}C]-methylcarbamates. Radiochemical yields were calculated as:

[Activity in vial/(Activity in vial + Activity in NaOH trap)] × HPLC conversion yield.

Radiosynthesis of [^{11}C-*carbonyl*]-GR103545

Complete experimental details are given in the ESI section.† Briefly, a Valco™ 6-port, 2-position valve equipped with 1 mL stainless steel sample loop and a 0.4 mL PTFE sample loop was used. Before release of [^{11}C]-CO_2, the PTFE sample loop was charged with a solution of degassed DMS (4 μL) in DMF. The 1 mL steel loop was charged with a solution of norcarbomethoxy GR103545 (0.1 mg) and carbon dioxide fixation agent (DBU or BEMP, 5 μL) in DMF (40 μL). [^{11}C]-$CO_2$was then swept through the steel loop and when activity in the loop had peaked the contents of the steel loop washed into a holding vial by the alkylating solution using N_2 pressure. The reaction was quenched and purified by semi-prep HPLC.

Conclusions

Sequential trapping of [^{11}C]-CO_2 in amine solutions followed by reaction with methylating electrophiles provides [^{11}C]-*carbonyl*] methylcarbamates rapidly and in high radiochemical yields. Biologically interesting molecules which contain carbamate groups are relatively rare (nevertheless, for recent examples see ref. 28) but it can be anticipated that their numbers will grow as libraries of carbamates are easily accessible *via* combinatorial techniques. The method described herein should be useful to expand the type and number of radiotracers available for PET imaging and other radiotracer applications.

Acknowledgements

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