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Research Article

Reductive *N*-alkylation of secondary amines with [2-^{11}C]acetone

Margaretha van der Meij[1,*], Nicholas I. Carruthers[2], Jacobus D.M. Herscheid[1], Jill A. Jablonowski[2], Josee E. Leysen[3,4] and Albert D. Windhorst[1]

[1] *Radionuclide Center, Vrije Universiteit, De Boelelaan 1085c, 1081 HV Amsterdam, The Netherlands*
[2] *Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121, USA*
[3] *Johnson & Johnson Pharmaceuticals, Turnhoutseweg 30, 2340, Beerse, Belgium*
[4] *Onderzoekinstituut Neurowetenschappen/Research Institute Neurosciences, Vrije Universiteit, c/o Dept. of Anatomy, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands*

Summary

The development of a labeling method for secondary amines with [2-^{11}C]acetone is described since the R_2N-isopropyl moiety is present in many biologically active compounds. The influence of a variety of parameters (e.g. reagents, solvents, temperature, and time) on the reaction outcome is discussed. Under the optimal reaction conditions, [^{11}C]1-isopropyl-4-phenylpiperazine ([^{11}C]iPPP) was synthesized from [2-^{11}C]acetone and 1-phenylpiperazine in a decay-corrected radiochemical yield of 72%. The overall synthesis time, from EOB to HPLC analysis of [^{11}C]iPPP, was 20 min. Specific activity was 142–208 GBq/μmol at the end of synthesis. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: [2-^{11}C]acetone; reductive alkylation; reductive amination; secondary amines; [2-^{11}C]*tert*-butanol

*Correspondence to: M. van der Meij, Radionuclidencentrum, Vrije Universiteit, De Boelelaan 1085c, 1081 HV Amsterdam, The Netherlands. E-mail: mmeij@rnc.vu.nl

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Introduction

The reactions of ammonia, primary amines, or secondary amines with aldehydes or ketones in the presence of reducing agents to give primary, secondary or tertiary amines, respectively, known as reductive alkylations (of the amines) or reductive aminations (of the carbonyl compounds) are among the most useful and important tools for the synthesis of amines.[1–3] Reductive alkylation reactions with ^{11}C-labeled carbonyl compounds such as formaldehyde,[4] acetaldehyde[5,6] and acetone[7] have been applied for the synthesis of potential positron emission tomography (PET) tracers and ligands.[8–15] In contrast to the [^{11}C]aldehydes, reductive alkylation reactions using [2-^{11}C]acetone are limited to primary aliphatic amines (Figure 1),[7–15] which generally react faster than primary aromatic and secondary aliphatic amines.[1] Nevertheless, it would be useful to develop a labeling method for secondary amines with [2-^{11}C]acetone since the R_2N-isopropyl moiety is present in many biologically active compounds (Figure 2).[16–18]

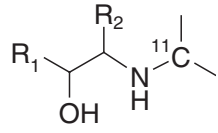


Figure 1. General molecular structure of *N*-[^{11}C]isopropyl labeled β-blockers prepared via reductive alkylation of the primary amine precursors

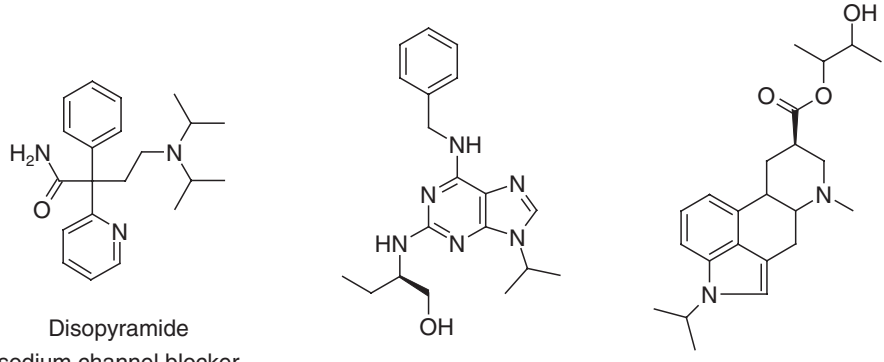
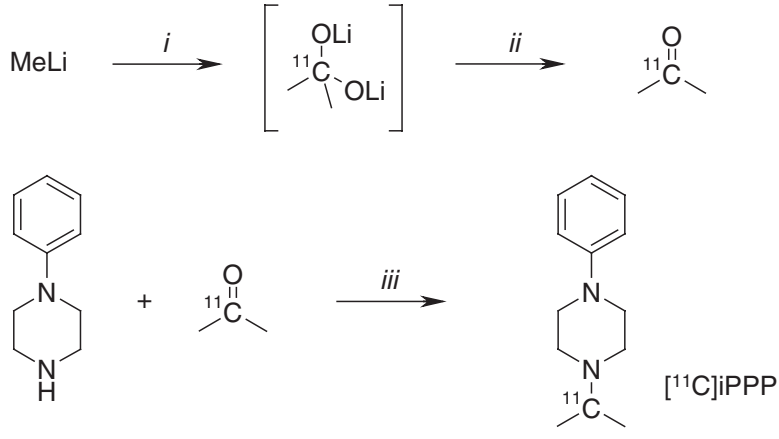


Figure 2. *N*-Isopropyl substituted biologically active compounds

This paper describes the fully automated synthesis of [2-^{11}C]acetone and the following reductive alkylation of 1-phenylpiperazine as an example for the reductive alkylation of secondary amines (Scheme 1). In the literature the $(CH_3)_2{}^{11}C(OLi)_2$ salt, prepared by trapping of [^{11}C]CO_2 in a solution of methyllithium, is usually converted into [2-^{11}C]acetone with water.[9,11–14,19] Subsequently, the [2-^{11}C]acetone is distilled into the precursor solution via a calcium chloride column. In order to eliminate this drying procedure we examined the application of non-aqueous quenching agents. In addition, the influence of various parameters including solvent and temperature on the reaction outcome is discussed.



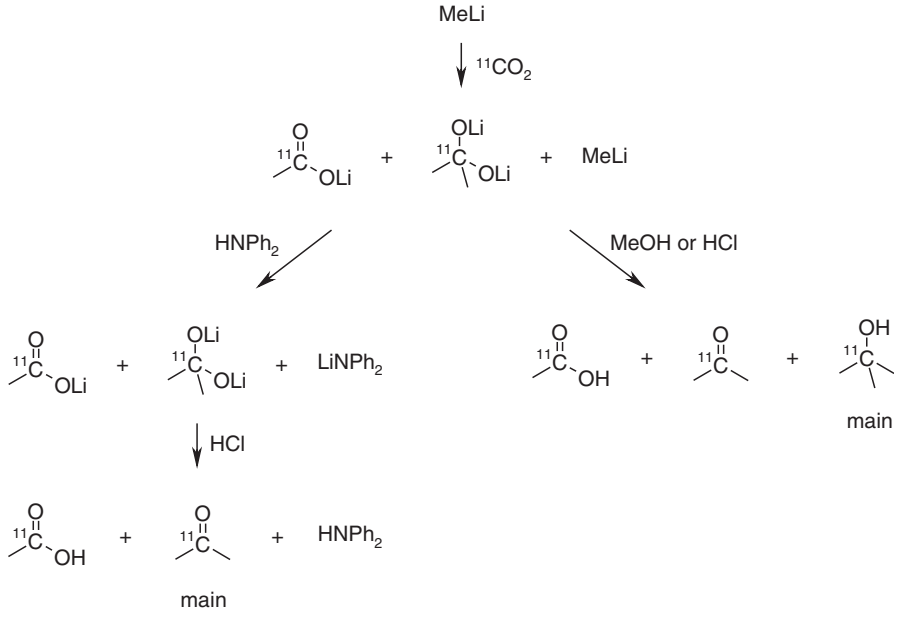
Scheme 1. Reductive alkylation of 1-phenylpiperazine with [2-^{11}C]acetone. The optimal reaction conditions are: (i) [^{11}C]CO_2, Et_2O; (ii) 1. 2 M $HNPh_2$, THF, 2. 1 M HCl, Et_2O; (iii) $NaB(O_2CCH_3)_3H$, DCE, 120–140°C, 10 min

Results and discussion

In the literature, a one-pot procedure for the reductive alkylation of primary amines with [2-^{11}C]acetone has been described.[7,8,10,15] In this procedure, a solution of primary amine in methanol and acetic acid is added to the (unquenched) MeLi solution. Unfortunately, we were not able to prepare [^{11}C]1-isopropyl-4-phenyl-piperazine ([^{11}C]iPPP) via this route. Therefore, we turned our attention to the common two-pot reductive alkylation as described below.

[2-^{11}C]Acetone synthesis

One problem in the synthesis of ketones from carboxylates using organolithium reagents is the undesired formation of tertiary alcohols (Scheme 2). The latter is caused by a kinetically controlled reaction of unhydrolyzed organolithium reagent with the newly formed ketone



Scheme 2. Preparation of [2-^{11}C]acetone vs [2-^{11}C]*tert*-butanol under non-aqueous quenching conditions

during the hydrolysis step. Therefore, before distillation, an initial quenching agent (e.g. alcohol, amine, ketone) is used to destroy excess MeLi, leaving the lithium diolate complex intact. A second reagent, usually water, then liberates acetone.[19] In these syntheses, the [2-^{11}C]acetone is distilled via a calcium chloride column as water inhibits the reductive alkylation reaction. However, in our hands, a high percentage of [2-^{11}C]acetone was trapped on the calcium chloride column. Therefore, to avoid distillation via a calcium chloride column we examined the application of non-aqueous quenching agents. The reaction conditions and the decay-corrected radiochemical yields of [2-^{11}C]*tert*-butanol and [2-^{11}C]acetone are listed in Table 1. The reaction routes including intermediate products are depicted in Scheme 2.

Table 1. Preparation of [2-^{11}C]acetone *vs.* [2-^{11}C]*tert*-butanol under non-aqueous quenching conditions

Entry	Quenching 1.	Quenching 2.	[2-^{11}C]acetone (%)[a]	[2-^{11}C]*tert*-butanol (%)[a]
1	MeOH[b]		18 ± 3	21 ± 3
2	AcOH in THF[c]		0	0
3	HCl in 1,4-dioxane-THF[d]		2 ± 2	67 ± 3
4	HCl in ether[d]		10 ± 2	81 ± 4
5	$HNPh_2$ in THF[c]	HCl in 1,4-dioxane-THF[d]	39 ± 3	0
6	$HNPh_2$ in THF[c]	HCl in ether[d]	80 ± 5	0
7	$HNPh_2$ in THF[c]	HCl in ether,[d] dist. via $NaHCO_3$ column	51 ± 6	0
8	$HNPh_2$ in THF[c]	HCl in ether,[d] dist. via NaOH column	8 ± 4	0

[a] Decay-corrected yield of the products after distillation calculated from the amount of [^{11}C]CO_2. Entries 1–4 and 8, $n = 3$. Entries 5–7, $n = 10$.
[b] 200 µl.
[c] 2 M solution; 200 µl.
[d] 1 M solution; 400 µl.

First, the reaction mixture was quenched with methanol since methanol is one of the solvents used for the reductive alkylation reaction (Table 1, entry 1). The decay-corrected radiochemical yields of [2-^{11}C]acetone and [2-^{11}C]*tert*-butanol in the distillate (second vial) were 18 and 21%, respectively. About 60% of the initial amount of radioactivity resided in the initial reaction vial. After quenching the reaction with acetic acid and subsequent distillation, the second vial contained no radioactive products at all (Table 1, entry 2). The addition of a 1 M solution of hydrogen chloride in 1,4-dioxane-THF (1:3) or diethyl ether yielded mainly [2-^{11}C]*tert*-butanol (67 and 81%, respectively; Table 1, entries 3 and 4). Next, excess methyllithium was selectively quenched with diphenylamine in tetrahydrofuran (THF, 2 M solution).[19] Subsequently, hydrogen chloride in 1,4-dioxane-THF (1:3, 1 M) was added to liberate [2-^{11}C]acetone, which was obtained in 39% decay-corrected radiochemical yield after distillation (Table 1, entry 5). The use of hydrogen chloride in diethyl ether instead of 1,4-dioxane-THF led to a major increase in the decay-corrected radiochemical [2-^{11}C]acetone yield from 39 to 80% (Table 1, compare entries 5 and 6). In order to prevent excess hydrogen chloride accumulating in the second reaction vial, the volatile products were

passed through a column containing sodium hydrogencarbonate (Table 1, entry 7) or sodium hydroxide (Table 1, entry 8). Unfortunately, a large amount of the [2-^{11}C]acetone was trapped on the column, resulting in a large decrease in the amount of [2-^{11}C]acetone in the second reaction vial.

Consequently, the reductive alkylation reactions described in the following paragraph were performed with [2-^{11}C]acetone prepared according to entry 6 (Table 1) which gave the highest decay-corrected radiochemical yield following distillation (80%).

Reductive alkylation of 1-phenylpiperazine with [2-^{11}C]acetone

The influence of reducing agent, solvent, temperature, duration and pH on the reductive alkylation reaction of 1-phenylpiperazine with [2-^{11}C]Acetone was investigated. [2-^{11}C]Acetone, synthesized as described in entry 6 of Table 1, was therefore distilled into a solution containing 1-phenylpiperazine, a reducing agent and in some cases acetic acid (Scheme 1). The results are summarized in Tables 2 and 3.

Sodium cyanoborohydride ($NaCNBH_3$) and sodium triacetoxyborohydride ($NaB(O_2CCH_3)_3H$), the most general and frequently used reagents,[1–3] are employed for comparison. The successful use of sodium cyanoborohydride is due to its stability in acidic solutions (down to pH $\sim$ 3).[20] Furthermore, reactions with sodium cyanoborohydride may be performed in any protic solvent including methanol.[2] The use of sodium triacetoxyborohydride rather than sodium cyanoborohydride eliminates the risk of residual cyanide in the final product.

Table 2. Reductive alkylation of 1-phenylpiperazine using [2-^{11}C]acetone and $NaCNBH_3$

Entry	$NaCNBH_3$ (μmol)	AcOH (μmol)	Time (min)	*T* (°C)	[^{11}C]iPPP (%)[a]
1	16	35	5	120	25 ± 4
2	35	87	5	120	21 ± 3
3	35	0	5	120	4 ± 2
4	35	87	5	140	23 ± 3
5	16	35	10	120	41 ± 4 (35)
6	35	87	10	120	33 ± 2 (28)
7	35	0	10	120	5 ± 3 (4)
8	35	87	10	140	29 ± 3 (24)

[a] Decay-corrected yield calculated from the amount of [2-^{11}C]acetone ($n \geqslant 4$). For comparison of entries 1–4 with 5–8, the yields between brackets are corrected for 5 min extra reaction time (decay). Reactions were performed in methanol.

Table 3. Reductive alkylation of 1-phenylpiperazine using [2-^{11}C]acetone and $NaB(O_2CCH_3)_3H$

Entry	AcOH (μmol)	Solvent	Time (min)	*T* (°C)	[^{11}C]iPPP (%)[a]
1	0	THF	5	100	37 ± 4
2	0	THF	5	120	53 ± 6
3	0	THF	5	140	32 ± 4
4	0	THF	10	100	44 ± 6 (37)
5	0	THF	10	120	58 ± 5 (49)
6	0	THF	10	140	35 ± 4 (30)
7	35	THF	10	120	48 ± 5 (40)
8	0	DCE	5	100	39 ± 6
9	0	DCE	5	120	57 ± 4
10	0	DCE	5	140	61 ± 4
11	0	DCE	5	160	49 ± 5
12	0	DCE	10	100	56 ± 4 (47)
13	0	DCE	10	120	72 ± 6 (61)
14	0	DCE	10	140	71 ± 5 (60)
15	0	DCE	10	160	49 ± 7 (41)
16	35	DCE	10	120	60 ± 6 (51)

[a] Decay-corrected yield calculated from the amount of [2-^{11}C]acetone ($n \geqslant 4$). For comparison of the individual entries, the yields between brackets are corrected for 5 min extra reaction time (decay).

The data shown in Table 2 was obtained using sodium cyanoborohydride ($NaCNBH_3$) as reducing agent. In the first entry of Table 2 the reaction mixture containing sodium cyanoborohydride, acetic acid, 1-phenylpiperazine and [2-^{11}C]acetone in methanol was heated to 120°C for 5 min. Under these conditions, 25% of the [2-^{11}C]acetone was converted into [^{11}C]1-isopropyl-4-phenylpiperazine ([^{11}C]iPPP). A two-fold increase in the amount of sodium cyanoborohydride and acetic acid did not alter the decay-corrected radiochemical reductive alkylation yield significantly (Table 2, compare entries 1 and 2). However, in the absence of acetic acid only 4% of [^{11}C]iPPP was formed (Table 2, entry 3). Raising the temperature to 140°C did not affect the decay-corrected radiochemical [^{11}C]iPPP yield (Table 2, compare entries 2 and 4).

Next, the reaction conditions from entries 1–4 were applied whilst changing the reaction time to 10 min (Table 2, entries 5–8). At a temperature of 120°C in the presence of acetic acid, the longer reaction time had a beneficial effect on the radiochemical reductive alkylation yields (Table 2, entry 1 vs 5 and 2 vs 6). The highest decay-corrected radiochemical yield obtained was 41% (Table 2, entry 5). In conclusion, the optimal reaction conditions using sodium cyanoborohydride as a

reducing agent are 10 min heating of the reaction mixture at 120°C in the presence of acetic acid.

Sodium triacetoxyborohydride ($NaB(O_2CCH_3)_3H$) was used for the reductive alkylation reactions described in Table 3. Comparison of entries 1–3 (Table 3) shows that the optimal temperature for the reductive alkylation with sodium triacetoxyborohydride in THF was 120°C. Changing the reaction time from 5 to 10 min had no significant consequences for the optimal temperatures (Table 3, compare entries 1–3 with 4–6) and the radiochemical [^{11}C]iPPP yield. Comparison of entries 5 and 7 reveals that the addition of acetic acid to the reaction mixture was unfavourable. The use of 1,2-dichloroethane (DCE) as solvent instead of THF positively influenced the decay-corrected radiochemical [^{11}C]iPPP yield (Table 3, 1–3 vs 8–10 and 4–6 vs 12–14, respectively). The ideal temperature range for the formation of [^{11}C]iPPP in DCE was 120–140°C (Table 3, compare entries 8–11 and 12–15). This temperature range was optimal for reactions at both 5 and 10 min duration. No significant differences in the radiochemical yields were found going from 5 to 10 min reaction time taking into account the 5 min extra decay (Table 3, entries 9 vs 13 and 10 vs 14). The presence of acetic acid in the reaction mixture was found to be detrimental to the reaction (Table 3, entry 13 vs 16). Finally, the highest decay-corrected radiochemical [^{11}C]iPPP yield obtained was 72% using sodium triacetoxyborohydride as reducing agent in DCE at 120–140°C for 10 min (Table 3, entry 13, 14). The overall synthesis time, from EOB to HPLC analysis of [^{11}C]iPPP, was 20 min. Specific activity was 142–208 GBq/μmol at the end of synthesis.

Conclusions

We have shown that liberation of the [2-^{11}C]acetone can be effected using non-aqueous hydrogen chloride. Consequently, distillation of [2-^{11}C]acetone via a calcium chloride column is no longer necessary. The radiochemical decay-corrected [2-^{11}C]acetone yield after quenching excess methyllithium with diphenylamine and subsequent release of [2-^{11}C]acetone using hydrogen chloride in diethyl ether is 80% following distillation.

Furthermore, we have developed a general synthetic procedure for the reductive alkylation of secondary amines. The method is reliable, easy to automate and gives high radiochemical yields. Reaction

of 1-phenylpiperazine with [2-^{11}C]acetone afforded [^{11}C]1-isopropyl-4-phenylpiperazine in 72% radiochemical yield (decay-corrected). The specific activity was 142–208 GBq/μmol at the end of synthesis and the overall synthesis time, from EOB to HPLC analysis of [^{11}C]iPPP, was 20 min.

Experimental

Material and methods

All chemicals were obtained from Sigma-Aldrich Chemie BV (Zwijndrecht, The Netherlands) and used as received unless otherwise stated. 1-Isopropyl-4-phenylpiperazine[21] was used as a reference compound for chromatography. THF was freshly distilled from $LiAlH_4$ before use. [^{11}C]Carbon dioxide was produced by ^{14}N(p, α) ^{11}C nuclear reaction using an IBA Cyclone 18/9. HPLC for analysis purposes was performed with a LKB/Pharmacia 2249 pump, an in-line LKB/Pharmacia VWM 2141 UV detector (wavelength 254 nm), a flow-through NaI(Tl) crystal scintillation detection system, and Gina-Star version 2.12 (Raytest) for data acquisition and processing. Radioactivity was quantified with a VDC-405 dose calibrator (Veenstra Instruments, Joure, The Netherlands). All reactions were carried out in a home-made, remotely controlled apparatus.[22]

Preparation of [2-^{11}C]acetone

[^{11}C]Carbon dioxide, carried by a stream of helium with a flow of 10 ml/min, was bubbled into a solution of methyllitium (0.25 ml, 1.6 M, 400 μmol) in ether at room temperature. After 3 min, the reaction was quenched. The reaction mixture was then heated to 85°C under helium flow to transfer volatile products including [2-^{11}C]acetone to a second vial containing 200 μl of MeOH. The precise reaction conditions can be found in Table 1.

An aliquot of the content of the second vial was analyzed by HPLC using a mobile phase of MeOH/H_2O/*N,N*-diisopropylamine (DIPA) 40/60/0.2 1.0 ml/min (Kromasil C18, 10 μm, 250 × 4.6 mm; λ = 254 nm). Three radioactive peaks corresponding to [^{11}C]acetate, [2-^{11}C]acetone and [2-^{11}C]*tert*-butanol were observed (R_t = 2.42, 3.92 and 6.13 min, respectively).

Preparation of [^{11}C]1-isopropyl-4-phenylpiperazine

[2-^{11}C]Acetone was distilled into a second reaction vial containing either a solution of 1-phenylpiperazine (4.0 µl, 26.2 µmol), sodium cyanoborohydride (1–3.5 mg, 15.9–55.7 µmol), AcOH (0–5 µl, 0–87.3 µmol) in methanol (150 µl) or 1-phenylpiperazine (4.0 µl, 26.2 µmol), sodium triacetoxyborohydride (3–6 mg, 14.2–28.3 µmol), acetic acid (0–2 µl, 0–34.9 µmol) in THF or DCE (150 µl). The resulting mixture was then heated to 100–160°C for 5–10 min to furnish [^{11}C]1-isopropyl-4-phenylpiperazine ([^{11}C]iPPP). For the exact reaction conditions see Tables 2 and 3.

An aliquot was analyzed by HPLC using a mobile phase of MeOH/H_2O/DIPA 80/20/0.2 1.0 ml/min (Kromasil C18, 10 µm, 250 × 4.6 mm; λ = 254 nm). Two radioactive peaks corresponding to [2-^{11}C]acetone and [^{11}C]1-isopropyl-4-phenylpiperazine were observed (R_t = 3.52 and 6.97 min, respectively).

The decay-corrected radiochemical yield based on [2-^{11}C]acetone of [^{11}C]1-isopropyl-4-phenylpiperazine was 72% using the optimal conditions (Table 3, entry 13). The overall synthesis time, from EOB to HPLC analysis of [^{11}C]iPPP, was 20 min. The specific activity of [^{11}C]iPPP was 142–208 GBq/µmol at the end of synthesis.

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