Synthesis of (R) and (S) 3-Aminoquinuclidine-[3-^{14}C] Enantiomers, Important Components of a Variety of 5-HT$_3$ Ligands

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SUMMARY

3-Aminoquinuclidine, an important fragment associated with many 5-HT (serotonin) receptor ligands, has been synthesized using a ^{14}C-carbonation based sequence to prepare the starting material, isonicotinic ^{14}C-acid (**6**). Elaboration of (**6**) to α-bromoacetyl-[^{14}C] isonipecotic acid (**10**) via the corresponding diazoketone, followed by intamolecular cyclization, gave the key intermediate 3-quinuclidone-[3-^{14}C] (**3**). 3-quinuclidone-[3-^{14}C] was converted to a mixture of phenethylamine diastereomers. Carrier free crystallization and hydrogenolysis furnished both (R) and (S) 3-aminoquinuclidine-[3-^{14}C] enantiomers at >99% optical purity.

Key words: 3-aminoquinuclidine-^{14}C, 5-HT$_3$ antagonists

INTRODUCTION

The 5-HT$_3$ receptor has been implicated in a variety of pharmacological responses, including emesis, anxiety, cognition, and withdrawal from drugs of abuse[1]. Antagonists to this receptor have been reported to effectively mediate such effects and are, therefore, potentially useful therapeutic agents. This multiplicity of effects implies the presence of multiple receptor subtypes[2]. In an

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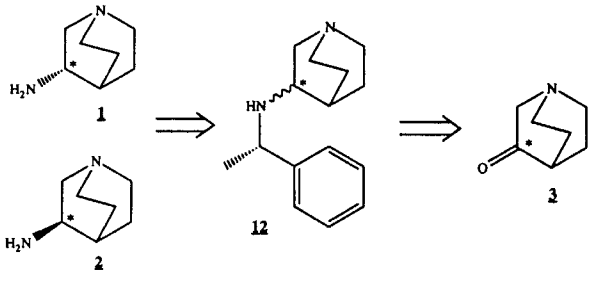
attempt to optimize response to a single subtype and thus isolate a singular pharmacological effect, a series of antagonists were prepared based on the concept of "virtual rings"[3]. More specifically, many 5-HT_3 antagonists contain an amide function linked to a nitrogen containing heterocycle. Hydrogen bonding in such molecules locks the conformation into a "virtual ring" which is thought to be the active pharmacophore. Several such conformationally restricted analogs, all of which contained the 3-aminoquinuclidine moiety, were highly active 5-HT_3 antagonists[3,4]. C-14 analogs of these compounds were required to more fully characterize their absorption and distribution properties. Since the 3-aminoquinuclidine group was a common structural feature, we decided to focus our efforts on the synthesis of that heterocycle in C-14 labelled form. Condensation with the appropriate precursors would then afford the desired series of labelled antagonists from a single batch of labelled reagent.

DISCUSSION

The most directly available presursor to 3-aminoquinuclidine enantiomers is 3-quinuclidone (**3**) as shown in **Retrosynthetic Scheme 1**. Reductive amination using a chiral amine, e.g., phenethylamine, followed by separation of

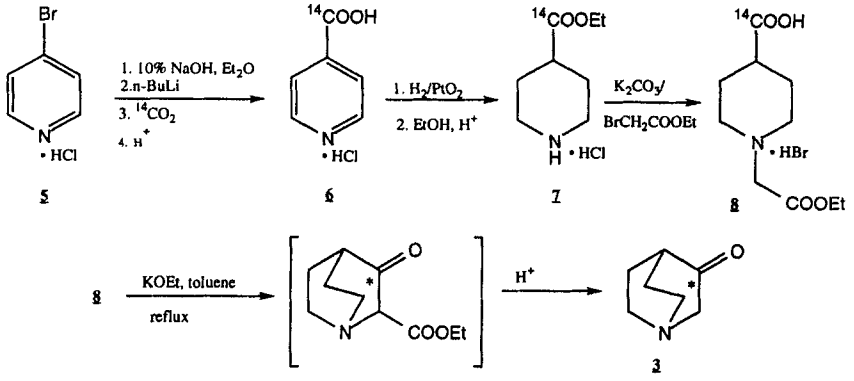
Retrosynthetic Scheme 1



diastereomers and hydrogenolysis would furnish the desired (S) and (R) enantiomers (**1**) and (**2**), respectively.

Thus, the challenge became to design an effective synthesis of 3-quinuclidone-^{14}C (**3**). Since our preference was to use a carbonation based labelling approach, the traditional Dieckmann route[5,6,7] to (**3**) (**Scheme 1**) seemed initially attractive.

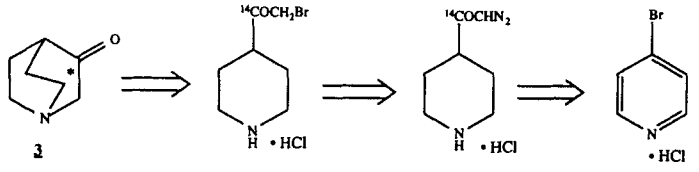
Scheme 1: Dieckmann Route to 3-Aminoquinuclidine



However, when this route was attempted in unlabelled trial reactions the overall yield to (**3**) was only 17%, despite several attempts to optimize reaction conditions.

Several years ago we described[8] the effectiveness and versatility of labelled diazoketones as synthetic intermediates. The disconnections shown in **Retrosynthetic Scheme 2** illustrate that such an intermediate could provide

Retrosynthetic Scheme 2

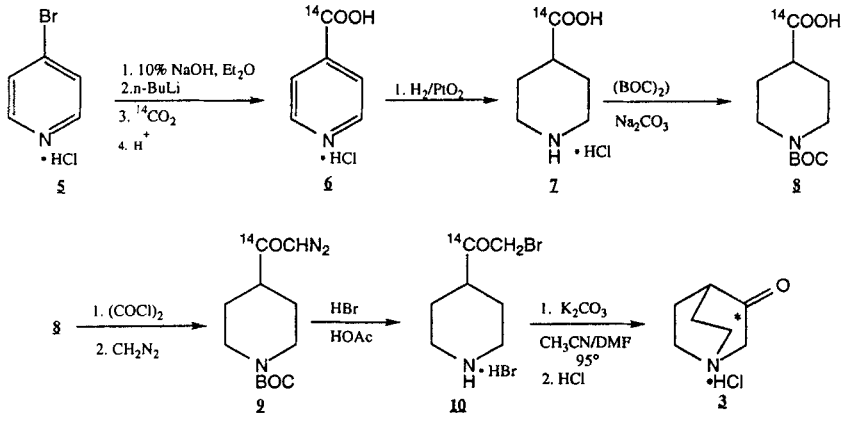


an alternative to the Dieckmann condensation, while retaining the original requirements. Such a route has been reported for the synthesis of several substituted 3-quinuclidones[9] in modest yields, but not for 3-quinuclidone itself. More recently[6], the cyclization of substituted 4-(α-bromoacetyl)-piperidines has been reported to proceed in 55% yield.

A synthesis, shown in **Scheme 2**, was initiated based on the "diazoketone route" since this strategy seemed to offer greater potential for higher yields.

Carbonation of 4-bromopyridine hydrochloride furnished isonicotinic-[^{14}C] acid in 97% yield (lit. yield 80%[10]). It was critical to liberate the free base with NaOH prior to lithium-halogen exchange. Simple addition of two equivalents of n-BuLi followed by treatment with CO_2 resulted in intractable mixtures. Reduction of **6**

Scheme 2: Diazoketone Route to 3-Aminoquinuclidine-[^{14}C]



with hydrogen over PtO_2 for two days gave a quantitative yield of isonipecotic-[^{14}C] acid **7**. Shorter reduction times gave lower yields. Treatment of **7** with di-t-butyldicarbonate afforded the BOC-protected analog **8** in 80% yield after extractive workup. Conversion of acid **8** to the acid chloride with oxalyl chloride followed by addition to diazomethane in ether furnished the diazoketone **9**.

Addition of HBr in acetic acid directly to **9** resulted in displacement of the diazo group by bromide with concomitant removal of the BOC group and reprotection of the basic piperidine nitrogen as the hydrobromide salt **10**. The yield from **7** to **10** was 83%.

After a review of the literature, it was uncertain whether an intramolecular cyclization of unsubstituted 4- α-haloacetylpiperidines could be achieved. For example, 3-carbomethoxy-4-(α-bromoacetyl)-piperidine was reported to cyclize to the corresponding substituted 3-quinuclidone in 55% yield[6]. However, other workers claimed that such cyclizations were unsuccessful unless there was geminal substituion at the 4-position or that the nitrogen was substituted[9]. Since **10** was a valuable intermediate and a good cyclization yield to 3-quinuclidone-[^{14}C] was essential, we undertook a study to evaluate the above reports and optimize the intramolecular cyclization conditions.

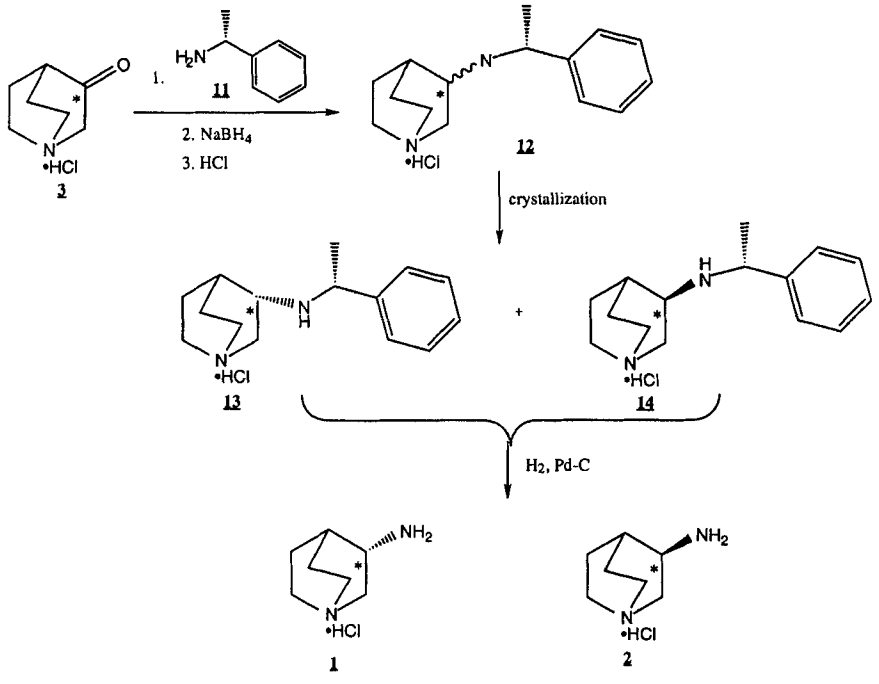
It was clearly important to liberate the free base of **10** at high dilution in order to minimize intermolecular displacement of bromide. Therefore, **10** was delivered via syringe pump, over periods ranging from 2 to 24 hr, into a large volume

solvent containing various bases. The best conditions on a 2.5 mmole scale, employing acetonitrile at 95° as the solvent, K_2CO_3 as the base, and a 9 hr syringe pump cycle to deliver **10**, furnished 3-quinuclidone-[3-^{14}C] in an average yield of 71%. These conditions resulted in significantly higher intramolecular cyclization yields than previously reported for this system despite the lack of substitution at either the piperidine nitrogen or C-4.

Isolation of 3-quinuclidone-[^{14}C] from the reaction mixture presented serious problems which deserve discussion. Because of its water solubility, **10** could not be isolated by the usual extraction procedures. An additional complication was the finding that **10** is fairly volatile, precluding chomatographic purification. Therefore, the cyclization reaction was filtered and acidified with HCl. Careful concentration resulted in the formation of a white precipitate. The remaining solvent was evaporated with a gentle stream of nitrogen. The product was then dried by evaporation from ethanol-toluene, followed by acetonitrile-toluene.

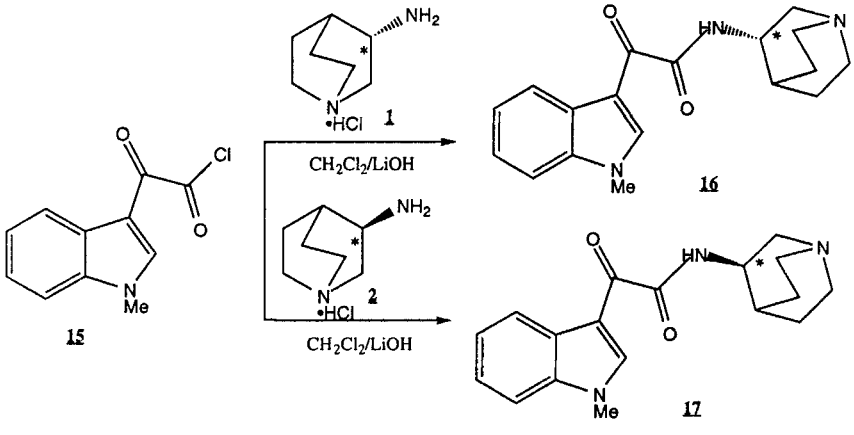
Transformation of **10** to both enantiomers of 3-aminoquinuclidine-^{14}C is shown in **Scheme 3**. Reaction with (R) phenethylamine (**11**) followed by addition of

Scheme 3



$NaBH_4$ gave the diastereomeric amine (12). Although column chromatography did not effect a separation of diastereomers, HPLC did. However, HPLC was not practical because of the large mass involved, about 2 mmoles. A separation of diasteromers was, however, achieved by crystallization from ethanol-isopropanol. The first crop furnished 28 mCi of 98.6% pure (S,R) diastereomer (13), which was the isomer of primary interest. A small amount of the mother liquors (from a previous experiment), enriched in (R,R) diastereomer (14), was purified by HPLC to give 1.7 mCi of >99% pure (R,R) diastereomer (14). Hydrogenolysis of each diastereomer over Pd-C furnished (S), and (R) 3-aminoquinuclidine-[3-^{14}C] at >98% optical purity as determined by HPLC analysis of their respective GITC derivatives[11]. Since (1) and (2) lack a chromophore, the specific activity was determined using 5-HT_3 ligands (16) and (17) prepared from these fragments (Scheme 4) and determined to be 58 mCi/mmole.

Scheme 4



In summary, an effective diazoketone based intramolecular cyclization has been developed which provides an entry to labelled 3-quinuclidone-[3-^{14}C]. This key intermediate was then readily converted to both enantiomers of 3-aminoquinuclidine-[3-^{14}C], an important component of many 5-HT ligands.

EXPERIMENTAL

$Ba^{14}CO_3$ was purchased from Nordion, International. Non-radioactive reagents were purchased from Aldrich Chemical Co. and used without purification. Solvents were HPLC grade. Only results of optimized conditions are reported where appropriate. Radiochromatography was performed on a Bioscan 200

scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. UV spectra were obtained using a Hitachi UV-265 spectrophotometer.

Isonicotinic-[carboxy-^{14}C] acid hydrochloride salt (6)

An aqueous solution of 4-bromopyridine hydrochloride (1.83g, 9.41 mmol) was made basic with 10% NaOH. The free base was extracted with ether which was dried over Na_2SO_4 then 3Å sieves.

A side-arm septum flask (100 mL) was charged with $Ba^{14}CO_3$ (204 mCi, 57 mCi/mmol, 708 mg, 3.58 mmol) and connected to a vacuum line and evacuated. The ether solution of 4-bromopyridine was transferred, under nitrogen, into a 250 mL side-arm septum flask which was also connected to the vacuum line, then cooled to -78°. n-BuLi (1.6M, 4.47 mL, 7.15 mmol) was injected and the system was stirred for 20 minutes at which point the orange solution was cooled further to -178° (liquid N_2). The entire system was evacuated . $^{14}CO_2$, liberated from $Ba^{14}CO_3$ by injection of conc. H_2SO_4, was vacuum transferred onto the frozen aryllithium solution. The reaction was warmed to -78° and stirred for 1 h, then quenched with water. The aqueous layer was extracted with ether three times and washed with water. The aqueous phase was acidified with 6N HCl and the acidic products were extracted with ether. The aqueous phase was evaporated to dryness. The residue, dissolved in 35 mL water-isopropanol (1:1), contained 198 mCi (97% yield) of (1).

Radio-TLC (silica gel): CH_2Cl_2-MeOH-HOAc (90:10:1), R_f 0.3.

Isonipecotic-[carboxy-^{14}C] acid hydrochloride salt (7)

A solution of (6) (135 mCi, 2.37 mmol, 57 mCi/mmol) in 25 mL water-isopropanol (1:1) was hydrogenated over PtO_2 (160 mg) for 5 days. The catalyst was removed by filtration and washed with water, isopropanol:water (1:1), and dilute HCl until no more activity could be removed. The product (7) was obtained in quantitative yield.

Radio-TLC (silica gel): CH_2Cl_2-MeOH-HOAc (90:10:1), 6 R_f 0.3, 7, R_f 0.15.

1-Butoxycarbonyl-4-carboxypiperidine-[carboxy-^{14}C] (8)

A solution of (7) (297 mCi, 5.2 mmol) in water (80 mL) was brought to pH 8 with Na_2CO_3. Di-t-butyldicarbonate (1.8 g, 8.25 mmol) was dissolved in 60 mL THF, added to the above solution of (7) and the reaction was stirred at ambient

temperature while pH 8-9 was maintained by periodic addition of Na_2CO_3. The reaction was complete after 48 h as indicated by radio-TLC. The reaction was concentrated by rotary evaporation and the basic aqueous solution was extracted with EtOAc to remove neutrals. Careful acidification to pH 2, first with 6N HCl then $NaHSO_4$, and extraction with EtOAc afforded a quantitative yield of (**8**).

Radio-TLC (silica gel): CH_2Cl_2-MeOH-HOAc (90:10:1), **7** R_f 0.3, **8** R_f 0.85; Hexane-EtOAc-HOAc (60:40:1), **7** R_f 0.05, **8** R_f 0.4.

1-t-Butoxycarbonyl-4-(9α-diazoacetyl-[^{14}C])-piperidine (9)

Water was removed from a solution of (**8**) (297 mCi, 5.2 mmol) by azeotropic evaporation from toluene. The residue was dissolved in CH_2Cl_2 (5 mL), cooled in an ice bath, and treated with oxalyl chloride (1.1 mL, 12.56 mmol) and a trace of DMF. After 1 h, the reaction was warmed to ambient temperature and evaporated to dryness. The residue was azeotroped to dryness from toluene and dissolved in ether. The ether solution of acid chloride was added to a large excess of freshly prepared diazomethane in ether which was cooled in an ice bath. After stirring for 1.5 h, radio-TLC showed complete conversion to a single, strongly uv absorbing spot.

Radio-TLC (silica gel): Hexane-EtOAc-HOAc (20:10:1), **8** R_f 0.4, **9** R_f 0.3.

4-(α-Bromoacetyl-[^{14}C])-piperidine hydrobromide (10)

To the solution of (**9**) prepared above was added 300 mL of toluene. The solution was cooled to 0° and treated with HOAc (5 mL) followed by 33% HBr/HOAc (5 mL) and allowed to stand overnight. The ether layer was separated and evaporated to dryness leaving 297 mCi of a pale orange crystalline residue, (containing @ 85% **10**), which was used in the next step without purification. Radio-TLC (silica gel): CH_2Cl_2-MeOH-HOAc (90:10:0.1), **9** R_f 0.85, **10** R_f 0.1; CH_2Cl_2-MeOH-NH_4OH (90:10:0.1), **9** R_f 0.95, **10** R_f 0.4.

3-Quinuclidone-[3-^{14}C] hydrochloride salt (3)

To a 5 L 3-neck flask equipped with stirring magnet, thermometer, and condenser was added CH_3CN (2500 mL, previously dried over 3Å sieves) and K_2CO_3 (732 mg, 5.3 mmole). The suspension was stirred rapidly while maintaining an internal temperature of 95°. Crude (**10**) (140 mCi, 2.45 mmol) was dissolved in 2 mL DMF and 43 mL CH_3CN and added to the above suspension via syringe pump over 9h. Radio-TLC showed a clean reaction

mixture consisting only of 73% product (**3**) and origin material. The reaction was cooled to ambient and filtered. Acidification with 2N HCl was followed by evaporation to dryness. The residue was dissolved in ethanol-toluene (1:1) and evaporated to dryness leaving a white crystalline solid which was used directly in the next step.

Radio-TLC (silica gel): CH_2Cl_2-MeOH-NH_4OH (90:10:0.1), **10** R_f 0.0.4, **3** R_f 0.7.

3-(N-(R)-α-methylbenzyl)-(R) and (S) 3-Aminoquinuclidine-[3-^{14}C] (13) and (14)

To a suspension of (**3**) (200 mCi, 3.5 mmol) in toluene (200 mL) and EtOAc (10 mL) was added Li_2O (128 mg, 4.26 mmol), freshly activated 4Å molecular sieves, and (R) methylbenzylamine (0.95 mL, 7.5 mmol). The reaction was stirred at 60° for 3 days. Additional 4Å sieves, (R) methylbenzylamine (0.95 mL, 7.5 mmol) and THF (20 mL) was added and stirring was continued for 3 days. The reaction was cooled and filtered.

To the filtrate was added ethanol (50 mL) and $NaBH_4$ (200 mg, 5.2 mmol) and the reaction was stirred overnight at which point radio-TLC showed no remaining starting material. The reaction was quenched with water and evaporated to dryness. Th residue was dissolved in CH_3CN-EtOH and evaporated to dryness again. The residue was dissolved in a minimum volume of toluene and applied to a flash chromatography column and eluted with toluene-EtOH (3:1), toluene-EtOH-MeOH (5:4:1), and toluene-EtOH-MeOH-NH_4OH (5:4:1:0.1). Fractions containing the diastereomeric amines (**12**) (97 mCi) were concentrated, dissolved in EtOH, acidified with 6N HCl, and evaporated to dryness. The residue was crystallized from EtOH-i-PrOH to yield 28 mCi of >98% pure (S,R) N-methylbenzyl-3-aminoquinuclidine-[3-^{14}C] (**13**). Less pure fractions (87% S,R diastereomer) containing 55 mCi were also isolated. Diastereomeric purity was confirmed by HPLC (Biotage alumina PBD column, eluted with 25% CH_3CN-0.2% NH_4OH at 1 mL/min, detected at 224 nm, ret 21 min).

The mother liquors, enriched in (R,R) diastereomer (**14**), were concentrated and purified by HPLC affording 1.8 mCi of >99% pure (R,R)) N-methylbenzyl-3-aminoquinuclidine-[3-^{14}C] (**14**). Diastereomeric purity was confirmed by HPLC (Biotage alumina PBD column, eluted with 25% CH_3CN-0.2% NH_4OH at 1 mL/min, detected at 224 nm, ret 17.5 min).

(S) 3-Aminoquinuclidine-[3^{14}C] (1) and (R) 3-Aminoquinuclidine-[3^{14}C] hydrochloride salt (2)

The (R,S) isomer (13) (21 mCi, 0.37 mmol) was dissolved in EtOH (10 mL) containing 10% HCl (2 mL) and hydrogenolyzed over 10% Pd-C (10 mg). After 2 days the reaction was complete by radio-TLC. The catalyst was filtered to furnish a quantitative yield of pure (S) 3-aminoquinuclidine-[^{14}C] (1). The enantiomeric purity was >98% as determined by HPLC analysis of the GITC[11] derivative.

In anidentical manner, the (R,R) isomer (14) (1.7 mCi, 0.03 mmol) was hydrogenolyzed to furnish 1.3 mCi of pure (R) 3-aminoquinuclidine-[^{14}C] (2). The enantiomeric purity was >98% as determined by HPLC analysis of the GITC[11] derivative.

Radio-TLC (silica gel): MeOH-NH_4OH (9:1) 1 R_f 0.45, 2 R_f 0.2

(R) an (S) isomers of RS-56812-^{14}C (16) and (17)

To a solution of (1) (2.7 mCi; 0.05 mmol) in CH_2Cl_2 (3 mL) was added LiOH (3 mg; 0.13 mmol) and 4Å molecular sieves. The solution was stirred at ambient temperature and N-methyl-3-chlorooxalylindole (15 mg; 0.07 mmole) dissolved in CH_2Cl_2 (1 mL) was added. After 24 h radio-TLC showed 12% product. Additional portions of the indole derivative, LiOH, and 4Å sieves were added. After 5 days the title compound was obtained in 95% yield. Following purification by flash column chromatography (EtOAc-EtOH-NH_4OH, 90:10:1), the specific activity was determined by HPLC to be 58.8 mCi/mmol. The (R) isomer (17) was prepared in an identical manner and had the same specific activity.

Radio-TLC: EtOAc-EtOH-NH_4OH (90:10:1) R_f 0.5

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