Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVI, No. 1

Synthesis of the Antiinflammatory Prodrug RS-42169-^{14}C

Howard Parnes and Glenn T. Huang
Institute of Organic Chemistry, Syntex Discovery Research
3401 Hillview Ave., Palo Alto, CA 94303

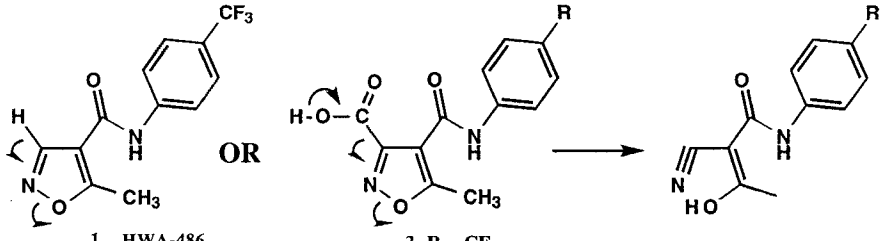
SUMMARY

A facile synthesis of the antiinflammatory agent RS-42169-^{14}C, based on a carbonation/cycloaddition sequence is described. The product was obtained in 36% yield from $Ba^{14}CO_3$ at a specific activity of 52 mCi/mmole. The design of the six step sequence was such that pure intermediates were isolated by extractive workup, and only a single chromatographic purification was required.

Key Words: RS-42619-^{14}C, HWA 486-^{14}C, cycloaddition, anti-inflammatory.

INTRODUCTION

The isoxazole compound (**1**), (HWA-486)[1] is rapidly converted, *in vivo*, to the putative antiinflammatory agent 2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]-2-butenamide[2] (**2**). It was theorized that a



1 HWA-486

3 R = CF_3
4 R = OCF_3

2 R = CF_3
2a R = OCF_3

Contribution #867 from the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, CA 94303

CCC 0362–4803/95/010051–06

Received 19 June, 1994
Revised 16 August, 1994

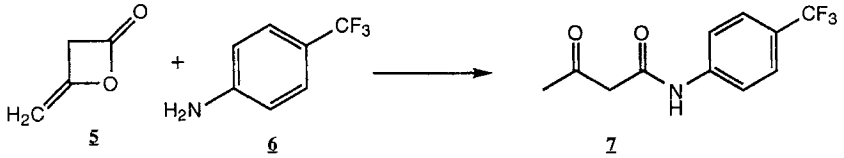
prodrug which had to undergo a more extended fragmentation to form **2** might result in more sustained plasma levels of **2** over a longer period of time. The carboxylic acid analog, (**3**), was synthesized[3] to demonstrate this concept. This compound also generates **2**. However, because of its more extended fragmentation, **3** does, indeed, show a much longer plasma half-life[3]. This result led to the discovery of a still superior compound, RS-42169 (**4**), the p-trifluoromethoxyaniline analog of **3**. The pharmacokinetic parameters of **4** were investigated by means of its ^{14}C-labelled analog, **4a**, whose synthesis is described in this report.

DISCUSSION

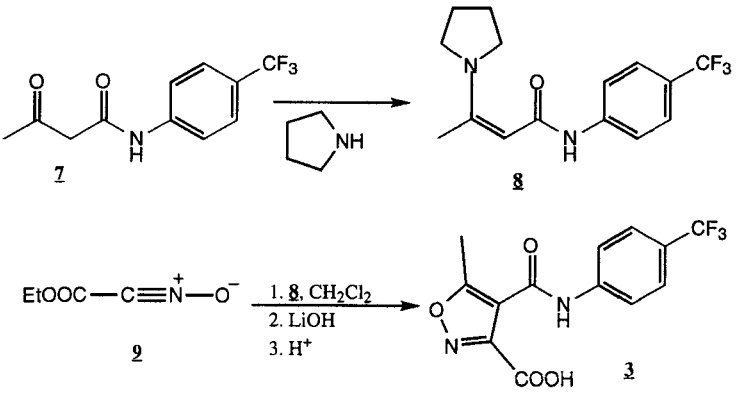
A molecule whose synthesis may be straightforward in its unlabelled form can become a significant challenge when considering the preparation of its labelled analog. This is often the case when the structure offers few opportunities for introducing a label[4]. At other times, perfectly ordinary reagents (which would make the synthesis easy) may not be available in labelled form or, their preparation may present special problems[5]. A case in point, RS-42169-^{14}C, (**4a**), is the subject of this paper.

It is clear from its structure that **4** offers limited opportunity for introducing a ^{14}C label. The carbonyl group linking the isoxazole to the p-trifluoromethoxyaniline seems to be the only practical center amenable to labelling. This center is *not* accessible in the reported synthesis[3] of **3**, (shown in **Scheme 1**) from the reaction of p-trifluoromethylaniline and diketene. Neither of these reagents are amenable to labelling. Another approach might involve the use of ethyl [^{14}C]-acetoacetate. The multistep synthesis of this intermediate affords low yields[6,7]. In addition, the volatility of ethyl acetoacetate makes its preparation operationally difficult. However, the direct nature of **Scheme 1** makes this approach extremely attractive. We therefore turned our attention to making this synthesis amenable to introduction of C-14 by focussing on alternate approaches to the key intermediate, enamine (**8**). More specifically, we wanted to introduce the labelled carbonyl group via carbonation with $^{14}CO_2$ since this approach is inexpensive, affords high yields, and is easy to implement[8,9]. The retrosynthetic scheme below shows our strategy. Thus, carbonation of propynyllithium to give butynoic-[1-^{14}C] acid (**11**),

Scheme 1

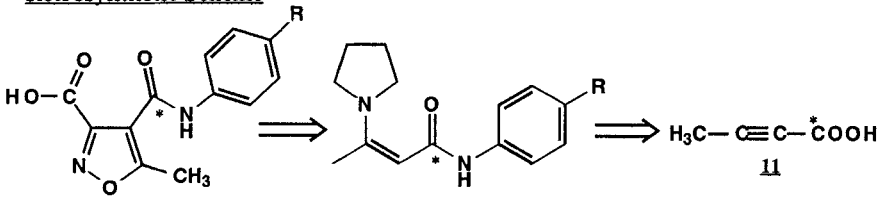


5 **6** **7**



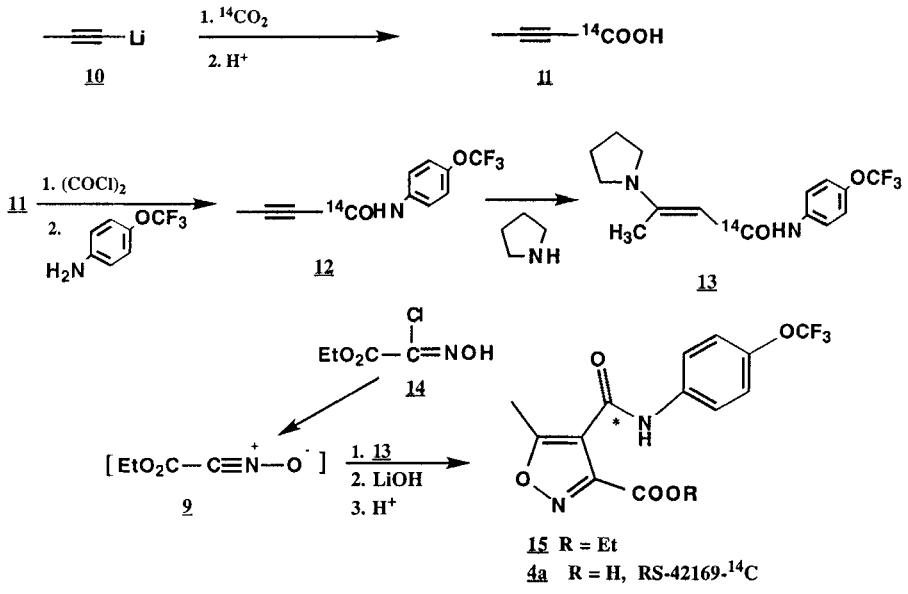
followed by Michael addition of pyrrolidine should afford the identical enamine obtained from the acetoacetanilide (**7**). The actual synthesis based on this thinking is depicted in **Scheme 2**.

Retrosynthetic Scheme



Initial attempts to prepare butynoic-[1-^{14}C] acid (**11**) centered around the deceptively simple lithiation of propyne with n-butyllithium in THF, followed by addition of $^{14}CO_2$. Despite several attempts, and very careful vacuum line work, no acidic products corresponding to (**11**) could be isolated. However, the use of commercially available, powdered propynyllithium (**10**) afforded the desired acid (**11**) in 66% yield. Conversion of **11** to the p-trifluoromethoxyanilide (**12**) via the acid chloride proceeded without event. The amide was isolated in 88% yield. In a one pot reaction, (**12**) was heated in pyrrolidine to give the enamine (**13**) resulting from Michael addition as expected. Addition of ethyl chlorooximidoacetate (**14**) in dichloromethane at 0° generated the nitrile oxide (**9**)[3,10], *in situ*, which resulted in concomitant cycloaddition to (**13**) to give the isoxazole ethyl ester (**15**) in 83% yield after purification. The ester was carefully hydrolysed with LiOH in methanol-water at 0°. Following addition of water and evaporation of methanol, neutrals were removed with ethyl acetate. The aqueous phase was acidified with $NaHSO_4$ and extracted with ethyl acetate. Sequential washes with bicarbonate, water, bisulfate, and brine, followed by drying over sodium sulfate afforded the isoxazole acid, RS-42169-^{14}C (**4**) in 75% yield (36% from $Ba^{14}CO_3$). The specific activity of (**4**) was found to be 52 mCi/mmole by the UV/radioassay method. Using the workup procedure described, (**4**) was obtained having a purity of >99% by HPLC analysis without additional purification.

Scheme 2: Synthesis of RS-42169-^{14}C



EXPERIMENTAL

$Ba^{14}CO_3$ was purchased from Nordion International. Unlabelled reagents were purchased from Aldrich Chemical Co. and were used without purification. Propynyllithium was purchased from Johnson Mathey Co., Woodhill, Mass. Solvents were HPLC grade. Radiochromatography was performed on a Bioscan 200 Scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. HPLC analyses were done using Beckman gradient HPLC systems equipped with Beckman uv and radioactivity flow detectors. UV spectra were obtained using a Hitachi UV-265 spectrophotometer. NMR spectra were recorded using a Varian EM 390 spectrometer. MS spectra were obtained on a Finning-MAT 8230 spectrometer.

Butynoic-[1-^{14}C] acid (11)

A 25 mL side-arm septum flask was charged with propynyllithium (84 mg; 1.83 mmole). The flask was connected to a vacuum line, evacuated, and cooled to -78°. THF (15mL) over $LiAlH_4$ was vacuum transferred into the reaction flask.

A second 25 mL side arm septum flask, containing $Ba^{14}CO_3$ (85 mCi; 1.5 mmole; 57 mCi/mmole) was attached to the vacuum line and evacuated. Concentrated H_2SO_4 (10 mL) was injected and the liberated $^{14}CO_2$ was vacuum transferred into the cooled reaction flask. After stirring for 40 min the carbonation reaction was quenched with aq. $NaHCO_3$ (5 mL),

warmed to ambient temperature, and any residual volatile radioactivity was vacuum transferred into an evacuated receiver cooled to -78°.

The reaction flask was removed from the vacuum line, diluted with water, and any remaining THF was evaporated. The aqueous residue was extracted with ethyl acetate-ether (1:1) to remove neutrals. The aqueous phase was acidified with HCl and the product extracted with ethyl acetate-ether (1:1) until no additional product could be extracted. The combined organic phase was washed with water, brine, dried over Na_2SO_4 and filtered to furnish 56 mCi of (66% yield) (**11**). Radio-HPLC (Vydac C-18, 2% CH_3CN in .03M triethylammonium phosphate, pH 3), 1 mL/min, uv detection at 220 nm, R_t 6.05 min) showed the product to be identical to authentic standard.

N-(4-Trifluoromethoxyphenyl)-2-butynoic-[1-^{14}C] acid carboxamide (12)

A solution of butynoic-[1-^{14}C] acid (**11**) (34 mCi; 0.6 mmole) in CH_2Cl_2 (10 mL) was treated with oxalyl chloride (60 µL, 4.4 mmole) and a trace of DMF. After 1h at ambient temperature the reaction was complete by radio-TLC (aliquot quench into excess 4-trifluoromethoxyaniline in CH_2Cl_2). The reaction was cooled to 0° and 4-trifluoromethoxyaniline (0.6 mL; 4.4 mmole) in CH_2Cl_2 (10 mL) was added dropwise. Stirring was continued at 0° for 30 min at which time radio-TLC (hexane-EtOAc, 2:1) indicated complete conversion to the amide (**12**). The reaction was diluted with CH_2Cl_2, then washed sequentially with 10% HCl, water, $NaHCO_3$, brine, and dried over $MgSO_4$ to furnish 30 mCi (88% yield) of (**12**). The radiochemical purity was found to be 97% by radio-TLC.

3-Carboethoxy-5-methyl-N-[4-trifluoromethoxyphenyl]-4-isoxazolecarbox-[^{14}C]-amide (15)

A solution of amide (**12**) (25 mCi; 0.44 mmole) in pyrrolidine (10 mL) was heated at reflux for 5 min then cooled to ambient temperature. The reaction was evaporated to dryness and the residue was dissolved in CH_2Cl_2 (30 mL). While stirring at ambient temperature, ethyl chlorooximidoacetate (135 mg; 0.89 mmole) was added in 3 portions over 2h. The reaction was diluted with CH_2Cl_2 and washed sequentially with 10% HCl, water, brine, and dried over Na_2SO_4. Purification by radial chromatography (Chromatotron®) on silica gel, eluted with hexane-EtOAc (2:1) afforded 20.7 mCi (83% yield) of isoxazole ester (**15**).

3-Carboxy-5-methyl-N-[4-trifluoromethoxyphenyl]-4-isoxazolecarbox-[^{14}C]-amide (4a)

The isoxazole ester (**15**) (20.7 mCi; 0.36 mmole), dissolved in MeOH (20 mL), was cooled to 0° and treated with a solution of LiOH (24 mg; 1 mmole) in MeOH-water (9:1). The base was added dropwise over 15 minutes. After stirring for 2 h, the reaction was diluted with water and the MeOH was evaporated. The aqueous residue was extracted with EtOAc

to remove neutrals, then acidified with $NaHSO_4$. The product was extracted three times with EtOAc. The combined extracts were washed with $NaHCO_3$, water, bisulfate, brine, and dried over Na_2SO_4. A total of 15.4 mCi (75% yield) of the title compound (**4a**) was isolated in this manner. The specific activity was 52 mCi/mmole, and the radiochemical purity was >99% without additional purification. The HPLC conditions used to determine radiochemical purity were: Beckman ultrasphere C-18, CH_3CN-0.1% trifluoroacetic acid (43:57), 1 mL/min, monitored at 220 nm, R_t = 12.4 min.

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