SYNTHESIS OF [^{35}S]-LABELLED MK-0571, A POTENT ANTAGONIST OF LTD_4

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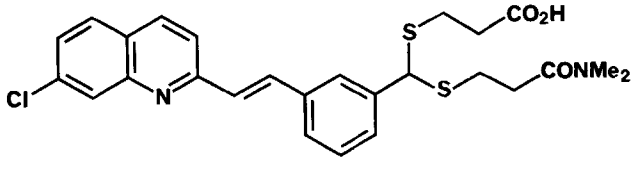
SUMMARY

The synthesis of 5-[3-{2-(7-chloroquinolin-2-yl)ethenyl}-phenyl]-8-dimethylcarbamyl-4,6-[6-^{35}S]dithiaoctanoic acid at a specific activity of 1350 Ci/mmol is reported. This compound is a reagent suited for selective affinity binding studies at the LTD_4 receptor.

KEYWORDS: sulphur-35, [6-^{35}S]MK-0571, dithioacetals, triphenylmethane[^{35}S]thiol, triphenylmethyl [^{35}S]thiopropanoic acid derivatives, leukotriene D_4 antagonist.

INTRODUCTION

MK-0571†, 5-[3-{2-(7-chloroquinolin-2-yl)-ethenyl}phenyl]-8-dimethylcarbamyl-4,6-dithiaoctanoic acid, 1) is an intrinsically potent antagonist of LTD_4, with an IC_{50} = 0.9 nM versus LTD_4 on Guinea Pig lung membrane, and this property, together with high in vivo antagonist activity and favourable pharmacodynamics[1] makes the compound a very interesting prospect as a clinical candidate to evaluate the role of leukotriene D_4 in human disease states.



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†Formerly known as L-660,711

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The high binding affinity of the compound also gives it the potential of acting as a biochemical tool for the characterization of the LTD_4 receptor; and the scope of its use in this respect would be enhanced significantly if the molecule carried a radioactive label. The compound has been synthesized with a tritium atom on the ethylene bridge connecting the quinoline and benzene rings,[††] providing a means of conducting metabolic experiments, but the intrinsic radioactivity available by this synthesis was not sufficient for receptor-binding studies.

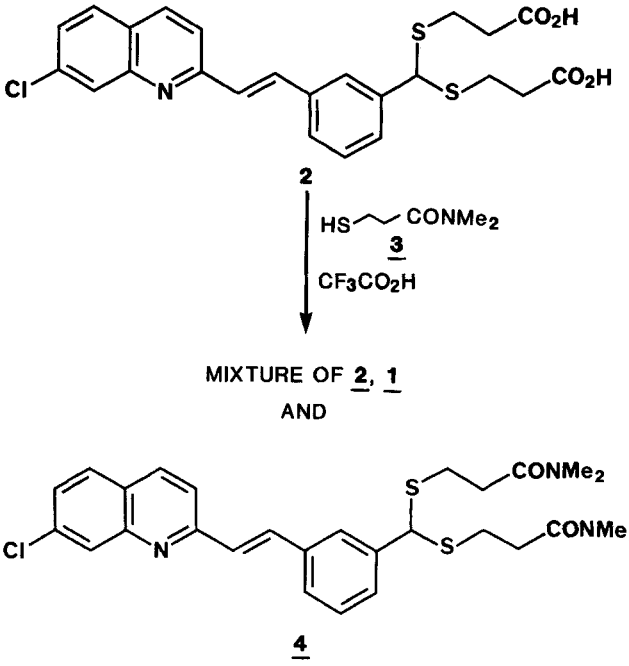
For the latter purpose, the idea of inserting a ^{35}S atom was attractive, because the half-life (87 days), and the energy (0.167 MeY) of the emitted β-particle of this isotope would provide a compound very suited to the needs of the biochemist as far as scintillation counting and autoradiography are concerned. However the laboratory synthetic procedure for preparing the compound was not a good model for making the labelled compound because it involved the introduction of the two sulphur-containing chains at too early a stage in the synthesis.[2] Accordingly, we have devized a new approach for the preparation, which may prove to have more general scope in the synthesis of ^{35}S-labelled compounds.

DISCUSSION AND RESULTS

The two problems to be overcome in the context of the synthesis were firstly the preparation of a ^{35}S-labelled 3-mercaptopropanoic acid derivative, and secondly, a means of introducing the labelled chain into the target molecule at a late stage, preferably the final step. An answer to the second problem was provided by the discovery that when an equimolar mixture of the diacid 2 and N,N-dimethyl-3-mercaptopropanoamide (3) was treated with trifluoroacetic acid, (or a suitable Lewis acid), an exchange reaction took place to give a mixture of 2, 1, and the diamide 4 presumably in a ratio of approximately 1:2:1, and in high theoretical yield (ca. 45% conversion to 1,). (Scheme 1)

[††]Unpublished results by S. Léger and R. Zamboni

SCHEME 1

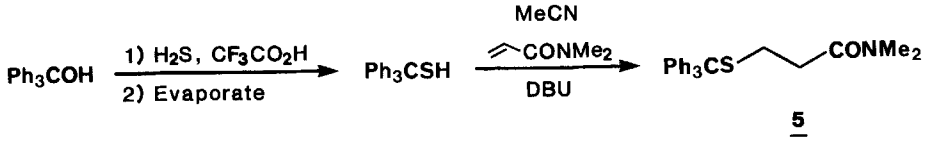


The usual route to 3-mercaptopropanoic acid derivatives involves the Michael addition of thiolacetic acid to an acrylic acid derivative followed by alkaline hydrolysis of the acetyl group.[3] The prospect of making thiolacetic acid from hydrogen [^{35}S]sulphide, and then doing a Michael addition and hydrolysis did not look a very attractive approach, and a new route was sought with the notion that a suitably protected derivative could be made which would generate the mercaptopropanoic acid derivative in situ under the acid conditions required for the last step of the synthesis i.e. in trifluoroacetic acid.

The objective was attained using the trityl group as the protecting group. Treatment of a mixture of triphenylmethanol and hydrogen sulphide with trifluoroacetic acid gave a crude product containing triphenylmethanethiol; then removal of the trifluoroacetic acid in a stream of nitrogen, and the additions of acetonitrile as solvent, 1,8-diazabicyclo[5.4.0]undec-7-ene as basic catalyst, and N,N-dimethylacrylamide as reactant gave N,N-dimethyl-3-mercaptopropanoamide in a two step, one pot reaction sequence (Scheme 2). One

benefit accruing from the use of the bulky trityl group may be to limit the formation of thioether in the first step, even when triphenylmethanol is in excess over the hydrogen sulphide.

SCHEME 2



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The procedure was put into practice using hydrogen [^{35}S]sulphide, and a radioactive product was isolated possessing the same retention times as authentic "cold" compound using two very different sets of hplc conditions as described in the Experimental Section.

In developing methodology to use this reagent to label MK-0571, the use of "cold" N,N-dimethyl-3-mercaptopropanoamide as carrier was examined first. Provided that the ethanol in which the labelled reagent was stored was removed before initiating the reaction by the addition of trifluoroacetic acid, the MK-0571 recovered from the reaction had a ^{35}S label. This result corroborates the authenticity of the labelled intermediate. However for the LTD_4 receptor studies, the requirement was for a no-carrier-added end-product. When the reaction was carried out with no N,N-dimethyl-3-mercaptopropanoamide added, the reaction again failed to proceed normally, and it was found necessary to add about 10 mol% of 3-mercaptopropanoic acid as a radical scavenger for the labelled reagent. Presumably this quantity of 3-mercaptopropanoic acid affects the final equilibrium adversely only to the extent by which the pool of $-SCH_2CH_2COOH$ groups is diluted, i.e. about 5%. For the purification of the product by hplc, two reversed phase solvent systems were examined. The first system employed, 65:35 acetonitrile/water containing 0.015% of propanoic acid 0.01% of mercaptoethanol and 0.01% of dithioerythritol, is an imperfectly buffered solvent, and when used for elution of the crude reaction mixture still containing a trace of trifluoroacetic acid, the product was eluted in two

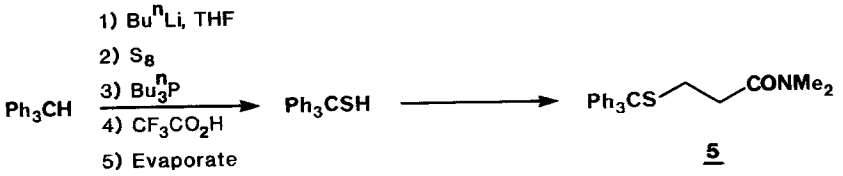
distinct, but rather wide, fractions. These fractions were pooled, and evaporated to dryness by freeze-drying before a second hplc was carried out. The product from this process was eluted as a single homogeneous fraction. A more satisfactory system was found to be a methanol/water mixture buffered with sodium dihydrogen phosphate and ammonium hydroxide, (described in detail in the Experimental Section). The buffering offered by this solvent copes with the trace of trifluoroacetic acid left in the reaction mixture, and gives a single sharp elution fraction: however the eluted fraction cannot be freeze-dried in this case, because the high salt concentration which developes in the later stages of the evaporation converts the compound to another material. Instead, the methanol was removed in a stream of nitrogen, and the product was absorbed in a C_{18} Sep Pak to remove inorganic salts. It was recovered by washing with methanol, and this solution was evaporated to a suitable volume for subsequent hplc.

The product obtained from these experiments coeluted with MK-0571 in three hplc systems, and its authenticity was supported by its behaviour in biochemical studies.

When further quantities of the compound were required, a significant improvement was made in the preparation of the labelled intermediate. It was felt that replacement of hydrogen [^{35}S]sulphide with elemental sulphur would confer two advantages; firstly, in ease of handling, (both from an experimental point of view and from safety considerations), and secondly, in providing a procedure that would be less sensitive to the imbalances in the stoichiometry which are unavoidable in such syntheses. One idea was to make the carbanion from triphenylmethane, and to react this with elemental sulphur to obtain triphenylmethanethiol, followed by Michael addition of the thiol to N,N-dimethylacrylamide as before. A search of the literature showed some prior art, in that the reaction of n-butyllithium with sulphur was reported to give butanethiol, the corresponding disulphide, and other minor products.[4] Because of the stability of the carbanion of triphenylmethane, it was possible to work out fairly simple methodology for the case at hand, e.g. it was not found necessary to carry out the reactions at low temperature, and the

addition of tri-n-butylphosphine to the reaction mixture augments the overall yield, presumably by reducing any disulphide that is formed (Scheme 3).

SCHEME 3



The labelled MK-0571 obtained by this route has proved to be surprisingly stable when stored at -78°C in the hplc solvent containing the antioxidants mentioned. At room temperature in the laboratory, a single new radioactive product appears over the course of several hours, and from the elution time it is deduced to be the cis-compound, arising as a consequence of photolysis of the ethylenic linkage. This compound has a much lower affinity for the LTD_4 receptor than the trans-isomer, and does not pose a problem in the biological experiments. Binding studies of the substrate at the LTD_4 receptor will be reported at a later date.

EXPERIMENTAL

Instrumentation

Infrared spectra were recorded using a Perkin Elmer 683 spectrophotometer, and proton magnetic resonance spectra were obtained using a Bruker EM250 instrument with tetramethylsilane as internal standard. High performance liquid chromatography was performed with Waters equipment, and a Flo-one Model HP radiation detector was employed for detecting radioactive fractions.

N,N-Dimethyl-3-triphenylmethylthiopropanoamide (3)

To a mixture of N,N-dimethylacrylamide (515 µL, 5.0 mmol), triphenylmethanethiol (1.38 g, 5.0 mmol) and acetonitrile (2.0 mL) was added

1,8-diazabicycloundec-7-ene (DBU) (75 µL, 0.5 mmol) initiating a rapid reaction, and giving a pale reddish-brown solution from which the product crystallized. The solid was collected, and washed with a little acetonitrile. 1.42 g (80%). On recrystallization from acetonitrile, the amide had mp 114-116°C. IR νmax (KBr) 1647 cm^{-1}.

^1H NMR δ 2.15 (t, 2H, J = 7.4Hz), 2.55 (t, 2H, J = 7.4Hz, CH_2), 2.78 (s, 3H, CH_3), 2.87 (s, 3H, CH_3), 7.28 (m, 9H, ArH), 7.42 (t, 6H, J = 7Hz, ArH).

Anal. $C_{24}H_{25}NOS$ requires: C 76.76, H 6.71, N 3.73, S 8.54: Found: C 77.08, H 6.81, N 3.76, S 8.75.

N,N-Dimethyl-3-triphenylmethyl[^{35}S]thiopropanoamide

First Procedure

A Kontes hydrolysis tube of about 1 mL capacity was charged with triphenylmethanol (26 mg, 0.1 mmol), and the air in the tube was swept out with hydrogen sulphide. Trifluoroacetic acid (200 mL) was added, and the tube was sealed. The reaction mixture was shaken occasionally, and after 30 minutes the trifluoroacetic acid was evaporated in a stream of nitrogen. Final traces of trifluoroacetic acid were removed under vacuum, then acetonitrile (200 µL), N,N-dimethylacrylamide (10 µL), and DBU (25 µL) were added. After a reaction time of 30 minutes, the solvent was evaporated in a stream of nitrogen.

Estimation of the product in the residual oil was performed by dissolving the oil in ethyl acetate (10 µL), and carrying out high performance liquid chromatography (hplc) using a solution of authentic product from Example 1 as a standard. Suitable hplc conditions employed a 7.8 mm x 30 cm silica gel column eluted at 3.0 mL/minute with 1:3 ethyl acetate/hexane. The uv detector was set at 270 nm. The retention time of the product was 4.9 minutes, and the yield was typically 40%.

The above procedure is amenable to adaption for the preparation of N,N-dimethyl-3-triphenylmethyl[^{35}S]thiopropanoamide by replacing hydrogen sulphide with hydrogen [^{35}S]sulphide with a specific activity of about

1350 Curie /mmol to provide a product of about the same specific activity; furthermore replacement of N,N-dimethylacrylamide with a lower alkyl acrylate in the above procedure provides an alkyl 3-triphenylmethyl[^{35}S]thiopropanoate.

N,N-Dimethyl-3-triphenylmethyl[^{35}S]thiopropanoamide

Second Procedure

To a solution of triphenylmethane (27 mg, 0.11 mmol) and tri-n-butylphosphine (3 μL)[5] in dry tetrahydrofuran (250 μL), contained in a 1 mL argon-filled Reactivial sealed with a septum, 1.6M butyllithium in hexane (60 μL) was added by syringe. The mixture was stirred at room temperature for 5 minutes, then it was withdrawn into a syringe containing a few μL of dry tetrahydrofuran, and transferred into another argon-filled reaction vessel containing sulphur (1.1 mg, 0.034 mmol). This mixture was stirred at room temperature for 10 minutes, then the reaction mixture was neutralized with trifluoroacetic acid (8 μL). After evaporation of the solvent in a stream of nitrogen, acetonitrile (100 μL), N,N-dimethylacrylamide (5 μL), and DBU (5 μL) were added in succession. The mixture was stirred at room temperature for 30 minutes, and the work-up and estimation of the product were performed as described in the First Procedure. The yield was typically 50% based on the amount of sulphur used.

This procedure too can be adapted for the preparation of ^{35}S-labelled propanoic acid derivatives by using [^{35}S]sulphur and either N,N-dimethylacrylamide, or a lower alkyl ester of acrylic acid as the reactants. After purification of the product by hplc, the eluting solvent was evaporated, and the residue was dissolved in ethanol containing 2,6-di-*tert*-butyl-4-cresol (0.5 mM%) as antioxidant. The purity of the compound was checked by normal and reversed phase hplc. The specific activity was 1350 Ci/mmol.

5-[3-{2-(7-Chloroquinolin-2-yl)ethenyl}phenyl]-8-dimethylcarbamyl-4,6-[6-^{35}S]dithiaoctanoic acid

To a nitrogen-filled Reactivial (volume 300 µL) containing 5-[3-{2-(7-chloroquinolin-2-yl)ethenyl}phenyl]-4,6-nonanedioic acid (2) (ca. 0.2 mg) was added a solution of 3-mercaptopropanoic acid (8 µg) in ether (10 µL), and then the ethanol solution of the labelled reagent (200 µL, ca. 600 µCi). The solvents were evaporated in a stream of nitrogen, and trifluoroacetic acid (50 µL) was added. The reaction mixture was allowed to stand for 1 h, and then the trifluoroacetic acid was evaporated in a stream of nitrogen. For injection into the hplc apparatus the residue was dissolved in the buffered hplc solvent, (this neutrallizes the remaining trifluoroacetic acid as can be seen by the fading of the yellow colour of the mixture). Hplc was performed using a 7.8 mm x 10 cm C_{18} µ-Bondapak column eluted at 7.0 mL/min with 165:95 methanol/water containing 1 g/L of sodium dihydrogen phosphate neutrallized to pH 6.5 with ammonium hydroxide, and 0.01% each of mercaptoethanol and dithioerythritol. For establishing the elution time of "cold" MK-0571, the UV detector was set at 280 nm. The elution time was adjusted to 5 to 6 minutes by the addition of a little methanol or water to the elution solvent prior to the prep. run. In the vicinity of the expected elution time, fractions were collected at 20 second intervals. Scintillation counting of the fractions indicated which fractions contained product, and these were pooled. Evaporation of these fractions in a stream of nitrogen was taken only as far as the removal of the methanol, then the solution was diluted with water containing 0.01% of mercaptoethanol, and this solution was passed through a C_{18} Sep Pak, which resulted in 100% retention of the radioactivity. On washing through the Sep Pak with methanol (2 mL aliquots), all of the radioactivity was eluted in the first two fractions which were combined and evaporated in nitrogen. This material was purified a second time using the hplc conditions described above, and the resulting product was found to be pure by analytical hplc, (3.9 mm x 30 cm C_{18} column eluted with 50:40:75:1:0.7 acetonitrile/methanol/water/sodium acetate trihydrate/acetic acid). The yield was 225 µCi.

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5. In two pairs of probe runs, the addition of tri-n-butylphosphine at this point gave better results than when the compound was added just prior to the acidification, where one might expect it to be effective in reducing any disulfide formed in the reaction.

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