A Short Radiosynthesis of 6-[C^3H_3]-Dorzolamide at Very High Specific Activity and Optical Purity

[1,*]W.-s. Eng, [1]H.D. Burns, [2]G.S. Ponticello, and [2]H.G. Selnick

[1]Department of Pharmacology and [2]Department of Medicinal Chemistry
Merck Research Laboratories, West Point, PA 19486

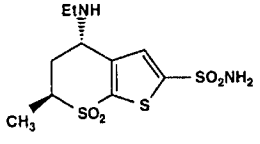
SUMMARY

6-[C^3H_3]-Dorzolamide# was prepared starting from N,N'-bis-Boc-6-desmethyl-dorzolamide. An efficient radiosynthesis was developed involving a regioselective and stereo-controlled ^{3}H-methylation of the α–sulfone carbanion in the presence of the monoprotected sulfonamide anion. The methylation led to a mixture (ca. 7 : 1 for 4S,6R : 4S,6S) of the diastereomeric, tritiated products. Removal of the Boc protecting groups followed by HPLC purification of the resulting diastereomers afforded 6-[C^3H_3]-dorzolamide with > 99 % radiochemical purity, > 98.4 % enantiomeric excess, and a specific activity of > 74 Ci/mmol.

Key Words: dorzolamide, tritium, methyl iodide, methylation, MK-507

INTRODUCTION

Dorzolamide is a carbonic anhydrase inhibitor developed for the treatment of glaucoma [1]. Radioisotopically labeled dorzolamide at the 6-methyl position with very high specific activity was needed for various biological and biochemical studies. The position of the radiolabel was essential since previous studies had shown that radiolabels at other positions, such as on the N-ethyl moiety or within the bicyclic ring, were either difficult to achieve synthetically or were unacceptable due to extensive degradative metabolism.



Dorzolamide

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* Please address correspondences to this author.

\# Dorzolamide is the generic name for MK-507 or TRUSOPT™ (5,6-dihydro-4*H*-(S)-4-ethylamino-(S)-6-methylthieno[2,3-*b*]-thiopyran-2-sulfonamide-7,7-dioxide, HCl salt)

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One of the isotopologs of the title radiotracer, 6-[$^{14}CH_3$]-dorzolamide (**5**), was first synthesized with a specific activity of 26.3 mCi/mmol in an eight-step sequence from a homochiral cyclic sulfone intermediate [2]. As shown in Fig. 1, the key feature of the label incorporation was an efficient diastereoselective methylation, with [^{14}C]methyl iodide, of an enantiomerically enriched (4R)-*t* -butyldimethylsilyl protected alcohol **1** to produce a 15:1 mixture of the **4R,6S : 4R,6R** diastereomers, **2** and **3**, in near quantitative yield. While this elegant asymmetric alkylation provided an efficient entry to the desired radiotracer, the inherent, low specific activity of ^{14}C-labeled radiotracers (only ca. 60 mCi/mmol for one ^{14}C atom per label compound molecule) is inadequate for some biological and biochemical studies. Tritium labeled compounds can be prepared at much higher specific activity since the inherent specific activity of one tritium atom is ca. 30 Ci/mmol (500 times higher than that of ^{14}C) and it is often convenient to incorporate more than one tritium atom/molecule into tritiated radiotracers.

Although the route used to prepare [^{14}C]dorzolamide is attractive, it was not convenient to adapt this sequence for the synthesis of a high specific activity, tritium-labeled version of the desired radiotracer. This was partially due to the fact that in the ^{14}C-sequence, the functional group elaborations after the methylation were still lengthy and involved procedures which were not easily amendable for use in a sub-milligram scale radiolabeling with tritium.

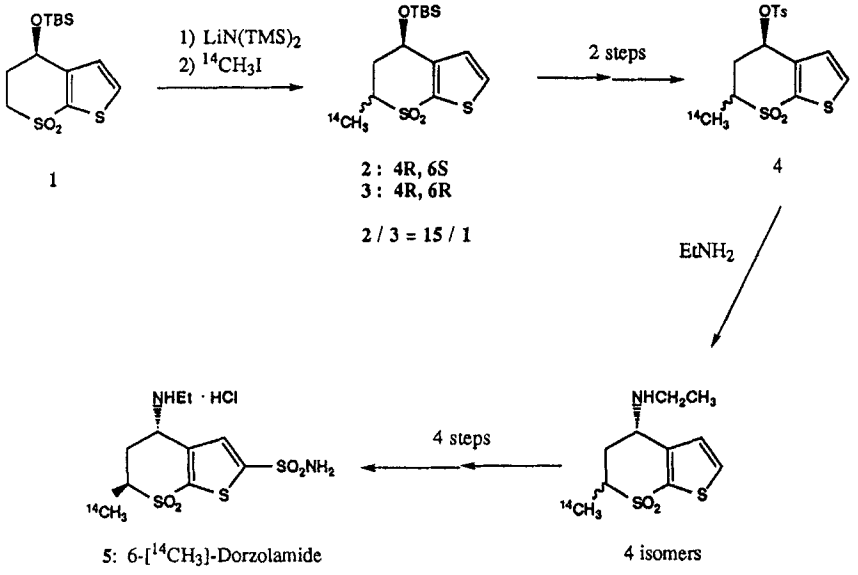

Figure 1. Reaction scheme for synthesis of 6-[$^{14}CH_3$]-dorzolamide.

We wish to report a two-step radiosynthesis of dorzolamide labeled with multiple tritium atoms at the 6-methyl position, starting from a protected 6-desmethyl-dorzolamide (**7**), as shown in Fig. 2. This route offered the required high specific activity (about 2000 - 3000 fold higher than the [^{14}C]dorzolamide). Additionally, unlike the radiosynthesis of the [^{14}C]dorzolamide, in which

sophisticated chiral resolution was involved, this synthesis of 6-[C^3H_3]-dorzolamide started with an optically enriched chiral 4S-ethylamino precursor (**6**). As a result, the methylated, diastereomeric products could be easily purified with conventional HPLC to afford the desired 6-[C^3H_3]-dorzolamide (**10**) in high optical purity.

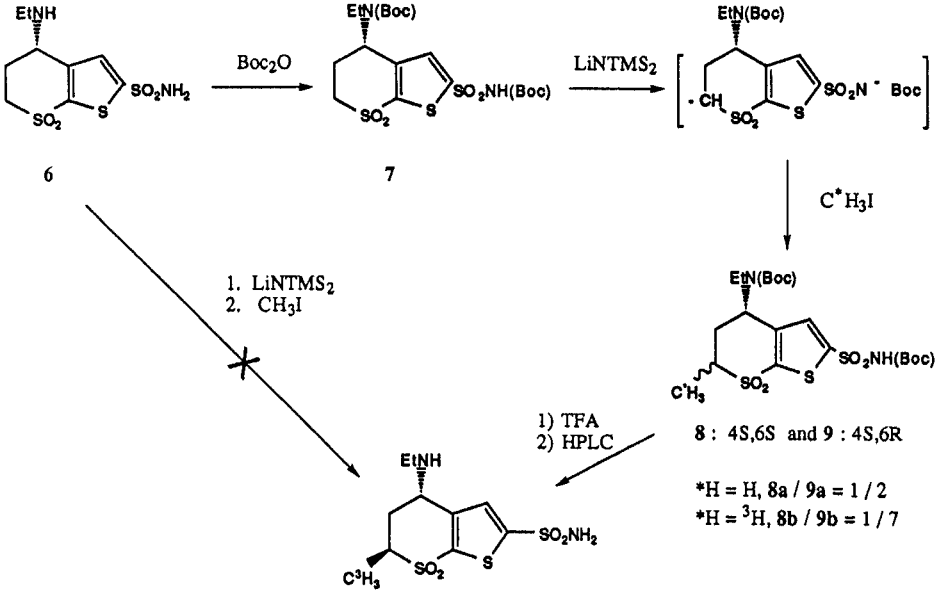

Figure 2. Reaction scheme for synthesis of high specific activity 6-[$^3CH_3$]-dorzolamide.

Results and Discussion

As shown in Fig. 2, the key feature of the short radiosynthesis of 6-[C^3H_3]-dorzolamide (**10**) was the regioselective and stereo-controlled methylation of the C-6 (α–sulfone) carbanion. Our initial attempts to develop conditions for the synthesis of high specific activity [^{3}H]dorzolamide focused on methylation of the multiple anions generated directly from desmethyldorzolamide (**6**, Fig 2), and resulted in the formation of complex mixtures. At least three competing sites (C-6, NHEt, and SO_2NH_2) in desmethyldorzolamide are capable of being directly methylated under the conditions used. Thus, an alternate synthetic strategy was investigated which involved protection of the amine and sulfonamide moieties in order to direct the methylation exclusively to C-6.

The *t*-Butoxycarbonyl (Boc) group was chosen to protect the two nitrogens. The Boc group has been extensively used in protecting amines as well as amides [3]. However, relatively few examples of the use of Boc-protected sulfonamides in organic synthesis are known [4]. In our approach, desmethyldorzolamide (**6**) was first treated with di-*t*-butyl dicarbonate in the presence of Et_3N/DMAP [5] to give the corresponding N, N'-bis-Boc-derivative **7** in 20 % yield (reaction conditions not optimized). As a result, the secondary amine in **7** was completely protected while the mono-N-Boc-protected sulfonamide was electronically and sterically deactivated enough to decrease

the chances of forming the corresponding N-alkylation product. In fact, such sulfonamide-methylated by-products (easily identifiable by NMR: $\delta \sim 3$ ppm $NC\underline{H}_3$) were never detected even under conditions where excess base and MeI were used.

The complete success of this synthetic strategy also requires that the stereochemistry at C-4 not be lost due to base catalyzed epimerization of the ethylamino substituent. This type of epimerization has been reported on acyl-derivatized analogs of dorzolamide when the acylamine was heated for an extended time in the presence of excess base [6]. However, in our synthesis, since the generation of the carbanion and subsequent methylation proceeded rapidly and at very low temperature, this epimerization was considered to be unlikely, as later evidenced by the high enantiomeric excess (>98.4%) of the final product, 6-[C^3H_3]-dorzolamide.

Thus, treatment of the protected desmethyldorzolamide (**7**) with a slight excess of base resulted in formation of the double anion as shown in Fig 2. Further treatment with a limited amount of methyl iodide gave a mixture of the C-6 methylated isomers (**8** and **9**). Here, the use of a limited amount of methyl iodide is a realistic model of the actual tritiation in which it is essential to make full use of the radioisotope source, as well as to prevent the formation of the C-6 dimethylated by-product. Similar to the [^{14}C]dorzolamide synthesis (2), the major methylation product **9** (4S, 6R) in the current synthesis corresponds to a conformation in which both C-4 and C-6 substituents are equatorial. This favored conformation was highly desirable for **2** (4R, 6S) in the ^{14}C-synthesis, since the desired stereochemical configuration of the final product (4S, 6S) was achieved by the subsequent inversion of the C-4 substituent via nucleophilic displacment of the tosyl group in **4** with ethylamine (Fig 1). However, the methylation product with the desired C-6 (S) configuration in the current synthetic sequence, bis-Boc-dorzolamide (**8**), is the less favored, hence a minor, product. In our model reaction with 2.5 eq of base and 0.9 eq of methyl iodide at -78 ^{o}C, a mixture of the C-6 methylated isomers (6R: 6S = 2:1, methylation yield ca. 20 - 30 %) was obtained, as indicated by NMR analysis of the crude reaction mixture (Fig 3). In the actual radiosynthesis, under very similar reaction conditions, a 35% radiochemical yield of the two methylated products was obtained with a ratio of 6R: 6S = 7:1 (determined by TLC analysis). Despite the low yield of the desired 6S isomer, a sufficient quantity was obtained for subsequent conversion to 6-[C^3H_3]-dorzolamide (**10**).

Attempts to separate **8a** and **9a** as the bis-Boc-derivatives under a variety of reverse phase HPLC conditions were unsuccessful. Therefore, the subsequent deprotection was carried out with TFA on the unpurified mixture of the tritium-methylated diastereomers (**8b** and **9b**), which also contained the unreacted, nonradioactive desmethyl precursor **7**. Although this non-aqueous deprotection was sluggish, it offered a convenient sample preparation for the final HPLC purification, since TFA could be simply removed *in vacuo*. Initial HPLC purification was performed on a semipreparative, reverse phase column to remove the majority of the unreacted desmethyl precursor and the undesired 4S, 6R product. The fraction containing the desired radiotracer **10** was collected and further purified on an analytical reverse phase column. A fraction was thus obtained which was found to contain > 99 % of the desired 4S, 6S isomer, [C^3H_3]dorzolamide (**10**), as well as < 1 % of its 4S, 6R diastereomer and other radioactive impurities (Fig. 4A).

The absence of a significant quantity of the nonradioactive desmethyldorzolamide (**6**, from **7**) was confirmed by the high specific activity of 6-[C^3H_3]-dorzolamide (> 74 Ci / mmol, essentially identical to that of C^3H_3I), determined by the UV analysis of the final fraction against a standard curve established with radioinert dorzolamide. If present, desmethyldorzolamide (**6**) would have

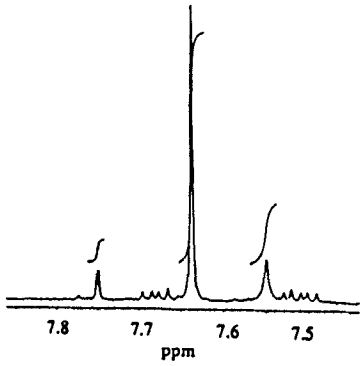

Figure 3. NMR (aromatic region) of crude reaction mixture from model reaction showing the presence of unreacted desmethyldorzolamide (7, δ = 7.64 ppm), 4S,6S product (8a, 7.75 ppm) and 4S,6R product (9a, 7.55 ppm).

lowered the effective specific activity of the radiotracer. The optical purity of 6-[C^3H_3]-dorzolamide was determined with chiral HPLC to be > 99.2 % (approaching the detection limit), which corresponds to > 98.4 % e.e. (Fig. 4B). This direct separation of the enantiomers of dorzolamide was the first achieved with a chiral HPLC column (Ciral-AGP). On the other hand, attempts of this separation on another chiral stationary phase, CYCLOBOND I RN, were unsuccessful, in spite of its ability to separate a close analog, MK-927 (a racemic mixture). Similar results were also reported by Matuszewski and Constanzer [7]. The very high chemical and optical purity of 6-[C^3H_3]-dorzolamide

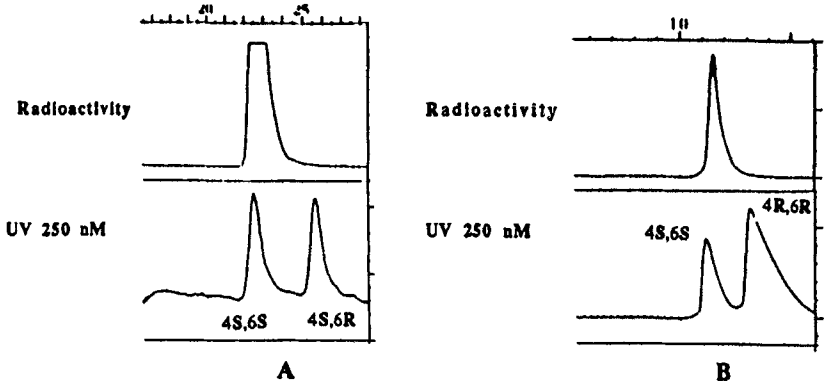

Figure 4. HPLC analysis of 6-[C^3H_3]-dorzolamide. Panel A shows the result of reverse phase HPLC analysis of 6-[C^3H_3]-dorzolamide co-injected with a mixture of authentic, non-radioactive 4S,6S-dorzolamide and its 4S,6R-diastereomer. Panel B shows the result of chiral HPLC analysis of 6-[C^3H_3]-dorzolamide co-injected with a mixture of authentic, non-radioactive 4S,6S-dorzolamide and its 4R,6R-enantiomermer. In both of these HPLC analyses, 6-[C^3H_3]-dorzolamide (shown by radioactivity, upper traces) coelutes with the authentic dorzolamide (shown by UV absorbance, lower traces). The HPLC results also reveal the high radiochemical and optical purities of the final product.

(**10**) obtained after a simple separation from a complex mixture reflected not only the efficiency of HPLC purification, but also the inherent advantages of this synthetic sequence. Finally, the exclusive retention of the C-4 stereochemistry throughout the synthesis, as shown by the retention of enantiomeric excess (> 99 % e.e. in **6** to > 98.4 % e.e. in **10**), indicated the absence of epimerization of C-4 under the current methylation conditions.

Although this radiochemical synthesis was carried out in two steps, the alkylation and the subsequent deprotection steps can, in principle, be carried out in the same reaction vessel to reduce the number of manipulations involving radioactive compounds. If the reaction time for the deprotection step can be shortened significantly, this short synthetic sequence may find applications in radiotracer syntheses involving short-lived isotopes, such as methylation with [^{11}C]methyl iodide.

EXPERIMENTAL

Material and Methods

Unless otherwise mentioned, solvents and reagents were purchased from either Aldrich-Sigma or Fisher Scientific. ^{1}H NMR spectra were obtained in a Varian Infinity-300 spectrometer in $CDCl_3$ with TMS (δ = 0 ppm) as an internal standard. High performance liquid chromatography (HPLC) analyses of radioinert compounds were performed on a system consisting of a Waters 600E gradient pump, a Rheodyne injector, and either a C_{18} μ-Bondapak reverse phase column (4 mm x 25 cm), a Vydac C_{18} peptide protein column (4 mm x 25 cm), or a Zorbax RX-C8 column (4 mm x 15 cm) for reverse phase HPLC and a Chromtech Chiral-AGP column (4 mm x 10 cm) for chiral HPLC. The pump and UV detection and data processing with a Waters 991 diode array detector were controlled via a Powerline^{TM} package. HPLC analyses and preparations of radioactive compounds were performed on a similar system with the exception that the on-line detection results from a Beckman 171 HPLC radioactivity detector were also collected and processed. Preparative HPLC purifications were performed on an Alltech C_{18} Econosil semi-preparative column (10 mm x 25 cm). Sample radioactivities were determined on a LKB Wallac 1410 scintillation counter and UV measurements were performed on a HP-8452A diode array spectrophotometer.

Synthesis

N,N'-Bis(t-butoxycarbonyl)-5,6-dihydro-4H-(S)-4-ethylamino-thieno[2,3-b]-thiopyran-2-sulfonamide-7,7-dioxide (**7**)

The HCl salt of 6-desmethyldorzolamide (**6**, α= 34.6 °, e.e.> 99 %, 300 mg, 1.1 mmol) was mixed with Et_3N (240 mg, 2.2 mmol) and the mixture was dissolved in 2 mL of dry CH_2Cl_2. Into the solution was added DMAP (260 mg, 2.2 mmol), followed by di-*t*-butyl dicarbonate (500 mg, excess) in batches (gas generated). The resulting yellow solution was stirred at room temperature for

72 h. The solvents were removed *in vacuo* and the residue was purified with silica gel flash chromatography (0 - 100% ethyl acetate/hexane) and 105 mg of purified 7 was obtained (0.22 mmol, 20 % yield).

HNMR (δ, ppm): 7.64 (s, 1H), 4.09 (dd, J = 6.4 and 3.9 Hz, 1H), 3.69 (ddd, J = 13.7, 9.8, and 2.7 Hz, 1H), 3.32 (ddd, J = 13.7, 9.3, and 2.5 Hz, 1H), 2.6 - 2.8 (m, 3H), 2.4 - 2.5 (m, 1H), 1.54 (s, 9H), 1.49 (s, 9H), 1.11 (t, J = 7.3 Hz, 3H)

N, N'-Bis(t-butoxycarbonyl)-5,6-dihydro-4H-4-ethylamino-6-methylthieno[2,3-b]-thiopyran-2-sulfonamide-7,7-dioxide (**8a** and **9a**)

To a dry ice cooled solution of N,N'-diBoc-desmethyldorzolamide (**7**, 17 mg, 0.036 mmol) in 1mL of anhydrous THF was added dropwise 0.11 mL of lithium bis(trimethylsilyl)amide (1 M in THF, 0.11 mmol). The bright yellow solution was stirred at -78 °C for 20 min before a solution of methyl iodide (5 mg, 0.033 mmol) in THF (anhydrous, approx. 0.2 mL) was added dropwise. The resulting mixture was further stirred at -78 °C for 4 h and then was quenched by the addition of 1 mL of wet ether (ether treated with NH_4Cl-saturated water solution) at -78 °C. The mixture was then concentrated *in vacuo* and analyzed by NMR. The resonance of the thiophene protons (**7**: δ = 7.64 ppm, **8a**: 7.75 and **9a** 7.55) were indicative of the reaction progress, which revealed that while the total methylation yield approached ca. 20 - 30 % with very few byproducts, the 6R:6S product ratio (**9a** : **8a**) was approximately 2:1, favoring the undesired 6R methylated product (**9a**). Attempts to separate the two isomers with reverse phase HPLC under a variety of conditions failed to yield satisfactory results. The identity of the two isomers was confirmed by a comparison with the products of independent syntheses from the authentic dorzolamide and its 6R isomer using a procedure similar to that described for **7**.

N,N'-Bis(t-butoxycarbonyl)-5,6-dihydro-4H-4-ethylamino-6-[$^3H_3$-methyl]-methylthieno[2,3-b]-thiopyran-2-sulfonamide-7,7-dioxide (**8b** and **9b**)

The actual tritium-methylation was carried out at NEN (DuPont). Into several milliliters of anhydrous THF in a 10 mL reaction flask was dissolved N,N'-diBoc-desmethyldorzolamide (**7**, 35 mg, 0.073 mmol). The solvent was then removed *in vacuo*, and the residue dried under high vacuum with warming to 35 °C. The dried residue was redissolved in 2 mL of anhydrous THF under Ar and cooled to -78° C. To the solution was added dropwise 0.22 mL of lithium bis(trimethylsilyl)amide (1 M in THF, 0.22 mmol) and the mixture stirred for 10 minutes, after which it was frozen. Freshly generated C^3H_3I (0.07 mmol, specific activity: ca. 80 Ci/mmol) was added to the reaction flask via vacuum transfer. The resulting mixture was allowed to warm to -78 °C and stirred at that temperature for 4 h. The reaction was quenched at -78 °C by the addition of 1 mL of wet ether (treated with NH_4Cl-saturated water solution). Volatiles were removed *in vacuo* and the residue redissolved in EtOH. A total of ca. 500 mCi of radioactivity was recovered. HPLC analysis (Zorbax RX-C8, 60 % CH_3CN / 1% TEAOAc buffer, pH = 4, 1 mL / min, UV 254 nm) of the resulting mixture revealed that the methylation yield was ca. 35 % (based on the absorbence of the unreacted precursor, k' = 1.7 and that of **8a** and **9a**, k' = 2). TLC analysis (SiO_2 G, 60 % ethyl acetate/hexane) of the mixture showed three radioactive peaks: 5 % at the origin, 83 % at R_f 0.24 (**9b**), and 12 % at R_f 0.32 (**8b**). This corresponded to a **9b**: **8b** ratio of 7 : 1.

5,6-Dihydro-4H-(S)-4-ethylamino-(S)-6-[$^3H_3$-methyl]-methylthieno[2,3-b]-thiopyran-2-sulfonamide-7,7-dioxide (6-[C^3H_3]-dorzolamide, **10**)

A fraction (30 mCi) of the mixture of **8b** and **9b** was concentrated *in vacuo* and 1 mL of TFA was added to the residue. The resulting solution was stirred overnight at room temperature. The resulting mixture was concentrated *in vacuo* and first purified with an Alltech semiprep HPLC column (15 % CH_3CN / H_2O, both containing 0.1 % TFA, 1.5 mL / min). The fraction containing the desired 4S, 6S isomer (10) was collected and further purified with a Vydac C_{18} column (6 % CH_3CN / H_2O, both containing 0.1 % TFA, 1 mL / min). Thus, a fraction (ca. 1.5 mCi) was obtained, the HPLC analysis of which (Vydac C_{18}, 6 % CH_3CN / H_2O, both containing 0.1 % TFA, 1 mL / min) revealed >99 % of the desired radiotracer (10, k' = 2.14) and < 1% of its 4S, 6R isomer (k' = 2.57) and other radioactive impurities. The optical purity of the fraction was determined by chiral HPLC (Chiral-AGP, 0.5 mL/min, 100% 0.1 M phosphate buffer, pH 7) and found to contain > 99.2 % of 4S, 6S isomer (**10**, k' = 4.7) and < 0.8 % of its 4S, 6R isomer (k' = 5.75). The specific activity of the radiotracer **10** was found to be > 74 Ci / mmol, based on the radioactivity and the mass, determined by UV analysis (254 nm) against a standard curve established with non-radioactive dorzolamide, of the final product **10**.

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