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Research Article

Preparation of tritium-labelled BIIL 260 of high specific radioactivity

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Summary

Various approaches to the synthesis of tritium-labelled BIIL 260 with high specific radioactivity were investigated. Attempts to incorporate tritium directly into BIIL 260 were made by solid-phase isotope exchange with tritium gas and by isotope exchange with tritiated water which yielded a final product with specific activities ranging from 2 to 7 Ci/mmol. However, the solid-phase and liquid-phase dehalogenations of an appropriate synthon fragment of BIIL 260 followed by its subsequent conversion to the final product via chemical synthesis yielded the desired tritium-labelled BIIL 260 with specific activities of 25 or 71 Ci/mmol, depending upon the precursors and methods used in the dehalogenation step. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: tritiation; synthesis of labelled BIIL 260

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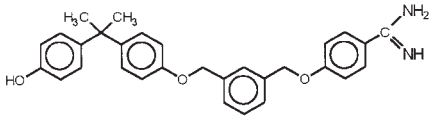
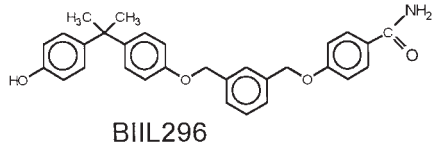
Introduction

BIIL 284 BS is a new LTB_4 receptor antagonist developed as a novel anti-inflammatory agent for several chronic inflammatory conditions. BIIL 284 is a pro-drug which is metabolized to the active metabolite BIIL 260. BIIL 260 has a high affinity for the LTB_4 receptor with a K_i value of 1.7 nM on human neutrophilic granulocytes.[1]

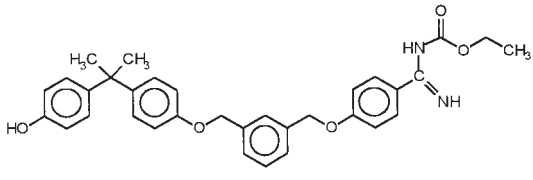
For direct characterization of BIIL 260 in its interaction with the LTB_4 receptor, the preparation of tritium-labelled BIIL 260 with a high molar radioactivity was required. Methods allowing the direct tritium labelling of the final product[2–6] are always preferable over the more time-consuming and complex synthetic methods[7,8] which need to be used if the compound is unstable under the given reaction conditions. The purpose of the present work was the synthesis of tritium-labelled BIIL 260 with a high specific radioactivity.



BIIL260



BIIL296



BIIL284 BS

Results and discussion

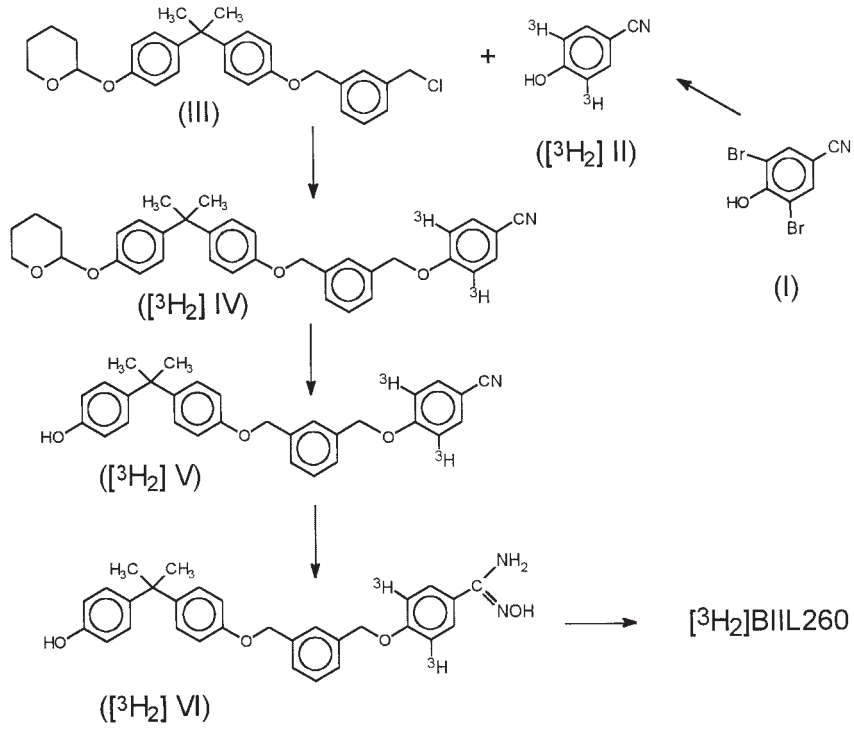
The first attempts to introduce tritium directly into BIIL 260 were made via the solid-phase isotope exchange method[2,3] (reaction times ranging from 15 to 120 min and temperatures from 140°C to 200°C; palladium catalysts on various carriers – carbon, barium sulphate, etc.) and via the isotope exchange method[4,5] with tritiated water of high specific radioactivity (reaction times from 0.5 to 16 h, reaction temperatures ranging from 23°C to 160°C, using various palladium catalysts). We found out that under these conditions only preparations with specific

radioactivities from 2 to 7 Ci/mmol were obtained, possibly due to the instability of the BIIL 260 molecule under the given reaction conditions. Further, treatment of BIIL 260 with gaseous tritium via the method [6] (using palladium catalysts; solvents such as methanol, ethanol, acetonitrile or their mixtures; and tritium pressure – 400 hPa) led to a complete destruction of the substance during reaction times ranging from 10 to 30 min. This was probably due to the previously described[9–11] phenomenon of hydrogenolysis of compounds with the general formula $R'O\text{-}CH_2\text{-}C_6H_4\text{-}R$ with the formation of R'-OH and $R''\text{-}C_6H_4\text{-}CH_3$ moieties. Thus, 2,2-bis-(4-hydroxyphenyl) propane, *m*-xylene, and $HO\text{-}C_6H_4\text{-}C(NH_2) = NH$ resulted from the degradation of the BIIL 260 molecule. Consequently, in order to achieve our goal to prepare BIIL 260 with a higher specific radioactivity we devised two labelling pathways described below (Schemes 1 and 2):

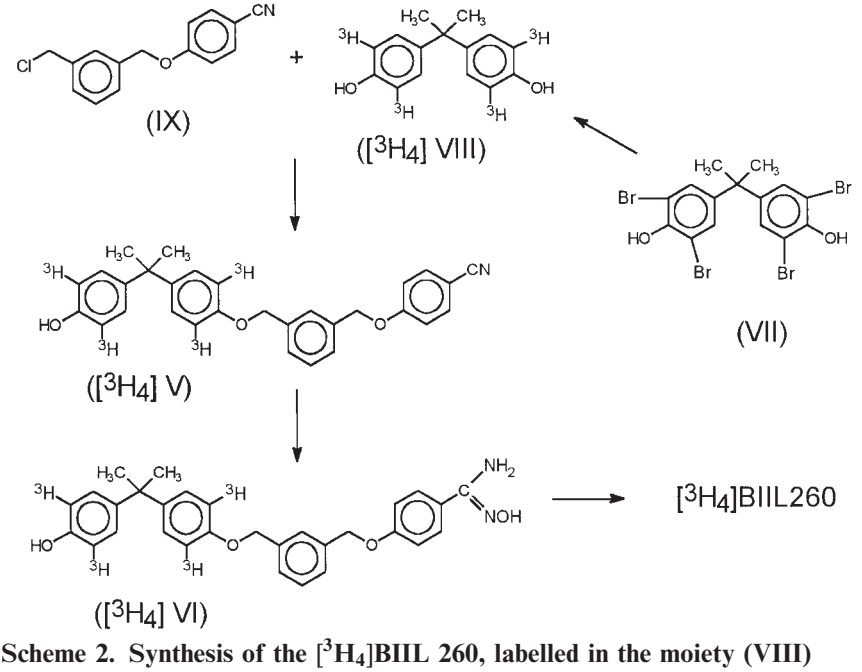
1. Preparation of the tritiated 4-hydroxybezonitrile ([^{3}**H**$_2$] **VIII**) intermediate via the dehalogenation of 4-hydroxy-3,5-dibromobezonitrile (**I**), with subsequent condensation of the resulting labelled product ([^{3}**H**$_2$] **II**) with the chloride (**III**) and conversion of the resulting nitrile ([^{3}**H**$_2$] **IV**) to [^{3}**H**$_2$] BIIL 260 as per Scheme 1.
2. Preparation of tritiated 2,2-bis-(4-hydroxyphenyl)propane ([^{3}**H**$_4$] **VIII**) via dehalogenation of (**VII**) and subsequent condensation of the labelled product with $n(m\text{-}ClCH_2C_6H_4CH_2O)C_6H_4CN$ (**IX**) and conversion of the resulting nilrile ([^{3}**H**$_4$] **V**) to [^{3}**H**$_4$]BIIL 260 as per Scheme 2.

The first reaction pathway yielded tritium-labelled BIIL 260 with a specific radioactivity of 25 Ci/mmol, while the second reaction pathway yielded tritium-labelled BIIL 260 with a specific radioactivity of 71 Ci/mmol. The key reactions in the overall process were the introduction of the label and the reduction of [^{3}**H**]amidoximes ([^{3}**H**$_2$] **VI** and [^{3}**H**$_4$] **VI**) to [^{3}**H**]BIIL 260 in the presence of Raney nickel.

The dehalogenation was carried out via both the solid-phase and the liquid-phase methods. In the case of the dehalogenation of nitrile (**I**), the best results were achieved via the liquid-phase method. Apparently, considerable loss of substrate occurred via sublimation of the unchanged reactant (high reaction temperature of above 100°C is needed for an efficient high-temperature solid-phase dehalogenation) making this method not practical in this case. In the case of the dehalogenation



Scheme 1. Synthesis of the [$^3H_2$]BIIL 260, labelled in the moiety (II)



Scheme 2. Synthesis of the [$^3H_4$]BIIL 260, labelled in the moiety (VIII)

of 2,2-bis-(4-hydroxy-3,5-dibromophenyl)propane (**VII**), the solid-phase method gave better results (Table 1).

The difficulty of completing the remaining reaction steps resided in the fact that only milligram quantities of the key starting labelled product could be prepared via the above reactions. Using the reaction pathway 1, high yields (up to 90% and above) of the product ([^{3}**H**$_2$] **IV**) were obtained when using a rather large excess of the chloride (**III**) by comparison with the nitrile ([^{3}**H**$_2$] **II**). We also found out that upon reacting ([^{3}**H**$_4$] **VIII**) with the chloride (**IX**), an equimolar ratio of these reagents should be used in order to minimize the formation of a dimeric condensation by-product. Due to this, the yield of the desired labelled product ([^{3}**H**$_4$] **VIII**) cannot exceed 50% of the theoretical value.

The removal of protective groups and the transformation of the tritium-labelled nitriles (**V**) to the labelled amidoximes (**VI**) did not cause problems since these reactions could be carried out with a large excess of non-labelled reagents. For reasons of safety, all reactions involving labelled intermediates were carried out in sealed ampoules. The stirring of the reaction mixtures was achieved by rotation of the ampoule in a rotary evaporator. A water bath provided the necessary temperature control.

The most complex reaction step was the reduction of the tritium-labelled amidoximes (**VI**) by Raney nickel. This reaction was carried out with microgram amounts of the labelled precursor (**VI**). The reaction yield was strongly influenced by the conditions used to prepare the Raney nickel. Thus, we found that the reduction yield of amidoximes (**VI**) was raised by up to 40% by treating the Raney nickel with acetic acid while also using a substance-to-catalyst ratio of 1:40 (by weight). In

Table 1. Dehalogenation of 2,2-bis-(4-hydroxy-3,5-dibromophenyl)propane (VII); tritium pressure 400 hPa, 5% Pd/C catalyst

Temp. (°C)	Reaction time (min)	Solvent	Yield (%)	Specific activity (Ci/mmol)	Method
140	15	—	68	54	Solid-phase
160	15	—	54	69	Solid-phase
160	30	—	50	71	Solid-phase
180	15	—	23	83	Solid-phase
200	15	—	17	88	Solid-phase
20	80	Ethylacetate-triethylamine (20:1)	73	63	Liquid-phase

addition, the yield of the final labelled product was strongly dependent on the reaction time (Table 2).

Experimental

The solvents, catalysts and other chemicals were commercial reagents. Initial preparations and standard compounds (including BIIL 296 – a hydrolysis product of BIIL 260) were commercial reagents or were prepared by Boehringer Ingelheim Pharma KG, Germany.

The analysis and/or purification of various intermediates and final products was carried out by high-performance liquid chromatography (HPLC) and by thin layer chromatography (TLC).

TLC analysis was carried out using silica gel plates and the following solvent system: ethylacetate–methylene chloride–acetic acid–water (40:20:20:5:5). The R_f values were (0.64) for BIIL 260, and (0.88) for BIIL 296.

Analytical HPLC was performed on a 4×150 mm column packed with Separon SGX C18, 7 μm, at a flow rate of 0.8 ml/min, using the following systems:

(A) methanol–water–trifluoroacetic acid (78:22:0.1);
(B) acetonitrile–50 mM potassium phosphate buffer, pH 3, (4:6);
(C) acetonitrile–50 mM potassium phosphate buffer, pH 3, (1:1);
(D) acetonitrile–50 mM potassium phosphate buffer, pH 3, (6:4);
(E) methanol–50 mM potassium phosphate buffer, pH 3, (7:3);
(F) methanol–50 mM potassium phosphate buffer, pH 3, (3:1).

Table 2. Kinetics of the conversion of [^{3}H] amidoximes (VI) to tritium-labelled BIIL 260

Reaction time	Composition of the reaction mixture		Amount of BIIL 260 in
(min)	[^{3}H]amidoximes (%)	[^{3}H]BIIL 260 (%)	the standard sample (μg)
30	81	11	0.2
55	69	15	0.3
60	66	20	0.5
75	39	52	1.1
80	30	70	2.2
90	16	74	1.9
135	11	62	1.2
180	8	17	0.3
300	3	8	0.1

Preparative HPLC purifications were carried out on a 10×250 mm column packed by

Silasorb C18, 13 μm, at a flow rate of 2.0 ml/min, using the following mobile phases:

(G) acetonitrile–50 mM potassium phosphate buffer, pH 3, (6:4);
(H) methanol–50 mM potassium phosphate buffer, pH 3, (4:1);
(I) methanol–water–trifluoroacetic acid (850:150:1).

Retention times are given in Table 3.

Bromination of phenolic precursors

In a typical run, 1.26 mmol of phenolic precursor dissolved in 3 ml of methanol and 2 ml of concentrated aqueous ammonia was stirred for 70 min with a solution of 2 ml of methanol containing 300 μl of bromine. Following this, the reaction mixture was evaporated to dryness, dissolved in 2 ml of methanol and acidified to pH 2 with a 1 N solution of HCl. The resulting solution was evaporated once more, dissolved in 5 ml of ethylacetate and washed with water (3×1 ml). The organic solvent was removed by evaporation and the resulting residue was dissolved in 4 ml of methanol, heated to 70°C after which 2 ml of water was added with stirring. The desired brominated derivative was formed upon slow cooling and the isolated yield was 60–65%.

Table 3. Retention times of BIIL 260 and its intermediates in various chromatographic systems

Compound	Retention time (min)								
	A	B	C	D	E	F	G	H	I
I	—	—	—	—	3.21	—	—	—	—
II	—	—	—	—	2.21	—	—	2.32	—
III	—	—	—	—	—	12.11	—	—	—
IV	8.52	—	—	—	—	—	—	—	—
V	—	—	—	—	—	14.16	—	—	9.76
VI	6.02	—	—	—	—	—	—	—	7.12
VII	6.20	—	—	—	—	—	—	—	—
VIII	2.65	—	—	—	—	—	—	—	2.68
IX	3.75	—	—	—	—	—	—	—	—
BIIL 260	9.46	10.94	8.24	4.31	8.86	—	13.36	—	10.79
BIIL 296	—	—	10.15	5.53	—	—	—	—	—

Dehalogenation of compound (I)

1. A batch of 13 mg of the substance (I), 20 μl of triethylamine in 500 μl of dioxane, and 40 mg of 5% $Pd/BaSO_4$ were placed in the reaction ampoule and the mixture was frozen in liquid nitrogen. After evacuation, the ampoule was filled with gaseous tritium up to a pressure of 400 hPa. The reaction mixture was stirred for 2.5 h at the given reaction temperature followed by freezing with liquid nitrogen and evacuation of the unreacted tritium gas. The catalyst was removed by filtration and washed with methanol (3 × 2 ml), and the filtrates were combined and evaporated 3 times with methanol (3 × 2 ml) to remove the labile tritium. The residue left, representing the crude reaction product, was purified by preparative HPLC. The yield of ([$^3H_2$] **II**) was 80–85%, with a specific radioactivity of 25 Ci/mmol.
2. In a typical run, 10 mg of the substance (I), coated on 100 mg of 5% $Pd/BaSO_4$ was placed in the reaction ampoule which was evacuated and filled with gaseous tritium at a partial pressure of 400 hPa. The reaction was carried out for 15 min at various temperatures such as 100°C, 120°C, 140°C, 160°C and the crude reaction mixture was processed as described above. The product resulting from the reaction carried out at 100°C contained 46% of ([^{3}**H**$_2$] **II**), 26% of mono-bromoderivative and 28% starting material (I) while at 160°C, the reaction mixture consisted of 54% ([^{3}**H**$_2$] **II**), 14% mono-bromoderivative, and 32% starting material. The specific radioactivity of the resulting products were in the range 25–30 Ci/mmol.

Synthesis of [$^3H_2$] *V*

1. 12.8 Mg of (**III**), K_2CO_3 (4 mg), KI (1.5 mg), 2.6 mg of ([^{3}**H**$_2$] **II**), and 0.2 ml of acetonitrile were placed in an ampoule. The latter was sealed and the reaction was kept at 85°C for 2 h while stirring. The reaction mixture was diluted with 1 ml of acetonitrile, the solution was filtered off and the residue was washed with acetonitrile (3 × 1 ml). The combined filtrates were evaporated to dryness and dissolved in 1.5 ml of ethylacetate–methanol (2:1) mixture.
2. 1.5 ml of the reactant solution was placed in the reaction ampoule, and 10 mg of toluenesulphonic acid was added. After 2 h the solvents were evaporated and the residue was dissolved in 6 ml of ethylacetate. The toluenesulphonic acid was extracted with 3 ml of 1 N sodium bicarbonate and twice with 3 ml of water. The resulting organic phase was evaporated to dryness and the remaining crude product was purified by HPLC. The isolated yield of ([^{3}**H**$_2$] V) was 80–85%, while the specific radioactivity remained the same.

Synthesis of [$^3H_4$] *VIII*

16 mg of (**VII**) coated on 160 mg 5% Pd/C was placed in a reaction ampoule, evacuated, filled with gaseous tritium at a partial pressure of

400 hPa and the reaction carried out at 160°C for 30 min after which the reaction mixture was processed as described above. The yield of ([^{3}**H**$_4$] **VIII**) was 50%, the specific radioactivity 71 Ci/mmol and a radiochemical purity greater than 98%.

Synthesis of [$^3H_4$] *V*

A suspension of 2 mg of ([^{3}**H**$_4$] **VIII**), 3 mg of (**IX**), 3.52 mg of K_2CO_3 and 2.8 mg of KI in 0.1 ml of acetonitrile was heated with stirring for 90 min at 80°C in a sealed ampoule. The reaction mixture was diluted with 1 ml of acetonitrile, filtered off, and the solid residue washed with acetonitrile (3 × 1 ml). The combined filtrates were evaporated to dryness and the rest was dissolved in 1.5 ml of ethylacetate–methanol (2:1) mixture. After careful HPLC purification the yield of the desired compound ([^{3}**H**$_4$] **V**) was about 40%, the specific radioactivity remained unchanged, and a radiochemical purity of 95–97% was achieved.

Synthesis of tritium-labelled amidoximes (VI)

A solution of 1.9 mg of tritium-labelled (**V**) in 1 ml of methanol was treated with 0.4 ml of an aqueous solution of sodium carbonate (70 mg) and hydroxylamine hydrochloride (62 mg) in a sealed ampoule at 100°C over 2 h. Following this, the ampoule was cooled to room temperature, opened, and its contents evaporated to dryness. The resulting product was extracted by shaking with 1.2 ml of a (1:1) mixture of ethylacetate and water followed by additional extraction of the aqueous layer with ethylacetate (3 × 0.6 ml). The resulting organic extracts were evaporated to dryness and dissolved in 1 ml of methanol containing 40 μl of acetic acid. The yield at this stage after chromatographic purification was 80–85% and the radiochemical purity 95–97%.

Synthesis of tritium-labelled BIIL 260

A suspension of 20 mg of fresh Raney nickel in 1 ml of methanol was treated with 0.1 ml of acetic acid for 1 h, after which the above methanolic solution of the labelled precursor containing (0.5 mg) of (**VI**) was added and the reaction left for 80 min. The yield of [^{3}H] BIIL 260 ranged from 10% to 40%, and the specific radioactivity of the final preparation was equal to that of the labelled precursor.

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