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Research Article

Synthesis of tritium-labelled BIBN4096, an experimental h-CGRP-antagonist

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Summary

Synthesis of tritium labelled BIBN 4096 – 1-piperidine-^{3}H-carboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2 H)-quinazolinyl)-, [R-(R*,S*)]-, – a h-CGRP-antagonist for the treatment of migraine is described. Selective tritiation of a heterocyclic aromatic fragment in the presence of aromatic ring in a precursor of BIBN4096 was successfully carried out using the solid state catalytic exchange method. Subsequent completion of the synthesis sequence gave the final [^{3}H]BIBN4096 with a specific activity of >170 Ci/mmol. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: tritium labelled BIBN4096; solid state isotope exchange

Introduction

Pharmaceuticals, in general, contain both aromatic and heterocyclic fragments. The most commonly used methods to introduce tritium are the isotope exchange reaction and the reduction or dehalogenation of appropriate precursors using tritium gas[1]. If the structure of the compound of interest proved to be labile under the reaction conditions when using any of the above

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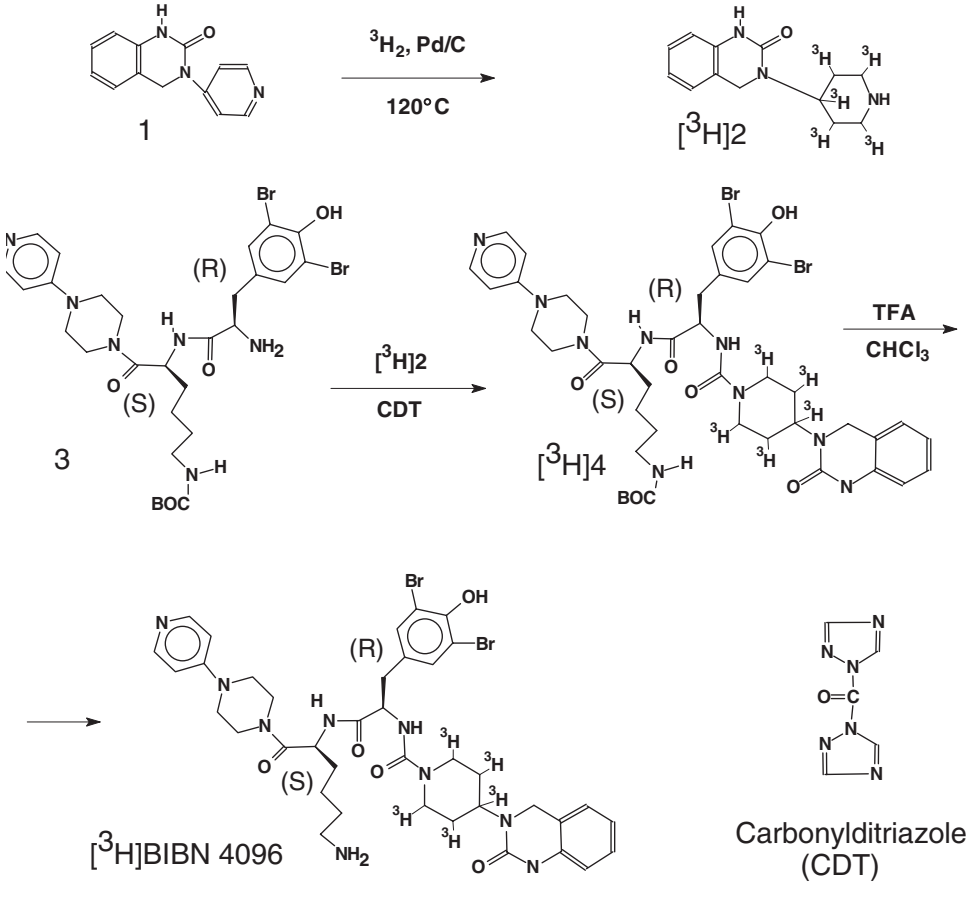
methods then tritium should be introduced into a suitable intermediate precursor of the given compound followed by subsequent synthesis of the target compound.

Piperidine and piperazine fragments are often present in the structure of potent drugs (that show a strong effect on the central nervous system) such as midocalm, pipotiazine, thioridazine, meridine. To study the biological properties of such compounds, labelled analogs with a specific activity above 100 Ci/mmol are often required. The following approach can be used for their synthesis: a precursor containing a pyridine fragment instead of piperidine is prepared followed by a subsequent hydrogenation with tritium gas[2] that will give a product containing tritium atoms in the piperidine ring.

The difficulty of this approach is that conventional procedures of heterocycle hydrogenation[3] require protonic solvents at very low pH values. Unfortunately, in the case of the tritiation reaction considerable isotopic



Scheme 1. The synthesis of [^{3}H]BIBN 4096

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dilution is observed due to isotope exchange between the mobile protons of the solvent and tritium gas. As a result, the specific activity of the products obtained are several times lower than expected. The catalytic tritiation of benzylamine to [^{3}H]cyclohexylmethylamine at room temperature (using tritium gas, 5% Rh/Al_2O_3, PtO_2, 5% 0.5 N HCl in methanol, 10 h) leads for example to a product with a specific activity of 40–50 Ci/mmol only. However, when the hydrogenation of benzylamine with tritium gas was carried out by heating a mixture of the substrate with 5% Rh/Al_2O_3 at 60°C for 3 h instead, the resulting [^{3}H]cyclohexylmethylamine had a specific activity of 215–225 Ci/mmol[1].

The goal of this study was to synthesize tritium labelled BIBN 4096, a new calcitonin gene-related peptide (CGRP) receptor antagonist, with a high specific activity.

The synthesis of [^{3}H]BIBN 4096 is shown in Scheme 1.

Experimental

The reagents and catalysts (5% Pd/C, 5% $Pd/CaCO_3$, PdO, PtO_2, 5% Rh/Al_2O_3, $RhCl_3$, 1,1'-carbonyl-di-(1,2,4-triazole) (**CDT**), trifluoroacetic acid) were commercial reagents from Sigma-Aldrich. Diethyl formamide with water content less than 0.02% was obtained from Merck. The precursors for the synthesis of BIBN4096 (i.e. **1**, **2**, **3**, **4** and **BIBN4096** were synthezised by Boehringer Ingelheim Pharma KG according to the method described in Switek and Braunger[4].

The analysis of the labelled intermediates and final product were carried out by HPLC using 4 × 150 mm column with Kromasil $100C_{18}$, 7 μm. A flow rate of 1.0 ml/min was used while the mobile phase consisted of various ratios of methanol and 50 mM ammonium phosphate buffer (pH = 2.8) as follows: a (33:67) mixture for **2** (elution time 3.22 min); a (45:55) mixture for BIBN4096 (elution time 11.60 min); a (65:35) mixture for **3** (elution time 2.09 min) and for **4** (elution time 8.38 min); and finally, a (20:80) mixture for **2** (elution time 7.73 min) and for **1** (elution time 8.76 min).

To acquire and process the chromatographic data, the Chrom&Spec Data Station (Ampersand, Ltd., Russia) was used. Radioactivity was measured using a LKB1215 scintillation counter.

The reaction conditions were optimized using a 1% tritium–protium mixture according to the procedure described in Shevchenko and Nagaev[2] in order to determine the relationship between the specific activity of the resulting product and the following parameters: (a) the reaction time (0.25–12 h), (b) the type of catalyst, (c) the catalyst-to-compound ratio (from 3:1 to 15:1), and (d) reaction temperature (40–210°C).

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Preparative synthesis of tritium labelled BIBN4096

[^{3}H]Compound **2**

A solution of **1** (21 mg) in methanol (0.8 ml) with 5% Pd/C (210 mg) was evaporated with stirring. The remainder was carefully dried and powdered, and 55 mg of the mixture were placed into a reaction ampoule that was afterwards evacuated and then filled with tritium gas under a 333 hPa. The reaction was carried out at 120°C for 15 min. Upon removal of excessive tritium gas under vacuum, the residue was extracted with methanol-1 N HCl (10:1) (5×1.0 ml) and then with 1 N HCl (5×0.5 ml). The filtrates were combined and then evaporated to dryness. The labile tritium was removed by repeated azeotropic distillation with methanol (3×2.0 ml). After HPLC purification of the resulting crude product (Figure 1) and desalting, about 0.8 Ci of [^{3}H]compound **2** with a specific activity of 180–185 Ci/mmol was isolated as a solution in methanol.

[^{3}H]Compound **4**

An aliquot (0.5 ml) of the methanol solution of [^{3}H] compound **2** (0.6 Ci) was introduced in a reaction ampoule and then evaporated to dryness. Separately a solution of compound 3 (25.5 mg) in diethylformamide (0.2 ml) was added to a cold solution of 1,1′-carbonyl-di-(1,2,4-triazol) (**CDT**, 6 mg) in diethylformamide (0.43 ml) and the mixture was stirred for 5 min at 0°C and then for an additional 15 min at room temperature. Following this a 0.38 ml aliquot of the prepared diethylformamide reaction mixture of compound 3 and **CDT** was

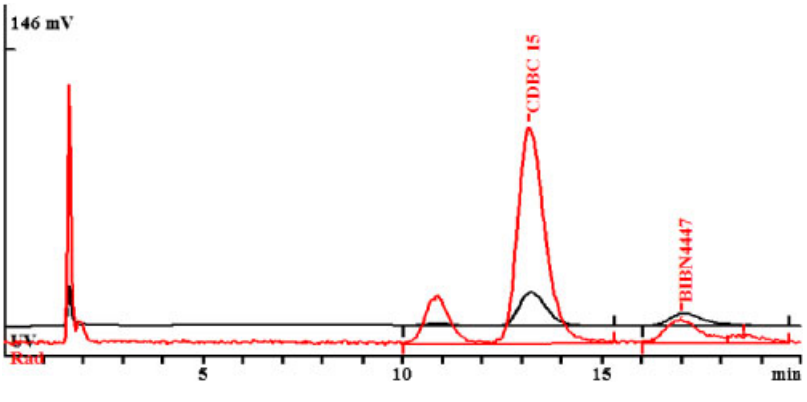

Figure 1. Preparative purification of [^{3}H]CDBC 15 on Kromasil 100 C_{18}, 8×150 mm, 15% MeOH-50 mM ammonium phosphate buffer (pH = 2.8), flow 2 ml/min (no more than 0.2 mg must be loaded onto the column, otherwise overloading observed; after methanol evaporation the sample was applied on SepPack C_{18} cartridge, washed with water and eluted with methanol)

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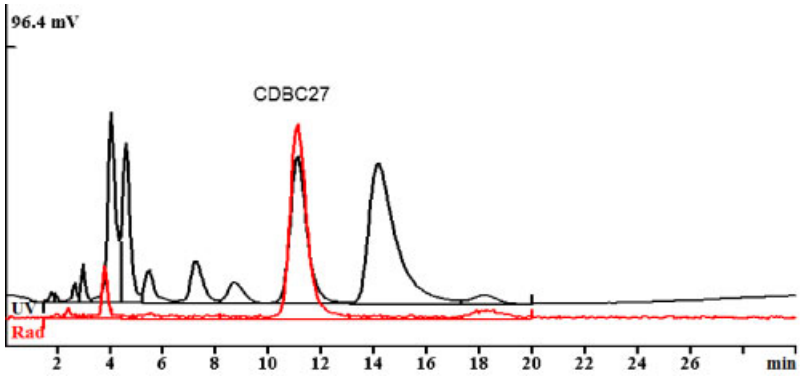

Figure 2. Preparative purification of [^{3}H]CDBC27 on Kromasil 100C_{18},8 × 150 mm, 65% MeOH-50 mM ammonium phosphate buffer (pH = 6), 2 ml/min (after evaporation of methanol, desalting was carried out on SepPack C_{18}; the cartridge was washed with 1 ml of 20% methanol and the target compound was eluted with 4 ml of methanol)

added to the evaporated residue of [^{3}H] compound **2**. The resulting molar ratio of the non-labelled components and of the labelled compound was 6:1. The ampoule was sealed and its contents were mixed at 70°C for 2.5 h. The ampoule was then cooled to + 5°C, opened, and treated with water (1 ml) and 10% potassium phosphate buffer (0.25 ml). Diethylformamide and volatile reaction by-products were removed by solid-phase extraction on SepPack C_{18} (loaded as 10% solution in MeOH, washed with 3 ml of MeOH–water, 35:65, and eluted with 4 ml of MeOH with 0.1% TFA), the reaction mixture was purified by HPLC (Figure 2). The resulting [^{3}H] compound **4** was desalted by solid-phase extraction on a SepPack C_{18} HPLC column and dissolved in 0.4 ml chloroform.

[^{3}H]BIBN4096

The [^{3}H] compound **4** was converted to [^{3}H] **BIBN4096** by acidic hydrolysis. The reaction was conducted by stirring for 75 minutes the solution of [^{3}H] compound **4** in chloroform (0.4 ml) as above with trifluoroacetic acid (70 µl). After purification and desalting stages, the isolated yield of the [^{3}H] **BIBN4096** was 45% (starting from [^{3}H] compound **2**), with a specific activity of 170–175 Ci/mmol and radiochemical purity of 98.5%.

Results and discussion

The synthesis of tritium labelled BIBN4096 – 1-piperidine-*^{3}H*-carboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl] amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2 H)-quinazolinyl)-, [R-(R*,S*)]-, – with a specific activity of no less than

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100 Ci/mmol was required for biological studies. According to the elaborated scheme of the synthesis (Scheme 1), only the compound **2** moiety could be taken as the starting intermediate for the labelling of BIBN4096, because the other part of the molecule (compound **3**) needed in the [^{3}H]BIBN4096 synthesis contained a fragment labile to hydrogenolysis.

Attempts to introduce the tritium label directly into compound **2**, using the solid state isotope exchange (reactions conducted during 0.25–12 h in the presence of 5% Pd/C, 5% Pd/$CaCO_3$, PdO, PtO_2, 5% Rh/Al_2O_3, $RhCl_3$, at temperatures between 40 and 210°C, were unsuccessful because the specific activities obtained did not meet the required specification. Therefore, compound **1** was used as the initial compound, synthesized according to the same procedure as compound **2**, except that the molecule contained the pyridine moiety in place of piperidine.

The catalysts listed above were used for the hydrogenation of compound **1**. The catalytic exchange reaction in solution was found not to be useful because the specific activity of the resulting [^{3}H] compound **2** did not exceed 50 Ci/mmol. Therefore, a selective solid state hydrogenation was applied for this class of compounds for the first time. The goal was to hydrogenate the pyridine fragment while preserving the aromatic ring intact. The best results were obtained at 120°C during 15 min on 5%Pd/C using 10:1 catalyst-to-compound ratio.

Probably, the main reason for the selectivity in the hydrogenation of a heterocyclic aromatic fragment is due to the nature of the activated tritium particles. If these particles are tritium cations [1], the selectivity of the process should be high since the protonation of the pyridine fragment proceeds more effectively than that of the benzene ring. If the aromatic structure of the pyridine fragment gets disturbed, its hydrogenation will be then facilitated. The fact that the amount of the desired intermediate (reduced pyridine ring in the presence of a benzene ring) in the reaction mixture was seven times higher than that of the other possible hydrogenation products, proving that the selectivity of the process was quite high. This is a clear evidence that tritium cations play an active role in the hydrogen spillover reactions.

Acknowledgements

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