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C-14 Labelling of NVP VID400 - A Specific Vitamin D_3-Hydroxylase Inhibitor

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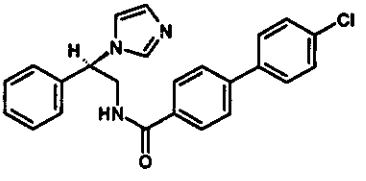
Summary

The synthesis and analysis of [^{14}C]**NVP VID400** **1**, a specific vitamin D_3-hydroxylase inhibitor is reported. The key intermediate is (R)-2-amino-1-phenyl-[1-^{14}C]ethanol **9** synthesized in an effective, enantioselective approach using a borane reduction of phenacyl chloride **6** in the presence of (R)-oxazaborolidine-catalyst **2**. After N-acylation of **9** the resulting amide **10** was converted to the oxazoline **12**, which when treated with imidazole ring-opened to the title compound **1**. Both reactions - ring-closure and ring-opening - went with complete configurational inversion. The secondary isotope effect induced splitting of ^{13}C-NMR signals enabling the quantification of the isotopic purity and thereby the specific activity of **1**.

***Key words*: NVP VID400**, (R)-2-amino-1-phenyl-[1-^{14}C]ethanol, enantioselective synthesis, isotope effect, ^{13}C-NMR spectroscopy

Introduction

2-(1H-Imidazol-1-yl)-2-phenyl-ethyl-amides are interesting sub-structures from a pharmacological point of view [1]. From a series of structurally related derivatives, **NVP VID400** **1**, a specific inhibitor of vitamin D_3-hydroxylases was selected for development.



1 (**NVP VID400** - (R)-antipode)

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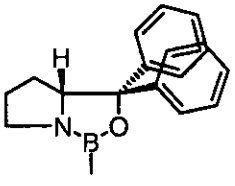
In order to study the pharmacokinetic and metabolic behavior of **NVP VID400 1** in animals a radiolabeled isotopomer had to be synthesized. To receive maximum metabolic information even after potential hydrolytic cleavage of the amide bond, it was decided to place the C-14 label in the pharmacologically more interesting amine moiety. From a synthetic point of view the benzylic position appeared to be the most appropriate.

Based on the synthetic pathway developed by H. Egger [2] for the unlabelled drug substance (1R)-2-amino-1-phenyl-ethanol **9** was also identified as the key intermediate for the radiosynthesis of [^{14}C]**NVP VID400 1**.

Synthesis (R)-2-amino-1-phenyl-[1-^{14}C]ethanol 9

For the synthesis of (R)- and (S)-2-amino-1-phenylethanol **9** there are different procedures described [3]. However, in most cases they contain a resolution step. For radio-economic reasons we concentrated on the enantioselective sequence given in Scheme 1.

[^{14}C]Benzoyl chloride **5** was reacted with diazomethane to obtain the diazomethyl phenyl [carbonyl-^{14}C]ketone, which upon treatment with ethereal HCl was converted *in situ* to the 2-chloro-[carbonyl-^{14}C]acetophenone **6** [4]. The key step of the overall procedure was the enantioselective borane reduction of **6** in the presence of the (R)-oxazaborolidine-catalyst **2** (Corey-Bakshi-Shibata = CBS-catalyst [5]).



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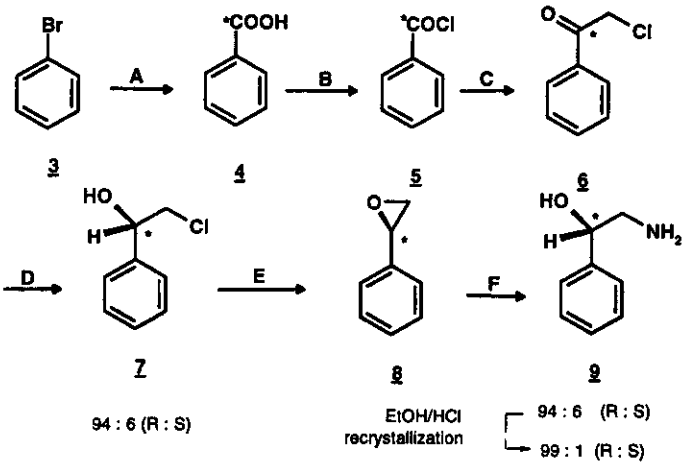
Freshly prepared catalyst **2**, obtained by condensation of methylboronic acid with (R)-(+)-2-(diphenylhydroxymethyl)pyrrolidine, and a slight excess of borane, afforded **7** in more than 90%ee. Base induced ring-closure converted **7** to the (R)-(+)-phenyl-[^{14}C]oxirane **8**, which was regioselectively ring-opened with aqueous ammonia to afford the key intermediate (R)-2-amino-1-phenyl-[1-^{14}C]ethanol **9**. Repeated recrystallization of pre-purified **9** separated traces of regio- and enantiomers. Chiral-HPLC finally confirmed the optical purity was 99%ee, which was sufficient for further reaction.

Scheme 1: Synthesis of (1R)-2-amino-1-phenyl-[1-^{14}C]ethanol **9**



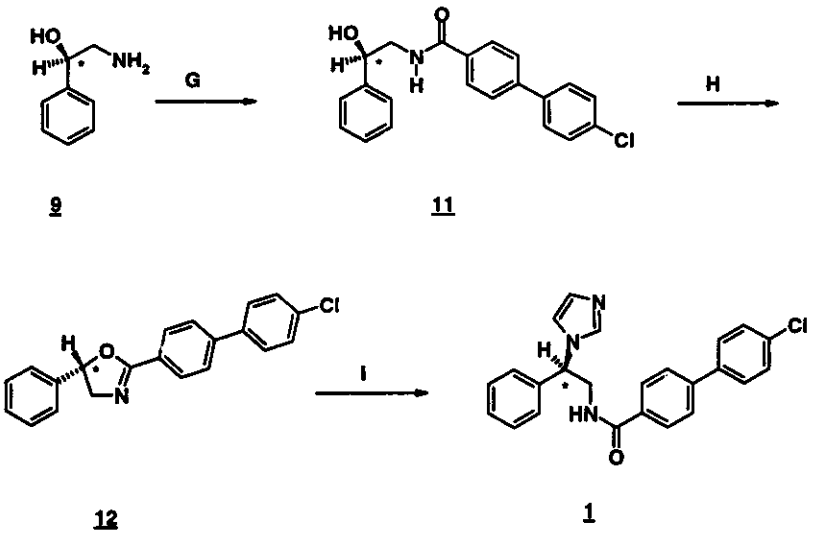
Reaction conditions: A) Mg, ether, $^{14}CO_2$, 2h, -10°C, 100%; B) $SOCl_2$, DMAP, benzene, 3h, 110°C, 89%; C) CH_2N_2, Et_2O, 2h, 0°C; HCl, 12h, r.t., 92%; D) 1M BH_3 in THF, **2**, THF, 1h, r.t., 100%; E) 2N NaOH, Et_2O, 1h, r.t.; F) aqu.NH_3, MeOH, 3.5h, 40°C;

Synthesis of [^{14}C]NVP VID400 **1**

The direct introduction of the imidazole moiety by substitution of the hydroxy-group of **9** under MITSUNOBU-conditions [6] suffers from significant racemization. Therefore, an alternative approach was chosen. N-acylation of **9** with 4'-chlorobiphenyl-4-carboxylic acid **10** using 1,1'-carbonyl-diimidizole as coupling reagent gave the respective amide **11**, which when treated with methanesulfonic anhydride cyclized to the (S)-2-oxazoline derivative **12** with complete configurational inversion [7]. It is interesting to note that methanesulfonic anhydride was essential for the cyclization. When p-toluenesulfonyl choride was used only starting material was isolated. Subsequent heating of **12** in the presence of imidazole ring-opened the oxazoline with additional complete configurational inversion to give the R-enantiomer of [^{14}C]NVP VID400 **1** [8].

In summary [^{14}C]NVP VID 400 **1** was prepared using a nine-step synthesis in an 18.4 % overall radiochemical yield calculated from the barium [^{14}C]carbonate employed. The optical purity was higher than 98%ee as determined by RA chiral-HPLC.

Scheme 2: Synthesis of [^{14}C]NVP VID400 1



Reaction conditions: G_1) 4'-chlorobiphenyl-4-carboxylic acid **10**, 1,1'-carbonyldiimidazole, DMF, 1h, r.t.,G_2) **9**, 0°C, 16h, r.t., 93%; H) NEt_3, $(CH_3SO_2)_2O$, 3h, 0°C, crystallization, 81%; I) imidazole, isopropyl acetate, 72h, 125°C, crystallization, 84%;

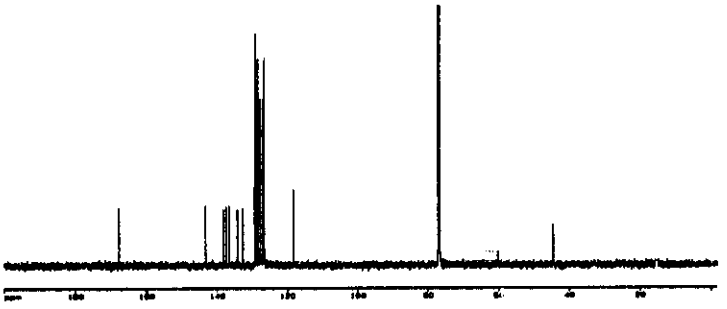
Specific Activity of [^{14}C]NVP VID400 1

The incorporation of a ^{14}C-isotope induces an upfield shift in the range of up to 20 ppb in the adjacent ^{13}C nuclei. This secondary isotope effect [9] results in an observable splitting of the corresponding signals, which can be used for the quantification of isotope ratios. In contrast to direct integration of the residual ^{13}C signal of the labelled position, this method is more robust when there is a weak signal-to-noise ratio. Due to the relatively large linewidth of ^{13}C-NMR signals compared to the small difference in shift, however, this phenomenon has rarely been used for the determination of specific activities [10].

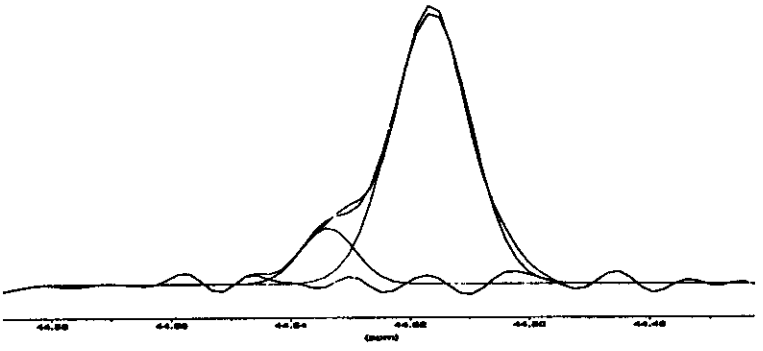
The ^{13}C-NMR spectrum of [^{14}C]NVP VID400 1 revealed a sufficient splitting ($\Delta\delta$ = 17 ppb) of the two signals of ^{13}C-3 attached to ^{14}C-2 and ^{12}C-2 (44.517 ppm and 44.534 ppm, respectively) to permit quantification of the isotope ratio. Despite their incomplete resolution (**<< 1.5**) an estimation of the individual peak areas reflecting the isotope ratio ^{14}C:^{12}C of the benzylic position was possible by peak simulation [11, see figure 2b]. Integration of the peaks resulted in an isotopic ratio ^{14}C :^{12}C of 88:12 corresponding to a specific activity of 54.9 mCi/mmol. This result was in excellent agreement with the specific activities determined by two independent methods (mass spectroscopy : 54.9 mCi/mmol and HPLC/LSC : 56.5 mCi/mmol).

Figure 2: ^{13}C-NMR of [^{14}C]**NVP VID400 1**

a) ^{13}C-NMR of [^{14}C]**NVP VID400 1** ($CDCl_3$, 500.133 MHz)



b) ^{13}C-NMR of [^{14}C]**NVP VID400 1** (detail: 44.48 - 44.58 ppm) - experimental and computer-based simulated peak



Experimental Details

Materials and Methods

Unless otherwise noted, chemicals and radiochemicals were purchased from commercial suppliers. The identity of intermediates and precursors was identified by either chromatographic and/or spectroscopic methods.

Barium [^{14}C]carbonate (1973 mg, 10 mmol, 562 mCi) was converted to [^{14}C]benzoylchloride according to standard procedures.

^{13}C NMR spectra were measured on a BRUKER DMX-500 AVANCE spectrometer at 125.771 MHz at 300 K. To achieve the required resolution of 0.06 Hz/pt, 64K time domain data were acquired at a spectral width of 7788 Hz and Fourier transformed after zero filling to yield 132K time domain data.

Synthesis of 2-chloro-[carbonyl-^{14}C]acetophenone 6

At -20°C [^{14}C]benzoyl chloride (1200 mg, 8.54 mmol, 480 mCi) was added to an excess of ethereal diazomethane-solution. After stirring the yellow solution at 0°C for 2h the reaction mixture was treated with ethereal HCl-solution and subsequently stirred at room temperature overnight. The reaction mixture was made alkaline by addition of aqueous $NaHCO_3$-solution and repeatedly extracted with ether. The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was removed using a Vigreux-column in order to prevent any contamination. Due to the sufficient radiochemical purity (>90%, RTLC) the residue (472mCi) was employed without further purification.

Synthesis of (1R)-2-chloro-1-phenyl-[1-^{14}C]ethanol 7

To a solution of freshly prepared oxazaborolidine complex 2 in dry THF (0.79 mmol in 2.0 ml) and borane-tetrahydrofuran complex (1M, 4.72 ml), 6 (472 mCi) dissolved in absolute THF (8 ml) was added at 25°C within 10 minutes. After completion of the reaction (stirring at room temperature for 1 h; TLC-control: methylene chloride) excess borane was destroyed by careful addition of water. The aqueous solution was extracted with ether. The combined organic phases were dried over $MgSO_4$ and finally concentrated using a Vigreux-column. The residue (464 mCi) showing a radiochemical purity higher than 91% was used without further purification.

HPLC-method for the determination of the optical purity: DAICEL OD, 250 x 4.6 mm, n-hexane : 2-propanol 97 : 3, 20°C, 1.5 ml/min., RA / UV (215 nm)

Synthesis of (R)-(+)-1-phenyl-[1-^{14}C]oxirane 8

(1R)-2-chloro-1-phenyl-[1-^{14}C]ethanol 7 (464 mCi) dissolved in ether (15 ml) was added to aqueous NaOH-solution (2N, 30 mmol, 15 ml) and stirred at room temperature for 1h. After repeated extraction with ether the combined organic phases were dried over $CaCl_2$, filtered and concentrated via a Vigreux-column. The residue was employed without further purification.

Synthesis of (1R)-2-amino-1-phenyl-[1-^{14}C]-ethanol 9

(R)-(+)-phenyl-[^{14}C]oxirane 8 dissolved in methanol (9.5 ml) was added to aqueous ammonia (25%, 27.4 ml) and stirred at 40°C for 3.5h. After completion of the reaction the mixture was concentrated under reduced pressure and purified by flash chromatography (silica gel;

methylene chloride:methanol: ammonia 950:45:5). The resulting material (642 mg, 4.68 mmol) was shown by HPLC to contain 6% S-enantiomer and 5% regioisomer.

In order to separate the enantiomers the crude material was repeatedly recrystallized as the respective hydrochloride from ethyl acetate.

1. Recrystallization: 667 mg of (1R)-2-amino-1-phenyl-[1-^{14}C]-ethanol hydrochloride
   radiochemical purity: 98% (2% S-enantiomer)
2. Recrystallization: 640 mg of (1R)-2-amino-1-phenyl-[1-^{14}C]-ethanol hydrochloride
   radiochemical purity: 98.8% (1.2% S-enantiomer)

Liberating the base yielded 474 mg (3.46 mmol, 191.4 mCi), which correlated to 34% of the barium [^{14}C]carbonate employed.

HPLC-method for the determination of the optical purity: DAICEL OD, 250 x 4.6 mm, n-hexane : 2-propanol 90 : 10, 20°C, 0.4 ml/min., RA / UV (210 nm).

Synthesis of 4'-chlorobiphenyl-4-carbonyl (2(R)-hydroxy-2-phenyl-[2-^{14}C]ethyl)-amide 11

To a suspension of 805 mg of 4'-chlorobiphenyl-4-carboxylic acid **10** (3.46 mmol) in DMF (8 ml) 590 mg of 1,1'-carbonyldiimidazole (3.46 mmol) was added and stirred at room temperature for 1h. The reaction mixture was cooled to 0°C and subsequently combined with a solution of **9** (474 mg, 3.46 mmol, 191.4 mCi) in DMF (2 ml). The mixture was stirred at room temperature for 16h. After completion of the reaction (RTLC-control; silica gel: methylene chloride:methanol:ammonia 100:10:1) the mixture was poured onto ice. The resulting precipitate was filtered off, washed with water and ethanol and finally dried under HV to obtain 1136 mg **11** (93.3% d.Th.).

Synthesis of 2-(4'-chlorobiphenyl-4-yl)-5(S)-phenyl-4,5-di-hydro[^{14}C]oxazole 12

To a solution of **11** (1136 mg, 3.23 mmol) in anhydrous THF (11 ml) combined with triethylamine (2.02 ml) and methanesulfonic anhydride (846 mg, 4.85 mmol) dissolved in anhydrous THF (7 ml) was added at 0°C within 30 minutes. After stirring at 0°C for 3h the reaction was complete (RTLC-control; silica gel, toluene : ethyl acetate 4 : 1) and therefore quenched by addition of aqueous ammonia solution (25%, 0.32 ml). After stirring at room temperature for an additional 15 minutes the mixture was concentrated and finally distributed between ethyl acetate and aqueous $NaHCO_3$-solution. After repeated extraction the organic

phases were dried and evaporated. Purification of the resulting residue (1259 mg) by crystallization from ethanol (7 ml) yielded 876 mg of 12 (81%).

Synthesis of [^{14}C]NVP VID400 1

A mixture of 12 (856 mg, 2.56 mmol) and imidazole (3472 mg, 51 mmol) dissolved in isopropyl acetate (0.3 ml) was heated at 125°C for 72h. After completion of the reaction (RTLC-control: silica gel, toluene : ethyl acetate 85 : 15) the reaction mixture was partitioned between water and ethyl acetate. The organic phase was separated, dried and evaporated. Purification by repeated crystallization from ethanol (4.7 ml) /water (2.9 ml) afforded 744 mg of **[^{14}C]NVP VID400 1** (72%).

HPLC-method for the determination of the optical purity: DAICEL OD, 250 x 4.6 mm, n-hexane : 2-propanol 97 : 3, 20°C, 1.5 ml/min., RA / UV (215 nm).

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