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Carbon-14 Labelling of DIOVAN™ in its Valine-Moiety

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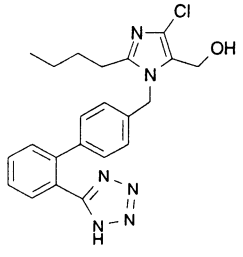
Summary

As a highly specific and non peptide AT_1-antagonist Valsartan **2** is marketed under the tradename DIOVAN™ for effective treatment of hypertension. This paper describes the synthesis of C-14 labelled Valsartan **2**, which incorporates two C-14 isotopes in the valine-moiety. Reaction of (-)-bromo-[1,2-^{14}C]acetyl bornane-10.2-sultam **8a** ((-)-[$^{14}C_2$]BABS) with benzophenone imine gave (-)-diphenyl-methylene[1,2-$^{14}C_2$]glycinyl bornane-10.2-sultam **9** ((-)-[$^{14}C_2$]DPMGBS), which was alkylated with 2-iodopropane to build-up the valine structure **10**. Initially the resulting sultam-protected valine **11** was treated with the benzyl bromide **12** to produce the precursor **13**. However, under conditions routinely used for sultam-cleavage deprotection resulted in the racemization of the amino acid. Successful cleavage was accomplished via N-Boc-protection of **11** followed by hydrolytic cleavage of the auxiliary and esterification to give the L-[$^{14}C_2$]valine benzyl ester **18**. Finally [$^{14}C_2$]Valsartan **2** was synthesised in a 10 step synthesis in an overall radiochemical yield of 10 % relative to the (-)-[1,2-^{14}C]BABS **8a** employed.

Keywords: C-14 Labelling, BABS, diastereoselective synthesis, DPMGBS, Diovan, L-[^{14}C]valine, Valsartan

Introduction

The octapeptide angiotensin II (AII), which is formed from its precursor the decapeptide angiotensin I by proteolytic cleavage by angiotensin-converting enzyme (ACE) has been shown to play a key role in the regulation of blood pressure and electrolytic homeostasis [1]. Blocking the formation of AII via inhibition of ACE has provided a powerful strategy for the treatment of hypertension and congestive heart failure. Attempts to develop therapeutic agents capable of blocking AII at its receptor failed in the past, due to the antagonists being peptides, that lacked oral activity [2]. More recently, starting from imidazole derivatives first described by Furukawa [3], it has been possible to identify and characterize specific, non peptide AII-receptor antagonists - one of the earliest being losartan **1** developed by Du Pont. Replacement of the imidazole-moiety by an acylated amino acid opened up an entire range of orally



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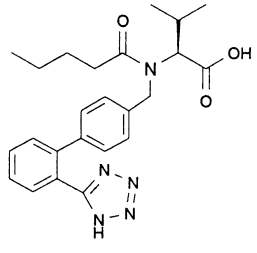

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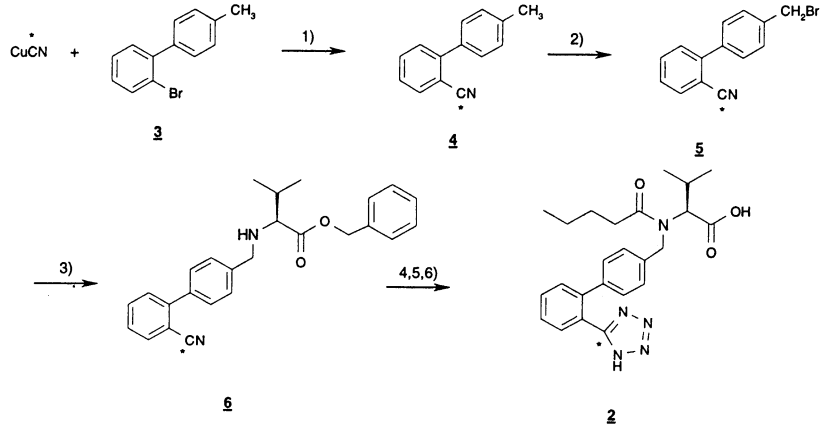
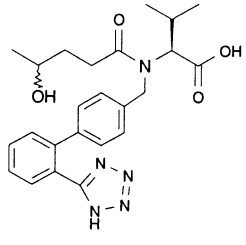
active derivatives [4]. Valsartan **2** belonging to this structural class is a highly specific and selective antagonist of AT_1-receptor (AII-subtype) [5]. For the superior treatment of hypertension Valsartan **2** is marketed as DIOVAN ™ by NOVARTIS Pharma AG. For pharmacokinetic and metabolic investigations (ADME-studies) there was a need for the C-14 labelled material. P. Bühlmayer developed a sequence for the synthesis of the cold drug substance [6], in which the key step utilizes the reaction of the cyanide **6** with tributyl tin azide in order to build-up the tetrazole-moiety. P. Ackermann et. al. [7] adapted this procedure for the mono-labelling of Valsartan **2**. Starting from Cu[^{14}C]CN introduction of the label in the metabolically stable position of the tetrazole-moiety was possible as outlined below. Experimental details of this procedure will be reported elsewhere.

Scheme 1: Synthesis of [^{14}C]Valsartan **2**



Reaction conditions: **1)** DMF, reflux, 32 h, 85 %; **2)** NBS, AIBN, CCl_4, reflux, 30 min., 79 %; **3)** valine benzyl ester, DMF, 80 °C, *N,N*-diisopropylethylamine, 90 min., 85%; **4)** valeryl chloride, *N,N*-diisopropylethylamine, toluene, 2 h, 98%; **5)** Bu_3SnN_3, 24 h, 140°C, 95 %; **6)** Pd/C 10 %, H_2, dioxane, 50 h, 65 %.

Based on investigations by F. Waldmeier [8] valeryl-(ω-1)-hydroxy-valsartan **7** was identified as the only major metabolite in healthy volunteers. Therefore when receiving a request for [$^{14}C_2$]Valsartan labelling of the valine moiety promised a two-fold advantage as it is metabolically stable and easily accessible synthetically.



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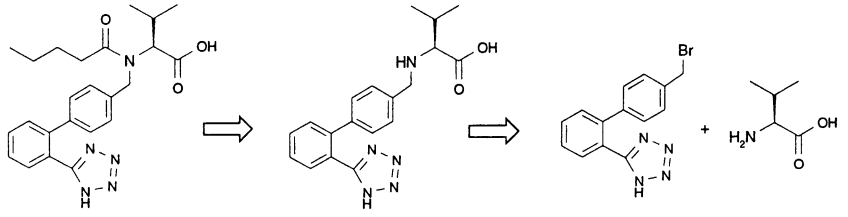
The following scheme illustrates the retrosynthesis of [$^{14}C_2$]Valsartan **2**.

Scheme 2: Retrosynthesis of [$^{14}C_2$]Valsartan **2**



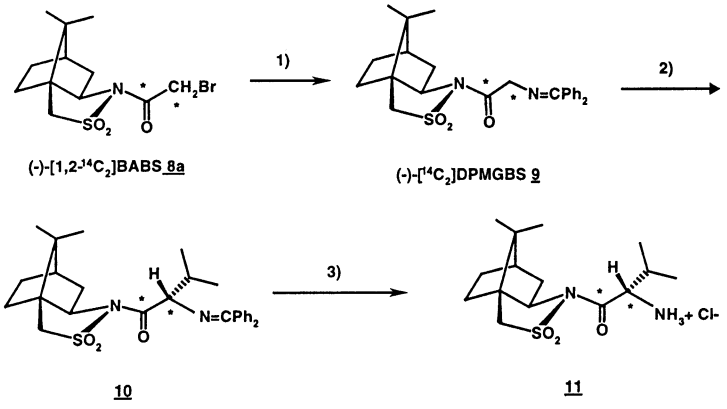
The retrosynthesis identifies L-[$^{14}C_2$]valine as a key intermediate for a straight forward synthesis of [$^{14}C_2$]Valsartan **2**.

Synthesis of L-[$^{14}C_2$]valine

Several stereoselective syntheses of amino acids have been applied to the C-14 labelling. One of them is based on bromo acetyl bornane-10.2-sultam **8** (**BABS**), which was identified as a highly valuable chiral synthon for the preparation of a broad variety of complex, homochiral, singly/multiply labelled compounds [9].

(-)-[1,2-^{14}C]BABS 8a was reacted with benzophenone imine in dry acetonitrile at 70°C to afford (-)-diphenylmethylene[$^{14}C_2$]glycinyl bornane-10.2-sultam **9** ((-)-[$^{14}C_2$]**DPMGBS**). To achieve yields in the range of 70 - 80% even after chromatographic purification triethylamine-mediated deactivation of the silica gel is important in order to avoid cleavage of the diphenylmethylene protecting group. Alkylation of its lithium enolate with 2-iodopropane in THF/DMPU produced **10**, which when treated with 1N HCl-THF (1:1) resulted in the carboxyl-protected L-[$^{14}C_2$]valine **11**. The radiochemical yield of **11** in relation to **8a** was 48 %.

Scheme 3: Synthesis of L-[$^{14}C_2$]valine

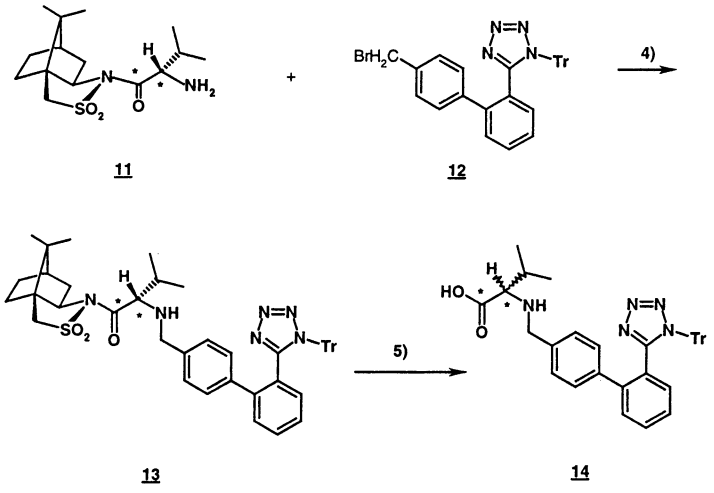


Reaction conditions: 1) Ph_2C=NH, CH_3CN, 70°C, 3.5 h, 75%; 2) n-BuLi, THF, DMPU, -78°C, 30 min., 2-iodopropane, -78°C/15 min, r.t./12 h, 76 %; 3) 1N HCl - THF 1 : 1, r.t., 2 h, 85 %.

Attempt at direct access to [^{14}C]Valsartan 2

For direct synthesis of [^{14}C]Valsartan **2** we reacted **11** with the corresponding benzylbromide **12**. At 80°C in DMF in the presence of N,N-diisopropylamine **13** was obtained in 70 % yield without any traces of bis-alkylated by-product. Under mild conditions [10], however, subsequent removal of the camphorsultam moiety failed. Only ultrasound assisted cleavage (LiOH, LiBr, Bu_4NBr, acetonitrile, RT, 6 h) produced **14** in reasonable yields. Moreover nearly complete racemization and significant (ca. 50 %) formation of a by-product occurred, which resulted most likely from the competitive N-S bond cleavage.

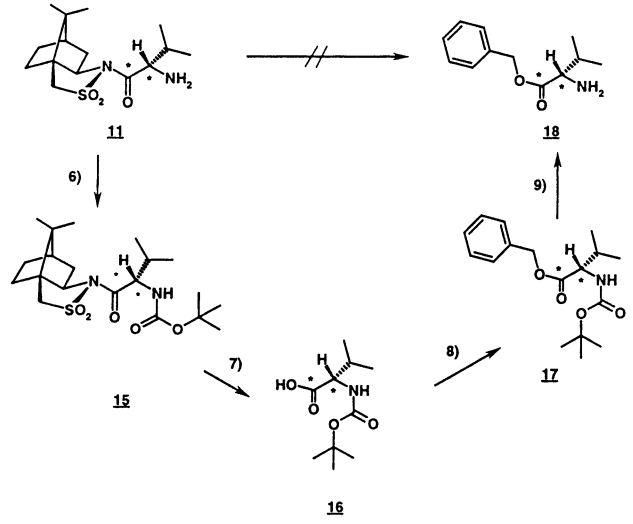
Scheme 4: Attempt at direct access to [^{14}C]Valsartan **2**



Reaction conditions: 4) DMF, *N,N*-diisopropylethylamine, 80°C, 150 min., 67 %; 5) ultrasound, LiOH, LiBr, Bu_4NBr, RT, 6 h.

In addition direct transesterification (LaI_3 [11], $TiCl_4$ [12]) of **11** to the corresponding benzylester **18** failed. Therefore the well-established procedure of N-protection, LiOH-mediated cleavage of the camphorsultam, EDCl-mediated benzyl ester formation and final TFA-mediated N-deprotection was sucessfully applied to the synthesis of L-[$^{14}C_2$]valine benzyl ester **18**. Despite these additional steps, the overall radiochemical yield was 64% relative to **11**.

Scheme 5: Synthesis of L-[$^{14}C_2$]valine benzyl ester **18**



Reaction conditions: 6) $(BOC)_2O$, THF, r.t., 12 h, 99 %; 7) 1N LiOH : THF 1 : 2, r.t., 2 h, 100 %;8) BzOH, DMAP, EDCl, CH_2Cl_2, r.t., 90 min., 65%; 9) TFA, CH_2Cl_2, r.t., 60 min., 100 %.

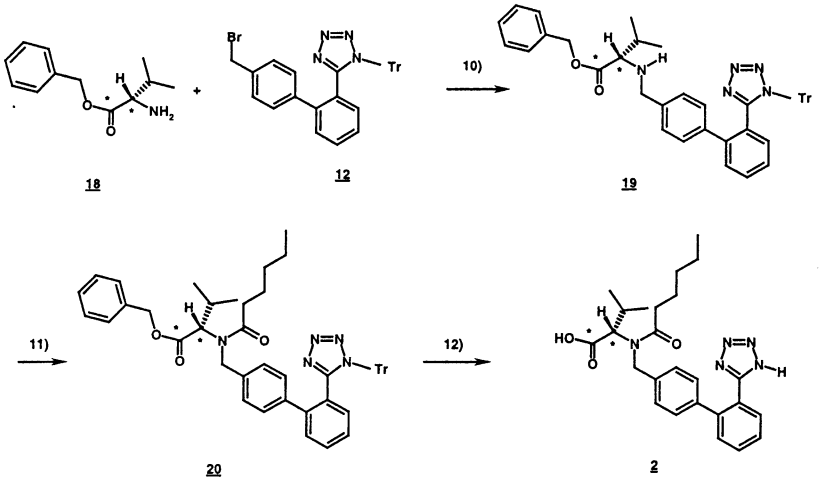
Synthesis of [$^{14}C_2$]Valsartan 2

Under basic conditions the L-[$^{14}C_2$]valine benzyl ester **18** was reacted with a stoichiometric amount of the benzyl bromide **12** to afford **19**. Acylation of the secondary amine **19** under standard conditions (*N.N*-diisopropylethylamine, toluene, RT, 18h) produced the amide **20** in a radiochemical yield of 81% after chromatographic purification. Hydrogenolysis of both protection groups (H_2, Pd/C 10%, EtOH, 40°C, 4h) produced a mixture of [$^{14}C_2$]Valsartan **2** and triphenylmethane, which could be separated by trituration with n-hexane. Final purification was accomplished by recrystallization from ethyl acetate and hexane.

Radiochemical purity and optical purity of [$^{14}C_2$]Valsartan **2** were determined by HPLC-analysis with >99 % and >99 %ee, respectively.

The overall radiochemical yield of this 10 step synthesis was 10 % relative to the **(-)-[1,2-^{14}C]BABS 8a**.

Scheme 6: Synthesis of [$^{14}C_2$]Valsartan **2**



Reaction conditions: 10) DMF, *N,N*-diisopropylethylamine, 80°C, 150 min., 67 %; **11)** valeryl chloride, *N,N*-diisopropylethylamine, toluene, RT, 18h, 81%; **12)** Pd/C 10%, EtOH, H_2, 40°C, 240 min., recrystallisation EtOAc - n-hexane 1 : 1, 60%.

Experimental Details

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

(-)-Diphenylmethylene[1,2-$^{14}C_2$]glycinyl bornane-10.2-sultam 9 ([$^{14}C_2$]DPMGBS) : A solution of carefully dried (-)-bromo[1,2-^{14}C]acetyl bornane-10.2.-sultame **8a** ((-)-[1,2-^{14}C]BABS, 1.13 g, 3.36 mmol), N,N-diisopropylethylamine (2.35 ml, 13.5 mmol), benzophenone imine (4.5 ml, 26.88 mmol) and freshly activated molecular sieves (3A°, 1.6 g) in dry acetonitrile was stirred at 70°C under argon for 3.5 h. After cooling to room temperature the reaction mixture was quenched with cold saturated $NaHCO_3$-solution and four times extracted with ethyl acetate. The combined organic

extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The resulting orange oil was purified by flash chromatography (silica gel 0.06-0.04 mm, n-hexane - ethyl acetate - triethylamine 85 : 15 : 0.5) to give (-)-[1,2-$^{14}C_2$]DPMGBS in 78% yield (1.145 g, 2.62 mmol). The silica gel was thoroughly pretreated with mobile phase before use to avoid hydrolytic cleavage of the diphenylmethylene group during the chromatographic process.

(2S)-Isopropyl-(-)-[1,2-$^{14}C_2$]DPMGBS 10 : Under argon a solution of (-)-[1,2-$^{14}C_2$]DPMGBS **9** (1.82 g, 4.17 mmol) in dry THF (25 ml) was treated dropwise at -78°C with 1.5M n-butyllithium in n-hexane (3.2 ml, 4.8 mmol). After 1 h freshly distilled 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone DMPU (6.6 ml) and isopropyl iodide (2.1 ml, 20.85 mmol) in THF were consecutively injected using a syringe. The reaction mixture was stirred at -78°C for an additional 30 min. and at 5°C overnight. Subsequently it was quenched with cold water and three times extracted with ether. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated. The resulting oil was purified by flash chromatography (silica gel 0.06 -0.04 mm, ethyl acetate - n-hexane - triethylamine 7 : 3 : 0.05) to give (2S)-isopropyl-(-)-[1,2-$^{14}C_2$]DPMGBS in 71 % yield (1.422 g, 2.97 mmol).

(S)-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam 11 : A solution of (2S)-isopropyl-(-)-[1,2-$^{14}C_2$]DPMGBS **10** (939 mg, 2.067 mmol) in THF (20 ml) was treated with 1N HCl (20 ml) and stirred at ambient temperature for 1 h. THF was evaporated, the resulting aqueous phase was three times extracted with dichloromethane, adjusted to pH 9 with cold 2N NaOH, and four times extracted with dichloromethane. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give (S)-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam **11** in 85.3 % yield (554.1 mg, 1.762 mmol).

N-Boc-(S)-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam 15 : A solution of S-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam **11** (554 mg, 1.762 mmol) and di-*tert*-butyl dicarbonate (1.163 g, 5.323 mmol) in dry THF (9 ml) was stirred at ambient temperature under argon overnight. The solution was evaporated and the residue purified by flash chromatography (silica gel 0.06 - 0.04 mm, n-hexane - ethyl acetate 4 : 1) affording N-Boc-(S)-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam **15** in 98.9 % yield (722 mg, 1.742 mmol).

N-Boc-(S)-[1,2-$^{14}C_2$]valine 16 : An aqueous solution of 1N LiOH (17.4 ml) was added dropwise to a stirred solution of N-Boc-(S)-[1,2-$^{14}C_2$]valinyl bornane-10.2-sultam **15** in THF (9 ml) at 0°C under argon. After 2 h at 0°C the resulting solution was extracted with 3 portions of dichloromethane, then acidified with ice-cold 2N HCl to pH 1.65 and repeatedly extracted with dichloromethane - methanol 10 : 2. The combined extracts were dried (Na_2SO_4) and evaporated affording N-Boc-(S)-[1,2-^{14}C]valine **16** in quantitative yield and an enantiomeric purity of > 99%.

N-Boc-(S)-[1,2-$^{14}C_2$]valine benzyl ester 17 : To a solution of N-Boc-(S)-[1,2-$^{14}C_2$]valine **16** in dichloromethane (7 ml) 4-dimethylaminopyridine DMAP (19 mg, 0.160 mmol) was added followed by benzyl alcohol (172 µl, 1.662 mmol). Subsequently the reaction mixture was cooled to 0°C. After addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimde hydrochloride EDCl (364 mg, 1.897 mmol) the solution was stirred for 90 min. at 0°C and for 60 min. at room temperature. After concentration to dryness the residue was distributed between ethyl acetate and water. The aqueous phase was extracted three times. The combined organic phases were washed with aqueous $NaHCO_3$-solution, with brine, dried over

anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography (silica gel 0.063 0.04 mm, n-hexane - acetone 9 : 1) to give N-Boc-(S)-[1,2-$^{14}C_2$]valine benzyl ester **17** (348 mg, 1.13 mmol) in 65% yield.

(S)-[1,2-$^{14}C_2$]valine benzyl ester 18 : At ambient temperature trifluoroacetic acid (2.2 ml) was added to a solution of N-Boc-(S)-[1,2-$^{14}C_2$]valine benzyl ester **17** (347 mg, 1.13 mmol) in dichloromethane (2.2 ml). After 1 h the cleavage of the the Boc-protecting group was complete (RTLC-control: silica gel 60 F_{254}, n-hexane - ethyl acetate 80 : 20). The solution was concentrated to dryness and distributed between saturated aqueous $NaHCO_3$-solution and ethyl acetate. After exhaustive extraction of the aqueous phase the organic phases were combined, washed with brine, dried over Na_2SO_4 and evaporated to give (S)-[1,2-$^{14}C_2$]valine benzyl ester **18** (251 mg, 107%). The crude material was accomplished for further reaction without additional purification.

(S)-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$]valine benzyl ester 19 : A suspension of the [2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl bromide **12** (630 mg, 1.129 mmol) in DMF (1.5 ml) was added to a solution of (S)-[1,2-^{14}C]valine benzyl ester **18** (234 mg, 1.129 mmol) in DMF (1.5 ml) at 80°C. After completion of the reaction (2 h) the suspension was cooled to room temperature and combined with water (11ml). After exhaustive extraction with ethyl acetate the combined organic phases were washed with 20% aqueous $KHCO_3$-solution, water (2x) and brine (3x). The resulting solution was dried over Na_2SO_4 and evaporated to give crude material, which was purified by flash chromatography (silica gel 0.063 - 0.04 mm, n-hexane - ethyl acetate 9 : 1) to give 519 mg (S)-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$]valine benzyl ester **19** in 67.2% yield.

(S)-N-Valeryl-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$] valine benzyl ester 20 : A solution of (S)-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-^{14}C]valine benzyl ester **19** (519 mg, 0.759 mmol) in toluene (2.6 ml) was cooled to 10°C and combined with N,N-diisopropylethylamine (273 µl, 1.596 mmol). After 10 min. valeryl chloride (110 µl, 0.912 mmol) was added at 5°C. After 18 h stirring at room temperature the reaction mixture was diluted with toluene (20 ml). The organic phase was washed with 1N HCl, water, saturated aqueous $NaHCO_3$-solution and brine. After evaporation of the solvent the residue was purified by flash chromatography (silica gel 0.068 - 0.04 mm, n-hexane - ethyl acetate 8 : 2) to give 473 mg (S)-N-Valeryl-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$]valine benzyl ester **20** in a 81% yield.

(S)-N-Valeryl-N-{[2'-(1H-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$]valine 2 : (S)-N-Valeryl-N-{[2'-(1-trityl-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-^{14}C]valine benzyl ester **20** (473 mg, 0.616 mmol) dissolved in EtOH (34 ml) was treated with hydrogen (1 atm) at 40°C for 4 h in the presence of Pd/C 10 % (192 mg). The reaction mixture was filtered over Hyflo, rinsed with EtOH, and evaporated to dryness.The residue was recrystallised from ethyl acetate : n-hexane 1 : 1 to give 150 mg of (S)-N-Valeryl-N-{[2'-(1H-tetrazol-5-yl-)biphenyl-4-yl]methyl}-[1,2-$^{14}C_2$]valine **2** in 62.5 % yield.

The product was identified by HPLC for its radiochemical and enantiomeric purity by comparision with an authentic sample of unlabelled reference material using the following systems:

System 1: Nucleosil 100-5 C-18 (Machery Nagel), 250 x 4 mm, A: water : acetonitrile 900 : 100, B: water : acetonitrile : trifluoro acetic acid 50 : 950 : 0.5, gradient : 20 % --> 80 % B : 0 min --> 30 min., 80 % : 30 min. --> 34 min., temperature 50°C, flow 1.0 ml/min., detection UV (227 nm), RA

System 2: Chiral-AGP (ChromTech), 100 x 4 mm, puffer pH 7 : 2-propanol 98 : 2, isocratic, temperature 25°C, flow 1.0 ml/min., detection UV (227 nm) RA

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