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Synthesis of ^{14}C- and $^{14}C_3$-labelled Org 37462

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Org 37462 (1) is the active ingredient in Orgalutran®, an innovative product that reduces the time of treatment in *in vitro* fertilization from four to less than two weeks. Org 37462 is a synthetic decapeptide containing several amino acids that are unnatural in stereochemistry and/or in structure. The synthesis, starting with a ProtectingGroup-*D*-Ala-resin, is a typical solid state synthesis. For the conduction of several metabolism studies, Org 37462 had to be labelled with carbon 14. It was decided to label *D*-3-(2-naphthyl)alanine, the last amino acid to be coupled to the resin. We report the synthesis of [^{14}C]- and [$^{14}C_3$]-Org 37462, starting from 2-bromo-[^{14}C-methyl]-naphthalene and [$^{14}C_2$]-*tert*-butyl glycinate.

Introduction

Org 37462 (**1**) (Figure 1) is the active ingredient in Orgalutran®, an innovative product that reduces the time of treatment in *in vitro* fertilization (IVF) from four to less than two weeks. Patients given Orgalutran® have a much simpler IVF procedure. Their exposure to drugs as well as the physical and emotional burdens, associated with IVF treatment, are reduced. Orgalutran® prevents the premature rise in levels of the luteinizing hormone (LH) during specific infertility treatments such as IVF and intracytoplasmic sperm injection. When the growth of multiple follicles is stimulated with gonadotropins such as follicle-stimulating hormone, an early increase in LH may cause unfavorable effects on the eggs and the possibility of getting pregnant.[1–4]

Org 37462 is a synthetic decapeptide containing several amino acids that are unnatural in stereochemistry and/or in structure. The synthesis of unlabelled Org 37462 is a linear process that starts with a protected-*D*-Ala-resin to which subsequently the amino acids are coupled in the right order. This is a typical solid state synthesis: removal of the protecting group, coupling of the protected amino acid, washing away the reagents and repetition of this sequence. After nine cycles, subsequently followed by removal of the remaining protecting groups, cleavage from the resin and purification by preparative high-performance liquid chromatography (HPLC), the final product, Org 37462, is isolated.

For the conduction of several metabolism studies, among others a human ADME study, the availability of Org 37462 labelled with carbon-14 was required. It was decided to label 3-(2-naphthyl)-*D*-alanine since this is the final amino acid to be coupled to the resin and thus the number of actions with carbon-14-labelled material is minimized. Another reason for this position of the label is that it is an unnatural amino acid and it might be interesting to follow this amino acid in the metabolic pathway. In this paper we report the synthesis of the mono- and the triple-[^{14}C]-labelled versions of 3-(2-naphthyl)-*D*-alanine and its conversion to the corresponding mono- and triple-[^{14}C]-labelled versions of Org 37462.

Results and Discussion

For the mono-labelled Org 37462 it was decided to synthesize *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (**8**) (Scheme 1) to be coupled to the peptide moiety. Because the *N*-acetyl group is already present, the use of **8** would minimize the number of reaction steps with radiolabelled material. In the case of the triple-labelled product it was decided to synthesize and use *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine (**10**) in order to follow the same coupling procedure as for unlabelled Org 37462 to increase the chemical yield.

The syntheses of these two compounds are outlined in Scheme 1. (1*R*)-(+)-Camphor (**2**) was used as an auxiliary group to introduce the correct stereochemistry in the alkylating step, as described by McIntosh and Mishra[4] and McIntosh and Leavitt[5] (1*R*)-(+)-Camphor (**2**) was converted to (1*R*)-(+)-thiocamphor (**3**) using phosphorus pentasulfide.[6] Condensation of **3** with *tert*-butyl glycinate and [$^{14}C_2$]-*tert*-butyl glycinate, leading to imines **4a** and **4b**, respectively, followed by alkylation with 2-bromo-[^{14}C-methyl]-naphthalene gave the corresponding imines **5a** and **5b** and their C-2 epimers. In test experiments with unlabelled materials imine **5** and its C-2 epimer were formed in a 9/1 ratio, according to ^1H NMR. The imines **5a** and **5b** and their C-2 epimers were converted to the corresponding final products **12** and **14**. In both cases the undesired diastereoisomer, Org 14735, could easily be separated by preparative HPLC from Org 37462 (Figure 2). This figure shows that the acid Org 14734, which was formed during the split-off of the resin, could also be removed in the same HPLC purification system.

Hydrolysis[7] of the imine **5a** and acetylation of the amino group of **6a** resulted in the fully protected amino acid **7**. The

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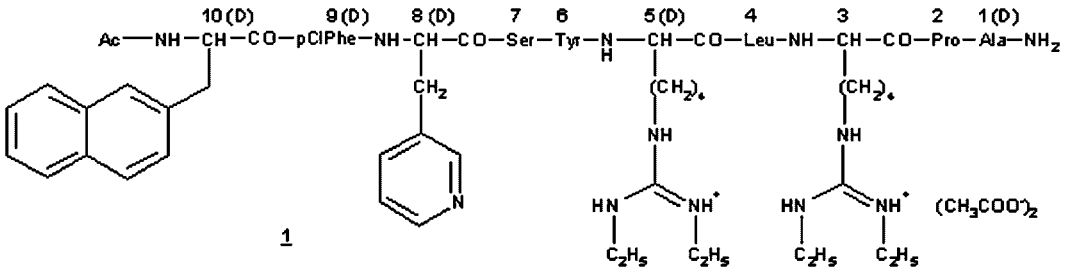

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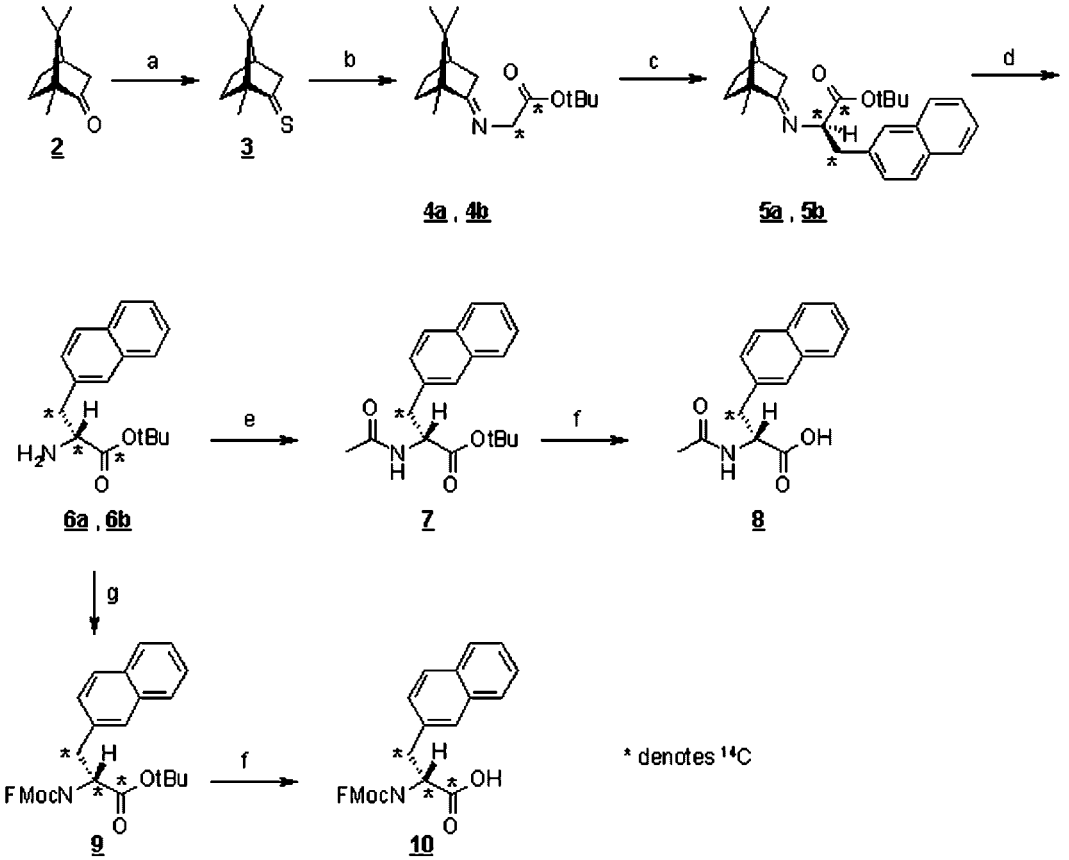
Figure 1. Structure of Org 37462.



2 3 4a, 4b 5a, 5b 6a, 6b 7 8 9 10

Scheme 1

amino acid was purified by column chromatography. Subsequently, the *tert*-butyl ester of **7** was cleaved yielding *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (**8**).

In a similar way, hydrolysis of the imine **5b**, Fmoc-protection of the amino group of **6b**, purification by column chromatography and cleavage of the *tert*-butyl ester **9**, gave the *N*-Fmoc-[1,2,3-^{14}C$_3$]-3-(2-naphthyl)-*D*-alanine (**10**).

The *N*-protected amino acids **8** and **10** were used without further purification in the coupling reaction with the peptide–resin moiety.

In the early stage, an 'acid-sensitive' resin was used to build up the decapeptide, Org 37462, in nine steps. From this route nonapeptide moiety **11** was built up and coupled to *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (**8**) in the presence of 1-hydroxybenzotriazole (HOBt), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and *N*-methylmorpholine (Scheme 2). After 24 hr the protected peptide was separated from the resin upon treatment with a mixture of trifluoroacetic acid, anisole and 1,2-ethanedithiol. Purification with reverse-phase HPLC resulted in the desired [^{14}C]-Org 37462 (**12**) in a yield of 1.74 mCi (3.1% from **5a**) with a specific activity of 58 mCi/mmol.

In a later stage, Org 37462 was developed starting with a 'base-sensitive' resin, which can be split off using a mixture of ammonia (10%) in methanol. Since the synthesis of [^{14}C$_3$]-Org 37462 (**14**) was performed in a later stage of development, nonapeptide moiety **13**, obtained from the production department, was used as the starting material (Scheme 3). This peptide was coupled to *N*-Fmoc-[1,2,3-^{14}C$_3$]-3-(2-naphthyl)-*D*-alanine (**10**) under the same conditions as mentioned above. After 24 hr the resin was split off upon treatment with a solution of 10% ammonia in methanol, the protecting groups were removed and the crude peptide was purified by reverse-phase HPLC, yielding 7.2 mCi (2.9% from **5b**) [^{14}C$_3$]-Org 37462 (**14**) with a specific activity of 161 mCi/mmol.

In the coupling reaction the isolated yield of **12** (5.0%) and **14** (4.0%), respectively, was comparable. Whereas the yield in the unlabelled procedure was significantly higher (>50%). The

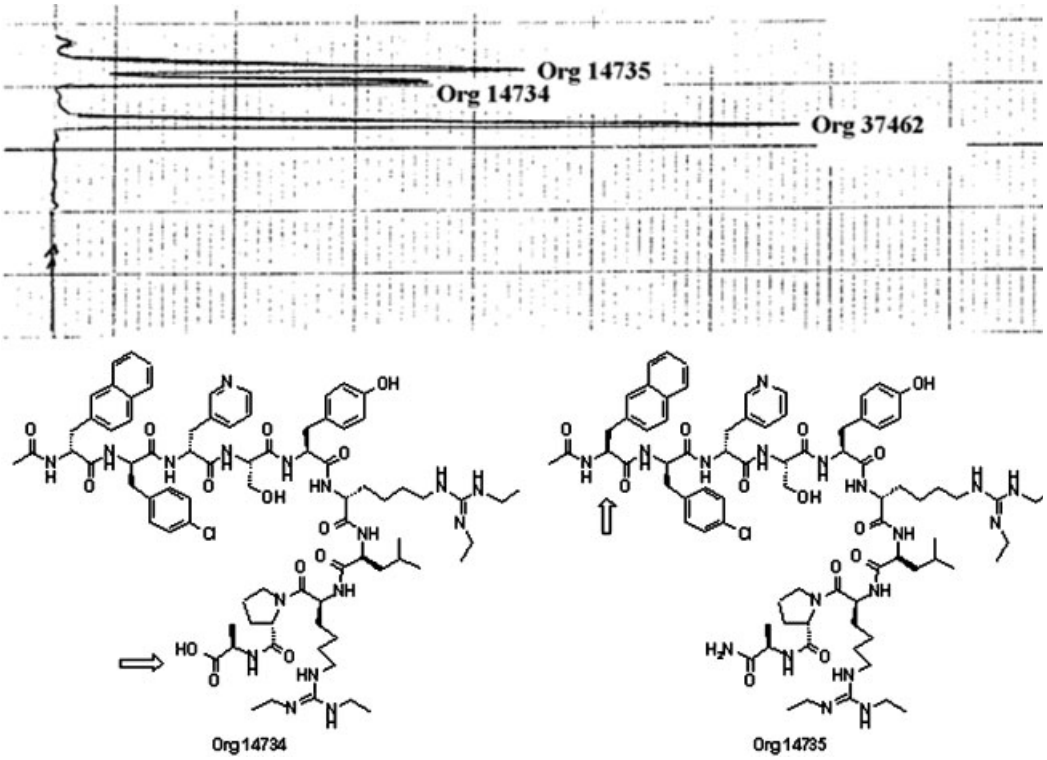
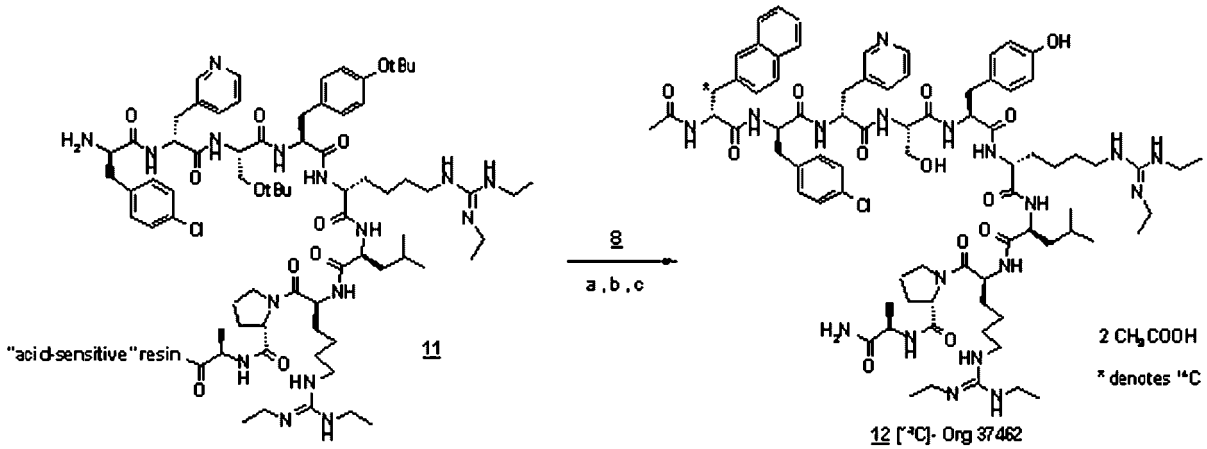


Figure 2. HPLC chromatogram Org 37462, Org 14734 and Org 14735 (HPLC: Symmetry C_{18} (5 µm 250 × 4.6 mm) with a gradient of water/acetonitrile/trifluoroacetic acid (90/10/0.1–10/90/0.1, V/V/V), 1 ml/min, 220 nm).



Scheme 2

lower yield can be ascribed to the fact that in both labelled coupling reactions two to three equivalents of the labelled amino acid were added to the resin, whereas in the unlabelled procedure 10 equivalents of the amino acid were added and not to the difference in *N*-protection group.

[^{14}C]-Org 37462 (**12**) was further processed and formulated and successfully applied in a human ADME study. [$^{14}C_3$]-Org 37462 (**14**) was successfully used in ADME studies, which required a higher specific activity.

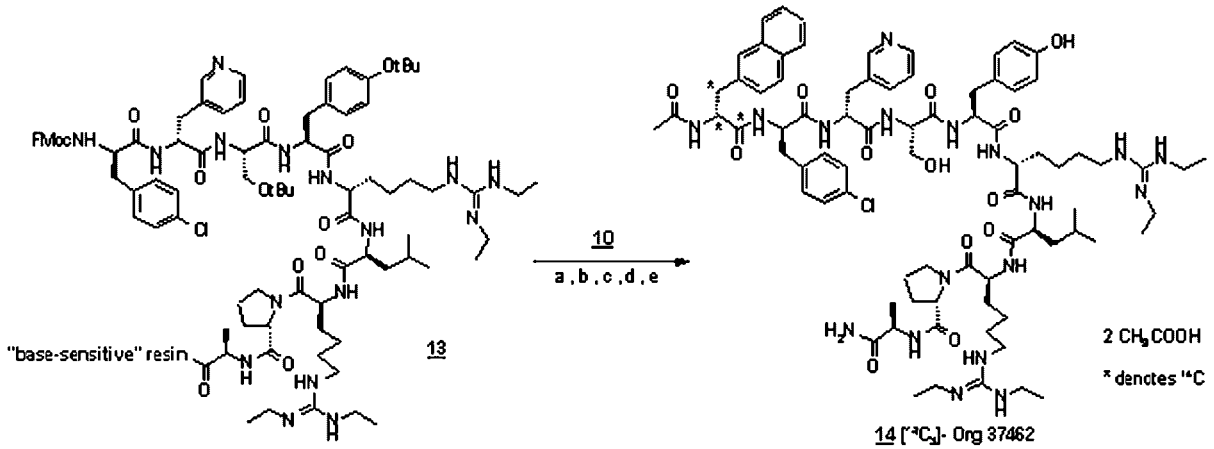
Experimental

Materials

2-Bromo-[^{14}C]-methylnaphthalene was purchased from BlyChem Ltd. [$^{14}C_2$]-Glycine *tert*-butyl ester hydrochloride and 2-bromo-[^{14}C]-methylnaphthalene were purchased from PerkinElmer Life and Analytical Sciences. Resin-bound peptides **11** and **13** were supplied by Organon, the Department of Development Chemistry Riom. The remaining solvents and reagents were purchased from Sigma-Aldrich or Acros Organics.

Instrumentation and equipment

The HPLC purification and purity controls were performed on a Waters 510 HPLC system equipped with an Applied Biosystems 785A UV-detector and a Lablogic β-RAM radioactivity detector and on a Waters 600 HPLC system equipped with an Applied Biosystems 757 UV-detector and a Lablogic β-RAM radioactivity detector Thin layer chromatography (TLC) plates, Merck silica gel 60 F-254, were scanned with a TLC scanner from Raytest, type RITA 92 and type RITA Star.



Scheme 3

Radioactivity was counted with a Packard Tri-CARB 2200 CA/4 liquid scintillation analyzer, using Ultima Gold mv6013159 scintillation fluid.

^{1}H NMR spectra were recorded in CD_3OD on a Bruker DRX400 spectrometer and on a Bruker AVANCE 600 spectrometer.

Mass spectra (ESI) were recorded on a PerkinElmer Sciex API 100 spectrometer and on a Thermo Fisher Scientific LTQ Orbitrap spectrometer.

Synthesis of [^{14}C]-Org 37462 (12) and [$^{14}C_3$]-Org 37462 (14)

Thiocamphor (3)

(1*R*)-(+)-Camphor (**2**) (5.05 g, 33.2 mmol) was dissolved in ethylene glycol dimethyl ether (50 ml) to give a colorless solution. A mixture of phosphorous pentasulfide (14 g, 63.0 mmol) and sodium hydrogen carbonate (14 g, 167 mmol) was prepared. Part of this mixture (3 g) was added to the solution mentioned above, which became a greenish suspension. After stirring at room temperature for 30 min, this suspension was slowly heated to 90°C. At 60°C gas formation was visible. The remaining amount of the mixture (25 g), mentioned above, was added in small portions within 30 min at 90°C. The suspension was stirred for an additional 60 min at 90°C, and subsequently cooled to room temperature. The reaction mixture, an orange solution containing a sticky precipitation, was poured into ice–water (200 ml), leading to the formation of an orange suspension. The suspension was stirred for 30 min and the formed crystals were filtered off. The crystals were dissolved in toluene (50 ml) and evaporated to dryness. This was repeated twice to remove residual water. In this way, thiocamphor **3** (3.7 g, 22.2 mmol) was isolated in 67% yield, which was used as such in the next step.

tert-Butyl *N*-[(1*R*)-bornylidene]-glycinate (4a)

tert-Butyl glycinate (470 mg, 3.6 mmol) was dissolved in toluene (10 ml). Thiocamphor **3** (600 mg, 3.6 mmol) was added. The reaction mixture was heated to reflux temperature and stirred for 16 hr. An additional amount of *tert*-butyl glycinate (280 mg, 2.1 mmol) was added and the mixture was again stirred for 16 hr at reflux temperature. The reaction mixture was cooled and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, heptane/ethyl acetate: 75/25, v/v), yielding *tert*-butyl-*N*-(1*R*)-bornylideneglycinate **4a** in 78% yield (730 mg, 2.8 mmol). ^{1}H NMR, $CDCl_3$, δ4.00 (q, J = 16 Hz, 2H), δ2.3 (dt, J_d = 18 Hz, J_t = 4.0 Hz, 1H), δ1.95 (t, J = 4.0 Hz, 1H), δ1.87 (μ, 1H), δ1.83 (t, J = 18 Hz, 1H), δ1.67 (m, 1H), δ1.47 (s, 9H), δ1.44 (m, 1H), δ1.22 (m, 1H), δ1.02 (s, 3H), δ0.94 (s, 3H), δ0.80 (s, 3H). MS (ESI) *m/z* 266 (M+1).

tert-Butyl *N*-[(1*R*)-bornylidene]-[1,2-$^{14}C_2$]-glycinate (4b)

Thiocamphor **3** (1.08 g, 6.4 mmol) was dissolved in toluene (10 ml). Subsequently, [$^{14}C_2$]-glycine *tert*-butyl ester hydrochloride (300 mCi) and triethylamine (0.65 ml, 0.65 mmol) were added. The formed orange suspension was slowly heated to 120°C and stirred for 5 hr. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, heptane/ethyl acetate: 8/2, v/v), giving the *tert*-butyl-*N*-(1*R*)-bornylidene-[1,2-$^{14}C_2$]-glycinate **4b** (225 mCi, 75% yield). Radiochemical purity 98% (TLC: silica gel, toluene/ethanol 9/1, v/v and heptane/ethyl acetate 8/2, v/v).

tert-Butyl *N*-[(1*R*)-bornylidene]-[3-^{14}C]-3-(2-naphthyl)-*D*-alanate (5a)

Diisopropylamine (125 μl) was dissolved in dry tetrahydrofuran (2.5 ml) at 0°C under nitrogen atmosphere. *n*-Butyllithium (560 μl, 1.6 M in hexane) was added and the mixture was cooled to −78°C. A solution of *tert*-butyl *N*-[(1*R*)-bornylidene]-glycinate (**4a**) (212 mg, 0.80 mmol) in tetrahydrofuran (1 ml) and hexamethylphosphoramide (155 μl) was added and the mixture was stirred for 15 min at −78°C. Next a solution of 2-bromo-[^{14}C]-methylnaphtalene (45 mCi) in tetrahydrofuran (1 ml) was added and the reaction mixture was stirred for 3 hr at −78°C. The mixture was quenched with water (1 ml) and allowed to warm up to room temperature. Brine (1 ml) was added and the mixture was extracted three times with diethyl ether. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to give the crude *tert*-butyl *N*-[(1*R*)-bornylidene]-[3-^{14}C]-(2-naphthyl)-*D*-alanate (**5a**) (34 mCi). In a similar experiment, starting from *tert*-butyl *N*-[(1*R*)-bornylidene]-glycinate (**4a**) (240 mg, 0.91 mmol) and 2-bromo-[^{14}C]-methylnaphtalene (55 mCi), a second amount of crude *tert*-butyl *N*-[(1*R*)-bornylidene]-[3-^{14}C]-(2-naphthyl)-*D*-alanate (**5a**) (54 mCi) was obtained. The two portions were

combined and purified by column chromatography (silica gel, toluene/ethyl acetate 95/5, v/v), yielding *tert*-butyl *N*-[(1*R*)-bornylidene]-[3-^{14}C]-(2-naphthyl)-*D*-alanate (**5a**) (57 mCi, 57% yield). Radiochemical purity 97% (TLC: silica gel, toluene/ethyl acetate 95/5, v/v).

tert-Butyl *N*-[(1*R*)-bornylidene]-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanate (5b)

tert-butyl-*N*-(1*R*)-bornylidene-[1,2-^{14}C]-glycinate **4b** (225 mCi) was dissolved in tetrahydrofuran (1 ml), under nitrogen atmosphere, and cooled to –78°C. Lithium diisopropylamide (1.19 g, 3.00 mmol) and hexamethylphosphoramide (0.5 ml) were added. The reaction mixture was stirred for 10 min at –78°C. Next a solution of 2-bromo-[^{14}C]-methylnaphtalene (100 mCi) in tetrahydrofuran (1 ml) was added. The reaction mixture was stirred for 3.5 hr at −78°C. The mixture was quenched with water (2.5 ml) and allowed to warm up to room temperature. Brine (2.5 ml) was added and the mixture was extracted three times with methyl *tert*-butyl ether. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to give the crude *tert*-butyl *N*-[(1*R*)-bornylidene]-[1,2,3-$^{14}C_3$]-(2-naphthyl)-*D*-alanate (309 mCi). Purification by column chromatography (silica gel, toluene/ethyl acetate 9/1, v/v) resulted in *tert*-butyl *N*-[(1*R*)-bornylidene]-[1,2,3-$^{14}C_3$]-(2-naphthyl)-*D*-alanate (**5b**) (260 mCi, 80% yield). Radiochemical purity 98% (TLC: silica gel, toluene/ethanol 9/1, v/v and heptane/ethyl acetate 8/2, v/v).

tert-Butyl [3-^{14}C]-3-(2-naphthyl)-*D*-alanate (6a)

Citric acid (5 ml of a 15% aqueous solution) was added to a solution of *tert*-butyl *N*-[(1*R*)-bornylidene]-[3-^{14}C]-(2-naphthyl)-*D*-alanate (**5a**) (57 mCi) in tetrahydrofuran (10 ml) and the reaction mixture was stirred for 4 hr at 80°C. The mixture was cooled to room temperature diluted with water (5 ml) and extracted once with methyl *tert*-butyl ether. Subsequently, the water layer was adjusted to pH 8 with a saturated aqueous solution of sodium carbonate and extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, dichloromethane/methanol/triethyl amine 80/20/1, v/v/v) to give *tert*-butyl [3-^{14}C]-3-(2-naphthyl)-*D*-alanate (**6a**) (46 mCi, 81% yield). Radiochemical purity 97% (TLC: silica gel, toluene/ethanol 85/15, v/v and dichloromethane/methanol 95/5, v/v).

tert-Butyl [1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanate (6b)

Citric acid (45 ml of a 15% aqueous solution) was added to a solution of *tert*-butyl *N*-[(1*R*)-bornylidene]-[1,2,3-$^{14}C_3$]-(2-naphthyl)-*D*-alanate (**5b**) (260 mCi) in tetrahydrofuran (20 ml) and the reaction mixture was stirred for 3.5 hr at 80°C. The mixture was cooled to room temperature. The tetrahydrofuran was removed via evaporation under reduced pressure. The residual water was extracted twice with methyl *tert*-butyl ether. Subsequently, the water layer was brought to pH 8 with a saturated aqueous solution of sodium carbonate and extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure, giving crude *tert*-butyl [1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanate (**6b**) (213 mCi, 82% yield). Radiochemical purity 98% (TLC: silica gel, dichloromethane/methanol 95/5 (v/v)+0.1% triethylamine). This compound was used directly in the following step.

tert-Butyl *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (7)

tert-Butyl [3-^{14}C]-3-(2-naphthyl)-*D*-alanate (**6a**) (46 mCi) was dissolved in acetic acid anhydride (0.5 ml). After the addition of pyridine (2.5 ml), the reaction mixture was stirred for 16 hr at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, dichloromethane/acetone 9/1, v/v) to give *tert*-Butyl *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine) (**7**) (39 mCi, 85% yield). Radiochemical purity 96% (TLC: silica gel, dichloromethane/acetone 9/1, v/v and heptane/ethyl acetate 6/4, v/v).

N-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (8)

tert-Butyl *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine) (**7**) (39 mCi) was dissolved in a mixture of trifluoroacetic acid (9 ml) and dichloromethane (1 ml). The reaction mixture was stirred for 3 hr at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane and again concentrated under reduced pressure to yield the crude *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine) (**8**). This compound was used directly in the following step.

tert-Butyl *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine (9)

tert-Butyl [1,2,3-^{14}C]-3-(2-naphthyl)-*D*-alanate (**6b**) (213 mCi) was dissolved in tetrahydrofuran (3.5 ml) and an aqueous solution of sodium carbonate (0.315 g in 3.5 ml) was added. The two-layer suspension was cooled to 0°C under thorough stirring. Next a suspension of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) (511 mg, 1.5 mmol) in tetrahydrofuran (2 ml) was added. The reaction mixture was allowed to warm up to room temperature and stirred for 15 min. The organic layer was separated from the water layer. The latter was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, toluene/ethyl acetate 9/1, v/v) obtaining *tert*-butyl *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine) (**9**) (188 mCi, 88% yield).

N-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine (10)

tert-Butyl *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine (**9**) (188 mCi) was dissolved in a mixture of dichloromethane (1.5 ml) and trifluoroacetic acid (10 ml). The reaction mixture was stirred for 2 hr at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (1 ml) and again concentrated under reduced pressure, this procedure was repeated twice.

The residue, *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine) (**10**), was used directly in the following reaction step.

N-Acetyl-[^{14}C]-3-(2-naphthyl)-*D*-alanyl-4-chloro-*D*-phenylalanyl-3-(3-pyridyl)-*D*-alanyl-*L*- seryl-*L*-tyrosyl-N^9,N^{10}-diethyl-*D*-homoarginyl-*L*-leucyl-N^9,N^{10}-diethyl-*L*-homoarginyl-*L*-prolyl-*D*-alanylamide acetate, [^{14}C]-Org 37462 (12)

To a solution of *N*-acetyl-[3-^{14}C]-3-(2-naphthyl)-*D*-alanine (**8**) (39 mCi) in 1-methyl-2-pyrrolidinone (NMP, 18 ml), subsequently 1-hydroxybenzotriazole hydrate (HOBt, 180 mg, 1.33 mmol) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate

(TBTU, 360 mg, 1.12 mmol) were added. The resulting mixture was added to the 'acid-sensitive' resin **11** (1.7 g, 0.22 mmol/g) and thoroughly shaken manually. After 10 min, *N*-methylmorpholine (90 μl) was added to the reaction mixture. After 4 h, additional amounts of HOBt (150 mg, 1.11 mmol) and TBTU (150 mg, 0.46 mmol) were added. After 16 h, the solvent was removed and the resin was washed, three times alternating with NMP (10 ml containing 0.1% HOBt) and with ethanol (10 ml), once with ethanol (45 ml) and once with dichloromethane (30 ml). The resin was dried under reduced pressure. The residue was suspended in a mixture of trifluoroacetic acid/anisole/1,2-ethanedithiol (15 ml, 95/2.5/2.5, v/v/v). The mixture was set aside for 2 hr and shaken occasionally. Next the solvent was removed, the residue was washed with trifluoroacetic acid and the soluble phases were lyophilized. The residue was dissolved in trifluoroacetic acid (6 ml) and precipitated with diethyl ether (15 ml) to give the crude [^{14}C]-Org 37462 (5.57 mCi). This crude product was purified by HPLC on Symmetry Prep C_{18} (7 μm, 19 × 150 mm) with a gradient of water/acetonitrile/trifluoroacetic acid (90/10/0.1–10/90/0.1, v/v/v), 10 ml/min, 300 nm, yielding the trifluoroacetic acid salt of **12**. This was converted into the acetic acid salt by dissolving it in water (15 ml), next an ion exchange resin (BioRad AG 1-X2, 1.0 g) was added and this suspension was stirred for 10 min before being filtered. To the filtrate a second amount of ion exchange resin (1.0 g) was added. After stirring for 10 min the suspension was filtered again. The filtrate was lyophilized to give *N*-acetyl-[^{14}C]-3-(2-naphthyl)-*D*-alanyl-4-chloro-*D*-phenylalanyl-3-(3-pyridyl)-*D*-alanyl-*L*- seryl-*L*-tyrosyl-N^9,N^{10}-diethyl-*D*-homoarginyl-*L*-leucyl-N^9,N^{10}-diethyl-*L*-homoarginyl-*L*-prolyl-*D*-alanylamide acetate, [^{14}C]-Org 37462 (**12**) (1.74 mCi, 5.0% yield) with a specific activity of 34 μCi/mg, 58 mCi/mmol. Radiochemical purity 95% (HPLC: Symmetry C_{18} (5 μm 250 × 4.6 mm) with a gradient of water/acetonitrile/trifluoroacetic acid (90/10/0.1–10/90/0.1, v/v/v), 1 ml/min, UV 220 nm and Supelcosil LC 18-DB (250 × 4.6 mm) with an aqueous 0.5 M NaH_2PO_4 (pH 2.1)/acetonitrile gradient, 1 ml/min, UV 220 nm. ^{1}H NMR spectrum is in agreement with the corresponding spectrum of the unlabelled Org 37462: ^{1}H NMR, CD_3OD, δ Naphthylalanine 1.83 (s, 3H), 2.92 (m, 1H), 3.14 (m, 1H), 4.68 (dd, 1H), 7.26 (dd, 1H), 7.39–7.44 (m, 2H), 7.58 (s, 1H), 7.71 (m, 1H), 7.74 (m, 1H), 7.78 (m, 1H). *p*-Cl-Phenylalanine, 2.86 (dd, 1H), 3.18 (dd, 1H), 4.59 (dd, 1H), 7.15 (d, 2H), 7.19 (d, 2H). Pyridylalanine, 3.05 (m, 1H), 3.14 (m, 1H), 4.48 (t, 1H), 7.35 (dd, 1H), 7.72 (m, 1H), 8.39 (m, 1H), 8.40 (s, 1H). Serine, 3.52 (dd, 1H), 3.73 (dd, 1H), 4.27 (m, 1H). Tyrosine, 3.01 (m, 1H), 3.07 (m, 1H), 4.38 (dd, 1H), 6.65 (d, 2H), 7.01 (d, 2H). Homo-arginine, 1.19 (t, 6H), 1.19–1.29 (m, 2H), 1.48–1.58 (m, 2H), 1.68 (m, 1H), 1.79 (m, 1H), 3.12–3.18 (m, 2H), 3.24 (q, 4H), 4.34 (t, 1H). Leucine, 0.85 (d, 3H), 0.91 (d, 3H), 1.61 (m, 1H), 1.64–1.65 (m, 2H), 4.50 (m, 1H). Homo-arginine, 1.20 (m, 1H), 1.30 (m, 1H), 1.35 (m, 1H), 1.42–1.50 (m, 2H), 1.67 (m, 1H), 3.08–3.16 (m, 2H), 4.28 (m, 1H). Proline, 1.88 (m, 2H), 2.05 (m, 1H), 2.15 (m, 1H), 3.50 (m, 1H), 3.87 (m, 1H), 4.30 (m, 1H). Alanine, 1.35 (d, 3H), 4.22 (q, 1H). Acetic acid, 1.90 (s, 6H). MS (ESI) unlabelled Org 27462 (**1**): *m/z* 1570; MS (ESI) [^{14}C]-Org 37462 *m/z* 1572 (8% $^{14}C_0$, 92% $^{14}C_1$).

N-Acetyl-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanyl-4-chloro-*D*-phenylalanyl-3-(3-pyridyl)-*D*-alanyl-*L*- seryl-*L*-tyrosyl-N^9,N^{10}-diethyl-*D*-homoarginyl-*L*-leucyl-N^9,N^{10}-diethyl-*L*-homoarginyl-*L*-prolyl-*D*-alanylamide acetate, [$^{14}C_3$]-Org 37462 (14)

'Base-sensitive' resin **13** (5.0 g, 0.079 mmol/g) was swollen in dichloromethane (20 ml). After 1 hr the suspension was filtered and the resin was washed with NMP (50 ml).

Next the resin was suspended in NMP (50 ml) and piperidine (10 ml) was added. After 10 min stirring this reaction mixture was filtered. This procedure was repeated twice. Next the resin was subsequently washed twice with a mixture of HOBt (20 μl) in NMP (20 ml), once with ethanol (20 ml), twice with a mixture of HOBt (20 μl) in NMP (20 ml), once with ethanol (20 ml) and again twice with a mixture of HOBt (20 μl) in NMP (20 ml).

The deprotected resin was added to a solution of *N*-Fmoc-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanine) (**10**) (188 mCi), HOBt (120 mg, 0.89 mmol) and TBTU (275 mg, 0.84 mmol) in NMP (10 ml). The reaction mixture was stirred for 2 min, before a mixture of *N*-methylmorpholine (130 μl) in NMP (660 μl) was added. The reaction mixture was stirred for 2 h, additional amounts of HOBt (70 mg, 0.52 mmol) and TBTU (152 mg, 0.46 mmol) and a mixture of *N*-methylmorpholine (40 μl) in NMP (200 μl) was added. The reaction mixture was stirred at room temperature. After 16 hr the mixture was filtered. The residual resin was subsequently washed with a mixture of HOBt (20 μl) in NMP (20 ml), with ethanol (10 ml), with a mixture of HOBt (20 μl) in NMP (20 ml), with ethanol (10 ml) and with a mixture of HOBt (20 μl) in NMP (20 ml).

Next the resin was suspended in NMP (50 ml) and piperidine (10 ml) was added. After stirring this reaction mixture for 10 min, it was filtered. This procedure was repeated twice. Next the resin was subsequently washed twice with a mixture of HOBt (10 μl) in NMP (10 ml), once with ethanol (10 ml), twice with a mixture of HOBt (10 μl) in NMP (10 ml), once with ethanol (10 ml) and again twice with a mixture of HOBt (10 μl) in NMP (10 ml). To the deprotected resin, a mixture of nitrophenyl acetate (160 mg, 0.88 mmol) and HOBt (125 mg, 0.93 mmol) in NMP (10 ml) was added and the reaction mixture was stirred for 5 min. Next a solution of *N*-methylmorpholine (40 μl) in NMP (200 μl) was added. This mixture (pH 6) was stirred for 90 min. After filtration, the residue was washed with dichloromethane (10 ml). This acetylation step with nitrophenyl acetate was repeated. The resin was dried on air for 64 hr.

Next a solution of 10% ammonia in methanol (v/v) (3.5 ml) was added to the resin. The reaction mixture was set aside for 16 h, before being filtered. The residue was washed twice with methanol (5 ml). The filtrate was dissolved in a solution of trifluoroacetic acid (3.5 ml), triisopropylsilane (100 μl) and water (100 μl). This mixture was stirred at room temperature for 1 hr. Next acetonitrile (15 ml) was added and the mixture was concentrated under reduced pressure. This was repeated twice, yielding the crude [$^{14}C_3$]-Org 37462 (29.1 mCi). The crude product was purified twice by HPLC on Symmetry Prep C_{18} (7 μm, 19 × 150 mm) with a gradient of water/acetonitrile/trifluoroacetic acid (95/5/0.1–5/95/0.1, v/v/v), 10 ml/min, UV 280 nm, yielding the trifluoroacetic acid salt of **14**. This was converted into the acetic acid salt by dissolving it in water (15 ml), next an ion exchange resin (BioRad AG 1-X2, 1.75 g) was added and this suspension was stirred for 10 min before being filtered. To the filtrate a second amount of ion exchange resin (1.75 g) was added. After stirring for 1.5 hr the suspension was filtered again. The filtrate was lyophilized to give pure *N*-acetyl-[1,2,3-$^{14}C_3$]-3-(2-naphthyl)-*D*-alanyl-4-chloro-*D*-phenylalanyl-3-(3-pyridyl)-*D*-alanyl-*L*- seryl-*L*-tyrosyl-N^9,N^{10}-diethyl-*D*-homoarginyl-*L*-leucyl-N^9,N^{10}-diethyl-*L*-homoarginyl-*L*-prolyl-*D*-alanylamide acetate, [$^{14}C_3$]-Org 37462 (**14**) (7.6 mCi, 4.0% yield), with a specific activity of 94.8 μCi/mg, 161 mCi/mmol. Radiochemical purity 96% (HPLC: Symmetry C_{18} (5 μm 250 × 4.6 mm) with a gradient of water/acetonitrile/trifluoroacetic acid (90/10/

0.1–10/90/0.1, v/v/v), 1 ml/min, 220 nm). ^{1}H NMR- and the ^{13}C NMR-spectra are in agreement with the corresponding spectra of the unlabelled Org 37462, with that respect that in the ^{13}C NMR spectrum of the labelled Org 37462 the signals of naphthylalanine, the carbonyl is 30%, the CH is 25% and the CH_2 is 5% meaning that the carbonyl is 70%, the CH is 75% and the CH_2 is 95% ^{14}C-labelled. ^{13}C NMR, CD_3OD: δ Napthylalanine, 22.6 (q, CH_3 ($C(O)CH_3$)), 173.3 (s, C═O ($C(O)CH_3$)), 56.3 (d, CH (^{14}C)), 38.9 (t, CH_2 (^{14}C)), 135.9 (s, 1-naphthyl), 128.9 (d, 2-naphthyl), 134.8 (s, 3-naphthyl), 128.6 (d, 4-naphthyl), 126.7 (d, 5-naphthyl), 127.1 (d, 6-naphthyl), 128.8 (d, 7-naphthyl), 133.8 (s, 8-naphthyl), 129.0 (d, 9-naphthyl), 128.5 (d, 10-naphthyl), 174.0 (s, C═O (^{14}C, amide)). P-Cl-Phenylalanine, 55.9 (d, CH), 37.8 (t, CH_2), 137.5 (s, 1-pCl-phenyl), 132.1 (d, 2+6-pCl-phenyl), 129.5 (d, 3+5-pCl-phenyl), 133.5 (s, 4-pCl-phenyl), 173.5 (s, C═O (amide)). Pyridylalanine, 56.5 (d, CH), 35.2 (t, CH_2), 134.9 (s, 1-pyridyl), 150.9 (d, 2-pyridyl), 148.5 (d, 3-pyridyl), 125.2 (d, 4-pyridyl), 139.2 (d, 5-pyridyl), 173.3 (s, C═O (amide)). Serine, 57.6 (d, CH), 62.5 (t, CH_2), 172.7 (s, C═O (amide)). Tyrosine, 57.7 (d, CH), 37.3 (t, CH_2), 129.0 (s, 1-phenol), 131.2 (d, 2+6-phenol), 116.4 (d, 3+5-phenol), 157.3 (d, 4-phenol), 174.0 (s, C═O (amide)). Homo-arginine, 52.3 (d, CH), 32.2 (t, CH_2), 24.2 (t, CH_2), 29.3 (t, CH_2), 42.4 (t, CH_2), 155.5 (s, C═N), 37.5 (t, CH_2), 14.6 (t, CH_3), 172.5 (s, C═O (amide)). Leucine, 52.8 (d, CH), 41.9 (t, CH_2), 25.9 (d, CH), 22.0 (q, CH_3), 174.1 (C═O (amide)). Homo-arginine, 54.6 (d, CH), 32.2 (t, CH_2), 23.3 (t, CH_2), 29.7 (t, CH_2), 42.5 (t, CH_2), 155.5 (s, C═N), 37.5 (t, CH_2), 14.6 (t, CH_3), 174.4 (s, C═O (amide)). Proline, 61.9 (d, CH), 30.3 (t, CH_2), 26.2 (t, CH_2), 49.5 (t, CH_2), 174.2 (s, C═O amide)). Alanine, 50.4 (d, CH), 17.7 (q, CH_3), 177.7 (s, C═O (amide)). Acetic acid, 24.0 (q, CH_3), 179.7 (s, C═O). MS (ESI) unlabelled Org 27462 (**1**): *m/z* 1576; MS (ESI) [$^{14}C_3$]-Org 37462 *m/z* 1576 (0.4% $^{14}C_0$, 13.5% $^{14}C_1$, 13.8% $^{14}C_2$, 72.3% $^{14}C_3$).

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References

[1] J. Itskov-Eldor, S. Kol, B. Mannaerts, H. Coelingh Bennink, *Hum. Reprod.* **1998**, *13*, 294–295.
[2] P. Devroey, K. Abyholm, K. De Jong, T. Hillensjo, B. Hedon, J. Itskovitz-Eldor, J. Kahn, O. Naether, F. Olivennes, S. Pavlou, B. Tarlatzis, L. Westergaard, B. Mannaerts, B. Van der Heijden, H. Coelingh Bennink, *Hum. Reprod.* **1998**, *13*, 3023–3031.
[3] H. J. Out, B. M. J. L. Mannaerts, *Hum. Fertil.* **2002**, *5*, G5–G12.
[4] J. M. McIntosh, P. Mishra, *Can. J. Chem.* **1985**, *64*, 726–731.
[5] J. M. McIntosh, R. K. Leavitt, *Tetrahedron Lett.* **1986**, *27*, 3839–3842.
[6] B. S. Pedersen, S. Scheibe, N. H. Nilsson, S. O. Lawesson, *Bull. Soc. Chim. Belg.* **1978**, *87*, 223–228.
[7] S. Hoarau, J. L. Fauchere, L. Pappalardo, M. L. Roumestant, P. Viallefont, *Tetrahedron Asymmetry* **1996**, *7*, 2585–2593.

