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Synthesis of [S-[1-^{14}C]Val^{7}]VALSPODAR Application of (+)/(-)-[$^{13,14}C_n$]BABS and (+)/(-)-[$^{13,14}C_n$]DPMGBS, *part 4*

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Summary

VALSPODAR **2**, *a cyclic undecapeptide anticancer drug derived from natural* **Cyclosporin D** **10**, *was labelled with carbon-14 in a nine step synthesis. The sequence started from (-)-[1-^{14}C]BABS* **1a**, *a highly versatile two-carbon synthon for a broad spectrum of singly/multiply labelled substance classes, which after conversion to (-)-[1-^{14}C]DPMGBS* **1b** *and subsequent alkylation with isopropyl iodide gave e.p. N-Boc-S-[1-^{14}C]valine* **7** *in 46% yield. Coupling to the respective linear decapeptide* $P_D^{8->6}$, *followed by cyclization and selective oxidation afforded the labelled drug substance in an overall radiochemical yield of 9%.*

Key words: [$^{14}C_n$]BABS; [$^{14}C_n$]DPMGBS, C-13/C-14 labelled chiral synthons, e.p. C-13/14 labelled amino acids, stereoselective synthesis, [^{14}C]VALSPODAR, [^{14}C]cyclosporins, peptide labelling

Introduction

Specifically as well as uniformly C-13/C-14 labelled (+) or (-)-**bromo[$^{13,14}C_n$]acetyl bornane-10.2-sultam** ((+) or (-)-[$^{13,14}C_n$]**BABS,** n = 1,2) **1a** has been shown in recent publications to be a highly valuable chiral synthon for the preparation of a broad variety of complex, enantiomerically pure, singly/multiply labelled compounds [1]. On the one hand its titanium enolate reacts with unlabelled or labelled aliphatic and aromatic aldehydes to give singly or multiply labelled syn-(2S,3R) and (2R,3S)-2-bromo-3-hydroxy[$^{13,14}C_m$]acyl sultams (m = 1,2,3), respectively, which can be easily converted to

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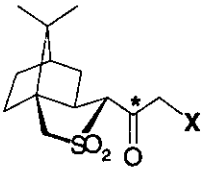
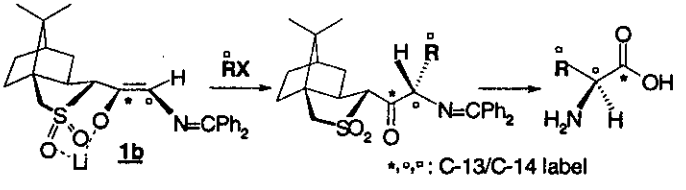
e.p. labelled β-hydroxy acids, α-amino-β-hydroxy acids (allothreonine type) and cis-(2S,3S) or (2R,3R)-epoxy acid derivatives. On the other hand it can be readily transformed to additional highly versatile chiral synthons **1b-d** by simple one step functional group interconversions which may serve as starting materials for e.p. singly/multiply C-13/C-14 labelled α-amino acids, α-amino-β-hydroxy acids (threonine typ) and chiral acrylates.



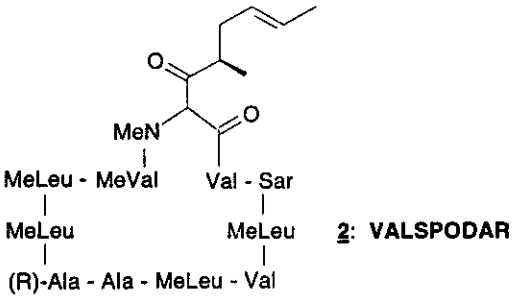
1a: X = Br -> (-)-[$^{13,14}C_n$]BABS
1b: X = N=CPh$_2$ -> (-)-[$^{13,14}C_n$]DPMGBS
1c: X = NCS -> (-)-[$^{13,14}C_n$]ITCABS
1d: X = PO(OEt)$_2$ -> (-)-[$^{13,14}C_n$]PABS

Reaction of **1a** with benzophenone imine at 70°C in acetonitrile for 3 h in the presence of diisopropylethylamine and molecular sieves 3A, for example, furnishes (+)/(-)-diphenylmethylene[$^{13,14}C_n$]glycinyl bornane-10.2-sultam (-)-[$^{13,14}C_n$]**DPMGBS**) **1b**, which was independently investigated in several labs in the late eighties and first introduced by Chassaing et al. in 1994 [2] for the preparation of e.p. carbon-13 labelled α-amino acids. The original procedure suffered, however, from the fact that for each individual labelling position different three to five 5 step protocols had to be followed. Reaction of lithiated (+) or (-)-[$^{13,14}C_n$]**DPMGBS** with alkyl halides, acidic removal of the diphenylmethylene function, and LiOH-mediated cleavage of the auxiliary furnish various respectively labelled α-amino acids in generally 40-80% yields and enantiomeric excesses of > 98%.



Unlike the commercially available Seebach and Schöllkopf chiral glycinates, which are by far less readily available in C-13/C-14-labelled form [3], (+)/(-)-**DPMGBS** also reacts with secondary alkyl

halides in high yields and so singly/multiply C-13/C-14 labelled e.p. β-branched α-amino acids can be conveniently obtained. This additional advantage was exploited for the synthesis of **N-Boc-S-[1-^{14}C]valine** 7, the key intermediate for the labelling of **VALSPODAR** 2, a semisynthetic cyclic undecapeptide drug derived from **Cyclosporin D** that has been demonstrated to reverse multidrug resistance to chemotherapy of cancer cells. Since for all cyclosporin derivatives investigated so far cleavage and degradation of the cyclic peptide ring system is only a minor metabolic pathway (if at all) [4], the incorporation of labelled S-α-amino acid(s) for ADME-investigations in animal and man is justified in this special case. Accordingly, the selection of the most suitable α-amino acid is better defined by the easiest accessible linear peptide precursor (i.e. for **VALSPODAR** the linear decapeptide $P_D^{8->6}$-methyl ester) than by biological stability considerations.



2: VALSPODAR

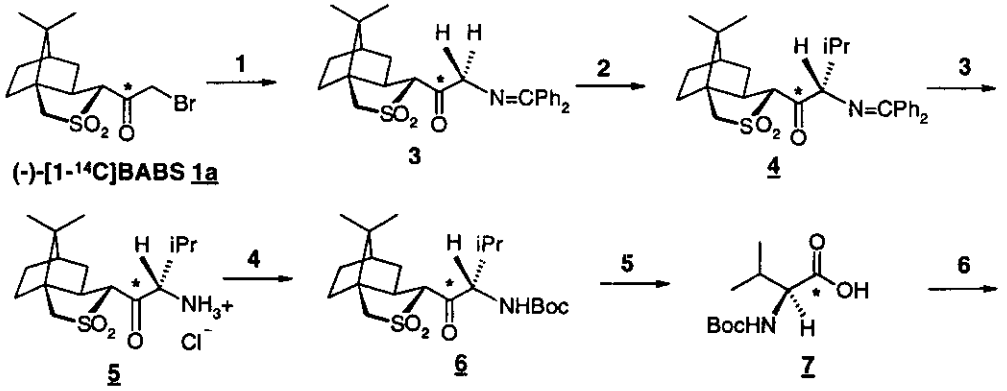
Results and Discussion

The lithium enolate of (-)-**[1-^{14}C]DPMGBS** 3 was generated by metallation with n-butyllithium in THF at -78°C. After the consecutive addition of HMPA (or DMPU alternatively) and isopropyl iodide the reaction mixture was kept at -78°C for an additional 30 min and at 5°C overnight to give (2S)-isopropyl (-)-[1-^{14}C]DPMGBS 4 in 71% yield. Removal of the diphenylmethylene group with 1N HCl-THF 1:1 (r.t., 1 h), protection of the liberated amino function by acylation of 5 with di-*tert*-butyl dicarbonate (THF; r.t., 16 h), and hydrolytic cleavage of the auxiliary with 1N LiOH-THF 1:1 (0°C, 2h) provided the requisite N-Boc-[1-^{14}C]amino acid 7 in 46% yield (referred to (-)-**[1-^{14}C]BABS**) and with an enantiomeric

excess of > 99%. The diastereomeric purity of precursor 6 (N-Boc-S-[1-^{14}C]valinyl bornan-10.2-sultam) was confirmed by HPLC - and ^{1}H-nmr spectroscopic comparison with authentic unlabelled reference materials (e.g. N-Boc-S-valinyl and N-Boc-R-valinyl (-)-bornane-10.2-sultam, respectively). Both were readily available by 1,3-dicyclohexylcarbodiimide-mediated coupling of the respective N-Boc amino acids to the deprotonated auxiliary in the presence of 1-hydroxybenztriazole and triethylamine (toluene, r.t., 2.5 h, 60%). The enantiomeric purity of 7 was ascertained by GC-analysis of the corresponding N-trifluoroacetyl ethyl ester on Chirasil-L-Val^{R} at 210°C.

The following sequence (steps 6 -> 8) was carried out following a strategy developed by Wenger [5] and Rich [6] for the synthesis of cyclosporin A and [R-Lys^{8}]cyclosporin with only minor modifications. Coupling of 7 to the linear decapeptide $\mathbf{P_D}^{8->6}$- methyl ester by BOP-reagent (benztriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) [7] in the presence of N-methylmorpholine (CH_2Cl_2; r.t., 16 h) gave the diprotected undecapeptide 8 in quantitative yield. Hydrolytic cleavage of the ester function with 0.2 N ethanolic NaOH (-5°C, 16 h) and smooth N-debocylation with

Scheme 1a: Synthesis of [S-[1-^{14}C]Val^{7}]VALSPODAR



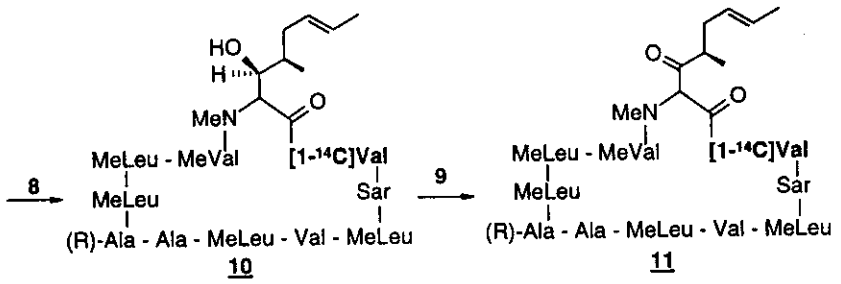
Boc-[S-[1-^{14}C]Val-Sar-MeLeu-Val-MeLeu-Ala-(R)-Ala-MeLeu-MeLeu-MeVal-MeBmt-OMe

8

7 → H-[S-[1-^{14}C]Val-Sar-MeLeu-Val-MeLeu-Ala-(R)-Ala-MeLeu-MeLeu-MeVal-MeBmt-OH

9

Scheme 1b: Synthesis of [S-[1-^{14}C]Val^{7}]VALSPODAR (continuation)



reactions and conditions:

1) $Ph_2C{=}NH$, i-Pr_2EtN, molecular sieves 3A°, acetonitrile; 70°C, 3.5 h **2)** BuLi, HMPA, iPrI, THF; -78°C, 1.5 h; 5°C, overnight **3)** 1N HCl, THF; r.t., 1 h **4)** Boc_2O, THF, r.t., overnight **5)** LiOH,THF-H_2O 1:1; 0°C, 2 h **6)** $P_D^{8->6}$- methyl ester, **BOP**, N-methylmorpholine, CH_2Cl_2; r.t.,16 h **7a)** NaOH, EtOH; -5°C, 16 h **7b)** CF_3COOH; -10°C, 1 h **8) BOP**, CH_2Cl_2; r.t., 18 h **9)** DMSO, $CHCl_2COOH$, DCCI, *tert*-butyl methyl ether; r.t., 4h

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$P_n^{8->6}$- methyl ester: H-Sar-MeLeu-Val-MeLeu-Ala-(R)-Ala-MeLeu-MeLeu-MeVal-MeBmt-OMe

trifluoroacetic acid (-10°C, 1 h) furnished the unprotected undecapeptide 9 in 95% yield. BOP-reagent mediated cyclization in the presence of 4-dimethylaminopyridine (dichloromethane, r.t., 18 h) and subsequent chromatographic purification finally afforded **[S-[1-^{14}C]-Val^{7}]cyclosporin D 10** in 51% yield. Modified Pfitzer-Moffat oxidation with dimethyl sulfoxide - DCC in *tert*-butyl methyl ether (25°C, 4 h) in the presence of dichloroacetic acid (1.2 equiv.) [8] converted **10** into **[S-[1-^{14}C]Val^{7}]VALSPODAR 11** in an overall radiochemical yield of 9% (referred to (-)-[1-^{14}C]**BABS 1a**.

Experimental Part:

Materials:

(-)-[1-^{14}C]BABS 1a at a specific activity of 58 mCi/mmol was purchased from NEN Life Science Products as well as from IICH. The material was prepared following procedures internally elaborated in our labs. The requisite linear decapeptide methyl ester $P_D^{8->10}$ and the following reference materials were synthesized and delivered by Dr. R. Wenger (Preclinical Research, NOVARTIS Pharma AG).

Unlabelled Cyclosporin D and **VALSPODAR** were obtained from the Chemical and Analytical Development, NOVARTIS. All reagents and solvents were of commercial grade and were used as supplied.

Analytics:

[S-[1-^{14}C]Val^{7}]VALSPODAR 11 and its immediate precusor **[S-[1-^{14}C]Val^{7}]Cyclosporin D** 10 were analyzed for their chemical and radiochemical **purity** as well as for their **identity** with authentic unlabelled reference materials by HPLC using the following systems:

system 1: RP 18, 5 µm (Merck). 4x125 mm, water – acetonitrile - *tert*-butyl methyl ether 515:375:110, isocratic, flow rate: 1 ml/min, concentration: 3 mg/ml in THF-H_2O 9:1, injection volume: 10 µl, temperature: 70°C

system 2: RP 18, 5 µm (Spheri, Brownlee Labs), 4.6x250 mm, water – acetonitrile - *tert*-butyl methyl ether-o-phosporic acid 390:570:40:1, isocratic, flow rate: 2 ml/min, concentration: 3 mg/ml in THF-H_2O 9:1, injection volume: 10 µl, temperature: 80°C

system 3: RP 18, 5 µm (Spheri, Brownlee Labs), 4.6x250 mm, water – acetonitrile - methanol- *tert*-butyl methyl ether 80:170:130:8, isocratic, flow rate: 2 ml/min, concentration: 3 mg/ml in THF-water 9:1, injection volume: 10 µl, temperature: 95°C

The chromatograms were monitored by UV (220 nm, Kontron 430) and a Berthold radioactivity detector LB 507A (yttrium glass cell, 150 µl).

The HPLC-results were confirmed by TLC/radio-TLC on silica gel 60, F254 (Merck No. 5729) using

system 1: ethyl acetate - methanol 95:5

system 2: n-hexane – acetone – dichloromethane -conc.NH_3 aq 40:30:5:1

as well as on reverse phase plates (RP 18, F254, Merck No. 15683) using

system 3: water saturated ethyl acetate.

UV-light (254 nm) in conjunction with Dragendorff's spray reagent and exposure to iodine vapour were applied to the detection of the spots. The radiochemical purity was monitored with a Berthold Thin-Layer Analyzer LB 2832.

The **identity** of the final compound and of all intermediates with the corresponding unlabelled reference materials was assayed by ^{1}H-nmr (360 MHz, AM 360 Bruker spectrometer; $CDCl_3$-solutions, internal

standard: TMS) as well as by ^{13}C-nmr (90.5 MHz, AM 60 Bruker). All spectra proved to be superimposable with the exception of the reduced C-13 signal for the carbon-14 labelled position.

The mass spectrum of **[S-[1-^{14}C]Val^{7}]VALSPODAR 11** was recorded on a VG 70-SE apparatus (Finnigan; ion source: FAB, Xe: 8 keV). It showed molecular peaks for the labelled and the unlabelled portions at m/z 1216 and 1214, respectively. The peaks of the unlabelled portion corresponded with the mass peaks of the unlabelled reference material. From the ratio of the mass peaks an **isotopic purity** of 91% was calculated which agreed well with the value calculated from liquid scintillation counting data (58.5 mCi/mmol = 88%).

Finally, the **diastereomeric purity** of **[S-[1-^{14}C]Val^{7}]VALSPODAR 11** was confirmed by comparison of its optical rotation with that of the unlabelled reference material (obtained from natural Cyclosporin D). The values observed proved to be in excellent agreement.

[S-[1-^{14}C]Val^{7}]VALSPODAR: $[\alpha]_D^{r.temp.}$: - 292° (c=1, $CHCl_3$)

reference material: $[\alpha]_D^{r.temp.}$: - 286° (c=1, $CHCl_3$)

Procedures:

(-)-Diphenylmethylene[1-^{14}C]glycinyl bornane-10.2-sultam ((-)- [1-^{14}C]DPMGBS) 3:

A solution of carefully dried (-)-bromo[1-^{14}C]acetyl bornane-10.2.-sultame ((-)-**[1-^{14}C]BABS 1a**, 195 mCi, 58 mCi/mmol, 3.36 mmol, 1.13 g), N,N-diisopropylethylamine (2.35 ml, 13.5 mmol), benzophenone imine (4.5 ml, 26.88 mmol) and 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, hexane - ethyl acetate - triethylamine 85:15:0.5) to give **(-)-[1-^{14}C]-DPMGBS** in 78% yield (1.145 g, 2.62 mmol, mean value of 2 separate runs). 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-^{14}C]DPMGBS 4: Under argon a solution of (-)-[1-^{14}C]DPMGBS 3 (1.82 g, 4.17 mmol, 242 mCi) in dry THF (25 ml) was treated dropwise at -78°C with 1.5M n-butyllithium in hexane (3,2 ml, 4.8 mmol). After 1 h freshly distilled HMPA (4.3 ml) and isopropyl iodide (2.1 ml, 20.85 mmol) in THF were consecutively injected via 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 – hexane - triethylamine 7:3:0.05) to give **(2S)-isopropyl-(-)-[1-^{14}C]DPMGBS 4** in 71% yield (1.422 g, 2.97 mmol).

S-[1-^{14}C]valinyl bornane-10.2-sultam 5: A solution of (2S)-isopropyl-(-)-[1-^{14}C]DPMGBS 4 (1.23 g, 2.57 mmol) in THF (15 ml) was treated with 1N HCl 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 (Na_2SO_4), and evaporated to give **S-[1-^{14}C]valinyl bornane-10.2-sultam 5** in 88% yield (710 mg, 2.26 mmol).

N-Boc-S-[1-^{14}C]valinyl bornane-10.2-sultam 6: A solution of S-[1-^{14}C]valinyl bornane-10.2-sultam 5 (710 mg, 2.26 mmol) and di-*tert*-butyl dicarbonate (1.485g, 6.8 mmol) in dry THF (10 ml) was stirred at ambient temperature under argon overnight. The solution was evaporated and the residue separated by flash chromatography (silica gel 0.06-0.04mm, hexane - ethyl acetate 4:1) affording **N-Boc-S-[1-^{14}C]valinyl bornane-10.2-sultam 6** in 94% yield (883 mg, 2.13 mmol).

N-Boc-S-[1-^{14}C]valine 7: An aqueous solution of 1N LiOH (15.5 ml) was added dropwise to a stirred solution of N-Boc-S-[1-^{14}C]valinyl bornane-10.2-sultam **6** in THF (20 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-^{14}C]valine 7** in quantitative yield (462 mg, 2.13 mmol) showing an enantiomeric purity of >99% (GC-analysis of the N-trifluoracetyl methyl ester on Chirasil-L-Val ^{R} at 210°C).

N-Boc-S-[1-^{14}C]Val-Sar-MeLeu-Val-MeLeu-Ala-(R)-Ala-MeLeu-MeLeu-MeVal-MeBmt-OMe 8: A solution of N-Boc-S-[1-^{14}C]-valine **A7** (273 mg, 1.25 mmol), linear cyclosporin-(3->1)-decapeptide methyl ester ($P_D^{8->6}$, 1.43 g, 1.25 mmol), N-methyl-morpholine (400 µl, 3.54 mmol), and benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate [7] (**BOP**-reagent: 600 mg, 1.36 mmol) in dry dichloromethane (100 ml) was stirred at ambient temperature for 16 h. The progress of the reaction was monitored by TLC (silica gel 60 F254, ethyl acetate - acetone 95:5). The solvent was evaporated and the residue purified by flash chromatography on silica gel (0.06-0.04 mm) with ethyl acetate - acetone 9:1 to give linear **[N-Boc-S-[1-^{14}C]Val]cyclosporin-(2->1)-undecapeptide methyl ester 8** in quantitative yield (1.7 g, 1.26 mmol).

H-S-[1-^{14}C]Val-Sar-MeLeu-Val-MeLeu-Ala-(R)-Ala-MeLeu-MeLeu-MeVal-MeBmt-OH (linear [S-[1-^{14}C]Val]cyclosporin-(2->1)undecapeptide 9: A solution of linear [N-Boc-S-[1-^{14}C]Val]-cyclosporin-(2->1)undecapeptide **8** (1.7 g, 1.26 mmol) in ethanol (30 ml) was cooled to -5°C. After addition of 0.2 N aqueous NaOH (6.4 ml, 1.28 mmol) stirring at -5°C was continued for an additional 16 h. The resulting reaction mixture was diluted with dichloromethane (100 ml) and brine (100 ml) and acidified with 2N HCl to pH 2.5. The organic layer was separated, the aqueous was extracted with 4 portions of dichloromethane. The combined organic extracts were

dried (Na_2SO_4), evaporated, and the resulting raw product purified by flash chromatography (silica gel 0.06-0.04 mm, ethyl acetate-acetone 9:1) affording linear **[N-Boc-S-[1-^{14}C]Val]cyclosporin-(2->1)-undecapeptide** in quantitative yield (1.45 g, 1.28 mmol). The isolated material was treated with trifluoroacetic acid (10 ml) at -10°C for 1 h. The reaction solution was diluted with dichloromethane (100 ml) and adjusted to pH 8.2 by addition of saturated aqueous $KHCO_3$-solution (250 ml). The organic phase was separated, the aqueous phase four times extracted with dichloromethane. The combined organic extracts were dried (Na_2SO_4), evaporated, and the residue was purified by flash chromatography on silica gel 0.06-0.04 mm, dichloromethane-increasing amounts of methanol (20->50%) to give deprotected **linear [S-[1-^{14}C]Val]cyclosporin-(2->1)undecapeptide 9** in 72% (1.13 g, 0.92 mmol).

[S-[1-^{14}C]Val^7]Cyclosporin D 10: A solution of linear [S-[1-^{14}C]Val]cyclosporin-(2->1)-undecapeptide **9** (389 mg, 0.315 mmol) in dichloromethane (1.26 l) was consecutively treated with 4-(dimethylamino)pyridine (5 equiv.,192 mg, 1.58 mmol) and benzotriazol-1-yl-O-tris-(dimethylamino)phosphoniumhexafluoro-phosphate (**BOP**-reagent: 4 equiv., 558 mg, 1.26 mmol). The solution was stirred at ambient temperature for 18 h, concentrated in vacuo to a yellow foam and purified in two steps by flash chromatography (silica gel 0.06-0.04 mm, ethyl acetate - acetone 9:1) and MPLC (LiChroprep RP-18, 40-63 µm, 400x25 mm, isocratic, acetonitrile - water 635:380, flow 9 ml/min, room temperature, detection UV 210 nm) followed by crystallization from acetone affording **[S-[1-^{14}C]Val^7]Cyclosporin D 10** in 51%yield (194 mg, 0.16 mmol, 48.1 µCi/mg, 9.34 mCi, mean value of 5 separate runs).

[S-[1-^{14}C]Val^7]VALSPODAR 11: A solution of [S-[1-^{14}C]-Val^7]Cyclosporin D **10** (485 mg, 0.4 mmol) and dimethyl sulfoxide (40 equiv., 16 mmol) in *tert*-butyl methyl ether (8 ml) was treated at 15°C with dichloroacetic acid (1.2 equiv., 72.2 mg, 46 µl). After 20 min 1.3-dicyclohexylcarbodiimide (6.5 equiv., 536 mg, 2.6 mmol) in *tert*-butyl methyl ether (5 ml) was added dropwise. The resulting

white suspension was warmed to room temperature and stirred for an additional 4 h. The progress of the reaction was monitored by HPLC (Sperisorb, RP 18, 5 µm, 250x4.6 mm (Brownlee Labs), acetonitrile – water - o-phosphoric acid 635:380:1, 80°C, 2.5 ml/min, detection: UV. 210 nm). The reaction was quenched with oxalic acid dihydrate (2 equiv., 101 mg, 0.8 mmol) in dimethyl sulfoxide (1.5 ml), kept at room temperature for 1 h and at -20°C for 16 h, and finally filtered. After addition of aqueous $KHCO_3$-solution (8%, 100 ml) the reaction product was exhaustively extracted with *tert*-butyl methyl ether. The combined organic extracts were dried (Na_2SO_4), evaporated and the isolated crude material 8475, 98%) was purified by flash chromatography (silica gel, 0.06-0.04 mm, diethyl ether - methanol 9:1) and crystallization from *tert*-butyl methyl ether providing **[S-[1-^{14}C]Val^{7}]VALSPODAR** in 54% yield (263 mg, 0.217 mmol, 48.2 mCi/mg, 58.5 mCi/mmol, 12.7 mCi, mean value of 2 separate runs) and > 98% chemical and radiochemical purity.

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