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Review Article

From chiral bromo[$^{13,14}C_n$]acetyl sultams to complex molecules singly/multiply labelled with isotopic carbon

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Summary

The chiral bromo[$^{13,14}C_n$]acetyl sultams (+)- and (−)-[$^{13,14}C_n$]BABS **1a**, **1b** have been demonstrated to be highly efficient, versatile and practical synthons to numerous enantiomerically pure singly and multiply labelled building blocks. The trichlorotitanium enolates derived from **1a**, **1b** undergo aldol addition reactions with aldehydes providing easily purified, crystalline *syn*-2-bromo-3-hydroxy [$^{13,14}C_n$]carboxylic acid derivatives with excellent diastereoselectivity. These can serve as starting materials for e.p. singly/multiply labelled α-substituted β-hydroxy acids, β-substituted/branched α-hydroxy acids and α-unsubstituted β-hydroxy acids. Furthermore, **1a**, **1b** can be easily converted to the (+)/(−)-[$^{13,14}C_n$]DPMGBS **6**, (+)/(−)-[$^{13,14}C_n$]ITCABS **8** and (+)/(−)-[$^{13,14}C_n$]PABS **10** synthons, which significantly enlarges the spectrum of readiliy accessible intermediates. **6** Provides e.p. labelled α-amino acids, **8** can be employed for the preparation of e.p. labelled α-amino-β-hydroxy acids (threonine type). Synthon **10** reacts with aldehydes to chiral *E*-configured enoyl sultams **11** which serve as starting materials for a broad variety of e.p. singly/multiply labelled α,β-substituted/branched, acyclic and cyclic carboxylic acid derivatives. Finally, aldehydes, generated by reductive cleavage of the auxiliary from the primary α,β-substituted acyl sultams, react with $Ph_3P=COOR$ to give γ,δ-substituted α,β,-unsaturated esters, which in turn can be readily converted to highly functionalized e.p. labelled intermediates. This methodology has been extensively exploited for the synthesis of a broad spectrum of carbon-14

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labelled drug substances e.g. Taxol, Valsartan, Everolimus, Lipid X, NVP IMM125, SDZ ISQ844, SDZ PRI05 and the cyclosporin derivatives Valspodar, NVP IMM125, NVP NIM811.

Key Words: Chiral bromo[$^{13,14}C_n$]acetyl sultams; Chiral synthons; Stereoselective syntheses; C-14 labelled BABS; DPMGBS; PABS; e.p. C-14 labelled drug substances

Review

In spite of the ever-growing need for enantiomerically pure singly and multiply ^{13}C or ^{14}C labelled drug substances for early pharmacokinetic and metabolic investigations, there is still a severe lack of commercially available, respectively labelled e.p. building blocks and chiral synthons. In order to at least partially fill this gap we allocated some internal capacity to the development of a few such synthons promising access to a broad spectrum of singly or multiply labelled target molecules. Criteria which need to be met by such synthons are outlined in Table 1.

A brief screening of potential candidates revealed, that even the simple singly or multiply labelled chiral bromoacetyl derivatives **1a** (bromo[$^{13,14}C_n$]acetyl (+)-bornane-10,2-sultam = (+)-[$^{13,14}C_n$]**BABS** ($n = 1,2$)), and **1b** (bromo[$^{13,14}C_n$]acetyl (−)-bornane-10,2-sultam = (−)-[$^{13,14}C_n$]BABS) mostly fulfill the requirements listed in Table 1.

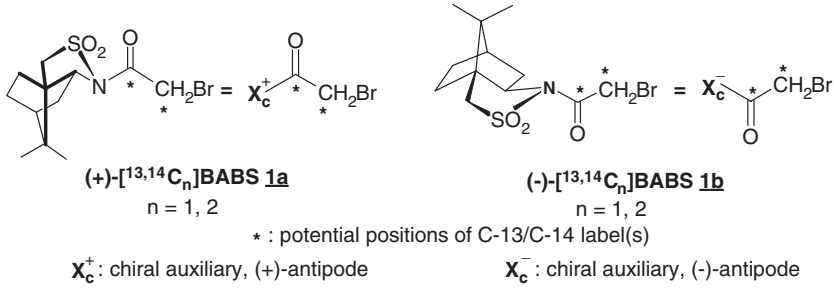


Table 1. Criteria for chiral synthons applicable to the single/multiple labelling of complex molecules with isotopic carbon

- Separable; both enantiomers available
- Label already incorporated; potential for alternative/multiple labelling following identical procedures
- Broad spectrum of applications
- Convenient, easily reproducible preparation procedures; potential for outsourcing
- Radiolytically stable; decomposition fragments easily separable

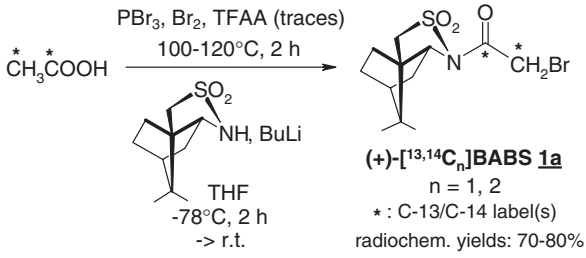


Figure 1. Synthesis of (+)-[$^{13,14}C_n$]BABS 1a

Both antipodal synthons **1a**, **1b** can be easily prepared in >70% yield by heating the corresponding labelled acetic acid with a 2:1 mixture of Br_2 and PBr_3 at 100°C for 2 h followed by vacuum transfer of the bromo[$^{13,14}C_n$]acetyl bromide intermediate into a suspension of lithiated (+)- or (−)-bornane-10,2-sultam in THF (Figure 1). Since both singly and doubly labelled acetic acid is commercially available, **1a** and **1b** are readily accessible at reasonable costs and without any special knowledge.

1a, **1b** Show excellent stability against radiolytic self-decomposition if stored in toluene solution at −80°C at a specific activity of 1 mCi/ml. Under these conditions even doubly C-14 labelled material shows a yearly decomposition rate of less than 10%. Furthermore, it can be conveniently re-purified by flash chromatography on silica gel using isopropyl acetate–hexane 2:1 as eluent.

Reaction of (+) or (−)-[$^{13,14}C_n$]**BABS** with equimolar amounts of $TiCl_4$ and *N*-ethyl- piperidine at −78°C in CH_2Cl_2 and trapping of the in situ generated chiral trichlorotitanium enolate with aldehydes furnishes singly or multiply labelled *syn*-(2*S*,3*R*)- or (2*R*,3*S*)-2-bromo-3-hydroxy[$^{13,14}C_m$]-acyl sultams **2** with d.e. >95% (Figure 2).[1–3] Only formaldehyde shows a much lower diastereoselectivity (d.e. >75%) due to significantly diminished steric interactions in the transition state for the corresponding aldol reaction. Since aldehydes can be readily labelled in their carbonyl positions, α,β-substituted carboxylic acid derivatives become available, either selectively or multiply labelled with ^{13}C or ^{14}C in at least three different positions without changing the synthesis route (Strategy 1).

Strategy 1

The chromatographically purified d.p. *syn*-bromoaldol sultams **2** are highly valuable intermediates since they can be readily converted into a broad variety of widely used e.p. singly or multiply labelled building blocks (e.g. β-hydroxy [$^{13,14}C_m$]carboxylic acids, α-amino-β-hydroxy

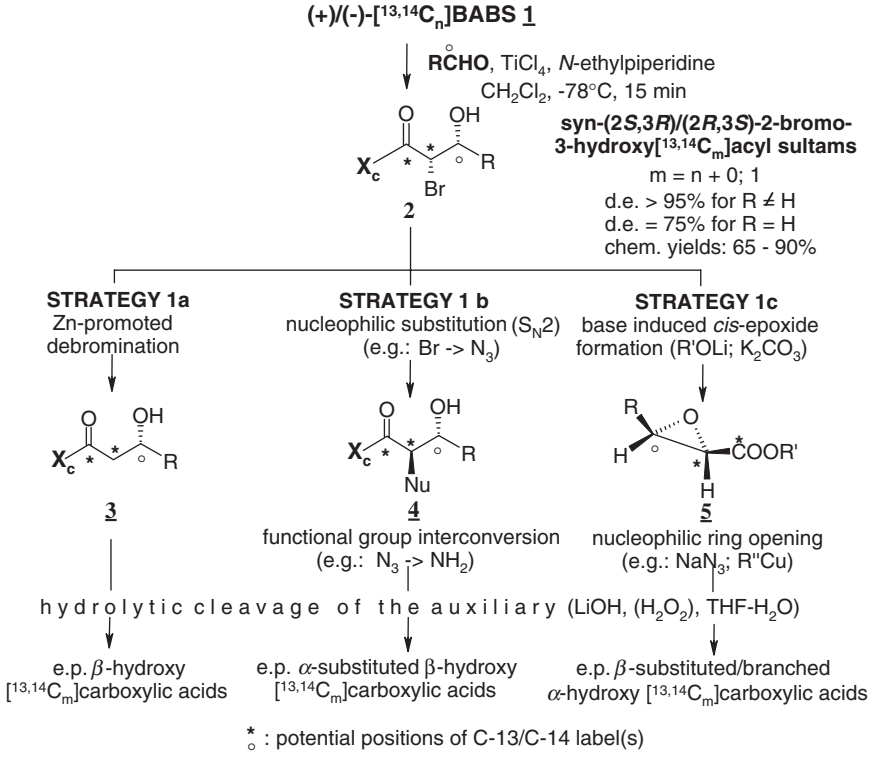


Figure 2. Aldol reaction of (+)/(−)-[$^{13,14}C_n$]BABS with unlabelled or [1-^{13,14}C]-aldehydes and subsequent functional group interconversions (Strategy 1)

[$^{13,14}C_m$]carboxylic acids, β-amino-α-hydroxy [$^{13,14}C_m$]-carboxylic acids etc.) [2,3a]following fundamentally different protocols as summarized under Strategy 1a–c (Figure 2).

Strategy 1a: Reductive dehalogenation with Zn, NH_4Cl (MeOH, r.t.) furnishes d.p. β-hydroxy [$^{13,14}C_m$]acyl sultams **3**. OH-Protection with TBDMS-triflate or benzyl trichloroacetimidate in order to suppress potential retroaldol reaction, and cleavage of the auxiliary with LiOH–H_2O_2 in aqueous THF gives e.p. *O*-protected or, after cleavage of the protecting group, unprotected β-hydroxy [$^{13,14}C_m$]carboxylic acids (see also Figure 5). This procedure circumvents the problems associated with the poor diastereoselectivities normally achieved when the unsubstituted (+)/(−)-acetyl sultam (or the respective oxazolidinone) is employed as starting material.[2c]

Strategy 1b: S_N2-displacement of the halogen by a suitable non-basic nucleophile provides α-substituted β-hydroxy [$^{13,14}C_m$]acyl sultams **4** with inversion of the configuration at the α-carbon. With NaN_3, for

example, (2*S*,3*S*)- or (2*R*,3*R*)-2-azido-3-hydroxy [$^{13,14}C_m$]acyl sultams **24** are obtained which can be easily reduced and cleaved to e.p. α-amino-β-hydroxy [$^{13,14}C_m$]acids of the allothreonine type (see also Figure 6).[3a,4]

Strategy 1c: Treatment of **2** with suitable bases (LiOR, THF; K_2CO_3, MeOH or wet DMF) affords *cis*-(2*S*,3*S*)- or (2*R*,3*R*)-epoxy [$^{13,14}C_m$]carboxylic acid derivatives **5** with or without simultaneous cleavage of the auxiliary depending on the reaction conditions applied. Subsequent ring opening by reaction with appropriate nucleophiles (N_3^-, RS^-, CuR) furnishes e.p. β-branched or substituted α-hydroxy [$^{13,14}C_m$]acid derivatives with simultaneous inversion of the configuration at the β-carbon. Thus, with NaN_3 the corresponding e.p β-azido-α-hydroxy derivatives are available which can be conveniently reduced to singly/multiply labelled e.p. (2*S*,3*R*)- or (2*R*,3*S*)-β-amino-α-hydroxy [$^{13,14}C_m$]acids **34** (see also Figure 7).[2,3a]

Strategy 2 (Figure 3): Alternatively, (+)- or (−)-[$^{13,14}C_n$]BABS can be readily converted to additional highly versatile C_2-synthons by simple

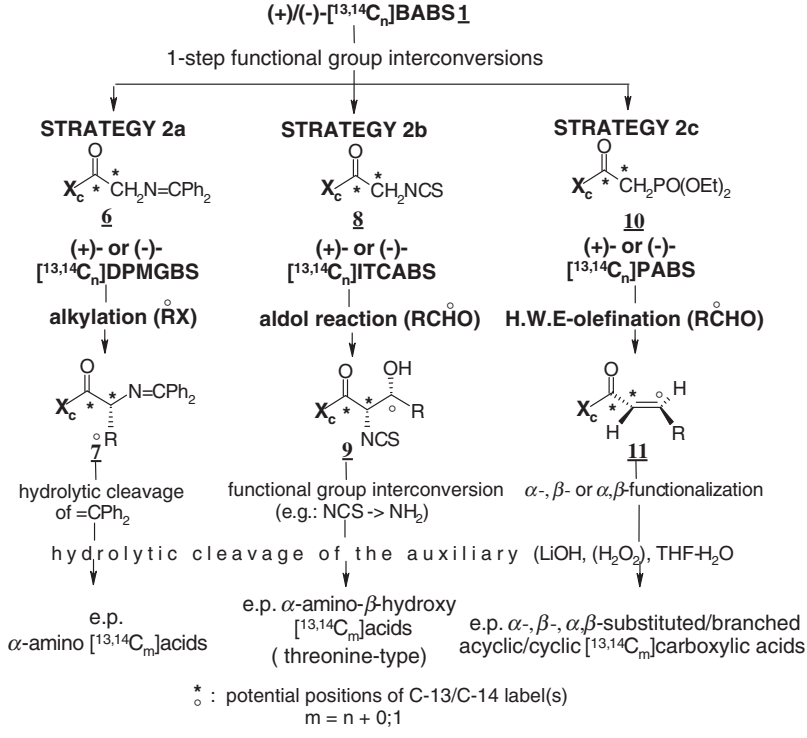


Figure 3. Conversion of (+)/(−)-[$^{13,14}C_n$]BABS into additional C_2-synthons labelled with isotopic carbon (Strategy 2)

one-step functional group interconversions, thus significantly broadening its spectrum of applications. Reaction with $Ph_2C=NH$ (MeCN, molecular sieves 3A, 70°C, 3 h, 70–80%) gives (+)- or (−)-diphenyl-

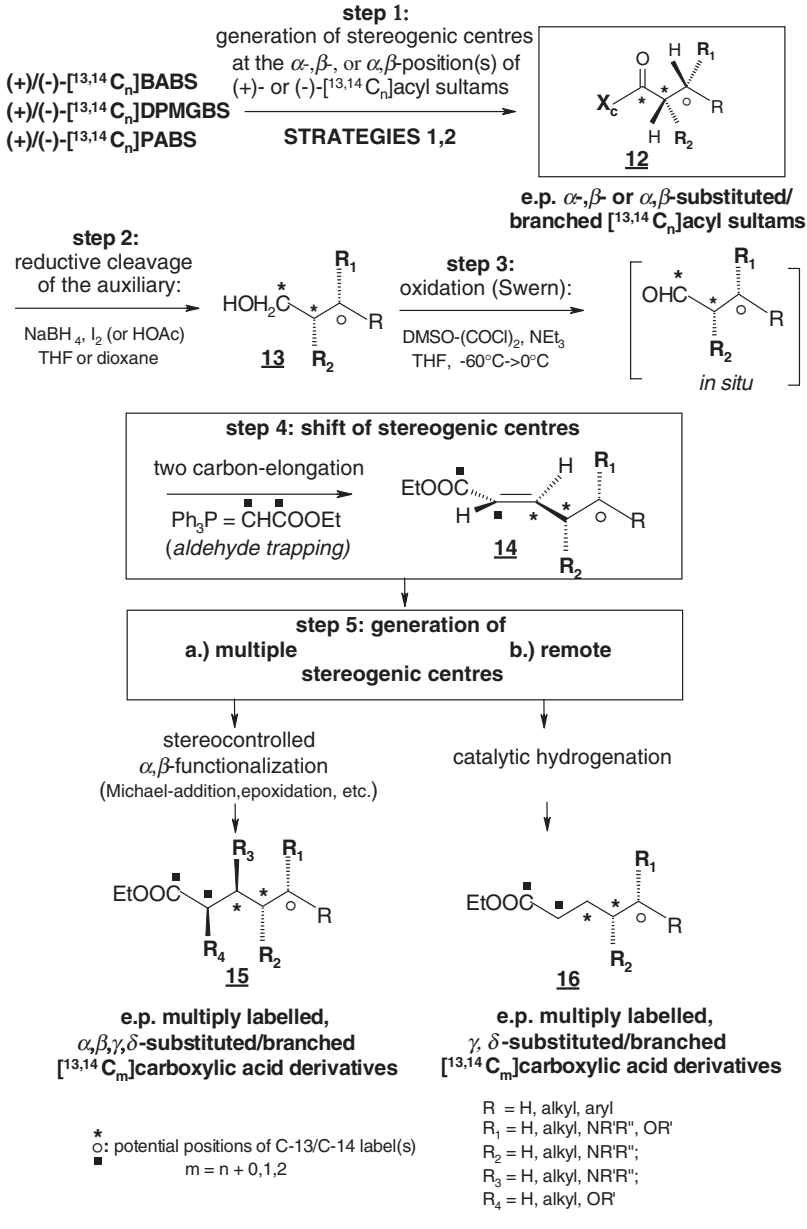


Figure 4. Generation of remote/multiple stereogenic centres from α-, β-, and α,β-functionalized or branched [$^{13,14}C_m$]acyl sultams (Strategy 3)

methylene[$^{13,14}C_n$]glycinyl bornane-10,2-sultam ((+)- or (−)-[$^{13,14}C_n$]**DPMGBS**) **6** which has been widely used for the preparation of e.p. ^{13}C and ^{14}C labelled (*R*)- and (*S*)-α-amino acids (see also Figure 9).[1,5] With KCNS (DMF, 60°C, 90%) isothiocyanato[$^{13,14}C_n$]acetyl bornane-10,2-sultam ((+)- or (−)-[$^{13,14}C_n$]-**ITCABS**) **8** is obtained, which may serve as a starting material for e.p. α-amino-β-hydroxy [$^{13,14}C_m$]acids of the threonine type (see also Figure 13).[6] Heating of (+)- or (−)-[$^{13,14}C_n$]**BABS** with triethyl phosphite (100°C, 3 h, >95%) furnishes (+)- or (−)-diethyl phosphono[$^{13,14}C_n$]acetyl bornane-10,2-sultam ((+)- or (−)-[$^{13,14}C_n$]**PABS**) **10**.[1,5b,7] This may be regarded as the key building block for chiral *E*-configured [$^{13,14}C_m$]enoyl sultams **11** which can be converted either by Michael addition or by cycloaddition to singly or multiply ^{13}C or ^{14}C labelled acyclic, alicyclic or heterocyclic α-, β-, or α,β-functionalized or branched carboxylic acid derivatives **82**, **84** (see also Figure 14).[1,7–9]

Strategy 3 (Figure 4): Appropriate diastereomerically pure α-, β- or α,β-functionalized acyl sultams **12** may serve as highly valuable starting materials for the synthesis of e.p multifunctionalized multiply labelled acid derivatives containing remote or multiple stereogenic centers. Reductive cleavage of the auxiliary to the corresponding alcohols **13**, subsequent oxidation of **13** to the respective aldehydes and their *in situ* trapping with alkoxycarbonylmethylenetriphenylphosphorane open up a convenient route to *E*-configured α,β-unsaturated γ,δ-substituted acid esters **14** elongated by two carbon atoms[1,10]. These in turn can be easily converted stereoselectively to the respective e.p. α,β,γ,δ-substituted acid derivatives **15**. Since the respective substituted acyl sultams and the phosporane reagents are readily available in multiply labelled form, derivatives containing up to five carbon-13/14 atoms and one ^{15}N can be conveniently prepared. On the other hand, catalytic reduction of **14** furnishes the respective e.p. γ,δ-functionalized or branched carboxylic acid derivatives **16**, thereby shifting the two stereogenic centres by two positions into the remote γ,δ-positions .

Applications

Strategy 1a (Figure 2): This is comprised of the synthesis of e.p. β-hydroxy [$^{13,14}C_m$]acids from e.p. *syn*-bromo[$^{13,14}C_m$]aldol sultams **2**. It was exploited for the carbon-14 labelling of the *N*-(*R*)-3-hydroxymyristic acid side chain of SDZ 89-366 (=Lipid X), a lipid A-analogue

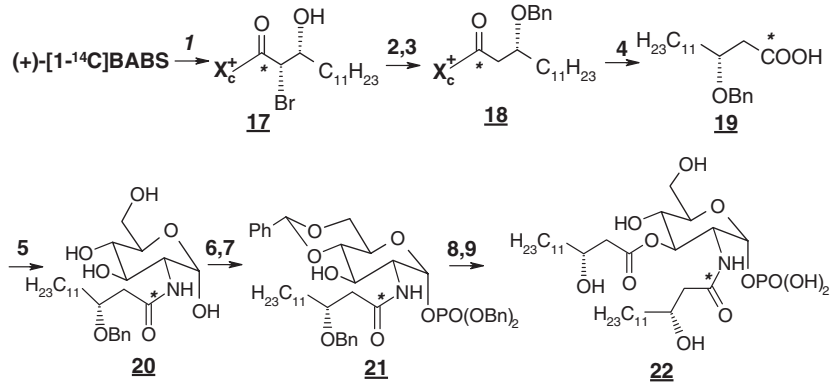


Figure 5. Synthesis of [^{14}C]SDZ 89-366 22: 1a. $TiCl_4$, *N*-ethylpiperidine, CH_2Cl_2; −78°C, 15 min b. $C_{11}H_{23}CHO$, CH_2Cl_2; −78°C, 1 h, −50°C, 30 min; 2. Zn, NH_4Cl, MeOH; r.t., 16 h; 3. $Cl_3CC(=NH)OBn$, CF_3SO_3H, cyclohexane; r.t., 2 h; 4. LiOH–H_2O_2, THF–H_2O 4:1; 0°C, 3 h; 5a. DCC, HOSu, EtOAc; 0°C, 4 h, b. D-glucosamine·HCl Et*i*Pr$_2$N, DMF; r.t.,16 h; 6. $PhCH(OMe)_2$, TsOH, DMF; 60°C, 2 h; 7a. BuLi, THF; −70°C, 10 min b. $(BnO)_2POCl$, toluene; −70°C, 15 min; 8. (*R*)-3-benzyloxymyristic acid, DCC, DMAP, CH_2Cl_2; 4°C, 4 h; 9. H_2 (10 atm), 10% Pd–C, THF-H_2O 5:1; 40°C, 2 h

investigated for its immunostimulating activity (Figure 5).[11] The requisite key intermediate, (2*S*,3*R*)-2-bromo-3-hydroxy[1-^{14}C]-myristoyl sultam **17**, was isolated in 72% yield and with d.e. >98% from the reaction of the trichlorotitanium enolate of (+)-[1-^{14}C]**BABS** with lauraldehyde. Traces of undesired diastereomers could be readily removed by crystallization from ether-hexane.

Debromination of **17** with Zn–NH_4Cl, protection of the hydroxy group by CF_3SO_3H-catalysed benzylation with benzyl trichloroacetimidate,[12] and hydrolytic cleavage of the auxiliary furnished (*R*)-3-benzyloxy[1-^{14}C]myristic acid **19**. Coupling of **19** to D-glucosamine with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide gave selectively the *N*-acylated intermediate **20**. Protection of the 4- and 6-hydroxy groups by TsOH-catalysed acetalation with benzaldehyde dimethyl acetal followed by phosphorylation of the 1-hydroxy group with dibenzyl chlorophosphonate converted **20** into precursor **21**. DCC-DMAP mediated acylation of the 3-hydroxy group of **21** with unlabelled (*R*)-3-benzyloxymyristic acid and, finally, hydrogenolytic cleavage of the protecting groups provided **22** in 8% overall yield (from (+)-[1-^{14}C]**BABS**).

Strategy 1b (Figure 2): The Br/N_3-displacement sequence leading to e.p. α-amino-β-hydroxy [$^{13,14}C_m$]-acids of the allothreonine type was applied to the preparation of *N*-Boc-(*R*)-[1,2-$^{14}C_2$]serine **26** (Figure 6).[4] Although the diastereoselectivity of the initial reaction between HCHO and (+)-[1,2-$^{14}C_2$]**BABS** to (2*S*)-2-bromo-3-hydroxy [1,2-$^{14}C_2$]propionyl sultam **23** was quite unsatisfactory (d.e. >78%), this procedure proved to be superior to all others investigated, especially since the undesired 2*R*-diastereomer could be easily separated by flash chromatography. Treatment with NaN_3 in DMSO converted **23** into the respective (2*R*)-azide **24** with inversion of the configuration at position 2. Pd-catalysed reduction of the azide function followed by hydrolytic

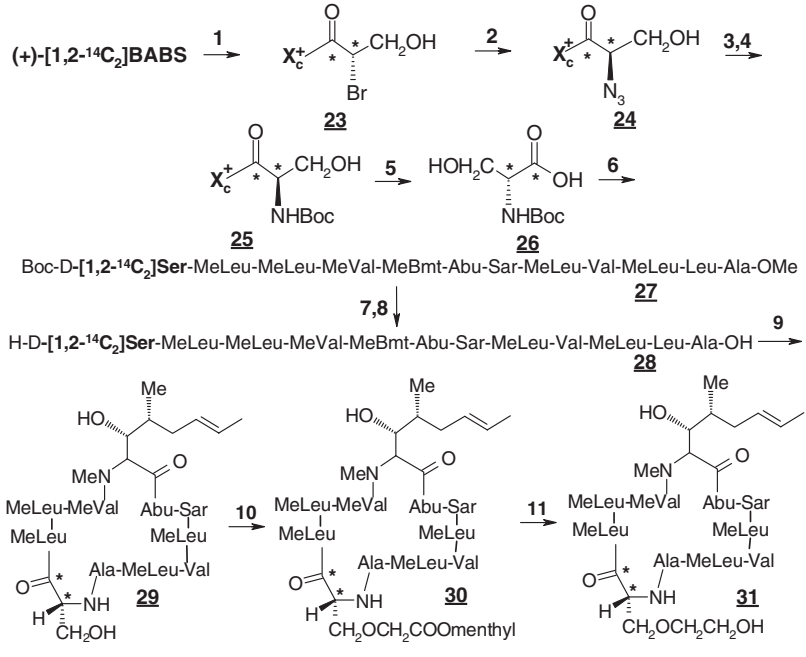


Figure 6. Synthesis of [*R*-[1,2-$^{14}C_2$]Ser^2]NVP IMM125 31: 1a. $TiCl_4$, *N*-ethylpiperidine, CH_2Cl_2, −78°C, 5 min, b. HCHO, −78°C, 1 h, −50°C, 0.5 h; 2. NaN_3, DMSO; 40°C, 4.5 h; 3. H_2, Pd/C 10%, MeOH-THF 10:1, r.t. 16 h; 4. Boc_2O, *tert*-BuOH–water; r.t., 4.5 h; 5. LiOH, THF–H_2O 1:1, r.t., 2.5 h; 6. P_A^{1-3}-OMe (= H-MeLeu–MeLeu–MeVal–MeBmt–Abu–Sar–MeLeu–Val–MeLeu–Ala–OMe), BOP, NMM, CH_2Cl_2, r.t., 1.5 days; 7. 0.2 N NaOH, EtOH, –5°C, 16 h; 8. TFA, CH_2Cl_2: –10°C, 1 h; 9. $(PrPO_2)_3$, DMAP, CH_2Cl_2, r.t., 16 h; 10. menthyl bromoacetate, Bu_4NBr (cat.); toluene–CH_2Cl_2–aq.NaOH 30% 15:5:1, r.t., 4 h; 11. $NaBH_4$, EtOH, r.t., 20 h;
Mebmt = (4*R*)-4-(*E*)-2-butenyl)–4,*N*-dimethyl–L-threonine; Abu = L-α-aminobutyric acid

cleavage of the auxiliary and protection of the amino group with $(Boc)_2O$ provided **26** in 46% overall yield (from (+)-[1,2-$^{14}C_2$]**BABS**). The substance was needed as a key intermediate for the labelling of the unnatural *R*-amino acid component of NVP IMM125 **31**, a Cyclosporin A-derivative showing antiasthmatic activities. For this purpose **26** was coupled with BOP (Castro reagent) and *N*-methylmorpholine to the free amino terminus of linear decapeptide methyl ester $P_D{}^{3->1}$-OMe. Sequential terminal deprotection of the resulting undecapeptide **27** with ethanolic NaOH and TFA furnished unprotected **28** which upon treatment with 1-propanephosphonic acid cyclic anhydride and DMAP cyclized to underivatized [(*R*)-[1,2-$^{14}C_2$]Ser^2]-cyclosporin A **29**.[13a–c] The concluding conversion of **29** into **31** followed the procedure developed for the unlabelled drug substance:[13d] etherification of the primary hydroxy group of the (*R*)-[1,2-$^{14}C_2$]Ser unit with menthyl bromoacetate to **30** and reductive cleavage of the menthyl group with sodium borohydride.

Strategy 1c (Figure 2): The third alternative involves base-induced conversion of d.p. *syn*-bromoaldol sultams **2** to e.p. *cis*-2,3-epoxy carboxylic acid esters **5** followed by regio- and stereoselective opening of the epoxide ring furnishing e.p. *β*-substituted α-hydroxy acid derivatives. This approach was applied to the C-14 labelling of the anticancer drug Taxol **38**[17] in its (2*R*,3*S*)-phenylisoserine side chain, an α-hydroxy-*β*-amino acid structural element (Figure 7). The requisite starting material, (2*R*,3*S*)-2-bromo-3-hydroxy-3-phenyl[1,2-$^{14}C_n$]propionyl (+)-sultam **32**, was obtained from the trichlorotitanium enolate of (+)-[1,2-$^{14}C_n$]**BABS** and PhCHO in 78% chemical yield and >98% diastereomeric excess. It was both cleanly and stereospecifically converted to the respective 2,3-*cis*-epoxy acyl sultam with potassium carbonate in wet DMF. The subsequent cleavage of the auxiliary with lithium benzoxide efficiently provided the (2*R*,3*R*)-2,3-epoxy benzyl ester **33** in 74% yield over the two steps without any detectable racemization. Regioselective opening[15] of the epoxide ring with sodium azide in aqueous methanol in the presence of methyl formate, protection of the hydroxy group with *tert*-butyldimethylsilyl triflate and 2,6-lutidine followed by reduction of the azide with Ph_3P gave (2*R*,3*S*)-*O*-*tert*-butyl-dimethylsilyl phenylisoserinate **35** in 81% yield. Exposure of **35** to 2.1 equivalents of *tert*-butylmagnesium chloride furnished (2*R*,3*S*)-[1,2-^{14}C]phenylisoserin lactam **36** in 40% overall radiochemical yield (from (+)-[1,2-$^{14}C_n$]BABS).[2a] Attachment of **36** to the baccatin skeleton was accomplished in 39% yield following literature procedures:[16] activation of the amide function of **36** by acylation with

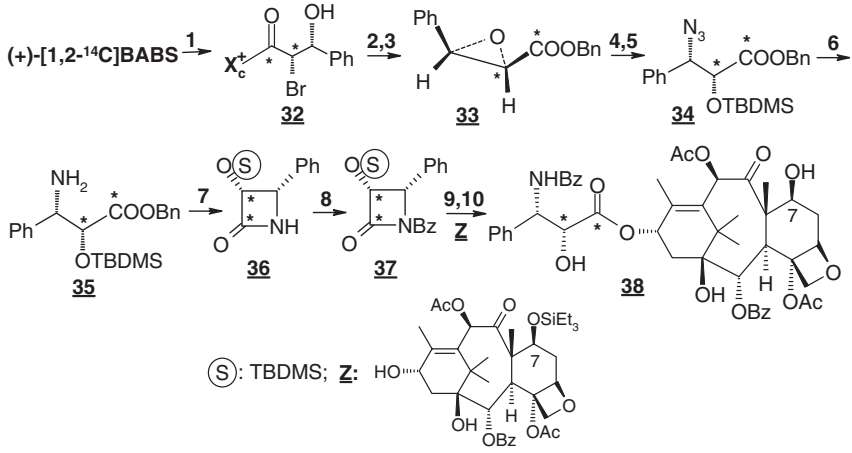


Figure 7. Synthesis of [$^{14}C_2$]Taxol 38: 1a. $TiCl_4$, *N*-ethylpiperidine, CH_2Cl_2; −78°C, 5 min, b. PhCHO, −78°C; 10 min; 2. K_2CO_3, DMF (traces of H_2O); 3. BnOLi, THF, –10°C, 4 h; 4. NaN_3, HCOOMe; MeOH–H_2O 8:1; 50°C, 24 h; 5. TBDMSOTf, 2,6-lutidine, CH_2Cl_2; 0°C–r.t., 1 h; 6. PPh_3, THF–H_2O 8:1; 60°C, 3 h; 7. *tert*-BuMgCl, ether, –10°C, 2 h; 8. PhCOCl, DMAP(cat.), Et_3N, CH_2Cl_2; −10°C, 1.5 h; 9a. BuLi, Z = (7-*O*-TES)-baccatin III, THF; −50°C, 0.5 h b. 37, 0°C, 1–5 h; 10. HF–pyridine; 0°C, 5 h

PhCOCl, reaction of the resulting *N*-benzoyl lactam **37** with deprotonated (7-triethylsilyl)-baccatin-III Z and, finally, desilylation upon treatment with HF-pyridine.

Strategy 2

One-Step functional group interconversions of (+)/(−)-[$^{13,14}C_n$]**BABS** **1a**, **1b** into (+)/(−)-[$^{13,14}C_n$]**DPMGBS** 6a,6b, (+)/(−)-[$^{13,14}C_n$]**ITCABS** **8a**, **8b** and (+)/(−)-[$^{13,14}C_n$]**PABS** **10a**, **10b** (see Figure 3) and applications:

Strategy 2a: (+)/(−)-[$^{13,14}C_n$]**DPMGBS** **6a**, **6b** were introduced by *Martin* and *Chassaing* in 1994 for the $^{13}C/^{15}N$ labelling of numerous e.p. amino acids.[5c]. The original preparation procedure for these synthons suffered from the drawback that for each labelling position different three- and five-step sequences, respectively, had to be followed. We found that they can be more readily obtained in one step and 75–80% yield by the reaction of (+) or (−)-[$^{13,14}C_n$]**BABS** with benzophenone imine at 70°C in acetonitrile in the presence of *N*-ethyldiisopropylamine and 3A molecular sieves under argon for 3−4 h irrespective of the requisite labelling position[5a,b] (Figure 8).

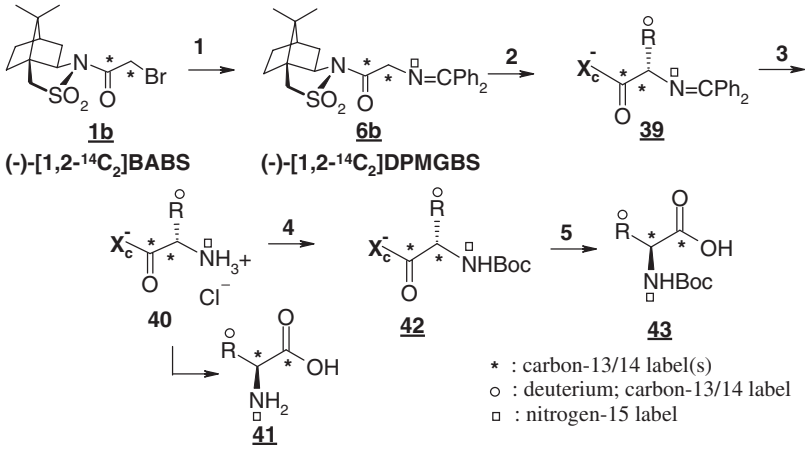


Figure 8. Preparation and application of (–)-[$^{13,14}C_n$]DPMGBS 6b to the synthesis of e.p. ^{13}C, ^{14}C, ^{15}N, ^{2}H labelled (*S*)-amino acids: 1. $Ph_2C{=}NH$, Et*i*Pr_2N, molecular sieves 3A, acetonitrile; 70°C, 3.5 h; 2a. BuLi, THF, –78°C, 30 min, b. RX**, HMPA (or DBU), THF, –78°C, 30 min; 0–r.t., 2–16 h; 3. 2 N HCl–THF 1:1; r.t., 1 h; 4. Boc_2O, THF; r.t. 5–10 h; 5. LiOH, H_2O–THF 1:1; 0°C, 2–5 h;**

RX**: MeI, BnBr, Me_2CHI, Me_2CHCH_2I, $BrCH_2COO$** *tert*-**Bu, $BrCH_2CN$, $I(CH_2)_mCOOMe$, ICH_2CH_2OBn, $ClCH_2SBn$, $Cl(CH_2)_4I$,** *N*-**Boc-3-bromomethylindole;** amino acids**: e.p. [1,2-^{13}C, ^{15}N]Ala, Phe, Val, Leu, Asp, Asn, Glu, Trp, Ser, CySH e.p. [1,2-^{14}C]Val, Leu,Phe, Asn, pipecolic acid**

The lithium enolate of **6a**/**6b** generated by metallation with *n*-BuLi in the presence of 30% HMPA or DMPU reacts with primary as well as with secondary alkyl bromides or iodides at $0° < T < 20°C$ to give the respective α-alkylated derivatives **39** with d.e. > 98%. Acid catalysed removal of the diphenylmethylene group followed by LiOH-mediated hydrolytic cleavage of the auxiliary in THF-water 1:1 furnishes the correspondingly labelled free e.p. amino acid **41** in 40–70% overall yield (starting from (+)- and (−)-[$^{13,14}C_n$]**DPMGBS**, respectively) and enantiomeric excesses of > 98%. Previous blocking of the free amine function of intermediate **40** with $(Boc)_2O$ gives the respective *N*-protected derivatives **43**.

Since both alkyl halides, labelled with isotopic carbon or deuterium at position 1, as well as [^{15}N]benzophenone imine are conveniently accessible, the **DPMGBS**-methodology can also be successfully applied to the synthesis of e.p. α-amino acids either multiply labelled with isotopic carbon or with a combination of isotopes of carbon, nitrogen, and/or hydrogen.

Unlike the commercially available Seebach and Schöllkopf chiral glycinate synthons, which are by far less readily available in $^{13}C/^{14}C$ labelled form[17a], (+)- or (−)-[$^{13,14}C_n$]**DPMGBS** reacts also with secondary alkyl halides in high yields,[17b] and therefore singly or multiply labelled e.p. β-branched α-amino acids can be conveniently obtained. Reaction of (−)-[1-^{14}C]**DPMGBS** **6b** with isopropyl iodide, for example, followed by acidic cleavage of the diphenylmethylene group, protection of the amino function with Boc_2O and hydrolytic removal of the auxiliary with LiOH provided *N*-Boc-(*S*)-[1-^{14}C]valine **46** in 46% yield and >98% e.e. (Figure 9).[5a]

Coupling of **46** to the free amino terminus of the linear decapeptide P_D $^{8->6}$-OMe methyl ester with BOP (benztriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluoro-phosphate)[13a–c] furnished the di-protected labelled undecapeptide **47**. Sequential cleavage of both protecting groups with ethanolic NaOH and TFA converted **47** to unprotected undecapeptide **48** which on treatment with BOP cyclized to [(*S*)-[1-^{14}C]Val^7]Cyclosporin D **49**. Finally, Moffat–Pfitzer oxidation of the

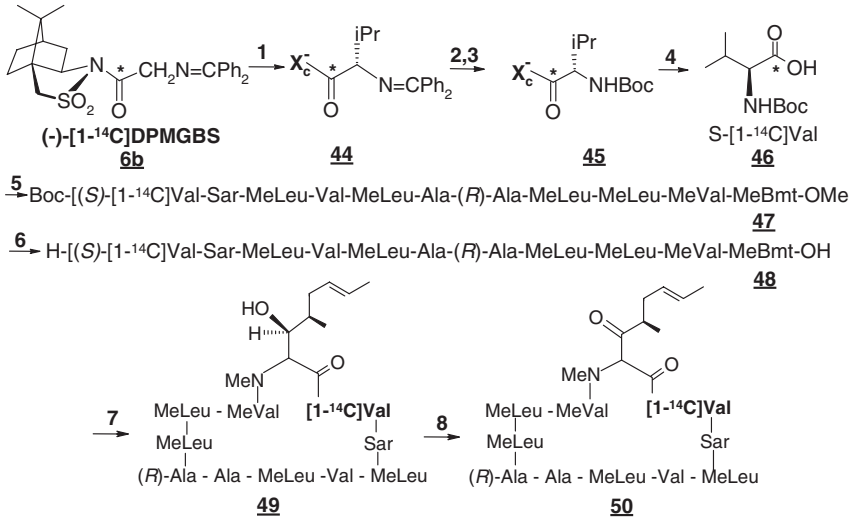


Figure 9. Synthesis of [(*S*)-[1-^{14}C]Val^7]Valspodar 50: 1. BuLi, HMPA, *i*PrI, THF; –78°C, 1.5 h, 5°C, overnight; 2. 1 N HCl, THF; r.t., 1 h; 3. Boc_2O, THF; r.t., overnight; 4. LiOH, THF–H_2O 1:1; 0°C, 2 h; 5. P_D $^{6-8}$-OMe, BOP, *N*-methylmorpholine, CH_2Cl_2; r.t., 16 h; 6a. NaOH, EtOH; −5°C, 16 h, b. CF_3COOH, CH_2Cl_2; −10°C, 1 h; 7. BOP, CH_2Cl_2; r.t., 18 h; 8. DMSO, $CHCl_2COOH$, DCC, *tert*-butyl methyl ether; r.t., 4 h; P_D $^{8->6}$-OMe: H–Sar–MeLeu–Val–MeLeuAla–(*R*)–Ala–MeLeuMeLeu–MeVal–MeBmt–OMe

secondary OH-group of **49** with DMSO–DCC in the presence of dichloroacetic acid gave [(*S*)-[1-^{14}C]Val^{7}]Valspodar **50**,[5a] a multidrug resistance modifying drug substance for cancer therapy.[18]

Doubly ^{14}C labelled *N*-Boc-L-valine **54**, prepared by an analogous procedure from (−)-[1,2-$^{14}C_2$]**BABS**, was selected as a key intermediate for the labelling of Valsartan **60**, a highly potent drug for the treatment of hypertension and congestive heart failure (Figure 10). The free carboxyl group of **54** was first blocked by esterification with benzyl alcohol and EDCI in the presence of catalytic amounts of DMAP. Deprotection of the amino group of the resulting benzyl ester **55** with CF_3COOH followed by *N*-alkylation of the released amine derivative **56** with *N*-trityl protected [2′-(tetrazol-5-yl)biphenyl-4-yl)]methyl bromide **57** led to intermediate **58**. *N*-acylation of **58** with valeryl chloride, and hydrogenolytic cleavage of both protecting groups furnished the labelled drug substance **60** in an overall yield of 3% starting from (−)-[1,2-$^{14}C_2$]**BABS**.[19]

Using suitable α,ω-dihalides selectively or multiply labelled e.p. cyclic α-amino acids are readily accessible as demonstrated by the synthesis of e.p. (*S*)-[2,7-$^{13,14}C_2$]-pipecolic acid **64** (Figure 11).[4b] Reaction of

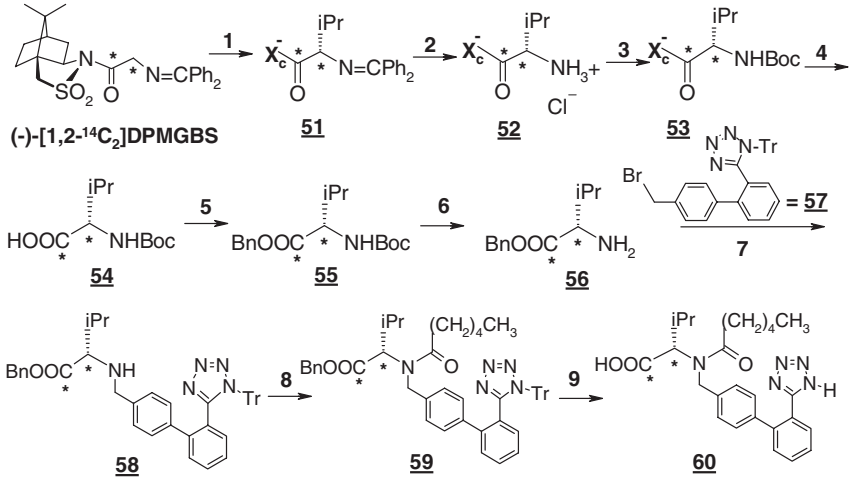


Figure 10. Synthesis of [(S)-[1,2-$^{14}C_2$]Val]Valsartan 60: 1a. BuLi, THF; −78°C, 30 min, b. *i*PrI, HMPA, THF; −78°C, 1.5 h; 5°C, 16 h; 2. 1 N HCl, THF; r.t., 1 h; 3. Boc_2O, THF; r.t., overnight; 4. LiOH, THF–H_2O 1:1; 0°C, 2 h; 5. BnOH, DMAP, EDCI, CH_2Cl_2; r.t., 90 min; 6. TFA, CH_2Cl_2; r.t., 60 min; 7. 57, Et*i*Pr$_2$N, DMF; 80°C, 150 min; 8. valeryl chloride, Et*i*Pr$_2$N, toluene; r.t., 18 h; 9. H_2, Pd/C 10%, EtOH; 40°C, 4 h

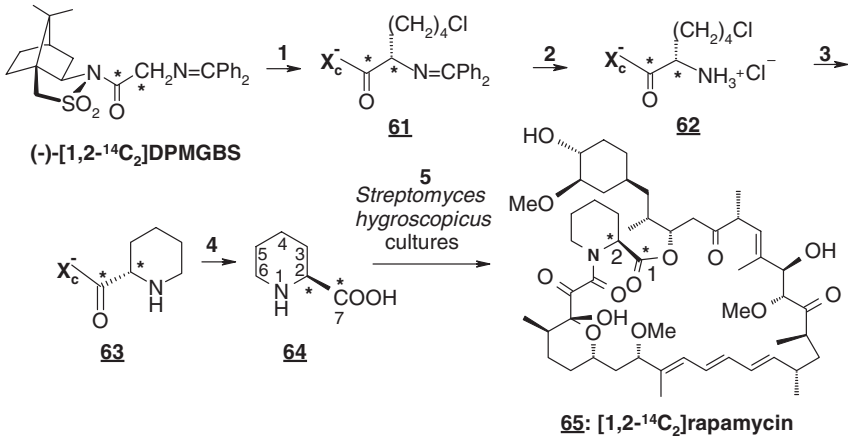


Figure 11. Synthesis of [1,2-$^{14}C_2$]rapamycin 65: 1a. BuLi, THF; −78°C, 1 h, b. DMPU, c. I$(CH_2)_4$Cl; −78°C–r.t., 120 min; 2a. 2 N HCl–THF 1:1; r.t., 18 h b. ether; 3. Et*i*Pr$_2$N, Bu$_4$NI, acetonitrile; 80°C 2 h; 4. LiOH, THF–H$_2$O r.t., 3 h; 5. *Streptomyces hygroscopicus* cultures, 24°C, 4 days

lithiated (−)-[1,2-$^{14}C_2$]DPMGBS with 4-chlorobutyl iodide gave the respective α-alkylated derivative **61** in 75% yield. Hydrolytic cleavage of the diphenylmethylene group with 2*N* HCl-THF, trituration of the dried crude reaction product with ether and separation of the precipitate afforded (2*S*)-2-amino-6-chloro[1,2-$^{14}C_2$]hexanoyl(–)-sultam hydrochloride **62** showing a chemical purity of >95%. Upon heating with *N*-ethyldiisopropylamine in the presence of Bu$_4$NI, **62** cyclized to the pipecolic acid derivative **63**. Subsequent hydrolytic cleavage of the auxiliary furnished the free α-amino acid **64** in 45% overall yield starting from (−)-[1,2-$^{14}C_2$]**BABS** and with an enantiomeric purity of >98%. Feeding of **64** to *Streptomyces hygroscopicus* bacteria cultures RSH 1701 followed by routine work up[20] gave rapamycin **65**, a highly potent immunosuppressive macrolide,[21] doubly labelled with carbon-14 at positions 9 and 10 in 15% radiochemical yield and with an extraordinarily high incorporation rate of 60%.[5b]

It was known from the literature that high yield alkylations of deprotonated (+)- or (−)-**DPMGBS** with sterically demanding halides (e.g. bromodiphenylmethane, 9-bromofluorene) require higher temperatures and significantly longer reaction times of up to 3 days.[22] Nevertheless, we were very surprised that even under these drastic conditions no reaction at all occurred with trimethylsilylmethyl halides (Br, I). The corresponding target molecule, *N*-protected D-trimethylsilyl[1-^{14}C]-alanine **71**, had been selected as a key intermediate for the

carbon-14 labelling of the unnatural amino acid subunit of NVP MTH958 **74**, a borolysine derived peptidomimetic drug for anticoagulant and antithrombotic therapy (Figure 12).[24a] To overcome these problems an alternative amino acid synthon, glycinyl (+)-pseudoephedrine (= (+)-**GPE**) **69**, was more closely investigated. The synthon was introduced by Myers in 1996 and described as less prone to steric effects.[23] Compound **69** was prepared in labelled form in three steps by reaction of (+)-pseudoephedrine **66** with bromo[1-^{14}C]acetyl bromide, followed by Br/N_3-exchange of the resulting *N*-acyl derivative **67** with guanidinium azide and concluding catalytic reduction of azide **68**.[24b,c] In fact, α-alkylation of doubly deprotonated (+)-[1-^{14}C]**GPE** with trimethylsilylmethyl bromide furnished (+)-(*R*)-trimethylsilyl-alaninyl (+)-pseudoephedrine **70** in 65% yield and >95% diastereomeric excess. Hydrolytic cleavage of the auxiliary with ethanolic NaOH followed by protection of the amino group by reaction using Boc_2O afforded *N*-Boc amino acid **71**. Activation of the free carboxyl group

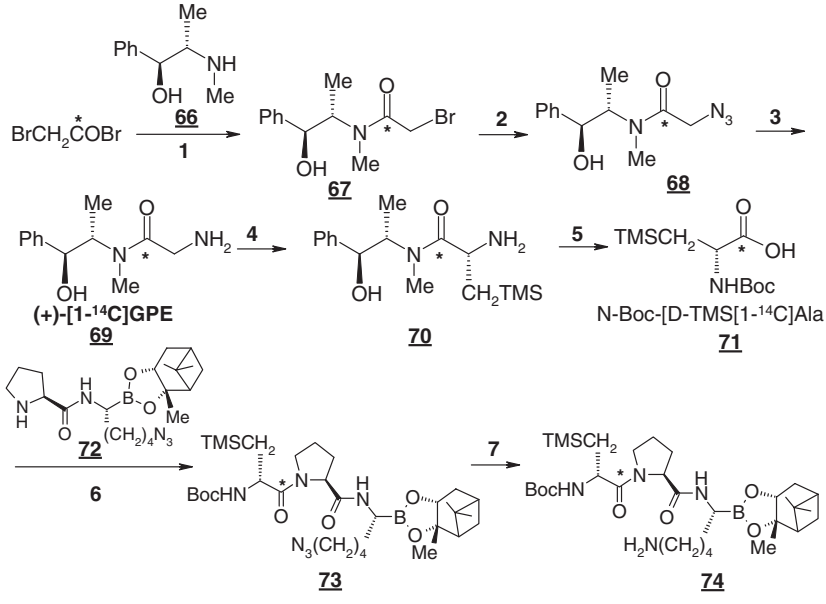


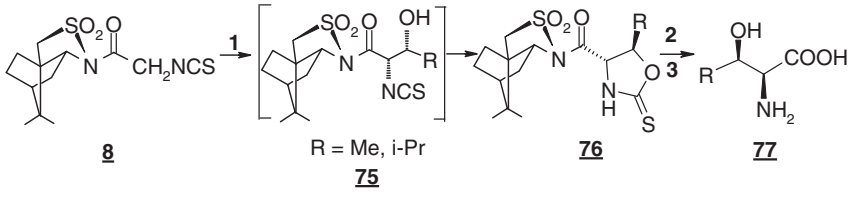
Figure 12. Synthesis of [D-TMS[1-^{14}C]Ala]NVP MTH958 74: 1. (+)-pseudoephedrine 66, Et_3N; CH_2Cl_2; 0°C, 1 h; 2. $(Me_2N)_2C{=}NH_2^+N_3^-$, CH_2Cl_2; r.t., 1 h; 3. H_2, 10% Pd-C, EtOH; r.t., 4 h; 4a. LDA, LiCl THF; −60°C, 15 min, −5°C, 15 min, b. $TMSCH_2Br$; r.t. 16 h; 5a. 1 N NaOH, EtOH–H_2O 1:1; reflux, 4.5 h, b. Boc_2O, THF-H_2O 1:1; 60°C, 4 h; 6a. ClCOO*i*-Bu, NMM, THF; r.t., 30 min, b. 72, NMM; r.t., 2 h; 7. H_2, 10% Pd–C, EtOAc; r.t., 4 h.

of **71** with isobutyl chloroformate and subsequent coupling of the resulting mixed anhydride to the respective pseudodipeptide **72** in the presence of *N*-methylmorpholine gave immediate precursor **73**. Finally, catalytic reduction of the azide function of **73** afforded the desired labelled drug substance **74** in an overall yield of 9%.[24b]

Strategy 2b (Figure 13): Analogous to the related Evans' oxazolidinone derivative,[6] the tin-II enolate of (+)- or (−)-isothiocyanatoacetyl bornane-10,2-sultam (=(+)- or (−)-**ITCABS**) **8**, generated *in situ* with $Sn(OTf)_2$ in the presence of *N*-ethylpiperidine, reacted with representative aliphatic aldehydes to form *syn*-(2*R*,3*S*)- or (2*S*,3*R*)-2-isothiocyanato-3-hydroxy-acyl derivatives **75**. These could not be isolated since spontaneous cyclization occurred to the corresponding (4*S*,5*R*)- or (*4R*,5*S*)-5-alkyl-2-thioxo-1,3-oxazolidinyl-4-carboximides **76** with d.e. of >80%.

Chromatographic separation of undesired diastereomers, hydrolytic removal of the auxiliary, and cleavage of the oxazolidinone system with refluxing 6 N HCl released the free e.p. *syn*-2-amino-3-hydroxy acids **77** in about 25–30% overall yield. As far as is known, this synthon has never been used for the preparation of labelled derivatives.

Strategy 2c (Figure 14): Unlike both (+)/(−)-[$^{13,14}C_n$]**DPMGBS** **6a**, **6b** and (+)/(−)-[$^{13,14}C_n$]**ITCABS** **8a**, **8b** which may only be used for the labelling of a limited number of structural classes, (+)/(−)-[$^{13,14}C_n$]**PABS** **10a** and **10b** (Figure 3), the third synthons under discussion, offers a much broader spectrum of applications. Horner-Wadsworth-Emmons reaction of **10a**, **10b** with unlabelled/labelled aldehydes furnishes the respective *E*-configured chiral enoyl sultams **11** in high stereoselectivity,[1,7] which have proved to be surprisingly stable towards radiation induced side reactions. These can be readily



8 R = Me, i-Pr **75** **76** **77**

Figure 13. Application of ITCABS to the synthesis of e.p. β-hydroxy-α-amino acids of the threonine type 77: 1a. $Sn(OTf)_2$, *N*-ethylpiperidine, CH_2Cl_2, −78°C, 10 min, b. RCHO, −78°C, 10 min; 2. LiOH, THF-H_2O 1:1, 0°C, 2 h; 3. 6 N HCl, reflux, 3 h

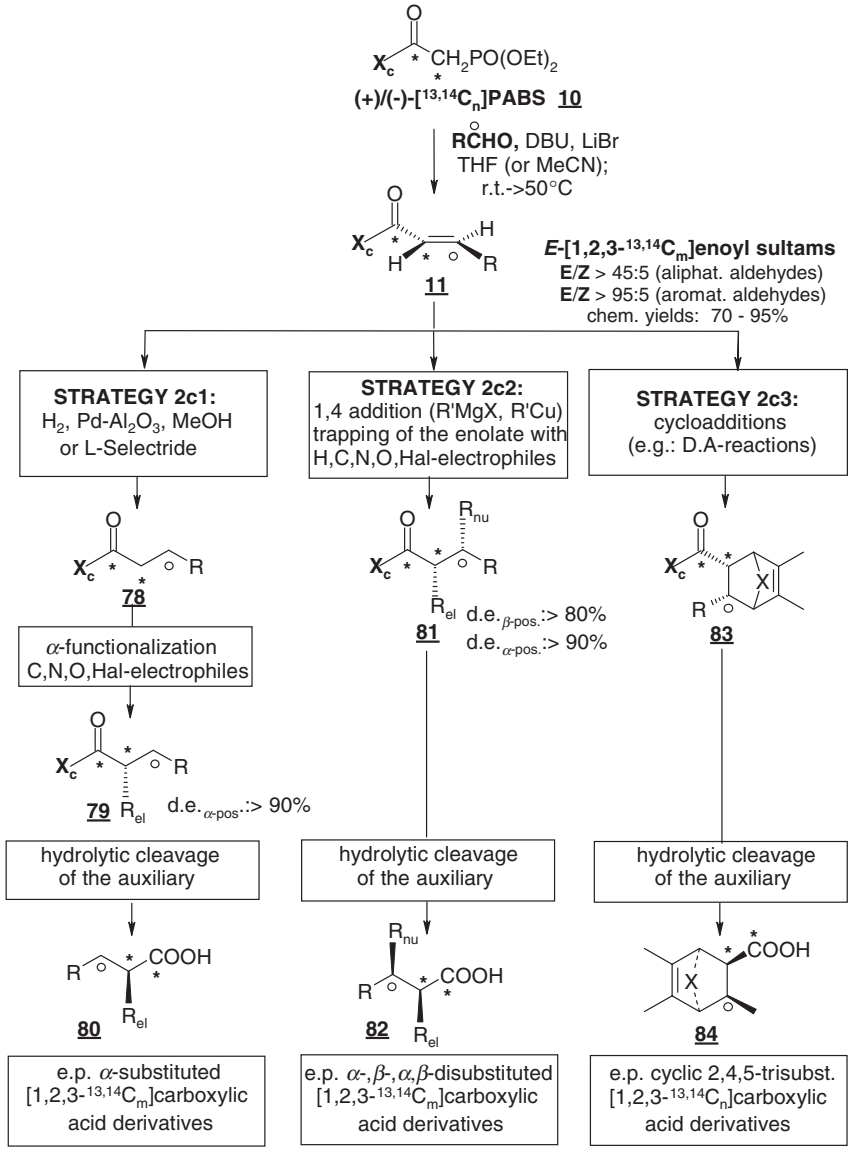


Figure 14. Application of [$^{13,14}C_n$]PABS for the synthesis of e.p. α,β, and α,β-substituted acyclic, alicyclic, heterocyclic [$^{13,14}C_m$]carboxylic acid derivatives (strategies 2c1–c3):

converted to a broad spectrum of selectively/multiply labelled building blocks[8,9] following three alternative strategies.[1]

Strategy 2c1: Catalytic hydrogenation[25] followed by α-functionalization of the saturated (+)/(−)-[$^{13,14}C_m$]acyl sultam **78** with C-, N-, O-,

Hal-electrophiles[7,26] and hydrolytic cleavage of the auxiliary yield the respective labelled e.p. α-branched or substituted carboxylic acids **80**.

By employing labelled aldehydes this approach facilitates the simultaneous incorporation of up to three carbon-13/carbon-14 atoms. Furthermore, it allows for the selective labelling of the positions 2 and/or 3, whenever position 1 (the carboxyl function) has to remain unlabelled (e.g. because of subsequent Curtius degradation or expected metabolic instability of this position in the target molecule).

Strategy 2c2: The Michael addition of appropriate C and N-nucleophiles (RMgX, CuR, $CuNSiMe_3Bn$, $PhMe_2SiCu$ etc.) and trapping of the enolates generated with H-, C-, N-, O-electrophiles[7] provide selectively/multiply labelled α-, β-, or α,β-branched or substituted derivatives **82** with diastereomeric excesses of $>80\%$ at the β-position and $>90\%$ at the α-position. In those cases, in which electrophile and nucleophile cross-react, the α-functionalization has to be performed in a separate second step.

Strategy 2c3: Cycloadditions (e.g. Lewis acid catalysed Diels-Alder reactions, 1,3-dipolar cycloadditions, OsO_4-catalysed dihydroxylations)[9] lead to e.p. selectively/multiply labelled alicyclic or heterocyclic carboxylic acid derivatives **84** and *cis*-diols, respectively.

Strategy 2c1: It was applied to the C-14 labelling of SDZ ISQ844 **91**,[1,26] an anti-asthmatic drug substance, in the metabolically stable position 4 (Figure 15). In order to facilitate labelling of the γ-position of the requisite key intermediate (S)-3′,4′-dimethoxy[3-^{14}C]phenylalanyl sultam **87**, the reaction sequence required subtle modification, namely use of unlabelled **PABS** and the respective carbonyl-^{14}C labelled aldehyde as reaction partners. Accordingly, [carbonyl-^{14}C]veratraldehyde **85** was reacted with **PABS** and the resulting cinnamoyl sultam catalytically reduced to the corresponding β-arylpropionyl derivative **86**. Diastereoselective α-hydroxyamination with 1-chloro-1-nitrosocyclohexane (CNC) and Zn-reduction of the hydroxyamine function[27] converted **86** into **87**. Reductive cleavage of the auxiliary and reaction of the resulting α-amino alcohol **88** with 3,5-dimethoxybenzoyl chloride furnished the bisacylated intermediate **89**. Bishler–Napieralski amide cyclization upon heating **89** in refluxing phosphorus oxychloride gave the *O*-acylated 1-aryl-3,4-dihydroisoquinoline **90**. Concluding cleavage of the ester function of **90** with LiOH in aqueous methanol released the labelled drug substance **91** in 18% overall yield (starting from **85**). To confirm the enantiomeric purity of **87** the substance was subjected to

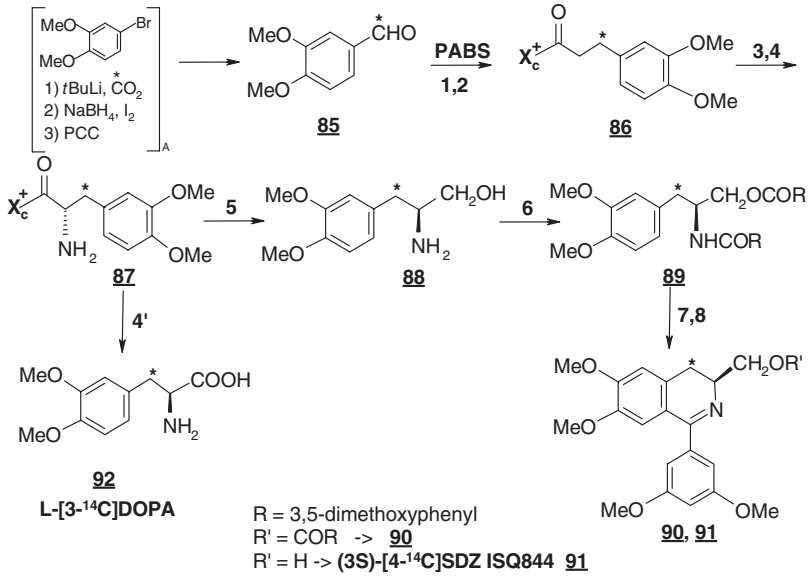


Figure 15. Synthesis of (3S)-[4-^{14}C]SDZ ISQ844 91: 1. (+)-PABS, DBU, THF; 50°C, 50 h; 2. H_2, Pd–Al_2O_3, MeOH–THF 2:1; r.t., 3 h; 3a. NHMDS, THF; -78°C, 45 min, b. 1-chloro-1-nitrosocyclohexane, −78°C, 45 min; 4. Zn, 1 N HCl–HOAc 1:1; 0°C, 1 h; 5. $NaBH_4$, I_2, EtOH–H_2O 3:1; 0, 90 min; r.t., 30 min; 6. 3,5-dimethoxybenzoyl chloride, DMAP, Et_3N, CH_2Cl_2 ; r.t. 3 h; 7. $POCl_3$; reflux, 3 h; 8. LiOH, MeOH–H_2O 20:1, r.t. 16 h; 4': LiOH, THF–H_2O 1:1; 0°C, 3 h

LiOH-mediated hydrolysis to give L-[3-^{14}C]DOPA **92** which was compared with commercially available unlabelled reference material.

Strategy 2c2: Unsymmetrically substituted chiral succinates **93** singly/multiply labelled with isotopic carbon are known to be of considerable interest as key intermediates in the stereoselective synthesis of labelled β^2- and β^3-amino acids (**96**, **99**).[28] This class of compounds has recently proved to be a useful structural element in modified peptides exhibiting remarkable stability towards proteolytic processes.[29] Depending on the nature of the β-amino acids (β^2 versus β^3 type) and the labelling pattern required, succinates **93** are readily accessible by either stereoselective α-alkylation of the respective unlabelled or singly labelled (+)/(−)-acyl sultams with singly or doubly labelled *tert*-butyl bromoacetate (pathway B, Figure 16) or use of unlabelled or singly labelled *tert*-butyl bromoacetate and singly or doubly labelled α-unsubstituted acyl sultams (pathway A, Figure 16). The latter ones can be conveniently generated from the corresponding labelled (+)/(−)-enoyl sultams **11** by either catalytic reduction (–> linear acyl derivatives) or 1,4-addition of

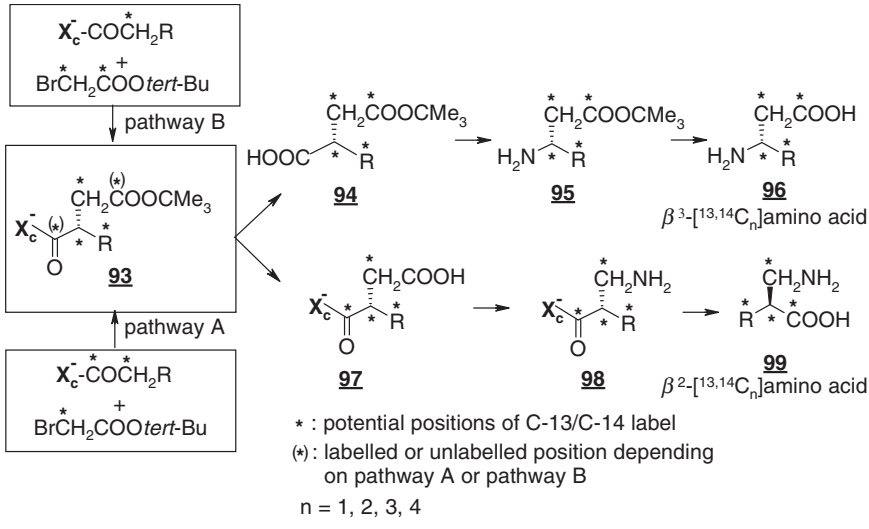


Figure 16. General synthesis of e.p. β^2-and β^3-amino acids singly/multiply labelled with isotopic carbon

Grignard or Cu-organic compounds (–>β-branched acyl derivatives) (see Figure 14). Cleavage of the *tert*-butyl ester function with TFA to **97** or, alternatively, hydrolytic removal of the auxiliary with LiOH to **94**, Curtius rearrangement of the liberated carboxylic acids to the respective N-protected or unprotected amino derivatives **95**, **98** and cleavage of the residual protecting group or the auxiliary furnish the respective β^3-[$^{13,14}C_n$]amino acids **96**, **99** in respectable overall radiochemical yields of 20–30%.

Pathway A was recently applied to the synthesis of β^2-[1,2-$^{14}C_2$]homovaline (=β^2-[1,2-$^{14}C_2$]HVal) **105**, required as a key intermediate for the labelling of linear β^2-decapeptides for investigations in their pharmacokinetic and metabolic behaviour (Figure 17). Employing *Strategy 2c2*, reaction of (–)-[1,2-$^{14}C_2$]**PABS** with acetaldehyde followed by 1,4-addition of Li[$CuMe_2$]Bu_3P to the resulting *trans*-crotonyl intermediate **100**[30] gave the requisite (–)-[1,2-$^{14}C_2$]valeryl sultam **101**. (The respective methyl-Grignard compound would have reacted in an 1,2-addition at the carbonyl group). Separate stereoselective α-alkylation of **101** with *tert*-butyl bromoacetate furnished the requisite unsymmetrically substituted succinate **102**. Acid-catalysed cleavage of the *tert*-butyl ester group, classical Curtius-rearrangement of the freed carboxyl group using ethyl chloroformate and sodium azide and trapping of the isocyanate generated *in situ* with *tert*-butyl alcohol converted **102** into the respective β-amino acyl sultam **104**. Finally,

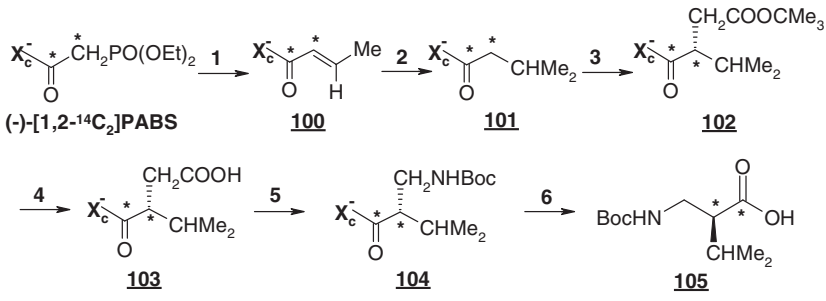


Figure 17. Synthesis of β^2-[1,2-$^{14}C_2$]HVal 105: 1. MeCHO, DBU, LiBr, acetonitrile; 0°C, 1 h; 2a. Li(Me_2Cu)Bu_3P (prepared from MeLi, CuI, Bu_3P, toluene; −5°C, 45 min), toluene; −78°C, 4 h, 2b. NH_4Cl, THF, −78°C–r.t.; 3a. NaHMDS, THF, −78°C, 1.5 h, 3b. $BrCH_2COO$ *tert*-Bu, HMPA, Bu_4NI, −78°C, 10 h; −50°C, 2 h; 4. TFA, CH_2Cl_2; 0°C, 2 h; 5a. ClCOOEt, Et_3N, THF; 0°C, 1 h, 5b. NaN_3, H_2O, THF; 0°C, 1 h, 5c. toluene; 100°C, 2 h, 5d. *tert*-BuOH, toluene; 100°C, 20 h; 6. LiOH, H_2O_2, THF-H_2O 3:1, r.t., 16 h

hydrolytic cleavage of the auxiliary gave the Boc-protected doubly labelled β^2-amino acid in 20% overall radiochemical yield.[28f]

The versatility of Strategy 2c2, which allows the creation of two stereogenic centres by a tandem reaction in one synthetic operation, was exemplified by the conjugate addition of EtMgBr to *E*-[1-^{14}C]crotonoyl (−)-sultam **106** and trapping of the resulting Mg-enolate with 1-chloro-1-nitrosocyclohexane to give the respective α-hydroxyamino acid derivative **107** with 85% d.e. (Figure 18). Chromatographic separation of the undesired diastereomers, followed by reductive *N*-methylation (HCHO, $NaBH_3CN$) of the α-hydroxyamino function and its subsequent reduction with zinc in acidic medium furnished *N*-methyl-(*S*)-[1-^{14}C]isoleucyl sultam **108**. Hydrolytic cleavage of the auxiliary converted **108** into the free *N*-methyl amino acid **109** in 34% yield (from (+)-[$^{14}C_2$]**PABS**).[1,8f,31]

N-methyl-(*S*)-[1-^{14}C]isoleucine **109** was needed for the C-14 labelling of NVP NIM811 **114**, a non-immunosuppressive cyclosporin derivative, following the strategies already outlined for NVP IMM125 **31** and Valspodar **50**[13a–c] with some minor modifications. (1) First, protection of the amino function of **109** with FmocOSU prior to coupling to the unlabelled linear decapeptide methyl ester $P_{Aac}{}^{8->6}$-OMe. (2) Protection of the secondary hydroxyl function of $P_{Aac}{}^{8->6}$-OMe by acetylation and (3). After the cyclization cleavage of the acetyl group upon treatment of **113** with sodium methoxide.

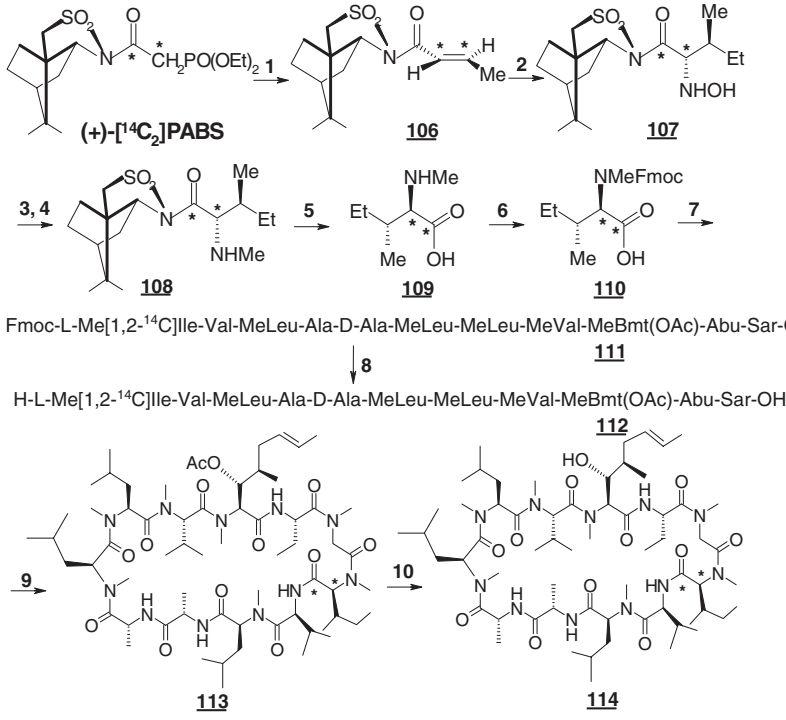


Figure 18. Synthesis of [L-Me[1,2-$^{14}C_2$]Ile^9]NVP NIM811 <u>114</u>: 1. MeCHO, DBU, LiBr, acetonitrile; r.t., 3 h; 2a. EtMgBr, THF; −80°C, 16 h, b. 1-chloro-1-nitrosocyclohexane, THF; −78°C, 1 h, c. 1N HCl; −78°C; 3. Aqu. HCHO (pH 4–6), $NaBH_3CN$, MeOH; r.t., 1.5 h; 4. Zn, 1 N HOAc-1 N HCl 2:1; 0°C, 30 min, r.t., 2 h; 5. LiOH, THF-H_2O, r.t., 16 h; 6. Fmoc–OSu, aqu. Na_2CO_3, THF; 0°C, 1 h; r.t., 20 h; 7. H–Val–MeLeu–Ala–D–Ala–MeLeu–MeLeu–MeVal–MeBmt(OAc)–Abu–Sar–OMe **(= $P_{Aac}{}^{8->6}$-OMe), $(PrPO_2)_3$, DMAP, CH_2Cl_2; r.t., 4.5 h; 8. 0.2 N NaOH, EtOH; 0°C, 16 h; 9. Castro-reagent, DMAP; r.t., 22 h; 10. NaOMe, MeOH; r.t., 3 h**

Strategy 2c3 (2,4-cycloadditions; Diels–Alder reaction, Figure 19):[7,9] Reaction of (+)-[1,2-$^{14}C_2$]**PABS** with paraformaldehyde in the presence of K_2CO_3 gave (+)-[1,2-$^{14}C_2$]acroyl sultam **<u>115</u>** in 52% yield. In contrast to what is known from other ^{14}C labelled acrylates, **<u>115</u>** proved to be unexpectedly stable towards radiation induced polymerization probably due to the bulky auxiliary. Subsequent treatment of the compound with excess butadiene at −70°C in the presence of $EtAlCl_2$ and 0.1 mol% of galvinoxyl as radical scavenger followed by chromatographic separation of the 3% of undesired stereoisomers provided d.p. (1*R*)-3-[1,7-^{14}C]cyclohex-3-enyl-carbonyl sultam **<u>116</u>**.[5b] Hydrolytic cleavage of the auxiliary, epoxidation of the free acid with

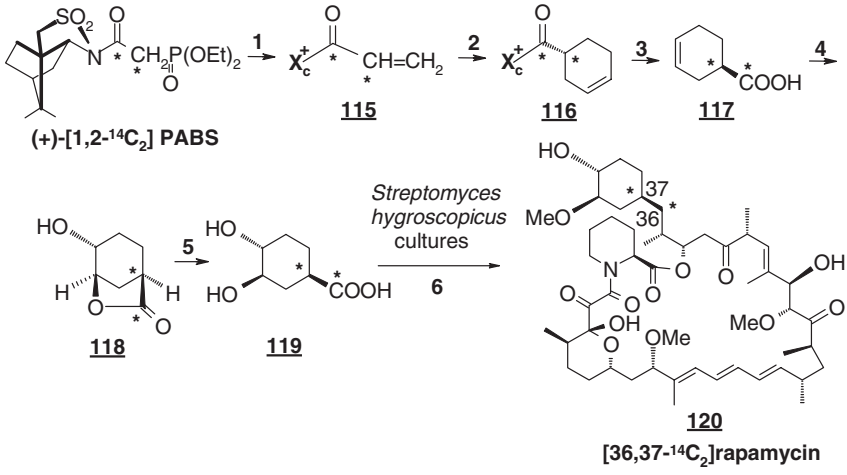


Figure 19. Synthesis of (1*R*,3*R*,4*R*)-3,4-dihydroxy-[1,7-$^{14}C_2$]cyclohexane-carboxylic acid 119 and [36,37-$^{14}C_2$]rapamycin 120: 1. $(HCHO)_n$, galvinoxyl, K_2CO_3, THF; 70°C, 30 min; 2. 1,3-butadiene, $EtAlCl_2$, CH_2Cl_2; -78°C, 4 h; 3. LiOH, THF–H_2O 2:1; r.t., 7 h; 4a. *m*-chloroperbenzoic acid, CCl_4; 0°C, 10 min; r.t., 16 h b. Et_3N, SiO_2; 70°C, 5 h; 5. 5 N HCl-THF 1:8; r.t., 16 h; 6. *Streptomyces hygroscopicus* cultures; 24°C, 4 days

m-chloroperbenzoic acid, and heating of the epoxy derivative in situ generated with triethylamine converted **116** into the respective lactone **118**. Acid-catalysed hydrolytic cleavage of the lactone ring finally afforded (1*R*,3*R*,4*R*)-3,4-dihydroxy[1,7-^{14}C]cyclohexane carboxylic acid **119** in 15% overall yield (starting from (+)-[1,2-$^{14}C_2$]**PABS**).[5b,32,33]

Compound **119** was investigated as an alternative to (*S*)-[2,7-$^{13,14}C_2$]pipecolic acid **64** (Figure 11) for the biological labelling of rapamycin with isotopic carbon at the positions **36** and **37**. Evaluation of analogous feeding experiments with ^{13}C- and ^{14}C-labelled **119** following isolation of **120** from the respective culture broths revealed an incorporation rate of 40% and a radiochemical yield of **120** of 10%.[5b] Since these numbers compare well with the 60% and 15%, respectively, observed when (*S*)-[1,7-$^{14}C_2$]pipecolic acid was employed, we were confident that both compounds would be suitable precursors for the biological preparation of larger quantities of [$^{14}C_n$]rapamycin with sufficiently high specific activity for the envisaged *in vivo* pharmacokinetic and metabolic studies.

In order to eliminate the considerable variation in the pharmacokinetic behaviour of rapamycin in man – a major weak point in its development as a conventional immunosuppressive drug – the

compound was converted in a two-step sequence into its 40-*O*-2-hydroxyethyl derivative **122** (Everolimus, NVP RAD001) (Figure 20) by selective alkylation of the hydroxy group of **121** at position 40 with 2-TBDMSO-ethyl triflate in toluene-dimethoxyethane (50°C, 3 h) followed by acid-catalysed cleavage of the protecting group with acetic acid.[34] Since first ADME-studies in animals had indicated a metabolic splitting of the molecule in two parts (hydrolysis of the lactone system; retro-aldol cleavage between positions 27 and 28),[35] each portion had to be labelled with comparable specific activities in order to trace all potential metabolites in the following human studies. This could be

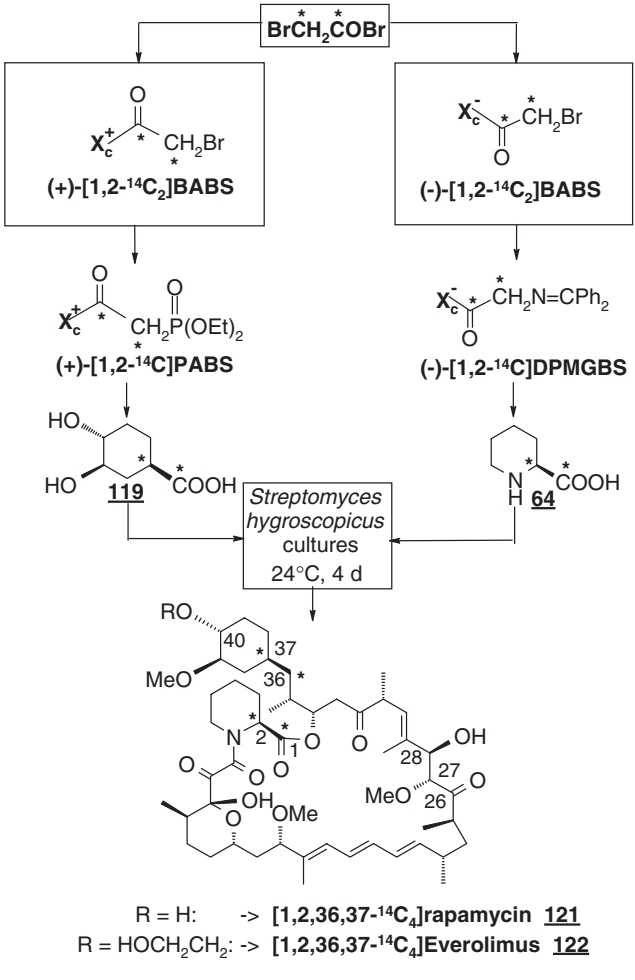


Figure 20. Synthesis of [1,2,36,37-$^{14}C_4$]-rapamycin 121 and [1,2,36,37-$^{14}C_4$]-Everolimus 122 at 100 mCi/mmol

easily achieved by employing a mixture of three equivalents of (1*R*,3*R*,4*R*)-3,4-dihydroxy-[1,7-$^{14}C_2$]cyclohexane carboxylic acid **119** and two equivalents of (*S*)-[1,7-$^{14}C_2$]pipecolic acid **64**, both with a specific activity of 110 mCi/mmol as substrate. Work up of the respective fermentation broth provided e.p. [1,2,36,37-$^{14}C_4$]rapamycin **121** in 15% radiochemical yield (from **64** and **119**) showing a specific activity of 104 mCi/mmol which could be easily converted into [1,2,36,37-$^{14}C_4$]Everolimus **122** following the two-step sequence described before.

Strategy 3 (Figure 4): The last synthetic sequence demonstrates the versatility of the 2-carbon elongation of appropriate [$^{13,14}C_n$]acyl sultams **12** which gives rise to e.p. singly or multiply labelled β,γ-unsaturated carboxylic acid derivatives bearing either remote or multiple stereogenic centers. This approach was applied to the synthesis

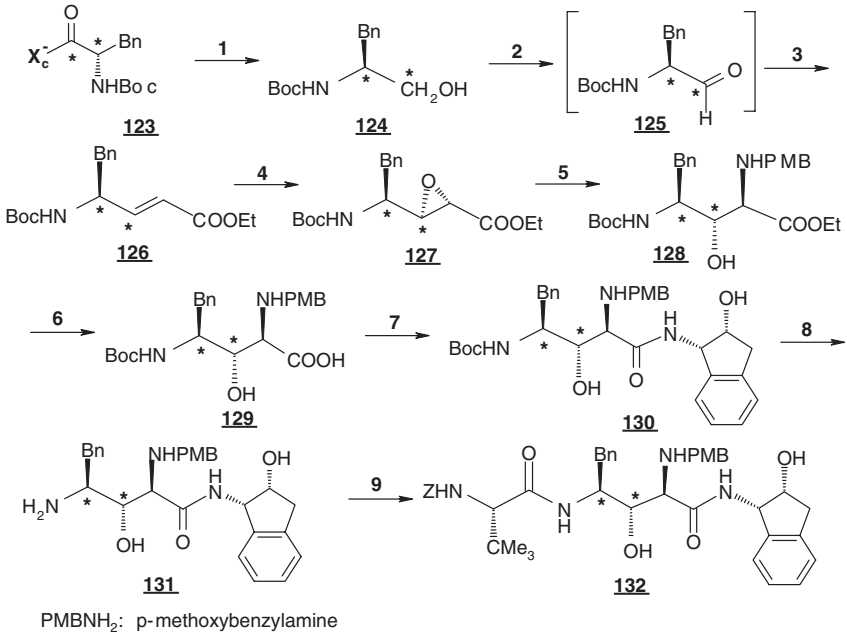


Figure 21. Synthesis of [$^{14}C_2$]SDZ PRI053 132: 1. $NaBH_4$–HOAc 1:1, dioxane; 80°C, 3 h; 2a. $(COCl)_2$, DMSO, THF; −60°C, 30 min,–0°C, b. Ph_3P=CHCOOEt, Et_3N; 0°C–r.t., 2 h; 3a. MCPBA, CH_2Cl_2; r.t., 8 days, b. LC-separation of 9:1-diastereomers; 4. p-methoxybenzylamine (= $PMBNH_2$), EtOH; 70°C, 20 h; 5. 2 N NaOH, THF; r.t., 16 h; 6. (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol, EDC, HOBT, DMF; r.t., 16 h; 7. 5 N HCl, ether; r.t., 3 h; 8. *N*-Z-(*S*)-*tert*-leucine, NMM, HOBT, EDC, DMF; r.t., 20 h

of [$^{14}C_2$]SDZ PRI053 **132**,[1,36b] a protease inhibitor of the β-hydroxy-α,γ-diamino acid type,[36a] which required double labelling with carbon-14 at the β- and γ-positions, both essential for the pharmacological activity of the compound (Figure 21).

N-Boc-(*S*)-[1,2-$^{14}C_2$]phenylalanyl (−)-bornane-10,2-sultam **123**, needed as a key intermediate for the envisaged sequence, was prepared by diastereoselective α-alkylation of (−)-[1,2-$^{14}C_2$]**DPMGBS** with benzyl bromide. Reduction of **123** with $NaBH_4$–HOAc 1:1 cleaved the auxiliary to give the *N*-protected amino alcohol **124** without any detectable racemization. Swern oxidation of **124** with DMSO-oxalyl chloride and trapping of the *in situ* generated aldehyde **125** with ethoxycarbonylmethylenetriphenylphosporane afforded the respective e.p. *N*-bocylated α,β-unsaturated γ-amino acid ester **126**. Epoxidation of **126** with *m*-chloroperbenzoic acid was followed by chromatographic separation of the resulting 9:1 mixture of the diastereomeric epoxides to give e.p. (2*R*,3*R*)-epoxide **127**. Stereoselective opening of the epoxide ring upon treatment of **127** with 4-methoxybenzylamine and saponification of the ester function with ethanolic NaOH furnished the N_4-bocylated (2*R*,3*R*,4*S*)-2,4-diamino-3-hydroxy-5-phenyl-[3,4-$^{14}C_2$]pentanoic acid derivative **129** in 10% overall non-optimized yield from (−)-[$^{14}C_2$]**BABS**.

Finally, intermediate **129** was converted into [$^{14}C_2$]SDZ PRI053 **132** using conventional peptide synthetic methodology. The free carboxyl group was coupled with EDC/HOBt to (1*S*,2*R*)-1-amino-2-hydroxyindane to give the respective amide **130**. After deprotection of the terminal amino group with 5 M ethereal HCl, intermediate **131** was coupled in the last step with EDC/HOBt to *N*-Z-(*S*)-*tert*-leucine to give **132**.

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