JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 361–370.
DOI: 10.1002/jlcr.558

Research Article

Regio- and stereoselective preparation of ascomycin-d_1 and FK 506-d_1

Murat Acemoglu[1]*, Hendrik Andres[2] and Thomas Moenius[2]
[1] *Chemical and Analytical Development, Novartis Pharma AG, CH-4002 Basel, Switzerland*
[2] *Preclinical Safety, Novartis Pharma AG, CH-4002 Basel, Switzerland*

Summary

The immunosuppressive macrolides ascomycin **1** and FK 506 **2** were stereoselectively deuteriated at C(32) using Curran's radical translocating method. Both AIBN and Et_3B/O_2 were tested as radical initiator for the radical translocation/reduction step with Bu_3SnD as reducing agent. Despite only minor structural differences, ascomycin and FK 506 showed remarkably different behaviour under the radical translocation/reduction conditions. Higher stereoselectivities were observed with Et_3B/O_2 as initiator, presumably due to lower reaction temperatures applied in this case. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: stereoselective deuteriation; radical translocation; ascomycin; FK506

Introduction

Translocation of radical sites by intramolecular H-atom transfer have found many applications in organic synthesis, e.g. remote functionalisation,[1] translocation with subsequent addition reaction[2] or more frequently, translocation with subsequent cyclisation.[3–7] Protecting/

*Correspondence to: M. Acemoglu, Chemical and Analytical Development, Novartis Pharma AG, WSJ-145.965, CH-4002, Basel, Switzerland. E-mail: murat.acemoglu@pharma.novartis.com

Received 11 October 2001
Revised 19 November 2001
Accepted 21 November 2001

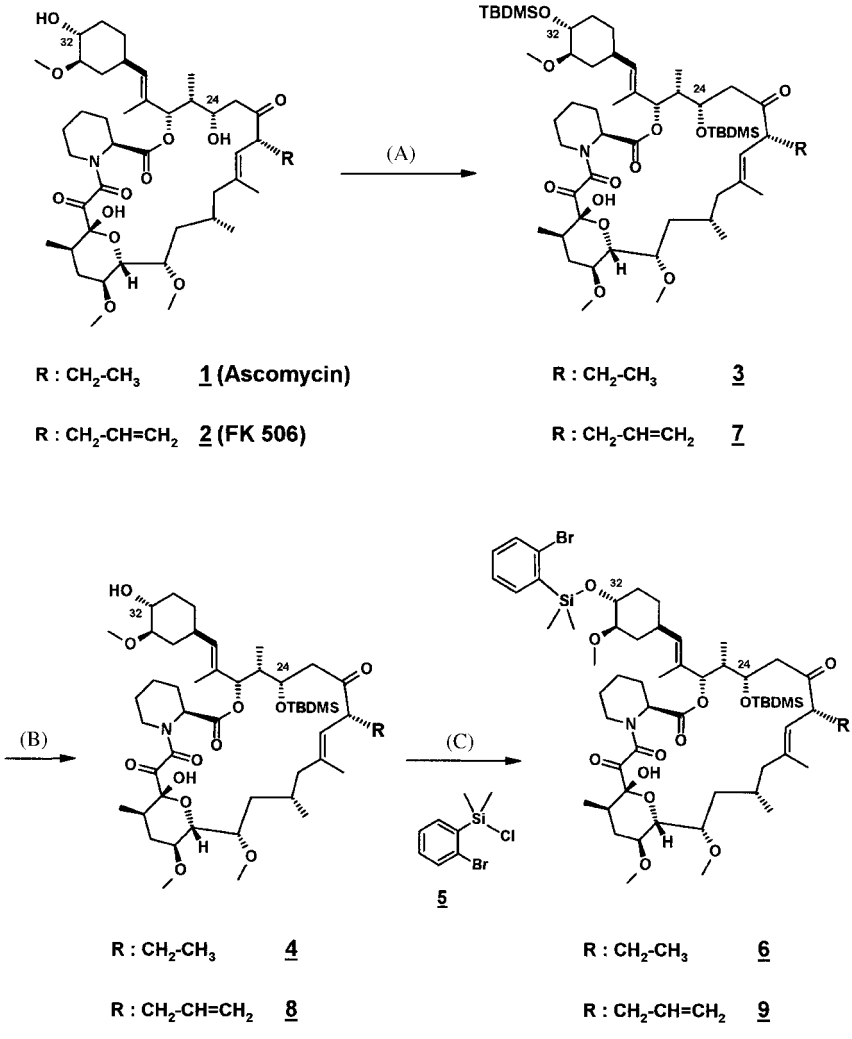
radical translocating (PRT) groups, such as (2-bromophenyl)dimethylsilyl ether were introduced by Curran *et al.*,[5,6] and were designed to serve a dual function, as a protecting group and as a group to selectively activate a remote C–H bond of the molecule for a radical bond forming reaction. Recently, the 1,5-hydrogen translocation was utilised for highly enantioselective synthesis of β-substituted β-amino acids by 1,3-asymmetric induction.[8]

Curran *et al.*[7] demonstrated the suitability of the (2-bromophenyl)dimethylsilyl derivatives for the regio- and stereoselective [^{2}H]-labelling of rapamycin at position C(42). Curran's method was adapted for tritium labelling of RAD001, a new immunosuppressant.[9] In this paper, the application of the method to the regio- and stereoselective preparation of [^{2}H]-labelled derivatives of ascomycin and FK 506 is reported.

Results and discussion

Synthesis of the (o-bromophenyl)dimethylsilylether derivatives of ascomycin and FK506

For the regioselective introduction of the radical translocating (2-bromophenyl)dimethylsilylether group, ascomycin (**1**) was silylated with TBDMS-Cl at positions O–C(24) and O–C(32), to give the bis-(TBDMS)-ether **3** (Scheme 1). The TBDMS-ether at O–C(32) was cleaved regioselectively with 40% aqueous HF in CH_3CN[10] to obtain the corresponding mono-(TBDMS)-ether **4** in 93% yield. The reaction of compound **4** with (2-bromophenyl)dimethylchlorosilane (**5**) proceeded smoothly with imidazole as base in dimethylformamide and gave the corresponding silyl ether **6** in 92.8% yield. An excess (2.0 moleq.) of the silylating agent **5** was necessary for complete conversion. The silylating agent, (2-bromophenyl)dimethylchlorosilane (**5**) was prepared from 1,2-dibromobenzene and dimethyldichlorosilane by a slight modification of a known procedure.[5,11] The process was modified to minimise side reactions (formation of benzyne and multiple metallation/polymerization processes) as well as to protect the highly sensitive product against hydrolysis. For this purpose, BuLi was added dropwise to a solution of 1,2-dibromobenzene at -110°C and the formed, monolithiated intermediate was allowed to react immediately by parallel slow addition of a solution of dimethyldichlorosilane. To avoid hydrolysis by uptake of water during work-up and to ensure dryness of the crude



Reaction conditions:(A)TBDMS-Cl, Imidazole, DMF, 95% for **3**, 96.7% for **7**;(B)40% aq. HF, CH_3CN, 93% for **4**, 90.3% for **8**. (C) Imidazole, DMF, 92.8% for **6**, 90.9% for **9**.

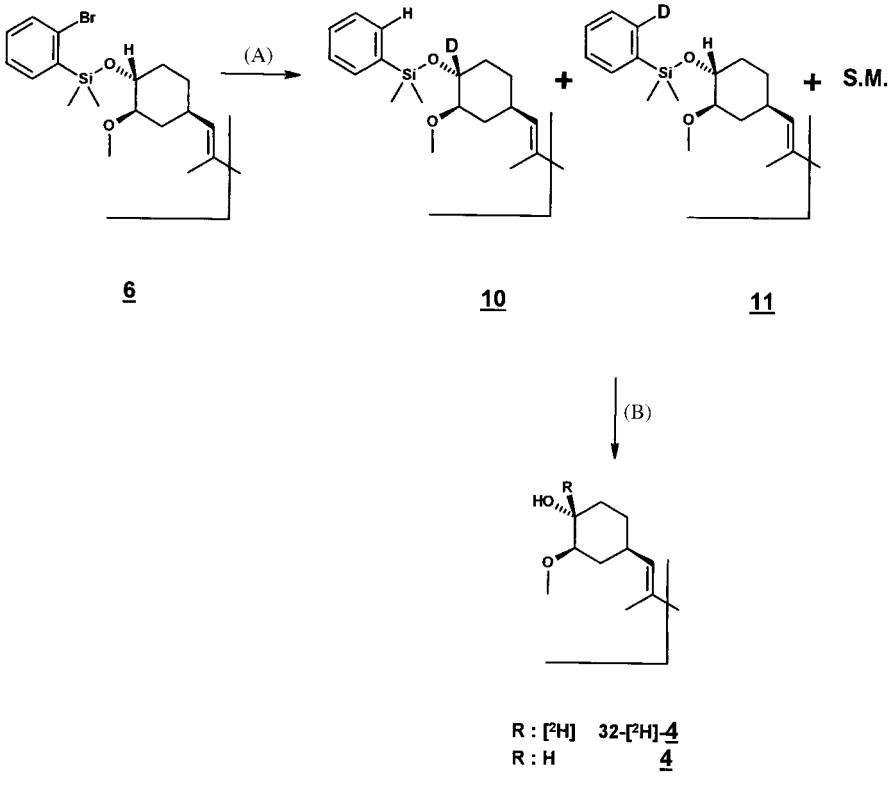
Scheme 1. Preparation of the (*o*-bromophenyl)dimethylsilylether derivatives of ascomycin and FK 506

product, a solvent exchange was performed by diluting the reaction mixture with cyclohexane, distillation of the ether solvents, filtration of the formed suspension and subsequent evaporation to dryness. The crude product was distilled in Kugelrohr to obtain compound **5** in 65% yield. If purified by Kugelrohr distillation, compound **5** was stable for several months in DMF-solution at 0–4°C.

Starting from FK 506 (**2**), the corresponding (*o*-bromophenyl)dimethylsilyl derivative **9** was prepared in comparable yields using the same procedures as described for the preparation of **6** (Scheme 1).

Synthesis of 24-O-TBDMS-32-[^{2}H]-ascomycin

When the ascomycin derivative **6** was exposed to the classical radical translocating/reducing conditions with AIBN and Bu_3SnD in refluxing benzene, slow decomposition of the starting material was observed without the formation of significant amounts of the desired product. However, with Et_3B/O_2 as radical initiator and THF as solvent at room temperature, 76% conversion to deuteriated products was achieved (Scheme 2). The ^{2}H-NMR spectrum of the crude product mixture revealed that ca 30% of the deuterium label was located at the aromatic ring (multiplet at 7.5–7.8 ppm) and ca 70% of it was on the ascomycin residue (broad multiplets at 3.2–3.8 and 1.0–1.5 ppm). It is worth mentioning that only one deuterium atom can be transferred to each molecule by this method. From the ^{2}H-NMR data given above it follows that ca 70% of the initially formed aryl radicals lead to translocation and in ca 30% of the occurrences, the aryl radicals were directly reduced by Bu_3SnD. Based on model studies with silylated cyclohexane-1,2-diols,[7] the hydrogen abstraction from position C(32) of ascomycin (1,5-hydrogen shift) was supposed to dominate over abstraction from positions C(31) and C(33) (1,6-hydrogen shift). Indeed, the integral of the signal at 1.0–1.5 ppm, corresponding to deuterium at C(31), showed $<5\%$ deuteriation at this C-atom. Due to overlapping broad signals, the exact ratio of deuteriation at C(33) could not be determined from the ^{2}H-NMR spectrum. However, although a higher ratio of deuteriation at C(33) by comparison to C(31) may be assumed as a consequence of oxy substitution at C(33), the order of magnitude of deuteriation must be similar in both cases, since both products are formed via a 7-ring transition state. Thus, minor deuteriation at C(33) and high regioselectivity of the desired radical translocation and deuteriation at C(32) can be concluded. After the radical translocation/reduction step, the phenyl(dimethyl)silyl ether was cleaved selectively (Scheme 2) by hydrolysis of the product mixture with acetic acid/H_2O in THF. As expected, a mixture of 24-O-TBDMS-32-[^{2}H]-ascomycin (32-[^{2}H]-**4**) and unlabelled 24-O-TBDMS-ascomycin (**4**) was obtained. 32-[^{2}H]-**4** was formed from the hydrolysis of compound



Reaction conditions:(A) Et_3B, O_2, Bu_3SnD, THF, 20°C, 76% conversion, **10**:**11** ca. 70:30; (B) AcOH, H_2O, 51% from **6**, **32-[²H]-4** : **4** = ca. 53:47.

Scheme 2. Deuterium labelling of ascomycin by radical translocation/reduction

10 whilst the hydrolysis of **11** and of the starting material **6** led to the formation of unlabelled **4**.

The product mixture was analysed by MS, ^{1}H-NMR, ^{2}H-NMR, and by HPLC. MS analysis revealed a ratio of 53:47 labelled to unlabelled product. The ^{2}H-NMR spectrum confirmed the high regioselectivity of the radical translocation/reduction step, showing a broad multiplet at 3.2–3.7 ppm for ^{2}H–C(32) as the only significant signal. The ^{1}H-NMR spectrum of the mixture was almost identical to that of unlabelled 24-O-TBDMS-ascomycin (**4**), exhibiting minor differences in the shape of the signal of H–C(33) at ca 3.05 ppm. The hydrogen abstraction and subsequent ^{2}H-incorporation at C(32) was also stereoselective, giving rise to minor epimerization at this carbon atom. In the HPLC, 24-O-TBDMS-32-[^{2}H]-epi-ascomycin (epi-32-[^{2}H]-**4**) appeared at ΔR_t = ca

2.0 min (4.7 area%) compared to 24-O-TBDMS-32-[^{2}H]-ascomycin (32-[^{2}H]-**4**) (95.3 area%) and was identified by LC–MS and by co-chromatography with an authentic sample. The epimerization must have occurred during the reduction of the intermediate radical at C(32) with Bu_3SnD and therefore, each epimer molecule must be deuteriated at position C(32), whilst the total ratio of deuteriated products including the epimer was 53 mol% according to MS.

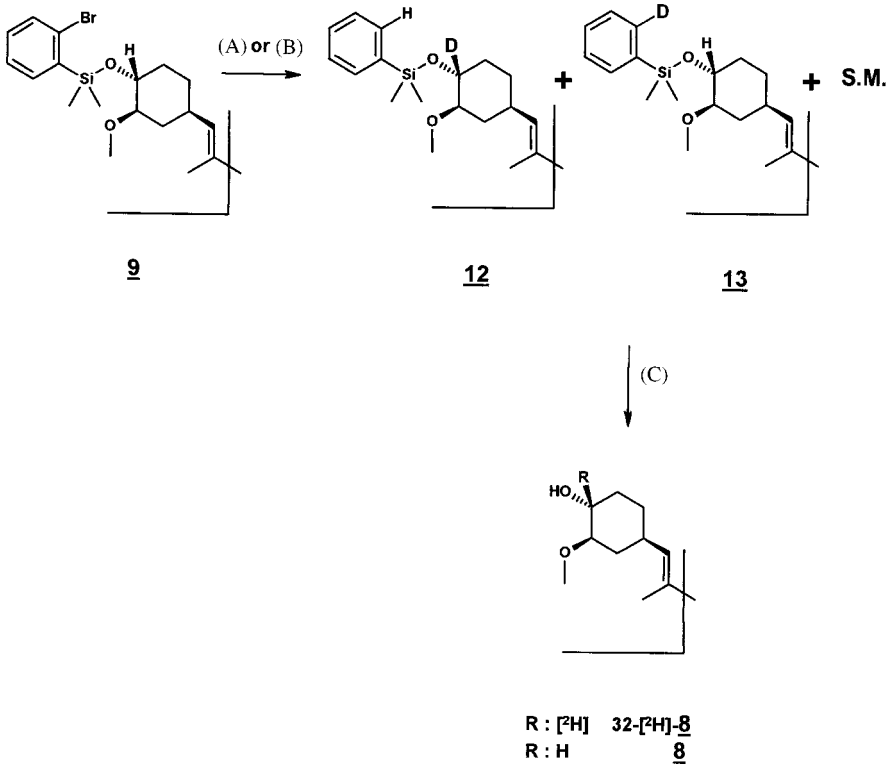
From the data given above, a ratio of ca 9% can be assumed for epimerization at C(32) and 91% of the radicals formed at C(32) must have been reduced stereoselectively to afford 32-[^{2}H]-**4**.

Synthesis of 24-O-TBDMS-32-[^{2}H]-FK 506

Under the radical translocation/reduction conditions, the FK 506 derivative **9** reacted differently to the ascomycin derivative **6**: Both Et_3B/O_2 and AIBN could be used as initiators in this case (Scheme 3). With Et_3B/O_2 as initiator, the conversion was 63%. According to the ^{2}H-NMR spectrum, ca 65% of the deuterium label was located on the FK 506 moiety of the molecule and the remainder on the aromatic ring. Regioselective desilylation of the mixture at position O–C(32) with $AcOH/H_2O$ gave a mixture of the corresponding 24-O-TBDMS-derivatives **8** and 32-[^{2}H]-**8** (Scheme 3). MS analysis of the product revealed a ratio of ca 41:59 for labelled:unlabelled product. The ^{2}H-NMR spectrum showed a significantly higher deuteriation (ca 7%) at C(31) and C(33) by comparison to the ascomycin series, where the deuteriations at these carbons were negligible. Finally, the ratio of epimerization at C(32) was determined to be ca 3% by HPLC analysis.

AIBN, which was rather ineffective as initiator in the ascomycin series, could be used in the case of FK 506 derivative **9** to initiate the radical translocation/reduction in refluxing benzene. The crude product mixture was then selectively desilylated at position O–C(32) to obtain a mixture of labelled/unlabelled 24-O-TBDMS-FK 506. MS analysis revealed a ratio of 68:32 for 32-[^{2}H]-**8** : **8**. The epimerization at C(32) was higher in this case (ca 10%) compared to Et_3B/O_2 as initiator, presumably due to the difference in the reaction temperature.

Complete desilylations of the mono- or bis-silylether derivatives of ascomycin and FK 506 were performed with 40% aqueous HF in CH_3CN at room temperature for 2.5 h, to obtain ascomycin or FK 506 in ca 70% yield.



Reaction conditions:(A)Et_3B, O_2, Bu_3SnD, THF, 20°C, 63% conversion, **12**:**13** ca. 65:35;
B) AIBN, Bu_3SnD, benzene, reflux, **12**:**13** n. d.;(C)AcOH, H_2O, THF, -5°C;
62% overall yield and **32-[²H]-8** : **8** = 41:59 for products from conditions (A) and(C);
45% overall yield and **32-[²H]-8** : **8** = 68:32 for products from conditions (A) and(C).

Scheme 3. Deuterium labelling of FK 506 by radical translocation/reduction

Conclusions

The protecting/radical translocating (2-bromophenyl)dimethylsilyl group can be used for regio- and stereoselective [^{2}H]-labelling of ascomycin and FK 506. Higher regio- and stereoselectivities can be achieved by performing the radical translocation/reduction step at lower temperatures. Despite only minor structural differences, the corresponding ascomycin and FK 506 derivatives behave significantly differently during the radical translocation/reduction step regarding reactivity and regio-, as well as stereoselectivity. These results suggest different conformations for the two macrocyclic substances.

Experimental

Chemicals were purchased from commercial suppliers. Intermediates and precursors were identified by either chromatographic and/or spectroscopic methods (NMR, MS). Mass spectra were measured on a VG 70-SE mass spectrometer in a nitrobenzylalcohol/lithium iodide matrix (FAB, Xe: 8 keV) or on a FINNIGAN TSQ 7000 spectrometer in acetonitrile/water (ESI +QlMS). NMR spectra were measured on a BRUKER AMX 400 spectrometer.

HPLC: HP Series 1050 with UV–VIS detector; stationary phase: Nucleosil 120/Cl8, 5 µm, 250 × 4.6 mm; mobile phase: gradient of *A* and *B*, consisting of H_2O/CH_3CN/*t*-butyl methyl ether /H_3PO_4, *A* = (650:240:60:0.2) v/v and *B* = (200:660:60:0.2) v/v. *B*:*A* = 5 min (70:30); 8–22 min (100:0); 27–32 min (70:30).

Modified synthesis of (2-Bromophenyl)dimethylchlorosilane (**5**)

BuLi (1.55 M solution in hexane, 56.46 g, 125 mmo1) was added dropwise to a solution of 1,2-dibromobenzene (30.4 g, 125 mmol) in 1000 ml of THF/diethyl ether (1:1) at −110°C and the formed, monolithiated intermediate was allowed to react immediately by parallel slow addition of a solution of dimethyldichlorosilane (24.30 g, 187 mmol) in THF/diethyl ether (1:1). When the addition was completed, the reaction mixture was stirred for 1 h at −110°C and allowed to warm up to room temperature. Then, a solvent exchange was carried out by diluting with cyclohexane and distilling off the ether/THF mixture. The formed precipitate was removed by filtration and the solvent was evaporated. The resulting crude liquid was purified by Kugelrohr-distillation at 80–100°C and 0.20–0.15 mbar. Yield: 19.5 g (65%) of (2-bromophenyl)dimethylchlorosilane (**5**) as colourless liquid.

Synthesis of 24-O-TBDMS-32-O-(2-bromophenyl)dimethylsilylether derivatives of ascomycin and FK 506 (compounds **6** *and* **9**)

A solution of **4** or **8**[10](3.17l mmol) and imidazole (0.876 g, 12.74 mmol) in DMF (100 ml) was cooled down to −9°C to −13°C and was treated dropwise with a solution of (2-bromophenyl)dimethylsilyl-chloride (**5**) in DMF (4.6 g of a 34.5% w/w solution, 6.35 mmol) while maintaining the temperature at −9°C to −13°C. Stirring was continued at this temperature for 90 min, until a TLC (silica gel, hexane/2-propanol 95:5

as eluent) indicated the completion of the conversion. The reaction mixture was then poured onto PBS-buffer, pH 7 (600 ml) and diethyl ether (200 ml) was added. The mixture was stirred for 45 min at room temperature, the phases were separated and the H_2O-phase was extracted with diethyl ether (2×150 ml). The organic phase was washed with H_2O (150 ml), dried over anhydrous Na_2SO_4 and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel with hexane/2-propanol/N-ethyl-diisopropylamine (95:5:0.1) as mobile phase, to obtain the pure compounds **6** (93% yield) or **9** (91% yield) as white solids. ^{1}H-NMR and MS spectra of the products were in accordance with the proposed structures.

Procedure for [^{2}H]-labelling by radical translocation/reduction with Et_3B/O_2 as initiator

O_2-gas (5 ml, 0.22 mmol) was bubbled through a solution of **6** or **9** (0.22 mmol), Bu_3SnD (0.132 g, 0.442 mmol) and Et_3B (0.22 ml of a 1 M THF solution, 0.22 mmol) in dry THF (9 ml) during 3 min at room temperature and the mixture was stirred for an additional 6 h. For work-up, the reaction mixture was diluted with diethyl ether (10 ml) and DBU (0.04 g, 0.257 mmol) was added. After addition of sufficient amounts of iodine (until coloured solution), the solution was filtered over silica gel and evaporated to obtain a crude product mixture including starting material. The crude product mixture was directly hydrolysed as follows: a solution of the product mixture (1 g) in THF (60 ml) was treated at -5°C with H_2O (22.5 ml) and subsequently with acetic acid (22.5 ml), while maintaining this temperature. The mixture was stirred for an additional 1 h at -5°C to complete the reaction. For work-up, the reaction mixture was diluted with diethyl ether (150 ml) and the solution was extracted with H_2O (2×300 ml). The H_2O-phases were combined and were extracted with diethyl ether (2×150 ml). The combined organic phases were washed with brine (250 ml), dried on Na_2SO_4 and the solvent was evaporated to obtain the crude product, which was purified by column chromatography on silica gel with hexane/2-propanol/*N*-ethyl-diisopropylamine (85:15:0.1) as mobile phase. Starting from the ascomycin derivative **6**, a 47:53 mixture of **4**/32-[^{2}H]-**4** was obtained as product in 51% overall yield. A 59:41 mixture of **8**/32-[^{2}H]-**8**, was obtained in 62% overall yield, when the FK 506 derivative **9** was used as starting material.

Procedure for radical translocation/reduction with AIBN as initiator

Essentially, the procedure of Curran *et al.*[7] was applied: a solution of 24-O-TBDMS-32-O-(*o*-bromophenyl)dimethylsilyl-FK 506 (**9**) (0.102 g, 0.09 mmol), AIBN (0.003 g, 0.018 mmol) and Bu_3SnD (0.0325 g, 0.108 mmol) in benzene (3 ml) was heated to reflux for 2.5 h. The reaction mixture was then cooled down to room temperature, the solution was filtered over silica gel and the silica gel was washed using THF (40 ml) for complete elution of the product/educt mixture. The mixture was hydrolysed with acetic acid/H_2O in THF as described above and the crude product was purified by column chromatography on silica gel with hexane/2-propanol/*N*-ethyl-diisopropylamine (85:15:0.1) as mobile phase to obtain a 32:68 mixture of **8**/32-[^{2}H]-**8** in 45% overall yield.

Acknowledgements

We thank Mr R. Schreiber and Miss N. Brändlin for technical assistance, Mr J. France for the NMR-spectra and Dr C. Guenat for the MS spectra and MS calculations.

References

1. Breslow R, Heyer D. *J Am Chem Soc* 1982; **104**: 2045.
2. Giese B, Dupuis J, Hasskerl T, Meixner J. *Tetrahedron Lett* 1983; **24**: 703.
3. Heiba EI, Dessau RM. *J Am Chem Soc* 1967; **89**: 3772.
4. Giese B. *Radicals in Organic Synthesis: Formation of Carbon–Carbon Bonds*. Pergamon Press: Oxford, 1986.
5. Curran DP, Kim D, Liu HT, Shen W. *J Am Chem Soc* 1988; **110**: 5900.
6. Curran DP, Xu J. *J Am Chem Soc* 1996; **118**: 3142.
7. Curran DP, Somayajula KV, Yu H. *Tetrahedron Lett* 1992; **17**: 2295.
8. Beaulieu F, Arora J, Veith U, Taylor NJ, Chapell BJ, Snieckus V. *J Am Chem Soc* 1996; **118**: 8727.
9. Moenius Th, Andres H, Acemoglu M, Kohler B, Schnelli P, Züger C. *J Labell Compd Radiopharm* 2000; **43**: 113–120.
10. Grassberger M. Novartis Research Institute, Vienna, unpublished results experimental details will be reported elsewhere.
11. Chen LS, Chen GJ, Tamborski C. *J Organomet Chem* 1980; **193**: 283.

