JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 25–30.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.892

Research Article

Stereoselective synthesis of radiolabelled avermectins

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Summary

The natural products avemectin B_{1a} **1a** and B_{1b} **1b** were each site-specifically ^{14}C labelled at carbon 23 in a convergent synthesis for metabolism, residue, and environmental studies. The 12-step radiosynthesis involved a stereoselective aldol condensation and later a coupling to a protected avermectin degradate **2** in a one-pot Wittig condensation/spiroketalization to reconstitute the dioxaspirane configuration found in the natural products. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: avermectins; Wittig condensation; *anti* aldol; ^{14}C labelled

Introduction

Abamectin^{TM}, an approximate 9:1 mixture of avermectin B_{1a} and B_{1b} components, is currently marketed as a miticides/insecticide owing to its potent anthelmintic and acaridal properties. The avermectins are a class of natural products composed of a disaccharide linked to a pentacyclic 16-membered lactone and are produced by the soil actinomycete *Streptomyces. Avermitilis*, with Avermectin B_{1a} **1a** being the major component of the homologous series. Label expansion and registration have created the need for the reliable preparation of both **1a** and **1b** with site-specific ^{14}C incorporation (Figure 1).

Previous labelled avermectins have been produced by feeding radiolabelled precursors along the biosynthetic pathway.[1] This approach suffers from the production of avermectins with low specific activity, low radiochemical yield, non-selective incorporation, racemization of key stereocenters, time constraints due to multiple batch runs, as well as the production of significant amounts of radiolabelled waste. As such, this approach is not practical for the preparation of significant quantities of avermectin B_{1a} or preparation at all of the minor constituent avermectin B_{1b}. The goal of our work was to design and

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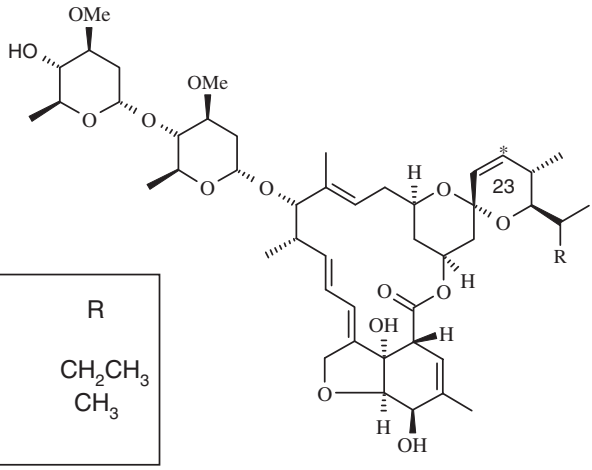
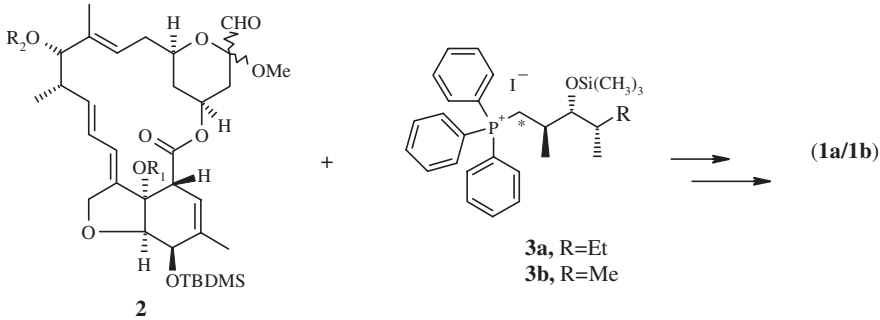
Received 26 August 2004
Accepted 8 September 2004



Entry	Compound	R
1a:	Avermectin B_{1a}	CH_2CH_3
1b:	Avermectin B_{1b}	CH_3

Figure 1. Naturally occurring avermectins



2

R_1=TMS, R_2=4''-OTBDMS-oleandrosyl-oleandrosyl

3a, R=Et
3b, R=Me

* denotes location of ^{14}C radiolabel

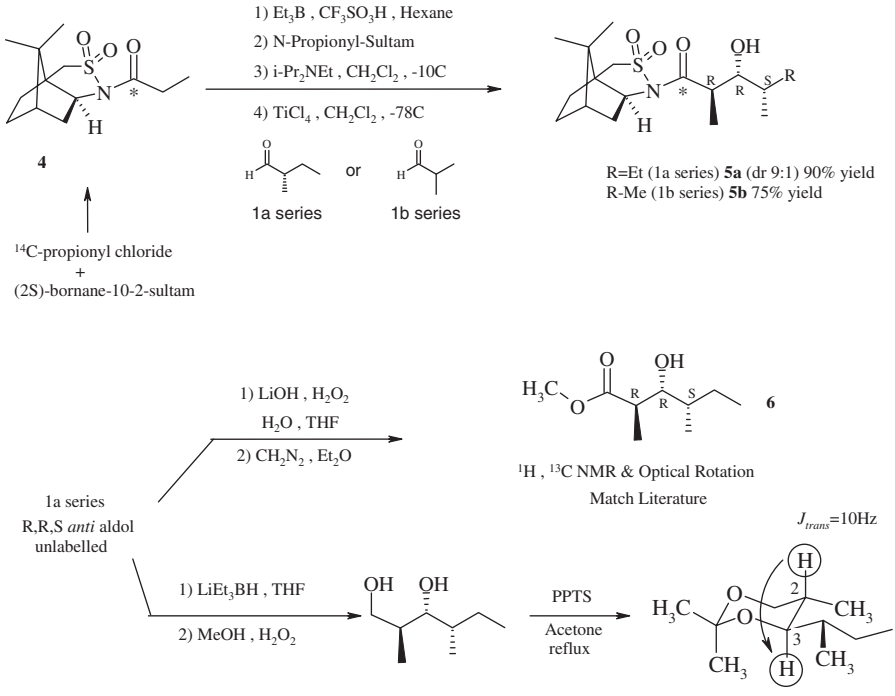
Scheme 1.

prepare any and all of the avermectin analogues with site-specific incorporation of a radiolabelled carbon without compromising the stereochemical integrity of the molecule. Our synthetic plan was based on early development work done by Merck Laboratories and involved chemical degradation of the structurally related avermectin B_{2a} to aldehyde intermediate **2**,[2] followed by Wittig condensation of an enantiomerically pure radiolabelled phosphonium salt **3** and spiroketalization to avermectin B_{1a} **1a** or avermectin B_{1b} **1b** (Scheme 1).[3]

Results and discussion

Chiral phosphonium salts **3a**,**b** were prepared in nine-step linear syntheses from 1-[^{14}C]-propionyl chloride.[†] The key step was an asymmetric aldol reaction coupling propionyl sultam **4**, prepared from (2S)-bornane-10,2-sultam, and 2S-

[†]1-[^{14}C]-propionyl chloride was prepared by NEN and Vitrax.

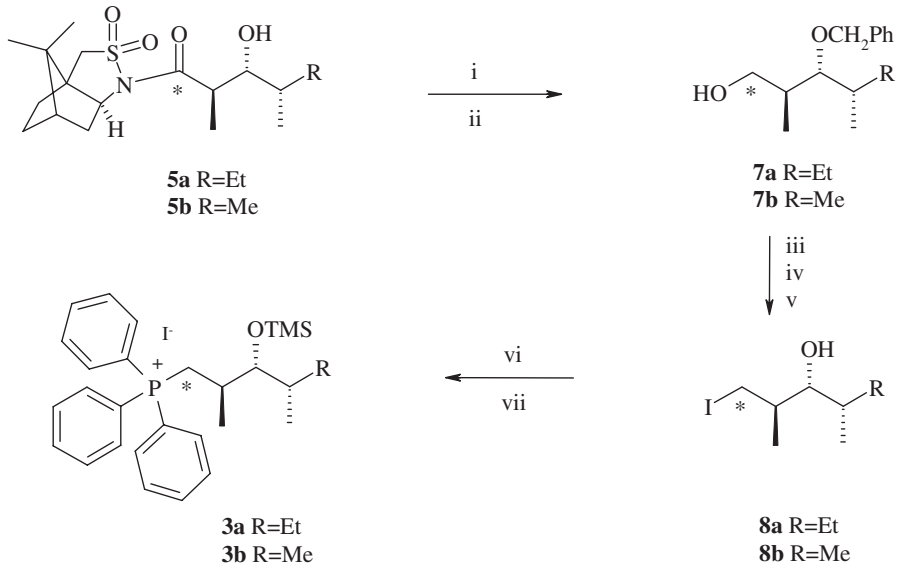


Scheme 2.

methylbutyraldehyde (1a series) or isobutyraldehyde (1b series) (Scheme 2). The aldol products **5a**,**b** was obtained in 90 and 75% yield, respectively, with greater than 99% *anti* aldol observed and with a diastereomeric ratio of up to R,R,S:R,R,R = 9:1 for the 1a series. The aldol condensation on the 1b series was in complete agreement with literature accounts (> 99% *anti*/*syn*).[4] Verification of the stereochemistry of the 1a series aldol product was done by oxidative removal of the chiral auxillary and formation of the hydroxy ester (**6**) which matched published NMR and optical rotation values.[1] Alternatively, the chiral auxillary was reductively removed and the resulting diol ketalized with acetone exhibiting the expected *trans* diaxial coupling relationship for the stereocenters, C-2 and C-3, formed during an *anti* aldol condensation. For comparison the *syn* aldol product was produced in the absence of $TiCl_4$ and then converted to its acetone ketal for analysis by ^1H NMR.‡

The secondary alcohol of the aldol products **5a,b** was quantitatively protected as its benzyl ether using benzyl 2,2,2-trichloroacetimidate[5] prior to reductive removal of the chiral auxiliary with $LiAlH_4$. Preparation of the benzyl ether was necessary to avoid loss of material following $LiAlH_4$ reduction. The resulting primary alcohols **7a,b** were then tosylated in the presence of pyridine and reacted with TMSI which simultaneously substitutes

‡The C-2,C-3 proton coupling constant for the acetal of the *syn* aldol product was $J = 2.3$ Hz, consistent with a *syn* axial/equatorial relationship.

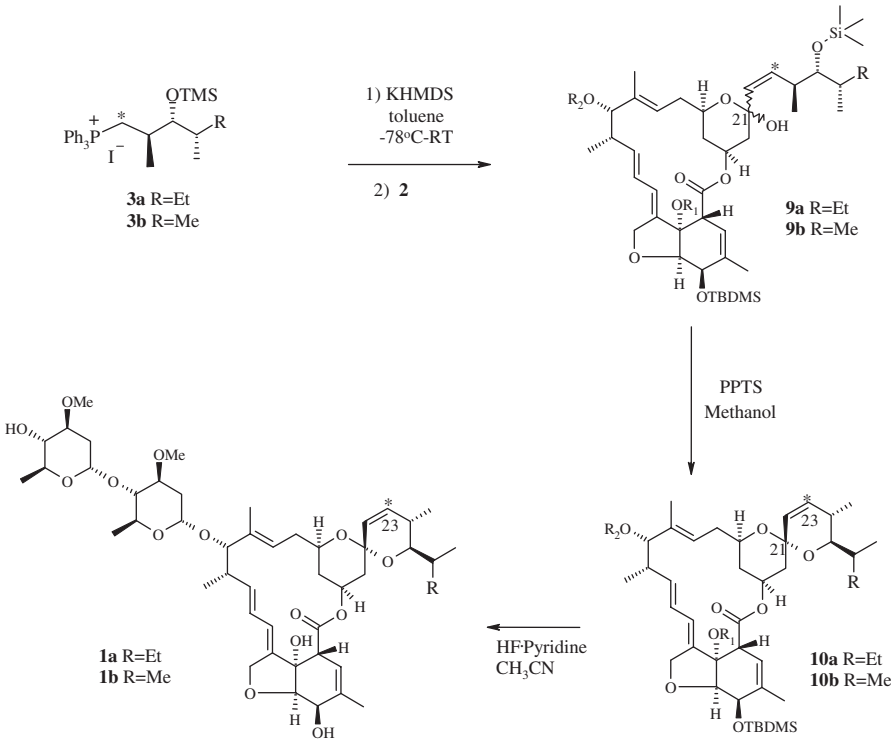


Scheme 3. (*denotes location of ^{14}C radiolabelled carbon). Reagents and conditions: (i) BTCA, CH2CI2, Cyclohexane. (ii) LiAIH4, THF, (96% for 7a over 2 steps, 68% for 7b over 2 steps). (iii) Tos-CI/Pyr. (iv) TMSI. (v) NaI/ Acetone, (87% for 7a to 8a, 73% for 7b to 8b). (vi) Ph_3P, $CaCO_3$, CAN. (vii) TMSI, 2,6 lutidine, (84% for 8a to 3a, 95% for 8b to 3b).

iodide for the tosylate and cleaves the benzyl ether[6] to form iodoalcohols **8a,b**. Incomplete displacement of the tosylate can be driven to completion by subjecting the TMSI reaction mixture to NaI in acetone. Formation of the phosphonium salts was effected with $Ph_3P/CaCO_3$ and the secondary alcohols were reprotected as the trimethylsilyl ether to furnish **3a,b**.

Ylid generation was done with KHMDS under scrupulously dry conditions at ambient temperature followed by immediate reaction with the persilylated aldehyde (**2**) at $-78^{\circ}C$ to RT to provide a mixture of Wittig products **9a,b** (Scheme 3).[§] In the presence of moisture phosphorane ylids **3a,b** are converted

[§] Representative Wittig Procedure: A 100 ml pear-shaped flask with stir bar, septum and dry argon needle inlet was charged with the radiolabelled substrate (/12.5 ml in toluene; 376 mCi total) and concentrated to dryness. The material was dried azeotropically with 2×15 ml dry EtOH, followed by 2×20 ml dry toluene, then placed under high vacuum parallel to P_2O_5 for an hour. The flask was charged with dry argon and dry toluene, then wrapped in foil in the dark. A 0.490 M solution of potassium *bis*(trimethylsilyl)amide (0.93 eq.) in toluene was added via over 2 min, and the deep orange solution was stirred at room temperature for 30 min. It was cooled to $-78^{\circ}C$ over 5 min, then quenched over 5 min, via syringe, with a solution of 4″,5-*bis*-*O*-TBDMS-7-*O*-TMS-21-methoxy-21-carboxaldehyde-seco-avermectin B_{1a} (**2**) (1.15 eq.) in dry toluene. The cooling bath was removed and the stirring was continued for 1.75 h, causing the solution to turn brown. After monitoring by β scanning TLC indicated a successful reaction, the reaction mass was loaded onto a 300 g column of silica gel and eluted with 4:1 hexanes:EtOAc to produce 188.5 mCi (50% yield) 21,25-deepoxy-4″,5-*bis*-*O*-[(1,1dimethylethyl)dimethylsilyl]-21-methoxy-7-*O*-(trimethylsilyl)-25-[(trimethylsilyl)oxy]-[23-^{14}C]-avermectin B_{1a} (**9a**) at 78.8% RCP. The column was later eluted with CH_3CN (1.5 l) to recover 191.4 mCi ([1-^{14}C]-2R,3R,4S-2,4-dimethyl-3-(trimethylsilyloxy)hexyl)triphenylphosphonium iodide (**8**) as a brown solution in CH_3CN (28.7% RCP).



Scheme 4.

to varying amounts of undesired diphenylphosphine oxides. Recovered from the Wittig reaction was mixtures of phophonium ylides **3a,b** and their desilylated analogues, both of which can be recycled in subsequent avermectin syntheses. Spiroketalization of the mixture was effected with PPTS in methanol which also allowed for thermodynamic equilibration of the Wittig products **9a,b** to the desired stereochemistry at C-21 prior to ring closure. The silyl protecting groups were removed with HF/Pyridine in acetonitrile to furnish either 23-^{14}C-Avermectin B_{1a} **1a** or 23-^{14}C-Avermectin B_{1b} **1b** with the natural configuration at C-21 (37% yield for 1a series yield from phosphonium salt **3a**, 13% yield for 1b series from **3b**).[¶] The overall yield of the longest linear route was 16% for the 1a series and the synthesis furnished 118 mCi of 23-^{14}C-Avermectin B_{1a} **1a** at 58 mCi/mmol or 66.4 μCi/mg. For the 1b series the overall yield was 5% furnishing 13 mCi of 23-^{14}C-Avermectin B_{1b} **1b** at 54 mCi/mmol or 63 μCi/mg (Scheme 4).

In summary, we have demonstrated reliable stereoselective syntheses of avermectin derivatives allowing for site-specific incorporation of a radiolabel

[¶] The final products are readily purified to radiochemical purities of >97.5% with no single impurity >1% and to chemical purities of >95% by external standard analysis.

to produce compounds with high specific activities without compromising the stereochemical integrity of the molecule.

References

1. (a) Ku C, Hwang S, Kaplan L, Nallin M, Jacob T. *J Label Compd Radiopharm* 1985; **XXII**: 451–459; (b) Wallace M, Dean D, Ellsworth R, Marks T, Melillo D, Masurekar P. *5th International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds,* Strasbourg, France, 20–24 June 1994 (Paper 108).
2. Shih T, Mrozik H, Holmes M, Fisher M. *Tetrahedron Lett* 1990; **31**: 3525–3528.
3. Shih T, Mrozik H, Holmes M, Fisher M. *Tetrahedron Lett* 1990; **31**: 3529–3532.
4. (a) Oppolzer W, Lienard P. *Tetrahedron Lett* 1993; **34**: 4321–4324; (b) Oppolzer W, Starkemann C, Rodriquez I, Bernardinelli G. *Tetrahedron Lett* 1991; **32**: 61–64.
5. Widmer U. *Synthesis* 1987; **6**: 568–570.
6. Jung M, Lyster M. *J Org Chem* 1977; **42**: 3761–3764.

