Chem. Soc. Rev., 1991, **20**, 211–269

Avermectins and Milbemycins Part I *

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1 Introduction

This review is an update of our earlier review published in 1986 [1] and, wherever possible, the duplication of references has been kept to a mininum. The format, however, has been retained in order to facilitate comparison.

The remarkable anthelmintic activity exhibited by a group of 16-membered ring macrolides first isolated in 1975 and later named the milbemycins, stimulated the interest of many pharmaceutical companies. Their discovery in 1975, by workers at Sankyo, was followed in 1979 by the isolation of the avermectins by Merck scientists. More recently, groups of related milbemycins have been disclosed by Glaxo, Cyanamid, Pfizer, Beecham, Kumiai Hokko, and ICI. The commercial importance and intricate structure of these macrolides has also encouraged many organic chemists to take up the challenge of their total synthesis.

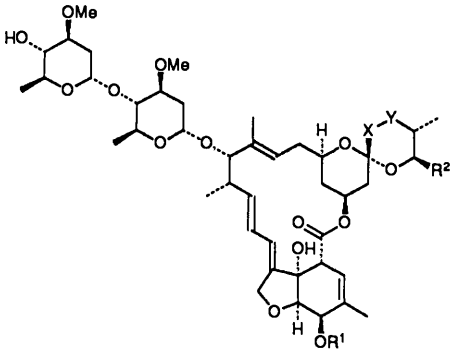
The structures of the naturally produced avermectins which were first isolated are (1)—(8). These fall into two main sections, designated A and B; the former possesses a methoxy group at C-5 and the latter possesses a 5-hydroxyl group. These sections are subdivided into the 1-series, with a C-22–C-23 double bond, and a 2-series, which has an axial hydroxyl group at C-23. A final subdivision into $_a$ and $_b$ series designates the presence of a *s*-butyl or isopropyl group, respectively, at C-25.

Whenever mixtures of $_a$ and $_b$ avermectins are to be described, it is common practice to use only the first two identifying characters, as in A2. This would refer to a mixture of $_a$ and $_b$ analogues. Where fully identified, as in $B2_b$, then this implies the use of pure compounds. This practice is adhered to in this review.

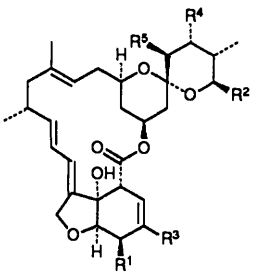
Milbemycins, while being structurally simpler than the avermectins, have a greater diversity of functionality, which has resulted in a less structured nomenclature. The only real subdivision of Sankyo's original milbemycins was into the α series [(9)—(23)], members of which possess a fused tetrahydropyranyl ring and are true analogues of avermectins, and the series [(24)—(28)], in which the tetrahydropyranyl ring is missing. The aromatic ring of milbemycin β_3 (28) is a notable feature in this otherwise alicyclic series, although it is now suspected that this is an artefact of the purification process and is not naturally produced.

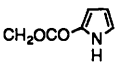
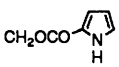
* Avermectins and Milbemycins Part II (Total Synthesis and General Chemistry) will be published in *Chemical Society Reviews*, 1991, Vol. 20, Issue 3 (September).

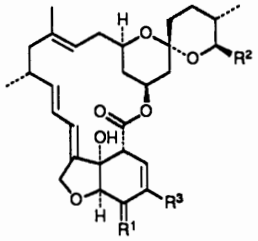
[1] H. G. Davies and R. H. Green, *Natural Product Reports*, 1986, **3**, 87.



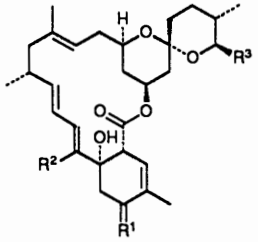
Avermectin		R^1	R^2	X—Y
(1)	$A1_a$	Me	Bu^s	CH=CH
(2)	$A1_b$	Me	Pr^i	CH=CH
(3)	$A2_a$	Me	Bu^s	CH_2—CH(OH)
(4)	$A2_b$	Me	Pr^i	CH_2—CH(OH)
(5)	$B1_a$	H	Bu^s	CH=CH
(6)	$B1_b$	H	Pr^i	CH=CH
(7)	$B2_a$	H	Bu^s	CH_2—CH(OH)
(8)	$B2_b$	H	Pr^i	CH_2—CH(OH)



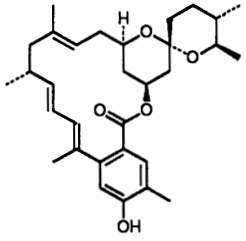
Milbemycin		R^1	R^2	R^3	R^4	R^5
(9)	α_1	OH	Me	Me	H	H
(10)	α_2	OMe	Me	Me	H	H
(11)	α_3	OH	Et	Me	H	H
(12)	α_4	OMe	Et	Me	H	H
(13)	α_5	OH	Me	Me	$OCOCHMeBu^n$	OH
(14)	α_6	OMe	Me	Me	$OCOCHMeBu^n$	OH
(15)	α_7	OH	Et	Me	$OCOCHMeBu^n$	OH
(16)	α_8	OMe	Et	Me	$OCOCHMeBu^n$	OH
(17)	α_9	OH	Me		H	H
(18)	α_{10}	OH	Et		H	H



Milbemycin		R^1	R^2	R^3
(19)	D	H, β-OH	Pr^i	Me
(20)	F	H, β-OH	Pr^i	CH_2OCO-(pyrrole)
(21)	G	H, β-OMe	Pr^i	Me
(22)	J	O	Me	Me
(23)	K	O	Et	Me



Milbemycin		R^1	R^2	R^3
(24)	β_1	H, β-OMe	CH_2OH	Me
(25)	β_2	H, β-OMe	CH_2OH	Et
(26)	E	H, β-OMe	CH_2OH	Pr^i
(27)	H	O	Me	Pr^i



(28) Milbemycin β_3

It is of interest to note that analogous aromatic avermectins and milbemycins have been described [2 3]

The nomenclature for milbemycins has become even less ordered with the advent of milbemycin analogues from other companies Glaxo's analogues have the descriptor S541, those from Cyanamid are designated LL-F (although LL-F28249α has recently been given the name nemadectin), while other companies use a variety of alphanumeric codes

Comparison of structures (1)—(23) reveals that the only major difference between avermectins and milbemycins is that the latter lack the disaccharide group at C-13 Confirmation of this interrelationship was provided by the conversion of 22,23-dihydroavermectin $B1_b$ aglycon into milbemycin D [4]

2 Fermentation and Isolation

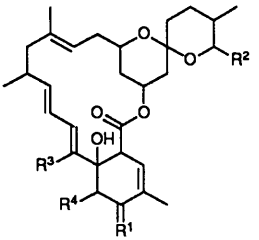
Since our earlier review, much work has concentrated on improving the yield and purification methods of the known anthelmintic macrolides by mutation of the producing organism and by studies of the fermentation itself This work is largely outside the scope of this review, but some examples may serve to illustrate the type of work involved

The addition of glucose in the early stages of fermentation of *S avermitilis* suppresses avermectin production Addition at a later stage of fermentation, however, increases avermectin production two-fold over that of a control batch [5]

The maximum yields of avermectins from *S avermitilis* C-18 were obtained with known concentrations of ammonium sulphate as the sole nitrogen source, although the yields were diminished with higher concentrations than optimal [6] The ratio of A type to B type avermectins produced was also dependent on the concentration of the nitrogen source, B type avermectins were predominant at < 15 mM ammonium sulphate, while the reverse was true at higher concentrations

Calcott and Fatig have recently reported avermectin $B1_a$ to be an antifungal agent, acting by interfering with chitin metabolism [7] Studies by Onishi and Miller, however, have shown that this activity was due to oligomycin and an unidentified agent 'of the methyl pentaene type', impurities present in the original work [8] Pure avermectin $B1_a$ had no antifungal activity and no effect on fungal chitin synthesis Avermectin $B1_a$ and milbemycin D have also been shown to be virtually inactive [9] against the chitinase of four insect species at doses of 10^{-5} to 10^{-6} M The oligomycin impurity has been isolated from ethanolic extracts of the fermentation broths of *S avermitilis* ATCC 31267 or ATCC 31272 and shown to

[2] B H Arison, R T Goegelman, and V P Gullo (Merck and Co Inc), US Patent, US 4 285 963 (1981)
[3] J A Pankavitch, G T Carter, M J Torrey, and M Greenstein (American Cyanamid Co), European Patent, EP 170 006 (1986)
[4] H Mrozik, J C Chabala, P Eskola, A Matzuk, F Waksmunski, M Woods, and M H Fisher *Tetrahedron Lett*, 1983, **24**, 5333
[5] H Ikeda, H Kotaki, H Tanaka, and S Omura, *Antimicrob Agents Chemother* 1988 **32** 282
[6] E Cimburkova, J Zima, J Novak, and Z Vanek, *J Basic Microbiol*, 1988, **28**, 491
[7] P H Calcott and R O Fatig III, *J Antibiot*, 1984, **37**, 253
[8] J C Onishi and T W Miller, *J Antibiot*, 1985, **38**, 1568
[9] P M Gordnier, J Brezner, and S W Tanenbaum, *J Antibiot*, 1987, **40** 110



	R^1	R^2	R^3	R^4
(29a)	H, OH	Me	Me	H
(29b)	H, OMe	Me	Me	H
(29c)	H, OH	Et	Me	H
(30)	H, OMe	Me	Me	OH
(31)	O	Me	Me	H
(32)	H, OMe	Me	CHO	H

be oligomycin A.[10] A *Streptomyces* mutant, designated NCIB 12197, which produces very low levels of oligomycins and thus simplifies the isolation of avermectins, was described in this patent. Improved methods of preparation of avermectins $B1_a$, $B2_a$, and 22,23-dihydroavermectin $B1_a$ directly from the fermentation broth have also been described by these workers.[11a,b]

Many new avermectin and milbemycin analogues have been produced by fermentation since the last review appeared. A novel *Streptomyces* species, given the ATCC number 53110, has been produced by protoplast fusion of *Streptomyces avermitilis* and *S. hygroscopicus*. Fermentation of this organism gives the novel milbemycins (29)—(32),[12] all of which have an incomplete tetrahydrofuran ring; in addition a range of 22-hydroxy-23-acyloxy milbemycins (33a—e) were isolated.[13] This patent contains a discrepancy in that the abstract describes the 23-acyloxy group to be heptanoyloxy whereas the experimental text describes this group as heptenoyloxy. The isomeric form of this acyloxy group is not described thus, by convention, it is assumed to be n-acyl. However, some caution is needed with this assumption as an n-acyl substituent is unusual from these fermentations. 22-Hydroxy-23-acyloxy milbemycin analogues (33f—i) have also been isolated by workers at ICI by fermentation of *Streptomyces* strain NCIB 11876.[14]

The organism *Streptomyces cyaneogriseus* ssp. *noncyanogenus* (NRRL 15773), isolated from mallee sand found in Southern Australia, produces fourteen

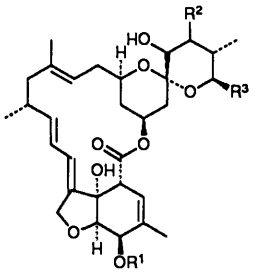
[10] L. M. C. Rodrigues, T. O. Izard, M. A. M. Valle, and J. L. Fernandez-Puentes (Antibioticos S.A.), British Patent, GB 2 185 480 (1986).

[11] (*a*) T. O. Izard, L. M. C. Rodrigues, J. L. F. Puentes, J. M. F. S. Faro, C. M. Maroto, M. A. M. Valle, R. N. Elvira, and A. P.-A. Ortega (Antibioticos SA), Spanish Patent, ES 549 856 (1986); (*b*) *ibid.* Spanish Patent, ES 550 588 (1986).

[12] R. T. Goegelman (Merck and Co. Inc.), European Patent, EP 205 251 (1986).

[13] P. A. McCormick and R. T. Goegelman (Merck and Co. Inc.), European Patent, EP 204 421 (1986).

[14] N. J. Poole, P. Hendley, M. W. Skidmore, and R. S. I. Joseph (ICI plc), British Patent, GB 2 170 499 (1985).



(33)	R^1	R^2	R^3
(a)	Me	Pentyloxy	Me
(b)	Me	H	Me
(c)	Me	H	Et
(d)	H	Heptanoyloxy	Me
(e)	Me	Heptanoyloxy	Me
(f)	H	2, 4-dimethylpentanoyl	Me
(g)	Me	2, 4-dimethylpentanoyl	Me
(h)	H	2, 4-dimethylpentenoyl	Me
(i)	Me	2, 4-dimethylpentenoyl	Me

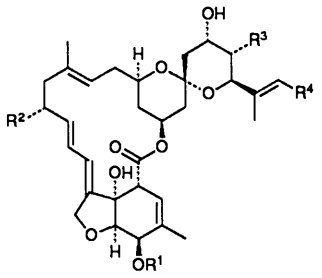
macrolides closely related in structure to the Sankyo milbemycins but with a trisubstituted alkenic substituent at C-23.[3,15] The four major milbemycins, designated LL-F28249 α, λ, β and γ (34a—d), were isolated by the normal processes of filtration, extraction with methanol and then with dichloromethane, and then normal phase chromatography followed by reverse phase chromatography. Structures of a further eight secondary metabolites (34e—1) have been claimed in the Cyanamid patent[3] but with the caveat that 'structures and stereochemistry have not been fully defined'. The conjugated structure (34j) is unusual among such metabolites and must lead to the supposition that the double bond moved into conjugation during the isolation of this metabolite. An interesting feature of this patent is that it claims a previously isolated metabolite of *S. griseochromogenes*, named Hondamycin,[16a–c] to be related to the LL-F structures.

Identical metabolites to Cyanamid's LL-F28249 compounds were simultaneously isolated by workers at Glaxo from *Streptomyces thermoarchaensis*, a soil isolate from Mexico, and given the trivial designation of S541 compounds.[17a,b] Six of these S541 metabolites, named Factors A—F, have been fully described. Correlation between the nomenclatures of Cyanamid and Glaxo is as follows: LL-F28249α (Factor A) (34a), LL-F28249γ (Factor B) (34d), LL-F28249β

[15] G T Carter, J A Nietsche, M R Hertz, D R Williams, M M Siegel, G O Morton, J C James, and D B Borders, *J Antibiot*, 1988, **41**, 519

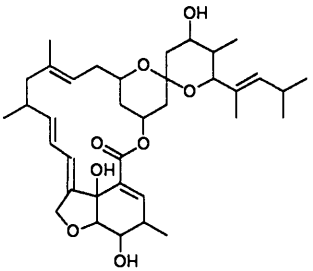
[16] (*a*) Y Sakegami, A Ueda, S Yamabayashi, Y Taurumaki, and S Kumon, *J Antibiot*, 1969, **22**, 521, (*b*) Y Sakagami, S Yamabayashi, and A Ueda, *J Antibiot*, 1969, **22**, 528, (*c*) A Ueda and Y Sakagami, *J Antibiot*, 1969, **22**, 536

[17] (*a*) J B Ward, H M Noble, N Porter, R A Fletton, and D Noble (Glaxo Group Ltd), European Patent, EP 2 166 436A (1986), (*b*) M V J Ramsay, S M Roberts, J C Russell, A H Shingler, A M Z Slawin, D R Sutherland, E P Tiley, and D J Williams, *Tetrahedron Lett*, 1987, **28**, 5353

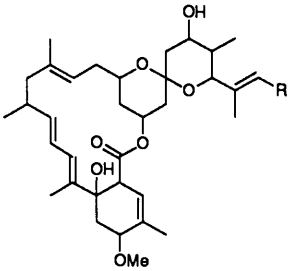


(34)

	R^1	R^2	R^3	R^4	LL-F 28249	S 541 Factor
(a)	H	Me	Me	Pr^j	α	A
(b)	Me	Me	Me	Pr^j	λ	F
(c)	H	Me	Me	Me	β	C
(d)	Me	Me	Me	Me	γ	B
(e)	H	H	Me	Pr^j	ε	
(f)	H	Me	Me	Et	ζ	D
(g)	H	Me	Et	Pr^j	θ	
(h)	H	Et	Me	Pr^j	ι	
(i)	Me	Me	Me	Et		E



(34j) LL-F 28249η

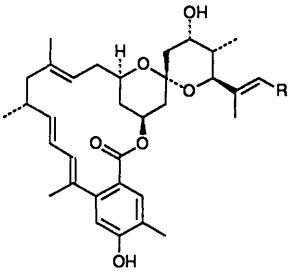


(34k) R = Me LL-F 28249κ
(34l) R = Pr^j LL-F 28249μ

(Factor C) (34c), LL-F28249ζ (Factor D) (34f) and LL-F28249λ (Factor F) (34b), Factor E (34i) is a C5 methylated homologue of LL-F28249ζ (34f).

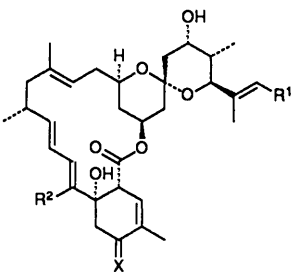
Mutants of *S. thermoarchaensis* categorized as NCIB 12015 and NCIB 12212 produce aromatic S541 analogues (35) reminiscent of milbemycin β_3 and analogues with incomplete tetrahydrofuran rings (36), reminiscent of milbemycin H.[18] *S. thermoarchaensis* strains NCIB 12015 and NCIB 12334 also give S541 analogues with hydroxymethyl and formyl groups at C-8 (37), while *S. avermitilis* ATCC 31272, *S. hygroscopicus* FERM F1438 and *S. thermoarchaensis* NCIB

[18] B. A. M. Rudd, R. A. Fletton, J. B. Ward, D. Noble, N. Porter, G. O. Lawrence, and H. M. Noble (Glaxo Group Ltd.), European Patent, EP 242 052 (1987).



R = Me; Et; Pr^i

(35)

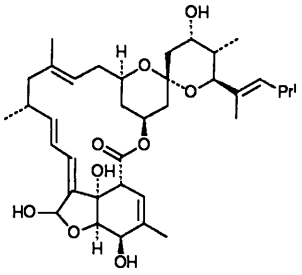


X = H, OH or O
R^1 = Me; Et; Pr^i
R^2 = Me

(36)

X = H, OH or O
R^1 = Me; Pr^i
R^2 = CHO; CH_2OH

(37)



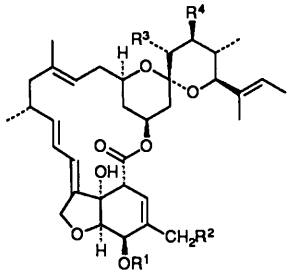
(38)

12213, will complete the process of tetrahydrofuran ring formation. Compounds hydroxylated at C-8a, reminiscent of those obtained in metabolism studies [*e.g.* (38)] are also obtained from these species.

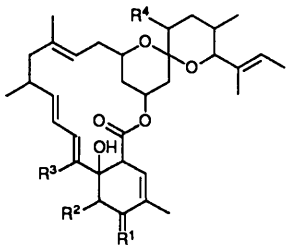
A recent Glaxo patent has described a mutant *Streptomyces* strain, NCIB 12334, which produces S541 Factor A oxidized at the 5-position.[19] As the resulting 5-keto compound is crystalline, its isolation in a high degree of purity is much more facile.

An improved method of isolation of the S541 components from the fermentation broth by alumina chromatography has been described,[20] as has a method of enhancing Factor A production relative to other S541 components by the addition of a mixture of valeric and isobutyric acids to the later stages of the fermentation.[21] The addition of 10% sulphuric acid to the fermentation mixture to adjust the pH to 7.2—7.4 also increases the percentage of Factor A in the crude fermentation product.[21]

[19] M V J Ramsay, D R Sutherland, B A M Rudd, R A Fletton, D A Noble, J B Ward, N Porter, and H M Noble (Glaxo Group Ltd), British Patent, GB 2 187 742 (1987)
[20] R Thornton, D T Eastlick, and K Briggs (Glaxo Group Ltd), European Patent, EP 237 340 (1987)
[21] M Todd, M A Haxell, and G O Lawrence (Glaxo Group Ltd), European Patent, EP 241 147 (1987)



(39)	R^1	R^2	R^3	R^4	
(a)	H	H	H	H	VM 44857
(b)	Me	H	OH	H	VM 44864
(c)	Me	OCOC(Me)=CHMe	OH	H	VM 44865
(d)	H	H	OH	H	VM 44866
(e)	H	H	OH	$OCOPr^i$	VM 48130
(f)	H	$OCOCH=C(Me)_2$	OH	H	VM 68633
(g)	H	$OCOBu^t$	OH	H	VM 47704
(h)	H	$OCOCH_2$ 	OH	H	VM 48642

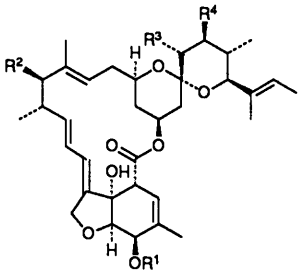


		R^1	R^2	R^3	R^4
(40)	VM 44867	H, OMe	OH	Me	OH
(41)	VM 44868	O	H	CH_2OH	H

Further milbemycin analogues [(39)—(41)], very similar to those isolated by Glaxo and Cyanamid, have been reported by workers at Beecham by fermentation of *Streptomyces* strains E225 (NCIB 12310) or E225B (NCIB 12509).[22a,b,c] The original culture, E225, was isolated from a sample of river mud collected from the River Mole in Surrey, England; the word 'ubiquitous' certainly applies to the sources of the milbemycins! One of the patents describing these compounds is notable in having the courage to describe an absolute configuration—a rare event!

In a case of history repeating itself, yet more Japanese soil samples have

[22] (*a*) R. M. Banks, M. E. Poulton, G. H. Baker, and R. J. Dorgan (Beecham Group plc), European Patent, EP 254 583 (1987); (*b*) R. M. Banks, S. E. Blanchflower, and P. R. Shelley (Beecham Group plc), European Patent, EP 325 462 (1989); (*c*) J. D. Hood, R. M. Banks, M. D. Brewer, J. P. Fish, B. R. Manger, and M. E. Poulton, *J. Antibiot.*, 1989, **42**, 1593.



(42)

	R^1	R^2	R^3	R^4	
(a)	Me	OH	OH	H	N 787-182-1
(b)	H	OH	OH	OCOPr^j	N 787-182-2
(c)	Me	OH	OH	OCOPr^j	N 787-182-3
(d)	H	OCOPr^j	OH	H	N 787-182-4
(e)	Me	OCOPr^j	OH	H	N 787-182-5
(f)	H	OCOPr^j	OH	OCOPr^j	N 787-182-6
(g)	Me	OCOPr^j	OH	OCOPr^j	N 787-182-7
(h)	H	H	OH	OCOPr^j	N 787-182-8
(i)	H	OCOPr^j	H	H	N 787-182-9
(j)	Me	OCOBu^i	OH	OCOPr^j	N 787-182-10
(k)	Me	H	OH	OCOPr^j	N 787-182-11
(l)	Me	OCOPr^j	H	H	N 787-182-12
(m)	H	OH	OH	H	
(n)	H	OH	H	H	
(o)	Me	OH	H	H	

yielded interesting macrolides. A sample from Kinashiki City yielded a new strain of *S. hygroscopicus* designated ATCC 53718. From this new strain, Pfizer workers isolated the macrolides (42a–l), many of which closely resemble the avermectin aglycon with a 13-oxygen substituent.[23] The parent macrolides (42m—o) were obtained by simple chemical deacylations.

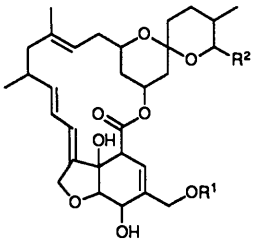
The Japanese firm Hokko have cultured a soil sample from Ohta-ku, Tokyo to produce a new strain of *S. hygroscopicus* ssp. *aureolacrimosus* identified as FERM BP-2059.[24] On fermentation this produces thirteen milbemycins acetoxylated at C-4a (43a—43m). By fermentation of another strain of this organism, SANK 60286 (FERM BP-1190), Sankyo isolated five of the same 4a-acetoxylated milbemycins which they named milbemycins α_{11}—α_{15},[25] and Kumiai utilised *S. hygroscopicus* KSB-1939 (FERM BP-1901) to produce four of these milbemycins (43a, c, e, g) and three milbemycins with different 4a-acyloxy groups (44a—c).[26] This latter patent is remarkable for the amount of detail presented in it.

[23] M. A. Haxell, H. Maeda, and J. Tone (Pfizer Ltd), European Patent, EP 334484 (1989).

[24] W. Tanaka, K. Dobashi, H. Naganawa, M. Hamada, T. Takeuchi, and K. Sato (Zaidan Hojin Biseibutsu Kagaku Kenkyu Kai and Hokko Chemical Ind. Ltd.), Australian Patent, AU 23602 (1988).

[25] T. Okazaki, S. Takahashi, S. Iwado, K. Tanaka, T. Yanai, and H. Kajino (Sankyo Co. Ltd.), European Patent, EP 274272 (1987).

[26] H. Katoh, R. Kobayashi, T. Shimazu, A. Suzuki, A. Isogai, and O. Tada (Kumiai Chem. Ind. Co. Ltd.), European Patent, EP 298423 (1989).



(43)

	R^1	R^2	Hokko	Sankyo	KSB
(a)	$COCH{=}CMe_2$	Me	M1 198-Z_1	α_{11}	KSB 1939 H_2
(b)	$COCH{=}CHMe$	Me	M1 198-Z_2		
(c)	$COCH{=}CMe_2$	Et	M1 198-Z_3	α_{14}	KSB 1939 S_5
(d)	$COCH{=}CMe_2$	Pr	M1 198-Z_4		
(e)	$COCH_2CHMe_2$	Me	M1 198-Z_5	α_{12}	KSB 1939 H_3
(f)	$COCH_2CHMe_2$	Et	M1 198-Z_6		
(g)	$COCH{=}C(Me)Et$	Me	M1 198-Z_7	α_{13}	KSB 1939 H_4
(h)	$COCH{=}C(Me)Et$	Et	M1 198-Z_8		
(i)	$COCH_2CH(Me)Et$	Me	M1 198-Z_9		
(j)	$COCH_2CH(Me)Et$	Et	M1 198-Z_{10}	α_{15}	
(k)	$COCH{=}CEt_2$	Me	M1 198-Z_{11}		
(l)	$COCH{=}CEt_2$	Et	M1 198-Z_{12}		
(m)	$COCH_2CHEt_2$	Me	M1 198-Z_{13}		

Sankyo have not been content to rest at milbemycin α_{15} but have produced further milbemycins, named α_{16}—α_{19} (45a—d) and β_8 (46) by fermentation of *Streptomyces* B-41-146 (FERM P1438).[27]

Elaboration of the C-25 substituent in avermectins and milbemycins requires branched-chain 2-oxo acid dehydrogenase activity and/or branched-chain amino acid transaminase in order to metabolize valine or isoleucine to the deaminated acid for incorporation. Single mutants of *Streptomyces* strains which lack this activity thus cannot produce macrolides with the 'natural' C-25 substituent. They can, however, utilise added carboxylic acids ($R.CO_2H$) to incorporate an unnatural R substituent at C-25. This process, aptly termed 'directed biosynthesis', has been used by several groups to produce new avermectins and milbemycins. As an example of this, the addition of cyclopentane carboxylic acid to the fermentation medium of *S. avermitilis* HL-026 (ATCC 53568) caused the production of a 25-cyclopentyl avermectin derivative.[28] A further refinement to this procedure is to add sinefungin, an *S*-adenosyl-methionine analogue (see footnote 1), to the fermentation in order to inhibit SAM-dependent *O*-methyl transferase activities, thus increasing the production of B type aglycons over

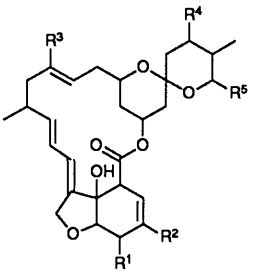
[27] F. Maruyama, S. Iwato, S. Tachibana, S. Hayashida, K. Sato, and K. Tanaka (Sankyo Co. Ltd.), Japanese Patent, JP 63 227 590 (1988).

[28] E. W. Hafner, K. S. Holdom, S.-J. E. Lee (Pfizer Inc.), European Patent, EP 276 131.

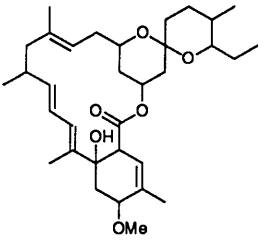
Footnote 1: *S*-adenosyl-methionine is the carbon source for the methyl groups at C-3′ and C-3″ and, for avermectins of the A series, the methyl group at C-5.

Footnote 2: For a further discussion of this point see the next section.

Footnote 3: For a further discussion of this point see the section on Biosynthesis.



	R^1	R^2	R^3	R^4	R^5	
(44a)	OH	$CH_2OCOCH{=}CH(Me)Et$	Me	H	Me	KSB-1939 H_4
(44b)	OH	CH_2OCOPr^i	Me	H	Me	KSB-1939 $L_{3\alpha}$
(44c)	OH	CH_2OCOPr^n	Me	H	Me	KSB-1939 $L_{3\beta}$
(45a)	OH	Me	H	H	Me	α_{16}
(45b)	OH	Me	H	H	Et	α_{17}
(45c)	=O	Me	Me	OH	Et	α_{18}
(45d)	OH	Me	Me	OH	Et	α_{19}



(46) β_8

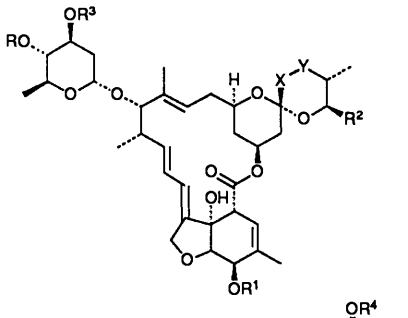
those of the A type and hence simplifying the isolation of products (see footnote 2). When sinefungin is added to a fermentation of *S. avermitilis* 08, a high producing strain which normally produces the eight known avermectins, eight new demethyl avermectins are produced (47).

Double mutants of *Streptomyces* lacking both 2-oxo acid dehydrogenase and *O*-methyl transferase activity are also known which obviate the addition of sinefungin (see footnote 3 and ref. 29).

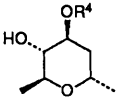
Other additives used in this directed biosynthesis approach include 2-methyl-4-pentenoic acid, 2-methylvaleric acid, thiophene-3-carboxylic acid, cyclobutane carboxylic acid, 3-cyclohexenoic acid, cyclohexane carboxylic acid, 2-methylthiopropionic acid and 2-methylcyclopropane carboxylic acid.[29,30] Carboxylic acid salts can also be used, as can esters, aldehydes and alcohols, which are converted *in situ* into the acids by attendant esterases and oxidoreductases.[31a–f]

[29] C L Ruby, M D Schulman, D L Zink, and S L Streicher, Symp Biol Hung, Vol 32, (Biol, Biochem Biomed Aspects Actinimycetes, Pt A), 1985, p 279

[30] C J Dutton and D A Perry (Pfizer Inc), European Patent, EP 308 145 (1987)



Disaccharide R =



R^1	R^2	R^3	R^4	X—Y
H	Bu^s	H	H	CH=CH
H	Bu^s	Me	H	CH=CH
H	Bu^s	H	Me	CH=CH
H	Bu^s	H	H	$CH_2CH(OH)$
H	Bu^s	H	Me	$CH_2CH(OH)$
H	Bu^s	Me	H	$CH_2CH(OH)$

Monosaccharide R = H

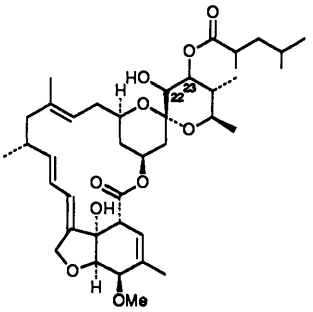
R^1	R^2	R^3	X—Y
Me	Bu^s	H	CH=CH
H	Bu^s	H	CH=CH

(47)

3 Structure Determination

Thanks to the excellent groundwork laid by the physical chemists at Sankyo and Merck, identification of new avermectins and milbemycins is easier than might be expected from such complex structures. As this early work was discussed extensively in our previous review[1] this section will concentrate solely on reassignments of data where the original was shown to be incorrect, and on the application of new spectroscopic and computational techniques. Spectroscopic

[31] (*a*) T. S. Chen, E. S. Inamine, C. D. Hensens, D. Zink, and D. A. Ostlind, *Arch. Biochem. Biophys.*, 1989, **269**, 544; (*b*) B. R. Gibson, K. S. Holdom, A. C. Goudie, and J. D. Bu'lock (Pfizer Inc.), European Patent, EP 214 731 (1986); (*c*) C. J. Dutton, S.-J. E. Lee and S. F. Gibson (Pfizer Inc.), European Patent, EP 317 148 (1987); (*d*) E. W. Hafner, K. S. Holdom, and S.-J. E. Lee (Pfizer Inc.), European Patent, EP 284 176 (1988); (*e*) E. W. Hafner, K. S. Holdom, and S.-J. E. Lee (Pfizer Inc.), European Patent, EP 276 103 (1988); (*f*) S. S. T. Chen (Merck and Co. Inc.), European Patent, EP 300 674 (1987).



(48) (22β, 23α)
(49) (22α, 23β)

details of all new milbemycins and avermectins can be found in the appropriate patent.

The 22-hydroxy-23-(2,4-dimethylpentanoyl) milbemycin (33g), isolated by workers at ICI,[14] was assigned the stereochemistry shown in (48) by analogy with the previously reported stereochemistry for milbemycins α_5 to α_8. This compound was also isolated by Beecham and the stereochemistry around the spiroacetal ring was studied by high-field NMR. With the aid of a 2D ^{1}H COSY-45 experiment, the proton spectrum was assigned and coupling constants for the relevant protons were extracted.[32a] These indicated that the C-22, C-23, C-24 and C-25 substituents were all equatorial (49), at variance to the assumed configuration. Unambiguous confirmation of this was obtained by the observation of a proton–proton NOE between the proton pairs at H-22 and H-24, and H-23 and H-25. The stereochemistry of the spiro ring fusion was also confirmed by the observation of a NOE enhancement between H-17 and H-25. This observation leads to the suggestion that the reported stereochemistry of milbemycins α_5 to α_8 may be incorrect, a conclusion for which the authors of this paper find support in the original reported data for milbemycin α_6 (see reference 1). A paper detailing the full structural elucidation of VM 44857, VM 44864, VM 44865, and VM 44866 by NMR experiments has recently been published.[32b] The results obtained fully confirmed the reported structures.

A full description of the structure determination of the Cyanamid LL-F28249α, β, γ, and λ milbemycins has been published.[15,33a,b] Definitive assignment of the ^{13}C spectrum was initiated by a DEPT polarization transfer technique[34a,b] to determine the number of attached protons. This confirmed the presence of seven

[32] (*a*) S. E. Blanchflower, R. J. J. Dorgan, J. R. Everett, and S. A. Readshaw, *Tetrahedron Lett.*, 1988, **29**, 6645; (*b*) G. H. Baker, R. J. J. Dorgan, J. R. Everett, J. D. Hood, and M. E. Poulton, *J. Antibiot.*, 1990, **43**, 1069.

[33] (*a*) G. T. Carter, J. A. Nietsche, and D. B. Borders, *J. Chem. Soc., Chem. Commun.*, 1987, 402; (*b*) S. Rajan and G. W. Stockton, *Magn. Reson. Chem.*, 1989, **27**, 437.

methyl groups, six methylenes, sixteen methines, and seven quaternary carbons. ^{13}C assignments were then made with the assistance of an INADEQUATE[35a,b] technique to identify connected carbon pairs. Assignment of the proton spectrum was made difficult by the presence of several overlapping multiplets, unsurprisingly in view of the complexity of the molecule, but this problem was circumvented by the use of 2D ^{13}C–^{1}H HETCOR and COSY experiments. Many of the proton spin–spin coupling constants for LL-F28249α were extracted by a spin simulation program. This work also led to a suggested reassignment for some of the ^{13}C signals for milbemycins D, α_2 and α_4; for milbemycins α_2 and α_4 the assignments for C-17 and C-19 were reversed, while for milbemycin D the signals for C-5, C-17, and C-19 were reassigned (see footnote in reference 15). In contrast, however, assignments of the ^{13}C spectrum of VM 44857 were in complete agreement with those for milbemycin D for C-1 to C-30.[32b] Dorgan and his co-workers have suggested that this anomaly is due to hydrogen bonding between the axial 23-hydroxy group and O-17 of the LL-F29249 structures affecting the shielding of C-17, and thus causing the apparent reversal of the signals for C-17 and C-19.

A HETCOR study of milbemycin β_1 has been reported by Ley and his co-workers,[36] and this group has also reported a reassignment[37] of the resonances of the spiroacetal ring of avermectin $B1_a$. By the combination of NOE difference spectra, 2D-INADEQUATE spectra and HETCOR spectra on a spiroacetal fragment, avermectin $B1_a$ aglycon and avermectin $B1_a$ itself it was shown that the assignments for C-22 and C-23 and thus the corresponding hydrogens, should be interchanged. Definitive assignments for the quaternary carbons at C-4 and C-14 were also possible.

X-Ray data for many avermectins and milbemycins to elucidate their solid-state conformations were available but, as always, it was questionable how closely this approximated to the solution conformation. For LL-F28249α this question has now been answered.[33b] The extracted spin–spin coupling constants qualitatively suggested the solution conformation of this compound to be similar to the solid-state conformation of the γ compound. For an accurate picture of the solution conformation, 2D phase-sensitive NOESY experiments were used to extract inter-proton distances, assuming isotropic tumbling and the usual sixth-power distance dependance. Calibration of the results was by measurements of cross-peak volumes from two different geminal proton pairs and from *X*-ray measurements of the distance between the protons at C-25 and C-27. Calculations of the RMS deviation between the NMR data and the *X*-ray data, together with the coupling constant data then suggested the solution conformation of LL-

[34] (*a*) T. D. Regg, D. M. Doddrell, and M. R. Bandall, *J. Chem. Phys.*, 1982, **77**, 2743; (*b*) O. W. Sorenson and R. R. Ernst, *J. Magn. Reson.*, 1983, **51**, 477.

[35] (*a*) A. Bax and R. Freeman, *J. Am. Chem. Soc.*, 1980, **102**, 4849; (*b*) A. Bax, R. Freeman, T. A. Frenkiel, and M. H. Levitt, *J. Magn. Reson.*, 1981, **43**, 478.

[36] S. V. Ley, N. J. Anthony, A. Armstrong, M. G. Brasca, T. Clarke, D. Culshaw, C. Greck, P. Grice, A. B. Jones, B. Lygo, A. Madin, R. N. Sheppard, A. M. Z. Slawin, and D. J. Williams, *Tetrahedron*, 1989, **45**, 7161.

[37] D. Diez-Martin, P. Grice, H. C. Kolb, S. V. Ley, and A. Madin, *Tetrahedron Lett.*, 1990, **31**, 3445.

F28249α was very close to the *X*-ray conformation of the γ analogue. A computer model of the conformation of the α compound was built, using CHEM-X®, based on the *X*-ray structure of the γ analogue and energy-minimized from a variety of distorted conformations. In most cases the resulting minimum energy conformation was virtually the same as the undistorted structure, strongly implying that the structure derived from both the *X*-ray and the NMR data was a global energy minimum. The conclusion from these studies must be that the conformation of these macrolides is decided very largely by intramolecular forces and not by crystal packing forces or solvation. A similar technique was used by workers at Beecham to confirm the equivalence of solution and crystalline conformations of VM 44857.[32b]

NMR based methods have also been used to assess the solution conformation of the disaccharide fragment of avermectin $B1_a$.[38] The necessary ^{13}C data were already available[39] and, in a similar manner to that described above, HETCOR and COSY experiments were used to unambiguously assign the proton spectrum. Coupling constants, determined from 600 mHz spectra and checked by a spin simulation programme, proved the monosaccharide subunits to be in a $^4C_1$ conformation. Contour maps for the conformational energies of both glycosidic linkages were calculated and showed, for each linkage, a well defined global minimum with only one local minimum. The conformation resulting from these calculations was then refined by data from NOE experiments at 400 mHz using the 1′-H–2′-H and 1″-H–2″-H distances as calibration. From these results it was demonstrated that, again, the average solution conformation was similar to that found in the solid-state *X*-ray structure. Intramolecular hydrogen bonds were not observed in the solution structure and it appears that the conformation is decided largely by non-bonded interactions.

4 Biosynthesis

The feeding of sodium [1-^{14}C]propionate to *S. avermitilis* MA5192 followed by semi-preparative HPLC has been described as a means of preparing ^{14}C-labelled avermectin $B1_a$, with a radiopurity of >99%, for biosynthetic studies.[40a,b] ^{13}C-Labelled avermectin $A1_a$ was prepared in the same manner and shown by ^{13}C NMR to be labelled at C-3, C-7, C11, C-13, and C-23, and from this it follows that the $B1_a$ is labelled at the same positions.

The biosynthetic assembly of the carbon skeleton of the avermectins has already been elucidated by Cane and his co-workers using [1-^{13}C]acetate and [1-^{13}C]propionate.[1,41] This has now been confirmed by further studies using [2-^{13}C]acetate and [3-^{13}C]propionate.[42] While the results from the labelled

[38] A Neszmelyi, D Machytka, A Kmety, P Sandor, and G Lukacs, *J Antibiot*, 1989, **42**, 1494
[39] G Albers-Schonberg, B H Arison, J C Chabala, A W Douglas, P Eskola, M H Fisher, A Lusi, H Mrozik, J L Smith, and R L Tolman, *J Am Chem Soc*, 1981, **103**, 4216
[40] (*a*) C C Ku, S C Hwang, and T A Jacob, *J Liq Chrom*, 1984, **7** 2905, (*b*) C C Ku, S C Hwang, L Kaplan, M K Nallin, and T A Jacob, *J Labelled Comp Radiopharm*, 1985, **22**, 451
[41] D E Cane, T-C Ziang, L Kaplan, M K Nallin, M D Schulman, O D Hensens, A W Douglas, and G Albers-Schonberg, *J Am Chem Soc*, 1983, **105**, 4110
[42] T S Chen, B H Arison, V P Gullo, and E S Inamine, *J Ind Microbiol*, 1989, **4**, 231

propionate were incontrovertible it was not clear from the acetate results whether there was direct conversion of acetate or whether there was some indirect incorporation of the label. This was clarified by the use of doubly-labelled [1,2-$^{13}C_2$]acetate, which gave avermectin labelled at C-1–C-2, C-5–C-6, C-9–C-10, C-15–C-16, C-17–C-18, C-19–C-20, and C-21–C-22, clearly indicating direct incorporation.

Studies using [1-^{14}C] and [2-^{14}C]acetate and propionate have demonstrated that acetate and propionate are poor precursors *per se* of avermectins, probably because the rate of oxidation of their CoA esters is high compared to the rate of avermectin biosynthesis. However, results obtained with [1-^{14}C], [2-^{14}C], [6-^{14}C], and [U-^{13}C]glucose demonstrated that both the oleandrose groups are directly derived from glucose and that the avermectin skeleton itself is derived entirely from glucose carbons.[43] Further confirmation of the direct conversion of glucose into the disaccharide moiety came from studies with doubly-labelled [6-^{14}C,6-^{3}H]glucose.[42] The hypothesis advanced for the production of the avermectin macrocycle from glucose carbons is that glucose is cleaved symmetrically by the Embden-Myerhoff pathway to give the required acetate for direct incorporation and that the required propionate is derived from this acetate by the tricarboxylic acid cycle.

The 2-methylbutyric acid required for biosynthesis of the C-25 isobutyl group of a-type avermectins is also derived from the acetate which enters the tricarboxylic acid cycle.[43] This produces oxaloacetate which is, in turn, converted into isoleucine and hence, by oxidative deamination, into 2-methylbutyric acid. That 2-methylbutyrate is the immediate precursor of the C-25 unit of the a-type avermectins, and that the b-type were derived from isobutyrate, was proved by feeding experiments with [1-^{13}C]2-methylbutyrate and [1-^{13}C]isobutyrate.[42] The appropriate butyrate then acts as a starter for the preparation of a single polyketide chain which is cyclized to the avermectin macrocycle.

Incorporation experiments with [*methyl*-^{14}C] and [2-^{14}C]methionine have demonstrated that the two methoxyl groups on the disaccharide and the methoxyl group of avermectins $A1_a$ and $A2_a$ were derived exclusively from the *S*-methyl group of methionine while the carbon backbone of methionine was not incorporated at all.[43]

The enzyme, avermectin B *O*-methyltransferase has been shown to transfer the methyl group of *S*-adenosyl methionine to the avermectin C-5 hydroxyl group in a non-rate limiting way.[44] Such methyltransferase enzymes are known to be inhibited by sinefungin,[45] so fermentation studies have been performed in the presence of this inhibitor.[46] *S. avermitilis* Agly-1 is a mutant strain of *S. avermitilis* which produces the aglycons of avermectins $A1_a$ and $A2_a$ almost

[43] M. D. Schulman, D. Valentino, and O. Hensens, *J. Antibiot.*, 1986, **39**, 541.
[44] M. D. Schulman, D. Valentino, M. Nallin, and L. Kaplan, *Antimicrob. Agents Chemother.*, 1986, **29**, 620.
[45] M. D. Schulman, D. Valentino, and C. Ruby, *Fed. Proc.*, 1985, **44**, 931.
[46] M. D. Schulman, D. Valentino, O. D. Hensens, D. Zink, M. Nallin, L. Kaplan, and D. A. Ostlind, *J. Antibiot.*, 1985, **38**, 1494.

exclusively (the ratio of A products to B products is approximately 40 1) but in the presence of sinefungin the B components exceeded the A components by a ratio of approximately 4 1 As the presence of sinefungin only reduced the overall yield of the fermentation by 10%, it is clear that sinefungin inhibits the methylation of avermectins of the B series quite specifically A similar experiment was performed on a high producing strain *S avermitilis* 08, which normally produces all eight major avermectin compounds The addition of sinefungin to this producing strain diverts the fermentation to produce the new demethyl avermectins detailed in the previous section

Thus sinefungin inhibits methylation at C-5, C-3′, and C-3″, but later work has demonstrated that methylation of the C-3′ and C-3″ positions occurs by a different *O*-methyltransferase enzyme [47] This was shown by studying six different mutants of *Streptomyces*, derived from *S avermitilis* WT by mutation with UV light and *N*-methyl-*N*-nitrosourethane Four of these strains, CR-1 to CR-4, lacked the avermectin B2 *O*-methyltransferase (B2OMT) activity and gave only avermectins of the B series, while CR-5 had normal levels of B2OMT activity and gave demethylavermectin A and B components The remaining strain, CR-6, was derived from the CR-1 strain and lacked B2OMT activity and gave only demethylavermectin B analogues Confirmation that this indicated the presence of two individual methylating enzymes was obtained by reacting 3′-*O*-demethylavermectin $B2_a$ and *S*-adenosyl methionine with cell extracts of *S avermitilis* WT or purified B2OMT These experiments yielded only 3″-*O*-demethylavermectin $A2_a$, corresponding to methylation at C-5 only

This work also indicates that methylation of the oleandrose occurs before the attachment of the disaccharide to the macrolide ring and thus cannot be a terminal step, in contrast to the biosynthesis of other macrolides such as tylosin and erythromycin [48a–c] This was confirmed in a later paper where [5-*methoxyl*-^{14}C]avermectin $A2_a$, not methylated on the disaccharide moiety, was fed to a 'wild' strain of *S Avermitilis* [49] The only radiolabelled compound recovered was the unaltered substrate, demonstrating that the oleandrose moiety is not methylated by this 'wild' strain In contrast, feeding of this strain with a mixture of unlabelled *bis*-dimethyl avermectin $B2_a$ and [*methyl*-^{14}C]methionine gave a mixture of the normal avermectin products and [5-*methoxy*-^{14}C]avermectin $A2_a$, thus confirming that the wild-type strain can methylate at C-5

Elucidation of the sequence of avermectin biosynthesis has been studied by Chen and Inamine [50] By feeding *S avermitilis* MA5502 with sodium [1-^{14}C]acetate, they prepared ^{14}C-labelled avermectins $A1_a$, $A2_a$, $B1_a$, and $B2_a$, and from the $B2_a$ they also prepared the corresponding monosaccharide and aglycon These avermectins were then individually fed to cultures of *S avermitilis*

[47] M D Schulman, D Valentino, S Streicher, and C Ruby, *Antimicrob Agents Chemother* 1987 **31** 744

[48] (*a*) J W Corcoran, *Methods Enzymol* 1975, **43**, 487 (*b*) E T Seno and R H Baltz, *Antimicrob Agents Chemother*, 1981, **20**, 370, (*c*) *ibid*, *Antimicrob Agents Chemother*, 1982, **21**, 758

[49] M D Schulman and C Ruby, *Antimicrob Agents Chemother*, 1987, **31**, 964

[50] T S Chen and E S Inamine, *Arch Biochem Biophys*, 1989, **270**, 521

Table 1

	Avermectin Produced					
Avermectin Fed	$A1_a$	$A2_a$	$B1_a$	$B2_a$	$B2_a$ AG	$B2_a$ MS
$A1_a$	100					
$A2_a$		93				
$B1_a$	2.7		93.6			
$B2_a$		29.7		68.3		
$B2_a$ AG		9.3		31.6	46.3	5.6
$B2_a$ MS		18.6		45.2		32.2

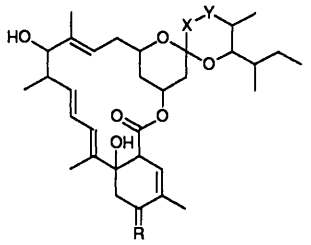
AG = Aglycon, MS = Monosaccharide

MA5502. After six days fermentation the crude avermectins produced, which accounted for approximately 98% of the administered radioactivity, were extracted and analysed by HPLC to give the results shown in Table 1. From this it can be seen that avermectins $A1_a$ and $A2_a$ are not converted into other avermectins and thus are end products to the biosynthetic sequence, whereas $B1_a$ was converted into $A1_a$ and $B2_a$ into $A2_a$. The results from $B2_a$ monosaccharide and aglycon show this part of the biosynthetic pathway to be $B2_a$ aglycon $\longrightarrow$ $B2_a$ monosaccharide $\longrightarrow$ $B2_a$ $\longrightarrow$ $A2_a$, and also indicate that they are not precursors to the '1' series of avermectins. In an additional experiment, avermectin $A2_a$ aglycon was fed to a mutant *S. avermitilis* strain, MA5542 (which is thought to be blocked at a very early stage of biosynthesis) and shown to produce avermectin $A2_a$. From this it can be deduced that the end products $A1_a$ and $A2_a$ may also be formed by the sequence $B1_a/B2_a$ aglycons $\longrightarrow$ $A1_a/A2_a$ aglycons $\longrightarrow$ $A1_a/A2_a$ monosaccharides $\longrightarrow$ $A1_a/A2_a$.

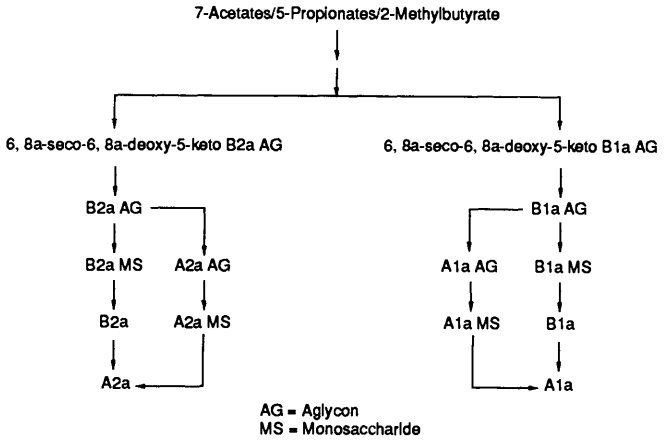
Avermectins lacking a tetrahydrofuran ring (50a—d) are known to be produced by the *S. avermitilis* mutant, MA5218,[1,51] Samples of these four compounds were separated and fed to cultures of *S. avermitilis* MA5542 (see above) when it was found that the two 5-keto compounds (50a and 50b) were converted into avermectins $B1_a/A1_a$ and $B2_a/A2_a$ respectively.[50] The other two metabolites (50c and 50d) did not produce avermectins but were converted into their corresponding mono and disaccharides. This indicates that formation of the carbonyl group at the C-5 position must precede formation of the tetrahydrofuran ring. That reduction of the 5-keto group occurs after tetrahydrofuran ring formation was demonstrated by feeding 5-keto avermectin $B2_a$ aglycon to MA5542, when avermectin $B2_a$ was produced. The accumulated information from all the biosynthetic work reported has led to the proposed biosynthetic scheme shown in Scheme 1. The only remaining step which is in question is the biosynthetic dehydration at C-22–C-23, the branching point for the production of '1' and '2' series avermectins.

Studies of the biosynthesis of the Cyanamid LL-F28249 compounds (34a—d)

[51] R. T. Goegelman, V. P. Gullo, and L. Kaplan (Merck and Co. Inc.), US Patent, US 4 378 353 (1983).



	R	X—Y
(50a)	O	CH=CH
(50b)	O	$CH_2CH(OH)$
(50c)	H, OH	CH=CH
(50d)	H, OMe	$CH_2CH(OH)$



Scheme 1

indicate, unsurprisingly, that the mode of formation of the macrocycle and spiroacetal rings is identical to that of the avermectins and milbemycins.[33a,52] The unique side chain at C-25, however, is derived from two acyl units. From studies with carbon labelled precursors, the terminal residue of LL-F28249α was shown to be derived from isobutyrate linked, by a double bond, to the propionate derived unit at C-25 (Figure 1). The origin of the oxygen atoms was also derived by experiments with oxygen-labelled acetate and propionate, and this again was commensurate with avermectin and milbemycin biosynthesis. This

[52] H.-R. Tsou, Z. H. Ahmed, R. R. Fiala, M. W. Bu'lock, G. Carter, J. J. Goodman, and D. B. Borders, *J. Antibiot.*, 1989, **42**, 398.

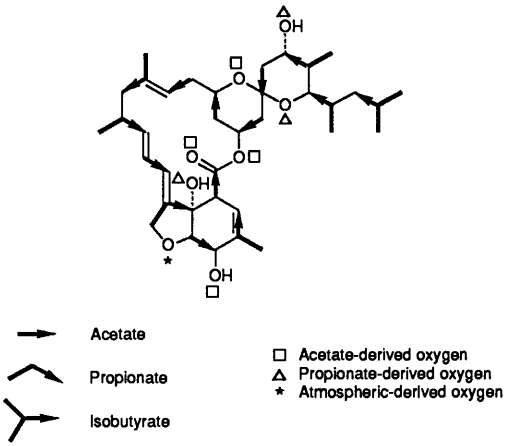


Figure 1

work accounted for all the incorporated oxygen except for that in the tetrahydrofuran ring which, by fermentation in the presence of molecular $^{18}O_2$, was proved to be derived from atmospheric oxygen.

5 Metabolism and Assay

It is known that a thin film of avermectins, as would be found on crops after spraying, decomposes rapidly, particularly in strong sunlight.[53] Avermectins which fall on the soil are similarly rapidly removed from the environment as they are very tightly bound to soil. However, Ivermectin is excreted largely unchanged in the faeces[54a,b] and this could cause environmental problems, since unmetabolized material left on the pasture could eradicate the fauna which normally biodegrades cattle droppings. This can lead in time to fouling of the pasture. Until this can be obviated, worries over this problem could lead to limitations on the agricultural use of these powerful anthelmintics.

Our previous review[1] reported *in vitro* studies on the metabolism of 22,23-dihydroavermectin $B1_a$, the major component of the commercially available Ivermectin, with rat and steer liver microsomes. This work has now been extended to *in vivo* studies of the metabolism of 22,23-^{3}H-Ivermectin (a 4:1 mixture of 22,23-dihydroavermectins $B1_a$ and $B1_b$) in rats, cattle, and sheep.[55] In all these species the metabolic disposition was essentially the same, with the unaltered drug being the major tissue residue in the livers, kidneys, muscles, and fat, the highest levels of radioactivity being found in the liver and fat. A subtle

[53] Y. Iwata, J. G. McConnell, J. E. Flor, I. Putter, and T. M. Dinoff, *J. Agric. Food Chem.*, 1985, **33**, 467.
[54] (*a*) A. Miller, S. E. Kunz, D. D. Oehler, and R. W. Miller, *J. Econ. Entomol.*, 1981, **74**, 608; (*b*) C. D. Schmidt, *Environ. Entomol.*, 1983, **12**, 455.
[55] S.-H. L. Chiu, E. Sestokas, R. Taub, R. P. Buhs, M. Green, R. Sestokas, W. J. A. Vendenheuvel, B. H. Arison, and T. A. Jacob, *Drug Metabl. Dispos.*, 1986, **14**, 590.

difference of metabolism was noted in the tissue samples from steers relative to those from sheep and rats. The initial sample of Ivermectin contained a 4:1 ratio of dihydroavermectin components, whereas the samples recovered from steer tissue showed ratios varying between 5:1 and 20:1; those from rat or sheep tissue, in contrast, showed ratios close to or less than 4:1. This suggests that steers metabolize (or excrete) the $B1_b$ component more rapidly than the $B1_a$ component, a surprising result given the close structural similarity between the two series.

Drug residues in steer and sheep liver tissue contained 20—22% of polar metabolites, while rat liver tissue contained 8%. The patterns of metabolites obtained in all three species, however, were closely similar, consisting of four components. The three major metabolites were isolated and identified as 24-hydroxymethyl-22,23-dihydroavermectin $B1_a$ (the major metabolite), its monosaccharide and 24-hydroxymethyl-22,23-dihydroavermectin $B1_b$. Confirmation of their identity was obtained by comparison with authentic samples obtained from the earlier *in vitro* work.

In vivo experiments with swine indicated a major difference in metabolic products from the liver, with five major metabolites being produced, only slightly more polar than the administered drug.[56] This is in contrast to the metabolites previously seen in other species. Isolation and identification of these metabolites proved to be troublesome because of the low residue levels, but the two major metabolites were proved to be 3″-demethyl-22,23-dihydroavermectin $B1_a$ and $B1_b$ by comparison with samples obtained from *in vitro* experiments with pig liver microsomes.

For metabolic studies on new compounds, work with large animals such as steers is made easier by the large doses needed, because this means that greater amounts of metabolites can be isolated for identification. The previously described paper,[56] however, points out that for such efficacious compounds as the avermectins this does not hold true, and thus they represent a major challenge for methods of assay. Studies of such methods have concentrated largely on Ivermectin, as this is the main avermectin at present on the market.

Reverse isotope dilution assay is a frequently used method to identify and quantitate radioactive metabolites in gross mixtures. The normal technique is to dilute the mixture with a known weight of a large excess of the non-radioactive carrier, and then to recrystallize to a constant specific activity. With the low levels of avermectin metabolites obtained in *in vivo* experiments this technique is impractical because, after dilution with a reasonable amount of unlabelled compound, the resulting specific activity would be at a level such as to make accurate measurement difficult. This has been circumvented by Chiu and his co-workers[57] by the use of an HPLC technique. Unlabelled Ivermectin was added to the tissue sample containing the radioactive metabolite (^{2}H-$B1_a$ and ^{2}H-$B1_b$ in this instance) and the mixture of Ivermectin and the radioactive metabolite was

[56] S.-H. L. Chiu, R. Taub, E. Sestokas, A. Y. H. Lu, and T. A. Jacob, *Drug Metab. Rev.*, 1987, **18**, 289.
[57] S.-H. L. Chiu, R. P. Buhs, E. Sestokas, R. Taub, and T. A. Jacob, *J. Agric. Food Chem.*, 1985, **33**, 99.

extracted by a multiple solvent extraction procedure. This was then separated by reverse phase HPLC (and normal phase, if necessary) to obtain the 22,23-dihydroavermectins $B1_a$ and $B1_b$. Ultraviolet spectra in ethanol solution were used to determine the concentrations of the samples by reference to their known absorbance coefficients. The specific activity of these solutions was then determined by scintillation counting and the proportion of each compound in the total radioactive residue was then determined by comparison of the specific activities before and after the addition of the cold Ivermectin.

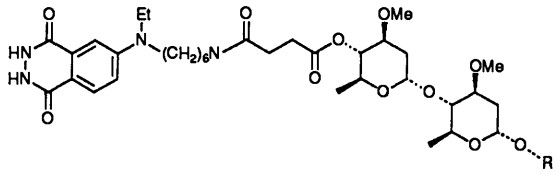
Two dimensional TLC methods have long been known to be advantageous in the elucidation of complex mixtures and an HPLC equivalent has been described.[58] After initial purification by reverse-phase HPLC, the samples obtained were checked by normal-phase chromatography for homogeneity and, in the case of radioactive samples, specific activity. The advantage of this procedure was shown in the examination of an *in vivo* metabolite from swine liver, when an initial identification by reverse-phase HPLC of a metabolite as 22,23-dihydroavermectin $B1_a$ monosaccharide was shown to be incorrect by normal-phase HPLC. A further advantage for very small samples of radioactive isolates is that this 2D HPLC method is very sensitive; only low levels of unlabelled carrier need to be added to the isolate, resulting in a higher level of specific activity for the purified component.

A rapid and reproducible method of determination of Ivermectin levels has been published by Stong.[59] Ivermectin was oxidized at C-5 with manganese dioxide and then aromatized by treatment with ammonium acetate to produce an intense blue–white fluorescence, ideal for quantitation by HPLC. This provides an overall procedure which is accurate over a range of 0.01—10 μg/ml.

Previous methods of measurement of Ivermectin or 22,23-dihydroavermectin $B1_a$ levels in plasma have relied upon solvent extraction, which can present practical difficulties. This has been overcome by Kojima and his co-workers, who deproteinized samples of dog plasma with trichloroacetic acid and then separated the avermectin by a solid-phase extraction procedure.[60] The avermectin was then quantitated with HPLC.

In response to the clinical trials in man which are now underway, workers in the Merck laboratories have developed an assay for Ivermectin in plasma or breast milk.[61] The sample was cleaned up by various solid-phase procedures, derivatized by a method based on that of Tolan *et al.*,[62] and the derivatized drug was then extracted by a further solid-phase extraction step. Quantification of the sample was then performed by reversed-phase HPLC with a xenon lamp fluorescence detector. As no solvent extraction steps are involved in this assay, this means that the volume of sample needed for this assay is minimal (0.5—1.0

[58] S.-H. L. Chiu, E. Sestokas, and R. Taub, *J. Chromatogr.*, 1988, **433**, 217.
[59] J. D. Stong, *Anal. Chem.*, 1987, **59**, 266.
[60] K. Kojima, K. Yamamoto, and Y. Nakanishi, *J. Chromatogr.*, 1987, **413**, 326.
[61] R. Chiou, R. J. Stubbs, and W. F. Bayne, *J. Chromatogr.*, 1987, **416**, 196.
[62] J. W. Tolan, P. Eskola, D. W. Fink, H. Mrozik, and L. A. Zimmerman, *J. Chromatogr.*, 1980, **190**, 367.



R = 13-substituted 22,23-dihydroavermectin B1a

(51)

ml of plasma or 2.5 ml of milk) while the detection limits are very low (0.2 ng/ml for plasma, 0.05 ng/ml for breast milk).

Assays involving the receptors which mediate the biological response of avermectins are limited in their sensitivity by the specific activity of the tritiated avermectins used in the assay. This has been circumvented by Schaeffer and his co-workers at Merck by the preparation of a 4″-luminol analogue of 22,23-dihydroavermectin $B1_a$ (51).[63] This coupling, by a succinoyl bridge, gives an avermectin which is equi-active with the avermectin precursor and loses none of its affinity for the specific binding sites. In the presence of hydrogen peroxide and excess horseradish peroxidase the luminol derivative emits light in proportion to its concentration, permitting quantitation at levels down to 3.5×10^{-16} M. This is approximately thirty fold more sensitive than is permitted by the use of tritiated avermectins.

6 Partial Synthesis

A. Spiroacetal Portion.—The milbemycins were first reported in 1974[64,65] and the avermectins in 1979,[66] but until 1981 very little was reported on either their partial or total synthesis. However, since then, and especially during the last five years, a wealth of publications has appeared on these subjects.

The spiroacetal sub-unit has been the subject of a number of synthetic approaches to avermectins and milbemycins. Model studies by DeShong and associates[67] have shown a general and highly stereoselective approach to spiroacetals involving the preparation of a pyranone by oxidation of a suitably substituted furfurol derivative. The pyranone is then treated with acid when a stereoselective, intramolecular ketalization occurs providing the requisite spiroacetal.

The spiroacetal unit of milbemycin β_3 in chiral form was first synthesized by

[63] J M Schaeffer, J H Stiffey, and H Mrozik, *Anal Biochem*, 1989, **177**, 291

[64] H Mishima, M Kurubayashi, C Tamura, S Sato, H Kuwano, A Saito, and A Aoki, 'Structures of Milbemycins α_1, α_2, α_3, α_4, α_5, α_6, α_7, α_8, α_9, α_{10} and β_1' Abstracts Papers 18th Symp Chem Natural Products, Kyoto, 1974, p 309

[65] H Mishima, M Kurubayashi, C Tamura, S Sato, H Kuwano, and A Saito, *Tetrahedron Lett*, 1975, 711

[66] R W Burg, B M Miller, E E Baker, J Birnbaum, S A Currie, R Hartmann, Y-L Kong, R L Monaghan, G Olson, I Putter, J B Tunac, H Wallick, E O Stapley, R Oiwa, and S Omura, *Antimicrob Agents Chemother*, 1979, **15**, 361

[67] P DeShong, R E Waltermire, and H L Ammon, *J Am Chem Soc*, 1988, **110**, 1901

Baker and colleagues[68–70,1] and this work was extended to the total synthesis of optically pure milbemycin β_3 (*vide infra*).

Kocienski and co-workers[71] have reported the use of metallated 3,4-dihydro-2*H*-pyran as a masked acyl anion equivalent in the synthesis of the optically pure spiroacetal (57). The key step involves the nucleophilic cleavage of the chiral oxirane (53), available in eight steps from (−)-(*S*)-malic acid (52), with two equivalents of the lithium cuprate (54) (Scheme 2). The resulting alcohol (55), being acid sensitive, was not purified but cyclized immediately using a catalytic amount of acid. The spiroacetal (56) was thus obtained as a single diastereoisomer in an overall yield of 70% from (53); routine manipulations then provided the requisite spiroacetal (57). Kocienski and colleagues[72] have also described an efficient synthesis of the aldehyde (64) (Scheme 3), a late stage synthon in their total synthesis of (+)-milbemycin β_3 (*vide infra*). Once again the key step involves cleavage of an oxirane with an organocuprate derived from a 3,4-dihydro-2*H*-pyran. (−)-(*S*)-Malic acid was transformed into the protected epoxide (58) (Scheme 3) in routine fashion, and this, on condensation with the aluminate (59), gave the epimeric alcohols (60) and (61). The unwanted isomer (61) was easily converted into the required alcohol by Mitsunobu inversion.[73] Simple manipulations then provided the required epoxide (62) in good yield. Treatment of this with the lithium cuprate (54), as described above, afforded the alkylation product which, without purification, was treated with a catalytic amount of acid in methanol when the spiroacetal (63) was obtained as a single diastereoisomer. Routine chemistry[74] then allowed the obtention of the required aldehyde (64).

More recently, workers in France[75] have reported a new strategy, involving a regiospecific Baeyer–Villiger oxidation, for the stereocontrolled synthesis of the spiroacetal portion of the milbemycins. Norbornadiene was converted into the 2,5-dione (65) using known methodology and this, on exposure to *m*-chloroperoxybenzoic acid, afforded the di-lactone (66) (Scheme 4). Methanolysis, followed by mono-lactonization and alcohol protection, then gave the lactone (67). Condensation of this with the lithium salt of the alkyne (68) afforded the acetylenic ketone (69) in high yield. The alkyne (68) was obtained from acetaldehyde by condensation with the Grignard reagent derived from 3-bromo-

[68] R. Baker , R. H. O. Boyes, D. M. P. Broom, J. A. Devlin, and C. J. Swain, *J. Chem. Soc., Chem. Commun.*, 1983, 829.

[69] R. Baker, R. H. O. Boyes, D. M. P. Broom, M. J. Mahoney, and C. J. Swain, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1613.

[70] R. Baker, C. J. Swain, and J. Head, 'Synthetic Studies towards the Avermectins and Milbemycins' in 'Recent Advances in the Chemistry of Insect Control,' ed. N. F. Janes, The Royal Society of Chemistry Special Publication No. 53, London, 1985, p. 245.

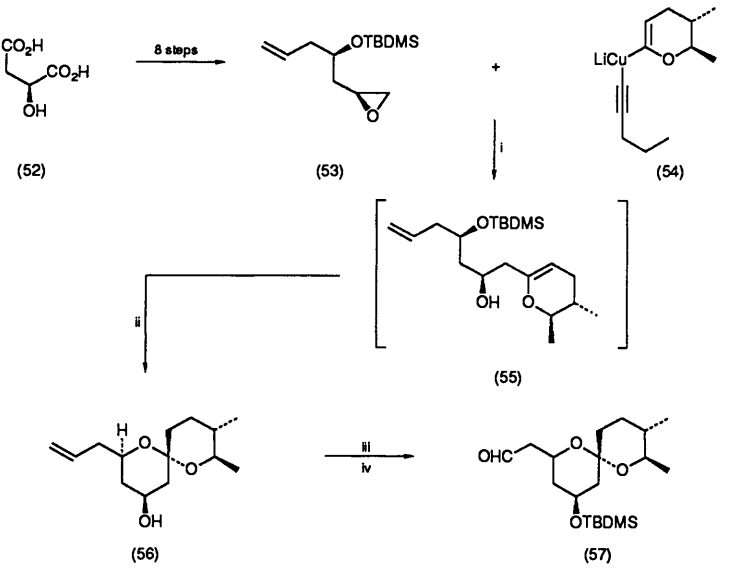
[71] P. Kocienski, C. Yeates, S. D. A. Street, and S. F. Campbell, *J. Chem. Soc., Perkin Trans. 1*, 1987, 2183.

[72] P. Kocienski, S. D. A. Street, C. Yeates, and S. F. Campbell, *J. Chem. Soc., Perkin Trans. 1*, 1987, 2189.

[73] O. Mitsunobu, *Synthesis*, 1981, **1**, 1.

[74] P. J. Kocienski, S. D. A. Street, C. Yeates, and S. F. Campbell, *J. Chem. Soc., Perkin Trans. 1*, 1987, 2171.

[75] N. Van Bac and Y. Langlois, *Tetrahedron Lett.*, 1988, **29**, 2819.



(56) (57)

Reagents: i, THF, $-30 \longrightarrow 20\,^{\circ}C$; ii, camphorsulphonic acid (trace)–MeOH; iii, Bu^tMe_2SiCl–DMF–NEt_3–DMAP; iv, (a) O_3–MeOH–pyridine, $-78\,^{\circ}C$, (b) Me_2S

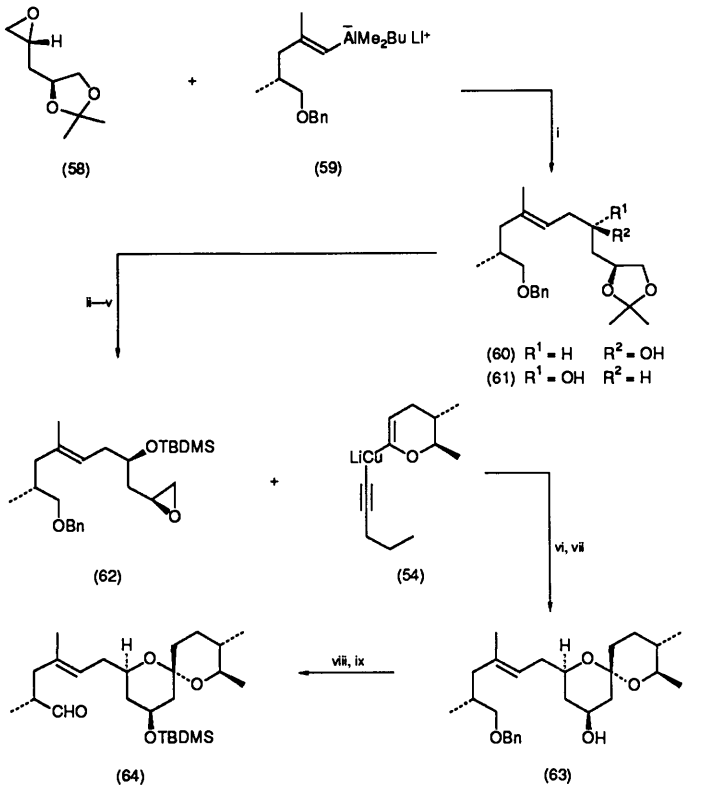
Scheme 2

1-propyne or, more directly, by condensation with the Grignard reagent derived from 3-bromo-1-butyne. Reduction of the triple bond in (69) and simultaneous debenzylation followed by cyclization and deprotection then gave the requisite spiroacetal (70). Conversion of this work into an enantioselective synthesis is reported to be in progress.

A number of other syntheses of spiroacetal containing portions of various other milbemycins have also been described in the literature. For example, workers in Japan[76] have described some work which culminated in the synthesis of the spiroacetal moiety of various milbemycins. They employed as their starting material the *threo*-alcohol (71) and by complete hydrogenation were able to obtain the diol (72). Treatment of this with the lithium acetylide of (73), followed by partial reduction and acid catalysed cyclization, provided the unsaturated spiroacetal (74) and on prolonged exposure of this to concentrated hydrochloric acid in aqueous tetrahydrofuran, they were able to obtain the requisite spiroacetal (75) in 30% yield. By far the major product of the reaction (53%) was the isomeric axial alcohol, which could be reprocessed to provide further amounts of (75) by an oxidation–reduction sequence.

Crimmins and colleagues[77] in America have reported a highly stereocontrolled

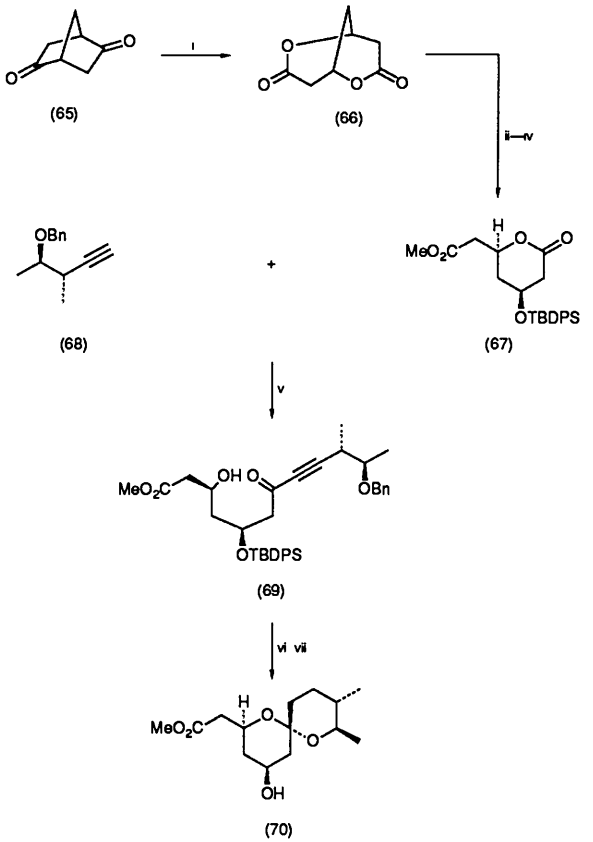
[76] S. Takano, Y. Sekiguchu, and K. Ogasawara, *Heterocycles*, 1989, **29**, 445.
[77] M. T. Crimmins, W. G. Hollis, and D. M. Bankaitis-Davis, *Tetrahedron Lett.*, 1978, **28**, 3651.



Reagents: i, Hexane, −30 to −10 °C; ii, Amberlite IR 120 (H^+), THF–H_2O 20 °C, 3 d; iii, TsCl–pyridine, 0 °C, 10 h; iv, K_2CO_3–MeOH, 20 °C, 25 min; v, Bu^tMe_2SiCl, DMAP, Et_3N–DMF, 20 °C, 5 h; vi, THF, −30 to 20 °C, 6 h; vii, H^+–MeOH, 20 °C, 1 h followed by K_2CO_3; viii, Bu^tMe_2SiCl, DMAP, Et_3N–CH_2Cl_2 20°, 3.5 h; ix, Ref. 74

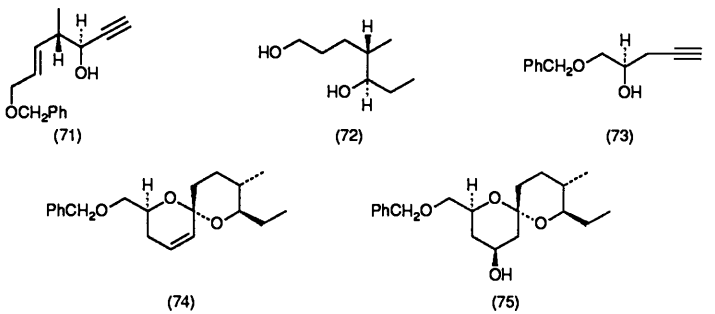
Scheme 3

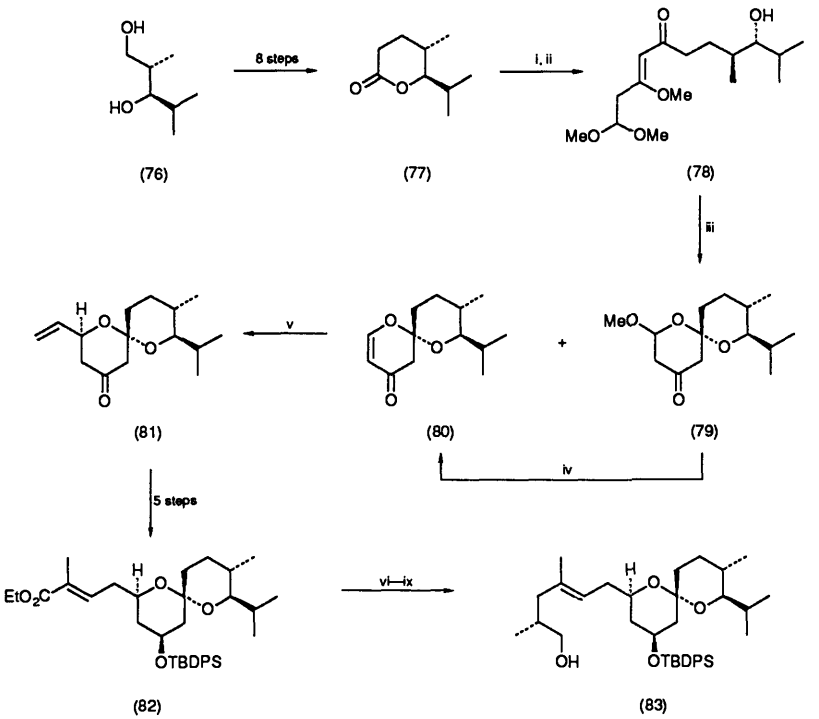
synthesis of the C-11 to C-31 fragment of milbemycin D (83) (Scheme 5). Sharpless asymmetric epoxidation of 4-methyl-2-penten-1-ol, followed by regioselective opening of the epoxide with lithium dimethylcopper, afforded a 90% yield of a 6:1 mixture of the diol (76) and the corresponding 1,2-diol. The unwanted isomer could be readily removed on treatment of the mixture with sodium metaperiodate. Protecting group manipulation of the diol (76) followed by Swern oxidation, Wittig coupling, deprotection, hydrogenation, and lactonization, afforded the lactone (77) in 88% yield. Ring opening of this lactone with the lithium acetylide of 1-methoxy-1-buten-3-yne followed by exposure of the product to potassium carbonate in methanol afforded the ketone (78). Treatment of this ketone with 30% aqueous perchloric acid in an ultrasound bath gave a 4:1 mixture of the spiroacetal (80) and the methanol adduct (79). (The use of



Reagents i, mcpba, $NaHCO_3$, CH_2Cl_2, r t , 48 h, ii, MeOH, HCl (trace) r t , 2 h, iii, C_6H_6, reflux, 20 h, iv, Bu^tPh_2SiCl, imidazole, DMF, v, THF, −30 °C, 30 min, vi, 10% Pd–C, H_2, MeOH, 4 h, vii, TBAF, THF, 20 °C, 24 h

Scheme 4



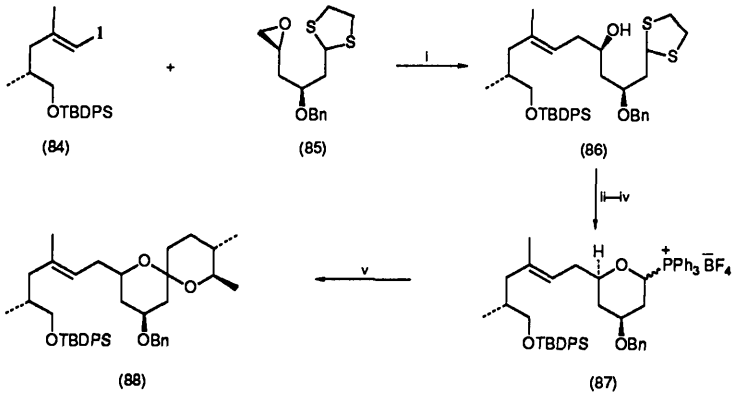


Reagents: i, MeOCH=CH·C≡C·Li, THF, −78 °C, 1 h; ii, K_2CO_3, MeOH, 18 h; iii, 30% $HClO_4$, CH_2Cl_2, ultrasound, 20 min; iv, moist Amberlyst, CH_2Cl_2, 40 °C; v, CH_2=CH·MgBr, Cu; vi, DIBAL, THF, −78 °C, 3 h; vii, CBr_4, Ph_3P, CH_3CN, 1 h; viii, Evans alkylation; ix, $LiAlH_4$, Et_2O, 0 °C, 1 h

Scheme 5

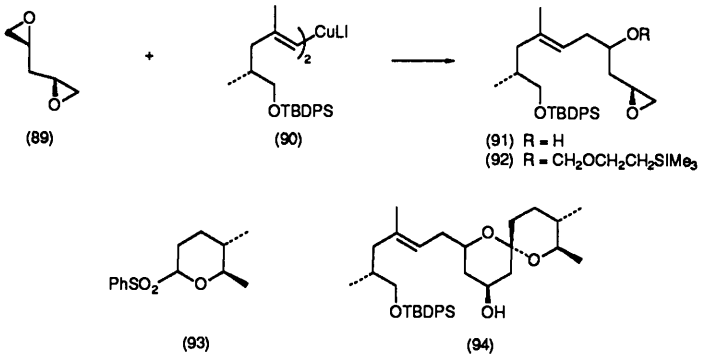
ultrasonic waves is essential for the efficient execution of this hydrolysis–cyclization). The components (79) and (80) were separated by chromatography and the unwanted product (79) converted into the spiroacetal (80) on treatment with moist Amberlyst resin. Copper catalysed conjugate addition of vinyl magnesium bromide to this spiroacetal exhibited a high preference for equatorial addition, resulting in a 78% yield of the adduct (81). Reduction of the ketone followed by t-butyldiphenylsilyl protection, hydroboration, Swern oxidation, and Wittig coupling afforded the olefin (82) as a single detectable isomer. This olefin was converted into its allylic bromide which was then subjected to Evans' alkylation using ten equivalents of the lithium enolate derived from (*L*)-valinol. After reductive cleavage of the intermediate oxazolidinone, the required C-11 to C-31 moiety of milbemycin D (83) was obtained in high yield. A similar intermediate for the synthesis of milbemycin β_1 has been described by Ley and associates,[78] who have reported the preparation of (88) in which a new procedure

[78] D. Culshaw, P. Price, S. V. Ley, and G. A. Strange, *Tetrahedron Lett.*, 1985, **26**, 5837.



Reagents: i, Homocuprate addition, −25 °C, 16 h; ii, MeI, MeCN, H_2O; iii, $(MeO)_2CMe_2/H^+$; iv, Ph_3PHBF_4; v, Ref. 79

Scheme 6

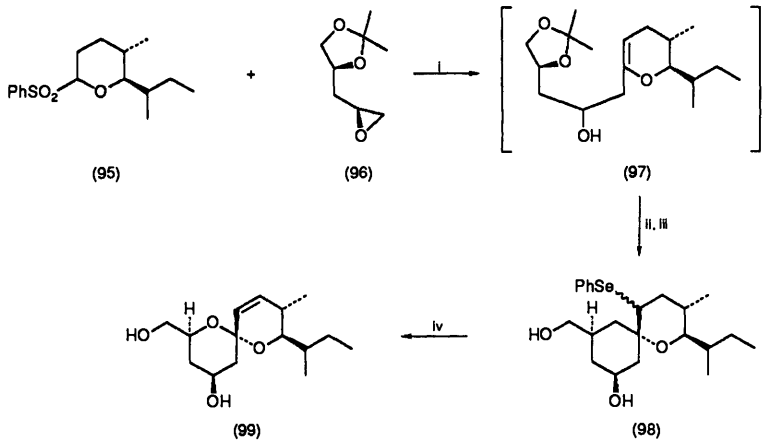


Scheme 7

for the formation of the C-15–C-16 double bond was employed (Scheme 6). The vinyl iodide (84), readily obtainable from commercially available (*S*)-(+)-methyl-3-hydroxy-2-methylpropionate in five steps, was coupled, *via* its homocuprate, with the epoxide (85) to afford the alcohol (86) in 96% yield. Hydrolysis, followed by phosphonium salt formation, gave the intermediate (87) which was transformed into the requisite milbemycin precursor (88) by a previously established sequence [79,1] in low yield.

Ley and colleagues [80] have also described an alternative synthesis of the C-11–C-25 fragment of milbemycin β_1. The key step of the synthesis involved the addition of a 2-benzenesulphonyltetrahydropyranyl anion to an epoxide (Scheme 7). The readily available bis-epoxide (89) on treatment with one equivalent of the

[79] J. Godoy, S. V. Ley, and B. Lygo, *J. Chem. Soc., Chem. Commun.*, 1984, 1381.
[80] C. Greck, P. Grice, S. V. Ley, and A. Wonnacott, *Tetrahedron Lett.*, 1986, **27**, 5277.



Reagents: i, Bu^{n}Li, THF, −78 °C; ii, PhSeCl, MeOH, Et_3N; iii, TsOH, MeOH; iv, *p*-nitrophenyl-*N*-sulphonyloxaziridine

Scheme 8

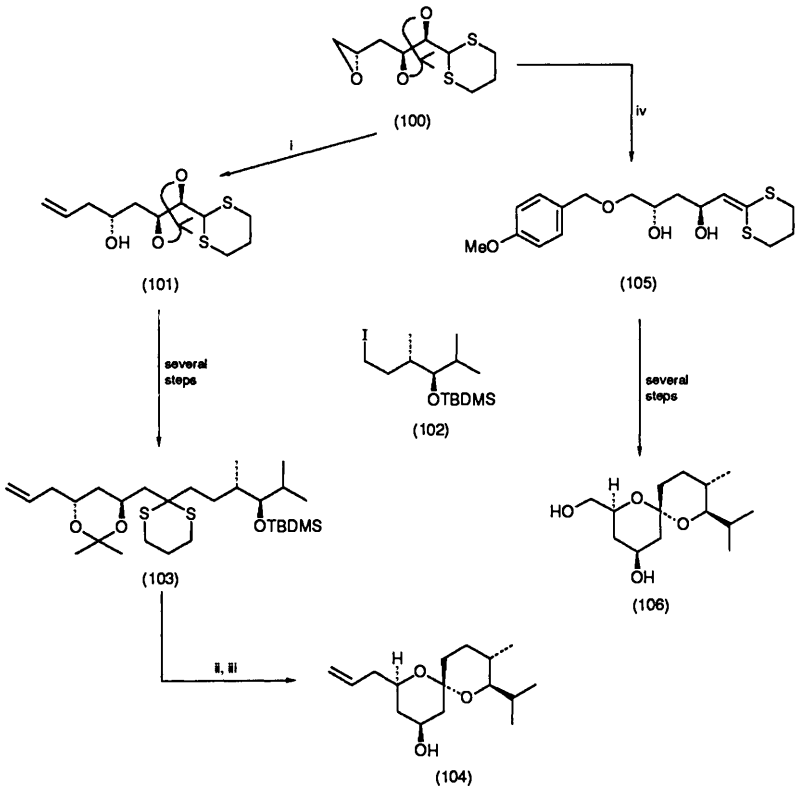
homocuprate (90)[78] at −65 °C provided the epoxy alcohol (91), which could be readily protected as its β-trimethylsilylethoxymethyl (SEM) ether (92). Preparation of the requisite milbemycin intermediate (94) could be accomplished in two ways. Reaction of the alcohol (91) with the anion of the sulphone (93) in the presence of titanium(IV)isopropoxide followed by work up with 5% sulphuric acid gave (94). Alternatively, reaction of the SEM ether (92) with the anion of (93) in the presence of boron trifluoride etherate followed by treatment with 5% hydrofluoric acid in acetonitrile also gave (94). Cyclization *via* the alcohol (91) gave an 80% yield of the spiroacetal (94); when the reaction was performed on the SEM ether a 72% yield was observed. Further work from this group has now demonstrated that this strategy can be readily employed in the synthesis of the spiroacetal portion of avermectin $B1_a$.[81] The sulphone (95) (Scheme 8) was allowed to react with the protected epoxide (96) when the enol ether (97) was obtained. This intermediate, whilst being highly reactive, could be isolated. After phenylselenylation, acid catalysed cyclization, with concomitant deprotection of the isopropylidene group, gave the substituted spiroacetal (98). Oxidation to the selenoxide led to *syn*-elimination and thus provided the desired spiroacetal portion of avermectin $B1_a$ (99) in reasonable yield.

The spiroacetal fragment of milbemycins D, E, F, G, and H have received attention by Thomas and colleagues[82,83] who employed methyl-α-D-

[81] D. Diez-Martin, P. Grice, H. C. Kolb, S. V. Ley, and A. Madin, *Tetrahedron Lett.*, 1990, **24**, 3445.

[82] G. Khandekar, G. C. Robinson, N. A. Stacey, P. G. Steel, E. J. Thomas, and S. Vather, *J. Chem. Soc., Chem. Commun.*, 1987, 877.

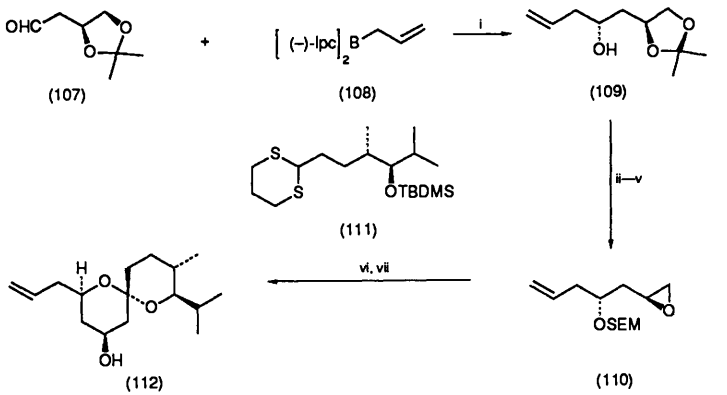
[83] E. J. Thomas, 'Approaches to the Synthesis of Antibacterial Compounds' in 'Topics in Medicinal Chemistry, 4th SCI–RSC Medicinal Chemistry Symposium', ed. P. R. Leeming, The Royal Society of Chemistry Special Publication No. 65, London, 1987, p. 284.



Reagents: i, vinylmagnesium bromide, CuI, −40 to −30 °C, 4 h; ii, 1M-HCl, MeOH, r.t., 12 h; iii, $HgCl_2$, THF, r.t., 12—24 h; iv, *p*-$MeOC_6H_4CH_2OH$, NaH, DMF, r.t., 2 h

Scheme 9

glucopyranoside as their starting material. The key epoxide (100) (Scheme 9), obtained in six routine steps from this pyranoside, was treated with vinylmagnesium bromide in the presence of a copper catalyst which effected ring opening of the epoxide and gave a good yield (82%) of the homoallylic alcohol (101). Protecting group manipulation of this alcohol (101) followed by alkylation with the optically active iodide (102) resulted in the formation of the dialkyldithiane (103). The iodide was readily obtained in four steps from 2-methylpropanal utilising *trans*-but-2-enyldi-(+)-isopinocamphenylborane to introduce chirality. This iodide was shown to possess an enantiomeric excess of >90% by formation of a Mosher derivative of its precursor. The dithiane (103), on deprotection, spontaneously cyclized to give the requisite milbemycin fragment (104). The epoxide (100) could also be reacted with the sodium salt of anisyl alcohol which simultaneously opened the epoxide and fragmented the

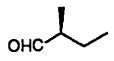


Reagents: i, −70 °C then H_2O_2, OH^-; ii, SEM-Cl, Pr^i_2NEt, CH_2Cl_2; iii, HCl, H_2O, THF; iv, TsCl, Et_3N, DMAP, CH_2Cl_2; v, K_2CO_3, MeOH; vi, Bu^tLi, tetramethylethylene diamine, THF, −20 °C; vii, HF, H_2O, MeCN, r.t., 3 h

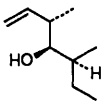
Scheme 10

acetonide group. The resulting diol (105) was then simply transformed into the hydroxyspiroacetal (106), a further intermediate for milbemycin synthesis, in an overall yield of about 10%. More recently, Thomas[83,84] has described an alternative synthesis of the spiroacetal moiety of milbemycin E, and by a modification of the conditions employed was also able to design an expedient and stereoselective synthesis of the spiroacetal portion of avermectins $A2_a$ and $B2_a$. The starting material for the milbemycin portion was (*S*)-dimethyl malate which was smoothly transformed into the aldehyde (107) (Scheme 10) employing standard methodology. This aldehyde had previously been employed by Kocienski[85] in a synthesis of a precursor for the total synthesis of (+)-milbemycin β_3 (*vide infra*). It was however stressed by Thomas and colleagues that due to the inherently weak diastereofacial selectivity of (107) towards nucleophilic attack, undesired adducts for milbemycin synthesis would usually predominate. Nevertheless, by the subtle employment of an optically active allylborane these workers were able to circumvent this problem. Thus, treatment of the aldehyde (107) with allyl di-isopinocamphenylborane (108), followed by oxidation with hydrogen peroxide, gave a 65% yield of (109) and its diastereoisomer, in the very favourable ratio of 9:1. Protecting group manipulation of (109) followed by selective tosylation and intramolecular cyclization afforded the epoxide (110) in excellent overall yield. Treatment of this epoxide with an excess of the lithium anion of the dithiane (111) effected ring opening of the epoxide and on treatment of the product with dilute acid, deprotection and spontaneous cyclization occurred producing the requisite spiroacetal (112) in

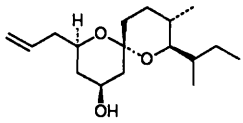
[84] E. Merifield, P. G. Steel, and E. J. Thomas, *J. Chem. Soc., Chem. Commun.*, 1987, 1826.
[85] C. Yeates, S. D. A. Street, P. Kocienski, and S. F. Campbell, *J. Chem. Soc., Chem. Commun.*, 1985, 1388.



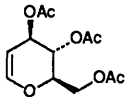
(113)



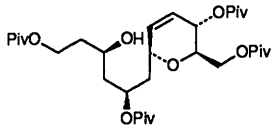
(114)



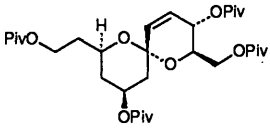
(115)



(116)



(117) Piv = Pivaloyl



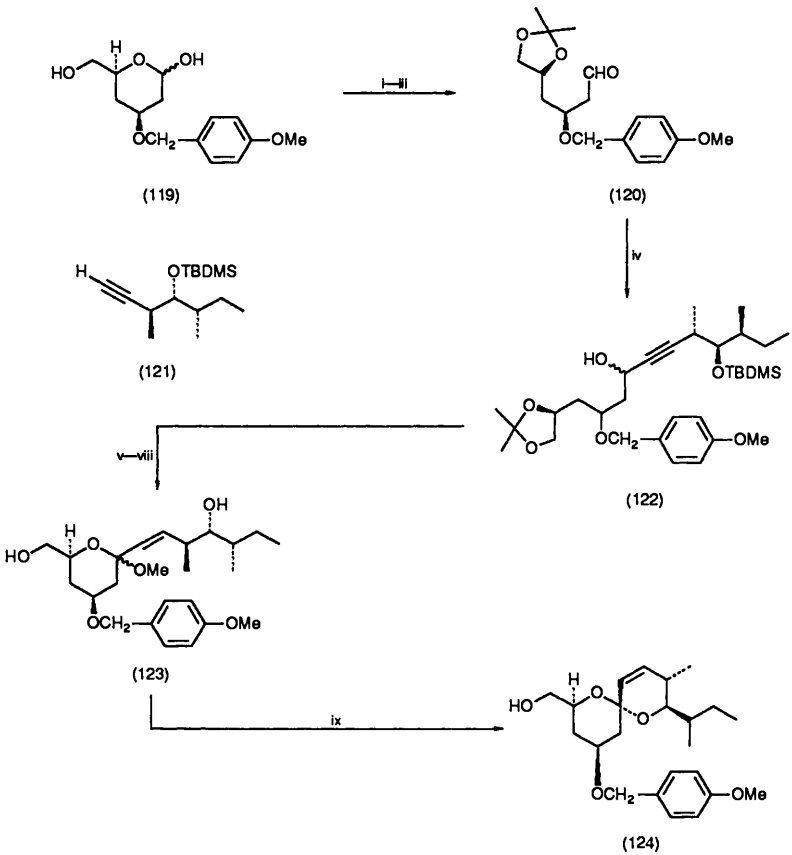
(118)

69% yield. This approach was then applied to the synthesis of the spiroacetal fragment of the avermectins with only minor modifications. Commercially available (*S*)-2-methylbutanol was oxidized with chromic acid to give the aldehyde (113) but although the chemical yield was high the reaction was accompanied by some racemization. However, treatment with allylborane as mentioned above gave two diastereoisomers in high yield. By far the major product was (114), and this was fortuitously obtained in >90% enantiomeric excess. Epoxidation, followed by similar transformations to those mentioned above, then gave the spiroacetal fragment of the 2a series of the avermectins (115).

The spiroacetal portions of the avermectins have also received attention from a number of other groups. Studies by Danishefsky and colleagues[86] have resulted in the synthesis of a model for the spiroacetal moiety of avermectins of the 1 series (those possessing a C-22–C-23 double bond). Starting from (D)-glucal triacetate (116), these workers were able to obtain the alcohol (117) in ten steps and this underwent spirocyclization on treatment with mercuric oxide and iodine to give the model spiroacetal (118). This methodology has now been adapted to the total synthesis of the aglycon of avermectin $A1_a$ (*vide infra*) and thence to the total synthesis of avermectin $A1_a$ itself.

An enantiospecific synthesis, starting from laevoglucosan, of the spiroacetal portion of avermectin $B1_a$ has recently been described by White and colleagues.[87] This material was stripped of its unnecessary hydroxyl groups and converted into

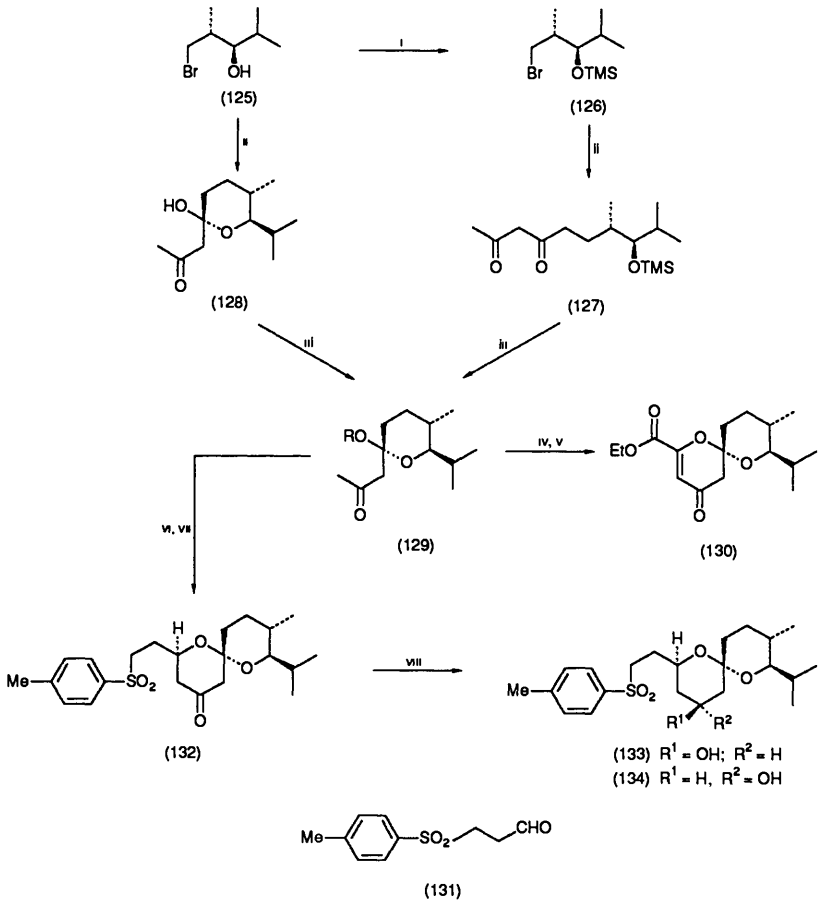
[86] F. E. Wincott, S. J. Danishefsky, and G. Shulte, *Tetrahedron Lett.*, 1987, **28**, 4951.
[87] C. M. J. Fox, R. N. Hiner, U. Warrier, and J. D. White, *Tetrahedron Lett.*, 1988, **29**, 2923.



Reagents: i, $LiAlH_4$, THF, 25 °C, 3 h; ii, $Me_2C(OMe)_2$, camphorsulphonic acid (cat.), CH_2Cl_2, 1 h; iii, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −60 °C; iv, Bu^nLi, THF, −78 °C then $CeCl_3$, 0.5 h, then (122); v, MnO_2, CH_2Cl_2, 2 h; vi, MeOH, camphorsulphonic acid (cat), 1 h; vii, TBAF, THF, 50 °C, 1 h; viii, H_2, $Pd/BaSo_4$, quinoline, MeOH, 0.5 h; ix, Et_2O, camphorsulphonic acid, 10 min

Scheme 11

the lactol (119) in four steps (Scheme 11). Reduction of this lactol produced a triol which could be selectively protected as an acetonide and the pendant primary alcohol oxidized under Swern conditions to give the aldehyde (120). Condensation of this aldehyde with the lithio-anion of (121) gave poor yields of the required product (122); however, by employment of the less basic alkynylcerium reagent these workers were able to obtain the requisite diastereomeric alcohol (122) in 67% yield. Oxidation of the alcohol followed by lactonization, desilylation, and semi-hydrogenation then produced the lactol (123). A final acid-catalysed cyclization gave the spiroacetal (124) as a single stereoisomer. This



Reagents i, TMSCl, HMDS, pyridine, pentane, 35 °C, ii, $CH_3COCH_2COCH_3$, LDA, HMPT, THF, −78 °C to 20 °C, iii, citric acid, MeOH or 2,2,2-triphenylethanol, iv, LDA, THF, −78 °C, $(CO_2Et)_2$ then −15 °C, v, 250 °C then distillation, vi, LDA, THF, −78 °C then (131), 1 h, vii, HCl, $CHCl_3$, viii, $LiAlH_4$, benzene, 20 °C or L-Selectride, THF, 0 °C

Scheme 12

synthesis has now been extended to provide a total synthesis of the aglycon of avermectin $B1_a$ (*vide infra*).

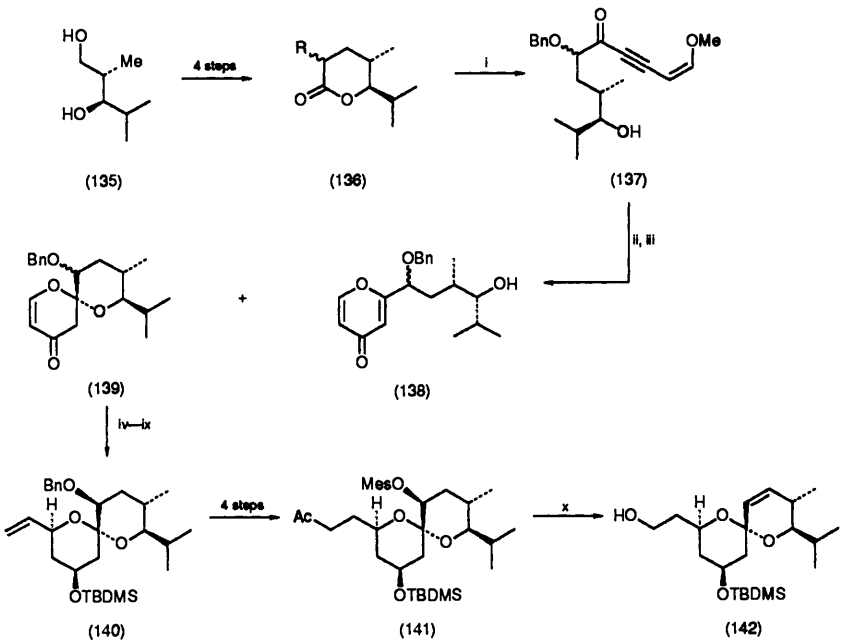
A diastereocontrolled synthesis of the spiroacetal subunit of 22,23-dihydroavermectin $B1_b$ has been reported by Julia and colleagues.[88] Reaction of the dianion of penta-2,4-dione with the bromoalcohol (125) resulted in the formation of the hemiketal (128) in 90% yield (Scheme 12). Similar treatment of

[88] J Ardisson, J P Ferezou, M Julia, L Lenglet, and A Pancrazi, *Tetrahedron Lett*, 1987, **28**, 1997

the silyl protected alcohol (126) furnished the diketone (127) and treatment of both (127) and (128) with citric acid in methanol yielded the methylketal (129; R = Me) in high yield. Acylation of this material with diethyl oxalate, followed by thermolysis and distillation, afforded the homochiral spiroacetal (130) in 54% yield. This material is similar to an intermediate in Barratt's milbemycin synthesis (*vide infra*) and the methodology for converting (130) into the corresponding hydroxyspiroacetal has been thoroughly investigated. In addition, it was shown that condensation of the kinetic enolate of (129; R = Me) with the aldehyde (131) gave a 55:45 mixture of diastereoisomeric alcohols. These were separated by flash chromatography and the minor, less polar isomer, subjected to acid catalysed cyclization when the crystalline spiroacetal (132) was obtained. Recently an improvement in the stereoselectivity of this intramolecular aldol reaction has been reported.[89] The hemiketal (128) was protected with a variety of alkyl groups of increasing bulk and treated with a selection of aldehydes. The diastereoselectivity of the process was found to be very variable in favour of either isomer with no obvious pattern emerging. The best results observed were when the hemiacetal (128) was protected as its triphenylethyl ether (129; R = Ph_3CCH_2–); the diastereoselectivity in this case being 72:28 in favour of the required isomer. This led to the formation of the spiroacetal (132) in a more favourable yield. Reduction of this ketone (132) with lithium aluminium hydride in benzene afforded an 80:20 mixture of the two epimers (133) and (134), which were separated by chromatography. In contrast, reduction of the ketone (132) with L-Selectride® exhibited opposite stereoselectivity and the two alcohols (133) and (134) were obtained as a 5:95 mixture.

The spiroacetal portion of avermectin $B1_b$ has been the subject of a study by Crimmins[90] in which the key steps involved ring opening of a suitably appended lactone followed by recyclization to a spiroacetal (Scheme 13). This was then routinely transformed into the spiroacetal portion of avermectin $B1_b$. The bis alcohol (135), readily available in two steps from trans-4-methyl-2-penten-1-ol, *via* a Sharpless epoxidation followed by epoxide opening using a dimethyl cuprate, was converted in four high yielding steps into the lactone (136; R = $PhCH_2O$–). Ring opening of this lactone proceeded smoothly to give the addition product (137) in high yield. Interestingly, very poor yields were observed when this ring opening was performed on the lactone (136; R = PhS–); this was thought to be due to significant deprotonation of the lactone, which led to incomplete reaction. Treatment of the addition product (137) gave a 1,3-bis addition of methanol to the ene–yne system, and the product, on exposure to acid, afforded a 1:1 mixture of (138) and (139). However, treatment of this mixture with trifluoroacetic acid, afforded a 1:1 mixture of diastereoisomers of (139) in high yield (82%). Addition of a two-carbon unit to the α,β-unsaturated ketone using vinylmagnesium bromide, followed by routine chemistry and chromatography, gave the suitably appended spiroacetal (140). Hydroboration,

[89] J. P Ferezou, J. Gauchet-Prunet, M. Julia, and A. Pancrazi, *Tetrahedron Lett.*, 1988, **29**, 3667
[90] M. T. Crimmins and R. O'Mahony, *Tetrahedron Lett.*, 1989, **30**, 5993.



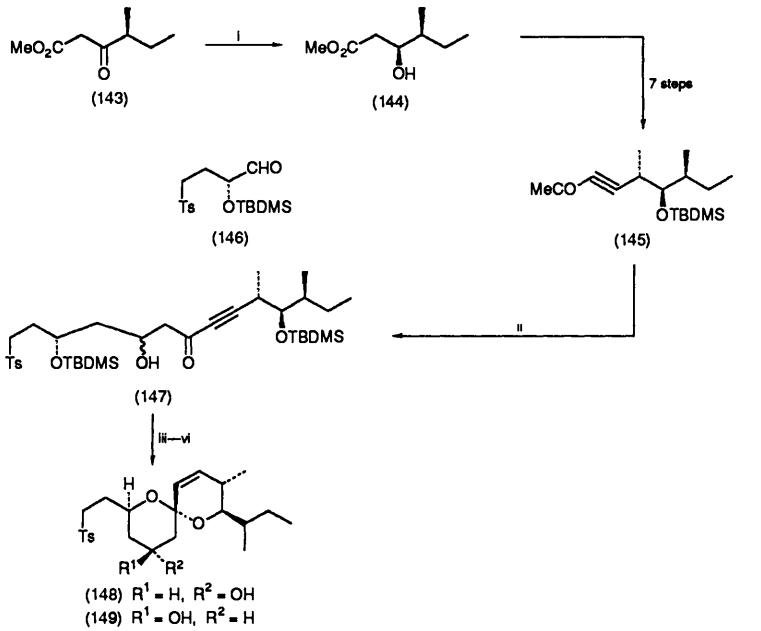
Reagents i, LiC≡C·CH=CH·OMe, THF, −78 °C, ii, K_2CO_3, MeOH, iii, ptsa, 4 1 THF H_2O, 65 °C, 12 h, iv, CH_2=CHMgBr, $[CuI(PBu_3)]_4$, THF, v, chromatography, vi, $NaBH_4$, DME, 0 °C, vii, chromatography, viii, Jones' reagent, acetone, ix, Bu^tMe_2SiOTf, lutidine, CH_2Cl_2, x, DBU, LiCl, moist DMSO, 150 °C, 2 h

Scheme 13

followed by protection, hydrogenation, and mesylation then gave the protected tri-alcohol (141), and conversion of this into the required spiroacetal (142) was accomplished by elimination of the axial mesylate using DBU in DMSO. It was noted, however, that if 'dry' DMSO was employed the primary acetate group was untouched and that wet DMSO was required for both elimination and deacetylation to occur. In addition, these workers have so far been unable to effect elimination of the equatorial alcohol derived from the other isomer of (139).

An enantiospecific synthesis of the spiroacetal moiety of avermectin $B1_a$ has been described[91] in which the chirality was introduced *via* a stereoselective bakers' yeast reduction of the β-ketoester (143) (Scheme 14). The major product (144), obtained in 99% enantiomeric excess, and shown to possess the (3*S*,4*S*) stereochemistry by comparison with an authentic sample, was transformed, using standard methodology, into the alkyne (145). Aldol condensation of this with the aldehyde (146) gave a 1:1 mixture of the epimeric alcohols (147). Stepwise

[91] M Hirama, T Nakamine, and S Ito, *Tetrahedron Lett*, 1986, **27**, 5281

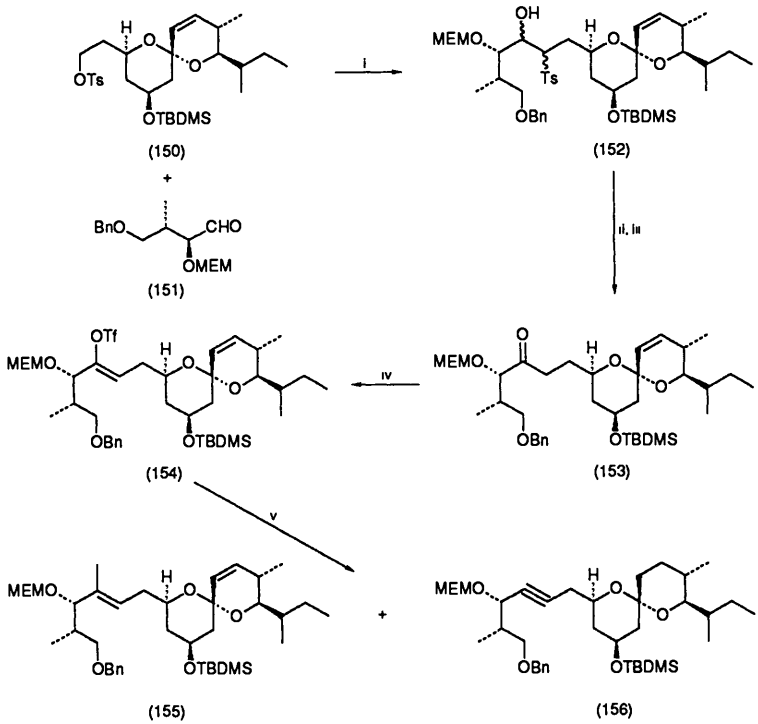


Reagents: i, Baker's yeast, glucose, 2 d; ii, LDA, THF, −78 °C, (146); iii, MeOH, $CH(OMe)_3$ (13:1), ptsa (cat), r.t., 5 h; iv, TBAF, THF, r.t., 12 h; v, $Pd/CaCO_3/Pb$, H_2, toluene; vi, camphor sulphonic acid, CH_2Cl_2

Scheme 14

removal of the silyl protecting groups followed by partial reduction and acid catalysed cyclization afforded the epimeric mixture of spiroacetals (148) and (149) in a 1:1.7 ratio. This ratio was improved to 1:4.1 by Collins oxidation of the mixture and subsequent reduction with lithium aluminium hydride. The isomers were readily separated by chromatography and the requisite spiroacetal (149) purified by crystallization. This work has recently been extended[92] to allow preparation of the C-11 to C-28 portions of avermectins $A1_a$ and $B1_a$ (155) (Scheme 15). The spiroacetal, protected as its t-butyl dimethylsilyl ether (150), was condensed with the aldehyde (151), utilizing an extension of the Julia–Kocienski procedure,[93] resulting in a quantitative yield of the alcohol (152) as a mixture of diastereoisomers. After conversion into the ketone (153), regio- and stereoselective enolization with lithium hexamethyldisilazide, followed by treatment with *N*-phenyltrifluoromethanesulphonimide, a mild triflating reagent, furnished the *Z*-enol triflate (154). Displacement of this triflate with methyl lithium/copper(I) cyanide finally afforded a 4:1 mixture of the required avermectin

[92] M. Hirama, T. Nakamine, and S. Ito, *Tetrahedron Lett.*, 1988, **29**, 1197.
[93] M. Julia and J.-M. Paris, *Tetrahedron Lett.*, 1973, 4833; P. J. Kocienski, B. Lythgoe, and S. Rushton, *J. Chem. Soc., Perkin Trans. 1*, 1978, 829.

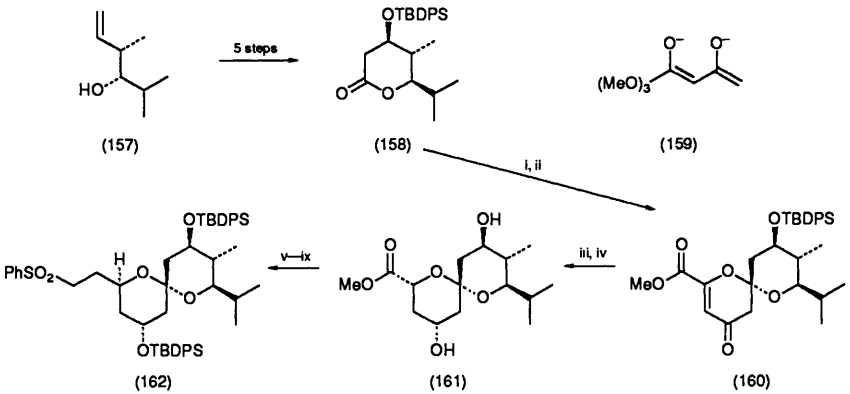


Reagents i, Bu^{n}Li, −78 °C, THF, ii, CrO_3·2py, iii, Al(Hg), 65 °C, THF, H_2O, iv, LiHMDS, $PhNTf_2$, THF, −78 °C, v, MeLi (30 eq), CuCN (20 eq), THF, −4 °C, 38 h

Scheme 15

precursor (155) and the alkyne (156). This work is an extremely valuable addition to the burgeoning field of avermectin chemistry, since many of the reported syntheses of milbemycin precursors could prove problematic if adapted to the synthesis of avermectins owing to the presence of the chiral C-13 oxygen functionality. To date, only four other total syntheses of similar C-11 to C-28 avermectin sub-units have been described,[94–102] and each of

[94] S Hanessian, A Ugolini, D Dube, P J Hodges, and C Andre, *J Am Chem Soc*, 1986, **108**, 2776
[95] S Hanessian, A Ugolini, D Dube, P J Hodges, C Andre, and P Beaulieu, *Pure Appl Chem*, 1987, **57**, 299
[96] S J Danishefsky, D M Armistead, F E Wincott, H G Selnick, and R Hungate, *J Am Chem Soc*, 1987, **109**, 8117
[97] S J Danishefsky, D M Armistead, F E Wincott, H G Selnick, and R Hungate, *J Am Chem Soc*, 1989, **111**, 2967
[98] J D White and G L Bolton, *J Am Chem Soc*, 1990, **112**, 1626
[99] A Armstrong and S V Ley, *SYNLETT*, 1990, **6**, 323
[100] D Diez-Martin, P Grice, H C Kolb, S V Ley, and A Madin, *SYNLETT*, 1990, **6**, 326
[101] A Armstrong, S V Ley, A Madin, and S Mukherjee, *SYNLETT*, 1990, **6**, 328
[102] M J Ford, J G Knight, S V Ley, and S Vile, *SYNLETT*, 1990, **6**, 331



Reagents: i, (159), THF, 0 °C; ii, AcOH, TsOH; iii, HF, pyridine, CH_2Cl_2, −23 °C; iv, H_2, Rh/Al_2O_3, EtOH, 25 °C; v, Bu^tPh_2SiCl, imidazole, DMAP, DMF, 25 °C; vi, LDA, THF, −78 °C, AcOH; vii, $LiAlH_4$, Et_2O, 25 °C; viii, $(PhO)_3P^+MeI^-$, DMF, 25 °C; ix, $PhSO_2CH_2Li$, THF, HMPT, 25 °C

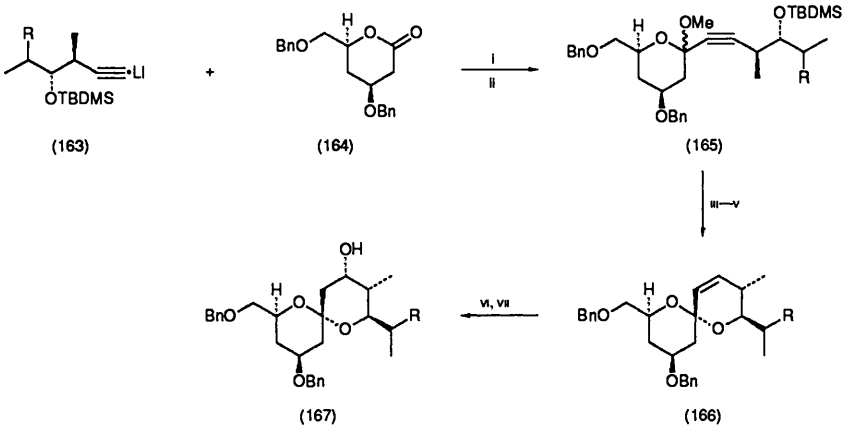
Scheme 16

these has been incorporated into a total synthesis of one of the avermectins (*vide infra*).

Employing methodology utilized in their synthesis of milbemycin β_3 (*vide infra*), Barrett and colleagues[103] have recently reported the enantiospecific synthesis of the spiroacetal fragment of avermectins $A2_b$ and $B2_b$. The homoallylic alcohol (157), readily available from isobutyraldehyde, was converted, in five steps and with high diastereoselectivity, into the lactone (158) (Scheme 16). Condensation of this with the β-dianion (159) at 0 °C, followed by acidification, gave the spirodihydropyrone (160) as a diastereoisomeric pair; this was as a consequence of the anomeric effect. Hydrogenation of the deprotected dihydropyrone over rhodium on alumina proceeded rapidly and with excellent C-4 diastereoselectivity to provide the diol (161). Resolution of this racemic diol by its bis-(*S*)-*O*-methyl mandelate ester gave the optically pure diol which was converted into the spiroacetal (162) as shown in the scheme. Conversion of the spiroacetal into the avermectins is reported to be under investigation.

Recently, Baker and colleagues[104,105] have described the synthesis of a series of spiroacetals corresponding to the requisite portions of all eight of the avermectins and two of the milbemycins (namely α_7 and α_8). The spiroacetal moieties of avermectins $A1_b$ and $B1_b$ and $A1_a$ and $B1_a$ were obtained (Scheme 17) by initial reaction of the appropriate chiral lithium acetylide (163; R = Me or Et) with the lactone (164), an important intermediate in Baker's milbemycin

[103] A. G. M. Barrett and T. M. Raynham, *Tetrahedron Lett.*, 1987, **28**, 5615.
[104] R. Baker, J. C. Head, and C. J. Swain, *J. Chem. Soc., Perkin Trans. 1*, 1988, 85.
[105] R. Baker, C. J. Swain, and J. C. Head, *J. Chem. Soc., Chem. Commun.*, 1986, 874.



Reagents I, THF, −60 °C, II, MeOH, H^+, III, TBAF, THF, r t, 8h, IV, MeOH, H_2, Lindlar's reagent, V, camphorsulphonic acid, ether, r t, 30 min, VI, Bu^{t}OCl, H_2O, acetone, VII, Bu_3SnH, AIBN, toluene

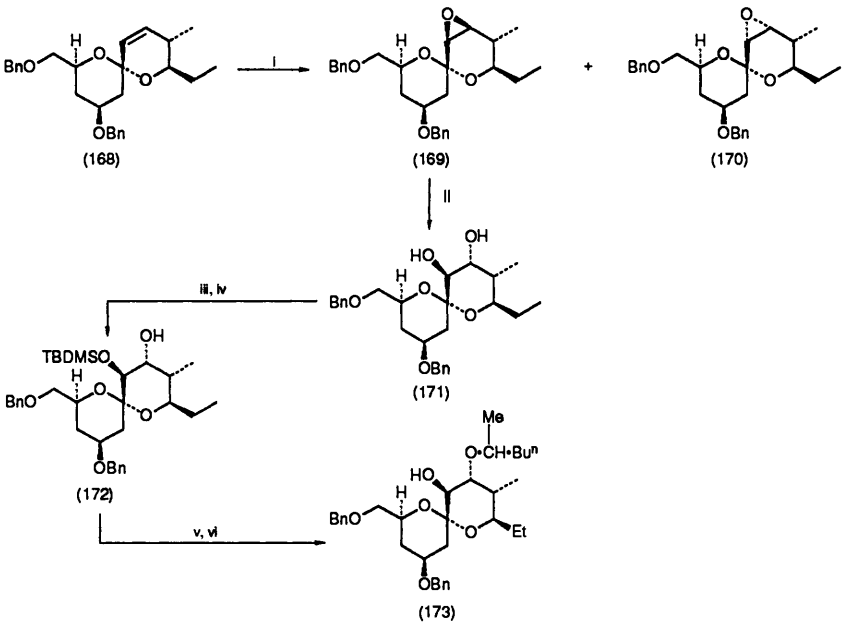
Scheme 17

synthesis (*vide infra*). The resulting mixture, on methanolysis, afforded the methoxyacetal (165) as a mixture of anomers. Deprotection, partial reduction and acid catalysed cyclization then gave the spiroacetal (166; R = Me or Et). Transformation of these spiroacetals into those of the 2a and 2b series of avermectins (167; R = Et or Me) was accomplished by chlorohydrin formation and dehydrochlorination with tributyltin hydride. Using similar methodology, the spiroacetal (168) was prepared using the appropriate lithium acetylide and this, on treatment with *m*-chloroperoxybenzoic acid, gave a mixture of two epoxides (169) and (170) in a 1.6:1 ratio (Scheme 18).[105] Chromatography allowed the obtention of the pure β-epoxide (169) in 56% yield, and this, on treatment with 5% aqueous perchloric acid, afforded the *trans*-diaxial diol (171). Diprotection of this diol followed by careful partial deprotection gave the alcohol (172), and this, on treatment with (*R*)-(−)-2-methylhexanoyl chloride followed by desilylation, gave the requisite spiroacetal moiety of milbemycins α_7 and α_8 (173).

Finally, workers in the Upjohn laboratories have claimed, in the patent literature, the syntheses of a variety of spiroacetals, useful as intermediates in the synthesis of avermectins and milbemycins.[106,107]

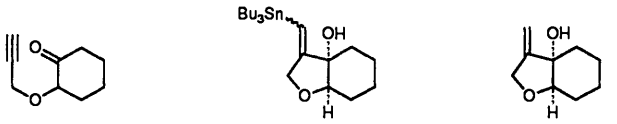
B. Oxahydrindene Portion.—Initially, the C-1 to C-8 portion of the avermectins and milbemycins received scant attention compared with the spiroacetal component, but the last four years have seen an upsurge of interest in this part of

[106] S J Nelson (Upjohn Co), US Patent, US 4 680 419 (1987)
[107] S J Nelson (Upjohn Co), US Patent, US 4 686 297 (1987)



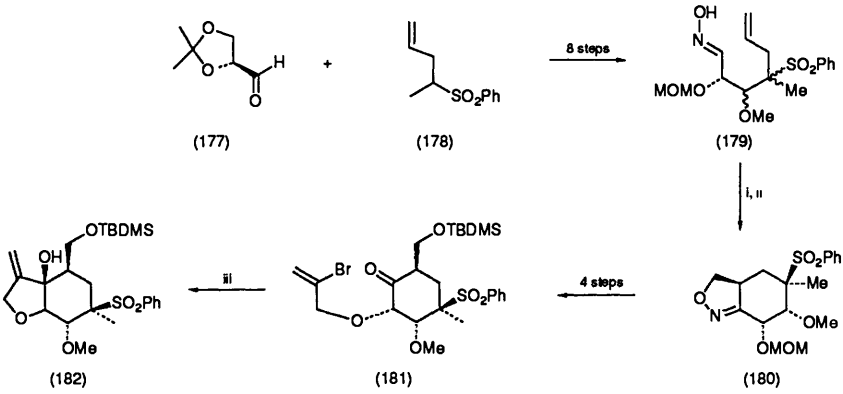
Reagents: i, mcpba, CH_2Cl_2, r.t., 30 h; ii, $HClO_4$, H_2O, THF, 55 °C 1 h; iii, Bu^tMe_2SiOTf, CH_2Cl_2, lutidine, 0 °C, 1 h; iv, camphorsulphonic acid, MeOH, r.t., 3 h; v, BuLi, THF, −80 °C then (*R*)-2-methyl hexanoylchloride, −60 °C to 0 °C, 1.25 h; vi, TBAF, THF, 2 h

Scheme 18



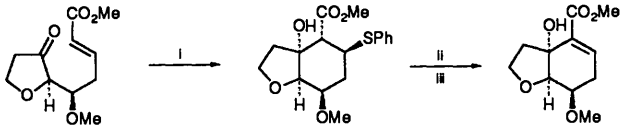
the molecule. In addition to a number of both chiral and achiral syntheses of the C-1 to C-8 portion of the macrolide ring, a few groups have described studies aimed towards the synthesis of model structures for this fragment. For example, Julia and his colleagues [108] have shown that octahydrobenzofurans, *e.g.* (176), are readily available *via* a free radical cyclization process. This involved the addition of tributyltin hydride to an alkyne, *e.g.* (174), followed by intramolecular reaction of the generated vinyl radical with the enolic double bond. By this method the stannyl derivative (175) was obtained in 30% yield. Acidic treatment then gave the octahydrobenzofuran (176) in 70% yield. Kozikowski [109] has reported the synthesis of a similar system but with a more complex substitution pattern.

[108] J. Ardison, J. P. Ferezou, M. Julia, and A. Pancrazi, *Tetrahedron Lett.*, 1987, **28**, 2001.
[109] A. P. Kozikowski and K. E. Maloney Huss, *Tetrahedron Lett.*, 1985, **26**, 5759.



Reagents i, NaOCl, Et_3N, ii, chromatography, iii, Bu^n_2CuLi, pentane, ether

Scheme 19

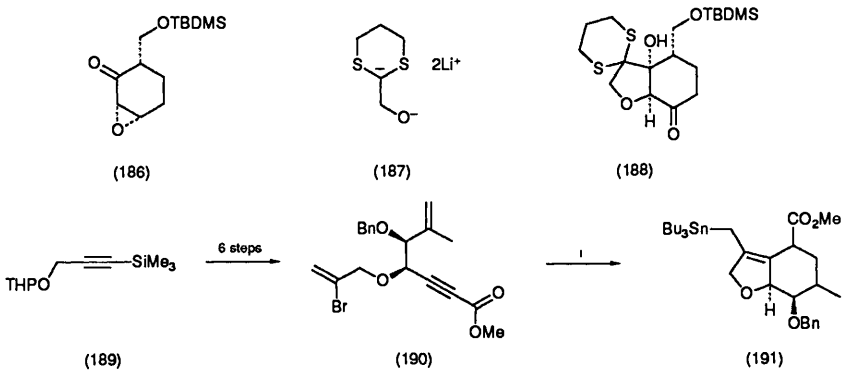


Reagents i, $Me_3AlSPhLi$, THF, 0 °C, ii, mcpba, iii, 110 °C

Scheme 20

Starting from the optically active glyceraldehyde acetonide (177), these workers were able to produce the phenylsulphonyl derivative (182) (Scheme 19). Whilst the (*S*)-isomer of the acetonide should be employed in order to obtain the avermectin fragment of correct absolute stereochemistry, this group utilised the (*R*)-isomer due to its greater accessibility. The aforementioned acetonide (177) was thus condensed with the anion of the sulphone (178) and the product transformed, in eight steps, into the oxime (179). An INOC cyclization, employing sodium hypochlorite, then provided a mixture of two major and two minor diastereoisomers from which the isoxazoline (180), required for elaboration, could be isolated in 37% yield. Replacement of the methoxymethyl group with a 2-bromo-2-propenyl group, followed by isoxazoline ring opening and alcohol protection, gave the ketone (181). On exposure of this to di-n-butylcopper lithium, cyclization occurred, affording the requisite octahydrobenzofuran derivative (182). Danishefsky and co-workers[110] have also described some studies which resulted in the formation of the model system (185) (Scheme 20). The key step involved the Michael–Aldol intramolecular condensation of the enoate-

[110] D M Armistead and S J Danishefsky, *Tetrahedron Lett*, 1987, **28**, 4959



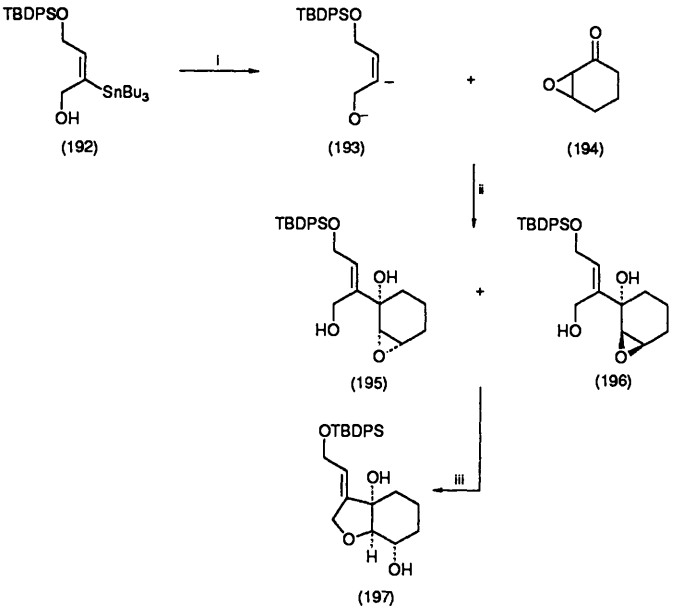
Reagents 1, Bu_3SnH, AIBN, C_6H_6, 80 °C

Scheme 21

ketone (183), available in ten high yielding steps from (*D*)-ribose. The resulting octahydrobenzofuran (184), isolated in 89% yield, was obtained as a single diastereoisomer with its three functionalities being equatorial to the cyclohexane ring. Oxidation and thermal elimination then provided the required model system (185). This elegant chemistry has recently been modified and incorporated into a total synthesis of avermectin $A1_a$ (*vide infra*).

Barrett and his colleagues in America[111] have also described a succinct method of acquiring the diastereoisomerically pure octahydrobenzofuran moiety of the avermectins, chirality being introduced in the first step of the synthesis by the enantioselective yeast reduction of commercially available ethyl-2-oxo cyclohexanecarboxylate. The key step of the synthesis involved the condensation of an appropriately functionalized cyclohexanone, *e.g.* (186), with the dilithio derivative of 2-hydroxymethyl-1,3-dithiane (187). The resulting alcohol was then transformed *via* intramolecular ring opening of the epoxide, followed by Swern oxidation into the octahydrobenzofuran analogue (188). Further work on the elaboration of this molecule is reported to be under investigation and will presumably be reported in due course. Recently Parsons and co-workers[112] have reported an alternative approach to this portion of the avermectins. Their method involved a tandem radical cyclization of the *erythro*-ether (190) (Scheme 21). This was constructed from the silylated acetylene (189) in six steps, employing standard methodology. Cyclization was then induced using tributyltin hydride, in the presence of a radical initiator, affording the allyl stannane (191) as a single isomer; the quality of the tin hydride was found to be crucial to the success of the reaction. These workers are hoping to be able to extend this chemistry to obtain a total synthesis of the avermectins which will not suffer from

[111] A. G. M. Barrett and N. K. Capps, *Tetrahedron Lett.*, 1986, **27**, 5571.
[112] P. J. Parsons, P. A. Willis, and S. C. Eyley, *J. Chem. Soc., Chem. Commun.*, 1988, 283



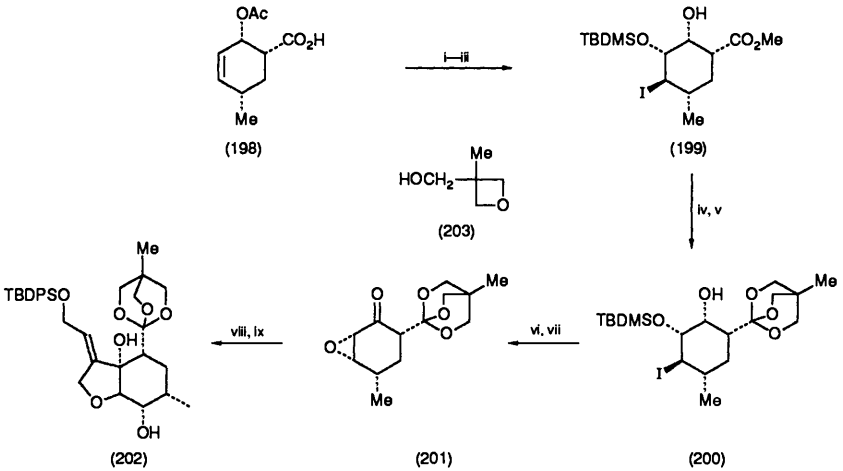
Reagents: i, BuLi, THF, −35 °C, 2 h; ii, THF; iii, TsOH(cat), CH_2Cl_2

Scheme 22

the problems of deconjugation which has beset other work in this area (*vide infra*) (see also Part II of this review).

A recent study by Barrett and colleagues,[113] culminating in the synthesis of a variety of substituted tetrahydrofurans, also demonstrated the availability of *cis*-fused octahydrobenzofurans. These workers were able to develop methodology by which diastereoisomeric control served to provide three contiguous asymmetric centres in addition to an exocyclic double bond suitable for further elaboration. The readily available tributylstannyl alcohol (192), on treatment with 2.1 equiv. of n-butyl-lithium at −35 °C, was converted into the dianion (193) (Scheme 22). The temperature required for the formation of this dianion was found to be crucial in that lower temperatures gave incomplete formation and higher temperatures led to appreciable decomposition. This dianion was shown to add to a variety of epoxy aldehydes and ketones, but in particular addition to the epoxy-cyclohexanone (194) led to the formation of a 2.2:1 mixture of the diastereoisomers (195) and (196) in 64% yield. Fortunately only one of these diastereoisomers possessed the necessary stereochemistry for cyclization, and thus treatment of the mixture with a catalytic amount of *p*-toluenesulphonic acid served to generate the required octahydrobenzofuran (197) along with the recovery of the unwanted diastereoisomer. Although this work

[113] A. G. M. Barrett, T. E. Barta, and J. A. Flygare, *J. Org. Chem.*, 1989, **54**, 4246.

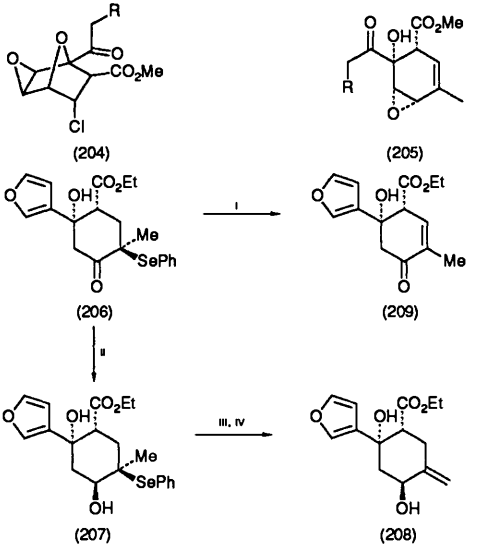


Reagents: i, NIS, THF, reflux, $NaHCO_3$; ii, MeOH, AcCl, reflux; iii, Bu^tMe_2SiCl, imidazole, DMF, DMAP; iv, $Ti(OPr^i)_4$, (203), 80 °C; v, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C; Et_3N, Et_2O, −78 °C; vi, TBAF, THF; vii, DMSO, TFAA, CH_2Cl_2, −78 °C; Et_3N, Pr^iOH; viii, BuLi, THF, −78 to −35 °C, (192), THF, −78 °C; ix, Bu^tOK, THF, r.t., 1 h

Scheme 23

represents a valuable addition to the field of avermectin chemistry, it nevertheless suffers from the inability to provide an octahydrobenzofuran with the required carboxylic acid functionality present at C-1 (avermectin numbering). However, further work in this area by the same workers has recently resulted in the resolution of this problem.[114] The carboxylic acid (198), readily available by Diels–Alder chemistry, was subjected to iodolactonization, and the resulting mixture of isomers treated with methanol under acidic conditions (Scheme 23). The mixture of methyl esters was separated by chromatography and the required major isomer protected as its t-butyldimethylsilyl ether (199). Transesterification of this material under titanium catalysis followed by boron trifluoride assisted rearrangement provided the orthoester (200), which on deprotection of the silyl ether underwent concomitant epoxide formation. Finally, exposure of the product to Swern oxidation afforded the ketone (201). It then only remained to transform this ketone into the required octahydrobenzofuran portion of the avermectins, and this was carried out using the conditions described in the earlier paper.[113] Thus, sequential treatment of the stannane (192) with n-butyl-lithium and the ketone (201) provided an adduct which on exposure to base provided the necessary fragment of the avermectins (202). A noteworthy feature of the synthesis is the fact that all intermediates were crystalline and, in addition, the synthesis serves not only to provide the Δ^8 olefin (avermectin numbering) with the

[114] A. G. M. Barrett, T. E. Barta, J. A. Flygare, M. Sabat, and C. D. Spilling, *J. Org. Chem.*, 1990, **55**, 2409.



Reagents I, H_2O_2, CH_2Cl_2, II, $NaBH(OAc)_3$, III, H_2O_2, CH_2Cl_2 then CCl_4, reflux

Scheme 24

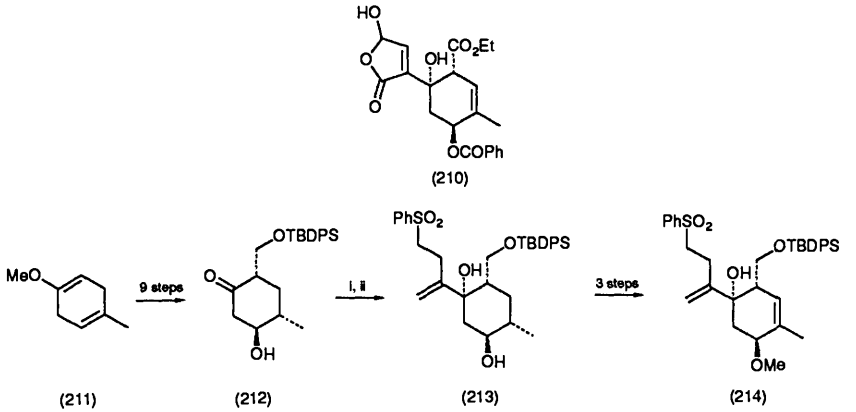
required *E*-geometry but also the C-1 carboxylic acid in the correct oxidation state.

Other approaches to the reduced benzofuran moiety by Jung[115] have involved the intramolecular cycloaddition of a suitably appended furan derivative, resulting in the formation of the epoxy ester (204; R = H). Reductive elimination of this then produced the epoxycyclohexene (205; R = H). Similarly, the compound (204; R = OCH_2Ph) could be easily prepared; the transformation of this material into the epoxide (205; R = OCH_2Ph), and thence into the required avermectin portion, is currently under investigation.

In addition to the reduced benzofuran portion of the avermectins, certain groups of collaborators have devoted their attention to the milbemycin cyclohexyl portion. For example, such a synthesis has been described by Thomas and colleagues[116] in which the key step involved a selenoxide elimination. However, contrary to expectations, a change in the regioselectivity of the reaction was observed (Scheme 24). When the reaction was performed on the hydroxy-selenoxide (207), mainly exocyclic elimination occurred, providing the cyclohexane derivative (208) as the major product (90%). However, when the reaction was carried out on the precursive ketone (206), endocyclic elimination predominated and the major product (85%) was the expected cyclohexenyl compound (209). This discrepancy was not fully investigated, but it was thought

[115] M E Jung and L J Street, *J Am Chem Soc*, 1984, **106**, 8327, M E Jung and L J Street, *Tetrahedron Lett*, 1985, **26**, 3639, M E Jung, L J Street, and Y Usui, *J Am Chem Soc*, 1986, **108**, 6810, M E Jung and L J Street, *Heterocycles*, 1988, **27**, 45

[116] S V Mortlock, N A Stacey, and E J Thomas, *J Chem Soc, Chem Commun*, 1987, 880



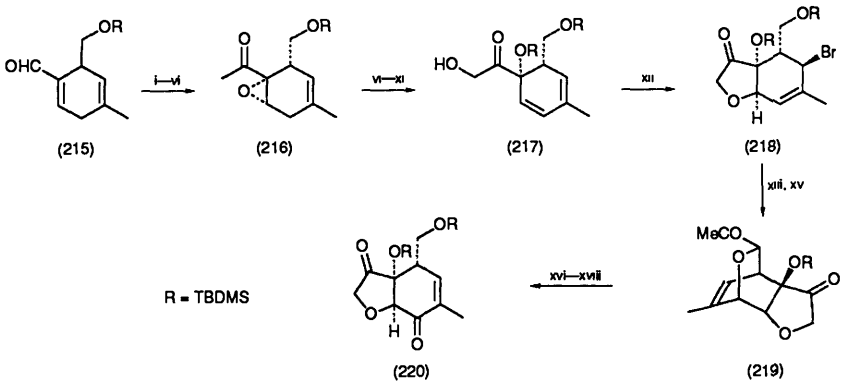
Reagents: i, 2.2 eq. $PhSCH_2CH_2C(Li)=CH_2$, Et_2O, THF, −78 °C, 2 h; ii, Oxone® THF, H_2O, r.t., 3 h

Scheme 25

that, in the intermediate transition state, the hydroxyl group interacted with the proton at C-2, causing the exocyclic elimination to prevail. In the corresponding keto derivative the interaction is much reduced and the more usual endocyclic elimination occurs. Employing similar methodology, these workers also prepared the hydroxybutenolide (210), and it is to be hoped that this can be incorporated into a total synthesis of a non-aromatic milbemycin utilizing similar procedures to those described by Thomas in his 1985 synthesis of a milbemycin analogue.[1,117]

The synthesis of the non-aromatic C-1 to C-10 fragment of the milbemycins and avermectins has also been the subject of a study by Ley and colleages,[118] who synthesized the racemic phenylsulphonyl derivative (214) depicted in Scheme 25. 2,5-Dihydro-4-methylanisole (211) was transformed in nine steps, including a resolution *via* a 1(*R*)-(−)-camphanoyl ester, into the optically pure alcohol (212). In spite of the ready availability of this optically active material, further transformations were carried out using racemic material in order to establish the methodology and to avoid wasting a valuable chiral synthon. Introduction of the C-7 substituent was then achieved by condensation with the lithio derivative of 4-phenylthiobut-1-ene followed by oxidation with Oxone®. Thus the dihydroxycyclohexane (213) was stereoselectively obtained in 80% yield. Whilst Julia coupling of this material with an appropriately appended spiroacetal would eventually lead to a macrolide system analogous to the avermectins and milbemycins, these workers, in the interests of completeness, also investigated the introduction of the requisite C-3–C-4 double bond (avermectin numbering). This they achieved by Swern oxidation of the secondary

[117] M. J. Hughes, E. J. Thomas, M. D. Turnbull, R. H. Jones, and R. Warner, *J. Chem. Soc., Chem. Commun.*, 1985, 755.
[118] N. J. Anthony, T. Clarke, A. B. Jones, and S. V. Ley, *Tetrahedron Lett.*, 1987, **28**, 5755.



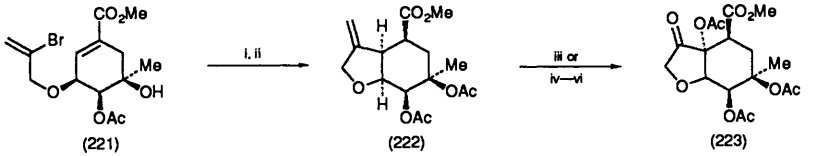
Reagents i, Dibal-H, ii, Bu^tOOH, $Ti(O\text{-}Pr^i)_4$, tartrate, iii, Swern's reagent, iv, MeLi, v, CrO_3·2pyr, vi, chromatography, vii, LDA, Bu^tMe_2SiCl, THF, viii, $LiNEt_2$, Et_2O, ix, LDA, TMSCl, x, mcpba, xi, K_2CO_3, MeOH, xii, NBS, CH_2Cl_2, xiii, HF, MeCN, xiv, PCC, xv, AgOAc, AcOH, xvi, $LiAlH_4$, xvii, Bu^tMe_2SiCl, xviii, Jones' reagent

Scheme 26

alcohol followed by a one-pot selenation and oxidative *syn*-elimination. Reduction of the C-5 ketone with sodium triacetoxyborohydride followed by methylation with diazomethane then gave the appropriately substituted cyclohexene (214) in 70% overall yield. The authors propose that this double bond introduction should be feasible at a later stage of a total synthesis should the need arise.

The hexahydrobenzofuran portion of milbemycins J and K has also been obtained in a seventeen stage synthesis beginning with a Diels–Alder reaction between t-butyldimethylsilyl protected 4-methyl-1,4-pentadienol and propiolaldehyde.[119] Reduction of the resulting aldehyde (215), followed by Sharpless asymmetric epoxidation, afforded a diastereoisomeric mixture of α-epoxides which was transformed, as shown in Scheme 26, into the methyl ketone (216). This material was obtained in 95% enantiomeric excess and smoothly converted into the α-hydroxy ketone (217) in 60% overall yield. Electrophilic ring closure was then brought about using *N*-bromosuccinimide, when the hexahydrobenzofuran (218) was obtained in 94% yield. Although a number of methods were investigated for this ring closure, only *N*-bromosuccinimide provided the required hexahydrobenzofuran in high yield. Selective removal of the protecting group on the primary alcohol followed by oxidation and silver acetate mediated cyclization resulted in the isolation of the acetal (219) in 70% yield. Routine manipulations then furnished the requisite milbemycin fragment (220). Although this molecule is a specific fragment of both milbemycins J and K it would be possible, by employing known chemistry, to synthesize fragments of other milbemycins and avermectins from it.

[119] M T Crimmins and J G Lever, *Tetrahedron Lett.*, 1986, **27**, 291



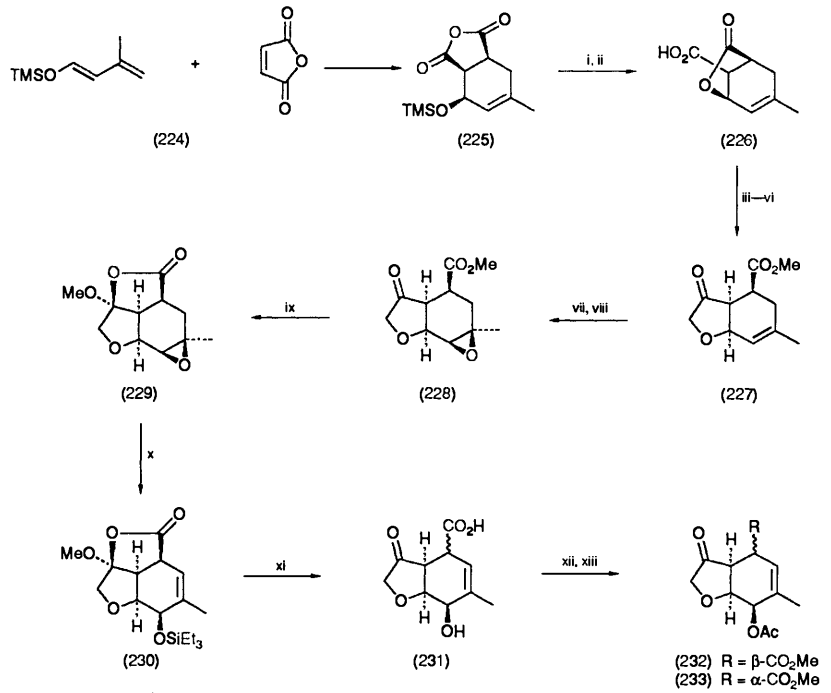
(221) (222) (223)

Reagents: i, Ac_2O, pyridine; ii, Ph_3SnH, AIBN; iii, $Pb(OAc)_4$, AcOH; iv, O_3; v, Me_2S; vi, $Pb(OAc)_4$, AcOH

Scheme 27

The oxahydrindene portion of the avermectins has also generated a vast amount of interest and a number of syntheses of this portion of the macrolide system have appeared in the literature. For example, Hanessian and colleagues[120] have described the synthesis of the racemic derivative (223). This was constructed using two critically important bond-forming reactions. Diels–Alder condensation of a 2-acetoxyfuran with maleic anhydride followed by routine manipulation of the product gave, after eight steps, the suitably appended cyclohexene ester (221) (Scheme 27). Acetoxylation, followed by treatment with triphenyltin hydride in the presence of a radical initiator caused an intramolecular Michael cyclization to occur, resulting in formation of the octahydrobenzofuran (222) in 65% yield. High field ^1H NMR spectroscopy and decoupling experiments established the structure unambiguously. The second step, which was critical to the successful accomplishment of the synthesis, was the introduction of an oxygen substituent at C-7. Whilst this was conveniently carried out in 75% yield by ozonolysis of the double bond followed by lead tetra-acetate treatment, it was surprisingly found that simple treatment of the olefin (222) with lead tetra-acetate led to the same product (223) in 30% yield. The authors presumed this to occur *via* cleavage of a transient diol, followed by α-acetoxylation of the resulting ketone. It was also demonstrated that one of the early intermediates in the synthesis was readily available in optically active form from commercially available (−)-quinic acid, thus rendering the avermectin moiety (223) also available in optically pure form. The Diels–Alder reaction was also the method of choice of White and colleagues[121] in their synthesis of the hexahydrobenzofuran nucleus of the avermectins and milbemycins. This work has recently been successfully extended (*vide infra*) to provide the third total synthesis of an avermectin. Condensation of the butadiene (224) with maleic anhydride gave the single *endo*-adduct (225) (Scheme 28). On desilylation and treatment with sodium hydride, the pendant hydroxyl group cleaved the anhydride ring and produced the lactone acid (226) in high yield. This material was resolved *via* its (*R*)-(+)-α-methylbenzylamine salt and the absolute configuration determined by *X*-ray analysis. Conversion of this acid to the diazoketone followed by acid hydrolysis and esterification provided the methyl ester (227), which on epoxidation under standard conditions provided

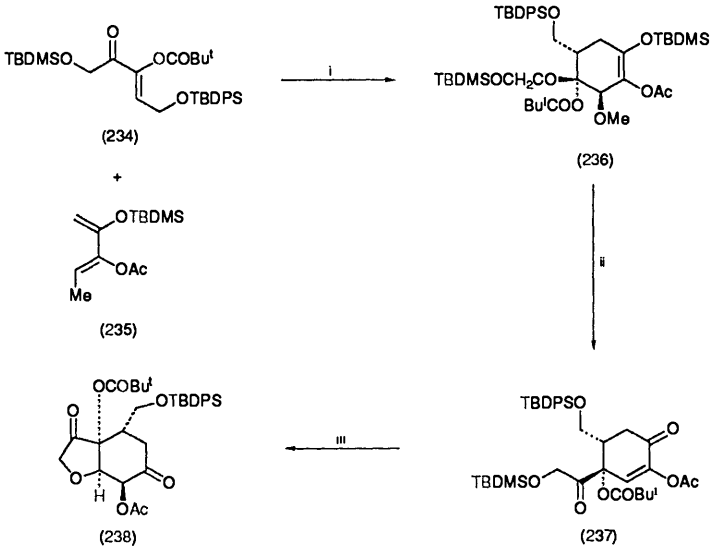
[120] S. Hanessian, P. Beaulieu, and D. Dube, *Tetrahedron Lett.*, 1986, **27**, 5071.
[121] J. D. White and A. P. Dantanarayana, *Tetrahedron Lett.*, 1987, **28**, 6417.



Reagents: i, $Bu_4^nN^+F^-$, THF, 0 C, 0.5 h; ii, NaH, C_6H_6; iii, $(COCl)_2$, 0 C to r.t., 1 h; iv, CH_2N_2, 0 C, 0.5 h; v, 10% H_2SO_4, dioxane, 50 °C, 0.5 h; vi, CH_2N_2, Et_2O, 0.5 h; vii, NBS, acetone, H_2O, 0 C, 1 h; viii, DBU, CH_2Cl_2, 10 min; ix, NaOMe, MeOH, 0 °C to r.t., 1.5 h; x, Et_3SiOTf, 2,6-lutidine, CH_2Cl_2, 2.5 h; xi, 3N-HCl, acetone, Δ, 5 h; xii, CH_2N_2, Et_2O, 1 h; xiii, Ac_2O, pyridine, DMAP, CH_2Cl_2, 6 h

Scheme 28

(228) in high yield. Whilst two methods were investigated for conversion of this epoxide into the required avermectin fragment, only one of these allowed for isolation of the pure fragment; the alternative method gave a 1:1 mixture of the required fragment and its exocyclic double bond isomer. Thus the favoured method involved treatment of the epoxide (228) with sodium methoxide, when the tricyclic lactone (229) was obtained in high yield. On exposure of this material to triethylsilyl triflate, a single allylic ether (230) was produced. Although acid hydrolysis of this ether then produced the α,β-unsaturated ester (231), it was unfortunately obtained as a mixture of diastereoisomers due to epimerization of the carboxyl substituent. Interestingly, basic hydrolysis of the lactone ring caused migration of the double bond into conjugation with the carboxyl group. Esterification of (231) followed by acetylation then provided a separable mixture of the α- and β-esters (233) and (232) in a 4:1 ratio. Although this compound serves as an excellent model system for the hexahydrobenzofuran portion of the avermectins it suffers from the disadvantage of not possessing the requisite 7-α-hydroxy substituent. Nevertheless, when this chemistry was adapted



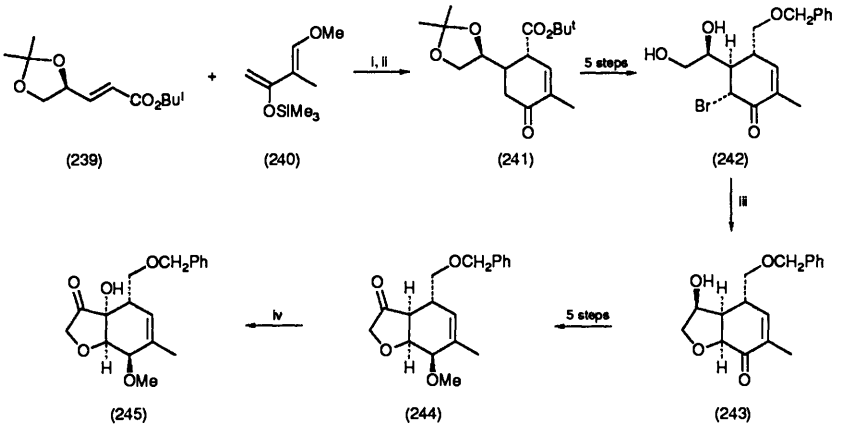
Reagents: i, benzene, 110 C; ii, BF_3-Et_2O, CH_2Cl_2, −78 C to 0 C; iii, THF: 1N-HCl (5: 1)

Scheme 29

to obtain a total synthesis of avermectin B_{1a} aglycon these workers were able to ingeniously correct this omission (*vide infra*).

An alternative approach to avermectin benzofuran moieties utilizing a Diels–Alder reaction has been the subject of a study by Ireland.[122] He was able to demonstrate that a highly regiospecific Diels–Alder reaction between dienophiles and dienes gave rise to highly functionalized t-α-hydroxy ketones. This study resulted in the development of a synthetic approach to the oxahydrindene portion of the avermectins and some of the milbemycins (Scheme 29). The Diels–Alder reaction between (234) and (235) was carried out in benzene in a sealed tube over two days, and the cyclohexane (236) was isolated, after chromatography, in a yield of 74%. Simple treatment of this product with boron trifluoride etherate effected removal of the enolate t-butyldimethylsilyl group, resulting in clean formation of the enone (237). This system was not amenable to deprotection with tetra-n-butylammonium fluoride since this induced aromatization of the molecule. However, on treatment with 1N-hydrochloric acid, the more labile t-butyldimethylsilyl group was selectively cleaved and the resulting free hydroxyl group cyclized intramolecularly on the enone portion of the molecule resulting in the formation of the avermectin synthon (238). Whilst this was not converted into the required avermectin oxahydrindene, it was shown that tosylhydrazone formation was possible and that conversion of

[122] R. E. Ireland and D M. Obrecht, *Helv. Chim. Acta*, 1986, **69**, 1273.



Reagents i, 115 °C, 26 h, ii, BF_3–Et_2O, −78 °C, 2 h, iii, 2,6-lutidine, $CHCl_3$, iv, LDA, −78 °C to −20 °C, cool to −70 °C then phenyl-*N*-phenylsulphonyloxaziridine

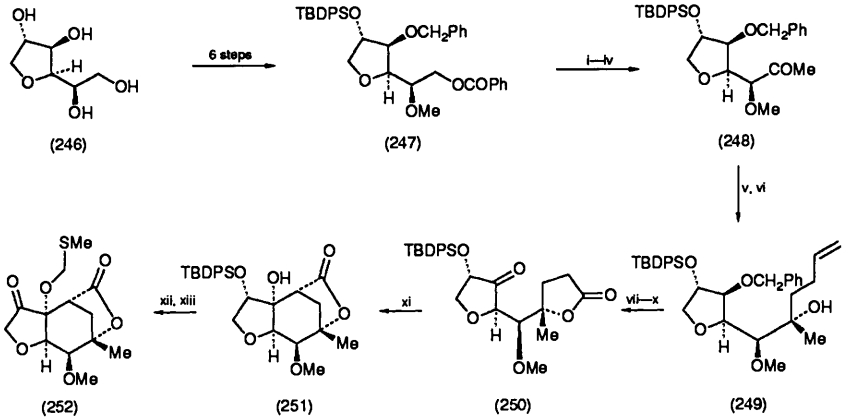
Scheme 30

this into the required C-4 methyl derivative could be achieved using known methodology.

An enantioselective synthesis of the hexahydrobenzofuran portion, also employing Diels–Alder chemistry, has recently been reported by workers in China.[123] The optically active α,β-unsaturated ester (239), prepared using a literature procedure, was allowed to react at high temperature with the diene (240) (Scheme 30). After treatment with a Lewis acid, a mixture of cyclohexenones was obtained, the major component being the diastereoisomer (241). Although separation by HPLC was possible at this stage, the mixture was carried through the synthesis until the required diastereoisomer could be purified by column chromatography. Transposition of the ester moiety, using standard methodology, into a primary alcohol, followed by bromination in the α-position to the ketone and isopropylidene ring opening, gave the diol (242). It was at this stage that chromatographic separation was performed and the major isomer transformed into the hexahydrobenzofuran (243) on treatment with 2,6-lutidine. The *trans* relationship between the protons at C-2 and C-7 were confirmed by NOE experiments; chemical oxidation of the secondary alcohol, to give a pair of enantiomers, confirmed the clean regiospecificity of the Diels–Alder process. Functional group manipulations of the hexahydrobenzofuran (243) gave the synthon (244), and introduction of the desired 7-hydroxyl group using Davis' reagent completed the synthesis of the required avermectin fragment (245).

An additional synthesis of the hexahydrobenzofuranone fragment in optically pure form has recently been described by Williams and colleagues.[124] Starting

[123] K -C Lee, J C C Wu, K -F Yen, and B -J Uang, *Tetrahedron Lett*, 1990, **25**, 3563
[124] D R Williams, F D Klinger, and U Dabral, *Tetrahedron Lett*, 1988 **29**, 3415



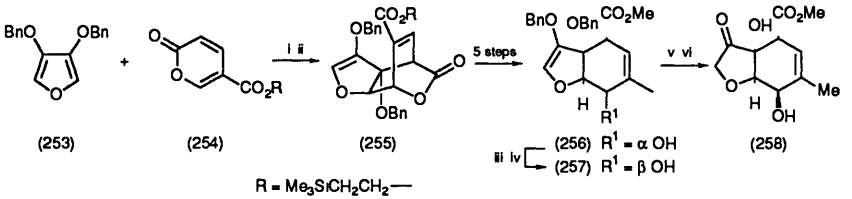
Reagents: i, LiOH, H_2O, THF; ii, PCC, Al_2O_3; iii, MeMgCl, THF, −78 °C; iv, PCC, Al_2O_3; v, 3-butenyl magnesium bromide, THF, −78 °C; vi, chromatography; vii, O_3, CH_2Cl_2, pyridine then Ph_3P; viii, PCC, Al_2O_3; ix, H_2, Pd-black, EtOH; x, PCC, Al_2O_3, CH_2Cl_2, 40 °C; xi, LDA, THF, −78 °C; xii, TBAF, THF, −30 °C; xiii, $(COCl)_2$, DMSO, −78 °C then Et_3N, −78 °C to 0 °C

Scheme 31

from 1,4-anhydrosorbitol (246), these workers were able to differentiate between each of the hydroxyl groups and prepare the fully protected derivative (247) in 51% overall yield (Scheme 31). This material was routinely converted into the methyl ketone (248), which on treatment with 3-butenylmagnesium bromide resulted in the formation of a 3:1 mixture of tertiary alcohol diastereoisomers. These were readily separated by chromatography, allowing the isolation of the major isomer (249) in 70% yield. Ozonolysis, followed by oxidation of the intermediate lactol, gave a lactone which on hydrogenolysis and oxidation furnished the keto-ester (250). Inverse addition of a tetrahydrofuran solution of lithium di-isopropylamide to this keto-ester initiated the crucial intramolecular Claisen condensation which culminated in the formation of the *cis*-fused oxahydrindane (251) in high yield. This was obtained as a single isomer through a highly regioselective and stereocontrolled reaction and the structure substantiated through an *X*-ray study of its derived C-8-monobenzoate. Conversion into the required ketone (252) was then accomplished by desilylation and Swern oxidation. It was also demonstrated that this molecule (252) underwent a Horner–Emmons reaction with trimethyl phosphonoacetate to give selective formation of an *E*-α,β-unsaturated ester. This would be an extremely useful intermediate for the construction of the diene system of the macrocyclic ring should the need arise.

Recently, Jung and his co-workers were one of the first groups to describe the synthesis of an avermectin oxahydrindene portion with all its functionalities in the correct oxidation state.[125] The key step of this synthesis was the thermally

[125] M. E. Jung, Y. Usui, and C. T. Wu, *Tetrahedron Lett.*, 1987, **28**, 5977.



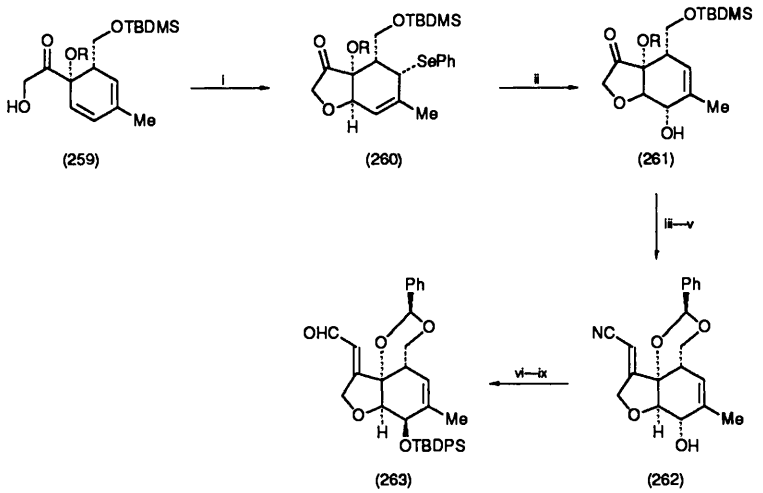
Reagents i MeOH Δ 3h ii chromatography iii DMSO TFAA Et_3N CH_2Cl_2 −65 C iv $NaBH_4$ $CeCl_3$ MeOH 0 C v 1 4 cyclohexadiene Pd C EtOH 25 C 2h vi 18% HCO_2H MeOH 10% Pd C −25 C 3h

Scheme 32

induced cycloaddition reaction between 3,4-bis(benzyloxy)furan (253) and 2-(trimethylsilyl)ethyl coumalate (254), as depicted in Scheme 32 This reaction provided a readily separable mixture of cycloadducts in an approximately 1 1 ratio Although, potentially, both could be converted into the required avermectin synthon, only the *endo*-adduct (255) was progressed Conversion of this into the tetrahydrobenzofuran (256) proceeded without event in five steps in an overall yield of *ca* 40% and epimerization of the secondary alcohol group was readily achieved by an oxidation and reduction sequence The resulting 5-β-hydroxy compound (257) was subjected to two catalytic transfer hydrogenations to give the requisite oxahydrindene portion (258) in approximately 60% yield The first step of this two-stage reduction process served to remove the benzyl group at the more accessible C-8 site, generating the required reduced furan-3-one, whilst more forcing conditions were required for the more sterically hindered benzyl group at C-7 Concurrently, a report by Crimmins also described a synthesis of the oxahydrindene portion in which all the functionalities were similarly in the correct oxidation state [126] The α-hydroxy ketone (259), an intermediate in Crimmins' earlier synthesis [119] of the lower portion of the avermectins and milbemycins, was treated with phenylselenyl chloride, which initiated an electrophilic intramolecular cyclization resulting in the formation of the hexahydrobenzofuran (260) (Scheme 33) Direct treatment of this material with hydrogen peroxide instigated a [2,3] sigmatropic rearrangement resulting in the formation of the 5-α-hydroxy compound (261) Olefination with cyanomethylenetriphenylphosphorane followed by protecting group adjustment provided the β-benzylidene compound (262) It is noteworthy that if benzylidene formation precedes Wittig coupling then the α-benzylidene compound predominates Epimerization of the secondary alcohol, utilizing an oxidation–reduction sequence, followed by reduction of the nitrile with di-isobutylaluminium hydride, then produced the avermectin precursor (263)

Recently a stereocontrolled synthesis of the hexahydrobenzofuran fragment has been reported in which the system was constructed using two successive,

[126] M T Crimmins W G Hollis Jr and J G Lever *Tetrahedron Lett* 1987 **28** 3647



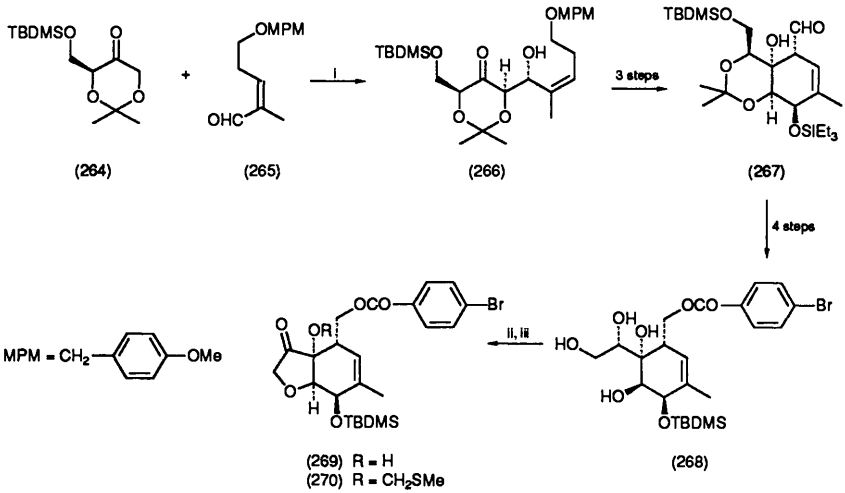
Reagents: i, PhSeCl, CH_2Cl_2, 0 °C, 15 min; ii, H_2O_2, CH_2Cl_2, pyr, 0 °C, 15 min; iii, $Ph_3P{=}CHCN$, toluene, 110 °C, 12 h; iv, 1% HF, MeCN, v, $PhCH(OMe)_2$, CH_2Cl_2, *p*-TsOH; vi, Jones' reagent, acetone, 0 °C, 5 min; vii, $NaBH_4$, MeOH, 10 min; viii, TBDPSCl, DMF, imidazole, DMAP; ix, Dibal-H, toluene, −78 °C, 3 h

Scheme 33

highly stereoselective, aldol reactions.[127] The first involved treatment of the freshly prepared acetonide (264) (Scheme 34) with lithium di-isopropylamide and quenching of the resulting anion with the aldehyde (265). This kinetic aldol reaction proceeded with good stereoselectivity, enabling the major alcohol (266) to be obtained in *ca.* 50% yield. Although the other diastereoisomer was obtained in this reaction, it was only a minor product and could be easily separated. Routine protecting group manipulation followed by Swern oxidation gave an intermediate which underwent a smooth intramolecular aldol reaction that proved to be a clean and highly selective process. The resulting aldehyde (267), after reduction and further protecting group manipulations, gave the tetrahydroxy compound (268). Sulphonation with excess 2,4,6-tri-isopropylbenzene sulphonyl chloride directly produced a hexahydrobenzofuran diol in 70% yield and, by simply subjecting this to Swern oxidation, the required avermectin fragment (269) was obtained in 45% yield. In addition, the methylthiomethyl ether (270) was isolated from this reaction in 30% yield.

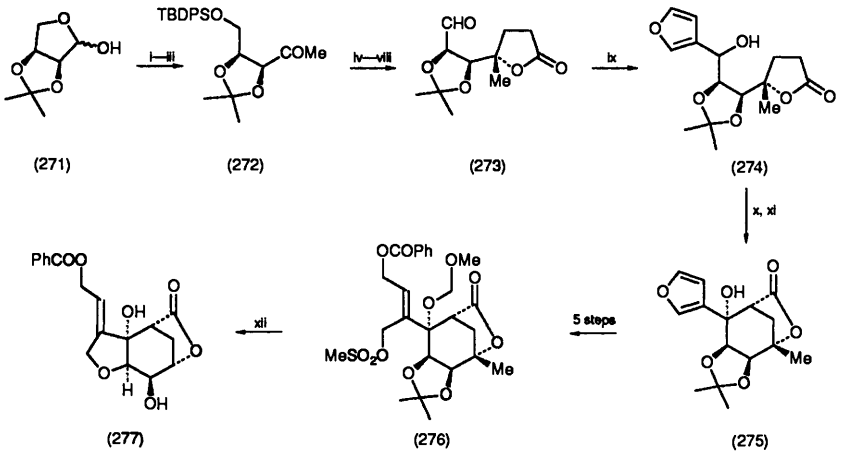
Finally, Williams and Klinger[128] have described their approaches to the hexahydrobenzofuran fragment, in which they sought to prevent epimerization at C-2 (*vide infra*) by tying up the ester group at C-2 as a bicyclic lactone. The erythrose (271), readily available in three steps from (*L*)-rhamnose, was treated

[127] (*a*) M. Hirama, T. Noda, S. Ito, and C. Kabuto, *J. Org. Chem.*, 1988, **52**, 706; (*b*) M. Hirama, T. Noda, S. Ito, and T. Nakamine, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, 1986, **28th**, 582.
[128] D. R. Williams and F. D. Klinger, *J. Org. Chem.*, 1988, **53**, 2134.



Reagents i, LDA, −78 °C, ii, 2,4,6-$Pr^i_3C_6H_2SO_2Cl$, pyr, 22 °C, 23 h, iii, $(COCl_2)$, DMSO, Et_3N, −60 °C, 20 min

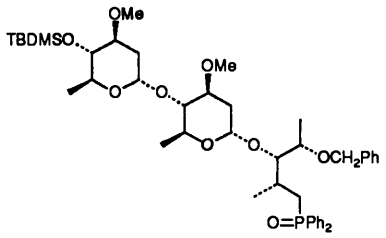
Scheme 34



Reagents i, MeMgCl, THF, −78 °C, ii, Bu^tPh_2SiCl, DMF, imidazole, iii, PCC, Al_2O_3, CH_2Cl_2, iv, $H_2C{=}CHCH_2CH_2MgBr$, THF, −78 °C, v, O_3, CH_2Cl_2 then Ph_3P, vi, PCC, Al_2O_3, CH_2Cl_2, vii, TBAF, THF, viii, PCC, Al_2O_3, CH_2Cl_2, NaOAc, ix, 3-bromofuran, Bu^sLi, Et_2O, −78 °C then $MgBr_2{\cdot}Et_2O$, x, $(COCl)_2$, DMSO, −78 °C then Et_3N, xi, THF, −78 °C then LDA, xii, THF–H_2O (1 1), Dowex 50W-X8, 70 °C

Scheme 35

with methylmagnesium chloride. The resulting diol, produced as a consequence of a highly stereoselective chelation-controlled addition, was selectively protected and then oxidized to give the ketone (272) (Scheme 35). Nucleophilic addition of



(278)

3-butenylmagnesium bromide gave the expected tertiary alcohol, and this, on ozonolysis, produced a lactol which could be readily converted into the aldehyde (273). This aldehyde proved to be very unstable and was thus utilized immediately in the next stage of the synthesis. Treatment with 2-bromofuran in the presence of s-butyl-lithium gave the secondary alcohol (274) as a 75:25 separable mixture of diastereoisomers. Swern oxidation of this alcohol mixture afforded a ketone which, on treatment with lithium di-isopropylamide, underwent an intramolecular Claisen condensation, resulting in the formation of the bicyclic lactone (275) in *ca.* 70% yield. This was produced as a 4:1 mixture with its C-7 diastereoisomer, but separation could be readily achieved by chromatography. Protection of the tertiary alcohol followed by oxidative cleavage of the furan ring gave a diol which was selectively protected to afford the mesylate (276). Treatment with an acidic ion-exchange resin then cleaved the acetonide, resulting in cyclization to give an excellent yield of the desired tricyclic derivative (277). The stereochemical assignments were confirmed by *X*-ray diffraction studies of the bis(methoxymethyl) ether of (277).

C. Miscellaneous.—With regard to the synthesis of derivatives of milbemycin β_3, two groups have described their approaches to the synthesis of the aromatic portion of this molecule. For example, Smith[129] has reported that dianions derived from 1,3-diketones react with 2-ethyl-3-bromopropenoate to afford unsaturated diketoesters which undergo facile conversion into a variety of *p*-hydroxybenzoates. In addition, Kotnis[130] has demonstrated that readily available Hagemanns esters are easily converted into *p*-methoxybenzoates on methanolic iodination.

Finally, a report by Barrett[131] has described the synthesis of the α-L-oleandrosyl-α-L-oleandroside derivative (278) which is potentially of use in the development of a new synthesis of an avermectin.

[129] A. B. Smith and S. N. Kilenyi, *Tetrahedron Lett.*, 1985, **26**, 4419.
[130] A. S. Kotnis, *Tetrahedron Lett.*, 1990, **31**, 481.
[131] A. G. M. Barrett and T. A. Miller, *Tetrahedron Lett.*, 1988, **29**, 1873.