Avermectin Acyl Derivatives with Anthelmintic Activity

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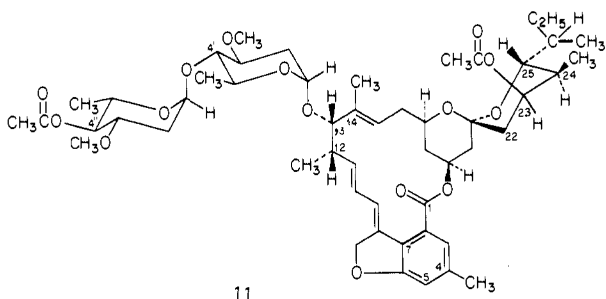
Avermectins A_{2a}, B_{1a}, and B_{2a} (1, 2, and 3) were acetylated to give 4″- and 23-acetates 4 and 5 and 4″,23-diacetate 6 from 1, the 4″- and 5-acetates 7 and 8 and 4″,5-diacetate 9 from 2, and triacetate 10 from 3. Structure proof by 300-MHz ^{1}H NMR and mass spectral fragmentation is discussed for 10. Forcing acetylation conditions generated from both 1 and 3 the identical aromatic diacetate 11. Good anthelmintic activities in gerbils and sheep for 4″-acetylated derivatives 4 and especially 7 prompted the preparation of additional 4″-acylated derivatives of 2 with pivaloyl, *n*-octanoyl, succinoyl, carbamoyl, dimethylcarbamoyl and *N*-acetylglycyl substituents, prepared from the 5-*O*-*tert*-butyldimethylsilyl-protected intermediate 12. Other key intermediates were the trichloroethoxysuccinoyl derivative 18 and 4-nitrophenyl carbonate 21. Anthelmintic activities against *Trichostrongylus colubriformis* in gerbils comparable in potency to the natural product 2 are shown by the more polar substituted derivatives 20, 23, and 27. Substitution of the 5-hydroxy group or its loss due to aromatization results in drastically reduced anthelmintic potency.

The avermectins are a group of closely related 16-membered macrocyclic lactones[1] which were recently obtained from the fermentation broth of *Streptomyces avermitilis*.[2,3] They are distinguished by extraordinarily potent anthelmintic[4] and insecticidal[5] activities, and a synthetically modified derivative, the 22,23-dihydroavermectin B_1, with the generic name ivermectin[6] has been selected as an antiparasitic drug for animals. The three major components of the fermentation broth, avermectin A_{2a}, B_{1a}, and B_{2a} (1, 2, and 3), differ by substitutions at the 5-position with a methoxy (1) vs. hydroxy group (2 and 3) and by a 23-hydroxy group (1 and 3) vs. the dehydration product (2) with a 22,23-double bond.[1] We were interested in the effect of selective acylation of these polyhydroxy compounds on their anthelmintic activity against the sheep parasite *Trichostrongylus colubriformis* in a model assay using gerbils (*Meriones unguiculatus*) as host animals.[7] Certain promising analogues were further evaluated against gastrointestinal nematode infections of sheep.[8]

Chemistry. Avermectin A_{2a} (1) contains a tertiary hydroxy group at C-7 and two secondary ones at C-4″ and C-23, of which the latter is axial and further hindered by the axial ether ring bond at C-21. Mild acetylation with acetic anhydride and pyridine at 0 °C for 5 h gave the expected 4″-*O*-acetyl derivative 4. The 4″,23-di-*O*-acetyl derivative 6 was obtained after heating the reaction mixture for 2 h at 100 °C. Careful base hydrolysis of this diacetate leaves the more hindered acetyl group intact and gives 23-*O*-acetylavermectin A_{2a} (5). Avermectin B_{2a} (3), containing an additional hydroxy group at C-5, gives the 4″,5,23-tri-*O*-acetyl derivative 10 upon acetylation at 100 °C for 2 h. Extending the reaction time to 24 h at 100 °C gave a product separated into 30% of above triacetate 10 and 30% of a new diacetate 11b, which has a benzene ring



11

comprising C-2 through C-7.[9] The identical aromatic diacetate 11a was obtained from avermectin A_{2a}, although only in 10% yield, after a 24-h reaction time. As expected, the elimination of the 5-methoxy group of 1 occurs at a slower rate than that of a 5-*O*-acetyl group of acetylated 3. Selective acetylation of avermectin B_{1a} (2), however, proved more difficult due to apparently similar reactivities of the two secondary hydroxy groups at carbon C-4″ and C-5. Acetylation with acetic anhydride in pyridine resulted in a mixture of acetates from which 4″-*O*-acetyl- and 4″,5-di-*O*-acetylavermectin B_{1a} (7 and 9) could be obtained in 39 and 21% yields, respectively, after chromatographic separation. The 5-*O*-acetylavermectin B_{1a} (8) could only be isolated in low yield by preparative layer chromatography of a mixed fraction containing 7 and 8. Brief treatment of the diacetate 9 with sodium methoxide in methanol gave 4″-*O*-acetylavermectin B_{1a} (7) in fair yield, but prolonged basic reaction conditions in an attempt to improve the yield of 7 or to recover 2 led only to extensive decomposition.

Preliminary tests of the acetyl derivatives against gastrointestinal nematode infections in sheep[8] indicated that the 4″-*O*-acetates, especially 4″-*O*-acetylavermectin B_{1a} (7), had the highest potency and widest spectrum of the analogues. We therefore undertook the synthesis of a number of 4″-*O*-acylated derivatives of avermectin B_{1a} (2) and looked for a procedure which provides these derivatives without the need for tedious separation of isomers. We expected a bulky substituent to react preferentially at the sterically less hindered 5-hydroxy group of 2 and so to furnish a suitably protected intermediate. *tert*-Butyldi-

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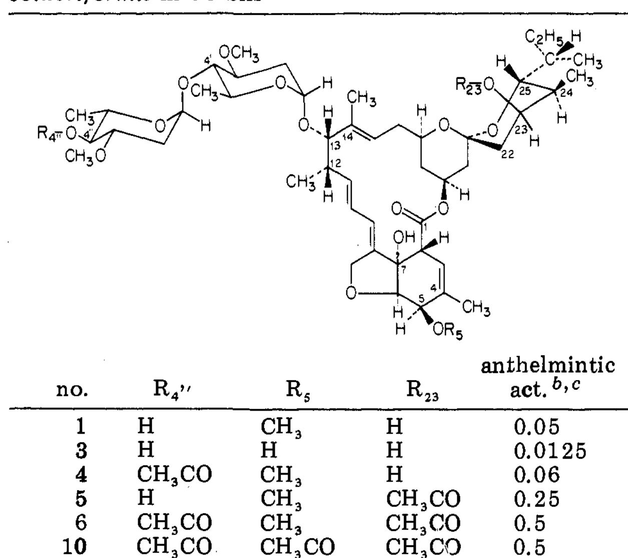
methylsilyl chloride (t-$BuMe_2SiCl$) indeed gave a 70% yield of 5-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (**12**), and only 11% of the disubstituted analogue **13** was formed. Acylation of **12** and deprotection with *p*-toluenesulfonic acid hydrate in methanol (1%, room temperature for 30 min) afforded the desired monoacyl derivatives pure and in good yield. Although the 4″-hydroxy group reacted readily with acetic anhydride and acid chlorides under basic conditions, it failed to react with succinic anhydride or potassium isocyanate, and alternate methods were required for the preparation of a hemisuccinate and a carbamate derivative. Trichloroethyl succinate[10] was reacted with thionyl chloride to its acid chloride, which gave readily the intermediate **18** in methylene chloride solution with 4-(dimethylamino)pyridine and diisopropylethylamine as catalyst. The usual removal of the silyl protecting group gave the trichloroethyl ester **19** and reduction with zinc in acetic acid afforded the hemisuccinate **20**. The 4″-*O*-(4-nitrophenyl)oxycarbonyl derivative **21** obtained from **12** and 4-nitrophenyl chloroformate served as a suitable intermediate for the syntheses of carbamates **23** and **25**. A recently published procedure[11] using 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide in methylene chloride as condensating agent allowed to react *N*-acetylglycine directly with **12** to give, after removal of the silyl protection group, the acetylaminoacetyl derivative **27**.

Detailed mass spectral fragmentation patterns for **1–3** and assignments for 59 of the 76 protons of **1** in the 300-MHz proton NMR spectrum have been reported[1] and facilitated the structure proof of the new avermectin derivatives and intermediates. Since the molecular ions of these high-molecular weight compounds and especially those of the polar derivatives are very weak or absent in the mass spectrum, we relied on specific fragments for the structure determinations. The disaccharide part of the molecule is eliminated, together with its 4″-O-substituent, and characteristic further fractionation[1] confirms a 4″-O-substituent. The 23-substituent is contained in a fragment obtained after an initial McLafferty rearrangement of the lactone and scission between C_{12} and C_{13}. A substituent at the 5-position can be observed in most instances through a weak fragment comprising C_1 through C_{12} and by comparison of the aglycon (C_1 through C_{28}) and the C_{13} to C_{28} fragments. Careful comparison of the 300-MHz proton NMR spectra confirmed the structure assignments obtained from the mass spectra data, allowed to rule out any double bond or other structural rearrangements, and assured the purity of the amorphous products. The protons of the hydroxy-bearing carbons 4″, 5, and 23 are particularly useful in assigning the structures of the acylated reaction products by their expected shifts. Avermectin B_{2a} (**3**), which contains these three secondary as well as the tertiary C-7-hydroxy groups, serves as an example. The 4″,5,23-tri-*O*-acetate **10** shows a new triplet at δ 4.70 (1 H, J = 9 Hz, $C_{4''}$ H, shifted from δ 3.18), a new multiplet at δ 4.89 (1 H, C_{23} H, shifted from δ 3.8), and a broad signal at δ 5.56 (2 H, C_3 H and C_5 H, C_5 H shifted from δ 4.30). Further subtle shifts are observed for $C_{3''}$ OCH_3 (δ 3.39, s, 3 H, shifted from δ 3.43), for C_3 H (δ 5.56, m, 2 H, including C_5 H, C_3 H shifted from δ 5.42), and for C_6 H (δ 4.11, d, J = 6 Hz, 1 H, shifted from δ 4.05). The two C_{8a} protons appear as doublets of doublets at δ 4.65 and 4.71 (J = 14 and 2 Hz) when C_5 OH is acylated (or methylated, cf. 1) but look almost like a broad singlet (δ 4.68) in the unsubstituted **3**. The three acetylmethyls appear as three distinct singlets at δ 2.03, 2.12, and 2.18. The complete identity of the remainder of the spectrum of **3** and **10** assures the absence of isomerizations or skeletal rearrangements and the purity of the product. Further confirmation is obtained from the comparison of the C-13 NMR spectra of **3**[1] and **10** (cf. Experimental Section). The 300-MHz proton NMR spectra of the two aromatic compounds **11a** and **11b** obtained from **1** and **3** are identical. Aromatization of the ring C_2 to C_7 is reflected by the following shifts: δ 6.88 and 6.74 (2 s slightly broadened, 2 H, C_3 H and C_5 H), 2.33 (s, 3 H, C_4 CH_3 shifted from δ 1.83), 6.16 (dt, 1 H, J = 11 and 2.5 Hz, C_9 H), 5.87 (dd, 1 H, J = 15 and 11 Hz, C_{10} H), 5.74 (dd, 1 H, J = 15 and 10 Hz, C_{11} H), 5.21 (br s, 2 H, C_{8a} H_2) and absence of protons from the usual position for C_2 H, C_3 H, C_4 CH_3, C_5 H, and C_6 H.

Table I. Derivatives of Avermectin A_{2a} and B_{2a} and Anthelmintic Activity against *Trichostrongylus colubriformis* in Gerbils[a]



no.	$R_{4''}$	R_5	R_{23}	anthelmintic act.[b,c]
1	H	CH_3	H	0.05
3	H	H	H	0.0125
4	CH_3CO	CH_3	H	0.06
5	H	CH_3	CH_3CO	0.25
6	CH_3CO	CH_3	CH_3CO	0.5
10	CH_3CO	CH_3CO	CH_3CO	0.5

[a] Reference 7. [b] Minimal doses (mg/kg) needed to remove >83% of the worm burden. [c] Compound 11: anthelmintic activity > 2.5.

Biological Results

The anthelmintic activities of the avermectin derivatives were determined against a *Trichostrongylus colubriformis* infection in gerbils.[7] The minimal doses necessary to eliminate at least 83% of the worm burden were used for comparative purposes. The 4″-*O*-acetate **4** of avermectin A_{2a} (**1**) was as potent as the unsubstituted compound **1**, while the 23-*O*-acetate **5** and the 4″,23-di-*O*-acetate **6** had reduced activities (Table I). Avermectin B_{2a} (**3**) exhibited the highest activity in this assay, but its triacetate **10** was 40 times less active. Avermectin B_{1a} (**2**) was later used as substrate for further modifications because of its high activity against *Haemonchus contortus* in sheep.[4] Again the 4″-*O*-acetate **7** had the same potency as the unsubstituted compound **2**, but 5-*O*-acetate **8** and 4″,5-*O*-diacetate **9** suffered a substantial loss of activity (Table II). It appears that acylation of the 5- or 23-hydroxy group results in a loss of anthelmintic activity, while the free 4″-hydroxy group is not necessary for high potency. In particular, the 5-hydroxy group is essential, as is also shown by lower activity of the natural product 1, which has a 5-methoxy substituent, and the total loss of activity with 5-O-*tert*-butyldimethylsilyl derivatives **12** and **13** and the aromatic analogue **11**. The efficacy of the acetates was further investigated against a broad spectrum of gas-

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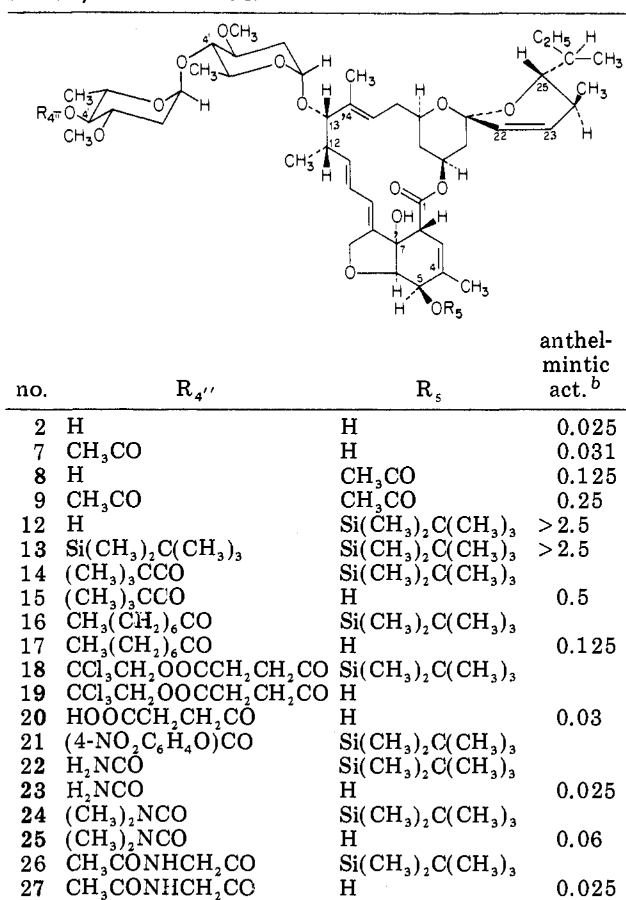
Table II. Derivatives of Avermectin B_{1a} and Anthelmintic Activity against *Trichostrongylus colubriformis* in Gerbils[a]



no.	$R_{4''}$	R_5	anthelmintic act.[b]
2	H	H	0.025
7	CH_3CO	H	0.031
8	H	CH_3CO	0.125
9	CH_3CO	CH_3CO	0.25
12	H	$Si(CH_3)_2C(CH_3)_3$	>2.5
13	$Si(CH_3)_2C(CH_3)_3$	$Si(CH_3)_2C(CH_3)_3$	>2.5
14	$(CH_3)_3CCO$	$Si(CH_3)_2C(CH_3)_3$	
15	$(CH_3)_3CCO$	H	0.5
16	$CH_3(CH_2)_6CO$	$Si(CH_3)_2C(CH_3)_3$	
17	$CH_3(CH_2)_6CO$	H	0.125
18	$CCl_3CH_2OOCCH_2CH_2CO$	$Si(CH_3)_2C(CH_3)_3$	
19	$CCl_3CH_2OOCCH_2CH_2CO$	H	
20	$HOOCCH_2CH_2CO$	H	0.03
21	$(4\text{-}NO_2C_6H_4O)CO$	$Si(CH_3)_2C(CH_3)_3$	
22	H_2NCO	$Si(CH_3)_2C(CH_3)_3$	
23	H_2NCO	H	0.025
24	$(CH_3)_2NCO$	$Si(CH_3)_2C(CH_3)_3$	
25	$(CH_3)_2NCO$	H	0.06
26	$CH_3CONHCH_2CO$	$Si(CH_3)_2C(CH_3)_3$	
27	$CH_3CONHCH_2CO$	H	0.025

[a] Reference 7. [b] Minimal doses (mg/kg) needed to remove >83% of the worm burden.

trointestinal parasites in sheep (Table III). The *T. colubriformis* results paralleled the relative potencies obtained in gerbils. The 4''-*O*-acetylavermectin B_{1a} (**7**) was the most potent derivative against gastrointestinal sheep parasites with only a slight apparent weakness against *Cooperia oncophora* and the immature (EL_4) stages of *Ostertagia circumcincta* compared to the unsubstituted natural compound **2**. Further substitutions of the 4''-hydroxy group of **2** were directed toward more polar derivatives to explore the effect of a decrease of the highly lipophilic character of the avermectins on their biological activities. The hemisuccinate **20**, carbamate **23**, and acetylaminoacetate **27** showed potencies in the gerbil assay comparable to **2** or **7**, while the lipophilic octanoate **17** and pivaloate **15** were substantially less active. No advantage over the natural products was shown, however, by any of the acylated derivatives, and eventually the 22,23-dihydroavermectin B_1[6] (ivermectin) was selected as a new anthelmintic agent.

Experimental Section

The natural products **1**–**3** used as starting materials contained up to 15% of a 27-demethyl analogue (the "b" series),[1] which could not readily be removed by chromatography and thus was carried through the reaction sequences.[12] The new derivatives, as well as starting materials **1** and **3**, were amorphous lyophilates or foams and were therefore vigorously purified by preparative layer chromatography (PLC) on silica gel GF (Uniplates, Analtech, 20 × 20 cm and of 0.25- to 2.0-mm thickness). Their purities were further demonstrated by analytical TLC on silica gel plates (Uniplate, Analtech, 25 × 100 mm) with hexane–EtOAc, CH_2Cl_2–EtOAc, toluene–2-propanol, $CHCl_3$–THF, CH_2Cl_2–THF–EtOH or CH_2Cl_2–MeOH as eluting solvents. The spots were observed in UV light and visualized by a ceric sulfate spray. The progress of all reactions was similarly followed by TLC. High-performance column chromatography (Waters Corasil A column, CH_2Cl_2–EtOAc solvent mixtures or Waters C_{18} μ-Bondapak reverse-phase columns with 75 to 98% aqueous MeOH as solvent) was carried out on certain selected compounds. Silica gel 60 (E. Merck, particle size 0.063–0.200 mm) was used for short column chromatography.[13] Usual workup means two to three extractions with the solvent specified and washing the extract with water, drying with $MgSO_4$, and concentration to a solid residue in vacuo and high vacuo. The analytical samples were dried for 16 h in high vacuo at 40 °C, which in most instances did not remove water completely, as was apparent from the NMR spectra. Therefore, analyses are calculated for a water content of 0.5 to 2 mol. Microanalyses were performed by the staff of Merck Sharp & Dohme Research Laboratories under the direction of J. Gilbert. All compounds were characterized by 300-MHz proton NMR spectra on a Varian SC300 in $CDCl_3$ solution with tetramethylsilane as internal standard, by mass spectra on an LKB Model 9000, and by UV spectra on a Cary 15 instrument and were in full agreement with the assigned structures.

4''-*O*-Acetylavermectin A_{2a} (4). A solution of **1** (20 mg, 0.022 mmol) in 0.3 mL of pyridine was treated with 0.1 mL of acetic anhydride and kept at 0 °C for 5 h. The reaction mixture was added to 2.0 mL of benzene, frozen, and lyophilized to give 21 mg of white foam. It was purified by PLC ($CHCl_3$–THF, 19:1) to give 13 mg of **4** (62%) as a white residue after lyophilization from benzene: NMR δ 4.70 (t, 1 H, J = 9 Hz, $C_{4''}$ H shifted from δ 3.18), 3.39 (s, 3 H, $C_{3''}$ OCH_3 shifted from δ 3.43), 2.11 (s, 3 H, CH_3CO); mass spectrum, m/e 598 (M^+ – 348, aglycon–H_2O), 323, 305, 275, 331 (disaccharide + 4''-OAc), 299, 187, 155; UV (MeOH) λ_{max} 243 nm (ϵ 30 300). Anal. ($C_{51}H_{78}O_{16}\cdot1.5H_2O$) C, H.

4'',23-Di-*O*-acetylavermectin A_{2a} (6). A solution of **1** (500 mg, 0.55 mmol) in 4.0 mL of pyridine was treated with 2.0 mL of acetic anhydride and heated in an oil bath for 2 h at 100 °C. The reaction mixture was poured into ice–water, and the precipitate was collected by filtration. It was dissolved in ether and gave, after usual workup, 560 mg (100%) of **6**: NMR δ 4.70 (t, 1 H, J = 9 Hz, $C_{4''}$ H shifted from δ 3.18), 4.90 (m, 1 H, C_{23} H shifted from δ 3.8), 3.40 (s, 3 H, $C_{3''}$ OCH_3 shifted from δ 3.43), 2.03 and 2.10 (2 s, 6 H, 2 CH_3CO); mass spectrum, m/e 640 (M^+ – 348, aglycon + C_{23} OAc–H_2O), 365, 275, 331 (disaccharide + 4''-OAc), 299, 187; UV (MeOH) λ_{max} 244 nm (ϵ 27 200). Anal. $C_{53}H_{80}O_{17}\cdot H_2O$) C, H.

23-*O*-Acetylavermectin A_{2a} (5). To a solution of **6** (100 mg, 0.1 mmol) in 2.5 mL of MeOH stirred in an ice bath was added 0.75 mL of a 0.16 N NaOH solution (0.12 mmol) in 50% aqueous MeOH. After a 4-h reaction time at 0 °C, the usual workup with ether gave 95 mg of crude reaction product. Purification by PLC (benzene–2-propanol, 9:1) gave, after freeze–drying of a benzene solution, 30.5 mg (30%) of **5** as white powder: NMR δ 4.86 (m, 1 H, C_{23} H shifted from δ 3.8), 2.02 (s, 3 H, CH_3CO); mass spectrum, m/e 640 (M^+ – 348, aglycon + C_{23} OAc–H_2O), 365, 275, 289 (disaccharide), 257, 145, 113; UV (MeOH λ_{max} 244 nm (ϵ 27 700). Anal. ($C_{51}H_{78}O_{16}\cdot H_2O$) C, H.

4'',5,23-Tri-*O*-acetylavermectin B_{2a} (10). A solution of **3** (250 mg, 0.28 mmol) in 4.0 mL of pyridine and 2.0 mL of acetic anhydride was heated in an oil bath to 100 °C for 2 h. The cooled reaction mixture was poured into ice–water, and the usual workup with ether gave 290 mg of brown foam. Purification by PLC (CH_2Cl_2–THF–EtOH, 96.8:3:0.2, two developments) afforded 190 mg (67%) of **10** as a white foam: ^1H NMR δ 4.70 (t, 1 H, J = 9 Hz, $C_{4''}$ H shifted from δ 3.18), ~5.56 (1 H, C_5 H shifted from δ 4.30), 4.89 (m, 1 H, C_{23} H shifted from δ 3.8), 3.39 (s, 3 H, $C_{3''}$ OCH_3 shifted from δ 3.43), 2.03, 2.12, and 2.18 (3 s, 3 × 3 H, 3 CH_3CO); mass spectrum, m/e 668 (M^+ – 348, aglycon + C_5 OAc + C_{23} OAc–H_2O), 365, 303, 331 (disaccharide + 4''-OAc), 299, 187, 155; UV (MeOH) λ_{max} 245 nm (ϵ 31 300). Anal. ($C_{54}H_{78}O_{17}\cdot1.5H_2O$) C, H.

(12) Biological activities of the compounds of the homologous "a" and "b" series are equivalent.

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Table III. Anthelmintic Efficacy by Oral Administration in Experimentally Infected Sheep[a]

treatment	dose, mg/kg	no. of sheep	*H.c.* EL_4	*H.c.* adult	*O.c.* EL_4	*O.c.* adult	*T.a.*	*T.c.*	*C.o.* EL_4	*C.o.* adult	*Oe.c.*
none		6	(70)[b]	(442)	(1421)	(1137)	(2852)	(4110)	(193)	(1761)	(61)
1	0.1	3	3[c]	3	0	3	3	3	1	0	3
	0.05	3	2	2	0	2	2	2	1	0	2
	0.025	3	1	0	0	2	0	0	1	0	2
4	0.25	1		3		3	3	3		3	3
5	0.25	1		1		0	0	0		2	1
6	0.25	1		3		2	1	2		2	3
2	0.1	3	3	3	3	3	3	3	3	3	3
	0.05	3	3	3	3	3	3	3	3	3	3
	0.025	3	3	3	1	2	2	2	0	1	3
7	0.1	3		3	2	3	3	3	3	2	3
	0.05	3		3	0	3	3	3	2	0	3
	0.025	3		3	0	3	1	3	2	0	3
9	0.25	1		3		3	3	3		3	3
	0.15	2		3	1	3	3	3		3	3
20	0.1 sc	1		3		0	2	3	3[d]	3[d]	3

[a] *H.c.* = *Haemonchus contortus*; *O.c.* = *Ostertagia circumcincta*; *T.a.* = *Trichostrongylus axei*; *T.c.* = *Trichostrongylus colubriformis*; *C.o.* = *Cooperia oncophora*; *Oe.c.* = *Oseophagostomum columbianum*. [b] Geometric mean of the number of worms per untreated experimentally infected lamb, representative of "typical" infections encountered under standard experimental procedures. [c] 3 = >90% efficacy; 2 = 60–89% efficacy; 1 = 20–59% efficacy; 0 = 0–19% efficacy. [d] *C. curticei*.

4″,23-Di-*O*-acetyl-5,7-dideoxy-2,5,6,7-tetradehydroavermectin B_{2a} (11). 11a from 1. A solution of 1 (100 mg, 0.11 mmol) in 1.0 mL of pyridine and 0.5 mL of acetic anhydride was heated in an oil bath at 100 °C for 24 h. The reaction mixture was concentrated to a brown oil under a stream of nitrogen and was separated by PLC ($CHCl_3$–THF, 97:3) into three bands. The major band (R_f 0.50) gave 63 mg (58%) of **6** as white foam identical by TLC, NMR, and mass spectrum with authentic **6**. The second band (R_f 0.55) gave 24 mg of an amorphous residue, which was not identified. The fastest (R_f 0.65) and highly fluorescent band gave 10 mg (9%) of **11a** as a white powder after lyophilization in benzene: NMR δ 6.88 and 6.74 (2 s, slightly broadened, 2 H, C_3 H and C_5 H), 6.16 (dt, 1 H, J = 11 and 2.5 Hz, C_9 H), 5.87 (dd, 1 H, J = 15 and 11 Hz, C_{10} H), 5.74 (dd, 1 H, J = 15 and 10 Hz, C_{11} H), 5.21 (br s, 2 H, C_{8a} H_2), 2.33 (s, 3 H, C_4 CH_3 shifted from δ 1.83), 2.03 and 2.10 (2 s, 2 × 3 H, 2 CH_3CO); mass spectrum, m/e 608 (M^+ − 330, aglycon + C_{23} OAc), 365, 243, 331 (disaccharide + 4″-OAc), 299, 187; UV (MeOH) λ_{max} 284 nm (ε 12 700), 360 (13 700). Anal. ($C_{52}H_{74}O_{15}$·1.5H_2O) C, H.

11b from 3. A solution of **3** (100 mg, 0.11 mmol) in 1.0 mL of pyridine and 0.5 mL of acetic anhydride was heated in an oil bath at 100 °C for 24 h under an atmosphere of nitrogen. Then the reaction mixture was allowed to come to room temperature and was poured into ice–water. This was extracted with ether, and the extract was washed with dilute aqueous HCl and $NaHCO_3$ solutions and water, dried, and concentrated. The crude product was separated by PLC (1.0 mm, hexane–EtOAc, 7:3, three consecutive developments) into a fraction with R_f 0.5: 33 mg (31%) of **11b**; identical by TLC, ^{1}H NMR, and mass spectrum with **11a**. A second fraction (R_f 0.35, 26 mg) was not identified, and the third fraction (R_f 0.25) amounted to 35 mg (31%) and was, according to TLC, ^{1}H NMR, and mass spectrum, identical with **10**.

4″-*O*-Acetyl- (7) and 4″,5-Di-*O*-acetylavermectin B_{1a} (9). A solution of **2** (5.0 g, 5.7 mmol) in 45 mL of anhydrous pyridine was stirred at 0 °C while 5.0 mL (52 mmol) of acetic anhydride was added rapidly. After 3.75 h at 0 °C, the reaction mixture was poured into 350 mL of ice–water, the mixture was filtered, and the white precipitate was washed twice with water. The moist product was dissolved in 400 mL of ether, and the solution was washed with dilute $NaHCO_3$ and water, dried, and concentrated in vacuo to 5.1 g of a white foam. TLC (SiO_2, $CHCl_3$–THF, 9:1) and HPLC (Waters C_{18} μ-Bondapak column, MeOH–H_2O, 85:15) showed a mixture containing mainly **7**, some **9**, and some recovered starting material. The crude product was dissolved in a mixture of CH_2Cl_2–EtOAc (8:2) and chromatographed on a column containing 300 g of silica gel. Elution with a total of 3.0 L of CH_2Cl_2–EtOAc, (8:2) solvent mixture gave first 1.11 g (20%) of **9** as a white foam: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ε 33 100). Anal. ($C_{52}H_{76}O_{16}$·0.5H_2O) C, H.

Next 0.47 g of a mixture of **9** and **7** was obtained, followed by 1.77 g (34%) of **7** as white foam: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH), λ_{max} 245 nm (ε 32 100). Anal. ($C_{50}H_{74}O_{15}$·0.5H_2O) C, H.

Further elution yielded 0.48 g of a mixture containing the monoacetates **7** and **8** and finally afforded 0.96 g of recovered starting material **2**.

5-*O*-Acetylavermectin B_{1a} (8). A mixture of **7** and **8** (obtained above), 250 mg, was applied to two SiO_2 PLC plates (20 × 20 × 0.2 cm) and developed twice with $CHCl_3$–THF, (92:8) and then once with CH_2Cl_2–EtOAc (8:2) (the plates were allowed to dry at ambient temperature after each elution). The front part of the band was extracted, giving 55 mg of pure **7** (TLC one spot) and 180 mg of a mixture. This was again applied to three SiO_2 PLC plates (20 × 20 × 0.1 cm), which were developed once with $CHCl_3$–THF (92:8) followed by three developments with CH_2Cl_2–EtOAc (8:2). The slower half of this band gave 136 mg of pure **8** (TLC one spot), which was lyophilized from dioxane: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 244 nm (ε 32 100). Anal. ($C_{50}H_{74}O_{15}$·H_2O) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)- (12) and 4″,5-Bis-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (13). To a solution of **2** (1.0 g, 1.15 mmol) in 10 mL of DMF was added 470 mg (6.9 mmol) of imidazole, followed by 520 mg (3.45 mmol) of *tert*-butyldimethylsilyl chloride, and the reaction mixture was kept at 18 °C for 2 h. Then ether (120 mL) and water (50 mL) were added, the aqueous layer was washed with ether, and then the combined ether extracts were washed with H_2O (3 times), dried, and concentrated in vacuo to 1.3 g of crude product. This was purified on a column containing 40 g of silica gel with CH_2Cl_2–THF (95:5) as solvent (total of 1.5 L) and gave 148 mg (10.8%) of **13** as white foam: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ε 33 200). Anal. ($C_{60}H_{100}O_{14}Si_2$·0.5H_2O) C, H. The next fractions gave 865 mg (70%) of **12** as a white foam: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ε 33 200). Anal. ($C_{54}H_{86}O_{14}Si$·H_2O) C, H. Finally, 207 mg (19%) of starting material was recovered.

4″-*O*-Pivaloyl-5-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (14). General Procedure A. A solution of **12** (100 mg, 0.1 mmol), 49 mg (0.4 mmol) of 4-(dimethylamino)pyridine, and 52 mg (0.4 mmol) diisopropylethylamine in 2.5 mL of CH_2Cl_2 was stirred in an ice bath while adding a solution of 36 mg (0.3 mmol) of pivaloyl chloride in 0.5 mL of CH_2Cl_2. After a 1-h reaction time at 0 °C, ice was added, and the reaction was worked up with CH_2Cl_2 to give a light foam. Purification by PLC (CH_2Cl_2–THF–EtOH, 95:4.75:0.25) afforded 98 mg (90%) of **14**: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ε 33 800). Anal. ($C_{59}H_{74}O_{15}Si$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)-4″-*O*-octanoylavermectin B_{1a} (16) was prepared according to general procedure A from **12**

Table IV. Characteristic 300-MHz ^{1}H NMR Chemical Shifts of Avermectin B_{1a} Derivatives

	chemical shift, δ (multiplicity, coupling constants[a])						
no.	C_4 CH_3	C_5 H	C_6 H	C_8 CH_2	$C_{4''}$ H	$C_{3''}$ OCH_3	
2	1.89 (s)	4.31 (t, 7)	3.98 (d, 7)	4.70 (br s)	3.18 (dt, 9, 2)	3.45 (s)	
7	1.89 (s)	4.31 (t)	3.99 (d, 6)	4.69 (br s)	4.70 (t)	3.38 (s)	2.10 (s, 3 H, CH_3CO)
8	1.77 (s)	5.54	4.06 (d, 6)	4.60 (dd, 14, 2), 4.69 (dd, 14, 2)	3.18 (dt)	3.43 (s)	2.15 (s, 3 H, CH_3CO)
9	1.78 (s)	5.56	4.10 (d, 6)	4.61 (dd, 15, 2), 4.71 (dd, 15, 2)	4.70 (t)	3.38 (s)	2.10, 2.17 (2 s, 6 H, 2 CH_3CO)
12	1.81 (s)	4.45 (br d)	3.84 (d)	4.60 (dd, 15, 2), 4.71 (dd, 15, 2)	3.19 (dt)	3.45 (s)	0.14 [s, $Si(CH_3)_2$], 0.93 [s, $SiC(CH_3)_3$]
13	1.80 (s)	4.47 (br m)	3.86 (d, 6)	4.62 (dd, 14, 2), 4.72 (dd, 14, 2)	3.17 (t, 9)	3.36 (s)	0.08 and 0.10 [2 s, $C_{4''}$ $OSi(CH_3)_2$], 0.13 [s, C_5 $OSi(CH_3)_2$], 0.88 and 0.92 [2 s, 2 $SiC(CH_3)_3$]
14	1.80	4.44 (br m)	3.85 (d)	4.61 (dd, 15, 3), 4.72 (dd, 15, 3)	4.69 (t, 9)	3.36 (s)	1.22 [s, $C(CH_3)_3$], 0.93 [s, $SiC(CH_3)_3$], 0.13 [s, $Si(CH_3)_2$]
15	1.90	4.38 (t)	4.06 (d)	4.71 (br s)	4.67 (t, 9.5)	3.37 (s)	1.22 [s, $C(CH_3)_3$]
16	1.80 (s)	4.45 (br d)	3.84 (d, 6)	4.60 (dd, 15, 2), 4.70 (dd, 15, 2)	4.70 (t, 9)	3.37 (s)	0.14 [s, $Si(CH_3)_2$], 0.92 [s, $SiC(CH_3)_3$]
17	1.89 (s)	4.34 (t, 7)	4.02 (d, 7)	4.70 (dd, 15, 2), 4.75 (dd, 15, 2)	4.73 (t, 9)	3.36 (s)	~1.27 [t, $CH_3(CH_2)_6CO$]
18	1.81 (s)	4.46 (br m)	3.85 (d, 6)	4.60 (br d, 15), 4.71 (br d, 15)	4.72 (t, 9)	3.39 (s)	4.77 (~3 H, $C_{1'}$ H and OCH_2CCl_3), 2.74 and 2.82 (2 m, 4 H, $COCH_2CH_2CO$), 0.93 [s, $SiC(CH_3)_3$], 0.13 [s, $Si(CH_3)_2$]
19	1.90 (s)	4.32 (t, 7)	3.99 (d, 7)	4.71 (br s)	4.72 (t, 9)	3.39 (s)	~4.80 (~3 H, $C_{1'}$ H and OCH_2CCl_3), 2.75 and 2.84 (2 t, 4 H, $COCH_2CH_2CO$)
20	1.90 (s)	4.32 (d, 7)	4.00 (d, 7)	4.70 (br s)	4.72 (t, 9)	3.38 (s)	2.74 (m, ~4 H, $COCH_2CH_2CO$)
21	1.83 (s)	4.47 (br d, 6)	3.83 (d, 6)	4.60 (dd, 15, 2), 4.70 (dd, 15, 2)	4.5 (t, 9)	3.46 (s)	7.46 and 8.33 (2 d, 4 H, 10, 2 ArH), 0.14 [s, $Si(CH_3)_2$], 0.94 [s, $SiC(CH_3)_3$]
23	1.92 (s)	4.34 (br m)	4.02 (d)	4.74 (br s)	4.56 (t)	3.40 (s)	4.68 (br s, NH_2)
24	1.80 (s)	4.46 (br m)	3.85 (d, 6)	4.61 (br d, 15), 4.72 (br d, 15)	4.60 (t, 9)	3.40 (s)	2.95 [s, $N(CH_3)_2$], 0.92 [s, $SiC(CH_3)_3$], 0.14 [s, $Si(CH_3)_2$]
25	1.92 (s)	4.34 (br s)	4.03 (d)	4.74 (br s)	4.62 (t)	3.40 (s)	2.98 [s, $N(CH_3)_2$]
27	1.88 (s)	4.35 (t, 8)	3.98 (d, 6)	4.72 (br s)	4.75 (t, 9)	3.36 (s)	6.73 (t, 5, NH), 4.09 (dd, 2 H, 12, 4, $NHCH_2CO$), 2.08 (s, 3 H, CH_3CO)

[a] Coupling constant (*J*) in hertz.

Table V. Prominent Mass Fragments[a] of Avermectin B_{1a} Derivatives[b]

	m/e						
fragments:	a	b	c	d	e	f	g
2	289	257	145	113	566	261	305
7	331	299	187	n.o.	566	261	305
8	289 (w)[c]	257	145	113	608	303	305
9	331	299	187	n.o.[c]	608	303	305
12	289	257	145	113	680	375	305
13	n.o.	371	259	227	680	375	305
14	373	341	229	197	680	375	305
15	373	341	229	197	566	261	305
16	415	383	271	239	680	375	305
17	415	383	271	239	566	261	305
18	519 (Cl_3, w)	487 (Cl_3)	375 (Cl_3)	n.o.	680	n.o.	305
19	n.o.	487 (w)	375 (Cl_3)	n.o.	566	261	305
20	n.o.	n.o.	245	213 (w)	566	261	305
21	n.o.	n.o.	310	278	680	375	305
23	n.o.	n.o.	n.o.	n.o.	566	261	305
24	360	328	216	184	680	375	305
25	360	328	216	184	566	261	305
27	388	356	244	212	566	261	305

[a] Fragments: a = disaccharide − OH + $R_{4''}$; b = a − MeOH; c = monosaccharide − OH + $R_{4''}$; d = c − MeOH; e = aglycon − H_2O + R_5; f = (C_1 to C_{12}) − H_2O + R_5; g = (C_{13} to C_{25}) − H_2O. [b] See Table II for $R_{4''}$ and R_5. [c] n.o. = fragment not observed; w = weak fragment.

with octanoyl chloride in 80% yield: NMR, see Table IV; mass spectrum, see Table V.

5-*O*-(*tert*-Butyldimethylsilyl)-4″-*O*-[3-[(2,2,2-trichloroethoxy)carbonyl]propanoyl]avermectin B_{1a} (18) was pre-

pared according to general procedure A from **12** with trichloroethylsuccinoyl chloride in nearly quantitative yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32100). Anal. ($C_{60}H_{91}Cl_3SiO_{17}$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)-4″-*O*-[[(4-nitrophenyl)-oxy]carbonyl]avermectin B_{1a} (21) was prepared according to general procedure A from **12** and 4-nitrophenyl chloroformate in 100% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 243 nm (ϵ 35600). Anal. ($C_{61}H_{89}O_{18}NSi$) C, H, N.

4″-*O*-Pivaloylavermectin B_{1a} (15). General Procedure B. A solution of **14** (95 mg, 0.088 mmol) in 8.0 mL of MeOH containing 80 mg (1%) of *p*-toluenesulfonic acid hydrate was stirred at 18 °C for 30 min. Then EtOAc was added, the solution was washed with dilute $NaHCO_3$ and H_2O (3 times), dried, and concentrated in vacuo. The crude product was purified immediately by PLC (CH_2Cl_2–THF–EtOH, 95:4.75:0.25) and gave 65 mg (75%) of **15**: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32300). Anal. ($C_{53}H_{80}O_{15}$·$0.5H_2O$) C, H.

4″-*O*-Octanoylavermectin B_{1a} (17) was prepared from **16** according to general procedure B in 80% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 31100). Anal. ($C_{56}H_{86}O_{15}$) C, H.

4″-*O*-[3-[(2,2,2-Trichloroethoxy)carbonyl]propanoyl]avermectin B_{1a} (19) was prepared from **18** according to general procedure B in 93% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32000). Anal. ($C_{54}H_{77}O_{17}Cl_3$) C, H, Cl.

4″-*O*-(Aminocarbonyl)avermectin B_{1a} (23) was prepared from **22** according to general procedure B in 73% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32000). Anal. ($C_{49}H_{73}O_{15}N$·$2H_2O$) C, H, N.

4″-*O*-[(Dimethylamino)carbonyl]avermectin B_{1a} (25) was prepared from **24** according to general procedure B in 89% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32700). Anal. ($C_{51}H_{77}O_{15}N$·$0.5H_2O$) C, H, N.

4″-*O*-[(Acetylamino)acetyl]avermectin B_{1a} (27) was prepared from **26** according to general procedure B in 82% yield: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 31700). Anal. ($C_{52}H_{77}O_{16}N$·$0.5H_2O$) C, H, N.

4″-*O*-Succinoylavermectin B_{1a} (20). A solution of **19** (100 mg, 0.09 mmol) in 3.5 mL of acetic acid was stirred with 400 mg (6 mmol) of zinc dust for 1 h at 18 °C. The reaction mixture was filtered, and the solids were washed well with EtOAc. The filtrate was concentrated in vacuo to a white glass, which was dissolved in EtOAc, washed with dilute HCl and water, dried, and evaporated to give 80 mg of a clear glass. This was further purified by PLC (CH_2Cl_2–THF–AcOH, 90:9.5:0.5) to give 62 mg (69%) of **20**: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 245 nm (ϵ 32200). Anal. ($C_{52}H_{76}O_{17}$·H_2O) C, H.

4″-*O*-[(Dimethylamino)carbonyl]-5-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (24). General Procedure C. A solution of **21** (50 mg, 0.044 mmol) in 4.0 mL of ether was cooled in an ice bath, and a stream of dimethylamine was bubbled into the solution for 1 min. The reaction mixture was kept 30 min at 0 °C, and then it was evaporated under a stream of N_2. The residue was taken up in ether, washed with H_2O (3 times), dried and again concentrated under a stream of N_2. The crude product was purified by PLC (CH_2Cl_2–THF, 95:5, two successive developments) to give 40 mg (87%) of **24** as a white foam: NMR, see Table IV; mass spectrum, see Table V; UV (MeOH) λ_{max} 244 nm (ϵ 31650). Anal. ($C_{57}H_{71}O_{15}NSi$) C, H, N.

4″-*O*-(Aminocarbonyl)-5-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (22) was prepared from **21** and NH_3 according to general procedure C in 87% yield, characterized after removal of the *tert*-butyldimethylsilyl protecting group (see **23**).

4″-*O*-(Acetylglycyl)-5-*O*-(*tert*-butyldimethylsilyl)avermectin B_{1a} (26). A solution of **12** (50 mg, 0.05 mmol), 4-(dimethylamino)pyridine (12.5 mg, 0.1 mmol), and *N*-acetylglycine (12 mg, 0.1 mmol) in 0.75 mL of CH_2Cl_2 was prepared at room temperature. After addition of a solution of dicyclohexylcarbodiimide (23 mg, 0.11 mmol) in 0.5 mL of CH_2Cl_2, the mixture was stirred for 90 min at room temperature, and then filtered and worked up with CH_2Cl_2. Purification by PLC (1.0 mm, CH_2Cl_2–MeOH, 95:5) gave 57 mg (100%) of amorphous **26**: NMR, see Table IV; mass spectrum, see Table V.

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Structure–Activity Correlations of Cytochalasins. Novel Halogenated and Related Cytochalasin C and D Derivatives

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A series of halogenated and related analogues of cytochalasin C (CC) and D (CD) has been synthesized, and the biological activities of the analogues as inhibitors in a cell-free contractility model system obtained from Ehrlich ascites tumor cells were evaluated. The reaction sequence involved treatment of CD with phenyltrimethylammonium perbromide to give 6,12-dibromo-CD (**2**), dehydrohalogenation of **2** to 12-bromo-CC (**3**), and the subsequent conversions of **3** to 12-azido- (**4**), 12-iodo- (**5**), and 12-cyano-CC (**6**). The ID_{50} values for **5**, **3**, **4**, **2**, and **6** are 6.0, 7.4, 8.8, 45, and 77×10^{-7} M, respectively, in comparison to ca. 2.8×10^{-7} M for the parental compounds. The potential cell and molecular biological applications of these compounds are delineated.

The cytochalasins, a group of secondary fungal metabolites[1] of widespread distribution, exhibit profound effects upon eukaryotic cells and cell systems.[2] These manifestations, many of which are essentially reversible with drug removal or dilution, take place rapidly and at quite low concentrations (10^{-8}–10^{-6} M). The availability of high specific activity radiolabeled congeners and of potential affinity-labelling species could prove useful to the further identification of macromolecular drug receptors, including cytoskeletal elements[3,4] and transport carriers.[5] Toward these objectives, we describe in this article the preparation of cytochalasin C and D derivatives, functionalized with halogens, which maintain complete or partial biological

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