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Avermectin Chemistry and Action: Ester- and Ether-type Candidate Photoaffinity Probes

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Abstract—Avermectin B_{1a} (**1**) is a potent anthelmintic, insecticide, miticide and chloride channel activator on interaction with a specific nerve membrane site analyzed by binding assays with [^{3}H]**1**. Candidate photoaffinity probes were prepared replacing the dioleandrosyl substituent with potential isosteric esters and ethers approximating the original overall atom length and terminating in a phenyl moiety substituted with azido, iodo or hydroxy. Several of the candidates met the goal of high potency on mouse, housefly and fruit fly brain chloride channels with IC_{50} values of 7–57 nM in competing for the [^{3}H]**1** binding site.

Introduction

The avermectins are potent anthelmintics, insecticides and miticides used to control pests of humans, veterinary animals and crops.[1,2] The prototype avermectin B_{1a} (**1**) consists of a macrocyclic lactone with a 13α-dioleandrosyl substituent (R) (Fig. 1). Compound **1** and many analogues bind to vertebrate and invertebrate γ-aminobutyric acid (GABA)-gated and invertebrate glutamate-gated chloride channels to increase ion conductance, thereby disrupting inhibitory or excitatory action. These mechanisms are established by physiological, biochemical and molecular biological investigations[3–8] with an important contribution from radioligand binding studies with [5α-^{3}H]**1** and its 22,23-ditritio derivative [^{3}H]ivermectin.[9–13] Photoaffinity labeling has been successfully applied by Merck scientists to the action of the avermectins, using radioligand **2** which is 4″-amino-4″-deoxy-**1** with 4-azido-3-^{125}iodosalicylic acid attached to the amino group through a β-alanyl-ε-aminocaproyl spacer (Fig. 1).[14,15] Photoaffinity probe **2** labels a 47-kDa polypeptide from head membranes of the fruit fly *Drosophila melanogaster* and 53-, 47- and 8-kDa polypeptides from the free-living nematode *Caenorhabditis elegans* but does not bind to rat brain membranes and, therefore, is selective for the insect and nematode membranes.[16–18] Although target site selectivity is a distinct advantage for safe use of a pharmaceutical or pesticide, it does not enable a single probe to be employed for comparing vertebrate and invertebrate targets and defining differences in their binding sites within the chloride channel, as has been successfully applied to chloride channel blockers.[19]

The goal of this investigation is to design a candidate photoaffinity probe effective for both mammals and insects. The failure of **2** to act in mammalian brain[16] is conceivably due to the far greater length of its R substituent than that of **1**. An alternative is that **2** has amide bonds versus only ether linkages in the R substituent of **1**. The candidate photoaffinity probes **3–6** prepared here, therefore, include ester and ether moieties more nearly isosteric with the dioleandrosyl substituent (Fig. 1). Potency is evaluated using the [^{3}H]**1** binding assay with membranes from mouse brain and housefly (*Musca domestica*) and fruit fly heads.

Structural modifications

Esters 3a–f of 1-aglycone (Figs 1 and 2). Key intermediate **9** was synthesized by a two-step reaction from 5-*O*-*tert*-butyldimethylsilylavermectin B_{1a} aglycone (**7**).[20] Treatment of **7** with the anhydride prepared in situ from *N*-(2,2,2-trichloroethoxycarbonyl)-β-alanine and

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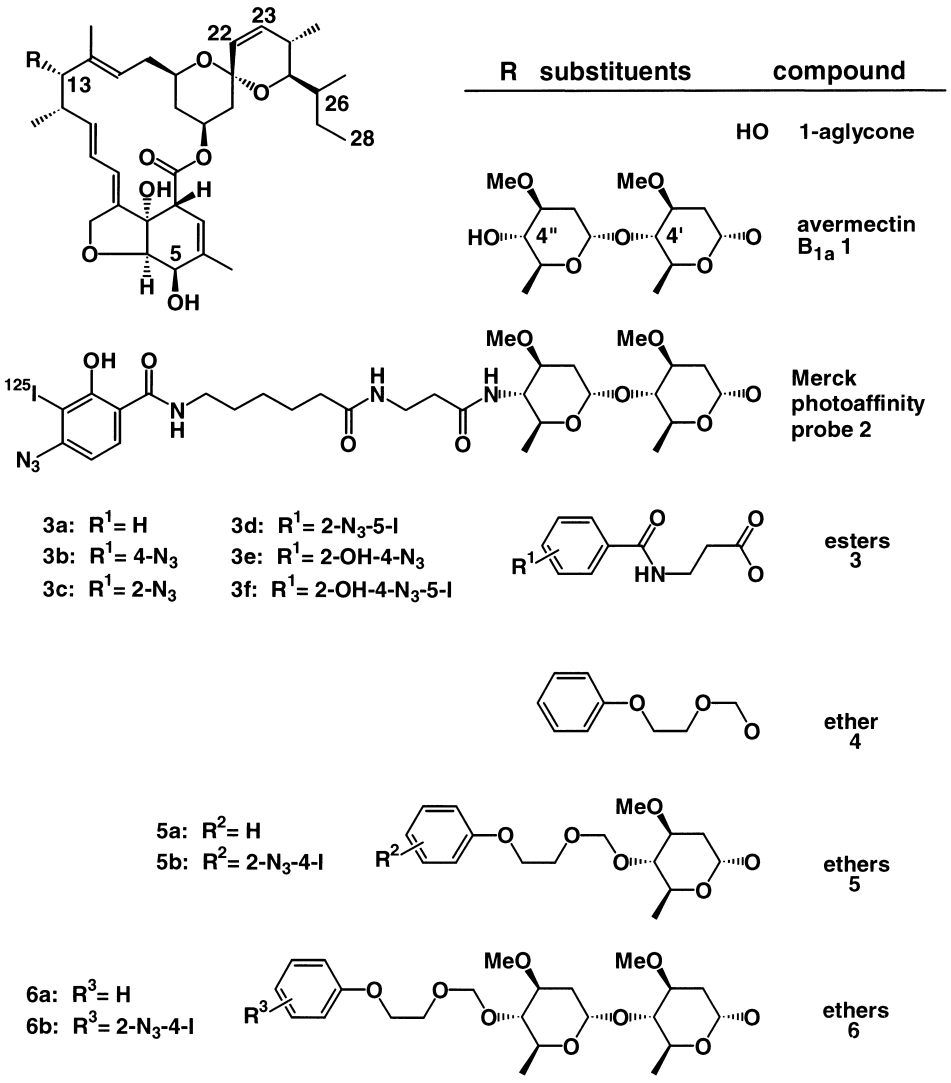


Figure 1. Structures of avermectin B_{1a} (**1**) and the corresponding aglycone (**1**-aglycone) and of an amide (**2**), esters (**3**) and ethers (**4**–**6**) derived therefrom.

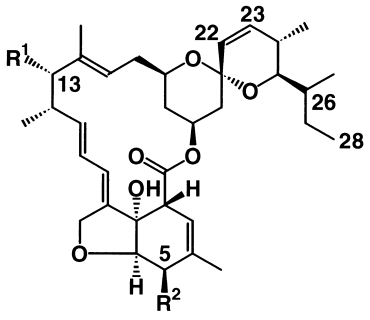
pivaloyl chloride in the presence of triethylamine gave ester **8** in high yield, and reductive cleavage of the 2,2,2-trichloroethoxycarbonyl group of this ester afforded **9**. Acylation of the deprotected amino group of **9** was carried out with benzoyl chloride for **3a** or with the previously known azidobenzoic acid derivatives in the presence of 1,3-dicyclohexylcarbodiimide (DCC) for **3b**–**d** or with the *N*-hydroxysuccinimidyl ester of 4-azido-2-hydroxybenzoic acid and 4-hydroxy-2-iodobenzoic acid for **3e** and **3f**, respectively. Finally, desilylation of the 5-hydroxy group gave the esters **3a**–**f**.[14,20,21]

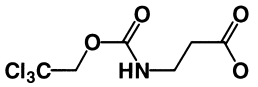
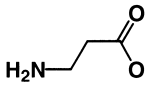
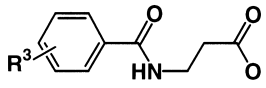
Ethers 4 of 1-aglycone, 5a and 5b of 1-monosaccharide and 6a and 6b of 1 (Figs 1 and 3). The ether-type derivatives (**4**–**6**) were obtained from **7**, 5-*O*-*tert*-butyldimethylsilylavermectin B_{1a} monosaccharide (**12**), and 5-*O*-*tert*-butyldimethylsilylavermectin B_{1a} (**14**)[22] by coupling with phenoxyethoxymethyl chloride or 2-(2-azido-4-iodophenoxy)ethoxymethyl chloride followed by deprotection of the 5-hydroxy group.

Structure–activity relationships

General (Fig. 1 and Table 1). Compound **1** differs only 3-fold in potency for the mouse, housefly and fruit fly brain [^{3}H]**1** binding site and **1**-aglycone is 18- to 68-fold less active. Photoaffinity probe **2** is similar to ivermectin in its affinity for the *C. elegans* membrane binding site despite the long spacer group between the macrocyclic lactone and the terminal photoreactive 4-azido-3-^{125}iodosalicylate moiety.[16] However, **2** does not bind to the rat brain [^{3}H]ivermectin binding site[16] suggesting that an R substituent more isosteric with that of **1** may be more appropriate. Several of the candidates in the present study met the goal of high potency on mouse, housefly and fruit fly brain chloride channels with IC_{50} values of 7–57 nM in competing for the [^{3}H]**1** binding site.

Esters 3a–f (Fig. 1 and Table 1). The goal was to replace the dioleandrosyl unit with an ester of approximately



compound	R^1	R^2
7	HO	$OSiMe_2Bu$-*t*
8		$OSiMe_2Bu$-*t*
9		$OSiMe_2Bu$-*t*
10a: R^3= H **10d:** R^3= 2-N_3-5-I **10b:** R^3= 4-N_3 **10e:** R^3= 2-OH-4-N_3 **10c:** R^3= 2-N_3 **10f:** R^3= 2-OH-4-N_3-5-I		$OSiMe_2Bu$-*t*

reaction conditions
7→**8**: $Cl_3CCH_2OCONHCH_2CH_2CO_2H$, Et_3N, *t*-BuCOCl, CH_2Cl_2, then **7**, *i*-Pr_2NEt, 4-DMAP (99%). **8**→**9**: Zn dust, AcOH, H_2O, THF (57%). **9**→**10a**: PhCOCl, Et_3N, CH_2Cl_2 (87%). **9**→**10b**: 4-N_3-$PhCO_2H$, DCC, CH_2Cl_2 (65%). **9**→**10c**: 2-N_3-$PhCO_2H$, DCC, CH_2Cl_2 (77%). **9**→**10d**: 2-N_3-5-I-$PhCO_2H$, DCC, CH_2Cl_2 (72%). **9**→**10e**: 2-HO-4-N_3-$PhCO_2$-Su, CH_2Cl_2 (66%). **9**→**10f**: 2-HO-4-N_3-5-I-$PhCO_2$-Su, CH_2Cl_2 (66%). **10**→**3**: HF-Py, MeCN; **3a** (86%), **3b** (100%), **3c** (99%), **3d** (79%), **3e** (69%), **3f** (91%).

Figure 2. 5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-(2-aminopropionyl)avermectin B_{1a} aglycone (**9**) and precursors (**7** and **8**) and derivatives (**10**) thereof. Su refers to *N*-hydroxysuccinimidyl ester of the acid.

10-atom equivalent length including an aryl moiety that would potentially bear azido, iodo and possibly hydroxy substituents. The β-alanyl group was used as a candidate isosteric spacer unit. All of the esters are potent in the housefly receptor assays (IC_{50} values 7–31 nM) while **3c** and **3e** are best for the mouse preparation (IC_{50} values 57 and 105 nM) and **3d–f** for the fruit fly membranes (IC_{50} values 26–56 nM). On this basis iodo-containing compounds **3d** and **3f** are candidates for radiolabeling, especially for the insect receptor. While a portion of the activity of the esters may be due to hydrolysis to **1**-aglycone, this is probably not a major factor since the IC_{50} values of **3e** for mouse brain and housefly membrane preparations were not changed by treating with a potent esterase inhibitor (phenyl saligenin cyclic phosphonate or phenylmethanesulfonyl fluoride, 10 μM, 30 min preincubation, data not detailed here) and the ester linkage can be substituted with a non-hydrolyzable ether moiety with enhanced potency relative to the aglycone (see below).

Ethers 4, 5a, 5b, 6a and 6b (Fig. 1 and Table 1). Ether linkages were examined as one way to rule out the possibility of esterases or amidases limiting the potency of candidate photoaffinity probes. The 13-*O*-(2-phenoxyethoxymethyl) derivatives as prepared from 1-aglycone (**4**), **1**-monosaccharide (**5a**) and **1** (**6a**) retain a high level of potency in the housefly receptor assay (IC_{50} values 19–97 nM). However, the 2-azido-4-iodophenoxy analogues (**5b** and **6b**) are ~100-fold less active than their phenoxy counterparts with mouse and 2–5-fold less active with housefly. Further optimization is required in the phenoxy substituents for a general photoaffinity probe.

Experimental

Chemistry

General. ^1H NMR spectra were recorded for $CDCl_3$ solutions with a Bruker AM-300 spectrometer. Fast atom bombardment (FABMS) (both low- and high-resolution, LR and HR) was conducted with the Fisons ZAB2-EQ spectrometer. Analytical thin-layer chromatography (TLC) was performed on silica gel with fluorescent indicator using precoated plastic sheets. Preparative TLC involved 20×20 cm plates coated with

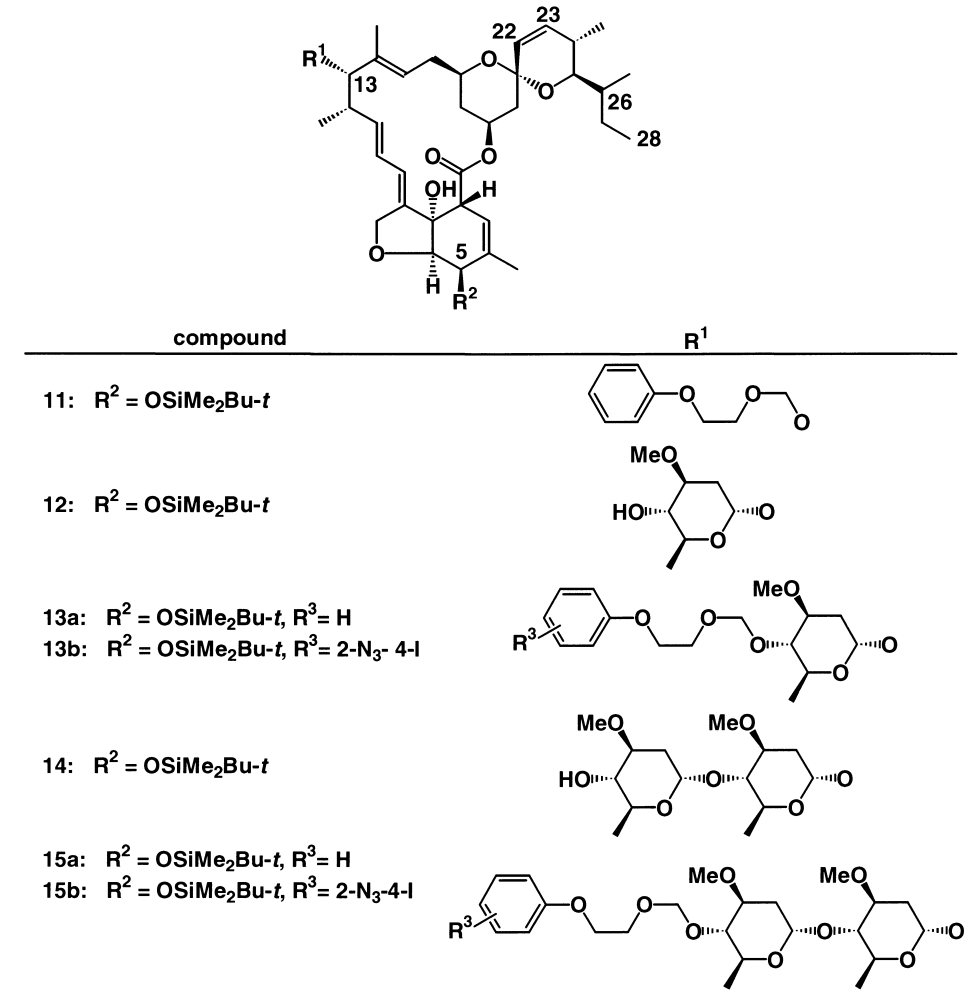


Figure 3. 5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-(2-phenoxyethoxymethyl)avermectin B_{1a} aglycone (**11**) and related compounds (**12**–**15**).

0.25 or 0.5 mm silica gel containing PF_{250} indicator. Compounds were visualized with short wavelength UV light. Column flash chromatography was performed using silica gel 60. All final products were purified to homogeneity as determined by TLC and ^1H NMR and had appropriate ^1H NMR spectra (Table 2) and molecular mass by FABMS.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(2,2,2-trichloroethoxycarbonylamino)propionyl]-avermectin B_{1a} aglycone (8). To a solution of *N*-(2,2,2-trichloroethoxycarbonyl)-β-alanine[23] (530 mg) in CH_2Cl_2 (7 mL) in an ice bath were added triethylamine (280 μL) and pivaloyl chloride (250 μL). The mixture was stirred for 30 min at 0 °C and a solution of **7**[20] (141 mg) in CH_2Cl_2 (3 mL), diisopropylethylamine (174 μL), and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) (123 mg) was added at 0 °C. The mixture was stirred at room temperature for 18 h and poured into ice-cold water (40 mL). The resulting mixture was extracted with ethyl acetate (40 mL, and then 3×20 mL) and the combined organic layer was washed with water (20 mL) and brine (20 mL), then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane-ethyl acetate gradient) to give 188 mg (99%) of **8**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-(2-aminopropionyl)-avermectin B_{1a} aglycone (9). A mixture of **8** (184 mg), water (10 μL), acetic acid (100 μL) and zinc dust (747 mg) in tetrahydrofuran (THF) (8 mL) was stirred for 4.5 h at room temperature. The reaction mixture was filtered through Celite and the solid was washed with ethyl acetate (80 mL). The filtrate and the ethyl acetate were combined and washed with water (20 mL) and

Table 1. Structure–activity relationships of avermectin derivatives as inhibitors of [^{3}H]avermectin binding in brain membranes of mouse, housefly and fruitfly

Compound	IC_{50}, nM ± SD (n)		
	Mouse	Housefly	Fruit fly
Avermectin series			
1	2.9 ± 0.9 (10)	1.5 ± 0.6 (9)	4.2 ± 1.3 (5)
1-aglycone	196 ± 46 (3)	64 ± 20 (4)	77 ± 18 (3)
Esters			
3a	653, 424[a]	13 ± 5 (3)	73 ± 28 (4)
3b	170 ± 98 (4)	15 ± 10 (3)	243 ± 86 (3)
3c	57 ± 34 (3)	15 ± 9 (3)	477, 304[a]
3d	185 ± 72 (3)	31 ± 12 (5)	44 ± 38 (3)
3e	105 ± 28 (3)	6.7 ± 3.7 (3)	54 ± 24 (3)
3f	130 ± 2 (3)	21 ± 15 (3)	56, 26[a]
Ethers			
4	29 ± 18 (5)	19 ± 8 (4)	—
5a	38 ± 7 (30)	33 ± 21 (3)	—
5b	3400, 506[a]	174, 154[a]	—
6a	33 ± 13 (3)	97, 62[a]	—
6b	3684, 1210[a]	201, 153[a]	—

[a]Values from separate experiments.

brine (20 mL), then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1) to give 85 mg (57%) of **9**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-(2-benzoylaminopropionyl)-avermectin B_{1a} aglycone (10a). To a solution of **9** (17.5 mg) in CH_2Cl_2 (1 mL) were added triethylamine (8 μL) and benzoyl chloride (3 μL) at 0°C. The reaction mixture was stirred at room temperature for 24 h and purified by preparative TLC (hexane:ethyl acetate, 2:1) to give 17.2 mg (87%) of **10a**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(4-azidobenzoyl)-aminopropionyl]avermectin B_{1a} aglycone (10b). A solution of **9** (16.9 mg), 4-azidobenzoic acid[24] (6.1 mg), and DCC (10 mg) in CH_2Cl_2 (1 mL) was stirred in the dark at room temperature for 15 h. The reaction mixture was purified by preparative TLC (hexane:ethyl acetate, 2:1) to give 13.1 mg (65%) of **10b**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(2-azidobenzoyl)-aminopropionyl]avermectin B_{1a} aglycone (10c). The above procedure with **9** (14.3 mg), 2-azidobenzoic acid[25] (6.0 mg) and DCC (10 mg) in the dark gave 13.1 mg (77%) of **10c**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(2-azido-5-iodobenzoyl)aminopropionyl]-avermectin B_{1a} aglycone (10d). The above procedure with **9** (17.0 mg), 2-azido-5-iodobenzoic acid[26] (11 mg) and DCC (10 mg) in the dark gave 16.7 mg (72%) of **10d**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(4-azido-2-hydroxybenzoyl)aminopropionyl]-avermectin B_{1a} aglycone (10e). A solution of **9** (17.8 mg) and *N*-hydroxysuccinimidyl-4-azido-2-hydroxybenzoate[26] (6.9 mg) in CH_2Cl_2 (1 mL) was stirred in the dark at 0°C for 30 min and then at room temperature for 25 h. The reaction mixture was purified by preparative TLC (hexane:ethyl acetate, 2:1) to give 14.2 mg (66%) of **10e**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-[2-(4-azido-2-hydroxy-5-iodobenzoyl)-aminopropionyl]avermectin B_{1a} aglycone (10f). The above procedure with **9** (17.8 mg) and *N*-hydroxysuccinimidyl-4-azido-2-hydroxy-5-iodobenzoate[27] (14.5 mg) in the dark gave 16.0 mg (66%) of **10f**.

5-*O*-(*tert*-Butyldimethylsilyl)-13-*O*-(2-phenoxyethoxymethyl)avermectin B_{1a} aglycone (11). To a solution of **7** (20 mg) and 2-phenoxyethoxymethyl chloride (106 mg) in CH_2Cl_2 (150 μL) was added diisopropylethylamine (120 μL). The reaction mixture was stirred for 3 days, poured into ice-cold saturated aqueous $NaHCO_3$ (20 mL), extracted with CH_2Cl_2 (4×10 mL) and the combined organic layer was washed with $NaHCO_3$ solution (2×10 mL) then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (hexane:ethyl acetate, 6:1) to give 16.3 mg (67%) of **11**.

5-*O*-(*tert*-Butyldimethylsilyl)avermectin B_{1a} monosaccharide (12). A solution of avermectin B_{1a} monosaccharide[28] (326 mg), imidazole (183 mg), and *tert*-butyldimethylsilyl chloride (202 mg) in DMF (4 mL) was stirred for 40 min. Then additional imidazole (183 mg) and *tert*-butyldimethylsilyl chloride (202 mg) were added followed by stirring for 75 min. The reaction mixture was poured into ice-cold water (50 mL) and extracted with hexane:ethyl acetate (1/1) (4×25 mL). The combined organic layer was washed with water (2×25 mL) and brine (25 mL) then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate gradient) to give 190 mg (50%) of **12**.

5-*O*-(*tert*-Butyldimethylsilyl)-4′-*O*-(2-phenoxyethoxymethyl)avermectin B_{1a} monosaccharide (13a). To a solution of **12** (21.9 mg) and 2-phenoxyethoxymethyl chloride (from treatment of 2-phenoxyethanol and paraformaldehyde in toluene with HCl gas) (260 mg) in CH_2Cl_2 (150 μL) was added diisopropylethylamine (243 μL). The reaction mixture was stirred for 1 day and poured into ice-cold saturated aqueous $NaHCO_3$ (10 mL), extracted with ethyl acetate (4×10 mL) and the combined organic layer was washed with aqueous $NaHCO_3$ (2×10 mL) then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (CH_2Cl_2:MeOH, 30:1) to give 12.7 mg (49%) of **13a**.

5-*O*-(*tert*-Butyldimethylsilyl)-4′-*O*-[2-(2-azido-4-iodophenoxy)ethoxymethyl]avermectin B_{1a} monosaccharide (13b). To a solution of **12** (40 mg) and 2-(2-azido-4-iodophenoxy)ethoxymethyl chloride (from 2-(2-azido-4-iodophenoxy)ethanol and paraformaldehyde with HCl gas as above) (101 mg) in CH_2Cl_2 (100 μL) was added diisopropylethylamine (243 μL). The reaction mixture was stirred for 3 days with work up as for **13a** to give 2.6 mg (4.7%) of **13b**.

5-*O*-(*tert*-Butyldimethylsilyl)-4″-*O*-(2-phenoxyethoxymethyl)avermectin B_{1a} (15a). The procedure for **11** but with **14**[22] and a reaction time of 1 day gave 14.7 mg (57%) of **15a**.

Table 2. Characteristic 300-MHz ^{1}H NMR chemical shifts ($CDCl_3$) of avermectin B_{1a} derivatives

Compound (s)	Chemical shift, δ (multiplicity, coupling constants)				
	C_5H	$C_{13}H$	$C_{4'}H$	$C_{4''}H$	13-Substituent other than saccharide
Avermectin series					
1	4.28 (brs)	3.93 (s)	3.24 (t, 9)	3.16 (t, 9)	
14	4.43–4.45 (m)	3.94 (s)	3.25 (t, 9)	3.17 (t, 9)	
1-monosaccharide	4.29 (brs)	3.96 (s)	3.17 (t, 9)	——	
12	4.43–4.45 (brs)	3.95 (s)	3.17 (t, 9)	——	
1-aglycone	4.29 (brs)	4.00 (s)	——	——	
7	4.41–4.44 (m)	4.01 (s)	——	——	
Intermediates					
8	4.41–4.44 (m)	5.19 (s)	——	——	2.62–2.73 (2H, m, N-C-CH_2CO), 3.55 (2H, q, J = 6 Hz, NCH_2-C-CO), 4.70 (1H, s, Cl_3CCHOCO-N), 4.71 (1H, s, Cl_3CCHOCO-N)
9	4.42–4.43 (m)	5.20 (s)	——	——	2.61–2.72 (2H, m, N-C-CH_2CO), 2.84 (2H, brs, NH_2), 3.08 (2H, t, J = 6 Hz, NCH_2-C-CO)
Esters					
3a	4.29 (brs)	5.20 (s)	——	——	2.63–2.81 (2H, m, N-C-CH_2CO), 3.70–3.86 (2H, m, NCH_2-C-CO), 6.85 (1H, t, J = 6 Hz, NH), 7.38–7.49 (3H, m, PhCO), 7.72–7.75 (2H, m, PhCO)
10a	4.41–4.43 (m)	5.20 (s)	——	——	2.64–2.81 (2H, m, N-C-CH_2CO), 3.70–3.86 (2H, m, NCH_2-C-CO), 6.86 (1H, t, J = 6 Hz, NH), 7.39–7.51 (3H, m, PhCO), 7.72–7.75 (2H, m, PhCO)
3b	4.28–4.32 (m)	5.21 (s)	——	——	2.64–2.81 (2H, m, N-C-CH_2CO), 3.71–3.86 (2H, m, NCH_2-C-CO), 6.84 (1H, t, J = 6 Hz, NH), 7.06 (2H, dd, J = 7 and 2 Hz, PhCO), 7.75 (2H, dd, J = 7 and 2 Hz, PhCO)
10b	4.41–4.44 (m)	5.21 (s)	——	——	2.64–2.81 (2H, m, N-C-CH_2CO), 3.71–3.85 (2H, m, NCH_2-C-CO), 6.84 (1H, t, J = 6 Hz, NH), 7.06 (2H, dd, J = 7 and 2 Hz, PhCO), 7.75 (2H, dd, J = 7 and 2 Hz, PhCO)
3c	4.29 (brs)	5.23 (s)	——	——	2.65–2.82 (2H, m, N-C-CH_2CO), 3.76–3.86 (2H, m, NCH_2-C-CO), 7.17 (1H, dd, J = 8 and 1 Hz, PhCO), 7.22 (1H, td, J = 8 and 1 Hz, PhCO), 7.48 (1H, td, J = 8 and 2 Hz, PhCO), 7.92 (1H, t, J = 5 Hz, NH), 8.11 (1H, dd, J = 8 and 2 Hz, PhCO)
10c	4.42–4.44 (m)	5.23 (s)	——	——	2.65–2.82 (2H, m, N-C-CH_2CO), 3.76–3.85 (2H, m, NCH_2-C-CO), 7.18 (1H, d, J = 9 Hz, PhCO), 7.22 (1H, td, J = 8 and 1 Hz, PhCO), 7.48 (1H, td, J = 8 and 2 Hz, PhCO), 7.93 (1H, t, J = 5 Hz, NH), 8.11 (1H, dd, J = 8 and 2 Hz, PhCO)
3d	4.27–4.31 (m)	5.23 (s)	——	——	2.63–2.80 (2H, m, N-C-CH_2CO), 3.73–3.86 (2H, m, NCH_2-C-CO), 6.92 (1H, d, J = 8 Hz, PhCO), 7.76 (1H, dd, J = 8 and 2 Hz, PhCO), 7.90 (1H, t, J = 6 Hz, NH), 8.41 (1H, d, J = 2 Hz, PhCO)
10d	4.41–4.31 (m)	5.23 (s)	——	——	2.65–2.80 (2H, m, N-C-CH_2CO), 3.75–3.86 (2H, m, NCH_2-C-CO), 6.92 (1H, d, J = 9 Hz, PhCO), 7.76 (1H, dd, J = 9 and 2 Hz, PhCO), 7.90 (1H, t, J = 6 Hz, NH), 8.41 (1H, d, J = 2 Hz, PhCO)
3e	4.27–4.31 (m)	5.21 (s)	——	——	2.67–2.80 (2H, m, N-C-CH_2CO), 3.75–3.86 (2H, m, NCH_2-C-CO), 6.47 (1H, dd, J = 9 and 2 Hz, PhCO), 6.62 (1H, d, J = 2 Hz, PhCO), 7.02–7.06 (1H, m, NH), 7.28 (1H, d, J = 9 Hz, PhCO), 12.30 (1H, s, HOPhCO)
10e	4.41–4.44 (m)	5.21 (s)	——	——	2.64–2.85 (2H, m, N-C-CH_2CO), 3.75–3.85 (2H, m, NCH_2-C-CO), 6.47 (1H, dd, J = 9 and 2 Hz, PhCO), 6.62 (1H, d, J = 2 Hz, PhCO), 7.03 (1H, t, J = 6 Hz, NH), 7.28 (1H, d, J = 9 Hz, PhCO), 12.30 (1H, s, HOPhCO)
3f	4.27–4.31 (m)	5.23 (s)	——	——	2.67–2.82 (2H, m, N-C-CH_2CO), 3.74–3.83 (2H, m, NCH_2-C-CO), 6.75 (1H, s, PhCO), 7.00 (1H, t, J = 6 Hz, NH), 7.67 (1H, s, PhCO), 12.32 (1H, s, HOPhCO)
10f	4.42–4.44 (m)	5.23 (s)	——	——	2.66–2.85 (2H, m, N-C-CH_2CO), 3.72–3.83 (2H, m, NCH_2-C-CO), 6.75 (1H, s, PhCO), 7.00 (1H, t, J = 6 Hz, NH), 7.67 (1H, s, PhCO), 12.32 (1H, s, HOPhCO)
Ethers					
4	4.27–4.31 (m)	3.96 (s)	——	——	3.80–4.18 (4H, m, OCH_2CH_2O), 4.65–4.68 (1H, obsc, OCH_2O), 4.71 (1H, d, J = 7 Hz, OCH_2O), 6.90–6.96 (2H, m, PhO), 7.25–7.31 (3H, m, PhO)
11	4.41–4.44 (m)	3.96 (s)	——	——	3.82–4.16 (4H, m, OCH_2CH_2O), 4.71 (1H, d, J = 7 Hz, OCH_2O), 4.77 (1H, d, J = 7 Hz, OCH_2O), 6.90–6.97 (2H, m, PhO), 7.26–7.30 (3H, m, PhO)
5a	4.30 (brs)	3.94 (s)	3.23 (t, 9)	——	3.83–4.20 (4H, m, OCH_2CH_2O), 4.89 (1H, d, J = 7 Hz, OCH_2O), 5.08 (1H, d, J = 7 Hz, OCH_2O), 6.91–6.97 (2H, m, PhO), 7.25–7.31 (3H, m, PhO)
13a	4.43–4.45 (m)	3.94 (s)	3.23 (t, 9)	——	3.82–4.16 (4H, m, OCH_2CH_2O), 4.89 (1H, d, J = 7 Hz, OCH_2O), 5.08 (1H, d, J = 7 Hz, OCH_2O), 6.91–6.97 (2H, m, PhO), 7.24–7.31 (3H, m, PhO)
5b	4.28–4.32 (m)	3.94 (s)	3.20 (t, 9)	——	3.92–4.02 (2H, m, OCH_2CH_2O), 4.17 (2H, t, J = 5 Hz, OCH_2CH_2O), 4.87 (1H, d, J = 7 Hz, OCH_2O), 5.06 (1H, d, J = 7 Hz, OCH_2O), 6.67 (1H, d, J = 9 Hz, PhO), 7.25 (1H, d, J = 2 Hz, PhO), 7.35 (1H, dd, J = 9 and 2 Hz, PhO)

(continued)

Table 2 (*continued*)

13b	4.43–4.45 (m)	3.94 (s)	3.20 (t, 9)	——	3.92–4.02 (2H, m, OCH_2CH_2O), 4.17 (2H, t, $J=5$ Hz, OCH_2CH_2O), 4.87 (1H, d, $J=7$ Hz, OCH_2O), 5.06 (1H, d, $J=7$ Hz, OCH_2O), 6.67 (1H, d, $J=9$ Hz, PhO), 7.25 (1H, d, $J=2$ Hz, PhO), 7.35 (1H, dd, $J=9$ and 2 Hz, PhO)
6a	4.30 (brs)	3.94 (s)	3,24 (t, 9)	3.22 (t, 9)	3.92–4.00 (2H, m, OCH_2CH_2O), 4.15 (2H, d, $J=5$ Hz, OCH_2CH_2O), 4.88 (1H, d, $J=7$ Hz, OCH_2O), 5.06 (1H, d, $J=7$ Hz, OCH_2O), 6.91–6.97 (3H, m, PhO), 7.25–7.30 (2H, m, PhO)
15a	4.43–4.45 (m)	3.93 (s)	3.24 (t, 9)	3.22 (t, 9)	3.80–4.00 (2H, m, OCH_2CH_2O), 4.15 (2H, t, $J=5$ Hz, OCH_2CH_2O), 4.88 (1H, d, $J=7$ Hz, OCH_2O), 5.06 (1H, d, $J=7$ Hz, OCH_2O), 6.91–6.97 (3H, m, PhO), 7.25–7.30 (2H, m, PhO)
6b	4.31 (brs)	3.94 (s)	3.23 (t, 9)	3.20 (t, 9)	3.90–4.05 (2H, m, OCH_2CH_2O), 4.16 (2H, t, $J=5$ Hz, OCH_2CH_2O), 4.87 (1H, d, $J=7$ Hz, OCH_2O), 5.05 (1H, d, $J=7$ Hz, OCH_2O), 6.67 (1H, d, $J=9$ Hz, PhO), 7.25 (1H, d, $J=2$ Hz, PhO), 7.36 (1H, dd, $J=9$ and 2 Hz, PhO)
15b	4.42–4.44 (m)	3.93 (s)	3.23 (t, 9)	3.20 (t, 9)	3.80–3.98 (2H, m, $OCHCH_2O$), 4.16 (2H, t, $J=5$ Hz, OCH_2CH_2O), 4.85 (1H, d, $J=7$ Hz, OCH_2O), 5.03 (1H, d, $J=7$ Hz, OCH_2O), 6.66 (1H, d, $J=9$ Hz, PhO), 7.24 (1H, d, $J=2$ Hz, PhO), 7.34 (1H, dd, $J=9$ and 2 Hz, PhO)

5-*O*-(*tert*-Butyldimethylsilyl)-4″-*O*-[2-(2-azido-4-iodophenoxy)ethoxymethyl]avermectin B_{1a} (15b). The procedure of **13b** but with **14** (40 mg) in the dark gave 3.6 mg (6.8%) of **15b**.

13-*O*-(2-Benzoylaminopropionyl)avermectin B_{1a} aglycone (3a). To a solution of **10a** (17.2 mg) in acetonitrile (1 mL) was added HF-pyridine (HF = 68%, 0.1 mL) and the mixture was stirred at room temperature. After 3 h, additional HF-pyridine (0.1 mL) was added and the mixture was stirred for 1 h. The reaction mixture was poured into ice-cold saturated aqueous $NaHCO_3$ (15 mL) and extracted with ethyl acetate (4×5 mL). The combined organic layer was washed with water (5 mL) and brine (5 mL) then dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (hexane:ethyl acetate, 2:3) to give 12.9 mg (86%) of **3a**. MS-LR: $C_{44}O_{57}O_{10}N$, (MH^+) 760.

13-*O*-[2-(4-Azidobenzoyl)aminopropionyl]avermectin B_{1a} aglycone (3b). The above procedure with **10b** (13.1 mg) but in the dark gave 11.5 mg (100%) of **3b**. MS-LR: $C_{44}H_{56}O_{10}N_4$, (MH^+) 801.

13-*O*-[2-(2-Azidobenzoyl)aminopropionyl]avermectin B_{1a} aglycone (3c). The above procedure with **10c** (13.1 mg) in the dark gave 11.4 mg (99%) of **3c**. MS-LR: $C_{44}H_{56}O_{10}N_4$, (MH^+) 801.

13-*O*-[2-(2-Azido-5-iodobenzoyl)aminopropionyl]avermectin B_{1a} aglycone (3d). The above procedure with **10d** (16.7 mg) in the dark gave 11.7 mg (79%) of **3d**. MS-LR: $C_{44}H_{55}O_{10}N_4I$, (MH^+) 927; HR (MH^+) calcd 927.3041, found 927.3020.

13-*O*-[2-(4-Azido-2-hydroxybenzoyl)aminopropionyl]avermectin B_{1a} aglycone (3e). The above procedure with **10e** (14.2 mg) in the dark gave 8.6 mg (69%) of **3e**. MS-LR: $C_{44}H_{56}O_{11}N_4$, (MH^+) 817.

13-*O*-[2-(4-Azido-2-hydroxy-5-iodobenzoyl)aminopropionyl]avermectin B_{1a} aglycone (3f). The above procedure with **10f** (16.0 mg) in the dark gave 13.0 mg (91%) of **3f** MS-LR: $C_{44}H_{55}O_{11}N_4I$, (MH^+) 943; HR (MH^+) calcd 943.2990, found 943.3015.

13-*O*-(2-Phenoxyethoxymethyl)avermectin B_{1a} aglycone (4). The procedure for **3a** but with **11** (16.3 mg) gave 10.0 mg (71%) of **4**. MS-LR: $C_{43}H_{58}O_{10}$, (MH^+) 735.

4′-*O*-(2-Phenoxyethoxymethyl)avermectin B_{1a} monosaccharide (5a). The procedure for **3a** but with **13a** (12.7 mg) gave 9.1 mg (81%) of **5a**. MS-LR: $C_{50}H_{70}O_{13}$, (MH^+) 879.

4′-*O*-[2-(2-Azido-4-iodophenoxy)ethoxymethyl]avermectin B_{1a} monosaccharide (5b). The procedure for **3a** but with **13b** (2.6 mg) in the dark gave 2.2 mg (94%) of **5b**. MS-LR HR: $C_{50}H_{68}O_{13}IN_3$, (MLi^+) 1052.

4″-*O*-(2-Phenoxyethoxymethyl)avermectin B_{1a} (6a). The procedure for **3a** but with **15a** (14.7 mg) gave 9.0 mg (68%) of **6a**. MS-LR: $C_{57}H_{82}O_{16}$, (MH^+) 1023.

4″-*O*-[2-(2-Azido-4-iodophenoxy)ethoxymethyl]avermectin B_{1a} (6b). The procedure for **3a** but with **15b** (3.3 mg) in the dark gave 1.4 mg (46%) of **6b**. MS-LR: $C_{57}H_{80}O_{16}IN_3$, (MLi^+) 1196.

Mouse and insect brain [^{3}H]1 binding assays

Brain and head preparations. The studies used whole brain from male mice (25–30 g) and frozen (−70 °C) heads from male and female houseflies (susceptible strain cultured in this laboratory, 4–7 days after emergence as adults) and male and female adult fruit flies (provided by Gerald Rubin of the Department of Molecular and Cell Biology on this campus). The frozen insects at dry ice temperature were shaken in a flask to break them into their body parts and the heads recovered by passing through a 2-mm sieve and recovery on a 1-mm sieve for houseflies or a 1-mm and 0.5-mm sieve, respectively, for fruit flies. Dry ice temperature was maintained for glassware and sieves and the frozen heads were held at −80 °C until used for membrane preparation. Subsequent steps were carried out at 4 °C.

Membrane preparations. Mouse brain homogenate at 10% (w/v) in 0.32 M sucrose was centrifuged at 1000×*g* for 10 min and the supernatant at 10,000×*g* for 20 min. The pelleted membranes were suspended in 1 mM

EDTA and dialyzed versus distilled water for 5–6 h at 4 °C. Centrifugation (25,000×*g*, 30 min) gave the final brain membrane fraction. Insect heads were homogenized in 0.25 M sucrose-10 mM Tris–HCl buffer, pH 7.5, and the homogenate was centrifuged at 1000×*g* for 10 min and the supenatant thereof at 130,000×*g* for 60 min. The pelleted mouse brain and insect head membranes were suspended in assay buffer (see below) and stored at −80 °C until used.

[^{3}H]1 binding assays. Procedures for the [^{3}H]**1** binding assays were based on earlier reports for mammalian brain[9–12] and houseflies.[13] The assay buffer was 10 mM phosphate, pH 7.5, containing 200 mM NaCl for mouse and 300 mM NaCl for insect membranes. The incubation mixtures were prepared by adding, in sequence, assay buffer (870 μL), the test compound in dimethyl sulfoxide (DMSO) (10 μL), [^{3}H]**1** in DMSO:assay buffer (1:3, 20 μL) and finally the membrane preparation (100 μL) with mixing. The protein level[29] was 50, 200 and 400 μg/assay for mouse, housefly and fruit fly, respectively. The final [^{3}H]**1** concentration was 2.9 nM (45,000 dpm) for mouse and 1.9 nM (30,000 dpm) for the insects. The mixtures were incubated for 90 min (mouse) or 70 min (insect) at 22 °C and then filtered on Whatman GF/C (mouse) or GF/B (insects) glass-fiber filters followed by three 5 mL rinses with ice-cold NaCl (0.9% w/v) solution containing ethanol (2% v/v). Specific binding was considered to be the difference between total ^{3}H bound with 1.9 or 2.9 nM [^{3}H]**1** and nonspecific ^{3}H bound on addition of 5 μM unlabeled **1**. The specifically bound [^{3}H]**1** with mouse and housefly membranes was directly proportional to protein level. Typical values for percentage and dpm specific binding were 67% and 7700 dpm for mouse, 67% and 7500 dpm for housefly and 86% and 12,000 dpm for fruit fly.

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References

1. Campbell, W. C. Ed.; *Ivermectin and Abamectin;* Springer–Verlag: New York, 1989.
2. Fisher, M. H. In *Recent Advances in the Chemistry of Insect Control II*; Crombie, L., Ed.; Royal Society of Chemistry: Cambridge, 1990; pp 52–68.
3. Fritz, L. C.; Wang, C. C.; Gorio, A. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2062.
4. Nicholson, R. A.; Robinson, P. S.; Palmer, C. L.; Casida, J. E. *Pestic. Sci.* **1988**, *24*, 185.
5. Turner, M. J.; Schaeffer, J. M. In *Ivermectin and Abamectin*, Campbell, W. C. Ed.; Springer–Verlag: New York, 1989; pp 73–88.
6. Fisher, M. H.; Mrozik, H. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 537.
7. Cully, D. F.; Paress, P. S.; Liu, K. K.; Schaeffer, J. M.; Arena, J. P. *J. Biol. Chem.* **1996**, *271*, 20187.
8. Huang, J.; Casida, J. E. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 261.
9. Pong, S.-S.; Wang, C. C. *Neuropharmacol.* **1980**, *19*, 311.
10. Drexler, G.; Sieghart, W. *Eur. J. Pharmacol.* **1984**, *99*, 269.
11. Schaeffer, J. M.; Haines, H. *Biochem. Pharmacol.* **1989**, *38*, 2329.
12. Arena, J. P.; Liu, K. K.; Paress, P. S.; Frazier, E. G.; Cully, D. F.; Mrozik, H.; Schaeffer, J. M. *J. Parasitol.* **1995**, *81*, 286.
13. Deng, Y.; Casida, J. E. *Pestic. Biochem. Physiol.* **1992**, *43*, 116.
14. Meinke, P. T.; Rohrer, S. P.; Hayes, E. C.; Schaeffer, J. M.; Fisher, M. H.; Mrozik, H. *J. Med. Chem.* **1992**, *35*, 3879.
15. Mrozik, H. In *Natural and Engineered Pest Management Agents*, Hedin, P. A.; Menn, J. J.; Hollingworth, R. M., Eds.; ACS Symposium Series, No. 551, American Chemical Society, Washington, DC, 1994; pp 54–73.
16. Rohrer, S. P.; Meinke, P. T.; Hayes, E. C.; Mrozik, H.; Schaeffer, J. M. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4168.
17. Rohrer, S. P.; Jacobson, E. B.; Hayes, E. C.; Birzin, E. T.; Schaeffer, J. M. *Biochem. J.* **1994**, *302*, 339.
18. Rohrer, S. P.; Birzin, E. T.; Costa, S. D.; Arena, J. P.; Hayes, E. C.; Schaeffer, J. M. *Insect Biochem. Mol. Biol.* **1995**, *25*, 11.
19. Cole, L. M.; Casida, J. E. *Pestic. Biochem. Physiol.* **1992**, *44*, 1.
20. Blizzard, T. A.; Margiatto, G. M.; Mrozik, H.; Shoop, W. L.; Frankshum, R. A.; Fisher, M. H. *J. Med. Chem.* **1992**, *35*, 3873.
21. Nicolaou, K. C.; Webber, S. E. *Synthesis* **1986**, *453*.
22. Mrozik, H.; Eskola, R; Fisher, M. H.; Egerton, J. R.; Cifelli, S.; Ostlind, D. A. *J Med. Chem.* **1982**, *25*, 658.
23. Lapatsanis L.; Milias G.; Froussios K.; Kolovos M. *Synthesis* **1983**, *671*.
24. Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *47*, 3125.
25. Okawa, T.; Sugimori, T.; Eguchi, S.; Kakehi, A. *Heterocycles* **1998**, *47*, 375.
26. Latli, B.; Tomizawa, M.; Casida, J. E. *Bioconjugate Chem.* **1997**, *8*, 7.
27. Kometani, T.; Watt, D. S.; Ji, T. *Tetrahedron Lett.* **1985**, *26*, 2043.
28. Mrozik, H.; Eskola, P.; Arison, B. H.; Albers-Schönberg, G.; Fisher, M. H. *J. Org. Chem.* **1982**, *47*, 489.
29. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.