# Inhibition of Delayed Hypersensitivity Reactions by Cinnamyl 1-Thioglycosides

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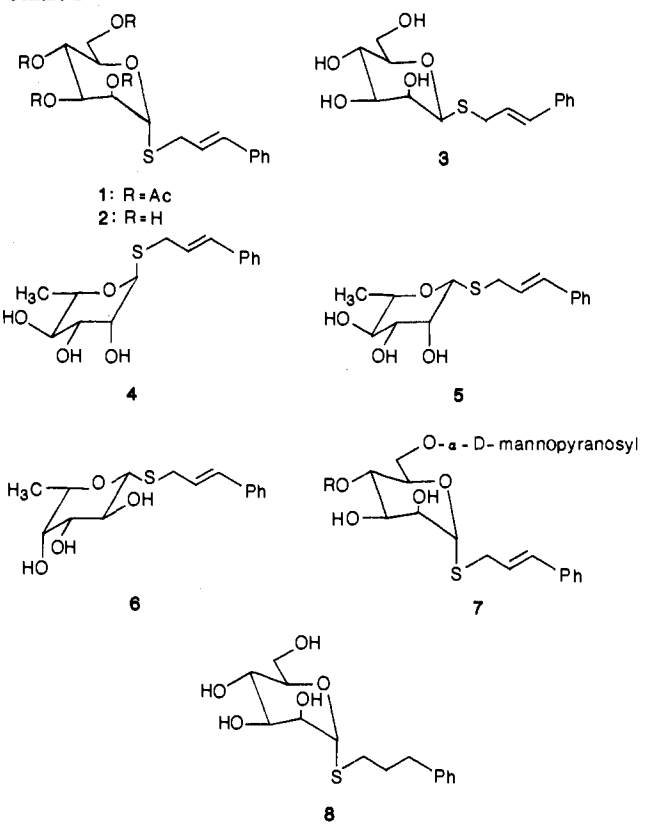
Cinnamyl 1-thio-α-D-manno(and L-rhamno)pyranosides have good inhibitory effects in an antigen-specific T cell proliferation assay. The β anomers are slightly less effective than the α anomers. The 6-substituted analogues of cinnamyl 1-thio-α-D-mannopyranoside such as 6-deoxy and 6-*O*-methyl derivatives also block macrophages in presenting the antigen to T cells. D-Mannose and L-rhamnose, when tested by themselves with no modifications, did not block at concentrations up to 1 mM. These cinnamyl 1-thioglycosides when given ip or po at 3–30 mg/kg to mice significantly inhibited the delayed type hypersensitivity reaction as measured by footpad swelling.

Macrophages can both degrade and present antigens. Some antigens characteristically stimulate an immune response that is mainly cellular. Such antigens are taken up by macrophages that deliver an immunogenic signal to the lymphocytes and, in particular, to T cells. The antigens, after being "presented" to T cells by macrophages, bind to receptors on the T cells, causing the cells to secrete lymphokines. Macrophage function is not restricted by the immune state of the animal from which the macrophage was obtained. However, macrophages and lymphocytes must share genetic identity at some portion of the major histocompatibility complex for successful detection of the antigenic signal borne by the macrophage.[1] Macrophage–lymphocyte interaction is a general requirement for T cell activation to cell surface antigens (alloantigens) as well as soluble protein antigens. Many of the biologic characteristics of antigen-specific T cell proliferation have been reviewed.[2] It was suggested that autologous macrophage–T cell interactions are at least in part mediated by sugar-specific cellular receptors.[3,4] With tetanus toxoid as an antigen and human peripheral blood mononuclear cells as a responder population, a variety of simple sugars were found to markedly inhibit antigen-specific proliferative responses. For example, D-mannose, L-rhamnose, and L-fucose gave 67%, 93%, and 55% inhibition at 25 mM concentration, respectively.[3,4] Interestingly, D-altrose, gentiobiose, and L-sorbose were also found to be inhibitory at the same concentration. These studies suggest that a variety of cellular interactions may indeed be mediated by receptors with specificity for simple sugars.

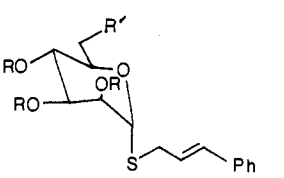
From our screening studies of D-mannose derivatives, previously prepared for macrophage binding and uptake studies,[5,6] D-mannosides bearing hydrophobic phenyl aglycons were found to be inhibitory at various concentrations in the guinea pig T cell proliferation assay. Thus it was thought of interest to prepare 1-thioglycosides having highly functionalized cinnamyl aglycons and examine their abilities to block the expression of T cell reactivity as measured by an in vitro antigen-specific proliferative assay and an in vivo delayed hypersensitivity. Thioglycosidic linkage was chosen because it is more stable toward acid

**Chart I**[a]




[a] Ph = phenyl.

**Chart II**[a]



**9**: R = Ac; R′ = OMs
**10**: R = Ac; R′ = I
**11**: R = Ac; R′ = $N_3$
**12**: R = H; R′ = $N_3$
**13**: R = H; R = $NH_2$
**14**: R = H; R′ = NHAc
**15**: R = H; R′ = $NHCOCH_2CH_2CH_3$
**16**: R = H; R′ = $CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
**17**: R = H; R′ = $NCO(CH_2)_4CHCH_2CH_2S{-}S$
**18**: R = Ac; R′ = $OCH_3$
**19**: R = H; R′ = $OCH_3$
**20**: R = Ac; R′ = H
**21**: R = R′ = H

[a] Ph = phenyl; Ms = methylsulfonyl.

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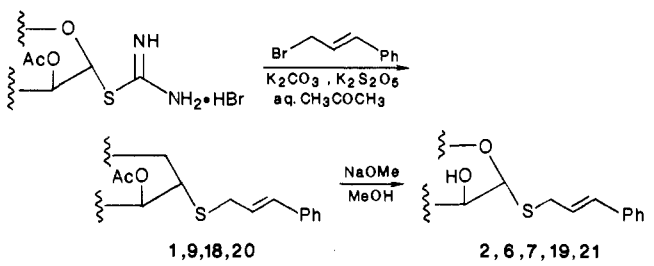
**Table I.** Proliferative Response of Cinnamyl 1-Thioglycosides ([$^3$H]Thymidine Uptake/Δcpm × $10^{-3}$)

| | concn, μM | | | | | | |
|---|---|---|---|---|---|---|---|
| compd | 1000 | 750 | 500 | 250 | 100 | 10 | 0 |
| **2**[a] | 0.18 ± 0.06 | 0 ± 0.04 | 0.04 ± 0.02 | 17.56 ± 8.65 | 63.65 ± 6.89 | 95.87 ± 2.25 | 82.37 ± 11.30 |
| **3**[b] | 42.05 ± 2.55 | 38.87 ± 3.86 | 62.69 ± 5.90 | 67.83 ± 6.98 | 66.74 ± 2.26 | 75.32 ± 5.48 | 80.26 ± 5.80 |
| **4**[b] | 0.06 ± 0.01 | 0.02 ± 0.01 | 1.51 ± 0.01 | 5.80 ± 1.59 | 62.13 ± 3.93 | 93.59 ± 3.54 | 72.80 ± 9.05 |
| **5**[c] | 17.52 ± 0.82 | 31.80 ± 10.33 | 66.62 ± 8.92 | 109.27 ± 3.18 | 129.87 ± 13.61 | 144.48 ± 7.64 | 147.35 ± 8.61 |
| **6**[d] | 5.97 ± 1.92 | 12.79 ± 7.23 | 36.42 ± 6.01 | 59.68 ± 7.32 | 101.06 ± 6.78 | 114.88 ± 7.04 | 93.46 ± 1.17 |
| **7**[d] | 3.85 ± 0.43 | 13.04 ± 4.43 | 28.94 ± 3.20 | 34.14 ± 3.45 | 54.88 ± 3.65 | 64.17 ± 6.39 | 86.88 ± 4.25 |
| **8**[c] | 0 ± 0.1 | 18.46 ± 5.42 | 34.23 ± 7.49 | 39.73 ± 7.45 | 59.03 ± 4.69 | 70.35 ± 3.90 | 72.81 ± 5.70 |
| **12**[c] | 0.06 ± 0.05 | 0 ± 0.06 | 2.26 ± 1.54 | 14.29 ± 6.38 | 21.10 ± 1.61 | 20.98 ± 1.57 | 33.45 ± 4.20 |
| **13**[c,e] | 0.01 ± 0.01 | 0.02 ± 0.01 | 1.23 ± 0.26 | 71.08 ± 1.37 | 101.31 ± 9.69 | 119.89 ± 9.02 | 81.05 ± 2.32 |
| **14**[c,e] | 36.03 ± 7.49 | 38.82 ± 0.09 | 39.27 ± 10.17 | 52.86 ± 12.39 | 66.58 ± 6.26 | 70.68 ± 0.10 | 79.84 ± 5.68 |
| **15**[c,e] | 0.07 ± 0.14 | 0 ± 0.02 | 0 ± 0.03 | 1.73 ± 0.79 | 44.98 ± 10.67 | 68.37 ± 2.28 | 85.76 ± 2.87 |
| **16**[b,e] | 0.02 ± 0.01 | 0.04 ± 0.02 | 0 ± 0.01 | 0 ± 0.01 | 0.61 ± 0.02 | 52.48 ± 3.00 | 72.27 ± 7.13 |
| **17**[b,e] | 0.08 ± 0.02 | 0 ± 0.01 | 0 ± 0.01 | 0 ± 0 | 2.97 ± 0.50 | 75.25 ± 6.5 | 93.24 ± 5.48 |
| **19**[b] | 0 ± 0.02 | 0 ± 0 | 0 ± 0.01 | 1.23 ± 0.38 | 36.21 ± 1.75 | 97.01 ± 2.18 | 87.98 ± 8.22 |
| **21**[c] | 0 ± 0.14 | 0 ± 0.05 | 0.11 ± 0.77 | 26.66 ± 7.30 | 66.34 ± 5.47 | 86.52 ± 5.79 | 89.06 ± 0.16 |

[a] Tested nine times in triplicate. [b] Tested twice in triplicate. [c] Tested thrice in triplicate. [d] Tested once in triplicate. [e] Tested as suspensions; thus the data acquired for these compounds may not be reliable. Data are shown as mean Δcpm × $10^{-3}$ ± SD. D-Mannose and L-rhamnose, when tested by themselves with no modifications, did not block at concentrations up to 1 mM.

**Scheme I**




1,9,18,20 2,6,7,19,21

and enzymatic hydrolysis than *O*-glycosides.

## Chemistry

Cinnamyl 1-thioglycosides **2**, **6**, **7**, **9**, **19**, and **21** (Charts I and II) were synthesized in good yields as outlined in Scheme I. Compounds **3–5** were prepared from cinnamyl bromide and the respective 1-thio-β-D-manno(and α,β-L-rhamno)pyranose sodium salts. In the preparation of the 6-oleamido derivative **16** from **13**, a substantial amount of the 6-acetamido analogue **14** was also isolated. The formation of **14** was apparently due to the contamination of oleic anhydride with acetic anhydride.

## Results and Discussion

In T cell proliferation assay, an enriched T cell population was derived from guinea pigs immunized with ovalbumin in complete Freunds adjuvant. Irradiated (2100 rads) exudate cells (macrophages) were used as a source of the antigen ovalbumin-presenting cells. Initially cinnamyl 1-thio-D-manno(and L-rhamno)pyranosides (**2–5**) were prepared and their effects on guinea pig T cell proliferation were tested by measuring the incorporation of [$^3$H]thymidine uptake (see Experimental Section). These compounds all appeared to block [$^3$H]thymidine uptake to various degrees (see Table I). In both cases the α anomers were more inhibitory than the corresponding β anomers (Table II). Since 6-*O*-α-D-mannopyranosyl-D-mannose was suggested to be capable of blocking early events necessary for an antigen-specific T cell response,[7] compound **7**, a cinnamyl 1-thioglycoside containing this disaccharide, was thus prepared and shown to have good inhibitory activity. It was, however, less potent than the monosaccharide **2** (Table II). Modification of the 6-OH group of **2** was undertaken to probe the effects of other substituents on T cell proliferation. Most of the 6-substituted analogues also have good inhibitory effects. The 6-*O*-methyl derivative (**19**) and the 6-deoxy analogue (**21**) appeared to be more potent than **2** and **4** (Table II). Unfortunately, compounds **13–17** were not very soluble in the testing medium, thus their biological data may not be reliable. Cinnamyl 1-thio-β-L-fucopyranoside (**6**) was also found to be inhibitory, but to a lesser degree than **2** and **4**. Compound **8**, the saturated analogue of **2**, was somewhat less inhibitory than the parent compound. D-Mannose and L-rhamnose, when tested by themselves without modifications, did not block T cell proliferation at concentrations up to 1 mM.

**Table II.** Inhibition of [$^3$H]Thymidine Uptake by Cinnamyl 1-Thioglycosides ($IC_{50}$, μM)[a]

| compd | anomeric config | $IC_{50}$, μM | compd | anomeric config | $IC_{50}$, μM |
|---|---|---|---|---|---|
| **2** | α | 198 | **7** | α | 340 |
| **3** | β | 871 | **8** | α | 402 |
| **4** | α | 160 | **12** | α | 240 |
| **5** | β | 525 | **19** | α | 41 |
| **6** | β | 750 | **21** | α | 102 |

[a] The guinea pig T cell proliferation assay was carried out as described in the Experimental Section. The data are expressed as concentration in μM of cinnamyl 1-thioglycosides required to exhibit 50% inhibition of maximum proliferative response.

The mechanism of blocking T cell proliferation is not clear. These sugar inhibitors may act on macrophages or T cells directly or they may block macrophages in presenting the antigen (ovalbumin) to T cells. Three compounds, **2**, **4**, and **21**, have been used to pretreat macrophage and peritoneal exudate lymphocyte (PELs) populations. Experimental design was such that each cell response to the pretreatment could be observed and reversibility of the effect could be studied (see Experimental Section). The lymphocytes (PELs) represent cells passed over a nylon wool column for T cell enrichment. The macrophage population did not receive such treatment. The conditions for pretreatment were incubation at 37 °C in 5% $CO_2$ for 18–20 h. The concentration of compounds used for pretreatment is indicated in Tables III–V. Macrophages were irradiated with 2100 rads and antigen pulsing was for 1 h at 37 °C with a concentration of 100 μg/mL. Data are shown as mean Δcpm × $10^{-3}$. Table III shows proliferative response of cells pretreated with **4**. Ovalbumin (OVA) pulsed macrophages pretreated with **4** shows effects on both populations of peritoneal exudate

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**Table III.** Proliferative Response[a] (Δcpm) of Cells Pretreated[b] with 4

| | PELs[c] | pretreated[b] PELs |
|---|---|---|
| macrophages (Mϕ) | 6.67 | 4.75 |
| OVA pulsed Mϕ[d] | 141.55 | 95.34 |
| 4 pretreated Mϕ[e] | 4.89 | 2.81 |
| OVA pulsed 4 pretreated Mϕ | 104.36 | 33.97 |
| Mϕ + OVA (10 μg/mL) | 129.51 | 45.99 |
| 4 pretreated Mϕ + OVA (10 μg/mL) | 139.97 | 49.95 |

[a] [$^3$H]Thymidine uptake was determined by liquid scintillation spectrometry, and data are shown as mean Δcpm × $10^{-3}$. [b] Pretreatment of cells with 1 mM of 4 at 37 °C for 18 h. [c] Peritoneal exudate lymphocytes. [d] Macrophages incubated with 100 μg/mL ovalbumin at 37 °C for 1 h. [e] Macrophages pretreated (incubated) with 1 mM 4 at 37 °C for 18 h.

**Table IV.** Proliferative Response[a] (Δcpm) of Cells Pretreated[b] with 2

| | PELs[c] | pretreated[b] PELs |
|---|---|---|
| macrophages (Mϕ) | 1.31 | 1.16 |
| OVA pulsed Mϕ[d] | 41.36 | 30.35 |
| 2 pretreated Mϕ[e] | 1.89 | 1.07 |
| OVA pulsed 2 pretreated Mϕ | 27.75 | 14.20 |
| 2 pretreated Mϕ + OVA (10 μg/mL) | 14.50 | 8.70 |
| 2 pretreated OVA pulsed Mϕ + OVA (10 μg/mL) | 18.94 | 12.06 |

[a] [$^3$H]Thymidine uptake was determined by liquid scintillation spectrometry and data are shown as mean Δcpm × $10^{-3}$. [b] Pretreatment of cells with 500 μM of 2 at 37 °C for 18 h. [c] Peritoneal exudate lymphocytes. [d] Macrophages incubated with 100 μg/mL ovalbumin at 37 °C for 1 h. [e] Macrophages pretreated (incubated) with 500 μM 2 at 37 °C for 18 h.

**Table V.** Proliferative Response[a] (Δcpm) of Cells Pretreated[b] with 21

| | PELs[c] | pretreated[b] PELs |
|---|---|---|
| macrophages (Mϕ) | 1.46 | 1.34 |
| OVA pulsed Mϕ[d] | 45.43 | 29.03 |
| 21 pretreated Mϕ[e] | 2.26 | 1.62 |
| OVA pulsed 21 pretreated Mϕ | 13.97 | 2.63 |
| 21 pretreated Mϕ + OVA (10 μg/mL) | 14.22 | 2.63 |
| 21 pretreated OVA pulsed Mϕ + OVA (10 μg/mL) | 11.14 | 3.82 |

[a] [$^3$H]Thymidine uptake was determined by liquid scintillation spectrometry and data are shown as mean Δcpm × $10^{-3}$. [b] Pretreatment of cells with 500 μM of 21 at 37 °C for 18 h. [c] Peritoneal exudate lymphocytes. [d] Macrophages incubated with 100 μg/mL of ovalbumin at 37 °C for 1 h. [e] Macrophages pretreated (incubated) with 500 μM of 21 at 37 °C for 18 h.

lymphocytes (PELs) (i.e., T cells that are both pretreated and untreated with 4). The effect of 4 on pretreated PELs is very significant (64%) and irreversible with addition of continuous antigen (cf. 49.95 with 95.34). The effect of 4 on PELs is significant (26%) and reversible with addition of continuous antigen (cf. 139.97 with 141.55). Tables IV and V show proliferative responses of cells pretreated with 2 and 21, respectively. Both populations of PELs show considerable inhibition by the pretreatment with either 2 or 21 (33% and 53% for 2; 69% and 91% for 21). These effects are all irreversible with addition of continuous antigen (cf. 18.94 with 41.36, and 12.06 with 30.35 for 2; and 11.14 with 45.43, and 3.82 with 29.03 for 21). The data in Tables IV and V show that the macrophage population's ability to process antigen is significantly inhibited and irreversible. Inhibition indicates possible interference with cellular metabolism.

**Table VI.** Effect of 2 and 4 Given Intraperitoneally[a] on Delayed Hypersensitivity Reaction in Mice

| compd | dose, mg/kg | increase in footpad thickness, mm × 10 | % reduction | P value[b] |
|---|---|---|---|---|
| control | | 5.62 ± 2.0 | | |
| 2 | 30 | 2.07 ± 1.2 | 63 | 0.005 |
| | 10 | 2.46 ± 0.5 | 56 | 0.005 |
| | 3 | 7.30 ± 1.5 | 0 | <0.05 |
| control | | 5.26 ± 2.0 | | |
| 4 | 30 | 2.34 ± 1.1 | 56 | 0.01 |
| | 10 | 2.78 ± 1.3 | 47 | 0.025 |
| | 3 | 4.60 ± 1.6 | 13 | <0.05 |

[a] Compounds were administered intraperitoneally to A/J female mice 20 min before footpad challenge with the antigen (see Experimental Section). Experiments were performed in triplicate with five mice per group. [b] Statistical analyses were performed by using Student's two-tailed $t$ test.

**Table VII.** Effect of 2 and 4 Given Orally[a] on Delayed Hypersensitivity Reaction in Mice

| compd | dose, mg/kg | increase in footpad thickness, mm × 10 | % reduction | P value[b] |
|---|---|---|---|---|
| control | | 1.17 ± 0.18 | | |
| 2 | 30 | 0.82 ± 0.16 | 30 | 0.005 |
| | 10 | 0.82 ± 0.23 | 30 | 0.01 |
| | 3 | 0.85 ± 0.14 | 27 | 0.01 |
| 4 | 30 | 0.77 ± 0.15 | 34 | 0.003 |
| | 10 | 0.96 ± 0.19 | 18 | 0.05 |
| | 3 | 0.98 ± 0.27 | 16 | <0.05 |
| Dex[c] | 2.5 | 0.30 ± 0.06 | 68 | <0.005 |

[a] Compounds were administered orally to A/J female mice 20 min before footpad challenge with the antigen (see Experimental Section). Experiments were performed in triplicate with six mice per group. [b] Statistical analyses were performed by using Student's two-tailed $t$ test. [c] Dexamethasone.

These sugar inhibitors also act on macrophages with other antigens such as PPD (protein purified derivative; a nonspecific antigen from cell-wall microbacteria) and concanavalin A (results not shown). We have so far demonstrated unequivocally that cinnamyl 1-thioglycosides such as 2 and 4 have an inhibitory effect in an antigen-specific T cell proliferation assay. It is thus of interest to see whether this in vitro effect can be translated into an in vivo effect such as blocking the delayed type hypersensitivity reaction (DHT) in mice. Table VI shows the results of typical experiments in which 2 and 4, administered ip 20 min before the antigen challenge, exhibit inhibitory effects in a dose-related response. For example, 2 gave about 63% and 56% inhibition at 30 and 10 mg/kg, respectively. Table VII shows that 2 and 4 are also effective when given orally, but the amount of inhibition is somewhat less. Readings after 48 h give the same pattern of response, but the swelling is less.

In summary, cinnamyl 1-thioglycosides such as 2 and 4 have an inhibitory effect in an antigen-specific T cell proliferation assay in vitro. D-Mannose and L-rhamnose without modifications did not inhibit the response up to concentrations of 1 mM. Both 2 and 4 inhibited DTH reactions in mice in a dose-related manner when administered ip or po. These inhibitors did not affect the viability of the cells as determined by trypan blue exclusion studies.

## Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter at 27 °C. Thin-layer chromatography (TLC) was performed on silica gel $GF_{254}$ (Analtech) plates, and the spots were detected by a ceric sulfate (1%)–sulfuric acid (10%) spray. Flash column chromatography was conducted on silica gel 60 (70–230 mesh ASTM).

High-pressure liquid chromatography (HPLC) was performed on PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min with use of developing solvents as indicated in the experiments. NMR spectra were recorded for solutions in chloroform-*d* (unless stated otherwise) at 200 MHz, with tetramethylsilane as the internal standard. Conventional processing consisted of drying organic solutions with anhydrous sodium sulfate, filtration, and evaporation of the filtrate under diminished pressure.

**Media.** RPMI 1640 and fetal calf serum (FCS) were obtained from Grand Island Biological Co., Grand Island, NY. The media was supplemental with L-glutamine (0.3 mg/mL), garamycin (0.02 μg/mL), penicillin (200 units/mL), 2-mercaptoethanol ($2.5 \times 10^{-5}$ M, Eastman Kodak Co., Rochester, NY) and 5% FCS. All washing procedures were performed with Hanks' balanced salt solution (HBSS)

**Antigens.** Ovalbumin (OVA) was obtained from Miles Laboratories, Kankakee, IL, and complete Freunds adjuvant (CFA) was from Difco Laboratories, Detroit, MI. Sheep red blood cells (SRBC) were from Colorado Serum Co., Denver, CO.

**Animals.** Inbred strain 2 female guinea pigs weighing 400–600 g were obtained from Biological Systems, Toms River, NJ. A/J female mice 20–23 g in weight were obtained from the Jackson Laboratory.

**Guinea Pig T Cell Proliferation Assay.** The in vitro proliferative response of guinea pig T cell was determined by [$^3$H]thymidine incorporation. Strain 2 guinea pigs were immunized with an emulsion of OVA in CFA by injection of a total 0.4 mL (0.1 mL/foot) which contained 100 μg of OVA. Fourteen days postimmunization, a light mineral oil (Marcal 52, Exxon Corp.) was injected intraperitoneally, followed 4 days later by a collection of an exudate. This exudate was washed with HBSS and passed over a nylon wool column. The cells obtained from this column represented an enriched T cell population (80–95%; PELs). The T cell proliferation assay[8] was carried out with some modification. Aliquots of T cell suspension (0.2 mL containing $2 \times 10^5$ cells) were pipetted into round-bottomed wells of microtiter plates (Flow Laboratories, McLean, VA). A suspension of irradiated (2100 rads) exudate cells (10%) were added to the T cell population as a source of antigen-presenting cells. To wells in triplicate was added 10 μL of the antigen (OVA 10 μg/mL) at appropriate dilution. This was followed by addition of cinnamyl 1-thioglycosides at appropriate concentrations, and the cultures were incubated for 72 h at 37 °C in a humidified atmosphere of 5% $CO_2$/95% air. Eighteen to 24 h before termination, 1 μCi of [$^3$H]thymidine (6.7 Ci/mmol, New England Nuclear) was added to each well. Cultures were terminated by aspiration onto glass fiber filter papers, and [$^3$H]thymidine incorporation was determined by liquid scintillation spectrometry. The data is expressed as mean Δcpm.

For the pretreatment experiments (Tables III–V) the PELs (enriched T cells) and irradiated exudate cells (macrophages) were each incubated with the indicated sugar derivative for 18 h at 37 °C and washed with HBSS to remove the unbound cinnamyl 1-thioglycoside. The macrophages were further incubated with OVA (100 μg/mL) at 37 °C for 1 h and washed with HBSS. The two populations of cells were then adjusted for viability by trypan blue before they were mixed together in microtiter plates. Ovalbumin (10 μg/mL) was added to appropriate wells containing untreated macrophages and macrophages previously treated with the indicated sugar derivative. The cultures were then incubated for 72 h at 37 °C and processed as described above.

**Delayed Type Hypersensitivity Assay.** A modification of the methods used by Hahn and co-workers[9] was employed. Briefly, A/J female mice were injected with 200 mg/kg of cyclophosphamide (sc) followed by sensitization (iv) with $10^8$ washed SRBC in 1.1 mL of saline 3 days later. On the sixth day postsensitization, each animal was challenged ($10^8$ SRBC in 20 μL) in one hind footpad with the other footpad receiving 20 μL of saline as a control. After 24 and 48 h footpad swelling was measured with the results being recorded as the difference between the saline-injected footpad vs. the antigen-challenged footpad. Swelling is reported in mm × 10. Constant-pressure calipers with a digital display were used for measuring the footpad swelling. In all instances the test compounds were administered either ip or po to animals fasted overnight at a time 20 min before footpad challenge.

**Cinnamyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (1).** A solution of cinnamyl bromide (4.0 g, 20.3 mmol) in acetone (20 mL) was added to a solution of 2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-thiopseudourea hydrobromide[10,11] (9.72 g, 20 mmol) in $H_2O$ (20 mL) containing $K_2CO_3$ (3.2 g, 230 mmol) and $K_2S_2O_5$ (4.0 g, 18 mmol), and the mixture was stirred vigorously for 30 min at room temperature. Chloroform (80 mL) and $H_2O$ (40 mL) were added, and the organic layer was washed with $H_2O$, dried, and evaporated to a syrup. Crystallization from $Et_2O$–petroleum $Et_2O$ gave 1 (9.3 g, 97%): mp 94–96 °C; $[\alpha]_D$ +185° (*c* 0.98, $CHCl_3$); MS, *m/z* 480 ($M^{\cdot+}$), 420 ($M^{\cdot+}$ – HOAc), 360 ($M^{\cdot+}$ – 2HOAc), 331 ($M^{\cdot+}$ – aglycon); NMR ($CDCl_3$) d 5.24 (br, H-1), 6.18 (4 d, *J* = 9.0, 6.0, and 16.0 Hz, C*H*=CHPh), 6.52 (d, *J* = 16.0 Hz, CH=C*H*Ph), 7.24–7.43 (Ar H). Anal. ($C_{23}H_{28}O_9S$) C, H, S.

**Cinnamyl 1-Thio-α-D-mannopyranoside (2).** A solution of 1 (1.0 g, 2.1 mmol) in MeOH (10 mL) was treated with NaOMe (0.05 g, 0.9 mmol) for 3 h at room temperature. The solution was deionized with ($H^+$) resin and filtered, and the filtrate was evaporated to a syrup, which was purified by silica gel column chromatography ($CHCl_3$–MeOH–$H_2O$, 90:10:1, v/v/v). Crystallization from aqueous 2-propanol gave **2** (0.54 g, 65%): mp 73–75 °C; $[\alpha]_D$ +331° (*c* 1.04, MeOH). Anal. ($C_{15}H_{20}O_5S$) C, H, S.

**Cinnamyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-mannopyranoside.** A solution of cinnamyl bromide (0.45 g, 2.28 mmol) in ethanol (4 mL) was added to a solution of 1-thio-β-D-mannopyranose sodium salt[12] (0.5 g, 2.29 mmol) in $H_2O$ (6 mL). After 20 min at room temperature, the solution was evaporated to a crystalline mass. Pyridine (5 mL) and acetic anhydride (5 mL) were added, and the solution was kept at room temperature for 3 h and poured into ice–water. The solid was collected and washed with cold $H_2O$. Recrystallization from MeOH gave the title compound (0.93 g, 85%): mp 113–114 °C; $[\alpha]_D$ –130° (*c* 1.0, $CHCl_3$); MS, *m/z* 480 ($M^{\cdot+}$), 420 ($M^{\cdot+}$ – HOAc), 360 ($M^{\cdot+}$ – 2HOAc), 331 ($M^{\cdot+}$ – aglycon); NMR ($CDCl_3$) δ 1.97, 2.03, 2.11, and 2.20 (12 H, 4 OAc), 4.15 (2 d, $J_{6',5}$ = 2.5 Hz, $J_{6',6}$ = 12.5 Hz, H-6'), 4.30 (2 d, $J_{6,5}$ = 6.0 Hz, H-6), 4.73 (br, H-1), 5.05 (2 d, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.28 (t, $J_{4,5}$ = 10.0 Hz, H-4), 5.51 (d, H-2), 6.21 (4 d, *J* = 6.0, 9.0, and 16.0 Hz, C*H*=CHPh), 6.50 (d, *J* = 16.0 Hz, CH=C*H*Ph), 7.28–7.45 (m, Ar H). Anal. ($C_{23}H_{28}O_9S$) C, H, S.

**Cinnamyl 1-Thio-β-D-mannopyranoside (3).** This compound was prepared in near-quantitative yield by de-*O*-acetylation of the above peracetate with NaOMe in MeOH similarly as **2**: mp 145–146 °C; $[\alpha]_D$ –215° (c 0.97, MeOH). ($C_{15}H_{20}O_5S$) C, H, S.

**Cinnamyl 1-Thio-L-rhamnopyranosides (4 and 5).** 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[13] (prepared from 32 g of 1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose and 32% HBr in glacial HOAc) was heated with potassium ethyl xanthate (14 g, 88 mmol) in dry acetone (125 mL) under reflux for 5 min. The mixture was cooled and poured into ice–$H_2O$. The solvent was decanted and the syrup was dried in high vacuo to give a mixture of anomers, α:β, 43:57 as estimated by NMR: NMR ($CDCl_3$) δ 6.12 (d, $J_{1,2}$ = 1.5 Hz, H-1α), 5.70 (d, $J_{1,2}$ = 1.0 Hz, H-1β), 5.63 (2 d, $J_{2,3}$ = 3.0 Hz, H-2β), 5.47 (2 d, $J_{2,3}$ = 3.0 Hz, H-2α), 3.92 (m, H-5α), 3.70 (m, H-5β).

A suspension of the above crude syrup (17 g) in methanol (100 mL) was cooled to –10 °C and treated with a solution of NaOMe (3 g) in MeOH (20 mL). The suspension gradually went into

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solution. After 90 min, EtOH (300 mL) and $Et_2O$ (1 L) were added, and the flocculent precipitate was filtered to give a mixture of 1-thiorhamnopyranose sodium salts (6.5 g, 75%), $\alpha$:$\beta$, 43:57 as estimated by NMR.

Cinnamyl bromide (3.0 g, 15.2 mmol) was added to a solution of 1-thiorhamnopyranose sodium salts (3.0 g, 14.8 mmol) in $H_2O$–EtOH (1:1, 30 mL), and the mixture was stirred at 50 °C for 1 h and concentrated to dryness. The residue was put on a column of silica gel and eluted with $CHCl_3$–MeOH–$H_2O$ (90:10:1, v/v/v). The more mobile component was identified as the $\alpha$ anomer **4** (700 mg, 16%): $[\alpha]_D$ −347° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) $\delta$ 5.23 (br, H-1), 1.34 (d, $J$ = 6.5 Hz, $CH_3$). Anal. ($C_{15}H_{20}O_4S$) C, H, S.

The $\beta$ anomer **5** crystallized upon standing. Recrystallization from $CH_2Cl_2$–$Et_2O$ afforded pure **4** (500 mg, 12%): mp 121–124 °C; $[\alpha]_D$ +176° (c 1.0, $CHCl_3$); NMR ($CDCl_3$) $\delta$ 4.62 (br, H-1), 1.38 (d, $J$ = 6.5 Hz, $CH_3$). Anal. ($C_{15}H_{20}O_4S$·0.5$H_2O$) C, H, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-1-thio-$\beta$-L-fucopyranoside.** This compound was prepared from 2-*S*-(2,3,4-tri-*O*-acetyl-$\beta$-L-fucopyranosyl)-2-thiopseudourea hydrobromide[10] similarly as **1**. The product was purified by flash column chromatography on silica gel with $CHCl_3$–EtOAc (98:2, v/v) as the eluant. It was isolated as a syrup (58%): $[\alpha]_D$ +44° (*c* 2.37, $CHCl_3$); NMR ($CDCl_3$) $\delta$ 1.21 (d, $CH_3$-5), 1.98, 2.07, and 2.15 (9 H, 3 OAc), 3.45 and 3.63 (2 q, $SCH_2$), 3.78 (q, $J$ = 6.5 Hz, H-5), 4.49 (d, $J_{1,2}$ = 10.0 Hz, H-1), 5.05 (2 d, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 5.29 (d, H-4), 5.31 (t, H-2), 6.25 (4 d, $J$ = 6.5, 8.0, and 16.0 Hz, C*H*=CHPh), 6.52 (d, $J$ = 16.0 Hz, CH=C*H*Ph), 7.24–7.44 (m, Ar H). Anal. ($C_{21}H_{26}O_7S$) C, H, S.

**Cinnamyl 1-Thio-$\beta$-L-fucopyranoside (6).** The above peracetate was de-*O*-acetylated similarly as **2** to give **6** in 60% yield: $[\alpha]_D$ +97.6° (*c* 0.93, $CHCl_3$); NMR ($CDCl_3$) $\delta$ 1.33 (d, $J$ = 6.0 Hz, $CH_3$-5), 3.70 (t, $J_{2,3}$ = 9.5 Hz, H-2), 3.78 (d, $J_{4,3}$ = 3.0 Hz, H-4), 4.33 (d, $J_{1,2}$ = 9.5 Hz, H-1). Anal. ($C_{15}H_{20}O_4S$) C, H, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-$\alpha$-D-mannopyranosyl)-1-thio-$\alpha$-D-mannopyranoside.** A solution of 1,2,3,4-tetra-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-$\alpha$-D-mannopyranosyl)-$\beta$-D-mannopyranose[14] (1.7 g, 2.5 mmol) in 33% HBr in glacial HOAc (10 mL) was kept at 0–5 °C for 1.5 h. The reaction mixture was processed to give 2,3,4-tri-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-$\alpha$-D-mannopyranosyl)-$\alpha$-D-mannopyranosyl bromide (1.8 g, 2.5 mmol), which was dissolved in dry acetone (5 mL) and reacted with thiourea (0.3 g, 3.9 mmol) at reflux temperature for 6 h. The solvent was evaporated and the syrup was partitioned between $Et_2O$ and $H_2O$. The 2-*S*-(per-*O*-acetylglycopyranosyl)-2-thiopseudourea hydrobromide in the aqueous layer was reacted directly with a solution of cinnamyl bromide (0.54 g, 2.7 mmol) in acetone (30 mL) containing $K_2CO_3$ (0.47 g, 3.4 mmol) and $K_2S_2O_5$ (0.66 g, 3.0 mmol) for 2 h at room temperature. The reaction mixture was worked up as usual to give a syrup (1.7 g), which was purified by flash column chromatography with $CHCl_3$–EtOAc (90:10, v/v) as the eluant. The title compound was isolated in 47% overall yield (0.91 g): $[\alpha]_D$ +150° (c 1.14, $CHCl_3$); MS, $m/z$ 768 ($M^{\cdot+}$), 708 ($M^{\cdot+}$ − HOAc), 648 ($M^{\cdot+}$ − 2HOAc), 617 ($M^{\cdot+}$ − $SCH_2CH$=$CHC_6H_5$); NMR ($CDCl_3$) $\delta$ 4.88 (br, H-1′), 5.22 (br, H-1). Anal. ($C_{35}H_{44}O_{17}S$) C, H, S.

**Cinnamyl 6-*O*-$\alpha$-D-Mannopyranosyl-1-thio-$\alpha$-D-mannopyranoside (7).** The above peracetate was de-*O*-acetylated similarly as **2** to give **7**: $[\alpha]_D$ +272° (*c* 1.03, MeOH). Anal. ($C_{21}H_{31}O_{10}S$) C, H, S.

**3-Phenylpropyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-$\alpha$-D-mannopyranoside.** A solution of **1** (800 mg, 1.67 mmol) in EtOAc (10 mL) and HOAc (10 $\mu$L) containing 10% palladium on charcoal (400 mg) was hydrogenated overnight at room temperature. The catalyst was filtered off and washed with EtOAc. The combined filtrates were evaporated in vacuo to give the title compound (780 mg, 97%): mp 73–74 °C ($Et_2O$–petroleum $Et_2O$); $[\alpha]_D$ +86° (c 1.08, $CHCl_3$); MS, $m/z$ 482 ($M^{\cdot+}$), 422 ($M^{\cdot+}$ − HOAc), 362 ($M^{\cdot+}$ − 2HOAc), 331 ($M^{\cdot+}$ − aglycon). Anal. ($C_{23}H_{30}O_9S$) C, H, S.

**3-Phenylpropyl 1-Thio-$\alpha$-D-mannopyranoside (8).** The above peracetate was de-*O*-acetylated similarly as **2** to give **8** in 92% yield: mp 122–123 °C ($H_2O$); $[\alpha]_D$ +185.4° (c 1.06, MeOH). Anal. ($C_{15}H_{22}O_5S$) C, H, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-*O*-(methylsulfonyl)-1-thio-$\alpha$-D-mannopyranoside (9).** Compound **9** was prepared in 70% yield from 2-*S*-[2,3,4-tri-*O*-acetyl-6-*O*-(methylsulfonyl)-$\alpha$-D-mannopyranosyl]-2-thiopseudourea hydrobromide[15] and cinnamyl bromide similarly as **1**: mp 111–113 °C ($Et_2O$–petroleum $Et_2O$); $[\alpha]_D$ +175° (*c* 1.0, $CHCl_3$); MS, $m/z$ 516 ($M^{\cdot+}$), 456 ($M^{\cdot+}$ − HOAc), 420 ($M^{\cdot+}$ − MsOH), 396 ($M^{\cdot+}$ − 2HOAc), 367 ($M^{\cdot+}$ − aglycon). Anal. ($C_{22}H_{28}O_{10}S_2$) C, H, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-iodo-1-thio-$\alpha$-D-mannopyranoside (10).** A suspension of **9** (2 g, 3.9 mmol) and NaI (1.0 g, 6.7 mmol) in DMF (15 mL) was heated with stirring at 70 °C (bath temperature) overnight. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ and $H_2O$. The organic layer was washed with $H_2O$, dried, and evaporated to a syrup, which was put on a flash column of silica gel and eluted with $CHCl_3$–EtOAc (95:5, v/v). Compound **10** was isolated in 94% yield (2.0 g): mp 91–92 °C (MeOH); $[\alpha]_D$ +203° (*c* 1.06, $CHCl_3$). Anal. ($C_{21}H_{25}IO_7S$) C, H, I, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-1-thio-$\alpha$-D-mannopyranoside (11).** A suspension of **10** (8.0 g, 14.6 mmol) and $NaN_3$ (2.0 g, 30.8 mmol) in DMF (100 mL) was heated with stirring at 85 °C (bath temperature) for 24 h. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ and $H_2O$. The organic layer was washed with $H_2O$, dried, and evaporated to a thick syrup (7.2 g, 98%): $[\alpha]_D$ +107° (c 1.0, $CHCl_3$); MS, $m/z$ 463 ($M^{\cdot+}$), 435 ($M^{\cdot+}$ − $N_2$), 314 ($M^{\cdot+}$ − aglycon). Anal. ($C_{21}H_{25}N_3O_7S$) C, H, N, S.

**Cinnamyl 6-Azido-6-deoxy-1-thio-$\alpha$-D-mannopyranoside (12).** Compound **11** (4.4 g, 9.5 mmol) was de-*O*-acetylated with NaOMe in MeOH similarly as **2** to give crude **12**, which was purified by column chromatography ($CHCl_3$–MeOH–$H_2O$, 95:5:0.5, v/v/v) to yield an oil (2.5 g, 78%): $[\alpha]_D$ +220° (*c* 1.06, MeOH); MS, $m/z$ 337 ($M^{\cdot+}$), 309 ($M^{\cdot+}$ − $N_2$). Anal. ($C_{15}H_{19}N_3O_4S$) C, H, N, S.

**Cinnamyl 6-Amino-6-deoxy-1-thio-$\alpha$-D-mannopyranoside (13).** Hydrogen sulfide was bubbled for 1 h into a solution of **12** (1.6 g, 4.74 mmol) in $CHCl_3$ (15 mL) containing $Et_3N$ (1 mL). The flask was sealed and kept for 18 h at room temperature. The solution was evaporated and the residue was put on a column of silica gel and eluted with $CHCl_3$–MeOH–$NH_4OH$ (70:30:2, v/v/v). Compound **13** was isolated in 68% yield (1.0 g): mp 166–167 °C (MeOH–$Et_2O$); $[\alpha]_D$ +385° (*c* 0.97, MeOH); MS, $m/z$ 311 ($M^{\cdot+}$). Anal. ($C_{15}H_{21}NO_4S$) C, H, N, S.

**Cinnamyl 6-Deoxy-6-oleamido-1-thio-$\alpha$-D-mannopyranoside (16) and the Corresponding 6-Acetamido Derivative (14).** A solution of **13** (200 mg, 0.64 mmol) and oleic anhydride (1 mL) in MeOH (10 mL) was kept at room temperature overnight. The solvent was evaporated and the residue was partitioned between $CHCl_3$ and $H_2O$. The organic layer was washed with aqueous $NaHCO_3$ and $H_2O$, dried, and evaporated to a syrup (two spots), which were separated by column chromatography ($CHCl_3$–MeOH–$H_2O$, 95:5:0.5, v/v/v). The more mobile compound (contaminated with oleic acid) was repurified to give **16** (70 mg, 19%): MS, $m/z$ 575 ($M^{\cdot+}$), 458 ($M^{\cdot+}$ − $CH_2CH$=$CHC_6H_5$), 426 ($M^{\cdot+}$ − $SCH_2CH$=$CHC_6H_5$).

The other product was identified as **14** (130 mg, 57%): mp 183–185 °C; $[\alpha]_D$ +338° (*c* 1.09, MeOH); MS, $m/z$ 353 ($M^{\cdot+}$), 236 ($M^{\cdot+}$ − $CH_2CH$=$CHC_6H_5$), 204 ($M^{\cdot+}$ − $SCH_2CH$=$CHC_6H_5$). Anal. ($C_{17}H_{23}NO_5S$) C, H, N, S.

**Cinnamyl 6-Butyramido-6-deoxy-1-thio-$\alpha$-D-mannopyranoside (15).** A solution of **13** (200 mg, 0.64 mmol) and butyric anhydride (1.0 g) in MeOH (10 mL) was kept overnight at room temperature. It was worked up as usual to give a crystalline mass. Recrystallization from MeOH–EtOAc afforded pure **15** (120 mg, 48%): mp 165–166 °C; $[\alpha]_D$ +338° (*c* 1.1, MeOH); MS, $m/z$ 381 ($M^{\cdot+}$), 264 ($M^{\cdot+}$ − $CH_2CH$=$CHC_6H_5$), 232 ($M^{\cdot+}$ − $SCH_2CH$=$CHC_6H_5$). Anal. ($C_{19}H_{27}NO_5S$) C, H, N, S.

***N*-(Lipoyloxy)succinimide.** *N*-Hydroxysuccinimide (1.15 g, 10 mmol) was added to a solution of lipoic acid (2.06 g, 10 mmol) and DCC (2.1 g, 10.2 mmol) in THF (10 mL). The reaction mixture was stirred for 65 h at room temperature and filtered,

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and the filtrate was evaporated to a crystalline mass. Recrystallization from toluene afforded the title compound (1.8 g, 59%): mp 85–87 °C.

**Cinnamyl 6-Deoxy-6-lipoamido-1-thio-α-D-mannopyranoside (17).** A solution of **13** (70 mg, 0.22 mmol) and *N*-(Lipoyloxy)succinimide (75 mg, 0.25 mmol) in THF (5 mL) containing $Et_3N$ (50 μL) was kept overnight at room temperature and evaporated to dryness. The residue (100 mg) was purified by chromatography ($CHCl_3$–MeOH–$H_2O$, 95:5:0.5, v/v/v) to give **17** (34 mg, 30%): mp 189–190 °C (MeOH); $[\alpha]_D$ +143° (c 1.0, DMF); MS, *m/z* 499 ($M^{\cdot+}$), 382 ($M^{\cdot+}$ – $CH_2CH{=}CHC_6H_5$), 358 ($M^{\cdot+}$ – $SCH_2CH{=}CHC_6H_5$). Anal. ($C_{23}H_{33}NO_5S_3$) C, H, N, S.

**1,2,3,4-Tetra-*O*-acetyl-6-*O*-methyl-β-D-mannopyranose.** A solution of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose[16] (2.94 g, 8.4 mmol) in $CH_2Cl_2$ (30 mL) at 0 °C was methylated with excess $CH_2N_2$ in $CH_2Cl_2$ containing boron trifluoride etherate (0.12 mL, 0.98 mmol). After 2 h, excess $CH_2N_2$ was destroyed with glacial HOAc and the mixture was filtered. The filtrate was washed with aqueous $NaHCO_3$ and $H_2O$, dried, and evaporated to a syrup (3.0 g, 98%). An analytical sample was crystallized from EtOH: mp 102–103 °C; $[\alpha]_D$ −16° (c 1.02, $CHCl_3$). Anal. ($C_{15}H_{22}O_{10}$) C, H.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (18).** Compound **18** was prepared from 1,2,3,4-tetra-*O*-acetyl-6-*O*-methyl-β-D-mannopyranose in 33% overall yield similarly as **1** and the peracetate of **7**. The product was purified by flash column chromatography on silica gel with $CHCl_3$–EtOAc (98:2, v/v) as the eluant. Compound **18** was isolated as an oil: $[\alpha]_D$ +147° (c 2.08, $CHCl_3$); MS, *m/z* 452 ($M^{\cdot+}$), 392 ($M^{\cdot+}$ – HOAc), 332 ($M^{\cdot+}$ – 2HOAc), 303 ($M^{\cdot+}$ – $SCH_2CH{=}CHC_6H_5$); NMR ($CDCl_3$) δ 1.98, 2.06, and 2.12 (9 H, 3 OAc), 3.38 (s, $OCH_3$), 4.34 (m, H-5), 5.27 (br, H-1). Anal. ($C_{22}H_{28}O_8S{\cdot}H_2O$) C, H, S.

**Cinnamyl 6-*O*-Methyl-1-thio-α-D-mannopyranoside (19).** Compound **18** was de-O-acetylated with NaOMe in MeOH similarly as **2** and the product was purified by preparative TLC with $CHCl_3$–MeOH (90:10, v/v) as an irrigant. Compound **19** was isolated as an oil: $[\alpha]_D$ +287° (c 1.17, MeOH). Anal. ($C_{16}H_{22}O_5S{\cdot}H_2O$) C, H, S.

**Cinnamyl 2,3,4-Tri-*O*-acetyl-6-deoxy-1-thio-α-D-mannopyranoside (20).** Compound **20** was prepared from 1,2,3,4-tetra-*O*-acetyl-6-deoxy-α-D-mannopyranose in 46% overall yield similarly as **1** and the peracetate of **7**. The product was purified by flash column chromatography on silica gel with $CHCl_3$–EtOAc (99:1, v/v) as the eluant: MS, *m/z* 422 ($M^{\cdot+}$), 362 ($M^{\cdot+}$ – HOAc), 302 ($M^{\cdot+}$ – 2HOAc), 273 ($M^{\cdot+}$ – $SCH_2CH{=}CHC_6H_5$).

**Cinnamyl 6-Deoxy-1-thio-α-D-mannopyranoside (21).** Compound **20** was de-O-acetylated with NaOMe in MeOH similarly as **2** to give **21** in near-quantitative yield: $[\alpha]_D$ +354° (c 1.0, $CHCl_3$); MS, *m/z* 296 ($M^{\cdot+}$), 278 ($M^{\cdot+}$ – $H_2O$), 147 ($M^{\cdot+}$ – $SCH_2CH{=}CHC_6H_5$). Anal. ($C_{15}H_{20}O_4S{\cdot}0.5H_2O$) C, H, S.

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# Structural Aspects of Ryanodine Action and Selectivity

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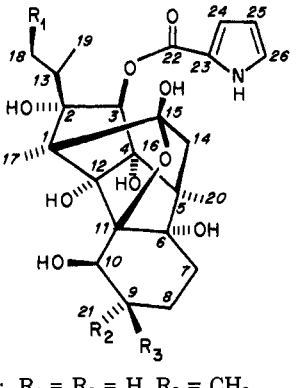
The topography and toxicological relevance of the $Ca^{2+}$-ryanodine receptor complex are evaluated with ryanodine and two natural analogues (9,21-didehydro and the new 18-hydroxy), 13 ryanoid derivatives (prepared from ryanodine and didehydroryanodine by functionalizing the available pyrrole, olefin, and hydroxyl substituents), and four degradation products. The potency of ryanoids at the skeletal muscle sarcoplasmic reticulum specific binding site generally parallels their toxicity to mice, supporting the toxicological relevance of the $Ca^{2+}$-ryanodine receptor. The optimal receptor potency of ryanodine and didehydroryanodine is reduced 3–14-fold by hydroxylation at an isopropyl methyl substituent, epimerization at $C_9$, oxidation or acetylation of the $C_{10}$-hydroxyl, or epoxidation at the 9,21-position; other ryanoids are less active. Ryanodol and didehydroryanodol, in contrast to ryanodine and didehydroryanodine, have low toxicity to mice and little activity at the mammalian receptor, yet they are potent knockdown agents for injected houseflies or cockroaches, suggesting a possible difference in the target sites of mammals and insects.

Ryanodine (**1**)[1,2] and 9,21-didehydroryanodine (**2**)[3-5] are the active principles of the botanical insecticide ryania, which is the ground stem wood of the shrub *Ryania speciosa* (family Flacourtiaceae).[6,7] Many degradation products and derivatives are known from studies on the characterization of **1**,[2] and other minor ryanoids are identified from *R. speciosa*,[5] but no information is available on their biological activity.

Ryanodine is a muscle poison that specifically uncouples the electrical signal of the transverse tubule from the $Ca^{2+}$-release mechanism of the sacroplasmic reticulum (SR).[8,9] The toxic action of ryanodine is attributed to binding to the $Ca^{2+}$-activated open state of the channel involved in the release of contractile $Ca^{2+}$ from the SR, resulting in a change of channel structure possibly preventing its complete closing.[10-13] This "$Ca^{2+}$-ryanodine receptor complex", as assayed with [$^3$H]ryanodine[3] in skeletal and cardiac preparations, is highly specific, $Ca^{2+}$-activated, biochemically relevant, and pharmacologically unique.[10,11] The [$^3$H]ryanodine binding site elutes from sepharose as a high-molecular-weight oligomer made



**1**: $R_1 = R_3 = H$, $R_2 = CH_3$
**2**: $R_1 = H$, $R_2 = R_3 = {=}CH_2$
**3**: $R_1 = OH$, $R_2 = CH_3$, $R_3 = H$

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(1) Rogers, E. F.; Koniuszy, F. R.; Shavel, J., Jr.; Folkers, K. *J. Am. Chem. Soc.* **1948**, *70*, 3086.

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