regards the lactone ring. This might account for the strong activity observed for racemic isopicrostegane (15). The enantiomer of isopicrostegane (15) shown in Figure 1 also bears some similarities with an enantiomer of stegane (11) (which is active in the racemic form). The fact that the lactone ring of this enantiomer of stegane occupies a position different from that of (-)-podophyllotoxin (2) might account for the comparatively low activity of stegane (11). The configuration of isopicrostegane (15) in Figure 1 is opposite to that of natural steganacin (4). Indeed, the latter is described as having an absolute configuration opposite to those of colchicine (1) and podophyllotoxin (2) on the basis of X-ray diffraction studies (without heavy atom). In our view, this is contradictory to the fact that steganacin (4) has a strong antitubulin activity.

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Synthesis and Antitumor Activity of Analogues of the Antitumor Antibiotic Chartreusin[1,2]

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First isolated in 1953 from a fermentation broth, chartreusin (1) has received renewed interest as a result of substantial antitumor activities recently demonstrated in several murine test systems. Poor water solubility frustrated formulation attempts, and rapid biliary excretion observed in mice made 1 an improbable candidate for clinical development but an excellent candidate for an analogue synthesis program. From a common intermediate, which was prepared from 1, three analogues were synthesized wherein the disaccharide moiety of 1 was systematically replaced with fucose (6), glucose (7), and the disaccharide maltose (8). Each of the three analogues had a cytotoxic potency against cultured L1210 cells which was equal to, or better than, that shown by 1. Based on the structural similarity with the parent, an improved water solubility, and a favorable accessibility through synthesis, maltoside 8 was chosen for further antitumor evaluation with an in vivo test system. Versus murine P388 leukemia, 8 showed reproducible activity comparable to chartreusin at similar dose levels. Although 8 caused no observable toxic effects at therapeutic dose levels when given ip, neither 1 nor 8 produced active indications when administered subcutaneously.

In 1953 the isolation of chartreusin (1) from the culture broth and mycelial cake of *Streptomyces chartreusis* was reported.[3] The assigned generic name was appropriate for the greenish-yellow crystalline antibiotic which exhibited a limited spectrum of antibacterial properties.[4] In a series of communications[5] from the University of Zurich the structure elucidation of chartreusin was set forth. However, the synthesis of 1 has not appeared in the literature.[6] Although no antineoplastic properties were originally found for the fermentation product, a recent reevaluation[7] of 1 using current antitumor test systems revealed significant activity in the murine L1210 and P388 leukemias, as well as in the B16 melanoma system. The latter can be considered a solid tumor model. In spite of the good activity shown in these three signal tumor systems, it is unlikely that chartreusin will receive further preclinical development because of two adverse factors. Although substantial activity was observed when tumor cells were inoculated intraperitoneally (ip) and when drug was also given ip, a rapid biliary excretion of 1 after intravenous (iv) administration resulted in plasma and tissue concentrations of drug below the necessary therapeutic level.[7] Oral (po) as well as subcutaneous (sc) administration of 1 failed to produce a positive antitumor response. In addition to the dependence of activity on the administration route, a second difficulty was encountered when a clinical formulation of the poorly soluble antibiotic (15 μg/mL H_2O) was attempted. Although formulation techniques resulted in a 300-fold enhancement of aqueous solubility, an acceptable clinical formulation was not achieved.[8]

Although chartreusin itself is an improbable candidate for clinical trials, its novel structure in combination with impressive activities in three experimental mouse tumor systems suggested the synthesis of chartreusin analogues. With the intention of using 1 as a synthetic starting material, a program was initiated to synthesize analogues of 1 which might possibly have (i) more favorable pharmacodynamic properties and (ii) improved water solubilities. Chartreusin, as indicated in structure 1, has a planar pentacyclic ring system which is linked by a glycosidic bond to a disaccharide moiety (2-*O*-α-D-digitaloxy-β-D-fucosyl). For initial structure modifications of 1, the approach selected required holding constant the aglycon of the glycoside (9, chartarin) while varying the carbohydrate

(1) Presented before the Division of Medicinal Chemistry at the 178th National Meeting of the American Chemical Society, Washington, D.C., Sept. 1979, Abstr MEDI 48.
(2) NIH Visiting Postdoctoral Fellows (a) from the Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan, and (b) from the Institute of Microbial Chemistry, Tokyo, Japan.
(3) Leach, B. E.; Calhoun, K. M.; Johnson, L. E.; Teeters, C. M.; Jackson, W. G. *J. Am. Chem. Soc.* **1953**, *75*, 4011.
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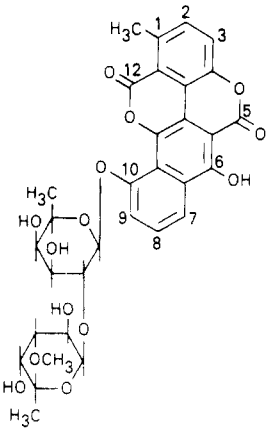

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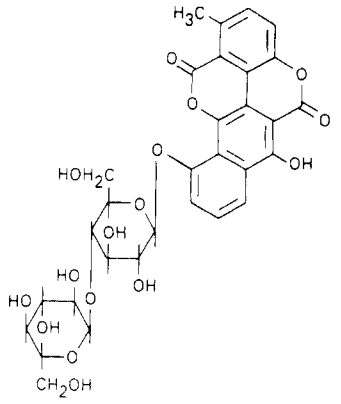


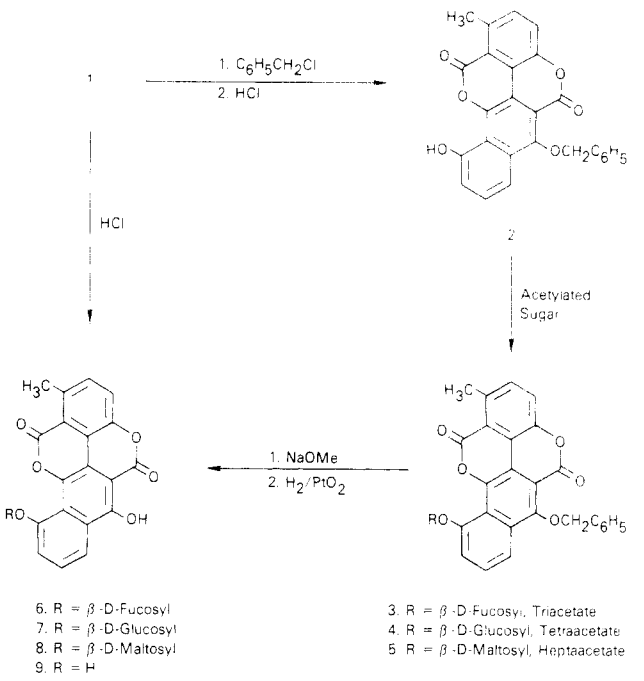
8

portion of the molecule. Since the supply of 1 which was available for chemical modification was limited, the scope of structure–activity relationship (SAR) studies was necessarily restricted. Nevertheless, it was hoped that some information concerning the influence of the disaccharide substituent on antitumor activity, solubility, and partition coefficient (log P) could be acquired. Therefore, a series of analogues was projected to determine if the unique disaccharide group of 1 is specific for cytotoxicity and/or transport or if more common sugar units, having a greater hydrophilicity, could be substituted for the digitalose–fucose assembly. Accordingly, work was begun to synthesize analogues wherein the aglycon (9) was substituted at the 10 position with β-D-fucosyloxy (6), β-D-glycosyloxy (7), or with the disaccharide, β-D-maltosyloxy (8). The maltosyloxy analogue was of particular interest because of its close resemblance to chartreusin. In similarity with 1, the disaccharide substituent of 8 has an α linkage between sugar units, which in turn are linked to the aglycon with a β-glycosidic bond. Since the disaccharide group of 8 has a greater percentage composition of oxygen than that of 1, there was the expectation that the maltosyloxy analogue would have a better water solubility than the parent.

Chemistry. The projected three analogues were synthesized from a common intermediate (2) which was prepared by exhaustive benzylation of chartreusin (1) in hot acetonitrile solution with benzyl bromide and silver oxide. The carbohydrate moiety was removed and discarded by acid cleavage of the glycosidic bond to give 6-O-benzylchartarin (2) in 68% overall yield. The infrared spectrum of 2 in chloroform showed a single carbonyl absorption at 1748 cm^{-1}. A chloroform solution of chartarin (9) not only gives the 1748-cm^{-1} peak but also gives a carbonyl absorption at 1690 cm^{-1}. The latter is assignable to a hydrogen bonded, or chelated, structure involving a lactone carbonyl and the neighboring phenolic hydroxyl to form a six-membered ring. Similar shifts to lower frequencies in the infrared can be observed for O-hydroxybenzoate esters. All attempts to synthesize intermediate 2 by selective benzylation of chartarin (9) failed. For example, treatment of 9 with benzyl bromide gave mixtures wherein alkylation of the undesired phenolic group predominated.

The Koenigs–Knorr synthesis was employed to provide the glycosidic intermediates. Accordingly, condensation of the 1-bromo derivatives of tri-O-acetylfucose, tetra-O-acetylglucose, or hepta-O-acetylmaltose with 6-O-benzylchartarin (2) in pyridine solution in which silver carbonate was slurried gave 3, 4, and 5, in yields of 11, 54, and 35%, respectively (Scheme I). Regardless of the stereochemistry of the parent halo sugar, the Koenigs–Knorr reaction generally gives the isomer in which the substituent at C-1′ is trans to the C-2′ acyloxy group. Therefore, the glycosidic linkages of condensation products 3–5 were assigned the β configuration. The mass spectra of 3 and 4 showed an intense ion at m/e 334 resulting from the loss of acetylated sugar residues and benzyl protection groups from the molecular ion with concomitant hydrogen transfers. Although the mass spectrum of 5 did not show a molecular ion, the m/e 334 peak was, nevertheless, prominent in the spectrum. The spectrum of 6-O-benzylchartarin (2) also had a strong peak at m/e 334 resulting from cleavage of the benzyl group and addition of a hydrogen atom.

Scheme I



For each of the intermediates, deblocking was accomplished in two steps. Acetyl groups were removed with stoichiometric amounts of sodium methylate in methanol solution; the benzyl blocking groups were, in turn, removed by hydrogenolysis. Since deacetylation gave materials which were not usefully soluble in solvents appropriate for hydrogenation, the benzyl derivatives of 6–8 were pertrimethylsilylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile solution. The resulting trimethylsilyl derivatives were sufficiently soluble in anhydrous tetrahydrofuran to give solutions which smoothly underwent hydrogenolysis. Over the two deprotection steps, 7 and 8 were obtained in yields of 57 and 50%, respectively. However, the fucose derivative (6) could only be obtained in 26% overall yield. Fucoside 6 was also prepared by controlled acid hydrolysis of chartreusin (1), but only a 12% yield of 6 was achieved, accompanied by a 58% yield of aglycon 9. The fucoside products, obtained from either the degradative or the synthetic method, were identical. Therefore, the β-configurational assignment for 6 was further supported and, by analogy, the β assignment for 7 and 8.

Partition Coefficients. In order to facilitate the comparison of analogues 6–8 with chartreusin (1) and chartarin (9), partition coefficients (log P) were determined for each and are recorded in Table I. Although an octanol/water partition coefficient for 1 was determined experimentally, the very poor solubility of 9 made a similar experimental determination impossible. Therefore, the tabulated log P for 9 was obtained by calculation using the fragment method.[9] The log P values for analogues 6–8, which ap-

Table I. Growth Inhibition of Cultured L1210 Cells after 48-h Drug Exposure

compd, mol/mL	% inhibn trial 1	% inhibn trial 2	log P
chartreusin (1)			1.80[a]
1.45×10^{-8}	100	100	
1.45×10^{-9}	21	32	
chartarin (9)			4.19[b]
1.65×10^{-8}	57	58	
1.65×10^{-9}	0	7	
fucoside (6)			2.07[c]
1.44×10^{-8}	100	100	
1.44×10^{-9}	71	65	
glucoside (7)			1.82,[c] 1.35[d]
1.45×10^{-8}	100	97	
1.45×10^{-9}	59	47	
maltoside (8)			1.68,[c] 0.95[d]
1.55×10^{-8}	98	97	
1.55×10^{-9}	84	61	

[a] Experimentally measured. [b] Calculated. A direct determination was not possible due to limited solubility. [c] Determined using a high-pressure LC method. [d] Calculated using π values.

pear in Table I, were measured using the liquid chromatography (LC) method of McCall[10] with an octadecylsilyl column eluted with acetonitrile–water (1:1). After calibration of the LC system with **1**, for which an octanol/water log P was in hand, relative log P values were obtained for **6–8** from elution time data.

Using a π value of 4.19 for **9** and the literature[11] value of −2.84 for the *O*-β-glucosyl group, a calculated log P of 1.35 for glucoside **7** was obtained which is in fair agreement with the value obtained by the LC method (Table I). For maltoside **8**, the LC method gave log $P = 1.68$, which indicated a greater lipophilicity than anticipated. However, a cross-check on the log P for **8** using π parameters was not possible because the π value for the *O*-β-maltosyl group was not available. Therefore, *p*-nitrophenol β-D-maltoside was synthesized using a literature[12] procedure. The octanol/water log P was found to be −1.39. Using $\pi = 1.85$ for the *p*-$NO_2C_6H_4$ substituent,[13] a π value of −3.24 for the *O*-β-maltosyl group was calculated. Bearing in mind that $\pi = -2.84$ for the *O*-β-glucosyl group, it is surprising that addition of a second glucose moiety to the first, as is the case for the maltosyl group, results in only a 0.4 increase in hydrophilicity. Apparently the terminal glucose unit of *p*-nitrophenol maltoside is not extended but tends to fold back in solution and, therefore, does not fully contribute to a hydrophilic effect.

Using $\pi = -3.24$ for the maltosyl substituent and $\pi = 4.19$ for aglycon **9**, a value of 0.95 was calculated for the log P of the maltoside analogue. The rather large disparity between the calculated log P and that found with the LC method (Δ 0.73) suggests that the maltosyl group is less effective in imparting hydrophilicity to the aglycon than might be expected. Apparently, a solution conformation of the disaccharide moiety of **8** exists which is more convoluted and hydrogen bonded to the aglycon than is the case for *p*-nitrophenol maltoside. As a result, the apparent π constant for the maltosyl group in **8** is probably less negative than that calculated for the *p*-nitrophenol derivative ($\pi = -3.24$). Nevertheless, the solubility determination of **8** was undertaken because the log P data indicated that **8** might have the best water solubility of the synthetic analogues. The UV detector of a liquid chromatograph was calibrated with solutions of known concentrations of **8** and found to give a linear response over the requisite analysis range. Analysis solutions were prepared by stirring an excess of **8** with water at room temperature, followed by a millipore filtration prior to LC analysis. The dissolution rate of **8** in water was quite slow. The maximum solubility of 45 μg/mL was reached only after stirring for 40 h. After stirring for 15 h the concentration of **8** was 25 μg/mL.

Table II. Comparison of Chartreusin (1) and Maltosylchartarin (8) against in Vivo P388 Murine Leukemia[a]

dose, mg/kg	% ILS (T − C) chartreusin trial 1	chartreusin trial 2	maltosylchartarin trial 1	maltosylchartarin trial 2
400				58 (1.0)
200	43 (−2.5)	58 (−1.8)	49 (0.8)	35 (0.8)
100	93 (−2.2)	45 (−1.5)	40 (−0.5)	31 (1.6)
50	60 (−3.7)	54 (0.3)	34 (1.5)	44 (0.5)
25	47 (−1.9)	56 (0.2)	47 (2.0)	37 (1.0)
12.5	46 (0.1)	17 (−0.1)	47 (2.1)	45 (1.4)

[a] Male CDF_1 mice (17–22 g) were given 10^6 P388 cells ip on day 0. Test compounds suspended in Emulphor[16]–0.9% saline were administered ip beginning on day 1 and continuing on days 5 and 9 (three injections). Six animals were used for each dose level. The median survival times for the untreated control groups in trials 1 and 2 were 11.4 and 11.7 days, respectively. Median increase in life span of the test animals beyond the survival time of the untreated control animals expressed as a percentage (% ILS) was used to evaluate antitumor activity. Activity is defined for this study as a % ILS of ≥25%. Values in parentheses give the difference of the average body weight change in grams of the test group (T) and the control group (C) as measured on days 1 and 5.

Cytotoxicity and Antitumor Evaluation. The range and thoroughness of biological evaluations of synthetic analogues resulting from a synthetic sequence that requires a scarce starting material are necessarily limited. Therefore, the strategy chosen to screen **6–8** was to use results from in vitro cytotoxicity studies to select one of the three for resynthesis in amounts large enough for in vivo testing. The growth-inhibition results of cultured L1210 cells[14] are given in Table I for chartreusin (**1**) and its aglycon (**9**) run in parallel experiments with the fucoside (**6**), glucoside (**7**), and maltoside (**8**) analogues. At a concentration of approximately 1.5×10^{-9} mol/mL, the three synthetic analogues inhibited cell growth to about the same extent. Although it was gratifying to observe an equivalent cytotoxicity for each of the three analogues at the same level of potency as the parent antibiotic, the cytotoxicity assay, unfortunately, did not allow a selection to be made from among the three.

In view of the cytotoxic equivalence, the maltoside analogue (**8**) was chosen for resynthesis and in vivo antitumor evaluation on the basis of (i) its structural similarity to the parent, (ii) its improved water solubility with respect to the parent, and (iii) the favorable overall percentage yield of maltoside given by the synthetic sequence. Table II shows the results of two in vivo experiments wherein chartreusin (**1**) and the maltoside (**8**), in a direct com-

(9) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979; Chapter 4. We are indebted to Dr. Leo for the calculated log P of **9**.
(10) McCall, J. M. *J. Med. Chem.* **1975**, *18*, 549.
(11) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(12) Babers, F. H.; Goebel, W. F. *J. Biol. Chem.* **1934**, *105*, 473.
(13) Reference 9, p 199.
(14) The cytotoxicity assay has been previously described: Takai, M.; Uehara, Y.; Beisler, J. A. *J. Med. Chem.* **1979**, *22*, 1380.

parison, were tested for antitumor activity against P388 mouse leukemia. The standard protocol[15] used by the National Cancer Institute for the P388 test system was followed. Drugs were suspended in saline with Emulphor[16] and were administered ip on days 1, 5, and 9 after tumor implantation. Although chartreusin consistently gave slightly greater percent increases in the life span of the test animals, comparable responses (% ILS = 50) were recorded for 1 and 8 at approximately the same dose levels. At the higher dose levels, chartreusin-treated animals showed drug-related toxicity in the form of weight loss with respect to an untreated control group (T - C). Substantial weight losses at therapeutic doses are characteristic of many antitumor drugs. However, maltoside 8 by contrast caused no weight loss toxicity indications even at 400 mg/kg. On the contrary, tumored animals treated with 8 showed net weight gains over the control group at all doses in both trials.

In order to determine if the activity of maltoside 8 is dependent on administration route as was observed for chartreusin, an in vivo P388 leukemia assay was carried out exactly as before, except that 1 and 8 were administered subcutaneously. In the sc assay, neither chartreusin (1) nor the maltoside analogue (8) showed activity against ip implanted P388 leukemia.

Discussion

None of the three synthetic analogues were clearly superior to chartreusin in terms of water solubility, cytotoxicity, and in vivo antitumor activity. In comparison to 1, analogue 8 showed a better water compatibility as evidenced by the relative ease with which saline-Emulphor suspensions were formed and by the improved water solubility. In the route-dependency experiment, when the animals in the higher dose level groups were prepared for sc drug administration on the 5th day, the day 1 dose of chartreusin could be palpated at the administration site. By contrast, the maltoside analogue was completely absorbed at all dose levels, including the 400 mg/kg level. However, the threefold solubility increase shown by 8, although an improvement over the parent, fell considerably short of expectations; it would seem unlikely that that modest solubility enhancement could lead to an acceptable clinical formulation.

The results of the direct comparison study given in Table II show the broad, essentially flat dose–response curves which are characteristic of chartreusin and its maltoside analogue. Although a number of interpretations can be given in explanation of these curves, the simplest, and the one which comes most readily to mind, is related to the slow dissolution rates and low solubilities of 1 and 8. Thus, the % ILS values produced by 1 and 8 did not show increases with increasing dose beyond threshold dose levels. Not only was the solubility of 8 found to be poor, but also a considerable amount of time was required to arrive at the maximum solubility. In the experiment in which 1 or 8 was administered subcutaneously, solubility characteristics apparently limited blood concentrations of drug to below the therapeutic level such that excretion mechanisms effectively competed for available drug and active indications were not observed. In experiments in which drugs are given intraperitoneally, available evidence suggests that % ILS values for 8 might be increased through a change in the administration schedule. McGovren et al. reported[7] that when chartreusin was administered on days 1–9 posttumor implant according to a QD 1–9 schedule (nine injections) a maximum % ILS was obtained which was 50% greater than that achieved with a Q4D schedule (days 1, 5, and 9, three injections). Therefore, solubility characteristics of chartreusin are not efficacy limiting in a Q4D assay. Since the maltoside analogue is biologically similar to the parent, it is likely that the % ILS values given for 8 in Table II could be increased by 50% if 8 were administered according to a QD 1–9 schedule.

Although none of the chartreusin analogues herein described appear to have clinical potential, the analogues have served to locate the active portion of the chartreusin molecule. The unusual disaccharide moiety of chartreusin can be replaced by more common sugar substituents, and the resulting analogues have retained in vitro as well as in vivo antileukemic effects with equivalent potency. Future efforts might explore the effect of oligosaccharide or amino sugar substituents on biological and physical properties.

Experimental Section

Routine purity analyses and the determination of relative partition coefficients were carried out with a Waters Associates, Inc., Model ALC/GPC-244 liquid chromatograph equipped with a fixed-wavelength (254 nm) UV detector and a 4 mm (i.d.) × μBondapak/C_{18} column which was eluted with acetonitrile-water mixtures. A Cary Model 15 spectrophotometer was used to obtain UV spectra, and a Perkin-Elmer Model 621 was used to record infrared spectra. Proton NMR spectra were recorded with a Varian HA-100D spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane, which was used as internal standard. Mass spectra were obtained by direct probe insertion with a DuPont 21-492 spectrometer operated with a 75-eV ionizing voltage. When necessary, samples were peracetylated with an acetic anhydride–pyridine (1:2) mixture. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytic Services and Instrumentation, NIAMDD, NIH, and by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbol were within ±0.3% of theoretical values.

10-Hydroxy-1-methyl-6-(phenylmethoxy)benzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (2). Chartreusin (1; 2.56 g, 4.0 mmol) in hot acetonitrile was treated with silver(I) oxide (4.6 g, 20 mmol) and benzyl bromide (3.4 g, 20 mmol). The mixture was stirred and heated at 80 °C (oil bath) for 2 h and then filtered through Celite without cooling. The filtrate was refluxed with 3 N aqueous HCl solution (160 mL) for 0.5 h. The reaction mixture was concentrated to 400 mL, and the precipitate (1.4 g) was collected by filtration and dried. Crystallization of the precipitate from chloroform gave 2 (1.16 g, 68%) as yellow needles: mp 236–238 °C; UV (EtOH) λ_{max} 237 nm (ϵ 41 000), 263 (38 000); IR ($CHCl_3$) 1748, 1490, 1369, 1351, 1252, 1108, 1056 cm^{-1}; MS *m/e* (relative intensity) 424 (42.5), 334 (77.8), 333 (83.3), 91 (100). Anal. ($C_{26}H_{16}O_6$, 424.4) C, H, O.

1-Methyl-6-(phenylmethoxy)-10-[(2,3,4-tri-*O*-acetyl-6-deoxy-β-D-galactopyranosyl)oxy]benzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (3). Tetra-*O*-acetyl-D-fucose[17] (2.0 g, 6 mmol) was converted to the bromide by the action of acetic acid saturated with hydrogen bromide (10 mL). After standing at room temperature for 1 h, the mixture was diluted with chloroform (30 mL) and shaken five times with water. The chloroform solution was dried ($CaCl_2$) and evaporated to give tri-*O*-acetyl-α-D-fucosyl bromide as an oil, which was used without further purification.

To a solution of 2 (850 mg, 2 mmol) in 80 mL of anhydrous pyridine was added 2.2 g of silver carbonate and the above bromide. After stirring the mixture at room temperature for 1.5

(15) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 9.

(16) Polyoxyethylated vegetable oil: GAF Corp., New York, N.Y. 10020.

(17) Levvy, G. A.; McAllan, A. *Biochem. J.* **1961**, *80*, 433.

h, chloroform (500 mL) was added and insoluble material was removed by filtration. The filtrate was washed with 1 N aqueous HCl (4 × 250 mL) followed by water and dried over $CaCl_2$. The chloroform solution was evaporated to dryness after the addition of a small amount of silica. The silica with adsorbed materials was loaded onto the top of a silica column which was eluted with benzene–acetone (20:1). The main fraction crystallized with benzene–cyclohexane to give 150 mg (11%) of **3**: mp 179–181 °C; UV (EtOH) λ_{max} 236 nm (ϵ 38000), 266 (42000); IR ($CHCl_3$) 1743, 1613, 1577, 1490, 1350, 1247, 1071 cm^{-1}; NMR ($CDCl_3$) δ 1.29 (d, J = 6 Hz, 3 H, fucose methyl), 1.96, 2.04, 2.24 (s, 3 H each, acetyl groups), 2.89 (s, 3 H, aromatic methyl), 4.08 (q, J = 6 Hz, $-CHCH_3$), 5.19 (s, 2 H, $-CH_2Ph$); MS m/e (relative intensity) 696 (0.5), 606 (1.6), 514 (0.8), 424 (79), 334 (100), 273 (37.1). Anal. ($C_{38}H_{32}O_{13}$, 696.6) C, H, O.

1-Methyl-6-(phenylmethoxy)-10-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]benzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (4). To a solution of **2** (454 mg, 1.07 mmol) in anhydrous pyridine (40 mL) was added silver carbonate (1.1 g, 4 mmol) and tetra-*O*-acetyl-β-D-glucosyl bromide[18] (1.6 g, 3.9 mmol). The mixture was stirred at room temperature for 2 h, then diluted with chloroform (400 mL), and filtered through a pad of Celite. The filtrate was shaken with 1 N aqueous HCl solution (3 × 160 mL) and water. After drying over Na_2SO_4, the chloroform solution was evaporated, and the residue was taken up in benzene and chromatographed on silica. Elution with benzene–acetone (20:1) gave **4** as yellow needles (434 mg, 54%) after crystallization from benzene: mp 233–234 °C; UV (EtOH) λ_{max} 236 nm (ϵ 39000), 267 (42000); IR ($CHCl_3$) 1743, 1612, 1576, 1490, 1349, 1070 cm^{-1}; NMR ($CDCl_3$) δ 1.98, 2.01, 2.07, 2.08 (s, 3 H each, acetyl groups), 2.90 (s, 3 H, aromatic $-CH_3$), 5.20 (s, 2 H, $-CH_2Ph$); MS m/e (relative intensity) 754 (0.3), 664 (0.7), 424 (7.9), 334 (50.6), 331 (16.2), 271 (2.3), 211 (1.9), 169 (65.2), 43 (100). Anal. ($C_{40}H_{34}O_{15}$, 754.7) C, H.

1-Methyl-6-(phenylmethoxy)-10-[[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]benzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (5). To a solution of **2** (1.27 g, 3.0 mmol) in anhydrous pyridine (120 mL) was added silver carbonate (1.7 g, 6 mmol) and hepta-*O*-acetyl-β-D-maltosyl bromide[19] (4.2 g, 6 mmol). The reaction conditions and workup procedures were identical with those described above for **4**. Crystallization of the main chromatography fraction from acetone gave 1.10 g (35%) of crystalline **5**: mp 224–225 °C; UV (EtOH) λ_{max} 236 nm (ϵ 38800), 266 (41700); IR ($CHCl_3$) 1741, 1610, 1573, 1488, 1367, 1349 cm^{-1}; NMR ($CDCl_3$) δ 1.96 (s, 3 H, –OAc), 2.03 (s, 9 H, –OAc × 3), 2.08 (s, 6 H, OAc × 2), 2.10 (s, 3 H, –OAc), 2.88 (s, 3 H, aromatic $-CH_3$), 5.20 (s, 2 H, $-CH_2Ph$). Anal. ($C_{52}H_{50}O_{23}$, 1042.9) C, H.

10-[(6-Deoxy-β-D-galactopyranosyl)oxy]-6-hydroxy-1-methylbenzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (6). Partial Hydrolysis of 1. Chartreusin (**1**; 1.0 g, 1.56 mmol) was dissolved in hot acetonitrile (250 mL), treated with 0.1 N aqueous HCl (250 mL), and stirred at 75 °C (oil bath) for 3 h. When the reaction solution was cooled to room temperature, chartreusin aglycon (**9**) separated and was collected by filtration. Evaporation of the filtrate gave a residue over which 200 mL of chloroform was refluxed briefly. On cooling to room temperature, additional aglycon (**9**) separated and was removed by filtration. The filtrate was evaporated to dryness after the addition of a small amount of silica. The silica with adsorbed materials was loaded onto a silica column from which a third crop of **9** was eluted with chloroform. The combined crops of **9** weighed 304 mg (58%): mp 308–310 °C (lit.[20] mp 310–311 °C); MS m/e (relative intensity) 334 (100), 306 (4.7), 250 (6.6), 165 (5.7), 167 (10.4).

Continued elution with chloroform–methanol (10:1) gave 60 mg (12%) of the fucoside (**6**), which melted at 266–268 °C after recrystallization from tetrahydrofuran: UV (EtOH) λ_{max} 236 nm (ϵ 32000), 263 (26000); MS (tetraacetate derivative) m/e (relative intensity) 648 (0.3), 606 (0.7), 376 (2.9), 334 (100), 273 (35.8), 213 (6.3), 171 (17.3), 153 (56.3), 111 (75.8). Anal. ($C_{25}H_{20}O_{10}$, 480.4) C, H, O.

Preparation from 3. A solution of **3** (350 mg, 0.5 mmol) in chloroform (20 mL) and methanol (10 mL) was cooled to 0 °C with an ice bath and, with stirring, 15 mL of 0.1 N sodium methoxide in methanol was added. The disappearance of **3** was monitored with TLC (silica, benzene–acetone, 10:1). After stirring for 80 min at 0 °C, protected from moisture with a $CaSO_4$ drying tube, the reaction was terminated by neutralization with 0.1 N aqueous HCl (15 mL). Solvent was removed at reduced pressure at 40 °C and the residue was stirred vigorously with methanol (100 mL). The resulting yellow solid was removed by filtration, dried, and boiled with acetone (400 mL). Following a hot filtration, the solution was concentrated on a steam cone to about 300 mL and then allowed to crystallize at room temperature, which gave 181 mg (63%) of yellow crystals: mp 261–262 °C. Anal. ($C_{32}H_{26}O_{10}$, 570.5) C, H, O.

A suspension of the benzyl derivative (57 mg, 0.1 mmol) in anhydrous acetonitrile (5 mL) was pertrimethylsilylated by the addition of BSTFA (1.0 mL), followed by stirring overnight at room temperature. The resulting solution was evaporated in vacuo to dryness. The residue was dissolved in anhydrous tetrahydrofuran (10 mL), slurried with platinum oxide (45 mg), and hydrogenated at room temperature and pressure. The progress of the hydrogenolysis was monitored by high-pressure LC analysis (CH_3CN–H_2O, 1:1). When the reaction was complete, the catalyst was removed by filtration and the filtrate evaporated. In order to remove trimethylsilyl groups, the filtrate residue was treated with 5 mL of methanol containing 5 drops of glacial acetic acid. After the solution was left standing at room temperature overnight, yellow crystals separated (20 mg, 42%), which were recrystallized from tetrahydrofuran, mp 264–265 °C. The mixture melting point with **6** obtained from the partial hydrolysis of chartreusin was 265–266 °C.

10-(β-D-Glucopyranosyloxy)-6-hydroxy-1-methylbenzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (7). A solution of **4** (434 mg, 0.57 mmol) in chloroform (60 mL)/methanol (40 mL) was cooled to 0 °C and was combined with 24 mL of 0.1 N sodium methoxide in methanol. The reaction was carried out and worked up as described above for the preparation of **6**. Crystallization of the product from tetrahydrofuran gave the 6-*O*-benzyl derivative of **7** (240 mg, 71%) as yellow needles, mp 255–257 °C. Anal. ($C_{32}H_{26}O_{11}\cdot 0.5H_2O$, 595.5) C, H, O.

The benzyl derivative (200 mg, 0.33 mmol) was subjected to trimethylsilylation and hydrogenolysis using the procedures described above for **6**. Crystallization of the product from tetrahydrofuran gave yellow needles (136 mg, 80%): mp 264 °C dec; UV (EtOH) λ_{max} 237 nm (ϵ 41000), 264 (34000); MS (pentaacetate derivative) m/e (relative intensity) 706 (0.15), 664 (0.42), 430 (0.51), 376 (1.66), 334 (74.9), 331 (16.3), 169 (72.1), 109 (51.4), 43 (100). Anal. ($C_{25}H_{20}O_{11}\cdot H_2O$, 514.4) C, H, O.

10-[(4-*O*-α-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]-6-hydroxy-1-methylbenzo[*h*][1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,12-dione (8). A chloroform (100 mL)/methanol (50 mL) solution of **5** (1.05 g, 1.0 mmol) was cooled to 0 °C and treated with 50 mL of 0.1 N sodium methoxide in methanol. The reaction was carried out as described above for compound **6**. Concentration of the neutralized reaction solution to half gave a crystalline product which was removed by filtration, washed with water, and dried to give the 6-*O*-benzyl derivative of **8** (0.6 g, 80%), mp 255–256 °C. Anal. ($C_{38}H_{36}O_{16}\cdot 2H_2O$, 784.7) C, H, O.

The benzyl derivative (525 mg, 0.67 mmol) was subjected to trimethylsilylation and hydrogenolysis using the procedures described above for **6**. Crystallization of the product from methanol gave 290 mg (63%) of **8**: mp 209 °C dec; UV (EtOH) λ_{max} 236 nm (ϵ 37000), 264 (30000). Anal. ($C_{31}H_{30}O_{16}\cdot 2H_2O$, 694.6) C, H, O.

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