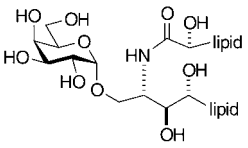
Biologically Active Compounds

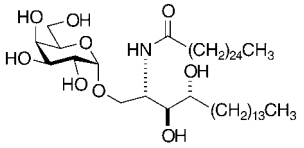
The C-Glycoside Analogue of the Immunostimulant α-Galactosylceramide (KRN7000): Synthesis and Striking Enhancement of Activity**

*Guangli Yang, John Schmieg, Moriya Tsuji, and Richard W. Franck**

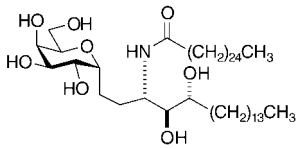
In the early 1990s a research group at Kirin Pharmaceuticals reported their results from screening lipophilic extracts from the Okinawan sponge *Agelas mauritianus*. When their extracts were tested in mice, but not in cell culture, they observed potent antitumor activity by glycolipids that they named the agelasphins (**1**).[1] Structure–activity studies of compounds available through synthesis revealed that a slightly simpler analogue of the natural agelasphins, an α-galactosyl ceramide named KRN7000 (**2**), had the best activity of the compounds tested, and that the functional groups and configuration of **2** were optimal. The only acceptable variables are the lengths of the lipid chains, the insertion of "taggants" at the terminus of the fatty amide chain through amide linkages, substitution at C6 of the galactose residue, the removal of the hydroxy group at C4 of the phytosphingosine moiety, and the presence or absence of an α hydroxy group in the fatty amide.[2]



1: agelasphins



2: KRN7000



3: C-glycoside analogue

Since the initial disclosures by the Kirin group, a multifaceted exploration of the biological effects of KRN7000 has unveiled its remarkable activity against a disparate group of diseases, such as cancer, including melanoma,[3] hepatic metastases of pancreatic cancer,[4] hepatic metastases of colon cancer,[5] and primary tumor formation in three different models,[6] as well as malaria,[7] juvenile diabetes,[8] hepatitis B,[9] and autoimmune encephalomyelitis,[10] in murine/whole animal versions of all the diseases. Although no activity against cell-culture versions of the diseases is ever detected, in vitro activity can be measured by incubating the glycolipid with antigen-presenting dendritic cells or their isolated CD1d receptors and then challenging NKT-cell hybridomas with the resulting complex.[11] This explosion of research has led to that finding that the unifying mechanism of action of KRN7000 is its remarkable ability to induce a potent expansion of Vα24+NKT cells. The sequence of events is: 1) the galactosyl ceramide binds to the CD1d receptor of antigen-presenting cells, then 2) the ceramide receptor complex binds to NKT cells, which stimulate a cascade of cytokines: signals for the processes that result in the suppression of the disease.[12] It is not yet known why an α-galactosyl ceramide of marine origin should be recognized by a mammalian receptor and elicit a spectacular immune response. It has also not been established whether an endogenous mammalian substance exists as the "natural" ligand for the CD1d receptor. Suffice to say that KRN7000 is a potent lead compound for the development of immunostimulant drugs.[13]

In our search for challenging targets that might showcase our application of the Ramberg–Bäcklund reaction for C-

[*] Dr. G. Yang, Prof. Dr. R. W. Franck
Department of Chemistry
Hunter College of CUNY
New York, NY 10021 (USA)
Fax: (+1) 212-772-5332
E-mail: rfranck@hunter.cuny.edu
J. Schmieg
Department of Medical and Molecular Parasitology
New York University School of Medicine
New York, NY (USA)
Prof. Dr. M. Tsuji
Department of Medical and Molecular Parasitology
New York University School of Medicine
New York and Aaron Diamond AIDS Research Center
The Rockefeller University
New York, NY (USA)

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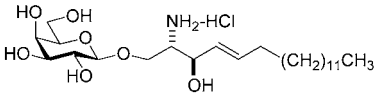
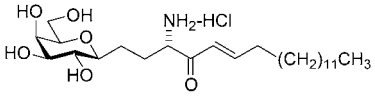
glycoside synthesis, we chose to prepare **3**, the nearest C-glycoside analogue of **2**, on the basis of the well-known rationale for the study of C-glycosides, namely that the O-glycoside may be susceptible to enzymatic degradation, whereas the corresponding C-glycoside is not. That the lability of **2** may be a problem is conjecture by the present authors, as little has been published about the metabolism of KRN7000 in mammals.[14] The C-glycoside concept has existed for many years and has been thoroughly reviewed.[15] It is fair to conclude that comparisons of bioactivities of synthetic C-glycosides with those of their O-glyco parents have uncovered some examples of similar activity,[16] many of diminished activity,[17] and none of significant improvement. The most apposite data for our ceramide project are those of Bertozzi et al., who compared the binding of the closely matched, but not identical β-O- and β-C-glycosides **4** and **5** to



4: 96% inhibition of binding of β-GalCer with gp120 at 1 mg mL^{-1}; IC_{50} = 160 μm



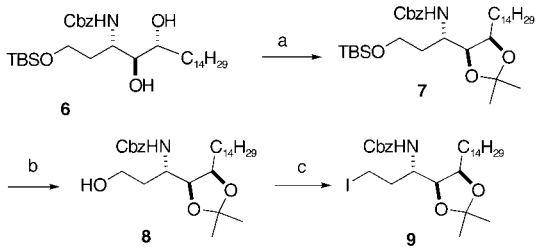
5: 86% inhibition of binding of β-GalCer with gp120 at 1 mg mL^{-1}; IC_{50} = 120 μm

a gp120 receptor. The binding constants of the two compounds were similar, and no kinetic data was presented to show that the longer lifetime of the C-glycoside had any effect.[18] There are a few examples of C-glycoside constructs that are not analogous to any specific O-glycoside. For example, Schmidt and co-workers recently described a C-glycoside mimetic of a complex proposed to be important in galactosyl transfer.[19] Although syntheses of C-glycosyl sphingosine substances somewhat related to **3** have been reported by two other research groups,[20] these groups have not reported the synthesis of the same analogue of KRN7000 prepared by us.

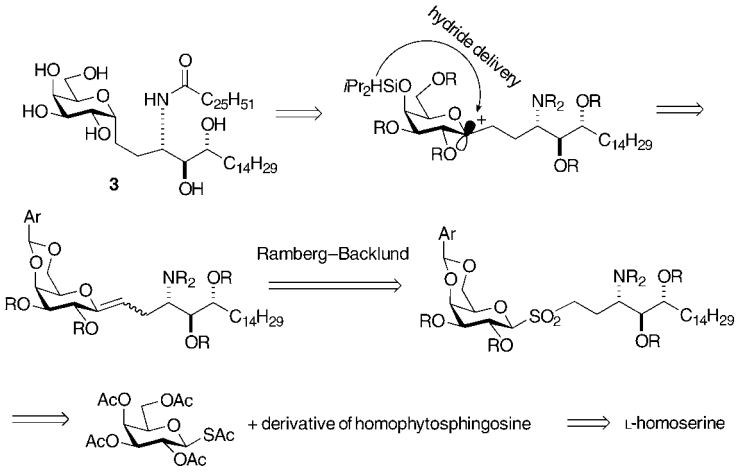
In our first approach to **3**, outlined retrosynthetically in Scheme 1, we embarked on a route with a Ramberg–Bäcklund (RB) reaction to "stitch" a homophytosphingosine side chain to galactose as the key feature, as our group had had previous success with this method for C-glycoside synthesis.[21] A second component of our plan was to establish the desired anomeric configuration through an intramolecular hydride transfer from a silyl group to an intermediate carbocation center.

The six-step synthetic route from commercially available L-homoserine to the homophytosphingosine component **6** is described in the Supporting Information. The sequence mimics a synthesis of phytosphingosine reported by Nakanishi and co-workers in which L-serine was used as the starting material.[22] The Cbz-substituted homophytosphingosine **6** was then converted into the iodo derivative **9** in anticipation of the ensuing S-glycoside formation (Scheme 2).[23]

The synthesis was continued by treatment of the galactose thioacetate **10** with hydrazinium acetate in DMF under nitrogen to deprotect the thiol,[24] which was subsequently treated with the electrophile **9** and triethylamine to provide the β-D-thiogalactoside **11** in 90% overall yield (Scheme 3). Treatment of **11** with NaOMe in MeOH to cleave the remaining four acetate groups followed by protection of the 4- and 6-hydroxy groups with *p*-methoxybenzaldehyde dimethyl acetal and *p*-toluenesulfonic acid produced the 4,6-*O*-(4-methoxybenzylidene)-β-D-1-thiogalactoside **12** in 86% yield.[25] Sodium hydride promoted benzylation of the remaining hydroxy groups of **12**,



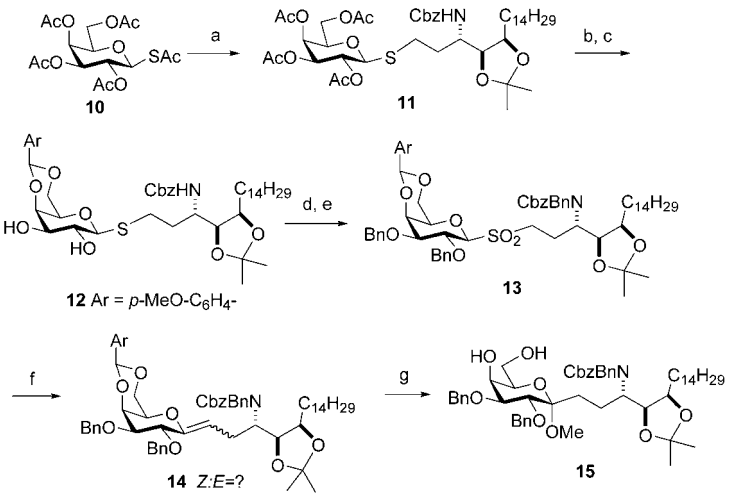
Scheme 2. Synthesis of an iodo derivative of homophytosphingosine: a) $(CH_3)_2C(OMe)_2$, PPTS, CH_2Cl_2, 95%; b) nBu_4NF, AcOH, THF, 86%; c) I_2, PPh_3, THF, reflux, 85%. Cbz = benzyloxycarbonyl, PPTS = pyridinium *p*-toluenesulfonate.



Scheme 1. Retrosynthesis of a C-glycoside analogue of KRN7000.

followed by oxidation of the thiogalactoside with MMPP, gave the sulfonyl galactoside **13** in good yield. We could not avoid *N*-benzylation in this step.

The RB reaction of **13** in the presence of $C_2F_4Br_2$, *t*BuOH, and KOH–Al_2O_3 at reflux afforded the product **14**. The *Z*:*E* ratio of alkene isomers was not determined because of the peak broadening in the NMR spectra. The 1-*O*-methyl-2,3-*O*-dibenzyl-β-galactoside **15** was prepared in one step from **14** by using chlorotrimethylsilane in methanol. At this stage, preliminary experiments with BF_3-catalyzed hydride transfer revealed that the isopropylidene protecting group was not sufficiently robust. It was therefore exchanged for a carbonate group. However, this carbonate protecting group would not be suitable for the RB step, in which KOH is required.

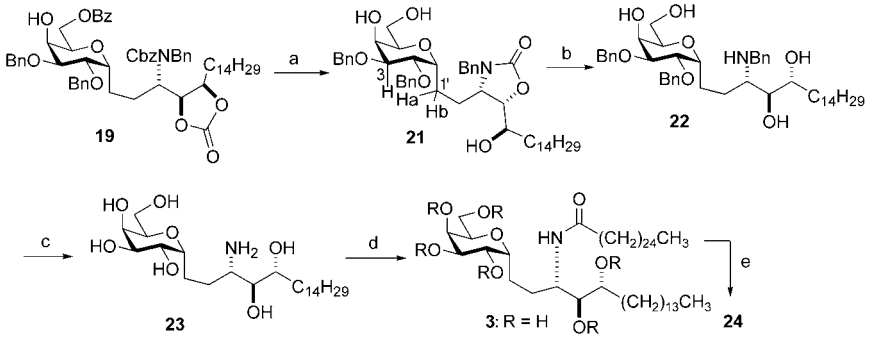


Scheme 3. Formation of the C-glycoside linkage: a) 1. NH_2NH_2, AcOH, DMF; 2. **9**, Et_3N, 90%; b) NaOMe, MeOH; c) *p*-$(MeO)C_6H_4CH(OMe)_2$, *p*-TsOH, DMF, CH_2Cl_2, 86% (2 steps); d) NaH, BnBr, THF, 83%; e) MMPP, THF, H_2O, EtOH, 60°C, 93%; f) $C_2F_4Br_2$, *t*BuOH, KOH, Al_2O_3, reflux, 70%; g) TMSCl, MeOH, 0°C, 66%. Bn = benzyl, DMF = *N*,*N*-dimethylformamide, MMPP = magnesium monoperoxyphthalate, TMS = trimethylsilyl, Ts = toluenesulfonyl.

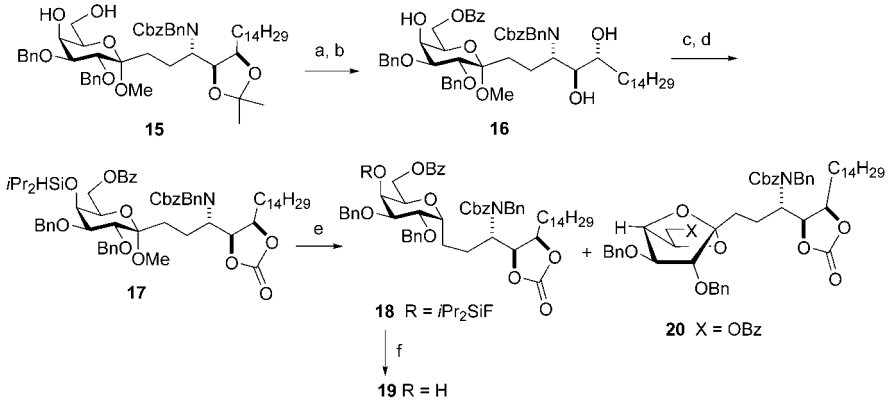
Our protecting-group manipulations began with **15**, whereby esterification of the primary hydroxy group at C6 and cleavage of the acetonide group afforded the corresponding diol **16** in good yield (Scheme 4). Cyclic carbonation of the diol in the presence of triphosgene followed by silylation of the axial hydroxy group at C4 then afforded the silyl ether **17**.[26] The controlled addition with a syringe pump of **17** as a solution in CH_2Cl_2 (0.01M) to $BF_3{\cdot}Et_2O$ (5 equiv) in CH_2Cl_2 afforded the α-C-galactosides **18** and **19**, as well as the 1,4-anhydro compound **20** (15%).[27] Treatment of the silyl ether **18** with Bu_4NF (1N) in THF afforded the product **19** (combined yield of 76%), which was identified by ^{1}H NMR spectroscopy (δ(anomeric H) = 3.95 ppm, J_{12} = 4.6 Hz).

The final deprotection steps prior to the ultimate amidation to form the target compound **3** were more challenging than anticipated. The total debenzylation of compound **19** failed under a variety of conditions, including with the Pearlman catalyst (H_2, 20% $Pd(OH)_2$, 30 psi), Birch reduction (Na/liquid NH_3), with 10% Pd/C, and with 4.4% formic acid in methanol. We therefore elected to remove the carbonyl protecting groups before the hydrogenolytic debenzylation process (Scheme 5).

Compound **19** was treated with NaOH at reflux in a mixture of dioxane and H_2O (1:1) to afford the oxazolidinone **21**, which was fully characterized. NOE effects were observed between 3-H and 1′-Ha or b, and between 5-H and 1′-Ha or b, which confirmed that the configuration of compound **21** (and hence **19**) was correctly assigned (α isomer). Hydrolysis of **21** (at reflux in KOH/EtOH) gave the *N*-benzylamine **22**, which was fully debenzylated by transfer hydrogenolysis (10% Pd/C, cyclohexene, reflux) to afford crude **23** in 80% overall yield.[28] The fatty amide chain was then introduced by using *p*-nitrophenyl hexadecanoate as the acylating agent[2a] to afford the target **3** in 60% yield. Final purification was carried out by flash chromatography on silica gel (eluant: $CHCl_3$/MeOH 4:1). The ^{1}H and ^{13}C NMR spectra, optical rotation value ($[\alpha]_D^{25} = +40.8$ (c = 0.13, pyridine)), m.p. (175–178°C), and high resolution MS (FAB) spectrum: m/z = 856.7601 ($C_{51}H_{101}O_8N+H^+$ requires 856.7605) served to fully characterize a sample of **3**. The mass spectrum and ^{1}H NMR spectrum of fully acylated **24** further confirmed the structure of **3**. Interestingly, the optical rotation value and NMR data for the O- and C-glycosides showed



Scheme 5. Removal of protecting groups followed by ceramidation: a) 1,4-dioxane, H_2O, NaOH, reflux, >90%; b) KOH, EtOH, reflux, 80%; c) 10% Pd/C, cyclohexene, HCl (1N), MeOH, reflux, >90%; d) *p*-nitrophenyl hexadecanoate, THF, DMAP, 48 h, room temperature, 60%; e) Ac_2O, DMAP, 80%. DMAP = 4-dimethylaminopyridine.



Scheme 4. Introduction of the α-C-glycoside linkage: a) BzCl, Et_3N, CH_2Cl_2, 88%; b) HCl (1N), Et_2O, MeOH, 80%, c) triphosgene, pyridine, CH_2Cl_2, 83%; d) iPr_2SiHCl, imidazole, DMF, 96%; e) $BF_3{\cdot}OEt_2$ (5 equiv), CH_2Cl_2, 0°C; f) TBAF, THF. Bz = benzoyl, TBAF = tetrabutylammonium fluoride.

a remarkable congruence, except for the chemical shifts of C1, C1′, and C2′.[29]

The coupling constant of 8.8 Hz between 2-H and 3-H of the galactose unit of **3** suggests that the $^4C_1$ chair (as drawn) is the principal conformer. Whereas the anomeric effect of the O-glycoside serves to compensate for the steric repulsions of the axial ceramide moiety, there is no such compensation in the C-glycoside analogue, so its actual conformation can not be assumed to be a chair. For example, Veyrières and co-workers reported two examples of α-C-galactosides for which the coupling constants clearly show that the standard chair can not be the principal conformer.[30]

A range of assays were carried out in which **3** was found to show outstanding activity, even in comparison with the very active O-glycoside **2**. In the following we summarize the results of these assays, which clearly demonstrate the excellent potential of C-glycoside analogues as therapeutic agents.

One assay that was carried out was a mouse malaria model, whereby mice were treated with galactosyl ceramide and then, after an interval, were challenged with malaria (*P. yoeli*).[31] After a further interval, the animals were sacrificed, and their livers were assayed for the sporozoite stage of malaria. Both the O- and the C-glycoside **2** and **3** were very effective at reducing sporozoite levels at a dosage level of 1 μg per mouse relative to the control. However, the C-glycoside **3** continued to show excellent activity at the 1-ng level. Thus, **3** is approximately 1000 times more protective than the O-glycoside (Figure 1).

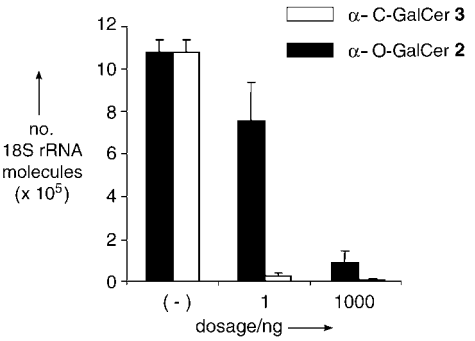


Figure 1. Assay for sporozoites in mouse liver with 1) no glycolipid, 2) 1 ng of **2** or **3**, and 3) 1 μg of **2** or **3**. Number of *P. Yoelii* specific 18S rRNA molecules (determined 42 h after challenge with 10000 viable sporozites) is plotted against dosage of **2** or **3**.

The results of this experiment do not show explicitly that the lifetime of the glycoside is a factor in the activity difference observed between **2** and **3**. Therefore, a "kinetics" experiment was carried out. The interval between the administration of the glycolipid and sporozoite challenge was varied. These experiments showed that the C-glycoside is effective with intervals of up to four days between drug dosage and challenge, whereas the O-glycoside is effective with only a one-day interval. One interpretation of these results is that the C-glycoside is not degraded by endogenous α-galactosidases, whereas the O-glycoside is; thus, the C-glycoside can remain active in the mouse for a longer period of time. Another argument could be that different forms of binding to the important receptors takes place, with the C-glycoside analogue favoring more effective signaling cascades in the immune system. Even though the former explanation is most consistent with the prevalent hypothesis of the merit of C-glycosides, we believe that our detailed bioassay data, presented elsewhere, favor the latter.[32]

A second assay carried out was a melanoma challenge in C57BL/6 mice. The animals were treated with either the C-glycoside or the O-glycoside 2 days before the challenge with B16 melanoma cells. In these mice, the melanoma attacks the lungs. The mice were sacrificed after two weeks and their lungs were examined visually for melanomas (black spots). Figure 2 hardly needs an explanation. It is clear that the C-glycoside at the 10-ng dosage level is far more effective than the O-glycoside at the same dose. In fact, detailed dose–response experiments showed that the C-glycoside **3** was 100 times more effective than the O-glycoside **2** in this assay.

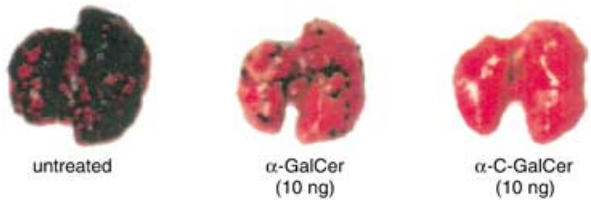


Figure 2. Melanoma appearance (black spots) on mouse lung after two weeks: untreated or treated with 10 ng of **2** or **3**.

These very promising initial results leave many important questions unanswered. More studies are required to extend these findings, including the preparation of more analogues in the search for compounds with greater potency, extensive bioassays to determine the effectiveness of these materials in other disease models, and modelling and biophysical studies to find an explanation for the dramatic difference between C- and O-glycosides in this series.

The experiments with animals were carried out with the permission of the Institutional Animal Care and Use Committee at New York University School of Medicine. The Laboratory Animal Protocol number is #4627-02 in the context of NIH/GM60271.

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[1] a) T. Natori, M. Morita, K. Akimoto, Y. Koezuka, *Tetrahedron* **1994**, *50*, 2771–2784; b) similar substances have been isolated from *Agelas Clathrodes* by the Mangoni research group: V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *J. Org. Chem.* **2004**, *69*, 1174–1179, and references therein.

[2] a) M. Morita, K. Motoki, K. Akimoto, T. Natori, T. Sakai, E. Sawa, K. Yamaji, Y. Koezuka, E. Kobayashi, H. Fukushima, *J. Med. Chem.* **1995**, *38*, 2176 – 2187; b) Y. Vo-Hoang, L. Micouin, C. Ronet, G. Gachelin, M. Bonin, *ChemBioChem* **2003**, *4*, 27 – 33; c) T. Sakai, O. Naidenko, H. Iijima, M. Kronenberg, Y. Koezuka, *J. Med. Chem.* **1999**, *42*, 1836 – 1841; d) T. Sakai, H. Ehara, Y. Koezuka, *Org. Lett.* **1999**, *1*, 359 – 361; e) X. T. Zhou, C. Forestier, R. D. Goff, C. Li, L. Teyton, A. Bendelac, P. B. Savage, *Org. Lett.* **2002**, *4*, 1267 – 1270; f) T. Sakai, M. Morita, N. Matsunaga, K. Akimoto, T. Yokoyama, H. Iijima, Y. Koezuka, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 697 – 702.

[3] A. Kikuchi, M. Nieda, C. Schmidt, Y. Koezuka, S. Ishihara, Y. Ishikawa, K. Tadokoro, S. Durrant, A. Boyd, T. Juji, A. Nicol, *Br. J. Cancer* **2001**, *85*, 741 – 746.

[4] G. Matsumoto, S. Nagai, M. Toi, S. Ishiyama, K. Kuroi, K. Turuta, A. Okamoto, T. Takahashi, *Nippon Geka Gakkai Zasshi* **2001**, *102*, 421.

[5] R. Nakagawa, K. Motoki, H. Ueno, H. Iijima, H. Nakamura, E. Kobayashi, A. Shimosaka, Y. Koezuka, *Cancer Res.* **1998**, *58*, 1202 – 1207.

[6] Y. Hayakawa, S. Rovero, G. Forni, M. J. Smyth, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9464 – 9469.

[7] G. Gonzalez-Aseguinolaza, C. de Oliveira, M. Tomaska, S. Hong, O. Bruna-Romero, T. Nakayama, M. Taniguchi, A. Bendelac, L. Van Kaer, Y. Koezuka, M. Tsuji, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8461 – 8466.

[8] a) S. Hong, M. T. Wilson, I. Serizawa, L. Wu, N. Singh, O. V. Naidenko, T. Miura, T. Haba, D. C. Scherer, J. Wei, M. Kronenberg, Y. Koezuka, L. Van Kaer, *Nat. Med.* **2001**, *7*, 1052 – 1056; b) S. Sharif, G. A. Arreaza, P. Zucker, Q. S. Mi, J. Sondhi, O. V. Naidenko, M. Kronenberg , Y. Koezuka, T. L. Delovitch, J. M. Gombert, M. Leite-De-Moraes, C. Gouarin, R. Zhu, A. Hameg, T. Nakayama, M. Taniguchi, F. Lepault, A. Lehuen, J. F. Bach, A. Herbelin, *Nat. Med.* **2001**, *7*, 1057 – 1062.

[9] K. Kakimi, L. G. Guidotti, Y. Koezuka, F. V. Chisari, *J. Exp. Med.* **2000**, *192*, 921 – 930.

[10] K. Miyamoto, S. Miyake, T. Yamamura, *Nature* **2001**, *413*, 531 – 534.

[11] a) L. Brossay, O. Naidenko, N. Burdin, J. Matsuda, T. Sakai, M. Kronenberg, *J. Immunol.* **1998**, *161*, 5124 – 5128; b) K. Benlagha, A. Weiss, A. Beavis, L. Teyton, A. Bendelac, *J. Exp. Med.* **2000**, *191*, 1895.

[12] a) D. B. Moody, G. S. Besra, I. A. Wilson, S. A. Porcelli, *Immunol. Rev.* **1999**, *172*, 285 – 296; b) M. T. Wilson, A. K. Singh, L. Van Kaer, *Trends Mol. Med.* **2002**, *8*, 225 – 231.

[13] a) For an overview of current research on glycosphingolipids in medicine, see: "Glycolipids in cell biology and medicine": *Philos. Trans. Biol. Sci.* **2003**, *358*, 845 – 983, organized and edited by R. A. Dwek, T. D. Butters, F. M. Platt, T. M. Cox; b) for a review of marine glycolipids, see: E. Fattorusso, A. Mangoni, *Fortschr. Chem. Org. Naturst.* **1997**, *72*, 215 – 301.

[14] M. Crul, R. A. Mathot, G. Giaccone, C. A. Punt, H. Rosing, M. X. Hillebrand, Y. Ando, N. Nishi, H. Tanaka, J. M. Schellens, J. H. Beijnen, *Cancer Chemother. Pharmacol.* **2002**, *49*, 287 – 293.

[15] "Synthesis of C-Glycosides: Stable Mimics of O-Glycosidic Linkages": a) C. R. Bertozzi, M. D. Bednarski in *Modern Methods in Carbohydrate Synthesis*, Harwood Academic Publishers, Reading, **1996**, pp. 316 – 351; b) Y. Du, R. J. Linhardt, I. R. Vlahov, *Tetrahedron* **1998**, *54*, 9913 – 9959; c) "C-Oligosaccharide Synthesis": T. Skrydstrup, B. Vauzailles, J.-M. Beau in *Glycoscience: Chemistry and Chemical Biology, Vol. 3* (Eds.: B. Fraser Reid, K. Tatsuta, J. Thiem), Springer, Heidelberg, **2001**, p. 2679.

[16] For a C-trisaccharide with lectin-binding behavior similar to that of the parent O-trisaccharide, see: L. M. Mikkelsen, M. J. Hernaiz, M. Martin-Pastor, T. Skrydstrup, J. Jimenez-Barbero, *J. Am. Chem. Soc.* **2002**, *124*, 14940 – 14951.

[17] a) For a C-glycoside with a tenfold loss of activity relative to phlorizin in an SGLT-inhibition assay, see: J. T. Link, B. K. Sorensen, *Tetrahedron Lett.* **2000**, *41*, 9213 – 9217; b) for a C-glycoside with a 500-fold reduction in antileukemia activity relative to the parent daunomycin, see: E. M. Acton, K. J. Ryan, M. Tracy, S. K. Arora, *Tetrahedron Lett.* **1986**, *27*, 4245 – 4248.

[18] C. R. Bertozzi, D. G. Cook, W. R. Kobertz, F. Gonzalez-Scarano, M. D. Bednarski, *J. Am. Chem. Soc.* **1992**, *114*, 10639 – 10641.

[19] B. Waldscheck, M. Streiff, W. Notz, W. Kinzy, R. R. Schmidt, *Angew. Chem.* **2001**, *113*, 4120 – 4124; *Angew. Chem. Int. Ed.* **2001**, *40*, 4007 – 4011.

[20] a) A. Dondoni, D. Perrone, E. Turturici, *J. Org. Chem.* **1999**, *64*, 5557 – 5564; b) M. K. Gurjar, R. Reddy, *Carbohydr. Lett.* **1997**, *2*, 293 – 298.

[21] For recent applications of the Ramberg–Bäcklund reaction to the synthesis of C-glycosides, see: a) D. E. Paterson, F. K. Griffin, M.-L. Alcaraz, R. J. K. Taylor, *Eur. J. Org. Chem.* **2002**, 1323; b) Y. Ohnishi, Y. Ichikawa, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 997; c) P. Pasetto, R. W. Franck, *J. Org. Chem.* **2003**, *68*, 8042 – 8060.

[22] O. Shirota, K. Nakanishi, N. Berova, *Tetrahedron* **1999**, *55*, 13643 – 13658.

[23] S. J. Spak, O. R. Martin, *Tetrahedron* **2000**, *56*, 217 – 224.

[24] W. K. Park, S. S. J. Meunier, D. Zanini, R. Roy, *Carbohydr. Lett.* **1995**, *1*, 179 – 184.

[25] R. Johansson, B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1* **1984**, *1*, 2371 – 2374.

[26] R. M. Burk, M. B. Roof, *Tetrahedron Lett.* **1993**, *34*, 395 – 398.

[27] M. Yu, B. L. Pagenkopf, *J. Org. Chem.* **2002**, *67*, 4553 – 4558.

[28] W. R. Roush, M. A. Adam, *J. Org. Chem.* **1985**, *50*, 3752 – 3757.

[29] This synthesis of the C-glycoside **3** has been disclosed at ACS meetings and is the subject of a patent application: R. W. Franck, G. Yang, J. Schmeig, M. Tsuji, ACS Meeting, Boston, August **2002**, ABS O-862; R. W. Franck, G. Yang, J. Schmeig, M. Tsuji, ACS Meeting, New Orleans, March **2003**, ABS Carbo-26; "Synthetic *C*-glycolipid and its use for treating cancer, infectious diseases and autoimmune diseases": M. Tsuji, R. W. Franck, G. Yang, G. Chen, US Patent Provisional App. June, **2002**, Full App. June **2003**.

[30] A. Jégou, C. Pacheco, A. Veyrières, *Tetrahedron* **1998**, *54*, 14779 – 14790.

[31] G. Gonzalez-Aseguinolaza, C. de Oliveira, M. Tomaska, S. Hong, O. Bruan-Romero, T. Nakayama, M. Taniguchi, A. Bendela, L. Van Kaer, Y. Koezuka, M. Tsuji, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8461 – 8466.

[32] J. Schmieg, G. Yang, R. W. Franck, M. Tsuji, *J. Exp. Med.* **2003**, *198*, 1631 – 1641.

