Synthesis and Evaluation of *N*-Acetyl-2-amino-2-deoxy-α-D-galactosyl 1-Thio-7-oxaceramide, a New Analogue of α-D-Galactosyl Ceramide

by **Roshini Rajan**[a]), **Thresen Mathew**[a]), **Radovan Buffa**[a]), **Frédéric Bornancin**[b]), **Marco Cavallari**[c]), **Peter Nussbaumer**[b]), **Gennaro De Libero**[c]), and **Andrea Vasella***[a])

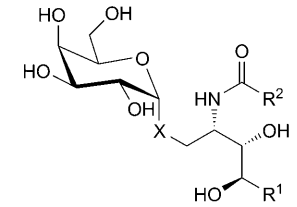
[a]) Laboratorium für Organische Chemie, Departement Chemie und Angewandte Biowissenschaften, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich (e-mail: vasella@org.chem.ethz.ch)
[b]) *Novartis Institute for BioMedical Research*, Brunner Strasse 59, A-1235 Vienna
[c]) Department of Biomedicine, University Hospital Basel, CH-4031 Basel

The *N*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl 1-thio-7-oxaceramide **1** was synthesized by substituting the 7-oxasphingosine triflate **3** with α-D-*N*-acetyl-1-thiogalactosamine (**2**). The triflate **3** was obtained from azide **4**. Thiol **2** was prepared according to a known procedure from α-D-galactosamine hydrochloride. As compared to ceramide (Cer), **1** is neither a substrate of ceramide kinase (CerK), consistent with the absence of the C(1)–OH group, nor an inhibitor of Cer phosphorylation by CerK. While **1** partially displaced CD1d-bound lipids, it failed to stimulate invariant natural killer T (iNKT) cells when presented by human CD1d-transfected cells. These results suggest that **1** binds weakly to recombinant CD1d, but does not form immunogenic complexes with CD1d.

Introduction. – Several α-D-galactopyranosyl ceramides [1–5], especially KRN-7000 [6][7], OCH [8], and RCAI-61[1]) [9], incorporating phytosphingosine or sphingosine moieties, possess important biological properties [10–13], comprising immunostimulating [14–16] and antitumor activities [17–24]. Several analogues of these α-D-galactopyranosides were synthesized, and they also display notable biological activities [8][14][25–37]. The synthesis and evaluation of a thioglycoside analogue of KRN7000 has recently been published [38], prompting us to report our results on the synthesis and evaluation of *N*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl 1-thio-7-oxaceramide **1**. Replacing the galactosyl by an *N*-acetyl-2-amino-2-deoxygalactosyl moiety was thought to contribute to elucidating the effect of C(2)–OH of the glycon on T-cell receptor (TCR) recognition, while the S-glycosyl bond will confer stability against chemical and enzymatic cleavage [39–42]. 7-Oxasphingosines behave very similarly to ceramides, and allow to independently modify the head group and lipid moiety of sphingosine, an advantage that has since been realized using olefin metathesis [43][44].

Conceivably, **1** may be obtained either by glycosidation [45] of a 1-thioceramide or of a 1-thiosphingosine, or by nucleophilic substitution by an *N*-acetyl α-D-galactopyranosyl thiol of a ceramide possessing a leaving group at C(1) and preferentially a non-participating neighbouring group, such as an N_3 substituent [46][47]. We opted for the

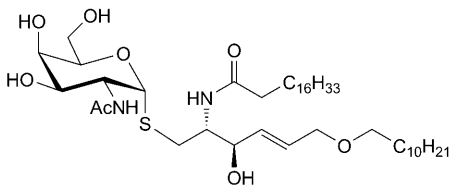
[1]) OCH is a truncated and RCAI-61 the 6′-*O*-methyl analogue of KRN7000.



KRN7000
X = O, $R^1 = C_{14}H_{29}$, $R^2 = C_{25}H_{51}$

OCH
X = O, $R^1 = C_5H_{11}$, $R^2 = C_{23}H_{47}$

S-Glycoside analogue of KRN7000
X = S, $R^1 = C_{14}H_{29}$, $R^2 = C_{25}H_{51}$



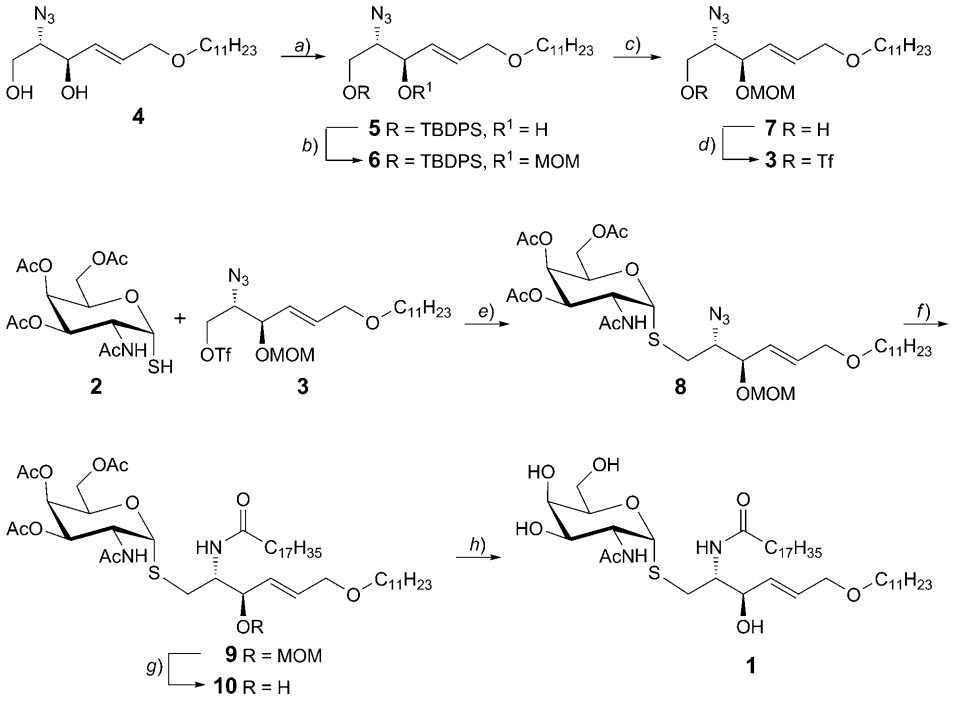
1

second strategy, assuming that nucleophilic substitution will prevail over single electron transfer from the thiolate to the N_3 group, and report on the synthesis of **1** and the results of its evaluation as substrate of Cer kinase [48] and as T cell-stimulatory antigen [49].

Synthesis. – The synthesis of the *N*-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl 1-thio-7-oxaceramide **1** is shown in the *Scheme*. The thiogalactoside **8** was obtained in 75% yield by the reaction between 2-acetamido-2-deoxy-α-D-galactopyranosyl thiol (**2**) [50] and the 7-oxasphingosine azido triflate (=trifluoromethylsulfonate) **3**. The thiol **2** was synthesized in three steps and in an overall yield of 99% from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose, according to the procedure of *Knapp* and *Myers* [50]. The azido triflate **3** was prepared from azide **4**, derived from 7-oxasphingosine [51], by first silylating the hydroxymethyl group with ^{t}BuPh$_2$SiCl (TBDPSCl) to provide **5** in 81% yield and then protecting the HO–C(3) group by treating it with MeOCH$_2$Cl (MOMCl) in the presence of *Hünig*'s base, to yield 91% of **6**. The alcohol **7** was obtained in a yield of 95% by desilylating **6**, and converted to the triflate **3**. The triflate, obtained as a pale yellow oil, was directly subjected to substitution by the thiol **2** in the presence of *Hünig*'s base, to provide 75% of the protected *S*-galactosaminide **8** ($J(1,2) = 5.4$). Reducing this galactosaminide by the action of polymer-bound Ph_3P [52], followed by *N*-acylation with stearic anhydride, yielded 86% of the protected ceramide **9** that was deprotected. The MOM group was removed by treating **9** with Tf_2O in MeCN to provide **10** in 87% yield [53][54]. Deacetylation of **10** was effected with 2M NH_3 in MeOH to yield 96% of the desired *N*-acetyl-α-D-galactopyranosyl 1-thio-7-oxaceramide **1**. The α-D configuration is evidenced by $J(1,2) = 5.4$ Hz.

Biological Studies. – 1. *Phosphorylation by Ceramide Kinase* (CerK). Phosphorylation of **1** by ceramide kinase (CerK) was assayed using an established *in vitro* radioassay [55]. As compared with C8-ceramide, **1** was not phosphorylated by CerK (*Fig. 1,a*), in agreement with previous observations showing the requirement for a free OH group on the first C-atom of ceramide to allow for phosphorylation by CerK [56].

Scheme



a) ^{t}BuPh$_2$SiCl (TBDPSCl), 1*H*-imidazole, 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; 81%. *b*) $MeOCH_2Cl$ (MOMCl), *Hünig*'s base, CH_2Cl_2; 91%. *c*) $Bu_4NF \cdot 3\,H_2O$, AcOH, THF; 95%. *d*) Tf_2O, py, CH_2Cl_2. *e*) *Hünig*'s base, CH_2Cl_2; 75%. *f*) 1. Polymer-bound PPh_3, 1,4-dioxane; 2. stearic anhydride; 86%. *g*) Tf_2O, MeCN; 87%. *h*) 2M NH_3 in MeOH; 96%.

In addition, **1** did not inhibit the phosphorylation of ceramide by CerK, suggesting that **1** does not compete with ceramide for binding to CerK (*Fig. 1,b*), in contrast to the recently identified CerK inhibitor NVP-231 [57].

2. *Human CD1d Binding and T-Cell Activation.* The galactosaminide **1** is neither cytotoxic to antigen-presenting cells (APC) nor to iNKT cells at the tested dosage (up to 20 μg/ml), as assessed by flow cytometry (data not shown).

We first investigated whether **1** binds recombinant human CD1d and displaces the strong agonist α-D-galactosyl ceramide (αGalCer) that activates iNKT cells and induces release of large amounts of cytokines from this cell population. CD1d was attached to the plastic and incubated for 4.5 h with a tenfold molar excess of **1** and then with αGalCer (2 μg/ml). Compound **1** partially reduced the activation of iNKT cells (*Fig. 2,a*), indicating a weak capacity to prevent binding of αGalCer to CD1d under these experimental conditions. Next, we investigated whether **1** activates iNKT cells when presented by living APC. Different APC were used to exclude cell type-specific effects. In all experiments, there was no activation of iNKT cells (*Fig. 2,b*). Lack of iNKT cell stimulatory activity may be ascribed to the replacement of the OH group at

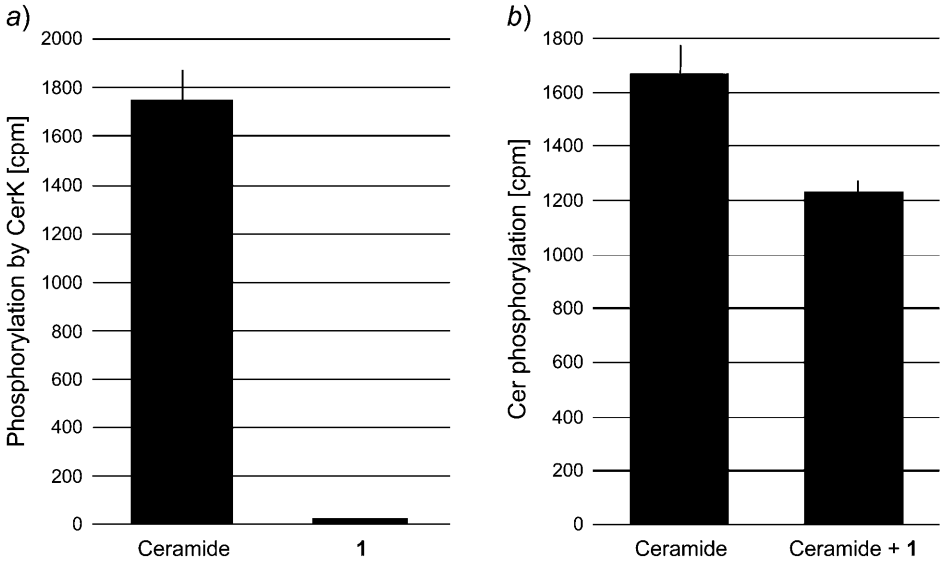


Fig. 1. *Phosphorylation of ceramide and the ceramide analogue* **1**, *assayed as described in the* Method *section. a*) Direct assay showing that **1** is not a substrate for CerK; *b*) indirect assay showing that **1** does not prevent phosphorylation of ceramide by CerK.

C(2) of the glycon by the acetamido group. Replacements of OH at C(2) of the glycon with an NH_2 group also abolishes iNKT cell activation [7]. The OH group at C(2) establishes H-bonding with two amino acids (R95 and G96) on the alpha chain of iNKT TCR [58] that is mandatory for T-cell activation, as shown by the CD1d: αGalCer binding footprint for the NKT TCR [59]. The observed weak displacement capacity of **1** may be ascribed to the different constitution and rigidity of the oxasphingosine moiety of **1** as compared to the phytosphingosine moiety of αGalCer. The different structure of oxasphingosine might impair the interaction between lipid **1** and the CD1d F' pocket, and influence the position of the sugar head, preventing an optimal interaction with the TCR.

We thank *Novartis AG*, Basel, the *ETH-Zürich*, and the *Swiss National Science Foundation* (grant 3100A0-109918) for generous support, and Dr. *Bruno Bernet* for checking the analytical data.

Experimental Part

1. *Synthesis. General*. THF was distilled from Na and benzophenone, CH_2Cl_2 from P_2O_5, and MeOH and MeCN from CaH_2. Reactions were carried out under Ar, unless stated otherwise. Qual. TLC: precoated silica-gel plates (*Merck* silica gel *60* F_{254}); detection by heating with 'mostain' (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6\,H_2O$, 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). Optical rotations: 1-dm cell at 25°, 589 nm. FT-IR spectra: KBr or *ca.* 2% soln. in $CHCl_3$, absorption in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: chemical shifts δ in ppm rel. to Me_4Si as external standard, and coupling constants J in Hz. HR-MALDI-MS: in gentisic acid (=2,5-dihydroxybenzoic acid, DHB) matrix.

*(4*E*)-2-Azido-1*-O-*[(*tert-*butyl)diphenylsilyl]-2,4,5-trideoxy-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**5**). A suspension of **4** [16] (570 mg, 1.74 mmol) and 1*H*-imidazole (154 mg, 2.26 mmol) in dry CH_2Cl_2

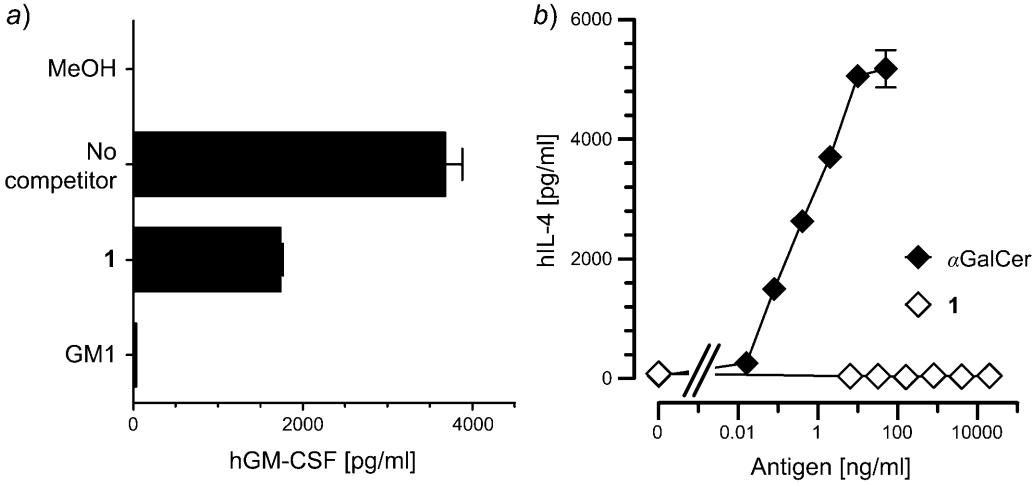


Fig. 2. *Binding of* **1** *to CD1d and failure to activate iNKT cells. N*-Acetyl-α-D-galactopyranosyl 1-thio-7-oxaceramide (**1**) was tested for its capacity to prevent binding of αGalCer to plate-bound human CD1d and subsequent T-cell response to αGalCer (*a*). Supernatants were taken after 24 h, and released human GM-CSF and human IL-4 (data not shown) were measured by ELISA and expressed as pg/ml ± SD of triplicates. Weak but significant competition of **1** with αGalCer was seen as compared to complete inhibition of αGalCer by ganglioside monosialic acid (GM1) used as control competitor. In contrast, **1** (◇) failed to activate iNKT cells when titrated on CD1d transfectants and compared to αGalCer (◆) (*b*). Supernatants were assessed for release of human IL-4, human IFN-γ, and human TNF-α giving similar results.

(10 ml) was treated with ^{t}BuPh$_2$SiCl (TBDPSCl; 0.58 ml, 2.26 mmol) and 4-(dimethylamino)pyridine (DMAP; 21 mg, 0.174 mmol), stirred for 4 h, diluted with H_2O, and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (AcOEt/hexane 1:9) gave **5** (797 mg, 81%). Colourless oil. R_f (AcOEt/hexane 1:9) 0.27. $[\alpha]_D^{25} = +13.1$ ($c = 2.0$, $CHCl_3$). IR ($CHCl_3$): 3451*w* (br.), 3073*w*, 3012*w*, 2930*s*, 2857*s*, 2105*s*, 1601*w*, 1590*w*, 1471*w*, 1428*w*, 1363*w*, 1264*w*, 1220*m*, 1112*s*, 1007*w*, 973*w*, 862*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.70–7.67 (*m*, 4 arom. H); 7.48–7.37 (*m*, 6 arom. H); 5.86 (*dtd*, J = 15.6, 5.1, 0.9, H–C(5)); 5.71 (*ddt*, J = 15.6, 6.3, 1.2, H–C(4)); 4.30 (*dtd*, J = 6.3, 5.1, 0.9, H–C(3)); 3.94 (br. *d*, J = 5.4, 2 H–C(6)); 3.85–3.78 (*dd*, J = 10.8, 6.0, H–C(1)); 3.80 (*dd*, J = 10.8, 4.5, H′–C(1)); 3.51 (*dt*, $J \approx 5.7$, 4.5, H–C(2)); 3.39 (*t*, J = 6.3, 2 H–C(1′)); 2.54 (br. *s*, J = 5.4, OH); 2.31 (br. *s*, J = 5.4, OH); 1.62–1.53 (*m*, 2 H–C(2′)); 1.35–1.26 (*m*, 16 H); 1.08 (*s*, Me_3C); 0.88 (*t*, J = 6.6, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 135.46 (4*d*); 132.53 (2*s*); 130.54 (*d*, C(4)); 130.08 (*d*, C(5)); 129.82 (2*d*); 127.73 (4*d*); 72.10 (*d*, C(3)); 70.70 (*t*, C(6)); 70.40 (*t*, C(1′)); 66.59 (*d*, C(2)); 64.06 (*t*, C(1)); 31.99, 29.82, 29.70, 29.60, 29.42, 26.26, 22.78 (several *t*); 26.73 (*q*, Me_3C); 19.23 (*s*, Me_3C); 14.32 (*q*, Me). HR-MALDI-MS: 588.3583 (100, $[M + Na]^+$, $C_{33}H_{51}N_3NaO_3Si^+$; calc. 588.3592). Anal. calc. for $C_{33}H_{51}N_3O_3Si$ (565.87): C 70.04, H 9.08, N 7.43; found: C 70.27, H 8.95, N 7.51.

*(4*E*)-2-Azido-1-*O*-[(*tert*-butyl)diphenylsilyl]-2,4,5-trideoxy-3-*O*-(methoxymethyl)-6-*O*-undecyl-*D*-erythro-hex-4-enitol* (**6**). A soln. of **5** (790 mg, 1.39 mmol) and *Hünig*'s base (0.69 ml, 4.19 mmol) in $ClCH_2CH_2Cl$ was treated with MOMCl (0.16 ml, 2.09 mmol), heated to 75°, stirred for 6 h, after which another 0.16 ml of MOMCl was added, stirred further for 16 h at 75°, diluted with H_2O, and extracted with CH_2Cl_2 (2 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (AcOEt/hexane 1:19) gave **6** (773 mg, 91%). Colourless oil. R_f (AcOEt/hexane 1:9) 0.30. $[\alpha]_D^{25} = -36.5$ ($c = 1.7$, $CHCl_3$). IR ($CHCl_3$): 3018*m*, 2930*s*, 2857*s*, 2103*m*, 1599*w*, 1471*w*, 1428*w*, 1362*w*, 1217*m*, 1152*m*, 1112*s*, 1005*s*, 1024*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.70–7.65 (*m*, 4 arom. H); 7.48–7.36 (*m*, 6 arom. H); 5.78 (*dtd*, J = 15.3, 5.3, 0.6, H–C(5)); 5.56 (*ddt*, J = 15.6, 8.1, 1.5, H–C(4)); 4.65 (*d*, J = 6.9, O–CH_a–O); 4.50

(*d*, *J* = 6.9, O–C*H*$_b$–O); 4.18 (*dd*, *J* = 7.8, 4.5, H–C(3)); 3.95 (*dd*, *J* = 5.1, 1.2, 2 H–C(6)); 3.77 (*dd*, *J* = 10.5, 5.4, H–C(1)); 3.72 (*dd*, *J* = 10.5, 6.6, H′–C(1)); 3.63 (*dt J* = 6.6, 5.1, H–C(2)); 3.37 (*t*, *J* = 6.6, 2 H–C(1′)); 3.28 (*s*, MeO); 1.60–1.50 (*m*, 2 H–C(2′)); 1.34–1.25 (*m*, 16 H); 1.08 (*s*, *Me*$_3$C); 0.88 (*t*, *J* = 6.3, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 135.50 (4*d*); 133.27 (*d*, C(4)); 132.91, 132.86 (2*s*); 129.75 (2*d*); 127.69 (4*d*); 127.20 (*d*, C(5)); 93.71 (*t*, OCH_2O); 75.45 (*d*, C(3)); 70.56 (*t*, C(6)); 70.35 (*t*, C(1′)); 66.59 (*d*, C(2)); 63.73 (*t*, C(1)); 55.66 (*q*, MeO); 32.03, 29.85, 29.75, 29.64, 29.46, 26.31, 22.83 (several *t*); 26.86 (*q*, *Me*$_3$C); 19.27 (*s*, Me$_3$*C*); 14.28 (*q*, Me)). HR-ESI-MS: 632.3848 (100, $[M + Na]^+$, $C_{35}H_{55}N_3NaO_4Si^+$; calc. 632.3854).

*(4*E*)-2-Azido-2,4,5-trideoxy-3*-O-*(methoxymethyl)-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**7**). A soln. of **6** (773 mg, 1.27 mmol) in THF (20 ml) was treated with $Bu_4NF \cdot 3\,H_2O$ (801 mg, 2.54 mmol) and AcOH (0.14 ml, 2.54 mmol) at 0°, warmed to r.t., stirred for 4 h, diluted with H_2O, and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layers were dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 3:7) gave **7** (449 mg, 95%). Colourless oil. R_f (AcOEt/hexane 3:7) 0.19. $[\alpha]_D^{25} = -104.7$ (*c* = 2.0, $CHCl_3$). IR ($CHCl_3$): 3448*w* (br.), 3018*m*, 2929*s*, 2856*s*, 2130*m*, 2106*s*, 1599*w*, 1466*w*, 1372*w*, 1354*w*, 1298*w*, 1274*w*, 1219*m*, 1152*m*, 1099*m*, 1068*w*, 1033*m*, 974*w*, 915*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.87 (*dtd*, *J* = 15.6, 5.1, 0.6, H–C(5)); 5.64 (*ddt*, *J* = 15.6, 8.1, 1.2, H–C(4)); 4.69 (*d*, *J* = 6.9, 1 H, OCH_2O); 4.55 (*d*, *J* = 6.6, 1 H, OCH_2O); 4.21 (*dd*, *J* = 7.8, 5.4, H–C(3)); 3.99 (*dd*, *J* = 5.1, 1.5, 2 H–C(6)); 3.76 (*dd*, *J* = 11.2, 4.2, H–C(1)); 3.71 (*dd*, *J* = 11.2, 6.0, H′–C(1)); 3.55 (*td*, *J* = 5.7, 4.2, H–C(2)); 3.41 (*t*, *J* = 6.6, 2 H–C(1′)); 3.38 (*s*, MeO); 2.40 (br. *s*, HO–C(1)); 1.62–1.52 (*m*, 2 H–C(2′)); 1.34–1.23 (*m*, 16 H); 0.87 (*t*, *J* = 6.9, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 133.48 (*d*, C(4)); 127.17 (*d*, C(5)); 93.80 (*t*, OCH_2O); 76.29 (*d*, C(3)); 70.75 (*t*, C(6)); 70.22 (*t*, C(1′)); 66.16 (*d*, C(2)); 62.12 (*t*, C(1)); 55.79 (*q*, OMe); 31.96, 29.75, 29.67, 29.56, 29.40, 26.33, 22.76 (several *t*); 14.21 (*q*, Me). HR-MALDI-MS: 394.2669 (100, $[M + Na]^+$, $C_{19}H_{37}N_3NaO_4^+$; calc. 394.2676). Anal. calc. for $C_{19}H_{37}N_3O_4$ (371.52): C 61.43, H 10.04, N 11.31; found: C 61.60, H 10.05, N 11.35.

*(4*E*)-2-Azido-2,4,5-trideoxy-3*-O-*(methoxymethyl)-1*-O-*[(trifluoromethyl)sulfonyl]-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**3**). Tf_2O (60 µl, 0.37 mmol) was added dropwise to a soln. of **7** (113 mg, 0.31 mmol) and pyridine (60 µl, 0.92 mmol) in dry CH_2Cl_2 (8 ml), cooled to −78°. The mixture was warmed to −20° within 3.5 h, diluted with NH_4Cl soln., and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated to give pale yellow oily **3**, which was used for the next step without further purification. R_f (AcOEt/hexane 2:8) 0.46. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.94 (*dtd*, *J* = 15.6, 5.1, 0.9, H–C(5)); 5.62 (*ddt*, *J* = 15.6, 8.1, 1.5, H–C(4)); 4.71 (*d*, *J* = 6.6, 1 H, O*C*H_2O); 4.63 (*dd*, *J* = 10.6, 3.5, H–C(1)); 4.53 (*d*, *J* = 6.9, 1 H, O*C*H_2O); 4.48 (*dd*, *J* = 10.5, 7.8, H′–C(1)); 4.22 (*dd*, *J* = 7.8, 5.7, H–C(3)); 4.02 (*dd*, *J* = 5.1, 1.8, 2 H–C(6)); 3.80 (*ddd*, *J* = 7.8, 6.0, 3.6, H–C(2)); 3.43 (*t*, *J* = 6.6, 2 H–C(1′)); 3.38 (*s*, MeO); 1.61–1.54 (*m*, 2 H–C(2′)); 1.38–1.20 (*m*, 16 H); 0.88 (*t*, *J* = 6.3, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 135.23 (*d*, C(4)); 125.43 (*d*, C(5)); 93.67 (*t*, OCH_2O); 75.24 (*d*, C(3)); 74.44 (*t*, C(1)); 70.95 (*t*, C(6)); 69.92 (*t*, C(1′)); 63.17 (*d*, C(2)); 56.00 (*q*, MeO); 31.96, 29.75, 29.67, 29.56, 29.40, 26.33, 22.76 (several *t*); 14.21 (*q*, Me). ^{19}F-NMR ($CDCl_3$): −74.22 (*s*, CF_3).

*(4*E*)-1*-S-*(2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-2-azido-2,4,5-trideoxy-3*-O-*(methoxymethyl)-1-thio-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**8**). A soln. of **2** (95 mg, 0.25 mmol) and crude **3** in dry CH_2Cl_2 (10 ml) was treated with *Hünig*'s base (63 µl, 0.38 mmol), stirred at r.t. for 2.5 h, diluted with NH_4Cl, and extracted with CH_2Cl_2. The combined org. layers were dried (Na_2SO_4) and evaporated. FC (AcOEt/hexane 1:1) gave **8** (136 mg, 75%). Colourless oil. R_f (AcOEt/hexane 7:3) 0.20. $[\alpha]_D^{25} = +38.5$ (*c* = 1.75, $CHCl_3$). IR ($CHCl_3$): 3018*w*, 2955*w*, 2929*m*, 2855*m*, 2120*m*, 1747*s*, 1680*m*, 1601*w*, 1511*m*, 1466*w*, 1371*m*, 1262*s*, 1250*s*, 1169*w*, 1151*w*, 1085*s*, 1053*m*, 1029*m*, 978*w*, 914*w*, 862*w*, 821*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 5.85 (*dtd*, *J* = 15.6, 5.1, 0.9, H–C(5)); 5.73 (*d*, *J* = 8.7, NH); 5.59 (*d*, *J* = 5.4, H–C(1‴)); 5.58 (*ddt*, *J* = 15.6, 8.1, 1.5, H–C(4)); 5.38 (*dd*, *J* = 3.3, 1.2, H–C(4‴)); 5.03 (*dd*, *J* = 12.0, 3.3, H–C(3‴)); 4.76 (*ddd*, *J* = 12.0, 8.7, 5.4, H–C(2‴)); 4.68 (*d*, *J* = 6.9, 1 H, O*C*H_2O); 4.56 (*td*, *J* = 6.6, 0.9, H–C(5‴)); 4.53 (*d*, *J* = 6.3, 1 H, O*C*H_2O); 4.13 (*dd*, *J* = 11.4, 6.0, H–C(6‴)); 4.15 (*dd*, *J* = 8.1, 4.5, H–C(3)); 4.04 (*dd*, *J* = 11.4, 6.9, H′–C(6‴)); 3.98 (*dd*, *J* = 5.4, 1.2, 2 H–C(6)); 3.68 (*dt*, *J* = 9.6, 4.2, H–C(2)); 3.39 (*t*, *J* = 6.6, 2 H–C(1′)); 3.36 (*s*, MeO); 2.79 (*dd*, *J* = 13.8, 4.2, H–C(1)); 2.67 (*dd*, *J* = 13.8, 9.6, H′–C(1)); 2.14 (*s*, AcNH); 2.04, 1.99, 1.97 (3*s*, 3 AcO); 1.60–1.51 (*m*, 2 H–C(2′)); 1.35–1.24 (*m*, 16 H); 0.86 (*t*, *J* = 6.3, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 170.82 (*s*, NHAc); 170.21, 170.04, (2*s*, 3 AcO); 134.38 (*d*, C(4)); 126.19 (*d*, C(5)); 93.66 (*t*, OCH_2O); 86.46 (*d*, C(1‴)); 77.83 (*d*, C(3)); 70.85, 70.20 (2*t*,

C(6), C(1′)); 68.29, 67.59, 67.28 (3*d*, C(3‴), C(4‴), C(5‴)); 65.81 (*d*, C(2)); 61.93 (*t*, C(6‴)); 55.86 (*q*, OMe); 48.43 (*d*, C(2‴)); 32.82, 31.99, 29.78, 29.70, 29.59, 29.43, 26.26, 22.78 (several *t*); 23.39 (*q*, AcNH); 20.84, 20.80, 20.77 (3*q*, 3 AcO); 14.24 (*q*, Me). HR-ESI-MS: 739.3548 (100, $[M+Na]^+$, $C_{33}H_{56}N_4NaO_{11}S^+$; calc. 739.3559). Anal. calc. for $C_{33}H_{56}N_4O_{11}S$ (716.89): C 55.29, H 7.87, N 7.82; S 4.47; found: C 55.14, H 8.07, N 7.70; S 4.44.

*(4*E*)-1*-S-*(2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-2,4,5-trideoxy-3*-O-*(methoxymethyl)-2-(octadecanoylamino)-1-thio-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**9**). A suspension of **8** (100 mg, 0.14 mmol) and polymer-bound PPh_3 (352 mg, 0.56 mmol) in dioxane (5 ml) was heated at 80° for 1 h. The temp. was then reduced to 60°, stearic anhydride (154 mg, 0.28 mmol) was added, the mixture was stirred for 48 h, filtered, and the filtrate was evaporated. FC (AcOEt/hexane 6:4) gave **9** (115 mg, 86%). White crystals. R_f (AcOEt/hexane 7:3) 0.31. M.p. 66–68.5°. $[\alpha]_D^{25} = +30.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3426*w*, 3019*m*, 2927*s*, 2855*s*, 1747*s*, 1676*m*, 1602*w*, 1511*m*, 1466*w*, 1371*m*, 1239*m*, 1151*w*, 1084*m*, 1050*m*, 1031*m*, 977*w*, 914*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 6.01 (*d*, $J = 9.0$, AcNH); 5.83 (*dtd*, $J = 15.6, 5.1, 0.9$, H–C(5)); 5.60 (*d*, $J = 8.7$, HN–C(2)); 5.58 (*ddt*, $J = 15.6, 6.9, 1.8$, H–C(4)); 5.45 (*d*, $J = 5.1$, H–C(1‴)); 5.38 (*dd*, $J = 3.0, 1.2$, H–C(4‴)); 5.01 (*dd*, $J = 12.0, 3.3$, H–C(3‴)); 4.77 (*ddd*, $J = 12.0, 9.0, 5.4$, H–C(2‴)); 4.65 (*d*, $J = 6.6$, 1 H, OCH_2O); 4.56 (*td*, $J \approx 6.6, 0.9$, H–C(5‴)); 4.54 (*d*, $J = 6.9$, 1 H, OCH_2O); 4.29–4.21 (*m*, H–C(2)); 4.17 (*dd*, $J = 11.4, 6.0$, H–C(6‴)); 4.14 (*t*, $J \approx 6.2$, H–C(3)); 4.07 (*dd*, $J = 11.4, 6.6$, H′–C(6‴)); 3.95 (br. *d*, $J = 5.1$, 2 H–C(6)); 3.38 (*t*, $J = 6.9$, 2 H–C(1′)); 3.37 (*s*, MeO); 3.00 (*dd*, $J = 14.1, 7.5$, H–C(1)); 2.83 (*dd*, $J = 13.8, 3.9$, H′–C(1)); 2.20–2.08 (*AB*, 2 H–C(2″)); 2.16 (*s*, AcNH); 2.04, 2.00, 1.97 (3*s*, 3 AcO); 1.62–1.51 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.38–1.19 (*m*, 44 H); 0.87 (*t*, $J = 6.6$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 172.66 (*s*, C(1″)); 170.82, 170.19, 170.05 (3*s*, 3 AcO); 169.92 (*s*, AcNH); 132.65 (*d*, C(4)); 128.01 (*d*, C(5)); 94.56 (*t*, OCH_2O); 86.79 (*d*, C(1‴)); 78.01 (*d*, C(3)); 70.83, 70.35 (2*t*, C(6) C(1′)); 68.35, 67.69, 67.25 (3*d*, C(3‴), C(4‴), C(5‴)); 61.79 (*t*, C(6‴)); 56.02 (*q*, MeO); 52.21 (*d*, C(2)); 48.41 (*d*, C(2‴)); 36.89, 33.08, 32.02, 29.82, 29.75, 29.67, 29.51, 29.47, 26.29, 25.75, 22.81 (several *t*); 23.47 (*q*, AcNH); 20.86 (*q*, 3 AcO); 14.24 (*q*, 2 Me). HR-MALDI-MS: 979.6244 (84, $[M + Na]^+$, $C_{51}H_{92}N_2NaO_{12}S^+$; calc. 979.6263). Anal. calc. for $C_{51}H_{92}N_2O_{12}S$ (957.36): C 63.98, H 9.69, N 2.93; found: C 64.19, H 9.68, N 2.96.

*(4*E*)-1*-S-*(2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-2,4,5-trideoxy-2-(octadecanoylamino)-1-thio-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**10**). An ice-cold soln. of **9** (90 mg, 0.09 mmol) in dry MeCN was treated with Tf_2O (32 µl, 0.19 mmol), slowly warmed to r.t. in 2 h, diluted with sat. NH_4Cl soln., and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (AcOEt/hexane 9:1) gave **10** (75 mg, 87%). White crystals. R_f (AcOEt/hexane 9:1) 0.23. M.p. 95–98°. $[\alpha]_D^{25} = +55.9$ ($c = 2.25$, $CHCl_3$). IR ($CHCl_3$): 3573*w*, 3485*w*, 3419*w*, 3021*w*, 2928*m*, 2855*w*, 1747*m*, 1675*w*, 1604*w*, 1512*w*, 1466*w*, 1372*w*, 1266*s*, 1170*m*, 1085*w*, 1044*m*, 1037*m*, 978 *w*, 929*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 6.14 (*d*, $J = 8.7$, AcNH); 5.87 (br. *dt*, $J = 15.6, 5.3$, H–C(5)); 5.75 (*d*, $J = 8.7$, HN–C(2)); 5.71 (br. *dd*, $J = 15.6, 5.7$, H–C(4)); 5.50 (*d*, $J = 5.4$, H–C(1‴)); 5.37 (*dd*, $J = 3.0, 0.9$, H–C(4‴)); 5.00 (*dd*, $J = 11.7, 3.3$, H–C(3‴)); 4.74 (*ddd*, $J = 11.8, 8.6, 5.1$, H–C(2‴)); 4.53 (br. *t*, $J = 6.6$, H–C(5‴)); 4.29 (br. *t*, $J \approx 4.9$, H–C(3)); 4.15 (*dd*, $J = 11.6, 6.2$, H–C(6‴)); 4.15–4.05 (*m*, H–C(2)); 4.08 (*dd*, $J = 11.3, 6.8$, H′–C(6‴)); 3.95 (br. *d*, $J = 5.4$, 2 H–C(6)); 3.39 (*t*, $J = 6.9$, 2 H–C(1′)); 3.00 (*dd*, $J = 14.0, 8.0$, H–C(1)); 2.80 (*dd*, $J = 14.0, 4.1$, H′–C(1)); 2.22–2.12 (*AB*, 2 H–C(2″)); 2.15 (*s*, AcNH); 2.05, 1.99, 1.97 (3*s*, AcO); 1.64–1.50 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.36–1.18 (*m*, 44 H); 0.87 (*t*, $J = 6.8$, 2 Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 173.86 (*s*, C(1″)); 170.80 (*s*, AcNH); 170.33, 170.07, 169.99 (3*s*, 3 AcO); 132.54 (*d*, C(4)); 130.05 (*d*, C(5)); 86.75 (*d*, C(1‴)); 73.51 (*d*, C(3)); 70.87, 70.45 (2*t*, C(6) C(1′)); 68.28, 67.63, 67.15 (3*d*, C(3‴), C(5‴), C(4‴)); 61.89 (*t*, C(6‴)); 54.30 (*d*, C(2)); 48.47 (*d*, C(2‴)); 36.73 (*t*); 32.65 (*t*); 31.98 (2*t*); 29.78–29.42 (several *t*); 26.25 (*t*); 25.78 (*t*); 23.39 (*q*, AcNH); 22.77 (2*t*); 20.90, 20.82, 20.77 (3*q*, 3 AcO); 14.21 (*q*, 2 Me). HR-MALDI-MS: 935.5984 (100, $[M + Na]^+$, $C_{49}H_{88}N_2NaO_{11}S^+$; calc. 935.6001). Anal. calc. for $C_{49}H_{88}N_2O_{11}S$ (913.29): C 64.44, H 9.71, N 3.07, S 3.51; found: C 64.18, H 9.74, N 3.11, S 3.36.

*(4*E*)-1*-S-*(2-Acetamido-2-deoxy-α*-D-*galactopyranosyl)-2,4,5-trideoxy-2-(octadecanoylamino)-1-thio-6*-O-*undecyl*-D-erythro-*hex-4-enitol* (**1**). A soln. of **10** (70 mg, 0.076 mmol) in 6 ml of 2M NH_3 in MeOH was heated to 40° for 4.5 h, and then evaporated. FC ($MeOH/CH_2Cl_2$ 3:17) gave **1** (58 mg, 96%). R_f (CH_2Cl_2/MeOH 9:1) 0.26. M.p. 207–209°. $[\alpha]_D^{25} = +56.3$ ($c = 1.08$, pyridine). IR ($CHCl_3$): 3407*m* (br.), 3289*s*, 3092*w*, 2953*m*, 2919*s*, 2850*s*, 1647*s*, 1551*s*, 1468*m*, 1435*m*, 1376*m*, 1298*w*, 1213*w*, 1168*w*,

1116*m*, 1084*m*, 1049*m*, 1020*w*, 977*w*, 960*w*. ^{1}H-NMR (CD_3OD, 300 MHz): 7.98 (*d*, *J* = 7.5, 0.4 H, AcNH); 7.88 (*d*, *J* = 9.0, 0.6 H, HN–C(2)); 5.78 (br. *dt*, *J* = 15.3, 5.2, H–C(5)); 5.71 (br. *dd*, *J* = 15.8, 5.9, H–C(4)); 5.52 (*d*, *J* = 5.4, H–C(1‴)); 4.40 (*dd*, *J* = 11.4, 5.4, H–C(2″)); 4.21 (*ddd*, *J* = 6.0, 4.8, 0.9, H–C(3)); 4.05 (br. *t*, *J* = 6.5, H–C(5‴)); 4.03–3.97 (*m*, H–C(2)); 3.94 (*d*, *J* = 4.5, 2 H–C(6)); 3.86 (*d*, *J* = 2.4, 2 H–C(4‴)); 3.79 (*dd*, *J* = 11.7, 7.2, H–C(6‴)); 3.73 (*dd*, *J* = 11.7, 5.1, H′–C(6‴)); 3.65 (*dd*, *J* = 11.6, 3.2, H–C(3‴)); 3.41 (*t*, *J* = 6.6, 2 H–C(1′)); 2.91 (*dd*, *J* = 13.8, 3.3, H–C(1)); 2.82 (*dd*, *J* = 13.7, 8.6, H′–C(1)); 2.18 (*t*, *J* = 7.4, 2 H–C(2″)); 1.96 (*s*, AcNH); 1.64–1.50 (*m*, 2 H–C(2′), 2 H–C(3″)); 1.36–1.26 (*m*, 44 H); 0.89 (*t*, *J* = 6.8, 2 Me). ^{13}C-NMR ((D$_5$)pyridine, 75 MHz): 173.46 (*s*, C(1″)); 170.44 (*s*, AcNH); 134.08 (*d*, C(4)); 128.69 (*d*, C(5)); 87.77 (*d*, C(1‴)); 73.61 (*d*, C(5‴)); 73.35 (*d*, C(3)); 71.08, 70.29 (2*t*, C(6), C(1′)); 70.17, 69.41 (2*d*, C(3‴), C(4‴)); 62.75 (*t*, C(6‴)); 55.26 (*d*, C(2)); 52.24 (*d*, C(2″)); 36.76 (*t*); 33.46 (*t*); 32.07 (2*t*); 30.21–29.56 (several *t*); 26.57 (*t*); 25.34 (*t*); 23.14 (*q*, AcNH); 22.89 (2*t*); 14.26 (2*q*, Me). HR-MALDI-MS: 809.5669 (100, $[M + Na]^+$, $C_{43}H_{82}N_2NaO_8S^+$; calc. 809.5684). Anal. calc. for $C_{43}H_{82}N_2O_8S \cdot H_2O$ (805.2001): C 64.14, H 10.52, N 3.48; found: C 64.10, H 10.69, N 3.45.

2. *Biological Tests. Phosphorylation of Ceramide Analogue by CerK.* Activity of CerK on C8-ceramide (*Cayman*) and the ceramide analogue **1** was measured according to published procedures [55] using 180 μM of either C8 ceramide or analogue **1** in the direct assay (*Fig. 1*,*a*), and 20 μM C8-ceramide and 100 μM **1** in the competitive assay (*Fig. 1*,*b*).

T-Cell Clones and APC. Human CD1d-restricted iNKT cell clones were derived and cultivated as described in [60]. Human CD1d-transfected C1R or THP1 cells were used as APC in the experiments.

Antigen-Presentation Assays. Human CD1d transfectants (2.5×10^4/well) in RPMI-1640 medium containing 10% FCS were incubated at 37° with sonicated compounds at the concentrations indicated in the *Figures* in the presence of T cells (7.5×10^4/well). Supernatants were harvested after 24 h, and released cytokines were measured by ELISA.

Plate-Bound Human CD1d Competition Assay. Plates were coated overnight at r.t. with 10 μg/ml BIR1.4 monoclonal antibody (mAb). Soluble recombinant human CD1d purified by isoelectric focusing was incubated overnight at r.t. on BIR1.4-coated plates at twofold molar concentration of BIR1.4 mAb. Sonicated compounds were added 4.5 h before αGalCer to plate-bound human CD1d and left overnight at r.t. to allow competition. Then, T cells (1.5×10^5/well) were plated in RPMI-1640 medium containing 5% human serum and 100 U/ml human IL-2 after extensive washing. Supernatants were harvested after 24 h of incubation at 37°, and released cytokines were measured by ELISA.

ELISA. Plates coated with 8D4-8 (anti-human IL-4 mAb, BD), MAb1 (anti-human TNF-α mAb, BD), 6804 (anti-human GM-CSF mAb, R&D), or HB-8700 (anti-human IFN-γ mAb, ATCC) were blocked and then incubated with the supernatants of the antigen presentation assays.

Detection of human IL-4, human TNF-α, or human IFN-γ was performed using MP4-25D2 (anti-human IL-4 biotin-labelled mAb, BD), MAb11 (anti-human TNF-α biotin-labelled mAb, BD), 3209 (anti-human GM-CSF biotin-labelled mAb, R&D), or γ69 [61] (anti-human IFN-γ biotin-labelled mAb, provided by *G. Garotta*) with streptavidin-HRP (*Zymed*, *Invitrogen*), and *o*-phenylenediamine dihydrochloride (*Sigma*, according to the manufacturer's instructions) as substrate. $OD_{490\,nm}$ was read on a spectrophotometer (*Molecular Devices*, Sunnyvale, CA) and converted to concentrations using the SoftMax Pro 5 program by comparison to standards of human IL-4 (human IL-4 secreting X63, kind gift of *U. Grawunder*), recombinant human TNF-α (*Immunokontact*), and recombinant human IFN-γ (*BenderMedSystems*).

REFERENCES

[1] R. X. Tan, J. H. Chen, *Nat. Prod. Rep.* **2003**, *20*, 509.
[2] S. Brodesser, P. Sawatzki, T. Kolter, *Eur. J. Org. Chem.* **2003**, 2021.
[3] N. S. Radin, *Bioorg. Med. Chem.* **2003**, *11*, 2123.
[4] Y. D. Vankar, R. R. Schmidt, *Chem. Soc. Rev.* **2000**, *29*, 201.
[5] M. Shimamura, Y.-Y. Huang, N. Okamoto, N. Suzuki, J. Yasuoka, K. Morita, A. Nishiyama, Y. Amano, T. Mishina, *Eur. J. Immunol.* **2007**, *37*, 1836.
[6] 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.

[7] K. Miyamoto, S. Miyake, T. Yamamura, *Nature* **2001**, *413*, 531.
[8] R. W. Franck, M. Tsuji, *Acc. Chem. Res.* **2006**, *39*, 692; R. M. Ndonye, D. P. Izmirian, M. F. Dunn, K. O. A. Yu, S. A. Porcelli, A. Khurana, M. Kronenberg, S. K. Richardson, A. R. Howell, *J. Org. Chem.* **2005**, *70*, 10260; K. Murata, T. Toba, K. Nakanishi, B. Takahashi, T. Yamamura, S. Miyake, H. Annoura, *J. Org. Chem.* **2005**, *70*, 2398.
[9] T. Tashiro, R. Nakagawa, S. Inoue, M. Shiozaki, H. Watarai, M. Tanigushi, K. Mori, *Tetrahedron Lett.* **2008**, *49*, 6827.
[10] D. I. Godfrey, S. P. Berzins, *Nat. Rev. Immunol.* **2007**, *7*, 505.
[11] E. Tupin, Y. Kinjo, M. Kronenberg, *Nat. Rev. Microbiol.* **2007**, *5*, 405.
[12] A. Bendelac, P. B. Savage, L. Teyton, *Annu. Rev. Immunol.* **2007**, *25*, 297.
[13] M. Kronenberg, *Annu. Rev. Immunol.* **2005**, *23*, 877.
[14] G. L. Yang, J. Schmieg, M. Tsuji, R. W. Franck, *Angew. Chem., Int. Ed.* **2004**, *43*, 3818.
[15] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *Tetrahedron* **2002**, *58*, 369.
[16] L. Barbieri, V. Costantino, E. Fattorusso, A. Mangoni, E. Aru, S. Parapini, D. Taramelli, *Eur. J. Org. Chem.* **2004**, 468.
[17] M. Maceyka, S. G. Payne, S. Milstien, S. Spiegel, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1585*, 193.
[18] O. Cuvillier, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1585*, 153.
[19] L. M. Obeid, C. M. Linardic, L. A. Karolak, Y. A. Hannun, *Science* **1993**, *259*, 1769.
[20] M. Kawase, M. Watanabe, T. Kondo, T. Yabu, Y. Taguchi, H. Umehara, T. Uchiyama, K. Mizuno, T. Okazaki, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1584*, 104.
[21] R. J. Bleicher, M. C. Cabot, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1585*, 172.
[22] B. J. Pettus, C. E. Chalfant, Y. A. Hannun, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1585*, 114.
[23] E. Gulbins, H. Grassme, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2002**, *1585*, 139.
[24] K. Shikata, H. Niiro, H. Azuma, K. Ogino, T. Tachibana, *Bioorg. Med. Chem.* **2003**, *11*, 2723.
[25] T. Tsujimoto, Y. Ito, *Tetrahedron Lett.* **2007**, *48*, 5513.
[26] Y. Liu, R. D. Goff, D. Zhou, J. Mattner, B. A. Sullivan, A. Khurana, C. Cantu III, E. V. Ravkov, C. C. Ibegbu, J. D. Altman, L. Teyton, A. Bendelac, P. B. Savage, *J. Immunol. Methods* **2006**, *312*, 34.
[27] T. Tashiro, R. Nakagawa, T. Hirokawa, S. Inoue, H. Watarai, M. Taniguchi, K. Mori, *Tetrahedron Lett.* **2007**, *48*, 3343.
[28] M. Fujio, D. G. Wu, R. Garcia-Navarro, D. D. Ho, M. Tsuji, C.-H. Wong, *J. Am. Chem. Soc.* **2006**, *128*, 9022.
[29] T. Lee, M. Cho, S.-Y. Ko, H.-J. Youn, D. J. Baek, W.-J. Cho, C.-Y. Kang, S. Kim, *J. Med. Chem.* **2007**, *50*, 585.
[30] T. Toba, K. Murata, K. Nakanishi, B. Takahashi, N. Takemoto, M. Akabane, T. Nakatsuka, S. Imajo, T. Yamamura, S. Miyake, H. Annoura, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2781.
[31] G.-W. Xing, D. Wu, M. A. Poles, A. Horowitz, M. Tsuji, D. D. Ho, C.-H. Wong, *Bioorg. Med. Chem.* **2005**, *13*, 2907.
[32] G.-T. Fan, Y.-S. Pan, K.-C. Lu, Y.-P. Cheng, W.-C. Lin, S. Lin, C.-H. Lin, C.-H. Wong, J.-M. Fang, C.-C. Lin, *Tetrahedron* **2005**, *61*, 1855.
[33] J.-J. Park, J. H. Lee, S. C. Ghosh, G. Bricard, M. M. Venkataswamy, S. A. Porcelli, S.-K. Chung, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3906.
[34] Y. Kinjo, B. Pei, S. Bufali, R. Raju, S. K. Richardson, M. Imamura, M. Fujio, D. Wu, A. Khurana, K. Kawahara, C.-H. Wong, A. R. Howell, P. H. Seeberger, M. Kronenberg, *Chem. Biol.* **2008**, *15*, 654.
[35] L. A. Augustin, J. Fantini, D. R. Mootoo, *Bioorg. Med. Chem.* **2006**, *14*, 1182.
[36] J. A. Morales-Serna, O. Boutureira, Y. Diaz, M. I. Matheu, S. Castillón, *Org. Biomol. Chem.* **2008**, *6*, 443.
[37] A. N. Rai, A. Basu, *J. Org. Chem.* **2005**, *70*, 8228; H. Tomiyama, T. Yanagisawa, M. Nimura, A. Noda, T. Tomiyama, 'Preparation of ceramidyl glycosides or C-glycosides for use as antitumor agents or immunostimulants', to Kotobuki Pharmaceutical Co., Ltd., Japan, Ger. Offen. DE 10128250-A1, 2001, 22 pp.
[38] R. T. Dere, X. Zhu, *Org. Lett.* **2008**, *10*, 4641.

[39] H. Driguez, *ChemBioChem* **2001**, *2*, 311.
[40] H. Driguez, in 'Glycoscience Synthesis of Substrate Analogs and Mimetics', Springer-Verlag, Berlin, 1997, 187, p. 85.
[41] J. Defaye, J. Gelas, 'Studies in Natural Products Chemistry', Elsevier, Amsterdam, 1991, p. 315.
[42] J. K. Fairweather, H. Driguez, 'Carbohydrates in Chemistry and Biology', Wiley-VCH, Toronto, 2000, p. 531.
[43] P. Nussbaumer, *ChemMedChem* **2008**, *3*, 543, and refs. cit. therein; A. N. Rai, A. Basu, *Org. Lett.* **2004**, *6*, 2861.
[44] T. Yamamoto, H. Hasegawa, T. Hakogi, S. Katsumura, *Org. Lett.* **2006**, *8*, 5569, and refs. cit. therein; S. Torssell, P. Somfai, *Org. Biomol. Chem.* **2004**, *2*, 1643; H. Hasegawa, T. Yamamoto, S. Hatano, T. Hakogi, S. Katsumura, *Chem. Lett.* **2004**, *33*, 1592.
[45] K. Pachamuthu, R. R. Schmidt, *Chem. Rev.* **2006**, *106*, 160.
[46] J. B. Schwarz, S. D. Kuduk, X.-T. Chen, D. Sames, P. W. Glunz, S. J. Danishefsky, *J. Am. Chem. Soc.* **1999**, *121*, 2662.
[47] H. Herzner, T. Reipen, M. Schultz, H. Kunz, *Chem. Rev.* **2000**, *100*, 4495.
[48] S. Spiegel, S. Milstien, *J. Biol. Chem.* **2007**, *282*, 2125.
[49] J. V. Staros, H. Bayley, D. N. Standring, J. R. Knowles, *Biochem. Biophys. Res. Commun.* **1978**, *80*, 568.
[50] S. Knapp, D. S. Myers, *J. Org. Chem.* **2002**, *67*, 2995.
[51] R. Rajan, K. Wallimann, A. Vasella, D. Pace, A. A. Genazzani, P. L. Canonico, F. Condorelli, *Chem. Biodiversity* **2004**, *1*, 1785.
[52] E. F. V. Scriven, K. Turnbull, *Chem. Rev.* **1988**, *88*, 297; S. J. Shuttleworth, S. M. Allin, P. K. Sharma, *Synthesis* **1997**, 1217.
[53] J. Pabba, B. P. Rempel, S. G. Withers, A. Vasella, *Helv. Chim. Acta* **2006**, *89*, 635.
[54] T. Morimoto, A. Yamazaki, K. Achiwa, *Chem. Pharm. Bull.* **2004**, *52*, 1367.
[55] P. Rovina, M. Jaritz, S. Höfinger, C. Graf, P. Dévay, A. Billich, T. Baumruker, F. Bornancin, *Biochem. J.* **2006**, *400*, 255.
[56] F. Bornancin, D. Mechtcheriakova, S. Stora C. Graf, A. Wlachos, P. Dévay, N. Urtz, T. Baumruker, A. Billich, *Biochim. Biophys. Acta* **2005**, *1687*, 31.
[57] C. Graf, M. Klumpp, M. Habig, P. Rovina, A. Billich, T. Baumruker, B. Oberhauser, F. Bornancin, *Mol. Pharmacol.* **2008**, *74*, 925.
[58] N. A. Borg, K. S. Wun, L. Kjer-Nielsen, M. C. Wilce, D. G. Pellicci, R. Koh, G. S. Besra, M. Bharadwaj, D. I. Godfrey, J. McCluskey, J. Rossjohn, *Nature* **2007**, *448*, 44.
[59] K. S. Wun, N. A. Borg, L. Kjer-Nielsen, T. Beddoe, R. Koh, S. K. Richardson, M. Thakur, A. R. Howell, J. P. Scott-Browne, L. Gapin, D. I. Godfrey, J. McCluskey, J. Rossjohn, *J. Exp. Med.* **2008**, *205*, 939.
[60] A. Shamshiev, H.-J. Gober, A. Donda, Z. Mazorra, L. Mori, G. De Libero, *J. Exp. Med.* **2002**, *195*, 1013.
[61] H. Gallati, I. Pracht, J. Schmidt, P. Häring, G. Garotta, *J. Biol. Regul. Homeost. Agents* **1987**, *1*, 109.

Received December 23, 2008