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Structure Assignment, Total Synthesis, and Evaluation of the Phosphatase Modulating Activity of Glucolipsin A

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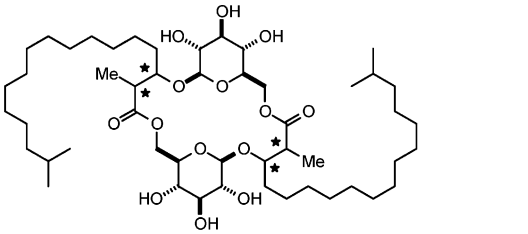
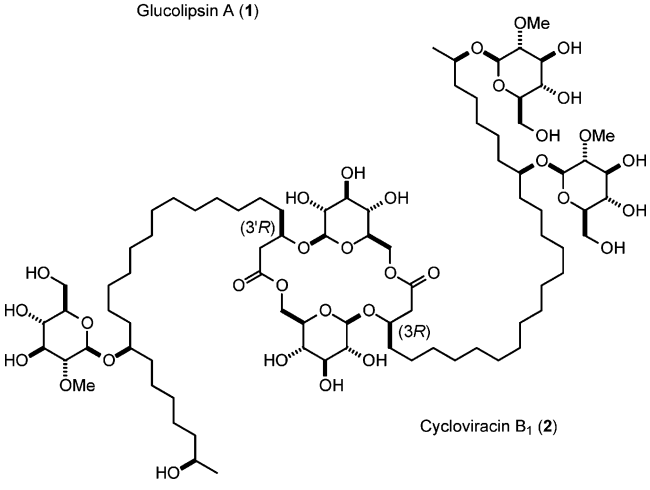
The previously unknown stereostructure of glucolipsin A (**1**), a complex glycolipid endowed with glucokinase-activating properties, was unambiguously elucidated as (2*R*,2′*R*,3*S*,3′*S*) by comparison of its spectroscopic and analytical data with those of all conceivable C_2-symmetric stereoisomers. This set of macrodiolides was prepared by a sequence comprising auxiliary guided aldol reactions, glycosidation of the resulting β-hydroxy acid derivatives with trichloroacetimidate **7**, followed by hydrolytic cleavage of the auxiliaries used. The hydroxy acids thus formed were subjected to a macrodilactonization reaction mediated by 2-chloro-1,3-dimethylimidazolinium chloride (**22**) as the activating agent; this transformation is highly productive only in the presence of admixed potassium cations which likely serve as templates to preorganize two substrate molecules in a favorable head-to-tail arrangement. Glucolipsin and analogues were subjected to enzymatic assays that revealed that glycoconjugates of this type effectively inhibit the activity of the dual specific phosphatase Cdc25A with IC_{50} values in the low micromolar range, while being hardly active against the tyrosine phosphatase PTP1B in vitro. This activity profile was compared to that of other glycolipids previously prepared in this laboratory, including cycloviracin B_1 (**2**), caloporoside (**38**), woodrosin I (**39**), sophorolipid lactone (**40**), and tricolorin G (**41**).

Introduction

Glucokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate which, in turn, plays a central role in the entire carbohydrate metabolism.[1] It is well established that this enzyme is allosterically inhibited by long-chain fatty acid- or stearoyl-CoA esters. Small molecules that either competitively bind to the fatty acid-CoA cofactor site or sequester these negative effectors would result in an upregulation of glucokinase activity and as such might ultimately lead to complementary drugs for therapeutic intervention in diabetes.[2]

During a search for natural products able to relieve the inhibitory effects of stearoyl-CoA on glucokinase, a team at Bristol-Myers Squibb discovered the structurally rather unusual glycoconjugate glucolipsin A (**1**) and a homologue thereof. These macrocyclic dilactones produced by *Streptomyces purpurogeniscleroticus* and *Nocardia vaccinii* strains exhibit RC_{50} values of 4.6−5.4 μM in the pertinent bioassays.[3] While spectroscopic investigations unraveled the symmetric structure of **1** and showed the presence of two β-glucose entities within its macrocyclic core, the absolute stereochemistry of the chiral centers (*) at the periphery remained elusive. It is tempting to speculate that glucolipsin might have the same configuration at the branching sites as cycloviracin B_1 (**2**), a constitutionally related glycoconjugate endowed





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‡ Max-Planck-Institut für Molekulare Physiologie.

(1) Nelson, D. L.; Cox, M. M. *Lehninger Biochemie*, 3rd ed.; Springer: Berlin, Germany, 2001.

(2) See the following for a leading reference and literature cited therein: Grimsby, J.; Sarabu, R.; Corbett, W. L.; Haynes, N.-E.; Bizzarro, F. T.; Coffey, J. W.; Guertin, K. R.; Hilliard, D. W.; Kester, R. F.; Mahaney, P. E.; Marcus, L.; Qi, L.; Spence, C. L.; Tegni, J.; Magnuson, M. A.; Chu, C. A.; Dvorozniak, M. T.; Matschinsky, F. M.; Grippo, J. F. *Science* **2003**, *301*, 370.

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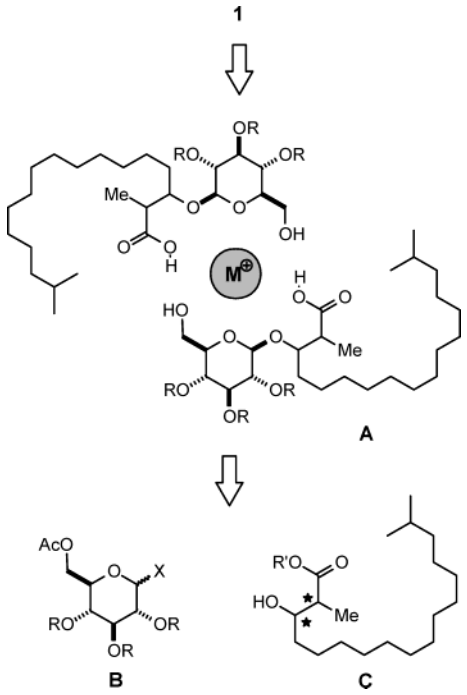
with antiviral properties.[4] It has recently been shown by total synthesis that compound **2** is (3*R*,3′*R*) configured.[5] The notion that **1** and **2** might share the same stereochemical pattern is corroborated by a seemingly consistent set of NMR data,[5] although possible effects of the adjacent methyl branch in **1** on the observed shifts leave some ambiguity in this regard. Since this methyl group is absent in **2**, no conclusions concerning the stereochemistry of the additional chiral centers at C-2/C-2′ in glucolipsin can be drawn and even their relative configuration remains unknown.

Outlined below is an unambiguous assignment of the stereostructure of glucolipsin A by comparison of the published data with those of all conceivable C_2-symmetrical dimers of this type. As part of a long-term project on bioactive glycoconjugates,[5−9] we prepared this set of compounds by a highly integrated synthesis route based on a template-directed cyclodimerization reaction as the key step. In contrast to previous assumptions, however, this study revealed that the fatty acid moiety of glucolipsin A comprises a *syn*-aldol with a (3*S*)-configured stereocenter, *opposite* the (3*R*)-configured motif found in cycloviracin **2**. Finally, synthetic **1** and congeners were subjected to a more detailed screening program that revealed an unusual profile as modulators of various phosphatases, allowing for the stimulation as well as the inhibition of enzymatic activity in a dose-dependent manner.

Results and Discussion

Strategy. On the basis of our previous experiences with sugar-based dilactones,[5] it was assumed that the specific array of oxygen atoms in the core region of glucolipsin A endows this compound with some degree of ionophoric character. This property can be exploited as a key design feature of an expeditious synthesis route, which allows the assembly of the target via a template-directed macrodilactonization reaction of a rather simple glycosylated aldol derivative **A** preorganized around a suitable metal cation. As can be seen from Scheme 1, this plan results in a considerable retrosynthetic simplification and should therefore be well suited for the preparation of the entire set of possible stereoisomers as required for the unambiguous structure elucidation of **1**.

SCHEME 1. Retrosynthetic Analysis of Glucolipsin A



Preparation of the Building Blocks. To meet this interim goal in a reliable and efficient way, recourse was taken to established auxiliary-based aldol methodology (Scheme 2).[10] The required aldehyde **4** was prepared from commercially available 12-bromo-1-dodecanol (**3**)[11] by reaction with isobutylmagnesium bromide in the presence of Li_2CuCl_4 [12] and subsequent PCC oxidation of the resulting alcohol. As expected, reaction of aldehyde **4** with the boron enolate derived from **5** and *n*-Bu_2BOTf under standard conditions delivered the (2*R*,3*S*)-configured *syn*-aldol derivative **6** in essentially diastereomerically pure form (de 99%) after purification of the crude product by flash chromatography.[13]

The subsequent glycosidation reaction, however, turned out to be far from routine.[14] In fact, the conversion of β-hydroxy carbonyl compounds to the corresponding β-glycosides constitutes a formidable challenge for which no general solution yet exists.[15] This is mainly caused by the poor glycosyl acceptor properties of aldol derivatives and by their insufficient chemical stability under the required acidic reaction conditions. In line with this,

(3) Qian-Cutrone, J.; Ueki, T.; Huang, S.; Mookhtiar, K. A.; Ezekiel, R.; Kalinowski, S. S.; Brown, K. S.; Golik, J.; Lowe, S.; Pirnik, D. M.; Hugill, R.; Veitch, J. A.; Klohr, S. E.; Whitney, J. L.; Manly, S. P. *J. Antibiot*. **1999**, *52*, 245.

(4) (a) Tsunakawa, M.; Komiyama, N.; Tenmyo, O.; Tomita, K.; Kawano, K.; Kotake, C.; Konishi, M.; Oki, T. *J. Antibiot*. **1992**, *45*, 1467. (b) Tsunakawa, M.; Kotake, C.; Yamasaki, T.; Moriyama, T.; Konishi, M.; Oki, T. *J. Antibiot*. **1992**, *45*, 1472.

(5) (a) Fürstner, A.; Albert, M.; Mlynarski, J.; Matheu, M.; DeClercq, E. *J. Am. Chem. Soc*. **2003**, *125*, 13132. (b) Fürstner, A.; Albert, M.; Mlynarski, J.; Matheu, M. *J. Am. Chem. Soc*. **2002**, *124*, 1168. (c) Fürstner, A.; Mlynarski, J.; Albert, M. *J. Am. Chem. Soc*. **2002**, *124*, 10274.

(6) (a) Fürstner, A.; Müller T. *J. Am. Chem. Soc*. **1999**, *121*, 7814. (b) Fürstner, A.; Müller, T. *J. Org. Chem*. **1998**, *63*, 424. (c) Lehmann, C. W.; Fürstner, A.; Müller, T. *Z. Kristallogr*. **2000**, *215*, 114.

(7) Fürstner, A.; Radkowski, K.; Grabowski, J.; Wirtz, C.; Mynott, R. *J. Org. Chem*. **2000**, *65*, 8758.

(8) (a) Fürstner, A.; Jeanjean, F.; Razon, P. *Angew. Chem*. **2002**, *114*, 2203; *Angew. Chem., Int. Ed*. **2002**, *41*, 2097. (b) Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J*. **2003**, *9*, 307. (c) Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J*. **2003**, *9*, 320.

(9) (a) Fürstner, A.; Konetzki, I. *J. Org. Chem*. **1998**, *63*, 3072. (b) Fürstner, A.; Konetzki, I. *Tetrahedron* **1996**, *52*, 15071. (c) Fürstner, A.; Konetzki, I. *Tetrahedron Lett*. **1998**, *39*, 5721.

(10) (a) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem*. **1982**, *13*, 1. (b) Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1984; Vol. 3, pp 111−212. (c) Cowden, C. J.; Paterson, I. *Org. React*. **1997**, *51*, 1.

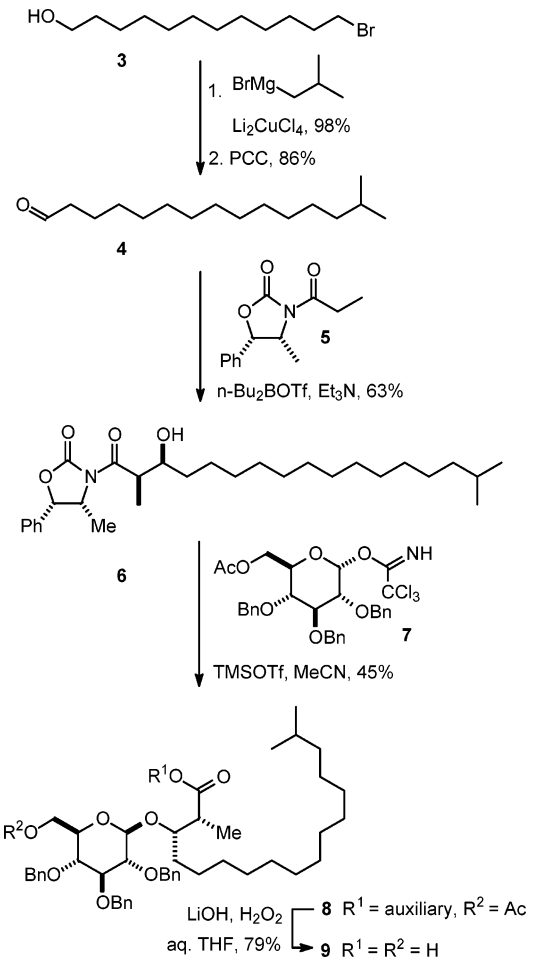
(11) This product can be prepared on a large scale from 1,12-dodecandiol and HBr in toluene according to the following: Chong, J. M.; Heuft, M. A.; Rabbat, P. *J. Org. Chem*. **2000**, *65*, 5837.

(12) Nunomoto, S.; Kawakami, Y.; Yamashita, Y. *J. Org. Chem*. **1983**, *48*, 1912.

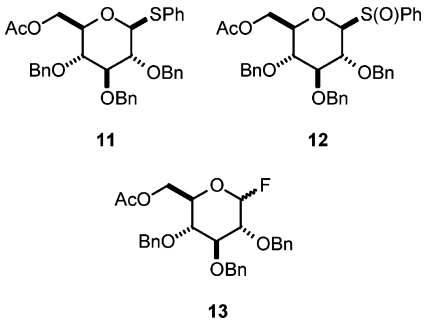
(13) (a) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc*. **1982**, *104*, 1737. (b) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc*. **1981**, *103*, 2127.

(14) General reviews see: (a) Paulsen, H. *Angew. Chem*. **1982**, *94*, 184; *Angew. Chem., Int. Ed. Engl*. **1982**, *21*, 155. (b) Schmidt, R. R. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, UK, 1991; Vol. 6, pp 33−61. (c) Toshima, K.; Tatsuta, K. *Chem. Rev*. **1993**, *93*, 1503.

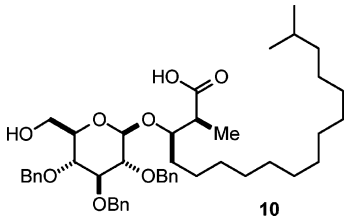
SCHEME 2



the effective yields obtained in the glycosidation of compound **6** remained rather low despite considerable experimentation with various glucosyl donors and different promoter systems.[16] Best results were obtained with use of an excess (3 equiv) of trichloroacetimidate **7**[5] activated in situ by TMSOTf (20 mol %) in MeCN.[17] Under these conditions, product **8** was obtained in 45% isolated yield; the undesired α-anomer (β:α = 5.2:1) was removed by routine flash chromatography. The use of other donors, including the phenyl thioglycoside **11**,[18] the anomeric sulfoxide **12**,[19] or the glycosyl fluoride **13** under Mukaiyama conditions ($SnCl_2$, $AgClO_4$, MeCN)[20,21] essentially met with failure.



Saponification of product **8** with aqueous LiOH in THF in the presence of H_2O_2[22] furnished hydroxy acid **9** as the required substrate for the cyclodimerization step by concomitant cleavage of the oxazolidinone and the residual acetate. The corresponding (2*S*,3*R*)-configured analogue **10** was obtained following the same reaction sequence employing the antipodal auxiliary (cf. Supporting Information).



In an attempt to probe whether the presence of the auxiliary in **6** or its enantiomer **14** is responsible for the low yield in the glycosidation reaction (e.g. by sequestering the Lewis-acidic promoter), the corresponding methyl ester **15** derived from **14** was reacted with donor **7** under similar conditions (Scheme 3). Although the yield of the desired glycoside **16** was raised to 60%, the significantly lower β:α ratio of 2.5:1 renders this detour unrewarding overall.

Access to the *anti*-aldol series was secured by the method developed by Abiko and Masamune employing a norephedrine-derived auxiliary (Scheme 4).[23] Specifically, enolization of **17** with dicyclohexylboryl triflate and Et_3N followed by addition of aldehyde **4** at low temperature gave the desired *anti*-aldol derivative **18** in good yield and excellent diastereoselectivity (de > 99% after flash chromatography). Subsequent reaction with trichloroacetimidate **7** in the presence of TMSOTf as the catalyst furnished glycoside **19** in a respectable yield of 74% as a 5:1 mixture of anomers. The cleavage of the auxiliary in **19**, however, could only be effected with (*n*-Bu)$_4$NOH in

(15) For representative examples illustrating this notion see: (a) Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001. (b) Paterson, I.; McLeod, M. D. *Tetrahedron Lett.* **1995**, *36*, 9065. (c) Toshima, K.; Misawa, M.; Ohta, K.; Tatsuta, K.; Kinoshita, M. *Tetrahedron Lett.* **1989**, *30*, 6417. (d) Mitchell, S. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *66*, 2327.

(16) Promotors screened include the following: TMSOTf, TBSOTf, triflic acid, BF_3·Et_2O, $SnCl_4$, Cl_3CCHO. Although TBSOTf has previously been recommended as a superior promotor for glycosidation reactions of sensitive substrates, we did not observe any improvement in our case upon replacing TMSOTf by TBSOTf; cf.: (a) Marzabadi, C. H.; Franck, R. W. *Tetrahedron* **2000**, *56*, 8385. (b) Roush, W. R.; Narayan, S. *Org. Lett.* **1999**, *1*, 899. (c) For the use of Cl_3CCHO as a particularly mild promotor for sensitive substrates see: Schmidt, R. R.; Gaden, H.; Jatzke, H. *Tetrahedron Lett.* **1990**, *31*, 327.

(17) (a) Schmidt, R. R. *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(18) (a) Nicolaou, K. C.; Ueno, H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 313−338. (b) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435.

(19) (a) Kahne, D.; Walker, S.; Cheng, Y.; Engen, D. V. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Sildersleeve, J.; Pascal, R. A.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 5961.

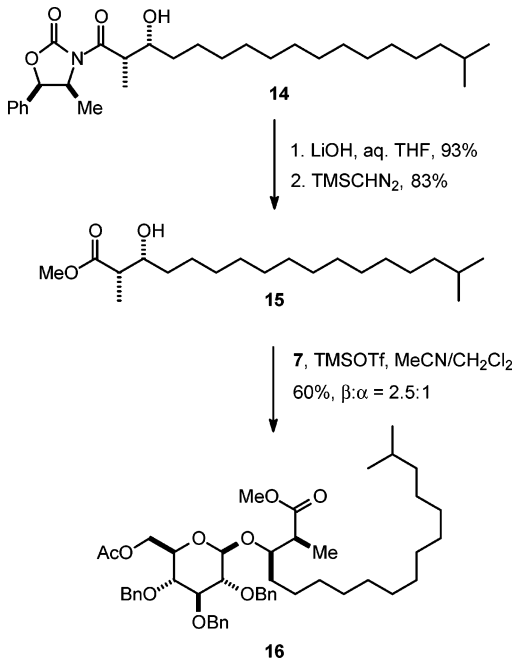
(20) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.

(21) For a recent successful application of a glycosyl fluoride to the formation of 2-deoxy-β-glycosides from β-hydroxy ketones and a detailed discussion see: Blanchard, N.; Roush, W. R. *Org. Lett.* **2003**, *5*, 81.

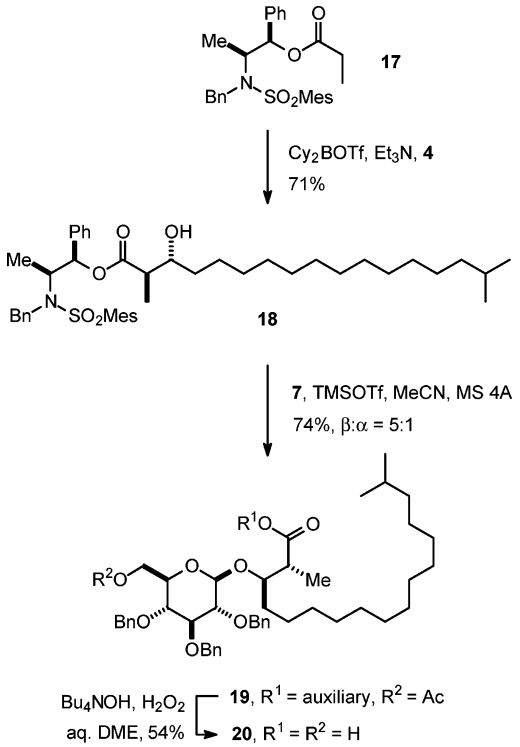
(22) Gage, J. R.; Evans, D. A. *Org. Synth.* **1990**, *68*, 83.

(23) (a) Abiko, A.; Liu, J.-F.; Masamune, S. *J. Org. Chem.* **1996**, *61*, 2590. (b) Abiko, A.; Liu, J.-F.; Masamune, S. *J. Am. Chem. Soc.* **1997**, *119*, 2586. (c) Inoue, T.; Liu, J.-F.; Buske, D. C.; Abiko, A. *J. Org. Chem.* **2002**, *67*, 5250. (d) Abiko, A. *Org. Synth.* **2002**, *79*, 116.

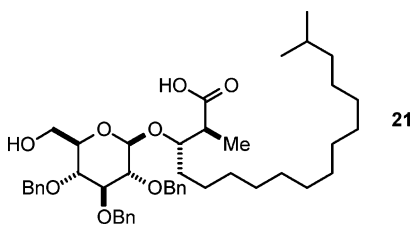
SCHEME 3



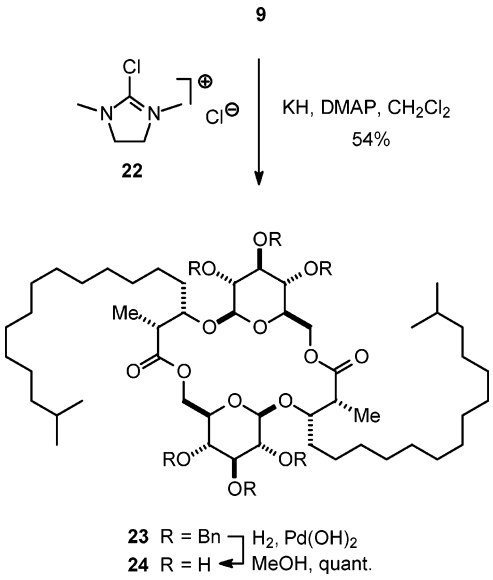
SCHEME 4



combination with H_2O_2,[24] whereas LiOH failed completely in this particular case. By following the same sequence of reactions using *ent*-**17** as the reagent in the crucial aldol step, access to the diastereomeric *anti*-aldol derivative **21** was secured (cf. Supporting Information).



SCHEME 5



Cyclodimerization Reactions and Completion of the Total Synthesis. With the complete set of stereoisomeric hydroxy acids in hand, the envisaged template directed cyclization reactions were investigated. In line with our expectations,[5] smooth macrodilactonization of substrate **9** was achieved when the reaction was performed with 2-chloro-1,3-dimethylimidazolinium chloride (**22**) as the activating agent[25,26] in the presence of admixed potassium cations derived from KH used as the base to deprotonate the starting material (Scheme 5). Under these conditions, the cyclic dimer **23** was obtained in 54% isolated yield.

As can be seen from Table 1, all other isomeric hydroxy acids behaved similarly well and afforded the corresponding C_2-symmetrical dimers in equally satisfactory yields. HPLC analyses of the crude reaction mixtures showed the striking preference for the formation of the macrodilactones over other conceivable products (cf. entry 1, footnote *b*), whereas in the absence of potassium cations an almost statistical mixture of the cyclic monomer, the desired cyclic dimer, and higher oligomers are formed.[5] Together with the fact that the reaction rate is considerably accelerated by the admixed cations, this result lends credence to the notion that the macrodilactonization reactions of **9** and analogues proceed in a template-directed manner in which the potassium cations help to preorganize the substrate in a suitable head-to-tail arrangement (cf. Scheme 1).[27,28]

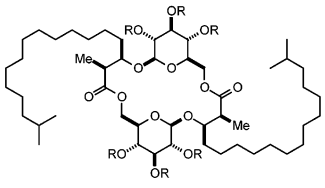
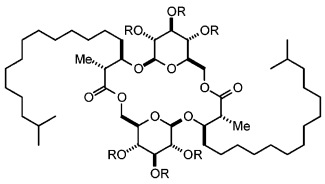
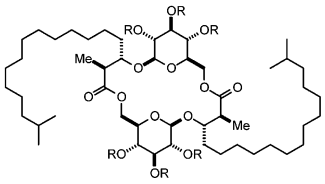
(24) Hasegawa, T.; Yamamoto, H. *Synlett* **1998**, 882.

(25) (a) Isobe, T.; Ishikawa, T. *J. Org. Chem*. **1999**, *64*, 5832. (b) Isobe, T.; Ishikawa, T. *J. Org. Chem*. **1999**, *64*, 6984. (c) Isobe, T.; Ishikawa, T. *J. Org. Chem*. **1999**, *64*, 6989. (d) See also: Garcia, D. M.; Yamada, H.; Hatakeyama, S.; Nishizawa, M. *Tetrahedron Lett*. **1994**, *35*, 3325. (e) Corey, E. J.; Gin, D. Y.; Kania, R. S. *J. Am. Chem. Soc*. **1996**, *118*, 9202. (f) Akaji, K.; Kurijama, N.; Kiso, Y. *J. Org. Chem*. **1996**, *61*, 3350.

(26) For an application of this reagent in organometallic chemistry see: Fürstner, A.; Seidel, G.; Kremzow, D.; Lehmann, C. W. *Organometallics* **2003**, *22*, 907.

(27) Fürstner, A. In *Templated Organic Synthesis*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, Germany, 2000; pp 249−273.

TABLE 1. Results of the Macrodilactonization Reactions Mediated by Imidazolinium Chloride 22 in CH_2Cl_2 (0.05 M) in the Presence of Potassium Cations as the Templates

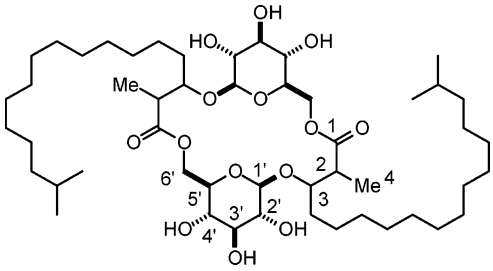
Substrate	Product	Yield (R = Bn)[a]	Yield (R = H)[g]
10		**25** (60%)[b,c]	**26** (quant.)
20		**27** (57%)[d]	**28** (quant.)
21		**29** (>90%)[e,f]	**30** (quant.)

[a] Isolated yield of analytically pure compound unless stated otherwise. [b] The product distribution in the crude mixture according to HPLC was as follows: cyclic monomer (11%), cyclic dimer **25** (87%), higher oligomers (3%). [c] $[\alpha]^{20}_D$ −19.4 (*c* 0.65, MeOH). [d] $[\alpha]^{20}_D$ −23.7 (*c* 0.75, CH_2Cl_2). [e] HPLC yield. [f] $[\alpha]^{20}_D$ −2.1 (*c* 0.65, MeOH). [g] Products formed upon hydrogenolytic cleavage of the benzyl ether protecting groups.

Hydrogenolytic cleavage of the benzyl ether groups in these products proceeded quantitatively in all cases with $Pd(OH)_2$ as the precatalyst. The spectroscopic data of the unprotected macrodiolides thus obtained were compared with those of glucolipsin A (**1**) reported in the literature.[3] Particularly diagnostic are the shifts of the anomeric carbon atom and the signals of the aldol region. As can be seen from Tables 2 and 3, it is the (2*R*,2′*R*,3*S*,3′*S*)-isomer **24** that shows an excellent match with the natural product. The characteristic pattern signature of its ^{1}H NMR spectrum is superimposable to that of authentic glucolipsin A depicted in ref 3. While these comparisons rigorously establish the syn arrangement of the aldol entity, it is surprising to find that the absolute configuration of C-3/C-3′ in **1** is *opposite* that one found in the otherwise closely related glycoconjugate cycloviracin B_1 (**2**).[5]

Phosphatase Activity-Modulating Properties of Glycolipids. Given the interesting biological properties of glucolipsin A, cycloviracin B_1, and related glycolipids and light of the fact that natural products frequently inhibit enzymes with significantly differing activity[29] we

TABLE 2. Comparison of Relevant Signals of the ^{13}C NMR Spectra (MeOH-d_4) of Glucolipsin A (1) with Those of the Synthetic Products[a]



position	glucolipsin A (1)	**24** (2*R*,3*S*)	**26** (2*S*,3*R*)	**28** (2*R*,3*R*)	**30** (2*S*,3*S*)
1	175.7	175.8	176.0	175.5	175.8
2	46.6	46.7	45.9	45.8	41.7
3	78.9	78.9	77.9	83.1	76.8
4	13.4	13.3	13.4	9.3	8.4
1′	104.5	104.5	102.0	106.7	100.0
2′	75.6	75.6*	75.1*	75.2*	75.1*
3′	78.0	78.0	78.2	78.0	78.1
4′	72.4	72.4	72.4	72.6	72.4
5′	75.4	75.5*	75.6*	75.1*	75.4*
6′	66.3	66.3	65.9	66.1	66.9

[a] Signals marked with an asterisk may be interchanged; arbitrary numbering scheme as shown.

subjected the stereoisomeric dilactones as well as selected key intermediates formed en route to **1** to biological screens established in our laboratories.[30] In particular, compounds **24**, **26**, **28**, and **30** were investigated as possible inhibitors of protein phosphatases.

Reversible phosphorylation of proteins is among the most important regulatory biological processes. It is vital to innumerable biological phenomena and involved in the establishment of diseases ranging from diabetes over immunological disorders to the development of cancer. While the phosphorylating kinases are established drug targets, their natural antagonists, i.e., the dephosphorylating phosphatases, have only recently moved into the focus of medicinal chemistry research, and new types of phosphatase inhibitors are in urgent demand.

The dual-specificity phosphatase Cdc25A and the tyrosine phosphatase PTP1B were chosen as representative enzymes for screening. The family of Cdc25 protein phosphatases is critically involved in cell cycle control.[31] Their physiological substrates are cyclin-dependent kinases which trigger key transitions in the process of eukaryotic cellular division. Their oncogenic properties together with the fact that Cdc25A and B are overexpressed in many human tumors render these isoenzymes molecular targets of utmost interest in the quest for anticancer drugs.[30−33] On the other hand, PTP1B is a key negative regulator of insulin-receptor activity, and PTP1B inhibitors are expected to enhance insulin sensitivity and act as effective therapeutics for the treatment of type II diabetes, insulin resistance, and obesity.[34]

The results of the screen for phosphatase-inhibiting activity are shown in Table 4. All four isomeric dilactones

(28) Attempts to monitor the complexation of K^+ by macrodiolide hosts via NMR titration experiments were inconclusive. However, it is known that the association constants between alkali metal cations and sugar-based crown ethers are usually rather low; see the following for a leading reference and literature cited therein: Shizuma, M.; Kadoya, Y.; Takai, Y.; Imamura, H.; Yamada, H.; Takeda, T.; Arakawa, R.; Takahashi, S.; Sawada, M. *J. Org. Chem*. **2002**, *67*, 4795.

(29) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed*. **2002**, *41*, 2878.

(30) For a recent study see: Fürstner, A.; Feyen, F.; Prinz, H.; Waldmann, H. *Angew. Chem*. **2003**, *115*, 5519; *Angew. Chem., Int. Ed*. **2003**, *42*, 5361.

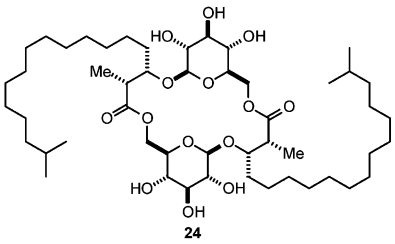
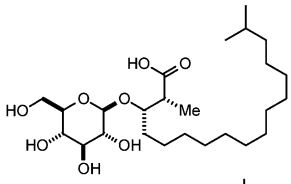
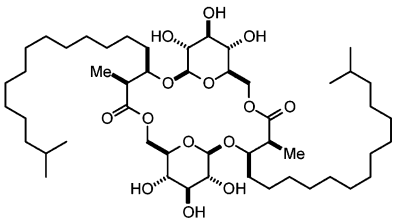
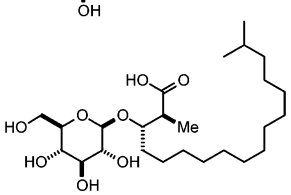
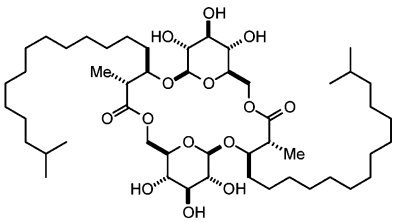
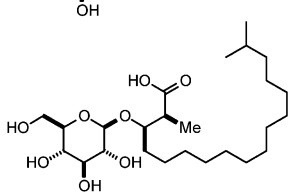
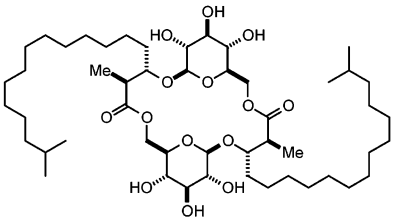
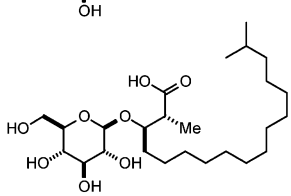
(31) Reviews: (a) Galaktinov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575. (b) Draetta, G.; Eckstein, J. *Biochim. Biophys. Acta* **1997**, *1332*, M53. (c) Nilsson, I.; Hoffmann, I. *Prog. Cell Cycle Res*. **2000**, *4*, 107.

TABLE 3. Comparison of Relevant Signals [δ (multiplicity, J (Hz))] of the ^{1}H NMR Spectra (MeOH-d_4) of Glucolipsin A (1) with Those of the Synthetic Products[a]

position	glucolipsin A	**24** (2*R*,3*S*)	**26** (2*S*,3*R*)	**28** (2*R*,3*R*)	**30** (2*S*,3*S*)
2	2.63 (m)	2.63 (quint, *7.2*)	2.60 (quint, *7.0*)	3.28 (qd, *6.8, 4.3*)	3.10 (qd, *6.8, 4.1*)
3	4.05 (m)	4.05 (dt, *7.2, 5.0*)	4.11 (q, *5.9*)	4.02−4.05 (m)	4.27−4.31 (m)
4	1.31 (d, *7.0*)	1.31 (d, *7.0*)	1.22 (d, *7.0*)	1.06 (d, *7.0*)	1.12 (d, *6.8*)
1′	4.36 (d, *7.7*)	4.36 (d, *7.6*)	4.31 (d, *7.7*)	4.37 (d, *7.8*)	4.37 (d, *7.7*)
2′	3.20 (dd, *9.0, 7.8*)	3.20 (dd, *9.1, 7.7*)	3.17 (dd, *9.1, 7.7*)	3.21 (dd, *9.1, 7.9*)	3.20 (dd, *9.2, 7.7*)
3′	3.35 (dd, *9.8, 9.1*)	3.35 (t, *9.0*)	3.34 (t, *9.0*)	3.36 (dd, *9.0, 7.8*)	3.37 (t, *9.0*)
4′	3.15 (t, *9.8*)	3.15 (dd, *9.7, 8.8*)	3.16 (dd, *9.8, 8.9*)	3.14 (dd, *9.2, 7.3*)	3.15 (dd, *9.6, 9.0*)
5′	3.46 (t, *9.9*)	3.46 (t, *9.8*)	3.36 (td, *9.0, 1.7*)	3.52 (td, *9.8, 1.6*)	3.55 (td, *9.8, 1.0*)
6a′	3.85 (t, *9.9*)	3.85 (dd, *11.4, 9.9*)	3.69 (dd, *11.5, 9.2*)	4.11 (t, *11.4*)	3.84 (dd, *11.4, 9.8*)
6b′	4.57 (d, *10.5*)	4.56 (bd, *10.3*)	4.73 (dd, *11.5, 1.7*)	4.32 (dd, *11.4, 1.6*)	4.49 (dd, *11.4, 1.0*)

[a] Arbitrary numbering scheme as shown at the top of Table 2.

TABLE 4. Inhibition of Cdc25A and PTP1B by Glucolipsin and Analogues (n.i.: less than 50% inhibition at 50 μM)

Compound	Cdc25A (IC_{50})	PTP1B (IC_{50})	Compound	Cdc25A (IC_{50})	PTP1B (IC_{50})
 **24**	2.2±1.1 μM	n.i.	 **31**	1.6±1.3 μM	15±7 μM
 **26**	2.6±1.3 μM	n.i.	 **32**	3.4±1.7 μM	23±11 μM
 **28**	2.6±1.3 μM	n.i.	 **33**	4.4±2.2 μM	23±11 μM
 **30**	2.3±1.1 μM	n.i.	 **34**	36±18 μM	n.i.

inhibit Cdc25A in the low micromolar range but they do not affect the activity of PTP1B. The IC_{50} values for the four diastereomers are identical within the error limit, indicating that the configuration of the stereocenters in the core region is not important for Cdc25A inhibition. The monomeric β-hydroxyacids **31**, **32**, and **33**, which

(32) See: (a) Cangi, M. G.; Cukor, B.; Soung, P.; Signoretti, S.; Moreira, G.; Ranashinge, M.; Cady, B.; Pagano M.; Loda, M. *J. Clin. Invest*. **2000**, *106*, 753. (b) Dixon, D.; Moyana, T.; King, M. J. *Exp. Cell Res*. **1998**, *240*, 236. (c) Gasparotto, D.; Maestro, R.; Piccinin, S.; Vukosavljigevic, T.; Barman, L.; Sulfaro, S.; Boiocchi, M. *Cancer Res*. **1997**, *57*, 2366. (d) Wu, W.; Fan, Y. H.; Kemp, B. L.; Walsh, G.; Mao, L. *Cancer Res*. **1998**, *58*, 4082.

(33) (a) For comprehensive literature coverage see: Lyon, M. A.; Ducruet, A. P.; Wipf, P.; Jazo, J. S. *Nature Rev. Drug Disc*. **2002**, *1*, 961. (b) For a representative study see: Brohm, D.; Philippe, N.; Metzger, S.; Bhargava, A.; Müller, O.; Lieb, F.; Waldmann, H. *J. Am. Chem. Soc*. **2002**, *124*, 13171.

(34) (a) Zhang, Z.-Y. *Curr. Op. Chem. Biol*. **2001**, *5*, 416. (b) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nature Rev. Drug Disc*. **2002**, *1*, 696.

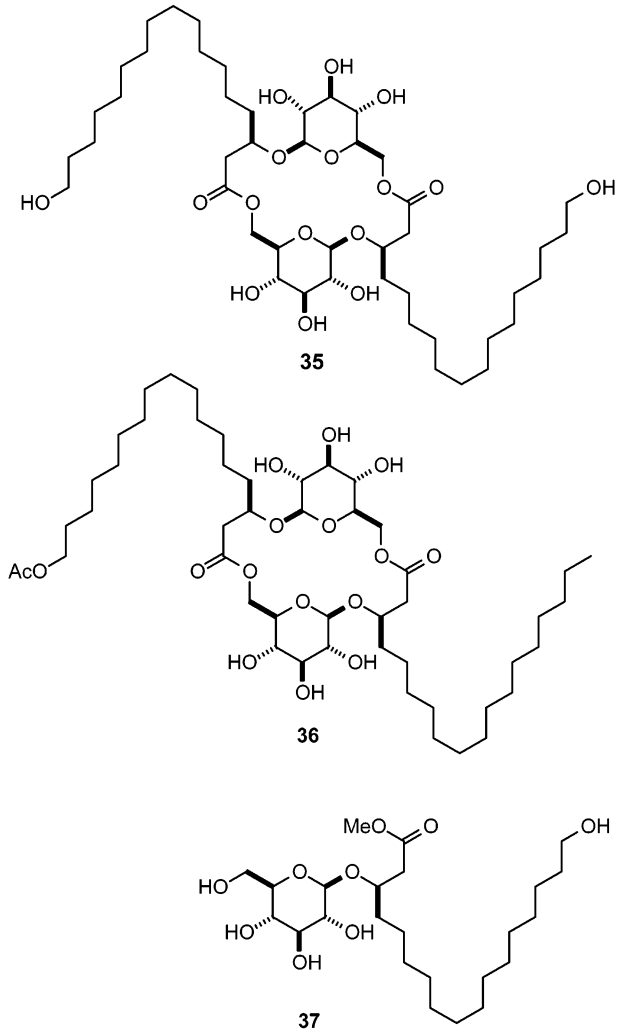
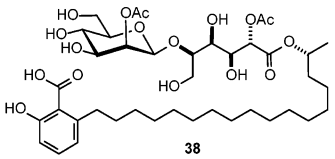
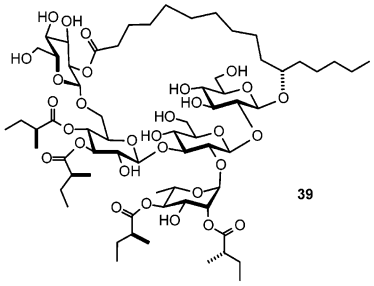
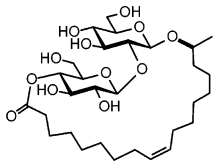
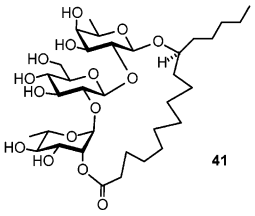


FIGURE 1. Glycolipids that did not inhibit Cdc25A and PTP1B.

were obtained from carboxylic acids **9**, **21**, and **10** by removal of the protecting groups, inhibit both Cdc25A and PTP1B. The isomeric compound **34**, however, deviates from this pattern as it is only a weak Cdc25A inhibitor and has no effect on the activity of the tyrosine phosphatase.

To obtain deeper insights into possible structure/activity relationships, other glycolipids obtained in previous synthesis projects were investigated. The results are compiled in Figure 1 and Table 5. Compounds **35**−**37** were obtained in the context of studies leading to the total synthesis of cycloviracin B_1 (**2**).[5] None of these turned out to be an inhibitor of Cdc25A or PTP1B. Notably, these compounds differ from the ones shown in Table 4 by only one stereogenic center missing in their β-hydroxyester segments and by the presence of at least one polar headgroup at the lipidic appendices; otherwise the structures are very similar to the glucolipsin series. Table 5 summarizes the results for the phospholipase inhibitor caloporoside (**38**),[9] woodrosin I (**39**),[8] sophorolipid lactone (**40**),[7] and tricolorin G (**41**).[6a] Caloporoside proved to be a strong inhibitor of both Cdc25A and even more potent against PTP1B. Woodrosin is a good Cdc25 inhibitor whereas compound **40** has only very weak inhibiting activity and tricolorin G (**41**) was devoid of any noticeable effects in our assays.

TABLE 5. Inhibition of Cdc25A and PTP1B by Different Types of Glycolipids (n.i.: less than 50% inhibition at 50 μM)

Compound	Cdc25A (IC_{50})	PTP1B (IC_{50})
 **38**	5.5±2.8 μM	1.6±0.8 μM
 **39**	7±1.1 μM	n.i.
 **40**	45±22 μM	n.i.
 **41**	n.i.	n.i.

Remarkably, in the course of the inhibition studies an unexpected phosphatase-*stimulating* activity was recorded for some of the compounds investigated. Thus, glycolipids **28** (but not **24**, **26**, and **30**), **31**−**33**, **35**, and **39**−**41** stimulate the activity of PTP1B at concentrations of 1−5 μM. Such an effect was not observed for the phosphatase Cdc25. We have not investigated this effect in detail; however, involvement of allosteric binding could be a possible explanation.

Given the amphiphilic character of the glycolipids investigated, the formation of micelles might also be a factor that influences the enzyme activity-modulating properties of this compound class.[35] However, the finding that structurally closely related compounds exhibit fairly differing inhibitory potency and that the observed stimulation is enzyme dependent (no effect on Cdc25A) and differs substantially even for compounds with very similar structure (compare compounds **28** with compounds **24**, **26**, and **30**) argue against a major influence of such a physical effect.

(35) Aggregate formation has for instance been found to influence the inhibitory activity of certain protein kinase inhibitors: McGovern, S. L.; Shoichet, B. K. *J. Med. Chem.* **2003**, *46*, 1478.

The results concerning the phosphatase-inhibiting activity of the glycolipids compiled in Tables 4 and 5 should be of general interest to this particular area of medicinal chemistry and chemical biology since glycolipids have not been identified as phosphatase inhibitors so far. Moreover, the observation that selectivity can be achieved between a dual specificity phosphatase and a tyrosine phosphatase even with the small set of compounds investigated suggests that further detailed study of this new class of inhibitors is worthwhile.

Experimental Section

14-Methylpentadecan-1-ol. To a stirred solution of 12-bromo-1-dodecanol (**3**) (10.2 g, 38 mmol) and Li_2CuCl_4 (0.1 M in THF, 11.5 mL) in THF (19 mL) was slowly added a solution of isobutylmagnesium bromide (2 M in Et_2O, 47.5 mL, 95 mmol) at 0 °C under Ar. Once the addition was complete, stirring was continued for 45 min at 0 °C before the mixture was allowed to reach ambient temperature. The reaction was then quenched with aq HOAc (20% w/w), the aqueous layer was repeatedly extracted with Et_2O, and the combined organic phases were washed with aq $NaHCO_3$ (5 mol %) and brine, then dried over Na_2SO_4 before the solvent was evaporated. Flash chromatography of the crude product (hexane/EtOAc 20/1 → 10/1) afforded 14-methylpentadecan-1-ol as a white solid (9.1 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (t, 2H, J = 6.7 Hz), 1.98 (br s, 1H, −OH), 1.57 (quint, 2H, J = 6.7 Hz), 1.54 (non., 1H, J = 6.6 Hz), 1.37−1.13 (m, 22H), 0.87 (d, 6H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.9, 39.0, 32.7, 29.9, 29.7, 29.6, 29.4, 27.9, 27.4, 25.7, 22.6; IR (film) 3157, 2918, 2849, 1471, 1363, 1052, 718 cm^{-1}; MS (EI) m/z (rel intensity) 224 (25), 196 (15), 168 (14), 82 (35), 69 (80), 68 (31), 67 (10), 57 (99), 56 (100), 55 (69), 43 (75), 41 (44); HRMS calcd for $C_{16}H_{34}O$ [$(M + H)^+$] 243.268790, found 243.268820. Anal. Calcd for $C_{16}H_{34}O$: C, 79.27; H, 14.14. Found: C, 79.18; H, 14.02.

14-Methylpentadecanal (4). To a solution of 14-methylpentadecan-1-ol (3.2 g, 13 mmol) in CH_2Cl_2 (150 mL) was added PCC (5.6 g, 26 mmol). After the mixture had been stirred for 2 h, it was filtered through a pad of Celite, the Celite was carefully rinsed with CH_2Cl_2, the combined filtrates were evaporated, and the residue was purified by flash chromatography (hexane:EtOAc 70/1) to yield product **4** as a white solid (2.7 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, 1H, J = 1.9 Hz), 2.42 (td, 2H, J = 7.3, 1.9 Hz), 1.65 (quint, 2H, J = 7.3 Hz), 1.52 (non., 1H, J = 6.6 Hz), 1.35−1.13 (m, 20H), 0.87 (d, 6H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 43.9, 39.1, 29.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.0, 27.4, 22.6; IR (film) 3200−2700, 2924, 2853, 2714, 1728, 1468, 1384, 1364, 1347, 1171, 718 cm^{-1}; MS (EI) m/z (rel intensity) 240 ([M^+], 3), 222 (14), 196 (7), 194 (7), 168 (4), 166 (6), 140 (6), 95 (39), 73 (3), 72 (6), 67 (34), 66 (9), 60 (3), 57 (94), 56 (51), 45 (9), 44 (22), 43 (100), 41 (63); HRMS calcd for $C_{16}H_{32}O$ [M^+] 240.245315, found 240.245396.

Compound 6. To a stirred solution of (4*R*,5*S*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone (**5**) (900 mg, 3.86 mmol) in CH_2Cl_2 (8.4 mL) at −5 °C were successively added *n*-Bu_2BOTf (1 M in CH_2Cl_2, 4.6 mL, 4.6 mmol) and triethylamine (0.68 mL, 5.0 mmol). The reaction mixture was stirred at −5 °C for 30 min before it was cooled to −78 °C. At that temperature, a solution of aldehyde **4** (1 g, 4.3 mmol) in CH_2Cl_2 (10 mL) was added dropwise, and the resulting mixture was stirred at −78 °C for 1 h and at room temperature for 15 min. The reaction was then quenched with aqueous phosphate buffer (pH 7), the aqueous layer was extracted with methyl *tert*-butyl ether (3×), and the combined organic layers were washed with saturated aqueous NH_4Cl and dried over Na_2SO_4, before the solvent was evaporated. The residue was purified by flash chromatography (hexane:EtOAc 30/1 → 9/1) to give aldol **6** as a white solid (1.2 g, 63%). $[\alpha]^{20}_D$ + 14 (*c* 1.41, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.27 (m, 5H), 5.69 (d, 1H, J = 7.3 Hz), 4.80 (quint, 1H, J = 6.6 Hz), 3.98−3.94 (m, 1H), 3.79 (qd, 1H, J = 7.0, 2.7 Hz), 3.50−3.00 (br s, 1H, −OH), 1.52 (non., 1H, J = 6.6 Hz), 1.65−1.13 (m, 24H), 1.24 (d, 3H, J = 7.1 Hz), 0.90 (d, 3H, J = 6.6 Hz), 0.87 (d, 6H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 152.6, 133.2, 128.8, 128.7, 125.6, 78.9, 71.6, 54.7, 42.2, 39.0, 33.9, 29.9, 29.7, 29.6, 27.9, 27.4, 26.0, 22.6, 14.3, 10.2; IR (KBr) 3445, 2922, 2851, 1797, 1688, 766, 698 cm^{-1}; MS (EI) m/z (rel intensity) 473 ([M^+], 1), 367 (16), 262 (14), 233 (100), 178 (11), 134 (15), 118 (27), 116 (11), 107 (37), 95 (11), 82 (18), 70 (14), 67 (10), 57 (50), 56 (12), 55 (18), 41 (16); HRMS calcd for $C_{29}H_{47}NO_4$ [M^+] 473.350509, found 473.350808. Anal. Calcd for $C_{29}H_{47}NO_4$: C, 73.53; H, 10.00; N, 2.96. Found: C, 73.02; H, 10.08; N, 2.91.

Compound 8. TMSOTf (5 μL, 0.027 mmol) was added to a solution of alcohol **6** (64 mg, 0.13 mmol) and trichloroacetimidate **7** (256 mg, 0.40 mmol)[5] in CH_3CN (6.7 mL) at −30 °C and stirring was continued for 2 h at that temperature. After neutralization with triethylamine and evaporation of the solvent, the residue was purified by flash chromatography (hexane:EtOAc 20/1 → 9/1) to yield glycoside **8** (48 mg, 38%, pure β-isomer) as a colorless syrup. $[\alpha]^{20}_D$ + 10 (*c* 5.11, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.00 (m, 20H), 5.34 (d, 1H, J = 7.1 Hz), 4.91 (d, 1H, J = 11.3 Hz), 4.88 (d, 1H, J = 11.0 Hz), 4.75 (d, 1H, J = 10.9 Hz), 4.73 (d, 1H, J = 11.0 Hz), 4.65 (d, 1H, J = 11.2 Hz), 4.49 (d, 1H, J = 10.9 Hz), 4.46 (d, 1H, J = 7.7 Hz), 4.43−4.41 (m, 1H), 4.27 (br d, 1H, J = 11.6 Hz), 4.10 (dd, 1H, J = 11.6, 4.8 Hz), 3.96 (quint, 1H, J = 6.6 Hz), 3.99−3.89 (m, 1H), 3.60 (tm, 1H, J = 8.8 Hz), 3.45−3.39 (m, 2H), 3.35 (dd, 1H, J = 9.0, 7.7 Hz), 1.95 (s, 3H), 1.69−1.05 (m, 27H), 1.44 (non., 1H, J = 6.6 Hz), 0.78 (d, 6H, J = 6.6 Hz), 0.73 (d, 3H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 170.6, 152.7, 138.6, 138.5, 137.8, 133.2, 128.7, 128.6, 128.4, 128.3 (2x), 128.0, 127.9, 127.7, 127.6, 127.5, 125.6, 103.0, 85.0, 82.5, 80.3, 78.7, 77.8, 75.6, 75.0, 74.9, 72.7, 63.3, 55.0, 42.1, 39.0, 33.8, 30.0, 29.9, 29.7, 29.6, 27.9, 27.4, 25.4, 22.6, 20.8, 14.1, 12.7; IR (film) 3031, 2925, 2853, 1780, 1743, 1703, 1235, 1196, 1090, 1072, 1029, 736, 699 cm^{-1}; MS (EI) m/z (rel intensity) 474 (3), 456 (33), 412 (6), 253 (11), 240 (8), 181 (7), 160 (8), 134 (12), 97 (5), 92 (10), 91 (100), 81 (5), 69 (7), 55 (7), 43 (16); HRMS (ESIpos) calcd for $C_{58}H_{77}NO_{10}$ [$(M + NH_4)^+$] 965.589122, found 965.58939. Anal. Calcd for $C_{58}H_{77}NO_{10}$: C, 73.47; H, 8.18; N, 1.48. Found: C, 73.44; H, 8.24; N, 1.46. A second fraction was collected that contained the corresponding α-anomer (9 mg, 7%).

Hydroxy Acid 9. A solution of compound **8** (93 mg, 0.098 mmol) in THF/H_2O (3:1, 2 mL) was treated with aq H_2O_2 (30% w/w, 0.61 mL, 0.07 mmol) and LiOH·H_2O (9 mg, 0.19 mmol). The mixture was stirred at 50−60 °C for 2 h. After treatment with aq Na_2SO_3 (1.6 M, 2 mL), the aqueous layer was acidified by addition of aq HCl (6 M) until pH 1 was reached. The aqueous phase was repeatedly extracted with methyl *tert*-butyl ether, the combined organic layers were dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (hexane:EtOAc:HOAc, 8/2/0.01) to give product **9** as a white solid (58 mg, 79%). $[\alpha]^{20}_D$ −3 (*c* 4.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.24 (m, 15H), 6.00−5.00 (br s, 2H), 4.90 (d, 1H, J = 11.1 Hz), 4.87 (d, 1H, J = 11.0 Hz), 4.83 (d, 1H, J = 11.0 Hz), 4.79 (d, 1H, J = 11.0 Hz), 4.71 (d, 1H, J = 11.2 Hz), 4.62 (d, 1H, J = 11.0 Hz), 4.55 (d, 1H, J = 7.8 Hz), 4.07−4.02 (m, 1H), 3.84 (dd, 1H, J = 11.9, 2.8 Hz), 3.68 (dd, 1H, J = 11.9, 4.8 Hz), 3.66 (t, 1H, J = 9.1 Hz), 3.53 (t, 1H, J = 9.1 Hz), 3.39 (dd, 1H, J = 9.1, 7.8 Hz), 3.39−3.33 (m, 1H), 2.67 (qd, 1H, J = 7.1, 4.4 Hz), 1.51 (non., 1H, J = 6.6 Hz), 1.65−1.12 (m, 24H), 1.22 (d, 3H, J = 7.1 Hz), 0.86 (d, 6H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 138.1−127.5, 102.5, 84.8, 82.1, 79.8, 77.8, 75.5, 75.0, 75.0, 62.2, 42.9, 39.1, 33.1, 30.0, 29.7, 29.6, 29.5, 28.0, 27.4, 25.7, 22.6, 11.2; IR (film) 3500−2700, 2924, 2853, 1708, 1497, 1455, 1071, 734, 697 cm^{-1}; HRMS (ESI-pos) calcd for $C_{46}H_{66}O_8$ [$(M + Na)^+$] 769.465538, found 769.46428. Anal. Calcd for $C_{46}H_{66}H_8$: C, 73.96; H, 8.91. Found C, 73.83, H, 8.88.

Compound 23. A solution of hydroxy acid **9** (40 mg, 0.053 mmol), 2-chloro-1,3-dimethylimidazolinium chloride (**22**) (22 mg, 0.13 mmol) and KH (5 mg, 0.11 mmol) in CH_2Cl_2 (1.1 mL) was stirred for 1 h at 0 °C. 4-(Dimethylamino)pyridine (15 mg, 0.12 mmol) was then added and stirring was continued for 16 h. The mixture was then filtered through Celite, the filtrate was evaporated, and the residue was purified by flash chromatography (hexane:EtOAc 20/1 → 10/1) to give cyclodimer **23** as a colorless syrup (21 mg, 54%). $[\alpha]^{20}_D$ +15 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.16 (m, 15H), 4.95 (d, 1H, J = 11.0 Hz), 4.91 (d, 1H, J = 10.9 Hz), 4.85 (d, 1H, J = 10.9 Hz), 4.78 (d, 1H, J = 11.0 Hz), 4.71 (d, 1H, J = 10.9 Hz), 4.55 (d, 1H, J = 10.9 Hz), 4.53 (d, 1H, J = 7.7 Hz), 4.29 (d, 1H, J = 10.6 Hz), 3.92−3.87 (m, 2H), 3.66 (t, 1H, J = 8.9 Hz), 3.57 (tm, 1H, J = 9.9 Hz), 3.41 (dd, 1H, J = 9.0, 7.7 Hz), 3.29 (dd, 1H, J = 9.9, 8.7 Hz), 2.71 (br quint, 1H, J = 7.1 Hz), 1.51 (non., 1H, J = 6.7 Hz), 1.58−1.12 (m, 24H), 1.32 (d, 3H, J = 6.9 Hz), 0.86 (d, 6H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 138.5−127.6, 103.7, 84.9, 82.6, 78.4, 75.7, 75.1, 72.9, 64.9, 45.7, 39.1, 30.2, 30.0, 29.8, 28.0, 27.5, 22.7, 14.4; IR (film) 2924, 2853, 1739, 1607, 1497, 1455, 1257, 1071, 750, 698 cm^{-1}; MS (EI) *m*/*z* (rel intensity) 1365 (<1), 513 (2), 279 (8), 253 (6), 240 (11), 193 (4), 181 (13), 109 (3), 97 (4), 91 (100), 81 (3), 57 (5), 55 (4), 43 (6), 41 (3); HRMS (ESI pos) calcd for $C_{92}H_{128}O_{14}$ [$(M + NH_4)^+$] 1474.964784, found 1474.96176.

Glucolipsin A (24). $Pd(OH)_2$ (13 mg) was added to a solution of cyclodimer **23** (21 mg, 0.014 mmol) in MeOH (5 mL) and the resulting suspension was stirred under H_2 (1 atm) for 3 d at ambient temperature. The catalyst was filtered off through a short pad of Celite, the Celite was carefully rinsed with MeOH, and the combined filtrates were evaporated giving compound **24** as an analytically pure white solid (13 mg, 100%). $[\alpha]^{20}_D$ +9.5 (*c* 0.55, MeOH) [ref 3: $[\alpha]^{25}_D$ +11.39 (*c* 0.05, MeOH)]; ^{1}H NMR (600 MHz, pyridine-d_5) δ 5.14 (d, 1H, J = 11.2 Hz), 5.06 (d, 1H, J = 7.7 Hz), 4.56−4.54 (m, 1H), 4.52 (t, 1H, J = 10.6 Hz), 4.22 (td, 1H, J = 8.8, 4.1 Hz), 4.18 (t, 1H, J = 9.9 Hz), 4.06 (td, 1H, J = 8.4, 4.4 Hz), 3.87 (td, 1H, J = 9.4, 4.7 Hz), 3.01 (m, 1H), 2.14 (m, 1H), 1.97 (m, 1H), 1.78 (m, 2H), 1.66 (d, 3H, J = 6.9 Hz), 1.49 (non., 1H, J = 6.6 Hz), 1.40−1.15 (m, 20H), 0.86 (d, 6H, J = 6.6 Hz); ^{13}C NMR (150 MHz, pyridine-d_5) δ 174.4, 105.1, 78.7, 78.6, 75.7, 75.2, 72.2, 66.4, 46.2, 39.3, 36.3, 30.7, 30.3, 30.2, 30.1, 28.2, 27.8, 24.2, 22.8, 14.4; ^{1}H NMR (300 MHz, CD_3OD) δ 4.56 (br d, 1H, J = 10.3 Hz), 4.36 (d, 1H, J = 7.6 Hz), 4.05 (dt, 1H, J = 7.2, 5.0 Hz), 3.85 (dd, 1H, J = 11.4, 9.9 Hz), 3.46 (t, 1H, J = 9.8 Hz), 3.35 (t, 1H, J = 9.0 Hz), 3.20 (dd, 1H, J = 9.1, 7.7 Hz), 3.15 (dd, 1H, J = 9.7, 8.8 Hz), 2.63 (quint, 1H, J = 7.2 Hz), 1.52 (non., 1H, J = 6.6 Hz), 1.31 (d, 3H, J = 7.0 Hz), 1.58−1.11 (m, 24H), 0.87 (d, 6H, J = 6.6 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 175.8, 104.5, 78.9, 78.0, 75.6, 75.5, 72.4, 66.3, 46.7, 40.3, 31.1 (2×), 31.0, 30.9 (2×), 30.8 (3×), 30.7, 29.1, 28.6, 25.2, 23.0, 13.3. For a comparison of the ^{1}H and ^{13}C NMR data of this compound with those of authentic glucolipsin A, see Tables 2 and 3.

Enzymatic Assays: (a) PTP1B Inhibition. PTP1B was purchased from Calbiochem (human recombinant). The enzyme (0.001 U) was preincubated with the inhibitors in a buffer (pH 7.2)[36] containing HEPES (25 mM), EDTA (2.5 mM), NaCl (50 mM), DTT (2 mM), and BSA (0.1%) for 15 min at room temperature. Then p-NPP was added (end concentration 50 μM) and the read-out (405 nm) was recorded on a microplate-reader at 37 °C continuously for 80 min. The reaction rate was determined from the absorption difference between 30 and 60 min reaction time.

(b) Cdc25A Inhibition. The clone pET9d/His-Cdc25A was expressed in the *E. coli* strain BL21-DE3 and purified in the presence of 8 M urea. A 30-μg sample of the purified enzyme was preincubated with the inhibitors in a pH 8.0 buffer containing 50 mM Tris, 50 mM NaCl, and 2 mM DTE for 15 min at room temperature.[37,38]

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Supporting Information Available: Full experimental and analytical details for the preparation of the stereoisomers of glucolipsin reported above and NMR spectra of all stereoisomeric macrodilactones in perbenzylated and deprotected form. This material is available free of charge via the Internet at http://pubs.acs.org.

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