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3,4-Dideoxy-3,3,4,4-tetrafluoro- and 4-OH epimeric 3-deoxy-3,3-difluoro- α -GalCer analogues: Synthesis and biological evaluation on human iNKT cells stimulation

Samuel Golten, Allan Patinec, Katy Akoumany, Jézabel Rocher, Jérôme Graton, Denis Jacquemin, Jean-Yves Le Questel, Arnaud Tessier, Jacques Lebreton, Virginie Blot, et al.

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1 tetrafluoro- α -GalCer analogues, and studied their ability to stimulate human *i*NKT cells. In
2 each case, deoxygenation at the indicated positions was accompanied by difluoro introduction
3 in order to evaluate the resulting electronic effect on the stability of the ternary
4 CD1d/Galcer/TCR complex which has been rationalized by modeling study. With deoxy-
5 difluorination at the 3-position, the two epimeric 4-OH analogues were investigated to
6 establish their capacity to compensate for the lack of the hydrogen bond donating group at the
7 3-position. The 3,4-dideoxytetrafluoro analogue was of interest to highlight the amide *NH*-
8 bond hydrogen bond properties.

9

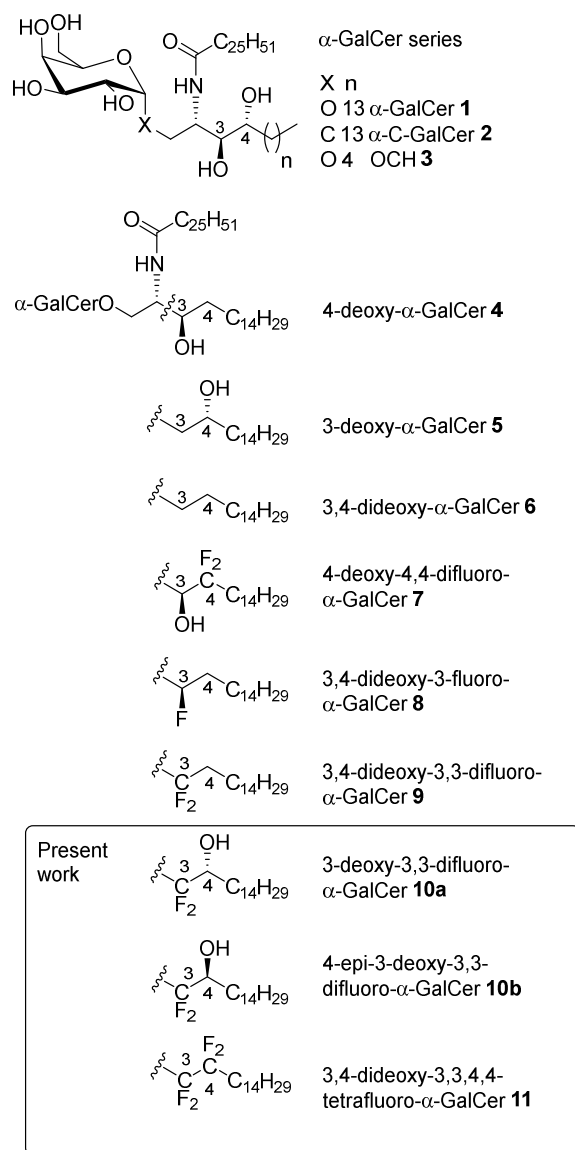
10 **Keywords**

11 Fluoro GalCer analogues, *i*NKT activation, immune response, modeling study.

12 **1. Introduction**

13 CD1d restricted T lymphocytes, a subclass of lymphocytes, play a pivotal role in the innate-
14 type immune response. A subpopulation of these CD1d restricted lymphocytes, called *i*NKT
15 cells, feature a semi-invariant T receptor (TCR) that recognizes a variety of glycolipids
16 antigens. In particular, recognition of glycosylceramides bound to CD1d protein receptors of
17 antigen presenting cells (APCs) by *i*NKT-TCR leads to tertiary complex formation inducing
18 expansion of their population and strong secretion of a large panel of T helper cytokines,
19 including IFN- γ , TNF- α , and several interleukins.[1-9] These cytokines can stimulate the
20 maturation of dendritic cells, activate the production of various cytokines, and stimulate other
21 by-stander immune cells as cytotoxic CD8 lymphocytes. These mechanisms contribute to the
22 inflammatory process, humoral immunity and antibody proliferation depending on two types
23 of helper T cells polarization (T_H1 or T_H2). It was found that T_H1 cytokines (e.g. IFN- γ , IL-2)
24 participate in cell-mediated immunity for tumor rejection and against infections,[10-15] while
25 T_H2 cytokines (e.g. IL-4, IL-13) promote auto-immune responses, associated with a variety of
26 diseases such as tuberculosis, type I diabetes, multiple sclerosis and rheumatoid arthritis.[16-
27 22] Disruption of the T_H1/T_H2 balance may lead to disease induction as T_H1 and T_H2 type
28 cytokines can antagonize each other's biological functions.[23-25] Synthetic α -
29 galactosylceramide α -GalCer **1** (also called KRN7000, Fig. 1)[26,27] has been considered as
30 a promising agent against cancer[28-34] despite some undesired side effects as well as long-
31 term NKT cell unresponsiveness following a first injection that restrict therapeutic
32 development as a free drug in human.[35-38] Recent clinical trials have highlighted its

1 therapeutic potency as a potent adjuvant for vaccines[39-42] and in anticancer
 2 immunotherapy when preloaded on dendritic cells (DCs) or CD1d co-effector, or in
 3 combination with programmed cell death 1 (PD-1) blockade proteins.[43-48]



4
 5 **Fig 1.** Structure of α -galactosylceramide analogues
 6

7 The use of synthetic analogues of α -GalCer **1** targeting the T_H1/T_H2 balance has been
 8 extensively studied and well documented in excellent reviews.[23,49-52] Combinations of
 9 computational and crystal data,[53-57] with several structure-activity relationship studies on
 10 CD1d/ α -GalCer analogues/TCR interactions established a relationship between stability of
 11 the ternary complex and T_H1/T_H2 polarisation of the immune response. After having shown
 12 the crucial importance of glycosidic α -configuration linkage,[58-60] replacement of the *O*-
 13 anomeric atom by a non-hydrolysable *C*-bond (α -C-GalCer **2**, Fig. 1) or ethylenic analogues

1 have produced potent derivatives for iNKT stimulation with T_{H1} gain.[61-66] Unfortunately
2 α -C-GalCer, appearing 1000-fold more potent than α -GalCer in mice, failed to satisfy clinical
3 trials due to weak antigenic character on human iNKT cells. Other osidic linkages, *e.g.*
4 thio[67-69] and amino analogues,[70,71] afforded versatile responses upon mouse or human
5 iNKT cells without offering significant improvement in T_{H1}/T_{H2} balance. Carbasugar[72-74]
6 and open chains mimicking sugar architectures[75,76] have been shown to reinforce the T_{H1}
7 bias and to diminish the anergy phenomenon encountered with α -GalCer **1**.

8 Following indications of the relative influence of 2''-OH and 3''-OH hydroxyl groups on the
9 sugar polar head interaction (2''-OH/Asp151-Cd1d and Gly96 α -TCR; 3''-OH/Ser30-TCR)
10 evidence was obtained for a preferential galactose configuration (4''-OH/Phe29 α -TCR).[77-
11 79] However, some recent data have emphasized a relative freedom for 6''-OH modifications
12 of galactosyl moiety presenting *O*-methyl or acetyl group without alteration of the bioactivity.
13 Furthermore, significant T_{H1} bias has been observed with various 6''-deoxy derivatives
14 bearing aromatic groups, such as phenyl, dansyl, biotinyl, pyridyl and naphthyl residues,
15 introduced either through 6''-*N*-amido, carbamoyl, ureido and triazole linkages.[80-88] With a
16 6''-naphthyl ureido group, it has been suggested that the formation of an extra anchor *NH*-
17 bond to the CD1d receptor results in a slight shift of the α -GalCer **1** ligand in the TCR grove
18 leading to the T_{H1}-bias observed *in vivo*. [82,85] Recently, Van Calenberg *et al.*[89] have
19 described a 6''-*O*-pyridinylcarbamoyl- α -C-GalCer analogue as potent iNKT agonist
20 displaying high antigenic properties. Studies have been completed with other 6''-modified α -
21 GalCer (6''-OMe, 6''-amidoalkyl and PEG chains...),[84,90] showing weak increase of iNKT
22 stimulation without significant outcome on cytokine bias.

23 The modification of the ceramide fragment of the glycolipid is broadly accepted as a sensitive
24 factor in terms of T_{H1}/T_{H2} polarity. Derivatives in which the initial linear C₂₆ acyl chain was
25 replaced by unsaturated fatty acids,[91,92] branched[93] or amide containing[94] chain have
26 been investigated and were suspected to use non-professional APC pathways to explain their
27 T_{H2} polarisation tendency.[95] Conversely, heterocyclic substitutions of shortened *N*-acyl
28 chain derivatives mostly promote T_{H1} orientation by installing adequate π - π stacking in the
29 CD1d pocket[96,97] as suggested by aromatic-ended alkyl chains.[98-103]

30 Galactosylceramides featuring a truncated sphingosine chain mostly improve T_{H2} response as
31 illustrated by the well know sphingosine shortened OCH **3** derivative (Fig. 1) and its 4-deoxy
32 analogues.[104-107] Indeed, the role of the two sphingosine hydroxyl groups at the C-3 and
33 C-4 positions in the stability of the CD1d/ α -GalCer/TCR complex was widely explored from

1 deoxy derivatives, 4-deoxy **4**, 3-deoxy **5** and 3,4-dideoxy **6** α -GalCer (Fig. 1)[78,103,104,108-
2 112], and from epimeric[103,113] and polyhydroxylated,[114,115] amino[116] and
3 amido[117] analogues. Although the role of the 4-OH group of α -GalCer in the interaction
4 with human CD1d (Asp80-hCD1d) remains debated, the importance of the 3-OH interaction
5 (Asp80-CD1d and Arg95 of the CDR3 α -loop of the TCR) is fully established. These
6 numerous efforts allowed to distinguish the effects of satellite hydrogen bonds on CD1d and
7 TCR receptor interactions.

8 Then, introduction of fluorine atoms on the acyl or sphingosine chains of the ceramide
9 appeared attractive to investigate modification of *H*-bonds in the interactions of GalCer
10 ligands with CD1d *vs* TCR. Fluorination of bioactive compounds is often used to optimize
11 properties.[118-122] While the blocking of metabolic sites is often achieved by fluorination,
12 the strong fluorine electronegativity induces modification of a range of relevant properties,
13 such as pK_a and hydrogen bond properties of adjacent functional groups, molecular
14 conformation, and lipophilicity.[123-132] Tetrafluoroethylene (CF₂-CF₂) groups have
15 received less attention as functional biological effectors,[133-137] relatively to -F and -CF₃
16 groups. CF₂ group has been shown to generate a widening of the C-CF₂-C angle (~111-118°)
17 and a narrowing of the F-C-F angle (~100-104°) relative to tetrahedral geometry.[138,139]
18 Linclau *et al.*[140] have reported the synthesis of a 4-deoxy-4,4-difluoro- α -GalCer **7** analogue
19 (Fig. 1) in which the *H*-bond donating capacity with CD1d was reinforced *vs*. a concomitant
20 decrease in its ability to accept the *H*-bond from Asp-95 of the NKT TCR showing a weak
21 loss on cytokine stimulation compared to α -GalCer **1** with a slight T_H1 bias. The latter
22 outcome was completed by suppression of the 3-OH hydrogen-bond replaced by one or two
23 fluorine atoms (3,4-dideoxy-3-fluoro- α -GalCer **8** and 3,4-dideoxy-3,3-difluoro- α -GalCer **9**
24 respectively, Fig. 1).[112] It was observed that introduction of fluorine groups at 3-position of
25 the sphinganine establishes a favourable *NH*-amide interaction from the acyl chain to the
26 hTrp154 of the TCR resulting from the withdrawing electronic effect. This modification aims
27 at partly compensate the lack of the 3-OH on the destabilisation of the CD1d/ α -GalCer/TCR
28 complex in human iNKT cells. The discrete role of *NH*-amide function, first suggested by
29 Calenberg,[81-85,87,88] has been also highlighted by Linclau *et al.*[141] who found that
30 amide neighbouring geminal (gem-) difluorine group, introduced at 2'-position of the acyl
31 chain, leads to a T_H2 polarisation while iNKT stimulation level remains similar to α -GalCer
32 **1**. This observation was confirmed by Hénon *et al.* in a molecular dynamic study[142] and
33 T_H2 orientation of α -GalCer analogues presenting amide alteration have comforted this

1 hypothesis, e.g., sulphonamide,[143] triazole,[84,87] ether and ester,[144] azetidine and
2 pyrrolidine.[145]

3 The results obtained with fluorinated analogues of α -GalCer **1** inspired us to continue
4 investigating this type of alteration on the CD1d/ α -GalCer/TCR complex stability. In
5 particular, the importance the 4-OH group will be evaluated through the two 3-deoxy-3,3-
6 difluoro- α -GalCer analogue **10a** and its 4-OH epimer **10b**, in which the 3-OH is replaced by a
7 *gem*- difluorine group. The 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11** analogue was also
8 proposed as the next member of the 3,4-dideoxy analogues and to compare increasing
9 fluorine's electronic effect with the 3,4-dideoxy-3,3-difluorinated **7** and 4-deoxy-4,4-difluoro
10 **9** analogues previously evaluated (Fig. 1). Here we report both the synthesis of polyfluoro- α -
11 GalCer analogues **10a**, **10b** and **11** and their *in vitro* biological evaluation on human iNKT
12 stimulation.

13

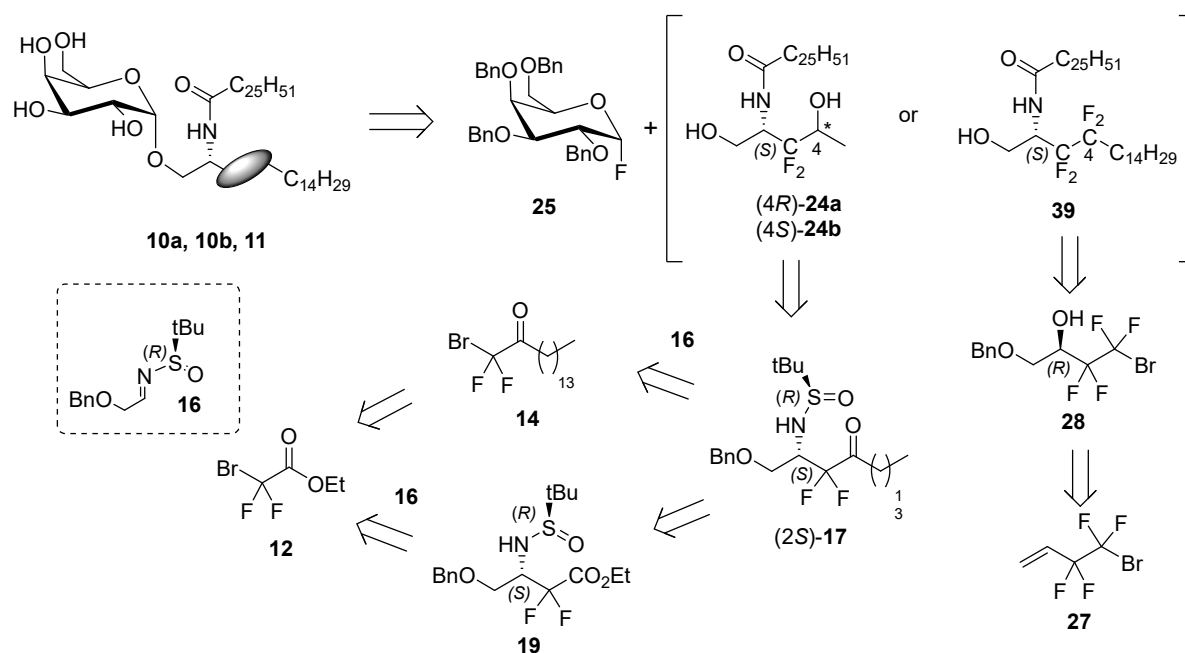
14 **2. Results and discussion**

15

16 2.1. Chemistry

17

18 A conventional retrosynthetic analysis of the galactosylceramide targets **10** and **11** leads to a
19 first disconnection between the galactose and the corresponding ceramide chains (Scheme 1),
20 leading to the well-known fluoro galactosyl donor[146] and the two respective modified
21 fluoroceramide analogues **24** and **39**. Functional group interconversion of the diastereomers
22 **24a** and **24b** leads to the same ketone **17**. Further analysis leads to two distinct pathways,
23 depending whether an imine addition disconnection is first executed, leading to **14** and the
24 known chiral (*R*)-sulfonamide **16** or whether the alkyl chain is first disconnected, leading to
25 the aminoester **19**.[147] Both intermediates **14** and **19** derive from the same
26 bromodifluoroester **12**.



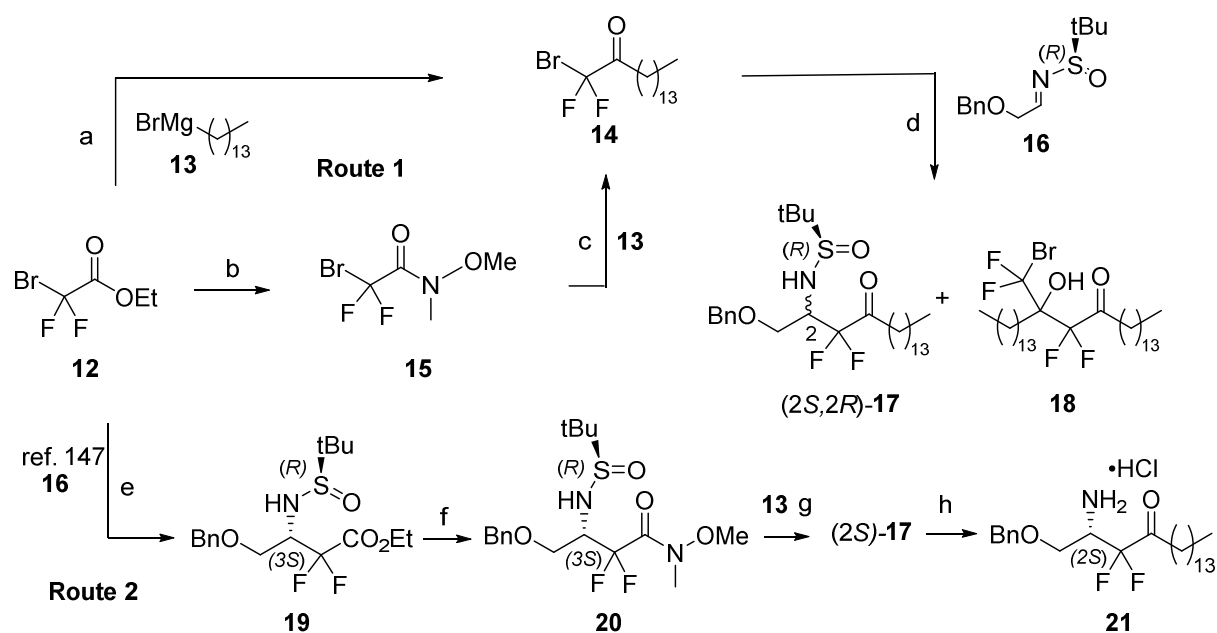
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2 **Scheme 1.** Retrosynthetic pathway for analogues **10a**, **10b** and **11**.

3 From **39**, further retrosynthetic analysis relies on a series of functional group interconversions
4 to introduce the amide bond and its amine precursor through dihydroxylation and alcohol to
5 amine conversion. This proceeds *via* **28** to the commercially available fluorinated building
6 block **27** by a known chemistry.[148]

7

8 2.1.1. Synthesis of 3-deoxy-3,3-difluoro- α -GalCer **10a** and its (4*S*)-OH epimer **10b**.

9 The synthesis of the sphingosine intermediate is shown in Scheme 2. The first approach
10 involved introduction of the long alkyl chain by reaction of bromodifluoroester **12** with
11 alkylmagnesiumbromide **13** to afford the bromoketone **14** (route 1).



1
 2 Reagents and Conditions: (a) Et₂O, -78 °C, 19%; (b) MeNHOMe.HCl, AlMe₃, THF, r.t., 3 h, 62%; (c) THF,
 3 25 °C, 30 min.; (d) RhCl(PPh₃)₃, Et₂Zn, THF, -20 to 0°C, 2 h, **17**: 27% [dr (2*S*):(2*R*), 82:18] and **18**: 36%; (e)
 4 RhCl(PPh₃)₃, Et₂Zn, THF, -20 to 0°C, 1 h, 43%; f) MeNHOMe.HCl, THF, *n*BuLi, -78°C for 4 h then -60 °C, 1
 5 h, 92%; (g) THF, 0 °C, 40 min., then r.t., 1 h, 90%. h) 3M aq. HCl, 1,4-dioxane, r.t., 14 h, 95%

6 Scheme 2. Synthesis of key keto intermediate **21**

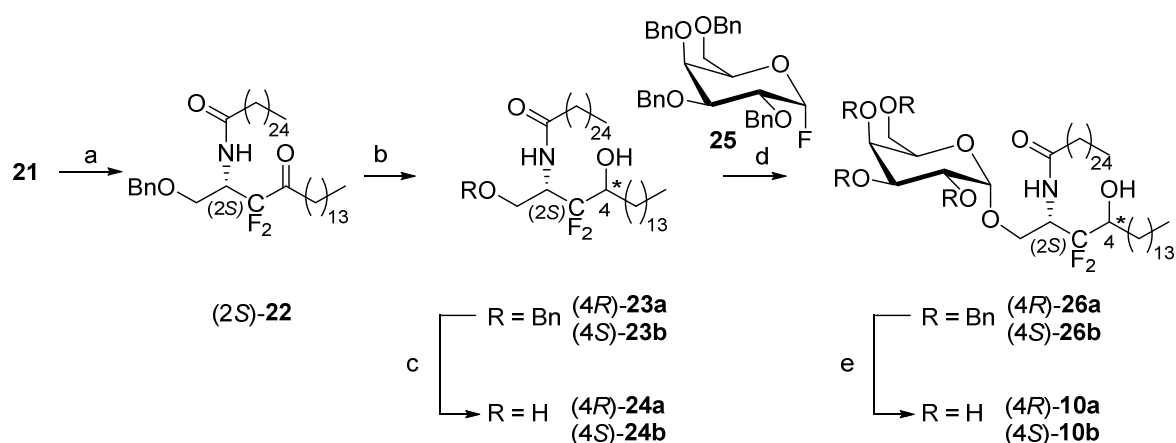
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 8 Hence, following the method of Kitazume *et al.*, [149] addition of ester **12** to Grignard reagent
 9 **13**, prepared *in situ* from tetradecanylebromide, afforded ketone **14** albeit in low yield (19%),
 10 while several side products prevailed. Consequently, addition of **13** to Weinreb amide
 11 intermediate **15** [150] was investigated. Unfortunately, while formation of the expected ketone
 12 **14** was observed in major amount, its isolation in pure form proved not possible.
 13 Nevertheless, Honda-Reformatsky reaction was attempted using the crude mixture with
 14 sulfinimine **16** [147] in the presence of RhCl(PPh₃)₃ and Et₂Zn. Following this route, the
 15 sulfinamine **17** was obtained in only 27% yield in an 82:18 3*S*:3*R* diastereomeric ratio, with
 16 the homocoupling product **18** being obtained as the major product (36%). Furthermore,
 17 attempts to separate the 2*S* and 2*R* isomers were unsuccessful.

18 An alternative route was thus investigated in which the introduction of the long alkyl chain
 19 would take place after installation of the chiral difluoroamine fragment (Scheme 2 - Route 2).
 20 Hence, Honda-Reformatsky reaction of difluoro ester **12** with (*R*)-sulfinimine ester **16** yielded
 21 difluorinated sulfinamine ester (3*S*)-**19** isolated in 43% yield. [147] This time, conversion of
 22 the ester moiety to the corresponding Weinreb amide and chain extension proved successful:
 23 reaction of **19** with *N,O*-dimethylhydroxylamine hydrochloride mediated by *n*BuLi instead of
 24 trimethyl aluminium, afforded the Weinreb amide **20** in excellent yield, as was the subsequent

1 chain extension with Grignard reagent **13**. Removal of the sulfinyl group in (2*S*)-**17** using aq.
 2 HCl in dioxane[151] gave the amine **21** as hydrochloride salt in 95% yield. Acylation of **21**
 3 with cerotic acid was initially attempted under benzotriazol-1-yl-
 4 oxytripyrrolidinophosphonium (PyBOP) activation in dichloromethane (DCM) (Scheme 3).
 5 Despite a prolonged reaction time (40 h), the ceramide **22** was only produced in a modest
 6 47% yield. An improved yield (73%) was obtained when carrying out the amide bond
 7 formation in refluxing DCM.

8 The two diastereomeric 3-deoxy-3,3-difluoride phytosphingosine analogues were then
 9 obtained by reduction of ketone **22** by NaBH₄, to give **23a** (52%) and **23b** (44%) which were
 10 separable by chromatography (Scheme 3). Establishment of the absolute alcohol
 11 configuration of **23a** and **23b** was achieved by ¹H NMR analysis of corresponding Mosher's
 12 ester derivatives (see Supporting Information SI1).

13



14

15 Reagents and Conditions: (a) Cerotic Acid, PyBOP, Et₃N, DCM, reflux, 20h, 73%; (b) NaBH₄, THF/EtOH
 16 (3:1), r.t., 2 h, **23a**: 52%, **23b**: 44%; (c) H₂, Pd(OH)₂/C, THF, r.t., 3 h, **24a**: 88% from **23a**, **24b**: 96% from **23b**;
 17 (d) AgClO₄, SnCl₂, 4Å MS, dark, THF, r.t., 2 h, **26a** from **24a**: 52%, **26b** from **24b**: 42%; (e) H₂, Pd(OH)₂/C,
 18 EtOH/CHCl₃ (4:1), r.t., 18 h, **10a**: 83% from **26a**, **10b**: 77% from **26b**.

19 **Scheme 3.** Synthesis of 3-deoxy-3,3-difluoro- α -GalCers **10a** and 4-OH epimer **10b**

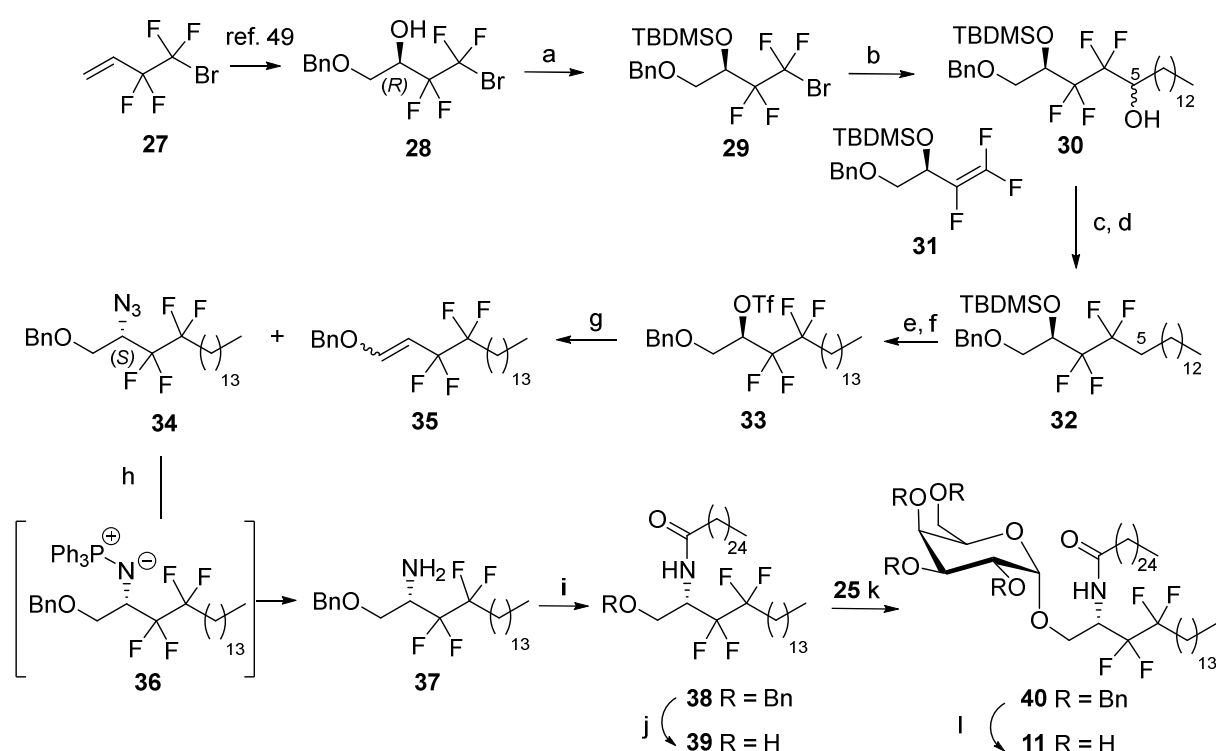
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21 Hydrogenolysis of the alcohols **23a** and **23b** cleanly delivered diols **24a** and **24b** in 88 and
 22 96% yield, respectively. The alcohols **24** were then individually glycosylated with the
 23 perbenzylated galactosyl fluoride donor **25**. The resulting protected glycosides **26a** and **26b**
 24 were debenzylated yielding both 3-deoxy-3,3-difluoro- α -GalCer analogues **10a** and **10b** in
 25 43% and 32% yield, respectively, over 2 steps.

26

27 2.1.2. Synthesis of 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11**

1 The synthesis of 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11** was performed in 12 reaction
 2 steps from the known enantiopure tetrafluoro (2*R*)-alcohol **28**[152] derived from alkene **27**
 3 *via* a stereoselective dihydroxylation and protection of the primary hydroxyl[148](Scheme 4).
 4 It was decided to carry out the chain extension before the amine introduction. Hence, the
 5 alcohol group in **28** was protected using tert-butyldimethylsilyl chloride (TBDMSCl) in the
 6 presence of imidazole and *N,N*-dimethylaminopyridine (DMAP). Due to the deactivating
 7 effect of the halogenated appendix, reaction for 4 days at 50 °C was required to give **29** in
 8 60% yield.
 9



10
11

12 Reagents and Conditions: (a) TBDMSCl, Imidazole, DMAP, DMF, 50 °C, 4 d, 60%; (b) MeLi, tetradecanal,
 13 THF, -74 to -69 °C, 45 min, then to -55 to -50 °C, 1.5 h, **30**: 81% and **31**: 10%; (c) TCDI, DCE, r.t., 18 h, 95%;
 14 (d) AIBN, Bu₃SnH, Toluene, 110 °C, 40 min, 94%; (e) TBAF·3H₂O, THF, r.t., 40 min, 98%; (f) Tf₂O, pyridine,
 15 -40 °C for 1 h, then -40 to -10 °C for 1 h 30, 91%; (g) NaN₃, DMF, 0 °C, 1 h, then 50 °C, 14 h, **34**: 77% and **35**:
 16 18%; (h) PPh₃, THF/H₂O, 60 °C for 13 h, 93%; (i) Cerotic acid, PyBOP, Et₃N, DCM, reflux, 21 h, 61%; (j)
 17 Pd(OH)₂/C, H₂, THF, r.t., 3 h, 94%; (k) **25**, AgClO₄, SnCl₂, 4Å MS, dark, THF, r.t., 2 h, **40**: 57%; (l)
 18 Pd(OH)₂/C, H₂, EtOH/CHCl₃ [4:1], r.t., 15 h, 85%.

19 **Scheme 4.** Synthesis of 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11**

20

21 Extension of the aliphatic chain was achieved through halogen-metal exchange followed by
 22 addition of tetradecanal. However, Li-halogen exchange from bromide **29** in the presence of
 23 long aliphatic aldehyde required an optimisation of the Konno's standard procedure[153,154]
 24 (Table 1).

1

2 **Table 1.** Optimization of conditions for the formation of alcohol **30** from **29**.

Entry	Method ^a	T (°C) [<i>t</i> (min)]	Yield (%)		
			29	30	31
1	A	-40 [120]	-	53	38
2	A	-78 [120]	61	21	-
3	B	-68 [45] to - 40[90]	-	43	48
4	B	-78[20] to -50[90]	-	74	19
5	B	-78[45] to -50[90]	-	81	10

3 ^aMethod A: MeLi (2.4 eq.) is added to a THF solution of **29** and tetradecanal; Method B: THF solution of **7**
4 and tetradecanal is added to MeLi (2.4 eq.).

5

6 Adding MeLi to a solution of bromide **29** and tetradecanal in THF at -40°C (Method A) led to
7 the desired alcohol **30** in 53 % yield (Table 1, entry 1). A byproduct **31** resulting from
8 fluoride elimination was isolated in 38 % yield. Reducing the elimination event by working at
9 lower temperature resulted in incomplete reaction due to precipitation of the aldehyde partner
10 (Table 1, entry 2). Furthermore, the reaction was found to be very sensitive to the addition
11 order of reagents and to the temperature (Table 1, entries 3-5). The best result was achieved
12 by adding a solution of bromide **29** and tetradecanal in THF at -78°C to the solution of MeLi
13 (Method B). After 45 min, the temperature was then increased up to -50°C and stirring was
14 continued for another 90 min. This led to the formation of alcohols **30** as a 1/1 diastereomeric
15 mixture in a reproducible 81% yield on 2 g scale along with a minor amount of alkene side
16 product **31** (10 %). Barton-McCombie deoxygenation[155] of alcohols **30** underwent 5-deoxy
17 intermediate **32** in 89% over 2 steps. Cleavage of the silylether with tetra-*n*-butylammonium
18 fluoride (TBAF•3H₂O), followed by activation of (2*R*)-OH group as triflate led to **33** which
19 was treated by NaN₃ in DMF to give the (2*S*)-azido derivative **34** in 68% yield over 3 steps.
20 Interestingly, the formation of a mixture of alkene side products resulting from elimination
21 process afforded a mixture of *E* and *Z*-tetrafluoro alkene **35** albeit in moderated yield (18%).
22 The reduction of azide **34** *via* the Staudinger reaction proved not straightforward. When the
23 reaction was conducted 2 h at 25 °C, the iminophosphorane **36** was isolated as the sole
24 product in 90 % yield. The stability of ylid **36** is likely due to a stabilization of the negative
25 charge by the electron withdrawing effect of the tetrafluoroethylene group.[156-158] The
26 addition of water to the reaction mixture (THF/H₂O [5:1]) did lead to the formation of the
27 desired amine **37** in a low 30% yield, even after prolonged reaction time (14 h at 25 °C).
28 However, by increasing temperature of the reaction to 60 °C for 13 h, complete hydrolysis of

1 the iminophosphorane **36** was performed and the target amine **37** was isolated as the sole
2 product in 93% yield.

3 The ceramide glycosylation acceptor **39** was then obtained by combining amine **37** with
4 hexacosanoic acid using PyBOP and coupling agent to afford **38** (61%) followed by
5 hydrogenolysis of the primary benzyl ether (94% yield). Glycosylation with the
6 perbenzylated galactosyl fluoride donor **25** using AgClO₄/SnCl₂ catalysis,[146] gave the
7 protected α -galactoceramide **40** in 57% yield and final hydrogenolysis (Pd(OH)₂/C) in
8 EtOH/CHCl₃ solution afforded the targeted 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11** in
9 85% yield.

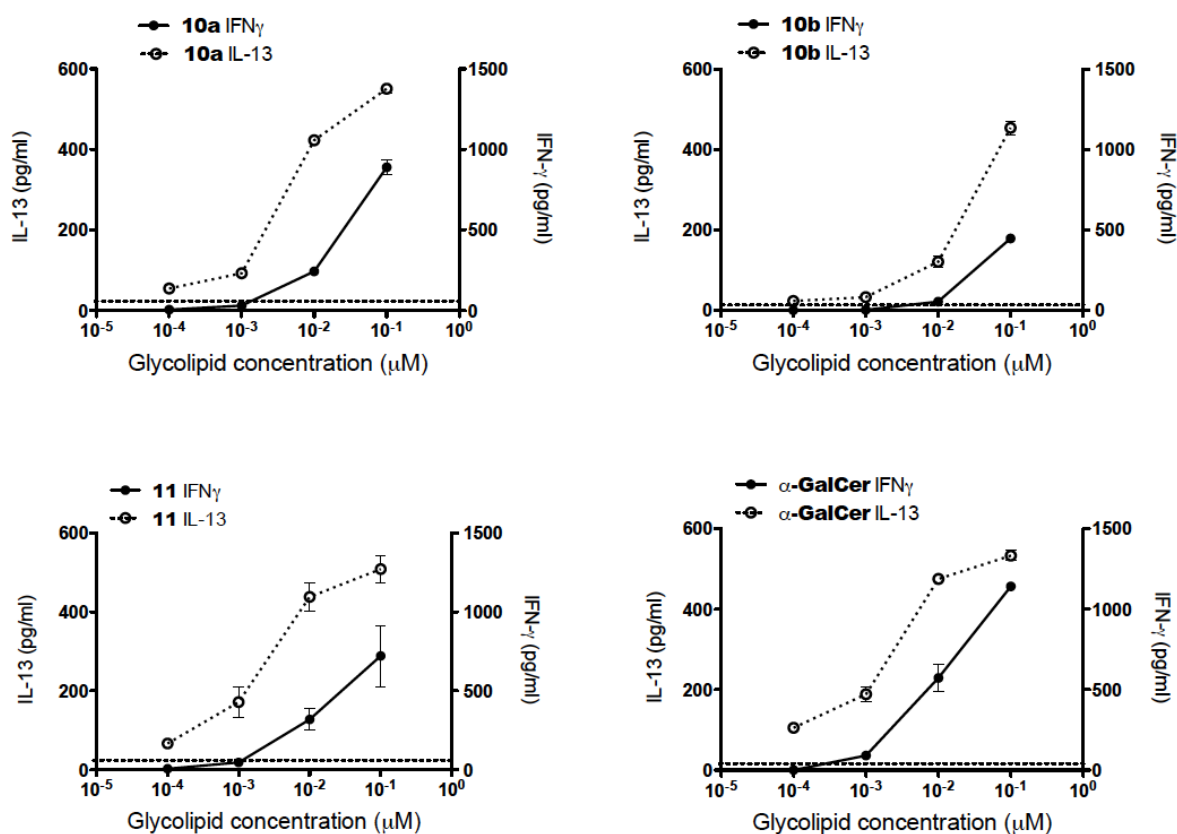
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11 2.2. Biological evaluation

12

13 The ability of the fluorinated GalCer analogues **10a**, **10b** and **11** to activate iNKT cells was
14 then evaluated *in vitro* using Hela antigen-presenting cell lines transduced to express human
15 CD1d (hHeLa-CD1d cells), and secretion of a T_H1 type cytokine IFN- γ , measured after a 6h
16 stimulation, and a T_H2 type cytokine IL-13, measured after a 24h stimulation, was analyzed
17 from human NKT cells (MAD11) prepared from bulk human peripheral lymphocytes (see
18 Supporting Information SI2). hHeLa-CD1d cells of epithelial origin are inherently CD1d
19 negative (result not shown) and IL-13 was chosen since IL-4 secretion was always extremely
20 low in our *in vitro* assay conditions. Figure 2 shows the IFN- γ and IL-13 secretions induced
21 by the iNKT MAD11 cell line after stimulation with antigen-presenting cells pulsed with
22 canonical ligand α -GalCer (i.e KRN7000) and fluorine α -GalCer analogues **10a**, **10b** and **11**.
23 A negative control confirms that when loaded to non-CD1d transduced HeLa cells the
24 glycolipids induced only background quantities of IFN- γ and IL-13 release.

25



1
 2
 3
 4 **Figure 2.** iNKT cell secretions of cytokines induced by fluorinated α -GalCer analogues **10a**, **10b** and **11** in
 5 hHeLa-CD1d cells of epithelial origin. Relative potencies of α -GalCer (KRN7000) and fluorinated compounds
 6 to stimulate IFN- γ (right y axis, solid lines), and IL-13 (left y axis, dashed lines) release by a human V α 24 iNKT
 7 cell line stimulated by CD1d-transfected Hela cells loaded with different concentrations of each glycolipid; a)
 8 top left: 3-deoxy-3,3-difluoro- α -GalCer **10a**, b) to right: 4-OH epimer **10b**, c) bottom left: 3,4-dideoxy-3,3,4,4-
 9 tetrafluoro- α -GalCer **11**, d) bottom right: α -GalCer. The mean release of cytokines into cell culture supernatants
 10 from triplicate wells were determined by ELISA and shown as pg/ml. Each graph is representative of at least
 11 two independent experiments. In absence of glycolipid or of CD1d, no IFN- γ secretion was detected and only
 12 very low IL-13 secretion was observed. The latter is shown by the horizontal dashed line.

13
 14 Surprisingly, both the di and tetrafluorinated compounds **10a** and **11** induced secretions of
 15 cytokines at level similar to those induced by the reference α -GalCer **1**. These performances
 16 in the absence of 3-OH group can be ascribed to an increasingly favorable *NH*-amide
 17 interaction with the hTrp154 of the TCR due to electron withdrawing effects. However, the
 18 behaviour of gem-3-difluoro-4-OH series **10** and its analogues 3,4-dideoxy-3,3-difluoro- α -
 19 GalCer **9**, previously reported,[112] displaying the same and only a 20 fold lower agonist
 20 potency on hiNKT stimulation, respectively, question the real participation of the 4-OH group
 21 in a conventional H-bond with the hCD1d receptor. This questions remains intriguing,
 22 especially when 4-epi-analogue **10b**, with unnatural 4-OH configuration, expressed only 10-

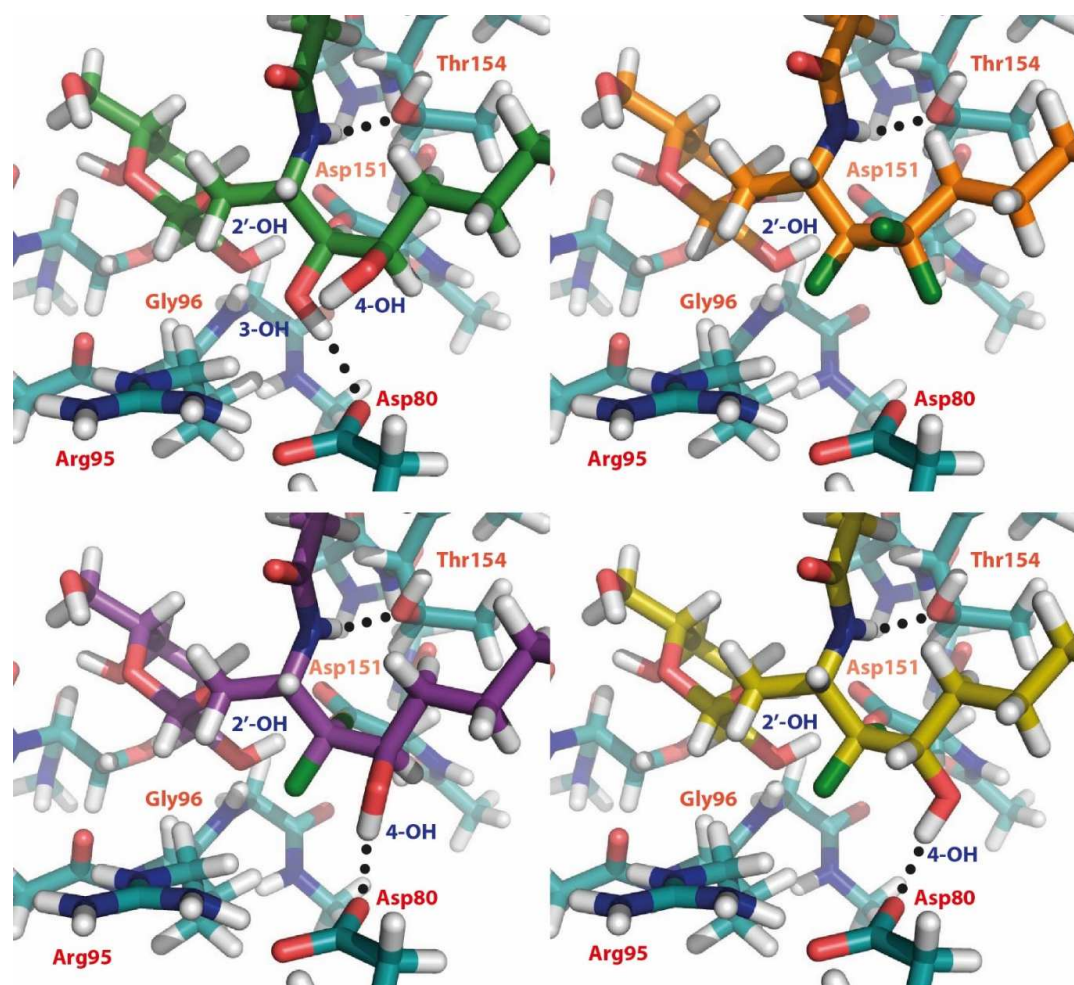
1 fold less potency than α -GalCer itself at inducing cytokines release. It is also noticeable that
2 in all cases, and contrary to the reported poor agonist activity of monofluorinated 3,4-
3 dideoxy-3-fluoro- α -GalCer **8** (Fig.1),[112] gem di- and tetrafluorinated groups introduced
4 either at 3- or/and 4-positions are able to fully restore the ability of deoxy-GalCer derivatives
5 to activate hiNKT cells.

6 7 2.3. Modelling study

8
9 The results obtained in human iNKT cells when presented by HeLa-CD1d transfected cells
10 with fluorinated analogues, highlight a versatile individual contribution of 4-OH in the
11 stability of the hCD1d/ α -GalCer/TCR complex, even when accompanied by the withdrawing
12 effect of vicinal 3,3-*gem*-difluoro group aimed at increase H-bond donating capacity. Beyond
13 the loss of the hydrogen bond donor and acceptor capacity of the sphingosine OH groups,
14 steric and/or conformational constraints induced by gem-difluoride group that could impair
15 OH availability cannot be ruled out to explain such results. Last but not least, the result with
16 3,3,4,4-tetrafluorinated analogue **11**, showing no significant loss on the iNKT stimulation
17 potency nor on the polarization of cytokines release, support the idea that a certain flexibility
18 regarding OH groups is allowed on the ceramide while retaining iNKT activation.

19
20 Intrigued by these results, we sought for structural information that would shed light on the
21 better understanding of the behavior of fluoro derivatives. A hybrid QM/QM' model (see the
22 Supporting Information SI3) has been applied for α -GalCer (used as reference) and its
23 fluorinated analogues, **10a**, **10b** and **11**, aiming to quantify their ability to interact with the
24 surrounding amino acid residues. Seven amino acid residues, in direct interaction with the
25 ligand in the CD1d/ α -GalCer/TCR trimolecular complex crystal structure (PDB-ID
26 2PO6),[56] have been selected to design the model. The main optimized distances are
27 reported in Table S1 (see SI3), whereas the energetic data are gathered in Table S2 (see SI3).
28 In the optimized structure, the tetrafluorinated analogue **11**, lacking 3-OH and 4-OH hydroxyl
29 groups, cannot exhibit hydrogen-bond interactions with Asp80, (Fig. 3) whereas in both
30 analogues **10a** and **10b**, the 4-OH group establishes a shorter intermolecular interaction for
31 which the distance appears to be dependant of the stereochemistry ($d_{(OH4...COO^-)} = 2.025 \text{ \AA}$ in
32 **10a** (4-*R*), and 1.808 \AA in **10b** (4-*S*)) and shorter than in parent α -GalCer **1**. Nevertheless, it is
33 worth noting that the interaction of Asp80 with **10a** and **10b** (ca. $-15 \text{ kcal mol}^{-1}$) remains

1 lower than with α -GalCer **1** (-22.0 kcal mol⁻¹), in which 3-OH hydroxyl group takes
 2 advantage from an intramolecular H-bond activation from 4-OH ($d_{\text{OH4}\dots\text{OH3}} = 2.044 \text{ \AA}$). [159]
 3 Conversely, a repulsive contribution prevails in the tetrafluorinated compound **11** (+3.5 kcal
 4 mol⁻¹). The presence of possible orthogonal multipolar C-F...C=O interactions as additional
 5 stabilizing interactions cannot be entirely ruled out. However, in the current optimized
 6 structures, the F...C distances, the C-F...C angles and the F...C=O angles (the criteria used to
 7 identify such interactions) are systematically outside the recommended ranges, indicating that
 8 such interactions cannot play a meaningful contribution. [126,160]
 9



10
 11 Legend: The designed QM/QM' model (PBE0/6-311G(d,p)/PBE0/6-31G) involves the Phe29, Ser30, Asp80,
 12 Arg95, Gly96, Asp151 and Thr154 residues. a) Top left: α -GalCer **1** in green. b) Top right: 3,4-dideoxy-3,3,4,4-
 13 tetrafluoro- α -GalCer **11** in orange. c) Bottom left: 3-deoxy-3,3-difluoro- α -GalCer **10a** in purple. d) Bottom
 14 right: 4-OH epimer **10b** in yellow. The dotted lines show the 3-OH...Asp80 in α -GalCer **1** and the 4-
 15 OH...Asp80 in **10a** and **10b**.

16 **Figure 3.** Comparison of the partially optimized structures of fluoro- α -GalCer derivatives
 17 within a model of the hCD1d/TCR binding site (Protein Data Bank code 2PO6)

18

1 In addition to its direct effect on the interaction with Asp80, we highlight that the ligand
2 fluorination also tunes the interaction energies with the other amino acid residues. The most
3 important pairwise interactions, ΔE , with the α -GalCer derivatives are observed with Asp151,
4 from $-43 \text{ kcal mol}^{-1}$ (for α -GalCer **1**) to $-35 \text{ kcal mol}^{-1}$ (for **10a** and **10b**) and an intermediate
5 value also being found ($-39 \text{ kcal mol}^{-1}$) for the tetrafluorinated derivative **11**. An enhancement
6 of ca. 3 kcal mol^{-1} of the interaction energies with the Arg95Gly96 residues is observed upon
7 difluorination, from α -GalCer **1** to **10a**, **10b** and to **11** (Table S2 in the SI3). A closer
8 examination reveals that the amide NH-bond of the ligands is interacting with the Thr154
9 hydroxyl group and despite the observed lengthening upon tetrafluorination, DFT indicates a
10 slight increase of the pairwise interaction energy, ΔE (**11**, Thr154). Finally, the pairwise
11 interaction energies with the Phe29Ser30 residues, which are interacting with the α -GalCer
12 analogues through their carbohydrate moieties, are almost unaffected by fluorination, the ΔE
13 values being almost unchanged ($-15.6 \text{ versus } -15.8 \text{ kcal mol}^{-1}$). In short, the computations
14 show that the interaction energies of these seven amino acid residues with the ligands
15 systematically decrease upon fluorination, the ΔE going from $-90 \text{ kcal mol}^{-1}$ with α -GalCer **1**
16 to $-80 \text{ kcal mol}^{-1}$ with **10a** and **10b**, and $-74 \text{ kcal mol}^{-1}$ with **11**. With all the necessary
17 precautions in interpreting the results of these simulations, it appears that the
18 polyfluorinations in position 3 and 4 lead to a destabilization of the α -GalCer energies of
19 interaction in the CD1d binding site.

20 Finally, it is worth noting that in their previous work, Baek *et al.* suggested, on the basis of
21 molecular docking results, that the absence of the 3-OH hydroxyl group could be
22 compensated by an interaction between the 4-OH group and the Tyr73 carbonyl group,
23 accompanied by a lateral shift of the galactose headgroup toward the center of the binding
24 groove.[110] Given Tyr73 is too far from the α -Galcer interacting sites, we initially did not
25 include this residue in our model. We have therefore modified our model adding this eighth
26 residue to investigate the interaction mode of analogue **10a** within the binding site defining a
27 first starting geometry as found above and a second starting-point geometry as proposed by
28 Baek *et al.* with the $4\text{-OH}\cdots\text{O}=\text{C}(\text{Tyr73})$ H-bond interaction ($d_{(\text{OH4}\cdots\text{O}=\text{C})} = 2.080 \text{ \AA}$).
29 Interestingly, it appears that after their geometry optimization the two final structures are very
30 close, the galactose moiety of the second geometry shifting back to the first geometry, losing
31 the $4\text{-OH}\cdots\text{O}=\text{C}(\text{Tyr73})$ H-bond interaction ($d_{(\text{OH4}\cdots\text{O}=\text{C})} = 3.297 \text{ \AA}$). In this case, the 4-OH
32 hydroxyl group does not interact with any residue, neither Asp80, nor Tyr73. Hence, it
33 appears that the recovery of the $2'\text{OH-COO}^-(\text{Asp151})$ and $3'\text{OH-COO}^-(\text{Asp151})$, but also of

1 the NH...OH(Thr154) H-bond is prevailing over the 4-OH...O=C(Tyr73) H-bond interaction.
2 The superposition of the two optimized structures is given in Figure S3 in SI3 (see supporting
3 information). Finally, in absence of 3-OH on the sphingosine the computed interaction
4 energies, $\Delta E = -86.4$ and -85.6 kcal mol⁻¹, corroborate the presence of a stabilizing interaction
5 between the 4-OH group and the Asp80, while, intramolecular H-bond between 4-OH and 3-
6 OH could prevail in α -GalCer **1** to strengthen 3-OH...Asp80 interaction. This latter outcome
7 is supported by a weak loss in cytokines release from iNKT previously observed by Linclau
8 with the 4-deoxy-4,4-difluoro- α -GalCer derivative **7** (Fig. 1), accompanied by a slight T_H1
9 bias.[140] The expected increase in H-bond donating capacity of the 3-OH group due to the
10 neighboring electron withdrawing gem-difluoro group at C4 could be attenuated by the loss
11 of the intramolecular H-bond from the 4-OH group, explaining the weak biological
12 improvement observed.

13

14 **3. Conclusion**

15 In conclusion, the synthesis of three novel α -GalCer analogues, 3-deoxy-3,3-difluoro- α -
16 GalCer **10a** and its 4-OH epimer **10b** and 3,4-dideoxy-3,3,4,4-tetrafluoro- α -GalCer **11**, was
17 achieved in ten and thirteen steps, respectively, to interrogate their molecular interactions at
18 the atomic level with CD1d and TCR receptors. The results confirm the ability of the
19 phytosphingosine fragment to adopt versatile conformational changes and to shift in the
20 hCD1d binding groove to accommodate new interactions when lacking one or two OH
21 structural ingredients. The only 10-fold lower potency of (4*S*)-OH epi-analogue **10b** to
22 stimulate hiNKT compared to 3-deoxy-3,3-difluoro- α -GalCer **10a** supports this observation
23 already mentioned by several authors from deoxy and diastereomeric analogues.
24 Nevertheless, our study using 3-gem-difluorine derivatives seems to confirm that reinforcing
25 the *NH*-amide donating capacity of 3-deoxy-phytosphinganine analogues tends to restabilize
26 the CD1d/ α -GalCer-analogue/TCR complex despite the loss of key contributing *H*-bonds on
27 the phytosphingosine fragment. Obviously, the potency of 3,3,4,4-tetrafluoro- α -GalCer
28 analogue **11** to stimulate hiNKT inducing IFN- γ and IL-13 secretions at the same level than
29 α -GalCer **1**, pleads for this statement. However, observed similar T_H1/T_H2 bias suggests the
30 lack of the *NH*-amide contribution on the polarisation of immune response. Although
31 polarizing effects may be more efficiently observed in the *in vivo* mouse model setting,

1 however, this model suffers to not reflect properly human context due to higher level of
2 available iNKT and can skew sensible information.

3 The observations made with fluorinated α -GalCer analogues may not only be due to a direct
4 binding effect of the compounds to CD1d, but may also involve differences in uptake and
5 subcellular localization owing to changes in hydrophobicity, especially upon polyfluorination.
6 Nevertheless, our previous studies on deoxyfluoro sphingosine modified GalCer derivatives,
7 supported by modeling along with the biological performances from these 3 new fluorinated
8 analogues, point to an unidentified assistance of the 4-OH group on the key 3-OH
9 contribution in the immune stimulation performance of α -GalCer (KRN7000), rather than a
10 direct involvement through a proper H-bond with the CD1d receptor.

11

12 **4. Experimental for Chemistry**

13 Solvents were purified and dried by standard methods prior to use. Alternatively, the MB
14 SPS-800-dry solvent system was used to dry dichloromethane and THF. Dry DMF solvent
15 was commercially available from Sigma Aldrich and was used without purification.
16 Glassware used for reaction was either flame dried under vacuum or under argon stream for
17 several minutes. Reactions were carried out under rigorous anhydrous conditions and argon
18 stream or positive pressure of argon. All reactions were monitored by TLC on commercially
19 available precoated plates (Kieselgel 60 F254), and the compounds were visualized by UV
20 (254 nm) when possible and with Ceric Ammonium Molybdate Solution [(NH₄)₆Mo₇O₂₄ (5g)
21 + Ce(SO₄)₂ (0.2g) in H₂SO₄ 5% solution (100 mL)] and heating. High purity grade (Merck
22 grade 9385) pore size 60Å, 230-400 mesh particle size silica gel (Sigma Aldrich) was used
23 for flash column chromatography. Solvents used for chromatography were prior distilled on a
24 Buchi rotavapor R-220-SE. Melting points were determined on a RCH (C. Reichert)
25 microscope equipped with a Kofler heating system. Optical rotations were measured at 20±1
26 °C with a Perkin–Elmer 341 instrument in the indicated solvents, and concentrations are
27 expressed in g/100 mL. FTIR spectra were obtained in the 500–4000 cm⁻¹ range with a
28 Bruker Vector 22 FTIR spectrometer. ¹H, ¹³C, ¹⁹F and ³¹P NMR spectra were recorded on a
29 *Bruker Avance 300* spectrometer fitted with a 5 mm i.d. BBO probe carefully tuned to the
30 recording frequency of 300.13 MHz (for ¹H), 75.47 MHz (for ¹³C) and 282.40 MHz (for ¹⁹F),
31 the temperature of the probe was set at room temperature (around 293-294 K), on a *Bruker*
32 *Avance 400* spectrometer fitted with a 5 mm i.d. BBFO+ probe carefully tuned to the
33 recording frequency of 400.13 MHz (for ¹H), 100.61 MHz (for ¹³C), 376.53 (for ¹⁹F) and

1 121.49 MHz (for ^{31}P). The spectra are referenced to the solvent in which they were run (7.27
2 ppm for ^1H CDCl_3 [idem for $\text{CDCl}_3/\text{CD}_3\text{OD}$ 2:1] and 77.16 ppm for ^{13}C CDCl_3 [idem for
3 $\text{CDCl}_3/\text{CD}_3\text{OD}$ 2:1], 3.58 and 1.73 ppm for ^1H $\text{THF-}d_8$ and 67.2 and 25.2 ppm for ^{13}C THF-
4 d_8). Chemical shifts (δ are given in ppm, and coupling constants (J) are given in Hz with the
5 following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m =
6 multiplet or massif, br = broad and app = appeared as. All assignments were confirmed with
7 the aid of two-dimensional ^1H , ^1H (COSY), or ^1H , ^{13}C (HSQC, HMBC) experiments using
8 standard pulse programs. Low resolution mass spectrometry (MS) were recorded on a
9 ThermoFinnigan DSQII quadrupolar spectrometer (coupled with a TracUltra GC apparatus)
10 for Chemical Ionization (CI) ; on a ThermoFinnigan LCQ Advantage spectrometer for
11 ElectroSpray Ionisation (ESI).

12 Low and High resolution mass spectrometry (HRMS) were recorded on a ThermoFisher
13 Scientific LTQ-Orbitrap spectrometer and on a Waters Xevo G2-XS Qtof spectrometer
14 (coupled with an HPLC Acquity H-Class) for ESI ; on a Waters Xevo G2-XS Qtof
15 spectrometer for ASAP+ ; on a Bruker Autoflex III spectrometer for MALDI+. Elemental
16 analyses were performed with a Thermo Fisher Scientific Flash 2000 Series CHNS analyser,
17 with detection by a catharometer (Thermal Conductivity Detector)

18

19 ***Synthesis of (2S,Rs)-1-Benzyloxy-3,3-difluoro-2-(tert-butyl)sulfinyloctadecan-4-one***
20 ***(2S)-17 via the route 1:***

21 **4.1. 1-bromo-1,1-difluorohexadecan-2-one 14.** Flame-dried Mg (82 mg, 3.36 mmol) was
22 suspended in Et_2O (1 mL) and treated with a few drops of 1-bromotetradecane. The mixture
23 was heated to reflux until effervescence was observed and then 1-bromotetradecane (1.00 mL,
24 3.36 mmol) was added over 10 min while heating and reflux was maintained an additional
25 hour. After cooled at r.t., the resulting Grignard solution was added to a solution, at -78°C , of
26 ester **12** (393 μL , 3.06 mmol) in Et_2O (3 mL). The mixture was stirred at -78°C for 3 h, and
27 then quenched with aq. HCl (3 M, 3 mL). The aqueous layer was extracted with Et_2O (3×3
28 mL) and the combined organic phases were dried (MgSO_4), filtered and concentrated to give
29 a yellow oil. Column chromatography (petroleum ether / Et_2O 100:0 to 80:20) gave ketone **14**
30 (202 mg, 19%) as colourless oil. *Data for 14:* ^1H NMR (300 MHz, CDCl_3) δ 2.78 (tt, $J = 0.9$,
31 7.3, 2H), 1.72–1.64 (m, 2H), 1.38–1.22 (m, 22H), 0.88 (t, $J = 6.7$, 3H). ^{13}C NMR (75 MHz,
32 CDCl_3) δ 192.2 (t, $J = 26$), 114.3 (t, $J = 319$), 34.7, 32.1, 29.8–28.9 (9C), 23.1, 22.9, 14.2. ^{19}F
33 NMR (376 MHz, CDCl_3) δ -64.7.

1
2 4.2. (2*S*,*R*_s)-1-Benzoyloxy-3,3-difluoro-2-(*tert*-butyl)sulfinylaminoctadecan-4-one (2*S*)-17
3 and 17-(bromodifluoromethyl)-16,16-difluoro-17-hydroxyhentriacontan-15-one **18**. To a
4 solution of sulfinylimine (*R*_s,*E*)-**16**[147] (105 mg, 0.414 mmol) and RhCl(PPh₃)₃ (12 mg, 13
5 μmol) in THF (2.8 mL), at -20 °C, was added a solution of ketone **14** (176 mg, 0.495 mmol)
6 in THF (0.5 mL) immediately followed by addition dropwise of Et₂Zn (1.0 M in hexane, 0.88
7 mL, 0.88 mmol). The mixture was warmed to 0 °C over 1 h, and then stirred for 1 h before
8 being quenched with aq. NH₄Cl (sat., 3 mL). The aqueous layer was extracted with EtOAc (3
9 × 5 mL) and the combined organic phases were dried (MgSO₄), filtered and concentrated to
10 give a brown oil. Column chromatography (petroleum ether /EtOAc/MeOH 90:10:0 to
11 0:90:10) gave compounds **18** (53 mg, 36%) as oily solid and (2*S*,2*R*)-**17** (59 mg, 27%) as a
12 colourless oil.

13 4.2.1. *Data for (2S)-17*: R_f 0.34 (petroleum ether/EtOAc 60:40). Mp 50–53 °C. [α]_D -17.5 (c
14 1.06, CHCl₃, 20 °C). IR (KBr) ν 3439, 3298, 2959, 2851, 1731, 1471, 1209, 1100, 1072 cm⁻¹.
15 ¹H NMR (400 MHz, CDCl₃) δ 7.38–7.24 (m, 5H), 4.55 (d, *J* = 11.7, 1H), 4.48 (d, *J* = 11.7,
16 1H), 4.06 (dddd, *J* = 13.2, 11.4, 9.5, 5.7, 3.5, 1H), 3.89 (d, *J* = 9.4, 1H), 3.87 (dd, *J* = 9.5,
17 3.5, 1H), 3.74 (ddd, *J* = 10.0, 5.6, 1.4, 1H), 2.60 (br. t, *J* = 7.3, 2H), 1.51–1.47 (m, 2H), 1.33–
18 1.19 (m, 22H), 1.22 (s, 9H), 0.89 (t, *J* = 7.1, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 200.4 (t, *J* =
19 29), 137.0, 128.3 (2C), 127.8, 127.7 (2C), 115.3 (t, *J* = 258), 73.5, 67.9, 57.8 (t, *J* = 25), 56.5,
20 37.5, 31.8, 29.7–28.7 (9C), 22.6, 22.3 (3C), 22.2, 14.0. ¹⁹F NMR (376 MHz, CDCl₃) δ -113.3
21 (dd, *J* = 270, 12, 1F), -116.0 (dd, *J* = 270, 14, 1F). MS (CI+) *m/z* 530.3 [M + H]⁺. HRMS
22 (ESI+) for C₂₉H₄₉F₂NO₃SNa⁺ [M+Na]⁺ calcd. 552.3299, found 552.3285.

23 4.2.2. *Data for 18*: ¹H NMR (300 MHz, CDCl₃) δ 2.80-2.71 (m, 2H), 1.66-1.54 (m, 2H),
24 1.35–1.20 (m, 22H), 0.88 (t, *J* = 8.0, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 38.1, 32.1 (2C),
25 30.3-28.8 (28C), 22.8 (2C), 22.4, 14.3 (2C) [loss of 4C related to fluorine due to bad
26 relaxation]. ¹⁹F NMR (282 MHz, CDCl₃) δ -51.5 (ddd, *J* = 172, 10, 2, 1F), -52.8 (ddd, *J* =
27 172, 18, 4, 1F), -109.0 (d, *J* = 285, 1F), -115.6 (ddd, *J* = 285, 18, 10, 1F). MS (ASAP+) *m/z*
28 631.3 [M + H]⁺. HRMS (ASAP+) for C₃₂H₆₀F₄O₂Br⁺ [M + H]⁺ calcd. 631.3713, found
29 631.3712.

30

31 4.3. 2-chloro-2,2-difluoro-*N*-methoxy-*N*-methylacetamide **15**. To a suspension of *N*,*O*-
32 dimethylhydroxylamine hydrochloride (2.28 g, 23.4 mmol) in THF (100 mL) at 0 °C was
33 added dropwise AlCl₃ (1M in heptane, 23.4 mL, 23.4 mmol). After 40 min. at 0 °C, the

1 mixture was cooled to -40°C to add ethyl bromodifluoroacetate **12** (1 mL, 7.8 mmol) then
2 warmed to r.t. and stirred for 3 h. Finally, the reaction was quenched at -40°C with aq. HCl
3 (1.5 M, 100 mL). The aqueous layer was extracted with Et_2O (3×100 mL) and the combined
4 organic phases were washed with brine (300 mL), dried (MgSO_4), filtered and concentrated to
5 give an oil. Column chromatography (pentane/ Et_2O 80:20) gave known compound **15**[161]
6 (1.06 g, 62%) as a pale yellow oil.

7
8 *Synthesis of (2S,RS)-1-Benzoyloxy-3,3-difluoro-2-(tert-butyl)sulfinylaminoctadecan-4-one*
9 *(2S)-17 via the route 2:*

10 4.4. (3S,RS)-Ethyl 4-(Benzyloxy)-3-(tert-butylsulfonamino)-2,2-difluorobutanoate **19**. To a
11 solution of sulfinylimine (*RS,E*)-**16**[147] (4.11 g, 16.20 mmol) and $\text{RhCl}(\text{PPh}_3)_3$ (450 mg, 0.49
12 mmol) in THF (120 mL), at -20°C , was added bromoester **12** (6.23 mL, 48.60 mmol) and
13 then dropwise Et_2Zn (1.0 M in hexane, 32.4 mL, 32.4 mmol). The mixture was warmed to 0
14 $^{\circ}\text{C}$ over 30 h and stirred for 1 h before being quenched with aq. NH_4Cl (sat., 90 mL). The
15 aqueous layer was extracted with EtOAc (3×160 mL) and the combined organic phases were
16 dried (MgSO_4), filtered and concentrated to give a brown oil. Column chromatography
17 (petroleum ether/ EtOAc/MeOH 90:10:0 to 0:90:10) gave sulfinylamide **19**[147] (2.67, 43%)
18 as yellow oil.

19
20 4.5. (3S,RS)-*N*-Methoxy-*N*-methyl-3-(tert-butyl)sulfinylamino-4-benzyloxy-2,2-difluorobutyryl-
21 *amide* **20**. $\text{MeNHOMe}\cdot\text{HCl}$ (3.40 g, 34.9 mmol) was suspended in THF (70 mL) and the
22 solution cooled to -78°C prior adding, dropwise *n*BuLi (2.4M in hexane, 29 mL, 69.7
23 mmol). The mixture was stirred at -78°C for 5 min then the cooling bath was removed for
24 approximately 15 min. The reaction was then re-cooled to -78°C and added with a solution
25 of ester **19** (2.63 g, 6.97 mmol) in THF (85 mL). The reaction was stirred at -78°C for 4 h
26 then at -60°C for 1 h before being quenched with aq. NH_4Cl (sat., 25 mL) and warmed to r.t.,
27 before addition of H_2O (50 mL). The aqueous layer was extracted with Et_2O (250 mL) and
28 EtOAc (3×250 mL). The combined organic layers were dried (MgSO_4), filtered and
29 concentrated to give a light brown oil. Column chromatography (petroleum ether/ EtOAc 70:30 to
30 50:50) gave the product **20** (2.52 g, 92%) as a pale yellow oil. R_f 0.19 (petroleum
31 ether/ EtOAc 50:50). $[\alpha]_D -29.7$ (c 1.07, CHCl_3 , 20°C). IR (neat) ν 3216, 2944, 2871, 1690,
32 1456, 1366, 1205, 1083 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 7.40–7.28 (m, 5H), 4.57 (d, $J =$
33 11.5, 1H), 4.53 (d, $J = 11.7$, 1H), 4.30 (m, 1H), 3.90–3.80 (m, 2H), 3.76 (dd, $J = 10.1$, 6.7,

1 1H), 3.72 (s, 3H), 3.11 (br. s, 3H), 1.23 (s, 9H). ¹³C NMR (75 MHz, CDCl₃) δ 137.4, 128.3
2 (2C), 127.9 (2C), 127.7, 115.8 (t, *J* = 256), 73.5, 68.1, 61.9, 58.5 (t, *J* = 24), 56.6, 33.0, 22.4
3 (3C)) [loss of 1C related to fluorine due to bad relaxation]. ¹⁹F NMR (376 MHz, CDCl₃) δ -
4 111.4 (dd, *J* = 262, 9, 1F), -112.7 (dd, *J* = 262, 14, 1F). MS (CI+) *m/z* 393.1 [M + H]⁺.
5 HRMS (ESI+) for C₁₇H₂₇N₂O₄SF₂ [M+H]⁺ calcd. 393.1660, found 393.1668.

6

7 4.6. (2*S*,*R*_S)-1-Benzoyloxy-3,3-difluoro-2-(*tert*butyl)sulfinyloctadecan-4-one (2*S*)-**17**.

8 Flame-dried Mg (305 mg, 12.5 mmol) was suspended in THF (74 mL) and treated with a few
9 drops of 1-bromotetradecane. The mixture was heated to reflux until effervescence was
10 observed then 1-bromotetradecane (3.69 mL, 12.4 mmol) was added over 60 min while
11 heating. Reflux was continued for 1 h then the reaction was cooled to 0 °C and a solution of
12 Weinreb amide **20** (974 mg, 2.48 mmol) in THF (15 mL) was added. Stirring was continued
13 at 0 °C for 40 min then at r.t. for 1 h. The mixture was then cooled to 0°C before being
14 quenched with aq. NH₄Cl (sat., 50 mL) then poured into H₂O (50 mL). The aqueous layer
15 was extracted with EtOAc (3 × 100 mL) and combined organic layers were washed with brine
16 (50 mL), dried (MgSO₄), filtered and concentrated to give an oil. Column chromatography
17 (petroleum ether /EtOAc 100:0 to 0:100) gave Sulfinylamine (2*S*)-**17** as a colorless oil which
18 became a white solid after storage (1.19 g, 90%). See data of **17** above.

19

20 4.7. (2*S*)-2-Amino-1-benzoyloxy-3,3-difluorooctadecan-4-one **21**. Sulfinylamine (2*S*)-**17** (1.55
21 g, 2.93 mmol) was dissolved in 1,4-dioxane (35 mL) and treated with aq. HCl (3M, 9.8 mL,
22 29.3 mmol). The mixture was stirred at r.t. for 14 h then extracted with pentane (3 × 90 mL).
23 A white solid was filtered from the pentane to give the product **21** (1.29 g, 95%) as the
24 hydrochloride salt. *R*_f 0.59 (petroleum ether/EtOAc 10:90). Mp 111–115 °C. [α]_D +6.4 (c 1.0,
25 CHCl₃, 20 °C). IR (KBr) ν 3432, 2918, 2850, 1744, 1123 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ
26 9.09 (br. s, 2H), 7.36–7.24 (m, 5H), 4.54 (d, *J* = 11.5, 1H), 4.47 (d, *J* = 11.5, 1H), 4.27 (m,
27 1H), 3.97 (br. s, 2H), 2.58 (td, *J* = 6.9, 3.9, 2H), 1.41–1.37 (m, *J* = 6.9, 3H), 1.34–1.11 (m,
28 22H), 0.89 (t, *J* = 7.0, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 199.0, 136.5, 128.4 (2C), 128.0,
29 127.9 (2C), 113.4 (t, *J* = 258), 73.9, 64.7, 52.3 (dd, *J* = 26, 23), 37.0, 31.9, 30.3–28.4 (9C),
30 22.7, 22.1, 14.1. ¹⁹F NMR (376 MHz, CDCl₃) δ -110.3 (dd, *J* = 280, 22, 1F), -116.3 (dq, *J* =
31 280, 14, 1F). MS (CI+) *m/z* 426.2 [M + H]⁺. HRMS (ESI+) for C₂₅H₄₂F₂NO₂⁺ [M+H]⁺ calcd.
32 426.3184, found 426.3185.

33

1 4.8. (2*S*)-1-Benzylxy-3,3-difluorooctadecan-4-one-2-yl hexacosanamide (2*S*)-**22**. Amine
2 hydrochloride **21** (1.27 g, 2.74 mmol) was dissolved in CHCl₃ (146 mL) and cerotic acid
3 (1.24 g, 3.12 mmol), PyBOP (1.62 g, 3.12 mmol) and Et₃N (0.83 mL, 5.94 mmol) were added
4 to the solution. The mixture was stirred at reflux for 20 h then diluted with DCM (130 mL).
5 The organic phase was washed with H₂O (130 mL) and brine (130 mL), dried (MgSO₄),
6 filtered and concentrated to give an off-white solid, which was suspended in MeOH and
7 filtered. The resultant white solid was purified by column chromatography (petroleum
8 ether/DCM 50:50 then 0:100) to give amide (2*S*)-**22** (1.60 g, 73%) as a white solid. R_f 0.50
9 (petroleum ether/EtOAc 80:20). Mp 93–97 °C. [α]_D +15.5 (c 0.96, CHCl₃, 20 °C). IR (KBr) ν
10 3311, 2917, 2849, 1736, 1657, 1546 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.40–7.23 (m, 5H),
11 6.12 (d, *J* = 9.3, 1H), 4.83 (dddd, *J* = 16.6, 12.0, 9.0, 4.2, 1H), 4.47 (s, 2H), 3.73 (dd, *J* = 10.2,
12 3.9, 1H), 3.58 (dd, *J* = 10.2, 4.7, 1H), 2.62 (dt, *J* = 7.3, 6.5, 2H), 2.21 (t, *J* = 7.6, 2H), 1.55–
13 1.40 (m, 6H), 1.37–1.17 (m, 64H), 0.89 (t, *J* = 6.7, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 173.0,
14 137.0, 128.5 (2C), 128.0, 127.7 (2C), 73.5, 67.1, 50.9 (t, *J* = 26), 37.3, 36.6, 31.9, 30.2–28.6
15 (31C), 25.5, 22.7, 22.4, 14.1 (2C) [loss of 2C related to fluorine due to bad relaxation]. ¹⁹F
16 NMR (376 MHz, CDCl₃) δ -113.1 (dd, *J* = 266, 12, 1F), -115.5 (dd, *J* = 266, 12, 1F). MS
17 (CI+) *m/z* 805.0 [M + H]⁺. HRMS (MALDI+) for C₅₁H₉₂F₂NO₃⁺ [M + H]⁺ calcd. 804.7040,
18 found 804.7026.

19

20 4.9. (2*S*)-1-Benzylxy-3,3-difluorooctadecan-4-ol-2-yl hexacosanamide **23**. Amide **22** (1.02 g,
21 1.26 mmol) was dissolved in THF/EtOH (3:1, 60 mL) and NaBH₄ (72 mg, 1.90 mmol) was
22 added to the solution. The mixture was stirred at r.t. for 2.5 h then quenched with H₂O (1.1
23 mL) and stirring was continued for 20 min before the solution was concentrated under
24 reduced pressure to give a white solid. Column chromatography (petroleum ether/EtOAc
25 95:5, 90:10) gave (4*R*)-**23a** (531 mg, 52%) and (4*S*)-**23b** (448 mg, 44%) both as white solids.

26 4.9.1. Data for isomer (4*R*)-**23a**: R_f 0.26 (petroleum ether/EtOAc 80:20). Mp 85–89 °C. [α]_D
27 +15.7 (c 0.7, CHCl₃, 20 °C). IR (KBr) ν 3432, 3304, 2917, 2850, 1654, 1551, 1464, 1100 cm⁻¹.
28 ¹H NMR (400 MHz, CDCl₃) δ 7.44–7.28 (m, 5H), 5.93 (d, *J* = 8.9, 1H), 4.85–4.68 (m,
29 1H), 4.58 (d, *J* = 11.8, 1H), 4.54 (d, *J* = 11.8, 1H), 3.84–3.67 (m, 2H), 3.63 (ddd, *J* = 10.2,
30 6.1, 1.0, 1H), 3.09 (d, *J* = 6.8, 1H), 2.22 (t, *J* = 7.6, 2H), 1.86–1.41 (m, 6H), 1.15–1.39 (m,
31 66H), 0.89 (t, *J* = 7.0, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 173.5, 137.0, 128.6 (2C), 128.1,
32 127.9 (2C), 122.0 (dd, *J* = 254, 249), 73.5, 71.8 (dd, *J* = 30, 26), 67.4, 50.1 (t, *J* = 25), 36.7,
33 31.9, 30.0–28.9 (32C), 25.7, 25.6, 22.7, 14.1 (2C). ¹⁹F NMR (376 MHz, CDCl₃) δ -114.9

1 (ddd, $J = 254, 15, 7, 1\text{F}$), -119.5 (dt, $J = 254, 14, 1\text{F}$). MS (CI+) m/z 807.0 $[\text{M} + \text{H}]^+$. HRMS
2 (ESI+) for $\text{C}_{51}\text{H}_{94}\text{F}_2\text{NO}_3$ $[\text{M} + \text{H}]^+$ calcd. 806.7202, found 806.7221.

3 4.9.2. *Data for isomer (4S)-23b*: R_f 0.53 (petroleum ether/EtOAc 80:20). Mp 88–90 °C. $[\alpha]_D$
4 -2.3 (c 0.8, CHCl_3 , 20 °C). IR (KBr) ν 3330, 2918, 2849, 1655, 1539, 1470, 1100 cm^{-1} . ^1H
5 NMR (400 MHz, CDCl_3) δ 7.43–7.29 (m, 5H), 6.11 (d, $J = 8.3, 1\text{H}$), 4.74 (d, $J = 4.5, 1\text{H}$),
6 4.56 (s, 2H), 4.60 (ddt, $J = 25.9, 8.5, 4.6, 1\text{H}$), 3.91 (dd, $J = 10.5, 4.7, 1\text{H}$), 3.75 (ddd, $J =$
7 $10.5, 4.2, 2.0, 1\text{H}$), 3.52 (ddd, $J = 23.6, 8.3, 4.0, 1\text{H}$), 2.26 (td, $J = 7.5, 2.2, 2\text{H}$), 1.70–1.55
8 (m, 6H), 1.35–1.21 (m, 66H), 0.89 (t, $J = 7.0, 6\text{H}$). ^{13}C NMR (75 MHz, CDCl_3) δ 175.5,
9 137.4, 128.5 (2C), 128.0, 127.7 (2C), 121.9 (dd, $J = 255, 252$), 73.4, 69.2 (dd, $J = 32, 24$),
10 65.6, 49.2 (dd, $J = 33, 23$), 36.5, 31.9, 30.0–29.0 (31C), 27.4 (d, $J = 4$), 25.9, 25.6, 22.7, 14.1
11 (2C). ^{19}F NMR (376 MHz, CDCl_3) δ -121.8 (dd, $J = 253, 25, 1\text{F}$), -124.7 (dd, $J = 253, 24,$
12 1F). MS (CI+) m/z 807.0 $[\text{M} + \text{H}]^+$. HRMS (MALDI+) for $\text{C}_{51}\text{H}_{93}\text{F}_2\text{NO}_3\text{Na}^+$ $[\text{M} + \text{Na}]^+$
13 calcd. 828.7016, found 828.6985.

14

15 4.10. *(2S,4R)-3,3-Difluorooctadecan-1,4-diol-2-yl hexacosanamide (4R)-24a*. Benzyl
16 protected ceramide (4R)-**23a** (477 mg, 0.592 mmol) was dissolved in THF (8.3 mL) and
17 treated with $\text{Pd}(\text{OH})_2/\text{C}$ (20%, 125 mg, 0.178 mmol). The reaction mixture was flushed with
18 H_2 then stirred under H_2 atmosphere for 3 h before being filtered through Celite®. The pad
19 was rinsed with warm THF. Concentration of the filtrate gave a white solid, which was
20 purified by column chromatography (DCM/MeOH 99:1 to 90:10) to give ceramide (4R)-**24a**
21 (372 mg, 88%) as a white solid. R_f 0.08 (petroleum ether/EtOAc 70:30). Mp 95–101 °C. $[\alpha]_D$
22 $+7.8$ (c 0.5, THF, 20 °C). IR (KBr) ν 3422, 3339, 2919, 2850, 1652, 1545, 1473, 1070 cm^{-1} .
23 ^1H NMR (400 MHz, $\text{THF}-d_8$) δ 7.10 (d, $J = 9.3, 1\text{H}$), 4.66–4.50 (m, 1H), 4.47 (d, $J = 7.8,$
24 1H), 4.12 (t, $J = 6.1, 1\text{H}$), 3.77–3.59 (m, 3H), 2.16 (t, $J = 7.4, 2\text{H}$), 1.69–1.40 (m, 6H), 1.38–
25 1.21 (m, 66H), 0.89 (t, $J = 7.1, 6\text{H}$). ^{13}C NMR (100 MHz, $\text{THF}-d_8$) δ 173.4, 123.9 (dd, $J =$
26 $252, 249$), 72.1 (dd, $J = 29, 26$), 61.1 (t, $J = 4$), 53.3 (t, $J = 24$), 36.8, 33.0, 30.9–30.3 (32C),
27 27.0, 26.7, 23.7, 14.6 (2C). ^{19}F NMR (376 MHz, $\text{THF}-d_8$) δ -119.4 (ddd, $J = 253, 16, 8, 1\text{F}$), $-$
28 121.4 (ddd, $J = 253, 16, 12, 1\text{F}$). MS (CI+) m/z 717.0 $[\text{M} + \text{H}]^+$. HRMS (MALDI+) for
29 $\text{C}_{44}\text{H}_{87}\text{F}_2\text{NO}_3\text{Na}^+$ $[\text{M} + \text{Na}]^+$ calcd. 738.6548, found 736.6532.

30

31 4.11. *(2S,4S)-3,3-Difluorooctadecan-1,4-diol-2-yl hexacosanamide (4S)-24b*. Benzyl
32 protected ceramide (4S)-**23b** (418 mg, 0.518 mmol) was dissolved in THF (8.3 mL) and
33 treated with $\text{Pd}(\text{OH})_2/\text{C}$ (20%, 125 mg, 0.178 mmol). The reaction mixture was flushed with

1 H₂ then stirred under H₂ atmosphere for 3 h before being filtered through Celite®. The pad
2 was rinsed with warm THF. Concentration of the filtrate gave a white solid, which was
3 purified by column chromatography (DCM/MeOH 99:1, 96:4) to give ceramide (4*S*)-**24b**
4 (358 mg, 96%) as a white solid. R_f 0.1 (petroleum ether/EtOAc 70:30). Mp 100–105 °C. [α]_D
5 –10.4 (c 0.87, CHCl₃, 20 °C). IR (KBr) ν 3343, 3303, 2915, 2850, 1623, 1472 cm⁻¹. ¹H NMR
6 (400 MHz, THF-*d*₈) δ 7.56 (d, *J* = 8.3, 1H), 5.10 (d, *J* = 4.3, 1H), 4.37 (app. dtd, *J* = 26.1, 8.3,
7 3.6, 1H), 3.90 (t, *J* = 5.3, 1H), 3.89–3.83 (m, 1H), 3.73–3.62 (m, 1H), 3.54–3.43 (m, 1H),
8 2.26 (t, *J* = 7.3, 2H), 1.69–1.43 (m, 6H), 1.39–1.21 (m, 66H), 0.89 (t, *J* = 6.9, 6H). ¹³C NMR
9 (100 MHz, THF-*d*₈) δ 176.5, 70.0 (dd, *J* = 32, 24), 59.0, 53.4 (dd, *J* = 32, 23), 36.4, 32.9,
10 31.1–30.5 (30C), 30.3 (t, *J* = 8), 28.5 (d, *J* = 4), 27.0, 26.6, 23.6, 14.5 (2C). ¹⁹F NMR (376
11 MHz, THF-*d*₈) δ -123.6 (dd, *J* = 249, 26, 1F), -126.3 (dd, *J* = 249, 24, 1F) [loss of 1C related
12 to fluorine due to bad relaxation]. MS (CI+) *m/z* 717.1 [M + H]⁺. HRMS (ESI+) for
13 C₄₄H₈₇F₂NO₃Na⁺ [M + Na]⁺ calcd. 738.6548, found 736.6531.

14

15 4.12. *1-O-(2,3,4,6-Tetra-O-benzyl-α-galactosyl)-(2*S*,4*R*)-3,3-difluorooctadecan-1,4-diol-2-yl*
16 *hexacosanamide (4*R*)-26a*. In the dark, SnCl₂ (230 mg, 1.21 mmol), AgClO₄ (251 mg, 1.21
17 mmol) and ground 4Å molecular sieves (1.66 g) were combined in THF (2.8 mL) and stirred
18 at r.t. for 90 min. In parallel, ceramide (4*R*)-**24a** (289 mg, 0.404 mmol) was dissolved in THF
19 (3.8 mL) and added to a solution of fluoro galactosyl donor **25**[162] (329 mg, 0.606 mmol)
20 dissolved in THF (5.3 mL). Then, the mixture containing Lewis acids was added with (4*R*)-
21 **24a** and **25**, *via* cannula, to the mixture of Lewis acids beforehand cooled to 0°C and stirring
22 was maintained, in the dark, for 20 min. The mixture was warmed to r.t., stirred for 2 h and
23 then filtered through Celite®. The mixture was stirred at r.t. in the dark for 2 h, then filtered
24 through Celite®. The pad was rinsed with EtOAc (~110 mL) and the filtrate was washed with
25 aq. NaHCO₃ (sat., 5 × 14 mL), dried (MgSO₄), filtered and concentrated to give a residue.
26 Column chromatography (petroleum ether/EtOAc 90:10 to 80:20) gave (4*R*)-**26a** (260 mg,
27 52%) as a white solid. R_f 0.50 (petroleum ether/EtOAc 70:30). Mp 85–86 °C. [α]_D +28.6 (c
28 1.0, CHCl₃, 20 °C). IR (KBr) ν 3629, 3317, 2919, 2850, 1652, 1617 cm⁻¹. ¹H NMR (400
29 MHz, CDCl₃) δ 7.51–7.09 (m, 20H), 6.07 (d, *J* = 8.7, 1H), 4.92 (d, *J* = 11.4, 1H), 4.87 (d, *J* =
30 3.9, 1H), 4.84 (d, *J* = 12.5, 1H), 4.80 (d, *J* = 11.8, 1H), 4.72 (d, *J* = 11.7, 2H), 4.82–4.68 (m,
31 1H), 4.56 (d, *J* = 11.5, 1H), 4.47 (d, *J* = 11.7, 1H), 4.39 (d, *J* = 11.8, 1H), 4.14 (d, *J* = 6.8,
32 1H), 4.06 (dd, *J* = 10.1, 3.6, 1H), 3.94 (d, *J* = 1.8, 1H), 3.91 (t, *J* = 6.5, 1H), 3.82 (dd, *J* =
33 10.1, 2.6, 1H), 3.87–3.74 (m, 2H), 3.68 (dd, *J* = 11.7, 8.0, 1H), 3.55–3.46 (m, 2H), 2.13 (td, *J*

1 = 7.8, 2.3, 2H), 1.77–1.47 (m, 5H), 1.45–1.06 (m, 67H), 0.90 (t, $J = 6.8$, 6H). ^{13}C NMR (100
2 MHz, CDCl_3) δ 173.1, 138.3, 138.3, 137.7, 137.5, 128.6–127.1 (20C), 122.4 (dd, $J = 255$,
3 248), 99.0, 79.0, 75.7, 74.6, 74.6, 73.8, 73.4, 73.0, 70.8 (dd, $J = 31$, 25), 70.0, 68.9, 66.1, 50.3
4 (t, $J = 24$ Hz), 36.4, 31.8, 29.8–29.0 (31C), 28.7, 25.9, 25.4, 22.6, 14.0 (2C). ^{19}F NMR (376
5 MHz, CDCl_3) δ -117.4 (dd, $J = 251$, 17, 1F), -120.1 (ddd, $J = 251$, 21, 7, 1F). MS (MALDI+)
6 m/z 1260.9 $[\text{M} + \text{Na}]^+$. HRMS (ESI+) for $\text{C}_{78}\text{H}_{121}\text{F}_2\text{NO}_8\text{Na}^+$ $[\text{M} + \text{Na}]^+$ calcd. 1260.8952,
7 found 1260.8998.

8

9 4.13. *1-O-(2,3,4,6-Tetra-O-benzyl- α -galactosyl)-(2S,4S)-3,3-difluorooctadecan-1,4-diol-2-yl*
10 *hexacosanamide (4S)-26b*. In the dark, SnCl_2 (199 mg, 1.05 mmol), AgClO_4 (218 mg, 1.05
11 mmol) and ground 4Å molecular sieves (1.47 g) were combined in THF (2.4 mL) and stirred
12 at r.t. for 90 min. In parallel, ceramide (4S)-**24b** (250 mg, 0.349 mmol) was dissolved in THF
13 (3.8 mL) and added to a solution of fluoro galactosyl donor **25**[162] (284 mg, 0.531 mmol)
14 dissolved in THF (4.5 mL). Then, the solution containing **25** and (4S)-**24b** was added, *via*
15 cannula, to the mixture of Lewis acids beforehand cooled to 0°C and stirring was maintained,
16 in the dark, for 20 min. The mixture was warmed to r.t., stirred for 2 h and then filtered
17 through Celite®. The pad was rinsed with EtOAc (~100 mL) and the filtrate was washed with
18 aq. NaHCO_3 (sat., 5×12 mL), dried (MgSO_4), filtered and concentrated to give an off-white
19 residue. Column chromatography (petroleum ether/EtOAc 90:10 to 70:30) gave (4S)-**26b**
20 (183 mg, 42%) as a white solid. R_f 0.56 (petroleum ether/EtOAc 70:30). Mp 74–76 °C. $[\alpha]_D$
21 +28.4 (c 1.1, CHCl_3 , 20 °C). IR (KBr) ν 3276, 2918, 2850, 1636, 1472, 1104 cm^{-1} . ^1H NMR
22 (400 MHz, CDCl_3) δ 7.42–7.26 (m, 20H), 6.74 (d, $J = 8.5$, 1H), 4.94 (d, $J = 9.4$, 1H), 4.92
23 (br. s, 1H), 4.84 (br. s, 1H), 4.84 (d, $J = 11.1$, 1H), 4.79 (d, $J = 11.9$, 1H), 4.75 (d, $J = 12.1$,
24 1H), 4.66 (d, $J = 11.3$, 1H), 4.58 (d, $J = 11.4$, 1H), 4.62–4.44 (m, 1H), 4.49 (d, $J = 11.9$, 1H),
25 4.41 (d, $J = 11.9$, 1H), 4.09 (dd, $J = 10.0$, 3.7, 1H), 4.03 (dd, $J = 12.0$, 4.2, 1H), 3.99 (d, $J =$
26 2.3, 1H), 3.95 (t, $J = 6.6$, 1H), 3.91–3.83 (m, 2H), 3.55 (dd, $J = 9.4$, 6.1, 1H), 3.51 (dd, $J =$
27 9.1, 6.9, 1H), 3.62–3.44 (m, 1H), 2.02–1.96 (m, 1H), 1.92–1.88 (m, 1H), 1.68–1.55 (m, 3H),
28 1.49 (dt, $J = 14.5$, 7.4, 2H), 1.40–1.09 (m, 67H), 0.89 (t, $J = 7.1$, 6H). ^{13}C NMR (100 MHz,
29 CDCl_3) δ 175.4, 138.4 (2C), 138.1, 137.8, 128.5–127.1 (20C), 121.8 (dd, $J = 256$, 251), 99.4,
30 78.9, 76.8, 74.8, 74.6, 74.1, 73.4, 72.7, 70.1, 69.4 (dd, $J = 32$, 25), 68.8, 65.2, 49.7 (dd, $J =$
31 33, 24), 36.0, 31.9, 29.9–29.0 (31C), 27.6 (d, $J = 4$), 25.9, 25.4, 22.6, 14.1 (2C). ^{19}F NMR
32 (376 MHz, CDCl_3) δ -119.6 (dd, $J = 253$, 25, 1F), -124.0 (dd, $J = 253$, 25, 1F). MS (ESI+)

1 m/z 1260.9 [M + Na]⁺. HRMS (ESI⁺) for C₇₈H₁₂₁F₂NO₈Na⁺ [M + Na]⁺ calcd. 1260.8952,
2 found 1260.8923.

3
4 4.14. *1-O-(α-Galactosyl)-(2S,4R)-3,3-difluorooctadecan-1,4-diol-2-yl hexacosanamide (4R)-*
5 **10a**. Galactosyl ceramide (4R)-**26a** (162 mg, 0.131 mmol) was dissolved in a mixture of
6 EtOH/CHCl₃ (8:2, 10 mL) and Pd(OH)₂/C (110 mg, 0.157 mmol) was added to the solution.
7 The latter was flushed with H₂ and stirred under H₂ atmosphere for 17 h. The mixture was
8 then filtered through Celite®, and the pad was rinsed with warm EtOH and warm CHCl₃.
9 Concentration of the filtrate gave a white solid which was purified by column
10 chromatography (DCM/MeOH 90:10) to give galactosyl ceramide (4R)-**10a** (95 mg, 83%) as
11 a white solid. R_f 0.22 (DCM/MeOH 90:10). Mp 155–156 °C. [α]_D +47.6 (c 0.46, CHCl₃,
12 20 °C). IR (KBr) ν 3410, 3274, 2919, 2851, 1646, 1471 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ
13 6.62 (br. s, 1H), 5.07 (br. s, 2H), 4.91 (br. s, 1H), 4.73 (br. s, 1H), 4.64 (br. s, 1H), 4.14 (br. s,
14 1H), 4.05 (br. s, 1H), 3.91 (br. s, 1H), 3.62–3.88 (m, 7H), 2.07–2.32 (m, 2H), 1.59 (br. s, 4H),
15 1.17–1.40 (m, 68H), 0.89 (t, *J* = 7.0, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 174.8, 122.2 (dd, *J*
16 = 253, 246), 99.2, 76.9, 70.5, 70.3, 70.2, 68.6, 65.3, 62.1, 50.4, 36.6, 32.0, 31.9, 30.0–29.3
17 (32C), 25.8, 22.7, 14.1 (2C). ¹⁹F NMR (376 MHz, CDCl₃) δ -118.9 (d, *J* = 258, 1F), -119.8
18 (d, *J* = 258, 1F). MS (CI⁺) m/z 879.1 [M + H]⁺. MS (CI⁻) m/z 877.1 [M – H]⁻. HRMS (ESI⁺)
19 for C₅₀H₉₈F₂NO₈ [M + H]⁺ calcd. 878.7255, found 878.7247. Elemental Analysis Calcd. C,
20 65.36; H, 11.13; N, 1.52 (**10a**+2.25H₂O); Found C, 64.95; H, 10.80; N, 1.37.

21
22 4.15. *1-O-(α-Galactosyl)-(2S,4S)-3,3-difluorooctadecan-1,4-diol-2-yl hexacosanamide (4S)-*
23 **10b**. Galactosyl ceramide (4S)-**26b** (49 mg, 0.040 mmol) was dissolved in a mixture of
24 EtOH/CHCl₃ (8:2, 3 mL) and Pd(OH)₂/C (33 mg, 0.048 mmol) was added to the solution.
25 The latter was flushed with H₂ and stirred under H₂ atmosphere for 18 h. The mixture was
26 then filtered through Celite, and the pad was rinsed with warm EtOH and warm CHCl₃.
27 Concentration of the filtrate gave a white solid which was purified by column
28 chromatography (CHCl₃/MeOH 95:5 then 90:10) to give galactosyl ceramide (4S)-**10b** (27
29 mg, 77%) as a white solid. R_f 0.28 (CHCl₃/MeOH 90:10). Mp 175–179 °C. [α]_D +32.3 (c 0.4,
30 THF, 20 °C). IR (KBr) ν 3417, 2915, 2850, 1653, 1468, 1076 cm⁻¹. ¹H NMR (300 MHz,
31 CDCl₃/CD₃OD 2:1) δ 4.70 (d, *J* = 3.6, 1H), 4.38 (m, 1H), 3.81 (m, 1H), 3.74 (d, *J* = 3.0, 1H),
32 3.66–3.45 (m, 6H), 3.36 (dd, *J* = 23.2, 8.8, 1H), 2.09 (t, *J* = 7.5, 2H), 1.52–1.23 (m, 4H),
33 1.17–1.00 (m, 68H), 0.67 (t, *J* = 6.4, 6H). ¹³C NMR (75 MHz, CDCl₃/CD₃OD 2:1) δ 176.6,

1 99.6, 76.9, 70.5, 69.8, 69.3, 68.6, 64.1, 61.3, 35.5, 31.5, 29.6–28.4 (31C), 27.2, 25.4, 25.3,
2 22.2, 13.5 (2C) [loss of 2C related to fluorine due to bad relaxation]. ¹⁹F NMR (376 MHz,
3 CDCl₃/CD₃OD 2:1) δ -122.0 (dd, J = 254, 24, 1F), -125.7 (dd, J = 254, 23, 1F). MS (CI+)
4 m/z 879.2 [M + H]⁺. MS (CI-) m/z 877.0 [M – H]⁻. HRMS (ESI+) for C₅₀H₉₈F₂NO₈ [M + H]⁺
5 calcd. 878.7255, found 878.7242. Elemental Analysis calcd. C, 66.67; H, 11.13; N, 1.55
6 (**10b**+1.25H₂O); Found C, 66.78; H, 11.19; N, 1.40.

7

8 4.16. (3*R*)-4-Benzoyloxy-1-bromo-3-*tert*-butyldimethylsilyloxy-1,1,2,2-tetrafluorobutane **29**.
9 To alcohol **28**[152] (8.35 g, 25.2 mmol) dissolved in DMF (120 mL) were added TBDMSCl
10 (4.56 g, 30.2 mmol), imidazole (5.15 g, 75.6 mmol) and DMAP (309 mg, 2.52 mmol). The
11 mixture was heated at 50 °C for 4 days and then quenched with brine (250 mL). The aqueous
12 layer was extracted with Et₂O (3 × 250 mL) and combined organic layers were dried
13 (MgSO₄), filtered and concentrated to give an orange oil. Column chromatography
14 (petroleum ether/Et₂O 99:1 to 70:30) gave compound **29** (6.71 g, 60%) as a colourless oil and
15 starting alcohol **28** (2.78 g, 33%) as a yellow oil. R_f 0.74 (petroleum ether/Et₂O 60:40). [α]_D
16 +4.9 (c 1.0, CHCl₃, 20 °C). IR (neat) ν 2931, 2860, 1254, 1160, 838 cm⁻¹. ¹H NMR (300
17 MHz, CDCl₃) δ 7.44–7.29 (m, 5H), 4.57 (d, J = 11.8, 1H), 4.52 (d, J = 11.8, 1H), 4.41 (dddd,
18 J = 12.1, 9.6, 7.0, 2.4, 1H), 3.79 (dd, J = 10.1, 2.6, 1H), 3.57 (dd, J = 9.5, 7.6, 1H), 0.89 (s,
19 9H), 0.11 (s, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 137.5, 128.4 (2C), 127.8, 127.7 (2C), 119.2–
20 110.1 (m, 2C), 73.6, 71.8 (t, J = 26), 70.4, 25.6 (3C), 18.1, -4.5, -5.3. ¹⁹F NMR (376 MHz,
21 CDCl₃) δ -61.2 (dd, J = 178, 7, 1F), -62.0 (dd, J = 178, 5, 1F), -113.4 (ddd, J = 272, 9, 5, 1F),
22 -118.6 (ddd, J = 272, 13, 8, 1F). MS (CI+) m/z 464.0 [M(⁸¹Br) + NH₄]⁺. HRMS (ESI+) for
23 C₁₇H₂₅O₂BrF₄SiNa⁺ [M + Na]⁺ calcd. 467.0636, found 467.0630.

24

25 4.17. (2*R*)-1-Benzoyloxy-2-*tert*-butyldimethylsilyloxy-3,3,4,4-tetrafluorooctadecan-5-ol **30**.
26 Bromide **29** (2.40 g, 5.39 mmol) and tetradecanal (2.82 g, 13.3 mmol) were independently
27 dissolved in DCM and filtered under nitrogen through Na₂SO₄. The two filtrates were dried
28 under high vacuum for several hours. The resultant dried tetradecanal was dissolved in THF
29 (9 mL) and added to the dried bromide **29**. In a separate flask, to THF (2 mL) at -74 °C was
30 added MeLi solution (1.35 M in Et₂O, 9.59 mL, 12.9 mmol) and then dropwise the THF
31 mixture of bromide **29** and aldehyde. The reaction was then stirred at -74 to -69 °C for 45 min
32 and to -55 to -50 °C for another 1.5 h. The mixture was quenched with aq. NH₄Cl (sat., 21
33 mL) then allowed to warm at r.t. over 20 min. H₂O (42 mL) was added and the aqueous layer

1 was extracted with EtOAc (3 × 100 mL). The combined organic phases were dried (MgSO₄),
2 filtered and concentrated to give a yellow oil. Column chromatography (petroleum ether/Et₂O
3 100:0 to 95:5) gave alcohols **30** (2.54 g, 81%) as a colourless oil and alkene **31** (186 mg,
4 10%).

5 Alcohols **30a/30b** were run as a mixture in the next step, but for analytical characterization,
6 the two diastereoisomers were separated by flash chromatography (petroleum ether/Et₂O
7 90:10).

8 4.17.1. *Data for diastereoisomer 30a*: R_f 0.09 (petroleum ether/Et₂O 95:5). [α]_D +10.1 (c 1.0,
9 CHCl₃, 20 °C). IR (KBr) ν 3439, 2927, 2855, 1497, 1257, 1107 cm⁻¹. ¹H NMR (400 MHz,
10 CDCl₃) δ 7.40–7.28 (m, 5H), 4.56 (d, *J* = 11.8, 1H), 4.52 (d, *J* = 11.7, 1H), 4.35 (m, 1H), 3.98
11 (m, 1H), 3.81 (d, *J* = 10.0, 1H), 3.62 (br. dd, *J* = 9.7, 8.2, 1H), 2.78 (d, *J* = 8.2, 1H), 1.74 (m,
12 1H), 1.66–1.52 (m, 2H), 1.30 (s, 21H), 0.97–0.88 (m, 12H), 0.17 (s, 6H). ¹³C NMR (100
13 MHz, CDCl₃) δ 137.5, 128.4 (2C), 127.8 (2C), 119.9–112.8 (m, 2C), 73.5, 73.2 (dd, *J* = 29,
14 23), 71.0 (t, *J* = 26), 70.3 (t, *J* = 6), 31.9, 29.9–29.1 (9C), 25.9 (3C), 25.7, 25.4, 22.7, 18.2,
15 14.1 (3C), –4.8, –4.9. ¹⁹F NMR (376 MHz, CDCl₃) δ -116.6 (dd, *J* = 277, 11, 1F), -117.7 (d, *J*
16 = 278, 1F), -119.4 (dt, *J* = 278, 9, 1F), -126.5 (dddd, *J* = 277, 12, 8, 4, 1F). MS (CI+) *m/z*
17 596.4 [M + NH₄]⁺. HRMS (ESI+) for C₃₁H₅₄O₃F₄SiNa⁺ [M + Na]⁺ calcd. 601.3671, found
18 601.3662.

19 4.17.2. *Data for diastereoisomer 30b*: R_f 0.19 (petroleum ether/Et₂O 95:5). [α]_D –3.8 (c 1.0,
20 CHCl₃, 20 °C). IR (KBr) ν 3430, 2932, 2860, 1258, 1109 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ
21 7.38–7.28 (m, 5H, H_{Ar}), 4.56 (d, *J* = 11.7, 1H), 4.49 (d, *J* = 11.7, 1H), 4.36–4.24 (m, 2H), 3.
22 99–3.86 (m, 1H), 3.79 (app. d, *J* = 10.4, 1H), 3.69 (br. dd, *J* = 10.4, 8.3, 1H), 1.70 (m, 1H),
23 1.65–1.55 (m, 2H), 1.50–1.14 (m, 21H), 0.99–0.81 (m, 12H), 0.18 (s, 3H), 0.16 (s, 3H). ¹³C
24 NMR (100 MHz, CDCl₃) δ 137.5, 128.4 (2C), 127.7 (2C), 120.1–113.0 (m, 2C), 74.1 (dd, *J* =
25 33, 23), 73.6, 70.0 (t, *J* = 7), 68.0 (dd, *J* = 28, 22), 31.9, 29.8–29.3 (9C), 27.8 (3C), 25.5, 25.2,
26 22.7, 18.1, 14.1, –4.7, –5.3. ¹⁹F NMR (376 MHz, CDCl₃) δ -117.2 (dq, *J* = 279, 11, 1F), -121.6
27 (dq, *J* = 279, 10, 1F), -122.1 (br. dt, *J* = 273, 11, 1F), -130.6 (ddt, *J* = 273, 22, 11, 1F). MS
28 (CI) *m/z* 596.4 [M + NH₄]⁺. HRMS (ESI+) for C₃₁H₅₅O₃F₄Si⁺ [M + H]⁺ calcd. 579.3851,
29 found 579.3842.

30 4.17.3. *Data for (3R)-4-Benzyloxy-3-tert-butyltrimethylsilyloxy-1,1,2-trifluorobutene 31*: ¹H
31 NMR (300 MHz, CDCl₃) δ 7.51–7.35 (m, 5H), 4.75–4.56 (m, 3H), 3.80–3.61 (m, 2H), 0.90
32 (s, 9H), 0.09 (s, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 138.0, 128.6 (2C), 127.9, 127.7 (2C),
33 73.6, 70.2, 65.9 (dt, *J* = 2, 21), 25.7 (3C), 18.2, –5.0, –4.9 [loss of 2C related to fluorine due to

1 bad relaxation]. ^{19}F NMR (282 MHz, CDCl_3) δ -102.3 (dd, $J = 79, 32, 1\text{F}$), -120.2 (dd, $J =$
2 115, 79, 1F), -189.3 (dd, $J = 32, 115, 1\text{F}$). MS (ESI+) m/z 369.1 $[\text{M} + \text{Na}]^+$. HRMS (ESI+)
3 for $\text{C}_{17}\text{H}_{25}\text{F}_3\text{O}_2\text{SiNa}^+ [\text{M} + \text{Na}]^+$ calcd. 369.1474, found 369.1472.

4

5 4.18. *(2R)-1-Benzoyloxy-2-tert-butyltrimethylsilyloxy-3,3,4,4-tetrafluorooctadecane* **32**.
6 Alcohols **30a** and **30b** (1.68 g, 2.91 mmol) were dissolved in dichloroethane (DCE) (12 mL)
7 and treated with thiocarbonyldiimidazole (TCDI) (1.56 g, 8.73 mmol). The mixture was
8 stirred at r.t. for 18 h then concentrated under reduced pressure to give an orange residue.
9 Flash chromatography (petroleum ether/ Et_2O 90:10) gave thiocarbamate intermediates, *O*-
10 *((2R)-1-Benzoyloxy-2-tert-butyltrimethylsilyloxy-3,3,4,4-tetrafluorooctadecan-5-yl)-1H*-
11 *imidazole-1-carbothioate*, (1.89 g, 95%) as a colourless oil.

12 For analytical characterization, the two diastereoisomers of thiocarbamate intermediates
13 were separated by flash chromatography (petroleum ether/ Et_2O 90:10).

14 4.18.1. *Data for diastereoisomer a*: $[\alpha]_{\text{D}} +12.2$ (c 1.0, CHCl_3 , 20 °C). IR (KBr) ν 2927, 2855,
15 1464, 1395, 1286, 1116 cm^{-1} . ^1H NMR (300 MHz, CDCl_3) δ 8.36 (br. t, $J = 0.9, 1\text{H}$), 7.64 (t,
16 $J = 1.5, 1\text{H}$), 7.40–7.28 (m, 5H), 7.06 (dd, $J = 1.7, 0.8, 1\text{H}$), 6.20 (dtd, $J = 13.5, 8.3, 4.7, 1\text{H}$),
17 4.55 (d, $J = 12.0, 1\text{H}$), 4.50 (d, $J = 11.9, 1\text{H}$), 4.34 (dddd, $J = 12.4, 9.7, 6.9, 3.1, 1\text{H}$), 3.77
18 (dd, $J = 10.1, 1.9, 1\text{H}$), 3.56 (dd, $J = 9.7, 7.4, 1\text{H}$), 2.11–1.91 (m, 2H), 1.56–1.18 (m, 22H),
19 0.98–0.82 (m, 12H), 0.19–0.04 (m, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 183.0, 137.5, 137.0,
20 130.9, 128.3 (2C), 127.7 (3C), 118.1, 78.2 (dd, $J = 30, 24$), 73.5, 71.9 (t, $J = 26$), 70.2, 31.9,
21 29.8–29.0 (8C), 27.8, 25.6, 24.6, 22.7, 18.1, 14.1 (3C), -4.5, -5.3 [loss of 2C related to
22 fluorine due to bad relaxation]. ^{19}F NMR (376 MHz, CDCl_3) δ -117.7 (dt, $J = 280, 13, 1\text{F}$), -
23 118.1 (ddd, $J = 282, 15, 9, 1\text{F}$), -119.8 (dtd, $J = 282, 14, 6, 1\text{F}$), -121.1 (dtd, $J = 280, 15, 15,$
24 6, 1F). MS (CI+) m/z 689.0 $[\text{M} + \text{H}]^+$. HRMS (ESI+) for $\text{C}_{35}\text{H}_{56}\text{F}_4\text{N}_2\text{O}_3\text{SSiNa}^+ [\text{M} + \text{Na}]^+$
25 calcd. 711.3609, found 711.3604.

26 4.18.2. *Data for diastereoisomer b*: $[\alpha]_{\text{D}} -6.8$ (c 0.9, CHCl_3 , 20 °C). ^1H NMR (300 MHz,
27 CDCl_3) δ 8.36 (t, $J = 0.9, 1\text{H}$), 7.65 (dd, $J = 1.7, 1.3, 1\text{H}$), 7.39–7.28 (m, 5H), 7.07 (dd, $J =$
28 1.7, 0.8, 1H), 6.34 (tt, $J = 11.2, 5.8, 1\text{H}$), 4.56 (d, $J = 11.9, 1\text{H}$), 4.50 (d, $J = 11.8, 1\text{H}$), 4.33
29 (m, 1H), 3.76 (br. d, $J = 9.5, 1\text{H}$), 3.59 (br. dd, $J = 9.3, 7.9, 1\text{H}$), 2.13–1.88 (m, 2H), 1.50–
30 1.15 (m, 22H), 0.98–0.83 (m, 12H), 0.13 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ
31 183.4, 137.5, 137.1, 131.0, 128.3 (2C), 127.7 (3C), 120.0–111.2 (m, 2C), 118.1, 78.5 (dd, $J =$
32 27, 21), 73.5, 72.5 (t, $J = 25$), 70.3, 31.9, 29.8–29.0 (8C), 27.9, 25.6, 24.6, 22.6, 18.1, 14.1
33 (3C), -4.8, -5.1. ^{19}F NMR (376 MHz, CDCl_3) δ -118.2 (ddd, $J = 280, 11, 7, 1\text{F}$), -118.8 (ddd,

1 $J = 271, 10, 7, 1\text{F}$), -119.6 (m, 1F), -120.3 (m, 1F). MS (CI) m/z 689.2 $[\text{M} + \text{H}]^+$. HRMS
2 (ESI+) for $\text{C}_{35}\text{H}_{56}\text{F}_4\text{N}_2\text{O}_3\text{SSiNa}^+ [\text{M} + \text{Na}]^+$ calcd. 711.3609, found 711.3605.

3
4 To a mixture of thiocarbamate intermediates (3.82 g, 5.55 mmol) dissolved in toluene (60
5 mL, degassed by bubbling of argon) was added AIBN (911 mg, 5.55 mmol). The reaction
6 was stirred at $110\text{ }^\circ\text{C}$ for 5 min then cooled at r.t. prior adding a solution of Bu_3SnH (5.97
7 mL, 22.2 mmol) in toluene (52 mL, degassed). The resultant mixture was stirred at $110\text{ }^\circ\text{C}$ for
8 40 min then concentrated to give a yellow oil. Column chromatography (pentane) gave alkane
9 **32** (2.92 g, 94%) as a colourless oil.

10 4.18.3 Data for **32**: R_f 0.74 (petroleum ether/ Et_2O 95:5). $[\alpha]_D +4.5$ (c 1.0, CHCl_3 , $20.0\text{ }^\circ\text{C}$). IR
11 (neat) ν 2297, 2856, 1465, 1128 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 7.38–7.28 (m, 5H), 4.55
12 (d, $J = 11.8$, 1H), 4.52 (d, $J = 11.8$, 1H), 4.28 (m, 1H), 3.78 (d, $J = 10.3$, 1H), 3.54 (dd, $J =$
13 9.6, 8.6, 1H), 2.12–1.91 (m, 2H), 1.56–1.48 (m, 2H), 1.40–1.20 (m, 22H), 0.95–0.86 (m,
14 12H), 0.14–0.07 (m, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 137.9, 128.3 (2C), 127.7 (2C), 127.6,
15 123.5–112.5 (m, 2C), 73.5, 72.4 (t, $J = 26$ Hz), 70.8, 32.0, 31.5 (t, $J = 23$), 29.1–29.9 (9C),
16 25.7, 22.7, 20.3, 18.2, 14.1 (3C), $-4.6, -5.2$. ^{19}F NMR (376 MHz, CDCl_3) δ -113.5 (dddd, $J =$
17 264, 26, 12, 3, 1F), -114.5 (ddd, $J = 264, 26, 13, 1\text{F}$), -119.9 (ddd, $J = 276, 12, 5, 1\text{F}$), -121.5
18 (ddt, $J = 276, 10, 5, 1\text{F}$). MS (CI+) m/z 580.5 $[\text{M} + \text{NH}_4]^+$. HRMS (ESI+) for
19 $\text{C}_{31}\text{H}_{54}\text{O}_2\text{F}_4\text{SiNa}^+ [\text{M} + \text{Na}]^+$ calcd. 585.3721, found 585.3714.

20
21 4.19. (2*R*)-1-Benzoyloxy-2-trifluoromethanesulfonyloxy-3,3,4,4-tetrafluorooctadecane **33**.
22 Alkane **32** (2.85 g, 5.05 mmol) was dissolved in THF (56 mL) and treated with TBAF.3H₂O
23 (3.98 g, 12.6 mmol). The mixture was stirred at r.t. for 40 min then concentrated to give a
24 green oil. Column chromatography (petroleum ether/ Et_2O 95:5 to 80:20) gave the alcohol
25 intermediate (2.23 g, 98%),

26 4.19.1. Data for intermediate alcohol (2*R*)-1-Benzoyloxy-3,3,4,4-tetrafluorooctadecan-2-ol, as
27 a colourless oil. R_f 0.29 (petroleum ether/ Et_2O 95:5). Mp $40\text{--}43\text{ }^\circ\text{C}$. $[\alpha]_D +2.0$ (c 0.9, CHCl_3 ,
28 $20\text{ }^\circ\text{C}$). IR (KBr) ν 3432, 2917, 2850, 1100 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 7.42–7.30
29 (m, 5H), 4.63 (d, $J = 12.3$, 1H), 4.60 (d, $J = 12.3$, 1H), 4.28 (m, 1H), 3.80 (dt, $J = 10.1, 2.5$,
30 1H), 3.73 (dd, $J = 9.8, 6.8$, 1H), 2.81 (d, $J = 5.5$, 1H), 2.17–1.92 (m, 2H), 1.58–1.50 (m, 2H),
31 1.38–1.23 (m, 22 H), 0.89 (t, $J = 6.5$, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 137.3, 128.5 (2C),
32 128.0, 127.8 (2C), 122.2–113.5 (m, 2C), 73.6, 68.7 (dd, $J = 27, 23$), 68.0, 31.9, 31.1 (t, $J =$
33 23), 29.0–29.9 (9C), 22.7, 20.4, 14.1. ^{19}F NMR (376 MHz, CDCl_3) δ -114.3 (dddd, $J = 264$,

1 23, 16, 4, 1F), -115.1 (dddd, $J = 264$, 22, 15, 5, 1F), -122.9 (d, $J = 274$, 1F), -126.1 (ddd, $J =$
2 275, 17, 5, 1F). MS (CI+) m/z 466.3 $[M + NH_4]^+$. HRMS (ESI+) for $C_{25}H_{40}F_4O_2Na^+$ $[M +$
3 $Na]^+$ calcd. 471.2857, found 471.2857.

4 Intermediate alcohol (2.11 g, 4.70 mmol) was dissolved in DCM (16 mL) and pyridine (761
5 μ L, 9.41 mmol) was added prior cooling the solution at -40 °C. Tf_2O (1M in DCM, 5.65 mL,
6 5.65 mmol) was then added. The mixture was stirred at -40 °C for 1 h, then warmed to -10 °C
7 and stirred for another 1.5 h before being quenched with aq. NH_4Cl (sat., 60 mL). The
8 aqueous layer was extracted with Et_2O (3×120 mL). The combined organic layers were
9 dried (Na_2SO_4), filtered and concentrated to give a yellow oil. Column chromatography
10 (petroleum ether/ Et_2O 100:0 to 97:3) gave triflate **33** (2.47 g, 91%) as a colourless oil.

11 4.19.2. Data for **33**: $[\alpha]_D +5.4$ (c 0.9, $CHCl_3$, 20.0 °C). IR (KBr) ν 2926, 2856, 1424, 1213,
12 1142, 937 cm^{-1} . 1H NMR (300 MHz, $CDCl_3$) δ 7.45–7.29 (m, 5H), 5.39 (dddd, $J = 15.1$, 8.7,
13 6.6, 2.2, 1H), 4.64 (s, 2H), 3.96 (dd, $J = 11.8$, 1.3 Hz, 1H), 3.84 (dd, $J = 11.5$, 8.8, 1H), 2.04
14 (br. ddd, $J = 27.0$, 17.9, 7.7, 2H), 1.60–1.56 (m, 2H), 1.43–1.21 (m, 22H), 0.92 (t, $J = 6.9$,
15 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.6, 128.5 (2C), 128.1, 127.8 (2C), 124.8–113.0 (m,
16 2C), 118.4 (q, $J = 315$), 81.2, 73.7, 66.0–65.8 (m), 31.9, 30.2 (t, $J = 23$), 29.0–29.7 (9C), 22.7,
17 20.1 (t, $J = 3$), 14.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ -74.0 (d, $J = 5$, 3F), -113.2 (qd, $J = 18$,
18 10, 2F), -116.3 (d, $J = 282$, 1F), -121.9 (ddd, $J = 282$, 13, 10, 1F). MS (CI+) m/z 598.4
19 $[M + NH_4]^+$. HRMS (ESI+) for $C_{26}H_{39}F_7O_4SNa^+$ $[M + Na]^+$ calcd. 603.2355, found 603.2355.

20

21 4.20. (2*S*)-2-Azido-1-benzyloxy-3,3,4,4-tetrafluorooctadecane **34**. Triflate **33** (2.47 g, 4.25
22 mmol) was dissolved in DMF (30 mL) and the solution was cooled to 0 °C. NaN_3 (1.38 g,
23 21.3 mmol) was then added and the mixture was stirred at 0 °C for 6 h, then heated to 50 °C
24 and stirred for another 14 h before being quenched with brine (35 mL). The aqueous layer
25 was extracted with Et_2O (3×55 mL) and the combined organic layers dried ($MgSO_4$), filtered
26 and concentrated to give an oil. Flash chromatography (pentane/ Et_2O 100:0 to 80:20)
27 afforded azide **34** (1.54 g, 77%) as a yellow oil, alkene (*Z*)-**35** (148 mg, 8%) as a white solid
28 and alkene (*E*)-**35** (195 mg, 10%) as a white solid.

29 4.20.1. Data for **34**: R_f 0.28 (petroleum ether/ Et_2O 99:1). $[\alpha]_D +3.1$ (c 0.96, $CHCl_3$, 20 °C).
30 IR (KBr) ν 2925, 2854, 2111, 1455, 1114 cm^{-1} . 1H NMR (300 MHz, $CDCl_3$) δ 7.47–7.30 (m,
31 5H), 4.67 (d, $J = 11.8$, 1H), 4.62 (d, $J = 11.8$, 1H), 4.18–4.01 (m, 1H), 3.96 (dd, $J = 10.3$, 2.7,
32 1H), 3.74 (dd, $J = 11.0$, 8.1, 1H), 2.16–1.90 (m, 2H), 1.59 (app. dq, $J = 7.6$, 7.0, 2H), 1.45–
33 1.20 (m, 22H), 0.93 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.2, 128.5 (2C),

1 127.9, 127.6 (2C), 124.2–112.2 (m, 2C), 73.6, 67.2, 60.7 (dd, $J = 25, 23$), 31.9, 30.7 (t, $J =$
2 23), 29.7–28.8 (9C), 22.7, 20.3 (t, $J = 4$), 14.1. ^{19}F NMR (376 MHz, CDCl_3) δ -113.4 (dtd, $J =$
3 266, 19, 10, 1F), -114.2 (dtd, $J = 266, 19, 19, 8, 1\text{F}$), -118.2 (dt, $J = 275, 10, 1\text{F}$), -119.2 (ddd,
4 $J = 275, 14, 10, 1\text{F}$). MS (CI+) m/z 491.3 $[\text{M} + \text{NH}_4]^+$. HRMS (ESI+) for $\text{C}_{25}\text{H}_{39}\text{F}_4\text{N}_3\text{ONa}^+$
5 $[\text{M} + \text{Na}]^+$ calcd. 496.2922, found 496.2928.

6 4.20.2. Data for (*Z*)-1-Benzylxy-3,3,4,4-tetrafluorooctadec-1-ene (*Z*)-**35**: R_f 0.28 (petroleum
7 ether/ Et_2O 99:1). Mp 35–37 °C. IR (KBr) ν 2920, 2850, 1675, 1457, 1378 cm^{-1} . ^1H NMR
8 (400 MHz, CDCl_3) δ 7.45–7.32 (m, 5H), 6.44 (dt, $J = 7.2, 1.8, 1\text{H}$), 4.98 (s, 2H), 4.60 (td, $J =$
9 14.9, 7.2, 1H), 2.03 (ddd, $J = 26.5, 18.1, 7.8, 2\text{H}$), 1.61 (dq, $J = 8.0, 7.2, 2\text{H}$), 1.44–1.26 (m,
10 22H), 0.94 (t, $J = 6.3, 3\text{H}$). ^{13}C NMR (75 MHz, CDCl_3) δ 152.7 (t, $J = 5$), 136.3, 128.6 (2C),
11 128.3, 127.2 (2C), 124.6–112.2 (m, 2C), 95.0 (t, $J = 25$), 75.4, 31.9, 30.2 (t, $J = 23$), 29.8–
12 29.0 (9C), 22.7, 20.6, 14.1. ^{19}F NMR (376 MHz, CDCl_3) δ -107.8 (d, $J = 15, 2\text{F}$), -116.3 (t, J
13 = 18, 2F). MS (CI) m/z 448.3 $[\text{M} + \text{NH}_4]^+$. HRMS (ESI+) for $\text{C}_{25}\text{H}_{38}\text{F}_4\text{ONa}^+$ $[\text{M} + \text{Na}]^+$
14 calcd. 453.2751, found 453.2762.

15 4.20.3. Data for (*E*)-1-Benzylxy-3,3,4,4-tetrafluorooctadec-1-ene (*E*)-**35**: R_f 0.50
16 (petroleum ether/ Et_2O 99:1). Mp 47–48 °C. IR (KBr) ν 2919, 2851, 1659, 1472, 1189 cm^{-1} .
17 ^1H NMR (400 MHz, CDCl_3) δ 7.46–7.32 (m, 5H), 7.05 (dt, $J = 12.8, 2.0, 1\text{H}$), 5.06 (q, $J =$
18 12.4, 1H), 4.86 (s, 2H), 2.00 (ddd, $J = 26.1, 18.8, 7.8, 2\text{H}$), 1.62–1.58 (m, 2H), 1.44–1.23 (m,
19 22H), 0.93 (t, $J = 7.0, 3\text{H}$). ^{13}C NMR (75 MHz, CDCl_3) δ 153.8 (t, $J = 11$), 135.5, 128.7 (2C),
20 128.4, 127.6 (2C), 124.6–112.8 (m, 2C), 94.3 (t, $J = 24$), 71.9, 31.9, 30.3 (t, $J = 24$), 29.8–
21 29.1 (9C), 22.7, 20.6, 14.1. ^{19}F NMR (376 MHz, CDCl_3) δ -109.3 (d, $J = 12, 2\text{F}$), -115.7 (m, J
22 = 18, 2F). MS (CI+) m/z 448.3 $[\text{M} + \text{NH}_4]^+$. HRMS (ESI+) for $\text{C}_{25}\text{H}_{38}\text{F}_4\text{ONa}^+$ $[\text{M} + \text{Na}]^+$
23 calcd. 453.2751, found 453.2762.

24

25 4.21. (*2S*)-1-Benzylxy-3,3,4,4-tetrafluorooctadecan-2-amine **37**. Azide **34** (1.396 g, 2.95
26 mmol) was dissolved in mixture of THF/ H_2O (5:1, 54 mL) and PPh_3 (1.161 g, 4.43 mmol)
27 was added. The mixture was stirred at 60 °C for 13 h and concentrated to give a white solid.
28 Flash chromatography (petroleum ether/ EtOAc 100:0 to 94:6) gave amine **37** (1.23 g, 93%)
29 as a white solid. R_f 0.38 (petroleum ether/ EtOAc 80:20). Mp 34–37 °C. $[\alpha]_D -9.3$ (c 0.98,
30 CHCl_3 , 20 °C). IR (KBr) ν 3432, 2918, 2851 cm^{-1} . ^1H NMR (300 MHz, CDCl_3) δ 7.42–7.28
31 (m, 5H), 4.58 (s, 2H), 3.79 (app. td, $J = 6.9, 6.2, 1\text{H}$), 3.68–3.52 (m, 2H), 2.03 (app. dddd, $J =$
32 26.7, 17.6, 7.6, 1.3, 2H), 1.56 (dt, $J = 14.9, 7.4, 2\text{H}$), 1.40–1.20 (m, 22H), 0.89 (t, $J = 6.4, 3\text{H}$)
33 NH_2 not observed. ^{13}C NMR (75 MHz, CDCl_3) δ 137.8, 128.4 (2C), 127.8, 127.7 (2C),

1 125.4–112.0 (m, 2C), 73.5, 69.0, 52.3 (t, $J = 23$), 31.9, 30.7 (t, $J = 23$), 29.8–29.0 (9C), 22.7,
2 20.3, 14.1. ^{19}F NMR (376 MHz, CDCl_3) δ -113.4 (t, $J = 16$, 2F), -120.8 (d, $J = 271$, 1F), -
3 121.9 (dd, $J = 271$, 13, 1F). MS (CI+) m/z 448.3 $[\text{M} + \text{H}]^+$. HRMS (ESI+) for $\text{C}_{25}\text{H}_{42}\text{F}_4\text{NO}^+$
4 $[\text{M} + \text{H}]^+$ calcd. 448.3197, found 448.3205.

5

6 4.22. (2*S*)-2-(*N*-(Triphenylphosphoranylidene))amino-1-benzyloxy-3,3,4,4-tetrafluoro-
7 octadecane **36**. Azide **34** (97 mg, 0.21 mmol) was dissolved in THF (3 mL) and PPh_3 (81 mg,
8 0.31 mmol) was added. The mixture was stirred at r.t. for 2 h and concentrated to give a white
9 solid. Flash chromatography (EtOAc) gave phosphoranyl **36** (130 mg, 90%) as a white solid.
10 ^1H NMR (300 MHz, CDCl_3) δ 7.75–7.61 (m, 6H), 7.54–7.32 (m, 10H), 7.26–7.18 (m, 2H),
11 7.05–6.92 (m, 2H), 4.25 (d, $J = 11.3$, 1H), 4.17 (d, $J = 11.2$, 1H), 3.93 (d, $J = 8.6$, 1H), 3.71 (t,
12 $J = 8.6$, 1H), 3.63 (m, 1H), 2.13–1.88 (m, 4H), 1.61–1.39 (m, 2H), 1.38–1.08 (m, 20H), 0.91 (t,
13 $J = 6.9$, 3H). ^{31}P NMR (121 MHz, CDCl_3) δ 14.3. MS (CI+) m/z 708.3 $[\text{M} + \text{H}]^+$. HRMS
14 (ESI+) for $\text{C}_{43}\text{H}_{55}\text{F}_4\text{NOP}^+$ $[\text{M} + \text{H}]^+$ calcd. 708.3949, found 708.3948.

15

16 4.23. (2*S*)-1-Benzyloxy-3,3,4,4-tetrafluorooctadecan-2-yl hexacosanamide **38**. The amine **37**
17 (943 mg, 2.11 mmol) in DCM (100 mL) was treated with cerotic acid (936 mg, 2.53 mmol),
18 PyBOP (2.42 g, 4.64 mmol) and Et_3N (0.59 mL, 4.22 mmol). The mixture was stirred at
19 reflux for 21 h and then diluted with DCM (100 mL). The organic layer was washed with
20 H_2O (100 mL) and brine (100 mL), dried (MgSO_4), filtered and concentrated to give a white
21 solid. Flash chromatography (petroleum ether/ Et_2O 98:2 to 0:100) gave amide **38** (1.06 g,
22 61%) as a white solid. R_f 0.53 (petroleum ether/ EtOAc 80:20). Mp 78–79 °C. $[\alpha]_D^{25} +11.6$ (c
23 1.0, CHCl_3 , 20 °C). IR (KBr) ν 3330, 2917, 2849, 1659, 1540, 1499 cm^{-1} . ^1H NMR (300
24 MHz, CDCl_3) δ 7.42–7.27 (m, 5H), 5.94 (d, $J = 9.9$, 1H), 4.94 (dddd, $J = 18.2$, 14.2, 9.9, 4.1,
25 1H), 4.54 (s, 2H), 3.82 (dd, $J = 10.4$, 4.0, 1H), 3.66 (br. d, $J = 10.1$, 1H), 2.21 (t, $J = 7.6$, 2H),
26 2.11–1.87 (m, 2H), 1.65–1.61 (m, 2H), 1.56–1.52 (m, 2H), 1.42–1.15 (m, 66H), 0.89 (t, $J =$
27 6.4, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 172.7, 137.5, 128.4 (2C), 127.8, 127.7 (2C), 73.3,
28 67.1, 47.9 (dd, $J = 26$, 22), 36.6, 31.9, 30.5 (t, $J = 23$), 29.9–28.9 (31C), 25.5, 22.7, 20.4, 14.1
29 (2C) [loss of 2C related to fluorine due to bad relaxation]. ^{19}F NMR (376 MHz, CDCl_3) δ -
30 113.6 (ddt, $J = 265$, 27, 10, 1F), -115.3 (ddtd, $J = 265$, 27, 11, 4, 1F), -117.9 (dt, $J = 274$, 9,
31 1F), -119.7 (m, 1F). MS (CI+) m/z 827.0 $[\text{M} + \text{H}]^+$. HRMS (ESI+) for $\text{C}_{51}\text{H}_{91}\text{F}_4\text{NO}_2\text{Na}^+$ $[\text{M} +$
32 $\text{Na}]^+$ calcd. 848.6884, found 848.6879.

33

1 4.24. (2*S*)-3,3,4,4-Tetrafluorooctadecan-1-ol-2-yl hexacosanamide **39**. Amide **38** (633 mg,
2 0.77 mmol) was dissolved in THF (11 mL) and Pd(OH)₂/C (20% wt, 161 mg, 0.23 mmol)
3 was added to the solution. The reaction mixture was flushed with H₂ then stirred under H₂
4 atmosphere for 3 h before being filtered through Celite®. The pad was rinsed with warm THF
5 and the filtrate was concentrated to give a white solid. Flash chromatography (DCM/MeOH
6 99:1 to 80:20) gave ceramide **39** (528 mg, 94%) as a white solid. R_f 0.27 (petroleum
7 ether/EtOAc 70:30). Mp 89–90 °C. [α]_D +3.8 (c 0.5, THF, 20 °C). IR (neat) ν 3427, 2921,
8 2850, 1661 cm⁻¹. ¹H NMR (400 MHz, THF-*d*₈) δ 7.25 (d, *J* = 9.8, 1H), 4.70 (m, 1H), 3.91 (t,
9 *J* = 6.3, 1H), 3.76 (m, 1H), 3.64 (m, 1H), 2.17 (t, *J* = 7.4, 2H), 2.13–1.86 (m, 2H), 1.65–1.56
10 (m, 2H), 1.56–1.51 (m, 2H), 1.39–1.24 (m, 66H), 0.89 (t, *J* = 7.0, 6H). ¹³C NMR (100 MHz,
11 THF-*d*₈) δ 173.0, 60.2, 51.4 (dd, *J* = 26, 21), 36.5, 32.9, 31.7 (t, *J* = 23), 30.9–30.1 (31C),
12 26.5, 23.6, 21.5, 14.5 (2C) [loss of 2C related to fluorine due to bad relaxation]. ¹⁹F NMR
13 (376 MHz, THF-*d*₈) δ -114.6 (ddd, *J* = 262, 29, 10, 1F), -116.7 (dddd, *J* = 262, 29, 10, 4, 1F),
14 -119.1 (dd, *J* = 271, 8, 1F), -123.9 (dd, *J* = 271, 20, 1F). MS (CI+) *m/z* 736.8 [M + H]⁺.
15 HRMS (ESI+) for C₄₄H₈₆F₄NO₂⁺ [M + H]⁺ calcd. 736.6589, found 736.6601.

16

17 4.25. 1-*O*-(2,3,4,6-Tetra-*O*-benzyl- α -galactosyl)-(2*S*)-3,3,4,4-tetrafluorooctadecan-1-ol-2-yl
18 hexacosanamide **40a**. In the Dark, SnCl₂ (89 mg, 0.47 mmol), AgClO₄ (98 mg, 0.47 mmol)
19 and ground 4Å molecular sieves (685 mg) were combined in THF (1.1 mL) and stirred at r.t.
20 for 90 min. In parallel, ceramide **39** (116 mg, 0.16 mmol) was dissolved in THF (2.7 mL) and
21 added to a solution of fluoro-galactosyl donor **25**[162] (128 mg, 0.24 mmol) dissolved in
22 THF (3 mL). Then, the solution containing **25** and **39** was added, *via* cannula, to the mixture
23 of Lewis acids beforehand cooled to 0°C and stirring was maintained, in the dark, for 20 min.
24 The mixture was warmed to r.t., stirred for 2 h and then filtered through Celite®, which was
25 rinsed with EtOAc (~40 mL). The filtrate was washed with aq. NaHCO₃ (sat., 5 × 5 mL),
26 dried (MgSO₄), filtered and concentrated to give a white solid. Flash chromatography
27 (petroleum ether/EtOAc 97:3 to 70:30) gave **40a** (112 mg, 57%) as a white solid. R_f 0.53
28 (petroleum ether/EtOAc 70:30). *Data for 40a*: Mp 89–90 °C. [α]_D +35.6 (c 1.1, CHCl₃,
29 20 °C). IR (KBr) ν 3427, 2921, 2850, 1661 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.48–7.16
30 (m, 20H), 6.44 (d, *J* = 9.7, 1H), 4.93 (d, *J* = 11.5, 1H), 4.81 (d, *J* = 11.9, 1H), 4.90–4.80 (m,
31 2H), 4.79 (d, *J* = 12.1, 1H), 4.73 (d, *J* = 12.1, 1H), 4.64 (d, *J* = 11.9, 1H), 4.56 (d, *J* = 11.5,
32 1H), 4.49 and 4.40 (AB syst. d, *J* = 12.0, 2H), 4.10 (dd, *J* = 10.9, 1.7, 1H), 4.05 (dd, *J* = 8.0,
33 1.9, 1H), 4.00 (t, *J* = 6.3, 1H), 3.92–3.86 (m, 2H), 3.71 (d, *J* = 11.8, 1H), 3.56 (dd, *J* = 9.5,

1 6.8, 1H), 3.40 (dd, $J = 9.5, 5.8$, 1H), 2.19–1.80 (m, 4H), 1.64–1.44 (m, 4H), 1.41–1.06 (m,
2 66H), 0.88 (t, $J = 6.2$, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 172.9, 138.6, 138.4 (2C), 137.7,
3 128.6–127.2 (20C), 100.0, 78.7, 76.7, 74.8, 74.6, 73.5, 73.3, 73.1, 70.1, 69.3, 67.9, 48.1 (dd, J
4 = 27, 21), 36.2, 31.9, 30.5 (t, $J = 23$), 29.9–28.9 (31C), 25.3, 22.7, 20.4, 14.1 (2C) [loss of 2C
5 related to fluorine due to bad relaxation]. ^{19}F NMR (376 MHz, CDCl_3) δ -113.5 (ddt, $J = 263$,
6 27, 9, 1F), -115.3 (m, 1F), -117.1 (br. d, $J = 274$, 1F), -119.7 (dd, $J = 274, 17$, 1F). HRMS
7 (MALDI+) for $\text{C}_{78}\text{H}_{119}\text{F}_4\text{NO}_7\text{Na}^+$ [$\text{M} + \text{Na}$] $^+$ calcd 1280.8815, found 1280.8786.

8

9 4.26. *1-O- α -Galactosyl-(2S)-3,3,4,4-tetrafluorooctadecan-1-ol-2-yl hexacosanamide 11.*
10 Protected Galactosyl ceramide **40a** (100 mg, 79 μmol) was dissolved in EtOH (4.6 mL) and
11 CHCl_3 (1.2 mL) prior adding Pd(OH) $_2$ /C (20%, 67 mg, 95 μmol) to the solution. The latter
12 was flushed with H_2 then stirred under H_2 atmosphere for 15 h before being filtered through a
13 pad of Celite[®]. The pad was rinsed with warm EtOH and warm CHCl_3 , and the solution was
14 concentrated to give a white solid. Flash chromatography (DCM/MeOH 90:10) gave
15 tetrafluorinated galactosyl ceramide **11** (60 mg, 85%) as a white solid. R_f 0.16 (DCM/MeOH
16 90:10). Mp 146–148 °C. $[\alpha]_D^{20} +47.1$ (c 0.6, CHCl_3 , 20 °C). IR (KBr) ν 3433, 2920, 2851,
17 1651, 1469 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 6.20 (d, $J = 9.9$, 1H), 4.98 (m, 1H), 4.95 (d, J
18 = 3.5, 1H), 4.09 (br. s, 1H), 4.02 (br. d, $J = 8.6$, 1H), 3.97–3.70 (m, 6H), 3.33 (br. s, 2H), 2.80
19 (br. s, 1H), 2.65 (br. d, $J = 6.5$, 1H), 2.25 (td, $J = 7.5, 3.6$, 2H), 2.09–1.86 (m, 2H), 1.66–1.58
20 (m, 2H), 1.58–1.51 (m, 2H), 1.42–1.16 (m, 66H), 0.89 (t, $J = 7.0$, 6H). ^{13}C NMR (100 MHz,
21 CDCl_3) δ 173.8, 100.4, 70.8, 70.3, 70.2, 69.3, 67.1, 62.9, 49.3 (dd, $J = 27, 21$), 36.6, 31.9,
22 30.5 (t, $J = 23$), 30.2–28.3 (31C), 25.5, 22.7, 20.3, 14.1 (2C) [loss of 2C related to fluorine
23 due to bad relaxation]. ^{19}F NMR (376 MHz, CDCl_3) δ -113.0 (ddt, $J = 267, 26, 9$, 1F), -114.5
24 (m, 1F), -117.2 (d, $J = 275$, 1F), -119.7 (d, $J = 275$, 1F). MS (MALDI+) m/z 920.7
25 [$\text{M} + \text{Na}$] $^+$. HRMS (MALDI+) for $\text{C}_{50}\text{H}_{95}\text{F}_4\text{NNaO}_7^+$ [$\text{M} + \text{Na}$] $^+$ calcd. 920.6937, found
26 920.6931. Elemental Analysis calcd. C, 65.86; H, 10.67; N, 1.54 (**11**+0.75 H_2O); found C,
27 65.92; H, 10.65; N, 1.45.

28

29 **5. Experimental for biological evaluation:**

30 ***In vitro* assays for human iNKT cell stimulation:** Human iNKT cells were prepared from
31 bulk human peripheral lymphocytes by two successive rounds of selection, using first an anti-
32 V α 24 and, second, an anti-V β 11 monoclonal antibody. At each round, cells were sorted using
33 anti-mouse IgG-coated magnetic beads (Dyna, Invitrogen Corp. Carlsbad, CA) and

1 cultivated in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2
2 mM glutamine, 50 U/mL penicillin, 50 mg/mL streptomycin (Gibco BRL, Carlsbad, CA) and
3 300 U/mL IL-2 (Chiron Corp. Emerville, CA). The iNKT cell line that was used (MAD11)
4 contained >90% V α 24/J α 18 positive cells. Human CD1d-transfected HeLa cells were
5 obtained from M. Kronenberg (La Jolla, CA). These antigen-presenting cells were cultivated
6 in DMEM or RPMI 1640, respectively, containing 1 g/L glucose, supplemented as described
7 above. Antigen-presenting cells HeLa-CD1d were plated at 30.000 per well, on 96-well flat
8 bottom plates in complete RPMI and incubated overnight at 37°C with varying concentrations
9 of glycolipids solubilized in DMSO. Synthetic KRN7000 was used as reference in all
10 experiments. The cells were then washed twice with RPMI. Fifteen thousand iNKT cells per
11 well in 200 μ L complete RPMI without IL-2 were then added for 6h at 37 °C for the IFN- γ
12 secretion analyses or 24h for the IL-13 secretion analysis. Cell-free supernatants were
13 collected and tested for the presence of either IFN- γ or IL-13 by ELISA (eBiosciences). No
14 glycolipid was added in control wells. Dependency on CD1d was tested using untransfected
15 HeLa cells devoid of CD1d as negative control presenting cells

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19 acknowledged for provision of computer time.

20

21 **Appendix A. Supporting data.**

22 Supplementary data to this article can be found on line at

23

24 **ABBREVIATIONS:**

25

26 AIBN, azobisisobutyronitrile ; AA, amino acids (Asp, aspartic acid; Arg, arginine; Thr,
27 threonine; Ser, Serine ; Phe, phenylalanine ; Gly, glycine); APCs, antigen presenting cells;
28 Boc, *tert*-butyloxycarbonyl; CD, cluster of differentiation (hCD1d, human CD1d; mCD1d,
29 mouse CD1d); DCs, dendritic *cells*; DFT, density functional theory; DMAP, *N,N*-
30 dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-

1 linked immunosorbent assay ; FCS, Fetal Calf Serum; α -GalCer and KRN7000, α -
2 galactosylceramide; *gem*, germinal; HOMO, highest occupied molecular orbital; IFN- γ ,
3 interferon γ ; IL, interleukin; *i*NKT, invariant natural killer T; PD-1 Programmed cell death 1;
4 PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate ; *QM*,
5 quantum mechanics; RPMI, Roswell Park Memorial Institute medium; TBAF, tetra-n-
6 butylammonium fluoride ; TBDMSCl, tert-butyldimethylsilyl chloride; TCDI,
7 thiocarbonyldiimidazole ; TCR, T cell receptor; T_H, T helper; TNF- α , tumor necrosis factor α ;
8

9 **References**

- 10
- 11 [1] D.I. Godfrey, S. Stankovic, A.G. Baxter, Raising the NKT cell family, *Nat. Immunol.*, 11
12 (2010) 197, <http://dx.doi.org/10.1038/ni.1841>.
- 13 [2] D.I. Godfrey, H.R. MacDonald, M. Kronenberg, M.J. Smyth, L.V. Kaer, NKT Cells:
14 What's in a Name?, *Nat. Rev. Immunol.*, 4 (2004) 231-237, <http://dx.doi.org/10.1038/nri1309>.
- 15 [3] D.I. Godfrey, M. Kronenberg, Going Both Ways: Immune Regulation via CD1d-
16 Dependent NKT Cells, *J. Clin. Invest.*, 114 (2004) 1379-1388, <Go to
17 ISI>://WOS:000225113200004
- 18 [4] G. Bricard, S.A. Porcelli, Antigen Presentation by CD1 Molecules and the Generation of
19 Lipid-specific T Cell Immunity, *Cell. Mol. Life Sci.*, 64 (2007) 1824-1840,
20 <https://doi.org/10.1007/s00018-007-7007-0>.
- 21 [5] A. Bendelac, P.B. Savage, L. Teyton, The Biology of NKT Cells, *Annu. Rev. Immunol.*,
22 25 (2007) 297-336.
- 23 [6] T. Yamamura, K. Sakuishi, Z. Illés, S. Miyake, Understanding the Behavior of Invariant
24 NKT Cells in Autoimmune Diseases, *J. Neuroimmunol.*, 191 (2007) 8-15,
25 <http://www.sciencedirect.com/science/article/pii/S0165572807003104>.
- 26 [7] L. Van Kaer, Alpha-Galactosylceramide Therapy for Autoimmune Diseases: Prospects
27 and Obstacles, *Nat. Rev. Immunol.*, 5 (2005) 31-42.
- 28 [8] P.J. Brennan, M. Brigl, M.B. Brenner, Invariant Natural Killer T Cells: An Innate
29 Activation Scheme Linked to Diverse Effector Functions, *Nat. Rev. Immunol.*, 13 (2013)
30 101-117, <http://dx.doi.org/10.1038/nri3369>.

- 1 [9] K.M. Murphy, S.L. Reiner, The Lineage Decisions of Helper T Cells, *Nat. Rev.*
2 *Immunol.*, 2 (2002) 933-944, <http://dx.doi.org/10.1038/nri954>.
- 3 [10] R.M. McEwen-Smith, M. Salio, V. Cerundolo, The Regulatory Role of Invariant NKT
4 Cells in Tumor Immunity, *Cancer Immunol. Res.*, 3 (2015) 425-435,
5 <http://cancerimmunolres.aacrjournals.org/content/3/5/425.abstract>.
- 6 [11] M. Terabe, J.A. Berzofsky, Chapter 8 The Role of NKT Cells in Tumor Immunity, in:
7 *Adv. Cancer Res.*, Academic Press, 2008, pp. 277-348.
- 8 [12] E. Tupin, Y. Kinjo, M. Kronenberg, The Unique Role of Natural Killer T Cells in the
9 Response to Microorganisms, *Nat. Rev. Microbiol.*, 5 (2007) 405-417,
10 <http://dx.doi.org/10.1038/nrmicro1657>.
- 11 [13] D.S. Hansen, M.-A. Siomos, L. Buckingham, A.A. Scalzo, L. Schofield, Regulation of
12 Murine Cerebral Malaria Pathogenesis by CD1d-Restricted NKT Cells and the Natural Killer
13 Complex, *Immunity*, 18 (2003) 391-402, [https://doi.org/10.1016/S1074-7613\(03\)00052-9](https://doi.org/10.1016/S1074-7613(03)00052-9).
- 14 [14] N.Y. Crowe, J.M. Coquet, S.P. Berzins, K. Kyparissoudis, R. Keating, D.G. Pellicci, Y.
15 Hayakawa, D.I. Godfrey, M.J. Smyth, Differential Antitumor Immunity Mediated by NKT
16 Cell Subsets In Vivo, *J. Exp. Med.*, 202 (2005) 1279-1288,
17 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1459911/>.
- 18 [15] J. Cui, T. Shin, T. Kawano, H. Sato, E. Kondo, I. Taura, Y. Kaneko, H. Koseki, M.
19 Kanno, M. Taniguchi, Requirement for V α 14 NKT Cells in IL-12-Mediated Rejection of
20 Tumors, *Science*, 278 (1997) 1623-1626,
21 <http://science.sciencemag.org/content/sci/278/5343/1623.full.pdf>.
- 22 [16] K. Sakuishi, S. Oki, M. Araki, S.A. Porcelli, S. Miyake, T. Yamamura, Invariant NKT
23 Cells Biased for IL-5 Production Act as Crucial Regulators of Inflammation, *J. Immunol.*, 179
24 (2007) 3452-3462, <http://www.jimmunol.org/content/jimmunol/179/6/3452.full.pdf>.
- 25 [17] A. Chackerian, J. Alt, V. Perera, S.M. Behar, Activation of NKT Cells Protects Mice
26 from Tuberculosis, *Infect. Immun.*, 70 (2002) 6302-6309.
- 27 [18] F. Dieli, M. Taniguchi, M. Kronenberg, S. Sidobre, J. Ivanyi, L. Fattorini, E. Iona, G.
28 Orefici, G. De Leo, D. Russo, N. Caccamo, G. Sireci, C. Di Sano, A. Salerno, An Anti-
29 Inflammatory Role for V α 14 NK T Cells in Mycobacterium Bovis Bacillus Calmette-Guérin-
30 infected Mice, *J. Immunol.*, 171 (2003) 1961-1968,
31 <http://www.jimmunol.org/content/jimmunol/171/4/1961.full.pdf>.
- 32 [19] N. Duarte, M. Stenström, S. Campino, M.-L. Bergman, M. Lundholm, D. Holmberg,
33 S.L. Cardell, Prevention of Diabetes in Nonobese Diabetic Mice Mediated by CD1d-

- 1 Restricted Nonclassical NKT Cells, *J. Immunol*, 173 (2004) 3112-3118,
2 <http://www.jimmunol.org/content/173/5/3112.abstract>.
- 3 [20] S. Hong, M.T. Wilson, I. Serizawa, L. Wu, N. Singh, O.V. Naidenko, T. Miura, T. Haba,
4 D.C. Scherer, J. Wei, M. Kronenberg, Y. Koezuka, L. Van Kaer, The Natural Killer T-Cell
5 Ligand α -Galactosylceramide Prevents Autoimmune Diabetes in Non-Obese Diabetic Mice,
6 *Nat. Med.*, 7 (2001) 1052-1056, <http://dx.doi.org/10.1038/nm0901-1052>.
- 7 [21] A. Chiba, S. Kaieda, S. Oki, T. Yamamura, S. Miyake, The Involvement of V α 14
8 Natural Killer T Cells in the Pathogenesis of Arthritis in Murine Models, *Arthritis Rheum.*, 52
9 (2005) 1941-1948, <https://onlinelibrary.wiley.com/doi/abs/10.1002/art.21056>.
- 10 [22] Z. Illés, T. Kondo, J. Newcombe, N. Oka, T. Tabira, T. Yamamura, Differential
11 Expression of NK T Cell V α 24J α Q Invariant TCR Chain in the Lesions of Multiple Sclerosis
12 and Chronic Inflammatory Demyelinating Polyneuropathy, *J. Immunol*, 164 (2000) 4375-
13 4381, <http://www.jimmunol.org/content/jimmunol/164/8/4375.full.pdf>.
- 14 [23] J.E. East, A.J. Kennedy, T.J. Webb, Raising the Roof: The Preferential Pharmacological
15 Stimulation of Th1 and Th2 Responses Mediated by NKT Cells, *Med. Res. Rev.*, 34 (2014)
16 45-76, <https://onlinelibrary.wiley.com/doi/abs/10.1002/med.21276>.
- 17 [24] M.-C. Rissoan, V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R.d.W. Malefyt, Y.-J.
18 Liu, Reciprocal Control of T Helper Cell and Dendritic Cell Differentiation, *Science*, 283
19 (1999) 1183-1186, <http://science.sciencemag.org/content/283/5405/1183.abstract>.
- 20 [25] S. Romagnani, The Th1/Th2 Paradigm, *Immunol. Today*, 18 (1997) 263-266, -
21 [http://www.sciencedirect.com/science/article/B6VHW-46T3NF8-](http://www.sciencedirect.com/science/article/B6VHW-46T3NF8-4/2/c44d5dce48040d523c20f7bd9dc72011)
22 [4/2/c44d5dce48040d523c20f7bd9dc72011](http://www.sciencedirect.com/science/article/B6VHW-46T3NF8-4/2/c44d5dce48040d523c20f7bd9dc72011)
- 23 [26] M. Morita, K. Motoki, K. Akimoto, T. Natori, T. Sakai, E. Sawa, K. Yamaji, Y.
24 Koezuka, E. Kobayashi, H. Fukushima, Structure-Activity Relationship of α -
25 Galactosylceramides against B16-Bearing Mice, *J. Med. Chem.*, 38 (1995) 2176-2187,
26 <http://dx.doi.org/10.1021/jm00012a018>.
- 27 [27] T. Natori, M. Morita, K. Akimoto, Y. Koezuka, Agelasphins, Novel Antitumor and
28 Immunostimulatory Cerebrosides from the Marine Sponge *Agelas Mauritianus*, *Tetrahedron*,
29 50 (1994) 2771-2784, <http://www.sciencedirect.com/science/article/pii/S004040200186991X>.
- 30 [28] Y. Hayakawa, S. Rovero, G. Forni, M.J. Smyth, α -Galactosylceramide (KRN7000)
31 Suppression of Chemical- and Oncogene-Dependent Carcinogenesis, *Proc. Natl. Acad. Sci.*
32 *USA*, 100 (2003) 9464-9469, <http://www.pnas.org/content/pnas/100/16/9464.full.pdf>.
- 33 [29] T. Kawano, J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, H. Sato, E. Kondo, M. Harada, H.
34 Koseki, T. Nakayama, Y. Tanaka, M. Taniguchi, Natural Killer-Like Nonspecific Tumor Cell

1 Lysis Mediated by Specific Ligand-Activated $V\alpha 14$ NKT Cells, *Proc. Natl. Acad. Sci. USA*,
2 95 (1998) 5690-5693, <http://www.pnas.org/content/95/10/5690.abstract>.

3 [30] R. Nakagawa, K. Motoki, H. Nakamura, H. Ueno, R. Iijima, A. Yamauchi, S. Tsuyuki,
4 T. Inamoto, Y. Koezuka, Antitumor Activity of α -Galactosylceramide, KRN7000, in Mice
5 with EL-4 Hepatic Metastasis and its Cytokine Production, *Onc. Res.*, 10 (1998) 561-568.

6 [31] T. Nishimura, H. Kitamura, K. Iwakabe, T. Yahata, A. Ohta, M. Sato, K. Takeda, K.
7 Okumura, L. Van Kaer, T. Kawano, M. Taniguchi, M. Nakui, M. Sekimoto, T. Koda, The
8 Interface Between Innate and Acquired Immunity: Glycolipid Antigen Presentation by CD1d-
9 Expressing Dendritic Cells to NKT Cells Induces the Differentiation of Antigen-specific
10 Cytotoxic T Lymphocytes, *Int. Immunol.*, 12 (2000) 987-994,
11 <http://dx.doi.org/10.1093/intimm/12.7.987>.

12 [32] F.C. Robertson, J.A. Berzofsky, M. Terabe, NKT Cell Networks in the Regulation of
13 Tumor Immunity, *Front. Immunol.*, 5 (2014) 543,
14 <https://www.frontiersin.org/article/10.3389/fimmu.2014.00543>.

15 [33] M.J. Smyth, K.Y.T. Thia, S.E.A. Street, E. Cretney, J.A. Trapani, M. Taniguchi, T.
16 Kawano, S.B. Pelikan, N.Y. Crowe, D.I. Godfrey, Differential Tumor Surveillance by Natural
17 Killer (NK) and NKT Cells, *J. Exp. Med.*, 191 (2000) 661-668,
18 <http://jem.rupress.org/content/191/4/661.abstract>.

19 [34] F.L. Schneiders, R.J. Scheper, B.M.E. von Blomberg, A.M. Woltman, H.L.A. Janssen,
20 A.J.M. van den Eertwegh, H.M.W. Verheul, T.D. de Gruijl, H.J. van der Vliet, Clinical
21 Experience with α -Galactosylceramide (KRN7000) in Patients with Advanced Cancer and
22 Chronic Hepatitis B/C Infection, *Clin.Immunol.*, 140 (2011) 130-141,
23 <http://www.sciencedirect.com/science/article/pii/S1521661610007709>.

24 [35] W.-S. Chang, J.-Y. Kim, Y.-J. Kim, Y.-S. Kim, J.-M. Lee, M. Azuma, H. Yagita, C.-Y.
25 Kang, Cutting Edge: Programmed Death-1/Programmed Death Ligand 1 Interaction
26 Regulates the Induction and Maintenance of Invariant NKT Cell Anergy, *J. Immunol.*, 181
27 (2008) 6707-6710, <http://www.jimmunol.org/content/jimmunol/181/10/6707.full.pdf>.

28 [36] V.V. Parekh, M.T. Wilson, D. Olivares-Villagomez, A.K. Singh, L. Wu, C.R. Wang, S.
29 Joyce, L. Van Kaer, Glycolipid Antigen Induces Long-Term Natural Killer T Cell Anergy in
30 Mice, *J. Clin. Invest.*, 115 (2005) 2572-2583,
31 <http://www.ncbi.nlm.nih.gov/pubmed/16138194>.

32 [37] B.A. Sullivan, M. Kronenberg, Activation or Anergy: NKT Cells Are Stunned by α -
33 galactosylceramide, *J. Clin. Invest.*, 115 (2005) 2328-2329,
34 <http://www.ncbi.nlm.nih.gov/pubmed/16138189>.

- 1 [38] S. Kojo, C. Elly, Y. Harada, W.Y. Langdon, M. Kronenberg, Y.-C. Liu, Mechanisms of
2 NKT Cell Anergy Induction Involve Cbl- β -Promoted Monoubiquitination of CARMA1, Proc.
3 Natl. Acad. Sci. USA, 106 (2009) 17847-17851,
4 <http://www.pnas.org/content/pnas/106/42/17847.full.pdf>.
- 5 [39] Y. Huang, A. Chen, X. Li, Z. Chen, W. Zhang, Y. Song, D. Gurner, D. Gardiner, S.
6 Basu, D.D. Ho, M. Tsuji, Enhancement of HIV DNA Vaccine Immunogenicity by the NKT
7 Cell Ligand, α -Galactosylceramide, Vaccine, 26 (2008) 1807-1816,
8 <http://www.sciencedirect.com/science/article/pii/S0264410X0800128X>.
- 9 [40] S. Kim, S. Lalani, V.V. Parekh, L. Wu, L. Van Kaer, Glycolipid Ligands of Invariant
10 Natural Killer T Cells as Vaccine Adjuvants, Expert Rev. Vaccines, 7 (2008) 1519-1532,
11 <https://doi.org/10.1586/14760584.7.10.1519>.
- 12 [41] Y.-S. Lee, K.-A. Lee, J.-Y. Lee, M.-H. Kang, Y.C. Song, D.J. Baek, S. Kim, C.-Y. Kang,
13 An α -GalCer Analogue with Branched Acyl Chain Enhances Protective Immune Responses
14 in a Nasal Influenza Vaccine, Vaccine, 29 (2011) 417-425,
15 <http://www.sciencedirect.com/science/article/pii/S0264410X10015999>.
- 16 [42] N.N. Padte, X. Li, M. Tsuji, S. Vasan, Clinical Development of a Novel CD1d-Binding
17 NKT Cell Ligand as a Vaccine Adjuvant, Clin.Immunol., 140 (2011) 142-151,
18 <http://www.sciencedirect.com/science/article/pii/S1521661610007692>.
- 19 [43] J.W. Molling, M. Moreno, H.J.J. van der Vliet, A.J.M. van den Eertwegh, R.J. Scheper,
20 B.M.E. von Blomberg, H.J. Bontkes, Invariant Natural Killer T Cells and Immunotherapy of
21 Cancer, Clin.Immunol., 129 (2008) 182-194,
22 <http://www.sciencedirect.com/science/article/pii/S1521661608007596>.
- 23 [44] E.-A. Bae, H. Seo, B.-S. Kim, J. Choi, I. Jeon, K.-S. Shin, C.-H. Koh, B. Song, I.-K.
24 Kim, B.S. Min, Y.D. Han, S.J. Shin, C.-Y. Kang, Activation of NKT Cells in an Anti-PD-1-
25 Resistant Tumor Model Enhances Antitumor Immunity by Reinvigorating Exhausted CD8 T
26 Cells, Cancer Res., 78 (2018) 5315-5326,
27 <http://cancerres.aacrjournals.org/content/canres/78/18/5315.full.pdf>.
- 28 [45] T. Uchida, S. Horiguchi, Y. Tanaka, H. Yamamoto, N. Kunii, S. Motohashi, M.
29 Taniguchi, T. Nakayama, Y. Okamoto, Phase I Study of α -Galactosylceramide-pulsed
30 Antigen Presenting Cells Administration to the Nasal Submucosa in Unresectable or
31 Recurrent Head and Neck cancer, Cancer Immunol, Immun., 57 (2008) 337-345,
32 <https://doi.org/10.1007/s00262-007-0373-5>.

- 1 [46] S. Motohashi, T. Nakayama, Clinical Applications of Natural Killer T Cell-based
2 Immunotherapy for Cancer, *Cancer Sci.*, 99 (2008) 638-645,
3 <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1349-7006.2008.00730.x>.
- 4 [47] M. Moreno, J.W. Molling, S. von Mensdorff-Pouilly, R.H.M. Verheijen, B.M.E. von
5 Blomberg, A.J.M. van den Eertwegh, R.J. Scheper, H.J. Bontkes, In vitro Expanded Human
6 Invariant Natural Killer T-Cells Promote Functional Activity of Natural Killer Cells,
7 *Clin.Immunol.*, 129 (2008) 145-154,
8 <http://www.sciencedirect.com/science/article/pii/S1521661608007171>.
- 9 [48] L. Zhang, A. Donda, Alpha-Galactosylceramide/CD1d-Antibody Fusion Proteins
10 Redirect Invariant Natural Killer T Cell Immunity to Solid Tumors and Promote Prolonged
11 Therapeutic Responses, *Front. Immunol.*, 8 (2017) 1417,
12 <https://www.frontiersin.org/article/10.3389/fimmu.2017.01417>.
- 13 [49] P.B. Savage, L. Teyton, A. Bendelac, Glycolipids for Natural Killer T Cells, *Chem. Soc.*
14 *Rev.*, 35 (2006) 771-779, <http://dx.doi.org/10.1039/B510638A>.
- 15 [50] M.M. Venkataswamy, S.A. Porcelli, Lipid and Glycolipid Antigens of CD1d-Restricted
16 Natural Killer T Cells, *Semin. Immunol.*, 22 (2010) 68-78,
17 <http://www.sciencedirect.com/science/article/pii/S1044532309000979>.
- 18 [51] X. Laurent, B. Bertin, N. Renault, A. Farce, S. Speca, O. Milhomme, R. Millet, P.
19 Desreumaux, E. Hénon, P. Chavatte, Switching Invariant Natural Killer T (iNKT) Cell
20 Response from Anticancerous to Anti-Inflammatory Effect: Molecular Bases, *J. Med. Chem.*,
21 57 (2014) 5489-5508, <http://dx.doi.org/10.1021/jm4010863>.
- 22 [52] T. Tashiro, Structure-Activity Relationship Studies of Novel Glycosphingolipids That
23 Stimulate Natural Killer T-cells, *Biosci. Biotechnol. Biochem.*, 76 (2012) 1055-1067,
24 <https://www.tandfonline.com/doi/abs/10.1271/bbb.120072>.
- 25 [53] M. Koch, V.S. Stronge, D. Shepherd, S.D. Gadola, B. Mathew, G. Ritter, A.R. Fersht,
26 G.S. Besra, R.R. Schmidt, E.Y. Jones, V. Cerundol, The Crystal Structure of Human CD1d
27 with and without α -Galactosylceramide, *Nature Immunol.*, 6 (2005) 819-826.
- 28 [54] D.M. Zajonc, P.B. Savage, A. Bendelac, I.A. Wilson, L. Teyton, Crystal Structures of
29 Mouse CD1d-iGb3 Complex and its Cognate $V\alpha 14$ T Cell Receptor Suggest a Model for
30 Dual Recognition of Foreign and Self Glycolipids, *J. Mol. Biol.*, 377 (2008) 1104-1116,
31 <http://www.sciencedirect.com/science/article/pii/S0022283608000624>.
- 32 [55] Y. Li, E. Girardi, J. Wang, E.D. Yu, G.F. Painter, M. Kronenberg, D.M. Zajonc, The
33 $V\alpha 14$ Invariant Natural killer T Cell TCR Forces Microbial Glycolipids and CD1d into a

- 1 Conserved Binding Mode, *J. Exp. Med.*, 207 (2010) 2383-2393,
2 <http://jem.rupress.org/content/207/11/2383.abstract>.
- 3 [56] N.A. Borg, K.S. Wun, L. Kjer-Nielsen, M.C.J. Wilce, D.G. Pellicci, R. Koh, G.S. Besra,
4 M. Bharadwaj, D.I. Godfrey, J. McCluskey, J. Rossjohn, CD1d-lipid-antigen Recognition by
5 the Semi-invariant NKT T-cell Receptor, *Nature*, 448 (2007) 44-49,
6 <http://dx.doi.org/10.1038/nature05907>.
- 7 [57] J. Nadas, C. Li, P.G. Wang, Computational Structure Activity Relationship Studies on
8 the CD1d/Glycolipid/TCR Complex Using AMBER and AUTODOCK, *J. Chem. Inf. Model.*,
9 49 (2009) 410-423, <https://doi.org/10.1021/ci8002705>.
- 10 [58] E. Kobayashi, K. Motoki, Y. Yamaguchi, T. Uchida, H. Fukushima, Y. Koezuka,
11 Enhancing Effects of α -, β -Monoglycosylceramides on Natural Killer Cell Activity, *Bioorg.*
12 *Med. Chem.*, 4 (1996) 615-619,
13 <http://www.sciencedirect.com/science/article/pii/0968089696000491>.
- 14 [59] K. Motoki, E. Kobayashi, M. Morita, T. Uchida, K. Akimoto, H. Fukushima, Y.
15 Koezuka, Radioprotective Effects of α -Galactosylceramides, *Bioorg. Med. Chem. Lett.*, 5
16 (1995) 2413-2416, <http://www.sciencedirect.com/science/article/pii/0960894X9500411L>.
- 17 [60] V.V. Parekh, A.K. Singh, M.T. Wilson, D. Olivares-Villagomez, J.S. Bezbradica, H.
18 Inazawa, H. Ehara, T. Sakai, I. Serizawa, L. Wu, C.R. Wang, S. Joyce, L. Van Kaer,
19 Quantitative and Qualitative Differences in the in vivo Response of NKT Cells to Distinct α -
20 and β -anomeric Glycolipids, *J. Immunol.*, 173 (2004) 3693-3706, <Go to
21 ISI>://WOS:000223878000016
- 22 [61] J. Schmieg, G. Yang, R.W. Franck, M. Tsuji, A Multifactorial Mechanism in the
23 Superior Antimalarial Activity of α -C-GalCer, *JBiomedBiotech*, 2010 (2010) 283612,
24 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2801455/>.
- 25 [62] J. Schmieg, G. Yang, R.W. Franck, M. Tsuji, Superior Protection against Malaria and
26 Melanoma Metastases by a C-glycoside Analogue of the Natural Killer T Cell Ligand α -
27 Galactosylceramide, *J. Exp. Med.*, 198 (2003) 1631-1641,
28 <http://jem.rupress.org/content/198/11/1631.abstract>.
- 29 [63] X. Li, G. Chen, R. Garcia-Navarro, R.W. Franck, M. Tsuji, Identification of C-glycoside
30 Analogues That Display a Potent Biological Activity Against Murine and Human Invariant
31 Natural Killer T Cells, *Immunology*, 127 (2009) 216-225, <http://dx.doi.org/10.1111/j.1365->
32 [2567.2008.02943.x](http://dx.doi.org/10.1111/j.1365-2567.2008.02943.x).
- 33 [64] X. Li, T. Shiratsuchi, G. Chen, P. Dellabona, G. Casorati, R.W. Franck, M. Tsuji,
34 Invariant TCR Rather Than CD1d Shapes the Preferential Activities of C-Glycoside

1 Analogues Against Human Versus Murine Invariant NKT Cells, *J. Immunol*, 183 (2009)
2 4415-4421, <http://www.jimmunol.org/content/jimmunol/183/7/4415.full.pdf>.

3 [65] X. Lu, L. Song, L.S. Metelitsa, R. Bittman, Synthesis and Evaluation of an α -C-
4 Galactosylceramide Analogue that Induces Th1-Biased Responses in Human Natural Killer T
5 Cells, *ChemBioChem*, 7 (2006) 1750-1756, <https://doi.org/10.1002/cbic.200600197>.

6 [66] G. Yang, J. Schmieg, M. Tsuji, R.W. Franck, The C-Glycoside Analogue of the
7 Immunostimulant α -Galactosylceramide (KRN7000): Synthesis and Striking Enhancement of
8 Activity, *Angew. Chem. Int. Ed.*, 43 (2004) 3818-3822,
9 <http://dx.doi.org/10.1002/anie.200454215>.

10 [67] A.E. Hogan, V. O'Reilly, M.R. Dunne, R.T. Dere, S.G. Zeng, C. O'Brien, S. Amu, P.G.
11 Fallon, M.A. Exley, C. O'Farrelly, X. Zhu, D.G. Doherty, Activation of Human Invariant
12 Natural Killer T Cells with a Thioglycoside Analogue of α -Galactosylceramide,
13 *Clin.Immunol.*, 140 (2011) 196-207,
14 <http://www.sciencedirect.com/science/article/pii/S1521661611001045>.

15 [68] M.L. Blauvelt, M. Khalili, W. Jaung, J. Paulsen, A.C. Anderson, S. Brian Wilson, A.R.
16 Howell, α -S-GalCer: Synthesis and Evaluation for iNKT Cell Stimulation, *Bioorg. Med.*
17 *Chem. Lett.*, 18 (2008) 6374-6376,
18 <http://www.sciencedirect.com/science/article/pii/S0960894X08012936>.

19 [69] R. Rajan, T. Mathew, R. Buffa, F. Bornancin, M. Cavallari, P. Nussbaumer, G. De
20 Libero, A. Vasella, Synthesis and Evaluation of N-Acetyl-2-amino-2-deoxy- α -D-galactosyl 1-
21 Thio-7-oxaceramide, a New Analogue of α -D-Galactosyl Ceramide, *Helv. Chim. Acta*, 92
22 (2009) 918-927, <https://onlinelibrary.wiley.com/doi/abs/10.1002/hlca.200800454>.

23 [70] T. Hiroshi, Y. Takashi, Masayuki Nimura, N. Atsushi, T. Tsuyaoshi, *Glycolipid*
24 *Derivatives*, in, japan, 2003.

25 [71] Y. Harrak, C.M. Barra, C. Bedia, A. Delgado, A.R. Castaño, A. Llebaria, Aminocyclitol-
26 Substituted Phytoceramides and their Effects on iNKT Cell Stimulation, *ChemMedChem*, 4
27 (2009) 1608-1613, <http://dx.doi.org/10.1002/cmdc.200900193>.

28 [72] T. Tashiro, R. Nakagawa, T. Hirokawa, S. Inoue, H. Watarai, M. Taniguchi, K. Mori,
29 RCAI-56, a Carbocyclic Analogue of KRN7000: Its Synthesis and Potent Activity for Natural
30 Killer (NK) T Cells to Preferentially Produce Interferon- γ , *Tetrahedron Lett.*, 48 (2007) 3343-
31 3347, <http://www.sciencedirect.com/science/article/pii/S0040403907005345>.

32 [73] T. Tashiro, R. Nakagawa, T. Hirokawa, S. Inoue, H. Watarai, M. Taniguchi, K. Mori,
33 RCAI-37, 56, 59, 60, 92, 101, and 102, Cyclitol and Carbasugar Analogs of KRN7000: Their
34 Synthesis and Bioactivity for Mouse Lymphocytes to Produce Th1-biased Cytokines, *Bioorg.*

1 Med. Chem., 17 (2009) 6360-6373,
2 <http://www.sciencedirect.com/science/article/pii/S0968089609006816>.
3 [74] T. Tashiro, E. Sekine-Kondo, T. Shigeura, R. Nakagawa, S. Inoue, M. Omori-Miyake, T.
4 Chiba, N. Hongo, S.-i. Fujii, K. Shimizu, Y. Yoshiga, T. Sumida, K. Mori, H. Watarai, M.
5 Taniguchi, Induction of Th1-Biased Cytokine Production by α -Carba-GalCer, a
6 Neoglycolipid Ligand for NKT Cells, *Int. Immunol.*, 22 (2010) 319-328,
7 <http://dx.doi.org/10.1093/intimm/dxq012>.
8 [75] B.G. Reddy, J.D. Silk, M. Salio, R. Balamurugan, D. Shepherd, G. Ritter, V. Cerundolo,
9 R.R. Schmidt, Nonglycosidic Agonists of Invariant NKT Cells for Use as Vaccine Adjuvants,
10 *ChemMedChem*, 4 (2009) 171-175,
11 <https://onlinelibrary.wiley.com/doi/abs/10.1002/cmdc.200800354>.
12 [76] J.D. Silk, M. Salio, B.G. Reddy, D. Shepherd, U. Gileadi, J. Brown, S.H. Masri, P.
13 Polzella, G. Ritter, G.S. Besra, E.Y. Jones, R.R. Schmidt, V. Cerundolo, Cutting Edge:
14 Nonglycosidic CD1d Lipid Ligands Activate Human and Murine Invariant NKT Cells, *J.*
15 *Immunol.*, 180 (2008) 6452-6456,
16 <http://www.jimmunol.org/content/jimmunol/180/10/6452.full.pdf>.
17 [77] N. Veerapen, F. Reddington, G. Bricard, S.A. Porcelli, G.S. Besra, Synthesis and
18 Biological Activity of α -L-Fucosyl Ceramides, Analogues of the Potent Agonist, α -D-
19 Galactosyl Ceramide KRN7000, *Bioorg. Med. Chem. Lett.*, 20 (2010) 3223-3226,
20 <http://www.sciencedirect.com/science/article/pii/S09680894X10005494>.
21 [78] S. Sidobre, K.J.L. Hammond, L. Bénazet-Sidobre, S.D. Maltsev, S.K. Richardson, R.M.
22 Ndonge, A.R. Howell, T. Sakai, G.S. Besra, S.A. Porcelli, M. Kronenberg, The T Cell
23 Antigen Receptor Expressed by V α 14i NKT Cells Has a Unique Mode of Glycosphingolipid
24 Antigen Recognition, *Proc. Natl. Acad. Sci. U.S.A.*, 101 (2004) 12254-12259,
25 <http://www.pnas.org/content/pnas/101/33/12254.full.pdf>.
26 [79] A. Uchimura, T. Shimizu, M. Morita, H. Ueno, K. Motoki, H. Fukushima, T. Natori, Y.
27 Koezuka, Immunostimulatory Activities of Monoglycosylated α -D-Pyranosylceramides,
28 *Bioorg. Med. Chem.*, 5 (1997) 2245-2249,
29 <http://www.sciencedirect.com/science/article/pii/S0968089697001697>.
30 [80] P.J. Jervis, L.M. Graham, E.L. Foster, L.R. Cox, S.A. Porcelli, G.S. Besra, New CD1d
31 Agonists: Synthesis and Biological Activity of 6"-Triazole-Substituted α -Galactosyl
32 Ceramides, *Bioorg. Med. Chem. Lett.*, 22 (2012) 4348-4352,
33 <http://www.sciencedirect.com/science/article/pii/S09680894X12005999>.

- 1 [81] P.J. Jervis, L.R. Cox, G.S. Besra, Synthesis of a Versatile Building Block for the
2 Preparation of 6-N-Derivatized α -Galactosyl Ceramides: Rapid Access to Biologically Active
3 Glycolipids, *J. Org. Chem.*, 76 (2011) 320-323, <https://doi.org/10.1021/jo102064p>.
- 4 [82] S. Aspeslagh, Y. Li, E.D. Yu, N. Pauwels, M. Trappeniers, E. Girardi, T. Decruy, K.
5 Van Beneden, K. Venken, M. Drennan, L. Leybaert, J. Wang, R.W. Franck, S. Van
6 Calenbergh, D.M. Zajonc, D. Elewaut, Galactose-Modified iNKT Cell Agonists Stabilized by
7 an Induced Fit of CD1d Prevent Tumour Metastasis, *Embo J.*, 30 (2011) 2294-2305,
8 <http://emboj.embopress.org/content/30/11/2294.abstract>.
- 9 [83] J.M.H. Cheng, S.H. Chee, D.A. Knight, H. Acha-Orbea, I.F. Hermans, M.S.M. Timmer,
10 B.L. Stocker, An Improved Synthesis of Dansylated α -Galactosylceramide and its Use as a
11 Fluorescent Probe for the Monitoring of Glycolipid Uptake by Cells, *Carbohydr. Res.*, 346
12 (2011) 914-926, <http://www.sciencedirect.com/science/article/pii/S0008621511000887>.
- 13 [84] T. Tashiro, R. Nakagawa, S. Inoue, M. Shiozaki, H. Watarai, M. Taniguchi, K. Mori,
14 RCAI-61, the 6'-O-Methylated Analog of KRN7000: Its Synthesis and Potent Bioactivity for
15 Mouse Lymphocytes to Produce Interferon- γ in vivo, *Tetrahedron Lett.*, 49 (2008) 6827-
16 6830, <http://www.sciencedirect.com/science/article/pii/S0040403908017322>.
- 17 [85] M. Trappeniers, K.V. Beneden, T. Decruy, U. Hillaert, B. Linclau, D. Elewaut, S.V.
18 Calenbergh, 6'-Derivatized α -GalCer Analogues Capable of Inducing Strong CD1d-Mediated
19 Th1-Biased NKT Cell Responses in Mice, *J. Am. Chem. Soc.*, 130 (2008) 16468-16469,
20 <http://dx.doi.org/10.1021/ja8064182>.
- 21 [86] T. Lee, M. Cho, S.-Y. Ko, H.-J. Youn, D.J. Baek, W.-J. Cho, C.-Y. Kang, S. Kim,
22 Synthesis and Evaluation of 1,2,3-Triazole Containing Analogues of the Immunostimulant α -
23 GalCer, *J. Med. Chem.*, 50 (2007) 585-589, <https://doi.org/10.1021/jm061243q>.
- 24 [87] Y. Liu, R.D. Goff, D. Zhou, J. Mattner, B.A. Sullivan, A. Khurana, C. Cantu, E.V.
25 Ravkov, C.C. Ibegbu, J.D. Altman, L. Teyton, A. Bendelac, P.B. Savage, A Modified α -
26 Galactosyl Ceramide for Staining and Stimulating Natural Killer T Cells, *J. Immunol.*
27 *Methods*, 312 (2006) 34-39,
28 <http://www.sciencedirect.com/science/article/pii/S0022175906000664>.
- 29 [88] X.-T. Zhou, C. Forestier, R.D. Goff, C. Li, L. Teyton, A. Bendelac, P.B. Savage,
30 Synthesis and NKT Cell Stimulating Properties of Fluorophore- and Biotin-appended 6'-
31 Amino-6"-deoxy-galactosylceramides, *Org. Lett.*, 4 (2002) 1267-1270,
32 <http://dx.doi.org/10.1021/ol025565+>.
- 33 [89] J. Guillaume, T. Seki, T. Decruy, K. Venken, D. Elewaut, M. Tsuji, S. Van Calenbergh,
34 Synthesis of C6"-modified- α -C-GalCer Analogues as Mouse and Human iNKT Cell

1 Agonists, Org. Biomol. Chem., 15 (2017) 2217-2225,
2 <http://dx.doi.org/10.1039/C7OB00081B>.

3 [90] P.-H. Liang, M. Imamura, X. Li, D. Wu, M. Fujio, R.T. Guy, B.-C. Wu, M. Tsuji, C.-H.
4 Wong, Quantitative Microarray Analysis of Intact Glycolipid-CD1d Interaction and
5 Correlation with Cell-Based Cytokine Production, *J. Am. Chem. Soc.*, 130 (2008) 12348-
6 12354, <https://doi.org/10.1021/ja8012787>.

7 [91] J.S. Im, P. Arora, G. Bricard, A. Molano, M.M. Venkataswamy, I. Baine, E.S. Jerud,
8 M.F. Goldberg, A. Baena, K.O.A. Yu, R.M. Ndonge, A.R. Howell, W. Yuan, P. Cresswell,
9 Y.-T. Chang, P.A. Illarionov, G.S. Besra, S.A. Porcelli, Kinetics and Cellular Site of
10 Glycolipid Loading Control the Outcome of Natural Killer T Cell Activation, *Immunity*, 30
11 (2009) 888-898, <http://www.sciencedirect.com/science/article/pii/S1074761309002386>.

12 [92] K.O.A. Yu, J.S. Im, A. Molano, Y. Dutronc, P.A. Illarionov, C. Forestier, N. Fujiwara, I.
13 Arias, S. Miyake, T. Yamamura, Y.-T. Chang, G.S. Besra, S.A. Porcelli, Modulation of
14 CD1d-Restricted NKT Cell Responses by Using N-acyl Variants of α -Galactosylceramides,
15 *Proc. Natl. Acad. Sci. U.S.A.*, 102 (2005) 3383-3388,
16 <http://www.pnas.org/content/pnas/102/9/3383.full.pdf>.

17 [93] D.J. Baek, Y.-S. Lee, C. Lim, D. Lee, T. Lee, J.-Y. Lee, K.-A. Lee, W.-J. Cho, C.-Y.
18 Kang, S. Kim, Rational Design and Evaluation of a Branched-chain-containing Glycolipid
19 Antigen That Binds to CD1d, *Chem. Asian J.*, 5 (2010) 1560-1564,
20 <https://onlinelibrary.wiley.com/doi/abs/10.1002/asia.201000120>.

21 [94] S. Inuki, E. Kashiwabara, N. Hirata, J. Kishi, E. Nabika, Y. Fujimoto, Potent Th2
22 Cytokine Bias of Natural Killer T Cell by CD1d Glycolipid Ligands: Anchoring Effect of
23 Polar Groups in the Lipid Component, *Angew. Chem. Int. Ed.*, 57 (2018) 9655-9659,
24 <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.201802983>.

25 [95] L. Bai, M.G. Constantinides, S.Y. Thomas, R. Reboulet, F. Meng, F. Koentgen, L.
26 Teyton, P.B. Savage, A. Bendelac, Distinct APCs Explain the Cytokine Bias of α -
27 Galactosylceramide Variants In Vivo, *J. Immunol.*, 188 (2012) 3053-3061,
28 <http://www.jimmunol.org/content/188/7/3053.abstract>.

29 [96] M. Fujio, D. Wu, R. Garcia-Navarro, D.D. Ho, M. Tsuji, C.-H. Wong, Structure-Based
30 Discovery of Glycolipids for CD1d-Mediated NKT Cell Activation: Tuning the Adjuvant
31 versus Immunosuppression Activity, *J. Am. Chem. Soc.*, 128 (2006) 9022-9023,
32 <https://doi.org/10.1021/ja062740z>.

1 [97] A. Schiefner, M. Fujio, D. Wu, C.-H. Wong, I.A. Wilson, Structural Evaluation of Potent
2 NKT Cell Agonists: Implications for Design of Novel Stimulatory Ligands, *J. Mol. Biol.*, 394
3 (2009) 71-82, <http://www.sciencedirect.com/science/article/pii/S002228360901081X>.

4 [98] Y. Vo-Hoang, L. Micouin, C. Ronet, G. Gachelin, M. Bonin, Total Enantioselective
5 Synthesis and In Vivo Biological Evaluation of a Novel Fluorescent BODIPY α -
6 Galactosylceramide, *ChemBioChem*, 4 (2003) 27-33,
7 <https://onlinelibrary.wiley.com/doi/abs/10.1002/cbic.200390009>.

8 [99] P.J. Jervis, P. Polzella, J. Wojno, J.-P. Jukes, H. Ghadbane, Y.R. Garcia Diaz, G.S.
9 Besra, V. Cerundolo, L.R. Cox, Design, Synthesis, and Functional Activity of Labeled CD1d
10 Glycolipid Agonists, *Bioconjugate Chem.*, 24 (2013) 586-594,
11 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3630740/>.

12 [100] T.-N. Wu, K.-H. Lin, Y.-J. Chang, J.-R. Huang, J.-Y. Cheng, A.L. Yu, C.-H. Wong,
13 Avidity of CD1d-Ligand-Receptor Ternary Complex Contributes to T-Helper 1 (Th1)
14 Polarization and Anticancer Efficacy, *Proc. Natl. Acad. Sci. USA*, 108 (2011) 17275-17280.

15 [101] Y.-J. Chang, J.-R. Huang, Y.-C. Tsai, J.-T. Hung, D. Wu, M. Fujio, C.-H. Wong, A.L.
16 Yu, Potent Immune-Modulating and Anticancer Effects of NKT Cell Stimulatory
17 Glycolipids, *Proc. Natl. Acad. Sci. USA*, 104 (2007) 10299-10304,
18 <https://www.pnas.org/content/pnas/104/25/10299.full.pdf>.

19 [102] M.-H. Hsieh, J.-T. Hung, Y.-W. Liw, Y.-J. Lu, C.-H. Wong, A.L. Yu, P.-H. Liang,
20 Synthesis and Evaluation of Acyl-Chain- and Galactose-6''-Modified Analogues of α -GalCer
21 for NKT Cell Activation, *ChemBioChem*, 13 (2012) 1689-1697,
22 <https://onlinelibrary.wiley.com/doi/abs/10.1002/cbic.201200004>.

23 [103] J.-J. Park, J.H. Lee, K.-C. Seo, G. Bricard, M.M. Venkataswamy, S.A. Porcelli, S.-K.
24 Chung, Syntheses and Biological Activities of KRN7000 Analogues Having Aromatic
25 Residues in the Acyl and Backbone Chains with Varying Stereochemistry, *Bioorg. Med.*
26 *Chem. Lett.*, 20 (2010) 814-818,
27 <http://www.sciencedirect.com/science/article/pii/S0960894X09018265>.

28 [104] K. Miyamoto, S. Miyake, T. Yamamura, A Synthetic Glycolipid Prevents Autoimmune
29 Encephalomyelitis by Inducing TH2 Bias of Natural Killer T Cells, *Nature*, 413 (2001) 531-
30 534, <http://dx.doi.org/10.1038/35097097>.

31 [105] K. Murata, T. Toba, K. Nakanishi, B. Takahashi, T. Yamamura, S. Miyake, H.
32 Annoura, Total Synthesis of an Immunosuppressive Glycolipid, (2S,3S,4R)-1-O-(α -D-
33 galactosyl)-2-Tetracosanoylamino-1,3,4-nonanetriol, *J. Org. Chem.*, 70 (2005) 2398-2401,
34 <https://doi.org/10.1021/jo048151y>.

- 1 [106] R.M. Ndonge, D.P. Izmirian, M.F. Dunn, K.O.A. Yu, S.A. Porcelli, A. Khurana, M.
2 Kronenberg, S.K. Richardson, A.R. Howell, Synthesis and Evaluation of Sphinganine
3 Analogues of KRN7000 and OCH, *J. Org. Chem.*, 70 (2005) 10260-10270,
4 <https://doi.org/10.1021/jo051147h>.
- 5 [107] G. Velmourougane, R. Raju, G. Bricard, J.S. Im, G.S. Besra, S.A. Porcelli, A.R.
6 Howell, Synthesis and Evaluation of an Acyl-chain Unsaturated Analog of the Th2 Biasing,
7 Immunostimulatory Glycolipid, OCH, *Bioorg. Med. Chem. Lett.*, 19 (2009) 3386-3388,
8 <http://www.sciencedirect.com/science/article/pii/S0960894X09007203>.
- 9 [108] L. Brossay, O. Naidenko, N. Burdin, J. Matsuda, T. Sakai, M. Kronenberg, Cutting
10 Edge: Structural Requirements for Galactosylceramide Recognition by CD1-Restricted NK T
11 Cells, *J. Immunol.*, 161 (1998) 5124-5128,
12 <http://www.jimmunol.org/content/161/10/5124.abstract>.
- 13 [109] V. Lacône, J. Hunault, M. Pipelier, V. Blot, T. Lecourt, J. Rocher, A.-L. Turcot-Dubois,
14 S. Marionneau, J.-Y. Douillard, M. Clément, J. Le Pendu, M. Bonneville, L. Micouin, D.
15 Dubreuil, Focus on the Controversial Activation of Human iNKT Cells by 4-Deoxy Analogue
16 of KRN7000, *J. Med. Chem.*, 52 (2009) 4960-4963, <http://dx.doi.org/10.1021/jm900290r>.
- 17 [110] D.J. Baek, J.-H. Seo, C. Lim, J.H. Kim, D.H. Chung, W.-J. Cho, C.-Y. Kang, S. Kim,
18 The 3-Deoxy Analogue of α -GalCer: Disclosing the Role of the 4-Hydroxyl Group for CD1d-
19 mediated NKT Cell Activation, *ACS Med. Chem. Lett.*, 2 (2011) 544-548,
20 <http://dx.doi.org/10.1021/ml2000802>.
- 21 [111] E.M. Dangerfield, J.M.H. Cheng, D.A. Knight, R. Weinkove, P.R. Dunbar, I.F.
22 Hermans, M.S.M. Timmer, B.L. Stocker, Species-Specific Activity of Glycolipid Ligands for
23 Invariant NKT Cells, *ChemBioChem*, 13 (2012) 1349-1356,
24 <http://dx.doi.org/10.1002/cbic.201200095>.
- 25 [112] J. Hunault, M. Diswall, J.-C. Frison, V. Blot, J. Rocher, S. Marionneau-Lambot, T.
26 Oullier, J.-Y. Douillard, S. Guillarme, C. Saluzzo, G. Dujardin, D. Jacquemin, J. Graton, J.-Y.
27 Le Questel, M. Evain, J. Lebreton, D. Dubreuil, J. Le Pendu, M. Pipelier, 3-Fluoro- and 3,3-
28 Difluoro-3,4-dideoxy-KRN7000 Analogues as New Potent Immunostimulator Agents: Total
29 Synthesis and Biological Evaluation in Human Invariant Natural Killer T Cells and Mice, *J.*
30 *Med. Chem.*, 55 (2012) 1227-1241, <http://dx.doi.org/10.1021/jm201368m>.
- 31 [113] M. Trappeniers, S. Goormans, K. Van Beneden, T. Decruy, B. Linclau, A. Al-
32 Shamkhani, T. Elliott, C. Ottensmeier, J.M. Werner, D. Elewaut, S. Van Calenbergh,
33 Synthesis and in vitro Evaluation of α -GalCer Epimers, *ChemMedChem*, 3 (2008) 1061-
34 1070, <http://dx.doi.org/10.1002/cmdc.200800021>.

- 1 [114] M. Shiozaki, T. Tashiro, H. Koshino, T. Shigeura, H. Watarai, M. Taniguchi, K. Mori,
2 Synthesis and Biological Activity of Hydroxylated Analogues of KRN7000 (α -
3 Galactosylceramide), *Carbohydr. Res.*, 370 (2013) 46-66,
4 <http://www.sciencedirect.com/science/article/pii/S0008621513000244>.
- 5 [115] Z. Zhang, W. Zhao, B. Wang, C. Xia, W. Zhang, P.G. Wang, The Total Synthesis of
6 Immunostimulant α -Galactosylceramides from Naturally Configured α -Galactoside
7 Raffinose, *Org. Lett.*, 13 (2011) 4530-4533, <http://dx.doi.org/10.1021/ol201695n>.
- 8 [116] M. Trappeniers, R. Chofor, S. Aspeslagh, Y. Li, B. Linclau, D.M. Zajonc, D. Elewaut,
9 S.V. Calenbergh, Synthesis and Evaluation of Amino-Modified α -GalCer Analogues, *Org.*
10 *Lett.*, 12 (2010) 2928-2931, <http://dx.doi.org/10.1021/ol100934z>.
- 11 [117] G.-T. Fan, Y.-s. Pan, K.-C. Lu, Y.-P. Cheng, W.-C. Lin, S. Lin, C.-H. Lin, C.-H. Wong,
12 J.-M. Fang, C.-C. Lin, Synthesis of α -Galactosyl Ceramide and the Related Glycolipids for
13 Evaluation of their Activities on Mouse Splenocytes, *Tetrahedron*, 61 (2005) 1855-1862,
14 <http://www.sciencedirect.com/science/article/pii/S0040402004020617>.
- 15 [118] E.P. Gillis, K.J. Eastman, M.D. Hill, D.J. Donnelly, N.A. Meanwell, Applications of
16 Fluorine in Medicinal Chemistry, *J. Med. Chem.*, 58 (2015) 8315-8359,
17 <http://dx.doi.org/10.1021/acs.jmedchem.5b00258>.
- 18 [119] W.K. Hagmann, The Many Roles for Fluorine in Medicinal Chemistry, *J. Med. Chem.*,
19 51 (2008) 4359-4369, <http://dx.doi.org/10.1021/jm800219f>.
- 20 [120] Y. Lu, T. Shi, Y. Wang, H. Yang, X. Yan, X. Luo, H. Jiang, W. Zhu, Halogen Bonding
21 - A Novel Interaction for Rational Drug Design?, *J. Med. Chem.*, 52 (2009) 2854-2862,
22 <https://doi.org/10.1021/jm9000133>.
- 23 [121] D. O'Hagan, Fluorine in Health Care: Organofluorine Containing Blockbuster Drugs, *J.*
24 *Fluorine Chem.*, 131 (2010) 1071-1081,
25 <http://www.sciencedirect.com/science/article/pii/S0022113910000722>.
- 26 [122] S. Purser, P.R. Moore, S. Swallow, V. Gouverneur, Fluorine in Medicinal Chemistry,
27 *Chem. Soc. Rev.*, 37 (2008) 320-330, <http://dx.doi.org/10.1039/B610213C>.
- 28 [123] J. Graton, G. Compain, F. Besseau, E. Bogdan, J.M. Watts, L. Mtashobya, Z. Wang, A.
29 Weymouth-Wilson, N. Galland, J.-Y. Le Questel, B. Linclau, Influence of Alcohol β -
30 Fluorination on Hydrogen-Bond Acidity of Conformationally Flexible Substrates, *Chem. Eur.*
31 *J.*, 23 (2017) 2811-2819, <http://dx.doi.org/10.1002/chem.201604940>.
- 32 [124] L. Hunter, The C-F Bond as a Conformational Tool in Organic and Biological
33 Chemistry, *Beilstein J. Org. Chem.*, 6 (2010) 38.

1 [125] B. Linclau, F. Peron, E. Bogdan, N. Wells, Z. Wang, G. Compain, C.Q. Fontenelle, N.
2 Galland, J.-Y. Le Questel, J. Graton, Intramolecular OH...Fluorine Hydrogen Bonding in
3 Saturated, Acyclic Fluorohydrins: The γ -Fluoropropanol Motif, *Chem. Eur. J.*, 21 (2015)
4 17808-17816, <http://dx.doi.org/10.1002/chem.201503253>.

5 [126] K. Müller, C. Faeh, F. Diederich, Fluorine in Pharmaceuticals: Looking Beyond
6 Intuition, *Science*, 317 (2007) 1881-1886,
7 <http://science.sciencemag.org/content/sci/317/5846/1881.full.pdf>.

8 [127] D. O'Hagan, Organofluorine Chemistry: Synthesis and Conformation of Vicinal
9 Fluoromethylene Motifs, *J. Org. Chem.*, 77 (2012) 3689-3699,
10 <http://dx.doi.org/10.1021/jo300044q>.

11 [128] D. O'Hagan, Understanding Organofluorine Chemistry. An Introduction to the C-F
12 Bond, *Chem. Soc. Rev.*, 37 (2008) 308-319, <http://dx.doi.org/10.1039/B711844A>.

13 [129] L.E. Zimmer, C. Sparr, R. Gilmour, Fluorine Conformational Effects in
14 Organocatalysis: An Emerging Strategy for Molecular Design, *Angew. Chem. Int. Ed.*, 50
15 (2011) 11860-11871, <http://dx.doi.org/10.1002/anie.201102027>.

16 [130] B. Linclau, Z. Wang, G. Compain, V. Paumelle, C.Q. Fontenelle, N. Wells, A.
17 Weymouth-Wilson, Investigating the Influence of (deoxy)Fluorination on the Lipophilicity of
18 Non-UV-active Fluorinated Alkanols and Carbohydrates by a New log P Determination
19 Method, *Angew. Chem. Int. Ed.*, 55 (2016) 674-678,
20 <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.201509460>.

21 [131] H.-J. Böhm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Müller, U. Obst-Sander, M.
22 Stahl, Fluorine in Medicinal Chemistry, *ChemBioChem*, 5 (2004) 637-643,
23 <https://onlinelibrary.wiley.com/doi/abs/10.1002/cbic.200301023>.

24 [132] B. Jeffries, Z. Wang, J. Graton, S.D. Holland, T. Brind, R.D.R. Greenwood, J.-Y. Le
25 Questel, J.S. Scott, E. Chiarparin, B. Linclau, Reducing the Lipophilicity of Perfluoroalkyl
26 Groups by CF₂-F/CF₂-Me or CF₃/CH₃ Exchange, *J. Med. Chem.*, 61 (2018) 10602-10618,
27 <https://doi.org/10.1021/acs.jmedchem.8b01222>.

28 [133] J.C. Biffinger, H.W. Kim, S.G. DiMugno, The Polar Hydrophobicity of Fluorinated
29 Compounds, *ChemBioChem*, 5 (2004) 622-627,
30 <https://onlinelibrary.wiley.com/doi/abs/10.1002/cbic.200300910>.

31 [134] L. Bonnac, S.E. Lee, G.T. Giuffredi, L.M. Elphick, A.A. Anderson, E.S. Child, D.J.
32 Mann, V. Gouverneur, Synthesis and O-Phosphorylation of 3,3,4,4-Tetrafluoroaryl-C-
33 nucleoside Analogues, *Org. Biomol. Chem.*, 8 (2010) 1445-1454,
34 <http://dx.doi.org/10.1039/B922442D>.

- 1 [135] H.W. Kim, P. Rossi, R.K. Shoemaker, S.G. DiMagno, Structure and Transport
2 Properties of a Novel, Heavily Fluorinated Carbohydrate Analogue, *J. Am. Chem. Soc.*, 120
3 (1998) 9082-9083, <https://doi.org/10.1021/ja9803714>.
- 4 [136] Y. Sakaguchi, S. Yamada, T. Konno, T. Agou, T. Kubota, Stereochemically Defined
5 Various Multisubstituted Alkenes Bearing a Tetrafluoroethylene (–CF₂CF₂–) Fragment, *J.*
6 *Org. Chem.*, 82 (2017) 1618-1631, <http://dx.doi.org/10.1021/acs.joc.6b02793>.
- 7 [137] K.E. van Straaten, J.R.A. Kuttiyatveetil, C.M. Sevrain, S.A. Villaume, J. Jiménez-
8 Barbero, B. Linclau, S.P. Vincent, D.A.R. Sanders, Structural Basis of Ligand Binding to
9 UDP-Galactopyranose Mutase from *Mycobacterium Tuberculosis* Using Substrate and
10 Tetrafluorinated Substrate Analogues, *J. Am. Chem. Soc.*, 137 (2015) 1230-1244,
11 <https://doi.org/10.1021/ja511204p>.
- 12 [138] M. Skibinski, Y. Wang, A.M.Z. Slawin, T. Lebl, P. Kirsch, D. O'Hagan, Alicyclic Ring
13 Structure: Conformational Influence of the CF₂ Group in Cyclododecanes, *Angew. Chem.*
14 *Int. Ed.*, 50 (2011) 10581-10584,
15 <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.201105060>.
- 16 [139] Y. Wang, R. Callejo, A.M.Z. Slawin, D. O'Hagan, The Difluoromethylene (CF₂)
17 Group in Aliphatic Chains: Synthesis and Conformational Preference of Palmitic Acids and
18 Nonadecane Containing CF₂ Groups, *Beilstein J. Org. Chem.*, 10 (2014) 18-25.
- 19 [140] L. Leung, C. Tomassi, K. Van Beneden, T. Decruy, D. Elewaut, T. Elliott, A. Al-
20 Shamkhani, C. Ottensmeier, S. Van Calenbergh, J. Werner, T. Williams, B. Linclau,
21 Synthesis and In Vivo Evaluation of 4-Deoxy-4,4-difluoro-KRN7000, *Org. Lett.*, 10 (2008)
22 4433-4436, <http://dx.doi.org/10.1021/ol801663m>.
- 23 [141] L. Leung, C. Tomassi, K. Van Beneden, T. Decruy, M. Trappeniers, D. Elewaut, Y.
24 Gao, T. Elliott, A. Al-Shamkhani, C. Ottensmeier, J.M. Werner, A. Williams, S. Van
25 Calenbergh, B. Linclau, The Synthesis and in vivo Evaluation of 2',2'-Difluoro KRN7000,
26 *ChemMedChem*, 4 (2009) 329-334, <http://dx.doi.org/10.1002/cmhc.200800348>.
- 27 [142] X. Laurent, N. Renault, A. Farce, P. Chavatte, E. Hénon, Relationships between Th1 or
28 Th2 iNKT Cell Activity and Structures of CD1d-Antigen Complexes: Meta-analysis of
29 CD1d-Glycolipids Dynamics Simulations, *PLOS Comput. Biol.*, 10 (2014) e1003902,
30 <https://doi.org/10.1371/journal.pcbi.1003902>.
- 31 [143] T. Tashiro, N. Hongo, R. Nakagawa, K.-i. Seino, H. Watarai, Y. Ishii, M. Taniguchi, K.
32 Mori, *RCAI-17*, 22, 24–26, 29, 31, 34–36, 38–40, and 88, the Analogs of KRN7000 with a
33 Sulfonamide Linkage: Their Synthesis and Bioactivity for Mouse Natural Killer T Cells to

1 Produce Th2-biased Cytokines, *Bioorg. Med. Chem.*, 16 (2008) 8896-8906,
2 <http://www.sciencedirect.com/science/article/pii/S0968089608008031>.

3 [144] M. Shiozaki, T. Tashiro, H. Koshino, R. Nakagawa, S. Inoue, T. Shigeura, H. Watarai,
4 M. Taniguchi, K. Mori, Synthesis and Biological Activity of Ester and Ether Analogues of α -
5 Galactosylceramide (KRN7000), *Carbohydr. Res.*, 345 (2010) 1663-1684,
6 <http://www.sciencedirect.com/science/article/pii/S0008621510001898>.

7 [145] K.-i. Fuhshuku, N. Hongo, T. Tashiro, Y. Masuda, R. Nakagawa, K.-i. Seino, M.
8 Taniguchi, K. Mori, RCAI-8, 9, 18, 19, and 49–52, Conformationally Restricted Analogues
9 of KRN7000 With an Azetidine or a Pyrrolidine Ring: Their Synthesis and Bioactivity for
10 Mouse Natural killer T Cells to Produce Cytokines, *Bioorg. Med. Chem.*, 16 (2008) 950-964,
11 <http://www.sciencedirect.com/science/article/pii/S096808960700853X>.

12 [146] K. Akimoto, T. Natori, M. Morita, Synthesis and Stereochemistry of Agelasphin-9b,
13 *Tetrahedron Lett.*, 34 (1993) 5593-5596,
14 <http://www.sciencedirect.com/science/article/pii/S0040403900738901>.

15 [147] C.Q. Fontenelle, M. Conroy, M. Light, T. Poisson, X. Pannecoucke, B. Linclau,
16 Stereoselectivity of the Honda–Reformatsky Reaction in Reactions with Ethyl
17 Bromodifluoroacetate with α -Oxygenated Sulfinylimines, *J. Org. Chem.*, 79 (2014) 4186-
18 4195, <https://doi.org/10.1021/jo500396p>.

19 [148] A.J. Boydell, V. Vinader, B. Linclau, Enantioselective Synthesis of
20 Tetrafluoroethylene-containing Monosaccharides, *Angew. Chem. Int. Ed.*, 43 (2004) 5677-
21 5679, <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.200460746>.

22 [149] T. Nihei, N. Iwai, T. Matsuda, T. Kitazume, Stereocontrolled Synthesis of β -
23 Difluoromethylated Materials, *J. Org. Chem.*, 70 (2005) 5912-5915,
24 <http://dx.doi.org/10.1021/jo050634u>.

25 [150] F.A. Davis, M.B. Nolt, Y. Wu, K.R. Prasad, D. Li, B. Yang, K. Bowen, S.H. Lee, J.H.
26 Eardley, Asymmetric Synthesis of β -Amino Carbonyl Compounds with N-Sulfinyl β -Amino
27 Weinreb Amides, *J. Org. Chem.*, 70 (2005) 2184-2190, <https://doi.org/10.1021/jo0402780>.

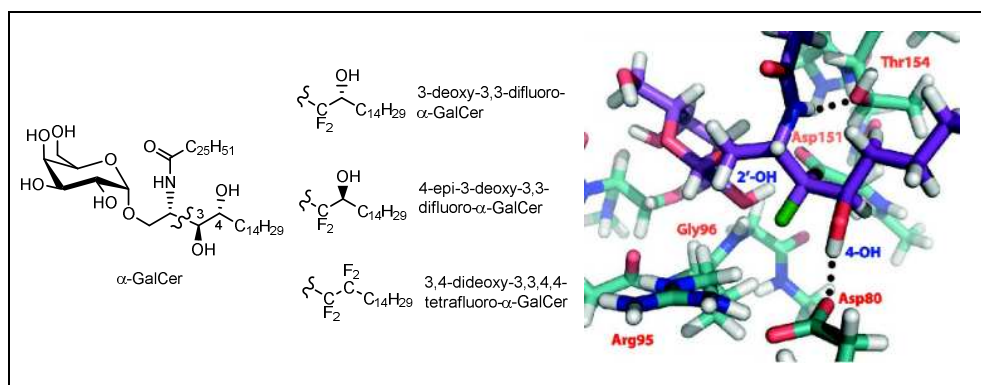
28 [151] H.T. Cao, T. Roisnel, A. Valleix, R. Grée, A Tandem Isomerization-Mannich Reaction
29 for the Enantioselective Synthesis of β -Amino Ketones and β -Amino Alcohols with
30 Applications as Key Intermediates for ent-Nikkomycins and ent-Funebrine, *Eur. J. Org.*
31 *Chem.*, 2011 (2011) 3430-3436,
32 <https://onlinelibrary.wiley.com/doi/abs/10.1002/ejoc.201100352>.

- 1 [152] B. Linclau, A.J. Boydell, R.S. Timofte, K.J. Brown, V. Vinader, A.C. Weymouth-
2 Wilson, Enantioselective Synthesis of Tetrafluorinated Ribose and Fructose, *Org. Biomol.*
3 *Chem.*, 7 (2009) 803-814, <http://dx.doi.org/10.1039/B817260A>.
- 4 [153] T. Konno, T. Hoshino, T. Kida, S. Takano, T. Ishihara, Short Synthetic Preparation of
5 Enantiomerically Pure Tetrafluoroethylenated Sugar Derivatives, *J. Fluorine Chem.*, 152
6 (2013) 106-113, <http://www.sciencedirect.com/science/article/pii/S0022113913000808>.
- 7 [154] T. Konno, S. Takano, Y. Takahashi, H. Konishi, Y. Tanaka, T. Ishihara, Novel
8 Introduction of a Tetrafluoroethylene (-CF₂CF₂-) Unit into Organic Molecules, *Synthesis*,
9 2011 (2011) 33-44.
- 10 [155] D.H.R. Barton, S.W. McCombie, A New Method for the Deoxygenation of Secondary
11 Alcohols, *J. Chem. Soc., Perkin Trans. 1*, (1975) 1574-1585,
12 <http://dx.doi.org/10.1039/P19750001574>.
- 13 [156] J.I.G. Cadogan, R.K. Mackie, Tervalent Phosphorus Compounds in Organic Synthesis,
14 *Chem. Soc. Rev.*, 3 (1974) 87-137, <http://dx.doi.org/10.1039/CS9740300087>.
- 15 [157] A. Deed, Some Reactions with 5-Chloro-3-Nethyl-4-Nitro-1-Nenylpyrazole, *Eur.*
16 *Chem. Bull.*, 2 (2013) 981-984.
- 17 [158] M.B. Smith, in: Wiley (Ed.) *March's Advanced Organic Chemistry: Reactions,*
18 *Mechanisms, and Structure*, 2013, pp. 1529.
- 19 [159] A.S. Mahadevi, G.N. Sastry, Cooperativity in Noncovalent Interactions, *Chem. Rev.*,
20 116 (2016) 2775-2825, <https://doi.org/10.1021/cr500344e>.
- 21 [160] R. Paulini, K. Müller, F. Diederich, Orthogonal Multipolar Interactions in Structural
22 Chemistry and Biology, *Angew. Chem. Int. Ed.*, 44 (2005) 1788-1805,
23 <https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.200462213>.
- 24 [161] W. Tang, S. Liu, D. Degen, R.H. Ebright, E.V. Prusov, Synthesis and Evaluation of
25 Novel Analogues of Ripostatins, *Chem. Eur. J.*, 20 (2014) 12310-12319,
26 <https://onlinelibrary.wiley.com/doi/abs/10.1002/chem.201403176>.
- 27 [162] T. Mukaiyama, Y. Murai, S.-i. Shoda, An Efficient Method for Glucosylation of
28 Hydroxy Compounds Using Glucopyranosyl Fluoride, *Chem. Lett.*, 10 (1981) 431-432,
29 <http://www.journal.csj.jp/doi/abs/10.1246/cl.1981.431>.

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31 Graphical abstract

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1

2 Highlights

- 3 • H-bonding of the 3-OH and the amide NH groups on the iNKT stimulation process.
- 4 • Synthesis of 3,4-dideoxy-3-fluoro- and 3,4-dideoxy-3,3-difluoro-KRN7000 analogues
- 5 • Co-participation of 4-OH on key 3-OH contribution in KRN7000 immune stimulation
- 6 • Potency of the tetrafluorinated analogue to highlight contribution of the NH group

7

Graphical abstract

