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Use of the NEO strategy (Nucleophilic addition/Epoxide Opening) for the synthesis of a new C-galactoside ester analogue of KRN 7000

Aline Banchet-Cadeddu [a,*], Agathe Martinez [a], Stéphane Guillarme [b], Véronique Parietti [c], Fanny Monneaux [c], Eric Hénon [a], Jean-Hugues Renault [a], Jean-Marc Nuzillard [a], Arnaud Haudrechy [a,*]

[a] ICMR UMR 6229, Université de Reims Champagne-Ardenne, BP 1039, 51687 REIMS Cedex, France
[b] UCO2M UMR 6011, Université du Maine, Av. Olivier Messiaen 72085 LE MANS Cedex 9, France
[c] CNRS UPR 9021 (IBMC), Université de Strasbourg, 67 084 STRASBOURG Cedex, France

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ABSTRACT

Our goal in the search for potentially bioactive analogues of KRN 7000 was to design an easy synthetic approach to a library of analogues using a strategy recently developed in our laboratory based on a Nucleophilic addition followed by an Epoxide Opening (the NEO strategy). Through the use of a common pivotal structure, a new C-galactoside ester analogue (**23**) was synthesized which showed an encouraging T_H2 biased response during preliminary biological tests.

KRN 7000 (**1**) is a synthetic glycolipid resulting from structure-activity studies based on compounds isolated from the Japanese marine sponge, Agelas mauritianus (Fig. 1).[1] It has shown promising bioactivity against diverse pathologies.[2] When associated with the protein CD1d,[3] this α-GalactosylCeramide (α-GalCer) interacts with the invariant Natural Killer T (*i*NKT) cells of the immune system,[3b] stimulating the production of signalling molecules involved in cellular communication called cytokines. According to the nature of the produced cytokines, a T_H1/T_H2 immune response profile is established and involves the activation of other immune cells (B cells, T cells…), to fight cancer (T_H1)[2a,4] or autoimmune diseases (T_H2).[5]

Biological tests have shown that certain parts of KRN 7000 must not be modified for an efficient stimulation of the *i*NKT cells. The configuration of the amide function,[6] that of the 3′-OH[6] and the α-glycoside bond[7] are all important. Certain structural modifications are possible in position 6 of the sugar moiety with no loss of activity.[8] Finally, the presence of lipid side chains is required to allow good contact with the CD1d protein. Various efforts have been made toward the synthesis of simple and more functionalized analogues (**2**–**5** for example) in order to selectively induce T_H1 or T_H2 - type cytokine production.[9,10] Among these compounds, few

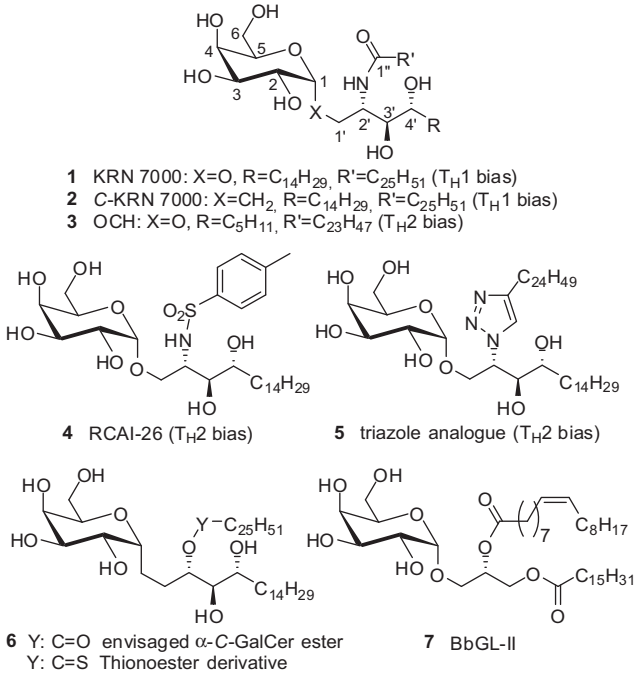

Figure 1. KRN 7000, several analogues, and the envisaged α-*C*-GalCer ester analogue **6**.

* Corresponding authors. Tel.: +33 (0)3 26 91 32 36 (A.H); fax: +33 (0)3 26 91 31 66 (ICMR).
E-mail addresses: A.Banchet@laposte.net (A. Banchet-Cadeddu), arnaud.haudrechy@univ-reims.fr (A. Haudrechy).

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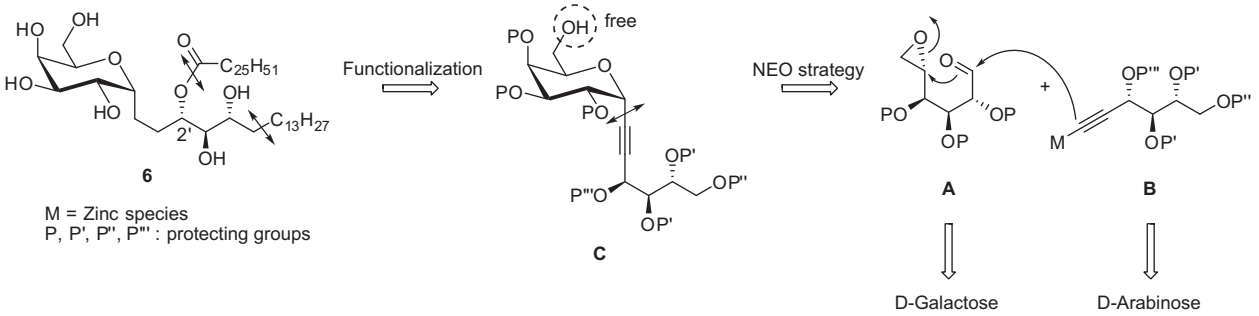


Scheme 1. Retrosynthetic approach.

modifications of the amide function have been reported in the literature (Fig. 1, analogues **4** and **5**).[10]

The C-glycoside of KRN 7000 (**2**) has also shown promising biological activity.[11] It is, in some cases, even more potent than its O-glycoside counterpart **1**. Using a strategy developed in our laboratory based on a Nucleophilic addition followed by an Epoxide Opening (the NEO strategy),[12] we wished to develop an easy synthetic access to the α-*C*-KRN 7000 skeleton. The replacement of the amide with an ester function was also planned, followed by an easy transformation into a thionoester or ether derivative.[13] Using this methodology, a new α-*C*-GalCer analogue (**6**, Fig. 1) was thus envisaged. The presence of the ester group on the side chain recalls the diacylglycerol structure of BbGL-II (**7**, Fig. 1), another potent glycolipid which stimulates mice and human NKT cells.[2b,14]

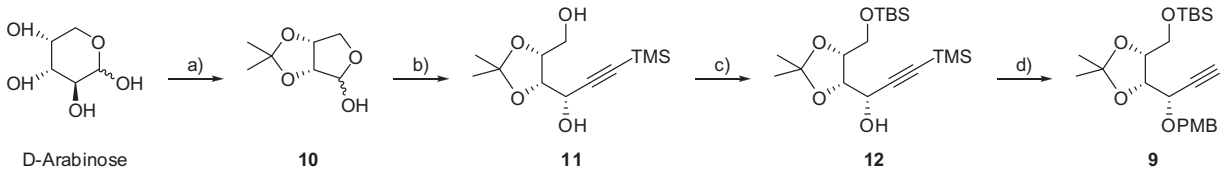
In order to generate a library of analogues, the insertion of lipid side chains was planned at an advanced stage in the synthesis. The functionalized C-galactoside framework **C** was thus chosen as a strategic intermediate. Using the NEO strategy, compound **C** could be prepared from an epoxyaldehyde **A** and a functionalized alkyne **B**, both obtained from inexpensive sugars. This method would also allow us to diversify position 6 of the sugar released during the NEO coupling (Scheme 1).

The synthesis of the functionalized alkyne **9** was achieved in 5 steps in 38–44% yield from D-Arabinose. Thus, after protection of the hydroxyl groups in positions 3 and 4 with an isopropylidene group, an oxidative cleavage gave the D-Erythrose derivative **10**. Addition of trimethylsilylethynyl magnesium bromide, in a key step, led exclusively to the 1,2-*anti* propargylic alcohol **11**.[15] After regioselective protection of the hydroxyl groups, a methanolic work-up gave the terminal alkyne **9** in a single step (Scheme 2).

The synthesis of the epoxyaldehyde **8** was carried out in 8 steps in 28% overall yield according to the procedure developed in our laboratory and previously described in the literature (Scheme 3).[12,16]

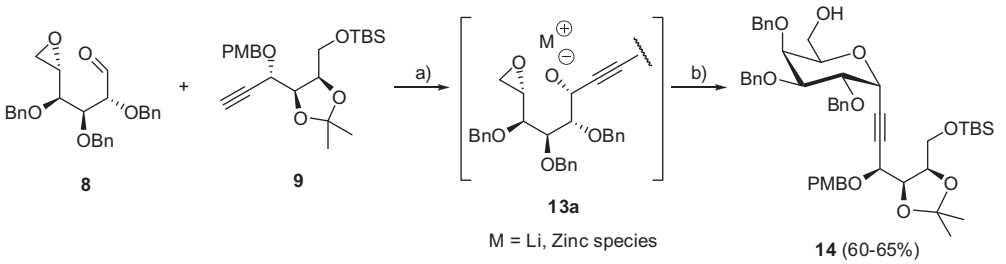
Next, coupling of compounds **8** and **9** using the optimized NEO strategy gave, in a one pot procedure, the key C-galactoside structure **14** in good yield (60–65%). The use of an excess of zinc chloride was necessary to activate epoxide opening and start the reaction cascade. The epoxyaldehyde **8** was mixed with the preformed zinc alkyne at low temperature and lead to the exclusive formation of the 1,2-*syn* intermediate alcoholate **13a**.[17] Lithium bromide was then added to achieve the cyclization at room temperature overnight (Scheme 3).

In our model study, the primary alcohol generated in this step was protected as a benzyl ether (Scheme 4). The sphingosine-like chain was then installed via a Wittig reaction with aldehyde **17**, this being obtained by a Swern oxidation of the primary alcohol **16**. At this stage of the synthesis, removal of the PMB group proved difficult. Indeed, the presence of the triple bond disturbs the deprotection performed with DDQ due to the formation of several by-products (presumably over-oxidation to a ketone followed by decomposition). To circumvent this problem, it was necessary to simultaneously reduce the alkene and the alkyne functions beforehand. The use of a large excess of tosylhydrazine and an aqueous solution of sodium acetate in dimethoxyethane under reflux allowed the reduction of the multiple bonds,[18] and then the PMB group was easily removed from compound **19** to give the free secondary alcohol **20**, ready for esterification. Classical coupling conditions were applied to attach the acyl chain in good yield (**21**). After several attempted deprotections under various conditions, the 3′-OH and 4′-OH were finally released using strongly acidic conditions, and surprisingly led to a mixture of the expected ester **22a** and the rearranged compound **22b** in which the long lipid ester chain had migrated to the alcohol in the beta position as previously described by Shiozaki et al. in the O-glycoside series.[19] Unfortunately, it was not possible to separate the products from the reaction mixture by chromatography. Compound **22a** was not cleanly obtained even in conditions used by Shiozaki[19] or by carefully controlling the time of the reaction. By NMR (Table 1 and supporting information for 2D analysis), the instability of **22a** was clearly demonstrated as the mixture **22a**/**22b** in a 1:4 ratio evolved over the time to give neatly the rearranged product **22b** almost exclusively in a 1:9 ratio after 3 days. The reason behind this



a) i) $(MeO)_2C(CH_3)_2$, DMF, PPTS, r.t.; ii) $NaIO_4$, $NaHCO_3$, H_2O, 0 °C to r.t., 12 h, 80 % over 2 steps; b) trimethylsilylethynyl magnesium bromide, THF, -78 °C to 6 °C, 12 h, 75-87 %; c) $^tBuMe_2SiCl$, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t., 3 h, 80 %; d) PMBBr, NaH, nBu_4NI, Imidazole, THF/DMF 4:1, 0 °C to r.t., 4 h, then MeOH, 80 %

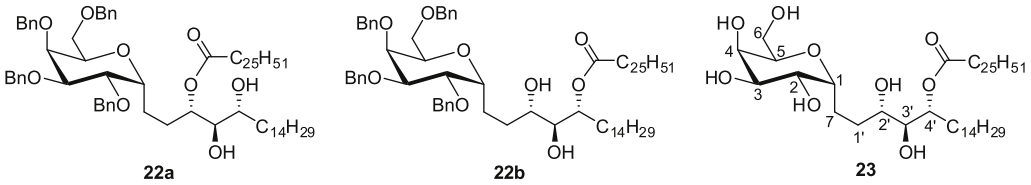
Scheme 2. Synthesis of alkyne **9**.



a) i) *n*BuLi, Et_2O, -78 °C to 0 °C; ii) $ZnCl_2$, Et_2O, 0 °C, 1 h; iii) **8**, 4 h; b) LiBr, r.t., o/n

Scheme 3. NEO coupling.

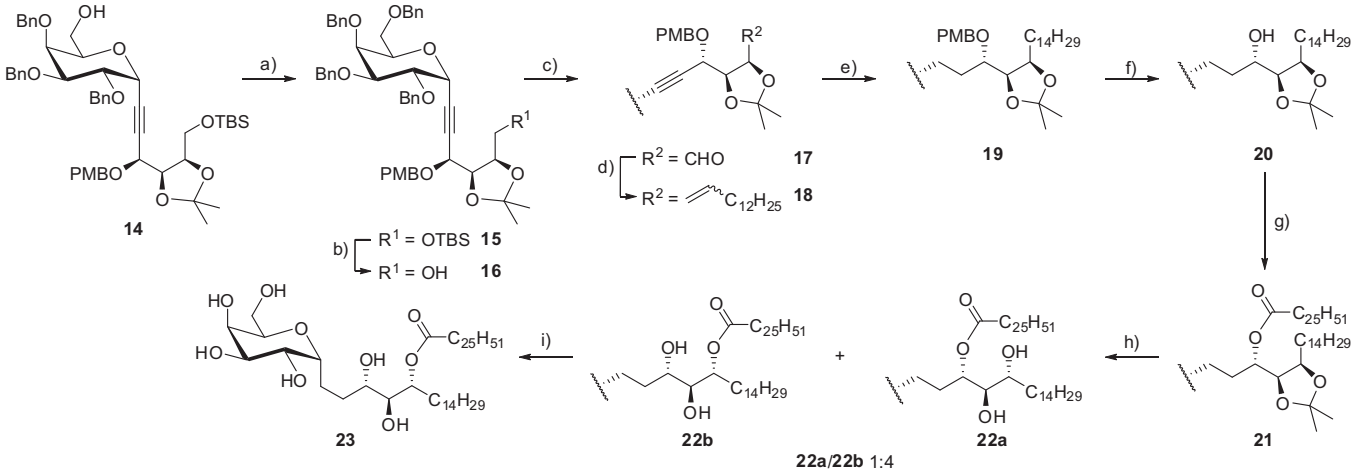
Table 1
NMR comparison of the rearranged products **22b** and **23** and the expected **22a** derivative



	Compounds 22a/22b		Compound 23	
	Proton	*Carbon*	*Proton*	*Carbon*
1	4.06	73.3	4.67 (ddd, *J* = 3.5, 6.1, 8.8 Hz)	79.7
2	3.89	76.9	4.82 (m, *J* = 6.1, 8.9 Hz)	72.9
3	3.67	77.5	4.39 (dd, *J* = 3.5, 8.9 Hz)	74.8
4	3.85	74.7	4.68 (m, *J* = 2.1, 3.5 Hz)	73.0
5	4.00	73.3	4.41 (ddd, *J* = 2.1, 4.2, 6.5 Hz)	76.2
6a	3.40	68.9	4.53 (ddd, *J* = 5.7, 6.5, 10.6 Hz)	64.9
6b	3.78	68.9	4.47 (m, *J* = 4.2, 10.6 Hz)	64.9
7a	1.85	29.6	2.85 (m)	24.3
7b	1.85	29.6	2.50 (m)	24.3
1'a	1.60	25.0	2.80 (m)	34.3
1'b	1.60	25.0	2.17 (m)	34.3
2'	5.03 – 3.75	72.2 – 70.6	4.33 (m, *J* = 6.6 Hz)	75.1
3'	3.61 – 3.52	75.0 – 75.7	4.37 (m, *J* = 5.4 Hz)	79.0
4'	3.54 – 5.00	75.6 – 74.8	6.06 (ddd, *J* = 3.1, 3.1, 10.1 Hz)	78.4
5'a	1.90		2.30 (m, *J* = 13.6 Hz)	31.5
5'b	1.90		2.22 (m, *J* = 13.6 Hz)	31.5
6'a			1.68 (m)	28.8
6'b			1.56 (m)	28.8
CO-*CH*H	2.30	34.5	2.48 (m)	37.4
CO-*CH*H	2.30	34.5	2.43 (m)	37.4
CO-CH_2-*CH*H			1.76 (m)	28.0
CO-CH_2-*CH*H			1.71 (m)	28.0

migration is not clearly understood although an ester group is more inclined to move than an amide function. We suppose that the ester shift might be privileged to better ensure the lipophilic layer formed by the two long carbon chains and to avoid some steric hindrance caused by hydroxyl groups. Total deprotection of the sugar was cleanly achieved by hydrogenation in the presence of palladium hydroxide ($CHCl_3$/MeOH 1:1). It is worth noting that the migration was totally completed at this stage when this last reaction was performed on the mixture **22a**/**22b** in a 1:4 ratio (Scheme 4). The chemical yield of the last step was considerably improved if the final product was centrifuged followed by extraction of the residual sediment with $CHCl_3$/MeOH (1:1). The unprotected ester **23** was fully identified by NMR analysis (Table 1).

First biological tests performed ex vivo demonstrated that ester **23** stimulates splenocytes from naïve BALB/c mice. Cell proliferation and induced cytokine secretion are dose-dependent and are abrogated by an anti-CD1d monoclonal antibody, indicating that the ester **23** stimulates CD1d-restricted NKT cells (Fig. 2A and B). Interestingly, the calculated ratio of IFN-γ (a typical pro-inflammatory T_H1 cytokine) to IL-4 (immunomodulatory T_H2 cytokine) was significantly reduced when NKT cells were stimulated ex vivo with compound **23** in comparison to KRN 7000 (1.6 vs 8.2, $p < 0.001$), showing that it induces an unexpected preferential T_H2 response. The biological activity of ester analogue **23** was then evaluated in vivo and the production of IL-4 and IFN-γ in sera of BALB/c mice administered with ester analogue **23** was determined. Compared to KRN 7000, the ester compound **23** induced a weaker secretion of IL-4 and IFN-γ (Table 2), but interestingly, the calculated IFN-γ/IL-4 ratio confirmed an enhanced T_H2-type response. Indeed, 6 h after administration, IL-4 levels were quasi equivalent to IFN-γ levels in the serum of mice that were treated with the ester **23** (mean of 83 ± 47 vs mean of 90 ± 27 pg/ml) whereas in the serum of mice that received KRN 7000, a 10-fold decrease of IL-4 levels compared to IFN-γ levels was observed (mean of 1450 ± 127 vs mean of 12145 ± 1131 pg/ml; Table 2).[20]



a) NaH, BnBr, DMF, 0 °C then r.t., 3 h; b) NH_4F, MeOH, reflux, 3 h, 76-82 % over 2 steps; c) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 1h, then Et_3N, -78 °C to r.t., 1.5 h; d) $nC_{13}H_{27}PPh_3Br$, nBuLi, THF, -40 °C, 1 h, then 17 at -5 °C to r.t., 1 h, 70-79 % over 2 steps; e) $TsNHNH_2$, NaOAc aq, DME, reflux, 5-6 h, 78-90 %; f) DDQ, CH_2Cl_2/H_2O 18:1, r.t., 40 min, 77-84 %; g) EDC.HCl, DMAP, cerotic acid, CH_2Cl_2, 28 °C, 18 h, 69-78 %; h) 12N HCl, MeOH, r.t., 2-3 days, 72-84 %; i) $Pd(OH)_2/C$ (20 %), H_2, $CHCl_3$/MeOH 1:1, r.t., 36 h, 80 %

Scheme 4. Functionalization of **14** to obtain the ester **23**.

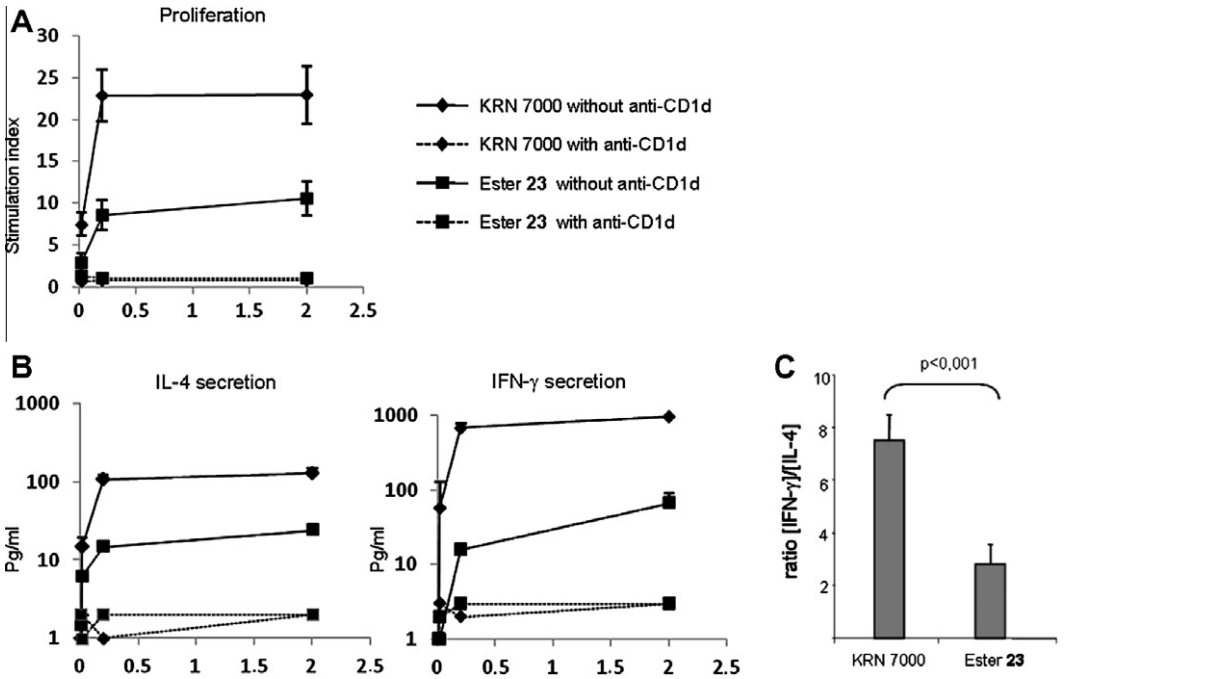


Figure 2. Ex vivo activation of NKT cells by the ester analogue **23**. (A) Cell proliferation was assessed at day 3 and results are expressed as stimulation index corresponding to counts per minutes (cpm) in the culture with ligand to cpm in culture without ligand; (B) IL-4 and IFN-γ levels were measured by ELISA and results are expressed as the mean concentration (pg/ml) or as a ratio.

Table 2
IL-4 and IFN-γ levels were determined in sera by ELISA and results, obtained 6 h after administration, and expressed as concentration (pg/ml) or as ratio

Mice	IL-4 (pg/ml)	IFN-γ (pg/ml)	Ratio
Ester **23**-1	59	76	1.3
Ester **23**-2	137	72	0.5
Ester **23**-3	53	120	2.3
mean	**83** ± **47**	**90** ± **27**	**1.37** ± **0.9**
KRN 7000-1	1540	12945	8.4
KRN 7000-2	1360	11345	8.3
mean	**1450** ± **127**	**12145** ± **1131**	**8.35** ± **0.07**

In conclusion, the synthesis of a new C-galactoside ester analogue of KRN 7000 (**23**) was achieved using the newly developed NEO strategy in 18 steps and 2.1–4.7% overall yield. The use of the pivotal α-C-galactoside structure **14** should allow access to a new series of KRN 7000 analogues. The ester analogue **23** has shown a promising T_H2 bias immune response during ex vivo tests. It induced, however, a weaker in vivo activity compared to KRN 7000. Following our previous theoretical work,[21] molecular modelling studies are currently being performed to evaluate the substitution of an ester in place of an amide on the stabilization and the orientation of the CD1d/glycolipid/TCR complex.

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Supplementary data

Supplementary data (Procedures and spectroscopic data for compounds **9**–**23**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.044.

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