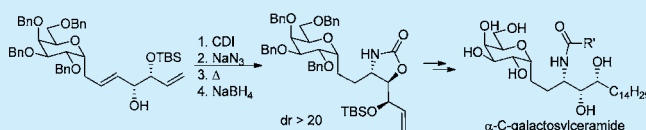


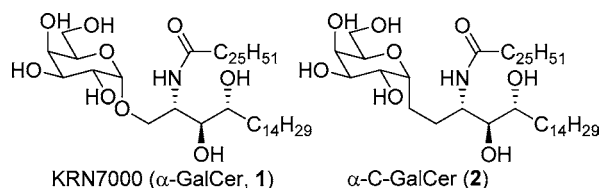
Synthesis of α -C-Galactosylceramide via Diastereoselective Aziridination: The New Immunostimulant 4'-*epi*-C-Glycoside of KRN7000Ya-Jen Chang,^{*,‡} Yi-Chen Hsuan,[†] Alan Chuan-Ying Lai,[‡] Yun-Chiann Han,[‡] and Duen-Ren Hou^{*,†}[†]Department of Chemistry, National Central University, No. 300 Jhong-Da Road, Jhong-li, Taoyuan, Taiwan 32001[‡]Institute of Biomedical Sciences, Academia Sinica, No. 128 Academia Road, Section 2, Nankang, Taipei, Taiwan 11529

S Supporting Information

ABSTRACT: A new immunostimulant, the 4'-epimer of α -C-GalCer, was synthesized from a C₂-symmetric dienediol and α -C-allyl galactoside. The intramolecular aziridination and the following reductive ring opening provided the core of the aliphatic amino alcohol with excellent regio- and stereocontrol. The new immunostimulants **3d** and **3e** gave a better polarized Th1-type cytokine response in murine NKT cells than the benchmarked α -C-GalCer.



In the 1990s, agelasphins, a family of glycosphingolipids isolated from the marine sponge *Agelas mauritianus*,¹ were found to prolong the life span of mice inoculated with B16 melanoma cells.² These active compounds were characterized as α -galactosylceramides, which were found to be effective immunostimulants for their antitumor activity.³ KRN7000 (α -GalCer, **1**), a synthetic analogue of the active agelasphins, was later developed by the Kirin Brewery Company to replace the scarce natural agelasphins as the prototype of a glycolipid antigen of invariant natural killer T (iNKT) cells.^{3,4} The binary complex of α -GalCer and CD1d, a histocompatibility complex class I-like protein, is recognized by the T-cell antigen receptor (TCR) of iNKT, and the activated iNKT cells release cytokines.⁵ The cytokines can be further classified as proinflammatory T-helper 1 (Th1) type and immunomodulatory T-helper 2 (Th2) type, such as interferon- γ (IFN- γ) and interleukin-4 (IL-4), respectively.⁶ In 2003, Franck and co-workers reported that α -C-GalCer (**2**), a C-glycoside analogue of KRN7000, is more potent than **1** in mouse malaria and melanoma assays, both disease models requiring a protective Th1-type immune response.⁷ These results suggest that it is possible that ligands for CD1d may lead to a selective immune response toward a Th1 or Th2 profile. Therefore, the synthesis of α -C-GalCer analogues is an attractive approach for new immunostimulants.^{8–14}

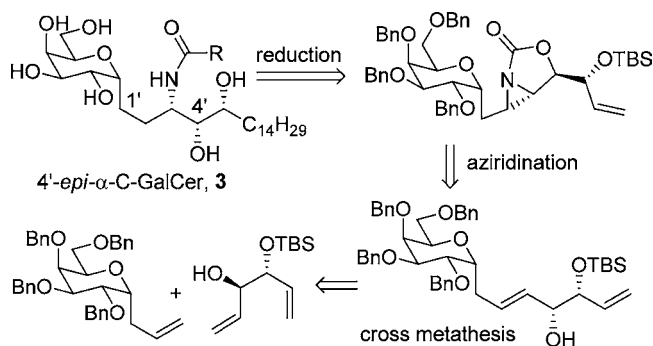


A general and convenient method to access α -C-GalCer is to utilize cross-metathesis between C-alkenylgalactose and optically active allyl alcohols.^{9a,11,13} However, further chemical

transformations on the internal alkene to install the aminodiol stereotriad of C-glycosphingolipids were often complicated by issues of regio- and diastereoselectivity.^{9,11–13}

Here we report that both issues can be resolved by the intramolecular aziridination and subsequent reductive ring opening to give C-glycosphingolipids (Scheme 1).¹⁵ The resulting 4'-*epi*-C-glycosides of KRN7000, **3d** and **3e**, show comparable potencies and better polarized Th1-type cytokine responses than **2**.

Scheme 1. Intramolecular Aziridination To Give C-Galactosyl Ceramide

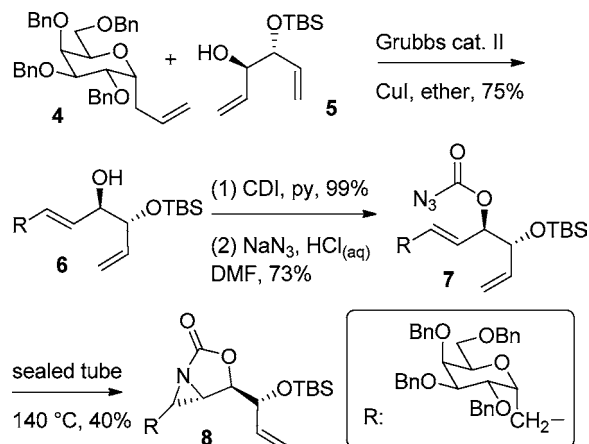


Our synthesis started with cross-metathesis between benzyl-protected α -C-allyl galactoside **4**¹⁶ and optically active hexa-1,5-diene **5**¹⁷ using Lipshutz's protocol¹⁸ to give compound **6** in 75% yield (Scheme 2). The hydroxyl group was then converted to the azidoformate **7** via a two-step sequence using 1,1'-carbonyldiimidazole (CDI) and then sodium azide. Intramolecular acylnitrene addition to the olefin was induced by

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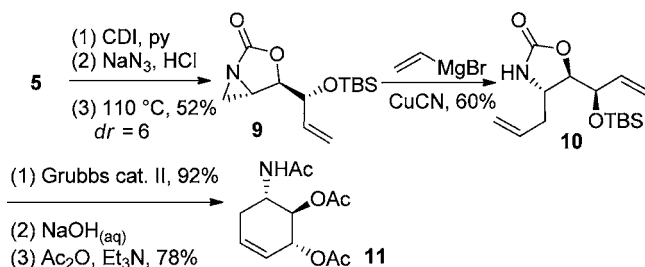
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Scheme 2. Synthesis of Aziridine 9



conventional heating, microwave-assisted heating, or ultraviolet light to give aziridine **8** (see the [Supporting Information](#)).^{15,19} To our delight, all of the reaction conditions screened provided diastereomerically pure *trans*-oxazolidinones **8** according to the ¹H NMR spectra of the crude products. Here the stereochemical assignment followed Bergmeier's studies using the azidoformates derived from 1-alkyl-substituted prop-2-en-1-ol^{15b,c} and was confirmed by the parallel synthesis of the known diacetate **11**²⁰ from **5** (Scheme 3). The improved diastereoselectivity of the aziridination may be a consequence of the synergistic influence of the bulky C-galactosyl group and the internal (*E*)-alkene.^{15b}

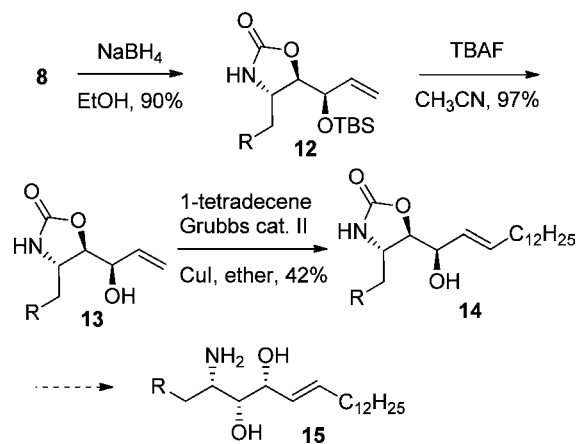
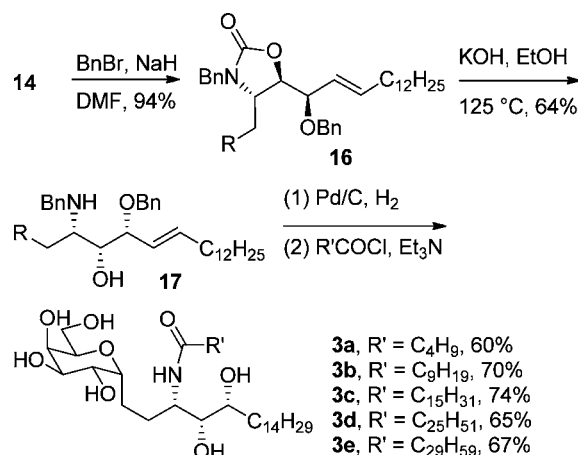
Scheme 3. Confirmation of the Stereochemistry of Aziridination



Reductive ring opening of the strained bicyclic compound **8** gave the stable oxazolidinone **12** as the sole product (Scheme 4). The TBS protecting group was then removed to facilitate the following cross-metathesis with 1-tetradecene.²¹ However, the intended hydrolysis of oxazolidinone **14** to generate **15** always led to decomposed mixtures under the reported basic or acidic reaction conditions.^{22,23} We suspect that the side reactions of allylic alcohol **14** were fostered by the remaining trace amounts of Grubbs catalyst, which is a common contaminant in the products of metathesis reactions.²⁴

This problem was resolved by replacing the labile –OH and –NH protons of **14** with benzyl groups (compound **16**; Scheme 5). The hydrolysis of the oxazolidinone moiety then succeeded at elevated temperatures to give amino alcohol **17**. Palladium-catalyzed hydrogenation, hydrogenolysis, and subsequent N-acylation provided the desired 4'-*epi*-α-C-GalCer products **3a–e**, whose difference is in the chain length of the N-acyl lipid.

Scheme 4. Synthesis of C-Glycosphingolipid

Scheme 5. Synthesis of 4'-*epi*-C-Glycosides

The *in vitro* assays to determine the stimulatory efficacies of the new α-C-GalCer analogues **3a–e** were performed with NKT cells sorted from Vα14tg BALB/c mice. Compounds **3a–e** were screened at a concentration of 1 μM using α-GalCer (**1**) (0.1 μM) and α-C-GalCer (**2**) (10 μM) as the controls (Figure 1). We observed a significant reduction of the IL-4 induced by **2** and **3a–e** in comparison with that induced by **1** (Figure 1A). A reduced function of these C-glycosides in inducing IFN-γ production was also observed, and the differences between them were minor (Figure 1B). However, the graph of the ratios derived from the induced IFN-γ/IL-4 shows that all of the C-glycosides have a significantly higher ratio than α-GalCer. More importantly, both **3d** and **3e** show a better Th1-biased response than **2** at a lower concentration (Figure 1C; also see the [Supporting Information](#) for a comparison of the immunostimulants at 0.1 μM). However, the efficacies of compounds **3d** and **3e** require further studies using an *in vivo* mouse model and/or an *in vitro* human NKT assay.

Compound **3d** has the same N-acyl group as in **2**. Compound **3e**, with the extended acyl chain, gave a slightly higher IFN-γ/IL-4 ratio than **3d**. On the other hand, less polarized Th1 responses were observed for the compounds with a truncated acyl lipid chain (**3a–c**), which is in agreement with the previous structure–activity relationship (SAR) study of KRN7000.^{8,25}

Comparing the potencies of the two stereoisomers **3d** and **2** is interesting. The corresponding (2'S,3'R,4'R) stereoisomer of

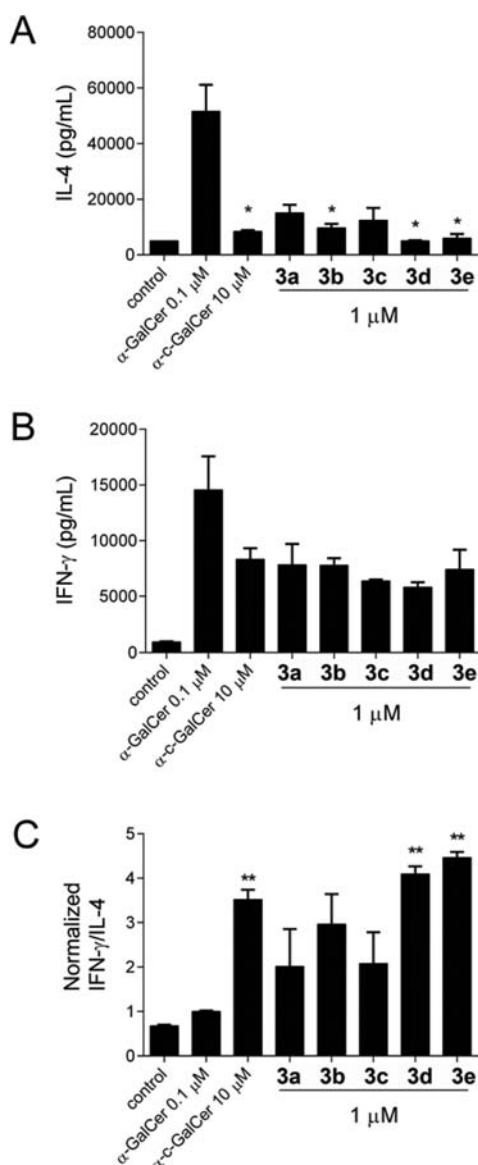


Figure 1. Potencies of α -C-GalCer analogues 3a–e in stimulating iNKT. NKT cells sorted from V α 14tg BALB/c mice were cultured with immunostimulants for 72 h, and the supernatants were collected and analyzed with ELISA for the cytokines IL-4 and IFN- γ . (A, B) Levels of IL-4 and IFN- γ , respectively. (C) IFN- γ /IL-4 ratios for the ligands normalized to the IFN- γ /IL-4 ratio for α -GalCer (1). Student's *t* analysis was performed by comparing all of the data to those for α -GalCer (**, *P* < 0.01; *, *P* < 0.05).

KRN7000 is known to give reduced cytokine secretion and weaker Th1 response in comparison with KRN7000.^{8c,26} However, this trend does not apply to the α -C-GalCer analogues, as a slightly more Th1-polarized response and comparable secretion of cytokines induced by 3d and 3e relative to 2 were observed. Previous studies indicated that the lower affinity of 2 for the TCR compared with that of 1 is partially due to the loss of the hydrogen-bonding interaction between Arg95 α of the TCR and the 3-OH of the phytosphingosine chain.²⁷ We speculate that the 4'-epimer of α -C-GalCer is beneficial to have this hydrogen-bonding interaction, leading to better potency.

In summary, a new route to prepare α -C-GalCer analogues has been developed and features intramolecular aziridination

and reductive ring opening to introduce the 3'-amino group with excellent stereo- and regiocontrol. Biological assays showed that the new 4'-*epi*- α -C-GalCer analogues 3d and 3e can generate a Th1-biased cytokine response at a lower concentration than that of the benchmarked α -C-GalCer (2).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00090.

Experimental procedures and spectral data of compounds described herein (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592.
- (2) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
- (3) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187.
- (4) Akimoto, K.; Natori, T.; Morita, M. *Tetrahedron Lett.* **1993**, *34*, 5593–5596.
- (5) (a) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, Y.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629. (b) Brossay, L.; Chioda, M.; Burdin, N.; Koezuka, Y.; Casorati, G.; Dellabona, P.; Kronenberg, M. *J. Exp. Med.* **1998**, *188*, 1521–1528. (c) Kim, H. S.; Garcia, J.; Exley, M.; Johnson, K. W.; Balk, S. P.; Blumberg, R. S. *J. Biol. Chem.* **1999**, *274*, 9289–9295. (d) Venkataswamy, M. M.; Porcelli, S. A. *Semin. Immunol.* **2010**, *22*, 68–78. (e) Rossjohn, J.; Pellicci, D. G.; Patel, O.; Gapin, L.; Godfrey, D. I. *Nat. Rev. Immunol.* **2012**, *12*, 845–857.
- (6) (a) Murphy, K. M.; Reiner, S. L. *Nat. Rev. Immunol.* **2002**, *2*, 933–944. (b) Steinman, L. *Nat. Med.* **2007**, *13*, 139–145. (c) Weaver, C. T.; Elson, C. O.; Fouser, L. A.; Kolls, J. K. *Annu. Rev. Pathol.: Mech. Dis.* **2013**, *8*, 477–512. (d) Hong, S.; Wilson, M. T.; Serizawa, I.; Wu, L.; Singh, N.; Naidenko, O. V.; Miura, T.; Haba, T.; Scherer, D. C.; Wei, J.; Kronenberg, M.; Koezuka, Y.; Van Kaer, L. *Nat. Med.* **2001**, *7*, 1052–1056.
- (7) (a) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631–1641. (b) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818–3822.
- (8) Reviews: (a) Tyznik, A. J.; Farber, E.; Girardi, E.; Birkholz, A.; Li, Y.; Chitale, S.; So, R.; Arora, P.; Khurana, A.; Wang, J.; Porcelli, S. A.

- Zajonc, D. M.; Kronenberg, M.; Howell, A. R. *Chem. Biol.* **2011**, *18*, 1620–1630. (b) Tashiro, T. *Biosci., Biotechnol., Biochem.* **2012**, *76*, 1055–1067. (c) Laurent, X.; Bertin, B.; Renault, N.; Farce, A.; Specia, S.; Milhomme, O.; Millet, R.; Desreumaux, P.; Hénon, E.; Chavatte, P. *J. Med. Chem.* **2014**, *57*, 5489–5508.
- (9) (a) Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077–4080. (b) Chen, G.; Chien, M.; Tsuji, M.; Franck, R. W. *ChemBioChem* **2006**, *7*, 1017–1022. (c) Pu, J.; Franck, R. W. *Tetrahedron* **2008**, *64*, 8618–8629.
- (10) (a) Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. *Tetrahedron Lett.* **2005**, *46*, 5043–5047. (b) Chaulagain, M. R.; Postema, M. H. D.; Valeriote, F.; Pietraszkewicz, H. *Tetrahedron Lett.* **2004**, *45*, 7791–7794.
- (11) (a) Liu, Z.; Courtney, A. N.; Metelitsa, L. S.; Bittman, R. *ChemBioChem* **2012**, *13*, 1733–1737. (b) Liu, Z.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2011**, *76*, 8588–8598. (c) Lu, X.; Song, L.; Metelitsa, L. S.; Bittman, R. *ChemBioChem* **2006**, *7*, 1750–1756.
- (12) Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375–3378.
- (13) Altiti, A. S.; Mootoo, D. R. *Org. Lett.* **2014**, *16*, 1466–1469.
- (14) (a) Colombel, S.; Van Hijfte, N.; Poisson, T.; Leclerc, E.; Pannecoucke, X. *Chem. - Eur. J.* **2013**, *19*, 12778–12787. (b) Colombel, S.; Van Hijfte, N.; Poisson, T.; Pannecoucke, X.; Monneaux, F.; Leclerc, E. *J. Fluorine Chem.* **2015**, *173*, 84–91.
- (15) (a) Rhouati, S.; Bernou, A. J. *Chem. Soc., Chem. Commun.* **1989**, 730–732. (b) Bergmeier, S. C.; Stanchina, D. M. *J. Org. Chem.* **1997**, *62*, 4449–4456. (c) Bergmeier, S. C.; Stanchina, D. M. *J. Org. Chem.* **1999**, *64*, 2852–2859. (d) Bergmeier, S. C.; Katz, S. J. *J. Comb. Chem.* **2002**, *4*, 162–166. (e) Yoshimitsu, T.; Ino, T.; Tanaka, T. *Org. Lett.* **2008**, *10*, 5457–5460.
- (16) Fletcher, S.; Jorgensen, M. R.; Miller, A. D. *Org. Lett.* **2004**, *6*, 4245–4248.
- (17) (a) Yadav, J. S.; Mysorekar, S. V.; Pawar, S. M.; Gurjar, M. K. J. *J. Carbohydr. Chem.* **1990**, *9*, 307–316. (b) Burke, S. D.; Sametz, G. M. *Org. Lett.* **1999**, *1*, 71–74. (c) Lu, K. J.; Chen, C. H.; Hou, D. R. *Tetrahedron* **2009**, *65*, 225–231.
- (18) Voigtritter, K.; Ghorai, S.; Lipshutz, B. H. *J. Org. Chem.* **2011**, *76*, 4697–4702.
- (19) (a) Kan, C.; Long, C. M.; Paul, M.; Ring, C. M.; Tully, S. E.; Rojas, C. M. *Org. Lett.* **2001**, *3*, 381–384. (b) Mendlik, M. T.; Tao, P.; Hadad, C. M.; Coleman, R. S.; Lowary, T. L. *J. Org. Chem.* **2006**, *71*, 8059–8070.
- (20) Doddi, V. R.; Kumar, A.; Vankar, Y. D. *Tetrahedron* **2008**, *64*, 9117–9122.
- (21) Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360–11370.
- (22) (a) Abad, J. L.; Nieves, I.; Rayo, P.; Casas, J.; Fabriàs, G.; Delgado, A. J. *Org. Chem.* **2013**, *78*, 5858–5866. (b) Katz, S. J.; Bergmeier, S. C. *Tetrahedron Lett.* **2002**, *43*, 557–559. (c) Johnson, D. V.; Felfer, U.; Griengl, H. *Tetrahedron* **2000**, *56*, 781–790.
- (23) (a) Rao, M. V.; Chandrasekhar, B.; Rao, B. V.; Swarnalatha, J. L. *Tetrahedron: Asymmetry* **2011**, *22*, 1342–1346. (b) Righi, G.; Ferrara, A.; Mari, A.; Bovicelli, P. *Synth. Commun.* **2010**, *40*, 1650–1670.
- (24) Maynard, H. D.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 4137–4140.
- (25) Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Am. Chem. Soc.* **2004**, *126*, 13602–13603.
- (26) (a) Park, J.-J.; Lee, J. H.; Ghosh, S. C.; Bricard, G.; Venkataswamy, M. M.; Porcelli, S. A.; Chung, S.-K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3906–3909. (b) Trappeniers, M.; Goormans, S.; Van Beneden, K.; Decruy, T.; Linclau, B.; Al-Shamkhani, A.; Elliott, T.; Ottensmeier, C.; Werner, J. M.; Elewaut, D.; Van Calenbergh, S. *ChemMedChem* **2008**, *3*, 1061–1070.
- (27) (a) Aspeslagh, S.; Li, Y.; Yu, E. W.; Pauwels, N.; Trappeniers, M.; Girardi, E.; Decruy, T.; Van Beneden, K.; Venken, K.; Drennan, M.; Leybaert, L.; Wang, J.; Franck, R. W.; Van Calenbergh, S.; Zajonc, D. M.; Elewaut, D. *EMBO J.* **2011**, *30*, 2294–2305. (b) Patel, O.; Cameron, G.; Pellicci, D. G.; Liu, Z.; Byun, H.-S.; Beddoe, T.; McCluskey, J.; Franck, R. W.; Castano, A. R.; Harrak, Y.; Llebaria, A.;