Rhamnose-based glycomimetic for recruitment of endogenous anti-rhamnose antibodies

Nives Hribernik [a,c], Fabrizio Chiodo [b,d], Roland J. Pieters [c], Anna Bernardi [a,*]

[a] Università degli Studi di Milano, Dipartimento di Chimica, via Golgi 19, 20133 Milano, Italy
[b] Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam 1081HV, The Netherlands
[c] Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, NL-3508 TB Utrecht, The Netherlands
[d] Institute of Biomolecular Chemistry, National Research Council (CNR), Pozzuoli 80078, Napoli, Italy

ARTICLE INFO

ABSTRACT

Recruitment of natural antibodies towards tumour cells for their elimination by the immune system could be a highly specific and efficacious therapeutic strategy. While natural L-rhamnose has already been explored as a suitable antigen for antibody recruitment, we here report the first rhamnose-based glycomimetic to be used for such purpose. The glycomimetic is designed to be more hydrolytically and enzymatically stable than natural rhamnosides, provides a site for easy further conjugation and proved to capture anti-rhamnose IgG antibodies in serological ELISA assay.

Introduction

Utilizing the immune system to combat diseases, such as cancer, can be a particularly specific and efficacious therapeutic strategy. Although significant progress has been made in surgery, chemo- and radiotherapy, we are still on the lookout for new cancer treatment approaches that could improve long-term survival and tolerability. An interesting novel idea is based on selective labelling of tumour cells with compounds able to recruit naturally produced antibodies, which then activates the immune system and ultimately leads to destruction of these cells.

The destruction of malignant cells happens through two antibody effector mechanisms: complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) [1–4]. CDC is based mainly on IgM type antibodies, their recruitment leads to activation of the classical complement cascade. Subsequently, signalling molecules such as cytokines and inflammatory mediators are released. These signalling molecules then attract neutrophils, macrophages, and natural killer cells that are involved in ADCC. Immune effector cells recognize IgG antibodies bound to the cell surface and initiate ADCC through activation of Fc receptors [4].

Endogenous antibodies could be recruited towards tumour cells with the help of a so called antibody recruiting molecule that consists of an antibody binding module and a tumour binding module as shown in Fig 1 [5]. The function of the tumour binding module is to specifically recognize and bind to epitopes only present on the surface of tumour cells. Upon binding of the antibody recruiting molecule to the tumour cells, the antibody binding module is then presented on the surface of those cells. The antibody binding module on the other hand should contain an antigen that is recognized by antibodies and subsequently directs them towards tumour cells. There are three main criteria for an ideal antigen for this purpose [4]. First, the antigen has to be readily accessible or easy to synthesize and it should be possible to conjugate it to the tumour binding module. Second, it is considered a significant advantage if the antigen binds endogenous antibodies, because those are present even in partially immunocompromised individuals. Finally, the antigen should preferably bind antibodies of both IgG and IgM isotype, so that it can use both CDC and ADCC effector mechanisms.

Several potential antigens have been identified, for example dinitrophenyl (DNP), galactose-α-1,3-D-galactose (αGal) and α-L-rhamnose (αRha) (Fig. 2). DNP is a small molecule that is immunogenic and can be easily manipulated, however natural anti-DNP antibodies are present only in low concentrations [6–8]. Additionally, DNP can non-specifically interact with membranes and albumins, which limits its potential as an antigen [9–10]. As an alternative, αGal is a natural antigenic epitope present in most
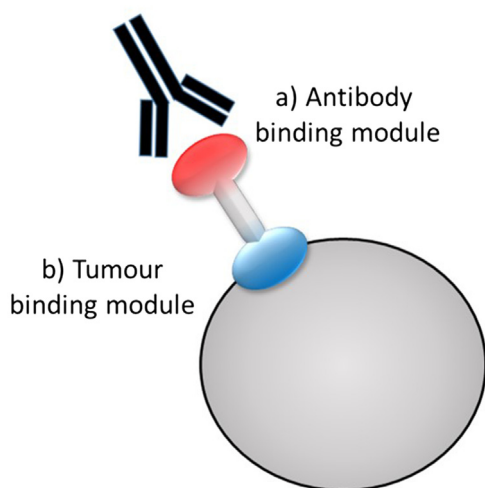
Fig. 1. The antibody recruiting molecule consists of two modules: a) antibody binding module and b) tumour binding module.
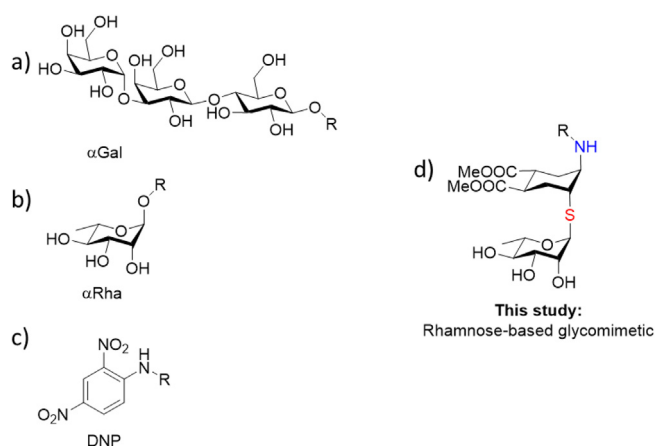

Fig. 2. Potential antigens for endogenous antibody recruitment: a) galactose-α-D-1,3-galactose (αGal), b) α-L-rhamnose (αRha), c) dinitrophenyl (DNP), d) rhamnose based glycomimetic used in this study.

mammals and bacteria, yet absent in humans and therefore recognized as foreign [11–12]. Due to constant exposure to endogenous gut bacteria, anti-Gal antibodies actually represent approximately 2% of circulating IgG and 3–8% of serum IgM in humans [13–15]. A significant drawback of utilizing αGal for antibody recruitment however is its synthetic complexity, both for chemical and chemoenzymatic synthesis [16–19]. Finally, the αRha epitope is, similar to αGal, common in microbes and plants and absent in humans [20–22]. In fact, it has been reported that anti-Rha antibodies are the most abundant and prevalent anti-carbohydrate antibodies in human serum [23–24]. The exposure of tumour cells to rhamnose-bearing glycolipids and human serum also promotes complement-mediated cytotoxicity [4]. This shows that αRha is an excellent candidate for further development in immune system recruitment strategies.

In addition to antibody recruitment for tumour cell elimination, it has been proposed that a rhamnose binding lectin is present on the surface of malignant cell lines [25]. Such lectin was found for instance on the surface of normal human dermal fibroblasts (NHDFs) and human umbilical venous endothelial cells (HUVEC) [26–27]. Therefore, αRha could also play an important role in anti-

tumor activity by binding to the rhamnose binding receptor of certain cell lines [25].

Previously, several groups successfully constructed rhamnosides for recruitment of natural antibodies. For example, αRha functionalized liposomes were developed for selective tumour cell targeting [28]. Such system works on the basis of multivalent amplification of low-affinity interactions between carbohydrates and antibodies that lead to efficient recognition of cancer cells by the immune system [29–30]. Further, αRha can be incorporated onto the scaffolds of cytotoxic agents, for example betulinol 3,28-di-O-α-L-rhamnopyranoside and betulinic acid 3-O-α-L-rhamnopyranoside display antitumor activity [31–33]. Rhamnose derivatives of anthracenes [34–35], steroids [36] and saponins [37] also showed cytotoxicity.

While rhamnose-based constructs clearly show the potential as anticancer therapeutics, the carbohydrate nature of αRha is inherently linked to certain drawbacks, such as low affinity and metabolic instability. To overcome the low affinity of rhamnose-antibody interactions, multivalent systems can amplify the impact with the so-called cluster effect [38–39]. In fact, the importance of multivalent presentation of αRha for efficient recognition with natural IgM antibodies was demonstrated [5], leading to the development of different multivalent rhamose-based glycoconjugates [5,40]. Still, all previously developed constructs utilized natural αRha, which is, as all natural saccharides, sensitive to enzymatic and acid-catalysed hydrolysis. Therefore, we here report the synthesis and serological evaluation of a rhamnose-based glycomimetic antigen (Fig. 2) with an improved metabolic stability, equipped with a site for easy conjugation to multivalent systems and/or tumour binding module.

Results and discussion

As described above, all previous constructs utilized natural αRha and conjugated it to the scaffold through an O-linkage, which is sensitive to enzymatic hydrolysis. We therefore decided to rather introduce an S-linkage which is known to be significantly more resistant towards glycosidases [41]. Our group has previously shown that pseudo-thio-dimannoside is an excellent mimic of the natural dimannoside epitope in the development of DC-SIGN ligands [42], thus confirming that due to similar bonding and geometry, the anomeric oxygen can in fact be replaced with a sulphur atom.

Working with S-glycosides is also synthetically advantageous as they are often easier to synthesize. In fact, we previously developed a one-pot synthetic procedure for synthesis of N-linked pseudo-thio-glycosides, including rhamnoside **3** as shown in Scheme 1 [43]. In this procedure the peracetylated rhamnosyl thioacetate **1** is deacetylated in situ at the anomeric position by excess amount of Et₂NH. This generates a thiolate able to open the aziridine **2**. The product **3** is formed as a single compound from a selective trans-diaxial aziridine opening in an excellent 92% yield. The reaction is carried out as a one-pot procedure at room temperature and is thus facile and operationally simpler than classic gly-
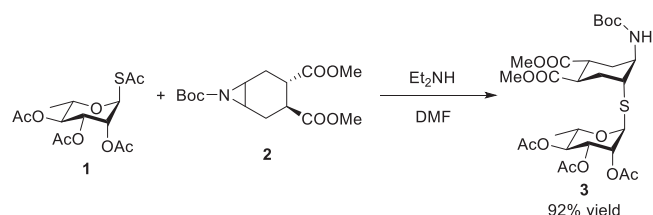
Scheme 1. One-pot aziridine opening reaction for the synthesis of rhamnoside **3**.

cosylation methods. The rhamnoside **3** is equipped with an agly-cone that can assist its binding and contains an *N*-linkage for simple conjugation to multivalent scaffolds or directly to the tumour binding module. The α-anomeric configuration of the Rha derivative **3** was established on the basis of the NOESY experiment and the measured J_{H1-C1} HSQC NMR without ^{13}C decoupling [43].

To prove that the glycomimetic **3** can in fact be used for further conjugation and for the purpose of biological evaluation by ELISA assay, a multivalent construct **10** was then synthesized from the *thio*-rhamno conjugate **3** as shown in Scheme 2. The design of **10** includes a hydrophobic tail, to allow immobilization of the structure on an ELISA plate, and three copies of the *thio*-rhamnoside ligand, to generate a minimal cluster effect. For its synthesis, after Boc deprotection of **3** (TFA, quant), the resulting amine **4** was coupled with 5-azido-valeric acid **5** using HATU as the coupling agent to give **6** in 87% yield. On the other hand, the tetravalent alkyne **8** was synthesized from pentaeritritol with propargyl bromide and treated with 10-azidodecane **7** to afford the trivalent alkyne **9** in 35% yield. The monovalent rhamnose-azide conjugate **6** was then

used in a click reaction with **9** using CuI/CH$_3$CN and the final multivalent construct **10** was obtained in 29% yield after deacetylation of hydroxyl groups with MeNH$_2$/THF and purification with size exclusion chromatography on P2 gel with eluent H$_2$O:MeOH 1:1.

As a negative control we synthesized a dendrimer **12** by esterification of **5** with triethylene glycol followed by a click reaction of **11** with **9** to afford **12** in 22% yield upon purification with size exclusion chromatography on P2 gel with eluent H$_2$O:MeOH 1:1 (Scheme 3).

A serological ELISA assay with 12 human samples from healthy donors was then performed adapting a protocol from a similar study [44]. This solid phase assay was designed to prove the ability of the rhamnosylated construct in capturing anti-Rha IgG antibodies.

Briefly, the ELISA plate was coated with product **10** or **12** and after a blocking step, the wells were incubated with human plasma. After washing, an anti-human IgG antibody conjugated with horseradish peroxidase was added to the wells. A last washing step was then performed followed by the addition of the chro-
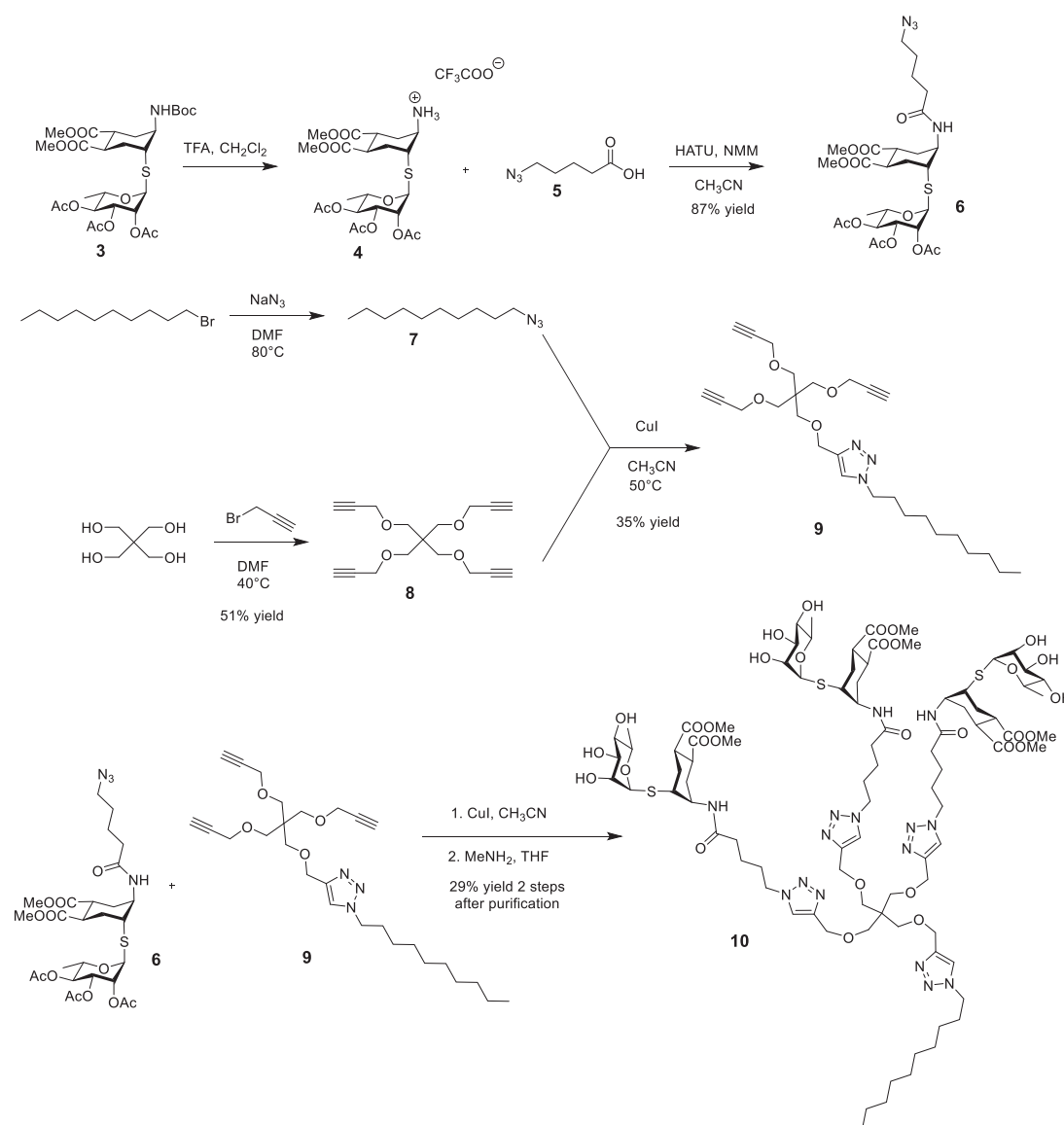
Scheme 2. Synthesis of multivalent construct **10** from the thio-rhamno conjugate **3**.
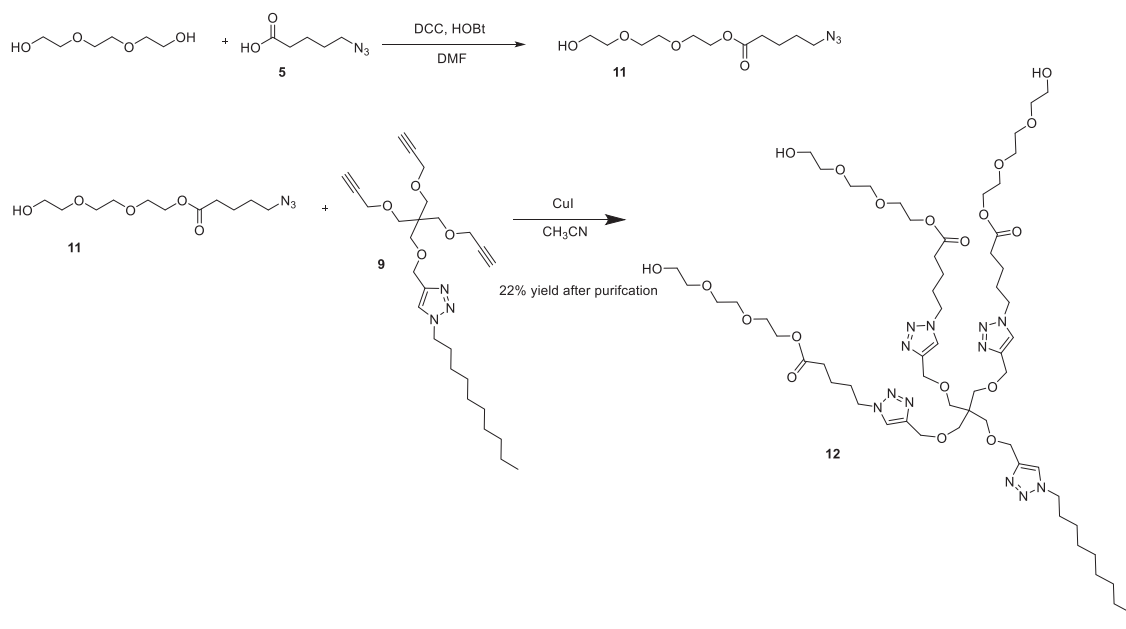
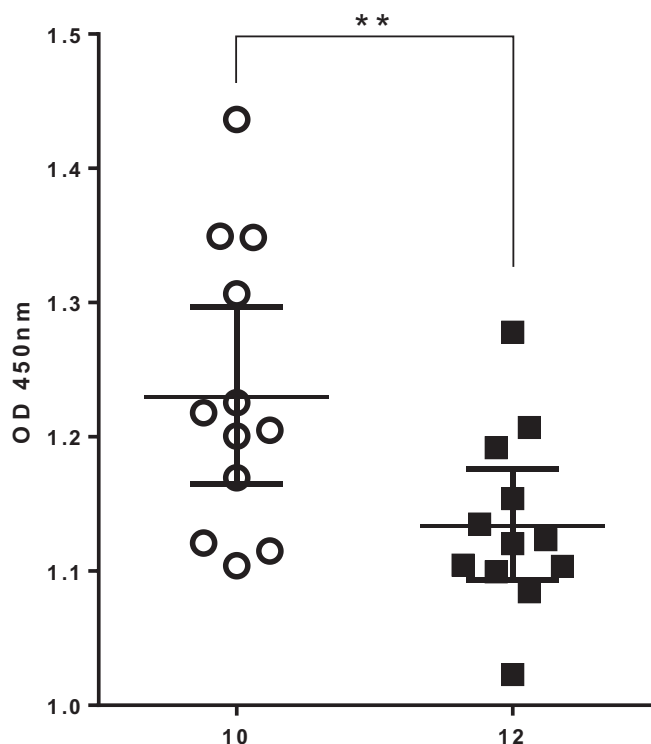**Scheme 3.** Synthesis of dendrimer **12** that was used as negative control in the ELISA experiment.

Fig. 3. Human IgG in plasma detected by coating ELISA plates with the mentioned dendrimer (n = 12, biologically independent samples). Ordinary two-way ANOVA with an alpha signification level of 0.05 was used with Prism GraphPad version 6·0. $P \leq 0.01$ is indicated with **. OD, optical density.

drimer **10** could be explored to target anti-Rha IgG for vaccination strategies.

Conclusions

As the search for novel strategies to combat cancer continues, immunotherapy certainly shines as one of the most promising options. The utility of synthetic chemistry in immunotherapy has already been demonstrated through development of antitumoral vaccines, yet immunization of immunocompromised cancer patients is often inefficient. Therefore, it appears to be more suitable to use a synthetic molecule that would direct the endogenous antibodies towards tumour cells. We herein report the first rhamnose-based glycomimetic that can be potentially used as the antibody binding module for such purpose. In comparison to the natural αRha epitope, the glycomimetic is designed to be more enzymatically and hydrolytically stable and equipped with an aglycone that allows simple conjugation to tumour binding module or multivalent scaffold. This study sets the stage for the development of a second generation of rhamnose-based glycomimetics to be used for antibody recruitment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2022.153843.

mogenic substrate. The readout of the wells was done reading the plate at 450 nm. Fig. 3 showed the binding results: dendrimer **12** was able to capture anti-Rha IgG while the control dendrimer **10** showed a significant lower binding. These results proved that den-

References

[1] A. Natsume, R. Niwa, M. Satoh, Drug Des. Dev. Ther. 3 (2009) 7–16.
[2] D. Berd, Vaccine 19 (2001) 2565–2570.
[3] U. Galili, M.R. Albertini, P.M. Sondel, K. Wigglesworth, M. Sullivan, G.F. Whalen, Cancers (2010) 2.
[4] R.T.C. Sheridan, J. Hudon, J.A. Hank, P.M. Sondel, L.L. Kiessling, ChemBioChem 15 (2014) 1393–1398.
[5] B. Liet, E. Laigre, D. Goyard, B. Todaro, C. Tiertant, D. Boturyn, N. Berthet, O. Renaudet, Chemistry – A European Journal 25 (2019) 15508–15515.
[6] F.S. Farah, Z.L. Awdeh, J. Immunol. Methods 1 (1972) 353–361.
[7] R.P. Murelli, A.X. Zhang, J. Michel, W.L. Jorgensen, D.A. Spiegel, J. Am. Chem. Soc. 131 (2009) 17090–17092.
[8] C.G. Parker, R.A. Domaoal, K.S. Anderson, D.A. Spiegel, J. Am. Chem. Soc. 131 (2009) 16392–16394.
[9] A.X. Zhang, R.P. Murelli, C. Barinka, J. Michel, A. Cocleaza, W.L. Jorgensen, J. Lubkowski, D.A. Spiegel, J. Am. Chem. Soc. 132 (2010) 12711–12716.
[10] H. Jung, T. Yang, M.D. Lasagna, J. Shi, G.D. Reinhart, P.S. Cremer, Biophys. J . 94 (2008) 3094–3103.
[11] U. Galili, M.R. Clark, S.B. Shohet, J. Buehler, B.A. Macher, Proc. Natl. Acad. Sci. 84 (1987) 1369.
[12] U. Galili, S.B. Shohet, E. Kobrin, C.L. Stults, B.A. Macher, J. Biol. Chem. 263 (1988) 17755–17762.
[13] U. Galili, R.E. Mandrell, R.M. Hamadeh, S.B. Shohet, J.M. Griffiss, Infect. Immun. 56 (1988) 1730–1737.
[14] U. Galili, E.A. Rachmilewitz, A. Peleg, I. Flechner, J. Exp. Med. 160 (1984) 1519–1531.
[15] W. Parker, D. Bruno, Z.E. Holzknecht, J.L. Platt, J. Immunol. 153 (1994) 3791.
[16] R.M. Owen, C.B. Carlson, J. Xu, P. Mowery, E. Fasella, L.L. Kiessling, ChemBioChem 8 (2007) 68–82.
[17] C. Gege, W. Kinzy, R.R. Schmidt, Carbohydr. Res. 328 (2000) 459–466.
[18] K.P. Naicker, H. Li, A. Heredia, H. Song, L.-X. Wang, Org. Biomol. Chem. 2 (2004) 660–664.
[19] J. Fang, J. Li, X. Chen, Y. Zhang, J. Wang, Z. Guo, W. Zhang, L. Yu, K. Brew, P.G. Wang, J. Am. Chem. Soc. 120 (1998) 6635–6638.
[20] V. Martinez, M. Ingwers, J. Smith, J. Glushka, T. Yang, M. Bar-Peled, J. Biol. Chem. 287 (2012) 879–892.
[21] S.D. Bentley, D.M. Aanensen, A. Mavroidi, D. Saunders, E. Rabbinowitsch, M. Collins, K. Donohoe, D. Harris, L. Murphy, M.A. Quail, G. Samuel, I.C. Skovsted, M.S. Kaltoft, B. Barrell, P.R. Reeves, J. Parkhill, B.G. Spratt, PLoS Genet. 2 (2006) e31.
[22] G. Samuel, P. Reeves, Carbohydr. Res. 338 (2003) 2503–2519.
[23] O. Oyelaran, L.M. McShane, L. Dodd, J.C. Gildersleeve, J. Proteome Res. 8 (2009) 4301–4310.
[24] M.E. Huflejt, M. Vuskovic, D. Vasiliu, H. Xu, P. Obukhova, N. Shilova, A. Tuzikov, O. Galanina, B. Arun, K. Lu, N. Bovin, Mol. Immunol. 46 (2009) 3037–3049.
[25] Y. Wang, J. Gao, G. Gu, G. Li, C. Cui, B. Sun, H. Lou, ChemBioChem 12 (2011) 2418–2420.
[26] H. Tateno, A. Saneyoshi, T. Ogawa, K. Muramoto, H. Kamiya, M. Saneyoshi, J. Biol. Chem. 273 (1998) 19190–19197.
[27] G. Faury, E. Ruszova, J. Molinari, B. Mariko, S. Raveaud, V. Velebny, L. Robert, BBA 1780 (2008) 1388–1394.
[28] X. Li, X. Rao, L. Cai, X. Liu, H. Wang, W. Wu, C. Zhu, M. Chen, P.G. Wang, W. Yi, ACS Chem. Biol. 11 (2016) 1205–1209.
[29] C.B. Carlson, P. Mowery, R.M. Owen, E.C. Dykhuizen, L.L. Kiessling, ACS Chem. Biol. 2 (2007) 119–127.
[30] L.L. Kiessling, J.E. Gestwicki, L.E. Strong, Curr. Opin. Chem. Biol. 4 (2000) 696–703.
[31] C. Gauthier, J. Legault, S. Lavoie, S. Rondeau, S. Tremblay, A. Pichette, J. Nat. Prod. 72 (2009) 72–81.
[32] M. Mihoub, A. Pichette, B. Sylla, C. Gauthier, J. Legault, PLoS ONE 13 (2018) e0193386.
[33] C. Gauthier, J. Legault, M. Lebrun, P. Dufour, A. Pichette, Bioorg. Med. Chem. 14 (2006) 6713–6725.
[34] G. Song, H. Liu, W. Zhang, M. Geng, Y. Li, Bioorg. Med. Chem. 18 (2010) 5183–5193.
[35] J.-Y. Xing, G.-P. Song, J.-P. Deng, L.-Z. Jiang, P. Xiong, B.-J. Yang, S.-S. Liu, PLoS ONE 10 (2015) e0144781.
[36] B.E. Cham, T.R. Chase, Planta Med 78 (2012) 349–353.
[37] Y. Wang, Y. Zhang, B. Yu, ChemMedChem 2 (2007) 288–291.
[38] C. Fasting, C.A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E.-W. Knapp, R. Haag, Angew. Chem. Int. Ed. 51 (2012) 10472–10498.
[39] S. Cecioni, A. Imberty, S. Vidal, Chem. Rev. 115 (2015) 525–561.
[40] J. Alsarraf, P. Gormand, J. Legault, M. Mihoub, A. Pichette, Tetrahedron Lett. 61 (2020) 151706.
[41] H. Driguez, ChemBioChem 2 (2001) 311–318.
[42] A. Tamburrini, S. Achilli, F. Vasile, S. Sattin, C. Vivès, C. Colombo, F. Fieschi, A. Bernardi, Bioorg. Med. Chem. 25 (2017) 5142–5147.
[43] N. Hribernik, A. Tamburrini, E. Falletta, A. Bernardi, Org. Biomol. Chem. 19 (2021) 233–247.
[44] X. Wang, H. Chen, F. Chiodo, B. Tefsen, Matters, 2019.