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N-Guanidino Derivatives of 1,5-Dideoxy-1,5-imino-D-xylitol are Potent, Selective, and Stable Inhibitors of β-Glucocerebrosidase

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A series of lipidated guanidino and urea derivatives of 1,5-dideoxy-1,5-imino-D-xylitol were prepared from D-xylose using a concise synthetic protocol. Inhibition assays with a panel of glycosidases revealed that the guanidino analogues display potent inhibition against human recombinant β-glucocerebrosidase with IC_{50} values in the low nanomolar range. Related urea analogues of 1,5-dideoxy-1,5-imino-D-xylitol were also synthesized and evaluated in the same fashion and found to be selective for β-galactosidase from bovine liver. No inhibition of human recombinant β-glucocerebrosidase was observed for the urea analogues. Computational studies provided insight into the potent activity of analogues bearing the substituted guanidine moiety in the inhibition of lysosomal glucocerebrosidase (GBA).

Creating potent and selective glycosidase inhibitors is an important goal in medicinal chemistry[1] due to their therapeutic potential in the treatment of a variety of carbohydrate-mediated diseases.[2–12] In this respect, iminosugars are privileged lead compounds because of their complementarity to glycosidase active sites and aspects of the relevant transition states in the hydrolysis processes catalyzed by glycosidases.[13] Glycomimetics that comprise an endocyclic nitrogen, such as the naturally occurring 1-deoxynojirimycin (DNJ, **1**, Figure 1) as well as 1,5-dideoxy-1,5-imino-D-xylitol (DIX, **4**) and their closely related unnatural relative isofagomine (IFG, **6**), are of particular interest[14,15] and a number of syntheses of these compounds have been reported.[16–18] It has also been demonstrated that synthetically modified N-substituted iminosugars often possess improved specificities and potent inhibition toward glycosidases.[19–21] In this context, some N-alkylated iminosugars, such as **2**, **3**, **5**, **7**, **8** and **9**, have already shown promise as potent glycosidase inhibitors.[22–32] Our research group's activities in this area have focused on preparing iminosugar analogues with an sp^2 hybridized endocyclic nitrogen.[33] In doing so both the conformation and charge delocalization of the endocyclic nitrogen atom is altered. These modifications have resulted in interesting specificity changes in comparison with the parent iminosugars.[34] To this end, we recently attempted the synthesis of a series of lipidated DNJ guanidine analogues (compounds **I**, Scheme 1).[35] Interestingly, we found that such N-alkylated guanidine DNJ analogues **I** spontaneously cyclized to generate the corresponding stable bicyclic isoureas **II**. Gratifyingly, the isoureas proved to be very potent and specific inhibitors of β-glucocerebrosidase.[35]

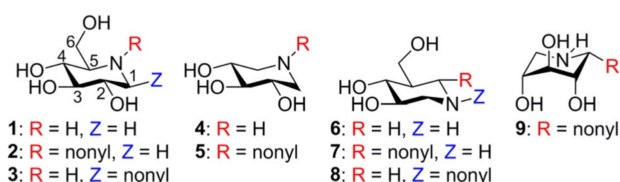

Figure 1. Chemical structures of selected iminosugar-based glycosidase inhibitors.

Our previous studies established that formation of the cyclic isourea **II** proceeds via the guanidine species, which is prone to cyclization by action of the 6-OH group. We here report a strategy designed to circumvent this process wherein N-substituted guanidine analogues of DIX (**4**), lacking the 6-OH group of DNJ, were prepared and found to be stable. Previous reports indicate that a DIX analogue bearing an unsubstituted guanidinium moiety (**10**) displays a 100-fold enhancement in the inhibition of almond β-glycosidase (Figure 2).[36] However, N-guanidino-alkylated variants of DIX (**A**) have not been studied. We here report the synthesis and testing of new guanidinium compounds of type **A** as well as the corresponding urea derivatives **B** (Figure 2) both derived from DIX and lacking the hydroxymethyl found in DNJ that causes the cyclization. Interestingly, it has also been shown that the hydroxymethyl of DNJ can have a detrimental effect on its GBA binding when compared with unsubstituted DIX.[32]

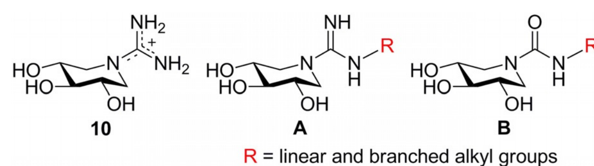

Figure 2. Unsubstituted guanidinium DIX derivative **10**[36] and general structures of **A** guanidine and **B** urea DIX derivatives prepared in this work.

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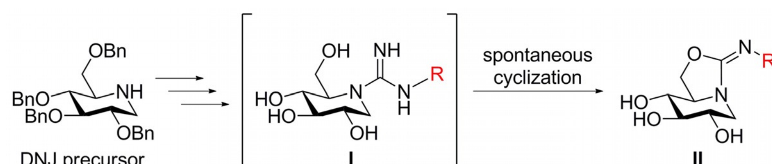
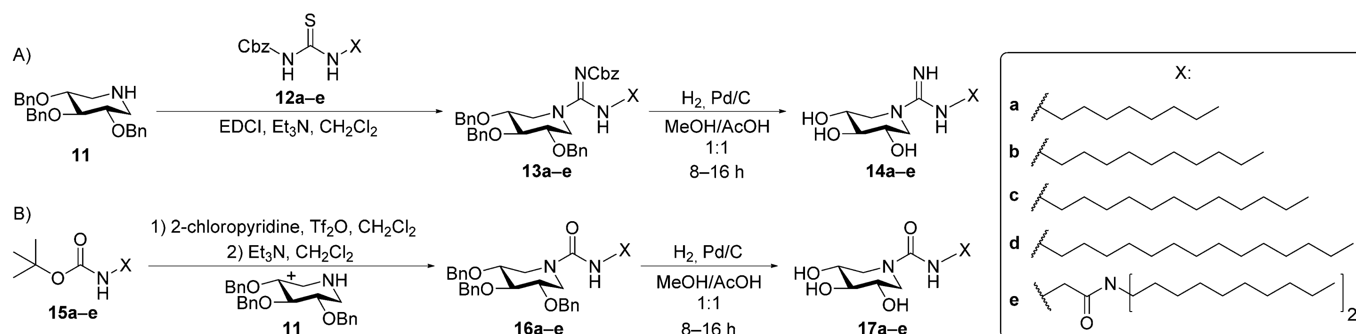


Scheme 1. Previously published spontaneous cyclization of guanidine (**I**) compounds to bicyclic isoureas (**II**) derived from DNJ precursor.[35]



Scheme 2. Synthetic route used to prepare 1,5-dideoxy-1,5-imino-D-xylitol derivatives with N^G-substituted A) guanidine and B) urea analogues.

The synthetic strategy used in preparing the guanidinium and urea analogues of DIX (**4**), is outlined in Scheme 2. Benzyl protected 1,5-dideoxy-1,5-imino-D-xylitol **11** was synthesized according to the literature procedure[37] and used as a starting material for the preparation of both the guanidine and urea analogues. As indicated in Scheme 2 A, treatment of **11** with the appropriate Cbz-protected thiourea (**12 a**–**e**) and EDCI led to clean formation of protected guanidines **13 a**–**e**.[38,39] Removal of Cbz and benzyl groups was achieved via hydrogenation to yield the guanidine products **14 a**–**e**. For the synthesis of the corresponding urea species, a series of Boc-protected amines (**15 a**–**e**) were generated according to a literature procedure.[40] Treatment of the Boc-protected amines with 2-chloropyridine followed by the addition of trifluoromethanesulfonic anhydride resulted in formation of the corresponding isocyanate intermediates that were immediately treated with **11** to yield the protected ureas **16 a**–**e**. Removal of the benzyl groups by hydrogenation provided ureas **17 a**–**e** (Scheme 2 B). The guanidine (**14 a**–**d**) and urea (**17 a**–**d**) series both incorporate different N^G-substituents composed of simple alkyl chains

Table 1. Glycosidase inhibition values obtained for guanidines **14 a**–**e** and ureas **17 a**–**e**.

Compd	IC$_{50}$ [μM][a]							
	α-glu[b]	α-gal[c]	β-glu[d]	β-gal[e]	Nar[f]	GBA[g] pH 7.0	GBA[g] pH 5.2	GALC[g]
14 a	>100	>100	38.14±1.47	24.21±0.14	52.98±2.19	0.245±0.02	0.999±0.092	>10
14 b	>100	>100	26.04±0.87	2.75±0.22	41.47±0.21	0.033±0.004	0.093±0.007	>10
14 c	>100	>100	10.73±0.28	0.68±0.04	37.23±1.67	0.020±0.003	0.038±0.003	>10
14 d	>100	>100	2.84±0.07	1.63±0.21	33.51±1.68	0.019±0.003	0.036±0.002	>10
14 e	>100	>100	>100 000	11.62±4.02	3.92±0.55	0.017±0.003	0.038±0.005	>10
17 a	>100	>100	>100	19.57±1.50	>100	>10	>10	>10
17 b	>100	>100	>100	10.44±0.88	>100	>10	>10	>10
17 c	>100	>100	>100	12.96±1.95	>100	>10	>10	>10
17 d	>100	>100	>100	34.20±1.90	>100	>10	>10	>10
17 e	>100	>100	>100	12.51±2.92	>100	>10	>10	>10
NN-DNJ	>100	>100	>100	>100	0.176±0.012	0.752±0.093	2.564±0.287	>10

[a] Values are averages obtained from triple independent duplicate analysis of each compound. For ease of comparison, IC$_{50}$ values are compared with those of the reference compound NN-DNJ. [b] α-glucosidase (from baker's yeast, Sigma G5003): 0.05 U mL^{-1}, the activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2). [c] α-galactosidase (from green coffee beans, Sigma G8507): 0.05 U mL^{-1}; α-galactosidase activity was determined with *p*-nitrophenyl-α-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 6.8). [d] β-glucosidase (from almond, Sigma G4511): 0.05 U mL^{-1}; the activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0). [e] β-galactosidase (from bovine liver, Sigma G1875): 0.05 U mL^{-1}; activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2). [f] Naringinase (from *Penicillium decumbens*, Sigma N1385): 0.06 U mL^{-1}. the activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0). [g] β-glucocerebrosidase (GBA) and β-galactocerebrosidase (GALC) activities were determined using 4-methylumbelliferyl-β-D-glucopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside, respectively, using assay conditions based on those previously reported.[41]

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ranging from eight to fourteen carbon atoms in length. In addition, bis-lipidated species **14e** and **17e** were also synthesized as more representative substrate mimics for β-glucocerebrosidase. We also confirmed that both the guanidine **14a**–**e** and the urea species **17a**–**e**, are stable in aqueous solution even after 12 days (see Supporting Information, Table S1).

The inhibitory potencies of DIX derivatives **14a**–**e** and **17a**–**e** were determined against a panel of readily available glycosidase enzymes as well as the human recombinant enzymes β-glucocerebrosidase (GBA) and β-galactocerebrosidase (GALC). With the plant enzymes, low micromolar inhibition of the β-glycosidases was observed for **14a**–**e** whereas no inhibition was observed for the α-glycosidases, indicating an interesting preference (Table 1). The corresponding ureas were even more selective displaying inhibition of only the β-galactosidase from bovine liver. We further evaluated the compounds against human recombinant β-specific enzymes. Strikingly, potent inhibition was observed for guanidinium analogues **14a**–**e** against the human recombinant GBA with inhibition constants measured in the low nanomolar range (IC_{50}: 17–245 nM). Despite the observed β-selectivity, the guanidinium compounds did not inhibit the human recombinant galactose specific GALC, indicating a high degree of selectivity among the human enzymes. In contrast, urea species **17a**–**e** did not inhibit any of the human recombinant enzymes. Although the reason for the dramatic difference between the inhibition profile of the guanidine and urea analogues is not clear, the positive charge of the guanidinium group may point to an explanation. As can be seen in Table 1, the length of the lipid appended to the guanidine moiety also has some effect on the inhibition. The longer alkyl tails led to more potent inhibition. To confirm the validity of our assays, we measured the often-used reference compound NN-DNJ (**2**) and found it to have an IC_{50} for GBA of 750 nM, which is similar to previous reports.[32] Also of note is the pH dependence observed for GBA inhibition by compounds **14a**–**e**. In general, the IC_{50} values measured at pH 7.0 were two- to three-fold lower than those measured at pH 5.2 (Table 1).

To evaluate the effect of the substituted guanidinium groups in comparison with a simple *N*-alkylated analogue of DIX, we compared the C8-functionalized guanidine analogue **14a** with the previously reported *N*-alkylated DIX derivative **5** bearing a C9 lipid. Using similar assay conditions, we measured a near 7-fold lower IC_{50} value for compound **14a** (245 nM) relative to that reported for **5** (1500 nm).[32] Similar IC_{50} values were measured for NN-DNJ (**2**) in both studies indicating that the above comparison is legitimate.[32] While previous studies have indicated that *N*-alkylated DIX analogues are moderate glycosidase inhibitors,[30] our data indicate that incorporating an *N*-alkylated guanidino moiety can drastically improve inhibitor potency.

To gain insight into the possible binding mode(s) of guanidinium compound **14a** within the GBA active site, molecular modeling was performed (Figure 3 A,B; see the Supporting Information for detailed description of docking experiments on page S29). A comparison was made to the reported complex of NN-DNJ (**2**). It is clear that the guanidinium of **14a** is capable of making additional cation–π interactions with the nearby Tyr313 in comparison to the smaller amino function of NN-DNJ (**2**). Furthermore, the guanidinium group engages in a hydrogen bond/salt bridge with nearby Glu235 that has no counterpart in the structure of NN-DNJ (**2**). Both features likely contribute to the enhanced binding of **14a**. This enhanced binding was also the predicted outcome of the performed local docking simulation, which resulted in a calculated K_i of 155 nM for **14a** and of 1150 nM for NN-DNJ (**2**).

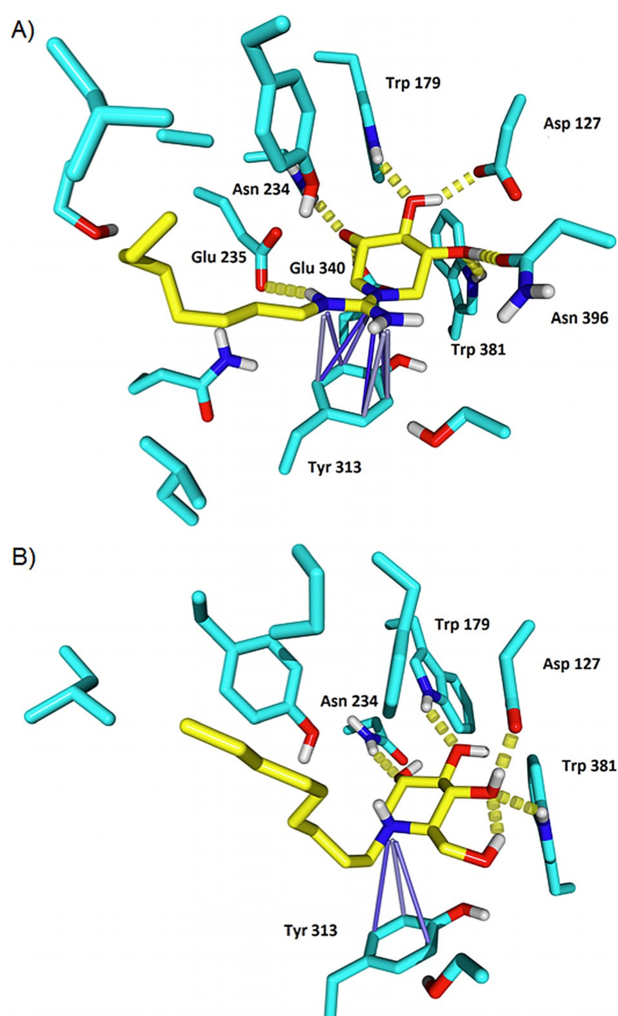

Figure 3. A) The polar interactions of compound **14a** (yellow carbon atoms) with GBA residues. In blue we see numerous cation–π interactions of the guanidinium group with Tyr313. Dashed yellow lines are the hydrogen bonds from the sugar hydroxy groups and the ion–ion interaction between the guanidinium group with Glu235. B) The polar interactions of NN-DNJ (**2**, yellow carbon atoms) with GBA residues after docking and minimization. We see fewer interactions of NN-DNJ with Tyr313 and none with Glu235.

Although much research is still needed to fully determine their pharmacological chaperone function,[10] preliminary data from experiments using Gaucher patient-derived fibroblasts homozygous for N370S mutation, indicate that **14a** possesses a minor chaperone activity, somewhat weaker than the known chaperone NN-DNJ (**2**) (see Supporting Information, Figure S1).

In conclusion, we report a series of stable iminosugar based glycosidase inhibitors that contain either an exocyclic *N*-alkylat-

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ed guanidinium or urea moiety. Interestingly, the DIX-derived ureas (**17a**–**e**) were selective inhibitors of β-galactosidase from bovine liver. By comparison, the guanidinium analogues (**14a**–**e**) were found to be highly selective inhibitors of the human β-glycosidase GBA. Our study clearly indicates that the addition of a guanidinium moiety leads to more potent inhibition of GBA when compared to the reported alkylated amine compound (**5**). The inhibitory potency is increased with longer alkyl substituents with the measured inhibition constants ranging from 245 nM to 19 nM for compounds **14a**–**d**. In addition, the bis-lipidated analogue **14e** served as a close substrate mimic for β-glucocerebrosidase and proved to be on par with our most potent inhibitors **14b**–**d** with an IC_{50} of 17 nM. Docking studies also point to additional cation–π interactions, as well as an extra hydrogen bond/salt bridge to the guanidinium group, as a plausible explanation for the enhanced glycosidase inhibition exhibited by **14a**–**e**. More comprehensive studies examining the potential for the DIX analogues reported here, to serve as pharmacological chaperones will be reported in due course.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Gaucher disease · glycosidase inhibitors · guanidinium · iminosugars · pharmacological chaperones · β-glucocerebrosidase

[1] G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams, R. Storer, *Drug Discovery Today* **2011**, *16*, 107–118.
[2] J. Churruca, V. Luis, E. Luna, J. Ruiz-Galiana, V. Manuel, *Diabetes Metab. Syndr. Obes.* **2008**, *1*, 3–11.
[3] X. Gao, H. Yang, Y. Xu, Y. Xiong, G. Wang, X. Ye, J. Ye, *Int. Immunopharmacol.* **2014**, *23*, 688–695.
[4] D. Ruhela, P. Chatterjee, R. A. Vishwakarma, *Org. Biomol. Chem.* **2005**, *3*, 1043–1048.
[5] S. Hussain, J. L. Miller, D. J. Harvey, Y. Gu, P. B. Rosenthal, N. Zitzmann, J. W. Mccauley, *J. Antimicrob. Chemother.* **2015**, *70*, 136–152.
[6] G. N. Wang, Y. Xiong, J. Ye, L. H. Zhang, X. S. Ye, *ACS Med. Chem. Lett.* **2011**, *2*, 682–686.
[7] D. A. Kuntz, S. Nakayama, K. Shea, H. Hori, Y. Uto, H. Nagasawa, D. R. Rose, *ChemBioChem* **2010**, *11*, 673–680.
[8] F. M. Platt, *Nature* **2014**, *510*, 68–75.
[9] G. Parenti, G. Andria, K. J. Valenzano, *Mol. Ther.* **2015**, *23*, 1138–1148.
[10] M. Convertino, J. Das, N. V. Dokholyan, *ACS Chem. Biol.* **2016**, *11*, 1471–1489.
[11] W. W. Kallemeijn, M. D. Witte, T. M. Voorn-Brouwer, M. T. C. Walvoort, K. Y. Li, J. D. C. Codée, G. A. Van Der Marel, R. G. Boot, H. S. Overkleeft, J. M. F. G. Aerts, *J. Biol. Chem.* **2014**, *289*, 35351–35362.
[12] K.-Y. Li, J. Jiang, M. D. Witte, W. W. Kallemeijn, W. E. Donker-Koopman, R. G. Boot, J. M. F. G. Aerts, J. D. C. Codée, G. A. van der Marel, H. S. Overkleeft, *Org. Biomol. Chem.* **2014**, *12*, 7786–7791.
[13] P. Compain, O. R. Martin, *Iminosugars: From Synthesis to Therapeutic Applications*, Wiley, Chichester, **2007**.
[14] M. Yagi, T. Kouno, Y. Aoyagi, H. Murai, *J. Agric. Chem. Soc. Jpn.* **1976**, *50*, 571–572.
[15] T. Sekioka, M. Shibano, G. Kusano, *Nat. Med.* **1995**, *49*, 332–335.
[16] A. B. Hughes, A. J. Rudge, *Nat. Prod. Rep.* **1994**, *11*, 135–162.
[17] R. C. Bernotas, G. Papandreou, J. Urbach, B. Oanem, *Tetrahedron Lett.* **1990**, *31*, 3393–3396.
[18] T. M. Jespersen, M. Bols, *Tetrahedron* **1994**, *50*, 13449–13460.
[19] E. M. Sánchez-Fernández, J. M. G. Fernandez, C. O. Mellet, *Chem. Commun.* **2016**, *52*, 5497–5515.
[20] A. T. Ghisaidoobe, R. J. B. H. N. Van Den Berg, S. S. Butt, A. Strijland, W. E. Donker-koopman, S. Scheij, A. M. C. H. Van Den Nieuwendijk, G. Koomen, A. Van Loevezijn, M. Leemhuis, et al., *J. Med. Chem.* **2014**, *57*, 9096–9104.
[21] A. Hottin, D. W. Wright, G. J. Davies, J. B. Behr, *ChemBioChem* **2015**, *16*, 277–283.
[22] A. R. Sawkar, W.-C. Cheng, E. Beutler, C.-H. Wong, W. E. Balch, J. W. Kelly, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15428–15433.
[23] G. Godin, P. Compain, O. R. Martin, K. Ikeda, L. Yu, N. Asano, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5991–5995.
[24] N. T. Patil, S. John, S. G. Sabharwal, D. D. Dhavale, *Bioorg. Med. Chem.* **2002**, *10*, 2155–2160.
[25] G. Pandey, M. Kapur, M. I. Khan, S. M. Gaikwad, *Org. Biomol. Chem.* **2003**, *1*, 3321.
[26] H. Han, *Tetrahedron Lett.* **2003**, *44*, 1567–1569.
[27] H. Ouchi, Y. Mihara, H. Takahata, *J. Org. Chem.* **2005**, *70*, 5207–5214.
[28] C. Parmeggiani, S. Catarzi, C. Matassini, G. D′Adamio, A. Morrone, A. Goti, P. Paoli, F. Cardona, *ChemBioChem* **2015**, *16*, 2054–2064.
[29] A. E. Mccaig, B. Chomier, R. H. Wightman, *J. Carbohydr. Chem.* **1994**, *13*, 397–407.
[30] G.-N. Wang, G. Reinkensmeier, S.-W. Zhang, J. Zhou, L.-R. Zhang, L.-H. Zhang, T. D. Butters, X.-S. Ye, *J. Med. Chem.* **2009**, *52*, 3146–3149.
[31] X. Zhu, K. A. Sheth, S. Li, H.-H. Chang, J.-Q. Fan, *Angew. Chem. Int. Ed.* **2005**, *44*, 7450–7453; *Angew. Chem.* **2005**, *117*, 7616–7619.
[32] P. Compain, O. R. Martin, C. Boucheron, G. Godin, L. Yu, K. Ikeda, N. Asano, *ChemBioChem* **2006**, *7*, 1356–1359.
[33] N. I. Martin, J. J. Woodward, M. A. Marletta, *Org. Lett.* **2006**, *8*, 4035–4038.
[34] R. Kooij, H. M. Branderhorst, S. Bonte, S. Wieclawska, N. I. Martin, R. J. Pieters, *MedChemComm* **2013**, *4*, 387.
[35] A. Sevšek, M. Čelan, B. Erjavec, L. Q. van Ufford, J. S. Toraño, E. E. Moret, R. J. Pieters, N. I. Martin, *Org. Biomol. Chem.* **2016**, *14*, 8670–8673.
[36] J. Lehmann, B. Rob, *Carbohydr. Res.* **1995**, *272*, C11–C13.
[37] R. G. Boot, M. Verhoek, W. Donker-Koopman, A. Strijland, J. Van Marle, H. S. Overkleeft, T. Wennekes, J. M. F. G. Aerts, *J. Biol. Chem.* **2007**, *282*, 1305–1312.
[38] N. I. Martin, W. T. Beeson, J. J. Woodward, M. A. Marletta, *J. Med. Chem.* **2008**, *51*, 924–931.
[39] N. I. Martin, R. M. J. Liskamp, *J. Org. Chem.* **2008**, *73*, 7849–7851.
[40] C. Helgen, C. G. Bochet, *J. Org. Chem.* **2003**, *68*, 2483–2486.
[41] A. Trapero, P. González-Bulnes, T. D. Butters, A. Llebaria, *J. Med. Chem.* **2012**, *55*, 4479–4488.

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