Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1713–1716

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design, Synthesis and Biological Evaluation of Hetaryl-Nucleoside Derivatives as Inhibitors of Chitin Synthase

Jean-Bernard Behr, Thierry Gourlain, Abdellatif Helimi and Georges Guillerm*

Laboratoire Réactions Sélectives et Applications UMR 6519, UFR Sciences, CNRS BP 1039, 51687 Reims Cedex 2, France

Received 3 January 2003; revised 4 February 2003; accepted 3 March 2003

Abstract—We report here the design, synthesis and biological evaluation of new models of sugar analogues for chitin synthase. These UDP-GlcNAc mimetics associate a sugar-mimicking hetaryl group and uridine, linked with different pyrophosphate bioisosteres. The compounds displayed weak inhibition activity on chitin synthase and their antifungal potencies have been assayed against a large variety of pathogenic fungi.

In the 25 past years, a steep increase in life-threatening fungal infections has occurred and resistance development to the currently administered treatments has urged the development of new antifungal agents.[1] In this context, chitin synthase (CS) a specific enzyme present in fungi and essential for cell viability was envisaged as an attractive target.[2] The first isolated CS inhibitors were the peptidyl nucleoside Polyoxins and Nikkomycins, among which Nikkomycin Z **1** (Fig. 1) was the most promising candidate as antifungal drug.[3] Some recent studies described new nucleoside analogues as potent competitive inhibitors of CS,[4] as well as non-competitive inhibitors, lacking any structural resemblance with the natural substrate UDP-GlcNAc, but displaying K_i's in the sub-nanomolar range.[5] However, the modest antifungal properties displayed by these new CS inhibitors, implied further research in this field.

In view of our interest in new antifungal agents, we designed hetaryl nucleoside derivatives **2**–**4** (Fig. 1) as new models of CS inhibitors, in the prospect of improving their antifungal properties. The easily synthesized heteroaromatic ring might serve as suitable glycopyranosyl surrogate[6] and might favourably interact with the hydrophobic binding area in the active site of CS.[4b] It is worth noting that Nikkomycin Z utilizes an analogous hydroxypyridine, providing conformational mimicry of the transition-state in the presumed glycosyl transfer reaction catalyzed by chitin synthase.[7]

In this study, malonic as well as tartaric acid or carbohydrate-based linkages were chosen as non-hydrolizable analogues of the pyrophosphate portion of UDP-GlcNAc, mimicking the probable six-membered ring pyrophosphate–M^{2+} complex formed in the active site (Fig. 1).[8] In this paper, we report the synthesis of these

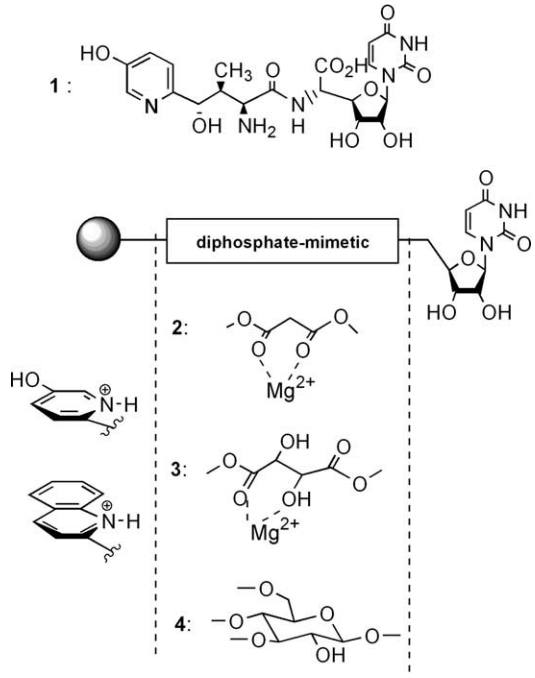


Figure 1. Design of new chitin synthase inhibitors **2**–**4**.

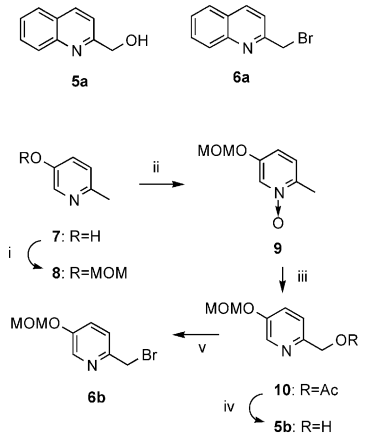
*Corresponding author. Tel.: +33-326-913-308; fax: +33-326-913-166; e-mail: georges.guillerm@univ-reims.fr

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00239-7

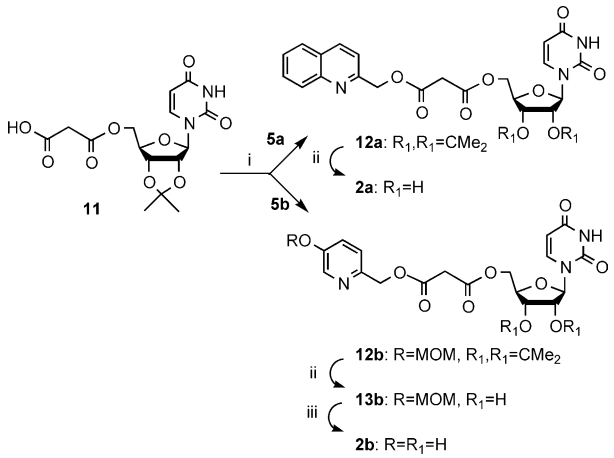
new sugar-nucleoside analogues, their interaction with CS at the active site and their antifungal activity.

The 2-hydroxymethylquinoline **5a**[9] and the corresponding bromide **6a**[10] were prepared in one step according to known procedures. The hydroxypyridine derivatives **5b** and **6b** were obtained starting from 2-methyl-5-hydroxypyridine **7** after protection of the free hydroxyl group with MOM-chloride (Scheme 1).[11]

Malonic models **2a**,**b** were obtained by coupling alcohols **5a**,**b** with the known uridinyl malonate **11** (Scheme 2).[7] This esterification was best achieved with DCC and HOBT as activators in the presence of a catalytic amount of pyrrolidinopyridine (PPY). Conversion was somewhat low and could be improved by adding an excess of DCC and acid **11** during the course of the reaction. After filtration to remove DCU, purification of the crude material by FC (ethyl acetate/diethyl ether, v/v, 1/1) gave malonic diesters **12a,b**. Deprotection of the acid-sensitive protective groups and purification by FC (ethyl acetate/CH_3CN, v/v, 9/1) gave pure **2a**,**b**. The partially deprotected intermediate **13b** was isolated and assayed to evaluate the influence of hydrophobic character of the pyridinyl moiety on enzyme's recognition.



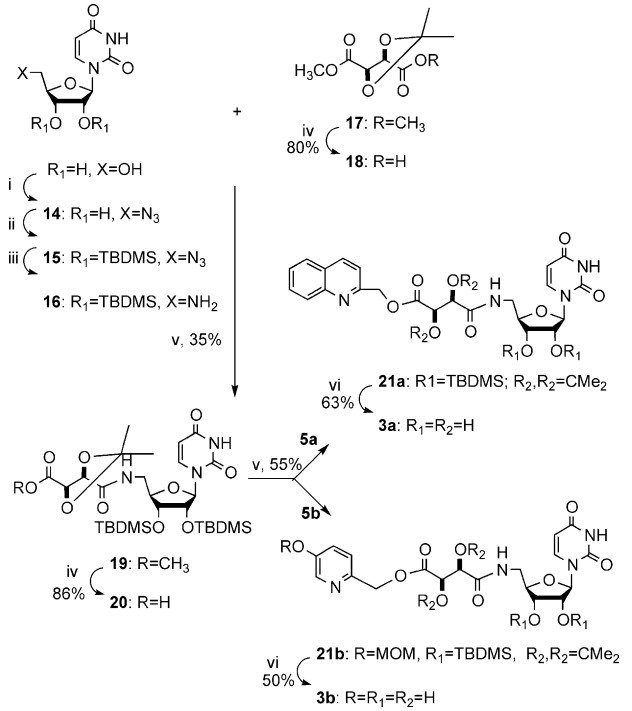
Scheme 1. (i) MOMCl, iPr_2NEt, CH_2Cl_2 (94%); (ii) *m*-CPBA, $CHCl_3$ (94%); (iii) Ac_2O, 120 °C (75%); (iv) KOH, MeOH (85%); (v) PPh_3, CBr_4, CH_2Cl_2 (100%).



Scheme 2. (i) DCC, HOBT, PPY, CH_2Cl_2/DMF (46%); (ii) 80% aq HCO_2H, CH_3CN (73%); (iii) 90% TFA (52%).

Synthesis of non-symmetrical L-tartaric acid derivatives **3a**,**b** (Scheme 3) implied the differentiation of both linkages. For this reason, we synthesized the known 5′-amino-5′-deoxy uridine derivative **16**[12] by direct azidation of uridine,[13] protection with TBDMS-Cl and azide reduction with a catalytic amount of 1,3-propanedithiol and $NaBH_4$ as a co-reagent. Tartaric monomethyl ester **18**, readily obtained by mono-saponification of commercial **17**, was reacted with amine **16** under the conditions stated above, to give tartaric nucleoside **19**. As expected, saponification with KOH/MeOH occurred specifically on the ester function without affecting the amide bond and yielded acid **20**, after neutralization with Dowex (H^+) resin. Esterification of **20** with alcohols **5a,b**, purification of the resulting esters **21a**,**b** (FC, ethyl acetate/petroleum ether, v/v, 7/3) and subsequent deprotection (90% aqueous TFA) gave pure crystalline UDP-GlcNAc analogues **3a**,**b** after subsequent washings in $CHCl_3$.

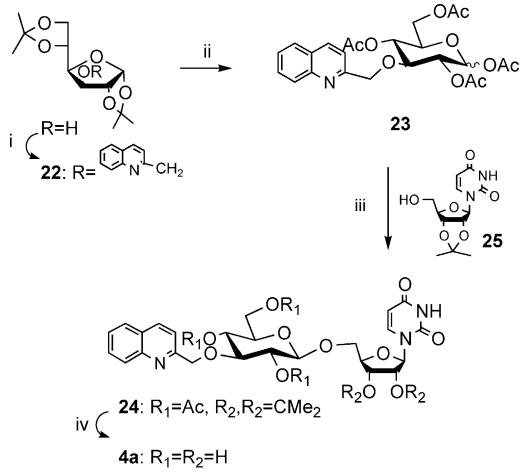
The structural similarity of a monosaccharide moiety and a pyrophosphate–M^{2+} complex was first pointed out to explain the high potency of tunicamycin to bind to the catalytic site of GlcNAc phosphotransferase.[14] In search for new galactosyltransferase inhibitors, Wong and co-workers demonstrated that (1,4)-linked glucose could also serve as a pyrophosphate mimic.[8] In this study, we investigated (1,3)-, (1,4)- or (1,6)-linked glucose units as pyrophosphate substitutes. In each proposed model, the chair-like carbohydrate could mimic the six-membered diphosphate–metal complex as



Scheme 3. (i) PPh_3, CBr_4, NaN_3, DMF (80%); (ii) TBDMSCl, imidazole, pyr. (89%); (iii) 1,3-propanedithiol, $NaBH_4$, NEt_3, *i*PrOH (90%); (iv) KOH (1.2 equiv), MeOH, then Dowex 50WX-8 (H^+) (80%); (v) DCC, HOBT, PPY, CH_2Cl_2/DMF; (vi) 90% TFA.

discussed above. In addition, comparison of the binding affinity of the corresponding **4a–c** regioisomers should give an evaluation of the distance fitting the O-P-O-P-O five-atom bridge. The (1,3)-substituted glucosyl residue **4a** was prepared starting from diacetone-D-glucose after alkylation with bromide **6a** (Scheme 4). Deprotection of acetonides (80% aqueous TFA) and subsequent acetylation gave **23**, which was glycosylated to **24** with 2′,3′-*O*-isopropylideneuridine **25**, using standard procedures. Pure **24** (silica gel FC, CH_2Cl_2/MeOH, v/v, 95/5) was treated with MeONa/MeOH and formic acid (80% aqueous solution) to remove protecting groups. Compound **4a** was purified by FC on a HP20 hydrophobic support, eluted with a gradient of water/methanol (from v/v, 50/50 to 20/80) before biological evaluation.

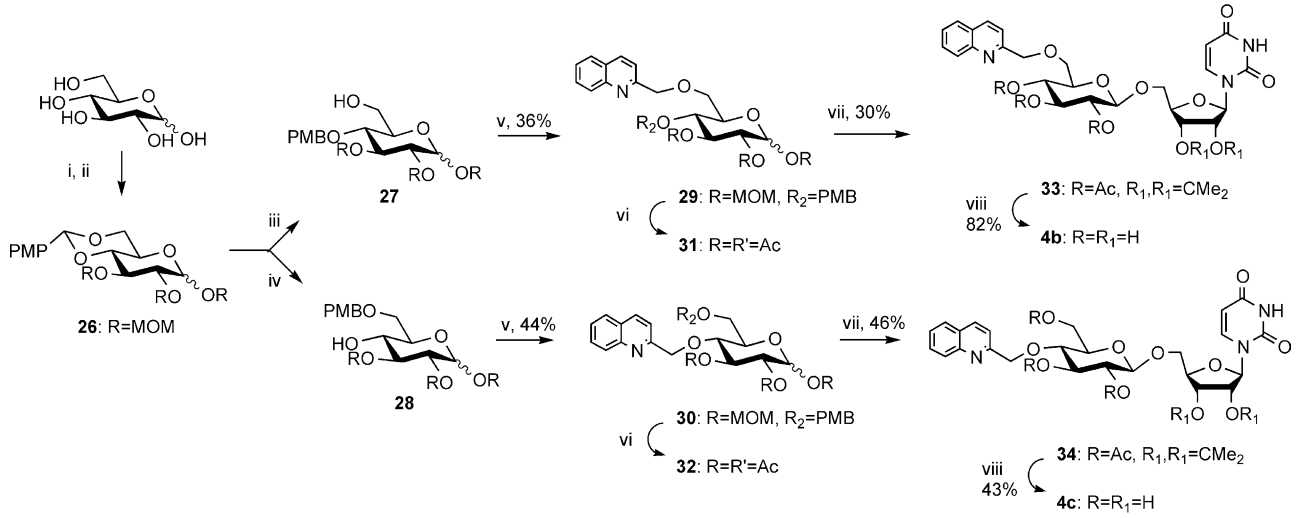
The (1,4)- and (1,6)-substituted monosaccharide linkages were prepared by regioselective ring opening of the readily prepared methoxybenzylidene-glucose derivative **26** (Scheme 5).[15] According to the reaction conditions,[16] either the 6-OH or the 4-OH was kept free to yield respectively **27** or **28** as major product. Subsequent alkylation with **6a** gave hetaryl-glucose compounds **29**, **30** which were deprotected (80% aqueous TFA) and further acetylated (Ac_2O, pyridine) to give **31**, **32** as suitable substrates for the glycosylation step with **25**. Deprotection and purification in the conditions described above, led to pure UDP-GlcNAc analogues **4b**,**c**. All the new compounds **2–4** gave satisfactory analytical data.[17]



Scheme 4. (i) **6a**, NaH, DMF (80%); (ii) 80% aq TFA, then Ac_2O, pyr. (63%); (iii) TMSOTf, 3 Å MS, CH_2Cl_2 (36%); (iv) MeONa/MeOH, then 80% HCOOH (63%).

For the inhibition studies, enzymatic assays were performed using *Saccharomyces cerevisiae* cells (X2180 strain), which were permeabilized according to a recently reported procedure.[18] Activity of Chitin Synthase I was assayed specifically by measuring the rate of formation of [^{14}C]-chitin from UDP-*N*-acetyl-[^{14}C]-glucosamine, according to the standard method reported by Choi and Cabib.[19] The reaction rate of chitin synthase showed normal Michaelis–Menten kinetics with UDP-GlcNAc as the variable substrate and GlcNAc as the fixed substrate. A K_m value of 0.5 mM was determined for UDP-GlcNAc from Lineweaver–Burk plot, which was in good agreement with earlier reported values. The standard Nikkomycin Z **1** strongly inhibited our chitin synthase preparation ($IC_{50} = 0.50$ μM, $K_i = 0.34$ μM, competitive). The CS inhibitory activity of **2–4** were examined under the same conditions and the corresponding IC_{50} values were determined by the Dixon method (Table 1). As shown on Table 1, compounds **2–4** displayed weak activity on CS, when compared to **1** used as reference.

Antifungal activity was also evaluated against *Candida albicans, Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* or *Cryptococcus neoformans*, according to the standard protocol.[20] No significant activity of compounds **2–4** was noted on this panel of strains. Growth inhibition was only detected with **4b**, at 256 μg/mL (80% inhibition for *C. neoformans*, 30% inhibition for *C. albicans*,



Scheme 5. (i) p-$CH_3OPhCH(OCH_3)_2$, TsOH, DMF, 70 °C, 150 torr (70%); (ii) MOMCl, *i*Pr_2NEt, CH_2Cl_2, 45 °C (90%); (iii) $NaBH_3CN$, TMS, 3 Å MS, CH_3CN (75%); (iv) $NaBH_3CN$, TFA, 3 Å MS, DMF (75%); (v) NaH, DMF, **6a**; (vi) 80% aq TFA, then Ac_2O, pyr.; (vii) **25**, TMSOTf, 3 Å MS, CH_2Cl_2; (viii) MeONa/MeOH, then 80% HCO_2H.

Table 1. Inhibition of chitin synthase with compounds **1**–**4**

Compd	CSI inhibition (IC_{50}, mM)
1	0.00034 (±0.00002)
2a	2.9 (±0.4)
13b	10.8 (±2)
2b	2.8 (±0.4)
3a	2.0 (±0.2) (K_i = 0.8 mM)[a]
3b	3.2 (±0.4)
4a	0.8 (±0.1) (K_i = 0.25 mM)[a]
4b	3.2 (±0.4)
4c	2.0 (±0.2)

[a]Competitive.

S. cerevisiae and *A. fumigatus*). Analogue **4c** showed some weak activity against *C. albicans* at the same high concentration.

In conclusion, the new hetaryl nucleosides **2**–**4**, designed to mimic the sugar-nucleoside donor at the transition-state during the glycosyl transfer process, did not improve the binding affinity as expected. From these results and others,[21] it appeared until now that attempts to inhibit the catalytic activity of CS with a single UDP-GlcNAc analogue, were mostly inefficient. Targeting, with a multicomponent adduct, the multi-binding sites suggested to be present in this processive glycosyl-transferase,[22] could help the discovery of potent inhibitors of this enzyme. Work is in progress to evaluate this new model.

Acknowledgements

The authors thank Dr. N. S. Ryder (Novartis Research Institute, A-1235 Vienna) for the antifungal evaluation. This work was supported by a grant (T.G.) from the *Conseil général de la Marne* and *Europol'Agro*.

References and Notes

1. (a) Turner, W. W.; Rodriguez, M. J. *Curr. Pharm. Des.* **1996**, *2*, 209. (b) Fostel, J. M.; Lartey, P. A. *Drug Discov. Today* **2000**, *5*, 25. (c) Hossain, M. A.; Ghannoum, M. A. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1797.
2. Maertens, J. A.; Boogaerts, M. A. *Curr. Pharm. Des.* **2000**, *6*, 225.
3. Zhang, D.; Miller, M. J. *Curr. Pharm. Des.* **1999**, *5*, 73.
4. (a) Suda, A.; Ohta, A.; Sudoh, M.; Tsukuda, T.; Shimma, N. *Heterocycles* **2001**, *55*, 1023. (b) Obi, K.; Uda, J.-I.; Iwase, K.; Sugimoto, O.; Ebisu, H.; Matsuda, A. *Bioorg. Chem. Med. Lett.* **2000**, *10*, 1451.
5. Sudoh, M.; Yamazaki, T.; Masubuchi, K.; Taniguchi, M.; Shimma, N.; Arisawa, M.; Yamada-Okabe, H. *J. Biol. Chem.* **2000**, *275*, 32901.
6. (a) Knapp, S.; Dong, Y.; Withers, S. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 763. (b) Bhattacharya, A. K.; Stolz, F.; Schmidt, R. R. *Tetrahedron Lett.* **2001**, *42*, 5393.
7. Behr, J.-B.; Gautier-Lefebvre, I.; Mvondo-Evina, C.; Guillerm, G. *J. Enz. Inhib.* **2001**, *16*, 107.
8. Wang, R.; Steensma, D. H.; Takaoka, Y.; Joanne, W. Y.; Kajimoto, T.; Wong, C. H. *Bioorg. Med. Chem.* **1997**, *5*, 661.
9. Chiu, Y.-H.; Dos Santos, O.; Canary, J. W. *Tetrahedron* **1999**, *55*, 12069.
10. Rieger, B.; Abu-Surrah, A. S.; Fawzi, R.; Steinman, M. *J. Organomet. Chem.* **1995**, *497*, 73.
11. Linderman, R. J.; Jaber, M.; Griedel, B. D. *J. Org. Chem.* **1994**, *59*, 6499.
12. Peterson, M. A.; Nilsson, B. L.; Sarker, S.; Doboszewski, B.; Zhang, W.; Robins, M. J. *J. Org. Chem.* **1999**, *64*, 8183.
13. Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1980**, 306.
14. Camarasa, M.-J.; Fernandez-Resa, P.; Garcia-Lopez, M.-T.; Heras, F.; Mendez-Castrillon, P. P.; Alarcon, B.; Carrasco, L. *J. Med. Chem.* **1985**, *28*, 40.
15. Fürstner, A.; Radkowski, K.; Grabowski, J.; Wirtz, C.; Mynott, R. *J. Org. Chem.* **2000**, *65*, 8758.
16. Johansson, R.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2371.
17. Selected data, **2a**: ^{1}H NMR (DMSO-d_6, 500 MHz, δ ppm, J = Hz) 8.25 (d, 1H, J = 8.6 Hz, Ar–H), 7.90 (d, 1H, J = 8.6 Hz, Ar–H), 7.85 (d, 1H, J = 8.6 Hz, Ar–H), 7.66 (t, 1H, J = 2×8.6, Ar-H), 7.50 (m, 3H, Ar–H, H-6), 5.80 (d, 1H, J = 3.7 Hz, H-1′), 5.60 (d, 1H, J = 8.0 Hz, H-5), 5.41 (s, 2H, Ar–CH_2O), 4.40 (m, 2H, H-5′), 4.10 (m, 2H, H-3′, H-4′), 4.06 (m, 1H, H-2′), 3.71 (s, 2H, C(O)CH_2C(O)). –^{13}C NMR (DMSO-d_6, 62.5 MHz) 175.5, 166.1, 165.7, 163.2, 150.5, 146.9, 140.2, 136.9, 129.6, 128.5, 127.6, 127.1, 126.5, 119.1, 102.0, 89.2, 80.9, 73.2, 69.5, 67.6, 64.5, 41.0. – CI-MS (NH_3): m/z = 472 (MH^+, 10%), 160 (100%). **3a**: ^{1}H NMR (CD_3OD, 500 MHz) 8.47 (d, 1H, J = 8.5 Hz, Ar-H), 8.07 (d, 1H, J = 8.5 Hz, Ar-H), 7.99 (d, 1H, J = 8.5 Hz, Ar-H), 7.82 (t, 1H, J = 2×8.5 Hz, Ar-H), 7.73 (d, 1H, J = 8.5 Hz, Ar-H), 7.68 (d, 1H, J = 8.1 Hz, H-6), 7.65 (t, 1H, Ar-H), 5.82 (d, 1H, J = 4.7 Hz, H-1′), 5.72 (d, 1H, J = 8.1 Hz, H-5), 5.60 (d, 1H, J_{AB} = 14.1 Hz, ArCH_2O), 5.53 (d, 1H, J_{AB} = 14.1 Hz, ArCH_2O), 4.79 (d, 1H, J = 2.2 Hz, CHOH), 4.64 (d, 1H, J = 2.2 Hz, CHOH), 4.21 (t, 1H, J = 2×4.9 Hz, H-2′), 4.10 (t, 1H, J = 2×5.0 Hz, H-3′), 4.02 (q, 1H, J = 3×4.9 Hz, H-4′), 3.75 (dd, 1H, J = 4.9 Hz 10.5, H-5′a), 3.51 (dd, 1H, J = 4.9 Hz 10.5, H-5′b).). –^{13}C NMR (CD_3OD, 125 MHz) 169.7, 169.6, 166.3, 159.4, 152.0, 147.5, 143.1, 139.9, 131.8, 129.0, 128.2, 127.8, 127.6, 120.5, 103.0, 91.1, 83.7, 74.3, 73.6, 73.5, 71.5, 67.3, 40.8. – CI-MS (NH_3): m/z = 517 (MH^+, 5%), 303 (60%), 160 (100%). **4a**: ^{1}H NMR (CD_3OD, 500 MHz) 8.48 (bs, 1H, NH), 8.34 (d, 1H, J = 8.5 Hz, Ar-H), 8.04 (d, 1H, J = 8.1 Hz, H-6), 8.00 (d, 1H, J = 8.5 Hz, Ar-H), 7.80 (d, 1H, J = 8.5 Hz, Ar-H), 7.77 (t, 1H, J = 2×8.5 Hz, Ar–H), 7.60 (m, 2H, Ar–H), 5.94 (d, 1H, J = 4.9 Hz, H-1′), 5.80 (d, 1H, J = 8.1 Hz, H-5), 5.22 (s, 2H, ArCH_2O), 4.49 (d, 1H, J = 7.8 Hz, H-1″), 4.35 (t, 1H, J = 2×4.9 Hz, H-3′), 4.30 (t, 1H, J = 4.9 Hz, H-2′), 4.24 (m, 2H, H-4′, H-5′a), 3.93 (dd, 1H, J = 2.0 Hz 12.2, H-6″a), 3.85 (m, 1H, H-5′b), 3.74 (dd, 1H, J = 5.9 Hz 12.2, H-6″b), 3.59 (m, 2H, H-4″, H-3″), 3.49 (t, 1H, J = 2×7.8 Hz, H-2″), 3.42 (m, 1H, H-5″). –^{13}C NMR (CD_3OD, 125 MHz) 166.5, 160.4, 152.6, 147.5, 142.6, 138.7, 131.0, 128.9, 128.7, 127.8, 127.6, 120.5, 103.5, 102.6, 89.9, 87.1, 84.5, 77.3, 75.2, 74.9, 74.6, 70.9, 70.8, 69.1, 61.9. – CI-MS (NH_3): m/z = 549 (MH^+, 15%), 220 (100%).
18. Crotti, L. B.; Drgon, T.; Cabib, E. *Anal. Biochem.* **2001**, *292*, 8.
19. Choi, W.-J.; Cabib, E. *Anal. Biochem.* **1994**, *219*, 368.
20. Ryder, N. S.; Wagner, S.; Leitner, I. *Antimicrob. Agents Chemother.* **1998**, *42*, 1057.
21. (a) Xie, J.; Thellend, A.; Becker, H.; Vidal-Cros, A. *Carbohydrate Res.* **2001**, *334*, 177. (b) Gautier-Lefevre, I.; Behr, J.-B.; Guillerm, G.; Ryder, N. S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1483.
22. Saxena, I. M.; Brown, R. M.; Fèvre, M.; Geremia, R. A.; Henrissat, B. *J. Bacteriol.* **1995**, *177*, 1419.