



Article First Synthesis of DBU-Conjugated Cationic Carbohydrate Derivatives and Investigation of Their Antibacterial and Antifungal Activity

Fruzsina Demeter¹, Patrik Török¹, Alexandra Kiss², Richárd Kovásznai-Oláh², Zsuzsa Máthéné Szigeti², Viktória Baksa², Fruzsina Kovács^{3,4}, Noémi Balla^{3,4}, Ferenc Fenyvesi⁵, Judit Váradi⁵, Anikó Borbás^{1,*} and Mihály Herczeg^{1,*}

- ¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary
- ² Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary
- ³ Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary
- ⁴ Doctoral School of Pharmaceutical Sciences, University of Debrecen, H-4032 Debrecen, Hungary
- ⁵ Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Nagyerdei Körút 98, H-4032 Debrecen, Hungary
- * Correspondence: borbas.aniko@pharm.unideb.hu (A.B.); herczeg.mihaly@pharm.unideb.hu (M.H.); Tel.: +36-52-512-900 (ext. 22472) (M.H.)

Abstract: The emergence of drug-resistant bacteria and fungi represents a serious health problem worldwide. It has long been known that cationic compounds can inhibit the growth of bacteria and fungi by disrupting the cell membrane. The advantage of using such cationic compounds is that the microorganisms would not become resistant to cationic agents, since this type of adaptation would mean significantly altering the structure of their cell walls. We designed novel, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)-derived amidinium salts of carbohydrates, which may be suitable for disturbing the cell walls of bacteria and fungi due to their quaternary ammonium moiety. A series of saccharide-DBU conjugates were prepared from 6-iodo derivatives of D-glucose, D-mannose, D-altrose and D-allose by nucleophilic substitution reactions. We optimized the synthesis of a D-glucose derivative, and studied the protecting group free synthesis of the glucose-DBU conjugates. The effect of the obtained quaternary amidinium salts against *Escherichia coli* and *Staphylococcus aureus* bacterial strains and *Candida albicans* yeast was investigated, and the impact of the used protecting groups and the sugar configuration on the antimicrobial activity was analyzed. Some of the novel sugar quaternary ammonium compounds with lipophilic aromatic groups (benzyl and 2-napthylmethyl) showed particularly good antifungal and antibacterial activity.

Keywords: cationic carbohydrate; amidinium salt; quaternary ammonium compound; antifungal; antibacterial

1. Introduction

In our rapidly evolving and changing world, in addition to viral infections, another serious problem is the resistance of bacteria to the currently used antibiotics, which means that these preparations must be continuously developed [1–5]. A less mentioned but at least as serious problem is that human pathogenic fungi can similarly become resistant to the antifungal agents used today [6].

The problem with the current treatment of bacterial and fungal diseases is that most of the drugs used affect the life cycle of microbes [7]. The pathogenic organism can adapt relatively easily to this type of intervention through spontaneous mutation [8]. Diseases caused by fungi can be divided into two large groups: (a) superficial mycosis (infection of



Citation: Demeter, F.; Török, P.; Kiss, A.; Kovásznai-Oláh, R.; Szigeti, Z.M.; Baksa, V.; Kovács, F.; Balla, N.; Fenyvesi, F.; Váradi, J.; et al. First Synthesis of DBU-Conjugated Cationic Carbohydrate Derivatives and Investigation of Their Antibacterial and Antifungal Activity. *Int. J. Mol. Sci.* **2023**, *24*, 3550. https://doi.org/10.3390/ijms24043550

Academic Editors: Magdalena Cal and Zsolt Szakonyi

Received: 29 December 2022 Revised: 31 January 2023 Accepted: 7 February 2023 Published: 10 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). skin, hair, nails, genitals), (b) systemic mycosis (fungal diseases of internal organs) [9,10]. In terms of their chemical structure, the antifungal agents can be imidazole ring compounds, derivatives containing a triazole group or antimycotics with a special chemical structure. The first group includes clotrimazole (Figure 1) [11,12], which is a broad-spectrum drug. By inhibiting the biosynthesis of ergosterol, it changes the membrane composition of the fungal cell wall, which leads to permeability disturbances and then cell lysis. Fluconazole (Figure 1) is an antifungal medicine containing a triazole group that can be used orally, intravenously, and vaginally [13,14]. The disadvantage is that it also has a liver-damaging effect. Among the active substances with a special structure is flucytosine, [15,16] which can be administered both orally and intravenously. However, this compound can also have serious side effects such as bone marrow suppression, loss of appetite, diarrhea, vomiting, and psychosis. Terbinafine is an allylamine derivative with highly lipophilic properties, which also inhibits the synthesis of ergosterol by inhibiting the enzyme squalene epoxidase, which plays an important role in the synthesis of the fungal cell wall [17,18]. Amphotericin B, isolated in 1955 from a culture of Streptomyces nodosus, is an antifungal polyene-macrolide antibiotic [19,20]. It is used for systemic infections, although it has many side effects. As can be seen from the examples so far, there are many effective agents for the treatment of fungal infections; however, the use of most of the products can have many side effects, i.e., the active ingredients damage human cells as well as microbial cells.



Figure 1. Structure of some antifungal drugs and antifungal cationic ammonium derivatives including carbohydrates and surfactants.

Positively charged structures can also have antibacterial and antifungal effects. Cationic ammonium surfactants, for example trimeric surfactant DTAD (tri(dodecyldimethylammonioacetoxy)diethyltriamine trichloride), excellently inhibits the growth of Gram-negative bacteria and fungi, but has no effect on mammalian cells [21]. Furthermore, there exist carbohydrate derivatives conjugated with DABCO (1,4-diazabicyclo[2.2.2]octane), which also have both antifungal and antibacterial effects [22,23]. A general characteristic of these positively charged compounds is that they exert their effect by destroying the cell wall of fungi and bacteria and inhibiting synthesis [24–26]. As a result, resistance does not develop against them, or only with difficulty, and since they do not affect the life cycle of the microorganism, they can have a much longer effect. The presented examples clearly show that the cationic nature of compounds is always ensured by one or more quaternary nitrogen atoms, which is decisive in terms of biological activities.

During our previous work, when we studied the elimination reaction of 6-iodohexopyranoside derivatives, completely new 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU)conjugated quaternary amidinium salts were isolated as side products (I–V, Figure 2) [27,28].



R¹ = Bz or Bn or H; R² = Bz or Bn or H; R³ = NAP or H

Figure 2. The structure of the synthesized quaternary amidinium salts.

These hitherto unknown DBU-carbohydrate conjugates show a high structural similarity to DABCO-sugar conjugates, which have been proven to have antifungal and antibacterial effects [22,23]. We therefore hypothesized that these new compounds also have antimicrobial effects, and we decided to test the antifungal and antibacterial activity of compounds I–V and also investigate the effect of sugar configuration and protecting group pattern on antimicrobial activity.

2. Results and Discussion

2.1. Synthesis of the DBU-Conjugated Derivatives

During our recent synthesis of L-hexoses based on C-5 epimerization of D-hexoses ($D \rightarrow L$ conversion), we used 5,6-unsaturated hexopyranosides as the key intermediates [27,28] These 5,6-unsaturated carbohydrates were prepared from the 6-iodo-hexopyranoside derivatives using different bases (Scheme 1). When the amidine base DBU was used as a reagent, in addition to the desired elimination reaction, nucleophilic substitution reaction also took place to some extent due to the nucleophilic behavior of DBU [29–34]. We investigated DBU-mediated elimination reactions of variously protected carbohydrates including D-gluco, D-manno, D-allo and D-altro configured derivatives [27,28]. We found that the elimination/substitution ratio strongly depended on the sugar configuration and, in the case of gluco derivatives, also on the protecting group pattern.



Scheme 1. The synthesis of the DBU-conjugated derivatives 3, 10–12, 21–24, 29, 30, 37, and 38 and the DABCO-conjugate 32.

In the case of D-*manno* and D-*altro* configurations, the amidinium salts were formed with moderate yields (**21**, **22**, **23**, **24**; **37**, **38**, 28–42%), while in the case of the D-*allo* configured

saccharides, the nucleophilic substitution products (**29**, **30**) were obtained in particularly high (63–76%) yields. In the case of the *D*-*allo* derivative (**31**), using DABCO as the reagent, the quaternary ammonium salt (**32**) was formed exclusively (yield 86%), which could be explained by the greater nucleophilicity of DABCO relative to DBU.

The fully ether-protected D-glucoside **4** gave the DBU conjugate in an acceptable yield, but in the presence of ester protecting groups (**1**, **5**, **6**), the yield of the amidinium salts was very low. We therefore attempted to increase the yield of the DBU-sugar conjugate **3** by shifting the DBU-mediated reaction of the ester-protected derivative **1** [28] towards nucleophilic substitution (Scheme 2). The optimization experiments are presented in Table 1. In our previous work, the reactions were carried out in tetrahydrofuran (THF), which is a preferred solvent for elimination [28]. During the optimization, we first replaced THF with acetonitrile, which, according to the literature, is a favorable solvent for nucleophilic substitution instead of elimination [22,23].



Scheme 2. Optimization of the synthesis of the cationic molecules.

Entry	Solvent	Reagent	Temp. (°C)	Time	Yield
1. ¹	THF	DBU (2.5 equiv.)	70	24	2 : 71% 3 : 26%
2.	CH ₃ CN	DBU (1.0 equiv.)	rt	16 days	1: 9% 2: 63% 3: 11%
3.	CH ₃ CN	DBU (1.5 equiv.)	rt	4 days	2 : 72% 3 : 14%
4.	CH ₃ CN	DBU (3.0 equiv.) + Glacial acetic acid (3.0 equiv.)	rt	3 days	2: 10% 3: 0% 39: 87%
5.	CH ₃ CN	DBU (6.0 equiv.) + <i>p</i> -TSA (6.0 equiv.)	rt to 85 °C	21 h at rt, 5 h 85 °C	1 : 84%
6.	CH ₃ CN	DBU (6.0 equiv.)	rt	6 days	2 : 70% 3 : 18%
7.	-	DBU (31.25 equiv.)	rt	24 h	2 : 32% 3 : 11% ²
8.	DMF	DBU (3.0 equiv.)	145	45 min	1: 2% 2: 58% 3: 33%

Table 1. Optimization trials to improve the yield of the DBU-conjugated derivatives.

¹ Original reaction conditions in Scheme 1, see ref. [28]. ² Other, not isolated, degradation products were also formed.

Performing the reaction at room temperature in the presence of 1 equiv. of DBU (entry 2.), the reaction was very sluggish, such that even after two weeks, the reaction mixture still contained traces of the starting material. The main product was the 5,6-unsaturated derivative (**2**, [28] 69%), and the expected amidinium salt **3** [28] was formed only with a very low yield (8%). By increasing the amount of DBU (1.5 eq., entry 2.), the reaction time was reduced to 4 days and the yield of the expected product slightly increased (**3**, 14%).

We tried to decrease the basicity of DBU by adding glacial acetic acid or *p*-toluene sulfonic acid (*p*-TSA) to the reaction mixture. Using 3 equiv. of DBU and 3 equiv. of glacial acetic acid (entry 3.), the reaction ended with a surprising result. The 6-OAc derivative

39 was isolated with a particularly good yield (76%), the 5,6-unsaturated compound (2) was formed with a low yield (10%), and the expected DBU-conjugated derivative was not observed. Using *p*-TSA as the acid, no reaction was observed (entry 4.). Increasing the amount of the reagents to 6 equiv. (entry 5.), the 5,6-unsaturated derivative (2) was isolated from the reaction mixture as the main product, and the expected product (3) was also obtained with a yield of 18%. Using DBU as a solvent (entry 6.), many degradation side-products were formed and the expected compound (3) could only be obtained with a low yield. The best result was finally achieved at high temperature in DMF, using 3 equiv. of DBU. The expected product (3) was formed in the reaction with a yield of 33%.

Since we also wanted to investigate the biological effect of the free amidinium salts, we attempted their direct production based on literature examples [23,35], by rapid synthesis without the use of protecting groups (Scheme 3). For this rapid synthetic method, we first synthesized the 6-O-tosyl derivative of methyl α -D-glucopyranoside (40) [36,37], then reacted this compound with DBU in dry acetonitrile. Neither the expected DBU-conjugated derivative nor the 5,6-eliminated derivative was formed in the reaction. The 3,6-anhydro derivative of the methyl α -D-glucopyranoside (**41**) [38] was identified as the main product, which was formed in excellent yield (80%). We also attempted to prepare the unprotected derivative from the 6-OTs derivative of phenyl 1-thio- α -D-mannopyranoside (42) [39,40]. We tested two types of reaction conditions (a, $CH_3CN + DBU$ and b, DMF + DBU), but unfortunately neither of the reactions produced the expected product. However, similar to the previous reaction, the 3,6-anhydro derivative of phenylthio-mannoside (43) was formed with excellent yield (97%). Finally, we attempted to carry out the reaction from the 6-iodo derivative of methyl α -D-glucopyranoside (44) [41], but these reactions did not lead to results either: here again, the 3,6-anhydro derivative (41) was formed along with other unidentified decomposition products. Based on these failed experiments, it can be concluded that the planned DBU-conjugated derivatives cannot be produced directly from the free derivatives under the conditions tested. Protection of the -OH groups of the carbohydrate part is essential for successful nucleophilic substitution reactions.



Scheme 3. Attempts at the direct preparation of the unprotected derivatives.

Based on the preliminary biological tests, we removed certain protective groups from the protected derivatives that seemed promising, thus examining the effect of each group on the biological activity, and in this way we were also able to increase the water solubility of our compounds. In initial studies, *D-gluco* and *D-manno* derivatives appeared to be active. Based on this, we first transformed the compounds with the *D-gluco* configuration (Scheme 4). The benzoyl groups were removed from compounds **3** and **11** under Zemplén conditions, thus releasing the hydroxyls at position 2 and 3 (**45**, **46**). The 2-naphthylmethyl group (2-NAP) was cleaved from derivatives **10** and **46** in CH_2Cl_2/H_2O using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) reagent, thus reaching compounds **47** and **48**.



Scheme 4. Removal of protecting groups of the D-gluco derivatives.

The D-*manno* derivatives were further modified in a similar manner (Scheme 5). The 2-NAP group was removed from derivatives **13**, **14** and **16** under oxidative conditions using DDQ, thus releasing one -OH per molecule (**49**, **50**, **52**). Furthermore, the benzoyl groups were removed from compounds **15** and **16**, thus reaching derivatives **51** and **53**.



Scheme 5. Partial deprotection of the D-manno derivatives.

After that, the antifungal and antibacterial effects of the synthesized derivatives were investigated, and the cytotoxicity, hemolytic activity and membrane permeabilizing effect of some compounds were also determined.

2.2. Biological Investigations of the DBU-Conjugated Derivatives

2.2.1. Antifungal and Antibacterial Studies

The antimicrobial activity of twenty-two cationic hexopyranoside-amidinium salts (3, 10, 11, 12, 21, 22, 23, 24, 29, 30, 32, 37, 38, 45, 46, 47, 48, 49, 50, 51, 52 and 53) were compared in susceptibility tests (Table 2). The effectiveness of all compounds was tested on *Staphylococcus aureus* ssp. *aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 10231). We tested the antimicrobial activity of the compounds at the concentration range of 6.25–100 μ g/mL.

	MIC in µg/mL			
Compound	Escherichia coli (ATCC 8739)	Staphylococcus aureus (ATCC 6538)	Candida albicans (ATCC 10231)	clogP
3	>100	≥6.25	≥ 50	7.413
10	≥ 100	6.25	≤ 6.25	9.672
11	>100	>100	>100	9.542
12	>100	6.25	≥ 50	9.547
21	>100	≥6.25	\geq 50	10.683
22	≥ 100	6.25	≤ 6.25	10.683
23	≥ 100	≥ 100	≥ 100	10.618
24	≥ 100	≥6.25	\geq 50	10.635
29	>100	<12.5	<6.25	9.673
30	≥ 100	≥ 25	≥ 25	9.542
32	≥ 100	≥6.25	≥ 100	6.373
37	>100	≥ 50	>100	10.683
38	>100	>100	>100	10.618
45	>100	>100	>100	2.661
46	>100	>100	>100	4.790
47	>100	>100	>100	1.826
48	>100	≥ 25	≥ 50	6.052
49	>100	>100	>100	7.070
50	>100	≥ 50	≥ 25	7.715
51	>100	≥12.5	≥ 50	5.800
52	≥ 100	≥ 100	>100	7.667
53	>100	≥6.25	≥ 6.25	8.240
Ceftazidime	8 [42]	8 [43-45]	-	-
Gentamicin	8 [42]	8 [43-45]	-	-
Amphotericin B	-	-	<0.5 [46,47]	-

Table 2. Antibacterial and antifungal activity. (MIC values given in μ g/mL, >larger than, <smaller than).

Compounds **10**, **12** and **22** showed a strong growth inhibitory effect on Gram-positive bacterium, *Staphylococcus aureus* ssp. *aureus*, their MIC values being smaller than 6.25 μ g/mL (Table 2). Compounds **3**, **21**, **24**, **29**, **30**, **32**, **37**, **48**, 50, **51**, and **53** also showed activity against the *S aureus* strain with MIC values of 6.25–50 μ g/mL. At the same time, compounds **11**, **23**, **38**, **45**, **46**, **47**, **49** and **52** had no anti Gram-positive effect even at 100 μ g/mL concentration.

Compounds **10**, **22** and **29** had an inhibitory effect on yeast, *Candida albicans*, with MIC values lower than 6.25 μ g/mL (Table 2). MIC values of **3**, **12**, **21**, **24**, **30**, **48**, **50**, **51** and **53** were in the range 6.25–50 μ g/mL on *Candida albicans*, while **11**, **23**, **32**, **37**, **38**, **45**, **46**, **47**, **49** and **52** were effective in concentration higher than 100 μ g/mL.

The Gram-negative bacteria, *Escherichia coli*, was less sensitive to the cationic carbohydrate derivatives. None of the tested compounds had any effect on *Escherichia coli* in the highest used concentration, $100 \mu g/mL$.

Compounds 10 and 22 were the most effective derivatives. Compounds 11, 23, 38, 45, 46, 47, 49 and 52 had no effect on any tested microorganism in concentrations as high as $100 \ \mu g/mL$.

The activity of the three most effective antifungal compounds was also determined at lower concentrations ($10 \times$ diluted) (Table 3). Testing the activity of the compounds against *C. albicans* at the concentration range of 0.625–10.0 µg/mL, we found that compounds **22** and **29** have excellent antifungal activity with 2.5 µg/mL MIC values, and derivative **10** is effective at a concentration of 5 µg/mL.

Compound	MIC in µg/mL Candida albicans (ATCC 10231)
10	5.0
22	2.5
29	2.5

Table 3. Antifungal activity of compounds 10, 22 and 29 in lower concentrations.

2.2.2. Cytotoxic Activity

The cytotoxic effect of six selected compounds (**3**, **10**, **12**, **21**, **22** and **29**) with promising antimicrobial activity was studied on HaCaT (human keratinocyte) cell lines. Using the MTT-test, we determined the viability of the cells (Figure 3), and the half maximal inhibitory concentration (IC₅₀) values of the compounds are summarized in Table 4. The duration of tests was 24 h; the examined concentrations were: 50–25–12.5–6.25–3.125 µg/mL.



Figure 3. The effect of **3**, **10**, **12**, **21**, **22** and **29** on viability of *HaCaT* cell line. (**A**) Cytotoxic activity of compound **3**; (**B**) Cytotoxic activity of compound **12**; (**C**) Cytotoxic activity of compound **21**; (**D**) Cytotoxic activity of compound **10**; (**E**) Cytotoxic activity of compound **22**; (**F**) Cytotoxic activity of compound **29**.

Table 4. Cytotoxic activity of compounds 3, 10, 12, 21, 22 and 29 on HaCaT cells.

Compound	HaCaT IC ₅₀ (µg/mL)	
3	0.13 ± 0.06	
10	15.3 ± 0.78	
12	4.26 ± 0.16	
21	1.02 ± 0.06	
22	10.3 ± 0.41	
29	11.9 ± 0.25	

Among the tested DBU-sugar conjugates, compound **3** showed the strongest cytotoxic effect on HaCaT cell line (Figure 3A), and its IC₅₀ value was as low as 0.13 µg/mL. Compounds **12** and **21** also had significant toxic effect (Figure 3B,C) with IC₅₀ values of 4.26 and 1.02 µg/mL, respectively.

Compounds **10**, **22**, **29** possessed only slight cytotoxicity (Figure 3D–F). Their IC₅₀ values were one order of magnitude higher than that of compounds **12** and **21** and two orders of magnitude higher than that of compound **3**. Most importantly, compounds **10** and **22** hardly affected the cell viability at their antibacterially and antifungally active concentrations of \leq 6.25 µg/mL (Figure 3D,E).

2.2.3. Hemolytic Activity

The hemolytic activity of compounds **10** and **22** was determined on freshly isolated rat red blood cells (Figure 4). Both compounds showed a dose-dependent hemolytic activity. At 6.25 µg/mL they did not cause significant hemolysis compared to the negative control PBS (p > 0.05); however, at 50 and 100 µg/mL they caused significant haemoglobin release (p < 0.0001) and their effect were comparable to the hemolytic effect of water used as positive control.



Figure 4. Hemolytic effect of compounds **10** and **22** on rat red blood cells. At 50 and 100 μ g/mL, both compounds significantly increased the hemolysis of red blood cells after 15 min of incubation. (n = 3–6, **** *p*< 0.001).

2.2.4. Membrane Permeabilization Study

In membrane permeabilization experiments, the Gram-positive *Lactobacillus plantarum* was used to test the effect of compounds **10** and **22** at 6.25 μ g/mL for 48 h. Bacterial cells were stained with SYTOX green (SG) fluorescent dye, which is impermeable on intact membranes but easily penetrates through compromised membranes. Test compounds **10** and **22** increased the ratio of SG-positive cells after 1 h of incubation and drastically increased it after 4 h of incubation (Figure 5), showing their membrane-compromising and toxic effects on *Lactobacillus plantarum*.



Figure 5. Compounds **10** and **22** caused increased membrane permeabilization on *Lactobacillus plantarum.* Both compounds caused membrane damage, which reflected in increased cellular fluorescence of SYTOX green (SG). The ratio of SG-positive cells was expressed in percent. **(A)** Membranepermeabilizing effect of compound **10**; **(B)** Membrane-permeabilizing effect of compound **22**.

3. Materials and Methods

3.1. General Information about the Syntheses

Optical rotations were measured at room temperature on a Perkin-Elmer 241 automatic polarimeter (PerkinElmer, Waltham, MA, US). TLC analysis was performed on Kieselgel 60 F₂₅₄ (Merck) silica-gel plates with visualization by immersing in a sulfuric-acid solution (5% in EtOH) followed by heating. Column chromatography was performed on silica gel 60 (Merck 0.063–0.200 mm). Organic solutions were dried over MgSO₄ and concentrated under vacuum. ¹H and J-modulated ¹³C NMR spectroscopy (¹H: 400 and 500 MHz; ¹³C: 100.28 and 125.76 MHz) were performed on Bruker DRX-400 and Bruker Avance II 500 spectrometers at 25 °C (Bruker, Billerica, MA, USA) Chemical shifts are referenced to SiMe4 or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS, $\delta = 0.00$ ppm for ¹H nuclei) and to residual solvent signals (CDCl₃: δ = 77.16 ppm, CD₃OD: δ = 49.15 ppm for ¹³C nuclei). You can find the NMR spectra of the new compounds in the supporting information. HRMS measurements were carried out on a maXis II UHR ESI-QTOF MS instrument (Bruker, Billerica, MA, USA) in positive ionization mode. The following parameters were applied for the electrospray ion source: capillary voltage: 3.6 kV; end plate offset: 500 V; nebulizer pressure: 0.5 bar; dry gas temperature: 200 °C; and dry gas flow rate: 4.0 L/min. Constant background correction was applied for each spectrum. The background was recorded before each sample by injecting the blank sample matrix (solvent). Na-formate calibrant was injected after each sample, which enabled internal calibration during data evaluation. Mass spectra were recorded by otofControl version 4.1 (build: 3.5, Bruker) and processed by Compass DataAnalysis version 4.4 (build: 200.55.2969).

3.1.1. General Methods

1. General Method A for Zemplén debenzoylation (45, 46, 51 and 53)

To a stirred solution of appropriate benzoyl protected compounds (3, 11, 15 and 16) (0.377 mmol) in MeOH (7.5 mL), NaOMe (25 mg, 0.472 mmol, 1.25 equiv.) was added and the reaction mixture was stirred at room temperature for 24 h. After 24 h, the mixture was neutralized with AcOH (500 μ L) and the mixture was concentrated under reduced pressure.

2. General Method B for cleavage of the 2-naphthylmethyl-group (NAP) (47–50 and 52)

The corresponding NAP-ether protected compounds (**10**, **13**, **14**, **16**, and **46**) (0.125 mmol) was dissolved in the mixture of CH_2Cl_2 (2.7 mL) and water (300 µL) then DDQ was added (71 mg, 0.313 mmol, 2.5 equiv.) and the reaction mixture was stirred at room temperature for 30 min. After 30 min, the mixture was neutralized with Cl^- exchange resin, filtered, washed with CH_2Cl_2 and concentrated under reduced pressure.

3.1.2. Optimization of the Synthesis of the DBU-Conjugated Methyl α -D-glucopyranoside derivative 3

 Methyl 2,3-di-O-benzoyl-4-O-benzyl-α-D-xylo-hex-5-enopyranoside (2) [28], 8-N-[methyl 6-deoxy-6-yl-2,3-di-O-benzoyl-4-O-benzyl-α-D-glucopyranoside]-1,8-diazabicyclo(5.4.0)undec-7-ene-iodide (3) [28] and methyl 6-O-acetyl-2,3-di-O-benzoyl-4-O-benzoyl-α-D-glucopyranoside (39).

Reaction 1: To the solution of compound **1** [28], (203 mg, 0.337 mmol) in dry CH₃CN (2.0 mL), DBU (50 μ L, 0.337 mmol, 1.0 equiv.) was added. The reaction mixture was stirred at room temperature for 16 days. After 16 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (9:1 CH₂Cl₂/*n*-hexane, then CH₂Cl₂ and finally 9:1 CH₂Cl₂/MeOH) to give **1** (18 mg, 9%) as a colorless syrup, **2** (101 mg, 63%) as a white crystal, and **3** (27 mg, 11%) as yellow foam.

Reaction 2: To the solution of compound **1** (503 mg, 0.835 mmol) in dry CH₃CN (5.0 mL), DBU (187 μ L, 1.253 mmol, 1.5 equiv.) was added. The reaction mixture was stirred at room temperature for 4 days. After 4 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂, then 9:1 CH₂Cl₂/MeOH) to give **2** (284 mg, 72%) as a colorless syrup and **3** (90 mg, 14%) as a yellow foam.

Reaction 3: To the solution of compound 1 (290 mg, 0.482 mmol) in dry CH₃CN (2.9 mL), DBU (216 μ L, 1.446 mmol, 3.0 equiv.) and glacial acetic acid (82 μ L, 1.446 mmol, 3.0 equiv.) were added. The reaction mixture was stirred at room temperature. After 3 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂, then 99:1 CH₂Cl₂/acetone, then 9:1 CH₂Cl₂/MeOH) to give **2** (23 mg, 10%) as a colorless syrup and **39** (223 mg, 87%) as a white foam.

Data of **39**: $[\alpha]_D^{25}$ +150.7 (*c* 0.15, CDCl₃); R_f 0.43 (CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) $\delta = 8.01-7.15$ (m, 15H, arom.), 6.07 (t, J = 9.4 Hz, 1H, H-3), 5.17 (dd, J = 3.5 Hz, J = 10.2 Hz, 1H, H-2), 5.12 (d, J = 3.5 Hz, 1H, H-1), 4.63 (d, J = 10.9 Hz, 1H, BnCH₂a), 4.54 (d, J = 10.9 Hz, 1H, BnCH₂b), 4.41 (t, J = 12.0 Hz, 1H, H-6a), 4.34 (dd, J = 4.0 Hz, J = 12.1 Hz, 1H, H-6b), 4.07–4.04 (m, 1H, H-5), 3.86 (t, J = 9.4 Hz, 1H, H-4), 3.40 (s, 3H, OCH₃), 2.11 (s, 3H, Ac-CH₃) ppm; ¹³C NMR (125 MHz, CDCl₃) $\delta = 170.7$ (1C, Ac C=O), 166.1, 165.6 (2C, 2 × Bz C=O), 137.1, 129.7, 129.1 (3C, 3 × C_q arom.), 133.4–128.1 (15C, arom.), 97.0 (1C, C-1), 75.8 (1C, OCH₃), 21.0 (1C, Ac-CH₃) ppm; UHR ESI-QTOF: m/z calcd for C₃₀H₃₀NaO₉ [M+Na]⁺ 557.1782; found: 557.1777.

Reaction 4: To the solution of compound **1** (305 mg, 0.506 mmol) in dry CH₃CN (3.0 mL), DBU (454 μ L, 3.034 mmol, 6.0 equiv.) and *p*-TSA (577 mg, 3.034 mmol, 6.0 equiv.) were added. The reaction mixture was stirred at room temperature for 21 h, then for 5 h at 85 °C. After 26 h, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂) to give **1** (256 mg, 84%) as a colorless syrup. The starting material was recovered.

Reaction 5: To the solution of compound **1** (528 mg, 0.877 mmol) in dry CH₃CN (5.3 mL), DBU (787 μ L, 5.261 mmol, 6.0 equiv.) was added. The reaction mixture was stirred at room temperature for 6 days. After 6 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂, then 9:1 CH₂Cl₂/MeOH) to give **2** (292 mg, 70%) as a white crystal and **3** (120 mg, 18%) as a yellow foam.

Reaction 6: To the compound **1** (254 mg, 0.422 mmol), DBU (2.0 mL, 13.2 mmol, 31.25 equiv.) was added at 0 °C. The reaction mixture was stirred at room temperature for 24 h. After 24 h, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂, then 9:1 CH₂Cl₂/MeOH) to give **2** (64 mg, 32%) as a colorless syrup, **3** (36 mg, 11%) as a yellow foam, and other, not isolated, degradation products.

Reaction 7: To the solution of compound **1** (288 mg, 0.479 mmol) in dry DMF (2.9 mL) heated to 150 $^{\circ}$ C, DBU (215 μ L, 1.437 mmol, 3.0 equiv.) was added. The reaction mixture

was stirred at 150 °C for 45 min. After 45 min, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (CH₂Cl₂, then 9:1 CH₂Cl₂/MeOH) to give **1** (5 mg, 2%) as a colorless syrup, **2** (133 mg, 58%) as a white crystal, and **3** (119 mg, 33%) as a yellow foam.

• Methyl 3,6-anhydro-α-D-glucopyranoside (41) [38].

Reaction 1: To the solution of compound **40** [36,37] (500 mg, 1.436 mmol) in dry CH₃CN (8.5 mL), DBU (1288 μ L, 8.618 mmol, 6.0 equiv.) was added. The reaction mixture was stirred at room temperature for 20 h and at 85 °C for 4 h. After 24 h the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (97:3 to 95:5 CH₂Cl₂/MeOH, then MeOH + 1% 60% aqueous solution of AcOH) to give **41** [38] (202 mg, 80%) as a colorless syrup and other, not isolated, degradation products.

Reaction 2: To the solution of compound 44 [41] (413 mg, 1.359 mmol) in dry CH₃CN (8.1 mL), DBU (1219 μ L, 8.152 mmol, 6.0 equiv.) was added. The reaction mixture was stirred at room temperature for 2 days at room temperature. After 2 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (9:1 CH₂Cl₂/MeOH, then MeOH + 1% 60% aqueous solution of AcOH) to give 41 [38] (151 mg, 62%) as a colorless syrup and other, not isolated, degradation products.

Reaction 3: To the solution of compound **44** (406 mg, 1.336 mmol) in dry DMF (8.1 mL) heated to 145 °C, DBU (599 μ L, 4.008 mmol, 3.0 equiv.) was added. The reaction mixture was stirred at 145 °C for 30 min. After 30 min, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (9:1 CH₂Cl₂/MeOH, then MeOH + 1% 60% aqueous solution of AcOH) to give **41** (136 mg, 58%) as a colorless syrup and other, not isolated, degradation products.

Phenyl 3,6-anhydro-1-thio-α-D-mannopyranoside (43)

Reaction 1: To the solution of compound **42** [**39**,**40**] (483 mg, 1.134 mmol) in dry CH₃CN (6.8 mL), DBU (1018 µL, 6.804 mmol, 6.0 equiv.) was added. The reaction mixture was stirred at room temperature for 5 days. After 5 days, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (9:1 CH₂Cl₂/MeOH, then MeOH + 1% 60% aqueous solution of AcOH) to give **43** (279 mg, 97%) as a white foam. $[\alpha]_D^{25}$ +85.5 (*c* 0.20, MeOH); *R*_f 0.56 (9:1 CH₂Cl₂/MeOH); ¹H NMR (400 MHz, CDCl₃) δ = 7.59–7.27 (m, 5H, arom.), 4.94 (d, *J* = 8.8 Hz, 1H, H-1), 4.28–4.27 (m, 1H, H-5), 4.26–4.25 (m, 1H, H-3), 4.21 (s, 1H, H-4), 4.11 (d, *J* = 11.0 Hz, 1H, H-6a), 3.98 (dd, *J* = 2.5 Hz, *J* = 10.9 Hz, 1H, H-6b), 3.78 (d, *J* = 8.6 Hz, 1H, H-2), 3.12–3.09 (m, 1H, H-2-OH), 2.86–2.83 (m, 1H, H-3-OH) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 132.5 (1C, 1 × C_q arom.), 132.8–128.3 (5C, arom.), 85.3 (1C, C-1), 77.8 (1C, C-3), 76.8 (1C, C-5), 71.3 (1C, C-4), 68.9 (1C, C-6), 67.6 (1C, C-2) ppm; UHR ESI-QTOF: m/z calcd for C₁₂H₁₄NaO₄S [M+Na]⁺ 277.0505; found: 277.0494.

Reaction 2: To the solution of compound **42** (497 mg, 1.167 mmol) in dry DMF (7.1 mL) heated to 145 °C, DBU (524 μ L, 3.501 mmol, 3.0 equiv.) was added. The reaction mixture was stirred at 145 °C for 4 h. After 4 h, the reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (9:1 CH₂Cl₂/MeOH, then MeOH + 1% 60% aqueous solution of AcOH) to give **43** (275 mg, 93%) as a white foam.

3.1.3. Synthesis and Transformation of the DBU-Conjugated Derivatives

8-N-[methyl 6-deoxy-6-yl-4-O-benzyl-α-D-glucopyranoside]-1,8-diazabicyclo(*5.4.0*)*undec-7ene-chloride* (**4**5). Compound **3 [28]** (110 mg, 0.146 mmol) was converted to **45** according to general **Method A**. The crude product was purified by silica gel chromatography (75:25 CH₂Cl₂/MeOH) to give **45** (48 mg, 73%) as a colorless syrup. $[\alpha]_D^{25}$ +60.0 (*c* 0.19, MeOH); *R*_f 0.63 (75:25 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CD₃OD) δ = 7.41–7.28 (m, 5H, arom.), 5.05 (d, *J* = 11.1 Hz, 1H, BnCH₂a), 4.70 (d, *J* = 3.7 Hz, 1H, H-1), 4.65 (d, *J* = 11.2 Hz, 1H, BnCH₂b), 3.85–3.82 (m, 2H, H-3, H-5), 3.75–3.51 (m, 8H, H-6a,b, 3 × NCH₂ DBU), 3.46 (dd, *J* = 3.7 Hz, *J* = 9.8 Hz, 1H, H-2), 3.38 (s, 3H, OCH₃), 3.27 (t, *J* = 8.8 Hz, 1H, H-4), 2.88 (t, $J = 5.5 \text{ Hz}, 2\text{H}, CH_2 \text{ DBU}, 2.07 \text{ (dq}, J = 6.4 \text{ Hz}, J = 13.1 \text{ Hz}, 2\text{H}, CH_2 \text{ DBU}, 1.77-1.58 \text{ (m}, 6\text{H}, 3 \times CH_2 \text{ DBU}) \text{ ppm}; {}^{13}\text{C} \text{ NMR} (125 \text{ MHz}, \text{CD}_3\text{OD}) \delta = 168.8 \text{ (1C, C}_q \text{ DBU}), 139.7 \text{ (1C, C}_q \text{ arom.)}, 129.6-128.9 \text{ (5C, arom.)}, 101.3 \text{ (1C, C-1)}, 80.8 \text{ (1C, C-4)}, 75.8 \text{ (1C, BnCH}_2), 75.4 \text{ (1C, C-5)}, 73.3 \text{ (1C, C-2)}, 71.1 \text{ (1C, C-3)}, 56.4 \text{ (1C, OCH}_3), 56.2 \text{ (1C, NCH}_2 \text{ DBU)}, 56.0 \text{ (1C, C-6)}, 50.3, 49.4 \text{ (2C, 2 \times \text{ NCH}_2 \text{ DBU})}, 29.4, 29.3, 27.0, 24.1, 21.3 \text{ (5C, 5 <math>\times \text{ CH}_2 \text{ DBU}) \text{ ppm}; UHR ESI-QTOF: m/z \text{ calcd for } C_{23}H_{35}N_2O_5 \text{ [M]}^+ 419.2540; \text{ found: } 419.2537.$

8-N-[ethyl 6-deoxy-6-yl-4-O-(2'-naphthyl)methyl-1-thio-α-D-glucopyranoside]-1,8diazabicyclo(5.4.0)undec-7-ene-chloride (46). Compound 11 [28] (315 mg, 0.377 mmol) was converted to 46 according to general Method A. The crude product was purified by silica gel chromatography (9:1 $CH_2Cl_2/MeOH$) to give 46 (190 mg, 91%) as a light yellow syrup. [α]_D²⁵+120.8 (*c* 0.13, MeOH); *R*_f 0.40 (9:1 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CD_3OD) δ = 7.88–7.46 (m, 7H, arom.), 5.35 (d, J = 5.4 Hz, 1H, H-1), 5.20 (d, J = 11.2 Hz, 1H, NAPCH₂a), 4.84 (d, J = 11.3 Hz, 1H, NAPCH₂b), 4.13 (t, J = 9.4 Hz, 1H, H-5), 3.86–3.81 (m, 2H, H-2, H-6a), 3.74 (t, J = 9.1 Hz, 1H, H-3), 3.67 (dd, J = 9.6 Hz, J = 15.4 Hz, 1H, H-6b), 3.57–3.38 (m, 6H, 3 × NCH₂ DBU), 3.35 (t, J = 9.1 Hz, 1H, H-4), 2.78–2.74 (m, 2H, CH₂ DBU), 2.52 (dd, J = 7.2 Hz, J = 14.5 Hz, 2H, SEt, CH₂), 1.98–1.95 (m, 2H, CH₂ DBU), 1.63–1.49 (m, 6H, $3 \times CH_2$ DBU), 1.25 (t, I = 7.4 Hz, 3H, SEt, CH_3) ppm; ¹³C NMR (125 MHz, CD_3OD) δ = 168.6 (1C, C_q DBU), 137.1, 134.6, 134.3 (3C, 3 × C_q arom.), 129.3–127.1 (7C, arom.), 87.0 (1C, C-1), 80.8 (1C, C-4), 76.1 (1C, C-3), 75.7 (1C, NAPCH₂), 72.7 (1C, C-2), 71.3 (1C, C-5), 56.1 (1C, NCH₂ DBU), 56.0 (1C, C-6), 50.2, 49.3 (2C, 2 × NCH₂ DBU), 29.3, 29.1, 26.9 (3C, $3 \times CH_2$ DBU), 25.9 (1C, SEt CH₂), 24.0, 21.0 (2C, 2 × CH₂ DBU), 15.5 (1C, SEt CH₃) ppm; UHR ESI-QTOF: m/z calcd for C₂₈H₃₉N₂O₄S [M]⁺ 499.2625; found: 499.2624.

8-N-[ethyl 6-deoxy-6-yl-1-thio-α-D-glucopyranoside]-1,8-diazabicyclo(5.4.0)*undec-7-ene-chloride* (47). Compound 46 (70 mg, 0.125 mmol) was converted to 47 according to general **Method B**. The crude product was purified by silica gel chromatography (7:3 CH₂Cl₂/MeOH) to give 47 (38 mg, 78%) as a light yellow syrup. $[\alpha]_D^{25}$ +142.7 (*c* 0.15, MeOH); *R*_f 0.37 (7:3 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CD₃OD) δ = 5.35 (d, *J* = 5.2 Hz, 1H, H-1), 4.13 (t, *J* = 8.8 Hz, 1H, H-5), 4.00 (d, *J* = 15.5 Hz, 1H, H-6a), 3.80 (dd, *J* = 9.1 Hz, *J* = 15.6 Hz, 1H, H-6b), 3.72–3.58 (m, 7H, H-2, 3 × NCH₂ DBU), 3.46 (t, *J* = 9.2 Hz, 1H, H-3), 3.19 (t, *J* = 9.1 Hz, 1H, H-4), 3.03 (dd, *J* = 7.7 Hz, *J* = 15.5 Hz, 1H, CH₂a DBU), 2.95 (dd, *J* = 9.5 Hz, *J* = 15.2 Hz, 1H, CH₂b DBU), 2.57 (td, *J* = 7.2 Hz, *J* = 11.5 Hz, 2H, SEt, CH₂), 2.09 (dd, *J* = 6.0 Hz, *J* = 11.6 Hz, 2H, CH₂ DBU), 1.81–1.73 (m, 6H, 3 × CH₂ DBU), 1.29 (t, *J* = 7.4 Hz, 3H, SEt, CH₃) ppm; ¹³C NMR (125 MHz, CD₃OD) δ = 169.1 (1C, NCH₂ DBU), 87.5 (1C, C-1), 75.5 (1C, C-3), 73.3 (1C, C-4), 72.7 (1C, C-2), 72.1 (1C, C-5), 56.1 (1C, NCH₂ DBU), 55.9 (1C, C-6), 50.3, 49.5 (2C, 2 × NCH₂ DBU), 29.4, 27.1 (3C, 3 × CH₂ DBU), 25.7 (1C, SEt CH₂), 24.3, 21.2 (2C, 2 × CH₂ DBU), 15.4 (1C, SEt CH₃) ppm; UHR ESI-QTOF: m/z calcd for C₁₇H₃₁N₂O₄S [M]⁺ 359.1999; found: 359.1999.

8-N-[ethyl 2,3-di-O-benzyl-6-deoxy-6-yl-1-thio-α-D-glucopyranoside]-1,8-diazabicyclo(5.4.0)undec-7-ene-chloride (48). Compound 10 [28] (218 mg, 0.270 mmol) was converted to 48 according to general **Method B**. The crude product was purified by silica gel chromatography (85:15 CH_2Cl_2 /MeOH) to give 48 (98 mg, 67%) as a light yellow syrup. [α] $_D^{25}$ +88.3 (*c* 0.12, CHCl₃); $R_{\rm f}$ 0.42 (85:15 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 7.40–7.21 (m, 10H, arom.), 5.74–7.71 (m, 1H, H-4-OH), 5.22 (d, J = 2.5 Hz, 1H, H-1), 4.95 (d, J = 11.3 Hz, 1H, BnCH₂a), 4.83, (d, J = 11.3 Hz, 1H, BnCH₂b), 4.68–4.63 (m, 2H, BnCH₂), 4.43 (d, J = 15.3 Hz, 1H, H-6a), 4.16 (t, J = 8.7 Hz, 1H, H-5), 3.69–3.68 (m, 2H, H-2, H-3), 3.58–3.54 (m, 7H, H-6a, 3 × NCH₂) DBU), 3.47–3.45 (m, 1H, H-4), 3.01–2.96 (m, 1H, CH₂a DBU), 2.92–2.88 (m, 1H, CH₂b DBU), 2.49–2.41 (m, 2H, SEt, CH₂), 2.01 (s, 2H, CH₂ DBU), 1.72–1.60 (m, 6H, 3 × CH₂ DBU), 1.26 (t, J = 7.3 Hz, 3H, SEt, CH₃) ppm; ¹³C NMR (125 MHz, CDCl₃) $\delta = 167.6$ (1C, C_q DBU), 139.3, 138.0 (2C, 2 × C_q arom.), 128.4–127.4 (10C, arom.), 83.8 (1C, C-1), 81.7 (1C, C-3), 78.6 (1C, C-2), 75.3, 72.6 (2C, 2 × BnCH₂), 72.0 (1C, C-5), 71.8 (1C, C-4), 55.5 (1C, NCH₂ DBU), 55.4 (1C, C-6), 49.4, 48.5 (2C, 2 × NCH₂ DBU), 28.8, 28.4, 26.2 (3C, 3 × CH₂ DBU), 24.4 (1C, SEt (CH_2) , 23.3, 20.2 (2C, 2 × CH_2 DBU), 14.9 (1C, SEt CH_3) ppm; UHR ESI-QTOF: m/z calcd for C₃₁H₄₃N₂O₄S [M]⁺ 539.2938; found: 539.2939.

8-*N*-[*phenyl* 2,4-*di*-O-*benzyl*-6-*deoxy*-6-*yl*-1-*thio*-α-D-*mannopyranoside*]-1,8-*diazabicyclo*(5.4.0)*undec*-7-*ene-chloride* (**49**). Compound **13** [27] (119 mg, 0.140 mmol) was converted to **49** according to general **Method B**. The crude product was purified by silica gel chromatography (8:2 CH₂Cl₂/MeOH) to give **49** (30 mg, 60%) as a light yellow syrup. $[\alpha]_D^{25}$ +84.5 (*c* 0.11, CHCl₃); *R*_f 0.50 (8:2 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 7.39–7.25 (m, 15H, arom.), 5.63 (s, 1H, H-1), 4.98–4.64 (m, 4H, 2 × BnCH₂), 4.02–3.99 (m, 2H, H-3, H-5), 3.96 (s, 1H, H-2), 3.85–3.82 (m, 1H, H-6a), 3.61 (s, 1H, H-3-OH), 3.51–3.49 (m, 2H, H-4, H-6b), 3.35–3.31 (m, 6H, 3 × NCH₂ DBU), 2.63 (s, 2H, CH₂ DBU), 1.72–1.22 (m, 8H, 4 × CH₂ DBU) ppm; ¹³C NMR (125 MHz, CDCl₃) δ = 167.3 (1C, C_q DBU), 137.9, 137.4, 133.1 (3C, 3 × C_q arom.), 130.7–127.8 (15C, arom.), 83.9 (1C, C-1), 79.3 (1C, C-2), 77.8 (1C, C-4), 75.4, 73.2 (2C, 2 × BnCH₂), 72.2 (1C, C-5), 71.3 (1C, C-3), 55.9 (1C, NCH₂ DBU), 55.2 (1C, C-6), 49.8, 49.7 (2C, 2 × NCH₂ DBU), 29.8, 28.9, 26.1, 25.2, 23.1 (5C, 5 × CH₂ DBU) ppm; UHR ESI-QTOF: m/z calcd for C₃₅H₄₃N₂O₄S [M]⁺ 587.2938; found: 587.2938.

8-N-[phenyl 2,3-di-O-benzyl-6-deoxy-6-yl-1-thio-α-D-mannopyranoside]-1,8-diazabicyclo(5.4.0)undec-7-ene-chloride (50). Compound 14 [27] (59 mg, 0.069 mmol) was converted to 50 according to general Method B. The crude product was purified by silica gel chromatography (8:2 CH₂Cl₂/MeOH) to give 50 (32 mg, 76%) as a light yellow syrup. $[\alpha]_D^{25}$ +73.0 (*c* 0.10, CHCl₃); R_f 0.45 (8:2 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 7.41–7.24 (m, 15H, arom.), 5.58 (s, 1H, H-1), 4.92–4.61 (m, 4H, 2 × BnCH₂), 4.40 (d, *J* = 15.4 Hz, 1H, H-6a), 4.24 (t, *J* = 8.5 Hz, 1H, H-5), 3.96–3.92 (m, 2H, H-2, H-4), 3.90–3.88 (m, 1H, H-3), 3.72–3.34 (m, 7H, H-6b, 3 × NCH₂ DBU), 2.94–2.89 (m, 1H, CH₂a DBU), 2.76–2.71 (m, 1H, CH₂b DBU), 1.81–1.53 (m, 8H, 4 × CH₂ DBU), 1.23 (t, *J* = 7.0 Hz, 1H, H-4-OH) ppm; ¹³C NMR (125 MHz, CDCl₃) δ = 167.6 (1C, C_q DBU), 138.9, 138.2, 134.1 (3C, 3 × C_q arom.), 130.7–127.2 (15C, arom.), 85.3 (1C, C-1), 79.3 (1C, C-3), 77.3 (1C, C-2), 73.6 (1C, C-5), 73.4, 73.1 (2C, 2 × BnCH₂), 68.9 (1C, C-4), 55.5 (1C, NCH₂ DBU), 55.4 (1C, C-6), 49.4, 48.3 (2C, 2 × NCH₂ DBU), 2.88, 28.5, 26.0, 23.2, 20.0 (5C, 5 × CH₂ DBU) ppm; UHR ESI-QTOF: m/z calcd for C₃₅H₄₃N₂O₄S [M]⁺ 587.2938; found: 587.2937.

8-*N*-[*phenyl* 6-*deoxy*-6-*y*]-4-*O*-(2'-*naphthyl*)*methyl*-1-*thio*-α-D-*mannopyranoside*]-1,8*diazabicyclo*(5.4.0)*undec*-7-*ene*-*chloride* (51). Compound **15** [27] (51 mg, 0.057 mmol) was converted to **51** according to general **Method A**. The crude product was purified by silica gel chromatography (85:15 CH₂Cl₂/MeOH) to give **51** (28 mg, 84%) as a light yellow syrup. $[\alpha]_D^{25}$ +153.0 (*c* 0.10, MeOH); *R*_f 0.37 (9:1 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 7.96–7.19 (m, 12H, arom.), 5.88 (s, 1H, H-1), 5.23 (d, *J* = 11.2 Hz, 1H, NAPCH₂a), 4.82 (d, *J* = 11.3 Hz, 1H, NAPCH₂b), 4.33 (s, 1H, H-2), 4.13–4.08 (m, 2H, H-3, H-5), 4.00–3.94 (m, 2H, H-4, H-6a), 3.69 (d, *J* = 14.2 Hz, 1H, H-6b), 3.46–3.22 (m, 6H, 3 × NCH₂ DBU), 2.78–2.75 (m, 1H, CH₂a DBU), 2.66–2.63 (m, 1H, CH₂b DBU), 2.45 (d, *J* = 3.8 Hz, 1H, H-3-OH), 1.64–1.26 (m, 9H, H-2-OH, 4 × CH₂ DBU) ppm; ¹³C NMR (125 MHz, CD₃OD) δ = 168.5 (1C, C_q DBU), 137.1, 134.8, 134.5, 134.0 (4C, 4 × C_q arom.), 132.1–127.2 (12C, arom.), 88.0 (1C, C-1), 78.2 (1C, C-4), 76.1 (1C, NAPCH₂), 73.6 (1C, C-3), 73.3 (1C, C-2), 72.3 (1C, C-5), 56.0 (1C, NCH₂ DBU), 55.8 (1C, C-6), 50.0, 49.3 (2C, 2 × NCH₂ DBU), 29.2, 29.1, 26.8, 23.8, 20.6 (5C, 5 × CH₂ DBU) ppm; UHR ESI-QTOF: m/z calcd for C₃₂H₃₉N₂O₄S [M]⁺ 547.2625; found: 547.2624.

8-N-[phenyl 2-O-benzoyl-3-O-benzyl-6-deoxy-6-yl-1-thio-α-D-mannopyranoside]-1,8diazabicyclo(5.4.0)undec-7-ene-chloride (52). Compound **16** [27] (50 mg, 0.058 mmol) was converted to **52** according to general **Method B**. The crude product was purified by silica gel chromatography (85:15 CH₂Cl₂/MeOH) to give **52** (32 mg, 76%) as a light yellow syrup. $[\alpha]_D^{25}$ +11.1 (*c* 0.56, CHCl₃); *R*_f 0.41 (85:15 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 8.07–7.24 (m, 15H, arom.), 5.74 (s, 1H, H-1), 5.68 (s, 1H, H-2), 4.76 (d, *J* = 11.5 Hz, 1H, BnCH₂a), 4.63 (d, *J* = 11.5 Hz, 1H, BnCH₂b), 4.32 (t, *J* = 8.4 Hz, 1H, H-5), 4.14 (d, *J* = 15.3 Hz, 1H, H-6a), 3.97 (t, *J* = 9.3 Hz, 1H, H-4), 3.89 (dd, *J* = 2.6 Hz, *J* = 9.0 Hz, 1H, H-3), 3.83 (dd, *J* = 8.2 Hz, *J* = 15.5 Hz, 1H, H-6b), 3.57–3.42 (m, 6H, 3 × NCH₂ DBU), 2.91 (dd, *J* = 6.6 Hz, *J* = 15.4 Hz, 1H, CH₂a DBU), 2.76 (dd, *J* = 8.6 Hz, *J* = 14.4 Hz, 1H, CH₂b DBU), 1.90–1.55 (m, 9H, H-4-OH, 4 × CH₂ DBU) ppm; ¹³C NMR (125 MHz, CDCl₃) δ = 167.9 (1C, C_q DBU), 165.8 (1C, C_q Bz), 137.5, 133.0 (3C, 3 × C_q arom.), 131.4–128.0 (15C, arom.), 85.4 (1C, C-1), 77.1 (1C, C-3), 72.7 (1C, C-5), 72.2 (1C, BnCH₂), 70.4 (1C, C-2), 68.5 (1C, C-4), 55.7 (1C, NCH₂) DBU), 55.2 (1C, C-6), 49.5, 48.4 (2C, $2 \times \text{NCH}_2$ DBU), 28.9, 28.5, 26.0, 23.0, 20.0 (5C, $5 \times \text{CH}_2$ DBU) ppm; UHR ESI-QTOF: m/z calcd for C₃₅H₄₁N₂O₅S [M]⁺ 601.2731; found: 601.2729.

8-N-[phenyl 3-O-benzyl-6-deoxy-6-yl-4-O-(2'-naphthyl)methyl-1-thio-α-D-mannopyranoside]-1,8-diazabicyclo(5.4.0)undec-7-ene-acetate (53). Compound 16²⁷ (70 mg, 0.081 mmol) was converted to 53 according to general Method A. The crude product was purified by silica gel chromatography (85:15 CH₂Cl₂/MeOH) to give 53 (47 mg, 85%) as a light yellow syrup. $[\alpha]_D^{25}$ +106.7 (*c* 0.12, MeOH); R_f 0.57 (85:15 CH₂Cl₂/MeOH); ¹H NMR (500 MHz, CDCl₃) δ = 7.85–7.26 (m, 17H, arom.), 5.86 (s, 1H, H-1), 5.08–4.65 (m, 4H, NAPCH₂, BnCH₂), 4.36 (s, 1H, H-2), 4.12 (t, *J* = 8.4 Hz, 1H, H-5), 3.90 (dd, *J* = 3.0 Hz, *J* = 8.9 Hz, 1H, H-3), 3.84 (t, *J* = 9.3 Hz, 1H, H-4), 3.81–3.77 (m, 2H, H-6a,b), 3.53–3.28 (m, 6H, 3 × NCH₂ DBU), 2.78–2.74 (m, 1H, CH₂a DBU), 2.66–2.62 (m, 1H, CH₂b DBU), 2.16 (s, 3H, CH₃COO⁻), 1.69–1.46 (m, 9H, H-2-OH, 4 × CH₂ DBU) ppm; ¹³C NMR (125 MHz, CDCl₃) δ = 167.3 (1C, C_q DBU), 137.4, 135.1, 133.3, 133.1, 132.9 (5C, 5 × C_q arom.), 130.6–126.2 (17C, arom.), 86.0 (1C, C-1), 79.8 (1C, C-3), 75.5 (1C, C-4), 75.4 (1C, NAPCH₂), 71.7 (1C, BnCH₂), 71.1 (1C, C-5), 68.5 (1C, C-2), 55.7 (1C, NCH₂ DBU), 55.0 (1C, C-6), 49.4, 48.0 (2C, 2 × NCH₂ DBU), 31.0 (1C, CH₃COO⁻), 29.0, 28.3, 25.8, 22.7, 19.7 (5C, 5 × CH₂ DBU) ppm; UHR ESI-QTOF: m/z calcd for C₃₉H₄₅N₂O₄S [M]⁺ 637.3095; found: 637.3095.

3.2. Antimicrobial Investigations of the DBU-Conjugated Derivatives

The detection of the antibacterial and antifungal effects of the tested compounds and the determination of the minimum inhibitory concentration (MIC) were carried out based on the method of CLSI [48] and Hungarian Pharmacopeia (chapters VIII. 2.6.12–13.) [49].

3.2.1. Materials

For the antimicrobial studies, we used three human pathogens as test organisms: *Candida albicans* (ATCC 10231), *Staphylococcus aureus* subspecies *aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739).

Staphylococcus aureus subspecies aureus and Escherichia coli were cultured on tryptic soya agar (TSA) for 18–24 h at 37 °C. Candida albicans was cultured on Sabouraud dextrose agar (SDA) for 18–24 h at 37 °C. To obtain cells for suspension, we made cell suspensions from Staph. aureus, E. coli and C. albicans with the cotton swab suspension technique. The dilution of suspension was then made with 0.85% NaCl solution, absorbance being set to $A_{600nm} = 0.06$. a volume of 10 µL of these suspensions contained ~4.5–6.0 × 10⁴ colony forming unit (CFU) (bacteria) and ~1.5–2.0 × 10³ CFU for yeast.

Tested agents were diluted with DMSO; the concentration of stock solutions was 4 mg/mL; and to ensure sterility, the closed sample containers were incubated at 35 °C for 1 h. The highest final concentration was 100 μ g/mL.

3.2.2. Methods

Testing was performed in 24-well plates, and cell suspensions were prepared with 0.85% NaCl solution. Stock solutions were made from the agents with DMSO (100%). Final concentrations of used compounds were 6.25–100 μ g/mL. The activity of the three most effective antibacterial (**10**, **12** and **22**) and antifungal (**10**, **22** and **29**) compounds was also determined at lower concentrations (10× diluted); the compounds were tested against *S. aureus* and *C. albicans* at the concentration range of 0.625–10.0 μ g/mL. After inoculation, plates were incubated at 35 °C, and results were then read visually after 18–24 h. If microbial growth could not be detected, results were read visually after 72 h. If there was no growth after 72 h, incubation was continued till the fifth day of the experiment and the results were read visually and with plate reader at 600 nm after 120 h.

3.2.3. Evaluation

The MIC value of a compound was defined as the minimal concentration that inhibits the growth of bacteria and yeasts comparing to the control. The results of the antibacterial

and antifungal tests were read visually after 24, 48, 72 and 120 h. The microbial growth was measured by plate reader at 600 nm after 120 h.

The IC₅₀ value of a compound was defined as the concentration that inhibits cell growth by 50% relative to the control. After the MTT test, the medium containing the inhibitor was removed, and 10 μ L MTT solution was added to the cells. The plates were then incubated for two hours at 37 °C. The medium from the wells was then carefully aspirated, and MTT formazan dissolved with 100 μ L of DMSO aided by gentle agitation on a shaker. After 10 min at room temperature, the absorbances were read at 570 nm by an automatic plate reader. The percentage of viability reflecting the respiratory potential of the cell population in each well was expressed as (absorbance of treated cells/absorbance of control cells) × 100. IC₅₀ values of tested agents were determined by Graphpad (Prism) semi-log line fitting (graphpad.com (8)) [50]. The MTT test was performed after treatment and removal of inhibitors followed by the addition of 10 μ L of MTT solution to the cells.

3.3. Viability Tests

We tested the toxic effect of compounds **3**, **10**, **12**, **21**, **22** and **29** on HaCaT cell line. HaCaT is a spontaneously transformed, aneuploid keratinocyte, an adherent cell type derived from the epithelium of Human Caucasian adult male skin. The cell line was immortalized in 1986 under low calcium and high-temperature conditions [51]. Cells were cultured in DMEM medium (iBiotech, Szigetszentmiklós, Hungary) containing 10% fetal bovine serum (FBS, iBiotech, Szigetszentmiklós, Hungary) and 1% antibiotic-antimycotic mix (Penicillin–Streptomycin–Neomycin) [52].

3.4. Hemolytic Activity

Blood was collected from a Sprague-Dawley rat into an EDTA tube. Erythrocytes were separated from blood by centrifugation at 1800 rpm for 8 min, washed 3 times with phosphate buffered saline (PBS), and resuspended in PBS. Aliquots of the cell suspension with the respective red blood cell number of 7×10^7 were added to PBS containing increasing concentrations of the test compounds or the vehicle DMSO. After gently mixing, each mixture was incubated at 37 °C for 15 min. The released hemoglobin into the supernatant was separated by a rapid centrifugation at 5000 rpm for 5 min. Finally, the absorbance of the released hemoglobin was measured at 540 nm using a Multiskan Go (ThermoFisher, Waltham, MA, USA) microplate reader. The absorbance values were compared to the absorbance of the sample kept in purified water during the incubation as positive control and given as percent hemolysis [53].

3.5. Membrane Permeabilization Study of Lactobacillus plantarum by SYTOX Green Staining

Lactobacillus plantarum subsp. *plantarum* (ATCC 14917) was purchased from ATCC (Manassas, VA, USA). The membrane permeabilization ability of compounds **10** and **22** was tested on *L. plantarum* and determined in the brain heart infusion medium (BHI) (CliniChem Ltd., Budapest, Hungary). Bacterial cells were treated with the test compounds at the final concentration of 6.25 μ g/mL and samples were taken at 1, 4, 8, 24 and 48 h. Samples were stained with SYTOX green fluorescent dye (ThermoFischer Scientific, Budapest, Hungary) at 1 μ M final concentration for 30 min at 37 °C, and flow cytometric measurements were carried out with a Guava Easy Cyte 6HT-2L flow cytometer (Merck Ltd., Darmstadt, Germany). Green (525/30 nm) and red (695/50 nm) fluorescence channels were used to gate out the cells on a green versus red dot plot. The ratio of the SYTOX green (SG) positive bacterial cells was evaluated [54].

4. Conclusions

In the course of our research, we produced novel DBU-conjugated cationic carbohydrate derivatives from D-hexopyranosides of various configurations (D-gluco, D-manno, D-allo and D-altro). The -OH groups of carbohydrates were masked with benzyl, benzoyl and/or 2-naphthylmethyl protecting groups. By varying these protecting groups and free hydroxyls on the sugar unit, we were able to tune the lipophilicity of the molecules. A total of 13 fully protected and 9 partially or completely deprotected cationic sugar amidinium salts were prepared. We optimized the synthesis of a D-gluco-DBU conjugate, and attempted to produce carbohydrate amidinium derivatives without the use of protecting groups.

The conjugation of the DBU unit to the sugar skeleton was carried out by nucleophilic substitution reactions on the fully protected 6-deoxy-6-iodohexopyranosides. The efficiency of the desired nucleophilic substitution was strongly dependent on the configuration of the sugar used due to competitive elimination reaction. We have found that the more the C-5 axial hydrogen of the carbohydrate is sterically hindered, the more favored the nucleophilic substitution reaction is. Accordingly, in the case of D-*altro* and D-*allo* configurations, where H-5 is sterically shielded, nucleophilic substitution was preferred, leading to the expected DBU-conjugated derivative in good yield. At the same time, in the case of the D-*gluco* and D-*manno* configurations, due to the good steric accessibility of H-5, elimination was the dominant reaction, and unfortunately the optimization experiments resulted in only a small, 7% increase in the yield of the DBU-conjugated glucoside.

Our attempts to prepare unprotected DBU-sugar conjugates without the use of protecting groups failed. In the presence of free hydroxyls, intramolecular nucleophilic substitution took place in all investigated cases, resulting in the 3,6-anhydro derivative of the carbohydrate.

Of the twenty-two compounds tested, nine showed promising activity against *Staphylococcus aureus* with MIC values of $6-25 \,\mu\text{g/mL}$, and six compounds showed potent antifungal activity against *Candida albicans*. The biological results clearly show that protecting groups, especially naphthylmethyl ether, are more favorable than ester protecting groups. The most effective compounds turned out to be the most lipophilic derivatives of highest clogP containing only ether protecting groups. Presumably, the lipophilic units facilitate the incorporation of the compounds into the lipid bilayer of the cell membrane and disrupt it. The positive results of membrane permeabilization experiments performed on a Gram-positive model bacterium with two active compounds (**10** and **22**) confirmed that the antimicrobial effect of our DBU-sugar conjugates may be related to their membrane-damaging ability. With the appearance of free hydroxyl groups, biological activity dropped drastically, and molecules containing more than one free hydroxyl group completely lost their activity. At the same time, the sugar configuration also plays a role in the effect, as evidenced by the ineffectiveness of the *altro*-configured compounds.

We found that the pattern of the protecting group also affects the cytotoxic properties. Compounds containing an ester group on the molecule or containing a benzyl group in position 4 (**3**, **12** and **21**) were cytotoxic on HaCaT cells. However, derivatives with exclusively ether groups on the molecule and a NAP group at position 4 (**10**, **22** and **29**) showed a greatly reduced toxicity. The latter three compounds had the best antifungal effect. They act against fungi at concentrations lower than their cytotoxicity value, thus giving the opportunity to use these derivatives as antifungal agents in either oral or dermal antifungal preparations. However, this still requires further investigations.

None of the 22 tested compounds had an effect against the Gram-negative *Escherichia coli* (ATCC 8739) strain in the applied concentration range of 6.25–100 μ g/mL. Considering the cationic, membrane-active structure of our compounds, this is a surprising result and worthy of further investigation.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi. com/article/10.3390/ijms24043550/s1.

Author Contributions: Conceptualization, M.H. and A.B.; investigation, F.D., P.T., A.K., R.K.-O., V.B., Z.M.S., F.K., N.B., F.F., J.V. and M.H.; writing—original draft preparation, F.D., A.K., Z.M.S., F.F., J.V., M.H. and A.B.; writing—review and editing, F.D., A.K., Z.M.S., F.F., J.V., M.H. and A.B.; funding acquisition, M.H. and A.B. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge financial support for this research from the National Research and Development and Innovation Office of Hungary (FK 137924 M. H. and K 132870 A.B), and from the EU co-financed by the European Regional Development Fund under the project GINOP-2.3.4-15-2020-00008.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Mariann Varga for excellent technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

4.

- Uçkay, I.; Pittet, D.; Vaudaux, P.; Sax, H.; Lew, D.; Waldvogel, F. Foreign body infections due to *Staphylococcus epidermidis*. *Ann. Med.* 2009, 41, 109–119. [CrossRef]
- Magill, S.S.; Edwards, J.R.; Bamberg, W.; Beldavs, Z.G.; Dumyati, G.; Kainer, M.A.; Lynfield, R.; Maloney, M.; McAllister-Hollod, L.; Nadle, J.; et al. Multistate point-prevalence survey of health care-associated infections. N. Engl. J. Med. 2014, 370, 1198–1208. [CrossRef]
- 3. Zhang, Q.; Lambert, G.; Liao, D.; Kim, H.; Robin, K.; Tung, C.-K.; Pourmand, N.; Austin, R.H. Acceleration of Emergence of Bacterial Antibiotic Resistance in Connected Microenvironments. *Science* **2011**, *333*, 1764–1767. [CrossRef]
 - Martínez, J.L. Antibiotics and Antibiotic Resistance Genes in Natural Environments. Science 2008, 321, 365–367. [CrossRef]
- Boucher, H.W.; Talbot, G.H.; Bradley, J.S.; Edwards, J.E.; Gilbert, D.; Rice, L.B.; Scheld, M.; Spellberg, B.; Bartlett, J. Bad Bugs, No Drugs: No Eskape! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2009, 48, 1–12. [CrossRef]
- 6. Loeffler, J.; Steven, D.A. Antifungal Drug Resistance. Clin. Infect. Dis. 2003, 36, S31–S41. [CrossRef]
- Stokes, J.M.; Lopatkin, A.J.; Lobritz, M.A.; Collins, J.J. Bacterial Metabolism and Antibiotic Efficacy. Cell Metab. 2019, 30, 251–259. [CrossRef]
- 8. Baracho, M.S.; Baracho, I.R. An analysis of the spontaneous mutation rate measurement in filamentous fungi. *Genet. Mol. Biol.* **2003**, *26*, 83–87. [CrossRef]
- 9. Ernst, E.J.; Rogers, P.D. Antifungal Agents: Methods and Protocols (Methods in Molecular Medicine), 1st ed.; Human Press: Totowa, NJ, USA, 2005; pp. 1–210.
- 10. Malayeri, F.A.; Rezaei, A.A.; Raiesi, O. Antifungal agents: Polyene, azole, antimetabolite, other and future agents. *J. Bas. Res. Med. Sci.* **2018**, *5*, 48–55. [CrossRef]
- 11. Sawyer, P.R.; Brogden, R.N.; Pinder, K.M.; Speight, T.M.; Avery, G.S. Clotrimazole: A Review of its Antifungal Activity and Therapeutic Efficacy. *Drugs* **1975**, *9*, 424–447. [CrossRef]
- Pelletier, R.; Peter, J.; Antin, C.; Gonzalez, C.; Wood, L.; Walsh, T.J. Emergence of Resistance of *Candida albicans* to Clotrimazole in Human Immunodeficiency Virus-Infected Children: In Vitro and Clinical Correlations. *J. Clin. Microbiol.* 2000, *38*, 1563–1568. [CrossRef]
- 13. Grant, S.M.; Clissold, S.P. Fluconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. *Drugs* **1990**, *39*, 877–916. [CrossRef]
- 14. Rex, J.H.; Rinaldi, M.G.; Pfaller, M.A. Resistance of *Candida* Species to Fluconazole. *Antimicrob. Agents Chemother.* **1995**, 39, 1–8. [CrossRef]
- 15. Polak, A.; Scholer, H.J. Mode of action of 5-fluorocytosine and mechanisms of resistance. *Chemotherapy* **1975**, 21, 113–130. [CrossRef]
- 16. Vermes, A.; Guchelaar, H.-J.; Dankert, J. Flucytosine: A review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J. Antimicrob. Chemother.* **2000**, *46*, 171–179. [CrossRef]
- 17. Balfour, J.A.; Faulds, D. Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. *Drugs* **1992**, *43*, 259–284. [CrossRef]
- 18. Abdel-Rahman, S.M.; Nahata, M.C. Oral terbinafine: A new antifungal agent. Ann. Pharmacother. 1997, 31, 445–456. [CrossRef]
- 19. Brajtburg, J.; Powderly, W.G.; Kobayashi, G.S.; Medoff, G. Amphotericin B: Current understanding of mechanisms of action. *Antimicrob. Agents Chemother.* **1990**, *34*, 183–188. [CrossRef]
- 20. Ostrosky-Zeichner, L.; Marr, K.A.; Rex, J.H.; Cohen, S.H. Amphotericin B: Time for a new "gold standard". *Clin. Infect. Dis.* 2003, 37, 415–425.
- Zhou, C.; Wang, F.; Chen, H.; Li, M.; Qiao, F.; Liu, Z.; Hou, Y.; Wu, C.; Fan, Y.; Liu, L.; et al. Selective Antimicrobial Activities and Action Mechanism of Micelles Self-assembled by Cationic Oligomeric Surfactants. ACS Appl. Mater. Interfaces 2016, 8, 4242–4249. [CrossRef]
- 22. Abel, T.; Cohen, J.L.I.; Engel, R.; Filshtinskaya, M.; Melkonian, A.; Melkonian, K. Preparation and investigation of antibacterial carbohydrate-based surfaces. *Carbohydr. Res.* **2002**, *337*, 2495–2499. [CrossRef] [PubMed]

- Engel, R.; Ghani, I.; Montenegro, D.; Thomas, M.; Klaritch-Vrana, B.; Castaño, A.; Friedman, L.; Leb, J.; Rothman, L.; Lee, H.; et al. Polycationic Glycosides. *Molecules* 2011, 16, 1508–1518. [CrossRef] [PubMed]
- Nikfarjam, N.; Ghomi, M.; Agarwal, T.; Hassanpour, M.; Sharifi, E.; Khorsandi, D.; Khan, M.A.; Rossi, F.; Rossetti, A.; Zare, E.N.; et al. Antimicrobial Ionic Liquid-based Materials for Biomedical Applications. *Adv. Funct. Mater.* 2021, *31*, 2104148. [CrossRef]
- Kwasniewska, D.; Chen, Y.-L.; Wieczorek, D. Biological Activity of Quaternary Ammonium Salts and Their Derivatives. *Pathogens* 2020, 9, 459. [CrossRef] [PubMed]
- 26. Jennings, M.C.; Minbiole, K.P.C.; Wuest, W.M. Quaternary Ammonium Compounds: An Antimicrobial Mainstay and Platform for Innovation to Address Bacterial Resistance. *ACS Infect. Dis.* **2015**, *1*, 288–303. [CrossRef]
- 27. Demeter, F.; Bereczki, I.; Borbás, A.; Herczeg, M. Synthesis of Four Orthogonally Protected Rare L-Hexose Thioglycosides from D-Mannose by C-5 and C-4 Epimerization. *Molecules* **2022**, 27, 3422. [CrossRef]
- 28. Demeter, F.; Bényei, A.; Borbás, A.; Herczeg, M. Synthesis of the three most expensive L-hexose thioglycosides from D-glucose. *Synthesis* 2023. [CrossRef]
- 29. Baidya, M.; Mayr, H. Nucleophilicities and carbon basicities of DBU and DBN. Chem. Commun. 2008, 15, 1792–1794. [CrossRef]
- Maji, B.; Breugst, M.; Mayr, H. N-Heterocyclic Carbenes: Organocatalysts with Moderate Nucleophilicity but Extraordinarily High Lewis Basicity. Angew. Chem. Int. Ed. Engl. 2011, 50, 6915–6919. [CrossRef]
- Li, Z.; Ji, P.; Cheng, J.-P. Brönsted Basicities and Nucleophilicities of *N*-Heterocyclic Olefins in Solution: *N*-Heterocyclic Carbene versus *N*-Heterocyclic Olefin. Which Is More Basic, and Which Is More Nucleophilic? *J. Org. Chem.* 2021, *86*, 2974–2985. [CrossRef]
- 32. Lammers, H.; Cohen-Fernandes, P.; Habraken, C.L. Nucleophilic behaviour of DBU and DBN in reactions with 4-halo-3,5-dimethyl-1-nitro-1H-pyrazoles. *Tetrahedron* **1994**, *50*, 865–870. [CrossRef]
- Johnson, M.G.; Foglesong, R.J. Nucleophilic behavior of DBU in a conjugate addition reaction. *Tetrahedron Lett.* 1997, 38, 7003–7006. [CrossRef]
- Németh, A.G.; Szabó, R.; Domján, A.; Keserű, G.M.; Ábrányi-Balogh, P. Chromatography-Free Multicomponent Synthesis of Thioureas Enabled by Aqueous Solution of Elemental Sulfur. *ChemistryOpen* 2021, 10, 16–27. [CrossRef] [PubMed]
- Thomas, M.; Montenegro, D.; Castaño, A.; Friedman, L.; Leb, J.; Huang, M.L.; Rothman, L.; Lee, H.; Capodiferro, C.; Ambinder, D.; et al. Polycations. 17. Synthesis and properties of polycationic derivatives of carbohydrates. *Carbohydr. Res.* 2009, 344, 1620–1627. [CrossRef]
- Suwinski, J.; Walczak, K. Salts of 5-Substituted Uracils with 1,8-Diazabicyclo[5.4.0]-undec-7-ene: Convenient Reagents for Nucleophilic Addition and Substitution Reactions. *Synthesis* 2001, 2, 225–228. [CrossRef]
- Cramer, F.; Otterbach, H.; Springmann, H. Eine Synthese der 6-Desoxy-6-amino-glucose. *Chem. Ber.* **1959**, *92*, 384–391. [CrossRef]
 Eskew, N.A.; Evans, S.A., Jr. Oxaphosphoranylation of Methyl α-D-Glucopyranoside with Diethoxytriphenylphosphorane. A
- Highly Stereoselective Route to Anhydropyranosides. J. Chem. Soc. Chem. Commun. 1990, 706–708. [CrossRef]
- Kerékgyártó, J.; Szurmai, Z.; Lipták, A. Synthesis of *p*-trifluoroacetamidophenyl 6-deoxy-2-O-(3-O-[2-O-methyl-3-O-(2-O-methylα-D-rhamnopyranosyl)-α-L-fucopyranosyl]-α-L-rhamnopyranosyl]-α-L-talopyranoside: A spacer armed tetrasaccharide glycopeptidolipid antigen of *Mycobacterium avium* serovar 20. *Carbohydr. Res.* 1993, 245, 65–80. [CrossRef]
- Gómez, A.M.; Casillas, M.; Rodríguez, B.; Valverde, S.; López, J.C. Synthesis of furanoid and pyranoid C-1 aryl glycals by reaction of glycosyl chlorides with organolithium reagents. *Arkivoc* 2010, *3*, 288–302. [CrossRef]
- 41. Skaanderup, P.R.; Poulsen, C.S.; Hyldtoft, L.; Jørgensen, M.R.; Madsen, R. Regioselective Conversion of Primary Alcohols into Iodides in Unprotected Methyl Furanosides and Pyranosides. *Synthesis* **2002**, *12*, 1721–1727. [CrossRef]
- 42. Benedict, K.M.; Gow, S.P.; Checkley, S.; Booker, C.W.; McAllister, T.A.; Morley, P.S. Methodological comparisons for antimicrobial resistance surveillance in feedlot cattle. *BMC Vet. Res.* **2013**, *9*, 216. [CrossRef] [PubMed]
- 43. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement;* M100-S22; Clinical and Laboratory Standards Institute (CLSI): Wayne, PA, USA, 2012.
- 44. European Committee on Antimicrobial Susceptibility Testing (Eucast) Documents: Document Version 2.0 2012-01-01. 2012. Available online: http://eucast.org/clinical_breakpoints (accessed on 2 January 2023).
- Marchese, A.; Esposito, S.; Barbieri, R.; Bassetti, M.; Debbia, E. Does the adoption of EUCAST susceptibility breakpoints affect the selection of antimicrobials to treat acute community-acquired respiratory tract infections? *BMC Inf. Dis.* 2012, 12, 181. [CrossRef] [PubMed]
- Berkow, E.L.; Lockhart, S.R.; Ostrosky-Zeichner, L. Antifungal Susceptibility Testing: Current Approaches. *Clin. Microbiol. Rev.* 2020, 33, e00069-19. [CrossRef] [PubMed]
- Koehling, H.L.; Willinger, B.; Buer, J.; Rath, P.M.; Steinmann, J. Comparative Evaluation of a New Commercial Colorimetric Microdilution Assay (SensiQuattro Candida EU) with MIC Test Strip and EUCAST Broth Microdilution Methods for Susceptibility Testing of Invasive Candida Isolates. J. Clin. Microbiol. 2015, 53, 255–261. [CrossRef]
- Wikler, M.A. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard, 10th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2006.
- Kőszeginé, S.H.; Török, I.; Ráfliné, R.Z.; Nagy, A.; Fejér, I.; Kertész, P.; Posgayné, K.E.; Vankó, É. Microbiological testing of non-sterile products, Chapter 2.6.12–2.6.13. In *Hungarian Pharmacopoeia*, 8th ed.; Vincze, J., Ed.; Medicina: Budapest, Hungary, 2003; Volume I, pp. 159–169.

- 50. Available online: https://www.graphpad.com/guides/prism/latest/curve-fitting/reg_fitting_lines_to_semilog.htm (accessed on 2 January 2023).
- 51. Boukamp, P.; Petrussevska, R.T.; Breitkreutz, D.; Hornung, J.; Markham, A.; Fusenig, N.E. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.* **1988**, *106*, 761–771. [CrossRef]
- 52. Boukamp, P.; Popp, S.; Altmeyer, S.; Hülsen, A.; Fasching, C.; Cremer, T.; Fusenig, N.E. Sustained nontumorigenic phenotype correlates with a largely stable chromosome content during long-term culture of the human keratinocyte line HaCaT. *Genes Chromosomes Cancer* **1997**, *19*, 201–214. [CrossRef]
- 53. Malanga, M.; Szemán, J.; Fenyvesi, É.; Puskás, I.; Csabai, K.; Gyémánt, G.; Fenyvesi, F.; Szente, J. "Back to the Future": A New Look at Hydroxypropyl Beta-Cyclodextrins. J. Pharm. Sci. 2016, 105, 2921–2931. [CrossRef]
- 54. Kovács, R.; Erdélyi, L.; Fenyvesi, F.; Balla, N.; Kovács, F.; Vámosi, G.; Klusóczki, Á.; Gyöngyösi, A.; Bácskay, I.; Vecsernyés, M.; et al. Concentration-Dependent Antibacterial Activity of Chitosan on *Lactobacillus plantarum*. *Pharmaceutics* **2023**, *15*, 18. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.