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Synthesis of *N*-(β-D-glucopyranosyl) monoamides of dicarboxylic acids as potential inhibitors of glycogen phosphorylase

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Dedicated to the memory of Professor Dr. Zoltán Györgydeák[✠]

Abstract—O-Peracetylated *N*-(β-D-glucopyranosyl)imino trimethylphosphorane obtained in situ from 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and PMe_3 was reacted with saturated and unsaturated aliphatic and aromatic dicarboxylic acids, or their anhydrides, or monoesters to give the corresponding *N*-(β-D-glucopyranosyl) monoamides of dicarboxylic acids or derivatives. The acetyl protecting groups were removed according to the Zemplén protocol to give a series of compounds which showed moderate inhibitory effects against rabbit muscle glycogen phosphorylase *b*. The best inhibitor was 3-(*N*-β-D-glucopyranosyl-carbamoyl)propanoic acid (**7**) with $K_i = 20\ \mu M$.

Keywords: *N*-Glycosylamides; Glucose analogues; Inhibitors; Glycogen phosphorylase

1. Introduction

Many efforts devoted to develop carbohydrate based therapeutics aim at finding inhibitors of glycoprocessing enzymes and discovering their structure–activity relationships (SAR). Special types of glycoenzymes are the glycogen phosphorylases[1] (GP), which transfer a glucose unit from the nonreducing end of the storage polysaccharide glycogen to an inorganic phosphate. This is a most important event in glycogen metabolism, and the liver GP, as part of a complex regulatory system is directly responsible for the regulation of hepatic glucose output and thereby of blood sugar levels. Therefore, inhibition of GP[2] as a new concept of a possible basis of a novel treatment[3] for type 2 diabetes[4,5] has emerged and intensively been studied both in academia and industry.[6] One of the most populated class of inhibitors of GP is represented by derivatives of D-glucose (Chart 1).[†,7–11]

N-(β-D-glucopyranosyl)amides **A** and **B** are among the first efficient glucose analogue inhibitors.[12] An important structural feature in binding the amides to the catalytic site of GP is a strong H-bond between the amide NH and the main chain carbonyl of His377.[2,13] A recent study on a series of various *N*-glucopyranosyl amides[14] has revealed that a properly

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✠ Passed away on October 20th, 2005 at the age of 63 years.

† For other inhibitors of GP the reader is kindly referred to recent review articles[2,7,8] and the literature quoted there.

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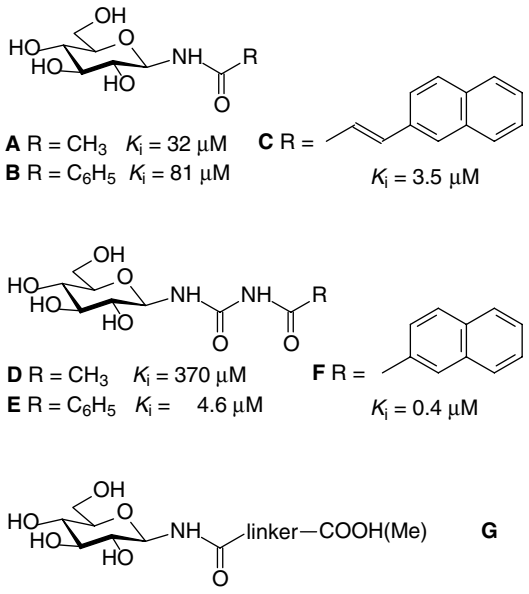


Chart 1. Inhibitors of glycogen phosphorylase.

positioned and large enough hydrophobic group attached to the amide moiety as in **C** makes the inhibition one order of magnitude stronger than that of **A**. On the other hand, this compound is still much less efficient than the best known glucose analogue inhibitor of GP *N*-(β-D-glucopyranosyl)-*N*′-(2-naphthoyl) urea **F**.[7,8] Other compounds of the urea type[15] (**D**, **E**) show weaker activity than that of **F**. Binding of the aglycon of these compounds takes place in the so called β-channel of the enzyme. This channel of mixed character is surrounded by both polar and apolar amino acid side chains.[2] Therefore, carefully designed inhibitors need proper arrangements of groups capable of interacting with the residues in the β-channel.

In this paper, we wish to map the β-channel with a series of *N*-(β-D-glucopyranosyl) monoamides of dicarboxylic acids (**G**). Such compounds offer the possibility to place a strongly polar group (COOH) at different distances from the sugar moiety while the ability to form the important H-bond from the amide can be maintained. The carbon skeleton (linker) of the diacid may also contribute to the binding and, furthermore, simple esterification (to COOMe) can modify the character of the polar group.

2. Results and discussion

2.1. Synthesis

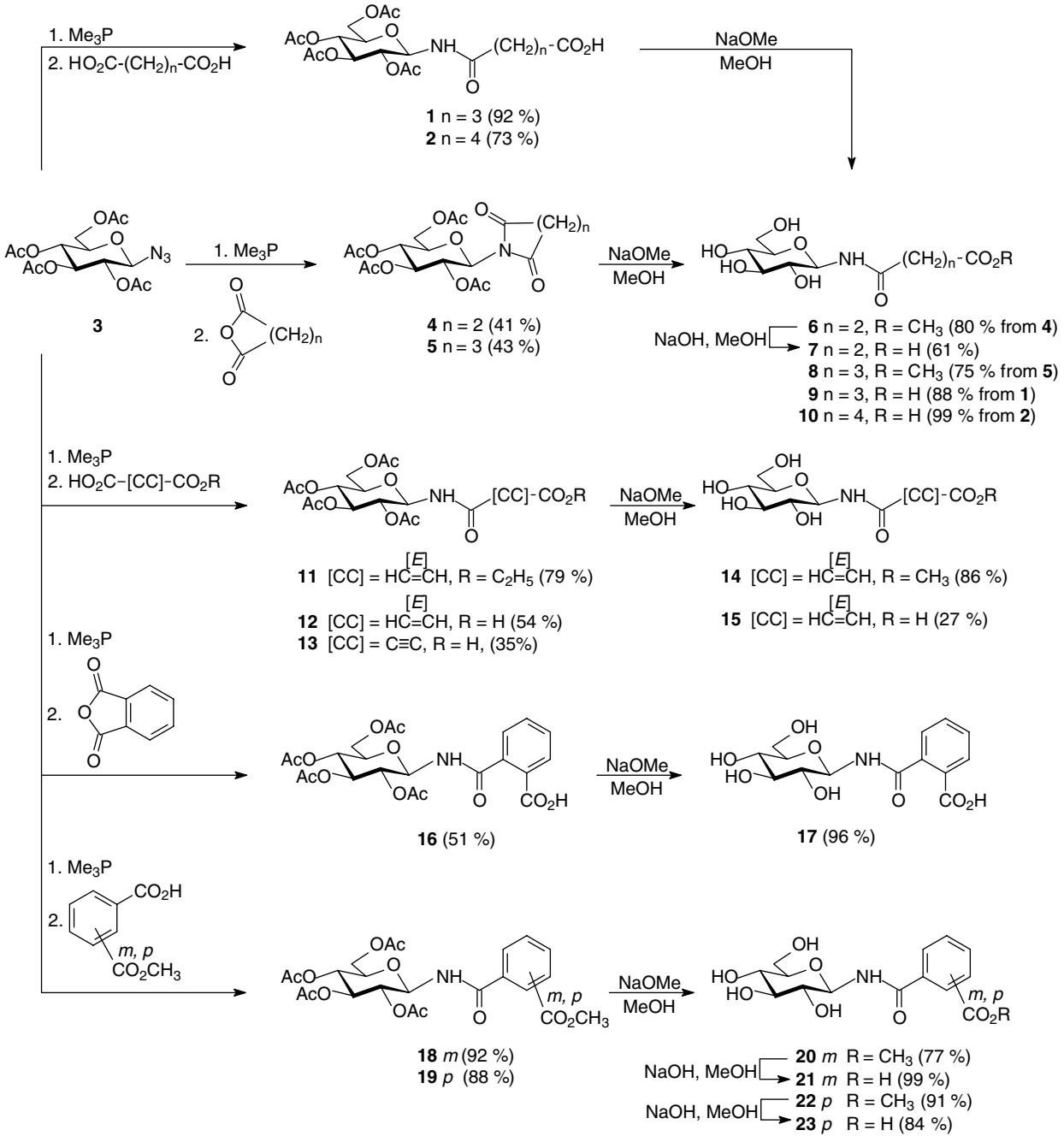
N-Glycosyl amides[‡] are frequently prepared by acylation of glycosylamines.[17] This method was applied for the preparation of *N*-glycosyl monoamides of some dicarboxylic acids: anhydrides of phthalic, succinic[18] and maleic acid[19,20] were used for the acylation of variously protected β-D-glucopyranosylamines; β-D-galactopyranosylamine was acylated by a mixed anhydride obtained from monomethyl succinate.[21] O-Peracetylated β-D-galactopyranosyl isothiocyanates were also transformed to N-galactosylated monoamides of C_3–C_6 and C_9 α,ω-dicarboxylic acids.[22]

A more advantageous method to produce *N*-glycosyl amides uses glycosyl azides[23] following the Staudinger protocol,[24] that is, reaction of an *N*-glycosyl iminophosphorane with activated carboxylic acid derivatives. An elegant modification of this method has recently been elaborated introducing the use of PMe_3 that results in enhanced nucleophilicity of the intermediate phosphorane[16] allowing carboxylic acids to be applied as the acylating agents. Easier work-up of the reaction mixtures due to the volatile nature of the by-product $P(=O)Me_3$ represents another advantage. Following the successful synthesis of several new *N*-glycosyl amides of monocarboxylic acids as well as α-amino acid derivatives[14] by this procedure we have now extended the method to dicarboxylic acids and their derivatives. It is to be noted that a similar reaction with phthalic anhydride resulted in several N-substituted phthalimides from diverse azido sugars excluding glycosyl azides.[25,26]

Formation of the intermediate *N*-glucosyl iminophosphorane was effected in each case at room temperature by reacting 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide[27] (**3**) dissolved in CH_2Cl_2 with a 1 M toluene or THF solution of PMe_3. Treatment of the iminophosphorane with glutaric or adipic acid (Scheme 1) gave the corresponding *N*-glucopyranosyl monoamides **1** and **2**, and subsequent removal of the protecting groups using the Zemplén method afforded **9** and **10**, respectively. Reaction of the iminophosphorane with succinic or glutaric anhydride gave *N*-glucopyranosyl succinimide[§] **4** or -glutarimide **5**, respectively. This contrasts with the reported formation of *N*-glycosyl monoamides of dicarboxylic acids in the reaction of glycosylamines with succinic anhydride[18] and in similar reactions mentioned in the first paragraph. During removal of the acetyl protecting groups by NaOMe/MeOH from **4** and **5** the cyclic imide moiety was opened to give methylester amides **6** and **8**, respectively. Free acid **7** was obtained from **6** by NaOH in MeOH followed by acidification with Amberlyst 15 (H^+).

[‡] For a critical survey of recent literature see Ref. 16.

[§] Compound **4** was prepared earlier by reacting succinimide (as the Ag salt) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide.[28] The benzoylated analogue of **4** was reported to form in a reaction of the corresponding ethyl 1-thioglucopyranoside with NIS–TfOH.[29] Diverse other *N*-glucopyranosyl succinimide derivatives were obtained by acylation of D-glucopyranosylamines with succinic anhydride followed by cyclization.[30]



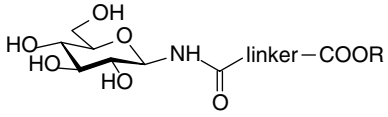
Scheme 1.

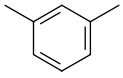
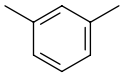
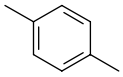
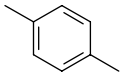
To get similar derivatives of unsaturated aliphatic diacids, reactions of the iminophosphorane with fumaric acid and butyndioic acid were investigated first. In both cases, the transformation was rather sluggish and gave complex mixtures from which the expected acids **12** and **13**, respectively, were isolated in moderate yields. On the other hand, in a reaction with monoethyl fumarate, ethylester amide **11** was formed in good yield. Deprotection according to the Zemplén method gave methylester amide **14** from **11**, free acid **15** from **12**, while in the case of **13** decomposition took place. With maleic anhydride, complex mixtures were formed from which no discrete product could be isolated. These observations with the unsaturated acids and derivatives might be due to the sensitivity of the electron poor double bonds toward nucleophilic attacks, and are in accord with the reported unstability of O-peracetylated *N*-glucopyranosyl maleimide.[¶,33]

Possibilities to obtain derivatives of aromatic dicarboxylic acids (phthalic-, isophthalic-, or terephthalic

[¶] For the preparation of other *N*-glucopyranosyl maleimide derivatives, see as leading references: 19,20,31,32.

Table 1. Inhibitory effects of *N*-(β-D-glucopyranosyl) monoamides of dicarboxylic acid derivatives tested with rabbit muscle GP*b*



Compound	–Linker–	R	K_i (μM)	IC_{50} (mM)
6	$-CH_2CH_2-$	CH_3	170	
7	$-CH_2CH_2-$	H	20	
8	$-CH_2CH_2CH_2-$	CH_3	83	
9	$-CH_2CH_2CH_2-$	H		No effect in 625 μM
10	$-CH_2CH_2CH_2CH_2-$	H		7.9
14		CH_3		1
15		H		No effect in 625 μM
17		H		No effect in 625 μM
20		CH_3	580	
21		H		4
22		CH_3	329	
23		H		No effect in 625 μM

acid) were investigated next. Reaction of either isomer of the diacids with the iminophosphorane resulted in decomposition. Phthalic anhydride furnished acid **16** in acceptable yield what is contrary to the formation of *N*-glycosyl imides **4** and **5** with the corresponding aliphatic anhydrides.[‖] This, we speculate, might be due to stereoelectronic reasons: attack of the iminophosphorane nucleophile may open the anhydro ring in each case; this can be followed by a ring closure to give the cyclic imide by an intramolecular nucleophilic attack of the nitrogen atom on the other carbonyl group; while with the flexible aliphatic derivatives this can occur along the favorable Bürgi–Dunitz trajectory,[36] in the conjugated and therefore planar aromatic derivative this stereoelectronic condition could only be met with significant loss of conjugative stabilization which cannot be provided energetically under the mild reaction conditions. Acylation of the iminophosphorane by monomethylesters of iso- and terephthalic acid gave good yields of methylester amides **18** and **19**, respectively. Zemplén deacetylations of **18** and **19** gave the deprotected methylester amides **20** and **22**, respectively, which were converted to acids **21** and **23**, respectively, by NaOH in MeOH followed by treatment with Amberlyst 15 (H^+) resin.

Stuctural elucidation of the new compounds was straightforward by NMR spectroscopy and needs no special comment. For assignments of the proton and carbon spectra, see Section 4.

2.2. Enzyme tests and evaluation of the inhibitors

Enzyme tests were carried out as described earlier[37] (see also Section 4). Compound **7** has proven the best inhibitor of the investigated series (Table 1) suggesting that a polar group with an ability to act as both hydrogen bond donor and acceptor (compare **6** and **7**) at three bond distance (compare **7**, **9** and **10**) from the amide moiety is beneficial for the strong binding of a glucose analogue. The flexibility of the linker seems also important since a less mobile carbon chain as in **15** results in loss of the activity. Changing the linker to aromatic residues again makes less efficient inhibitors (**17**, **20**–**23**). The role of the flexibility of the carbon chain cannot be evaluated on the basis of the present data. One can assess two possibilities: (a) the formation of intramolecular hydrogen bonds either with the amide NH or C=O to form ring shaped structures attached to the sugar

[‖] For the preparation of *N*-glycosyl phthalimide derivatives, see Refs. 28,31,34,35.

ring; (b) 'open chain' bound structures with more extensive interactions in the β-channel of the enzyme. Such binding properties can be revealed by protein crystallographic investigation of the enzyme–inhibitors complexes, and these studies are in progress.

3. Conclusion

Extension of the modified Staudinger methodology to the synthesis of *N*-(β-D-glucopyranosyl) monoamides of various dicarboxylic acids proved efficient and resulted in a new series of compounds which were tested as inhibitors of rabbit muscle glycogen phosphorylase *b*. The best inhibitor was compound **7** with a flexible linker between the amide and the carboxylic acid group.

4. Experimental

4.1. General methods

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter at room temperature. NMR spectra were recorded with a Bruker 360 (360/90 MHz for $^{1}H/^{13}C$) spectrometer. Chemical shifts are referenced to Me_4Si (^{1}H), or to the residual solvent signals (^{13}C). TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (E. Merck), and the plates were visualized under UV light and by gentle heating. For column chromatography, Kieselgel 60 (E. Merck, particle size 0.063–0.200 mm) was used. Organic solutions were dried over anhyd $MgSO_4$ and concentrated under diminished pressure at 40–50 °C (water bath). PMe_3 (1 M soln in toluene or THF) as well as dicarboxylic acids and their derivatives were purchased from Aldrich. The high resolution electrospray (ESI) mass spectra were obtained from a Bruker Apex III 70e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external APOLLO electrospray ion source (Agilent, off axis spray). Nitrogen was used as drying gas at 150 °C. The sample solns were introduced continuously via a syringe pump with a flow rate of 120 μL h^{-1}. All data were aquired with 256k data points and zero filled to 1024k by averaging 32 scans.

4.2. General procedure I for the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (3) with trimethyl phosphane and dicarboxylic acid derivatives

To a soln of **3** in dry CH_2Cl_2 (5–7 mL/mmol), PMe_3 (1.1 equiv of a 1 M toluene or THF soln) was added in one portion. The mixture was stirred at rt until nitrogen evolution had ceased and TLC (1:1 EtOAc–hexane) had indicated complete transformation of **3** (approx. 15 min). This soln was then reacted with a dicarboxylic acid, or its monoester, or anhydride (1.1 equiv) as indicated with the particular compounds till the disappearance of the iminophosphorane (~2 d, TLC 1:1 EtOAc–hexane). The volatiles were removed under diminished pressure and the residue purified by column chromatography.

4.3. General procedure II for the Zemplén-deacetylation

To a soln of an acetyl protected compound in dry MeOH, 1–2 drops of a ~1 M methanolic NaOMe soln were added, and the reaction mixture was kept at rt until completion of the transformation (TLC 1:1 $CHCl_3$–MeOH). Amberlyst 15 (H^+ form) was then added to remove sodium ions, the resin was filtered off, and the solvent removed under diminished pressure. If the residue was chromatographically not uniform it was purified by column chromatography or crystallization.

4.4. General procedure III for the hydrolysis of methylester groups

A methylester was suspended in distilled MeOH (2 mL/mmol), and NaOH (1.5 equiv of a 1 M methanolic soln) was added. The reaction mixture was stirred at rt or heated at 60 °C until disappearance of the starting material (TLC 1:1 $CHCl_3$–MeOH). Amberlyst 15 (H^+ form) was then added to remove sodium ions, the resin was filtered off, and the solvent removed under diminished pressure. The crude product was purified by crystallization or column chromatography.

4.5. 4-(*N*-2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosylcarbamoyl)butyric acid (1)

Prepared from **3** (1.5 g, 4.02 mmol) and glutaric acid (0.58 g, 4.42 mmol) according to General procedure I. Eluent for the column chromatography: 95:5 $CHCl_3$–MeOH. Yield: 1.7 g (92%) white crystalline product, mp: 119–122 °C; $[\alpha]_D$ +1 (*c* 0.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (ppm) 9.31 (1H, s, COO*H*), 6.74 (1H, d, $J = 9.3$ Hz, NH), 5.33, 5.28, 5.06, 4.95 (4H, 4 pseudo t, $J \sim 9.3$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.29 (1H, dd, $J = 12.4$, 3.9 Hz, H-6′a), 4.09 (1H, dd, $J = 12.4$, 1.5 Hz, H-6′b), 3.83 (1H, ddd, $J = 9.3$, 3.9, 1.5 Hz, H-5′), 2.39–2.27 (4H, m, $2 \times CH_2$), 2.08, 2.06, 2.03, 2.02 (12H, 4s, $4 \times CH_3$), 1.96–1.92 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$): δ (ppm) 177.1 (*C*OOH), 171.0, (NH*C*O), 170.6, 169.9, 169.8, 169.6 (*C*O), 169.6 (NH*C*O), 77.9 (C-1′), 73.4, 72.9, 70.6, 68.1 (C-2′ to C-5′), 61.7 (C-6′), 34.9, 32.5 (*C*H_2), 20.6, 20.5, 20.4, 20.3 (*C*H_3), 19.9 (*C*H_2); HRMS: *m*/*z* 484.14163

([M+Na]$^+$, calcd for $C_{19}H_{27}NO_{12}Na^+$ 484.14255). Anal. Calcd for $C_{19}H_{27}NO_{12}$ (461.43): C, 49.46; H, 5.90; N, 3.04. Found: C, 49.34; H, 5.86; N, 3.12.

4.6. 5-(*N*-2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)pentanoic acid (2)

Prepared from **3** (0.5 g, 1.34 mmol) and adipic acid (0.42 g, 1.46 mmol) according to General procedure I. Eluent for the column chromatography: 9:1 $CHCl_3$–MeOH. Yield: 0.46 g (73%) colourless oil, $R_f = 0.52$ (EtOAc); $[\alpha]_D$ +23 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (ppm) 6.51 (1H, d, $J = 9.5$ Hz, NH), 5.28, 5.24, 5.03, 4.88 (4H, 4 pseudo t, $J \sim 9.5$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.27 (1H, dd, $J = 12.6$, 4.4 Hz, H-6′a), 4.05 (1H, dd, $J = 12.6$, 2.0 Hz, H-6′b), 3.81 (1H, ddd, $J = 9.5$, 4.4, 2.0 Hz, H-5′), 2.33–2.20 (4H, m, $2 \times CH_2$), 2.05, 2.02, 2.01, 1.99 (12H, 4s, $4 \times CH_3$), 1.62–1.60 (4H, m, $2 \times CH_2$); ^{13}C NMR ($CDCl_3$): δ (ppm) 177.8, 172.3 (*C*OOH, NHCO), 170.8, 170.5, 169.7, 169.4 (*C*O), 77.9 (C-1′), 73.4, 72.7, 70.6, 68.1 (C-2′ to C-5′), 61.7 (C-6′), 36.0, 33.5, 24.4, 24.0 (CH_2), 20.8, 20.7, 20.6 (2) (CH_3); ESIMS: *m*/*z* 498 ([M+Na]$^+$).

4.7. *N*-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-succinimide (4)

Prepared from **3** (0.8 g, 2.14 mmol) and succinic anhydride (0.23 g, 2.30 mmol) according to General procedure I. Eluent for the column chromatography: 3:1 EtOAc–hexane. Yield: 0.28 g (41%) yellow crystalline product, mp: 203–204 °C; $[\alpha]_D$ +10 (*c* 0.43, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (ppm) 5.89, 5.28, 5.20 (3H, 3 pseudo t, $J \sim 10.0$ Hz in each, H-2′, H-3′, H-4′), 5.26 (1H, d, $J = 10.0$ Hz, H-1′), 4.19–4.16 (2H, m, H-6′a, H-6′b), 3.79 (1H, ddd, $J = 10.0$, 7.0, 3.5 Hz, H-5′), 2.70–2.68 (4H, m, $2 \times CH_2$), 2.13, 2.08, 2.05, 1.97 (12H, 4s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 175.1 (2), 170.4, 169.8, 169.3, 169.1 (*C*O), 78.2 (C-1′), 74.6, 73.2, 67.9, 67.8 (C-2′ to C-5′), 61.6 (C-6′), 27.9 (2) (CH_2), 20.8, 20.7, 20.6, 20.5 (CH_3); ESIMS: *m*/*z* 452 ([M+Na]$^+$). Anal. Calcd for $C_{18}H_{23}NO_{11}$ (429.38): C, 50.35; H, 5.40; N, 3.26. Found: C, 49.98; H, 5.26; N, 3.32.

4.8. *N*-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-glutarimide (5)

Prepared from **3** (0.8 g, 2.14 mmol) and glutaric anhydride (0.27 g, 2.37 mmol) according to General procedure I. Eluent for the column chromatography: 3:1 EtOAc–hexane. Yield: 0.41 g (43%) white crystalline product, mp: 129–132 °C; $[\alpha]_D$ +14 (*c* 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (ppm) 5.83 (1H, d, $J = 9.4$ Hz, H-1′), 5.90, 5.28, 5.20 (3H, 3 pseudo t, $J \sim 9.4$ Hz in each, H-2′, H-3′, H-4′), 4.21 (1H, dd, $J = 12.5$, 4.7 Hz, H-6′a), 4.15 (1H, dd, $J = 12.5$, 2.3 Hz, H-6′b), 3.77 (1H, ddd, $J = 9.4$, 4.7, 2.3 Hz, H-5′), 2.66–2.62 (6H, m, $3 \times CH_2$), 2.08, 2.05, 2.04, 1.99 (12H, 4s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 172.1, 170.5, 170.4, 169.8, 169.6, 169.2 (*C*O), 78.7 (C-1′), 74.5, 73.4, 68.8, 67.9 (C-2′ to C-5′), 61.8 (C-6′), 33.9, 32.9 (CH_2) 20.8, 20.7, 20.6, 20.5 (CH_3), 16.8 (CH_2); ESIMS: *m*/*z* 466 ([M+Na]$^+$). Anal. Calcd for $C_{19}H_{25}NO_{11}$ (443.41): C, 51.47; H, 5.68; N, 3.16. Found: C, 51.22; H, 5.26; N, 3.10.

4.9. Methyl 3-(*N*-β-D-glucopyranosyl-carbamoyl)-propanoate (6)

Prepared from **4** (0.13 g, 0.30 mmol) according to General procedure II. Eluent for the column chromatography: 7:3 $CHCl_3$–MeOH. Yield: 0.07 g (80%) white crystalline product, mp: 160–163 °C; $[\alpha]_D$ −19 (*c* 0.41, H_2O); ^{1}H NMR (D_2O): δ (ppm) 4.95 (1H, d, $J = 9.3$ Hz, H-1′), 3.87 (1H, dd, $J = 12.4$, 2.2 Hz, H-6′a), 3.71 (1H, dd, $J = 12.4$, 5.3 Hz, H-6′b), 3.70 (3H, s, OCH_3), 3.54 (1H, pseudo t, $J = 9.3$, 9.1 Hz, H-2′), 3.50 (1H, ddd, $J = 9.3$, 5.3, 2.2 Hz, H-5′), 3.44–3.38 (2H, m, H-3′, H-4′), 2.71–2.62 (4H, m, $2 \times CH_2$); ^{13}C NMR (D_2O): δ (ppm) 176.8, 176.4 (*C*OOCH$_3$, NH*C*O), 80.2 (C-1′), 78.5, 77.5, 72.7, 70.2 (C-2′ to C-5′), 61.5 (C-6′), 53.2 (O*C*H$_3$) 31.2, 29.9 (CH_2); ESIMS: *m*/*z* 316 ([M+Na]$^+$). Anal. Calcd for $C_{11}H_{19}NO_8$ (293.28): C, 45.05; H, 6.53; N, 4.78. Found: C, 45.14; H, 6.66; N, 4.52.

4.10. 3-(*N*-β-D-Glucopyranosyl-carbamoyl)propanoic acid (7)

Prepared from **6** (0.05 g, 0.17 mmol) according to General procedure III. Eluent for the column chromatography: 1:1 $CHCl_3$–MeOH. Yield: 0.03 g (61%) colourless oil, $R_f = 0.21$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +2 (*c* 0.38, MeOH); ^{1}H NMR (D_2O): δ (ppm) 4.96 (1H, d, $J = 9.1$ Hz, H-1′), 3.87 (1H, dd, $J = 12.4$, 2.2 Hz, H-6′a), 3.72 (1H, dd, $J = 12.4$, 5.1 Hz, H-6′b), 3.55 (1H, pseudo t, $J = 9.1$, 8.9 Hz, H-2′), 3.52 (1H, ddd, $J = 9.1$, 5.1, 2.2 Hz, H-5′), 3.45–3.36 (2H, m, H-3′, H-4′), 2.60–2.56 (4H, m, $2 \times CH_2$); ^{13}C NMR (D_2O): δ (ppm) 179.6 (*C*OOH), 177.4 (NH*C*O), 80.2 (C-1′), 78.5, 77.4, 72.8, 70.2 (C-2′ to C-5′), 61.5 (C-6′), 31.5, 32.1 (CH_2); ESIMS: *m*/*z* 302 ([M+Na]$^+$).

4.11. Methyl 4-(*N*-β-D-glucopyranosyl-carbamoyl)-butyrate (8)

Prepared from **5** (0.10 g, 0.22 mmol) according to General procedure II. Eluent for the column chromatography 7:3 $CHCl_3$–MeOH. Yield: 0.05 g (75%) colourless oil, $R_f = 0.67$ (7:3 $CHCl_3$–MeOH); $[\alpha]_D$ −8 (*c* 0.33, H_2O); ^{1}H NMR (D_2O): δ (ppm) 4.96 (1H, d, $J =$

8.9 Hz, H-1′), 3.89 (1H, dd, $J = 12.4$, 1.8 Hz, H-6′a), 3.72 (1H, dd, $J = 12.4$, 4.9 Hz, H-6′b), 3.71 (3H, s, OCH_3), 3.56 (1H, pseudo t, $J = 9.1$, 8.9 Hz, H-2′), 3.52 (1H, ddd, $J = 9.1$, 4.9, 1.8 Hz, H-5′), 3.42–3.38 (2H, m, H-3′, H-4′), 2.46–1.93 (6H, m, $3 \times CH_2$); ^{13}C NMR (D_2O): δ (ppm) 178.0, 177.1 (*C*OOCH$_3$, NH*C*O), 80.2 (C-1′), 78.5, 77.5, 72.7, 70.2 (C-2′ to C-5′), 61.5 (C-6′), 53.2 (O*C*H$_3$) 35.7, 33.8, 21.3 (*C*H$_2$); HRMS: *m*/*z* 330.11593 ([M+Na]$^+$, calcd for $C_{12}H_{21}NO_8Na^+$ 330.11594).

4.12. 4-(*N*-β-D-Glucopyranosyl-carbamoyl)butyric acid (9)

Prepared from **1** (0.40 g, 0.86 mmol) according to General procedure II. Eluent for the column chromatography: 1:1 $CHCl_3$–MeOH. Yield: 0.26 g (88%) yellowish oil, $R_f = 0.11$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +1 (*c* 0.69, MeOH); ^1H NMR (D_2O): δ (ppm) 4.95 (1H, d, $J = 8.9$ Hz, H-1′), 3.87 (1H, dd, $J = 11.4$, 2.2 Hz, H-6′a), 3.70 (1H, dd, $J = 11.4$, 3.8 Hz, H-6′b), 3.56 (1H, pseudo t, $J = 9.4$, 8.9 Hz, H-2′), 3.51 (1H, ddd, $J = 9.4$, 3.8, 2.2 Hz, H-5′), 3.42–3.38 (2H, m, H-3′, H-4′), 2.37–2.28 (4H, m, $2 \times CH_2$), 1.89–1.85 (2H, m, $2 \times CH_2$); ^{13}C NMR (D_2O): δ (ppm) 181.1 (*C*OOH), 178.1 (NH*C*O), 79.8 (C-1′), 78.1, 77.0, 72.3, 69.8 (C-2′ to C-5′), 61.1 (C-6′), 35.0 (2), 21.8 (*C*H$_2$); HRMS: *m*/*z* 316.10037 ([M+Na]$^+$, calcd for $C_{11}H_{19}NO_8Na^+$ 316.10029).

4.13. 5-(*N*-β-D-Glucopyranosyl-carbamoyl)pentanoic acid (10)

Prepared from **2** (0.25 g, 0.52 mmol) according to General procedure II. Eluent for the column chromatography: 1:1 $CHCl_3$–MeOH. Yield: 0.16 g (99%) colourless oil, $R_f = 0.37$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ −16 (*c* 0.39, H_2O); ^1H NMR (D_2O): δ (ppm) 4.94 (1H, d, $J = 9.1$ Hz, H-1′), 3.86 (1H, dd, $J = 12.4$, 2.0 Hz, H-6′a), 3.71 (1H, dd, $J = 12.4$, 5.3 Hz, H-6′b), 3.53 (1H, pseudo t, $J = 9.1$, 8.9 Hz, H-2′), 3.52 (1H, ddd, $J = 9.1$, 5.3, 2.0 Hz, H-5′), 3.43–3.37 (2H, m, H-3′, H-4′), 2.34–1.61 (8H, m, $4 \times CH_2$); ^{13}C NMR (D_2O): δ (ppm) 182.3 (*C*OOH), 178.9 (NH*C*O), 80.2 (C-1′), 78.5, 77.5, 72.7, 70.2 (C-2′ to C-5′), 61.6 (C-6′), 36.7, 36.5, 25.8, 25.7 (*C*H$_2$); ESIMS: *m*/*z* 330 ([M+Na]$^+$).

4.14. Ethyl 3-(*N*-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)-(*E*)-propenoate (11)

Prepared from **3** (1.0 g, 2.68 mmol) and fumaric acid monoethyl ester (0.42 g, 2.91 mmol) according to General procedure I. Eluent for the column chromatography: 1:1 EtOAc–hexane. Yield: 1.0 g (79%) white crystalline product, mp: 123–126 °C; $[\alpha]_D$ −8 (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 7.27 (1H, d, $J = 9.2$ Hz, NH) 6.92 (1H, d, $J = 15.8$ Hz, CH), 6.83 (1H, d, $J = 15.8$ Hz, CH), 5.39, 5.32, 5.06, 4.98 (4H, 4 pseudo t, $J \sim 9.2$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.28 (1H, dd, $J = 11.9$, 5.3 Hz, H-6′a), 4.24 (2H, q, $J = 6.6$ Hz, CH_2), 4.09 (1H, dd, $J = 11.9$, 1.5 Hz, H-6′b), 3.88 (1H, ddd, $J = 9.5$, 5.3, 1.5 Hz, H-5′), 2.08, 2.04 (2), 2.03, (12H, 3s, $4 \times CH_3$), 1.45 (3H, t, $J = 6.6$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.6, 170.5, 169.8, 169.5, 165.0, 163.9 (*C*O, NH*C*O, *C*OOCH$_2$CH$_3$), 135.0, 131.9 (*C*H), 78.0 (C-1′), 73.5, 72.7, 70.5, 68.0 (C-2′ to C-5′), 61.6 (C-6′), 61.3 (*C*H$_2$CH$_3$), 20.5, 20.4 (3) (*C*H$_3$), 13.9 (CH$_2$*C*H$_3$); HRMS: *m*/*z* 496.14226 ([M+Na]$^+$, calcd for $C_{20}H_{27}NO_{12}Na^+$ 496.14255). Anal. Calcd for $C_{20}H_{27}NO_{12}$ (473.44): C, 50.74; H, 5.75; N, 2.96. Found: C, 50.43; H, 5.86; N, 3.00.

4.15. 3-(*N*-2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)-(*E*)-propenoic acid (12)

Prepared from **3** (0.8 g, 2.14 mmol) and fumaric acid (0.27 g, 2.35 mmol) according to General procedure I. Eluent for the column chromatography: 4:1 EtOAc–hexane. Yield: 0.52 g (54%) yellowish oil, $R_f = 0.12$ (EtOAc); $[\alpha]_D$ −21 (*c* 0.22, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 7.42 (1H, d, $J = 9.2$ Hz, NH) 7.08 (1H, d, $J = 15.8$ Hz, CH), 6.91 (1H, d, $J = 15.8$ Hz, CH), 5.39, 5.31, 5.10, 5.03 (4H, 4 pseudo t, $J \sim 9.2$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.30 (1H, dd, $J = 13.2$, 5.3 Hz, H-6′a), 4.11 (1H, dd, $J = 13.2$, 2.6 Hz, H-6′b), 3.88 (1H, ddd, $J = 9.2$, 5.3, 2.6 Hz, H-5′), 2.08, 2.05, 2.03 (2) (12H, 3s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.4, 170.7, 169.6, 167.6, 167.4, 164.0 (*C*OOH, NH*C*O, *C*O), 136.8, 131.3 (*C*H), 78.0 (C-1′), 73.7, 73.0, 70.6, 68.0 (C-2′ to C-5′), 61.7 (C-6′), 20.5 (4) (*C*H$_3$); HRMS: *m*/*z* 468.11154 ([M+Na]$^+$, calcd for $C_{18}H_{23}NO_{12}Na^+$ 468.11125).

4.16. 3-(*N*-2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)propynoic acid (13)

Prepared from **3** (0.5 g, 1.34 mmol) and acetylenedicarboxylic acid (0.17 g, 1.47 mmol) according to General procedure I. Eluent for the column chromatography: 3:1 EtOAc–hexane. Yield: 0.20 g (35%) colourless oil, $R_f = 0.40$ (3:1 EtOAc–hexane); $[\alpha]_D$ −33 (*c* 0.27, Me_2SO); ^1H NMR ($CDCl_3$): δ (ppm) 7.30 (1H, d, $J = 9.1$ Hz, NH), 5.40, 5.30, 5.07, 4.99 (4H, 4 pseudo t, $J \sim 9.5$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.31 (1H, dd, $J = 12.4$, 3.8 Hz, H-6′a), 4.11 (1H, dd, $J = 12.4$, 2.2 Hz, H-6′b), 3.85 (1H, ddd, $J = 9.5$, 3.8, 2.2 Hz, H-5′), 2.09, 2.08, 2.04, 2.03 (12H, 4s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.4, 169.7 (2), 169.4 (2) (*C*OOH, *C*O), 152.0 (NH*C*O), 77.5 (C-1′, ≡*C*–), 75.5 (≡*C*–), 73.5, 72.6, 70.1, 67.9 (C-2′ to C-5′), 61.5 (C-6′), 20.4 (2), 20.2 (2) (CH$_3$); HRMS: *m*/*z* 422.10615 ([M+Na−CO$_2$]$^+$, calcd for $C_{17}H_{21}NO_{10}Na^+$ 422.10577).

4.17. Methyl 3-(*N*-β-D-glucopyranosyl-carbamoyl)-(*E*)-propenoate (14)

Prepared from **11** (0.2 g, 0.42 mmol) according to General procedure II. Crystallized from MeOH to give 0.11 g (86%) white solid, mp: 176–179 °C; $[\alpha]_D$ −54 (*c* 0.22, H_2O); ^1H NMR (D_2O): δ (ppm) 7.04 (1H, d, $J = 15.8$ Hz, CH), 6.84 (1H, d, $J = 15.8$ Hz, CH), 5.07 (1H, d, $J = 9.2$ Hz, H-1′), 3.87 (1H, dd, $J = 11.9$, 2.6 Hz, H-6′a), 3.83 (3H, s, OCH_3), 3.73 (1H, dd, $J = 11.9$, 5.3 Hz, H-6′b), 3.57 (1H, t, $J = 9.2$, 9.2 Hz, H-2′), 3.55 (1H, ddd, $J = 9.2$, 5.3, 2.6 Hz, H-5′), 3.47–3.42 (2H, m, H-3′, H-4′); ^{13}C NMR (D_2O): δ (ppm) 168.1, 167.6 (*C*OOCH$_3$, NH*C*O), 136.0, 131.7 (*C*H), 80.0 (C-1′), 78.3, 77.0, 72.4, 69.8 (C-2′ to C-5′), 61.1 (C-6′), 53.40 (O*C*H$_3$); HRMS: *m*/*z* 314.08493 ([M+Na]$^+$, calcd for $C_{11}H_{17}NO_8Na^+$ 314.08464). Anal. Calcd for $C_{11}H_{17}NO_8$ (291.26): C, 45.36; H, 5.88; N, 4.81. Found: C, 45.20; H, 5.56; N, 4.62.

4.18. 3-(*N*-β-D-Glucopyranosyl-carbamoyl)-(*E*)-propenoic acid (15)

Prepared from **12** (0.1 g, 0.22 mmol) according to General procedure II. Eluent for the column chromatography: 2:1 $CHCl_3$–MeOH. Yield: 0.03 g (27%) colourless oil, $R_f = 0.47$ (2:1 $CHCl_3$–MeOH); $[\alpha]_D$ −22 (*c* 0.37, H_2O); ^1H NMR (D_2O): δ (ppm) 6.77 (1H, d, $J = 15.8$ Hz, CH), 6.61 (1H, d, $J = 15.8$ Hz, CH), 5.00 (1H, d, $J = 9.2$ Hz, H-1′), 3.81 (1H, dd, $J = 11.9$, 2.6 Hz, H-6′a), 3.67 (1H, dd, $J = 11.9$, 5.3 Hz, H-6′b), 3.53 (1H, t, $J = 9.1$, 9.1 Hz, H-2′), 3.52 (1H, ddd, $J = 9.1$, 5.3, 2.6 Hz, H-5′), 3.41–3.36 (2H, m, H-3′, H-4′); ^{13}C NMR (D_2O): δ (ppm) 173.5, 169.2 (*C*OOH, NH*C*O), 138.6, 131.0 (CH), 80.1 (C-1′), 78.2, 77.0, 72.4, 69.7 (C-2′ to C-5′), 61.1 (C-6′); HRMS: *m*/*z* 276.07305 ([M−H]$^-$, calcd for $C_{10}H_{14}NO_8^-$ 276.07249).

4.19. 2-(*N*-2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)benzoic acid (16)

Prepared from **3** (0.8 g, 2.14 mmol) and phthalic anhydride (0.35 g, 2.36 mmol) according to General procedure I. Eluent for the column chromatography: 95:5 $CHCl_3$–MeOH. Yield: 0.54 g (51%) white crystalline product, mp: 158–160 °C; $[\alpha]_D$ +5 (*c* 0.83, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 7.94–7.51 (4H, m, aromatics), 7.46 (1H, d, $J = 10.4$ Hz, NH), 5.46, 5.38, 5.05, 4.97 (4H, 4 pseudo t, $J \sim 10$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.30 (1H, dd, $J = 12.5$, 3.6 Hz, H-6′a), 4.11 (1H, ddd, J=10.4, 3.6, 2.1 Hz, H-5′), 3.95 (1H, dd, $J = 12.5$, 2.1 Hz, H-6′b), 2.17 (2), 2.10, 2.05 (12H, 3s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.4, 171.0, 169.9 (2), 169.6 (2) (*C*OOH, NH*C*O, *C*O), 136.8 (C-1) 132.1 (C-2) 132.0, 130.7, 130.2, 127.4 (C-3 to C-6), 78.2 (C-1′), 73.3, 72.8, 70.6, 68.2 (C-2′ to C-5′), 61.8 (C-6′), 20.6, 20.5 (3) (*C*H$_3$); HRMS: *m*/*z* 518.12714 ([M+Na]$^+$, calcd for $C_{22}H_{25}NO_{12}Na^+$ 518.12690). Anal. Calcd for $C_{22}H_{25}NO_{12}$ (495.44): C, 53.34; H, 5.09; N, 2.83. Found: C, 53.14; H, 5.16; N, 2.92.

4.20. 2-(*N*-β-D-Glucopyranosyl-carbamoyl)benzoic acid (17)

Prepared from **16** (0.2 g, 0.40 mmol) according to General procedure II. Eluent for the column chromatography: 1:1 $CHCl_3$–MeOH. Yield: 0.13 g (96%) colourless oil, $R_f = 0.2$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +4 (*c* 0.21, MeOH); ^1H NMR (D_2O): δ (ppm) 7.76–7.52 (4H, m, aromatics), 5.10 (1H, d, $J = 9.5$ Hz, H-1′), 3.89 (1H, dd, $J = 11.6$, 1.3, Hz, H-6′a), 3.74 (1H, dd, $J = 11.6$, 4.8 Hz, H-6′b), 3.70–3.43 (4H, m, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (D_2O): δ (ppm) 174.2, 173.3 (*C*OOH, NH*C*O), 135.4 (C-1), 133.7 (C-2), 131.8, 130.3, 129.9, 128.0 (C-3 to C-5), 80.3 (C-1′), 78.3, 77.0, 72.6, 69.9 (C-2′ to C-5′), 61.2 (C-6′); HRMS: *m*/*z* 350.08501 ([M+Na]$^+$, calcd for $C_{14}H_{17}NO_8Na^+$ 350.08464).

4.21. Methyl 3-(*N*-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)benzoate (18)

Prepared from **3** (0.5 g, 1.34 mmol) and isophthalic acid monomethyl ester (0.17 g, 1.47 mmol) according to General procedure I. Eluent for the column chromatography: 1:1 EtOAc–hexane. Yield: 0.63 g (92%) white crystalline product, mp: 148–150 °C; $[\alpha]_D$ −10 (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 8.44–7.52 (4H, m, aromatics), 7.27 (1H, d, $J = 9.0$ Hz, NH), 5.48, 5.41, 5.11, 5.05 (4H, 4 pseudo t, $J \sim 9.0$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.34 (1H, dd, $J = 12.6$, 4.1 Hz, H-6a′), 4.12 (1H, dd, $J = 12.6$, 1.5 Hz, H-6b′), 3.95 (3H, s, O*C*H$_3$), 3.94 (1H, ddd, $J = 9.0$, 4.1, 1.5 Hz, H-5′), 2.07, 2.06 (2), 2.03 (12H, 3s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.3, 170.6, 169.8, 169.6, 166.3, 166.0 (NH*C*O, *C*OOCH$_3$, *C*O), 133.2 (C-1), 131.5 (C-3), 133.1, 130.8, 128.9, 128.2 (C-2, C-4 to C-6), 78.9 (C-1′), 73.7, 72.6, 70.8, 68.2 (C-2′ to C-5′), 61.7 (C-6′), 52.4 (COO*C*H$_3$) 20.6 (2), 20.5 (2) (*C*H$_3$); HRMS: *m*/*z* 532.14262 ([M+Na]$^+$, calcd for $C_{23}H_{27}NO_{12}Na^+$ 532.14255). Anal. Calcd for $C_{23}H_{27}NO_{12}$ (509.47): C, 54.22; H, 5.34; N, 2.75. Found: C, 54.34; H, 5.11; N, 2.66.

4.22. Methyl 4-(*N*-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-carbamoyl)benzoate (19)

Prepared from **3** (0.5 g, 1.34 mmol) and terephthalic acid monomethyl ester (0.17 g, 1.47 mmol) according to General procedure I. Eluent for the column chromatography: 1:1 EtOAc–hexane. Yield: 0.60 g (88%) white

crystalline product, mp: 187–190 °C; $[\alpha]_D$ −16 (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 8.11 (2H, d, $J = 7.8$ Hz, H-2, H-6), 7.83 (2H, d, $J = 7.8$ Hz, H-3, H-5), 7.21 (1H, d, $J = 9.0$ Hz, NH), 5.44, 5.40, 5.11, 5.06 (4H, 4 pseudo t, $J \sim 9.3$ Hz in each, H-1′, H-2′, H-3′, H-4′), 4.34 (1H, dd, $J = 12.3$, 3.9 Hz, H-6′a), 4.11 (1H, dd, $J = 12.3$, 2.5 Hz, H-6′b), 3.95 (3H, s, OCH_3), 3.94 (1H, ddd, $J = 9.0$, 3.9, 2.5 Hz, H-5′), 2.17, 2.08, 2.05, 2.05 (12H, 4s, $4 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.5, 170.5, 169.8, 169.6, 166.2, 166.0 ($COOCH_3$, $NHCO$, CO), 136.5 (C-1), 133.5 (C-4) 129.9 (C-2, C-6), 127.3 (C-3, C-5), 78.9 (C-1′), 73.6, 72.5, 70.8, 68.2 (C-2′ to C-5′), 61.5 (C-6′), 52.4 ($COOCH_3$) 20.6 (2), 20.5 (2) (CH_3); HRMS: *m/z* 532.14257 ($[M+Na]^+$, calcd for $C_{23}H_{27}NO_{12}Na^+$ 532.14255). Anal. Calcd for $C_{23}H_{27}NO_{12}$ (509.47): C, 54.22; H, 5.34; N, 2.75. Found: C, 54.14; H, 5.24; N, 2.80.

4.23. Methyl 3-(*N*-β-D-glucopyranosyl-carbamoyl)-benzoate (20)

Prepared from **18** (0.2 g, 0.39 mmol) according to General procedure II. Crystallized from Et_2O to give 0.10 g (77%) white crystalline product, mp: 222–224 °C; $[\alpha]_D$ +10 (*c* 0.44, MeOH); ^1H NMR (D_2O): δ (ppm) 8.33–7.56 (4H, m, aromatics), 5.23 (1H, d, $J = 8.0$ Hz, H-1′), 3.95 (3H, s, OCH_3), 3.93 (1H, dd, $J = 11.1$, 2.5 Hz, H-6′a), 3.83 (1H, dd, $J = 11.1$, 3.7 Hz, H-6′b), 3.68–3.59 (4H, m, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (D_2O): δ (ppm) 170.4, 168.1 ($COOCH_3$, $NHCO$), 133.2 (C-1), 129.9 (C-3), 133.1, 132.2, 129.2, 128.4 (C-2, C-4 to C-6), 79.9 (C-1′), 77.7, 76.5, 71.8, 69.2 (C-2′ to C-5′), 60.5 (C-6′), 52.8 (OCH_3); HRMS: *m/z* 364.09963 ($[M+Na]^+$, calcd for $C_{15}H_{19}NO_8Na^+$ 364.10029). Anal. Calcd for $C_{15}H_{19}NO_8$ (341.32): C, 52.79; H, 5.61; N, 4.16. Found: C, 52.54; H, 5.41; N, 4.06.

4.24. 3-(*N*-β-D-Glucopyranosyl-carbamoyl)benzoic acid (21)

Prepared from **20** (0.17 g, 0.49 mmol) according to General procedure III. Eluent for the column chromatography: 1:2 $CHCl_3$–MeOH. Yield: 0.16 g (99%) colourless oil, $R_f = 0.17$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +8 (*c* 0.29, MeOH); ^1H NMR (D_2O): δ (ppm) 8.22–7.45 (4H, m, aromatics), 5.20 (1H, d, $J = 8.2$ Hz, H-1′), 3.91 (1H, dd, $J = 11.3$, 1.5 Hz, H-6′a), 3.79 (1H, dd, $J = 11.3$, 3.8 Hz, H-6′b), 3.65–3.49 (4H, m, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (D_2O): δ (ppm) 174.9, 171.9 ($COOH$, $NHCO$), 136.6 (C-1), 132.9 (C-3), 132.8, 130.0, 128.8, 127.7 (C-2, C-4 to C-6), 80.5 (C-1′), 78.3, 76.2, 72.3, 69.8 (C-2′ to C-5′), 61.2 (C-6′); HRMS: *m/z* 326.08746 ($[M-H]^-$, calcd for $C_{14}H_{16}NO_8^-$ 326.08814).

4.25. Methyl 4-(*N*-β-D-glucopyranosyl-carbamoyl)-benzoate (22)

Prepared from **19** (0.2 g, 0.39 mmol) according to General procedure II. Crystallized from MeOH to give 0.115 g (91%) white crystalline product, mp: 245–248 °C; $[\alpha]_D$ +12 (*c* 0.38, Me_2SO); ^1H NMR (Me_2SO-d_6): δ (ppm) 8.04 (4H, br s, aromatics), 4.98 (1H, d, $J = 8.9$ Hz, H-1′), 3.88 (3H, s, OCH_3), 3.67 (1H, dd, $J = 12.1$, 3.7 Hz, H-6′a), 3.40 (1H, dd, $J = 12.1$, 5.3 Hz, H-6′b), 3.36–3.10 (4H, m, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (Me_2SO-d_6): δ (ppm) 165.7 (2) ($COOCH_3$, $NHCO$), 138.1 (C-1), 131.9 (C-4), 128.9 (C-2, C-6), 127.9 (C-3, C-5), 80.2 (C-1′), 78.7, 77.4, 71.9, 69.9 (C-2′ to C-5′), 60.9 (C-6′), 52.3 (OCH_3); HRMS: *m/z* 364.10027 ($[M+Na]^+$, calcd for $C_{15}H_{19}NO_8Na^+$ 364.10029). Anal. Calcd for $C_{15}H_{19}NO_8$ (341.32): C, 52.79; H, 5.61; N, 4.16. Found: C, 52.60; H, 5.55; N, 4.22.

4.26. 4-(*N*-β-D-Glucopyranosyl-carbamoyl)benzoic acid (23)

Prepared from **22** (0.17 g, 0.49 mmol) according to General procedure III. Eluent for the column chromatography: 1:2 $CHCl_3$–MeOH. Yield: 0.14 g (84%) colourless oil, $R_f = 0.17$ (1:1 $CHCl_3$–MeOH); $[\alpha]_D$ −5 (*c* 0.67, MeOH); ^1H NMR (D_2O): δ (ppm) 7.92 (2H, d, $J = 7.9$ Hz, H-2, H-6), 8.40 (2H, d, $J = 7.9$ Hz, H-3, H-5), 5.20 (1H, d, $J = 7.7$ Hz, H-1′), 3.89 (1H, dd, $J = 12.5$, 1.1, Hz, H-6′a), 3.69 (1H, dd, $J = 12.5$, 4.2 Hz, H-6′b), 3.66–3.45 (4H, m, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (D_2O): δ (ppm) 180.9 ($COOH$), 172.1 ($NHCO$), 140.4 (C-1), 135.5 (C-4), 129.5 (C-2, C-6), 128.0 (C-3, C-5), 80.6 (C-1′), 78.3, 77.1, 72.4, 69.9 (C-2′ to C-5′), 61.2 (C-6′); HRMS: *m/z* 350.08403 ($[M+Na]^+$, calcd for $C_{14}H_{17}NO_8Na^+$ 350.08464).

4.27. Enzyme assays

Glycogen phosphorylase *b* was prepared from rabbit skeletal muscle according to the method of Fischer and Krebs,[38] using 2-mercaptoethanol instead of L-cysteine, and recrystallized at least three times before use. The kinetic studies with glycogen phosphorylase were performed as described previously.[37] Kinetic data for the inhibition of rabbit skeletal muscle glycogen phosphorylase by monosaccharide compounds were collected using different concentrations of α-D-glucose-1-phosphate (4, 6, 8, 10, 12 and 14 mM) and constant concentrations of glycogen (1% w/v) and AMP (1 mM). The enzymatic activities were presented in the form of double-reciprocal plots (Lineweaver–Burk) applying a non-linear data-analysis programme. The inhibitor constants (K_i) were determined by Dixon plots, by replotting the

slopes from the Lineweaver–Burk plots against the inhibitor concentrations. The means of standard errors for all calculated kinetic parameters averaged to less than 10%.[39,40] IC_{50} values were determined in the presence of 4 mM glucose 1-phosphate, 1 mM AMP, 1% glycogen, and varying concentrations of an inhibitor.

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