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First general synthesis of 2-*C*-(β-D-glycopyranosyl)pyrimidines and their evaluation as inhibitors of some glycoenzymes

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Abstract

A systematic study was performed on the preparation of unknown 2-*C*-(β-D-glucopyranosyl)pyrimidines. Pinner type cyclisation of *O*-perbenzylated *C*-(β-D-glucopyranosyl)formamidine with β-ketoesters, dimethyl malonate, and β-diketone derived α,β-unsaturated β-chloroketones followed by catalytic hydrogenation resulted in variously substituted 2-*C*-(β-D-glucopyranosyl)-pyrimidin-4(3*H*)-ones, and 2-*C*-(β-D-glucopyranosyl)-

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4,6-disubstituted-pyrimidines, respectively, in moderate to good yields. The above pyrimidine derivatives were also achieved by ring closure of the unprotected *C*-(β-D-glucopyranosyl)formamidine with the same 1,3-dielectrophiles. In addition, a continuous one-pot three-step procedure starting from *O*-peracylated D-glycopyranosyl cyanides was also elaborated to give representatives of the aforementioned pyrimidines with various sugar configurations in acceptable to excellent overall yields (25-94 %). Due to the versatility of the applied 1,3-dielectrophiles these synthetic routes represent the first expansible method to get the target compounds. The new *C*-glycopyranosyl pyrimidines showed moderate inhibition against α-glucosidase and β-galactosidase enzymes had, however, no activity against glycogen phosphorylase. The obtained molecule library is ready for further biological testing.

Keywords

Pyrimidine, Amidine, 1,3-Dicarbonyl compounds, Pinner synthesis, *C*-Glucopyranosyl derivative

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Introduction

Today's chemical biology and drug discovery is in an urgent need to identify new small molecules and molecular architectures to extend chemical space in order to modulate the functions of target proteins. The traditional scenario of active ingredients comprising heterocyclic assemblies is continuously being broadened towards unconventional conjugates, among which especially sugar derivatives may open up ways to new targets, new ways of action and unprecedented selectivities.[1]

C-Glycosyl compounds[2] in general and *C*-glycopyranosyl arenes[3] and hetarenes[4] in particular continuously attract intense interest of synthetic and medicinal chemists due to many types of biological activities as well as to the use of such compounds as glycomimetics and also as active ingredients of marketed drugs. While *C*-glycosyl derivatives of five-membered heterocycles are widely represented, much less attention has been devoted to six-membered heterocyclic *C*-glycosyl compounds.[4] In the latter class *C*-glycofuranosyl heterocycles[5, 6] make the overwhelming majority of the known derivatives. This general trend is also valid for pyrimidines and especially for their 2-*C*-glycosylated derivatives. Thus, among 2-*C*-glycosyl pyrimidines, 2-(3',5'-di-*O*-benzoyl-2'-deoxy-α-D-ribofuranosyl)pyrimidine (together with its 4-substituted counterpart and the β-D-anomer of the latter in 56 % yield for the three products)[7] as well as 2-(1'-hydroxy-1',2';3',5'-di-*O*-isopropylidene-β-L-arabinofuranosyl)-4-methyl-pyrimidine (together with the 6-glycosyl derivative in 65 % combined yield with a ratio of ~7 : 3 for the 2-and 6-glycosyl isomers)[8] were obtained by Minisci type radical glycosylations of protonated pyrimidines. *C*-Glycofuranosyl formamidines (2,5-anhydro-aldonimidamides) were condensed with 1,3-dielectrophiles (e. g. $Me_2NCH=CHCOCOOEt$,[9] $RCOCH_2COR$[10] to the expected 2-*C*-glycosyl pyrimidine derivatives. A 2-*C*-ribofuranosyl-4-hydroxy-6-methyl-pyrimidine was obtained by a ring transformation of a related 1,3-oxazine

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derivative.[11] *C*-Glycofuranosylation in other positions of the pyrimidine ring is surveyed in excellent reviews.[5, 6]

On the other hand, to the best of our knowledge, *C*-glycopyranosyl derivatives of pyrimidine are even more scarce in the literature. Thus, only one report described a 2-(3',4'-di-*O*-benzoyl-2'-deoxy-β-D-ribopyranosyl)pyrimidine as a minor product beside the 4-substituted counterpart (combined yield 56 %, ratio 3:7).[7] Some 5-*C*-glycopyranosyl pyrimidines were achieved by cyclocondensations of *C*-glycopyranosylated enaminoketones with guanidine or acetamidine.[12, 13] A set of *C*-glycopyranosylated dihydropyrimidines (having the sugar moieties at C-4 or C-6, or in both positions) was synthetized by Biginelli-type cyclocondensations of *C*-glycopyranosyl formaldehydes and β-ketoesters, respectively.[4]

In the light of the practical non-existence of 2-*C*-glycopyranosylated pyrimidine type compounds we envisaged a series of investigations to obtain this class of compounds by reactions of *C*-(β-D-glycopyranosyl)formamidines which became available by our recently published procedures.[14-16] In addition, we also wished to study these compounds as inhibitors of different enzymes acting on carbohydrate substrates, such as glycogen phosphorylase (GP) since *N*-(β-D-glucopyranosyl)pyrimidines were shown to have low micromolar inhibition of rabbit muscle GP*b*.[17-19] In this work the reactions of *C*-(β-D-glycopyranosyl)formamidines with β-dicarbonyl derivatives and analogous 1,3-dielectrophiles to give the expected *C*-glucosyl pyrimidines and the study of the latter as inhibitors of GP and some glycosidases are described.

Results and Discussion

The construction of the pyrimidine ring from various precursors is a very active field of research and within this the archetypal Pinner method (reaction of an amidine with 1,3-

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dicarbonyl compounds) is being intensively investigated even today.[20-22] The Pinner synthesis of pyrimidines generally requires strongly basic conditions,[10, 23, 24] therefore, the recently prepared 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*gulo*-heptonimidamide[15, 16] (*O*-perbenzylated *C*-(β-D-glucopyranosyl)formamidine, **1**) was chosen as the starting material. The reactions of **1** with 3-ketoesters in the presence of 3 equiv of NaOMe in MeOH gave the corresponding ring-closed products **3** in acceptable to good yields (Scheme 1). Removal of the *O*-benzyl protecting groups was effected by catalytic hydrogenation over $Pd(OH)_2$, however, the use of elevated temparature was necessary to obtain the expected products **4a** and **4d** in good yields. To avoid possible reductive hydrodehalogenation[25] of **3b** and **3c** during deprotection, hydrogenolytic *O*-debenzylation of **1** was performed in an acidified mixture of EtOAc and EtOH at room temperature to give the unprotected amidine **2** as a hydrochloride salt in quantitative yield. The ring closure of **2** with 3-ketoesters was achieved under the same conditions as with the protected **1** and pyrimidines **4** were obtained in good to excellent yields. Besides facilitating to avoid hydrodehalogenation, this latter route resulted in significantly higher overall yields of the target products (**1→3a→4a**: 49 % *vs* **1→2→4a**: 87 %; **1→3d→4d**: 33 % *vs* **1→2→4d**: 64 %).

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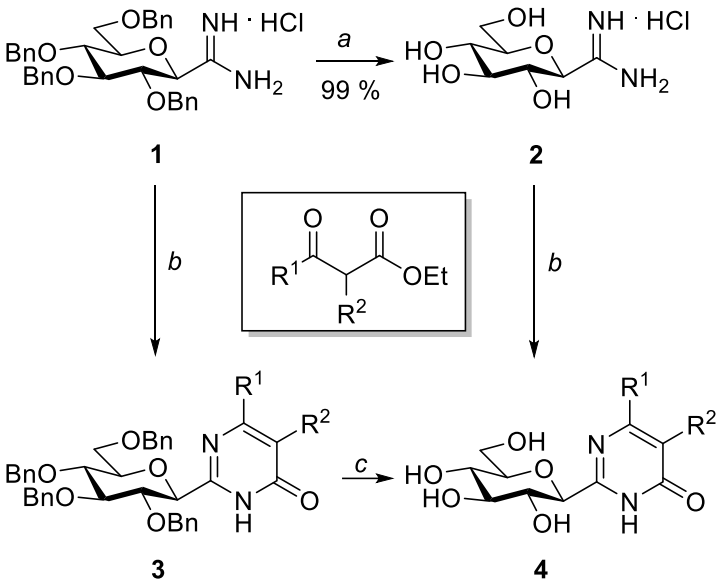

	Yield (%)	R^1	R^2		Yield (%) from **2**	Yield (%) from **3**
3a	79	CH_3	H	**4a**	88	62
b	87	CH_2Cl	H	**b**	59	
c	60	CH_3	Cl	**c**	73	
d	43	Ph	H	**d**	65	77

Scheme 1. *a*) H_2, $Pd(OH)_2$/C, dry EtOAc-EtOH (1 : 2), 1 drop of ccHCl, rt; *b*) 3-ketoester (2 equiv), 1M NaOMe in dry MeOH (3 equiv), dry MeOH, rt; *c*) H_2, $Pd(OH)_2$/C, dry EtOAc-EtOH (1 : 2), reflux.

The reaction of **1** with an excess of dimethyl malonate (Scheme 2) proceeded similarly to give pyrimidine **5** which was *O*-deprotected by catalytic hydrogenolysis to **6**. The unprotected amidine **2** and dimethyl malonate gave **6** to show again that in terms of overall yields debenzylation followed by ring closure is more efficient than the opposite reaction sequence (**1**→**2**→**6**: 70 % *vs* **1**→**5**→**6**: 39 %).

When malononitrile or a cyanoacetic acid ester were used as the dielectrophile to react with **1** or **2** no ring closure could be achieved. With **2** complex mixtures were formed while with **1** these reagents replaced one of the nitrogens in the amidine moiety to give enamino

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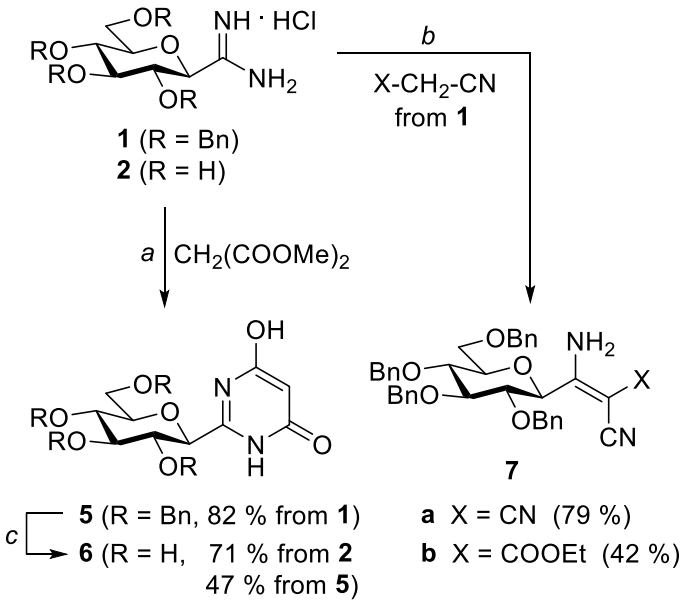
ester/nitrile type derivatives **7**. A similar transformation was observed with simple aliphatic or aromatic substrates.[26] In **7b** the (*Z*)-configuration of the double bond unequivocally followed from the different chemical shifts of the NH_2 protons (8.89 and 6.37 ppm) due to formation of a H-bond with the COOEt group.[27]



Scheme 2. Reagents and conditions: *a*) dimethyl malonate (10 equiv), 1 M solution of NaOMe in dry MeOH (10 equiv), dry MeOH, rt; *b*) malononitrile (2 equiv) or ethyl-2-cyanoacetate (10 equiv), 1M NaOEt in dry EtOH (2 or 10 equiv), dry EtOH, rt; *c*) H_2, $Pd(OH)_2/C$, dry EtOAc-EtOH (1 : 2), reflux.

The experiences of reactions of amidines **1** and **2** with 1,3-diketones are summarized in Table 1. Since the applied 1,3-diketones gave no reaction with the above substrates activation of the reagents was necessary as described previously.[10] The diketones were treated with a chlorination agent (PCl_5, $(COCl)_2$ or $SOCl_2$) and the so obtained crude α,β-unsaturated β-chloroketones were reacted with **1** or **2** to give the expected **8** or **10**, respectively. In both series the yields were in the 62-75 % range. *O*-Debenzylation of compounds **8** was effected

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by catalytic hydrogenation. While at room temperature and under neutral conditions no reaction took place, boiling of the reaction mixtures gave very good yields of the desired **10a** and **10b**. On the other hand, these conditions resulted in a mixture of compounds which contained partially saturated pyrimidines **9c,d** and **10c,d** (*vide infra* Fig 1 for stereochemical assignments). When these hydrogenolyses were carried out under acidic conditions only the formation of inseparable mixtures of **9c,d** could be observed in keeping with the known general behaviour of pyrimidines to take up two moles of hydrogen under catalytic hydrogenation conditions in acidic media.[28]

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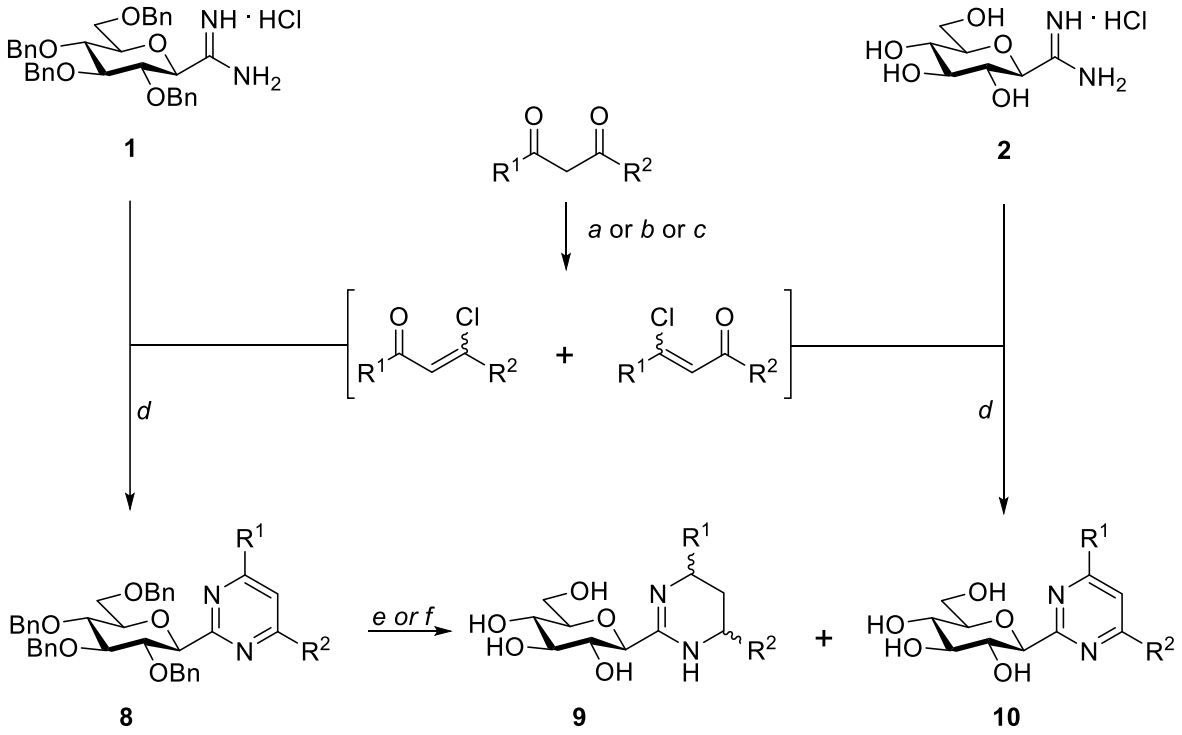
Table 1. Reactions of amidines **1** and **2** with 1,3-diketones



a) PCl_5 (1 equiv), dry Et_2O, rt; *b*) $(COCl)_2$ (1.2 equiv), DMF (1.3 equiv), dry CH_2Cl_2, 0 °C to rt; *c*) $SOCl_2$ (3 equiv), dry $CHCl_3$, cat. DMF, reflux; *d*) K_2CO_3 (4 equiv), α,β-unsaturated chloroketone (1.2 equiv), 4 Å molecular sieves, dry DMF, 0 °C to rt; *e*) H_2, $Pd(OH)_2/C$, dry EtOAc-EtOH, (1 : 2), reflux; *f*) H_2, $Pd(OH)_2/C$, 1 drop of ccHCl, dry EtOAc-EtOH (1 : 2), rt.

			Conditions and yields (%)							
	R^1	R^2	[a]	**8**		**9**[b]		**10**		
								from **2**	from **8**	
a	CH_3	CH_3	*a*	*d*	65			*d*	62	
						e	--			79
b	CH_3	CF_3	*b*	*d*	68			*d*	63	
						e	--			92
c	Ph	CH_3	*c*	*d*	74			*d*	69	
						e	27			36
						f	82			--
d	Ph	CF_3	*c*	*d*	72			*d*	75	
						e	43			19
						f	79			--

[a] Conditions for the activation of the 1,3-diketone.
[b] Mixture of two diastereomers (see Fig 1).

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The formation of tetrahydropyrimidines **9** was clearly indicated by the mass spectra of the compounds showing an increase of the molecular masses by ~4 Da. The ^{1}H NMR spectra exhibited two series of resonances in a ratio of ~5 : 1 for **9c** and 1 : 1 for **9d** (see supporting material). These were supposed to be originated from the presence of diastereomers rather than tautomers that were not to be detected in CD_3OD applied as the solvent. The coupling constants identified in the proton spectra (e. g. for **9d**: 4.62-4.57 ppm, 2 dd, $J_{4,5ax}$ = 11.3 Hz, $J_{4,5eq}$ = 3.8 Hz in each, 2 × Py-H-4; 2.31-2.25 ppm, 2 dt, $J_{5ax,5eq}$ = 13.0 Hz, $J_{4,5eq} = J_{5eq,6}$ = 3.8 Hz in each, 2 × Py-H-5_{eq}; 1.81-1.67 ppm, 2 dt, $J_{5ax,5eq}$ = 13.0 Hz, $J_{4,5ax} = J_{5ax,6}$ = 11.3 Hz in each, 2 × Py-H-5_{ax}) indicated that the tetrahydropyrimidine rings were 4,6-*cis*-disubstituted diastereomers, and this was in accord with the expected suprafacial addition of the hydrogens under the heterogenous catalytic conditions. In addition, these coupling constants were also consistent with the existence of the tetrahydropyrimidine moieties in E_5 and ^5E conformations. The envelope-like conformations of 1,4,5,6-tetrahydropyrimidine derivatives were firmly determined by NMR[29] and X-ray[29-32] studies and it was also pointed out that the conformations were the same in solution and in the solid phase.[29] The established structures for compounds **9** are shown in Fig. 1.

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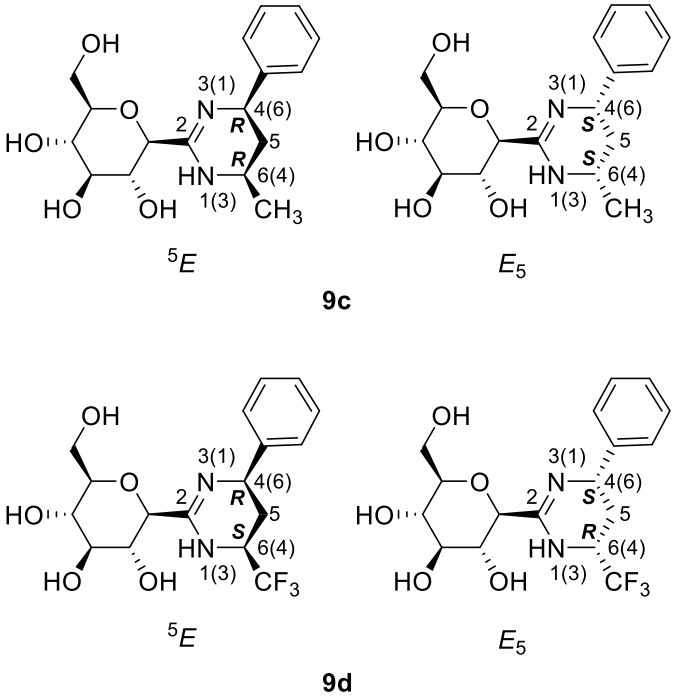

Figure 1. Stereochemical assignment of tetrahydropyrimidines **9**.

Since in the above studies the best results were obtained with the unprotected amidine **2**, experiments were also directed to getting this compound from the easily available *O*-perbenzoylated β-D-glucopyranosyl cyanide **13**.[33] To this end the Pinner-type conversion of **13** to the imidate **11** was investigated first (Scheme 3). Contrary to the experiences with the analogous *O*-benzoyl protected ribofuranosyl cyanide, where the crystalline imidate could be isolated from a reaction with catalytic NaOMe in MeOH,[34] under similar conditions **13** gave a crude mixture from which the isolation of **11** by crystallization failed. A trial to treat the crude mixture by NH_3/NH_4Cl transformed the imidate to the expected amidine **2**, however, a minor amount of the unsaturated **12** inseparable from **2** was also detected by the presence of a resonance[35] at 6.07 ppm of H-2 (d, J = 2.6 Hz) in the ^{1}H NMR spectrum of the mixture. The elimination most probably took place from molecules still having the 2-OBz group since it is well known that base-induced eliminations can be brought about from *O*-peracylated glycosyl cyanides[36, 37] and related compounds.[38] Following these experiences, we attempted the

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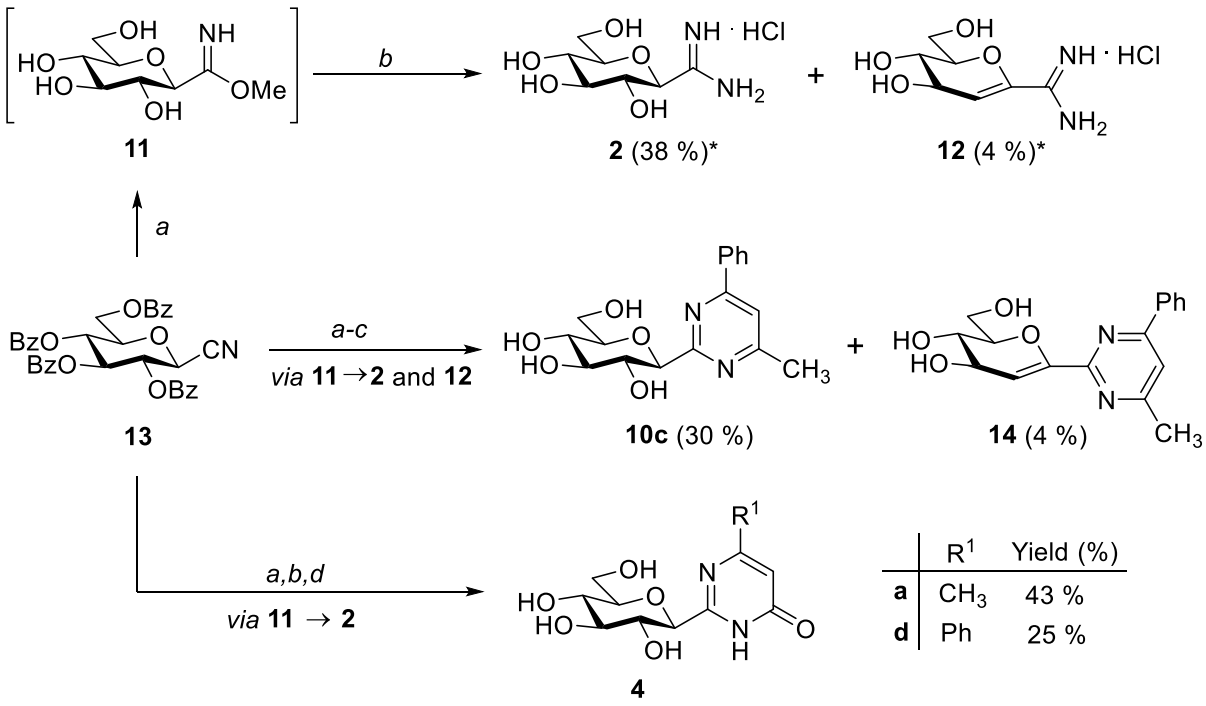
transformation of **13** to pyrimidines **4** and **10** in continuous operations by addition of NaOMe in MeOH, followed by NH_3/NH_4Cl and subsequent reaction with the corresponding 1,3-dielectrophile. This procedure gave **4a** and **4b** in 43 % and 25 % isolated yield, respectively, for the three steps. From the final reaction mixture of **13** with benzoylacetone besides the expected **10c** (30 % isolated overall yield) the unsaturated by-product **14** could also be obtained (4 %) by column chromatography.



*Inseparable mixture of **2** and **12**. Calculated yields based on the ^{1}H-NMR spectra.

Scheme 3. Reagents and conditions: *a*) 1M NaOMe in dry MeOH (20 mol %), dry, MeOH, dry $CHCl_3$, rt; *b*) NH_4Cl (1 equiv), sat. NH_3 in dry MeOH; *c*) K_2CO_3 (4 equiv), α,β-unsaturated chloroketone (1.2 equiv) freshly prepared from 1-phenylbutane-1,3-dione (see in Table 1.), 4 Å molecular sieves, dry DMF, 0 °C to rt; *d*) $RCOCH_2COOEt$ (2 equiv), 1M NaOMe in dry MeOH (3 equiv), dry MeOH, rt.

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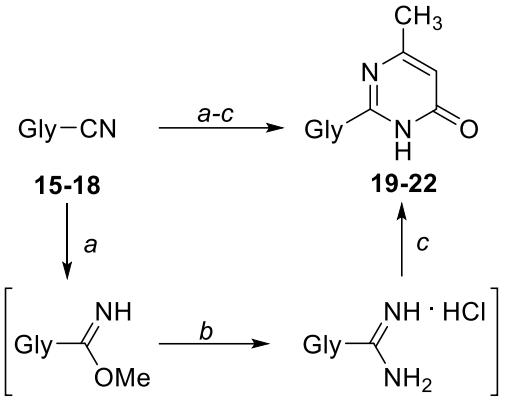
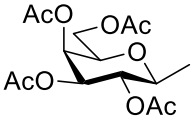
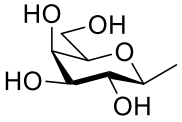
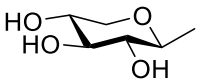
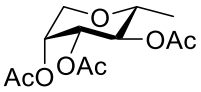
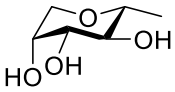
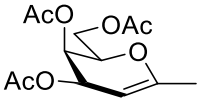
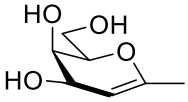
This method was also extended to the preparation of further 2-*C*-glycopyranosyl-pyrimidin-4(3*H*)-ones (Table 2). Starting from various *O*-peracylated glycosyl cyanides **15**,[39] **16**,[40] **17**,[41] and **18**[36, 42-44] the one-pot three-step procedures were smoothly accomplished to get the corresponding *C*-glycopyranosyl heterocycles **19-22** in moderate to excellent yields.

Table 2. Synthesis of 6-methyl-2-*C*-glycopyranosyl-pyrimidin-4(3*H*)-ones



a) 1M NaOMe in dry MeOH (20 mol %), dry MeOH, dry $CHCl_3$, rt; *b*) NH_4Cl (1.2 equiv), dry MeOH; *c*) CH_3COCH_2COOEt (2 equiv), 1M NaOMe in dry MeOH (3 equiv), dry MeOH, rt.

	Gly			Yield (%)
	Starting material		Product	
15		**19**		70
16		**20**		27
17		**21**		43
18		**22**		94

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The unprotected pyrimidines were assayed as inhibitors of some glycoenzymes. Compounds **4**, **6**, and **10** had no inhibitory effect against rabbit muscle glycogen phosphorylase *b* up to 625 μM concentration. Pyrimidines **4**, **10**, **19** and **20** did not inhibit almond β-glucosidase in a low mM concentration range, while weak inhibitions of yeast α-glucosidase and bovine liver β-galactosidase by the same molecules were observed (Table 3). Among the tested molecules, **4d** and **10d** proved to be the most potent ones, displaying submillimolar inhibitory activities (IC_{50} = 0.7 and 0.34 mM, respectively) against the indicated glycosidase enzymes.

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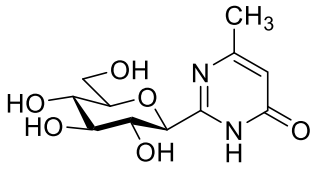
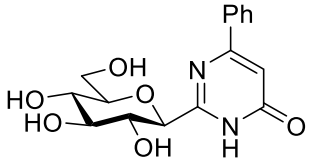
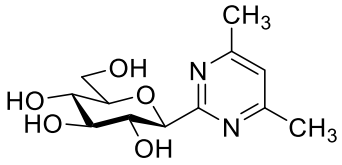
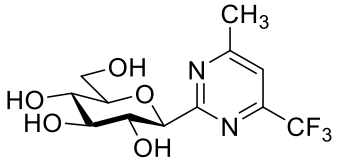
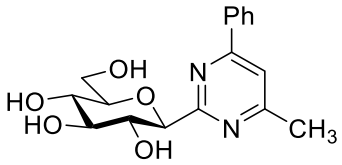
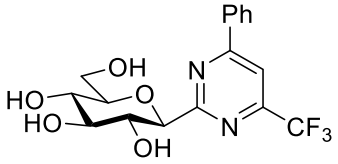
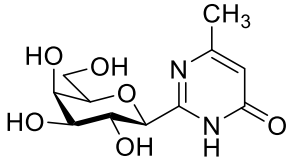
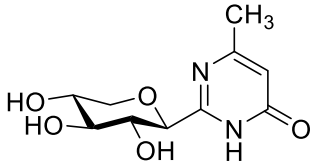
Table 3. Inhibitory effects of 2-*C*-(β-D-glycopyranosyl)-pyrimidines against glycosidase enzymes

Code	Chemical structure	Inhibition	
		α-glucosidase (yeast)	β-galactosidase (bovine liver)
4a		27 % at 2.1 mM	no inh. at 2.1 mM
4d		IC_{50} = 0.7 mM	56 % at 3.2 mM
10a		33 % at 3.1 mM	45 % at 3.1 mM
10b		30 % at 1.6 mM	20 % at 1.6 mM
10c		90 % at 5.7 mM	56 % at 5.7 mM
10d		54 % at 6.8 mM	IC_{50} = 0.34 mM
19		10 % at 1.3 mM	no inh. at 1.3 mM
20		14 % at 0.78 mM	no inh. at 0.78 mM

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Although the inhibitory potency of the newly synthesized componds was not outstanding against the studied enzymes, the observed activities demonstrate the interaction of 2-*C*-(β-D-glucopyranosyl)pyrimidines with proteins, notably with glycosidase enzymes, whose analogues play important roles in human diseases such as lysosomal disorders, diabetes, and several neurological disorders.[45]

Conclusion

This work represents the first systematic study on the synthesis of 2-*C*-(β-D-glucopyranosyl)pyrimidines. The reactions of *O*-perbenzylated *C*-(β-D-glucopyranosyl)formamidines with β-ketoesters, dimethyl malonate, and β-diketone derived α,β-unsaturated β-chloroketones gave the expected pyrimidines in good yields. Removal of the *O*-benzyl protecting groups under catalytic hydrogenation in neutral media gave the unprotected 2-*C*-glucosyl-4-substituted-6-hydroxy-pyrimidines and 2-*C*-glucosyl-4,6-dihydroxy-pyrimidine in their respective keto tautomeric forms as well as some 2-*C*-glucosyl-4,6-disubstituted-pyrimidines in good to excellent yields. Under similar hydrogenolytic conditions 2-*C*-glucosyl-4-phenyl-6-substituted-pyrimidines gave mixtures of two 4,6-*cis*-disubstituted diastereomers of the corresponding 1,4,5,6-tetrahydropyrimidines. Unprotected *C*-(β-D-glucopyranosyl)formamidine proved also a suitable substrate to give good to high yields of the aforementioned pyrimidines. Preparation of other 2-*C*-(β-D-glycopyranosyl)-pyrimidines was also achieved from *O*-peracylated D-glycopyranosyl cyanides in a one-pot three-step continuous operation to give the expected products in 25-94 % overall yields. The compounds showed no activity against glycogen phosphorylase, however, weak inhibition of some glycosidase enzymes was observed. By this work a new compound class has been made

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available for biological investigations. Further studies for the synthesis of other types of *C*-glycopyranosyl pyrimidines as well as their biological assays are in progress in our laboratory.

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Experimental

Syntheses

General Methods

Melting points were measured on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Jasco P-2000 polarimeter at rt. NMR spectra were recorded with Bruker 360 (360/90 MHz for $^{1}H/^{13}C$) or Bruker 400 (400/100 MHz for $^{1}H/^{13}C$) spectrometers. Chemical shifts are referenced to the internal TMS (^{1}H), or to the residual solvent signals (^{13}C). Proton-signal assigments for compounds **3, 5, 7** are based on COSY correlations. Microanalyses were performed on an Elementar Vario Micro cube instrument. Mass spectra were obtained by Thermo Scientific LTQ XL or 284 MicroTOF-Q type Qq-TOF MS (Bruker Daltonik, Bremen, 285 Germany) instruments. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (Merck), and the plates were visualised under UV light and by gentle heating (generally no spray reagent was used but, if more intense charring was necessary, the plate was sprayed with the following solution: abs. EtOH (95 mL), ccH_2SO_4 (5 mL) anisaldehyde (1 mL)). For column chromatography Kieselgel 60 (Merck, particle size 0.063-0.200 mm) was used. EtOAc, $CHCl_3$ and CH_2Cl_2 were distilled from P_4O_{10} and stored over 4 Å molecular sieves. MeOH was purified by distillation after refluxing for a couple of hours with magnesium turnings and iodine. EtOH and DMF were purchased from Sigma-Aldrich. 1M solution of NaOMe in anhydrous MeOH and NaOEt in anhydrous EtOH was freshly prepared before using. Organic solutions were dried over anhydrous $MgSO_4$ and concentrated under diminished pressure at 40-60 ºC (water bath). *C*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)formamidine hydrochloride[15, 16] (**1**) and *O*-

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peracylated glycosyl cyanides (**11**,[33] **15**,[39] **16**,[40] **17**,[41] **18**[43]) were synthesized based on literature procedures. The α,β-unsaturated β-chloroketones were obtained by chlorination of the corresponding 1,3-diketones (pentane-2,4-dione/PCl_5,[46] 1,1,1-trifluoropentane-2,4-dione/$(COCl)_2$,[10] 1-phenylbutane-1,3-dione/$(COCl)_2$,[47] 4,4,4-trifluoro-1-phenylbutane-1,3-dione/$SOCl_2$[10]) according to the cited protocols.

General procedure I for the synthesis of 2-(β-D-glucopyranosyl)-pyrimidin-4(3*H*)-ones (3, 4) by cyclocondensation of *C*-(β-D-glucopyranosyl)formamidines (1, 2)

The corresponding *C*-(β-D-glucopyranosyl)formamidine hydrochloride (**1**[15, 16] or **2**) was dissolved in anhydrous MeOH (2 mL/100 mg amidine), and a 1M solution of NaOMe in MeOH (3 equiv) was added. After 10 min, the appropriate 3-ketoester (2 equiv) was added to the reaction mixture and the stirring was continued at rt until the TLC showed complete conversion of the starting material (9 : 1 $CHCl_3$-MeOH and 1 : 1 hexane-EtOAc for compounds **1**, **3**, and 7 : 3 $CHCl_3$-MeOH for compounds **2**, **4**, respectively). The reaction mixture was then neutralized with glacial AcOH, and concentrated under diminished pressure. The crude product was purified by column chromatography.

General procedure II for the removal of *O*-benzyl protecting groups by catalytic hydrogenation under neutral conditions to get compounds 4, 6, 10

To a solution of the corresponding *O*-perbenzylated *C*-(β-D-glucopyranosyl)pyrimidine (**3**, **5** or **8**) in a mixture of anhydrous EtOAc (2 mL/100 mg substrate) and EtOH (4 mL/100 mg substrate) 20 % $Pd(OH)_2/C$ (50 weight % of substrate) was added. The reaction mixture was then vigorously stirred at reflux temperature under H_2 atmosphere. After completion of the reaction monitored by TLC (1 : 1 hexane-EtOAc and 3 : 1 EtOAc-MeOH), the hot mixture was filtered through a pad of celite, and washed thoroughly with MeOH. The solvent was then

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evaporated under reduced pressure and the crude product was purified by column chromatography.

General procedure III for the reaction of *C*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)formamidine (1) with malononitrile and ethyl cyanoacetate to get compounds 7a,b

To a solution of *C*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)formamidine hydrochloride[15, 16] (**1**, 200 mg, 0.33 mmol) in anhydrous EtOH (5 mL) a 1M solution of NaOEt in EtOH (2 or 10 equiv, depending on the quantity of the reagent, see below) was added. After stirring the reaction mixture for 15 min at rt malononitrile (2 equiv) or ethyl cyanoacetate (10 equiv) was added. The stirring was continued at rt until the TLC (9 : 1 $CHCl_3$-MeOH, 1 : 1 hexane-EtOAc) indicated complete conversion of the starting material. The reaction mixture was then neutralized with glacial AcOH, and evaporated under diminished pressure. The resulting crude product was purified by column chromatography.

General procedure IV for the synthesis of 4,6-disubstituted-2-(β-D-glucopyranosyl)-pyrimidines (8, 10) by cyclocondensation of *C*-(β-D-glucopyranosyl)formamidines (1, 2)

The corresponding *C*-(β-D-glucopyranosyl)formamidine hydrochloride (**1**[15, 16] or **2**) and K_2CO_3 (4 equiv) in the presence of activated molecular sieves (4 Å) were suspended in anhydrous DMF (3 mL/100 mg substrate). This mixture was then cooled to 0 °C and the freshly prepared α,β-unsaturated β-chloroketone (1.2 equiv) obtained from the corresponding 1,3-diketone was added. The reaction mixture was allowed to warm up to rt and the stirring was continued. After completion of the reaction (~ 2 d), monitored by TLC (9 : 1 $CHCl_3$-MeOH and 1 :1 hexane-EtOAc for compounds **1**, **8**, and 7 : 3 $CHCl_3$-MeOH for compounds **2**, **10**, respectively), the

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inorganic precipitates were filtered off, washed with MeOH, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography.

General procedure V for the removal of *O*-benzyl protecting groups by catalytic hydrogenation under acidic conditions to get compounds 9c,d

A degassed, vigorously stirred suspension of 20 % $Pd(OH)_2/C$ (50 weight % of substrate) in a mixture of anhydrous EtOAc (2 mL/100 mg substrate) and EtOH (5 mL/100 mg substrate) was saturated with H_2. To this mixture a solution of the corresponding *O*-perbenzylated *C*-β-D-glucopyranosyl derivative in anhydrous EtOAc (3 mL/100 mg substrate), and a drop of ccHCl were added. The reaction mixture was then stirred under H_2 atmosphere at rt overnight. The completion of the reaction was judged by TLC (1 :1 hexane-EtOAc and 7 : 3 $CHCl_3$-MeOH) and the mixture was neutralized by the addition of $NaHCO_3$. The catalyst and the inorganic salts were filtered off through a pad of celite, and washed with MeOH. The solvent was removed under diminished pressure, and the resulting crude product was purified by column chromatography.

General procedure VI for the synthesis of 2-glycosyl-6-methylpyrimidin-4(3*H*)-ones (19-22) from glycosyl cyanides (15-18) by a *one-pot* three-step procedure

To a stirred solution of the corresponding cyanide (**15-18**) in a mixture of anhydrous $CHCl_3$ (2 mL / 1 g substrate) and MeOH (15 mL / 1 g substrate) a 1M solution of NaOMe in MeOH (20 mol %) was added at rt. After conversion of the starting material into the unprotected methyl *C*-glycopyranosyl formimidate (1 day) NH_4Cl (1.2 equiv) was added to the reaction mixture and the stirring was continued at rt. When TLC (7 : 3 $CHCl_3$-MeOH) indicated complete conversion of formimidate into the appropriate formamidine derivative (1 day) ethyl acetoacetate (2 equiv) and a 1M solution of NaOMe in MeOH (3 equiv) were added to the

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stirred reaction mixture. After 8 h the solution was neutralized with glacial acetic acid and the solvents were removed under reduced pressure. The residual crude product was purified either by column chromatography or by crystallization.

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Enzyme assays

Glycogen phoshorylase *b* was obtained from rabbit skeletal muscle by some modifications (application of 2-mercaptoethanol instead of L-cysteine, and recrystallization at least three times before use) of the purification protocol developed by Fischer and Krebs.[48] The kinetic measurements were carried out in the direction of glycogen synthesis as described earlier[49] with maximal inhibitor concentrations of 625 μM.

The glycosidase enzymes used were purchased from Sigma-Aldrich.

In the glycosidase assays typically a 10 μL aliquot of each inhibitor's stock solution was mixed with 370 μL of the buffer and 20 μL enzyme stock solution in a plastic UV cuvette. After equilibration at 37 °C for 5 min, a 100 μL aliquot of the substrate stock solution was added. The resulting solutions were thoroughly mixed, and the change in absorbance was followed at 400 nm over 24s in 2s intervals using the Parallel Kinetics Analysis program of a JASCO V550 (JASCO Tokyo, Japan) spectrophotometer. Progress curves were plotted and fitted to a straight line. ΔA/min values, proportional to initial rate, were considered to be enzyme activities. In a control experiment, the aliquot of the inhibitor solution was replaced by the same amount of buffer. The initial rate data for the enzymatic substrate hydrolysis in presence and absence of inhibitor were transferred into percentages of overall inhibition, and plotted against the inhibitor concentration in logarithmic scale for IC_{50} determination.

Further details of the experimental procedures and compound characterization can be found in the supporting material.

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Conflicts of interest

There are no conflicts of interest to declare.

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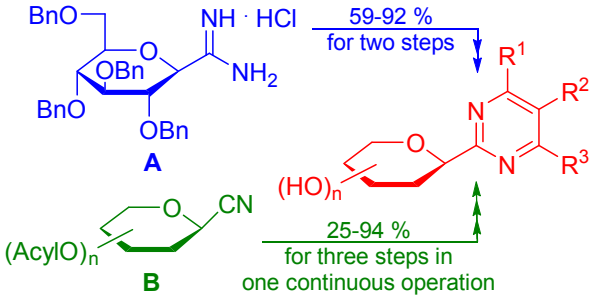

The first systematic study on the synthesis of 2-*C*-(β-D-glycopyranosyl)pyrimidines either from amidine **A** or glycosyl cyanides **B** and 1,3-dicarbonyl compounds.

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