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Original article

Synthesis of glycose carbamides and evaluation of the induction of erythroid differentiation of human erythroleukemic K562 cells

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Abstract

A series of carbamides derived from 1,2:5,6-di-*O*-isopropylidene-D-gluco- (**1**) and D-allofuranose (**3**) as well as their 5,6-*O*-deprotected analogues (**2** and **4**) and methyl 3,4-*O*-isopropylidene-α- and β-D-galactopyranosides (**5** and **6**) have been prepared in order to evaluate their ability to induce erythroid differentiation of human erythroleukemic K562 cells. Twenty out of 51 carbamides tested exhibit an appreciable activity as inducers of erythroid differentiation and have been fully characterized and described.

Keywords: Carbamides; Monosaccharides; Erythroid differentiation; K562 cells

1. Introduction

The K562 cell line, isolated and characterized by Lozzio and Lozzio [1] from a patient with chronic myelogenous leukemia in blast crisis, has been proposed as a very useful experimental model system to identify inducers of γ-globin gene expression of possible interest in the therapy of several haematological diseases, including β-thalassemia and sickle cell anaemia [2].

K562 cells exhibit a low proportion of hemoglobin-synthesizing cells under standard cell growth conditions, but are able to undergo erythroid differentiation when treated with a variety of compounds, including short fatty acids, 5-azacytidine, mithramycin, and chromomycin, cisplatin and cisplatin analogues, tallimustine, rapamycin, everolimus, psoralens and resveratrol [3]. Following erythroid induction, a sharp increase of expression of human ϵ- and γ-globin genes is observed in K562 cells, leading to a cytoplasmic accumulation of Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\epsilon_2$) [4–7].

Among possible biological response modifiers, one of the most studied classes of compounds is represented by short fatty acids, especially butyric and pivalic acids and related esters. Glycide esters have been initially proposed merely as convenient prodrugs, able to gradually release the pharmacophore following the in vivo action of esterases [8].

In recent papers regarding the synthesis and evaluation of some partially acetonated monosaccharide esters of several fatty acids we have highlighted the role of the structure of either the ester or the carbohydrate residue in the antiproliferative effects and erythroid differentiation activity of K562 cells [9]. Furthermore, it was observed that some glycose isobutyrates and pivaloates stimulate erythroid differentiation to higher levels than the corresponding free fatty acids [9]. Consequently, we assumed that, at least in the case of isobutyrates and pivaloates, the biological activity of monosaccharide esters might be related to the whole structure prior to the occurrence of the hydrolysis, rather than to the release of the free fatty acid acting as biologically active component.

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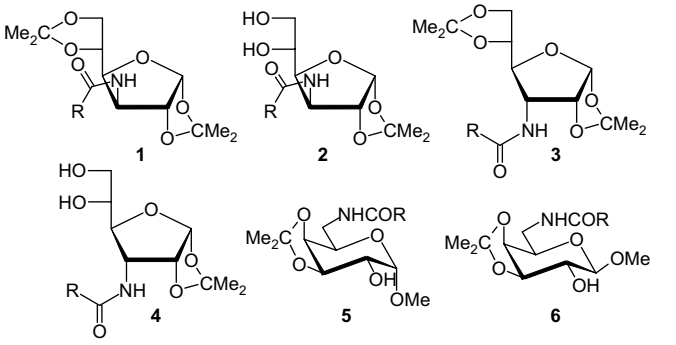
Unfortunately, fast in vivo ester hydrolysis on rabbit [10] precludes the possibility of any therapeutic use of these glycide esters. Therefore, we turned our efforts to investigate some more stable isosters of active glycose esters. Among various possibilities, we decided to focus on the substitution of the ester group with an amide one, which would offer the advantage of a simple synthetic access.

The present paper deals with the synthesis and the biological evaluation of part of a library of glycose carbamides **1**–**6** (Chart 1) in which the carbohydrate scaffoldings were the same in the previously described biologically active glycide esters [9]. Besides butyrates and pivaloates, we have selected a wide set of acyl residues classified into three groups (Chart 1): the first one includes linear and branched fatty acids (group A), the second one contains residues characterized by the presence of aromatic or heteroaromatic rings (group B) and the third one is a miscellaneous group (group C) including residues with heteroatoms or unsaturations within the chain.

A subset of 51 compounds, out of 174 glycose carbamides represented in Chart 1, were synthesized and tested toward for biological activity on the K562 cellular system, a subset, 20 of them showed an appreciable erythroid differentiation inducing activity on K562 cells. Synthesis, characterization and ability to induce erythroid differentiation of these biologically active compounds are herein presented.

2. Chemistry

3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-hexofuranose scaffolds **7** [11] and **8** [12] (Scheme 1) were prepared according to the literature procedures. The synthesis of methyl α- and β-6-amino-6-deoxy-3,4-*O*-isopropylidene-D-galactopyranosides **15**



GROUP A

a: R = CH_3, **b**: R = CH_2CH_3, **c**: R = $(CH_2)_2CH_3$, **d**: R = $(CH_2)_3CH_3$,
e: R = $(CH_2)_4CH_3$, **f**: R = $CH_2CH(CH_3)_2$, **g**: R = $CH_2CH(CH_3)CH_2C(CH_3)_3$,
h: R = $CH_2C(CH_3)_3$, **i**: R = $CH(CH_3)_2$, **j**: R = $C(CH_3)_3$,
k: R = $CH_2CH_2(CH_2)_5$,**l**: R = $(CH_2)_3$, **m**: R = $(CH_2)_4$,
n: R = $(CH_2)_5$, **o**: R = $(CH_2)_6$

GROUP B

a: R = CH_2-C_6H_5, **b**: R = CH_2-(3-OMe)C_6H_4, **c**: R = CH_2-(4-OMe)C_6H_4,
d: R = CH_2-(2,5-di-OMe)C_6H_3, **e**: R = $CH(C_6H_5)_2$, **f**: R = CH_2CH_2-C_6H_5,
g: R = C_6H_5, **h**: R = (4-C_6H_5)-C_6H_4, **i**: R = (4-OMe)C_6H4, **j**: R = C_6H_4N

GROUP C

a: R = C≡CCH_3, **b**: R = CH_2OCH_3, **c**: R = $CH_2OC_6H_5$,
d: R = $CH_2OCH_2(C_6H_5)$

Chart 1.

and **16** was easily achieved starting from the corresponding anomeric diols **9** [13] and **10** [13] and employing the same reaction sequence. Reactions of both **9** and **10** with a slight excess of *p*-toluenesulphonyl choride caused the expected selective [14,15] tosylation of primary OH group. The resulting 6-sulphonates were subjected to nucleophilic displacement with NaN_3, followed by reduction of resulting azides to furnish **15** and **16** in satisfactory overall yield (39 and 45%, respectively).

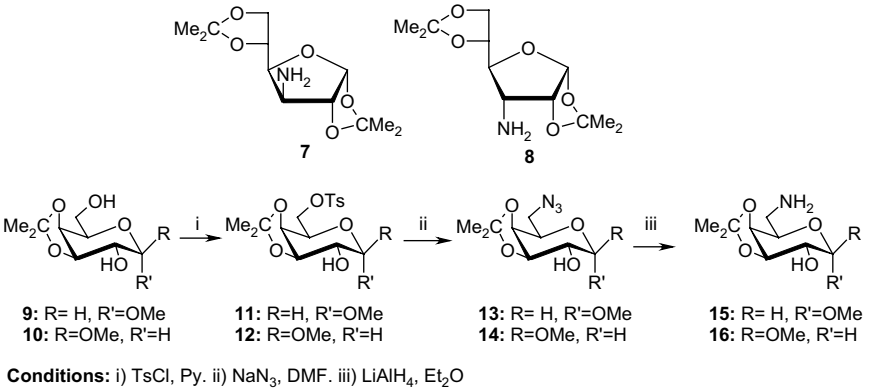
The glycose carbamides of type **1**, **3**, **5** and **6** were prepared through parallel synthesis by treatment of the four aminated scaffolds **7**, **8**, **15** and **16** with an excess of the appropriate commercial acyl chlorides in methylene chloride in the presence of PS-piperidinomethyl resin (Scheme 2).

Partially protected furanosic carbamides of type **2** and **4** (Scheme 2) were obtained from protected ones (**1** and **3**) through selective hydrolytic removal of 5,6 acetonide group with 80% aqueous AcOH. In the case of the D-allose series (**3**) acid hydrolysis was conducted under milder temperature conditions (45 °C) with respect to that used for D-glucose analogues (60 °C), in order to prevent complete removal of the two acetal groups. As pointed out by Collins [16], in fact, the hydrolysis rate of the 1,2-*O*-isopropylidene functionality in the D-allose series is higher with respect to that observed for analogous acetonide functionality in the D-glucose series [16]. All the compounds were fully characterized by ^1H and ^{13}C NMR and standard analytical parameters (see Section 4).

3. Biological activity

The human leukemia K562 cell line [17] was kept in a humidified atmosphere of 5% CO_2/air in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Celbio, MI, Italy), 50 units/mL penicillin and 50 μg/mL streptomycin [18]. In order to determine the ability of the tested compounds to inhibit the cell growth and induce the erythroid differentiation, K562 cells (30,000 cells/mL) were cultured both in the absence and presence of the indicated concentrations of compounds and the cell number/mL was determined with an ZF Coulter Counter (Counter Electronics, Hialeah, FL, USA) at different days from the culture set-up. In order to verify possible effects on erythroid differentiation, the proportion of benzidine-positive K562 cells was determined and compared to the values obtained employing other known inducers of erythroid differentiation, including cytosine arabinoside (ara-C) [19], mithramycin [3], rapamycin [3] and butyric acid [3].

Among the tested carbamides (**1Ca**, **1Ac**, **1Al**, **2Aa**, **2Ab**, **2Ac**, **2Ad**, **2Ae**, **2Af**, **2Ag**, **2Ah**, **2Ai**, **2Aj**, **2Ak**, **2Am**, **2An**, **2Ao**, **2Be**, **2Ca**, **2Cb**, **3Ab**, **3Ad**, **3Af**, **3Ah**, **3Al**, **3An**, **3Ba**, **3Bb**, **3Bc**, **3Bd**, **3Bf**, **3Bg**, **3Bi**, **3Bj**, **3Ca**, **4Ac**, **4Ac**, **4Ai**, **4Aj**, **4Al**, **4An**, **4Bb**, **4Bg**, **4Bh**, **4Bi**, **4Bj**, **4Cc**, **4Cd**, **5Ac**, **6Al**, **6Ac**) 14 (**1Ca**, **2Ab**, **2Ac**, **2Ad**, **2Ae**, **2Af**, **2Ah**, **3Ab**, **3An**, **4Ac**, **4Aj**, **4Bi**, **5Ac**, **6Al**) were found to exhibit appreciable (≥15%) erythroid differentiation effect, while nine (**2Ac**, **2Aj**, **3Bb**, **3Bf**, **3Bd**, **4Ac**, **4Ai**, **4Bh**, **4Aj**), three of which (**2Ac**, **4Ac** and **4Aj**) active on their own, were the compounds



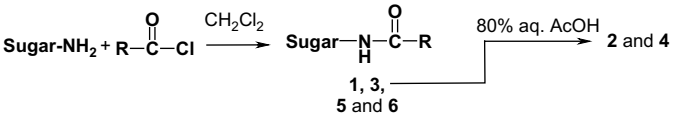
Scheme 1. Conditions: (i) TsCl, Py; (ii) NaN_3, DMF; (iii) $LiAlH_4$, Et_2O.

resulted active in synergism, potentiating erythroid induction of K562 cells treated with sub-optimal concentrations of ara-C. The data obtained on erythroid differentiation are shown in Tables 1 and 2.

When the analysis of the structure of carbamides active in the stimulation of erythroid differentiation was performed, it was found that seven of those exhibited linear saturated fatty acid residues, three branched saturated fatty acid residues and two alicyclic residues. However, we underline that many amides of linear, branched and most of alicyclic fatty acid residues are present in the list of inactive tested amide (i.e. **2Ak**, **3Af**, **5Ac**, **2An**). In addition, it should be noted that, despite the well-known activity of phenylbutyrates and phenylacetates [20], only one of the 14 aromatic rings containing the tested amides exhibits activity. In contrast, among the nine carbamides active in synergism with ara-C, four bear aromatic rings.

With respect to the role of sugar scaffold, we did not recognise any clear structure–activity relationship either related to the protection/deprotection of glycide [compare couples **1Ac** (inactive)/**2Ad** (active) and **1Ca** (active)/**2Ca** (inactive)] or to the stereochemistry of carbon bearing the pharmacophore [compare couples **2Ac** (active)/**4Ac** (active), **2Ai** (inactive)/**4Ai** (active in synergism) and **2Aj** (active in synergism)/**4Aj** (active)].

In conclusion, the majority of active glycose carbamides act with a mechanism resembling ara-C (no synergism observed with ara-C); three carbamides (**2Ac**, **4Ac** and **4Aj**) display a mechanism of action presumably different compared to that exhibited by sub-optimal concentrations of ara-C; six (**2Aj**, **3Bf**, **3Bb**, **3Bd**, **4Ai** and **4Bh**), which are not active by themselves, are, however, able to enhance ara-C mediated erythroid induction. Whether they induce a part of the erythroid differentiation program complementary to that stimulated by sub-optimal concentrations of ara-C remains to be investigated.



Scheme 2.

As far as structure–activity relationship (SAR) analysis, we like to underline that compounds of type **2** display the highest probability (35% in our set) to induce differentiation (6/17, compared to 2/15 and 3/13 of compounds of type **3** and **4**, respectively). Compounds carrying residues of group **A** display the highest probability (36%) to induce erythroid differentiation (11/31, compared to 1/14 and 1/6 of compounds carrying residues of group **B** and **C**, respectively).

More in detail, all the compounds carrying the residue **Ab** ($R = CH_2CH_3$) and three out of five compounds carrying residue **Ac** [$R = (CH_2)_2CH_3$] were found able to induce differentiation (two of them, **2Ac** and **4Ac**, also in synergism with ara-C). With respect to synergism with ara-C, only the compounds carrying the residue $R = C(CH_3)_3$ (**2Aj** and **4Aj**) were found to be active in inducing high level of differentiation.

Table 1
Effects of active carbamides on *in vitro* growth and erythroid differentiation of human leukemic K562 cells

Compound	IC_{50} value	Erythroid induction[a] (% of benzidine-positive cells)
1Ca	0.5 mM	19 ± 3.5
2Ab	30.0 mM	20 ± 4.4
2Ac	0.5 mM	20 ± 4.5
2Ad	5.0 mM	18 ± 3.2
2Ae	10.0 mM	28 ± 6.5
2Af	5.0 mM	25 ± 5.2
2Ah	5.0 mM	15 ± 3.3
3Ab	7.5 mM	19 ± 4.5
3An	1.0 mM	21 ± 3.2
4Ac	20.0 mM	20 ± 3.7
4Aj	20.0 mM	25 ± 7.2
4Bi	2.5 mM	24 ± 4.7
5Ac	0.1 mM	18 ± 4.4
6Al	0.25 mM	27 ± 5.5
Ara-C	500 nM	78 ± 24.5
Mithramycin	100 nM	86 ± 48.3
Rapamycin	1.0 mM	75.5 ± 7.5
Butyric acid	2.0 mM	32.5 ± 3.4

[a] Results are presented as average ± SD (three independent experiments performed) of % of benzidine-positive (hemoglobin-containing) cells after 6 days induction period at the indicated concentrations of the tested compounds.

Table 2
Synergism between ara-C and carbamides on *in vitro* growth and erythroid differentiation of human leukemic K562 cells

Compound	Concentration	Erythroid induction[a] (% of benzidine-positive cells)
2Ac	0.5 mM	35 ± 3.8
2Aj	5.0 mM	68 ± 5.5
3Bf	0.75 mM	49 ± 6.1
3Bb	1.0 mM	43 ± 4.4
3Bd	0.5 mM	50 ± 4.3
4Ac	20 mM	40 ± 3.5
4Ai	8.0 mM	66 ± 7.8
4Aj	20 mM	40 ± 5.6
4Bh	0.1 mM	33 ± 4.4
Ara-C	500 nM	22 ± 2.8

[a] Results are presented as average ± SD (three independent experiments performed) of % of benzidine-positive (hemoglobin-containing) cells after 6 days induction period at the indicated concentrations of the tested compounds. Suboptimal concentrations of ara-C (200 nm) were used in combination with the tested compounds.

Despite being limited, this SAR analysis suggests that the most promising molecules able to induce differentiation are glycose carbamides of type **2** or those displaying residues **Ab** ($R = CH_2CH_3$) and **Ac** [$R = (CH_2)_2CH_3$]. Residue **Aj** [$R = C(CH_3)_3$] appears to be involved in the property to act in synergism with ara-C. Obviously, this hypothesis is not conclusive, due to our choice of a diversity oriented selection of compounds within the complete library of carbamides. This choice has allowed to explore some representatives of each class of acyl residue, but it has prevented the performance of a systematic sight on the effect of glycide scaffold in biological activity when a same acyl residue is considered. The SAR analysis here discussed should be considered a starting point for further synthetic activity toward the generation of other oriented set of analogues, thus helping to verify this hypothesis.

4. Experimental section

4.1. General methods

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 20 ± 2 °C. ^{1}H NMR spectra were recorded in appropriate solvents (internal standard Me_4Si) with a Bruker AC 200 instrument at 200 MHz and with a Bruker AvanceII operating at 250 MHz. ^{13}C NMR spectra were recorded with the spectrometers operating at 50 and 62.9 MHz. Assignments were made with the aid of DEPT, HETCOR and COSY experiments and by comparison with values for known compounds and applying the known additivity rules [21]. All reactions were followed by TLC on Kieselgel 60 F_{254} (E. Merck) with detection by UV light and/or with ethanolic 10% phosphomolybdic or sulphuric acid and heating. Kieselgel 60 (E. Merck, 70–230 and 230–400 mesh, respectively) was used for column and flash chromatography. Parallel reactions were followed by HPLC–MS analyses (Waters Acquity UPLC, with Waters Acquity PAD detector and Micromass ZQ 2000 mass analyzer, controlled by PC with MassLynx TM 4.1, column Waters Acquity UPLC BEH C18 2.1 × 50 mm, 1.7 μm, eluent: $H_2O/CH_3CN/HCOOH$ 95/5/0,05 v/v/v). Solvents were dried by distillation according to standard procedures [22], and storage over 4 Å molecular sieves activated for at least 24 h at 250 °C. $MgSO_4$ was used as the drying agent for solutions. Acyl chlorides were purchased from Aldrich, with the exception of cyclopropanecarbonyl chloride prepared from corresponding acid according to the literature procedure [23]. PS-piperidinomethyl resin and polyamine resin were purchased from Novabiochem.

4.2. Amino scaffolds

3-Ammino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [11] (**7**) and 3-ammino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose [12] (**8**) were synthesized as reported.

4.2.1. Methyl-3,4-O-isopropylidene-6-O-methylsulphonyl-α-D-galactopyranoside (11)

A solution of **9** [13] (1.00 g, 4.27 mmol) in pyridine (25 mL) was treated at 0 °C under stirring with commercial methylsulphoyl chloride (896 mg, 4.70 mmol). The solution was allowed to warm to room temperature and stirred until the TLC analysis (EtOAc) showed the complete disappearance of the starting material (74 h) and the formation of one component (R_f 0.51). The reaction mixture was repeatedly coevaporated with toluene (5 × 15 mL) under diminished pressure. The crude residue was partitioned between CH_2Cl_2 (60 mL) and H_2O (30 mL), the aqueous phase was extracted with CH_2Cl_2 (4 × 40 mL) and the organic ones were collected, dried ($MgSO_4$) and concentrated under diminished pressure. Flash chromatography on silica gel (petroleum ether–EtOAc 2:1) of the crude solid led to pure **15** (1.07 g, 64%) as a white solid. R_f 0.51 (EtOAc); mp (EtOAc) 127–129 °C; lit. [24] 129 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.82 (AA′XX′, 2H, Ar-H), 7.35 (AA′XX′, 2H, Ar-H), 4.70 (d, 1H, $J = 3.9$ Hz, H-1), 4.28–4.12 (m, 5H, H-6a, H-6b, H-2, H-3, H-4), 3.79 (m, 1H, H-5), 3.41 (s, 3H, O*Me*), 2.45 (s, 3H, *Me*Ph), 1.42, 1.29 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 133.2, 144.8 (2 × Ar-C), 128.0, 129.8 (4 × Ar-CH), 110.0 (*C*Me$_2$), 97.8 (C-1), 75.6 (C-3), 72.4 (C-4), 68.7, 69.2 (C-2, C-5), 66.5 (C-6), 55.5 (O*Me*), 25.7, 27.4 (C*Me*$_2$), 21.6 (*Me*Ph). Compound **11** was stored at 4 °C or lower temperature to prevent degradation.

4.2.2. Methyl-3,4-O-isopropylidene-6-O-methylsulphonyl-β-D-galactopyranoside (12)

This compound was prepared starting from **10** [13] (3.10 g, 13.3 mmol) by a procedure analogous to that of **11**. White solid, 3.61 g (70%). R_f 0.57 (EtOAc); mp (EtOAc) 157–159 °C; lit. [15] 154–155 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +1.0; lit. [15] (*c* 2.3, $CHCl_3$): $[\alpha]_D$ 1.0. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.81 (AA′XX′, 2H, Ar-H), 7.35 (AA′XX′, 2H,

Ar-H), 4.28 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.23, 4.07 (2m, 5H, H-3, H-4, H-5, H-6a, H-6b), 3.47 (dd, 1H, $J_{2,3} = 6.5$ Hz, H-2), 3.47 (s, 3H, OMe), 2.46 (s, 3H, *Me*Ph), 1.44, 1.29 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 145.1, 132.1 (2 × Ar-C), 127.9, 128.9 (4 × Ar-CH), 110.5 (*C*Me$_2$), 103.0 (C-1), 78.5 (C-3), 73.4, 72.9, 71.0 (C-2, C-4, C-5), 68.6 (C-6), 57.0 (OMe), 27.9, 26.2 (*Me*$_2$C), 21.6 (*Me*Ph). Compound **12** has to be stored at 4 °C or lower temperature to prevent degradation.

4.2.3. Methyl-6-azido-6-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (13)

A solution of **11** (1.00 g, 2.58 mmol) in DMF (35 mL) was treated with commercial sodium azide (355 mg, 5.15 mmol). The suspension was warmed to 120 °C and stirred until the TLC analysis (petroleum ether–EtOAc 1:1) showed the complete disappearance of the starting material (28 h) and the formation of one component (R_f 0.35). The reaction mixture was allowed to cool to room temperature and concentrated under diminished pressure. The crude residue was partitioned between CH_2Cl_2 (35 mL) and H_2O (30 mL), the aqueous phase was extracted with CH_2Cl_2 (5 × 25 mL) and the organic ones were collected, dried ($MgSO_4$) and concentrated under diminished pressure. Flash chromatography on silica gel (petroleum ether–EtOAc 1:1) of the crude syrup led to pure **13** (677 mg, 77%) as a syrup. R_f 0.35 (petroleum ether–EtOAc 1:1); optical rotation (*c* 1.2, $CHCl_3$) $[\alpha]_D$ +89.4. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.78 (d, 1H, $J_{1,2} = 3.9$ Hz, H-1), 4.29 (t, 1H, $J_{3,4} = 6.1$ Hz, H-3), 4.14 (m, 2H, H-5, H-4), 3.85 (m, 1H, H-2), 3.59 (dd, 1H, $J_{5,6b} = 8.3$ Hz, H-6b), 3.50 (s, 3H, OMe), 3.33 (dd, 1H, $J_{6a,6b} = 12.9$ Hz, $J_{5,6a} = 4.3$ Hz, H-6a), 2.53 (d, 1H, $J_{2,OH} = 5.7$ Hz, OH), 1.51, 1.35 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 109.9 (*C*Me$_2$), 98.1 (C-1), 75.6 (C-3), 73.1 (C-4), 68.8, 67.9 (C-5, C-2), 55.6 (OMe), 51.2 (C-6), 27.4, 25.7 (C*Me*$_2$). Anal. for $C_{10}H_{17}N_3O_5$. Calcd (%): C, 46.33; H, 6.61; N, 16.21. Found (%): C, 44.66; H, 6.47; N, 15.66.

4.2.4. Methyl-6-azido-6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside (14)

This compound was prepared starting from **12** (1.50 g, 3.87 mmol) by a procedure analogous to that of **13**. White solid, 792 mg (79%). R_f 0.22 (petroleum ether–EtOAc 1:1); mp (hexane) 80–82 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ −16.6. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.14 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.10 (m, 2H, H-3, H-4), 3.94 (ddd, 1H, $J_{4,5} = 1.7$ Hz, H-5), 3.73 (dd, 1H, $J_{5,6b} =$ 8.3 Hz, H-6b), 3.57 (s, 3H, OMe), 3.54 (m, 1H, H-2), 3.33 (dd, 1H, $J_{6a,6b} = 12.9$ Hz, $J_{5,6a} = 4.2$ Hz, H-6a), 2.85 (br s, 1H, OH), 1.35, 1.53 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 110.4 (*C*Me$_2$), 103.1 (C-1), 78.6 (C-3), 73.5, 73.7, 72.9 (C-2, C-5, C-4), 57.0 (OMe), 51.0 (C-6), 26.2, 27.9 (C*Me*$_2$). Anal. for $C_{10}H_{17}N_3O_5$. Calcd (%): C, 46.33; H, 6.61; N, 16.21. Found (%): C, 44.28; H, 6.32; N, 16.32.

4.2.5. Methyl-6-amino-6-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (15)

To a suspension of $LiAlH_4$ (93 mg, 2.5 mmol) in dry Et_2O (10 mL) at 0 °C a solution of **13** (260 mg, 1.0 mmol) in dry Et_2O (6 mL) was added drop wise and under stirring. The suspension was warmed to reflux and stirred until the TLC analysis (petroleum ether–EtOAc 1:1) showed the complete disappearance of the starting material (30 min) and the formation of one component (R_f 0). The reaction mixture was cooled to 0 °C, diluted with Et_2O and treated in sequence with 1 mL of water, 2 mL of 10% aqueous NaOH and 3 mL of water, obtaining the formation of a white precipitate. The mixture was filtered, the solid was washed with CH_2Cl_2 (4 × 5 mL) and the organic ones were collected, dried ($MgSO_4$) and concentrated under diminished pressure to provide crude **15** (184 mg, 79%). Crystallization (EtOAc) of the crude solid furnished **15** as a white crystalline solid. Mp 152–157 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +145.7. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.76 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.21 (m, 2H, H-4, H-5), 3.91 (m, 1H, H-2), 3.79 (t, 1H, $J_{2,3} = J_{3,4} = 5.7$ Hz, H-3), 3.45 (s, 3H, OMe), 3.05 (dd, 1H, $J_{5,6b} = 7.0$ Hz, H-6b), 2.91 (dd, 1H, $J_{6a,6b} = 13.3$ Hz, $J_{5,6a} = 4.9$ Hz, H-6a), 1.35, 1.50 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 109.5 (*C*Me$_2$), 98.7 (C-1), 76.4 (C-3), 74.0 (C-4), 69.3, 69.7 (C-5, C-2), 55.4 (OMe), 42.8 (C-6), 25.9, 27.8 (C*Me*$_2$). Anal. for $C_{10}H_{19}NO_5$. Calcd (%): C, 51.49; H, 7.95; N, 6.00. Found (%): C, 50.97; H, 8.25; N, 5.77.

4.2.6. Methyl-6-amino-6-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside (16)

This compound was prepared starting from **14** (630 mg, 2.44 mmol) by a procedure analogous to that of **15**. White solid 463 mg (81%); mp (hexane) 146–153 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ −18.9. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.11 (d, 1H, $J = 8.3$ Hz, H-1), 4.08 (m, 2H, H-3, H-4), 3.73 (ddd, 1H, $J_{4,5} = 1.9$ Hz, H-5), 3.55 (s, 3H, OMe), 3.49 (t, 1H, $J = 7.8$ Hz, H-2), 3.14 (dd, 1H, $J_{5,6'} = 7.6$ Hz, H-6b), 2.96 (dd, 1H, $J_{6,6'} = 13.3$ Hz, $J_{5,6} = 4.7$ Hz, H-6a), 2.48 (br s, 1H, OH), 1.50, 1.34 (2s, each 3H, C*Me*$_2$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 110.0 (*C*Me$_2$), 103.4 (C-1), 79.3 (C-3), 74.4, 74.1, 73.3 (C-2, C-5, C-4), 56.8 (OMe), 42.6 (C-6), 28.1, 26.2 (C*Me*$_2$). Anal. for $C_{10}H_{19}NO_5$. Calcd (%): C, 51.49; H, 7.95; N, 6.00. Found (%): C, 50.93; H, 7.83; N, 5.85.

4.3. General method for the parallel synthesis of amides of type 1, 3, 5 and 6

A 0.2 M solution of amine scaffold in CH_2Cl_2 and a 0.4 M solution of the selected acyl chloride in CH_2Cl_2 were prepared under anhydrous conditions.

In each reactor 1.00 g of resin PS-piperidinomethyl was put and in sequence 5 mL of the solution containing the amine and 5 mL of the solution containing the acyl chloride were added. The reaction mixture was stirred at room temperature for 12 h. The reaction proceeding was checked by HPLC–MS. A 1.00 g of resin polyamine (loading = 3.2 mmol/g) to extract the excess acyl chloride was added after dilution with

CH_2Cl_2 (5 mL) and vortically stirred for 2 h. The suspension was filtered on paper and the clear solutions were evaporated under diminished pressure to give oils that were purified by a Combi Flash chromatographic system (ISCO, using columns Redisep of 10 g eluting with a CH_2Cl_2–MeOH gradient from 98:2 to 95:5). After evaporation the solvent pure products were obtained.

4.4. General method of the synthesis of selectively deprotected furanosic amides of type *1* and *3*

The fully protected amides of type **1** and **3** (1.5–3.0 mmol), prepared following the general procedure reported above, were dissolved in 80% aqueous AcOH (20 mL) and the resulting solutions were warmed to 60 °C in the case of hydrolysis of amides of type **1** and to 45 °C in the case of amides of type **3**. Reaction mixtures were maintained under stirring until the TLC analysis (Hexane–EtOAc) revealed the disappearance of starting material (1–4 h). The solution was repeatedly coevaporated with toluene (5 × 10 mL) under diminished pressure. The crude residues were purified by a Combi Flash chromatographic system (ISCO, using columns Redisep of 10 g and as eluted a CH_2Cl_2–MeOH gradient from 98:5 to 90:10). After evaporation the solvent pure products were obtained.

4.5. Active carbamides

4.5.1. 3-N-(2-Butynoyl)-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (*1Ca*)

From **7** (778 mg, 3.00 mmol) by acylation with 2-butynoyl chloride, white solid 705 mg (72%); R_f 0.32 (hexane–EtOAc 3:7); mp (chrom) 160–162 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ −47.3; ^{1}H NMR (250 MHz, CD_3CN): see Table 5 and δ 1.93 (s, 3H, *Me*C≡), 1.44, 1.36, 1.28, 1.26 (4s, each 3H, 2 × C*Me*$_2$); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 6 and δ 153.7 (*CO*), 112.6, 110.0 (2 × *C*Me$_2$), 84.9 (≡*C*CO); 75.2 (Me*C*≡), 27.9, 27.8, 26.3, 25.4 (2 × C*Me*$_2$), 3.5 (*Me*C≡); Anal. for $C_{16}H_{23}NO_6$. Calcd (%): C, 59.07; H, 7.13; N, 4.30. Found (%): C, 58.87; H, 7.16; N, 4.28.

4.5.2. 3-N-Propanoyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (*2Ab*)

From **7** (778 mg, 3.00 mmol) by acylation with propanoyl chloride and subsequent hydrolysis; colourless syrup 504 mg (61%); R_f 0.27 (EtOAc–MeOH 9:1); optical rotation (*c* 1.0, MeOH): $[\alpha]_D$ +15.4; ^{1}H NMR (250 MHz, CD_3CN): see Table 3 and δ 2.30 (br s, 2H, OH-5, OH-6), 2.25 (t, 2H, *J* = 7.5 Hz, *CH*$_2$CO), 1.45, 1.27 (2s, each 3H, C*Me*$_2$), 1.08 (t, 3H, *J* = 7.5 Hz, *Me*); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 176.6 (*CO*), 112.4 (*C*Me$_2$), 29.7 (*CH*$_2$CO), 26.7, 26.3 (C*Me*$_2$), 10.2 (*Me*). Anal. for $C_{12}H_{21}NO_6$. Calcd (%): C, 52.35; H, 7.69; N, 5.09. Found (%): C, 52.16; H, 7.72; N, 5.07.

4.5.3. 3-N-Butanoyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (*2Ac*)

From **7** (778 mg, 3.00 mmol) by acylation with butanoyl chloride and subsequent hydrolysis; colourless syrup 564 mg (65%); R_f 0.43 (EtOAc–MeOH 9:1); optical rotation (*c* 1.0, MeOH): $[\alpha]_D$ +19.8; ^{1}H NMR (200 MHz, $CDCl_3$): see Table 3 and δ 3.60 (br s, 2H, OH-5, OH-6), 2.23 (t, 2H, *J* = 7.0 Hz, *CH*$_2$CO), 1.58 (m, 2H, *CH*$_2$Me), 1.50, 1.30 (2s, each 3H, C*Me*$_2$), 0.91 (t, 3H, *J* = 7.2 Hz, *Me*); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 4 and δ 175.2 (*CO*), 111.7 (*C*Me$_2$), 37.8 (*CH*$_2$CO), 26.2, 25.8 (C*Me*$_2$), 18.9 (*CH*$_2$Me), 13.5 (*Me*). Anal. for $C_{13}H_{23}NO_6$. Calcd (%): C, 53.97; H, 8.01; N, 4.84. Found (%): C, 54.18; H, 8.04; N, 4.86.

4.5.4. 3-N-Pentanoyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (*2Ad*)

From **7** (778 mg, 3.00 mmol) by acylation with pentanoyl chloride and subsequent hydrolysis; colourless syrup 574 mg (52%); R_f 0.25 (EtOAc); optical rotation (*c* 1.4, MeOH): $[\alpha]_D$ +25.7; ^{1}H NMR (250 MHz, CD_3CN): see Table 3 and δ 4.30, 2.38 (2br s, each 1H, OH-5, OH-6), 2.20 (t, 2H, *J* = 7.4 Hz, *CH*$_2$CO), 1.55 (m, 2H, *CH*$_2$CH$_2$CO), 1.34 (m, 2H, *CH*$_2$Me), 1.45, 1.27 (2s, each 3H, C*Me*$_2$), 0.90 (t, 3H, *J* = 7.3 Hz, *Me*); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 176.0 (*CO*), 112.5 (*C*Me$_2$), 36.3 (*CH*$_2$CO), 28.6 (*CH*$_2$CH$_2$CO), 26.7, 26.4 (C*Me*$_2$), 23.0 (*CH*$_2$Me), 14.1 (*Me*).

Table 3
^{1}H NMR data (δ, ppm; *J*, Hz) for compounds of type **2** and **4**

Compound	Solvent	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	NH	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{3,NH}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
2Ab	CD_3CN	5.84	4.59	4.20	4.01	3.50	3.50	3.64	6.87	3.7	0	3.2	7.6	8.4	n.d.	2.2	10.4
2Ac	$CDCl_3$	5.89	4.58	4.36	4.10	3.70	3.70	3.70	7.49	3.7	0	3.2	7.1	7.3	n.d.	n.d.	n.d.
2Ad	CD_3CN	5.83	4.50	4.21	4.01	3.49	3.49	3.66	6.96	3.7	0	3.2	7.7	8.3	n.d.	n.d.	n.d.
2Ae	CD_3CN–D_2O	5.83	4.49	4.21	4.02	3.55	3.55	3.62	–	3.6	0	3.2	–	7.9	n.d.	2.7	10.7
2Af	CD_3CN	5.83	4.50	4.20	4.00	3.53	3.53	3.53	6.84	3.6	0	3.1	7.8	8.2	n.d.	n.d.	n.d.
2Ah	CD_3CN	5.84	4.50	4.21	4.02	3.48	3.48	3.64	6.90	3.7	0	3.2	7.4	8.3	n.d.	2.6	10.3
2Aj	$CDCl_3$	5.89	4.56	4.31	4.15	3.90	3.70	3.70	6.84	3.7	0	3.4	6.4	6.4	n.d.	n.d.	n.d.
4Ac	CD_3CN	5.76	4.59	4.23	3.77	3.65	3.42	3.51	6.62	3.8	5.1	9.2	8.9	4.8	6.6	4.0	11.3
4Ai	CD_3CN	5.76	4.59	4.23	3.80	3.66	3.40	3.51	6.56	3.8	5.1	9.2	9.0	4.6	6.7	3.8	11.5
4Aj	CD_3CN	5.79	4.62	4.17	3.82	3.66	3.40	3.52	6.46	3.8	5.3	9.2	8.2	4.9	6.7	4.0	11.2
4Bh	CD_3CN	5.84	4.76	4.47	4.04	3.79	3.48	3.58	7.15	3.8	5.0	9.4	8.1	4.7	6.6	4.2	11.4
4Bi	CD_3CN–D_2O	5.81	4.72	4.45	4.08	3.80	3.44	3.55	–	3.8	4.9	9.7	–	4.1	7.1	4.2	11.5

Table 4
^{13}C NMR data (δ, ppm) for compounds of type **2** and **4**

Compound	Solvent	C-1	C-2	C-3	C-4	C-5	C-6
2Ab	CD_3CN	105.6	84.8	57.0	79.8	70.0	64.4
2Ac	$CDCl_3$	104.2	83.7	56.3	78.5	69.4	63.7
2Ad	CD_3CN	105.5	84.8	57.1	79.8	70.1	64.4
2Ae	CD_3CN-D_2O	105.5	84.8	56.7	79.4	70.0	64.1
2Af	CD_3CN	105.6	84.8	57.1	79.9	70.2	64.5
2Ah	CD_3CN	105.6	84.8	57.1	79.7	70.2	64.4
2Aj	$CDCl_3$	104.0	84.1	56.7	78.1	69.9	63.5
4Ac	CD_3CN	105.1	80.6	53.3	80.5	73.2	63.8
4Ai	CD_3CN	105.2	80.7	53.3	80.5	73.1	63.8
4Aj	CD_3CN	105.2	80.9	53.6	80.3	73.1	63.8
4Bh	CD_3CN	105.3	80.4	54.1	80.2	73.1	63.8
4Bi	CD_3CN-D_2O	105.2	80.3	53.5	79.6	72.7	63.4

Anal. for $C_{14}H_{25}NO_6$. Calcd (%): C, 55.43; H, 8.31; N, 4.62. Found (%): C, 55.21; H, 8.34; N, 4.58.

4.5.5. 3-N-Hexanoyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**2Ae**)

From **7** (778 mg, 3.00 mmol) by acylation with hexanoyl chloride and subsequent hydrolysis; colourless syrup 628 mg (66%); R_f 0.42 (EtOAc–MeOH 95:5); optical rotation (*c* 1.0, MeOH): $[\alpha]_D$ +23.2; ^{1}H NMR (250 MHz, CD_3CN-D_2O): see Table 3 and δ 2.18 (t, 2H, $J = 6.4$ Hz, CH_2CO), 1.54 (m, 2H, CH_2CH_2CO), 1.27 [m, 4H, $(CH_2)_2Me$], 1.44, 1.26 (2s, each 3H, CMe_2), 0.89 (t, 3H, $J = 6.7$ Hz, *Me*); ^{13}C NMR (62.9 MHz, CD_3CN-D_2O): see Table 6 and δ 176.5 (*CO*), 112.7 (CMe_2), 36.5 (CH_2CO), 32.0 (CH_2CH_2CO), 26.6, 26.2 (CMe_2), 26.1, 23.0 [$(CH_2)_2Me$], 14.2 (*Me*). Anal. for $C_{15}H_{27}NO_6$. Calcd (%): C, 56.77; H, 8.57; N, 4.41. Found (%): C, 57.02; H, 8.61; N, 4.39.

4.5.6. 3-N-(3-Methylbutanoyl)-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**2Af**)

From **7** (778 mg, 3.00 mmol) by acylation with 3-methylbutanoyl chloride and subsequent hydrolysis; white solid 501 mg (55%); R_f 0.28 (EtOAc); mp (hexane) 115–116 °C; optical rotation (*c* 0.4, $CHCl_3$): $[\alpha]_D$ +46.3; ^{1}H NMR (200 MHz, CD_3CN): see Table 3 and δ 4.23 (br s, 1H, OH-5), 2.77 (br t, 1H, OH-6), 2.06 (m, 2H, CH_2CO), 2.02 (m, 1H, $CHMe_2$), 1.45, 1.27 (2s, each 3H, CMe_2), 0.92 (d, 6H, $J = 6.2$ Hz, $2 \times Me$); ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ 175.2 (*CO*), 112.5, (CMe_2), 45.7 (CH_2CO), 26.9 ($CHMe_2$), 26.8, 26.3 (CMe_2), 22.6 ($2 \times Me$). Anal. for $C_{14}H_{25}NO_6$. Calcd (%): C, 55.43; H, 8.31; N, 4.62. Found (%): C, 55.30; H, 8.34; N, 4.60.

4.5.7. 3-N-(3,3-Dimethylbutanoyl)-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**2Ah**)

From **7** (778 mg, 3.00 mmol) by acylation with 3,3-dimethylbutanoyl chloride and subsequent hydrolysis; colourless syrup, 505 mg (53%); R_f 0.47 (EtOAc–MeOH 95:5); optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +35.4; ^{1}H NMR (250 MHz, CD_3CN): see Table 3 and δ 4.31, 2.85 (2br s, each 1H, OH-5, OH-6), 2.08 (m, 2H, CH_2CO), 1.45, 1.27 (2s, each 3H, CMe_2), 1.01 (s, 9H, CMe_3); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 174.5 (*CO*), 112.5 (CMe_2), 50.0 (CH_2CO), 31.4 (CMe_3), 30.1 (CMe_3), 26.8, 26.4 (CMe_2). Anal. for $C_{15}H_{27}NO_6$. Calcd (%): C, 56.77; H, 8.57; N, 4.41. Found (%): C, 56.59; H, 8.55; N, 4.43.

4.5.8. 3-N-Pivaloyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**2Aj**)

From **7** (778 mg, 3.00 mmol) by acylation with 2,2-dimethylbutanoyl chloride and subsequent hydrolysis; white solid, 821 mg (90%); R_f 0.44 (EtOAc–MeOH 95:5); mp (chrom) 161–163 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +22.3; compound **2Aj** ^{1}H NMR (200 MHz, $CDCl_3$): see Table 3 and δ 4.35, 3.02 (2br s, each 1H, OH-5, OH-6), 1.51, 1.31 (2s, each 3H, CMe_2), 1.21 (s, 9H, CMe_3); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 4 and δ 180.3 (*CO*), 111.9 (CMe_2), 38.8 (CMe_3), 27.4 (CMe_3), 26.4, 26.2 (CMe_2). Anal. for $C_{14}H_{25}NO_6$. Calcd (%): C, 55.43; H, 8.31; N, 4.62. Found (%): C, 55.62; H, 8.35; N, 4.60.

4.5.9. 3-N-Propanoyl-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**3Ab**)

From **8** (778 mg, 3.00 mmol) by acylation with propanoyl chloride; white solid, 833 mg (88%); R_f 0.60 (EtOAc–MeOH 9:1); mp (hexane) 93–95 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +71.1; ^{1}H NMR (200 MHz, CD_3CN): see Table 5 and δ 2.15 (q, 2H, $J = 7.5$ Hz, CH_2CO), 1.50, 1.34, 1.29, 1.27 (4s, each 3H, $2 \times CMe_2$), 1.05 (t, 3H, *Me*); ^{13}C NMR (50 MHz, CD_3CN): see Table 6 and δ 174.3 (*CO*), 112.9, 109.9 ($2 \times CMe_2$), 29.6 (CH_2CO), 26.9, 26.6, 26.5, 25.4 ($2 \times CMe_2$), 10.1 (Me). Anal. for $C_{15}H_{25}NO_6$. Calcd (%): C, 57.13; H, 7.99; N, 4.44. Found (%): C, 57.39; H, 8.02; N, 4.42.

Table 5
^{1}H NMR data (δ, ppm; *J*, Hz) for compounds of type **1**, **3**, **5** and **6**

Compound	Solvent	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	NH	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{3,NH}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1Ca	CD_3CN	5.81	4.46	4.33	4.10	4.20	3.83	4.05	7.05	3.7	0	3.7	8.8	7.2	5.5	6.1	8.4
3Ab	CD_3CN	5.75	4.55	4.13	3.90	4.13	3.90	3.90	6.43	3.7	4.3	n.d.	8.1	n.d.	n.d.	n.d.	n.d.
3An	CD_3CN	5.75	4.54	4.19	3.91	4.11	3.91	3.91	6.43	3.6	4.3	9.5	8.8	n.d.	n.d.	n.d.	n.d.
3Bd	CD_3CN	5.75	4.53	4.09	3.84	4.12	3.79	3.94	6.60	3.8	4.6	9.3	8.4	3.5	6.8	6.6	8.0
3Bf	CD_3CN	5.75	4.52	4.10	3.86	4.07	3.82	3.95	6.48	3.8	4.7	9.9	8.8	3.4	6.3	6.6	7.9
3Bb	CD_3CN	5.75	4.56	4.13	3.87	4.11	3.81	3.96	6.59	3.8	4.9	9.8	8.4	4.0	6.6	6.6	8.2
5Ac	CD_3CN	4.59	3.57	4.02	4.15	3.96	3.23	3.48	6.62	3.6	7.5	5.5	–	2.4	7.8	4.4	13.7
6Al	CD_3CN	4.02	3.28	3.94	4.10	3.80	3.28	3.53	6.90	8.2	7.0	5.5	–	2.1	8.4	4.2	13.8

Table 6
^{13}C NMR data (δ, ppm) for compounds of type **1**, **3**, **5** and **6**

Compound	Solvent	C-1	C-2	C-3	C-4	C-5	C-6
1Ca	CD_3CN	105.6	85.2	56.4	79.5	73.7	67.5
3Ab	CD_3CN	105.3	80.1	53.6	78.9	76.4	65.4
3An	CD_3CN	105.3	80.1	53.7	79.0	76.5	65.6
3Bd	CD_3CN	105.3	80.0	53.5	79.2	76.3	65.2
3Bf	CD_3CN	105.4	80.0	53.7	78.8	76.3	65.3
3Bb	CD_3CN	105.4	80.0	54.0	79.1	76.5	65.6
5Ac	CD_3CN	100.4	70.8	77.4	74.7	66.8	40.7
6Al	CD_3CN	104.4	74.2	80.3	75.1	72.1	41.1

*4.5.10. 3-N-Cyclopentanecarbonyl-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**3An**)*

From **8** (778 mg, 3.00 mmol) by acylation with cyclopentanecarbonyl chloride; white solid, 960 mg (90%); R_f 0.33 (hexane–EtOAc 1:1); mp (chrom) 102–104 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +65.5; ^{1}H NMR (200 MHz, CD_3CN): see Table 5 and δ 2.61 (m, 1H, *CH*), 1.63 (m, 8H, cyclopentyl H), 1.50, 1.34, 1.29, 1.26 (4s, each 3H, 2 × C*Me*$_2$); ^{13}C NMR (50 MHz, CD_3CN): see Table 6 and δ 176.8 (*CO*), 113.0, 110.0 (2 × *C*Me$_2$), 45.8 (CH); 31.3, 30.7 (2 × CH_2), 26.9, 26.7, 26.6, 25.6 (2 × C*Me*$_2$), 26.7 (2 × CH_2). Anal. for $C_{18}H_{29}NO_6$. Calcd (%): C, 60.83; H, 8.22; N, 3.94. Found (%): C, 60.58; H, 8.25; N, 3.94.

*4.5.11. 3-N-(3-Methoxyphenylacetyl)-3-deoxy-1,2:3,4-di-O-isopropylidene-α-D-allofuranose (**3Bb**)*

From **8** (778 mg, 3.00 mmol) by acylation with 3-methoxyphenylacetyl chloride; colourless syrup, 1004 mg (82%); R_f 0.50 (EtOAc); optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +70.1; ^{1}H NMR (250 MHz, CD_3CN): see Table 5 and δ 7.23 (dd, 1H, $J_{6',5'} = 8.0$ Hz, $J_{4',5'} = 8.2$ Hz, H-5′), 6.86 (m, 2H, H-2′, H-6′), 6.82 (ddd, 1H, $J = 2.5$ Hz, $J = 1.0$ Hz, H-4′), 3.77 (s, 3H, OMe), 3.47 (s, 2H, PhC*H*$_2$), 1.48, 1.31, 1.28, 1.25 (4s, each 3H, 2 × C*Me*$_2$); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 6 and δ 171.3 (*CO*), 160.8 (C-3′), 138.3 (C-1′), 130.5 (C-5′), 122.4, 115.8, 113.1 (C-2′, C-4′, C-6′), 113.0, 110.0 (2 × *C*Me$_2$), 55.8 (OMe), 43.4 (Ph*CH*$_2$), 26.7, 26.6, 26.5, 25.5 (2 × C*Me*$_2$); Anal. for $C_{21}H_{29}NO_7$. Calcd (%): C, 61.90; H, 8.31; N, 3.44. Found (%): C, 61.87; H, 8.35; N, 3.43.

*4.5.12. 3-N-(2,5-Dimethoxyphenylacetyl)-3-deoxy-1,2:3,4-di-O-isopropylidene-α-D-allofuranose (**3Bd**)*

From **8** (778 mg, 3.00 mmol) by acylation with 2,5-dimethoxyphenylacetyl chloride; colourless syrup, 998 mg (76%); R_f 0.39 (hexane–EtOAc 1:9); optical rotation (*c* 1.2, $CHCl_3$): $[\alpha]_D$ +57.1; ^{1}H NMR (200 MHz, CD_3CN): see Table 5 and δ 6.91–6.78 (m, 3H, H-3′, H-4′, H-6′), 3.78, 3.72 (2s, each 3H, 2 × OMe), 3.44 (s, 2H, *CH*$_2$CO), 1.47, 1.29, 1.28, 1.25 (4s, each 3H, 2 × C*Me*$_2$); ^{13}C NMR (50 MHz, CD_3CN): see Table 6 and δ 171.4 (*CO*), 154.5, 152.4 (C-3′, C-5′), 126.0 (C-1′), 118.1, 113.4, 112.6 (C-3′, C-4′, C-6′), 113.0, 110.0 (2 × *C*Me$_2$), 56.6, 56.2 (2 × OMe), 38.9 (*CH*$_2$CO), 26.8, 26.6, 26.5, 25.5 (2 × C*Me*$_2$); Anal. for $C_{22}H_{31}NO_8$. Calcd (%): C, 60.40; H, 7.14; N, 3.20. Found (%): C, 60.37; H, 7.14; N, 3.19.

*4.5.13. 3-N-Hydrocinnammoyl-3-deoxy-1,2:3,4-di-O-isopropylidene-α-D-allofuranose (**3Bf**)*

From **8** (778 mg, 3.00 mmol) by acylation with hydrocinnammoyl chloride; colourless syrup, 846 mg (72%); R_f 0.72 (EtOAc–MeOH 9:1); optical rotation (*c* +1.0, $CHCl_3$): $[\alpha]_D$ +66.6; ^{1}H NMR (200 MHz, CD_3CN): see Table 5 and δ 7.25 (m, 5H, Ar-H), 2.89, 2.47 (2t, each 2H, $J = 8.1$ Hz, 2 × CH_2), 1.49, 1.34, 1.28, 1.28 (4s, each 3H, 2 × C*Me*$_2$); ^{13}C NMR (50 MHz, CD_3CN): see Table 6 and δ 172.9 (*CO*), 142.3 (Ar-C), 129.3–127.0 (Ar-CH), 113.0, 110.0 (2 × *C*Me$_2$), 38.2, 32.1 (2 × CH_2), 26.9, 26.6, 26.5, 25.5 (2 × C*Me*$_2$); Anal. for $C_{21}H_{29}NO_6$. Calcd (%): C, 64.43; H, 7.47; N, 3.58. Found (%): C, 64.38; H, 7.50; N, 3.57.

*4.5.14. 3-N-Butanoyl-3-deoxy-1,2-O-isopropylidene-α-D-allofuranose (**4Ac**)*

From **8** (778 mg, 3.00 mmol) by acylation with butanoyl chloride and subsequent hydrolysis; white solid, 556 mg (64%); R_f 0.32 (EtOAc); mp (chrom) 123–124 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +4.4; ^{1}H NMR (200 MHz, CD_3CN): see Table 3 and δ 3.76 (d, 1H, $J_{5,OH} = 4.6$ Hz, OH-5), 2.80 (dd, 1H, $J_{6a,OH} = 5.4$ Hz, $J_{6b,OH} = 6.5$ Hz, OH-6), 2.17 (t, 2H, $J = 7.2$ Hz, *CH*$_2$CO), 1.53 (sestetto, 2H, *CH*$_2$Me); 1.50, 1.30 (2s, each 3H, C*Me*$_2$), 0.90 (t, 3H, $J = 7.4$ Hz, *Me*); ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ 175.0 (*CO*), 113.0 (*C*Me$_2$), 38.5 (*CH*$_2$CO), 26.9, 26.7 (C*Me*$_2$), 19.7 (*CH*$_2$Me), 13.9 (*Me*); Anal. for $C_{13}H_{23}NO_6$. Calcd (%): C, 55.97; H, 8.01; N, 4.84. Found (%): C, 55.88; H, 8.04; N, 4.86.

*4.5.15. 3-N-(2-Methylpropanoyl)-3-deoxy-1,2-O-isopropylidene-α-D-allofuranose (**4Ai**)*

From **8** (778 mg, 3.00 mmol) by acylation with 2-methylpropanoyl chloride and subsequent hydrolysis; white solid, 460 mg (53%); R_f 0.26 (EtOAc–MeOH 95:5); mp (chrom) 105–107 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +4.6; ^{1}H NMR (250 MHz, CD_3CN): see Table 3 and δ 3.73 (d, 1H, $J_{5,OH} = 4.8$ Hz, OH-5), 2.84 (dd, 1H, $J_{6a,OH} = 5.3$ Hz, $J_{6b,OH} = 6.6$ Hz, OH-6), 2.45 (ept, 1H, $J = 6.8$ Hz, *CH*Me$_2$), 1.51, 1.30 (2s, each 3H, C*Me*$_2$), 1.06, 1.07 (2d, each 3H, $J = 6.8$ Hz, CH*Me*$_2$); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 178.8 (*CO*), 113.0 (*C*Me$_2$), 35.6 (*CH*Me$_2$), 26.9, 26.7 (C*Me*$_2$), 19.7 (CH*Me*$_2$). Anal. for $C_{13}H_{23}NO_6$. Calcd (%): C, 53.97; H, 8.01; N, 4.84. Found (%): C, 53.99; H, 7.99; N, 4.82.

*4.5.16. 3-N-Pivaloyl-3-deoxy-1,2-O-isopropylidene-α-D-allofuranose (**4Aj**)*

From **8** (778 mg, 3.00 mmol) by acylation with pivaloyl chloride and subsequent hydrolysis; white solid, 510 mg (56%); R_f 0.36 (EtOAc–MeOH 95:5); mp (chrom) 107–108 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +8.5; ^{1}H NMR (200 MHz, CD_3CN): see Table 3 and δ 3.58 (br s, 1H, OH-5), 2.76 (br t, 1H, OH-6), 1.51, 1.31 (2s, each 3H, C*Me*$_2$), 1.16 (s, 9H, *CMe*$_3$); ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ 172.6 (*CO*), 113.0, (*C*Me$_2$), 39.4 (*C*Me$_3$), 27.5 (*CMe*$_3$),

26.8, 26.7 (CMe_2). Anal. for $C_{14}H_{25}NO_6$. Calcd (%): C, 55.43; H, 8.31; N, 4.62. Found (%): C, 55.47; H, 8.32; N, 4.64.

*4.5.17. 3-N-(Biphenyl-4-carbonyl)-3-deoxy-1,2-O-isopropylidene-α-D-allofuranose (**4Bh**)*

From **8** (778 mg, 3.00 mmol) by acylation with biphenyl-4-carbonyl chloride and subsequent hydrolysis; white solid, 685 mg (57%); R_f 0.29 (EtOAc); mp (chrom) 176–178 °C; optical rotation (*c* 1, $CHCl_3$): $[\alpha]_D$ +107.0; ^{1}H NMR (250 MHz, CD_3CN): see Table 3 and δ 7.94 (m, 2H, Ar-H), 7.71 (m, 4H, Ar-H), 7.45 (m, 3H, Ar-H), 3.65 (d, 1H, $J_{5,OH} = 4.7$ Hz, OH-5), 2.88 (dd, 1H, $J_{6a,OH} = 5.7$ Hz, $J_{6b,OH} = 6.3$ Hz, OH-6), 1.55, 1.33 (2s, each 3H, CMe_2); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 168.8 (*CO*), 145.1, 140.7, 133.7 (3 × Ar-C), 130.0–128.0 (Ar-CH), 113.1 (CMe_2), 27.0, 26.7 (CMe_2). Anal. for $C_{22}H_{25}NO_6$. Calcd (%): C, 66.15; H, 6.31; N, 3.51. Found (%): C, 66.17; H, 6.32; N, 3.50.

*4.5.18. 3-N-(4-Methoxybenzoyl)-3-deoxy-1,2-O-isopropylidene-α-D-allofuranose (**4Bi**)*

From **8** (778 mg, 3.00 mmol) by acylation with 4-methoxybenzoyl chloride and subsequent hydrolysis; white solid, 583 mg (55%); R_f 0.35 (EtOAc–MeOH 9:1); mp (chrom) 180–182 °C; optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +26.7; ^{1}H NMR (250 MHz, CD_3CN–D_2O): see Table 3 and δ 7.70 (AA′XX′, 2H, H-2′, H-6′), 6.97 (AA′XX′, 2H, H3′, H-5′), 3.81 (s, 3H, OMe), 1.51, 1.28 (2s, each 3H, CMe_2); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 168.7 (*CO*), 163.5 (C-4′), 126.8 (C-1′), 130.3 (C-2′, C-6′), 114.8 (C-3′, C-5′), 113.3 (CMe_2), 56.2 (OMe), 26.9, 26.6 (CMe_2). Anal. for $C_{17}H_{23}NO_7$. Calcd (%): C, 57.78; H, 6.56; N, 3.96. Found (%): C, 67.72; H, 6.59; N, 3.95.

*4.5.19. Methyl-6-N-butanoyl-6-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (**5Ac**)*

From **15** (700 mg, 3.00 mmol) by acylation with butanoyl chloride; colourless syrup, 737 mg (81%); R_f 0.30 (EtOAc–MeOH 95:5); optical rotation (*c* 1.0, $CHCl_3$): $[\alpha]_D$ +111.0; ^{1}H NMR (250 MHz, CD_3CN–D_2O): see Table 5 and δ 3.32 (s, 3H, OMe), 2.70 (br s, 1H, OH), 2.10 (t, 2H, $J = 7.2$ Hz, CH_2CO), 2.02 (m, 2H, CH_2CH_2CO), 1.43, 1.29 (2s, each 3H, CMe_2), 0.89 (t, 3H, $J = 7.4$ Hz, *Me*); ^{13}C NMR (62.9 MHz, CD_3CN): see Table 4 and δ 173.9 (*CO*), 109.8 (CMe_2), 55.7 (OMe), 38.7 (CH_2CO), 28.3, 26.5 (CMe_2), 19.9 (CH_2CH_2CO), 14.0 (Me). Anal. for $C_{10}H_{19}NO_5$. Calcd (%): C, 51.49; H, 8.21; N, 6.00. Found (%): C, 51.50; H, 8.23; N, 5.58.

*4.5.20. Methyl-6-N-cyclopropanecarbonyl-6-deoxy-3,4-O-isopropylidene-β-D-galctopyranoside (**6Al**)*

From **16** (700 mg, 3.00 mmol) by acylation with cyclopropanecarbonyl chloride; white solid, 679 mg (75%); R_f 0.40 (EtOAc–MeOH 9:1); mp (chrom) 65–67 °C; optical rotation (*c* 1.1, $CHCl_3$): $[\alpha]_D$ +37.2; ^{1}H NMR (200 MHz, CD_3CN–D_2O): see Table 5 and δ 3.47 (s, 3H, OMe), 3.28 (br s, 1H, OH), 1.48 (m, 1, *CH*), 1.43, 1.30 (2s, each 3H, CMe_2), 0.85–0.66 (m, 4H, CH_2CH_2); ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ 174.6 (*CO*), 110.3 (CMe_2), 56.9 (OMe), 28.4, 26.6 (CMe_2), 14.6 (*CH*), 7.1 (CH_2CH_2). Anal. for $C_{14}H_{25}NO_6$. Calcd (%): C, 55.43; H, 8.31; N, 4.62. Found (%): C, 55.45; H, 8.33; N, 4.62.

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