Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 2291–2299
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

# Synthesis and structural characterization of 1-(D-glycosyloxy)phthalazines

Abdellfattah Z. Haikal,[a] El Sayed H. El Ashry,[b,*] Joseph Banoub[c,d]

[a] Department of Chemistry, Faculty of Science, University of United Arab Emirates, P.O. Box 17551, Alain, United Arab Emirates
[b] Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt
[c] Department of Fisheries and Oceans Canada, Science, Oceans and Environment Branch. St John's, P.O. Box 5667, Newfoundland, Canada A1C 5X1
[d] Biochemistry Department, Memorial University, St. John's, Newfoundland, Canada A1C 5S7

Received 16 May 2003; accepted 29 August 2003

The authors dedicate this paper to Professor Hassan El Khadem on the occasion of his 80th birthday

**Abstract**

Coupling of the trimethylsilyl derivative of (2*H*)phthalazin-1-one with 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose and 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose in the presence of stannic chloride gave the respective glycosides, 2-(per-*O*-acetyl-D-glycosyloxy)phthalazines, which upon deacetylation gave the respective unprotected analogues. Under the same conditions 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose gave 1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyloxy)phthalazine. Electrospray mass spectrometry aided the structural characterization of this series of 1-(D-glycosylyloxy)phthalazines. Low energy collisionally-induced dissociation tandem mass spectrometry of the protonated molecules confirmed the MS fragmentation routes and the structural identities of this novel series of glycosides.


*Keywords:* Synthesis; Structural characterization; Electrospray tandem mass spectrometry; 1-(D-Glycosylyloxy)phthalazines

## 1. Introduction

Glycosyl heterocycles and their nucleoside analogues have multiple potential applications. Significant progress with such analogues has led to advances in cancer chemotherapy and anti-HBV and HIV applications. The lack of an effective therapy to treat hepatitis B virus and HIV infections, particularly in chronic cases, has focused considerable effort into the synthesis of nucleoside analogues possessing antiviral activity.[1–3] Some analogues having either modified bases and/or glycosyl residues have shown promise in antiparasite chemotherapy,[4,5] for cytokinin activities,[6–8] as antihypertensive agents,[9] as biochemical tools[10,11] and as inhibitors of cellular enzymes.[12,13] The fluorescent base, wyosine, was found in the anticodon loop of some species of RNA.[14]

An area of intensive research is in the design of nucleoside analogues wherein the aglycone moieties are altered while biological activity is retained. This type of novel design of nucleoside analogue pertains to modified nucleobases which are of neither the purine nor pyrimidine types.

We have reported the synthesis and biological activity of nucleoside analogues incorporating modified nucleobases.[15–19] In this regard, the (2*H*)phthalazin-1-one ring has attracted our attention in regard to synthesis of glycosyl derivatives wherein the sugar moiety is attached to oxygen rather than the anticipated nitrogen of the phthalazinone ring.

## 2. Results and discussion

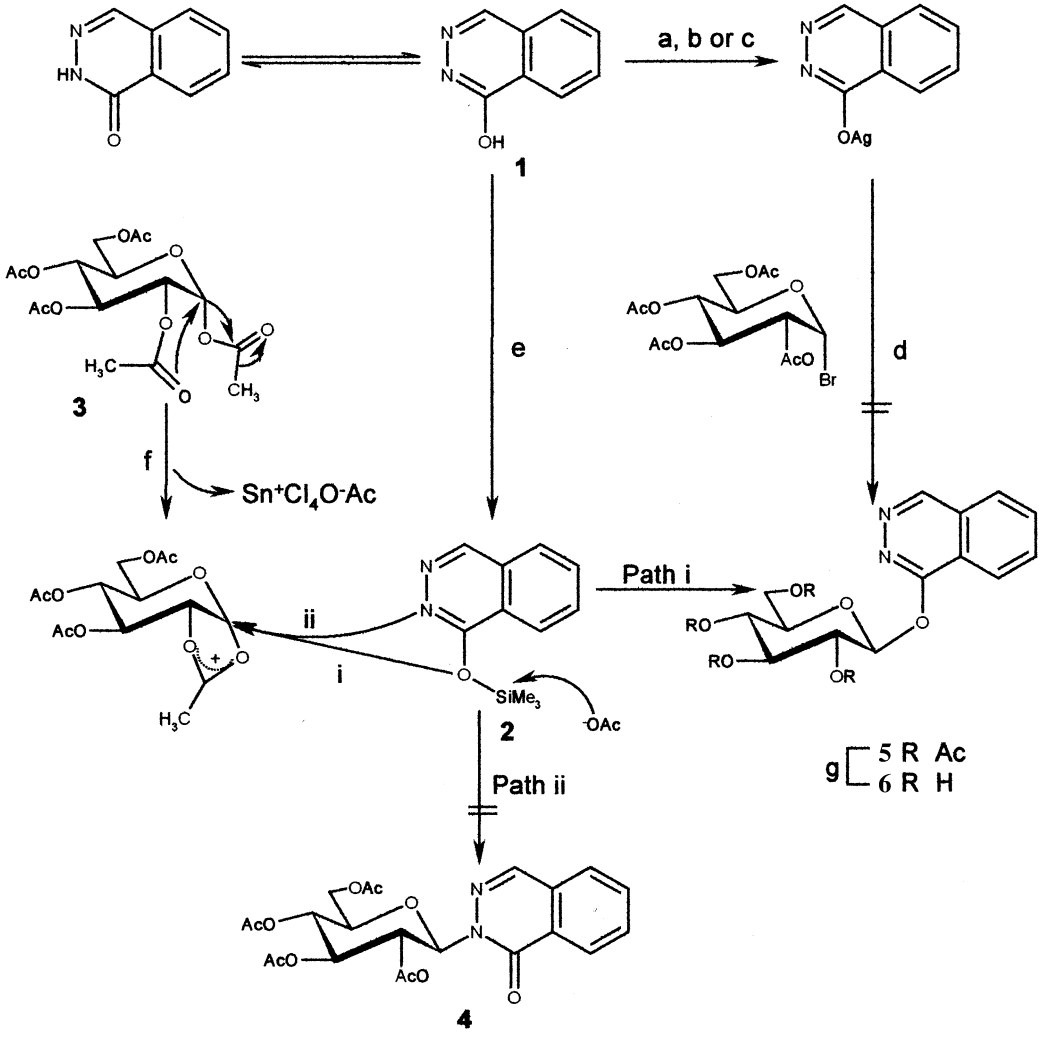
Glucosylation of the silver salt of (2*H*)phthalazin-1-one (**1**) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide did not give the corresponding 1-*O*-glucosyl derivative **5**.[20] The 4-chloro- and the 4-phenyl- derivatives of **1** gave,

* Corresponding author. Fax: +20-3-427-1360.
*E-mail addresses:* eelashry60@hotmail.com, eelashry@link.net (E.S.H. El Ashry).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.08.016

under similar conditions, the 1-glucosyloxy derivative in low and high yields, respectively.[20] The use of the sodium or potassium salts of **1** or its 4-chloro derivative in acetone–water mixture did not react with tetra-*O*-acetyl-α-D-glucopyranosyl bromide. On the other hand, the 4-phenyl derivative of **1** reacted to give a good yield of the corresponding *N*-glucosyl derivative. These results led us to explore the synthesis of nucleosides of **1** via its silyl derivative where the nature of the silylated base and the catalyst are important for the outcome of nucleoside synthesis.[21,22] (2*H*)Phthalazin-1-one (**1**) can exist as both the lactam and lactim tautomers with the lactam tautomer being the most prevalent[23] (Scheme 1). The change from the lactam to lactim form takes place during the salt formation with metal ions, where the metal ion is located on oxygen rather than nitrogen, regardless of the electropositive nature of the metal ion.

Silylation of **1** with hexamethyldisilazane (HMDS) catalyzed by ammonium sulfate would give 2-(trimethyl)silyloxyphthalazin-1-one (**2**), which can be expected to readily afford the respective nucleoside by coupling with per-*O*-acetyl sugars via activation with stannic chloride as catalyst to give the 1,2-acetyloxonim ion with the formation of $SnCl_4OAc^-$. A nucleophilic attack of the silylated phthalazine on the oxonium ion from the top side would then give exclusively the β anomer.[21,22] Thus the reaction of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**3**) with 1-(trimethyl)silyloxyphthalazine (**2**) in the presence of stannic chloride and in 1,2-dichloroethane as solvent gave a crystalline product (**5**) in good yield, deacetylation of which gave **6**. In the infrared (IR) spectrum of **6**, the carbonyl amide group band was absent, indicating that glycosylation had taken place on the oxygen and not on the nitrogen




Scheme 1.

of the aglycone of **2**, to give **5** (Scheme 1, path ii). The expected nucleoside **4** was therefore not formed in this reaction. A similar O-glycosylation was reported in the treatment of the β anomer of **3** with 2-ethoxy-3,4,5,6-tetrahydro-2*H*-azepine in refluxing dichloromethane, which did not give the expected *N*-glycosyl derivative.[24]

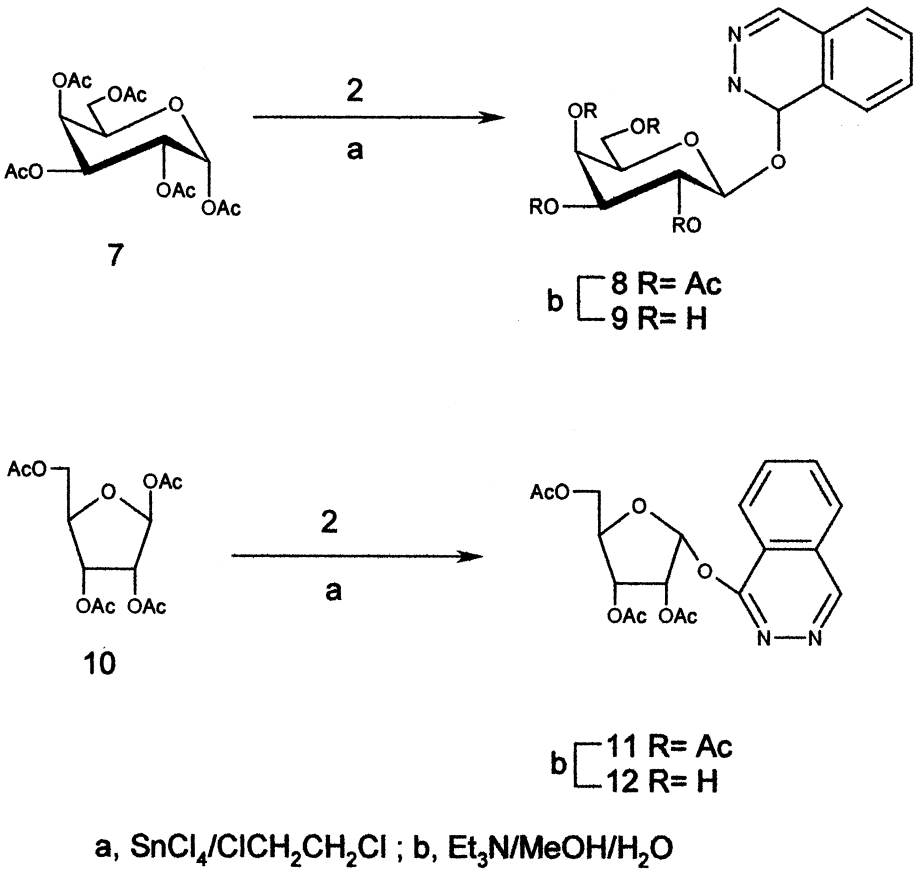
The structures were confirmed on the basis of heteromultiple bond correlation $^{1}H-^{1}H$ DQFCOSY and $^{1}H-^{13}C$ HMQC experiments, which facilitated the spectral assignments. In the $^{1}H$ NMR spectrum of **5**, there are four signals in the downfield region corresponding to the four acetoxy groups. The anomeric purity was apparent from the spectrum, in which the signal for H-1 appeared as a doublet at $\delta$ 5.58. The $J_{1,2}$ coupling constant of 8.9 Hz and its correlation with the chemical shift at $\delta_c$ 99.2 for C-1 are characteristic of a β anomer. The large $J_{2',3'}$, $J_{3',4'}$, and $J_{4',5'}$ coupling constants confirmed the axial relationship of the respective protons. The assignment of a signal in the upper field region at $\delta$ 4.00 to H-5′ indicated its linkage to the carbon-bearing oxygen of the pyranose ring. Moreover, it can be correlated in its $^{13}C$ NMR spectrum with a signal in the downfield region at $\delta_c$ 75.4 due to C-5′. The C-2′ and C-3′ signals appeared at higher field, $\delta_c$ 71.8 and 71.7, respectively, in accord with the assigned structure.

The same sequence of reactions were applied with α-D-galactopyranose pentaacetate and the β-D-ribopyranose tetraacetate analogues **7** and **10**, to give **8** and **11**, respectively whose deacetylation gave the corresponding glycosides **9** and **12** (Scheme 2). The anomeric proton of **8** appeared as a doublet at $\delta$ 5.58 with a $J_{1,2}$ coupling constant of 8.8 Hz. The relatively large coupling constant of **8** is characteristic for a β-D-galactopyranosyl anomer with having interaction of H-1′ and H-2′. The $^{4}C_1$ conformation can be readily deduced from the coupling constants. On the other hand, the small coupling constant of **11** could be due to the α configuration; the β anomer would be about 5.0 Hz. Moreover, that signal is correlated in the COSY spectrum with the C-1′ signal at $\delta_c$ 99.4 Hz, confirming the α-anomeric configuration; the β anomer should show C-1′ at $\delta_c$ 90.0 Hz. The same conclusions could also be drawn also from the deacetylation product. The assignment of a signal in the upperfield region at $\delta$ 4.59 to H-4 indicated its linkage to the carbon-bearing oxygen of the furanose ring. Its $^{13}C$ NMR spectrum showed a downfield shift of C-4 as compared with those of C-2′ and C-3′.

The electrospray mass spectra (positive ion mode) of this series of per-*O*-acetylated 1-(D-glycosyloxy)phthalazine, **5**, **8**, and **11**, and the de-O-acetylated derivatives **6**, **9**, and **12**, were recorded and are summarized in Table 1. The ESIMS showed the characteristic ions expected for such structurally simple *O*-glycosides. In all cases, we noted the presence of the protonated molecule $[M+H]^+$ and sodiated molecule $[M+Na]^+$. The formation of the sugar oxonium ion $[A]^+$ was observed at *m/z* 331 from **5** and **11**, and at *m/z* 259 in the case of **8**. These fragment ions eliminated molecules of acetic acid and ketene, as indicated in Table 1. Another fragmentation route, which leads to the formation of the fragment ion $[BOH+H]^+$, is governed by the heterocyclic cleavage of the $C_1$-*O* aglycone base with the transfer of a hydrogen atom from the glycosyl moiety.[24,25]

The CID tandem mass spectra of the precursor protonated molecule $[M+H]^+$ at *m/z* 477 for the acetylated 1-(D-glycosyloxy)phthalazines **5** and **8** and of *m/z* 309 for the deprotected corresponding analogues **6** and **9** were recorded and are shown in Fig. 1. One of the main benefits of the MS/MS technique is that all ambivalence concerning the origin of the ions produced is removed and this confirms the proposed fragmentation routes obtained from the $[M+H]^+$ precursor ions. The product-ion spectrum of the protonated molecule $[M+H]^+$ of the peracetylated *gluco* analogue **5** indicates the formation of the glycosyloxonium ion $[A_1]^+$ at *m/z* 331. The $[A_1]^+$ ion fragments further, losing, by a stepwise mechanism, molecules of acetic acid, to form the $[A_1-AcOH]^+$ and $[A_1-2AcOH]^+$ ions at *m/z* 271 and 211, respectively. The glycosyl $[A_1]^+$ ion can also, concertedly, lose two molecules of acetic acid and one molecule of ketene to afford the $[A_1-2AcOH-CH_2CO]^+$ ion at *m/z* 169 (base peak). This ion can either lose an additional molecule of acetic acid to afford the $[A_1-3AcOH-CH_2CO]^+$ ion at *m/z* 109 or a molecule of ketene to produce the $[A_1-2AcOH-2CH_2CO]^+$ ion at *m/z* 127. The formation of the nucleobase $[B-OH+H]^+$ ion at *m/z* 147 is also noted (Fig. 1A). It is noteworthy that the $[B-OH+H]^+$ ion is not the base peak ion, as would have been the case of glycosylically N-linked nucleosides. The product-ion mass spectrum of the protonated molecule $[M+H]^+$ for the peracetylated *galacto* analogue **8** is shown in Fig. 1B. This MS/MS is similar to that obtained for the *gluco* analogue **5**. However, we noted that the intensities of the fragment ions produced were quite different, thus indicating distinct fragmentation patterns for each diastereomeric structure. These MS/MS differences in fragmentation, however, are not noted in the product-ion mass spectra obtained for the protonated molecule $[M+H]^+$ at *m/z* 309 for the deacetylated *gluco* and *galacto* analogues **6** and **9**, respectively (Fig. 1C and 1D). In these two MS/MS experiments we note that only the base peak $[B-OH+H]^+$ product ion at *m/z* 147 is formed. Once more, in contrast to the MS/MS behavior of N-linked nucleosides, we observe the complete absence of the $[A_1]^+$ glucosyl oxonium ion at *m/z* 163.[25–27]

The CID tandem mass spectra of the precursor protonated molecules $[M+H]^+$ at *m/z* 407 for the acetylated 1-(D-ribosyloxy)phthalazine **11** and of *m/z*




Scheme 2.

279 were recorded and are shown in Fig. 2. The product ion mass spectrum of the precursor ion at *m/z* 407 (Fig. 2A) indicates the formation of the glycosyloxonium ion $[A_1]^+$ at *m/z* 259. This ion can lose, by a stepwise elimination, either one molecule of acetic acid to form the $[A_1-AcOH]^+$ ion at *m/z* 199, or one molecule of acetic acid and one of ketene to produce the $[A_1-AcOH-CH_2CO]^+$ ion at *m/z* 156. The glycosyl ion $[A_1]^+$ can also lose two molecules of acetic acid to afford the $[A_1-2AcOH]^+$ ion at *m/z* 139. This ion can lose a molecule of ketene to afford the $[A_1-2AcOH-CH_2CO]^+$ ion at *m/z* 97. It was, once more, noted that the $[B-OH+H]^+$ ion at *m/z* 147 is not the base peak, as would be the case for the N-linked analogues (nucleosides). The CID MS/MS of the $[M+H]^+$ ion at *m/z* 279 (Fig. 2B) indicates the presence of the

Table 1
Electrospray mass spectra (positive-ion mode) recorded with a cone voltage of 20 V for the 1-(D-glycosylyloxy)phthalazine compounds **5**, **8**, and **9**

| Characteristic ion | **5** *m/z* (%) | **6** *m/z* (%) | **8** *m/z* (%) | **9** *m/z* (%) | **11** *m/z* (%) | **12** *m/z* (%) |
|---|---|---|---|---|---|---|
| $[M+Na]^+$ | 499.23 (10.6) | 331.28 (20.1) | 499.21 (12.5) | 331.20 (10.1) | 427.17 (15.1) | |
| $[M+H]^+$ | 477.12 (100.0) | 309.04 (62.4) | 477.01 (100.0) | 309.02 (65.6) | 405.15 (100.0) | 279.09 (100.0) |
| $[A]^+$ | 331.01 (25.6) | | 331.01 (27.8) | | 259.01 (86.4) | |
| $[A-AcOH]^+$ | 271.06 (10.1) | | 271.01 (12.1) | | 199.05 (10.1) | |
| $[A-2AcOH]^+$ | 211.04 (9.4) | | 211.00 (10.1) | | 139.00 (90.5) | |
| $[A-2AcOH-CH_2CO]^+$ | 168.00 (40.5) | | 168.01 (48.6) | | 97.00 (16.2) | |
| $[BOH+H]^+$ | 147.06 (14.1) | 147.03 (100.0) | 147.00 (14.1) | 147.02 (100.0) | 147.06 (30.1) | 147.03 (76.8) |
| $[A-3AcOH-CH_2CO]^+$ | 108.03 (10.1) | | 108.01 (10.0) | | | |

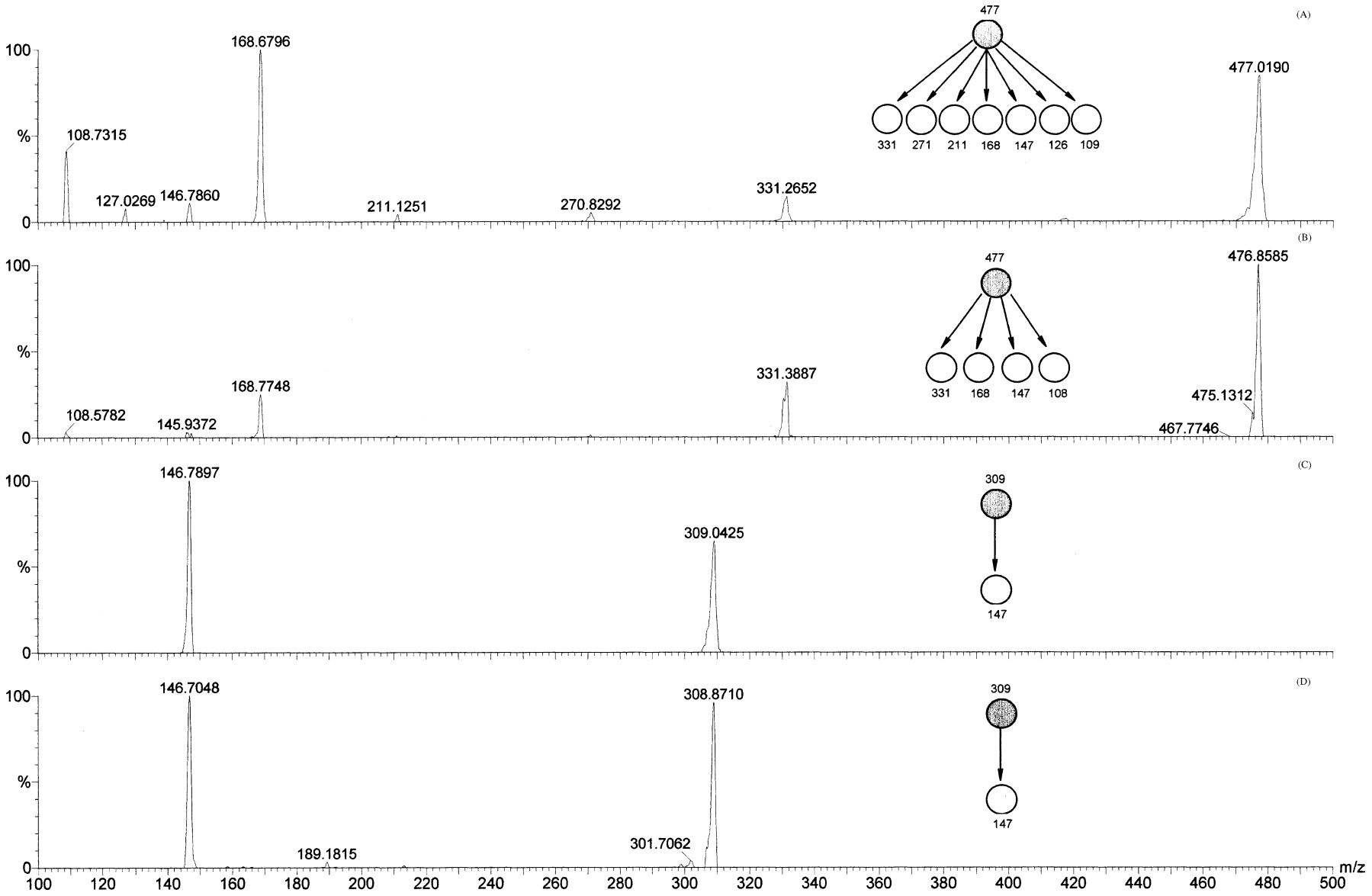



Fig. 1. The CID tandem mass spectra of the protonated molecule $[M+H]^+$ at *m/z* 477 obtained from the acetylated *gluco* and *galacto* analogues of 1-(D-glycosyloxy)phthalazines (Fig. 1A and B), and of *m/z* 309 isolated from the analogues **6** and **9** (Fig. 1C and D).

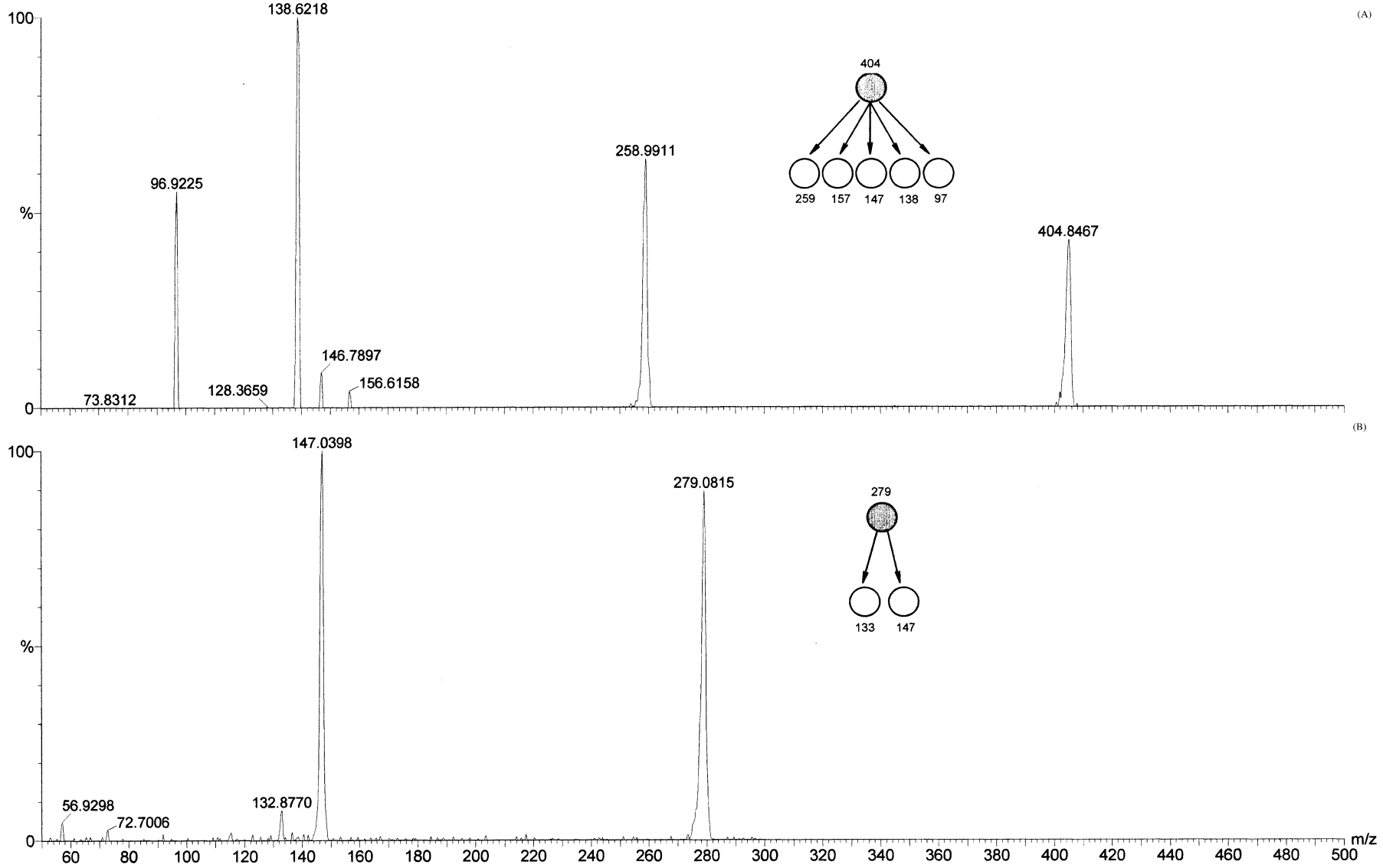



Fig. 2. The CID tandem mass spectra of the protonated molecule $[M+H]^+$ at *m/z* 407 obtained from the acetylated 1-(D-ribosyloxy)phthalazine **11** and of *m/z* 279 isolated from the de-O-acetylated analogue **12**.

diagnostic ion $[B-OH+H]^+$ ion at *m/z* 147 and a minute amount of the ribosyl oxonium ion $[A_1]^+$ at *m/z* 133.

In conclusion, the glycosidation of (2*H*)phthalazin-1-one can take place readily with peracetylated glycosyl derivatives via their trimethylsilyl derivatives and in the presence of stannic chloride as catalyst to give the respective β-glucoside and β-galactoside, whereas the riboside was found to have the α-anomeric configuration. Electrospray tandem mass spectrometry of the precursor protonated molecules of a novel series of *O*-glycosides confirmed their proposed structures and the fragmentation routes obtained in conventional electrospray mass spectra.

## 3. Experimental

### 3.1. Synthesis

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded with a Unicam SP 1025 spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded with a Bruker Avance DRX 600 MHz spectrometer. The chemical shifts are expressed on the $\delta$-scale using $Me_4Si$ as the standard, and coupling-constant values are given in Hz. The assignments of $^1$H NMR spectra were based on chemical-shift correlation (DQFCOSY) spectra. Assignments of the $^{13}$C NMR spectra were based on carbon–proton shift-correlation spectra HMQC and HMBC. TLC was performed on Merck Silica Gel 60F254 with detection by charring by $H_2SO_4$ and by UV light; using solvent A: 1:19 MeOH–$CH_2Cl_2$ and solvent B; 1:5:14 $H_2O$–MeOH–$CH_2Cl_2$ as eluents. High-resolution MS analysis were performed at the Northwest Atlantic Fisheries Centre, St. John's, NL, Canada.

**3.1.1. 1-(Trimethyl)silyloxyphthalazine (2).** A solution of phthalazinone (2.92 g, 0.02 mol) in hexamethyldisilazane (50 mL) and in the presence of catalytic amount of ammonium sulfate was heated under reflux for 5 h with exclusion of humidity. The excess HMDS was evaporated under diminished pressure, followed by coevaporation with anhydrous xylene (2 × 20 mL).The residue was used directly for the next step.

### 3.2. Synthesis of 1-(per-*O*-acetyl-D-glycosyloxy)phthalazines

**3.2.1. General procedure.** To a solution of the silylated phthalazinone (0.01 mol) in dry 1,2-dichloroethane (30 mL) was added β-D-ribopyranose tetracetate, α-D-galactopyranose, or α-D-glucopyranose pentaacetate (0.011 mol) and $SnCl_4$ (0.012 mol). The stirred mixture was heated under reflux for 2 h, cooled, neutralized with satd aq $NaHCO_3$, and extracted with $CHCl_3$. The extract was washed with water (3 × 30 mL) dried ($Na_2SO_4$) and evaporated to dryness. The residue was crystallized from water.

**3.2.2. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)phthalazine (5).** Yield: 85%; $R_f$ 0.3 (solvent A); mp 105–107 °C; $^1$H NMR ($CDCl_3$): 2.00, 2.06, 2.09, 2.10 (4 s, 12 H, 4 $CH_3$), 4.00 (m, 1 H, H-5′), 4.22 (dd, 1 H, $J_{5',6'}$ 1.6, $J_{6',6'}$ 12.7 Hz, H-6′′), 4.41 (dd, 1 H, $J_{5',6'}$ 5.3, $J_{6',6''}$ 12.7 Hz, H-6′), 5.25 (t, 1 H, $J_{3',4'}$ 9.6, $J_{4',5'}$ 9.9 Hz, H-4′), 5.31 (t, 1 H, $J_{2',3'}$ 9.3 Hz, H-2′), 5.48 (t, 1 H, $J_{3'4'}$ 9.5 Hz, H-3′), 5.58 (d, 1 H, $J_{1',2'}$ 8.9 Hz, H-1′), 7.81 (t, 1 H, *J* 7.2 Hz, H-7), 7.85 (d, 1 H, $J_{7,8}$ 7.8 Hz, H-8), 7.91 (t, 1 H, H-6), 8.40 (d, 1 H, $J_{5,6}$ 8.0, H-5), 8.69 (s, 1 H, H-3). $^{13}$C NMR: $\delta$ 20.2, 20.4, 20.5, 20.7 (4 Ac), 61.5 (C-6′), 67.7 (C-4′), 71.7 (C-3′), 71.8 (C-2′), 75.4 (C-5′), 94.2 (C-1′), 127.2, 127.8, 128.5, 128.7, 130.9, 132.4, 135.2, (Ar-C), 168.3, 169.4, 169.6, 169.8 (4CO), 170.4 (N CO). High-resolution ESI-TOF-MS (positive-ion mode) calcd for $C_{22}H_{24}N_2O_{10}$, $[M+H]^+$ at *m/z* 477.1509, found *m/z* 477.0926.

**3.2.3. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)phthalazine (8).** Yield: 85%; $R_f$ 0.3 (solvent A); mp 133–135 °C; $^1$H NMR ($CDCl_3$): $\delta$ 2.04, 2.06. 2.07, 2.26 (3 s, 12 H, 4 Ac), 4.29 (dd, 1 H, $J_{5',6'}$ 7.0, $J_{6',6''}$ 10.5 Hz, H-6′), 4.3 (dd, 1 H, $J_{5',6''}$, 6.8, $J_{6',6''}$ 10.5 Hz, H-6′′), 4.39 (m, 1 H, H-5′), 5.35 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.3 Hz, H-3′), 5.43 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{1',2'}$ 8.8 Hz, H-2), 5.57 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1′), 5.62 (d, 1 H, $J_{4',5'}$ 2.5 Hz, H-4′), 7.84 (t, 1 H, $J_{6,7}$ 7.2 Hz, H-7), 7.89 (d, 1 H, $J_{7,8}$ 7.8 Hz,H-8), 7.93 (t, 1 H, H-6), 8.51 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 8.71 (s, 1 H, H-4). $^{13}$C NMR: $\delta$ 20.3, 20.4, 20.57, 20.58 (4 Ac), 61.2 (C-6′), 66.8 (C-4′), 69.3 (C-2′), 69.9 (C-3′), 74.4 (C-5′), 94.7 (C-1′), 127.2, 127.8, 128.5, 128.7, 130.8, 132.3, 135.1 (Ar-C), 168.3, 169.6, 169.7, 170.2, (OCO), 170.3 (NCO). High-resolution ESI-TOF-MS (positive-ion mode) calcd for $C_{22}H_{24}N_2O_{10}$, $[M+H]^+$ at *m/z* 477.1509, found *m/z* 477.2226.

**3.2.4. 1-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyloxy)phthalazine (11).** Yield: 55%; $R_f$ 0.4 (solvent A); mp 87–89 °C; $^1$H NMR ($CDCI_3$): $\delta$ 2.05, 2.07, 2.14 (3 s, 9 H, 3 $CH_3$), 4.49, 4.50 (2 d,2 H, $J_{4',5''}$ 4.7, $J_{4',5'}$ 2.7, $J_{5',5''}$ 8.5 Hz, H-5′,5′′), 4.59 (m, 1 H, H-4′), 5.46 (dd, 1 H, $J_{2',3'}$ 5.5, $J_{3',4'}$ 7.2 Hz, H-3′), 5.84 (dd, 1 H, $J_{1',2'}$ 1.3, $J_{2',3'}$ 5.0 Hz, H-2′), 5.91 (d, 1 H, H-1′), 7.79 (m, 2 H, H-7,8), 7.91 (t, 1 H, $J_{6,7}$ 8.1, $J_{5,6}$ 8.0 Hz, H-6), 8.49 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 8.79 (s, 1 H, H-4); $^{13}$C NMR: $\delta_c$ 20.3, 20.4, 20.7 (3 Ac), 62.3 (C-5′), 69.4 (C-3′), 75.3 (C-2′), 81.7 (C-4′), 99.4 (C-1′), 127.3, 127.5, 128.4, 130.1, 132.4, 135.1 (Ar-C). 168.6 (NCO), 169.1, 169.2, 170.2 (3CO). High-resolution ESI-TOF-MS (positive-ion mode) calcd for $C_{19}H_{20}N_2O_8$, $[M+H]^+$ at *m/z* 405.1298, found *m/z* 405.0923.

### 3.3. 1-(Glycopyranosyloxy)phthalazines

**3.3.1. General procedure.** Triethylamine (1 mL) was added to a 0.5 M solution of the protected phthalazinone glycosides **5**, **8**, and **11** in 10:1 MeOH–$H_2O$. The mixture was stirred overnight at room temperature. It was evaporated under reduced pressure and coevaporated with MeOH until removal of excess $Et_3N$ was complete. The residue was crystallized from MeOH–$H_2O$ to give the deacetylated product in $>90\%$ yield.

**3.3.2. 1-(1-β-D-galactopyranosyloxy)phthalazine (9).** $R_f$ 0.24 (solvent B); mp 155–156 °C; $^1$H NMR ($Me_2SO$-$d_6$+$D_2O$): $\delta$ 3.56 (m, 3 H, H-3′,6′,6′), 3.76 (m, 2 H, H-4′,5′), 4.12 (t, 1 H, $J_{2',3'}$ 9.2 Hz, H-2′), 5.21 (d, 1 H, $J_{1',2'}$ 8.8 Hz, H-1′), 7.90 (t, 1 H, $J_{6,7}$ 7.3 Hz, H-7), 7.99 (d, 1 H, $J_{7,8}$ 7.3 Hz, H-8), 8.07 (t, H-6), 8.22 (d, 1 H, $J_{5,6}$ 7.9 Hz, H-5), 9.21 (s, 1 H, H-4). $^{13}$C NMR: $\delta$ 60.6 (C-6′), 68.4 (C-4′), 69.2 (C-2′), 73.2 (C-3′), 79.0 (C-5′), 99.5 (C-1′), 126.1, 128.0, 129.0, 129.2, 133.1, 135.5, 135.7 (Ar-C), 168.5 (NCO). High-resolution ESI-TOF-MS (positive-ion mode) calcd for $C_{14}H_{16}N_2O_6$, $[M+H]^+$ at $m/z$ 309.1086, found $m/z$ 309.0925.

**3.3.3. 1-(α-D-Ribofuranosyloxy)phthalazine (12).** $R_f$ 0.4 (solvent B); mp 163–164 °C; $^1$H NMR ($Me_2SO$-$d_6$+$D_2O$): $\delta$ 3.66 (dd, 1 H, $J_{4',5'}$ 3.6, $J_{5',5''}$ 12.5 Hz, H-5′′), 3.84 (dd, 1 H, $J_{4',5'}$ 2.4, $J_{5',5''}$ 12.5 Hz, H-5′), 4.10 (dd, 1 H, $J_{3',4'}$ 2.4, $J_{2',3'}$ 4.4 Hz, H-3′), 4.12 (m, 1 H, H-4′), 4.40 (dd, $J_{1',2'}$ 1.1, $J_{2',3'}$ 4.4 Hz, H-2′), 5.66 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1′), 7.88 (t, 1 H, $J_{6,7}$ 7.7 Hz, H- 7), 7.96 (d, 1 H, $J$ 7.4 Hz, H-8), 8.00 (t, 1 H, $J$ 8.3 Hz, H-6), 8.20 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 9.36 (s, 1 H, H-4). $^{13}$C NMR: $\delta$ 60.6 (C-5′), 69.1 (C-3′), 76.1 (C-2′), 86.6 (C-4′), 102.0 (C-1′), 126.4, 127.9, 129.1, 129.5, 133.5, 136.0 (Ar-C), 168.8 (N-CO). High-resolution ESI-TOF-MS (positive-ion mode) calcd for $C_{13}H_{14}N_2O_5$, $[M+H]^+$ at $m/z$ 279.0981, found $m/z$ 279.1346.

### 3.4. Mass spectrometry

The ESI mass spectra (positive-ion mode) were recorded with a Micromass Quattro quadrupole–hexapole–quadrupole mass spectrometer equipped with a mega-flow ESI source and capable of analyzing ions up to $m/z$ 4000. A personal computer (Compaq, PIII 300 MHz processor, running Windows NT 4, service pack 3) equipped with Micromass MASSLYNX 3.3 Mass Spectrometry Data System software was used for data acquisition and processing. The temperature of the ESI source was maintained at 75 °C. The operating voltage of the ESI capillary was 3.00 kV and the high-voltage lens was set at 0.40 kV throughout the whole operation. ESIMS were recorded with a cone voltage of 25 V. Conventional ESI mass spectra were obtained by scanning in the Multi Channel Analysis mode (MCA) with a scan time of 1 s per 250 mass numbers. Spectra are an average of 20–30 scans. The mass scale was calibrated in the positive ion mode using a polyethylene glycol mixture. MaxEnt automatically disentangles the $m/z$ spectrum produced by the mass spectrometer and presents the data for each individual 1-(D-glycosylyloxy)phthalazine in a single peak on a true molecular weight scale. Product ion MS/MS experiments were conducted using the same instrument. Product ion spectra of mass-selected precursor molecular ion species were induced by collision with argon in the (r.f.-only) hexapole. The resulting fragment ions were analyzed by the second quadrupole. A cone voltage varying from 20 to 35 V, collision energies varying from 10 to 40 eV, and a collision gas pressure in the collision cell varying from $3.5\times10^{-4}$ to $6.5\times10^{-4}$ mbar (1 bar $=10^5$ Pa) were used in all MS/MS experiments. The collision gas pressure was increased to induce the dissociation of the sodiated adduct anions (typical settings were around $6.0\times10^{-4}$ mbar). Precursor ion MS/MS scans were obtained by scanning the first quadrupole while selecting a given $m/z$ value with the second quadrupole.

High-resolution ESI mass spectra were recorded with a Applied Biosystems QSTAR XL quadrupole, time-of-flight hybrid LC-MS/MS-TOF mass spectrometer, equipped with an ion-spray source capable of analyzing a mass range of $m/z$ 5 to 40,000, with a TOF resolution of 10,000 in positive-ion mode. The TOF analyzer was of the reflectron type with an effective path of 2.5 m.

### Acknowledgements

We would like to express our thanks to Dr. R. R. Schmidt for conducting some of the spectral measurements at Konstanz University. The partial support from AvH foundation and DFG are highly appreciated. Joseph Banoub acknowledges the Natural Science and Engineering Research Council of Canada for financial support in the form of a Discovery Grant.

### References

1. Perigaud, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides Nucleotides* **1992**, *11*, 903–945.
2. Chu, C. K.; Cutler, S. J. *J. Heterocycl. Chem.* **1986**, *23*, 289.
3. Remy, R. J.; Secrist, J. A., III *Nucleosides Nucleotides* **1985**, *4*, 411–439.
4. (a) MacCoss, M.; Tolman, R. L.; Ashton, W. T.; Wagner, A. F.; Hannah, J.; Field, A. K.; Karkas, J. D.; Germershausen, J. I. *Chem. Scr.* **1986**, *26*, 113–121;
(b) Yokoyama, M.; Watanabe, S. *Yuki Gosei Kagaku Kyokaishi* **1989**, *47*, 694–706 (*Chem. Abstr.* **1990**,11, 98984C);

(c) Balzarini, J.; De Clereq, E. *Pharmacochem. Libr.* **1990**, *14*, 175–194;
(d) Chu, C. K.; Baker, D. C. *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Plenum Press: New York, 1993;
(e) De Clereq, E. *Int. J. Immunopharmacol.* **1991**, *13*, 91–98.
5. Kim, H. O.; Shanmuganathan, K.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Shinazi, R. F.; Chang, C. N.; Cheng, Y.-C.; Chu, C. K. *Tetrahedron Lett.* **1992**, *33*, 6899–6902.
6. Wang, C. C. *Trends Biochem. Sci.* **1982**, *7*, 354–356.
7. Mittelman, A.; Evans, J. T.; Chheda, G. B. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 234.
8. Hashizuma, T.; Yoshida, K. *Agr. Biol. Chem.* **1976**, *40*, 2001–2004.
9. Shibata, H.; Ohnishi, N.; Takeda, K.; Fukunaga, H.; Shimamura, K.; Yasunobu, E.; Tani, I. *Can. J. Microbiol.* **1986**, *32*, 186–189.
10. Trivedi, B. K.; Blankley, C. J.; Bristol, J. A.; Hamilton, H. W.; Patt, W. C.; Kramer, W. J.; Johnson, S. A.; Bruns, R. F.; Cohen, D. M.; Ryan, M. J. J. *Med. Chem.* **1991**, *34*, 1043–1049.
11. Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463–5467.
12. 11.Prober, J. M.; Trainor, G. L.; Dam, R. J.; Hobbs, F. W.; Robertson, C. W.; Zagursky, R. J.; Cocszza, A. J.; Jensen, M. A.; Baumeister, K. *Science* **1987**, *238*, 336–341.
13. Meggio, F.; Shugar, D.; Pinna, L. A. *Eur. J. Biochem.* **1990**, *187*, 89–94.
14. Stevens, A.; Maupin, M. K. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 508–515.
15. Bluckburn, G. M.; Gail, M. J. *Nucleic Acids in Chemistry and Biology*; 2nd ed; Oxford University Press, 1997; p 94.
16. El Ashry, E. S. H.; Abdel-Rahman, A. A.-H.; Rashed, N.; Rasheed, H. A. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 327–333.
17. El Ashry, E. S. H.; Abdel-Rahman, A. A.; Rashed, N.; Rasheed, H. *Pharmazie* **1999**, *54*, 893–897.
18. El Ashry, E. S. H.; El Kilany, Y. *Adv. Heterocycl. Chem.* **1997**, *67*, 391–432.
19. El Ashry, E. S. H.; El Kilany, Y. *Adv. Heterocycl. Chem.* **1997**, *68*, 1–88.
20. El Ashry, E. S. H.; El Kilany, Y. *Adv. Heterocycl. Chem.* **1998**, *69*, 129–215.
21. Wagner, G.; Heller, D. *Arch. Pharm.* **1966**, *299*, 768–777.
22. Niedballa, U.; Vorbruggen, H. J. *Org. Chem.* **1974**, *39*, 3654–3660, 3664–3668.
23. Watanabe, K. A.; Hollenberg, D. H.; Fox, J. J. *J. Carbohydr. Nucl. Nucleot.* **1974**, *1*, 1–14.
24. Patel, N. R. In *The Heterocyclic Compounds*; Castles, R. N., Ed. Condensed Pyridazines Including Cinnolines and Phthalazines, 1927; pp 323–760.
25. Hanessian, S.; Banoub, J. *Adv. Chem. Ser.* **1976**, *39*, 36–63.
26. Banoub, J.; Becchi, M.; Descotes, G.; Fraisse, D.; Humble, R.; Mackenzie, G. *J. Carbohydr. Chem.* **1992**, *11* (6), 471–484.
27. Len, C.; MacKenzie, G.; Ewing, D. F.; Sheppard, G.; Banoub, J. *J. Carbohydr. Res.*, this issue; see following communication, doi: S0008-6215(03)00452-X.