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Structural characterization of a series of 10-carbon sugar derivatives by electrospray-ionization MS^n mass spectrometry

Da-Peng Zou, Shu-Xia Cao, Wei-Chao Xu and Hong-Min Liu*

Department of Chemistry, Zhengzhou University, Zhengzhou 450052, PR China

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Abstract—Electrospray-ionization MS^n mass spectrometry (ESI-MS^n) with low-energy, collision-induced dissociation (CID) was used to establish the fragmentation behavior of sodium ion adducts of higher-carbon amino spiro-sugar derivatives. Their fragmentation pathways are proposed on the basis of the MS^n studies and deuteration experiments. Some of the rings of these derivatives opened under the conditions of electrospray ionization. Novel fragmentations were observed and their mechanisms are proposed. This study demonstrates the power of modern mass spectrometry for rapid elucidation of the structure of higher-carbon sugar derivatives.

Keywords: Higher-carbon sugars; Amino spiro-sugars; Structural characterization; Electrospray ionization; MS^n mass spectrometry

1. Introduction

Higher-carbon sugar derivatives having backbones longer than the usual five or six carbon atoms are an important class of carbohydrates. The presence of such units in natural products confers unique biological properties. Several of these 7–11 carbon carbohydrates play prominent roles in biological processes.[1,2] Thus, the higher-carbon sugars are interesting synthetic targets, and several approaches towards their synthesis have been reported.[3–7] In our previous work, an efficient and economical synthetic method for C_{10} higher-carbon sugars from D-xylose was reported.[8] Hence, we employ substituted anilines in C_{10} higher-carbon sugars to alter their stereochemistry and search structure–activity relationships, and some higher-carbon amino spiro-sugars useful as chiral synthons were therefore synthesized. Some of these have glycosidase-inhibitor activities.

For structural elucidation of these compounds, studies on their ESI-MS fragmentation characteristics are important. Electrospray ionization (ESI), a soft ionization technique, is a powerful tool for structure elucidation of nonvolatile and thermally labile compounds, especially biological materials[9–12] and carbohydrate derivatives.[13–17] Many investigations concerning metal-ion adducts of carbohydrates in ESI-MS experiments have been reported recently,[18–20] but to the best of our knowledge, the ESI-MS fragmentation characteristics of higher-carbon amino spiro-sugar derivatives have not been studied. Here, we report the ESI-MS^n spectra of a series of such compounds (**4a–h**, **5a–h**, **6a–f**, and **7a–f** in Scheme 1) to study their fragmentation behavior and establish characteristic fragments suitable for the identification of similar carbohydrates.

2. Results and discussion

Under the experimental conditions, it was found that the sodium ion adducts were the base peaks in the ESI-MS spectra of all compounds in Scheme 1. The sodium ion was probably derived from glass containers and as an impurity picked up during the preparation of the sample. Figure 1a shows the ESI mass spectrum of a methanol

* Corresponding author. Tel./fax: +86 371 7767200; e-mail: liuhm@zzu.edu.cn

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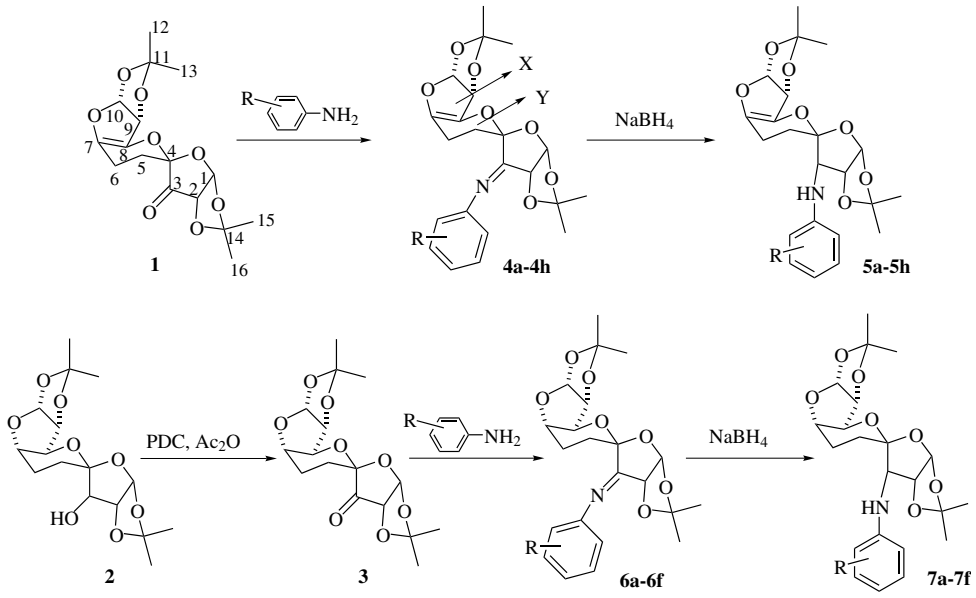


a R = *p*-CH_3; **b** R = *o*-CH_3; **c** R = *p*-F; **d** R = *p*-Cl; **e** R = *p*-Br; **f** R = *p*-OCH_3; **g** R = H; **h** R = *m*-CH_3

Scheme 1. Structures of compounds **1**–**7**.

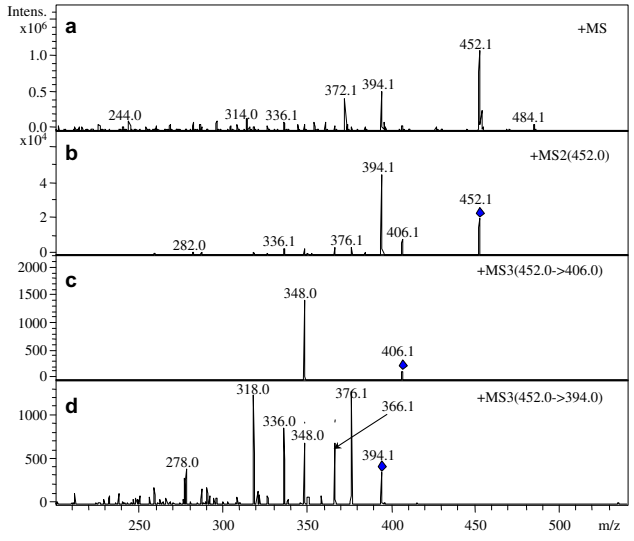
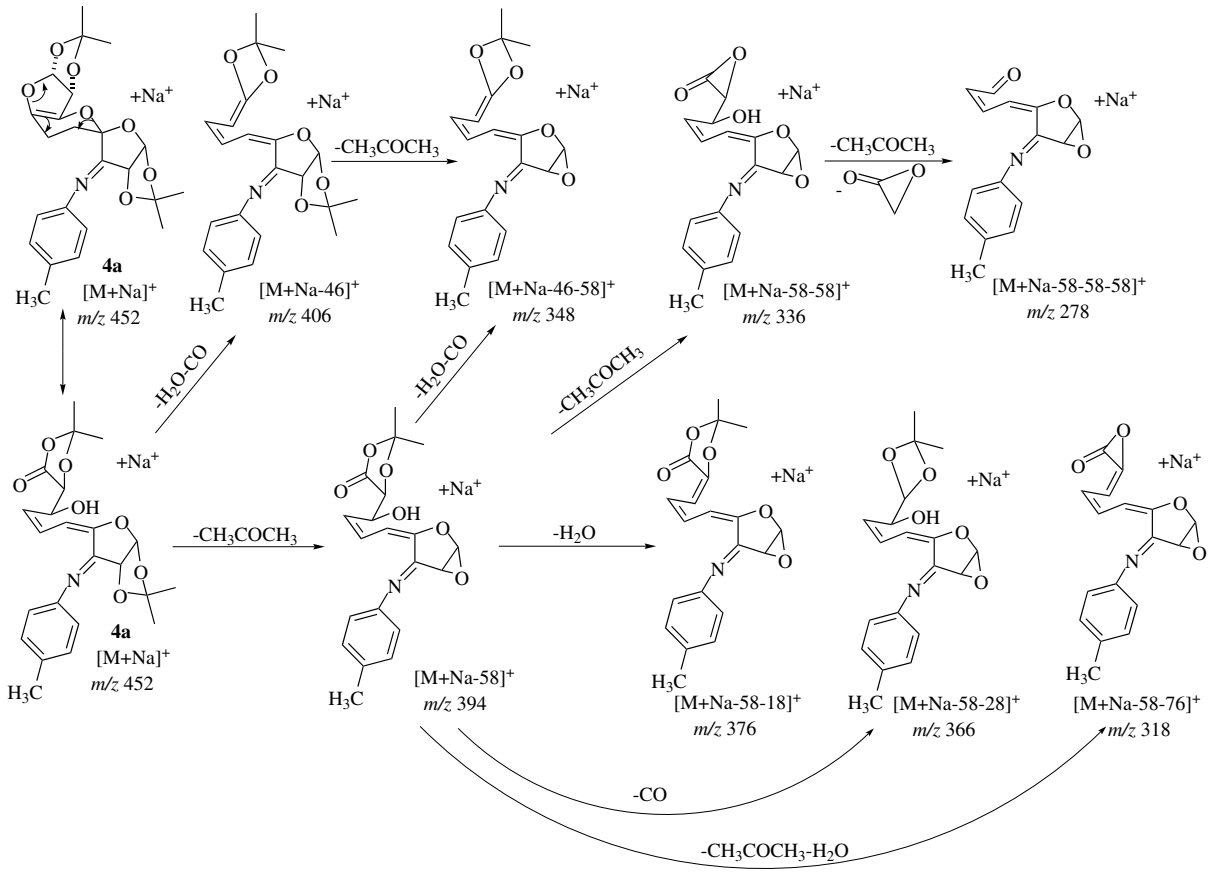


Figure 1. (a) ESI-MS spectrum of a methanol solution of compound **4a**; (b) MS^2 spectrum of the $[M+Na]^+$ (*m*/*z* 452) of compound **4a**; (c) MS^3 spectrum of the *m*/*z* 406 of compound **4a**; (d) MS^3 spectrum of the *m*/*z* 394 of compound **4a**.

solution of (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-tri-deoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (**4a**) without any other additive. Like all other compounds studied here, no other significant molecular ions beside the base peak, which was the sodium ion adduct, appeared in the spectrum except for the solvated molecular ion $[M+CH_3OH+Na]^+$. Therefore, the $[M+Na]^+$ ions were studied in detail to investigate some new general rules for the interpretation of the fragment ion spectra of higher-carbon amino spiro-sugar derivatives.

2.1. MS^n spectra analysis of compounds 4a–h

The compounds **4a**–**h** obviously followed a common dissociation pathway. Studies by MS^n mass spectrometry showed losses of 58 and 46 Da from the precursor $[M+Na]^+$ ion. For example, the mass spectrum of compound **4a** showed the base peak $[M+Na]^+$ at *m*/*z* 452. In the ESI-MS^2 spectrum of compound **4a** (Fig. 1b), the $[M+Na]^+$ ion of the most abundant fragment-ion appeared at *m*/*z* 394, obviously formed by the loss of 58 Da, the molecular mass of acetone. According to the structure of compound **4a** (Scheme 1), there were two possibilities to yield the fragment ion at *m*/*z* 394, namely by the loss of a molecule of acetone, either from the 1,2-*O*- or the 9,10-*O*-isopropylidene group of the molecule. In addition to the ion at *m*/*z* 394, a weak ion peak at *m*/*z* 406 in the ESI-MS^2 spectrum was also observed. This signal can be rationalized in terms of an $[M+Na-H_2O-CO]^+$ ion. We presumed that the presence of the double bond in the C-7–C-8 position may cause the X and Y rings (Scheme 1) to be unstable under the experimental conditions and they would cleave to an open-chain structure. Therefore, we proposed that the $[M+Na-H_2O-CO]^+$ ion was probably formed by elimination of carbon monoxide and water simultaneously after the X and Y rings were opened, as shown in Scheme 2. In addition, the presence of the C=N double bond would stabilize the $[M+Na-H_2O-CO]^+$ fragment ion. The ESI-MS^3 spectrum of the $[M+Na-H_2O-CO]^+$ ion shows the elimination of 58 Da to produce the single ion corresponding to loss of a molecule of acetone. Furthermore, the ESI-MS^3 spectrum of the $[M+Na-CH_3COCH_3]^+$ ion at *m*/*z* 394 shows the formation of an



Scheme 2. Proposed fragmentation pathway of compound **4** during electrospray MS^n mass spectrometry with CID.

$[M+Na-CH_3\text{-}COCH_3-H_2O-CO]^+$ ion at *m/z* 348 as shown in Figure 1d. Simultaneously, the loss of 18, 28, 58, or 76 Da is also observed in the ESI-MS^3 spectrum of *m/z* 394. The major fragmentation pathways for compound **4** can be summarized and depicted as shown in Scheme 2, using compound **4a** as a representative example.

2.2. MS^n spectra analysis of compounds 5a–h

Compound **5** has active hydrogen at the nitrogen atom in its structure, thus differing from compound **4**. In the MS^2 spectra of the $[M+Na]^+$ ion of compound **5**, the base peak was the peak corresponding to the ion $[M+Na-CH_3COCH_3]^+$, as with compound **4**, whereas, the fragment ion $[M+Na-H_2O-CO]^+$ was not found in the MS^2 spectra. After elimination of a molecule of acetone from the precursor ion, the ESI-MS^3 spectra of $[M+Na-CH_3COCH_3]^+$ ion showed the sequential elimination of 58 and 18 Da, corresponding to loss of a molecule of acetone and water, respectively. For example, the first stage MS of (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (**5a**) showed the base peak $[M+Na]^+$ at *m/z* 454. In the ESI-MS^2 spectrum, the most abundant fragment ion $[M+Na-CH_3COCH_3]^+$ at *m/z* 396 was derived from the $[M+Na]^+$ ion of compound **5a** by the elimination of a molecule of acetone. In the ESI-MS^3 spectra, the $[M+Na-CH_3COCH_3]^+$ ion then lost 18, 58, and 76 Da, successively. Furthermore, the ESI-MS^4 spectrum of the $[M+Na-2CH_3COCH_3]^+$ ion at *m/z* 338 showed the fragment ions at *m/z* 320 and 310.

In order to confirm the fragmentation pathways and to investigate the mechanism of formation of the $[M+Na-58-76]^+$ ion in the spectra of compound **5**, the deuterated analogues of compounds **4** and **5** under the same experimental conditions were studied in detail. As shown in Figure 2a, the MS^2 spectrum of the deuterium-exchanged $[M+Na]^+$ (*m/z* 452) ion of compound **4a** was the same as that in Figure 1b, because there was no active hydrogen in the structure of compound **4**. In the CID spectra of deuterated analogues of compound **5a** (Fig. 2b), the $[M+Na]^+$ ion produced the most abundant fragment ion at *m/z* 397 by the elimination of a molecule of acetone. Figure 2c and d shows the ESI-MS^3 and ESI-MS^4 spectra of deuterated analogues of compound **5a**, respectively. The ESI-MS^3 spectrum of the $[M+Na-CH_3COCH_3]^+$ ion at *m/z* 397 showed elimination of 58 and 19 Da fragments, corresponding to the loss of a molecule of acetone and of water (DHO). Simultaneously, the elimination of 18 Da was also

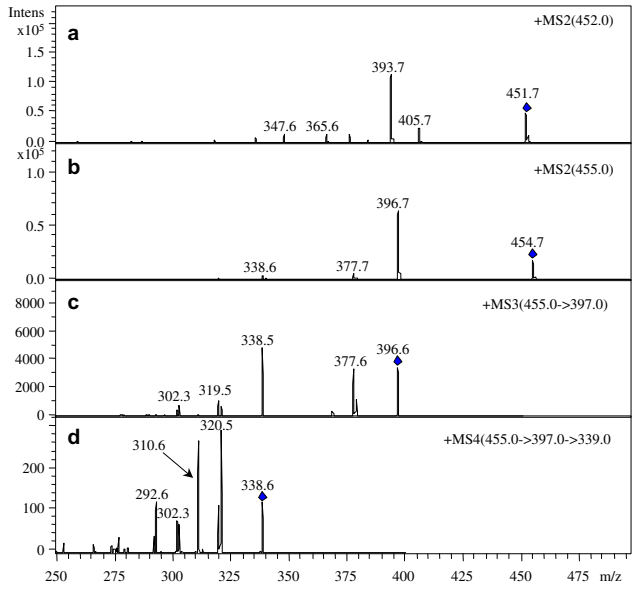
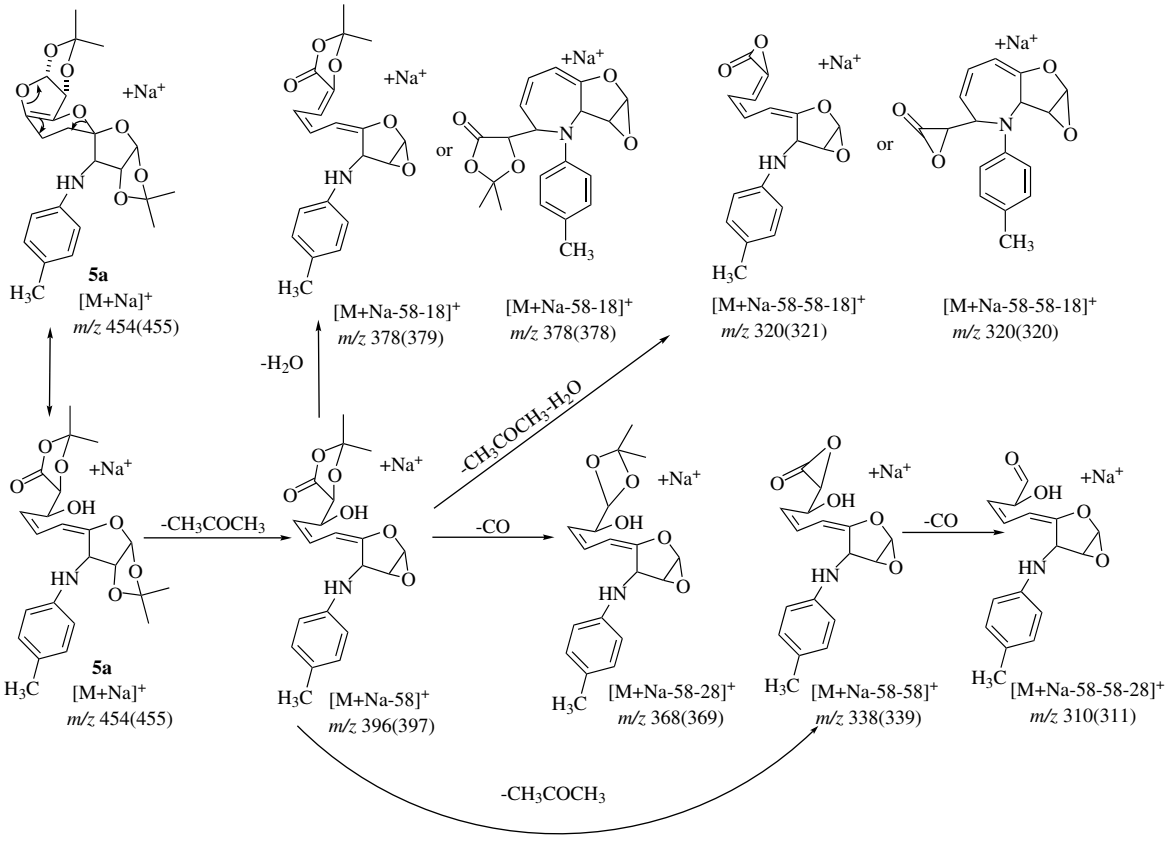


Figure 2. (a) MS^2 spectrum of the $[M+Na]^+$ (m/z 452) ion of compound **4a** after treatment with deuterated methanol; (b) MS^2 spectrum of the $[M+Na]^+$ (m/z 455) ion of compound **5a** after treatment with deuterated methanol; (c) MS^3 spectrum of m/z 397 of the deuterated analogue of **5a**; (d) MS^4 spectrum of the m/z 339 ion of the deuterated analogue of **5a**.

observed in the ESI-MS^3 spectrum of the $[M+Na-CH_3COCH_3]^+$ ion. This indicated that there might be two pathways for the dehydration process, and the exchangeable hydrogen at the nitrogen atom participated in one of the dehydration processes of compound **5a**. Therefore, as shown in Scheme 3, the deuterated $[M+Na-CH_3COCH_3]^+$ ion at m/z 397 lost a molecule of deuterium-exchanged water (DHO) and of water (H_2O) to form the $[M+Na-CH_3COCH_3-DHO]^+$ ion at m/z 378 and the $[M+Na-CH_3COCH_3-H_2O]^+$ ion at m/z 379, respectively. In Scheme 3, we propose a fragmentation mechanism for compound **5** using compound **5a** as a representative example. The values in parentheses are the observed m/z values of the corresponding peaks in the CID spectra of deuterium-exchanged molecules. This is also applicable in all the other schemes depicting fragmentation of the sodium adduct ions. The corresponding product ions observed for deuterium-exchanged compounds also match the suggested formation pathways (Schemes 2 and 3).

2.3. MS^n spectra analysis of compounds 6a–f

The structure of compound **6** differs from compound **4** by the absence of a double bond in the X ring. As expected, there was almost no $[M+Na-H_2O-CO]^+$ ion in the CID spectrum of compound **6**, because cleavage of the X and Y rings to an open-chain structure is not as favorable as that in the compound **4**. The MS^3



Scheme 3. Proposed fragmentation pathway of compound **5** during electrospray MS^n mass spectrometry with CID.

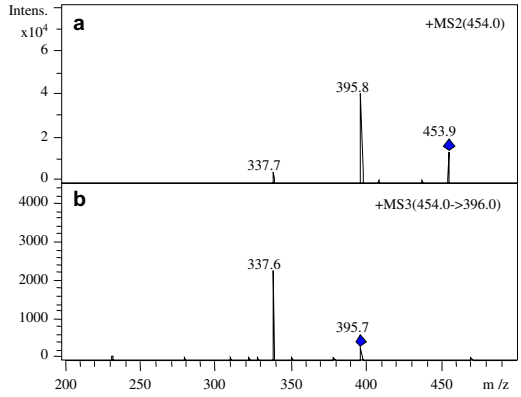


Figure 3. (a) MS^2 spectrum of the $[M+Na]^+$ ion (m/z 454) of compound **6a**; (b) MS^3 spectrum of the m/z 396 ion of compound **6a**.

spectra of $[M+Na-CH_3COCH_3]^+$ of compound **6** was considerably simpler than that of compounds **4** and **5**. This could be explained by the absence of a double bond in the X ring and the absence of active hydrogen at the nitrogen atom. The presence of a double bond in the X ring would result in an intense peak corresponding to the open-chain structure, and the active hydrogen at the nitrogen atom would result in the formation of the $[M+Na-CH_3COCH_3-H_2O]^+$ and $[M+Na-CH_3COCH_3-CO]^+$ ions. For example, the $[M+Na]^+$ ion of (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxy-dec-4-uloaldose 1,4:7,10-difuranose-4,8-pyranose (**6a**) yielded the most abundant fragment ion at m/z 396 by the loss of a molecule of acetone, as shown in Figure 3a. Furthermore, Figure 3b shows the ESI-MS^3 spectrum of m/z 396, and the single product ion is $[M+Na-2CH_3COCH_3]^+$ ion at m/z 338. The fragmentation mechanism for compound **6** is shown in Scheme 4 with compound **6a** as a representative example.

2.4. MS^n spectra analysis of compounds 7a–f

Compound **7** does not contain a double bond in the X ring, but there is an active hydrogen at the nitrogen atom, as in the structure of compound **5**. Hence, the MS^n spectra of the $[M+Na]^+$ ion of compound **7** is not so simple as the spectra of compound **6**. The main fragmentation pathway of the $[M+Na]^+$ ion is still the sequential elimination of 58 Da to form the $[M+Na-CH_3COCH_3]^+$ and $[M+Na-2CH_3COCH_3]^+$ ions. In addition to the loss of 58 Da, the losses of 76, 100, and 116 Da were also observed in the ESI-MS^2 spectrum of the $[M+Na]^+$ ion. From the ESI-MS^3 spectrum of the $[M+Na-CH_3COCH_3]^+$ ion, it can be deduced that a $[M+Na-CH_3COCH_3-H_2O]^+$ ion and a $[M+Na-2CH_3COCH_3]^+$ ion were formed by elimination of 18 and 58 Da from the $[M+Na-CH_3COCH_3]^+$ ion. Formation of the $[M+Na-100]^+$ ion may be rationalized by the presence of active hydrogen at the nitrogen atom. The deuterated analogues of compound **7** under the same experimental conditions were also investigated in detail. For example, Figure 4c showed the MS^2 spectrum of deuterium-exchanged $[M+Na]^+$ (m/z 473) of (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-di-*O*-isopropylidene-3-(*p*-methoxyanilino)-3,5,6-trideoxydecos-4-ulose

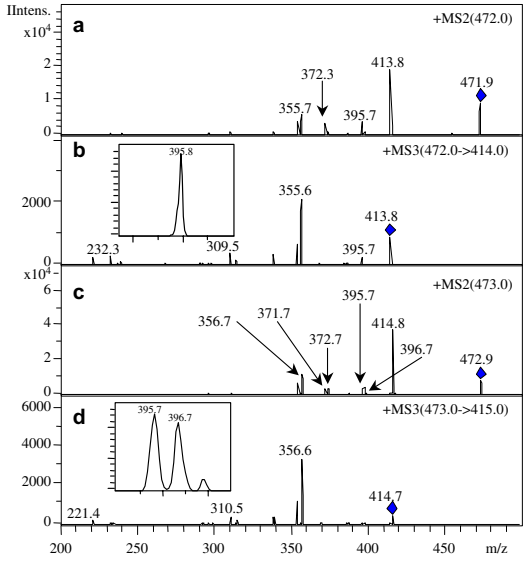
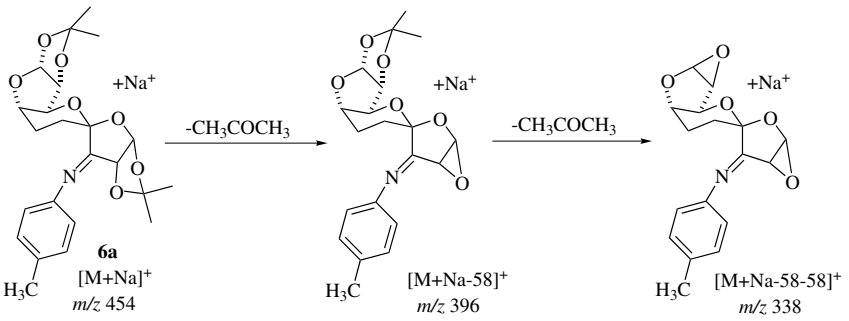


Figure 4. (a) MS^2 spectrum of the $[M+Na]^+$ (m/z 472) ion of compound **7f**; (b) MS^3 spectrum of the m/z 414 ion of compound **7f**; (c) MS^2 spectrum of the deuterium-exchanged $[M+Na]^+$ (m/z 473) ion of compound **7f**; (d) MS^3 spectrum of the m/z 415 ion of deuterium-exchanged compound **7f**.



Scheme 4. Proposed fragmentation pathway of compound **6** during electrospray MS^n mass spectrometry with CID.

1,4:7,10-difuranose-4,8-pyranose (**7f**). The most abundant fragment ion at *m/z* 415 was formed by the elimination of acetone, as in Figure 4a. Because of the presence of both the product ion $[M+Na-100]^+$ at *m/z* 373 and the $[M+Na-101]^+$ at *m/z* 372 in the MS^2 spectrum of the deuterium-exchanged $[M+Na]^+$ (*m/z* 473) ion of compound **7f**, we could presume that there are two pathways to yield the $[M+Na-100]^+$ ion by the loss of a molecule of 1,2-*O*-isopropylideneethenediol. One pathway involves the active hydrogen and the other does not, as shown in Scheme 5. After loss of acetone, the $[M+Na-CH_3COCH_3]^+$ ion at *m/z* 414 also had two pathways for elimination of a water molecule. Therefore, in the MS^3 spectrum of the *m/z* 415 ion of deuterium-exchanged compound **7f**, there were two ions at *m/z* 396 and at *m/z* 397 as shown in Figure 4d. The major fragmentation pathways for compound **7** are summarized and depicted as shown in Scheme 5. Compound **7f** is an example.

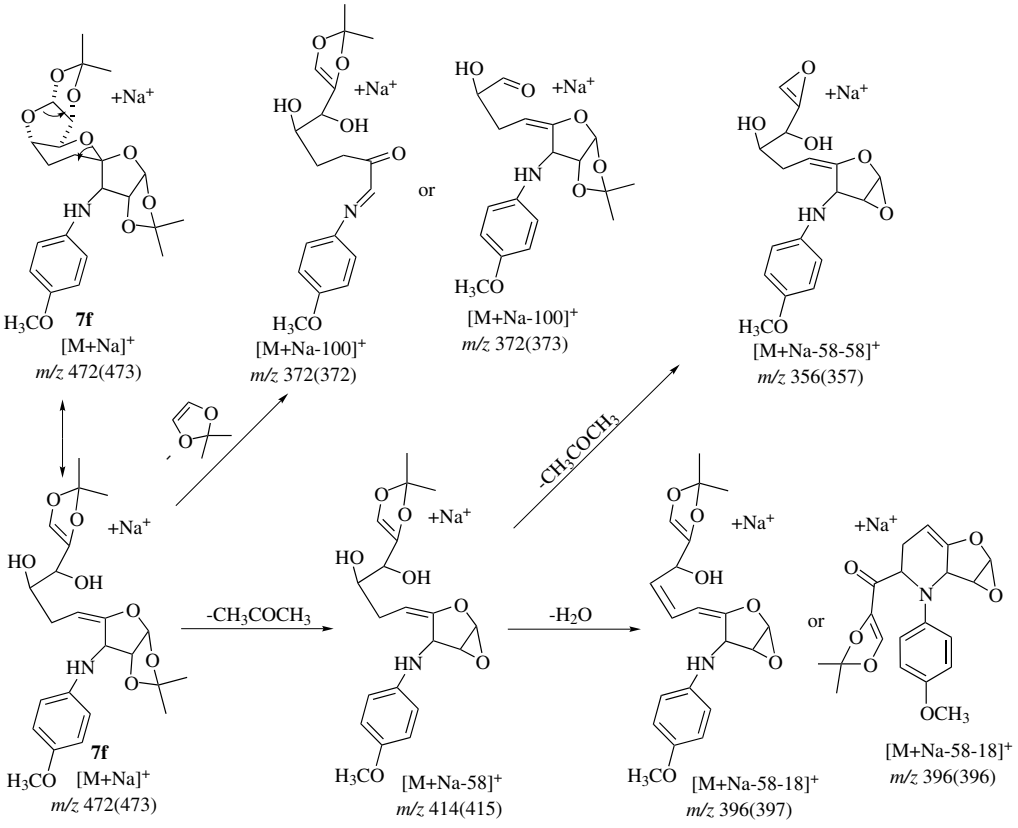
3. Experimental

3.1. Chemicals

Melting points were determined on a WC-1 melting-point apparatus and are uncorrected. Elemental analyses were carried out on a MOD 1106 analyzer. Infrared spectra were recorded on a Bio-Rad FTS-40 instrument using KBr disks in the 400–4000 cm^{-1} region. The ^1H and ^{13}C NMR spectra were acquired using a Bruker AVANCE DPX-400 spectrometer with chemical shifts (δ) given in parts per million relative to Me_4Si as the internal standard. Liquid secondary-ion mass spectrometry spectra were taken with a ZAB-2SE double-focusing mass spectrometer (VG Analytical, Manchester, UK).

3.1.1. General procedure for the preparation of compounds 4a–h.[21] A solution of (1α*S*,2α*S*,4*S*,7*E*,9α*R*,10α*R*)-5,6-dideoxy-1,2:9,10-di-*O*-isopropylidene-dec-7-enos-3,4-diulose 1,4:7,10-difuranose-4,8-pyranose (**1**) (340 mg, 1.0 mmol) and a substituted aniline (1.1 mmol) in benzene (10 mL) was stirred at rt for 48 h in the presence of *p*-toluenesulfonic acid (3 mg). The mixture was filtered through a short silica-gel column and the solvents evaporated. The residue was crystallized from MeOH to afford **4a**–**h** as white needles.

3.1.1.1. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4a). Obtained from **1** (340 mg, 1.0 mmol); 236 mg (55%); mp 154–156 °C; IR (KBr); *ν* 1504 and 1380 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.23; H, 6.19; N, 3.25.



Scheme 5. Proposed fragmentation pathway of compound **7** during electrospray MS^n mass spectrometry with CID.

3.1.1.2. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4b). Obtained from **1** (340 mg, 1.0 mmol); 219 mg (51%); mp 154–155 °C; IR (KBr); *ν* 1505 and 1379 cm^{-1}(Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.13; H, 6.14; N, 3.15.

3.1.1.3. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Fluoroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4c). Obtained from **1** (340 mg, 1.0 mmol); 303 mg (70%); mp 138–140 °C; IR (KBr); *ν* 1503 and 1380 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{22}H_{24}FNO_7$: C, 60.96; H, 5.58; N, 3.23. Found: C, 60.85; H, 5.45; N, 3.14.

3.1.1.4. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Chloroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4d). Obtained from **1** (340 mg, 1.0 mmol); 301 mg (67%); mp 152–153 °C; IR (KBr); *ν* 1481 and 1382 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{22}H_{24}ClNO_7$: C, 58.73; H, 5.38; N, 3.11. Found: C, 58.65; H, 5.41; N, 3.22.

3.1.1.5. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Bromoanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4e). Obtained from **1** (340 mg, 1.0 mmol); 296 mg (60%); mp 159–160 °C; IR (KBr); *ν* 1478 and 1382 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{22}H_{24}BrNO_7$: C, 53.45; H, 4.89; N, 2.83. Found: C, 53.36; H, 4.91; N, 2.94.

3.1.1.6. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(4-methoxyanilino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4f). Obtained from **1** (340 mg, 1.0 mmol); 320 mg (72%); mp 58–60 °C; IR (KBr); *ν* 1507 and 1380 cm^{-1} (Ph); ^1H NMR (400 MHz, $CDCl_3$): *δ* 7.21 (d, 2H, *J* 8.8 Hz, H-arom), 6.92 (d, 2H, H-arom), 6.02 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.94 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.30 (m, 1H, H-9), 4.80 (d, 1H, H-2), 3.83 (s, 3H, OCH_3), 2.51–2.50 (m, 1H, H-6b), 2.45–2.30 (m, 2H, H-5b, 6a), 2.15–2.05 (m, 1H, H-5a), 1.56 (s, 6H, CH_3), 1.48 (s, 3H, CH_3), 1.41 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{27}NO_8$: C, 62.01; H, 6.11; N, 3.14. Found: C, 62.17; H, 6.21; N, 3.38.

3.1.1.7. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-3-Anilino-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4g). Obtained from **1** (340 mg, 1.0 mmol); 224 mg (54%); mp 158–160 °C (dec); IR (KBr); *ν* 1589 and 1380 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.77; H, 6.20; N, 3.28.

3.1.1.8. (1α*S*,2α*R*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*m*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (4h). Obtained from **1** (340 mg, 1.0 mmol); 287 mg (67%); mp 125–126 °C; IR (KBr); *ν* 1592 and 1381 cm^{-1} (Ph); ^1H and ^{13}C NMR data were in agreement with those published;[22] Anal. Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.21; H, 6.19; N, 3.22.

3.1.2. General procedure for the preparation of compound 5a–h. A solution of **4a**–**h** (0.1 mmol) and $NaBH_4$ (11.0 mg, 0.29 mmol) in EtOH (5 mL) was stirred at rt for 3 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in water (5 mL), extracted with EtOAc (3 × 10 mL), dried (Na_2SO_4), and the solvents evaporated. After recrystallization from MeOH, the products **5a**–**h** were obtained as colorless needles.

3.1.2.1. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5a). Obtained from **4a** (43 mg, 0.1 mmol); 40 mg (93%); mp 182–184 °C; IR (KBr); *ν* 3369 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): *δ* 6.90 (d, 2H, *J* 8.4 Hz, H-arom), 6.73 (d, 2H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.83 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.21 (m, 2H, H-9), 5.18 (d, 1H, NH), 4.83 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.25 (dd, 1H, $J_{3,NH}$ 9.6 Hz, H-3), 2.15 (s, 3H, CH_3), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 1.70–1.60 (m, 1H, H-5a), 1.50 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 64.23; H, 6.79; N, 3.25.

3.1.2.2. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*o*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5b). Obtained from **4b** (43 mg, 0.1 mmol); 41 mg (95%); mp 86–88 °C; IR (KBr); *ν* 3443 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): *δ* 7.04 (m, 2H, H-arom), 6.70 (d, 1H, *J* 8.0 Hz, H-arom), 6.60 (t, 1H, H-arom), 5.94 (d, 1H, $J_{10,9}$ 5.6 Hz, H-10), 5.87 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.25 (m, 1H, H-9), 4.96 (m, 1H, H-2), 4.31 (m, 2H, H-3, NH), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 2.12 (s, 3H, CH_3), 1.70–1.60 (m, 1H, H-5a), 1.53 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 64.15; H, 6.82; N, 3.28.

3.1.2.3. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Fluoroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5c). Obtained from **4c** (43 mg, 0.1 mmol); 41 mg (95%); mp

186–188 °C; IR (KBr); ν 3384 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.93 (m, 2H, H-arom), 6.82 (m, 2H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.84 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.45 (d, 1H, NH), 5.21 (m, 1H, H-9), 4.82 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.25 (dd, 1H, $J_{3,NH}$ 9.6 Hz, H-3), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 1.70–1.60 (m, 1H, H-5a), 1.51 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}FNO_7$: C, 60.68; H, 6.02; N, 3.22. Found: C, 60.78; H, 6.11; N, 3.28.

3.1.2.4. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Chloroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5d). Obtained from **4d** (45 mg, 0.1 mmol); 41 mg (91%); mp 183–185 °C (dec); IR (KBr); ν 3390 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.11 (d, 2H, J 8.8 Hz, H-arom), 6.85 (d, 2H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.85 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.72 (d, 1H, NH), 5.21 (m, 1H, H-9), 4.83 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.28 (dd, 1H, $J_{3,NH}$ 9.2 Hz, H-3), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 1.65–1.55 (m, 1H, H-5a), 1.51 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}ClNO_7$: C, 58.47; H, 5.80; N, 3.10. Found: C, 58.52; H, 5.91; N, 3.20.

3.1.2.5. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-3-(4-Bromoanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5e). Obtained from **4e** (50 mg, 0.1 mmol); 48 mg (96%); mp 175–176 °C; IR (KBr); ν 3404 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.22 (d, 2H, J 8.8 Hz, H-arom), 6.81 (d, 2H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 4.4 Hz, H-10), 5.84 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.73 (d, 1H, NH), 5.21 (m, 1H, H-9), 4.82 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.28 (dd, 1H, $J_{3,NH}$ 9.2 Hz, H-3), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 1.65–1.55 (m, 1H, H-5a), 1.51 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}BrNO_7$: C, 53.24; H, 5.28; N, 2.82. Found: C, 53.41; H, 5.22; N, 2.85.

3.1.2.6. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(4-methoxyanilino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5f). Obtained from **4f** (45 mg, 0.1 mmol); 43 mg (95%); mp 155–156 °C; IR (KBr); ν 3405 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.77 (d, 2H, J 9.2 Hz, H-arom), 6.71 (d, 2H, H-arom), 5.92 (d, 1 H $J_{10,9}$ 5.2 Hz, H-10), 5.83 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.21 (m, 1H, H-9), 5.07 (d, 1H, NH), 4.82 (dd, 1H, $J_{2,3}$ 4.8 Hz, H-2), 4.20 (dd, 1H, $J_{3,NH}$ 9.2 Hz, H-3), 3.64 (s, 3H, OCH_3), 2.20–2.10 (m, 3H, H-5b, 6a, 6b), 1.70–1.60 (m, 1H, H-5a), 1.50 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{29}NO_8$: C, 61.73; H, 6.53; N, 3.13. Found: C, 61.95; H, 6.59; N, 3.21.

3.1.2.7. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-3-Anilino-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5g). Obtained from **4g** (42 mg, 0.1 mmol); 40 (94%); mp 174–176 °C; IR (KBr); ν 3384 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.09 (t, 2H, J 7.6 Hz, H-arom), 6.82 (t, 2H, H-arom), 6.59 (t, 1H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.84 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.40 (d, 1H, NH), 5.22 (m, 1H, H-9), 4.84 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.29 (dd, 1H, $J_{3,NH}$ 9.2 Hz, H-3), 2.20–2.05 (m, 3H, H-5a, 6a, 6b), 1.70–1.60 (m, 1H, H-5a), 1.51 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{27}NO_7$: C, 63.30; H, 6.52; N, 3.36. Found: C, 63.51; H, 6.49; N, 3.25.

3.1.2.8. (1α*S*,2α*R*,3α*S*,4*S*,7*E*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*m*-toluidino)-3,5,6-trideoxy-dec-7-enos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (5h). Obtained from **4h** (43 mg, 0.1 mmol); 39 mg (90%); mp 146–148 °C; IR (KBr); ν 3381 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.97 (t, 1H, J 8.0 Hz, H-arom), 6.66 (s, 1H, H-arom), 6.62 (d, 1H, H-arom), 6.42 (d, 1H, H-arom), 5.92 (d, 1H, $J_{10,9}$ 5.2 Hz, H-10), 5.84 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.28 (d, 1H, NH), 5.21 (m, 1H, H-9), 4.82 (dd, 1H, $J_{2,3}$ 5.6 Hz, H-2), 4.29 (dd, 1H, $J_{3,NH}$ 9.6 Hz, H-3), 2.18 (s, 3H, CH_3), 2.20–2.05 (m, 3H, H-5b, 6a, 6b), 1.60–1.50 (m, 1H, H-5a), 1.51 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 64.21; H, 6.79; N, 3.27.

3.1.3. (1α*S*,2α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-5,6-Dideoxy-1,2:9,10-di-*O*-isopropylidene-decosdialdos-3,4-diulose 1,4:7,10-difuranose-4,8-pyranose (3). A solution of (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-5,6-dideoxy-1,2:9,10-di-*O*-isopropylidene-decosdialdos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (**2**)[8] (1.0 g, 2.9 mmol) and PDC (pyridinium dichromate) (0.8 g, 2.0 mmol) in CH_3CN (8 mL) was stirred at 80 °C for 6 h in the presence of Ac_2O (0.9 mL). After evaporation of the solvent under reduced pressure, the residue was dissolved in EtOAc (30 mL), filtered through a short silica-gel column, washed with satd aq $NaHCO_3$ (3 × 20 mL) and the solvents evaporated. Recrystallization from Et_2O yielded the product **3** as white needles (0.9 g, 90%); mp 128–130 °C; IR (KBr); ν 1778 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ 6.19 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.71 (d, 1H, H-10), 4.74 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.65 (d, 1H, H-2), 3.96 (dd, $J_{8,7}$ 4.8, $J_{8,9}$ 5.2 Hz, 1H, H-8), 3.88 (m, 1H, H-7), 2.37 (m, 1H, H-6b), 2.13 (m, 2H, H-5b, 6a), 1.85 (m, 1H, H-5a), 1.65 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.42 (s, 3H, CH_3); Anal. Calcd for $C_{16}H_{22}O_8$: C, 56.13; H, 6.48. Found: C, 56.35; H, 6.62.

3.1.4. General procedure for the preparation of compound 6a–f. A solution of (1α*S*,2α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-5,6-dideoxy-1,2:9,10-di-*O*-isopropylidene-decosdialdos-3,4-diulose 1,4:7,10-difuranose-4,8-pyranose (**3**) (342 mg, 1.0 mmol) and substituted aniline (1.1 mmol) in EtOH (10 mL) was stirred at 70 °C for 48 h. After evaporation of the solvent under reduced pressure, the residue was recrystallized from EtOH and afforded **6a–f** as white needles.

3.1.4.1. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6a). Obtained from **3** (342 mg, 1.0 mmol); 181 mg (42%); mp 164–165 °C; IR (KBr); *v* 1504 and 1375 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): *v* 7.18 (d, 2H, *J* 8.4 Hz, H-arom), 6.97 (d, 2H, H-arom), 5.95 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.63 (d, 1H, H-10), 5.14 (d, 1H, H-2), 4.75 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.22 (dd, $J_{8,9}$ 5.2, $J_{8,7}$ 9.2 Hz, H-8), 3.89 (m, 1H, H-7), 2.38–2.32 (m, 1H, H-6b), 2.30 (s, 1H, CH_3), 2.00–1.85 (m, 3H, H-5a, 5b, 6a), 1.52 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 62.24; H, 6.61; N, 3.40.

3.1.4.2. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*o*-toluidino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6b). Obtained from **3** (342 mg, 1.0 mmol); 194 mg (45%); mp 122–124 °C (dec); IR (KBr); *v* 1505 and 1378 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.22 (t, 1H, *J* 7.2 Hz, H-arom), 7.17 (d, 1H, H-arom), 7.06 (t, 1H, H-arom), 6.89 (d, 1H, H-arom), 5.99 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.64 (d, 1H, H-10), 5.14 (d, 1H, H-2), 4.77 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.20 (dd, 1H, $J_{8,7}$ 4.0, $J_{8,9}$ 5.6 Hz, H-8), 3.91 (m, 1H, H-7), 2.45–2.35 (m, 1H, H-6b), 2.12 (s, 3H, CH_3), 2.00–1.85 (m, 3H, H-6a, 5a, 5b), 1.54 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.23 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 62.23; H, 6.56; N, 3.39.

3.1.4.3. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Fluoroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6c). Obtained from **3** (342 mg, 1.0 mmol); 196 mg (45%); mp 162–164 °C; IR (KBr); *v* 1504 and 1378 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.21 (m, 2H, H-arom), 7.12 (m, 2H, H-arom), 5.96 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.64 (d, 1H, H-10), 5.22 (d, 1H, H-2), 4.74 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.21 (dd, 1H, $J_{8,7}$ 4.4, $J_{8,9}$ 5.6 Hz, H-8), 3.89 (m, 1H, H-7), 2.40–2.30 (m, 1H, H-6b), 2.00–1.85 (m, 3H, H-5a, 5b, 6a), 1.53 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}FNO_7$: C, 60.68; H, 6.02; N, 3.22. Found: C, 60.84; H, 6.23; N, 3.42.

3.1.4.4. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Chloroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6d). Obtained from **3** (342 mg, 1.0 mmol); 284 mg (63%); mp 192–194 °C; IR (KBr); *v* 1503 and 1379 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.43 (d, 2H, *J* 8.8 Hz, H-arom), 7.09 (d, 2H, H-arom), 5.96 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.64 (d, 1H, H-10), 5.23 (d, 1H, H-2), 4.75 (t, 1H, $J_{9,10}$ 5.6 Hz, H-9), 4.20 (dd, $J_{8,7}$ 4.4, $J_{8,9}$ 5.6 Hz, H-8), 3.89 (m, 1H, H-7), 2.40–2.30 (m, 1H, H-6b), 2.00–1.80 (m, 3H, H-5a, 5b, 6a), 1.53 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}ClNO_7$: C, 58.47; H, 5.80; N, 3.10. Found: C, 58.35; H, 6.01; N, 3.15.

3.1.4.5. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Bromoanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6e). Obtained from **3** (342 mg, 1.0 mmol); 253 mg (51%); mp 220–222 °C; IR (KBr); *v* 1480 and 1379 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.55 (d, 2H, *J* 8.4 Hz, H-arom), 7.02 (d, 2H, H-arom), 5.96 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.64 (d, 1H, H-10), 5.22 (d, 1H, H-2), 4.75 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.19 (dd, 1H, $J_{8,7}$ 4.0, $J_{8,9}$ 5.2 Hz, H-8), 3.89 (m, 1H, H-7), 2.40–2.30 (m, 1H, H-6b), 2.00–1.80 (m, 3H, H-5a, 5b, 6a), 1.53 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{26}BrNO_7$: C, 53.24; H, 5.28; N, 2.82. Found: C, 53.28; H, 5.32; N, 2.90.

3.1.4.6. (1α*S*,2α*R*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(4-methoxyanilino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (6f). Obtained from **3** (342 mg, 1.0 mmol); 188 mg (42%); mp 134–136 °C; IR (KBr); *v* 1513 and 1376 cm^{-1} (Ph); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.12 (d, 2H, *J* 8.8 Hz, H-arom), 6.94 (d, 2H, H-arom), 5.96 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.63 (d, 1H, H-10), 5.18 (d, 1H, H-2), 4.74 (dd, $J_{9,10}$ 4.4 Hz, H-9), 4.22 (dd, $J_{8,9}$ 5.2, $J_{8,7}$ 9.6 Hz, H-8), 3.90 (m, 1H, H-7), 3.76 (s, 3H, OCH_3), 2.40–2.30 (m, 1H, H-6b), 2.00–1.80 (m, 3H, H-5a, 5b, 6a), 1.52 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{29}NO_8$: C, 61.73; H, 6.53; N, Found: C, 61.62; H, 6.35; N, 3.33.

3.1.5. General procedure for the preparation of compound 7a–f. A solution of compound **6a–f** (0.1 mmol) and $NaBH_4$ (11.0 mg, 0.29 mmol) in EtOH (5 mL) was stirred at rt for 3 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in water (10 mL), extracted with EtOAc (3 × 10 mL), dried (Na_2SO_4), and the solvents evaporated. After recrystallization from EtOH, the product **7a–f** was obtained as colorless needles.

3.1.5.1. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*p*-toluidino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7a). Obtained from **6a** (43 mg, 0.1 mmol); 40 mg (92%); mp 132–134 °C; IR (KBr); ν 3415 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.90 (d, 2H, J 8.4 Hz, H-arom), 6.73 (d, 2H, H-arom), 5.79 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.57 (d, 1H, H-10), 5.04 (d, 1H, NH), 4.78 (dd, 1H, $J_{2,3}$ 5.6 Hz, H-2), 4.64 (dd, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.07 (dd, 1H, $J_{3,NH}$ 10.4 Hz, H-3), 3.98 (dd, 1H, $J_{8,7}$ 5.2, $J_{8,9}$ 5.6 Hz, H-8), 3.76 (m, 1H, H-7), 2.15 (s, 3H, CH_3), 1.90–1.70 (m, 4H, H-5, 6), 1.50 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{31}NO_7$: C, 63.73; H, 7.21; N, 3.23. Found: C, 63.86; H, 7.33; N, 3.20.

3.1.5.2. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(*o*-toluidino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7b). Obtained from **6b** (43 mg, 0.1 mmol); 41 mg (95%); mp 80–81 °C; IR (KBr); ν 3439 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.04 (m, 2H, H-arom), 6.79 (d, 1H, J 8.0 Hz, H-arom), 6.60 (t, 1H, J 7.2 Hz, H-arom), 5.85 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.61 (d, 1H, H-10), 4.89 (t, 1H, $J_{2,3}$ 4.0 Hz, H-2), 4.67 (t, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.16 (m, 2H, H-3, NH), 4.11 (t, 1H, $J_{8,9}$ 4.8 Hz, H-8), 3.84 (m, 1H, H-7), 2.12 (s, 3H, CH_3), 1.90–1.60 (m, 4H, H-5, 6), 1.51 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{31}NO_7$: C, 63.73; H, 7.21; N, 3.23. Found: C, 63.80; H, 7.42; N, 3.32.

3.1.5.3. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Fluoroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7c). Obtained from **6c** (43 mg, 0.1 mmol); 41 mg (95%); mp 92–94 °C; IR (KBr); ν 3428 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.92 (m, 2H, H-arom), 6.83 (m, 2H, H-arom), 5.80 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.58 (d, 1H, H-10), 5.27 (d, 1H, NH), 4.79 (dd, 1H, $J_{2,3}$ 4.8 Hz, H-2), 4.64 (t, 1H, $J_{9,10}$ 4.8 Hz, H-9), 4.06 (dd, 1H, $J_{3,NH}$ 9.6 Hz, H-3), 3.98 (dd, 1H, $J_{8,9}$ 4.8, $J_{8,7}$ 5.2 Hz, H-8), 3.77 (m, 1H, H-7), 1.90–1.70 (m, 4H, H-5, 6), 1.50 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{28}FNO_7$: C, 60.40; H, 6.45; N, 3.20. Found: C, 60.54; H, 6.49; N, 3.34.

3.1.5.4. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Chloroanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7d). Obtained from **6d** (45 mg, 0.1 mmol); 40 mg (89%); mp 130–132 °C; IR (KBr); ν 3412 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.09 (d, 2H, J 8.4 Hz, H-arom), 6.85 (d, 2H, H-arom), 5.80 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.58 (d, 1H, H-10), 5.55 (d, 1H, NH), 4.81 (t, 1H, $J_{2,3}$ 3.6 Hz, H-2), 4.65 (t, 1H, $J_{9,10}$ 4.4 Hz, H-9), 4.10 (dd, 1H, $J_{3,NH}$ 10.0 Hz, H-3), 3.99 (t, 1H, $J_{8,9}$ 4.4 Hz, H-8), 3.76 (m, 1H, H-7), 1.90–1.70 (m, 4H, H-5, 6), 1.50 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{28}ClNO_7$: C, 58.21; H, 6.22; N, 3.09. Found: C, 63.86; H, 7.33; N, 3.20.

3.1.5.5. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-3-(4-Bromoanilino)-1,2:9,10-di-*O*-isopropylidene-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7e). Obtained from **6e** (50 mg, 0.1 mmol); 48 (96%); mp 122–124 °C; IR (KBr); ν 3412 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 7.21 (d, 2H, J 8.8 Hz, H-arom), 6.81 (d, 2H, H-arom), 5.80 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.59 (d, 1H, H-10), 5.58 (d, 1H, NH), 4.65 (t, $J_{9,10}$ 4.8 Hz, H-9), 4.81 (dd, 1H, $J_{2,3}$ 5.6 Hz, H-2), 4.10 (dd, 1H, $J_{3,NH}$ 10.4 Hz, H-3), 3.98 (t, $J_{8,9}$ 4.8 Hz, H-8), 3.76 (m, 1H, H-7), 1.90–1.70 (m, 4H, H-5, 6), 1.50 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{22}H_{28}BrNO_7$: C, 53.02; H, 5.66; N, 2.81. Found: C, 53.22; H, 5.58; N, 2.92.

3.1.5.6. (1α*S*,2α*R*,3α*S*,4*S*,7α*S*,8β*R*,9α*R*,10α*R*)-1,2:9,10-Di-*O*-isopropylidene-3-(4-methoxyanilino)-3,5,6-trideoxydecos-4-ulose 1,4:7,10-difuranose-4,8-pyranose (7f). Obtained from **6f** (45 mg, 0.1 mmol); 43 mg (95%); mp 102–104 °C; IR (KBr); ν 3429 cm^{-1} (NH); ^1H NMR (400 MHz, Me_2SO-d_6): δ 6.77 (d, 2H, J 9.2 Hz, H-arom), 6.72 (d, 2H, H-arom), 5.79 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.58 (d, 1H, H-10), 4.82 (d, 1H, NH), 4.77 (dd, 1H, $J_{2,3}$ 5.6 Hz, H-2), 4.64 (dd, 1H, $J_{9.10}$ 4.8, $J_{9,8}$ 5.6 Hz, H-9), 4.10–3.90 (m, 2H, H-3, 8), 3.77 (m, 1H, H-7), 3.65 (s, 3H, OCH_3), 1.70–1.90 (m, 4H, H-5,6), 1.50 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); Anal. Calcd for $C_{23}H_{31}NO_8$: C, 61.46; H, 6.95; N, 3.12. Found: C, 61.65; H, 6.80; N, 3.23.

3.2. Mass spectrometry

The chemicals used were commercially available and of analytical grade. HPLC grade MeOH was used to dissolve the samples. MeOH-*d* was purchased from the Sigma Company. Mass spectra were recorded on a Bruker Esquire 3000 ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an electrospray ion source. Samples were typically dissolved in MeOH at a concentration of about 10^{-5} mol/L. The deuterated analogues were prepared by dissolving the sample in MeOH-*d*. They were introduced into the electrospray needle by a Cole–Parmer 74900 syringe pump (Cole–Parmer Instrument Company) at a flow rate of 4 μL/min. The ESI source potentials were capillary 4.0 kV,

lens 1 5.0 V, lens 2 60.0 V, and capillary exit offset is 75.9 V. The mass spectrometer was scanned at the rate 300 mass units per second. At least 10 scans were averaged to obtain each spectrum. Nitrogen as nebulizer gas with a flow of 4 L/min (nebulizer pressure of 7 psi) at 300 °C was used. The MS^n spectra were obtained by low-energy CID with helium after isolation of the appropriate precursor ions. An isolation width of 2.0 m/z was used to isolate the selected peaks. The fragmentation low mass cut-off was set to 27% of the precursor ion mass. The collision conditions were ramped from 0.60% to 1.20% of the fragmentation amplitude and set over the 40 ms fragmentation time. Data acquisition and processing were carried out using Data analysis 5.0 software supplied with the instrument.

4. Conclusions

Electrospray-ionization MS^n mass spectrometry (ESI-MS^n) with CID was used to establish the fragmentation behavior of four series of sodium ion adducts of higher-carbon amino spiro-sugar derivatives. In the ESI-MS spectra of compounds **4**–**7**, the base peaks are the sodium ion adducts and a dominant fragmentation pathway is the loss of one or two molecule of acetone. Their fragmentation pathways have been proposed on the basis of the MS^n studies. Some interesting ions at $[M+Na-46]^+$, $[M+Na-58-76]^+$, and $[M+Na-100]^+$ have been found and the mechanism of formation is proposed. The $[M+Na-46]^+$ ion in the spectra of compounds **4a**–**h** was probably formed by the opening of two rings followed by simultaneous elimination of carbon monoxide and water. The MS^n spectra of the $[M+Na-58]^+$ ion of compound **6** was considerably simpler than that of compounds **4** and **5**. Formation of the $[M+Na-100]^+$ ion of compounds **7a**–**f** may be rationalized by the presence of active hydrogen at the nitrogen atom. Deuterated analogues were prepared for confirmation of the proposed mechanism. The observations may assist in the interpretation of fragment ion spectra of related derivatives.

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References

1. (a) Iwasa, T.; Kusuka, T.; Suetomi, K. *J. Antibiot.* **1978**, *31*, 511–518; (b) Harada, S.; Kishi, T. *J. Antibiot.* **1978**, *31*, 519–524; (c) Harada, S.; Mizuta, E.; Kishi, T. *Tetrahedron* **1981**, *37*, 1317–1327.
2. (a) Takatsuki, A.; Arima, K.; Tamura, G. *J. Antibiot.* **1971**, *24*, 215–223; (b) Takatsuki, A.; Tamura, G. *J. Antibiot.* **1971**, *24*, 224–231; (c) Takatsuki, A.; Tamura, G. *J. Antibiot.* **1971**, *24*, 232–238; (d) Takatsuki, A.; Kawamura, K.; Okina, M.; Kodama, Y.; Ito, T.; Tamura, G. *Agric. Biol. Chem.* **1977**, *41*, 2307–2309.
3. (a) Mootoo, D. R.; Fraser-Reid, B. *J. Org. Chem.* **1987**, *52*, 4511–4517; (b) Mootoo, D. R.; Fraser-Reid, B. *J. Org. Chem.* **1989**, *54*, 5548–5550; (c) Jørgensen, M.; Iversen, E. H.; Madsen, R. *J. Org. Chem.* **2001**, *66*, 4625–4629; (d) Jarosz, S.; Skóra, S.; Szewczyk, K.; Ciunik, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 1895–1905; (e) Jarosz, S. *J. Carbohydr. Chem.* **2001**, *20*, 93–107; (f) Marco-Cotelles, J.; Opazo, E. D.; Arroyo, N. *Tetrahedron* **2001**, *57*, 4729–4739; (g) Junker, H.-D.; Phung, N.; Fessner, W.-D. *Tetrahedron Lett.* **1999**, *40*, 7063–7066; (h) Jarosz, S.; Salański, P.; Mach, M. *Tetrahedron* **1998**, *54*, 2583–2594.
4. Araki, Y.; Endo, T.; Arai, Y.; Tanji, M.; Ishido, Y. *Tetrahedron Lett.* **1989**, *30*, 2829–2832.
5. Kim, K. S.; Cho, I. H.; Joo, Y. H.; Yoo, I. Y.; Song, J. H.; Ko, J. H. *Tetrahedron Lett.* **1992**, *33*, 4029–4032.
6. Eyrisch, O.; Keller, M.; Fessner, W.-D. *Tetrahedron Lett.* **1994**, *35*, 9013–9016.
7. Haines, A. H.; Lamb, A. J. *Carbohydr. Res.* **2000**, *325*, 323–339.
8. Liu, H. M.; Zou, D. P.; Zhang, F.; Zhu, W. G.; Peng, T. *Eur. J. Org. Chem.* **2004**, *10*, 2103–2106.
9. Przybylski, M.; Glocker, M. O. *Angew. Chem., Int. Ed.* **1996**, *35*, 806–813.
10. Loo, J. A. *Int. J. Mass Spectrom.* **2000**, *200*, 175–186.
11. Hua, Y.; Wainhaus, S. B.; Yang, Y.; Shen, L.; Xiong, Y.; Xu, X.; Zhang, F.; Bolton, J. L.; Breemen, R. B. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 80–87.
12. Jerić, I.; Versluis, C.; Horvat, Š.; Heck, A. J. R. *J. Mass Spectrom.* **2002**, *37*, 803–811.
13. Desaire, B.; Leary, J. A. *Anal. Chem.* **1999**, *71*, 1997–2002.
14. Penn, S. G.; Cancilla, M. T.; Lebrilla, C. B. *Anal. Chem.* **1996**, *68*, 2331–2339.
15. Banoub, J.; Thibault, P.; Gouéth, P. Y.; Ronco, G.; Villa, P. *J. Mass Spectrom.* **1997**, *32*, 109–121.
16. Young, M. K.; Dinh, N.; Williams, D. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1462–1467.
17. Sánchez-Rabaneda, F.; Jáuregui, O.; Casals, I.; Andrés-Lacueva, C.; Izquierdo-Pulido, M.; Lamuela-Raventós, M. L. *J. Mass Spectrom.* **2003**, *38*, 35–42.
18. Levery, S. B.; Toledo, M. S.; Straus, A. H.; Takahashi, H. K. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 2240–2258.
19. Madhusudanan, K. P.; Raj, K.; Bhaduri, A. P. *J. Mass Spectrom.* **2000**, *35*, 901–911.
20. Cui, M.; Song, F.; Liu, Z.; Liu, S. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 586–595.
21. Liu, H. M.; Zhang, F.; Wang, S. *Org. Biomol. Chem.* **2003**, *1*, 1641–1642.
22. Zou, D. P.; Zhu, W. G.; Xu, W. C.; Liu, H. M.; Zhang, H. J.; Li, X. *Bopuxue Zazhi* **2004**, *3*, 305–310.