Pergamon

Tetrahedron, Vol. 52, No. 33, pp. 10903-10920, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00612-6

Synthesis and structural characterization of Macrocyclic Carbohydrate Derivatives obtained from Catalytic Metathesis Reaction with Chloro-Aryloxide Complexes of Tungsten.

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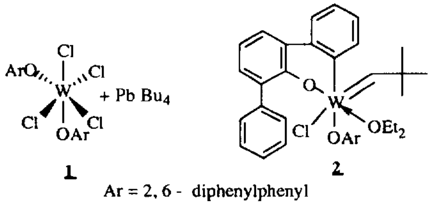
Abstract: Tungsten aryloxo-complex **1** catalyses the intramolecular metathesis reactions of di- and tri-substituted ω-unsaturated glucose, and glucosamine derivatives, yielding bicyclic carbohydrate-based compounds **12**, **13**, **14**, **15**, containing 12-, and 14-atoms rings. Electrospray tandem mass spectrometry was used for the structural characterization of these novel bicyclic carbohydrate compounds. Copyright © 1996 Elsevier Science Ltd

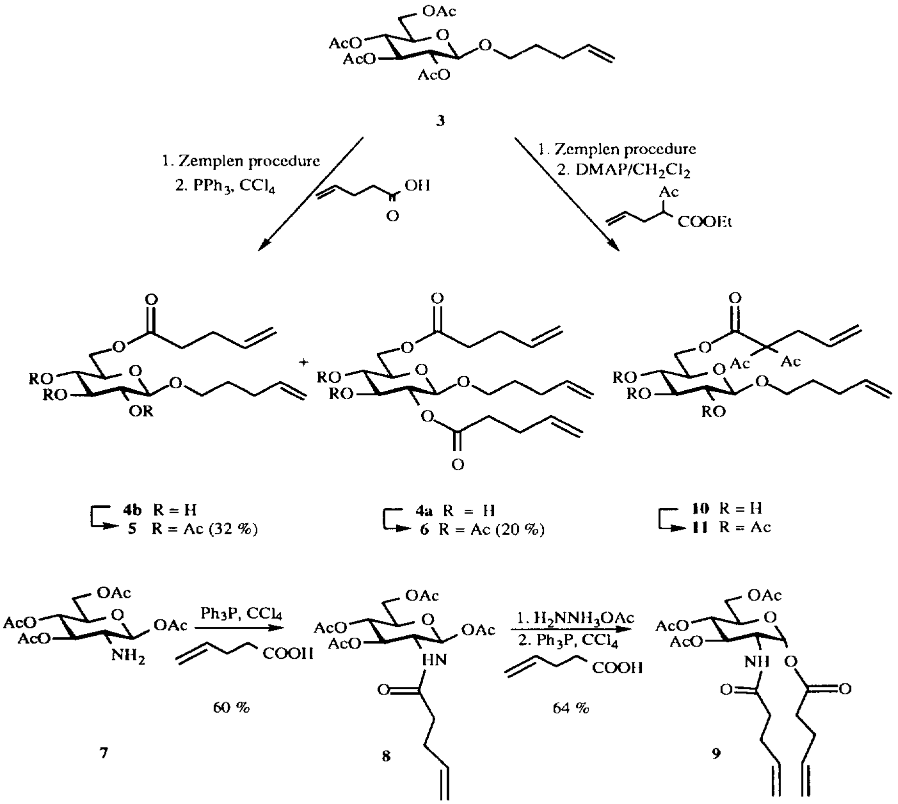
Metathesis is a well known catalytic reaction which allows the interconversion of olefins[1,2] by coupling of terminal double bonds followed by the production of ethylene. As we previously reported [3,4] this reaction can be successfully applied to carbohydrate derivatives. Due to their huge steric hindrance, the recently developed tungsten-based catalysts[5,6,7], are able to carry out metathesis reaction of olefins bearing a wide range of functional groups (phosphanes, sulfides, esters, ethers and silanes) (scheme 1). Other workers have completed metathesis of olefins containing oxygen and nitrogen with either molybdenum[7a] or, more recently, ruthenium based catalysts[7b]. For example, carbohydrate-substituted polymers have been synthesized by metathesis reaction with ruthenium trichloride in water[7c] and a recent review reported many applications of the catalytic ring-closing metathesis reactions in organic synthesis[7d]. Cyclization by intramolecular metathesis reactions with ruthenium catalysts have been described[8] in the literature to form, six-, seven- and eight-membered carbocycles and heterocycles.



Scheme 1

So far, our research efforts have mainly focused on the intermolecular metathesis reactions leading to the formation of unsaturated bolaforms. Now we wish to report the application of this very powerful reaction to carbohydrate intramolecular macrocyclization.

First, we have prepared from 4-penten-1-yl β-D-glucoside **3** a serie of substrates (**4**, **5**, **6**, **11**) bearing two or three terminal olefins (scheme 2).



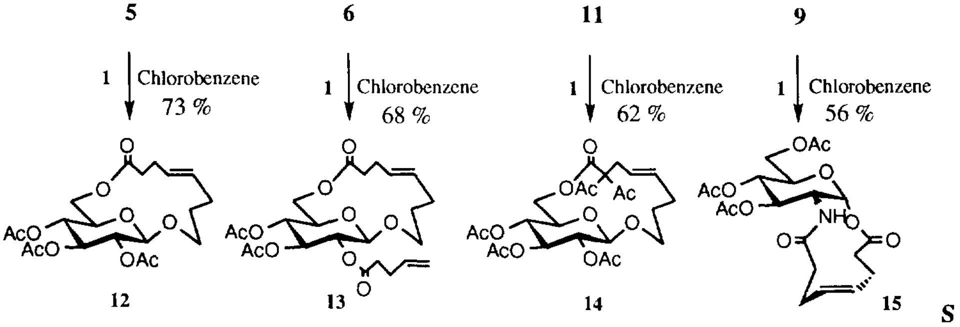
Scheme 2

Esterification of the deprotected 4-penten-1-yl β-D-glucoside **3**[4] with acyl chloride generated *in situ* [9] from pent-4-enoic acid, afforded a mixture of mono- and di-esters (**4a** + **4b**) easily separable by column chromatography. Positions of the unsaturated ester substituents were confirmed by the chemical shifts of H-6 and H-2 in the ^{1}H NMR spectra of intermediates **4a**, and **4b** [4.86 ppm (dd, H-2), 4.52 ppm (dd, H-6), 4.40 ppm (dd, H-6') and 4.50 ppm (dd, H-6), 4.39 ppm (dd, H-6') respectively]. Acetylation of **4a** + **4b** gave the desired compounds **5** and **6**.

The conversion of **3** was realised into the more sterically hindered ester **11** by transesterification with 2-(methylcarbonyl)-pent-4-enoic acid ethyl ester followed by reacetylation under standard conditions. The position of the olefinic substituent was also confirmed by the chemical shifts of H-6 in the ^{1}H NMR spectrum of intermediate **10** [4.48 ppm (dd, H-6), 4.36 ppm (dd, H-6')]. The acetylation on the active α position of the unsaturated keto-ester substituent was confirmed by ^{13}C NMR signal of the quaternary carbon atom (119.6 ppm) as well as by an additional methyl group signal in the ^{1}H NMR spectrum.

Acylation of the glucosamine derivative **7**[10] yielded intermediate **8**, which, after selective deprotection of the anomeric position with hydrazine acetate[11], and further acylation was converted into the α-amido ester **9**, which contained two unsaturated side-chains with a *cis*-configuration.

Catalyst **1** was employed for metathesis experiments. In diluted solutions of the unsaturated substrates in chlorobenzene, in the presence of ~7% of catalyst at 80-85°C, cyclic products were formed with reasonable yields. It was noticed that no products resulting from intermolecular reactions were formed. The results for the metathesis reactions are summarised in the scheme 3.



Scheme 3

Substrate **5** yielded the bicyclic product **12**, containing a 14-membered ring. Similarly, compound **13** was formed, when substrate **6** was submitted to the same conditions. We had anticipated that the configuration at the anomeric center controls the chemoselectivity of this reaction. Thus the cyclisation took place between the two substituents at C_1 and C_6 which are in a *cis*-configuration.

This hypothesis was confirmed by the successful reaction of the amido-ester **9** containing *cis* substituents at positions 1 and 2 . As expected the 12-membered ring **15** was formed as the only reaction product.

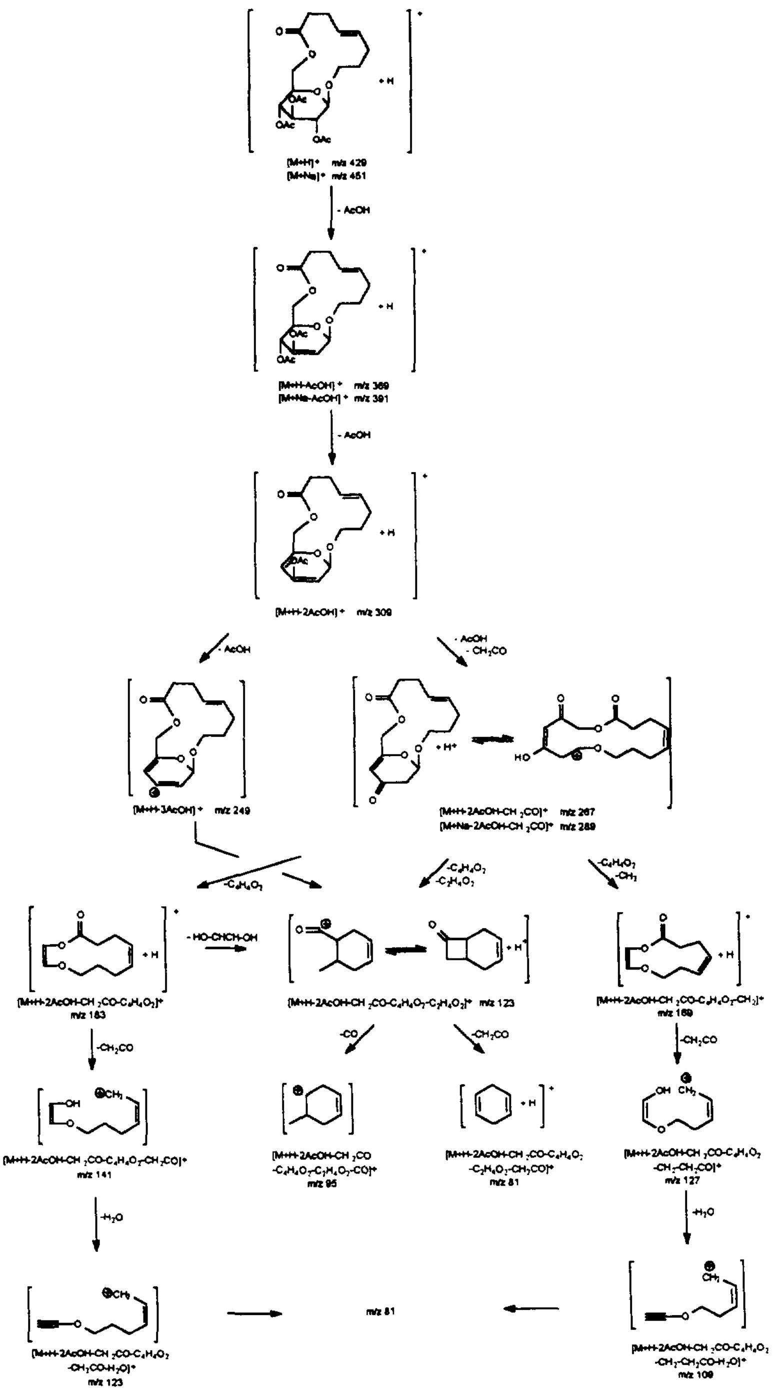
Finally substrate **11**, with two additional acetyl substituents close to the reaction center was easily cyclized to give product **14**. This result confirmed the resistance of the oxophilic catalyst to deactivation by the functional groups.

All obtained compounds were characterised by their ^1H and ^{13}C NMR spectra. The proton and carbon atoms signals of the terminal double bonds of the substrates were indeed replaced in the products by signals corresponding to an endocyclic olefin (Table 1 and 2). For compounds **14** and **15** the *trans* configuration of the endocyclic double bonds was confirmed by the spectral data ($J_{H,H\ olefin}$ = 13.9Hz and 15.1Hz respectively). For the other products, we could not determine this coupling constant, because the olefinic protons showed the same chemical shifts (experiments with different solvents and chemical shift reagents were unsuccessful). We supposed that the more stable *trans* isomers were formed in each case.

Additional structural characterization of the bicyclic products **12-15** was achieved using electrospray mass spectrometry[13-15].

The electrospray mass spectra (positive ion mode) were recorded for this series of novel compounds. The ES MS recorded with a low focus voltage afforded the protonated molecules $[M+H]^+$ at *m/z* 442 for compound **15** and the sodiated adducts $[M+Na]^+$ at *m/z* 451, 491, 535 and 463 for compounds **12**, **13**, **14** and **15**, respectively. Evidence of dimers was not found at higher m/z. The characteristic monocharged fragment ions and their relative intensities are presented in table 4. In the ES MS of compound **12** we noticed a series of ions derived from the sodiated adducts $[M+Na]^+$ at *m/z* 451, the [M+Na-AcOH] ion at *m/z* 391 and the $[M+Na-2AcOH-CH_2CO]^+$ ion at *m/z* 289, in addition to other fragment ions derived from the protonated molecule at m/z 429 (which was not apparent in the conventional ES MS). These were assigned as $[M+H-2AcOH-CH_2CO]^+$ and $[M+H-3AcOH]^+$ at *m/z* 267 and 249, respectively. It is also worthwhile to mention the presence of a serie of ions which we have tentatively assigned as the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2]^+$ ion at *m/z* 169, the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2CO]^+$ ion at *m/z* 141, the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2-CH_2CO]^+$ ion at *m/z* 127, the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-C_2H_4O_2]^+$ ion at *m/z* 123 and the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2CO-CH_2-H_2O]^+$ ion at *m/z* 109. This series of ions seems to be derived from the $[M+H-2AcOH-CH_2CO]^+$ ion at *m/z* 267. Note that this series of ions is common for products **12**, **13** and **14**. Low energy MS/MS analyses were conducted to rationalize the pathways leading to the various fragmentations observed in the conventional electrospray mass spectrum of compound **12**. Product-ion spectra arising from fragmentation in the RF-only hexapole collision cell of the instrument were observed. The $[M+Na]^+$ ion at m/z 451 was selected for the recording of product ions formed by collisionally activated dissociations (CAD). One of the main benefits of the CAD MS/MS technique is that all uncertainties concerning the origin of the fragment ion are removed. A summary of the proposed modes of formation and suggested structures of the CAD MS/MS fragment ions formed during the dissociation of the sodiated adducts $[M+Na]^+$ and protonated molecules $[M+H]^+$ are shown in Scheme 4.

The CAD MS/MS spectrum of the sodiated adducts $[M+Na]^+$ at *m/z* 451 confirmed the formation of the product ions at *m/z* 391, 289, 267, 249, 169, 141, 127,123, 109, 95 and 81 as shown in figure 1a. The assignments of the product ions at m/z 169, 141, 127, 109, 95 and 81 were performed in a different set of experiments using the precursor ion scan technique[13-15]. It was thus established that the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2-CH_2CO]+$ ion at *m/z* 127 was either produced from the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2]^+$ ion at *m/z* 169 by the loss of a molecule of ketene, or from the $[M+H-2AcOH-CH_2CO]^+$ ion at *m/z* 267 which loses the $C_4H_4O_2$ portion of the macrocycle by simultaneous fissions between C_1-C_2 and C_5-C_6, followed by elimination of a methylene group followed by a ketene molecule. The ion at *m/z* 127 is also formed from the sodiated adduct at *m/z* 451 (Figure 2a). In summary, the ion at *m/z* 127 is formed from the precursor ions at *m/z* 169, 267 and 451 either by consecutive individual losses of the various fragments ($C_4H_4O_2$, CH_2 and CH_2CO), directly from each of the individual precursor ions or by concerted losses between the individual precursors (Scheme 4). Therefore, it is obvious that the ion at *m/z* 169 is formed also from the ion at *m/z* 267 and the sodiated adduct at *m/z* 451. Note that the protonated cyclic ion at *m/z* 169, which is formed from the ion at *m/z* 267, is a diagnostic fragment ion characteristic of the presence of the 14-membered macrocyclic ring as it is present in all CAD MS/MS analyses of this novel series of bicyclic compounds (**12**, **13** and **14**) which have different sodiated adducts. The precursor ion scan of the ion at *m/z* 123 indicated that it originates from the $[M+H-2AcOH-CH_2OH-CH_2CO-C_4H_4O_2-CH_2CO]^+$ ion at *m/z* 141 by loss of a molecule of water (Figure 2b). The ion at *m/z* 141 is formed from the $[M+H-2AcOH-CH_2CO-C_4H_4O_2]^+$ ion at *m/z* 183 by loss of a ketene molecule. The formation of the ion at *m/z* 183 is tentatively assigned in Scheme 4. As expected, it was noted that the ion at *m/z* 123 can also be formed either directly from the ion at *m/z* 183 by loss of a neutral molecule of $C_2H_4O_2$ (60 Da) or from each of the individual precursor ions at *m/z* 249, 267, 309 and 429 (Scheme 4).

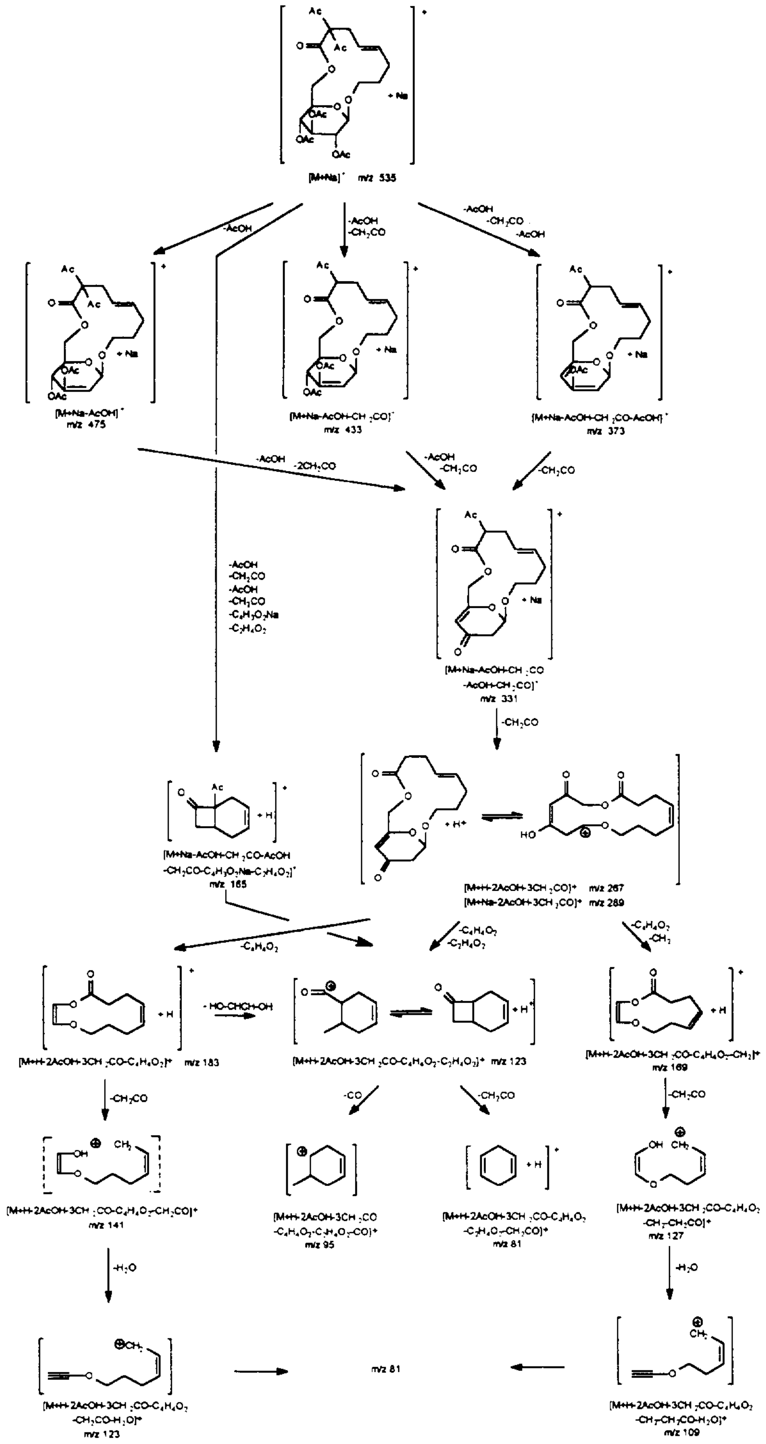


Scheme 4

Major fragmentations routes of compound **12** observed by CAD MS/MS

It is imperative to mention that there are two isomeric forms for the ion at *m/z* 123. Please note that the ions $[M+H-3AcOH]^+$ at *m/z* 249, $[M+H-2AcOH]^+$ at *m/z* 309 and the protonated molecules at *m/z* 429 are absent in the conventional ES MS. The precursor ion scan of the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CO]^+$ ion at *m/z* 95 showed that it originated from the ion at *m/z* 123 by loss of a carbonyl group. The precursor ion scan of the ion at *m/z* 81 showed that it originated from the ions at *m/z* 109, 123 and their precursors (Figure 2c). All "concerted losses" reported for the various MS/MS experiments simply means that these were lost within the time window of the same reaction region of the tandem mass spectrometer. No conclusions about "concerted" reactions on the molecular timescale can be drawn and is beyond the scope of the present work.

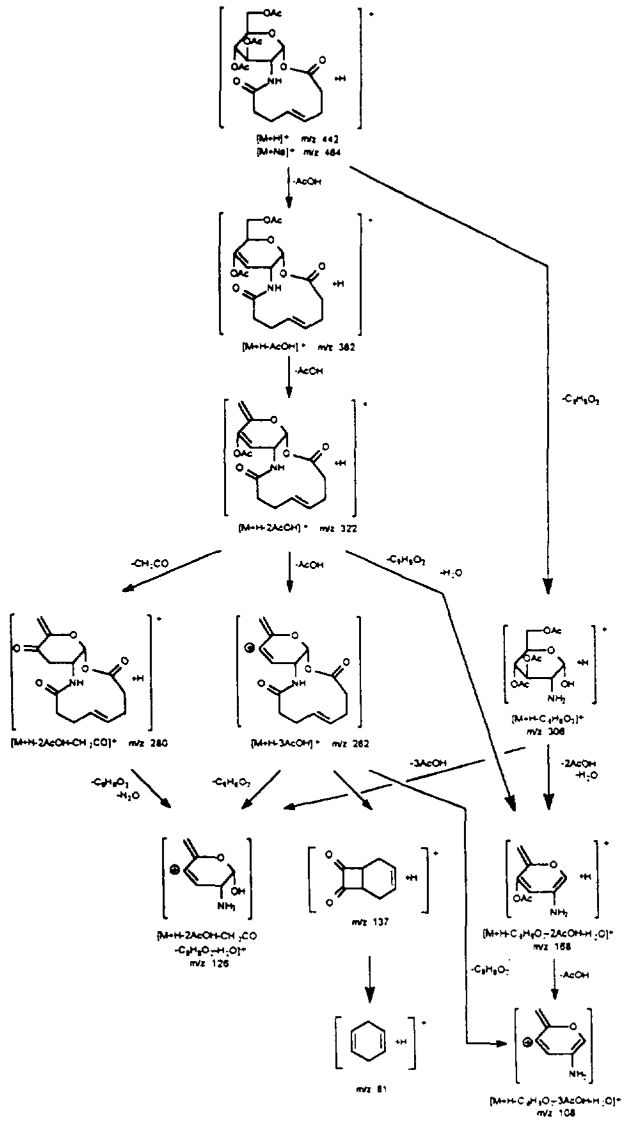
Therefore, by using low energy CAD MS/MS analyses (product ion and precursor ion scans) we have established that, indeed, the ions at *m/z* 183, 169, 127, 123, 109, 95 and 81 are formed from the 14-membered macrocycle ring portion of the product **12** and in the following part of this rationale, we will show that these ions are also present for the other bicyclic products **12**, **13** and **14**. The characteristic monocharged fragment ions and their relative intensities observed in the ES MS of compound **13** are shown in table 4. The CAD MS/MS spectrum of the sodiated adducts $[M+Na]^+$ at *m/z* 491 showed an identical series of product ions (figure 1b) to that of product **12**. This is not surprising since as soon as the sodiated adduct loses an acidic molecule $C_5H_8O_2$ from C-2 to form the $[M+Na-C_5H_8O_2]^+$ ion at *m/z* 391, all of the remaining CAD MS/MS product ions are identical to that of product **12**. However, this is not true in the case of the bicyclic product **14** which contains geminal acetate groups located on the 14-membered macrocycle ring. Indeed, in the ES MS of compound **14**, we noticed the formation of the diagnostic $[M+Na-AcOH-CH_2CO]^+$ ion at *m/z* 433, which is obtained by the favored loss of one of the geminal acetate groups. This type of ion is unique to compound **14** and was absent in the ES MS of compounds **12** and **13** (Table 4). The CAD MS/MS of the sodiated adducts $[M+Na]^+$ at *m/z* 535 gave a similar series of product ions as the one obtained from the sodiated adducts of compounds **12** and **13**, in addition to the diagnostic$[M+Na-AcOH-CH_2CO-AcOH-CH_2CO-C_4H_3O_2Na-C_2H_4O_2]^+$ ion at *m/z* 165 (figure 1d). It should be noted that this ion is formed by elimination of two molecules of acetic acid, 3 molecules of ketene, loss of the $C_4H_3O_2Na$ by fission between C1 - C2 and C5 - C6 of the macrocycle ring and loss of $C_2H_4O_2$ (not necessarily in this order) accounting for five acetate groups. The precursor ion scan of the ion at *m/z* 165 indicated that this ion was produced solely from the sodiated adduct precursor $[M+Na]^+$ at *m/z* 535 (Figure 2d). The major fragmentation routes of the sodiates adduct $[M+Na]^+$ at *m/z* 535, obtained by CAD MS/MS, are depicted in Scheme 5.



Scheme 5

Major fragmentations routes of compound **14** observed by CAD MS/MS

Finally, the characteristic fragment ions observed in the ES MS of the nitrogen analog of the bicyclic product **15** and their relative intensities are described in table 4. The ES MS of this bicyclic compound **15** showed the formation of protonated molecules $[M+H]^+$ at *m/z* 442, the sodiated adduct $[M+Na]^+$ at *m/z* 463 and the potassiated adduct $[M+K]^+$ at *m/z* 480. CAD MS/MS of the protonated molecules $[M+H]^+$ at *m/z* 442, shown in figure 1c, confirmed the formation of the product ions originally obtained in the conventional ES mass spectrum and afforded the series of product ions at *m/z* 382, 322, 280, 262, 168, 137, 126, 108 and 81, whose formation and structures are tentatively depicted in Scheme 6. For more confirmatory evidence on the genesis of the product ions, precursor ion scans were recorded for all of this series of ions (in the interest of brevity, we will only report some of these). Therefore, precursor ion scan of the ion at *m/z* 262 showed that it originated from the series of precursor ions at *m/z* 322, 382, 442 and 464 (Figure 3a). Precursor ion scan of the ion at *m/z* 168 showed that it was formed from the series of ions at *m/z* 306, 322, 382, 442 and 464 (Figure 3b). The precursor ion scan of the ion at *m/z* 137 showed that it was produced by the series of precursor ions at *m/z* 262, 322, 382, 442 and 464 (Figure 3c). The precursor ion scan of the ion at *m/z* 126 indicated that it was produced by the series of precursor ions at *m/z* 262, 280, 306, 322, 382, 442 and 464 (Figure 3d). The precursor ion scan of the ion at *m/z* 108 indicated that it was formed from the series of precursor ions at *m/z* 168, 262, 322, 382, 442 and 464 (Figure 3e). Finally, the precursor ion scan of the ion at *m/z* 81 indicated that it originated from a series of precursor ions at *m/z* 137, 262, 322, 442 and 464. It should be noted that during the various low energy MS/MS analyses we have identified the ions at *m/z* 306 and 137 as two complementary portions of compound **15** accounting for the exact molecular mass and characteristic of the bicyclic structure (carbohydrate portion at *m/z* 306 and macrocycle portion at *m/z* 137). Mass spectral analysis of this series of novel macrocyclic carbohydrate derivatives was performed using electrospray ionization. Abundant signals corresponding to the sodiated adducts $[M+Na]^+$ were observed in all cases. MS/MS spectra obtained using low energy collisional activation permitted the rationalization of the unique fragmentation pathways obtained for this series of bicyclic compounds. Furthermore, product ion and precursor ion MS/MS of selected intermediates allowed rationalization and confirmation of the novel fragmentation behavior of these compounds and established the presence of the macrocyclic rings.



Scheme 6

Major fragmentations routes of compound **15** observed by CAD MS/MS

In conclusion we have developed a new general and powerful method for macrocyclization in carbohydrate series. The catalytic metathesis reaction, with sterically hindered tungsten complexes as **1**, can be employed for cyclization of substrates bearing numerous functional groups (esters, amides, ketones, acetals).

EXPERIMENTAL:

Organic preparation

General methods: All reactions were performed under argon atmosphere in flame dried reaction flasks. All solvents were distilled prior to use. Chlorobenzene and methylene chloride were dried according to standard procedures. For column chromatography silica gel 60, 400-230 mesh (E. Merck) was employed. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM-200 spectrometer with TMS and $CDCl_3$ as internal standard respectively, with $CDCl_3$, as a solvent. For some cases decoupling experiments were done to ensure protons assignments. IR spectra were obtained on Perkin Elmer 681 Infrared Spectrofotometer. Optical rotations were measured on Perkin Elmer 241 Polarimeter.

Pent-4-enyl 2,3,4-tri-O-acetyl-6-O-(3'-buten-1'-yl-carbonyl)-β-D-glucopyrano-side **5** and *Pent-4-enyl 3,4-di-O-acetyl-2,6-di-O-(3'-buten-1'-yl-carbonyl)-β-D-gluco pyranoside* **6**: to the solution of compound **3** (500 mg, 1.2 mmol) in methanol (10 mL) catalytic amount of sodium methoxide was added. The resulting mixture was stirred at rt. for 6 hrs. After neutralisation (acetic acid), and solvent evaporation, the residue was added to the previously prepared solution of Ph_3P (525 mg, 2 mmol), CCl_4 (0.19 mL, 2 mmol) and 4-pentenoic acid (0.204 mL, 2 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred overnight at rt. Solvent evaporation and column chromatography (dichloromethane/methanol 9:1) aforded two products: **4a** [^{1}H NMR, δ: 5.78 (m, 3H, 3xCH=), 4.90 (m, 6H, $3xCH_2$=), 4.86 (dd, $J_{2,3}$ 9.0, $J_{1,2}$ 7.6, 1H, H-2), 4.52 (dd, $J_{5,6}$ 2.2, $J_{6,6'}$ 12.3, 1H, H-6), 4.40 (dd, $J_{6,6'}$ 12.3, $J_{5,6'}$ 2.4, 1H, H-6'), 4.32 (d, $J_{1,2}$ 7.6, 1H, H-1), 4.10 (bs, 3H, 3xOH), 3.84 (m, 1H, H-α), 3.50 (m, 4H, H-α, H-3, H-4, H-5), 2.42 (m, 4H, $2xCH_{2ester}$), 2.0 (m, 2H CH_2 allyl), 1.62 (m, 2H, CH_2 β)], and **4b** [^{1}H NMR, δ: 5.78 (2xdddd, $2xJ_{cis}$ 10.3, $2xJ_{trans}$ 17.0, $4xJ_{allyl}$ 6.6, 2H, 2xCH=), 4.90 (m, 4H, CH_2=), 4.50 (dd, $J_{5,6}$ 2.2, $J_{6,6'}$ 12.3, 1H, H-6), 4.39 (dd, $J_{6,6'}$ 12.3, $J_{5,6'}$ 2.4, 1H, H-6'), 4.25 (d, $J_{1,2}$ 7.6, 1H, H-1), 4.10 (bs, 3H, 3xOH), 3.84 (m, 1H, H-α), 3.50 (m, 5H, H-α, H-2, H-3, H-4, H-5), 2.42 (m, 4H, $2xCH_{2ester}$), 2.0 (m, 2H CH_2 allyl), 1.62 (m, 2H, CH_2 β)] which were separately acetylated with excess of acetic anhydride and triethylamine. Column chromatography (hexane/ethyl acetate 9:1) of the residue after acetylation of **4b** and solvent evaporation afforded compound **5** (180 mg, 32 %) as an oil $[\alpha]_D^{20}$ -20.0° (c: 0,06 , chloroform); IR ν $_{max}$: 2930, 1740, 1640, 1225, 1040 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data see table 1 and 2.

Anal. Calcd. for $C_{22}H_{32}O_{10}$: C, 57.89; H, 7.07. Found: C, 58.14; H, 7.23.

The same procedure after acetylation of **4a** gave compound **6** (124 mg, 20 %) as an oil $[\alpha]_D^{20}$ -4.9° (c: 0,32 , chloroform) ; IR ν $_{max}$: 2930, 1740, 1225, 1040 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data : see table 1 and 2.

Anal. Calcd. for $C_{25}H_{36}O_{10}$: C, 60.47; H, 7.31. Found: C, 60.49; H, 7.34.

1,3,4,6-tetra-O-acetyl-2-deoxy-2-(3'-buten-1'-yl-carbonylamino)-β-D-glucopy-ranose **8** : to the solution of Ph_3P (525 mg, 2 mmol), CCl_4 (0.19 mL, 2 mmol) and 4-pentenoic acid (0.204 mL, 2 mmol) in CH_2Cl_2 (10 mL) compound **7**^{9} (500 mg, 1.45 mmol) was added, and the mixture was stirred overnight at rt. Solvent evaporation and column chromatography (hexane/ethyl acetate/methanol 20:5:1) afforded compound **8** (368 mg, 60 %) as an oil $[\alpha]_D^{20}$ 3.6° (c: 1,22 , chloroform) ; IR ν $_{max}$: 3450, 2930, 1760, 1225, 1040 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data see : table 1 and 2.

Anal. Calcd. for $C_{19}H_{27}O_{10}N$: C, 54.14; H, 6.34; N, 3.26. Found: C, 54.43; H, 6.43; N, 2.89.

Pent-4-enyl 3,4,6-*tri-O-acetyl-2-deoxy-2-(3'-buten-1'-yl-carbonylamino)-α-D-glucopyranoside* **9** : to the solution of compound **8** (350 mg, 0.815 mmol) in DMF (10 mL) hydrazine acetate (92 mg, 1 mmol) was added. The resulting mixture was stirred 30 min. at 50°C. After extraction (CH_2Cl_2/H_2O), drying and solvent evaporation, the residue was added to the previously prepared solution of Ph_3P (525 mg, 2 mmol), CCl_4 (0.19 mL, 2 mmol) and 4-pentenoic acid (0.204 mL, 2 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred overnight at rt. Solvent evaporation and column chromatography (hexane/ethyl acetate 5:1) afforded compound **9** (260 mg, 64 %) as an oil $[\alpha]_D^{20}$ 77.2° (c: 4,4 , chloroform) ; IR ν $_{max}$: 3450, 2930, 1760, 1220, 1030 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data see table 1 and 2.

Anal. Calcd. for $C_{22}H_{31}O_{10}N$: C, 56.28; H, 6.66; N, 2.98. Found: C, 56.36; H, 6.52; N, 3.03.

Pent-4-enyl 2,3,4-tri-O-acetyl-6-O-(3'-buten-1',1'-di-methylcarbonyl-1'-yl-carbonyl)-β-D-glucopyranoside **11**: to the solution of compound **3** (500 mg, 1.2 mmol) in methanol (10 mL) catalytic amount of sodium methoxide was added. The resulting mixture was stirred at rt. 6 hrs. After neutralisation (acetic acid), and solvent evaporation, the residue was dissolved in CH_2Cl_2 (10 mL) and 2-(methylcarbonyl)-pent-4-enoic acid ethyl ester (340 mg, 2 mmol) and DMAP (10 mg) were added. The mixture was stirred 48 hrs under reflux. Solvent evaporation and column chromatography (hexane/ethyl acetate 4:1) afforded intermediate product: **10** [^{1}H NMR, δ: 5.80 (m, 3H, 3xCH=), 4.90 (m, 6H, 3xCH_2=), 4.48 (dd, $J_{6,6'}$ 12.3, $J_{6,5}$ nd, 1H, H-6), 4.36 (dd, $J_{6,6'}$ 12.3, $J_{6',5}$ nd, 1H, H-6'), 4.30 (d, $J_{1,2}$ 7.6, 1H, H-1), 4.10 (bs, 3H, 3xOH), 3.82 (m, 1H, H-α), 3.50 (m, 6H, H-α, H-2, H-3, H-4, H-5, CH_{alkyl}), 2.65 (m, 4H, 2xCH_{2ester}), 2.2 (s, 3H,CH_3CO), 2.0 (m, 2H, $CH_{2\ allyl}$), 1.62 (m, 2H, $CH_{2\ \beta}$)] which was acetylated with excess of acetic anhydride and triethylamine and catalytic amount of DMAP. Column chromatography (hexane/ethyl acetate 9:1) of the residue after acetylation and solvent evaporation afforded compound **11** (292 mg, 45 %) as an oil $[\alpha]_D^{20}$ 3.06° (c: 2,22 , chloroform) ; IR ν $_{max}$: 2930, 1740, 1620, 1225, 1040 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data : see table 1 and 2.

Anal. Calcd. for $C_{26}H_{36}O_{12}$: C, 57.77; H, 6.71. Found: C, 57.54; H, 6.43.

General procedure for metathesis reaction: to the solution of tetrachloro-bis-(2,6-diphenylphenoxy) tungsten (8.2 mg, 0.01 mmol) in dry chlorobenzene (2 mL) 2 eq. of $PbBu_4$ (6.9 mL) were added by syringe and the solution was stirred 15 min. at 80°C. The unsaturated substrate **5**, **6**, **9**, or **11** (~15 eq.), dissolved in chlorobenzene (5 mL), was added at the same temperature, and stirring was continued overnight. Products **12**, **13**, **14** and **15** were separated by column chromatography (hexane/ethyl acetate 9:1) after evaporation of solvent under reduced pressure. Experimental data for each product are presented in tables 1, 2, and 3.

Table 1: ^{1}H NMR Data for Substrates and Products

a: chemical shifts (δ ppm, in $CDCl_3$, TMS as internal standard, methyl groups signals from acetyl protecting groups omitted)

	Sugar unit							Side chain, or macrocycle						
Comp	H1	H2	H3	H4	H5	H6	H6	CH=	CH=	CH_2=	CH_2			
											α		β	γ
5	4.49	5.05	5.15	5.19	3.71	4.23	4.19	5.80		5.01	3.88	3.51	2.01	1.68
								5.80		5.01	2.45			
6	4.49	5.02	5.12	5.21	3.69	4.25	4.17	5.79		5.00	3.88	3.49	2.08	1.67
								5.79		5.00	2.37			
								5.79		5.00	2.37			
8	5.68	4.32	5.12	5.19	3.82	4.26	4.12	5.80		5.03	2.28			
9	6.20	4.49	5.09	5.22	4.00	4.24	4.06	5.81		5.15	2.24			
								5.81		5.07	2.30			
11	4.51	5.00	5.00	5.20	3.76	4.33	4.18	5.79		5.02	3.85	3.47	2.09	1.69
								5.80		5.02	3.02			
12	4.50	4.95	4.99	5.20	3.72	4.31	4.23	5.38	5.38		3.71	3.56	2.06	1.65
13	4.49	4.97	4.99	5.21	3.72	4.29	4.22	5.38	5.38		3.70	3.56	2.05	1.65
								5.77		5.05	2.38			
14	4.19	5.27	5.01	5.38	3.55	4.28	4.19	5.49	5.39		3.54	3.10	1.98	1.50
15	6.22	4.38	5.21	5.26	4.03	4.28	4.19	5.61	5.40		2.43			

b: coupling constants (Hz)

	Sugar unit						Side chain, or cycle		
Comp	J_{12}	J_{23}	J_{34}	J_{45}	J_{56}	J_{56}	$J_{CH=CH2}$	$J_{CH=CH}$	J_{NH-2}
5	7.9	9.5	9.5	9.3	2.4	4.6	17.0, 10.2		
							nd		
6	7.9	9.4	9.4	9.7	2.7	4.6	nd		
							nd		
							nd		
8	8.8	9.4	9.4	nd	1.3	4.8	nd		9.2
9	3.6	9.3	9.4	9.2	2.7	4.3	16.7, 10.3		8.6
							16.8, 10.2		
11	7.9	9.4	9.4	9.7	2.5	5.8	17.3, 10.2		
							16.9, 10.2		
12	7.9	9.2	9.2	9.0	3.3	6.9		nd	
13	7.9	9.4	9.3	9.4	3.3	6.8	16.9, 10.2	nd	
14	7.4	8.9	8.9	9.9	2.6	8.2		13.9	
15	3.7	10.4	nd	nd	2.2	4.0		15.1	8.9

Table 2: ^{13}C NMR Data for Substrates and Products

a: chemical schifts (δ ppm, TMS as internal standard, methyl groups and carbonyl carbons from acetyl groups omitted)

	Sugar unit						Side chain, or macrocycle					
Comp	C1	C2	C3	C4	C5	C6	CH=	CH=	CH_2=	CH_2		
										α	β	γ
5	100.9	71.5	73.0	68.6	71.9	62.0	137.9		115.2	69.4	29.9	28.7
							136.6		115.7	169.4	33.3	28.7
6	100.3	70.8	72.3	67.9	71.2	61.44	137.2		114.5	68.7	29.3	27.8
							136.0		115.0	168.8	32.7	28.1
							135.6		115.2	168.7	32.6	28.7
8	92.6	52.7	72.9	67.9	72.6	61.7	136.5		115.7	169.1	35.7	29.2
9	90.6	51.0	70.6	67.6	69.9	61.6	136.6		116.2	169.1	35.5	29.2
							136.3		115.8	170.7	33.4	28.7
11	100.7	71.4	72.9	69.0	71.8	62.7	137.8		115.1	69.0	29.9	28.5
							134.9		115.7	158.7	119.6	31.4
12	99.2	71.1	73.3	69.6	71.6	61.3	130.4	129.6		65.5	28.2	27.0
										169.5	34.3	27.3
13	99.2	71.1	73.1	69.6	71.5	61.3	130.4	129.6		65.5	28.2	27.0
										169.5	34.2	28.2
							136.3		115.7	169.5	33.2	27.3
14	99.3	71.0	73.2	69.8	71.6	62.5	130.6	127.6		66.1	27.1	26.8
										156.1	120.9	30.3
15	90.4	50.8	70.7	67.2	70.2	61.5	132.2	130.7		169.1	35.3	29.9
										170.7	34.4	27.8

Table 3: Results Obtained for Catalytic Metathesis Cyclizations of Carbohydrate-containing Substrates:

compound	yield	$[\alpha]_D$ $CHCl_3$		Elementary analysis							IR_{max}
					calcd			found			
N°	%	$[\alpha]_D$	c	formula	%C	%H	%N	%C	%H	%N	cm^{-1}
12	73	20.8	1.55	$C_{20}H_{28}O_{10}$	56.07	6.59	-	55.74	6.62	-	1745
13	68	14.5	0.85	$C_{23}H_{32}O_{10}$	58.97	6.88	-	59.31	6.79	-	1750
14	62	2.26	1.15	$C_{24}H_{32}O_{12}$	55.25	6.29	-	55.05	6.22	-	1750
15	56	29.2	2.95	$C_{20}H_{27}O_{10}N$	54.42	6.17	3.17	54.18	6.32	3.05	1750

Mass spectrometry

Sample preparation : The standard solutions used for LC-MS and LC-MS/MS were prepared with HPLC solvent grade methanol at a concentration of 50 pmol/µl. A 10 µl aliquot of sample was then introduced into the electrospray ion source by a continuous flow of acetonitrile : water (CH_3CN : H_2O, 1 : 1) at a flow rate of 20 µl/min using a Shimadzu LC-10 AD pump connected to the Rheodyne injector with a 20 µl loop.

Mass spectrometry conditions : The electrospray MS spectra (positive-ion mode) were recorded using a Fisons VG-Quattro quadrupole-hexapole-quadrupole mass spectrometer, equiped with an electrospray ionization source and capable of analyzing ions up to *m/z* 4000. A personal computer (486 -66 MHz processor) equipped with Fisons MASSLYNX mass spectrometry data system software, was used for data acquisition and processing. The temperature of the ES ionisation source was maintained at 75°C. The operating voltage of the ES capillary was 3.50 KV, the focus voltage was 50 V and the HV lens was at 0.50 KV throughout the whole operation. Conventional ES 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 3-4 scans. The mass scale was calibrated in the positive ion mode using a polyethylene glycol mixture. MS/MS experiments were conducted on the same instrument. Fragment ion spectra of mass-selected ions were induced by collision with argon in the second (RF only) hexapole. Argon collision gas was added in the enclosed chamber of the hexapole to give a pressure of 2×10^{-5} mbar for collisional activation of the sample ions. The resulting fragments were analyzed by the second quadrupole. Collision energies of approximately 50 eV and a focus voltage of 50 V were used in all MS/MS experiments. Precursor ion scan were obtained by scanning the first quadrupole while selecting a given *m/z* value with the second quadrupole.

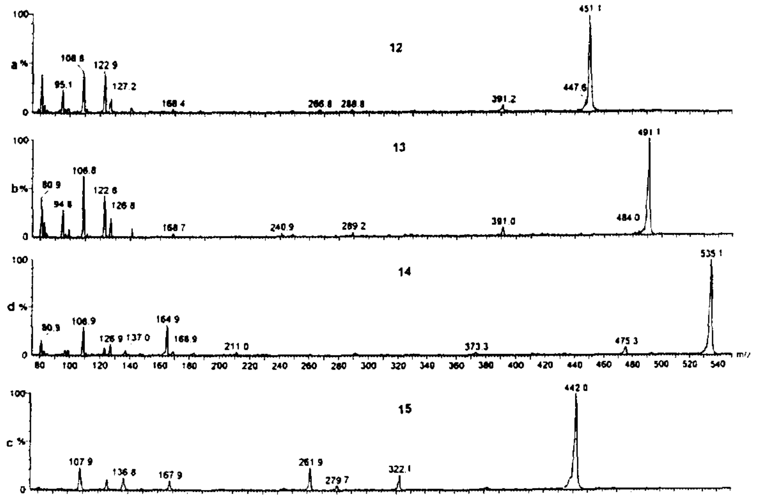


Figure 1 :

Low energy CAD MS/MS spectra of (a) the $[M+Na]^+$ ion of **12**, (b) the $[M+Na]^+$ ion of **13**, (c) the $[M+H]^+$ ion of **15** and (d) the $[M+Na]^+$ ion of **14**.

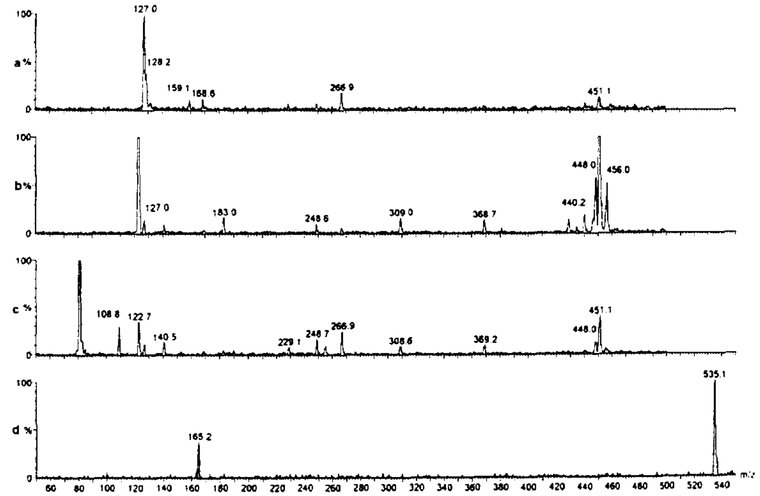


Figure 2 :

Precursor ion scan of (a) the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-CH_2-CH_2CO]^+$ ion at *m/z* 127 from **12**, (b) the $[M+H-2AcOH-CH_2CO-C_4H_4O_2-C_2H_4O_2]^+$ ion at *m/z* 123 from **12**, (c) the ion at *m/z* 81 from **12**, (d) the $[M+Na-2AcOH-2CH_2CO-C_4H_3O_2Na-C_2H_4O_2]^+$ ion at *m/z* 165 from **14**.

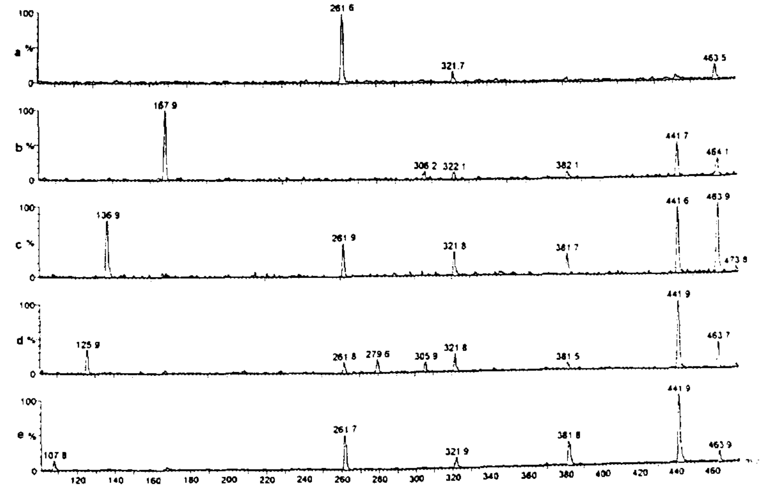


Figure 3 :

Precursor ion scan of (a) the $[M+H-3AcOH]^+$ ion at *m/z* 262 from **15**, (b) the $[M+H-C_8H_8O_2-2AcOH-H_2O]^+$ ion at *m/z* 168 from **15**, (c) the ion $[M+H-3AcOH-C_6H_7NO_2]^+$ at *m/z* 137 from **15**, (d) the $[M+H-2AcOH-CH_2CO-C_8H_8O_2-H_2O]^+$ ion at *m/z* 126 from **15**, (e) the $[M+H-C_8H_8O_2-2AcOH-H_2O-AcOH]^+$ ion at *m/z* 108 from **15**.

Table 4. Relative intensities (%) of the characteristic ions of compounds **12-15** observed by electrospray mass spectrometry.

Compound	Characteristic ion	m/z	(%)
12	[M+Na]+	451	100
	[M+Na-AcOH]$^+$	391	36
	[M+Na-2AcOH-CH$_2$CO]$^+$	289	6
	[M+H-2AcOH-CH$_2$CO]$^+$	267	5
	[M+H-3AcOH]$^+$	249	7
	[M+H-2AcOH-CH2CO-C4H4O2-CH2]+	169	8
	[M+H-2AcOH-CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$CO]$^+$	141	15
	[M+H-2AcOH-CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$-CH$_2$CO]$^+$	127	26
	[M+H-2AcOH-CH$_2$CO-C$_4$H$_4$O$_2$-C$_2$H$_4$O$_2$]$^+$	123	81
	[M+H-2AcOH-CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$-CH$_2$CO-H$_2$O]$^+$	109	
13	[M+Na]$^+$	491	100
	[M+Na-C$_5$H$_8$O$_2$]$^+$	391	16
	[M+Na-C$_5$H$_8$O$_2$-AcOH-CH$_2$CO-C$_4$H$_4$O$_2$-C$_2$H$_4$O$_2$]$^+$	123	10
14	[M+Na]$^+$	535	100
	[M+Na-AcOH]$^+$	475	25
	[M+Na-AcOH-CH$_2$CO]$^+$	433	4
	[M+Na-AcOH-CH$_2$CO-AcOH]$^+$	373	6
	[M+Na-2AcOH-2CH$_2$CO-C$_4$H$_3$O$_2$Na-C$_2$H$_4$O$_2$]$^+$	165	35
	[M+H-2AcOH-2CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$-CH$_2$CO]$^+$	127	16
	[M+Na-2AcOH-3CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$CO-H$_2$O]$^+$	123	8
	[M+H-2AcOH-3CH$_2$CO-C$_4$H$_4$O$_2$-CH$_2$-CH$_2$CO-H$_2$O]$^+$	109	34
15	[M+K]$^+$	480	12
	[M+Na]$^+$	464	50
	[M+H]$^+$	442	100
	[M+H-AcOH]$^+$	382	33
	[M+H-2AcOH]$^+$	322	30
	[M+H-3AcOH]$^+$	262	24
	[M+H-C$_8$H$_8$O$_2$-2AcOH-H$_2$O]$^+$	168	3
	[M+H-3AcOH-C$_6$H$_7$NO$_2$]$^+$	137	6
	[M+H-C$_8$H$_8$O$_2$-2AcOH-H$_2$O-AcOH]$^+$	108	2

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Acknowledgement : One of co-authors (J.R.) thanks Conseil Général du Rhône for granting the postdoctorate position.

(*Received in Belgium* 20 *December* 1994; *accepted* 1 *July* 1996)