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Differentiating Isomeric Deprotonated Glucuronide Drug Metabolites via Ion/Molecule Reactions in Tandem Mass Spectrometry

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Abstract

Isomeric *O*- and *N*-glucuronides are common drug metabolites produced in phase II of drug metabolism. Distinguishing these isomers by using common analytical techniques has proven challenging. A tandem mass spectrometric method based on gas-phase ion/molecule reactions of deprotonated glucuronide drug metabolites with trichlorosilane (HSiCl₃) in a linear quadrupole ion trap mass spectrometer is reported here to readily enable differentiation of the *O*- and *N*-isomers. The major product ion observed upon reactions of HSiCl₃ with deprotonated *N*-glucuronides is a diagnostic HSiCl₃ adduct that has lost two HCl molecules ([M – H + HSiCl₃ – 2HCl][–]). This product ion was not observed for deprotonated *O*-glucuronides. Reaction mechanisms were explored with quantum chemical calculations at the M06-2X/6-311++G(d,p) level of theory.

Graphical Abstract

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The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b02083.

Experimental details; table of studied glucuronides; ion/ molecule reaction MS/MS spectra; HPLC/MS/MS ion/ molecule reaction chromatograms and MS/MS spectra; computational data (PDF)

Glucuronidation is a common pathway of drug metabolism for xenobiotic drugs and endobiotic compounds.¹ Glucuronidation is catalyzed by the superfamily of uridine diphosphate glucuronosyl transferase (UGT) enzymes that transfer glucuronic acid from uridine 5'-diphosphoglucuronic acid (UDP-GlcA) to target substrates.² Glucuronidation occurs on functional groups that contain a nucleophilic *O*- or *N*-atom, such as amino and hydroxyl groups.³ Even parent drugs that do not possess such groups can have *O*- and/or *N*-atoms added through prior oxidative metabolism, making them susceptible to glucuronidation.^{4,5} When a parent drug contains both *O*- and *N*-heteroatoms, glucuronidation can occur at either site, which can produce isomeric glucuronide metabolites. For instance, carvedilol (**1**), used for treatment of hypertension, angina, and congestive heart failure, contains three sites susceptible to glucuronidation (**2–4**) (Scheme 1).^{2,5}

More than 20% of current drugs are glucuronidated by UGTs to produce water-soluble glucuronide metabolites that are more easily excreted in bile or urine.⁶ Glucuronidation shortens the half-life of drugs,⁷ which is often compensated for by administering a higher dosage of the drug. This, however, can lead to undesirable side effects.⁸ Knowing the glucuronidation site allows targeted chemical modifications of the drug to prohibit glucuronidation, thereby improving the efficacy of the drug.⁸ The various approaches appearing in the literature for the identification of the glucuronidation sites of drugs are limited in scope and practicality. For example, selective acetylation of the hydroxyl and/or amino groups within a glucuronide has been explored for the identification of the site of glucuronidation based on the number of acetyl groups added.² However, this approach, which requires timeconsuming isolation and derivatization of the metabolite, has only proven reliable for carvedilol.⁵ In another study, the pH stability of ¹⁴C-labeled glucuronides was used to differentiate *O*- and *N*-glucuronides.^{4,9} Although this method is simple, the extensive modification of glucuronides by radiolabeling prevents its application to high-throughput analysis. While NMR is the gold standard for elucidating the structures of organic molecules, it requires high quantities of relatively pure compounds and therefore is not suitable for elucidating the structures of trace compounds in complex metabolite mixtures.¹⁰

Mass spectrometry (MS) combined with chromatography is a powerful approach for identifying minor components of complex mixtures. However, electron ionization mass

spectrometry often requires authentic compounds for unambiguous identification and even then sometimes fails to differentiate isomeric compounds. The same is true for tandem mass spectrometry (MS/MS) based on collision-activated dissociation (CAD). For example, the major fragmentation pathway for many positively and negatively charged glucuronides corresponds to the elimination of the glucuronyl moiety, which hinders the differentiation of isomeric glucuronides.^{2,11} In some cases, the CAD mass spectra of ionized isomeric glucuronides are essentially identical, as for example, those of deprotonated carvedilol *N*- and *O*-glucuronides shown in Figure 1. Another example of uninformative CAD mass spectra is shown in Figure S1 for deprotonated carvedilol isomers. On the other hand, gas-phase ion/molecule reactions have been successfully utilized in MS/MS experiments to elucidate the structures of many isomeric compounds that cannot be differentiated by CAD,^{12–29} including drug metabolites. We report here the discovery of a diagnostic reaction between trichlorosilane (HSiCl₃) and deprotonated *N*-glucuronides that can be used for the unambiguous differentiation of *N*- and *O*-glucuronides.

EXPERIMENTAL SECTION

Reagents and Materials

Darunavir *O*- β -D-glucuronide was purchased from Sussex Research. 4-Nitrophenol *O*- β -D-glucuronide and trichlorosilane were purchased from SigmaAldrich. The remaining *O*- and *N*-glucuronides were purchased from Toronto Research Chemicals. All purchased chemicals were used as received.

Ion/Molecule Reactions

The experiments were carried out in a Thermo Scientific linear quadrupole ion trap (LQIT) mass spectrometer modified with an external reagent mixing manifold.¹⁸ The studied glucuronides were ionized by negative mode electrospray ionization (ESI). The deprotonated glucuronides were isolated in the ion trap with an isolation width of 2 mass units and allowed to react with HSiCl₃ for 30–100 ms. SiHCl₃ was introduced into the ion trap via the external reagent mixing manifold at a flow rate 3 μ L/hour. No harmful effects to the instrumentation have been observed, likely because of the very small amount of the reagent introduced. A detailed description of the instrumentation used here for performing ion/molecule reactions can be found in the literature.¹⁵

Quantum Chemical Calculations

All density functional calculations were performed at the M06-2X/6-311++G(d,p) level of theory by using the Gaussian 09 program.^{30,31} All transition state structures were confirmed to possess exactly one negative eigenvalue corresponding to the reaction coordinate. Intrinsic reaction coordinate (IRC) calculations were performed for all transition states. The free energies used to construct the potential energy surfaces were calculated using ideal gas statistical mechanics.

HPLC/MS/MS

HPLC/MS/MS experiments were performed on a Thermo Surveyor HPLC coupled to an LQIT. The samples were injected via an autosampler with full-loop injection (25 μ L). The

mobile phases used were water (A) and methanol (B), both containing 0.1% formic acid. The column used was an Agilent ZORBAX SB-C18 5 μ m, 4.6 $\times$ 250 mm column. The eluate was subsequently ionized by ESI in negative ion mode, and the selected ions were isolated and allowed to react with the reagent for 30 ms.

RESULTS AND DISCUSSION

Reactions of all deprotonated *O*-glucuronides and some *N*-glucuronides generate an HSiCl_3 adduct that has lost one HCl molecule ($[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$) as a primary product ion (Table 1 and Figures S1–S17). However, only deprotonated *N*-glucuronides yield a diagnostic dominant HSiCl_3 adduct that has lost two HCl molecules ($[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$). This reaction allows the differentiation of *O*- and *N*-glucuronides. For example, deprotonated carvedilol *N'*- β -D-glucuronide formed the diagnostic product ion $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ while its *O*-glucuronide isomer did not (Figure 1). The *N*-glucuronide also formed a product ion $[\text{M} - \text{H} + \text{HSiCl}_3 - 3\text{HCl}]^-$, which is likely to be a dissociation product of the $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion (for reaction kinetics, see Figure S24). Therefore, this product ion may also be diagnostic for *N*-glucuronides.

Quantum chemical calculations were performed on simple model compounds to obtain insights into the mechanisms of the reactions of HSiCl_3 with deprotonated glucuronides. In these model compounds, the complex drug moiety was replaced by an *O*-methyl (for *O*-glucuronides) or *N*-methyl moiety (for *N*-glucuronides) to obtain representative results within a reasonable amount of computation time. On the basis of the calculations, the reactions are initiated by binding of HSiCl_3 to the carboxylate group of the deprotonated glucuronic acid, generating a covalently bound pentacoordinated silicon anion that is in close proximity to the 4-OH group (Figure 2). The electron withdrawing nature of the chloro-substituents in HSiCl_3 enhances the electrophilicity of the silicon atom, which promotes hypervalency and also enhances the reactivity of the Si–Cl bonds.^{32–34} In the second step, a chloride anion cleaves off from the silicon atom and forms a hydrogen bond with the 4-OH group (as indicated by O in Figure 2) of the *O*- or *N*-methylglucuronide, concerted with formation of a Si–O bond. Elimination of an HCl molecule yields the ionic product $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$ (Figure 2).

Calculations further suggest that the formation of the diagnostic product ion $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ for deprotonated secondary *N*-glucuronides can be explained by an alternative pathway that is in competition with the formation of the primary product ion $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$. This new pathway begins with adduct formation in a conformation wherein the silicon atom is in close proximity with the ring oxygen (as opposed to the 4-OH), as shown in the blue pathway in Figure 3 (this conformation is more stable than that shown in Figure 2). This is followed by the breaking of a Si–Cl bond followed by bond formation between the chloride anion and the hydrogen atom bound to the anomeric nitrogen of the *N*-glucuronides (Figure 3). This transfer does not occur for *O*-glucuronides, since they do not have a hydrogen attached to the anomeric oxygen, but it may occur for glucuronic acid, because it has a hydrogen at the anomeric oxygen, as shown in Figure 3 (black pathway).

Hydrogen chloride is then eliminated in concert with opening of the ring and formation of two bonds, a C=N bond at the anomeric position (or a C=O bond in the case of glucuronic acid) and an O–Si bond with the endocyclic oxygen (Figure 3). The flexibility afforded by the ring-opening enables a hydroxyl group to add to the silicon atom. According to calculations, the kinetically most favorable attack involves the O2 atom (as indicated by a red O in Figure 3). This leads to a concerted breaking of a Si–Cl bond and formation of a Si–O bond, which accounts for the loss of the second HCl molecule. The importance of ring-opening in this pathway is substantiated by the fact that underivatized deprotonated glucuronic acid also produces the characteristic $[M - H + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion upon reactions with HSiCl_3 , since deprotonated glucuronic acid can undergo ring-opening via the same mechanism as deprotonated *N*-glucuronides (Figure 3). It is important to note here that the mere presence of a second protic nucleophilic group is not enough to cause the second loss of HCl in the pathways shown in Figure 3. Even for deprotonated *O*-glucuronides with nearby protic nucleophilic groups (e.g., 2-aminophenol, dopamine, and carvedilol *O*-glucuronides, Table 1), no $[M - H + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion was observed.

A deviation from the above behavior was observed for deprotonated tertiary *N*-glucuronides. They do not have a hydrogen atom bound to the anomeric nitrogen atom, but they nevertheless form the characteristic $[M - H + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion. Calculations suggest that tertiary *N*-glucuronides can form this diagnostic product through a four-step reaction pathway (Figure 4, black trace). After the deprotonated carboxylic acid moiety is added to the silicon atom, a chloride anion migrates and forms a hydrogen bond with the hydrogen atom at the 2-OH group. This transition corresponds to the highest-energy transition state. The positioning of the chloride anion facilitates ring-opening, as it stabilizes the resulting iminium cation via a loose interaction with the partially positively charged carbon. This ring-opening occurs in concert with formation of a Si–O bond between Si and the ether oxygen. Rotation of the molecule brings the chloride ion in proximity to the 4-OH group, and the oxygen atom of this group adds to the iminium carbon, which leads to furanosyl cyclization and loss of the first HCl. The second HCl is lost in the same manner as the second loss of HCl in the pathways of Figure 3—nucleophilic attack of a hydroxyl to the silica to form a bicyclic trioxosilane.

To account for the lack of formation of $[M - H + \text{HSiCl}_3 - 2\text{HCl}]^-$ in reactions of deprotonated *O*-glucuronides with HSiCl_3 , calculations analogous to those discussed above were carried out for deprotonated methyl *O*-glucuronide (Figure 4, red trace). This ion is able to readily proceed through the first two steps of the reaction pathway discussed above. However, the energy of the transition state for the furanosyl cyclization is much higher than for the deprotonated *N*-glucuronide. This is accounted for by the fact that the chloride forms a covalent bond with the anomeric carbon during the ring-opening step. However, the transition state is still below the total energy level of the system. Indeed, when deprotonated methyl-*O*-glucuronide was allowed to react with HSiCl_3 , it showed the product ion $[M - H + \text{HSiCl}_3 - 2\text{HCl}]^-$ expected to be diagnostic only for *N*-glucuronides. Therefore, a model compound more representative of drug glucuronides, deprotonated phenyl-*O*-glucuronide, was examined. The barrier calculated for the furanosyl cyclization of this compound was found to be 7.2 kcal/mol *above* the energy level of the system, which prevents this

compound from forming the product ion of interest. Steric hindrance caused by the phenyl group is the likely cause for the high transition state energy. The same situation is expected to apply to real drug glucuronides, as none of them are as simple as methyl *O*-glucuronide. Steric hindrance will have a less profound effect on *N*-glucuronides than on *O*-glucuronides, since the barriers for furanosyl cyclization are much lower (e.g., -20.3 kcal/mol for dimethyl *N*-glucuronide compared to -2.3 for methyl *O*-glucuronide). Thus, no false positive results are expected due to *O*-glucuronides other than methyl *O*-glucuronide.

Quaternary *N*-glucuronides are special cases that are generally not ionized in negative ion mode because of their cationic nature. However, when doped with ammonium hydroxide, they generate a triply charged ion, overall with one negative charge (Scheme 2). This ion was found to react with HSiCl_3 to form the $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$ product ion (Scheme 2).

The absence of the $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion diagnostic for *N*-glucuronides supports the proposed mechanism involving ring-opening, as quaternary *N*-glucuronides cannot undergo ring-opening. Fortunately, quaternary *N*-glucuronides can be distinguished from other *O*- and *N*-glucuronides by being easily ionized in positive ion mode but not in negative ion mode without a base dopant.

High-Throughput HPLC/MS/MS/Ion/Molecule Reactions

To demonstrate the practicality of above analytical approach, an HPLC/LQIT was equipped with a reagent mixing manifold to carry out HPLC/MS/MS experiments based on ion/molecule reactions. A mixture of six glucuronidated drug metabolites, consisting of two pairs of isomers, carvedilol and darunavir *O*- and *N*- β -D-glucuronides as well as acetaminophen *O*- β -D-glucuronide and dapsone *N*- β -D-glucuronide, were separated by reversed-phase HPLC (Figure 5). The observation of glucuronic acid (eluted at 5.5 min) is likely a result of hydrolysis of the *N*-glucuronides, which is known to occur under acidic conditions.⁴ The eluted compounds were ionized by ESI in negative ion mode in the LQIT, isolated in the ion trap and allowed to react with HSiCl_3 for 30 ms. The formation or absence of the $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ions diagnostic of *N*-glucuronides, as well as the $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$ ions formed for both *O*- and *N*-glucuronides, was monitored. Each analyte reacted as expected on the basis of the pure model compound studies. As carvedilol is a racemic mixture, two peaks eluting at 29.2 and 30.8 min were observed. Notably, carvedilol *N'*- β -D glucuronide was found in the valley between these two peaks. More remarkably, peaks corresponding to darunavir *O*- and *N*- β -D-glucuronides were unambiguously identified even though the HPLC resolution was not high enough to resolve the two peaks.

CONCLUSIONS

Unambiguous differentiation of *O*- and *N*-glucuronides was achieved by gas-phase ion/molecule reactions of deprotonated glucuronides with HSiCl_3 in a quadrupole ion trap mass spectrometer. The diagnostic $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ion was observed for deprotonated *secondary* and *tertiary* *N*-glucuronides. Several mechanistic pathways that underlie the formation of the diagnostic product ion were identified via quantum chemical

calculations. Coupling of this ion/molecule reaction MS/MS experiment with HPLC demonstrates the practicality of this approach in high-throughput analysis of complex metabolite mixtures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Tephly TR; Green MD; Coffman BL; King C; Cheng Z; Rios G *Adv. Pharmacol* 1997, 42, 343–346.
- (2). Schaefer WH; Goalwin A; Dixon F; Hwang B; Killmer L; Kuo G *Biol. Mass Spectrom* 1992, 21 (4), 179–188.
- (3). Jancova P; Anzenbacher P; Anzenbacherova E *Biomed. Pap* 2010, 154 (2), 103–116.
- (4). Babu SR; Lakshmi VM; Huang GP-W; Zenser TV; Davis BB *Biochem. Pharmacol* 1996, 51 (12), 1679–1685. [PubMed: 8687483]
- (5). Schaefer WH; Politowski J; Hwang B; Dixon F Jr; Goalwin A; Gutzait L; Anderson K; DeBrosse C; Bean M; Rhodes GR *Drug Metab. Dispos.* 1998, 26 (10), 958–969. [PubMed: 9763400]
- (6). Fujiwara R; Yoda E; Tukey RH *Drug Metab. Pharmacokinet* 2018, 33 (1), 9–16. [PubMed: 29079228]
- (7). Blum MR; Liao SH; Good SS; de Miranda P *Am. J. Med* 1988, 85 (2A), 189–194.
- (8). Basu NK; Kole L; Basu M; McDonagh AF; Owens IS *Biochem. Biophys. Res. Commun* 2007, 360 (1), 7–13. [PubMed: 17586469]
- (9). Ciotti M; Lakshmi VM; Basu N; Davis BB; Owens IS; Zenser TV *Carcinogenesis* 1999, 20 (10), 1963–1969. [PubMed: 10506112]
- (10). Spraul M; Freund AS; Nast RE; Withers RS; Maas WE; Corcoran O *Anal. Chem* 2003, 75 (6), 1536–1541. [PubMed: 12659219]
- (11). Shimizu A; Ohe T; Chiba M *Drug Metab. Dispos* 2012, 40 (8), 1456–1459. [PubMed: 22611068]
- (12). Watkins MA; Price JM; Winger BE; Kenttamaa HI *Anal. Chem* 2004, 76 (4), 964–976. [PubMed: 14961727]
- (13). Campbell KM; Watkins MA; Li S; Fiddler MN; Winger B; Kenttamaa HI *J. Org. Chem* 2007, 72 (9), 3159–3165. [PubMed: 17397220]
- (14). Duan P; Gillespie TA; Winger BE; Kenttamaa HI *J. Org. Chem* 2008, 73 (13), 4888–4894. [PubMed: 18522416]
- (15). Habicht SC; Vinuesa NR; Archibold EF; Duan P; Kenttamaa HI *Anal. Chem* 2008, 80 (9), 3416–3421. [PubMed: 18363408]
- (16). Fu M; Duan P; Li S; Eismann RJ; Kenttamaa HI *J. Am. Soc. Mass Spectrom* 2009, 20 (7), 1251–1262. [PubMed: 19345113]
- (17). Somuramasami J; Winger BE; Gillespie TA; Kenttamaa HI *J. Am. Soc. Mass Spectrom* 2010, 21 (5), 773–784. [PubMed: 20189411]
- (18). Habicht SC; Vinuesa NR; Amundson LM; Kenttamaa HI *J. Am. Soc. Mass Spectrom* 2011, 22 (3), 520–530. [PubMed: 21472570]
- (19). Fu M; Duan P; Gao J; Kenttamaa HI *Analyst* 2012, 137 (24), 5720–5722. [PubMed: 23115775]
- (20). Jarrell T; Riedeman J; Carlsen M; Replogle R; Selby T; Kenttamaa H *Anal. Chem* 2014, 86 (13), 6533–6539. [PubMed: 24897424]

- (21). Sheng H; Williams PE; Tang W; Zhang M; Kenttamaa HI. *Analyst* 2014, 139 (17), 4296–4302. [PubMed: 24968187]
- (22). Tang W; Sheng H; Kong JY; Yerabolu R; Zhu H; Max J; Zhang M; Kenttamaa HIRapid Commun. Mass Spectrom 2016, 30 (12), 1435–1441. [PubMed: 27197036]
- (23). Brodbelt JS *Mass Spectrom. Rev* 1997, 16 (2), 91–110.
- (24). Gronert S *Chem. Rev* 2001, 101 (2), 329–360. [PubMed: 11712250]
- (25). O’Hair RAJ; Freitas MA; Gronert S; Schmidt JAR; Williams TD J. *Org. Chem* 1995, 60 (7), 1990–1998.
- (26). Lam AKY; Li C; Khairallah G; Kirk BB; Blanksby SJ; Trevitt AJ; Wille U; O’Hair RAJ; da Silva G *Phys. Chem. Chem. Phys* 2012, 14 (7), 2417–2426. [PubMed: 22249506]
- (27). Reid GE; Tichy SE; Pérez J; O’Hair RAJ; Simpson RJ; Kenttamaa HI J. *Am. Chem. Soc* 2001, 123 (6), 1184–1192. [PubMed: 11456672]
- (28). Thomas MC; Mitchell TW; Harman DG; Deeley JM; Murphy RC; Blanksby S J. *Anal. Chem* 2007, 79 (13), 5013–5022.
- (29). Thomas MC; Mitchell TW; Harman DG; Deeley JM; Nealon JR; Blanksby S J. *Anal. Chem* 2008, 80 (1), 303–311.
- (30). Frisch MJ; Trucks GW; Schlegel HB; Scuseria GE; Robb MA; Cheeseman JR; Scalmani G; Barone V; Mennucci B; Petersson GA; Nakatsuji H; Caricato M; Li X; Hratchian HP; Izmaylov AF; Bloino J; Zheng G; Sonnenberg JL; Hada M; Ehara M; Toyota K; Fukuda R; Hasegawa J; Ishida M; Nakajima T; Honda Y; Kitao O; Nakai H; Vreven T; Montgomery JA Jr.; Peralta JE; Ogliaro F; Bearpark M; Heyd JJ; Brothers E; Kudin KN; Staroverov VN; Kobayashi R; Normand J; Raghavachari K; Rendell A; Burant JC; Iyengar SS; Tomasi J; Cossi M; Rega N; Millam JM; Klene M; Knox JE; Cross JB; Bakken V; Adamo C; Jaramillo J; Gomperts R; Stratmann RE; Yazyev O; Austin AJ; Cammi R; Pomelli C; Ochterski JW; Martin RL; Morokuma K; Zakrzewski VG; Voth GA; Salvador P; Dannenberg JJ; Dapprich S; Daniels AD; Farkas O; Foresman JB; Ortiz JV; Cioslowski J; Fox DJ *Gaussian 09*, revision A.02; Gaussian, Inc.: Wallingford, CT, 2009.
- (31). Zhao Y; Truhlar DG *Theor. Chem. Acc* 2008, 120 (1–3), 215–241.
- (32). Wilhite DL; Spialter L J. *Am. Chem. Soc* 1973, 95 (7), 2100–2104.
- (33). Buckner SW; Gord JR; Freiser BS J. *Am. Chem. Soc* 1988, 110 (20), 6606–6612.
- (34). Pestunovich VA; Kirpichenko SV; Lazareva NF; Albanov AI; Voronkov MG J. *Organomet. Chem* 2007, 692 (11), 2160–2167.

Figure 1.

Mass spectra measured after 30 ms reaction of deprotonated carvedilol O-β-D-glucuronide (top) and carvedilol N'-β-D-glucuronide (bottom) with HSiCl₃. The chlorine isotopes support the identification of the products. As a result of the existence of trace levels of water in the ion trap, the primary product ion $[M - H + \text{HSiCl}_3 - \text{HCl}]^-$ is sometimes partially hydrolyzed to form a secondary product ion $[M - H + \text{HSiCl}_3 - \text{HCl} - \text{Cl} + \text{OH}]^-$ (indicated above as *Hydrolysis). Also, a simple HCl adduct ($[M - H + \text{HCl}]^-$) is sometimes formed due to the generation of HCl via decomposition of HSiCl₃ upon reactions with water in the ion trap. Inserts show the CAD mass spectra of isolated deprotonated carvedilol O-β-D-glucuronide (top) and N'-β-D-glucuronide (bottom).

Figure 2.

Calculated free energy surfaces for the reactions of HSiCl_3 with deprotonated glucuronic acid (black) and *O*- (red) and *N*-glucuronides (blue) via addition followed by elimination of one HCl molecule containing the hydrogen atom from the O4 position (indicated by O). Calculations were performed at the M06-2X/6-311++G(d,p)//M06-2X/6-311++G(d,p) level of theory; free energies (in kcal/mol) are relative to the deprotonated analyte and HSiCl_3 .

Figure 3.

Calculated free energy surfaces for the reactions of HSiCl_3 with deprotonated glucuronic acid (black) and deprotonated methyl *N*-glucuronide (blue) via addition followed by elimination of two HCl molecules (note that the product complex is not shown). Calculations were performed at the M06-2X/6-311++G(d,p) level of theory; free energies (in kcal/mol) are relative to the deprotonated analyte and HSiCl_3 .

Figure 4.

Calculated free energy surfaces for the reactions of HSiCl₃ with deprotonated dimethyl *N*-glucuronide (black) and deprotonated methyl *O*-glucuronide (red) via addition followed by elimination of two HCl molecules (note that the product complex is not shown).

Calculations were performed at the M06-2X/6-311++G(d,p) level of theory; free energies (in kcal/mol) are relative to the deprotonated analyte and HSiCl₃.

Figure 5.

HPLC/MS/MS chromatogram of 10–20 μM mixture of six model compounds, including one racemic mixture. HPLC analysis was performed using an analytical C18 column (4.6×250 mm) and gradient elution (solution A: 0.1% formic acid in water; solution B: 0.1% formic acid in methanol; flow rate: 4 mL min^{-1} ; B%: 5–80% within 40 min). The eluates were ionized by using ESI in negative ion mode and allowed to react with HSiCl_3 in the ion trap. The formation of the $[\text{M} - \text{H} + \text{HSiCl}_3 - 2\text{HCl}]^-$ product ions diagnostic for *N*-glucuronides and $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$ product ions formed for both *O*- and *N*-glucuronides were monitored as a function of time. In the chromatogram, the peaks corresponding to analyte compounds that generated the diagnostic product ions are colored blue and those that generated $[\text{M} - \text{H} + \text{HSiCl}_3 - \text{HCl}]^-$ product ions but not the diagnostic ions are colored red. Glucuronic acid was formed upon hydrolysis of *N*-glucuronides.

Scheme 1.

Carvedilol (1) and Its Possible *O*-Glucuronide (2) and *N*-Glucuronide (3,4) Metabolites

Scheme 2.

No Electron Lone Pairs on Nitrogen in Cationic Quaternary *N*-Glucuronides To Facilitate Ring-Opening

Table 1.

Primary Product Ions and Their Branching Ratios^a along with Their Observed Secondary Product Ions for Reactions of Deprotonated *O*- and *N*-Glucuronides and Glucuronic Acid with HSiCl₃

<i>O</i> -Glucuronides (<i>m/z</i> of deprotonated analyte)	1° ionic reaction products (<i>m/z</i> and their branching ratios)	2° ionic reaction products
2-Aminophenol <i>O</i> -β-D-glucuronide (284)	$\frac{[M+H+HCl]^+ (320)}{[M+H+HSiCl_3+HCl]^+ (382)} = \frac{29\%}{71\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HSiCl_3+2HCl]^+ (455)}{[M+H+HSiCl_3+HCl]^+ (491)} = \frac{94\%}{6\%}$
Dopamine 4- <i>O</i> -β-D-glucuronide (328)	$\frac{[M+H+HCl]^+ (364)}{[M+H+HSiCl_3+HCl]^+ (426)} = \frac{10\%}{90\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HCl]^+ (459)}{[M+H+HSiCl_3+2HCl]^+ (485)} = \frac{2\%}{97\%}$ $\frac{[M+H+HSiCl_3+HCl]^+ (521)}{[M+H+HSiCl_3+HCl]^+ (521)} = \frac{1\%}{1\%}$
Propafenone <i>O</i> -β-D-glucuronide (516)	$\frac{[M+H+HCl]^+ (552)}{[M+H+HSiCl_3+HCl]^+ (614)} = \frac{32\%}{68\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HCl]^+ (382)}{[M+H+HSiCl_3+2HCl]^+ (408)} = \frac{25\%}{53\%}$ $\frac{[M+H+HSiCl_3+HCl]^+ (444)}{[M+H+HSiCl_3+HCl]^+ (444)} = \frac{22\%}{22\%}$ Hydrolysis ^a (2°)
Raloxifene 4- <i>O</i> -β-D-glucuronide (648)	$\frac{[M+H+HCl]^+ (683)}{[M+H+HSiCl_3+HCl]^+ (746)} = \frac{22\%}{78\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HCl]^+ (434)}{[M+H+HSiCl_3+2HCl]^+ (461)} = \frac{1\%}{99\%}$
Ezetimibe <i>O</i> -β-D-glucuronide (584)	$\frac{[M+H+HCl]^+ (619)}{[M+H+HSiCl_3+HCl]^+ (682)} = \frac{13\%}{87\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HCl]^+ (513)}{[M+H+HSiCl_3+2HCl]^+ (540)} = \frac{1\%}{99\%}$
Carvedilol <i>O</i> -β-D-glucuronide (581)	$\frac{[M+H+HCl]^+ (617)}{[M+H+HSiCl_3+HCl]^+ (679)} = \frac{22\%}{78\%}$ Hydrolysis ^a (2°)	$\frac{[M+H+HCl]^+ (617)}{[M+H+HSiCl_3+2HCl]^+ (643)} = \frac{5\%}{71\%}$ $\frac{[M+H+HSiCl_3+HCl]^+ (679)}{[M+H+HSiCl_3+HCl]^+ (679)} = \frac{25\%}{25\%}$ $\frac{[M+H+HSiCl_3+3HCl]^+ (72)}{[M+H+HSiCl_3+3HCl]^+ (72)} = \frac{1\%}{1\%}$

O-Glucuronides (<i>m/z</i> of deprotonated analyte)	1° ionic reaction products (<i>m/z</i> and their branching ratios)		<i>N</i> -Glucuronides (<i>m/z</i> of deprotonated analyte)	2° ionic reaction products	
	1° ionic reaction products (<i>m/z</i> and their branching ratios)			2° ionic reaction products
Darunavir <i>O</i> -β- <i>D</i> -glucuronide (722)	Darunavir <i>O</i> -β- <i>D</i> -glucuronide (722)	$[M+H+HCl]^+$ (758) 1% $[M+H+HSCl_2+HCl]^+$ (820) 99% Hydrolysis ^a (2°)	Darunavir <i>N</i> -β- <i>D</i> -glucuronide (722)	$[M+H+HSCl_2+2HCl]^+$ (784) 75% $[M+H+HSCl_2+HCl]^+$ (820) 25% $[M+H+HSCl_2+3HCl]^+$ (2°)
4-Hydroxyamoxifen <i>O</i> -β- <i>D</i> -glucuronide (582)	4-Hydroxyamoxifen <i>O</i> -β- <i>D</i> -glucuronide (582)	$[M+H+HCl]^+$ (598) 74% $[M+H+HSCl_2+HCl]^+$ (660) 26% Hydrolysis ^a (2°)	4-Hydroxyamoxifen <i>N</i> -β- <i>D</i> -glucuronide (582)	$[M+H+HCl]^+$ (598) 59% $[M+H+HSCl_2+HCl]^+$ (660) 41% Hydrolysis ^a (2°)
Morphine 6- <i>O</i> -β- <i>D</i> -glucuronide (460)	Morphine 6- <i>O</i> -β- <i>D</i> -glucuronide (460)	$[M+H+HCl]^+$ (496) 36% $[M+H+HSCl_2+HCl]^+$ (558) 64% Hydrolysis ^a (2°)	Glucuronic acid (193)	$[M+H+HSCl_2+2HCl]^+$ (555) 98% $[M+H+HSCl_2+HCl]^+$ (291) 2% $[M+H+HSCl_2+3HCl]^+$ (2°)	

^a Branching ratios were obtained after reaction with HSiCl₃ for 100 ms.

^b Partial hydrolysis of [M – H + HSiCl₃ – HCl][–] forms [M – H + HSiCl₃ – HCl – Cl + OH][–].

^c Doped with a base to promote deprotonation.