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**Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethan-amine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) in human and rat urine by GC-MS, LC-MSn, and LC-HR-MS/MS approaches.**

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# Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) in human and rat urine by GC-MS, LC-MS$^n$, and LC-HR-MS/MS approaches

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ABSTRACT

25B-NBOMe and 25C-NBOMe are potent 5-$HT_{2A}$ receptor agonists that have been associated with inducing hallucinogenic effects in drug users and severe intoxications. This paper describes the identification of their metabolites in rat and human urine by liquid chromatography (LC)-high resolution (HR)-MS/MS, the comparison of metabolite formation in vitro and in vivo and in different species, the general involvement of human cytochrome-P450 (CYP) isoenzymes on their metabolism steps, and their detectability by standard urine screening approaches (SUSAs) using GC-MS, LC-$MS^n$, or LC-HR-MS/MS. Both NBOMe derivatives were mainly metabolized by *O*-demethylation, *O,O*-bis-demethylation, hydroxylation, and combinations as well as by glucuronidation and sulfation of the main phase I metabolites. For 25B-NBOMe, 66 metabolites could be identified and 69 for 25C-NBOMe. After application of low doses of both substances to rats, they were detectable mainly via their metabolites by both LC-based SUSAs. In case of acute intoxication, it was possible to detect 25B-NBOMe and its metabolites in an authentic human urine sample when using the GC-MS SUSA in addition to the LC-based SUSAs. Initial CYP activity screening revealed the involvement of CYP1A2 and CYP3A4 in hydroxylation and CYP2C9 and CYP2C19 in *O*-demethylation. The presented study demonstrated that 25B-NBOMe and 25C-NBOMe were extensively metabolized and detectable by both LC-based SUSAs.

*Keywords:*

25B-NBOMe

25C-NBOMe

new psychoactive substance

metabolism

cytochrome-P450

LC-$MS^n$

LC-HR-MS/MS

## 1. Introduction

According to annual drug reports published by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and United Nations Office on Drugs and Crime (UNODC) [1-4], the availability and abuse of new psychoactive substances (NPS) increased during the last few years. Besides synthetic cannabinoids, cathinones, opioids, and tryptamines, the group of phenethylamines gained more importance in the last years [5]. Among others, the so-called 2C-type phenethylamines have been a constant feature in the detection of NPS [6]. They were first described by Alexander Shulgin in his book PIHKAL [7]. Like many phenethylamines, they have powerful psychoactive and stimulating effects [7,8]. Although many of them have been scheduled, new and uncontrolled alternatives have emerged. Structure-activity relationship studies revealed that derivatization of the primary amine of the 2C partial structure with a 2-methoxybenzyl substituent significantly increased the affinity toward the serotonin 5-$HT_{2A}$ receptor, thus, mediating potent hallucinogenic effects [9-12]. The resulting 2C derivatives, the so-called NBOMes (*N*-2-methoxybenzyl phenethylamines), represent a new group of potent phenethylamine hallucinogens with high abuse potential. 2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxy¬phenyl)methyl]ethanamine (25B-NBOMe, 2C-B-NBOMe), 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxy¬phenyl)methyl]ethanamine (25C-NBOMe, 2C-C-NBOMe), and 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine, 25I-NBOMe, 2C-I-NBOMe) are among the most prevalent NBOMes. They are consumed depending on desired effects in reported dosages between 200-1,000 µg, administered orally, sublingually, bucally or insufflated as powder or in solution as nose spray [8,13-20]. In recent years, NBOMe consumption was described in the context of acute and severe intoxications and fatalities [8,14-18,21,22]. In some cases, an unintentional intake of NBOMes, sold as LSD or 2Cs, were found to be responsible for adverse events [8,15,19,22]. However, 25B-NBOMe has also been employed in positron emission tomography (PET) in human volunteers to assess binding of this ligand in distinct brain areas and at non-psychoactive dosage levels [23,24].

Due to high receptor affinity and functional activity as full agonists, comparatively low doses, comparable to LSD, are needed to induce psychoactive effects. Consequently, the resulting low blood plasma or urine concentrations can make it challenging to identify and characterize the intake of NBOMes. In urine, the concentration of compounds is generally higher than in blood, but in many cases, metabolites rather than the parent compounds are the targets. Therefore, metabolism studies are needed for the development of urine screening approaches. The comprehensive metabolism study for 25I-NBOMe revealed that it was extensively metabolized and that the parent compound was found in urine only in small amounts [25].

Recently, Wohlfarth et al. [26] described the metabolism of 25C-NBOMe and 25I-NBOMe in mice and human urine as well as in human hepatocytes, and the reported results were consistent with previously published human and rat data for 25I-NBOMe [25]. For 25B-NBOMe, only limited data on its biotransformation are available [27,28], and for both compounds, no comprehensive data appear to be available on their detectability. Therefore, the aims of the present study were to investigate the metabolism of 25B-NBOMe and 25C-NBOMe in rats and humans with LC-HR-MS/MS, to compare the results with in vitro and in vivo data and between different species, and to investigate their detectability by the authors' standard urine screening approaches (SUSA) by GC-MS, LC-$MS^n$, and LC-HR-MS/MS, respectively.

## 2. Experimental

### *2.1. Chemicals and reagents*

25B-NBOMe hydrochloride and 25C-NBOMe hydrochloride were purchased by LGC Standards (Wesel, Germany). Isolute HCX cartridges (130 mg, 3 mL) were obtained from Biotage (Uppsala, Sweden), isocitrate and isocitrate dehydrogenase from Sigma (Taufkirchen, Germany), $NADP^+$ from Biomol (Hamburg, Germany), acetonitrile (LC-MS grade), ammonium formate (analytical grade),

104 formic acid (LC-MS grade), methanol (LC-MS grade), mixture (100,000 Fishman units/mL) of
105 glucuronidase (EC No. 3.2.1.31) and arylsulfatase (EC No. 3.1.6.1) from Helix Pomatia, and all other
106 chemicals and reagents (analytical grade) from VWR (Darmstadt, Germany). The baculovirus-
107 infected insect cell microsomes (Supersomes) containing 1 nmol/mL of human cDNA-expressed
108 CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 (2 nmol/mL),
109 CYP3A4, or CYP3A5 (2 nmol/mL) were obtained from Corning (Amsterdam, The Netherlands).
110 After delivery, the CYPs were thawed at 37°C, aliquoted, snap-frozen in liquid nitrogen, and stored at
111 -80°C until use.
112
113 *2.2. Urine samples*
114
115 According to an established study design [29], the investigations were performed using rat urine
116 samples from male Wistar rats (Charles River, Sulzfeld, Germany) for toxicological diagnostic
117 reasons according to German law. Both compounds were administered in an aqueous suspension by
118 gastric intubation of a single 10 mg/kg body weight (BW) dose for identification of the metabolites
119 and of 0.1 mg/kg BW for screening (dose calculated based on common single dose reported in trip
120 reports (https://www.erowid.org) and scaled by dose-by-factor approach from man to rat according to
121 Sharma and McNeill [30]), respectively. The rats were housed in metabolism cages for 24 h, having
122 water ad libitum. Urine was collected separately from feces over a 24 h period. Blank urine samples
123 were collected before drug administration to verify that the samples were free of interfering
124 compounds. The samples were directly analyzed and then stored at -20°C.
125 In addition, for 25B-NBOMe, an authentic ante mortem human urine sample after unintentional
126 intake of an unknown dose of 25B-NBOMe (declared as 2C-B) submitted to the authors' laboratory
127 for toxicological diagnostics was also analyzed.
128
129 *2.3. Sample preparation*

130 *2.3.1. Sample preparation for identification of phase I metabolites by LC-HR-MS/MS*

131

132 According to a published procedure [29], 2 mL of urine was adjusted to pH 5.2 with acetic acid (1 M,
133 approximately 50 µL) and incubated at 50 °C for 2 h with 50 µL of a mixture of glucuronidase and
134 arylsulfatase. The urine sample was then loaded on an HCX cartridge previously conditioned with 1
135 mL of methanol and 1 mL of water. After passage of the sample, the cartridge was washed with 1 mL
136 of water, 1 mL of 0.01 M hydrochloric acid, and again with 1 mL of water. The acidic and neutral
137 compounds were eluted with 1 mL of methanol into a 1.5 mL reaction vial and evaporated to dryness
138 under a stream of nitrogen. In the same reaction vial, the basic compounds were eluted with 1 mL of
139 a freshly prepared mixture of methanol/aqueous ammonia 32% (98:2, v/v). After another evaporation
140 step the residues were reconstituted with 50 µL of a mixture of eluent A and B (1:1, v/v) for LC-HR-
141 MS/MS analysis. A 5-µL aliquot was then injected onto the LC-HR-MS/MS.

142

143 *2.3.2. Sample Preparation for the identification of phase I metabolites and MBPs*

144

145 According to a published procedure [29], 100 µL of urine was mixed with 500 µL of acetonitrile for
146 precipitation. After shaking and centrifugation, the supernatant was gently evaporated to dryness and
147 reconstituted in 50 µL of a mixture of 10 mM aqueous ammonium formate buffer and acetonitrile
148 (1:1, *v*/*v*) and 5 µL injected onto the LC-HR-MS/MS system.

149

150 *2.4. Incubations for initial CYP activity screening studies*

151

152 According to standard procedures [29,31], microsomal incubations were performed at 37°C at a
153 concentration of 25 µM 25B-NBOMe and 25C-NBOMe, respectively, with the CYP isoenzymes (75
154 pmol/mL, each) CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1,
155 CYP3A4, or CYP3A5 for 30 min. Besides enzymes and substrates, the incubation mixtures (final

volume, 50 µL) contained 90 mM phosphate buffer (pH 7.4), 5 mM $Mg^{2+}$, 5 mM isocitrate, 1.2 mM $NADP^{+}$, 0.5 U/mL isocitrate dehydrogenase, and 200 U/mL superoxide dismutase. For incubations with CYP2A6 and CYP2C9, phosphate buffer was replaced with 45 mM or 90 mM Tris buffer, respectively, according to the Gentest manual. Reactions were initiated by addition of the CYP enzymes and stopped with 50 µL of ice-cold acetonitrile. The solution was centrifuged for 2 min at 14,000 rpm; 70 µL of the supernatant phase were transferred to an autosampler vial and 5 µL injected onto the LC-HR-MS/MS system.

*2.5. LC-HR-MS/MS instrumentation for identification of phase I and II metabolites and CYP initial screening*

According to a published procedure [32], the extracts were analyzed using a ThermoFisher Scientific (TF, Dreieich, Germany) Dionex UltiMate 3000 RS pump consisting of a degasser, a quaternary pump, and an UltiMate autosampler, coupled to a TF Q-Exactive Plus system equipped with a heated electrospray ionization (HESI)-II source. The instrument was used in positive or in negative ionization mode. Mass calibration was performed prior to analysis according to the manufacturer's recommendations using external mass calibration.

Gradient elution was run on a TF Accucore PhenylHexyl column (100 mm x 2.1 mm, 2.6 µm). The mobile phases consisted of 2 mM aqueous ammonium formate containing formic acid (0.1%, *v*/*v*) and acetonitrile (1%, *v*/*v*) (pH 3, eluent A) and 2 mM ammonium formate solution with acetonitrile:methanol (50:50, *v*/*v*) containing formic acid (0.1%, *v*/*v*) and water (1%, *v*/*v*) (eluent B). The gradient and flow rate were programmed as follows: 0-1 min hold 99% A, 1-16 min 95% A to 5% A, 16-18 min hold 5% A, and 18-20 min hold 99% A, constantly at 500 µL/min.

The HESI-II source conditions were as follows: sheath gas, 60 arbitrary units (AU); auxiliary gas, 10 AU; spray voltage, 3.00 (positive polarity) and -4.00 kV (negative polarity); heater temperature, 320°C; ion transfer capillary temperature, 320°C; and S-lens RF level, 60.0. Mass spectrometry was

performed in positive and negative polarity mode using full scan (FS) data and a subsequent data dependent acquisition (DDA) mode with an inclusion list on the masses of interest (phase I or phase II metabolites). Additionally, DDA runs without inclusion list (positive and negative mode) were performed to detect unexpected metabolites.

The settings for FS data acquisition were as follows: resolution, 35,000; microscans, 1; automatic gain control (AGC) target, 1e6; maximum injection time (IT), 120 ms; and scan range, *m/z* 100–700. The settings for the DDA mode with and without an inclusion list were as follows: resolution, 17,500; microscans 1, AGC target, 2e5; maximum IT, 250 ms; isolation window, 1.0 *m/z*, HCD with stepped normalized collision energy (NCE), 17.5, 35, and 52.5%; spectrum data type, profile; and underfill ratio, 0.5%. For the run without inclusion list, the five most intense precursor ions were transferred to an exclusion list for 1 s (dynamic exclusion).

For analyzing the initial CYP activity screening, the MS settings and the mobile phases as well as the gradient and flow rate were the same with the same inclusion list as for identification of phase I metabolites.

### *2.6. Standard urine screening procedures (SUSAs)*

The SUSAs were performed as described in the following references: GC-MS SUSA [33,34], LC-$MS^n$ SUSA [25,35], and LC-HR-MS/MS SUSA [25,36].

## 3. Results and discussion

### *3.1. Identification of metabolites*

#### *3.1.1. Identification of 25B-NBOMe and 25C-NBOMe and their phase I metabolites via HR-MS/MS fragmentation*

The HR-MS/MS fragmentation patterns and metabolite formation of 25B-NBOMe and 25C-NBOMe were similar to those described for 25I-NBOMe [25]. Briefly, and for discussion purposes, the molecules were viewed as two distinct parts, i.e. the 4-halogenated 2,5-dimethoxyphenethylamine (2C) part and the *N*-(2-methoxybenzyl) (NBOMe) part. Due to the high number of metabolites, the fragmentation patterns could not be discussed in detail for all metabolites and only the typical fragment ions used for identification will be discussed.

In general, for both compounds and their metabolites, the precursor masses and the most abundant fragment ions formed from unmodified or modified NBOMe parts were used to identify the corresponding metabolites. As expected, the fragment ions formed by the NBOMe part were identical for 25B-NBOMe and 25C-NBOMe. To confirm the predicted chemical structure of the metabolites, the corresponding 2C fragment ions (Table S1 in the electronic supplementary data for 25B-NBOMe and Table S2 for 25C-NBOMe) were used. For the *N*-dealkylated metabolites, no fragment ions of the NBOMe part could be detected, but characteristic 2C fragmentation patterns for the bromo and chloro analogues (Tables S1 and S2). The precursor masses (PM) are given with the calculated exact masses.

25B-NBOMe (B1; PM at *m/z* 380.0856, $M+H^+$) showed a fragmentation pattern characteristic also for most of the detected metabolites. The most abundant fragment ion of *m/z* 121.0653 represented the cleavage of the NBOMe moiety, followed by the loss of the methoxy group (-30.0105 u) producing the tropylium ion of *m/z* 91.0548. The fragment ions representing the 2C part showed low abundances of at least 1 % (Table S1). The fragment ion of *m/z* 258.0124 representing the 2C-B iminium ion resulted from benzyl cleavage. A loss of NH (- 15.0109 u) formed the fragment ion of *m/z* 243.0021 followed by a loss of a methyl radical (- 15.0235 u) of one of the two methoxy groups in the 2C part resulting in fragment ion of *m/z* 227.9786. For the $MS^2$ spectrum of 25I-NBOMe, a rearrangement was described in the literature [25]. In the parent spectrum of 25B-NBOMe, one fragment ion could also be formed by the same rearrangement. The fragment ion of *m/z* 363.0596 resulted from a loss of ammonia (-17.0263 u) and appeared consistent with the postulated

234 rearrangement reaction. Few $MS^2$ spectra of metabolites also showed possible rearranged fragment
235 ions.
236 The fragmentation patterns of 25C-NBOMe (C1; PM at *m/z* 336.1361, $M+H^+$) corresponded to those
237 of 25B-NBOMe and 25I-NBOMe. Similarly, the most abundant fragment ions in $MS^2$ were formed
238 by cleavage of the NBOMe moiety producing fragment ions of *m/z* 91.0548 and 121.0653. Also, the
239 fragment ions representing the 2C part showed a lower abundance of about 1 % (Table S2). The
240 fragment ions of *m/z* 214.0629, *m/z* 199.0526, and *m/z* 184.0291 represented the 2C-C iminium ion,
241 the subsequent loss of NH (- 15.0109 u), and the loss of a methyl radical (- 15.0235 u) of one of the
242 two methoxy groups, respectively. In the spectrum of 25C-NBOMe, no fragment ions indicating the
243 rearrangement were detected, possibly due to low relative abundance. However, in the $MS^2$ spectra of
244 some 25C-NBOMe metabolites (e.g. *O*-demethyl metabolite isomers 1 and 2, C16 and C17), some
245 rearranged fragment ions could also be detected. Overall, 35 phase I metabolites could be detected for
246 25B-NBOMe in urine and 36 for 25C-NBOMe, respectively. All phase I metabolites are listed in
247 Tables S1 and S2 in the electronic supplementary data.
248 For metabolite identification based on the $MS^2$ spectra, in most cases the representative fragment ion
249 for the NBOMe part was used. Unmodified NBOMe parts led to a fragment ion of *m/z* 121.0653. The
250 presence of this fragment ion led to the suggestion that the expected modification took place at the 2C
251 part based on the predicted precursor mass for the metabolite. An unchanged fragment ion of *m/z*
252 121.0653 could be seen for the parent compounds (B1 and C1) as well as for mono- and bis-
253 demethylated (B13, B14, B8 and C16, C17, C10), mono-hydroxylated (B29, B32 and C31, C32), bis-
254 hydroxylated (B35), combined mono-demethylated with mono-hydroxylated (B22), dehydrogenated
255 (B20 and C24), dehydrogenated combined with mono-demethylated (B12 and C13), and mono-
256 hydroxylated (B27 and C30) metabolites. On the other hand, the fragment ion of *m/z* 137.0603
257 represented mono-hydroxylation at the NBOMe part (B16, B23, B28, B30, B31 and C19, C25, C27,
258 C33, C35). At the NBOMe moiety, bis-hydroxylation led to the fragment ion of *m/z* 153.0552 (B33,
259 B34 and C34, C36) and *O*-demethylation to fragment ion of *m/z* 107.0497 (B7, B9, B10, B11, B15,

B19, B21 and C8, C9, C11, C12, C14, C18, C21, C23, C26). The fragment ion of *m/z* 107.0497 could also be found for the NBOMe mono-hydroxylated (B16, B23, B28, B31 and C19, C25, C27, C33) or NBOMe bis-hydroxylated (B34 and C34, C35, C36) metabolites, but consistently in combination with the fragment ions of *m/z* 137.0603 or *m/z* 153.0552 as mentioned above. Therefore, the absence of the fragment ions of *m/z* 137.0603 and 153.0552 indicated *O*-demethylation at the NBOMe part. *O*-Demethylation combined with mono-hydroxylation led to fragment ion of *m/z* 123.0446 (B17, B18, B24, B25 and C20, C22, C28, C29). As mentioned above, all *N*-demethoxybenzyl metabolites were identified based on the 2C part fragmentation patterns (B2–B6 and C2–C7).

In summary, the fragmentation patterns of both NBOMes corresponded to those of 25I-NBOMe. Some compound-related characteristics were found for the bromo and chloro analogues as already described for 25I-NBOMe. All metabolites, which were *O*-demethylated at the NBOMe part (*m/z* 107.0497), showed higher abundances for fragment ions representing the 2C part probably due to a hydrogen bond between the nitrogen and the hydroxy group resulting from *O*-demethylation at the NBOMe part [25]. In addition, for these metabolites, the corresponding 2C fragment ion carrying the nitrogen was represented by the 2C primary amine instead of the 2C iminium ion found for the parent compounds or metabolites, which were not *O*-demethylated at the NBOMe part.

It was not possible in this study to identify the demethylated position of the methoxy group (2'- or 5'-position) or the position at which the NBOMe part was hydroxylated. Nevertheless, Wohlfarth et al. [26] synthesized six potential 25C-NBOMe metabolites (2'- and 5'-*O*-demethyl-25C-NBOMe and 3-/4-/5- and 6-hydroxy-25C-NBOMe) to confirm the exact position of the metabolic reaction. They observed that both in vivo samples (mouse and human urine) showed prevalence for *O*-demethylation at the 5'-position. Furthermore, they observed that the most intense signal for a mono-hydroxylated metabolite was detected for 5-hydroxy-25C-NBOMe in human urine as well as in mouse urine. In general, Wohlfarth et al. described the same main metabolic steps compared to the present study and

25I-NBOMe [25]. In accordance, Leth-Petersen et al. [28] described that the main metabolic step of 25B-NBOMe was also the 5'-*O*-demethylation in humans and pigs.

*3.1.2. Identification of 25B-NBOMe and 25C-NBOMe and their phase I metabolites via HR-MS/MS fragmentation*

The phase II metabolite formation and fragmentation patterns were very similar for both compounds and comparable with those described for 25I-NBOMe. For both compounds, the precursor masses and the most abundant fragment ions formed from unmodified or modified NBOMe part were used to identify the corresponding phase II metabolites. 2C fragment ions were used to confirm the predicted metabolites.

Overall, 31 phase II metabolites could be identified for 25B-NBOMe and 33 for 25C-NBOMe. A list of all phase II metabolites is given in Tables S3 and S4 in the electronic supplementary data. All glucuronides eliminated glucuronic acid (- 176.0321 u) and all sulfates sulfuric acid (- 79.9568 u). Thus, the rest of the spectra of phase II conjugates was in accordance with the spectrum of the corresponding phase I metabolite. Also, for some phase II metabolites, fragment ions formed by conjugated partial structures could be used to elucidate the position of the conjugation.

As already described for 2C derivatives [37] and 25I-NBOMe the metabolites formed after *N*-demethoxybenzylation could further be conjugated by acetylation, glucuronidation, sulfation, or even combinations of them. Furthermore, in accordance to 25I-NBOMe, an *O,O*-bis-demethylation of the 2C part led to a hydroquinone partial structure, which could further be conjugated with glutathione (GSH). The degradation products of GSH conjugated metabolites could be found for both compounds. Also the described conjugation catalyzed by catechol-*O*-methyl-transferase (COMT) could be found for both NBOMes forming *O*-methyl metabolites (B33ME and C24ME, C36ME), after bis-hydroxylation at the NBOMe part (*m/z* 167.0708) producing a catecholic partial structure.

311 *3.2. Initial CYP activity screening*

312

313 For identification of the CYPs catalyzing the initial metabolic steps, the ten most abundant human
314 hepatic CYPs were incubated under conditions allowing a statement on the general involvement of a
315 particular CYP enzyme. It should be kept in mind that these qualitative data did not reflect a
316 quantitative contribution of a CYP to the hepatic clearance that would require the collection of
317 enzyme kinetic data [38], which was beyond the scope of this study. As summarized in Tables 1 and
318 2, CYP2C9 and CYP2C219 were involved in *O*-demethylation for both, 25B-NBOMe and 25C-
319 NBOMe, respectively, CYP1A2 and CYP3A4 in hydroxylation, and CYP3A4 in *N*-
320 demethoxybenzylation.

321

322 *3.3. Proposed metabolic pathways*

323

324 According to the 25B-NBOMe metabolites identified in human and rat urine after cleavage of
325 conjugates and 25C-NBOMe metabolites identified in rat urine after cleavage of conjugates (Tables
326 S1 and S2), the following metabolic pathways, depicted in Figs. 1 and 2, could be proposed.
327 As expected, both compounds underwent the same main metabolic steps. *O*-Demethylation led to the
328 most abundant peaks in human and rat urine followed by *O*-bis-demethylation and /or by *O*-
329 demethylation plus hydroxylation. *N*-Demethoxybenzylation led to only small peaks in both species.
330 However, the relative abundance of the different metabolites varied between the species, but it should
331 also be kept in mind that the rat urines were pooled over 24 h and the human urine was collected at an
332 unknown time after administration of an unknown dose. Finally, the relation of the metabolites may
333 vary over the time of excretion.
334 For both derivatives, the following phase I pathways could be found: mono-demethylation (B13–B15
335 and C16–C18), bis-demethylation (B8–B10 and C10–C12), tris-demethylation (B7 and C8) of the
336 methoxy groups, mono- and bis-hydroxylation (B29–B32, B34, B35, and C31–C33, C35, C36), *N*-

337 demethoxybenzylation (B6 and C5), and combinations of mono-hydroxylation with mono-
338 demethylation (B22–B25 and C26–C29), and bis-demethylation (B16–B19 and C19–C23) as well as
339 bis-hydroxylation with mono-demethylation (B33 and C34), and *N*-demethoxybenzylation with
340 mono-demethylation (B3, B4 and C2, C3) followed by oxidative deamination (B2) and oxidation to
341 the corresponding carboxylic acid (B5 and C4). In addition, for 25C-NBOMe also *N*-
342 demethoxybenzylation with mono-hydroxylation (C7), and oxidation forming an amide structure
343 (C6) could be predicted. Also, dehydro metabolites (B20 and C24) were found for both compounds.
344 The presence of this metabolic step was already described for 25I-NBOMe [25].
345 Nielsen et al. [39] described dehydrogenation as a CYP-catalyzed reaction. The resulting double
346 bond was located at the 2C moiety and not between the nitrogen and the α-carbon of the 2C moiety as
347 confirmed with reference standard of the 25I-NBOMe imine. These compounds could further be
348 metabolized by mono-demethylation (B11, B12 and C13–C15), bis-demethylation (C9),
349 hydroxylation (B26–B28 and C30), and combination of mono-demethylation and hydroxylation
350 (B21 and C25). However, the possibility could not be excluded that the dehydro compound could
351 also be formed by artificial dehydration of the corresponding hydroxy metabolite. If hydroxylation
352 took place at the α-position to the nitrogen forming an unstable hemiaminal, then this metabolite
353 could further eliminate water under the ESI conditions described above. In summary, the metabolic
354 pathways for 25B-NBOMe and 25C-NBOMe corresponded to those described for 25I-NBOMe, i.e.
355 showing the same main phase I metabolism reactions.
356 The following phase II pathways could be proposed for humans and/or rats as given in Tables S3 and
357 S4 and Figs. 1 and 2: sulfation (S) glucuronidation (G) and/or of the *O*-demethyl metabolites
358 (B13/14S, B15S, B13G–B15G and C16/17S, C18S, C16G–C18G), of the *O,O*-bis-demethyl
359 metabolites (B8S, B9/10S, B8G, B9/10G and C10S–C12S, C10G–C12G), of *O,O,O*-tris-demethyl
360 metabolite (B7S, B7G and C8S, C8G), of the *O*-demethyl-hydroxy metabolites (B22S, B24/25S,
361 B22G, B23, B24/25G and C22S, C27S, C28/29S), of the *O,O*-bis-demethyl-hydroxy metabolites
362 (B16S, B17/18S, B16G, B19G and C20S, C19G–C22G), and of the hydroxy metabolites (B30G,

363 B31G and C31/32G, C33G). Glutathione (GSH) conjugation could be proposed for the *O,O*-bis-
364 demethyl metabolite isomer 1 (B8-GSH-1, B8-GSH-2 and C10-GSH-1, C10-GSH-2), *N*-acetylation
365 (AC) for the *N*-demethoxybenzyl-*O*-demethyl metabolites (B3, B4 and C2, C3) followed by further
366 sulfation and/or glucuronidation (B3AC+S, B4AC+S, B3/4AC+G and C3/4AC+S, C2/3AC+G), and
367 *O*-methylation (ME) of the bis-hydroxy metabolite (C36ME) and the *O*-demethyl-bis-hydroxy
368 metabolites (B33ME and C34ME). In summary, all phase II pathways could be proposed for both
369 species except for glutathione conjugation, which was observed only in rats after administration of
370 the high dose.

371

372 *3.4. Comparison of metabolite formation in vitro and in vivo and in different species*

373

374 In contrast to the development of new therapeutics drug, pharmacokinetic data are not routinely
375 collected for NPS before emergence on the market. For ethical reasons, controlled human studies are
376 not possible. Therefore, animal studies under controlled conditions are common in combination with
377 human in vivo assays as described e.g. in refs. [25,26]. Both data can be confirmed by authentic
378 human samples of e.g. intoxication cases. For development of urine screening approaches, it is
379 important to know the possible target. Thus, any metabolites identified first in animal urine can
380 become the main target in human urine considering e.g. inter-species and/or genetic variations in
381 drug metabolism and transport. For this reason, Tables 3 and 4 list the phase I and II metabolites
382 identified in this study compared to those detected in human liver microsomes (HLM) incubation,
383 porcine liver microsomes (PLM) incubation, mouse urine (MU), authentic human urines (HU), or
384 human hepatocyte (HP) incubation. Differences could be explained by species differences, higher
385 doses, and/or different sampling time after administration.

386

387 *3.4.1. 25B-NBOMe*

388

389 Boumrah et al. [27] described 21 phase I and II metabolites of 25B-NBOMe identified only in vitro
390 after incubation with HLM and cofactors for CYPs and glucuronyl transferases. Leth-Petersen et al.
391 [28]compared formation of phase I metabolites in HLM and PLM incubations. In the present study,
392 35 phase I and 31 phase II metabolites have been identified in human and rat urine. According to
393 Table 3, in both urine samples, various metabolites could be identified not described by Boumrah et
394 al. or Leth-Petersen et al. Most of them were isomers of metabolites formed by combined metabolic
395 reactions such as mono- and bis-*O*-demethylation with hydroxylation or *O*-demethylation with *N*-
396 dealkylation. Species differences occurred for the hydroxylation step because rats seemed to prefer
397 hydroxylation at the 2C part whereas human biotransformation might result in preferential
398 hydroxylation at the NBOMe part. Concerning phase II metabolism, Boumrah et al. investigated only
399 the glucuronide formation. In the present study, sulfation, *N*-acetylation, and *O*-methylation were
400 found in rat and human urine as further reactions. In addition, rats showed GSH conjugation and
401 combinations of *N*-acetylation with sulfation or glucuronidation. In contrast, the *N*-glucuronide of the
402 parent compound detected in HLM could not be found in the human or rat urine.
403
404 *3.4.2. 25C-NBOMe*
405
406 Table 4 summarizes the data obtained in rat urine and those in human hepatocytes and urines of
407 humans and mice [26]. Concerning phase I metabolism, most metabolites were common for all
408 species while the highest number was found in the rat urine probably due to the high dosage, urine
409 collection time, sample preparations, and/or chromatographic separation. Some metabolites were
410 only detected in rat urine such as the combined *N*-dealkylated and *O*-demethylated metabolites or
411 various isomers of *O,O*-bis-demethyl-hydroxy metabolites. Wohlfarth et al. [26] described *N*-
412 oxidation and carbonylation in the hepatocyte incubation although it was not clear why this could not
413 be found in their human and mice urine. As already described for 25B-NBOMe, rats seemed to
414 preferentially hydroxylated at the 2C part and humans at the NBOMe part. Most phase II pathways

415 could be proposed for all three species with the exception of *O*-acetylation, *N*-acetylation, GSH
416 conjugation, and *O*-methylation. Again, the highest number of metabolites was identified in rat urine
417 probably due to the reasons described above.
418

419 *3.5. Toxicological detection of 25B-NBOMe and 25C-NBOMe by SUSAs*

420 *3.5.1. GC-MS SUSA*

421

422 Unfortunately, 25B-NBOMe and 25C-NBOMe and/or their metabolites could not be detected in rat
423 urine after low dose administration (0.1 mg/kg BW). However, 25B-NBOMe and metabolites (Table
424 5) could be detected in the human urine sample by GC-MS SUSA. The compound ingested by the
425 user was believed to be 2C-B, which typically requires a ten-fold higher dose compared to 25B-
426 NBOMe [7]. Therefore, for acute and/or severe poisonings with NBOMes an intake could also be
427 detected by GC-MS SUSA. 25C-NBOMe could only be detected after the high dose, enzymatic
428 cleavage of conjugates, solid-phase extraction, and acetylation according to Welter et al. [31].
429

430 *3.5.2. LC-MS$^n$ SUSA*

431

432 The LC-MS$^n$ approach could detect 25B-NBOMe and 25C-NBOMe and/or their metabolites in rat
433 urine after low dosage (0.1 mg/kg BW) as well as in the authentic human urine sample. A list of the
434 detected metabolites is given in Table 6. As already mentioned above, the differences of detected
435 analytes in the human and rat urine samples could be caused by different doses and urine collection
436 times.
437

438 *3.5.3. LC-HR-MS/MS SUSA*

439

As expected, this approach was also able to reveal 25B-NBOMe and 25C-NBOMe and/or their metabolites in rat urine after low dosage (0.1 mg/kg BW) as well as in the authentic human urine sample. A list of the detected metabolites is given in Table 7. Again, the differences of detected analytes in the human and rat urine samples could be caused by different doses and urine collection times. Mostly due to the lethal overdose, the parent compound gave one of the most abundant signals in the human urine sample. However, low dose rat urine studies showed that the parent compound should not be expected in high amounts after recreational use. Therefore, it should not be used as the only target for NBOMe urine screening.

## 4. Conclusions

Both, 25B-NBOMe and 25C-NBOMe were extensively metabolized similar to 25I-NBOMe including *O*-demethylation, *O,O*-bis-demethylation, and hydroxylations as predominant pathways in humans and rats. This was in accordance to published human and animal in vitro and in vivo data. Several CYP isoenzymes were involved in formation of the main metabolites. An intake could be detected mainly via their metabolites by low and high resolution LC-MS SUSAs and by GC-MS SUSA only in overdose cases.

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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**Table 1** General involvement of the CYP isoenzymes on the formation of the given 25B-NBOMe metabolites, numbering according to Table S1

| **Metabolite** | **CYP 1A2** | **CYP 2A6** | **CYP 2B6** | **CYP 2C8** | **CYP 2C9** | **CYP 2C19** | **CYP 2D6** | **CYP 2E1** | **CYP 3A4** | **CYP 3A5** |
|---|---|---|---|---|---|---|---|---|---|---|
| *N*-Demethoxybenzyl (B6) | + | | + | | | | | | + | + |
| *O*-Demethyl isomer 1 (B13) | + | | | | + | + | | | + | |
| *O*-Demethyl isomer 2 (B14) | + | | | | + | + | + | | + | |
| *O*-Demethyl isomer 3 (B15) | + | | | + | + | + | + | | + | |
| Dehydro- (B20) | | | | | | + | + | | + | |
| Hydroxy isomer 2 (B30) | + | | | | | + | | | + | + |
| Hydroxy isomer 3 (B31) | + | | | | | + | | | + | + |
| Hydroxy isomer 4 (B32) | | | + | | | | | | + | |

**Table 2** General involvement of the CYP isoenzymes on the formation of the given 25C-NBOMe metabolites, numbering according to Table S2

| **Metabolite** | **CYP 1A2** | **CYP 2A6** | **CYP 2B6** | **CYP 2C8** | **CYP 2C9** | **CYP 2C19** | **CYP 2D6** | **CYP 2E1** | **CYP 3A4** | **CYP 3A5** |
|---|---|---|---|---|---|---|---|---|---|---|
| *N*-Demethoxybenzyl (C5) | + | | + | | | | | | + | + |
| *O*-Demethyl isomer 1 (C16) | + | | | | + | + | | | + | |
| *O*-Demethyl isomer 2 (C17) | + | | | | + | + | + | | + | |
| *O*-Demethyl isomer 3 (C18) | + | | | + | + | + | + | | + | |
| Dehydro- (C24) | | | | | | + | + | | + | |
| Hydroxy isomer 3 (C33) | + | | | | | + | | | + | + |

**Table 3** 25B-NBOMe phase I and II metabolites detected in rat (RU) and human (HU) urine compared to those detected in human liver microsome (HLM) incubation published by Boumrah et al. [27] and in HLM and porcine liver microsome (PLM) incubations published by Leth-Petersen et al. [28]. Numbering according to Tables S1 and S3, * = metabolite only described in references [27,28]

| No. | Metabolite | RU | HU | HLM [27] | HLM [28] | PLM [28] |
|---|---|---|---|---|---|---|
| B1 | 25B-NBOMe | + | + | + | + | + |
| B2 | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-hydroxy-) | + | + | | | |
| B3 | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 1 | + | + | | | |
| B4 | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 2 | + | + | | | |
| B5 | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-) | + | + | | | |
| B6 | 25B-NBOMe-M (*N*-demethoxybenzyl-) | + | + | + | + | + |
| B7 | 25B-NBOMe-M (*O,O,O-tris*-demethyl-) | + | + | | | |
| B8 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 1 | + | + | + | + | + |
| B9 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 | + | + | + | + | |
| B10 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 3 | + | + | + | + | + |
| B11 | 25B-NBOMe-M (*O*-demethyl-dehydro-) isomer 1 | + | + | | | |
| B12 | 25B-NBOMe-M (*O*-demethyl-dehydro-) isomer 2 | + | | | | |
| B13 | 25B-NBOMe-M (*O*-demethyl-) isomer 1 | + | + | + | + | + |
| B14 | 25B-NBOMe-M (*O*-demethyl-) isomer 2 | + | + | + | + | + |
| B15 | 25B-NBOMe-M (*O*-demethyl-) isomer 3 | + | + | + | + | + |
| B16 | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 1 | + | | | | |
| B17 | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 2 | + | + | | | |
| B18 | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 3 | + | + | | | |
| B19 | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 4 | + | | | | |
| B20 | 25B-NBOMe-M (dehydro-) | + | + | | | + |
| B21 | 25B-NBOMe-M (*O*-demethyl-dehydro-hydroxy-) | + | | | | |
| B22 | 25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 1 | + | | | | + |
| B23 | 25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 2 | + | + | | + | + |
| B24 | 25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 3 | | + | + | + | + |
| B25 | 25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 4 | + | | + | + | + |
| B26 | 25B-NBOMe-M (dehydro-hydroxy-) isomer 1 | | + | | | |
| B27 | 25B-NBOMe-M (dehydro-hydroxy-) isomer 2 | + | | | | |
| B28 | 25B-NBOMe-M (dehydro-hydroxy-) isomer 3 | + | + | | | |
| B29 | 25B-NBOMe-M (hydroxy-) isomer 1 | + | | | | |
| B30 | 25B-NBOMe-M (hydroxy-) isomer 2 | | + | + | + | + |
| B31 | 25B-NBOMe-M (hydroxy-) isomer 3 | + | + | + | | |
| B32 | 25B-NBOMe-M (hydroxy-) isomer 4 | | + | + | + | |
| B33 | 25B-NBOMe-M (*O*-demethyl-*bis*-hydroxy-) | + | | | | |
| B34 | 25B-NBOMe-M (*bis*-hydroxy-) isomer 1 | | + | | | |
| B35 | 25B-NBOMe-M (*bis*-hydroxy-) isomer 2 | + | + | | | |
| M11 | 25B-NBOMe-M (carbonyl) * | | | | | + |
| B3 AC | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 1 | + | + | | | |
| B4 AC | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 2 | + | + | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| B3<br>AC+S | 25B-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 1 | + | | | | |
| B4<br>AC+S | 25B-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 2 | + | | | | |
| B8<br>GSH-1 | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) S-methyl | + | | | | |
| B33<br>ME | 25B-NBOMe-M<br>(*O*-demethyl-*bis*-hydroxy-) *O*-methyl | + | + | | | |
| B7<br>S | 25B-NBOMe-M<br>(*O,O,O-tris*-demethyl-) sulfate | + | | | | |
| B3/4<br>G | 25B-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) glucuronide | + | | | | |
| B8<br>S | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) sulfate isomer 1 | + | + | | | |
| B9/10<br>S | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) sulfate isomer 2 | | + | | | |
| B13/14<br>S | 25B-NBOMe-M<br>(*O*-demethyl-) sulfate isomer 1 | + | + | | | |
| B15<br>S | 25B-NBOMe-M<br>(*O*-demethyl-) sulfate isomer 2 | + | | | | |
| B16<br>S | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) sulfate isomer 1 | + | | | | |
| B17/18<br>S | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) sulfate isomer 2 | | + | | | |
| B22<br>S | 25B-NBOMe-M<br>(*O*-demethyl-hydroxy-) sulfate isomer 1 | + | | | | |
| B24/25<br>S | 25B-NBOMe-M<br>(*O*-demethyl-hydroxy-) sulfate isomer 2 | + | + | | | |
| B3/4<br>AC+G | 25B-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide | + | | | | |
| B8<br>GSH-2 | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) acetylcysteine | + | | | | |
| B7<br>G | 25B-NBOMe-M<br>(*O,O,O-tris*-demethyl-) glucuronide | + | | | | |
| B8<br>G | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) glucuronide isomer 1 | + | + | + | | |
| B9/10<br>G | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-) glucuronide isomer 2 | + | + | + | | |
| B13<br>G | 25B-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 1 | + | + | + | | |
| B14<br>G | 25B-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 2 | + | + | + | | |
| B15<br>G | 25B-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 3 | + | + | + | | |
| B16<br>G | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1 | + | | | | |
| B19<br>G | 25B-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2 | + | | | | |
| B23<br>G | 25B-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer 1 | + | + | | | |
| B22<br>G | 25B-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer 2 | + | | | | |
| B24/25<br>G | 25B-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer 3 | | + | + | | |
| B30<br>G | 25B-NBOMe-M<br>(hydroxy-) glucuronide isomer 1 | | + | + | | |
| B31<br>G | 25B-NBOMe-M<br>(hydroxy-) glucuronide isomer 2 | | + | + | | |
| M21 | 25B-NBOMe-M<br>*N*-glucuronide * | | | + | | |

**Table 4** 25C-NBOMe phase I and II metabolites detected in rat (RU) urine compared to those in authentic human urines (HU), mouse urine (MU) and human hepatocyte (HP) incubation as published by Wohlfarth et al. [26]. Numbering according to Tables S2 and S4, * = metabolite only described in reference [26]

| No. | Metabolite | RU | HU [26] | MU [26] | HP [26] |
|---|---|---|---|---|---|
| C1 | 25C-NBOMe | + | + | + | + |
| C2 | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 1 | + | | | |
| C3 | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 2 | + | | | |
| C4 | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-) | + | | | |
| C5 | 25C-NBOMe-M (*N*-demethoxybenzyl-) | + | + | | + |
| C6 | 25C-NBOMe-M (*N*-demethoxybenzyl-oxo-) | + | | | |
| C7 | 25C-NBOMe-M (*N*-demethoxybenzyl-hydroxy-) | + | | | |
| C8 | 25C-NBOMe-M (*O,O,O-tris*-demethyl-) | + | | | |
| C9 | 25C-NBOMe-M (*O,O-bis*-demethyl-dehydro-) | + | | | |
| C10 | 25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 1 | + | | + | |
| C11 | 25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 | + | + | + | |
| C12 | 25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 3 | + | + | + | |
| C13 | 25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 1 | + | | | |
| C14 | 25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 2 | + | | | |
| C15 | 25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 3 | + | | | |
| C16 | 25C-NBOMe-M (*O*-demethyl-) isomer 1 | + | + | + | + |
| C17 | 25C-NBOMe-M (*O*-demethyl-) isomer 2 | + | + | + | + |
| C18 | 25C-NBOMe-M (*O*-demethyl-) isomer 3 | + | + | | + |
| C19 | 25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 1 | + | | | |
| C20 | 25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 2 | + | | + | |
| C21 | 25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 3 | + | | | |
| C22 | 25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 4 | + | | | |
| C23 | 25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 5 | + | | | |
| C24 | 25C-NBOMe-M (dehydro-) | + | | | |
| C25 | 25C-NBOMe-M (*O*-demethyl-dehydro-hydroxy-) | + | | | |
| C26 | 25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 1 | + | | | |
| C27 | 25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 2 | + | + | + | |
| C28 | 25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 3 | + | + | + | + |
| C29 | 25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 4 | + | | | |
| C30 | 25C-NBOMe-M (dehydro-hydroxy-) | + | | | |
| C31 | 25C-NBOMe-M (hydroxy-) isomer 1 | + | | | |
| C32 | 25C-NBOMe-M (hydroxy-) isomer 2 | + | | | |
| C33 | 25C-NBOMe-M (hydroxy-) isomer 3 | + | + | + | + |
| C34 | 25C-NBOMe-M (*O*-demethyl-*bis*-hydroxy-) | + | + | | |
| C35 | 25C-NBOMe-M (*bis*-hydroxy-) isomer 1 | + | | | |
| C36 | 25C-NBOMe-M (*bis*-hydroxy-) isomer 2 | + | | | |
| C-Hp-21 | 25C-NBOMe-M (*N*-oxide) * | | | | + |
| C-Hp-22 | 25C-NBOMe-M (carbonyl) * | | | | + |
| C2 AC | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 1 | + | | | |
| C3 AC | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 2 | + | | | |
| C7 AC | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-hydroxy-) *N*-acetyl | + | | | |
| C3/4 AC+S | 25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate | + | | | |
| C10 GSH-1 | 25C-NBOMe-M (*O,O-bis*-demethyl-) S-methyl | + | | | |
| C34 | 25C-NBOMe-M | + | | | |

| | | | | | |
|---|---|---|---|---|---|
| ME | (*O*-demethyl-*bis*-hydroxy-) *O*-methyl | | | | |
| C8<br>S | 25C-NBOMe-M<br>(*O,O,O-tris*-demethyl-) sulfate | + | | | |
| C2/3<br>G | 25C-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) glucuronide | + | | | |
| C36<br>ME | 25C-NBOMe-M<br>(*bis*-hydroxy-) *O*-methyl | + | | | |
| C10<br>S | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) sulfate isomer 1 | + | | | |
| C11<br>S | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) sulfate isomer 2 | + | | | |
| C12<br>S | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) sulfate isomer 3 | + | + | | + |
| C16/17<br>S | 25C-NBOMe-M<br>(*O*-demethyl-) sulfate isomer 1 | + | + | + | + |
| C18<br>S | 25C-NBOMe-M<br>(*O*-demethyl-) sulfate isomer 2 | + | | | + |
| C20<br>S | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) sulfate | + | | | |
| C22<br>S | 25C-NBOMe-M<br>(*O*-demethyl-hydroxy-) sulfate | + | | | |
| C2/3<br>AC+G | 25C-NBOMe-M<br>(*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide | + | | | |
| C10<br>GSH-2 | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) acetylcysteine | + | | | |
| C8<br>G | 25C-NBOMe-M<br>(*O,O,O-tris*-demethyl-) glucuronide | + | | + | |
| C10<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) glucuronide isomer 1 | + | + | + | |
| C11<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) glucuronide isomer 2 | + | + | + | |
| C12<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-) glucuronide isomer 3 | + | | + | |
| C16<br>G | 25C-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 1 | + | + | + | + |
| C17<br>G | 25C-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 2 | + | | | + |
| C18<br>G | 25C-NBOMe-M<br>(*O*-demethyl-) glucuronide isomer 3 | + | | | + |
| C19<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1 | + | | | |
| C20<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2 | + | | | |
| C21<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 3 | + | | | |
| C22<br>G | 25C-NBOMe-M<br>(*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 4 | + | | | |
| C27<br>G | 25C-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer 1 | + | | | |
| C28/29<br>G | 25C-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer 2 | + | | | + |
| C31/32<br>G | 25C-NBOMe-M<br>(hydroxy-) glucuronide isomer 1 | + | | | |
| C33<br>G | 25C-NBOMe-M<br>(hydroxy-) glucuronide isomer 2 | + | + | + | + |
| C-Hp-6 | 25C-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer * | | | | + |
| C-Hp-8 | 25C-NBOMe-M<br>(hydroxy-) glucuronide isomer * | | | | + |
| C-Hp-10 | 25C-NBOMe-M<br>(*O*-demethyl-hydroxy-) glucuronide isomer * | | | | + |

| | | | | | |
|---|---|---|---|---|---|
| C-Hp-18 | 25C-NBOMe-M<br>(hydroxy-) sulfate * | | | | + |
| C-Hp-19 | 25C-NBOMe-M<br>(hydroxy-) sulfate * | | | | + |
| C-MH-21 | 25C-NBOMe-M<br>(*O*-demethyl-) *O*-acetyl * | | | + | |

**Table 5** 25B-NBOMe and its metabolites, molecular mass, five most abundant EI-GC-MS fragment ions, retention indices (RI), and detectability in rat urine (RU) or human urine (HU) by GC-MS SUSA. The numbers correspond to those of Table S1.

| No. | Target for SUSA | Molecular mass, u | GC-MS fragment ions, *m/z* and their relative intensities, % | RI | Detected in urine sample |
|---|---|---|---|---|---|
| B1 | 25B-NBOMe AC | 421 | 121 (100), 150 (9), 229 (12), 242 (33), 421 (2) | 2920 | HU |
| B2 | 25B-NBOMe-M (*N*-demethoxybenzyl-deamino-*O*-demethyl-hydroxy-) 2AC | 330 | 215 (55), 228 (100), 246 (10), 288 (15), 330 (4) | 2160 | HU |
| B3/B4 | 25B-NBOMe-M (*N*-demethoxybenzyl -*O*-demethyl-) isomer 1 / isomer 2 2AC | 329 | 215 (17), 228 (100), 270 (10), 287 (21), 329 (8) | 2440 | HU |
| B6 | 25B-NBOMe-M (*N*-demethoxybenzyl -) AC | 301 | 148 (39), 199 (12), 229 (31), 242 (100), 301 (15) | 2180 | HU |
| B9/10 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 / isomer 3 3AC | 477 | 107 (78), 178 (100), 228 (42), 270 (12), 477 (1) | 3020 | HU |
| B13/14 | 25B-NBOMe-M (*O*-demethyl-) isomer 1 / isomer 2 2AC | 449 | 121 (100), 192 (22), 228 (19), 270 (3), 449 (2) | 3000 | HU |

**Table 6** 25B-NBOMe, 25C-NBOMe, and their metabolites, protonated precursor ions, characteristic $MS^2$ and $MS^3$ fragment ions, retention time (RT), and detectability in rat urine (RU) or human urine (HU, 25B-NBOMe) by LC-$MS^n$ SUSA. The numbers correspond to those of Tables S1-S4.

| No. | Target for SUSA | Precursor ions, *m/z* | $MS^2$ fragment ions, *m/z* and relative intensity, % | $MS^3$ fragment ions, *m/z* and relative intensity, % on the ion given in bold | RT, min | Detected in urine sample |
|---|---|---|---|---|---|---|
| B1 | 25B-NBOMe | 380 | 121 (100), 179 (10), 243 (10), 255 (18), 258 (14), 269 (10), 284 (15) | **121:** 91 (30), 93 (100)<br>**255:** 148 (10), 176 (100), 225 (44) | 14.6 | HU |
| B9 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 | 352 | 107 (1), 229 (56), 246 (100) | **229:** 135 (5), 150 (100)<br>**246:** 135 (3), 150 (51), 214 (100) | 9.7 | HU |
| B13 | 25B-NBOMe-M (*O*-demethyl-) isomer 1 | 366 | 121 (100), 229 (3), 241 (7), 244 (12), 270 (26) | **121:** 91 (24), 93 (100)<br>**270:** 145 (6), 224 (7), 239 (100) | 11.5 | HU |
| B14 | 25B-NBOMe-M (*O*-demethyl-) isomer 2 | 366 | 121 (88), 241 (100), 257 (92), 258 (37), 270 (51) | **241:** 147 (5), 162 (100)<br>**257:** 149 (46), 162 (55), 225 (100) | 12.3 | HU, RU |
| B8 G | 25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1 | 528 | 227 (8), 244 (4), 335 (7), 352 (100) | **352:** 121 (100), 227 (55), 244 (18), 256 (21), 273 (7) | 5.9 | RU |
| B14 G | 25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 2 | 542 | 244 (2), 270 (2), 349 (3), 366 (100) | **349:** 241 (41), 255 (22), 270 (100)<br>**366:** 121 (100), 241 (4), 244 (6), 270 (17) | 9.4 | HU, RU |
| C16 | 25C-NBOMe-M (*O*-demethyl-) isomer 1 | 322 | 91 (9), 121 (100), 197 (9), 200 (11), 214 (5) | **121:** 91 (22), 93 (100) | 13.1 | RU |
| C10 G | 25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1 | 484 | 183 (15), 200 (4), 291 (11), 308 (100) | **291:** 121 (47), 183 (100), 255 (23)<br>**308:** 121 (100), 183 (51), 200 (21) | 6.4 | RU |
| C11 G | 25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2 | 484 | 185 (38), 202 (47), 308 (100), 378 (20) | **202:** 150 (21), 157 (10), 170 (100)<br>**308:** 185 (84), 202 (100) | 8.2 | RU |
| C17 G | 25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 2 | 498 | 185 (1), 200 (2), 305 (2), 322 (100) | **322:** 121 (100), 197 (11), 200 (10), 214 (5) | 9.9 | RU |
| C28/29 G | 25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2 | 514 | 216 (2), 321 (5), 338 (100) | **338:** 121 (100), 198 (5), 216 (3), 230 (1), 303 (7) | 8.6 | RU |

**Table 7** 25B-NBOMe, 25C-NBOMe, and their metabolites, calculated masses of their precursor ions, retention times (RT) recorded in rat urine or human urine (25B-NBOMe, 25C-NBOMe not tested, n.t.) by LC-HR-MS/MS SUSA. The numbers correspond to those of Tables S1-S4 (D = detection of the precursor ion in $MS^1$, I = identification via $MS^1$ and $MS^2$).

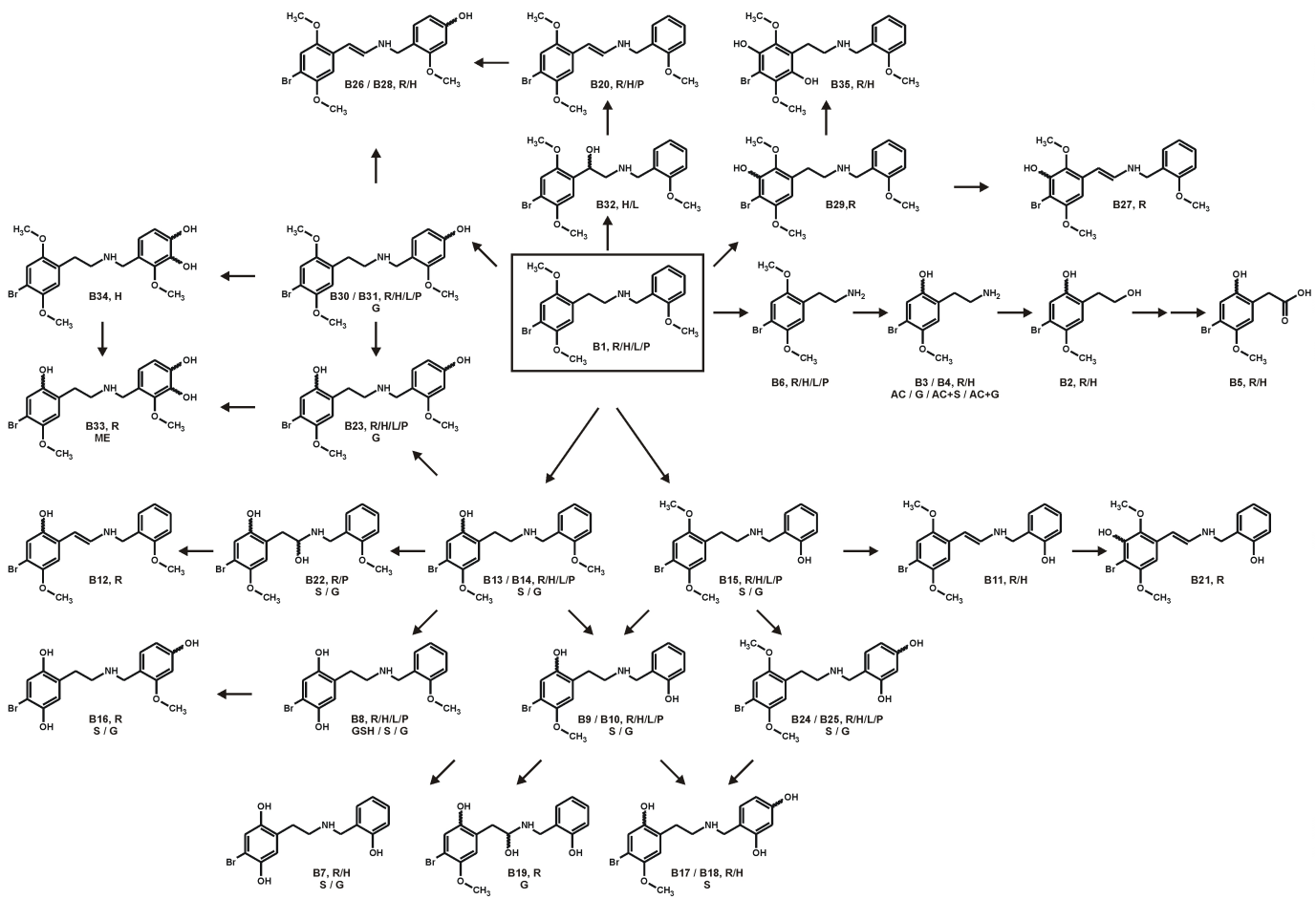
| No. | Targets in SUSA | Calculated exact masses of precursor ions, *m/z* | RT, min | Human urine | Rat urine 0.1 mg/kg BW |
|---|---|---|---|---|---|
| B1 | 25B-NBOMe | 380.0856 | 6.0 | I | |
| B5 | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-) | 258.9606 | 5.3 | D | D |
| B8 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 1 | 352.0543 | 5.0 | I | D |
| B9 | 25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 | 352.0543 | 5.3 | I | D |
| B13 | 25B-NBOMe-M (*O*-demethyl-) isomer 1 | 366.0699 | 5.3 | I | |
| B14 | 25B-NBOMe-M (*O*-demethyl-) isomer 2 | 366.0699 | 5.8 | I | I |
| B30 | 25B-NBOMe-M (hydroxy-) isomer 2 | 396.0805 | 5.4 | D | |
| B31 | 25B-NBOMe-M (hydroxy-) isomer 3 | 396.0805 | 5.9 | D | |
| B3 AC | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 1 | 288.0230 | 5.4 | | D |
| B4 AC | 25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 2 | 288.0230 | 5.5 | | D |
| B8 S | 25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 1 | 432.0111 | 4.9 | D | D |
| B9/10 S | 25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 2 | 432.0111 | 5.7 | I | |
| B13/14 S | 25B-NBOMe-M (*O*-demethyl-) sulfate isomer 1 | 446.0267 | 5.8 | I | |
| B24/25 S | 25B-NBOMe-M (*O*-demethyl-hydroxy-) sulfate isomer 2 | 462.0217 | 5.5 | D | |
| B7 G | 25B-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide | 514.0707 | 3.8 | | D |
| B8 G | 25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1 | 528.0864 | 4.2 | I | I |
| B9/10 G | 25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2 | 528.0864 | 4.8 | D | D |
| B13 G | 25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 1 | 542.1020 | 4.6 | I | |
| B14 G | 25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 2 | 542.1020 | 5.2 | I | I |
| B19 G | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2 | 544.0813 | 4.5 | | D |
| B23 G | 25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 1 | 558.0969 | 4.7 | | D |

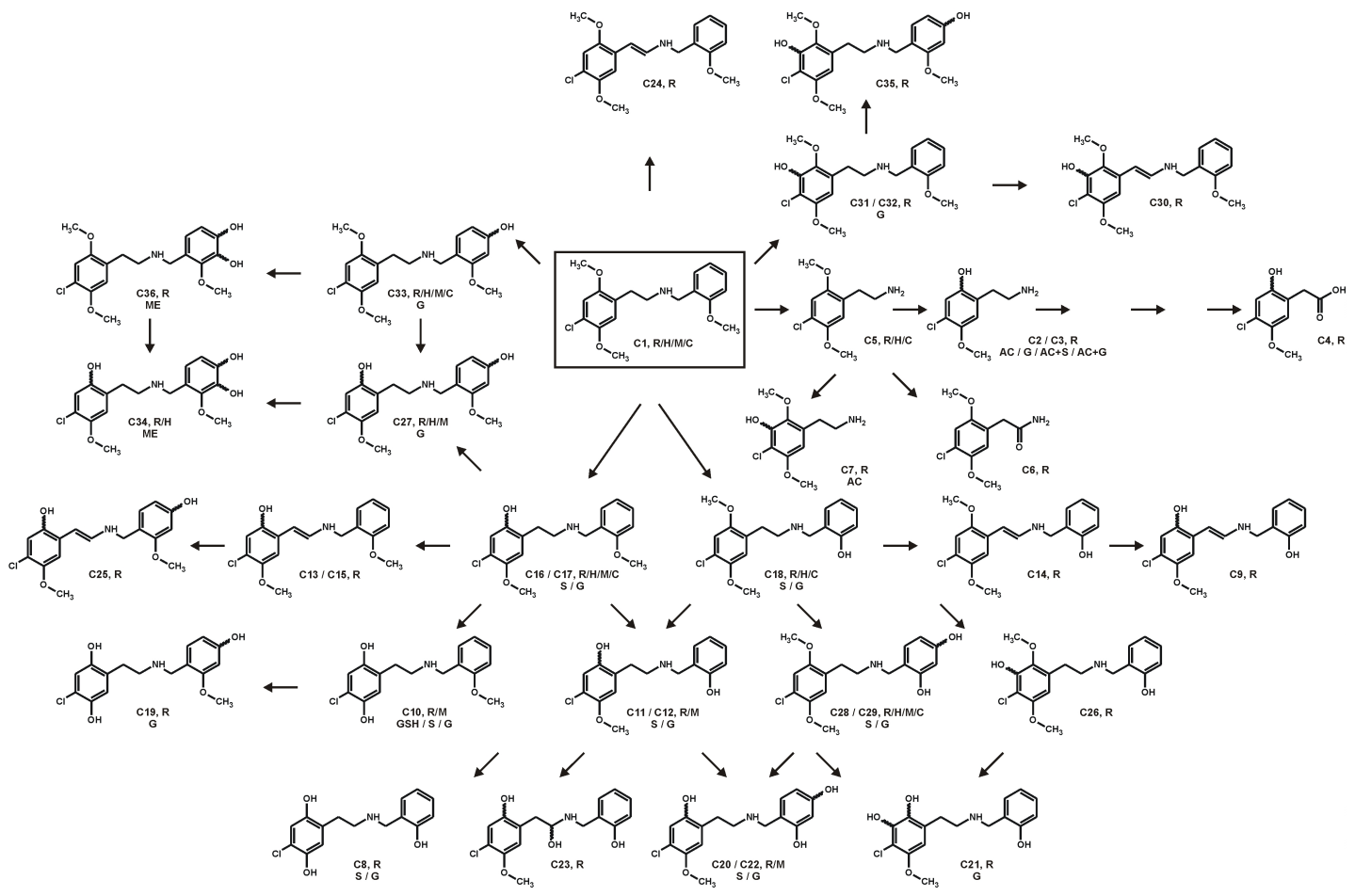
| | | | | | |
|---|---|---|---|---|---|
| B22 G | 25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2 | 558.0969 | 4.8 | | D |
| C11 | 25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 2 | 308.1048 | 4.6 | n.t. | D |
| C12 | 25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 3 | 308.1048 | 4.8 | n.t. | D |
| C17 | 25C-NBOMe-M (*O*-demethyl-) isomer 2 | 322.1204 | 5.1 | n.t. | D |
| C8 G | 25C-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide | 470.1212 | 3.5 | n.t. | I |
| C10 G | 25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1 | 484.1369 | 3.8 | n.t. | I |
| C11 G | 25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2 | 484.1369 | 4.4 | n.t. | I |
| C16 G | 25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 1 | 498.1525 | 4.3 | n.t. | D |
| C17 G | 25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 2 | 498.1525 | 4.8 | n.t. | I |
| C18 G | 25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 3 | 498.1525 | 5.2 | n.t. | D |
| C21 G | 25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 3 | 500.1318 | 4.1 | n.t. | D |
| C28/29 G | 25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2 | 514.1475 | 4.5 | n.t. | I |
| C31/32 G | 25B-NBOMe-M (hydroxy-) glucuronide isomer 1 | 528.1631 | 4.1 | n.t. | D |

**Legends to Figures**

**Fig. 1** Metabolic pathways of 25B-NBOMe studied in rat (R) or human (H) urine as well as in incubations with human (L) or porcine (P) liver microsomes. Phase II metabolites: glucuronides (G), sulfates (S), glutathione conjugates (GSH), acetyl conjugates (AC), O-methyl conjugates (ME), acetyl conjugates combined with glucuronidation (AC+G), acetyl conjugates combined with sulfation (AC+S). Undefined position of *O*-demethylation or hydroxylation indicated by tildes. Numbering according to Tables S1 and S3.

**Fig. 2** Metabolic pathways of 25C-NBOMe studied in rat (R), mouse (M), or human (H) urine as well as in incubations with human hepatocytes (C). Phase II metabolites: glucuronides (G), sulfates (S), glutathione conjugates (GSH), acetyl conjugates (AC), *O*-methyl conjugates (ME), acetyl conjugates combined with glucuronidation (AC+G), acetyl conjugates combined with sulfation (AC+S). Undefined position of *O*-demethylation or hydroxylation indicated by tildes. Numbering according to Tables S2 and S4.


B26 / B28, R/H
B20, R/H/P
B35, R/H
B32, H/L
B29,R
B27, R
B34, H
B30 / B31, R/H/L/P
G
B1, R/H/L/P
B6, R/H/L/P
B3 / B4, R/H
AC / G / AC+S / AC+G
B2, R/H
B5, R/H
B33, R
ME
B23, R/H/L/P
G
B12, R
B22, R/P
S / G
B13 / B14, R/H/L/P
S / G
B15, R/H/L/P
S / G
B11, R/H
B21, R
B16, R
S / G
B8, R/H/L/P
GSH / S / G
B9 / B10, R/H/L/P
S / G
B24 / B25, R/H/L/P
S / G
B7, R/H
S / G
B19, R
G
B17 / B18, R/H
S


C24, R
C35, R
C31 / C32, R
G
C30, R
C36, R
ME
C33, R/H/M/C
G
C1, R/H/M/C
C5, R/H/C
C2 / C3, R
AC / G / AC+S / AC+G
C4, R
C34, R/H
ME
C27, R/H/M
G
C7, R
AC
C6, R
C25, R
C13 / C15, R
C16 / C17, R/H/M/C
S / G
C18, R/H/C
S / G
C14, R
C9, R
C19, R
G
C10, R/M
GSH / S / G
C11 / C12, R/M
S / G
C28 / C29, R/H/M/C
S / G
C26, R
C8, R
S / G
C23, R
C20 / C22, R/M
S / G
C21, R
G

**Highlights**

- First detailed Orbitrap-based study on the metabolism of two New Psychoactive Substances (NPS) and on detectability in urine by GC-MS and low and high resolution LC-MS techniques.

- The analytical novelty consists of the description of the identification power of various GC-MS and LC-(HR) MS techniques.

- The corresponding reference spectra and their interpretation are basis for routine drug testing worldwide of these NPS and thus of great relevance for all toxicologists.

- First comparison of metabolism data obtained from in vivo studies with three different species and from human in cellulo and in vitro studies.

**Electronic Supplementary Data**

# Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) in human and rat urine by GC-MS, LC-$MS^n$, and LC-HR-MS/MS approaches

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**Table S1** List of 25B-NBOMe and its phase I metabolites together with the precursor mass (PM) recorded in $MS^1$, the corresponding characteristic fragment ions (FI) in $MS^2$, the calculated exact masses, the corresponding elemental composition, the deviation of the measured from the calculated masses, given as errors in parts per million (ppm), and the retention times (RT) in minutes (min). The metabolites were sorted by mass and RT.

| No. | Metabolite and characteristic ions Measured accurate mass, *m/z* | Relative intensity in $MS^2$, % | Calculated exact mass, *m/z* | Elemental composition | Error, ppm | RT, min |
|---|---|---|---|---|---|---|
| **B1** | **25B-NBOMe** | | | | | 8.8 |
| | **$MS^1$** PM at *m/z* 380.0859 (M+H) | 7 | 380.0856 | $C_{18}H_{23}O_3NBr$ | 0.84 | |
| | **$MS^2$** FI at *m/z* 91.0549 | 58 | 91.0548 | $C_7H_7$ | 1.37 | |
| | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | FI at *m/z* 227.9777 | 1 | 227.9786 | $C_9H_9O_2Br$ | -3.91 | |
| | FI at *m/z* 243.0010 | 1 | 243.0021 | $C_{10}H_{12}O_2Br$ | -4.39 | |
| | FI at *m/z* 258.0126 | 0.2 | 258.0124 | $C_{10}H_{13}O_2NBr$ | 0.71 | |
| | FI at *m/z* 363.0597 | 0.3 | 363.0596 | $C_{18}H_{20}O_3Br$ | 0.33 | |
| **B2** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-hydroxy-)** | | | | | 6.5 |
| | **$MS^1$** PM at *m/z* 244.9820 (M-H) | 5 | 244.9820 | $C_9H_{10}O_3Br$ | 2.73 | |
| | **$MS^2$** FI at *m/z* 78.9176 | 100 | 78.9183 | Br | -9.33 | |
| | FI at *m/z* 199.9474 | 9 | 199.9473 | $C_7H_5O_2Br$ | 0.55 | |
| | FI at *m/z* 211.9476 | 12 | 211.9473 | $C_8H_5O_2Br$ | 1.46 | |
| | FI at *m/z* 229.9584 | 76 | 229.9584 | $C_8H_7O_3Br$ | 2.37 | |
| **B3** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 1** | | | | | 3.9 |
| | **$MS^1$** PM at *m/z* 246.0123 (M+H) | 1 | 246.0124 | $C_9H_{13}O_2NBr$ | -0.47 | |
| | **$MS^2$** FI at *m/z* 135.0442 | 24 | 135.0446 | $C_8H_7O_2$ | -3.00 | |
| | FI at *m/z* 150.0677 | 47 | 150.0681 | $C_9H_{10}O_2$ | -2.53 | |
| | FI at *m/z* 213.9626 | 82 | 213.9629 | $C_8H_7O_2Br$ | -1.59 | |
| | FI at *m/z* 228.9861 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -1.38 | |
| **B4** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 2** | | | | | 4.0 |
| | **$MS^1$** PM at *m/z* 246.0130 (M+H) | 3 | 246.0124 | $C_9H_{13}O_2NBr$ | 2.37 | |
| | **$MS^2$** FI at *m/z* 135.0442 | 22 | 135.0446 | $C_8H_7O_2$ | -3.00 | |
| | FI at *m/z* 150.0676 | 40 | 150.0681 | $C_9H_{10}O_2$ | -3.20 | |
| | FI at *m/z* 213.9625 | 90 | 213.9629 | $C_8H_7O_2Br$ | -2.06 | |
| | FI at *m/z* 228.9861 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -1.38 | |
| **B5** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-)** | | | | | 6.3 |
| | **$MS^1$** PM at *m/z* 258.9610 (M-H) | 1 | 258.9606 | $C_9H_8O_4Br$ | 1.56 | |
| | **$MS^2$** FI at *m/z* 78.9176 | 100 | 78.9183 | Br | -9.33 | |
| | FI at *m/z* 199.9473 | 81 | 199.9473 | $C_7H_5O_2Br$ | 0 | |
| | FI at *m/z* 214.9709 | 5 | 214.9708 | $C_8H_8O_2Br$ | 0.62 | |
| **B6** | **25B-NBOMe-M (*N*-demethoxybenzyl-)** | | | | | 5.6 |
| | **$MS^1$** PM at *m/z* 260.0273 (M+H) | 1 | 260.0281 | $C_{10}H_{15}O_2NBr$ | -2.95 | |
| | **$MS^2$** FI at *m/z* 164.0830 | 22 | 164.0837 | $C_{10}H_{12}O_2$ | -4.45 | |
| | FI at *m/z* 212.9543 | 39 | 212.9551 | $C_8H_6O_2Br$ | -3.83 | |
| | FI at *m/z* 227.9776 | 100 | 227.9786 | $C_9H_9O_2Br$ | -4.35 | |
| | FI at *m/z* 243.0013 | 90 | 243.0021 | $C_{10}H_{12}O_2Br$ | -3.15 | |
| **B7** | **25B-NBOMe-M (*O,O,O-tris*-demethyl-)** | | | | | 5.1 |
| | **$MS^1$** PM at *m/z* 338.0392 (M+H) | 9 | 338.0386 | $C_{15}H_{17}O_3NBr$ | 1.68 | |
| | **$MS^2$** FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | FI at *m/z* 136.0520 | 33 | 136.0524 | $C_8H_8O_2$ | -3.16 | |
| | FI at *m/z* 214.9703 | 81 | 214.9708 | $C_8H_8O_2Br$ | -2.17 | |
| | FI at *m/z* 231.9968 | 32 | 231.9968 | $C_8H_{11}O_2NBr$ | 0 | |
| **B8** | **25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 1** | | | | | 6.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS**[1] | PM at *m/z* 352.0542 (M+H) | 5 | 352.0543 | $C_{16}H_{19}O_3NBr$ | -0.23 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 57 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 226.9700 | 1 | 226.9708 | $C_9H_8O_2Br$ | -3.37 | |
| | | FI at *m/z* 335.0267 | 0.5 | 335.0283 | $C_{16}H_{16}O_3Br$ | -4.72 | |
| **B9** | **25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 2** | | | | | | 6.8 |
| | **MS**[1] | PM at *m/z* 352.0540 (M+H) | 11 | 352.0543 | $C_{16}H_{19}O_3NBr$ | -0.80 | |
| | **MS**[2] | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 213.9630 | 31 | 213.9629 | $C_8H_7O_2Br$ | 0.28 | |
| | | FI at *m/z* 228.9858 | 90 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0124 | 29 | 246.0124 | $C_9H_{13}O_2NBr$ | 0 | |
| **B10** | **25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 3** | | | | | | 6.9 |
| | **MS**[1] | PM at *m/z* 352.0541 (M+H) | 12 | 352.0543 | $C_{16}H_{19}O_3NBr$ | -0.52 | |
| | **MS**[2] | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 213.9623 | 32 | 213.9629 | $C_8H_7O_2Br$ | -3.00 | |
| | | FI at *m/z* 228.9858 | 89 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0124 | 29 | 246.0124 | $C_9H_{13}O_2NBr$ | 0 | |
| **B11** | **25B-NBOMe-M (*O*-demethyl-dehydro-) isomer 1** | | | | | | 6.7 |
| | **MS**[1] | PM at *m/z* 364.0540 (M+H) | 25 | 364.0543 | $C_{17}H_{19}O_3NBr$ | -0.77 | |
| | **MS**[2] | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 228.9857 | 16 | 228.9864 | $C_9H_{10}O_2Br$ | -3.13 | |
| | | FI at *m/z* 258.0122 | 71 | 258.0124 | $C_{10}H_{13}O_2NBr$ | -0.84 | |
| **B12** | **25B-NBOMe-M (*O*-demethyl-dehydro-) isomer 2** | | | | | | 7.4 |
| | **MS**[1] | PM at *m/z* 364.0540 (M+H) | 3 | 364.0543 | $C_{17}H_{19}O_3NBr$ | -0.77 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 57 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 227.9655 | 13 | 227.9655 | $C_8H_7O_2NBr$ | 0 | |
| | | FI at *m/z* 242.9890 | 12 | 242.9890 | $C_9H_{10}O_2NBr$ | 0 | |
| **B13** | **25B-NBOMe-M (*O*-demethyl-) isomer 1** | | | | | | 7.6 |
| | **MS**[1] | PM at *m/z* 366.0700 (M+H) | 4 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 0.19 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 58 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 228.9861 | 0.5 | 228.9864 | $C_9H_{10}O_2Br$ | -1.38 | |
| | | FI at *m/z* 257.1167 | 1 | 257.1178 | $C_{16}H_{17}O_3$ | -4.16 | |
| | | FI at *m/z* 349.0433 | 0.6 | 349.0439 | $C_{17}H_{18}O_3Br$ | -1.81 | |
| **B14** | **25B-NBOMe-M (*O*-demethyl-) isomer 2** | | | | | | 7.7 |
| | **MS**[1] | PM at *m/z* 366.0703 (M+H) | 6 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 1.01 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 59 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0649 | 100 | 121.0653 | $C_8H_9O$ | -3.63 | |
| | | FI at *m/z* 228.9855 | 0.4 | 228.9864 | $C_9H_{10}O_2Br$ | -4.00 | |
| | | FI at *m/z* 243.9961 | 0.3 | 243.9968 | $C_9H_{11}O_2NBr$ | -2.73 | |
| | | FI at *m/z* 270.1254 | 0.1 | 270.1256 | $C_{17}H_{18}O_3$ | -0.72 | |
| **B15** | **25B-NBOMe-M (*O*-demethyl-) isomer 3** | | | | | | 8.0 |
| | **MS**[1] | PM at *m/z* 366.0696 (M+H) | 3 | 366.0699 | $C_{17}H_{21}O_3NBr$ | -0.91 | |
| | **MS**[2] | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 227.9779 | 50 | 227.9786 | $C_9H_9O_2Br$ | -3.03 | |
| | | FI at *m/z* 243.0014 | 92 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.74 | |
| | | FI at *m/z* 260.0280 | 26 | 260.0281 | $C_{10}H_{15}O_2NBr$ | -0.26 | |
| **B16** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 1** | | | | | | 5.1 |
| | **MS**[1] | PM at *m/z* 368.0495 (M+H) | 4 | 368.0492 | $C_{16}H_{19}O_4NBr$ | 0.82 | |
| | **MS**[2] | FI at *m/z* 107.0496 | 44 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 137.0598 | 100 | 137.0603 | $C_8H_9O_2Br$ | -3.32 | |
| | | FI at *m/z* 228.9858 | 1 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 351.0238 | 0.4 | 351.0232 | $C_{16}H_{16}O_4Br$ | 1.72 | |
| **B17** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 2** | | | | | | 5.6 |
| | **MS**[1] | PM at *m/z* 368.0481 (M+H) | 9 | 368.0492 | $C_{16}H_{19}O_4NBr$ | -2.98 | |
| | **MS**[2] | FI at *m/z* 123.0441 | 100 | 123.0446 | $C_7H_7O_2$ | -4.10 | |
| | | FI at *m/z* 213.9620 | 26 | 213.9629 | $C_8H_7O_2Br$ | -4.40 | |
| | | FI at *m/z* 228.9853 | 96 | 228.9864 | $C_9H_{10}O_2Br$ | -4.87 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | FI at *m/z* 246.0122 | 30 | 246.0130 | $C_9H_{13}O_2NBr$ | -3.11 | |
| **B18** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 3** | | | | | | 6.3 |
| | **MS**[1] | PM at *m/z* 368.0489 (M+H) | 13 | 368.0492 | $C_{16}H_{19}O_4NBr$ | -0.91 | |
| | **MS**[2] | FI at *m/z* 123.0443 | 100 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 213.9624 | 24 | 213.9629 | $C_8H_7O_2Br$ | -2.53 | |
| | | FI at *m/z* 228.9859 | 72 | 228.9864 | $C_9H_{10}O_2Br$ | -2.25 | |
| | | FI at *m/z* 246.0124 | 22 | 246.0130 | $C_9H_{13}O_2NBr$ | -2.30 | |
| **B19** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 4** | | | | | | 6.6 |
| | **MS**[1] | PM at *m/z* 368.0495 (M+H) | 10 | 368.0492 | $C_{16}H_{19}O_4NBr$ | 0.82 | |
| | **MS**[2] | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 229.9573 | 61 | 229.9579 | $C_8H_7O_3Br$ | -2.42 | |
| | | FI at *m/z* 244.9808 | 86 | 244.9813 | $C_9H_{10}O_3Br$ | -2.17 | |
| | | FI at *m/z* 262.0074 | 33 | 262.0073 | $C_9H_{13}O_3NBr$ | 0.26 | |
| **B20** | **25B-NBOMe-M (dehydro-)** | | | | | | 7.4 |
| | **MS**[1] | PM at *m/z* 378.0697 (M+H) | 16 | 378.0699 | $C_{18}H_{21}O_3NBr$ | -0.61 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 58 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 239.9647 | 1 | 239.9655 | $C_9H_7O_2NBr$ | -3.19 | |
| | | FI at *m/z* 255.9966 | 8 | 255.9968 | $C_{10}H_{11}O_2NBr$ | -0.65 | |
| **B21** | **25B-NBOMe-M (*O*-demethyl-dehydro-hydroxy-)** | | | | | | 5.7 |
| | **MS**[1] | PM at *m/z* 380.0494 (M+H) | 20 | 380.0494 | $C_{17}H_{19}O_4NBr$ | 0.54 | |
| | **MS**[2] | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 244.9809 | 9 | 244.9813 | $C_9H_{10}O_3Br$ | -1.76 | |
| | | FI at *m/z* 274.0072 | 55 | 274.0073 | $C_{10}H_{13}O_3NBr$ | -0.48 | |
| **B22** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 1** | | | | | | 6.2 |
| | **MS**[1] | PM at *m/z* 382.0665 (M+H) | 7 | 382.0648 | $C_{17}H_{21}O_4NBr$ | 4.33 | |
| | **MS**[2] | FI at *m/z* 91.0548 | 53 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 228.9859 | 4 | 228.9864 | $C_9H_{10}O_2Br$ | -2.25 | |
| | | FI at *m/z* 365.0403 | 0.2 | 365.0388 | $C_{10}H_{15}O_3NBr$ | 3.98 | |
| **B23** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 2** | | | | | | 6.7 |
| | **MS**[1] | PM at *m/z* 382.0643 (M+H) | 7 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -1.43 | |
| | **MS**[2] | FI at *m/z* 107.0494 | 44 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 228.9861 | 1 | 228.9864 | $C_9H_{10}O_2Br$ | -1.38 | |
| | | FI at *m/z* 243.9975 | 0.2 | 243.9968 | $C_9H_{11}O_2NBr$ | 3.01 | |
| | | FI at *m/z* 365.0375 | 0.1 | 365.0388 | $C_{17}H_{18}O_4Br$ | -3.69 | |
| **B24** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 3** | | | | | | 6.8 |
| | **MS**[1] | PM at *m/z* 382.0644 (M+H) | 11 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -1.17 | |
| | **MS**[2] | FI at *m/z* 123.0442 | 85 | 123.0446 | $C_7H_7O_2$ | -3.29 | |
| | | FI at *m/z* 227.9780 | 55 | 227.9786 | $C_9H_9O_2Br$ | -2.59 | |
| | | FI at *m/z* 243.0014 | 100 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.74 | |
| | | FI at *m/z* 260.0281 | 28 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0 | |
| **B25** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) isomer 4** | | | | | | 7.2 |
| | **MS**[1] | PM at *m/z* 382.0646 (M+H) | 8 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -0.65 | |
| | **MS**[2] | FI at *m/z* 123.0442 | 100 | 123.0446 | $C_7H_7O_2$ | -3.29 | |
| | | FI at *m/z* 227.9779 | 56 | 227.9786 | $C_9H_9O_2Br$ | -3.03 | |
| | | FI at *m/z* 243.0014 | 99 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.74 | |
| | | FI at *m/z* 260.0280 | 28 | 260.0281 | $C_{10}H_{15}O_2NBr$ | -0.26 | |
| **B26** | **25B-NBOMe-M (dehydro-hydroxy-) isomer 1** | | | | | | 6.5 |
| | **MS**[1] | PM at *m/z* 394.0652 (M+H) | 30 | 394.0648 | $C_{18}H_{21}O_4NBr$ | 0.90 | |
| | **MS**[2] | FI at *m/z* 109.0651 | 100 | 109.0653 | $C_7H_9O$ | -2.20 | |
| | | FI at *m/z* 137.0597 | 26 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 239.9647 | 1 | 239.9655 | $C_9H_7O_2NBr$ | -3.19 | |
| | | FI at *m/z* 255.9965 | 9 | 255.9968 | $C_{10}H_{11}O_2NBr$ | -1.04 | |
| **B27** | **25B-NBOMe-M (dehydro-hydroxy-) isomer 2** | | | | | | 6.6 |
| | **MS**[1] | PM at *m/z* 394.0650 (M+H) | 19 | 394.0648 | $C_{18}H_{21}O_4NBr$ | 0.39 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS²** | FI at *m/z* 91.0548 | 56 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 256.9681 | 2 | 256.9681 | $C_9H_8O_3NBr$ | -0.41 | |
| | | FI at *m/z* 271.9916 | 7 | 271.9917 | $C_{10}H_{11}O_3NBr$ | -0.30 | |
| **B28** | **25B-NBOMe-M (dehydro-hydroxy-) isomer 3** | | | | | | 6.7 |
| | **MS¹** | PM at *m/z* 394.0658 (M+H) | 6 | 394.0648 | $C_{18}H_{21}O_4NBr$ | 2.42 | |
| | **MS²** | FI at *m/z* 107.0495 | 38 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 239.9657 | 1 | 239.9655 | $C_9H_7O_2NBr$ | 0.97 | |
| | | FI at *m/z* 255.9967 | 6 | 255.9968 | $C_{10}H_{11}O_3NBr$ | -0.26 | |
| **B29** | **25B-NBOMe-M (hydroxy-) isomer 1** | | | | | | 7.1 |
| | **MS¹** | PM at *m/z* 396.0806 (M+H) | 12 | 396.0805 | $C_{18}H_{23}O_4NBr$ | 0.26 | |
| | **MS²** | FI at *m/z* 91.0548 | 62 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 243.9732 | 4 | 243.9735 | $C_9H_9O_3Br$ | -1.25 | |
| | | FI at *m/z* 258.9967 | 11 | 258.9970 | $C_{10}H_{12}O_3Br$ | -1.08 | |
| **B30** | **25B-NBOMe-M (hydroxy-) isomer 2** | | | | | | 7.3 |
| | **MS¹** | PM at *m/z* 396.0800 (M+H) | 17 | 396.0805 | $C_{18}H_{23}O_4NBr$ | -1.25 | |
| | **MS²** | FI at *m/z* 109.0651 | 100 | 109.0653 | $C_7H_9O$ | -2.20 | |
| | | FI at *m/z* 137.0596 | 23 | 137.0603 | $C_8H_9O_2$ | -4.78 | |
| | | FI at *m/z* 243.0015 | 4 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.33 | |
| | | FI at *m/z* 258.0123 | 1 | 258.0124 | $C_{10}H_{13}O_2NBr$ | -0.45 | |
| **B31** | **25B-NBOMe-M (hydroxy-) isomer 3** | | | | | | 7.8 |
| | **MS¹** | PM at *m/z* 396.0805 (M+H) | 10 | 396.0805 | $C_{18}H_{23}O_4NBr$ | 0 | |
| | **MS²** | FI at *m/z* 107.0494 | 53 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 243.0010 | 2 | 243.0021 | $C_{10}H_{12}O_2Br$ | -4.39 | |
| | | FI at *m/z* 258.0137 | 1 | 258.0124 | $C_{10}H_{13}O_2NBr$ | 4.97 | |
| **B32** | **25B-NBOMe-M (hydroxy-) isomer 4** | | | | | | 8.4 |
| | **MS¹** | PM at *m/z* 396.0800 (M+H) | 6 | 396.0805 | $C_{18}H_{23}O_4NBr$ | -1.25 | |
| | **MS²** | FI at *m/z* 91.0548 | 54 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 258.0128 | 4 | 258.0124 | $C_{10}H_{13}O_2NBr$ | 1.49 | |
| | | FI at *m/z* 378.0704 | 2 | 378.0699 | $C_{18}H_{21}O_3NBr$ | 1.24 | |
| **B33** | **25B-NBOMe-M (*O*-demethyl-*bis*-hydroxy-)** | | | | | | 6.2 |
| | **MS¹** | PM at *m/z* 398.0590 (M+H) | 1 | 398.0598 | $C_{17}H_{21}O_5NBr$ | -1.91 | |
| | **MS²** | FI at *m/z* 138.0312 | 39 | 138.0317 | $C_7H_6O_3$ | -3.59 | |
| | | FI at *m/z* 153.0547 | 100 | 153.0552 | $C_8H_9O_3$ | -3.07 | |
| | | FI at *m/z* 228.9861 | 71 | 228.9864 | $C_9H_{10}O_2Br$ | -1.38 | |
| | | FI at *m/z* 246.0123 | 25 | 246.0124 | $C_9H_{13}O_2NBr$ | -0.47 | |
| **B34** | **25B-NBOMe-M (*bis*-hydroxy-) isomer 1** | | | | | | 7.4 |
| | **MS¹** | PM at *m/z* 412.0750 (M+H) | 1 | 412.0754 | $C_{18}H_{23}O_5NBr$ | -1.00 | |
| | **MS²** | FI at *m/z* 107.0495 | 54 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 153.0544 | 63 | 153.0552 | $C_8H_9O_3$ | -5.03 | |
| | | FI at *m/z* 243.0010 | 100 | 243.0021 | $C_{10}H_{12}O_2Br$ | -4.39 | |
| | | FI at *m/z* 260.0282 | 32 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0.51 | |
| **B35** | **25B-NBOMe-M (*bis*-hydroxy-) isomer 2** | | | | | | 7.7 |
| | **MS¹** | PM at *m/z* 412.0756 (M+H) | 6 | 412.0754 | $C_{18}H_{23}O_5NBr$ | 0.46 | |
| | **MS²** | FI at *m/z* 91.0548 | 60 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 274.9907 | 1 | 274.9919 | $C_{10}H_{12}O_4Br$ | -4.35 | |
| | | FI at *m/z* 290.0018 | 0.3 | 290.0022 | $C_{10}H_{13}O_4NBr$ | -1.54 | |

**Table S2** List of 25C-NBOMe and its phase I metabolites together with the precursor mass (PM) recorded in $MS^1$, the corresponding characteristic fragment ions (FI) in $MS^2$, the calculated exact masses, the corresponding elemental composition, the deviation of the measured from the calculated masses, given as errors in parts per million (ppm), and the retention times (RT) in minutes (min). The metabolites were sorted by mass and RT.

| No. | Metabolite and characteristic ions Measured accurate mass, *m/z* | Relative intensity in $MS^2$, % | Calculated exact mass, *m/z* | Elemental composition | Error, ppm | RT, min |
|---|---|---|---|---|---|---|
| **C1** | **25C-NBOMe** | | | | | 8.5 |
| | **$MS^1$** PM at *m/z* 336.1360 (M+H) | 8 | 336.1361 | $C_{18}H_{23}O_3NCl$ | -0.29 | |
| | **$MS^2$** FI at *m/z* 91.0548 | 56 | 91.0548 | $C_7H_7$ | 0 | |
| | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | FI at *m/z* 184.0286 | 0.3 | 184.0291 | $C_9H_9O_2Cl$ | -2.76 | |
| | FI at *m/z* 199.0522 | 1 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -1.92 | |
| | FI at *m/z* 214.0627 | 0.2 | 214.0629 | $C_{10}H_{13}O_2NCl$ | -1.09 | |
| **C2** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 1** | | | | | 3.4 |
| | **$MS^1$** PM at *m/z* 202.0629 (M+H) | 1 | 202.0629 | $C_9H_{13}O_2NCl$ | 0 | |
| | **$MS^2$** FI at *m/z* 135.0441 | 6 | 135.0446 | $C_8H_7O_2$ | -3.74 | |
| | FI at *m/z* 150.0676 | 23 | 150.0681 | $C_9H_{10}O_2$ | -3.20 | |
| | FI at *m/z* 170.0129 | 83 | 170.0135 | $C_8H_7O_2Cl$ | -3.28 | |
| | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| **C3** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) isomer 2** | | | | | 3.5 |
| | **$MS^1$** PM at *m/z* 202.0628 (M+H) | 1 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | **$MS^2$** FI at *m/z* 135.0441 | 5 | 135.0446 | $C_8H_7O_2$ | -3.74 | |
| | FI at *m/z* 150.0675 | 18 | 150.0681 | $C_9H_{10}O_2$ | -3.86 | |
| | FI at *m/z* 170.0129 | 84 | 170.0135 | $C_8H_7O_2Cl$ | -1.84 | |
| | FI at *m/z* 185.0364 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.88 | |
| **C4** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-)** | | | | | 5.9 |
| | **$MS^1$** PM at *m/z* 215.0108 (M-H) | 1 | 215.0111 | $C_9H_8O_4Cl$ | -1.46 | |
| | **$MS^2$** FI at *m/z* 155.9976 | 100 | 155.9978 | $C_7H_5O_2Cl$ | -1.33 | |
| | FI at *m/z* 171.0210 | 2 | 171.0213 | $C_8H_8O_2Cl$ | -1.65 | |
| **C5** | **25C-NBOMe-M (*N*-demethoxybenzyl-)** | | | | | 5.2 |
| | **$MS^1$** PM at *m/z* 216.0781 (M+H) | 1 | 216.0786 | $C_{10}H_{15}O_2NCl$ | -2.24 | |
| | **$MS^2$** FI at *m/z* 164.0829 | 20 | 164.0837 | $C_{10}H_{12}O_2$ | -5.06 | |
| | FI at *m/z* 169.0048 | 28 | 169.0056 | $C_8H_6O_2Cl$ | -4.93 | |
| | FI at *m/z* 184.0284 | 100 | 184.0291 | $C_9H_9O_2Cl$ | -3.85 | |
| | FI at *m/z* 199.0517 | 86 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -4.44 | |
| **C6** | **25C-NBOMe-M (*N*-demethoxybenzyl-oxo-)** | | | | | 6.6 |
| | **$MS^1$** PM at *m/z* 230.0581 (M+H) | 1 | 230.0578 | $C_{10}H_{13}O_3NCl$ | 1.09 | |
| | **$MS^2$** FI at *m/z* 155.0257 | 42 | 155.0264 | $C_8H_8OCl$ | -4.31 | |
| | FI at *m/z* 173.0364 | 2 | 173.0369 | $C_8H_{10}O_2Cl$ | -3.08 | |
| | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| **C7** | **25C-NBOMe-M (*N*-demethoxybenzyl-hydroxy-)** | | | | | 3.8 |
| | **$MS^1$** PM at *m/z* 232.0730 (M+H) | 1 | 232.0735 | $C_{10}H_{15}O_3NCl$ | -2.15 | |
| | **$MS^2$** FI at *m/z* 185.0000 | 42 | 185.0005 | $C_8H_6O_3Cl$ | -2.96 | |
| | FI at *m/z* 200.0234 | 30 | 200.0240 | $C_9H_9O_3Cl$ | -3.11 | |
| | FI at *m/z* 215.0469 | 100 | 215.0475 | $C_{10}H_{12}O_3Cl$ | -2.78 | |
| **C8** | **25C-NBOMe-M (*O*,*O*,*O*-*tris*-demethyl-)** | | | | | 5.0 |
| | **$MS^1$** PM at *m/z* 294.0879 (M+H) | 6 | 294.0891 | $C_{15}H_{17}O_3NCl$ | -4.25 | |
| | **$MS^2$** FI at *m/z* 107.0494 | 100 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | FI at *m/z* 136.0518 | 6 | 136.0524 | $C_8H_8O_2$ | -4.63 | |
| | FI at *m/z* 171.0206 | 80 | 171.0213 | $C_8H_8O_2Cl$ | -3.99 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | FI at *m/z* 188.0472 | 27 | 188.0473 | $C_8H_{11}O_2NCl$ | -0.44 | |
| **C9** | **25C-NBOMe-M (*O,O-bis*-demethyl-dehydro-)** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 306.0893 (M+H) | 4 | 306.0891 | $C_{16}H_{17}O_3NCl$ | 0.49 | |
| | **MS²** | FI at *m/z* 107.0495 | 36 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 184.0160 | 46 | 184.0160 | $C_8H_7O_2NCl$ | 0 | |
| | | FI at *m/z* 199.0395 | 41 | 199.0395 | $C_9H_{10}O_2NCl$ | 0 | |
| | | FI at *m/z* 200.0474 | 100 | 200.0473 | $C_9H_{11}O_2NCl$ | 0.58 | |
| **C10** | **25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 1** | | | | | | 5.8 |
| | **MS¹** | PM at *m/z* 308.1046 (M+H) | 6 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.64 | |
| | **MS²** | FI at *m/z* 91.0547 | 51 | 91.0548 | $C_7H_7$ | -0.82 | |
| | | FI at *m/z* 121.0649 | 100 | 121.0653 | $C_8H_9O$ | -3.63 | |
| | | FI at *m/z* 185.0360 | 7 | 185.0369 | $C_9H_{10}O_2Cl$ | -5.04 | |
| | | FI at *m/z* 202.0624 | 2 | 202.0629 | $C_9H_{13}O_2NCl$ | -2.64 | |
| **C11** | **25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 2** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 308.1051 (M+H) | 13 | 308.1048 | $C_{16}H_{19}O_3NCl$ | 0.98 | |
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 170.0129 | 13 | 170.0135 | $C_8H_7O_2Cl$ | -3.28 | |
| | | FI at *m/z* 185.0364 | 77 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.88 | |
| | | FI at *m/z* 202.0632 | 22 | 202.0629 | $C_9H_{13}O_2NCl$ | 1.32 | |
| **C12** | **25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 3** | | | | | | 6.6 |
| | **MS¹** | PM at *m/z* 308.1048 (M+H) | 10 | 308.1048 | $C_{16}H_{19}O_3NCl$ | 0 | |
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 170.0130 | 25 | 170.0135 | $C_8H_7O_2Cl$ | -2.69 | |
| | | FI at *m/z* 185.0365 | 87 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0629 | 22 | 202.0629 | $C_9H_{13}O_2NCl$ | 0 | |
| **C13** | **25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 1** | | | | | | 5.7 |
| | **MS¹** | PM at *m/z* 320.1047 (M+H) | 17 | 320.1048 | $C_{17}H_{19}O_3NCl$ | -0.31 | |
| | **MS²** | FI at *m/z* 91.0548 | 57 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 198.0317 | 5 | 198.0316 | $C_9H_9O_2N$ | -3.14 | |
| **C14** | **25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 2** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 320.1049 (M+H) | 24 | 320.1048 | $C_{17}H_{19}O_3NCl$ | 0.32 | |
| | **MS²** | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 185.0365 | 17 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 214.0629 | 73 | 214.0629 | $C_{10}H_{13}O_2NCl$ | 0 | |
| **C15** | **25C-NBOMe-M (*O*-demethyl-dehydro-) isomer 3** | | | | | | 7.1 |
| | **MS¹** | PM at *m/z* 320.1050 (M+H) | 3 | 320.1048 | $C_{17}H_{19}O_3NCl$ | 0.63 | |
| | **MS²** | FI at *m/z* 91.0548 | 49 | 91.0548 | $C_7H_7$ | 0.27 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 184.0160 | 10 | 184.0160 | $C_8H_7O_2NCl$ | 0 | |
| | | FI at *m/z* 199.0394 | 11 | 199.0395 | $C_9H_{10}O_2NCl$ | -0.29 | |
| **C16** | **25C-NBOMe-M (*O*-demethyl-) isomer 1** | | | | | | 7.2 |
| | **MS¹** | PM at *m/z* 322.1206 (M+H) | 10 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.47 | |
| | **MS²** | FI at *m/z* 91.0548 | 54 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 185.0368 | 1 | 185.0369 | $C_9H_{10}O_2Cl$ | -0.72 | |
| | | FI at *m/z* 200.0473 | 1 | 200.0473 | $C_9H_{11}O_2NCl$ | 0 | |
| | | FI at *m/z* 305.0931 | 0.1 | 305.0944 | $C_{17}H_{18}O_3Cl$ | -4.42 | |
| **C17** | **25C-NBOMe-M (*O*-demethyl-) isomer 2** | | | | | | 7.3 |
| | **MS¹** | PM at *m/z* 322.1199 (M+H) | 6 | 322.1204 | $C_{17}H_{21}O_3NCl$ | -1.70 | |
| | **MS²** | FI at *m/z* 91.0549 | 58 | 91.0548 | $C_7H_7$ | 1.37 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 185.0364 | 0.3 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.88 | |
| | | FI at *m/z* 200.0473 | 0.2 | 200.0473 | $C_9H_{11}O_2NCl$ | 0 | |
| | | FI at *m/z* 305.0934 | 0.1 | 305.0944 | $C_{17}H_{18}O_3Cl$ | -3.44 | |
| **C18** | **25C-NBOMe-M (*O*-demethyl-) isomer 3** | | | | | | 7.7 |
| | **MS¹** | PM at *m/z* 322.1198 (M+H) | 10 | 322.1204 | $C_{17}H_{21}O_3NCl$ | -2.01 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS²** | FI at *m/z* 107.0494 | 100 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | | FI at *m/z* 184.0285 | 44 | 184.0291 | $C_9H_9O_2Cl$ | -3.30 | |
| | | FI at *m/z* 199.0518 | 90 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -3.93 | |
| | | FI at *m/z* 216.0784 | 21 | 216.0786 | $C_{10}H_{15}O_2NCl$ | -0.85 | |
| **C19** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 1** | | | | | | 5.0 |
| | **MS¹** | PM at *m/z* 324.1006 (M+H) | 4 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 2.73 | |
| | **MS²** | FI at *m/z* 107.0495 | 44 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2Cl$ | -4.05 | |
| | | FI at *m/z* 185.0365 | 4 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0637 | 1 | 202.0629 | $C_{19}H_{13}O_2NCl$ | 3.79 | |
| | | FI at *m/z* 307.0722 | 1 | 307.0737 | $C_{16}H_{16}O_4Cl$ | -4.93 | |
| **C20** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 2** | | | | | | 5.3 |
| | **MS¹** | PM at *m/z* 324.0997 (M+H) | 12 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 0 | |
| | **MS²** | FI at *m/z* 123.0443 | 95 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 170.0129 | 19 | 170.0135 | $C_8H_7O_2Cl$ | -3.28 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0630 | 26 | 202.0635 | $C_9H_{13}O_2NCl$ | -2.39 | |
| **C21** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 3** | | | | | | 5.6 |
| | **MS¹** | PM at *m/z* 324.0995 (M+H) | 3 | 324.0997 | $C_{16}H_{19}O_4NCl$ | -0.66 | |
| | **MS²** | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 186.0078 | 41 | 186.0084 | $C_8H_7O_3Cl$ | -3.08 | |
| | | FI at *m/z* 201.0312 | 94 | 201.0318 | $C_9H_{10}O_3Cl$ | -3.22 | |
| | | FI at *m/z* 218.0578 | 31 | 218.0578 | $C_9H_{13}O_3NCl$ | 0 | |
| **C22** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 4** | | | | | | 5.8 |
| | **MS¹** | PM at *m/z* 324.0995 (M+H) | 11 | 324.0997 | $C_{16}H_{19}O_4NCl$ | -0.66 | |
| | **MS²** | FI at *m/z* 123.0443 | 100 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 170.0130 | 12 | 170.0135 | $C_8H_7O_2Cl$ | -2.69 | |
| | | FI at *m/z* 185.0365 | 65 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 17 | 202.0635 | $C_9H_{13}O_2NCl$ | -3.38 | |
| **C23** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) isomer 5** | | | | | | 6.2 |
| | **MS¹** | PM at *m/z* 324.0990 (M+H) | 1 | 324.0997 | $C_{16}H_{19}O_4NCl$ | -2.20 | |
| | **MS²** | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 200.0473 | 99 | 200.0473 | $C_9H_{11}O_2NCl$ | 0 | |
| | | FI at *m/z* 218.0581 | 1 | 218.0578 | $C_9H_{13}O_3NCl$ | 1.15 | |
| | | FI at *m/z* 306.0890 | 9 | 306.0891 | $C_{16}H_{17}O_3NCl$ | -0.49 | |
| **C24** | **25C-NBOMe-M (dehydro-)** | | | | | | 7.2 |
| | **MS¹** | PM at *m/z* 334.1202 (M+H) | 20 | 334.1204 | $C_{18}H_{21}O_3NCl$ | -0.74 | |
| | **MS²** | FI at *m/z* 91.0548 | 53 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 196.0160 | 1 | 196.0160 | $C_9H_7O_2NCl$ | 0 | |
| | | FI at *m/z* 212.0472 | 7 | 212.0473 | $C_{10}H_{11}O_2NCl$ | -0.39 | |
| **C25** | **25C-NBOMe-M (*O*-demethyl-dehydro-hydroxy-)** | | | | | | 6.0 |
| | **MS¹** | PM at *m/z* 336.0983 (M+H) | 3 | 336.0997 | $C_{17}H_{19}O_4NCl$ | -4.21 | |
| | **MS²** | FI at *m/z* 107.0495 | 42 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 184.0160 | 11 | 184.0160 | $C_8H_7O_2NCl$ | 0 | |
| | | FI at *m/z* 199.0394 | 11 | 199.0395 | $C_9H_{10}O_2NCl$ | -0.29 | |
| **C26** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 1** | | | | | | 6.1 |
| | **MS¹** | PM at *m/z* 338.1145 (M+H) | 9 | 338.1154 | $C_{17}H_{21}O_4NCl$ | -2.55 | |
| | **MS²** | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 200.0235 | 78 | 200.0240 | $C_9H_9O_3Cl$ | -3.32 | |
| | | FI at *m/z* 215.0469 | 86 | 215.0475 | $C_{10}H_{12}O_3Cl$ | -2.78 | |
| | | FI at *m/z* 232.0733 | 26 | 232.0735 | $C_{10}H_{15}O_3NCl$ | -0.86 | |
| **C27** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 2** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 338.1151 (M+H) | 7 | 338.1154 | $C_{17}H_{21}O_4NCl$ | -0.78 | |
| | **MS²** | FI at *m/z* 107.0495 | 41 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0598 | 100 | 137.0603 | $C_8H_9O_2$ | -3.32 | |
| | | FI at *m/z* 185.0365 | 1 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 200.0465 | 0.2 | 200.0473 | $C_9H_{11}O_2NCl$ | -3.92 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | FI at *m/z* 321.0878 | 0.2 | 321.0894 | $C_{17}H_{18}O_4Cl$ | -4.87 | |
| **C28** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 3** | | | | | | 6.5 |
| | **MS¹** | PM at *m/z* 338.1146 (M+H) | 10 | 338.1154 | $C_{17}H_{21}O_4NCl$ | -2.26 | |
| | **MS²** | FI at *m/z* 123.0442 | 100 | 123.0446 | $C_7H_7O_2$ | -3.29 | |
| | | FI at *m/z* 184.0286 | 47 | 184.0291 | $C_9H_9O_2Cl$ | -2.76 | |
| | | FI at *m/z* 199.0521 | 91 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.43 | |
| | | FI at *m/z* 216.0788 | 22 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 1.00 | |
| **C29** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) isomer 4** | | | | | | 7.1 |
| | **MS¹** | PM at *m/z* 338.1164 (M+H) | 5 | 338.1154 | $C_{17}H_{21}O_4NCl$ | 3.07 | |
| | **MS²** | FI at *m/z* 123.0443 | 100 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 184.0287 | 39 | 184.0291 | $C_9H_9O_2Cl$ | -2.22 | |
| | | FI at *m/z* 199.0521 | 76 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.43 | |
| | | FI at *m/z* 216.0786 | 15 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 0 | |
| **C30** | **25C-NBOMe-M (dehydro-hydroxy-)** | | | | | | 6.3 |
| | **MS¹** | PM at *m/z* 350.1148 (M+H) | 17 | 350.1154 | $C_{18}H_{21}O_4NCl$ | -1.61 | |
| | **MS²** | FI at *m/z* 91.0547 | 54 | 91.0548 | $C_7H_7$ | -0.82 | |
| | | FI at *m/z* 121.0649 | 100 | 121.0653 | $C_8H_9O$ | -3.63 | |
| | | FI at *m/z* 213.0185 | 2 | 213.0187 | $C_9H_8O_3NCl$ | -1.05 | |
| | | FI at *m/z* 228.0421 | 6 | 228.0422 | $C_{10}H_{11}O_3NCl$ | -0.43 | |
| **C31** | **25C-NBOMe-M (hydroxy-) isomer 1** | | | | | | 6.9 |
| | **MS¹** | PM at *m/z* 352.1303 (M+H) | 10 | 352.1310 | $C_{18}H_{23}O_4NCl$ | -2.03 | |
| | **MS²** | FI at *m/z* 91.0546 | 51 | 91.0548 | $C_7H_7$ | -1.92 | |
| | | FI at *m/z* 121.0649 | 100 | 121.0653 | $C_8H_9O$ | -3.63 | |
| | | FI at *m/z* 200.0231 | 2 | 200.0240 | $C_9H_9O_3Cl$ | -4.61 | |
| | | FI at *m/z* 215.0465 | 2 | 215.0475 | $C_{10}H_{12}O_3Cl$ | -4.64 | |
| **C32** | **25C-NBOMe-M (hydroxy-) isomer 2** | | | | | | 7.1 |
| | **MS¹** | PM at *m/z* 352.1304 (M+H) | 13 | 352.1310 | $C_{18}H_{23}O_4NCl$ | -1.74 | |
| | **MS²** | FI at *m/z* 91.0549 | 61 | 91.0548 | $C_7H_7$ | 1.37 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 200.0236 | 3 | 200.0240 | $C_9H_9O_3Cl$ | -2.11 | |
| | | FI at *m/z* 215.0472 | 10 | 215.0475 | $C_{10}H_{12}O_3Cl$ | -1.39 | |
| **C33** | **25C-NBOMe-M (hydroxy-) isomer 3** | | | | | | 7.6 |
| | **MS¹** | PM at *m/z* 352.1305 (M+H) | 9 | 352.1310 | $C_{18}H_{23}O_4NCl$ | -1.46 | |
| | **MS²** | FI at *m/z* 107.0494 | 43 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | | FI at *m/z* 137.0596 | 100 | 137.0603 | $C_8H_9O_2$ | -4.78 | |
| | | FI at *m/z* 184.0283 | 2 | 184.0291 | $C_9H_9O_2Cl$ | -4.39 | |
| | | FI at *m/z* 199.0520 | 5 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.93 | |
| **C34** | **25C-NBOMe-M (*O*-demethyl-*bis*-hydroxy-)** | | | | | | 5.7 |
| | **MS¹** | PM at *m/z* 354.1090 (M+H) | 2 | 354.1103 | $C_{17}H_{21}O_5NCl$ | -3.61 | |
| | **MS²** | FI at *m/z* 107.0494 | 60 | 107.0497 | $C_7H_7O$ | -2.71 | |
| | | FI at *m/z* 153.0550 | 60 | 153.0552 | $C_8H_9O_3$ | -1.11 | |
| | | FI at *m/z* 185.0362 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -3.96 | |
| | | FI at *m/z* 202.0629 | 23 | 202.0629 | $C_9H_{13}O_2NCl$ | 0 | |
| **C35** | **25C-NBOMe-M (*bis*-hydroxy-) isomer 1** | | | | | | 6.0 |
| | **MS¹** | PM at *m/z* 368.1241 (M+H) | 6 | 368.1259 | $C_{18}H_{23}O_5NCl$ | -4.97 | |
| | **MS²** | FI at *m/z* 107.0495 | 40 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 215.0467 | 1 | 215.0475 | $C_{10}H_{12}O_3Cl$ | -3.71 | |
| | | FI at *m/z* 230.0570 | 1 | 230.0578 | $C_{10}H_{13}O_3NCl$ | -3.69 | |
| **C36** | **25C-NBOMe-M (*bis*-hydroxy-) isomer 2** | | | | | | 6.9 |
| | **MS¹** | PM at *m/z* 368.1247 (M+H) | 1 | 368.1259 | $C_{18}H_{23}O_5NCl$ | -3.34 | |
| | **MS²** | FI at *m/z* 107.0495 | 48 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 153.0546 | 58 | 153.0552 | $C_8H_9O_3$ | -3.72 | |
| | | FI at *m/z* 199.0521 | 100 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.43 | |
| | | FI at *m/z* 216.0782 | 17 | 216.0786 | $C_{10}H_{15}O_2NCl$ | -1.78 | |

**Table S3** List of all 25B-NBOMe phase II metabolites together with the precursor mass (PM) recorded in $MS^1$, the corresponding characteristic fragment ions (FI) in $MS^2$, the calculated exact masses, the corresponding elemental composition, the deviation of the measured from the calculated masses, given as errors in parts per million (ppm), and the retention times (RT) in minutes (min). The metabolites were sorted by mass and RT. Numbering according to Table 1 (AC = *N*-acetylation, GSH = glutathione conjugation, ME = *O*-methylation, G = glucuronidation, S = sulfation, AC+G = acetylation combined with glururonidation, AC+S = acetylation combined with sulfation)

| No. | Metabolite and characteristic ions<br>Measured accurate mass, *m/z* | | Relative intensity in $MS^2$, % | Calculated exact mass, *m/z* | Elemental composition | Error, ppm | RT, min |
|---|---|---|---|---|---|---|---|
| **B3 AC** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 1** | | | | | | 6.6 |
| | **$MS^1$** | PM at *m/z* 288.0226 (M+H) | 5 | 288.0230 | $C_{11}H_{15}O_3NBr$ | -1.32 | |
| | **$MS^2$** | FI at *m/z* 150.0674 | 58 | 150.0681 | $C_9H_{10}O_2$ | -4.53 | |
| | | FI at *m/z* 213.9621 | 35 | 213.9629 | $C_8H_7O_2Br$ | -3.93 | |
| | | FI at *m/z* 228.9857 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -3.13 | |
| | | FI at *m/z* 246.0123 | 13 | 246.0124 | $C_9H_{13}O_2NBr$ | -0.47 | |
| **B4 AC** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 2** | | | | | | 6.7 |
| | **$MS^1$** | PM at *m/z* 288.0226 (M+H) | 6 | 288.0230 | $C_{11}H_{15}O_3NBr$ | -1.32 | |
| | **$MS^2$** | FI at *m/z* 150.0674 | 32 | 150.0681 | $C_9H_{10}O_2$ | -4.53 | |
| | | FI at *m/z* 213.9621 | 64 | 213.9629 | $C_8H_7O_2Br$ | -3.93 | |
| | | FI at *m/z* 228.9857 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -3.13 | |
| | | FI at *m/z* 246.0123 | 13 | 246.0124 | $C_9H_{13}O_2NBr$ | -0.47 | |
| **B3 AC+S** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 1** | | | | | | 5.4 |
| | **$MS^1$** | PM at *m/z* 367.9798 (M+H) | 4 | 367.9798 | $C_{11}H_{15}O_6NBrS$ | 0 | |
| | **$MS^2$** | FI at *m/z* 228.9858 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0125 | 8 | 246.0124 | $C_9H_{13}O_2NBr$ | 0.34 | |
| | | FI at *m/z* 288.0229 | 7 | 288.0230 | $C_{11}H_{15}O_3NBr$ | -0.28 | |
| | | FI at *m/z* 308.9424 | 24 | 308.9432 | $C_9H_{10}O_5BrS$ | -2.70 | |
| | | FI at *m/z* 325.9692 | 18 | 325.9692 | $C_9H_{13}O_5NBrS$ | 0 | |
| **B4 AC+S** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 2** | | | | | | 5.5 |
| | **$MS^1$** | PM at *m/z* 367.9798 (M+H) | 4 | 367.9798 | $C_{11}H_{15}O_6NBrS$ | 0 | |
| | **$MS^2$** | FI at *m/z* 228.9858 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0123 | 12 | 246.0124 | $C_9H_{13}O_2NBr$ | -0.47 | |
| | | FI at *m/z* 288.0226 | 11 | 288.0230 | $C_{11}H_{15}O_3NBr$ | -1.32 | |
| | | FI at *m/z* 308.9425 | 16 | 308.9432 | $C_9H_{10}O_5BrS$ | -2.37 | |
| | | FI at *m/z* 325.9692 | 17 | 325.9692 | $C_9H_{13}O_5NBrS$ | 0 | |
| **B8 GSH-1** | **25B-NBOMe-M (*O,O-bis*-demethyl-) *S*-methyl** | | | | | | 7.7 |
| | **$MS^1$** | PM at *m/z* 398.0405 (M+H) | 1 | 398.0420 | $C_{17}H_{21}O_3NBrS$ | -3.78 | |
| | **$MS^2$** | FI at *m/z* 91.0548 | 53 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 259.9503 | 6 | 259.9507 | $C_9H_9O_2BrS$ | -1.40 | |
| | | FI at *m/z* 381.0149 | 0.4 | 381.0160 | $C_{17}H_{18}O_3BrS$ | -2.84 | |
| **B33 ME** | **25B-NBOMe-M (*O*-demethyl-*bis*-hydroxy-) *O*-methyl** | | | | | | 6.8 |
| | **$MS^1$** | PM at *m/z* 412.0760 (M+H) | 1 | 412.0754 | $C_{18}H_{23}O_5NBr$ | 1.43 | |
| | **$MS^2$** | FI at *m/z* 137.0597 | 39 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 167.0704 | 100 | 167.0708 | $C_9H_{11}O_3$ | -2.51 | |
| | | FI at *m/z* 228.9859 | 18 | 228.9864 | $C_9H_{10}O_2Br$ | -2.25 | |
| | | FI at *m/z* 246.0125 | 6 | 246.0124 | $C_9H_{13}O_2NBr$ | 0.34 | |
| **B7 S** | **25B-NBOMe-M (*O,O,O-tris*-demethyl-) sulfate** | | | | | | 4.7 |
| | **$MS^1$** | PM at *m/z* 417.9959 (M+H) | 6 | 417.9954 | $C_{15}H_{17}O_6NBrS$ | 1.08 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 214.9702 | 80 | 214.9708 | $C_8H_8O_2Br$ | -2.63 | |
| | | FI at *m/z* 294.9271 | 18 | 294.9276 | $C_8H_8O_5BrS$ | -1.64 | |
| | | FI at *m/z* 311.9538 | 27 | 311.9536 | $C_8H_{11}O_5NBrS$ | 0.69 | |
| | | FI at *m/z* 338.0387 | 10 | 338.0386 | $C_{15}H_{17}O_3NBr$ | 0.20 | |
| **B3/4 G** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) glucuronide** | | | | | | 3.0 |
| | **MS¹** | PM at *m/z* 422.0446 (M+H) | 4 | 422.0445 | $C_{15}H_{21}O_8NBr$ | 0.22 | |
| | **MS²** | FI at *m/z* 150.0674 | 24 | 150.0681 | $C_9H_{10}O_2$ | -3.86 | |
| | | FI at *m/z* 213.9623 | 43 | 213.9629 | $C_8H_7O_2Br$ | -3.00 | |
| | | FI at *m/z* 228.9858 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0126 | 28 | 246.0124 | $C_9H_{13}O_2NBr$ | 0.75 | |
| **B8 S** | **25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 1** | | | | | | 6.0 |
| | **MS¹** | PM at *m/z* 432.0113 (M+H) | 2 | 432.0111 | $C_{16}H_{19}O_6NBrS$ | 0.47 | |
| | **MS²** | FI at *m/z* 91.0548 | 61 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 352.0541 | 2 | 352.0543 | $C_{16}H_{19}O_3NBr$ | -0.52 | |
| **B9/10 S** | **25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 2** | | | | | | 6.8 |
| | **MS¹** | PM at *m/z* 432.0120 (M+H) | 24 | 432.0111 | $C_{16}H_{19}O_6NBrS$ | 2.09 | |
| | **MS²** | FI at *m/z* 107.0496 | 99 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 228.9860 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -1.82 | |
| | | FI at *m/z* 308.9428 | 34 | 308.9432 | $C_9H_{10}O_5BrS$ | -1.40 | |
| | | FI at *m/z* 325.9696 | 38 | 325.9692 | $C_9H_{13}O_5NBrS$ | 1.12 | |
| | | FI at *m/z* 352.0546 | 5 | 352.0543 | $C_{16}H_{19}O_3NBr$ | 0.90 | |
| **B13/14 S** | **25B-NBOMe-M (*O*-demethyl-) sulfate isomer 1** | | | | | | 7.6 |
| | **MS¹** | PM at *m/z* 446.0268 (M+H) | 2 | 446.0267 | $C_{17}H_{21}O_6NBrS$ | 0.12 | |
| | **MS²** | FI at *m/z* 91.0549 | 60 | 91.0548 | $C_7H_7$ | 1.37 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 349.0431 | 1 | 349.0439 | $C_{17}H_{18}O_3Br$ | -2.38 | |
| | | FI at *m/z* 366.0703 | 4 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 1.01 | |
| **B15 S** | **25B-NBOMe-M (*O*-demethyl-) sulfate isomer 2** | | | | | | 8.5 |
| | **MS¹** | PM at *m/z* 446.0264 (M+H) | 3 | 446.0267 | $C_{17}H_{21}O_6NBrS$ | -0.78 | |
| | **MS²** | FI at *m/z* 107.0495 | 59 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 243.0015 | 100 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.33 | |
| | | FI at *m/z* 260.0282 | 20 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0.51 | |
| | | FI at *m/z* 366.0702 | 30 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 0.73 | |
| **B16 S** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate isomer 1** | | | | | | 4.8 |
| | **MS¹** | PM at *m/z* 448.0061 (M+H) | 5 | 448.0060 | $C_{16}H_{19}O_7NBrS$ | 0.19 | |
| | **MS²** | FI at *m/z* 107.0496 | 41 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 137.0598 | 100 | 137.0603 | $C_8H_9O_2$ | -3.32 | |
| | | FI at *m/z* 217.0164 | 15 | 217.0171 | $C_8H_9O_5S$ | -3.10 | |
| | | FI at *m/z* 368.0491 | 5 | 368.0492 | $C_{16}H_{19}O_4NBr$ | -0.26 | |
| **B17/18 S** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate isomer 2** | | | | | | 5.6 |
| | **MS¹** | PM at *m/z* 448.0052 (M+H) | 3 | 448.0060 | $C_{16}H_{19}O_7NBrS$ | -1.82 | |
| | **MS²** | FI at *m/z* 123.0443 | 86 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 228.9860 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -1.82 | |
| | | FI at *m/z* 308.9426 | 32 | 308.9432 | $C_9H_{10}O_5BrS$ | -2.05 | |
| | | FI at *m/z* 325.9695 | 38 | 325.9692 | $C_9H_{13}O_5NBrS$ | 0.82 | |
| | | FI at *m/z* 368.0499 | 5 | 368.0492 | $C_{16}H_{19}O_4NBr$ | 1.91 | |
| **B22 S** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) sulfate isomer 1** | | | | | | 6.2 |
| | **MS¹** | PM at *m/z* 462.0212 (M+H) | 2 | 462.0217 | $C_{17}H_{21}O_7NBrS$ | -1.00 | |
| | **MS²** | FI at *m/z* 91.0548 | 65 | 91.0548 | $C_7H_7$ | 1.37 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 258.9974 | 1 | 258.9970 | $C_{10}H_{12}O_3Br$ | 1.62 | |
| | | FI at *m/z* 382.0654 | 4 | 382.0648 | $C_{17}H_{21}O_4NBr$ | 1.45 | |
| **B24/25 S** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) sulfate isomer 2** | | | | | | 7.2 |
| | **MS¹** | PM at *m/z* 462.0215 (M+H) | 9 | 462.0217 | $C_{17}H_{21}O_7NBrS$ | -0.35 | |
| | **MS²** | FI at *m/z* 123.0442 | 58 | 123.0446 | $C_7H_7O_2$ | -3.29 | |
| | | FI at *m/z* 203.0007 | 9 | 203.0014 | $C_7H_7O_5S$ | -3.56 | |
| | | FI at *m/z* 243.0014 | 100 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.74 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | FI at *m/z* 260.0282 | 19 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0.51 | |
| | | FI at *m/z* 382.0640 | 11 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -2.22 | |
| **B3/4 AC+G** | **25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide** | | | | | | 5.3 |
| | **MS¹** | PM at *m/z* 464.0564 (M+H) | 1 | 464.0551 | $C_{17}H_{23}O_9NBr$ | 2.86 | |
| | **MS²** | FI at *m/z* 150.0674 | 18 | 150.0681 | $C_9H_{10}O_2$ | -4.53 | |
| | | FI at *m/z* 228.9858 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 246.0121 | 20 | 246.0124 | $C_9H_{13}O_2NBr$ | -1.29 | |
| | | FI at *m/z* 288.0225 | 41 | 288.0230 | $C_{11}H_{15}O_3NBr$ | -1.67 | |
| **B8 GSH-2** | **25B-NBOMe-M (*O,O-bis*-demethyl-) acetylcysteine** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 513.0695 (M+H) | 10 | 513.0689 | $C_{21}H_{26}O_6N_2BrS$ | 1.08 | |
| | **MS²** | FI at *m/z* 91.0548 | 60 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 384.0264 | 6 | 384.0264 | $C_{16}H_{19}O_3NBrS$ | 0 | |
| | | FI at *m/z* 407.0273 | 1 | 407.0271 | $C_{14}H_{20}O_5N_2BrS$ | 0.53 | |
| **B7 G** | **25B-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide** | | | | | | 4.0 |
| | **MS¹** | PM at *m/z* 514.0714 (M+H) | 5 | 514.0707 | $C_{21}H_{25}O_9NBr$ | 1.32 | |
| | **MS²** | FI at *m/z* 107.0495 | 71 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 214.9701 | 100 | 214.9708 | $C_8H_8O_2Br$ | -3.10 | |
| | | FI at *m/z* 338.0383 | 24 | 338.0386 | $C_{15}H_{17}O_3NBr$ | -0.98 | |
| | | FI at *m/z* 408.0289 | 10 | 408.0289 | $C_{14}H_{19}O_8NBr$ | 0 | |
| **B8 G** | **25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1** | | | | | | 4.8 |
| | **MS¹** | PM at *m/z* 528.0867 (M+H) | 5 | 528.0864 | $C_{22}H_{27}O_9NBr$ | 0.62 | |
| | **MS²** | FI at *m/z* 91.0548 | 47 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 352.0544 | 16 | 352.0543 | $C_{16}H_{19}O_3NBr$ | 0.34 | |
| **B9/10 G** | **25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2** | | | | | | 5.9 |
| | **MS¹** | PM at *m/z* 528.0872 (M+H) | 5 | 528.0864 | $C_{22}H_{27}O_9NBr$ | 1.57 | |
| | **MS²** | FI at *m/z* 107.0496 | 60 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 228.9860 | 100 | 228.9864 | $C_9H_{10}O_2Br$ | -1.82 | |
| | | FI at *m/z* 246.0127 | 32 | 246.0124 | $C_9H_{13}O_2NBr$ | 1.15 | |
| | | FI at *m/z* 352.0548 | 26 | 352.0543 | $C_{16}H_{19}O_3NBr$ | 1.47 | |
| | | FI at *m/z* 422.0448 | 7 | 422.0445 | $C_{15}H_{21}O_8NBr$ | 0.70 | |
| **B13 G** | **25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 1** | | | | | | 5.6 |
| | **MS¹** | PM at *m/z* 542.1024 (M+H) | 9 | 542.1020 | $C_{23}H_{29}O_9NBr$ | 0.70 | |
| | **MS²** | FI at *m/z* 91.0548 | 57 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 228.9858 | 1 | 228.9864 | $C_9H_{10}O_2Br$ | -2.69 | |
| | | FI at *m/z* 366.0705 | 12 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 1.55 | |
| **B14 G** | **25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 2** | | | | | | 6.6 |
| | **MS¹** | PM at *m/z* 542.1022 (M+H) | 4 | 542.1020 | $C_{23}H_{29}O_9NBr$ | 0.33 | |
| | **MS²** | FI at *m/z* 91.0549 | 56 | 91.0548 | $C_7H_7$ | 1.37 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 228.9856 | 1 | 228.9864 | $C_9H_{10}O_2Br$ | -3.56 | |
| | | FI at *m/z* 366.0699 | 21 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 0 | |
| **B15 G** | **25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 3** | | | | | | 7.3 |
| | **MS¹** | PM at *m/z* 542.1020 (M+H) | 8 | 542.1020 | $C_{23}H_{29}O_9NBr$ | 0 | |
| | **MS²** | FI at *m/z* 107.0495 | 100 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 243.0015 | 45 | 243.0021 | $C_{10}H_{12}O_2Br$ | -2.33 | |
| | | FI at *m/z* 260.0282 | 14 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0.51 | |
| | | FI at *m/z* 366.0704 | 21 | 366.0699 | $C_{17}H_{21}O_3NBr$ | 1.28 | |
| **B16 G** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1** | | | | | | 4.1 |
| | **MS¹** | PM at *m/z* 544.0806 (M+H) | 4 | 544.0813 | $C_{22}H_{27}O_{10}NBr$ | -1.26 | |
| | **MS²** | FI at *m/z* 107.0495 | 36 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 368.0493 | 12 | 368.0492 | $C_{16}H_{19}O_4NBr$ | 0.28 | |
| **B19 G** | **25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2** | | | | | | 5.3 |
| | **MS¹** | PM at *m/z* 544.0817 (M+H) | 4 | 544.0813 | $C_{22}H_{27}O_{10}NBr$ | 0.76 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS²** | FI at *m/z* 107.0495 | 52 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 244.9808 | 100 | 244.9813 | $C_9H_{10}O_3Br$ | -2.17 | |
| | | FI at *m/z* 262.0074 | 35 | 262.0073 | $C_9H_{13}O_3NBr$ | 0.26 | |
| | | FI at *m/z* 368.0493 | 31 | 368.0492 | $C_{16}H_{19}O_4NBr$ | 0.28 | |
| | | FI at *m/z* 438.0394 | 3 | 438.0394 | $C_{15}H_{21}O_9NBr$ | 0 | |
| **B23 G** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 1** | | | | | | 5.7 |
| | **MS¹** | PM at *m/z* 558.0969 (M+H) | 4 | 558.0969 | $C_{23}H_{29}O_{10}NBr$ | 0 | |
| | **MS²** | FI at *m/z* 107.0495 | 36 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 228.9856 | 1 | 228.9864 | $C_9H_{10}O_2Br$ | -2.25 | |
| | | FI at *m/z* 382.0645 | 18 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -0.38 | |
| **B22 G** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2** | | | | | | 5.9 |
| | **MS¹** | PM at *m/z* 558.0970 (M+H) | 5 | 558.0969 | $C_{23}H_{29}O_{10}NBr$ | 0.11 | |
| | **MS²** | FI at *m/z* 91.0548 | 48 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 382.0647 | 22 | 382.0648 | $C_{17}H_{21}O_4NBr$ | -0.38 | |
| **B24/25 G** | **25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 3** | | | | | | 6.0 |
| | **MS¹** | PM at *m/z* 558.0968 (M+H) | 8 | 558.0969 | $C_{23}H_{29}O_{10}NBr$ | -0.24 | |
| | **MS²** | FI at *m/z* 123.0443 | 84 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 243.0019 | 100 | 243.0021 | $C_{10}H_{12}O_2Br$ | -0.68 | |
| | | FI at *m/z* 260.0282 | 32 | 260.0281 | $C_{10}H_{15}O_2NBr$ | 0.51 | |
| | | FI at *m/z* 299.0758 | 2 | 299.0767 | $C_{13}H_{15}O_8$ | -2.99 | |
| | | FI at *m/z* 382.0663 | 5 | 382.0648 | $C_{17}H_{21}O_4NBr$ | 3.80 | |
| **B30 G** | **25B-NBOMe-M (hydroxy-) glucuronide isomer 1** | | | | | | 6.6 |
| | **MS¹** | PM at *m/z* 572.1151 (M+H) | 7 | 572.1126 | $C_{24}H_{31}O_{10}NBr$ | 4.39 | |
| | **MS²** | FI at *m/z* 109.0653 | 100 | 109.0653 | $C_7H_9O$ | -0.37 | |
| | | FI at *m/z* 137.0598 | 37 | 137.0603 | $C_8H_9O_2$ | -3.32 | |
| | | FI at *m/z* 313.0920 | 30 | 313.0923 | $C_{14}H_{17}O_8$ | -1.10 | |
| | | FI at *m/z* 396.0792 | 5 | 396.0805 | $C_{18}H_{23}O_4NBr$ | -3.27 | |
| **B31 G** | **25B-NBOMe-M (hydroxy-) glucuronide isomer 2** | | | | | | 6.9 |
| | **MS¹** | PM at *m/z* 572.1134 (M+H) | 4 | 572.1126 | $C_{24}H_{31}O_{10}NBr$ | 1.42 | |
| | **MS²** | FI at *m/z* 107.0496 | 24 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 137.0599 | 100 | 137.0603 | $C_8H_9O_2$ | -2.59 | |
| | | FI at *m/z* 313.0918 | 26 | 313.0923 | $C_{14}H_{17}O_8$ | -1.74 | |
| | | FI at *m/z* 396.0808 | 4 | 396.0805 | $C_{18}H_{23}O_4NBr$ | 0.77 | |

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**Table S4** List of all 25C-NBOMe phase II metabolites together with the precursor mass (PM) recorded in $MS^1$, the corresponding characteristic fragment ions (FI) in $MS^2$, the calculated exact masses, the corresponding elemental composition, the deviation of the measured from the calculated masses, given as errors in parts per million (ppm), and the retention times (RT) in minutes (min). The metabolites were sorted by mass and RT. Numbering according to Table 2 (AC = *N*-acetylation, GSH = glutathione conjugation, ME = *O*-methylation, G = glucuronidation, S = sulfation, AC+G = acetylation combined with glururonidation, AC+S = acetylation combined with sulfation)

| No. | Metabolite and characteristic ions<br>Measured accurate mass, *m/z* | Relative intensity in $MS^2$, % | Calculated exact mass, *m/z* | Elemental composition | Error, ppm | RT, min |
|---|---|---|---|---|---|---|
| **C2 AC** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 1** | | | | | 6.3 |
| | **$MS^1$** PM at *m/z* 244.0736 (M+H) | 3 | 244.0735 | $C_{11}H_{15}O_3NCl$ | 0.42 | |
| | **$MS^2$** FI at *m/z* 150.0676 | 25 | 150.0681 | $C_9H_{10}O_2$ | -3.20 | |
| | FI at *m/z* 170.0130 | 30 | 170.0135 | $C_8H_7O_2Cl$ | -2.69 | |
| | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | FI at *m/z* 202.0630 | 9 | 202.0629 | $C_9H_{13}O_2NCl$ | 0.33 | |
| **C3 AC** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl isomer 2** | | | | | 6.5 |
| | **$MS^1$** PM at *m/z* 244.0732 (M+H) | 4 | 244.0735 | $C_{11}H_{15}O_3NCl$ | -1.22 | |
| | **$MS^2$** FI at *m/z* 150.0676 | 12 | 150.0681 | $C_9H_{10}O_2$ | -3.20 | |
| | FI at *m/z* 170.0129 | 48 | 170.0135 | $C_8H_7O_2Cl$ | -3.28 | |
| | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | FI at *m/z* 202.0629 | 11 | 202.0629 | $C_9H_{13}O_2NCl$ | 0 | |
| **C7 AC** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-hydroxy-) *N*-acetyl** | | | | | 5.1 |
| | **$MS^1$** PM at *m/z* 260.0681 (M+H) | 4 | 260.0684 | $C_{11}H_{15}O_4NCl$ | -1.21 | |
| | **$MS^2$** FI at *m/z* 166.0625 | 7 | 166.0630 | $C_9H_{10}O_3$ | -2.98 | |
| | FI at *m/z* 186.0078 | 82 | 186.0084 | $C_8H_7O_3Cl$ | -3.08 | |
| | FI at *m/z* 201.0312 | 100 | 201.0318 | $C_9H_{10}O_3Cl$ | -3.22 | |
| | FI at *m/z* 218.0577 | 14 | 218.0578 | $C_9H_{13}O_3NCl$ | -0.68 | |
| **C3/4 AC+S** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate** | | | | | 5.1 |
| | **$MS^1$** PM at *m/z* 324.0305 (M+H) | 4 | 324.0303 | $C_{11}H_{15}O_6NClS$ | 0.57 | |
| | **$MS^2$** FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | FI at *m/z* 202.0628 | 8 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | FI at *m/z* 244.0736 | 7 | 244.0735 | $C_{11}H_{15}O_3NCl$ | 0.42 | |
| | FI at *m/z* 264.9933 | 26 | 264.9938 | $C_9H_{10}O_5ClS$ | -1.70 | |
| | FI at *m/z* 282.0199 | 18 | 282.0198 | $C_9H_{13}O_5NClS$ | 0.53 | |
| **C10 GSH-1** | **25C-NBOMe-M (*O,O-bis*-demethyl-) *S*-methyl** | | | | | 7.5 |
| | **$MS^1$** PM at *m/z* 354.0922 (M+H) | 6 | 354.0925 | $C_{17}H_{21}O_3NClS$ | -0.91 | |
| | **$MS^2$** FI at *m/z* 91.0547 | 55 | 91.0548 | $C_7H_7$ | -0.82 | |
| | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | FI at *m/z* 232.0197 | 0.5 | 232.0194 | $C_9H_{11}O_2NClS$ | 1.48 | |
| | FI at *m/z* 337.0650 | 0.5 | 337.0665 | $C_{17}H_{18}O_3ClS$ | -4.51 | |
| **C34 ME** | **25C-NBOMe-M (*O*-demethyl-*bis*-hydroxy-) *O*-methyl** | | | | | 6.5 |
| | **$MS^1$** PM at *m/z* 368.1250 (M+H) | 1 | 368.1259 | $C_{18}H_{23}O_5NCl$ | -2.52 | |
| | **$MS^2$** FI at *m/z* 137.0597 | 40 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | FI at *m/z* 167.0704 | 100 | 167.0708 | $C_9H_{11}O_3$ | -2.51 | |
| | FI at *m/z* 185.0362 | 14 | 185.0369 | $C_9H_{10}O_2Cl$ | -3.96 | |
| | FI at *m/z* 202.0631 | 2 | 202.0629 | $C_9H_{13}O_2NCl$ | 0.82 | |
| **C8 S** | **25C-NBOMe-M (*O,O,O-tris*-demethyl-) sulfate** | | | | | 4.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **MS¹** | PM at *m/z* 374.0463 (M+H) | 7 | 374.0460 | $C_{15}H_{17}O_6NClS$ | 0.89 | |
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 171.0210 | 78 | 171.0213 | $C_8H_8O_2Cl$ | -1.65 | |
| | | FI at *m/z* 250.9778 | 21 | 250.9781 | $C_8H_8O_5ClS$ | -1.20 | |
| | | FI at *m/z* 268.0043 | 26 | 268.0041 | $C_8H_{11}O_5NClS$ | 0.74 | |
| | | FI at *m/z* 294.0894 | 10 | 294.0891 | $C_{15}H_{17}O_3NCl$ | 0.85 | |
| **C2/3 G** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) glucuronide** | | | | | | 2.6 |
| | **MS¹** | PM at *m/z* 378.0949 (M+H) | 5 | 378.0950 | $C_{15}H_{21}O_8NCl$ | -0.33 | |
| | **MS²** | FI at *m/z* 150.0675 | 9 | 150.0681 | $C_9H_{10}O_2$ | -3.86 | |
| | | FI at *m/z* 170.0130 | 35 | 170.0135 | $C_8H_7O_2Cl$ | -2.69 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 24 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| **C36 ME** | **25C-NBOMe-M (*bis*-hydroxy-) *O*-methyl** | | | | | | 7.6 |
| | **MS¹** | PM at *m/z* 382.1410 (M+H) | 1 | 382.1416 | $C_{19}H_{25}O_5NCl$ | -1.51 | |
| | **MS²** | FI at *m/z* 137.0596 | 39 | 137.0603 | $C_8H_9O_2$ | -4.78 | |
| | | FI at *m/z* 167.0702 | 100 | 167.0708 | $C_9H_{11}O_3$ | -3.71 | |
| | | FI at *m/z* 199.0519 | 22 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -3.43 | |
| | | FI at *m/z* 216.0785 | 6 | 216.0786 | $C_{10}H_{15}O_2NCl$ | -0.39 | |
| **C10 S** | **25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 1** | | | | | | 5.8 |
| | **MS¹** | PM at *m/z* 388.0614 (M+H) | 2 | 388.0616 | $C_{16}H_{19}O_6NClS$ | -0.56 | |
| | **MS²** | FI at *m/z* 91.0548 | 54 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 308.1046 | 2 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.64 | |
| **C11 S** | **25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 2** | | | | | | 6.6 |
| | **MS¹** | PM at *m/z* 388.0617 (M+H) | 6 | 388.0616 | $C_{16}H_{19}O_6NClS$ | 0.22 | |
| | **MS²** | FI at *m/z* 107.0496 | 96 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 185.0366 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -1.80 | |
| | | FI at *m/z* 264.9934 | 40 | 264.9938 | $C_9H_{10}O_5ClS$ | -1.32 | |
| | | FI at *m/z* 282.0200 | 42 | 282.0198 | $C_9H_{13}O_5NClS$ | 0.88 | |
| | | FI at *m/z* 308.1047 | 6 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.32 | |
| **C12 S** | **25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 3** | | | | | | 7.0 |
| | **MS¹** | PM at *m/z* 388.0619 (M+H) | 11 | 388.0616 | $C_{16}H_{19}O_6NClS$ | 0.73 | |
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 185.0366 | 99 | 185.0369 | $C_9H_{10}O_2Cl$ | -1.80 | |
| | | FI at *m/z* 187.0060 | 6 | 187.0065 | $C_7H_7O_4S$ | -2.71 | |
| | | FI at *m/z* 202.0629 | 28 | 202.0629 | $C_9H_{13}O_5NClS$ | 0 | |
| | | FI at *m/z* 308.1048 | 37 | 308.1048 | $C_{16}H_{19}O_3NCl$ | 0 | |
| **C16/17 S** | **25C-NBOMe-M (*O*-demethyl-) sulfate isomer 1** | | | | | | 7.4 |
| | **MS¹** | PM at *m/z* 402.0778 (M+H) | 3 | 402.0773 | $C_{17}H_{21}O_6NClS$ | 1.33 | |
| | **MS²** | FI at *m/z* 91.0548 | 61 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 305.0938 | 1 | 305.0944 | $C_{17}H_{18}O_3Cl$ | -2.12 | |
| | | FI at *m/z* 322.1205 | 4 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.16 | |
| **C18 S** | **25C-NBOMe-M (*O*-demethyl-) sulfate isomer 2** | | | | | | 8.2 |
| | **MS¹** | PM at *m/z* 402.0785 (M+H) | 5 | 402.0773 | $C_{17}H_{21}O_6NClS$ | 3.07 | |
| | **MS²** | FI at *m/z* 107.0495 | 59 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 199.0521 | 100 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.43 | |
| | | FI at *m/z* 216.0787 | 19 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 0.54 | |
| | | FI at *m/z* 322.1205 | 32 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.16 | |
| **C20 S** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate** | | | | | | 5.0 |
| | **MS¹** | PM at *m/z* 404.0569 (M+H) | 9 | 404.0565 | $C_{16}H_{19}O_7NClS$ | 0.91 | |
| | **MS²** | FI at *m/z* 123.0443 | 80 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 264.9934 | 26 | 264.9938 | $C_9H_{10}O_5ClS$ | -1.32 | |
| | | FI at *m/z* 282.0200 | 22 | 282.0198 | $C_9H_{13}O_5NClS$ | 0.88 | |
| | | FI at *m/z* 324.0998 | 8 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 0.27 | |
| **C22 S** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) sulfate** | | | | | | 6.9 |
| | **MS¹** | PM at *m/z* 418.0726 (M+H) | 12 | 418.0722 | $C_{17}H_{21}O_7NClS$ | 1.00 | |
| | **MS²** | FI at *m/z* 123.0443 | 55 | 123.0446 | $C_7H_7O_2$ | -2.48 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | FI at *m/z* 199.0522 | 100 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -1.92 | |
| | | FI at *m/z* 203.0008 | 10 | 203.0014 | $C_7H_7O_5S$ | -3.06 | |
| | | FI at *m/z* 216.0788 | 17 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 1.00 | |
| | | FI at *m/z* 338.1154 | 11 | 338.1154 | $C_{17}H_{21}O_4NCl$ | 0 | |
| **C2/3 AC+G** | **25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide** | | | | | | 5.0 |
| | **MS¹** | PM at *m/z* 420.1060 (M+H) | 1 | 420.1056 | $C_{17}H_{23}O_9NCl$ | 0.98 | |
| | **MS²** | FI at *m/z* 150.0675 | 6 | 150.0681 | $C_9H_{10}O_2$ | -3.86 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 19 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | | FI at *m/z* 244.0736 | 36 | 244.0735 | $C_{11}H_{15}O_3NCl$ | 0.42 | |
| **C10 GSH-2** | **25C-NBOMe-M (*O,O-bis*-demethyl-) acetylcysteine** | | | | | | 6.2 |
| | **MS¹** | PM at *m/z* 469.1200 (M+H) | 14 | 469.1195 | $C_{21}H_{26}O_6N_2ClS$ | 1.14 | |
| | **MS²** | FI at *m/z* 91.0548 | 55 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 340.0770 | 6 | 340.0769 | $C_{16}H_{19}O_3NClS$ | 0.38 | |
| | | FI at *m/z* 363.0781 | 0.5 | 363.0776 | $C_{14}H_{20}O_5N_2ClS$ | 1.38 | |
| **C8 G** | **25C-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide** | | | | | | 3.8 |
| | **MS¹** | PM at *m/z* 470.1219 (M+H) | 8 | 470.1212 | $C_{21}H_{25}O_9NCl$ | 1.41 | |
| | **MS²** | FI at *m/z* 107.0495 | 69 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 171.0208 | 100 | 171.0213 | $C_8H_8O_2Cl$ | -2.82 | |
| | | FI at *m/z* 294.0891 | 28 | 294.0891 | $C_{15}H_{17}O_3NCl$ | 0 | |
| | | FI at *m/z* 364.0796 | 12 | 364.0794 | $C_{14}H_{19}O_8NCl$ | 0.62 | |
| **C10 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1** | | | | | | 4.6 |
| | **MS¹** | PM at *m/z* 484.1377 (M+H) | 10 | 484.1369 | $C_{22}H_{27}O_9NCl$ | 1.68 | |
| | **MS²** | FI at *m/z* 91.0548 | 41 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 308.1047 | 18 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.32 | |
| **C11 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2** | | | | | | 5.5 |
| | **MS¹** | PM at *m/z* 484.1375 (M+H) | 23 | 484.1369 | $C_{22}H_{27}O_9NCl$ | 1.26 | |
| | **MS²** | FI at *m/z* 107.0495 | 87 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0629 | 61 | 202.0629 | $C_9H_{13}O_2NCl$ | 0 | |
| | | FI at *m/z* 308.1046 | 28 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.64 | |
| | | FI at *m/z* 378.0953 | 30 | 378.0950 | $C_{15}H_{21}O_8NCl$ | 0.73 | |
| **C12 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 3** | | | | | | 5.6 |
| | **MS¹** | PM at *m/z* 484.1375 (M+H) | 8 | 484.1369 | $C_{22}H_{27}O_9NCl$ | 1.26 | |
| | **MS²** | FI at *m/z* 107.0495 | 55 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 30 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | | FI at *m/z* 308.1047 | 29 | 308.1048 | $C_{16}H_{19}O_3NCl$ | -0.32 | |
| | | FI at *m/z* 378.0952 | 9 | 378.0950 | $C_{15}H_{21}O_8NCl$ | 0.47 | |
| **C16 G** | **25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 1** | | | | | | 5.5 |
| | **MS¹** | PM at *m/z* 498.1526 (M+H) | 15 | 498.1525 | $C_{23}H_{29}O_9NCl$ | 0.12 | |
| | **MS²** | FI at *m/z* 91.0548 | 52 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 185.0365 | 1 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 322.1205 | 13 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.16 | |
| **C17 G** | **25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 2** | | | | | | 6.4 |
| | **MS¹** | PM at *m/z* 498.1524 (M+H) | 8 | 498.1525 | $C_{23}H_{29}O_9NCl$ | -0.28 | |
| | **MS²** | FI at *m/z* 91.0548 | 45 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0650 | 100 | 121.0653 | $C_8H_9O$ | -2.81 | |
| | | FI at *m/z* 185.0365 | 1 | 185.0369 | $C_9H_{10}O_2Cl$ | 2.34 | |
| | | FI at *m/z* 322.1205 | 25 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.16 | |
| **C18 G** | **25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 3** | | | | | | 7.0 |
| | **MS¹** | PM at *m/z* 498.1525 (M+H) | 14 | 498.1525 | $C_{23}H_{29}O_9NCl$ | 0 | |
| | **MS²** | FI at *m/z* 107.0496 | 100 | 107.0497 | $C_7H_7O$ | -0.84 | |
| | | FI at *m/z* 199.0522 | 46 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -1.92 | |
| | | FI at *m/z* 216.0788 | 14 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 1.00 | |
| | | FI at *m/z* 322.1206 | 26 | 322.1204 | $C_{17}H_{21}O_3NCl$ | 0.47 | |

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|---|---|---|---|---|---|---|---|
| **C19 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1** | | | | | | 3.9 |
| | **MS¹** | PM at *m/z* 500.1320 (M+H) | 6 | 500.1318 | $C_{22}H_{27}O_{10}NCl$ | 0.39 | |
| | **MS²** | FI at *m/z* 107.0495 | 36 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0598 | 100 | 137.0603 | $C_8H_9O_2$ | -3.32 | |
| | | FI at *m/z* 324.0998 | 15 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 0.27 | |
| **C20 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2** | | | | | | 4.5 |
| | **MS¹** | PM at *m/z* 500.1318 (M+H) | 12 | 500.1318 | $C_{22}H_{27}O_{10}NCl$ | 0 | |
| | **MS²** | FI at *m/z* 123.0442 | 89 | 123.0446 | $C_7H_7O_2$ | -3.29 | |
| | | FI at *m/z* 185.0365 | 100 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 32 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | | FI at *m/z* 299.0762 | 5 | 299.0767 | $C_{13}H_{15}O_8$ | -1.66 | |
| | | FI at *m/z* 324.0997 | 12 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 0 | |
| **C21 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 3** | | | | | | 5.1 |
| | **MS¹** | PM at *m/z* 500.1321 (M+H) | 7 | 500.1318 | $C_{22}H_{27}O_{10}NCl$ | 0.59 | |
| | **MS²** | FI at *m/z* 107.0495 | 52 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 201.0313 | 100 | 201.0318 | $C_9H_{10}O_3Cl$ | -2.73 | |
| | | FI at *m/z* 218.0578 | 36 | 218.0578 | $C_9H_{13}O_3NCl$ | 0 | |
| | | FI at *m/z* 324.0997 | 31 | 324.0997 | $C_{16}H_{19}O_4NCl$ | 0 | |
| | | FI at *m/z* 394.0906 | 4 | 394.0899 | $C_{15}H_{21}O_9NCl$ | 1.68 | |
| **C22 G** | **25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 4** | | | | | | 5.3 |
| | **MS¹** | PM at *m/z* 500.1315 (M+H) | 7 | 500.1318 | $C_{22}H_{27}O_{10}NCl$ | -0.61 | |
| | **MS²** | FI at *m/z* 123.0443 | 100 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 185.0365 | 25 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 202.0628 | 11 | 202.0629 | $C_9H_{13}O_2NCl$ | -0.66 | |
| | | FI at *m/z* 324.0996 | 9 | 324.0997 | $C_{16}H_{19}O_4NCl$ | -0.35 | |
| | | FI at *m/z* 378.0945 | 1 | 378.0950 | $C_{15}H_{21}O_8NCl$ | -1.38 | |
| **C27 G** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 1** | | | | | | 5.5 |
| | **MS¹** | PM at *m/z* 514.1483 (M+H) | 6 | 514.1475 | $C_{23}H_{29}O_{10}NCl$ | 1.65 | |
| | **MS²** | FI at *m/z* 107.0495 | 33 | 107.0497 | $C_7H_7O$ | -1.77 | |
| | | FI at *m/z* 137.0597 | 100 | 137.0603 | $C_8H_9O_2$ | -4.05 | |
| | | FI at *m/z* 185.0365 | 1 | 185.0369 | $C_9H_{10}O_2Cl$ | -2.34 | |
| | | FI at *m/z* 313.0918 | 4 | 313.0923 | $C_{14}H_{17}O_8$ | -1.74 | |
| | | FI at *m/z* 338.1152 | 17 | 338.1154 | $C_{17}H_{21}O_4NCl$ | -0.48 | |
| **C28/29 G** | **25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2** | | | | | | 6.5 |
| | **MS¹** | PM at *m/z* 514.1479 (M+H) | 8 | 514.1475 | $C_{23}H_{29}O_{10}NCl$ | 0.87 | |
| | **MS²** | FI at *m/z* 123.0443 | 100 | 123.0446 | $C_7H_7O_2$ | -2.48 | |
| | | FI at *m/z* 199.0521 | 26 | 199.0526 | $C_{10}H_{12}O_2Cl$ | -2.43 | |
| | | FI at *m/z* 216.0787 | 11 | 216.0786 | $C_{10}H_{15}O_2NCl$ | 0.54 | |
| | | FI at *m/z* 338.1152 | 10 | 338.1154 | $C_{17}H_{21}O_4NCl$ | -0.48 | |
| **C31/32 G** | **25C-NBOMe-M (hydroxy-) glucuronide isomer 1** | | | | | | 5.2 |
| | **MS¹** | PM at *m/z* 528.1640 (M+H) | 7 | 528.1631 | $C_{24}H_{31}O_{10}NCl$ | 1.70 | |
| | **MS²** | FI at *m/z* 91.0548 | 46 | 91.0548 | $C_7H_7$ | 0 | |
| | | FI at *m/z* 121.0651 | 100 | 121.0653 | $C_8H_9O$ | -1.98 | |
| | | FI at *m/z* 352.1313 | 18 | 352.1310 | $C_{18}H_{23}O_4NCl$ | 0.81 | |
| **C33 G** | **25C-NBOMe-M (hydroxy-) glucuronide isomer 2** | | | | | | 6.3 |
| | **MS¹** | PM at *m/z* 528.1638 (M+H) | 7 | 528.1631 | $C_{24}H_{31}O_{10}NCl$ | 1.32 | |
| | **MS²** | FI at *m/z* 109.0652 | 73 | 109.0653 | $C_7H_9O$ | -1.28 | |
| | | FI at *m/z* 137.0598 | 100 | 137.0603 | $C_8H_9O_2$ | -3.32 | |
| | | FI at *m/z* 313.0921 | 17 | 313.0923 | $C_{14}H_{17}O_8$ | -0.78 | |
| | | FI at *m/z* 352.1324 | 3 | 352.1310 | $C_{18}H_{23}O_4NCl$ | 3.94 | |