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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

156 volume, 50 µL) contained 90 mM phosphate buffer (pH 7.4), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM
157 $NADP^{+}$, 0.5 U/mL isocitrate dehydrogenase, and 200 U/mL superoxide dismutase. For incubations
158 with CYP2A6 and CYP2C9, phosphate buffer was replaced with 45 mM or 90 mM Tris buffer,
159 respectively, according to the Gentest manual. Reactions were initiated by addition of the CYP
160 enzymes and stopped with 50 µL of ice-cold acetonitrile. The solution was centrifuged for 2 min at
161 14,000 rpm; 70 µL of the supernatant phase were transferred to an autosampler vial and 5 µL injected
162 onto the LC-HR-MS/MS system.
163
164 *2.5. LC-HR-MS/MS instrumentation for identification of phase I and II metabolites and CYP initial*
165 *screening*
166
167 According to a published procedure [32], the extracts were analyzed using a ThermoFisher Scientific
168 (TF, Dreieich, Germany) Dionex UltiMate 3000 RS pump consisting of a degasser, a quaternary
169 pump, and an UltiMate autosampler, coupled to a TF Q-Exactive Plus system equipped with a heated
170 electrospray ionization (HESI)-II source. The instrument was used in positive or in negative
171 ionization mode. Mass calibration was performed prior to analysis according to the manufacturer's
172 recommendations using external mass calibration.
173 Gradient elution was run on a TF Accucore PhenylHexyl column (100 mm x 2.1 mm, 2.6 µm). The
174 mobile phases consisted of 2 mM aqueous ammonium formate containing formic acid (0.1%, *v*/*v*) and
175 acetonitrile (1%, *v*/*v*) (pH 3, eluent A) and 2 mM ammonium formate solution with
176 acetonitrile:methanol (50:50, *v*/*v*) containing formic acid (0.1%, *v*/*v*) and water (1%, *v*/*v*) (eluent B).
177 The gradient and flow rate were programmed as follows: 0-1 min hold 99% A, 1-16 min 95% A to
178 5% A, 16-18 min hold 5% A, and 18-20 min hold 99% A, constantly at 500 µL/min.
179 The HESI-II source conditions were as follows: sheath gas, 60 arbitrary units (AU); auxiliary gas, 10
180 AU; spray voltage, 3.00 (positive polarity) and -4.00 kV (negative polarity); heater temperature,
181 320°C; ion transfer capillary temperature, 320°C; and S-lens RF level, 60.0. Mass spectrometry was

182 performed in positive and negative polarity mode using full scan (FS) data and a subsequent data
183 dependent acquisition (DDA) mode with an inclusion list on the masses of interest (phase I or phase
184 II metabolites). Additionally, DDA runs without inclusion list (positive and negative mode) were
185 performed to detect unexpected metabolites.
186 The settings for FS data acquisition were as follows: resolution, 35,000; microscans, 1; automatic
187 gain control (AGC) target, 1e6; maximum injection time (IT), 120 ms; and scan range, *m/z* 100–700.
188 The settings for the DDA mode with and without an inclusion list were as follows: resolution, 17,500;
189 microscans 1, AGC target, 2e5; maximum IT, 250 ms; isolation window, 1.0 *m/z*, HCD with stepped
190 normalized collision energy (NCE), 17.5, 35, and 52.5%; spectrum data type, profile; and underfill
191 ratio, 0.5%. For the run without inclusion list, the five most intense precursor ions were transferred to
192 an exclusion list for 1 s (dynamic exclusion).
193 For analyzing the initial CYP activity screening, the MS settings and the mobile phases as well as the
194 gradient and flow rate were the same with the same inclusion list as for identification of phase I
195 metabolites.
196
197 *2.6. Standard urine screening procedures (SUSAs)*
198
199 The SUSAs were performed as described in the following references: GC-MS SUSA [33,34], LC-
200 MS^n SUSA [25,35], and LC-HR-MS/MS SUSA [25,36].
201
202 **3. Results and discussion**
203
204 *3.1. Identification of metabolites*
205 *3.1.1. Identification of 25B-NBOMe and 25C-NBOMe and their phase I metabolites via HR-MS/MS*
206 *fragmentation*
207

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

rearrangement reaction. Few MS^2 spectra of metabolites also showed possible rearranged fragment ions.

The fragmentation patterns of 25C-NBOMe (C1; PM at *m/z* 336.1361, $M+H^+$) corresponded to those of 25B-NBOMe and 25I-NBOMe. Similarly, the most abundant fragment ions in MS^2 were formed by cleavage of the NBOMe moiety producing fragment ions of *m/z* 91.0548 and 121.0653. Also, the fragment ions representing the 2C part showed a lower abundance of about 1 % (Table S2). The fragment ions of *m/z* 214.0629, *m/z* 199.0526, and *m/z* 184.0291 represented the 2C-C iminium ion, the subsequent loss of NH (- 15.0109 u), and the loss of a methyl radical (- 15.0235 u) of one of the two methoxy groups, respectively. In the spectrum of 25C-NBOMe, no fragment ions indicating the rearrangement were detected, possibly due to low relative abundance. However, in the MS^2 spectra of some 25C-NBOMe metabolites (e.g. *O*-demethyl metabolite isomers 1 and 2, C16 and C17), some rearranged fragment ions could also be detected. Overall, 35 phase I metabolites could be detected for 25B-NBOMe in urine and 36 for 25C-NBOMe, respectively. All phase I metabolites are listed in Tables S1 and S2 in the electronic supplementary data.

For metabolite identification based on the MS^2 spectra, in most cases the representative fragment ion for the NBOMe part was used. Unmodified NBOMe parts led to a fragment ion of *m/z* 121.0653. The presence of this fragment ion led to the suggestion that the expected modification took place at the 2C part based on the predicted precursor mass for the metabolite. An unchanged fragment ion of *m/z* 121.0653 could be seen for the parent compounds (B1 and C1) as well as for mono- and bis-demethylated (B13, B14, B8 and C16, C17, C10), mono-hydroxylated (B29, B32 and C31, C32), bis-hydroxylated (B35), combined mono-demethylated with mono-hydroxylated (B22), dehydrogenated (B20 and C24), dehydrogenated combined with mono-demethylated (B12 and C13), and mono-hydroxylated (B27 and C30) metabolites. On the other hand, the fragment ion of *m/z* 137.0603 represented mono-hydroxylation at the NBOMe part (B16, B23, B28, B30, B31 and C19, C25, C27, C33, C35). At the NBOMe moiety, bis-hydroxylation led to the fragment ion of *m/z* 153.0552 (B33, 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,

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

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

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

3.4.1. 25B-NBOMe

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 AC+S	25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 1	+				
B4 AC+S	25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl sulfate isomer 2	+				
B8 GSH-1	25B-NBOMe-M (*O,O-bis*-demethyl-) S-methyl	+				
B33 ME	25B-NBOMe-M (*O*-demethyl-*bis*-hydroxy-) *O*-methyl	+	+			
B7 S	25B-NBOMe-M (*O,O,O-tris*-demethyl-) sulfate	+				
B3/4 G	25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) glucuronide	+				
B8 S	25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 1	+	+			
B9/10 S	25B-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 2		+			
B13/14 S	25B-NBOMe-M (*O*-demethyl-) sulfate isomer 1	+	+			
B15 S	25B-NBOMe-M (*O*-demethyl-) sulfate isomer 2	+				
B16 S	25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate isomer 1	+				
B17/18 S	25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate isomer 2		+			
B22 S	25B-NBOMe-M (*O*-demethyl-hydroxy-) sulfate isomer 1	+				
B24/25 S	25B-NBOMe-M (*O*-demethyl-hydroxy-) sulfate isomer 2	+	+			
B3/4 AC+G	25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide	+				
B8 GSH-2	25B-NBOMe-M (*O,O-bis*-demethyl-) acetylcysteine	+				
B7 G	25B-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide	+				
B8 G	25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1	+	+	+		
B9/10 G	25B-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2	+	+	+		
B13 G	25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 1	+	+	+		
B14 G	25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 2	+	+	+		
B15 G	25B-NBOMe-M (*O*-demethyl-) glucuronide isomer 3	+	+	+		
B16 G	25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1	+				
B19 G	25B-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2	+				
B23 G	25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 1	+	+			
B22 G	25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2	+				
B24/25 G	25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 3		+	+		
B30 G	25B-NBOMe-M (hydroxy-) glucuronide isomer 1		+	+		
B31 G	25B-NBOMe-M (hydroxy-) glucuronide isomer 2		+	+		
M21	25B-NBOMe-M *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 S	25C-NBOMe-M (*O,O,O-tris*-demethyl-) sulfate	+			
C2/3 G	25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) glucuronide	+			
C36 ME	25C-NBOMe-M (*bis*-hydroxy-) *O*-methyl	+			
C10 S	25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 1	+			
C11 S	25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 2	+			
C12 S	25C-NBOMe-M (*O,O-bis*-demethyl-) sulfate isomer 3	+	+		+
C16/17 S	25C-NBOMe-M (*O*-demethyl-) sulfate isomer 1	+	+	+	+
C18 S	25C-NBOMe-M (*O*-demethyl-) sulfate isomer 2	+			+
C20 S	25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) sulfate	+			
C22 S	25C-NBOMe-M (*O*-demethyl-hydroxy-) sulfate	+			
C2/3 AC+G	25C-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-) *N*-acetyl glucuronide	+			
C10 GSH-2	25C-NBOMe-M (*O,O-bis*-demethyl-) acetylcysteine	+			
C8 G	25C-NBOMe-M (*O,O,O-tris*-demethyl-) glucuronide	+		+	
C10 G	25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 1	+	+	+	
C11 G	25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 2	+	+	+	
C12 G	25C-NBOMe-M (*O,O-bis*-demethyl-) glucuronide isomer 3	+		+	
C16 G	25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 1	+	+	+	+
C17 G	25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 2	+			+
C18 G	25C-NBOMe-M (*O*-demethyl-) glucuronide isomer 3	+			+
C19 G	25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 1	+			
C20 G	25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 2	+			
C21 G	25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 3	+			
C22 G	25C-NBOMe-M (*O,O-bis*-demethyl-hydroxy-) glucuronide isomer 4	+			
C27 G	25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 1	+			
C28/29 G	25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2	+			+
C31/32 G	25C-NBOMe-M (hydroxy-) glucuronide isomer 1	+			
C33 G	25C-NBOMe-M (hydroxy-) glucuronide isomer 2	+	+	+	+
C-Hp-6	25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer *				+
C-Hp-8	25C-NBOMe-M (hydroxy-) glucuronide isomer *				+
C-Hp-10	25C-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer *				+

C-Hp-18	25C-NBOMe-M (hydroxy-) sulfate *				+
C-Hp-19	25C-NBOMe-M (hydroxy-) sulfate *				+
C-MH-21	25C-NBOMe-M (*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) **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) **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) **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) **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) **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) **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) **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).

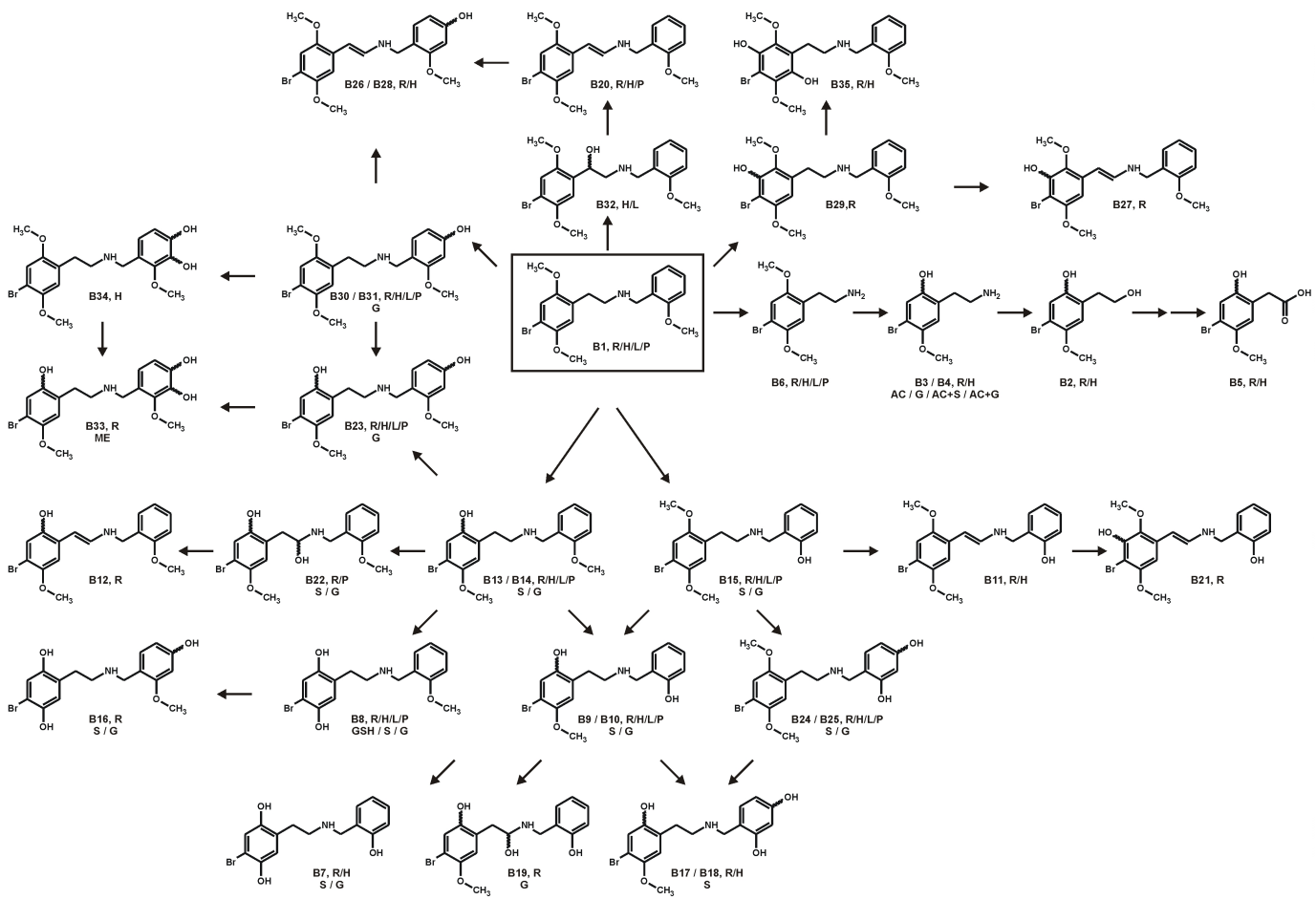
No.	Targets in SUSA	Calculated exact masses of precursor ions, *m/z*	RT, min	Human urine	Rat urine 0.1 mg/kg BW
B1	25B-NBOMe	380.0856	6.0	I	
B5	25B-NBOMe-M (*N*-demethoxybenzyl-*O*-demethyl-deamino-carboxy-)	258.9606	5.3	D	D
B8	25B-NBOMe-M (*O,O-bis*-demethyl-) isomer 1	352.0543	5.0	I	D
B9	25B-NBOMe-M (*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

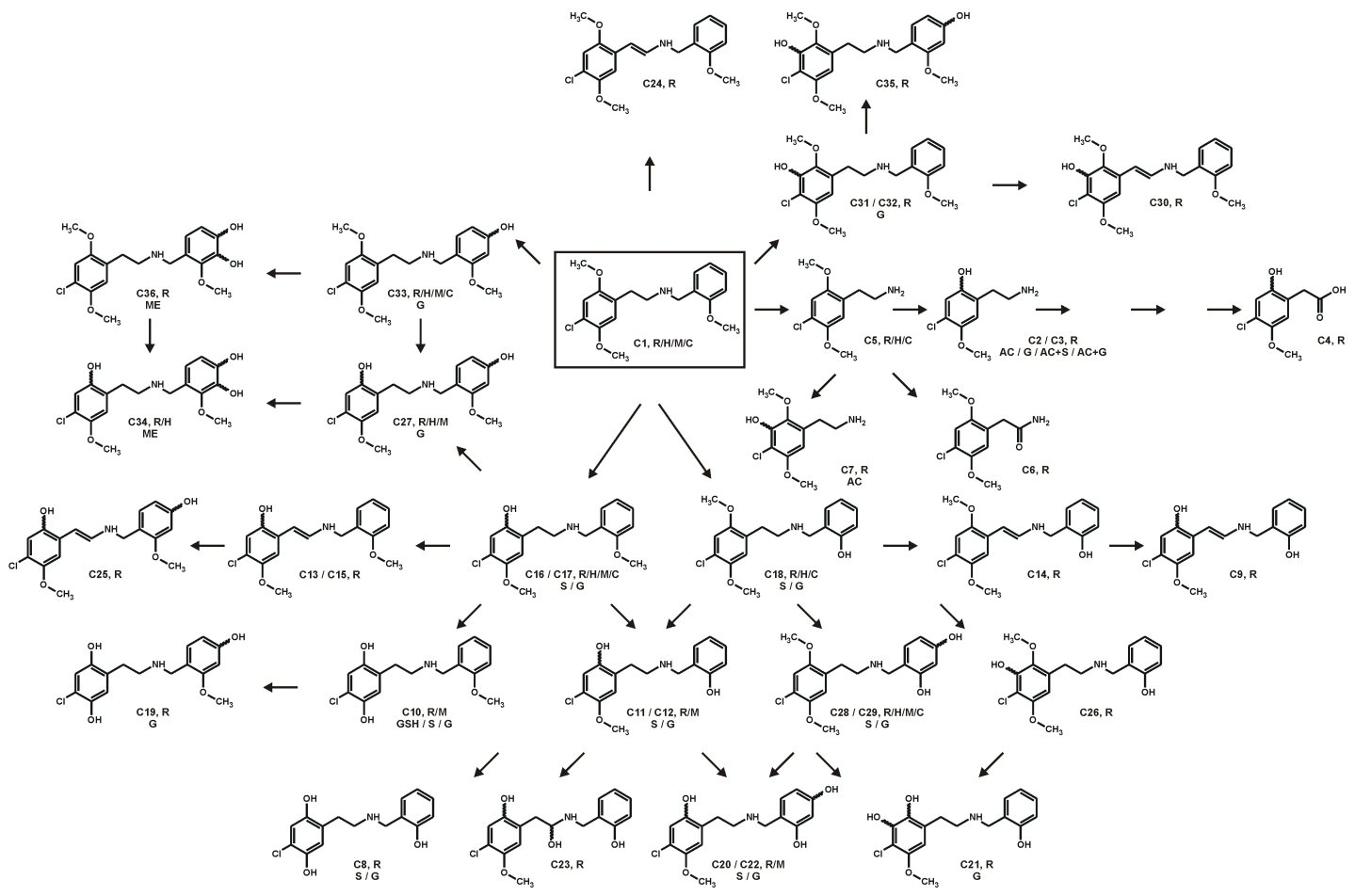
B22 G	25B-NBOMe-M (*O*-demethyl-hydroxy-) glucuronide isomer 2	558.0969	4.8		D
C11	25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 2	308.1048	4.6	n.t.	D
C12	25C-NBOMe-M (*O,O-bis*-demethyl-) isomer 3	308.1048	4.8	n.t.	D
C17	25C-NBOMe-M (*O*-demethyl-) isomer 2	322.1204	5.1	n.t.	D
C8 G	25C-NBOMe-M (*O,O,O-tris*-demethyl-) 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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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 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 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	

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	