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Metabolism, Excretion and Pharmacokinetics of [^{14}C]Tigecycline, a First-In-Class Glycylcycline Antibiotic, after Intravenous Infusion to Healthy Male Subjects

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Metabolic Disposition of [¹⁴C]Tigecycline in Humans

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Abbreviations used are: HPLC, high performance liquid chromatography; LC/MS, liquid chromatography / mass spectrometry; LC/MS/MS, liquid chromatography / tandem mass spectrometry; AUC, area under the concentration-time curve.

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ABSTRACT:

Tigecycline, a novel, first-in-class glycylcycline antibiotic, has been approved for the treatment of complicated intra-abdominal infections and complicated skin and skin structure infections. The pharmacokinetics, metabolism and excretion of [^{14}C]tigecycline were examined in healthy male volunteers. Tigecycline has been shown to bind to bone, so in order to minimize the amount of radioactivity binding to bone and to maximize the recovery of radioactivity, tigecycline was administered intravenously (30-min infusion) as a single 100 mg dose, followed by six 50 mg doses, every 12 h, with the last dose being [^{14}C]tigecycline (50 μCi). Following the final dose, the pharmacokinetics of tigecycline in serum showed a long half-life (55.8 h) and a large volume of distribution (21.0 L/kg), while radioactivity in serum had a shorter half-life (6.9 h) and a smaller volume of distribution (3.3 L/kg). The major route of elimination was feces, containing 59% of the radioactive dose, while urine contained 32%. Unchanged tigecycline was the predominant drug-related compound in serum, urine and feces. The major metabolic pathways identified were glucuronidation of tigecycline and amide hydrolysis followed by *N*-acetylation to form *N*-acetyl-9-aminomincycline. The glucuronide metabolites accounted for 5-20% of serum radioactivity, and approximately 9% of the dose was excreted as glucuronide conjugates within 48 h. Concentrations of *N*-acetyl-9-aminomincycline were approximately 6.5% and 11% of the tigecycline concentrations in serum and urine, respectively. Excretion of unchanged tigecycline into feces was the primary route of elimination, and the secondary elimination pathways were renal excretion of unchanged drug and metabolism to glucuronide conjugates and *N*-acetyl-9-aminomincycline.

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Tigecycline [GAR-936, (4S,4aS,5aR,12aS)-9-(2-tert-butylamino-acetylamino)-4,7-bis-dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide] (Fig. 1), a novel, first-in-class glycylicycline and an analog of the semisynthetic antibiotic minocycline, is a potent, broad-spectrum antibiotic that acts by inhibition of protein translation in bacteria. Glycylicycline antibiotics, including tigecycline and others (*N,N*-dimethylglycylamido-9-aminominocycline and *N,N*-dimethylglycylamido-9-amino-6-demethyl-6-deoxytetracycline), are active in vitro against multiple antibiotic-resistant pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* (Fraise et al., 1995; Weiss et al., 1995). Tigecycline is also active against bacterial strains carrying the two major forms of antibiotic resistance, active efflux and ribosomal protection (Schnappinger and Hillen, 1995). In patients, intravenous tigecycline (50 mg, twice daily) was effective against complicated skin and skin-structure infections and complicated intra-abdominal infections with an acceptable safety profile (Murray et al., 2003; Postier et al., 2004).

The pharmacokinetic profile of tigecycline in healthy human subjects is characterized by a long half-life of 37 to 67 h and a large volume of distribution (V_{SS}) of 7 to 10 L/kg (Muralidharan et al., 2005). The metabolism of glycylicycline antibiotics has not previously been investigated in humans. However, other antibiotics containing the tetracycline ring structure, such as doxycycline, tetracycline, chlortetracycline and demethylchlorotetracycline, generally undergo little or no metabolism (Kelly and Buyske, 1960; Kelly et al., 1961; Eisner and Wulf, 1963; Swarz, 1976; Nelis and De Leenheer, 1981). Minocycline is an exception to this, since it

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undergoes metabolism in humans via hydroxylation and *N*-demethylation (Nelis and De Leenheer, 1982; Böcker et al., 1991).

The present study was conducted to evaluate the pharmacokinetics, metabolic disposition and mass balance of a 50 mg intravenous dose of [¹⁴C]-labeled tigecycline in healthy male volunteers. [¹⁴C]Tigecycline has been shown to distribute extensively into bone following a single IV dose to rats, with bone to plasma ratios for radioactivity as high as 2000 (Tombs, 1999). Even with this extensive distribution into bone, the estimated exposure to radioactivity following a 50 µCi (50 mg) intravenous dose of [¹⁴C]tigecycline to humans was 0.023 rem or 0.46% of the maximum allowable exposure from radioactive drugs for human research subjects (5 rem). However, in order to minimize the amount of [¹⁴C]-labeled material binding to bone and to maximize the recovery of radioactivity, the [¹⁴C]-labeled tigecycline dose was administered following multiple doses (350 mg over 3 days) of unlabeled tigecycline. Using this dosing regimen, previous studies have shown that steady-state tigecycline serum concentrations were achieved on day 4 (Sun et al., 2005). Thus, for the current study, it was assumed that by the time of the [¹⁴C]tigecycline dose steady-state or close to steady-state conditions had been achieved in serum, as well as in other tissues such as bone.

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Materials and Methods

Materials. Tigecycline, as a lyophilized powder in vials containing a 50-mg dose of drug, was provided by Wyeth Research. The manufacturing and packaging of [^{14}C]tigecycline were carried out at the Parenteral Medications Laboratories, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN. Non-radiolabeled tigecycline drug substance was used to dilute radiolabeled tigecycline to make the final ^{14}C drug substance. Radiolabeled tigecycline for injection was prepared in 5 mL clear glass vials, each containing 53 mg of lyophilized, sterilized free tigecycline powder. The final [^{14}C]tigecycline drug product had a specific activity of 9.2 mCi/mmol (1.00 $\mu\text{Ci}/\text{mg}$) with a radiochemical purity of 98.6% and a chemical purity of 99.2%. Appearance, strength, identity, specific activity and purity testing of [^{14}C]tigecycline for injection was performed by ABC Laboratories, Columbia, MO. Bacterial endotoxin and sterility testing of [^{14}C]tigecycline for injection was carried out by Wyeth Research.

An additional batch of [^{14}C]tigecycline (95.3 $\mu\text{Ci}/\text{mg}$, 97.2% radiochemical purity), used in control samples, and [*t*-butyl- d_9]tigecycline, the internal standard for the determination of tigecycline in serum and urine, were received from Amersham Pharmacia Biotech, Buckinghamshire, England. Tigecycline reference standard (98.4% chemical purity), 9-aminominocycline-hydrochloride salt reference standard (CL-318614, 97% chemical purity), and *N*-acetyl-9-aminominocycline (WAY-188749, batch L23566-162) were received from Wyeth Research. All other reagents and chemicals were obtained from commercial sources.

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Study Design. This open-label, inpatient, multiple-dose tigecycline, single-dose [¹⁴C]tigecycline metabolic disposition and mass balance study was performed in healthy men. Eligible subjects were selected based on inclusion/exclusion criteria, medical history, physical examination and additional procedures outlined in the study protocol. Subjects using any investigational or prescription drug within 30 days of test article administration were excluded from the study. Prior to initiation of the study, the protocol and consent form were reviewed and approved by an institutional review board. Study subjects gave written informed consent before the screening process was initiated.

Dose Administration. The study was initiated with 12 healthy adult male volunteers to ensure six subjects would receive the radiolabeled dose. Each subject received a 100 mg loading dose on the morning of Day 1, followed by a 50 mg maintenance dose every 12 h for an additional 5 doses. On the morning of study day 4, six subjects (ranging from 22 to 40 years of age, with body weights of 70.1 kg to 90.8 kg) each received a single 50 mg dose of [¹⁴C]tigecycline, approximately 50 μCi/subject. Each tigecycline dose was administered via a 30-min intravenous infusion.

Throughout the study, subjects received a high fiber meal approximately 2 h prior to tigecycline administration. Study subjects received two tablets of FiberCon daily from Day 1 through Day 13 to facilitate regular bowel movements. The dosing and “inpatient-confinement portion” of the study were conducted at Quintiles, Kansas City, MO. Aliquots of the [¹⁴C]tigecycline dosing solution obtained before and after dose administration, dosing vials and dosing apparatus were shipped to ABC Laboratories for radioactivity analysis.

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Clinical Sample Collection. Whole blood samples (3 mL) from each subject, for the determination of total radioactivity, were collected into Vacutainer tubes containing EDTA within 2 h before [¹⁴C]tigecycline dosing and approximately 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h after the [¹⁴C]tigecycline dose. Additional whole blood samples (7 mL) were collected within 2 h prior to the first tigecycline dose and at the same collection times as the 3 mL whole blood samples. From these whole blood samples, serum was obtained after clot formation and centrifugation of the sample, and used to determine tigecycline and total radioactivity concentrations in serum. Whole blood samples (50 mL) for serum collection, were drawn approximately 2 h prior to the first tigecycline dose, within 2 h before [¹⁴C]tigecycline dosing and approximately 1, 4, 8, 24 and 48 h after the [¹⁴C]tigecycline dose. These serum samples were stored at -70°C until being analyzed for metabolite profiles. Urine samples were collected within 2 h before the [¹⁴C]tigecycline dose and all urine was collected from study subjects following the [¹⁴C]tigecycline dose at intervals of 0-4, 4-8, 8-24 h and every 24 h thereafter up to 240 h. Samples were kept refrigerated during the collection intervals, then separated into samples for total radioactivity determination, tigecycline concentration determination and metabolite profiling (samples up to 48 h following the [¹⁴C]tigecycline dose), and stored at ≤-20°C until analysis. When possible, a fecal sample was collected from subjects prior to the [¹⁴C]tigecycline dose, and all fecal samples up to 240 h following the [¹⁴C]tigecycline dose were also collected. Samples were homogenized in 3 volumes of water using a Stomacher® (3500, Seward Limited) and stored in plastic containers at ≤4°C until analysis. Aliquots of each sample were analyzed for

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total radioactivity concentrations and samples collected up to 48 h following the [¹⁴C]tigecycline dose were analyzed for metabolite profiles.

For two subjects, there were incomplete fecal collections following the [¹⁴C]tigecycline dose, due to non-compliance, and another subject withdrew from the study following sample collections at 24 h. Data from these subjects were not used in the mass balance portion of the study, but were used in the pharmacokinetic analysis and metabolite profiling portions of the study.

Radioanalysis. All radioactivity determinations were made using either a Tri-Carb Model 3100TR liquid scintillation counter (Perkin Elmer, Wellesley, MA) or Beckman/LS6000SC or LS6500 liquid scintillation counters (Beckman Coulter, Inc., Fullerton, CA). For dose, serum and urine analysis, Ultima Gold™ scintillation fluid (Perkin Elmer) was added to a known volume or weight of sample. Samples were then directly analyzed by liquid scintillation counting. Fecal homogenates were weighed, allowed to dry, and combusted using a Model 307 sample oxidizer (Perkin Elmer). The resultant [¹⁴C]CO₂ was trapped in Carbosorb® in combination with Permafluor® and radioassayed by liquid scintillation counting. For all sample matrices, a quench curve was used to convert cpm values to dpm values.

Measurement of Tigecycline in Serum and Urine. Serum and urine tigecycline concentrations were determined using validated LC/MS/MS assays. For serum, the assay was validated at concentrations between 10 and 2000 ng/ml. Briefly, tigecycline and an internal standard ([*t*-butyl-d9]tigecycline) were extracted from serum using 0.1% trifluoroacetic acid in acetonitrile. The denatured protein was removed by centrifugation and the supernatant was

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removed and evaporated to dryness at 35°C under a stream of air. Samples were reconstituted in 200 µl and an aliquot injected for LC/MS/MS analysis using a Keystone Scientific AQUASIL C₁₈, 50 x 2.1 mm, 5 µm analytical column. Tigecycline and the internal standard were measured using the transition of the positive ions m/z 586.5→513.2 and 595.3→514.3, respectively. For the QC samples, the overall precision (CV) was ≤10.5% and accuracy ranged from 104 to 108%.

For urine, the assay was validated at concentrations between 0.20 and 80 µg/ml. Briefly, tigecycline and an internal standard in urine were diluted, the samples were mixed, transferred to fresh tubes and an aliquot injected for LC/MS/MS analysis using a Keystone Scientific AQUASIL C₁₈, 100 x 2.0 mm, 5 µm analytical column. For the QC samples, the overall precision (CV) was ≤11.3% and accuracy ranged from 90.4 to 100%.

Metabolite Profiling by HPLC. Individual serum samples collected 1, 4 and 8 h following the [¹⁴C]tigecycline dose were analyzed for metabolite profiles. The 24 and 48 h samples were not analyzed because the concentration of radioactivity was too low. Each serum sample was divided into two samples of equal volume (approximately 9 mL each) to provide duplicate analyses. EDTA (final concentration of 40 mM) and three volumes of acetone were then added to the samples. Samples were mixed, and then centrifuged to remove the denatured protein. The supernatant was transferred to a fresh tube containing 20 µL glacial acetic acid. The pellet was re-extracted with 2 mL water, 160 µL 0.2 M EDTA and 6 mL acetone and processed as described above. The supernatants were combined and the pellet extraction repeated. Supernatant samples were evaporated to dryness under a stream of nitrogen at room temperature.

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The residue was reconstituted in 300 μ L of acetonitrile/water (1:9). The reconstituted residue was centrifuged to remove particulates and the supernatant transferred to an autosampler vial. The supernatant was assayed for radioactivity content and analyzed for metabolite profiles by HPLC. Extraction of control serum or fecal samples spiked with varying concentrations of [14 C]tigecycline showed that the extraction process resulted in a reproducible percentage of tigecycline in samples being converted to its epimer during the extraction process. Therefore, the amount of epimer in the samples prior to extraction could be determined based on the amount of epimer in the final serum or fecal extracts.

Aliquots (1 mL) of the individual urine samples collected up to 48 h following the [14 C]tigecycline dose were transferred to clean tubes and EDTA (final concentration of 40 mM) was added. The pH of the urine remained constant at pH 4.5-5.5. Samples were mixed, centrifuged and the supernatants transferred to autosampler vials. Separate aliquots of the supernatant were assayed for radioactivity content and analyzed for metabolite profiles by HPLC.

Fecal homogenates prepared from samples collected within 48 h following the [14 C]tigecycline dose and that contained greater than 8000 dpm/g were analyzed for metabolite profiles.

Aliquots of the fecal samples (approximately 1 g) were transferred to 15 mL tubes and three volumes of acetone were added and the samples mixed and centrifuged. The supernatant was transferred to a fresh tube and the pellet re-suspended with 40 mM EDTA in water, and re-extracted as described above. The pellet was re-extracted in this manner a total of three times and the supernatants pooled. The combined supernatants were evaporated to dryness under a

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stream of nitrogen. The residue was re-suspended in 500 μ L of water, centrifuged to remove particulates and the supernatant was assayed for radioactivity content and analyzed for metabolite profiles by HPLC.

For LC/MS quantitation of metabolites M5 (*N*-acetyl 9-aminomincycline) and its epimer M4 in serum and urine samples from three subjects, standard curves were generated for each metabolite in each matrix. In serum, the standard curves ranged from 2.1-514.0 ng/mL for M4 and 2.8-568.6 ng/mL for M5, while in urine, the ranges were 5.7-632.5 ng/mL for M4 and 4.3-367.8 ng/mL for M5. For quantitation of these metabolites, urine samples were prepared in the same manner as those for metabolite profiling. For the serum samples, EDTA (final concentration of 40 mM) was added to each sample and the samples vortex mixed. One volume of acetonitrile was then added to each sample, samples were vortex mixed and denatured protein separated by centrifugation. The supernatant was transferred to a fresh tube and concentrated under a stream of nitrogen to approximately 1 mL. The remaining supernatant was then assayed for M4 and M5 concentrations.

HPLC analyses for metabolite profiling were performed using a Waters 2695 Alliance Separation Module (Waters Corp., Milford, MA) with the sample temperature set to 4°C and a Waters Model 2487 dual wavelength UV absorbance detector set to monitor 350 nm. The system was in-line with a Gilson 215 liquid handler (Gilson, Middleton, WI) equipped to collect fractions at 20 second intervals. Fractions were collected into 96-well deep well Luma plates and analyzed for radioactivity using TopCount NXT (PerkinElmer, Shelton, CT). Flo-One analytical software (version 3.65) was utilized to integrate the radioactive peaks. Separation of

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tigecycline and drug-derived products was achieved on a Phenomenex Luna C18(2) column (150 x 2.1 mm, 5 μ m; Phenomenex, Torrance, CA) equipped with a Phenomenex SecurityGuardTM guard cartridge (5 μ m) using two mobile phases, 10 mM ammonium acetate in water and acetonitrile. The column was at an ambient temperature of approximately 20°C and the flow rate was 0.2 mL/min. The linear gradient was delivered as follows: 0 to 25 min, from 98% aqueous to 90% aqueous; 25 to 50 min, from 90% aqueous to 70% aqueous; hold at 70% aqueous until 60 min; return to initial conditions at 61 min.

Additional HPLC conditions were used to characterize a polar metabolite that was not retained on the C18 column. Separations were accomplished on a Waters Atlantis HILIC Silica column (150 x 2.1 mm, 5 μ m) using two mobile phases, 0.02% trifluoroacetic acid in acetonitrile (v/v) and 0.02% trifluoroacetic acid in water (v/v). The column was at an ambient temperature of approximately 20°C and the flow rate was 0.2 mL/min. The linear gradient was delivered as follows: 0 to 10 min, hold at 100% organic; 10 min to 66% organic; 5 min to 34% organic; hold for 15 min; return to initial conditions over one min.

Metabolite Characterization by Mass and NMR Spectroscopy. The HPLC system and separation conditions used for mass spectrometric analysis were similar to those described for metabolite profiling. MS and MS/MS experiments were performed on a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters Corp.) equipped with an electrospray source and operated in the positive ionization mode. The electrospray needle potential was set at 2.75 kV and the orifice potential was set at 44 V. The ion source was held at 80°C and the desolvation temperature was 250°C. In MS/MS experiments, the collision-activated dissociation

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of selected precursor ions was conducted using argon as the collision gas. The collision energy was 30 eV.

Serum extracts were analyzed for tigecycline and selected metabolites by LC/MS/MS in the selected reaction monitoring mode to reduce interference from endogenous components. These experiments were conducted with a dwell time setting of 200 ms. The following tigecycline-related components were monitored: tigecycline and its epimer, m/z 586→513; hydroxy tigecycline and tigecycline *N*-oxide, m/z 602→585 and m/z 602→472; *N*-desmethyl tigecycline, m/z 572→499; tigecycline glucuronide, m/z 762→569; 9-aminomincycline, m/z 473→456; and *N*-acetyl-9-aminomincycline, m/z 515→498. Urine and fecal samples were analyzed for tigecycline metabolites by LC/MS/MS analysis for precursors of product ions characteristic of tigecycline. Additionally, potential metabolites of tigecycline were searched for in the LC/MS data for subsequent MS/MS analysis.

The site of glucuronidation was further investigated using chemical oxidation of tigecycline and its glucuronide metabolite with Fremy's salt (potassium nitrosodisulfonate). Fremy's salt selectively oxidizes phenols, aromatic diols, aminophenols and diamines to the corresponding quinones (Zimmer et al., 1971). The glucuronide metabolite used for these incubations was isolated from human urine following collection of the HPLC column effluent that contained the glucuronide metabolite. Three sets of incubations with Fremy's salt were prepared: tigecycline alone, tigecycline plus the isolated glucuronide metabolite, and the isolated glucuronide metabolite alone. Incubations with tigecycline contained 10 µg/mL tigecycline in 10 mM ammonium acetate with 20 µM EDTA and incubations with the tigecycline glucuronide

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contained the isolated tigecycline glucuronide with EDTA added (final concentration of 20 μM). Incubations were prepared in duplicate and to one replicate Fremy's salt was added (2 $\mu\text{g/mL}$), to the other replicate nothing was added. Samples were incubated for at least 5 min at an ambient temperature of approximately 20°C prior to being directly analyzed by LC/MS. Tigecycline glucuronide conjugates were synthesized using modified Koenigs-Knorr reaction conditions ($\text{Ag}_2\text{CO}_3/\text{MeCN}$ in the presence of EDTA and molecule sieves) with an acyl-protected 1 α -bromo-sugar as the donor to generate protected tigecycline glucuronides followed by de-protection in the presence of 0.1 N lithium hydroxide (in methanol: water 1:1). Co-chromatography of the synthetic tigecycline glucuronides with tigecycline glucuronide (M7) isolated from human urine was used to determine the desired reaction product. NMR spectroscopy of the synthetic glucuronide was performed with a 500 MHz Bruker DRX spectrometer equipped with a Cap-NMR flow cell probe. Proton NMR spectra were acquired with 128K data points. The heteronuclear single quantum correlation (HSQC) spectrum was acquired with 2K data points in F2 and 256 increments in F1. Heteronuclear multiple bond correlation (HMBC) experiments were conducted with 4K data points in F2 and 512 increments in F1. One of the HMBC spectra was recorded with the $J_{\text{H/C}}$ setting at 8 Hz and another one was recorded with the $J_{\text{H/C}}$ setting at 5 Hz for three-bond long-range correlation detection.

Pharmacokinetic Analysis. The radioactivity concentration data in serum (expressed in ng-equivalent of tigecycline/mL), and the tigecycline serum concentration data for each subject were analyzed by using empirical, model-independent pharmacokinetic methods (Jusko, 1992). Peak concentration (C_{max}) was directly determined from the observed data. The apparent

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terminal-phase disposition rate constant (λ_Z) for tigecycline in serum was estimated by a log-linear regression of the last 3 to 7 observed concentrations that were determined to be in log-linear elimination by visual inspection. Due to low concentrations and rapid elimination, the concentration of radioactivity in serum could be determined only in samples collected within 24 h of dosing and therefore the λ_Z for this parameter was estimated using only 2 or 3 observed concentrations. The apparent terminal-phase disposition half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/\lambda_Z$.

Since only one dose of [^{14}C]tigecycline was administered, the serum radioactivity concentration data were analyzed as single-dose data. The single dose area under the concentration-time curve (AUC_T) to the last observable concentration (C_T) at time T was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. The total single-dose AUC was estimated by $\text{AUC} = \text{AUC}_T + C_T/\lambda_Z$. The single dose mean residence time (MRT) was calculated as $\text{AUC}/\text{AUMC} - T_{\text{inf}}/2$, where AUMC is total area under the first moment curve and T_{inf} is the duration of infusion (30 min).

The unlabeled tigecycline serum concentration data represent multiple-dose administration, and the steady-state AUC over one dose interval ($\text{AUC}_{0-\tau}$, where $\tau=12$ h) was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. Additionally, the steady-state MRT was calculated as $\text{AUC}_{0-\tau}/(\text{AUMC}_{0-\tau} + \tau \cdot \text{AUC}_{\tau-\infty}) - T_{\text{inf}}/2$. Systemic clearance (Cl) was calculated as dose/AUC , and the apparent steady-state volume of distribution (V_{SS}) was estimated as $\text{Cl} \cdot \text{MRT}$.

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Calculations. The amount of total radioactivity (dpm) in the urine, feces and serum was determined by multiplying the weight of samples by the radioactivity concentration (dpm/g) of each sample. The dose recovered was determined by total dpm in the sample at any given time point, divided by total radioactivity (in dpm) of the dose received by each subject, and multiplied by 100%. These calculations and the calculations of means and standard deviations were performed using Microsoft Excel® 2000 software. If the dpm in any post-dose sample aliquot was less than or equal to 2 x dpm in the pre-dose sample aliquot (background), the value was presented as not detectable (ND).

The concentration of tigecycline-related components in serum, urine and feces was calculated based on the total radioactivity concentrations. These concentrations were converted to tigecycline equivalent using the specific activity of the dose (1.00 $\mu\text{Ci}/\text{mg}$). Using this value, the concentrations of the specific components were then estimated based on the distribution of radioactivity in the radiochromatograms. These metabolite concentrations reflect only the disposition of the final [^{14}C] tigecycline dose, and do not account for tigecycline or its metabolites remaining from the unlabeled doses. For the concentration determinations of the non-radiolabeled metabolites M4 and M5, the LC/MS peak area from study samples was compared to a standard curve generated using peak areas of control samples spiked with synthetic standards of M4 and M5.

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Results

Excretion of Radioactivity. The final [^{14}C]tigecycline intravenous dose contained 45.9 ± 0.9 μCi (mean \pm SD), equivalent to 45.9 ± 0.9 mg. Recovery of radioactivity in urine and feces was calculated for five subjects (Table 1), with the excluded subject having withdrawn from the study following the 24 h sample collections. For two subjects that had incomplete sample collection, the recovery of radioactivity was $<55\%$ and these subjects were not used in the final mass balance calculations. The overall recovery of radioactivity by 240 h following administration of [^{14}C]tigecycline was $91.8 \pm 5.6\%$ (mean \pm SD, $n=3$), with feces being the major route of elimination. The majority of the radioactive dose ($87.5 \pm 5.1\%$) was eliminated within 120 h after dosing (Fig. 2).

Pharmacokinetic Analysis. The concentration-time curve and pharmacokinetic parameters for tigecycline in serum following the final tigecycline dose were different than those for radioactivity in serum (Table 2 and Fig. 3). The tigecycline concentrations in serum represent tigecycline present from all doses, while the radioactivity measurements represent only the final, radiolabeled dose of tigecycline. Tigecycline in serum had a long $t_{1/2}$, a large V_{SS} and tigecycline were measurable for 7 to 9 days following the final dose. Tigecycline Cl from serum was 22 L/h (0.29 L/h/kg), while mean renal clearance (Cl_r) was 4.6 L/h (data not shown). For radioactivity in serum, the $t_{1/2}$ was much shorter and the V_{SS} smaller than for tigecycline in serum. Additionally, both Cl of radioactivity from serum (41 L/h, 0.54 L/h/kg), and Cl_r (13 L/h, data not shown) were significantly higher than for tigecycline. Radioactivity concentrations in serum were measurable for approximately 12 to 24 h after the radiolabeled dose. The blood:serum

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ratios of radioactivity were between 0.7 to 1.1 at all time points tested (data not shown), which indicated no preferential binding of radioactivity to whole blood constituents.

Metabolic Profiles. HPLC chromatograms of extracted serum, urine and fecal homogenate from a representative subject are shown in Fig. 4. The metabolite profiles were similar between subjects and at the different time points. Unchanged [^{14}C]tigecycline was the predominant radiolabeled component in the serum samples at all time points (Table 3). The values for tigecycline and the epimer of tigecycline take into account the amount of tigecycline that was converted to the epimer during the extraction process, as described in the methods section. In serum, M1 (*t*-butylaminoacetic acid) and the glucuronide conjugates of tigecycline and its epimer (M3 and M2, respectively) increased in abundance, relative to tigecycline, from 1 to 8 h following the radioactive dose. Since the radiolabel had been cleaved from *N*-acetyl-9-aminominocycline (M5) and its (M4), the metabolites were detected and quantified in serum and urine by LC/MS analysis (Table 4). Therefore, the amount of M4 and M5 detected was the result of all of the tigecycline doses and not just the final radiolabeled dose. Metabolite M5 was detected in all serum samples analyzed at concentrations approximately 5-7% of the tigecycline concentrations in the same serum samples. The trace metabolite 9-aminominocycline (M6) was detected by LC/MS analysis of the serum, but was not quantified.

In urine collected up to 48 h following the [^{14}C]tigecycline dose, the predominant radiolabeled component was unchanged [^{14}C]tigecycline (Table 5). The epimer of tigecycline represented 2% of the radioactive dose and increased relative to tigecycline, over time. Some of the epimer may have formed in the bladder, as stability analysis demonstrated that tigecycline degraded to

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the epimer when incubated in human urine at 37°C (data not shown). In the 0-48 h urine samples, M1 represented 6.3% of the radioactive dose, while M3 represented 4.1%; M2 was not detected in urine. Within 48 h of the final tigecycline dose, approximately 1.5 mg of unlabeled metabolites M4 and M5 had been excreted in urine (Table 4). Additional trace metabolites in urine, detected by LC/MS, were 9-aminomincycline (M6) and two hydroxy tigecycline metabolites (M7 and M8).

For the fecal samples collected up to 48 h following the [¹⁴C]tigecycline dose, tigecycline was the predominant drug-related component (Table 5). The epimer of tigecycline, glucuronide conjugates of tigecycline and its epimer (M4 and M5), and *t*-butylaminoacetic acid (M1) were all observed in feces. Additional uncharacterized radioactive peaks were observed in some fecal samples, and represented less than 2.5% of the radioactive dose. The unlabeled metabolites 9-aminomincycline (M6), *N*-acetyl-9-aminomincycline (M5) and its epimer (M4) were not detected in any fecal samples.

Metabolite Structural Characterization. Fig. 5 shows the proposed structures for each of the metabolite peaks observed by HPLC with radioactivity flow detection and/or LC/MS.

Tigecycline generated a protonated molecular ion, [M+H]⁺, at *m/z* 586. The product ions of *m/z* 586 mass spectrum included *m/z* 569, 513, 456, 211, 154, 126 and 86 (Fig. 6, Table 6). Loss of NH₃ from [M+H]⁺ generated *m/z* 569. The product ion at *m/z* 513 represented loss of the *t*-butylamino group from the *t*-butylaminoacetylamino side chain. Loss of the entire *t*-butylaminoacetylamino side chain generated *m/z* 456. The product ions at *m/z* 211, 154 and 126 originated from the A ring of the tetracycline ring system as indicated in the fragmentation

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scheme. The product ion at m/z 86 represented the *t*-butylaminomethylene group. This proposed fragmentation scheme was consistent with the reported fragmentation of structurally similar antibiotics (Kamel et al., 2002).

The epimer of tigecycline generated a $[M+H]^+$ at m/z 586. Its product ions of m/z 586 mass spectrum was identical to the mass spectrum for tigecycline (Table 6). Identification as the epimer was made based on its relative retention time being shorter than that of tigecycline.

The $[M+H]^+$ for M2 and M3 was observed at m/z 762, which was 176 Da larger than tigecycline (Table 6). Neutral loss of 176 Da generated m/z 586, also the $[M+H]^+$ for tigecycline, which indicated a glucuronide of tigecycline. Product ions at m/z 569, 513, 456, 211, 154 and 86 were also observed for tigecycline and did not indicate the site of conjugation. The product ion at m/z 551 was formed by fragmentation of ring B, which indicated that the hydroxy group at either position 10 or 12 was the site of glucuronidation. LC/MS analysis of the reaction product formed following chemical oxidation of tigecycline with Fremy's salt showed that tigecycline was oxidized to the quinone product for tigecycline, with subsequent oxidation of the hydroxyl group at the 12 position (Fig. 7). Consistent with the mechanism of oxidation by the nitrosodisulfonate radical generated from Fremy's salt, the initial site of reaction was position 7 (Zimmer et al., 1971). Oxidation of the D ring phenol to a quinone required the presence of a free hydroxyl group at position 10 in order to generate the cyclohexadienone intermediate. The hydroxyl group at position at 12 was subsequently oxidized to a keto group, but this would not have occurred without prior oxidation of the D ring phenol to a quinone. Following incubations of M3 with Fremy's salt, no reaction products were observed. If the 10-hydroxyl group were the

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site of glucuronidation, then no cyclohexadienone intermediate could be formed and no oxidation products of tigecycline glucuronide (M3) would be expected. However, if the 12-hydroxyl group were the site of glucuronidation, then oxidation products of tigecycline glucuronide (M3) would be expected. The inability of tigecycline glucuronide to be oxidized by Fremy's salt indicated that a free phenolic hydroxyl group was no longer present at position 10 on account of position 10 being the site of glucuronidation. In the $^1\text{H-NMR}$ spectrum of the synthetic tigecycline glucuronide, the proton signal for the C10-OH expected at $\delta 12.3$ ppm for tigecycline was absent (data not shown), suggesting a 10-*O*-glucuronide structure. This suggestion was supported by a three-bond long-range correlation between H1' (anomeric proton of the sugar moiety at $\delta 4.47$) with C10 ($\delta 141.7$) observed in the HMBC spectrum (Fig. 8). The assignment of C10 at $\delta 141.7$ was confirmed by the three-bond long-range correlation of H8 ($\delta 8.26$) to C10. Therefore, the synthetic tigecycline glucuronide and tigecycline glucuronide isolated from human urine (M3) were both identified as tigecycline 10-*O*- β -D-glucuronide. Metabolite M2 was proposed to be the epimer of M3 based on its HPLC retention time being earlier than that of M3.

Metabolites M4 and M5 were observed in serum and urine, and only by LC/MS. The $[\text{M}+\text{H}]^+$ for these metabolites was observed at m/z 515, which was 71 Da smaller than tigecycline. The product ions of m/z 515 mass spectrum for M5 included m/z 498, 456, 154, 126 and 58 (Table 6). Mass spectral data for M4 and M5 were similar and indicated the tetracycline ring was intact. The lack of radiochromatographic peaks for M4 and M5 was consistent with loss of the radiolabeled carbonyl group of the *t*-butylaminoacetyl amino side chain. The most likely

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mechanism for formation of M5 was amide hydrolysis to generate *t*-butylaminoacetic acid (M1, which contained the radiolabel) and 9-aminominocycline (no ¹⁴C label). *N*-Acetylation of 9-aminominocycline was proposed to generate M5. The 58 Da difference between the *m/z* 456 product ion and molecular weight (514) was consistent with the presence of a non-radiolabeled acetylamino group and the observation of a *m/z* 58 product ion for M4 and M5 (Table 6).

Confirmation of these proposed structures was obtained by co-chromatography of a urine extract spiked with synthetic *N*-acetyl-9-aminominocycline (data not shown). *N*-Acetylation of 9-aminominocycline to form M5 was also demonstrated in human liver cytosol (data not shown). Metabolite M4 was proposed to be the epimer of M5 based on its HPLC retention time being earlier than that of M5. Therefore, M4 and M5 were identified as the epimer of *N*-acetyl-9-aminominocycline and *N*-acetyl-9-aminominocycline, respectively.

9-Aminominocycline (M6) was observed in serum and urine in trace amounts and only by LC/MS due to loss of the radiolabel. The [M+H]⁺ for 9-aminominocycline was observed at *m/z* 473. The product ions of *m/z* 473 mass spectrum included *m/z* 456 (Table 6), generated from loss of NH₃, which was characteristic of tigecycline related compounds. In serum samples, this metabolite was observed by monitoring the *m/z* 473→456 SRM transition (data not shown).

Confirmation of the identification of 9-aminominocycline was obtained by comparison with a synthetic standard. 9-Aminominocycline was proposed to have been generated by amide hydrolysis of the *t*-butylaminoacetylamino side chain with radiolabeled *t*-butylaminoacetic acid as a byproduct. Metabolite M1 was observed in serum, urine and feces, as an early eluting radiochromatographic peak. Isolation of this early eluting M1 peak from human urine and

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subsequent LC/MS analysis with normal phase HPLC resulted in observation of $[M+H]^+$ and $[MH+CH_3CN]^+$ for M1 at m/z 132 and 173, respectively (data not shown). This indicated a molecular weight of 131, consistent with the identification as *t*-butylaminoacetic acid.

Hydroxylated tigecycline metabolites M7 and M8 were observed in urine in trace amounts.

These metabolites produced a $[M+H]^+$ at m/z 602. Mass spectral data for both of the hydroxy metabolites were similar (Table 6). For both M7 and M8, product ions at m/z 529 and 472 were 16 Da larger than the corresponding ions at m/z 513 and 456, respectively, for tigecycline.

These data indicated that the tetracycline ring was the site of metabolism rather than the *t*-butylamino-acetylamino group. For M8, the product ions at m/z 211 and 154 indicated an unchanged A ring. Therefore, these metabolites were the product of oxidation of the tetracycline moiety.

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Discussion. Intravenous administration of tigecycline for 3 days (total dose of 350 mg), followed by a single IV dose of [^{14}C]tigecycline (50 mg, 50 μCi), was well tolerated. The rationale for preceding the [^{14}C]tigecycline dose with multiple doses of non-radiolabeled drug was based upon the ability of tigecycline to bind to bone (Tombs, 1999). This study design was meant to minimize the amount of radioactivity binding to bone while maximizing the recovery of radioactivity.

Another consequence of this study design was that the pharmacokinetic parameters for unlabeled tigecycline in serum were somewhat different than those for radioactivity in serum (Table 2). Tigecycline pharmacokinetics in serum for the current study, including C_{max} , AUC, Cl, V_{SS} and $t_{1/2}$, were similar to previously reported data under steady-state conditions (Muralidharan et al., 2005). Also consistent with previous data, the concentration vs time curve for tigecycline in serum, as well as for radioactivity in serum, appeared to be bi-phasic with rapid distribution into tissues followed by a longer elimination phase, likely caused by the continual release of tigecycline from slowly equilibrating tissues such as bone. However, the pharmacokinetic profile of the radioactivity in serum exhibited higher Cl, a smaller V_{SS} and a shorter $t_{1/2}$ as compared to the pharmacokinetic parameters for unlabeled tigecycline. These differences may have been caused by a significant amount of unlabeled tigecycline binding to the tissues prior to administration of the [^{14}C]tigecycline, and blocking subsequent tissue uptake of [^{14}C]tigecycline. This so-called “last-in, first-out” phenomenon has been reported previously for drugs administered with loading doses of unlabeled drug followed by a single dose of radiolabeled drug (Colburn and Matthews, 1979). Therefore, the “last-in, first-out”

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phenomenon accounts for the much smaller V_{SS} and the much higher Cl for total radioactivity as compared to these parameters for tigecycline.

The mass balance data from three subjects showed that recovery of the radioactivity was nearly complete, with $91.7 \pm 5.5\%$ of the dose recovered within 240 h (Table 1). One subject withdrew from the study approximately 36 h following the [^{14}C]tigecycline dose and two subjects had incomplete fecal sample collections and were not used in the mass balance calculations. Feces was the major route of excretion, containing $58.6 \pm 4.5\%$ of the dose, with the majority ($55.6 \pm 3.9\%$) excreted within 120 h (Fig. 2). Excretion in urine was a secondary route of elimination, with $33.2 \pm 1.9\%$ of the dose recovered within 240 h and a majority of the urinary radioactivity recovered within 72 h ($29.8 \pm 1.5\%$). There was minimal excretion of radioactivity in feces or urine after Day 5, with less than 1.0% of the dose excreted on Day 6 through Day 10.

Following intravenous administration of [^{14}C]tigecycline, unchanged drug was the predominant radiolabeled component in serum, urine and feces, with more than half of the radioactivity excreted within 48 h of dosing identified as [^{14}C]tigecycline. The epimer of tigecycline was also observed in each matrix, accounting for approximately 5-10% of the radioactivity in serum, urine and feces. The epimer of tigecycline was shown to be a degradant rather than a metabolite, as it was formed when tigecycline was incubated in control human serum and urine at 37°C (data not shown). Epimerization at the C-4 position has also been reported for structurally similar antibiotics (Remmers et al., 1963; Nelis and De Leenheer, 1982).

Metabolites M3 (tigecycline glucuronide) and its epimer, M2, were observed in serum, urine (M3 only) and feces. These metabolites represented up to 15% of the radioactivity in serum and

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nearly 10% of the dose was excreted as glucuronide conjugates within 48 h of dosing. There are no reports in the literature that identify glucuronidation as a metabolic pathway for antibiotics with similar structures. In fact, these structurally similar antibiotics generally undergo little or no metabolism in humans (Kelly et al., 1961; Eisner and Wulf, 1963; Swarz, 1976; Nelis and De Leenheer, 1981). One exception to this is minocycline, which is metabolized to 9-hydroxyminocycline, N₄-demethylminocycline and N₇-demethylminocycline in humans (Nelis and De Leenheer, 1982). The 9-hydroxyminocycline and N-demethylminocycline (site of demethylation not determined) metabolites can each represent up to 8% of the dose in urine (Böcker et al., 1991). Additional metabolites of minocycline have been reported in human urine, but these metabolites have not been characterized (Nelis and De Leenheer, 1982). No N-demethyl metabolites of tigecycline were observed in the current study. Trace amounts of two hydroxy tigecycline metabolites were detected by LC/MS analysis in urine, but the site of hydroxylation and detection by radiochromatography were not possible because the metabolites were present at very low concentrations.

Metabolite M1 (*t*-butylaminoacetic acid) was observed in each biological matrix, accounting for up to 20% of the radioactivity in serum and approximately 8% of the dose was excreted as M1 within 48 h. Metabolism of tigecycline to M1, via amide hydrolysis of the *t*-butylaminoacetylamino group, also led to the formation of 9-aminominocycline (M6, not radiolabeled). However, only trace amounts of M6 were detected in serum and urine. The difference in the amount of M1 and M6 observed can be accounted for because a large proportion of the M6 was further metabolized via *N*-acetylation to *N*-acetyl-9-aminominocycline

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(M5) and its epimer (M4). This metabolic pathway was demonstrated using human liver cytosol incubated with M6 (data not shown). While both M5 and M6 demonstrated some antibacterial activity *in vitro*, their activities were generally less than that of tigecycline and were effective against a narrower range of infectious organisms (data on file at Wyeth Research). Metabolite M4 was not tested for antibacterial activity since epimerization at the C-4 position in tigecycline and structurally similar antibiotics significantly decreases activity (Zhanel et al., 2004).

It is important to recognize that the excretion data and most of the metabolite profiling performed in this study assess only the disposition of the final [¹⁴C]-labeled tigecycline dose, and does not account for tigecycline-related products remaining from the unlabeled doses.

Therefore, the estimated concentrations of the tigecycline-related products that were based on radioactivity concentration data may underestimate the actual concentrations of these components in serum, urine and feces. The exception to this is the concentrations of the unlabeled metabolites M4 and M5, which were determined by LC/MS analysis rather than radioactivity concentrations. The amounts of [¹⁴C]tigecycline in serum and urine reported are somewhat less (generally 10 to 75% less) than the total tigecycline amounts reported.

Additionally, the contribution of the [¹⁴C]tigecycline to the total tigecycline amount in serum and urine decreased with time; as mentioned above, this is consistent with the last-in, first-out phenomenon.

In summary, following IV administration of tigecycline for 4 days (total dose of 400 mg), including a single IV dose of [¹⁴C]tigecycline (50 mg, 50 μ Ci), excretion of unchanged tigecycline into feces was the primary route of elimination, and the secondary elimination

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pathways were renal excretion of unchanged drug, and metabolism to the glucuronide conjugates and the *N*-acetyl-9-aminomincycline metabolite. While unchanged drug was the predominant radiolabeled component in serum, urine and feces, three radiolabeled metabolites were detected by radiochromatography and a total of eight metabolites characterized by LC/MS.

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Footnotes.

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Legends for figures.

Figure 1. Structure of tigecycline. The asterisk (*) indicates the site of the ^{14}C label.

Figure 2. Mean (SD) for cumulative elimination of radioactivity in urine and feces following a single intravenous 50 mg dose of [^{14}C]tigecycline ($n=3$).

Figure 3. Mean (SD) tigecycline and radioactivity concentrations in serum over time.

Figure 4. Metabolic profiles of [^{14}C]tigecycline in 4 h serum (A), 0-4 h urine (B) and 24-48 h fecal homogenate (C) from a representative subject.

Figure 5. Tigecycline-related compounds detected in human serum (S), urine (U) and feces (F), the asterisk (*) indicates the site of the ^{14}C label.

Figure 6. Product ions of m/z 586 mass spectrum for tigecycline.

Figure 7. Product ions of m/z 555 mass spectrum for the tigecycline Fremy salt reaction product.

Figure 8. An Expanded Region of the HMBC Spectrum for Tigecycline Glucuronide (arrows indicate the important three-bond long range $^1\text{H}/^{13}\text{C}$ correlations)

TABLE 1

Elimination of radioactivity in urine and feces following a single intravenous 50 mg dose of [¹⁴ C]tigecycline							
Subject # ^a	1	2	3	5	6	Mean (n=5)	Mean (n=3) ^b
Urine	31.8	30.1	34.6	34.0	31.0	32.3 ± 1.9	33.2 ± 1.9
Feces	22.0	16.1	56.4	63.7	55.7	42.8 ± 22.0	58.6 ± 4.5
Total	53.7	46.2	90.9	97.7	86.7	75.0 ± 23.4	91.7 ± 5.5

^a Subject 4 withdrew from the study following the 24 h sample collection and recovery was not determined for this subject.

^b Values exclude data from subjects 1 and 2 because of incomplete sample collection due to non-compliance.

TABLE 2

Pharmacokinetic parameters of tigecycline or radioactivity in subjects receiving a single intravenous 50 mg dose of [¹⁴C]tigecycline ^a

Analyte	C _{max} (ng-eq/mL)	t _{1/2} (h)	AUC _T (ng-eq*h/mL)	AUC _{0-∞} (ng-eq*h/mL)	Cl (L/h/kg)	V _{SS} (L/kg)
Tigecycline in serum	940 (201) ^b	55.8 (7.3)	2394 (583) ^c	NA	0.29 (0.06)	21.0 (22.6)
Radioactivity in serum	839 (191)	6.9 (3.6)	1230 (452)	1330 (458)	0.54 (0.17)	3.3 (1.2)

^a Pharmacokinetics of tigecycline are based non-radioactive analysis and takes into account all tigecycline doses. Pharmacokinetics of radioactivity in serum based on radioactivity analysis and takes into account only the final [¹⁴C]tigecycline dose.

^b All values are expressed as arithmetic mean (SD).

^c AUC₀₋₁₂ for tigecycline in serum.

NA indicates not applicable.

TABLE 3

Relative percent distribution (and estimated concentrations in ng/mL) of [^{14}C]tigecycline and [^{14}C]tigecycline metabolites in human serum samples following intravenous administration of a single 50 mg dose of [^{14}C]tigecycline

Time (h)	Tigecycline-related component			
	<i>t</i> -Butylaminoacetic acid (M1)	Glucuronide Metabolites (M2 and M3)	Tigecycline Epimer ^a	Tigecycline ^a
1	9.8 ± 3.2	0.7 ± 1.2	8.3 ± 2.1	81 ± 23
	(14 ± 4.1)	(0.8 ± 1.3)	(12 ± 3.0)	(118 ± 33)
4	17 ± 4.5	12 ± 8.0	6.8 ± 3.7	64 ± 29
	(13 ± 5.5)	(8.4 ± 5.5)	(5.0 ± 2.7)	(47 ± 21)
8	19 ± 6.2	15 ± 10	5.4 ± 2.1	58 ± 28
	(8.2 ± 2.8)	(7.4 ± 5.8)	(2.3 ± 0.9)	(25 ± 12)

^a Corrected for the epimer formed during the extraction process.

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TABLE 4

N-Acetyl-9-aminomincycline metabolites in serum and urine following tigecycline administration ^a

Matrix and Collection Time (h) ^b	M4	M5	Tigecycline
Serum			
-2	ND	10.5 ± 3.9 ^c	155 ± 39.0
24	ND	7.4 ± 2.6	120 ± 18.2
48	ND	3.9 ± 1.3	77.1 ± 21.4
Urine			
0-4	12.7 ± 10.0 ^d	124 ± 112	4420 ± 715
4-8	19.6 ± 9.58	181 ± 93.7	2720 ± 1215
8-24	67.7 ± 29.9	555 ± 255	4910 ± 1540
24-48	59.3 ± 33.7	476 ± 297	2920 ± 1150
Total Urine (0-48)	159 ± 77.6	1340 ± 695	15000 ± 2980

^a For quantification of *N*-acetyl 9-aminomincycline (M5) and its epimer (M4), only samples from three subject were used.

^b Time is expressed relative to the final tigecycline dose.

^c Values for serum are mean ± SD of concentration in serum (ng/mL).

^d Values for urine are mean ± SD of amount of compound excreted in each urine collection (μg).

ND indicates metabolite was below the limit of detection (2.1 ng/mL).

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TABLE 5

Percent of total [¹⁴C]tigecycline dose excreted as tigecycline and tigecycline metabolites within
 48 h of dosing

Matrix and Collection Time (h)	Percentage of Dose			
	M1	Glucuronide Metabolites (M2 and M3) ^a	Tigecycline Epimer	Tigecycline
Urine				
0-4	1.4 ± 0.5	0.4 ± 0.2	0.5 ± 0.2	7.3 ± 0.8
4-8	1.2 ± 0.2	1.0 ± 0.3	0.3 ± 0.1	2.5 ± 1.1
8-24	2.4 ± 0.6	1.9 ± 0.5	0.6 ± 0.2	3.3 ± 1.0
24-48 ^b	1.6 ± 0.3	0.8 ± 0.4	0.6 ± 0.1	1.7 ± 0.7
Total Urine (0-48)	6.3 ± 0.9	4.1 ± 1.4	2.0 ± 0.3	14.8 ± 2.9
Feces (0-48)	1.5 ± 1.0	5.4 ± 4.2	2.3 ± 1.9 ^c	12.1 ± 8.4 ^c
Total (0-48)	7.8 ± 0.7	9.4 ± 5.1	4.3 ± 3.8	26.9 ± 9.3

^a M3, but not M2, was detected in urine.

^b For the 24-48 h urine, the total urine, the fecal samples and the total *n*=5, because of incomplete urine and fecal collections from one subject.

^c Corrected for the epimer formed during the extraction process.

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TABLE 6

Mass spectral data for tigecycline metabolites in humans

Metabolite	Ret. Time		Product Ions of [M+H] ⁺ Mass Spectral Data
	(min) ^a	[M+H] ⁺	
Tigecycline	44.8	586	569, 513, 456, 211, 154, 126, 86
Tigecycline epimer	42.6	586	569, 513, 456, 211, 154, 126, 86
M1	4.5	132	
M2	27.3	762	586, 569, 513, 456, 86
M3	30.4	762	586, 569, 551, 513, 456, 211, 154, 86
M4	33.9	515	498, 456 154, 126 58
M5	36.8	515	498, 456 154, 126 58
M6	35.4	473	456
M7	34.7	602	585, 529, 472, 211. 154, 86
M8	38.3	602	585, 529, 472, 86

^a Retention times were normalized to retention times of metabolites from serum extracts analyzed by radiochromatography.

Figure 1

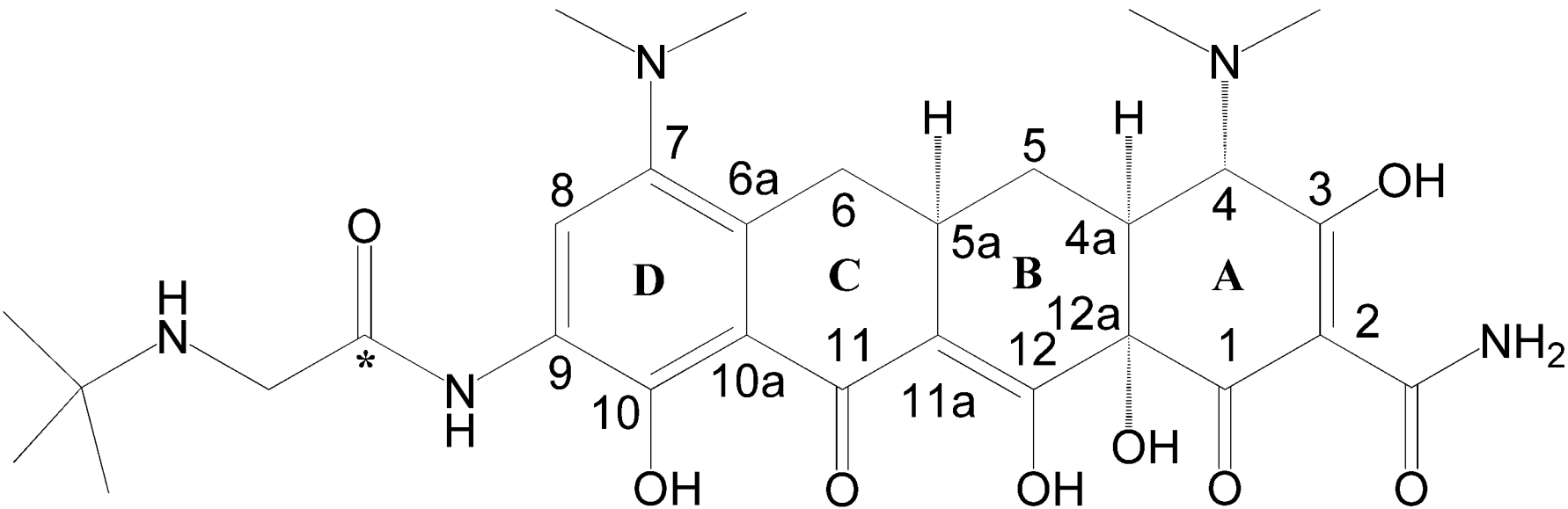


Figure 2

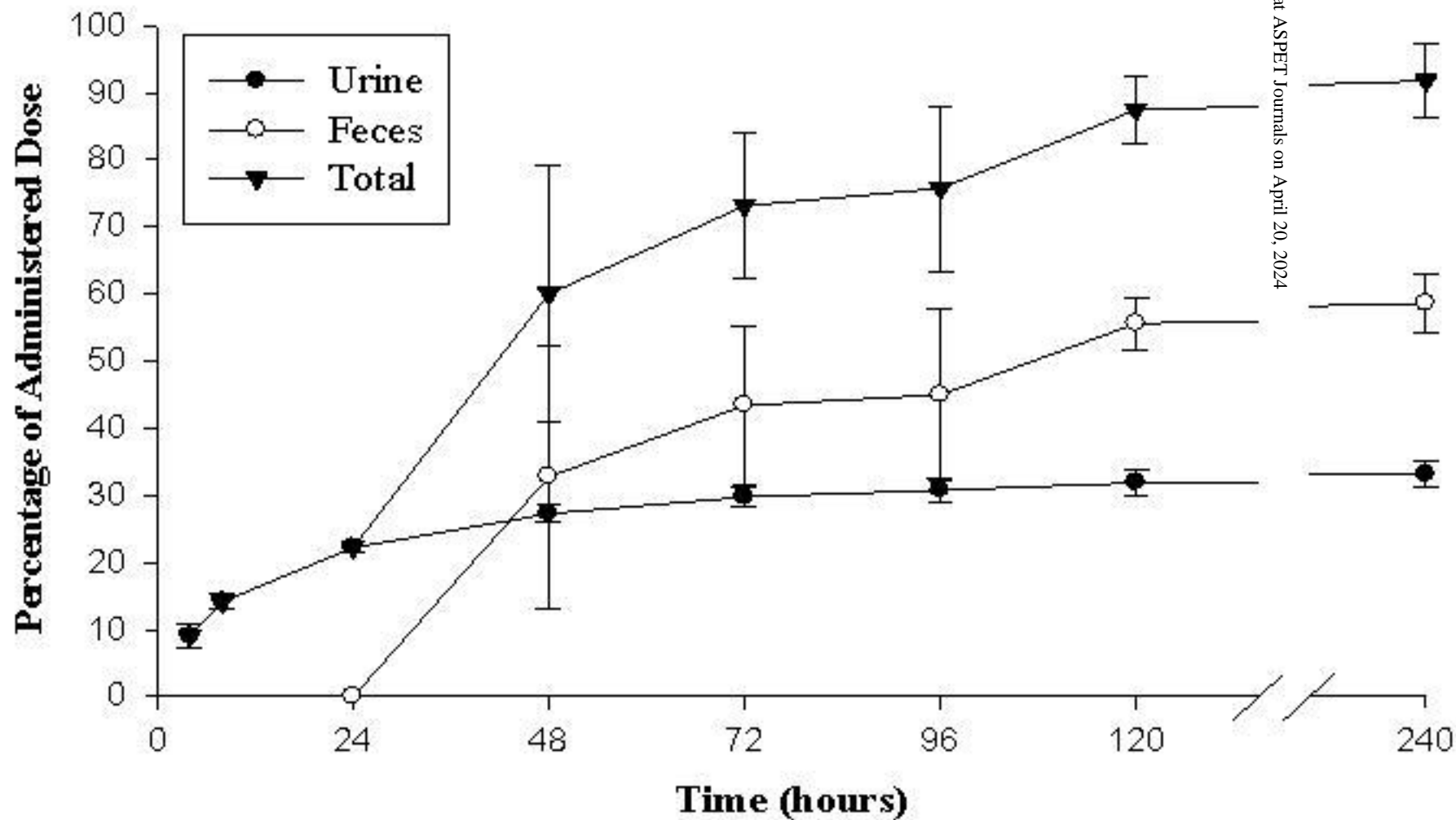


Figure 3

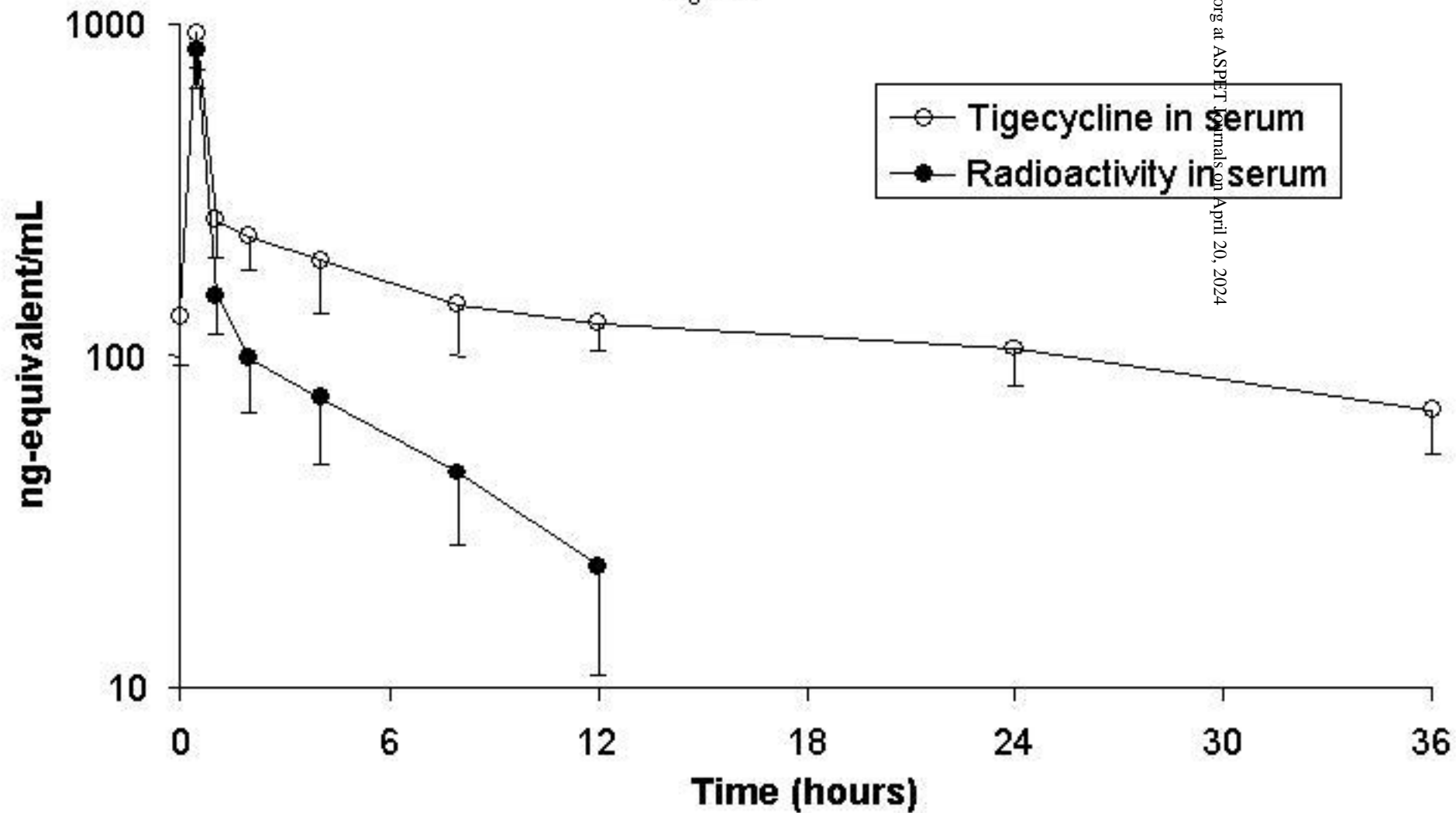


Figure 4

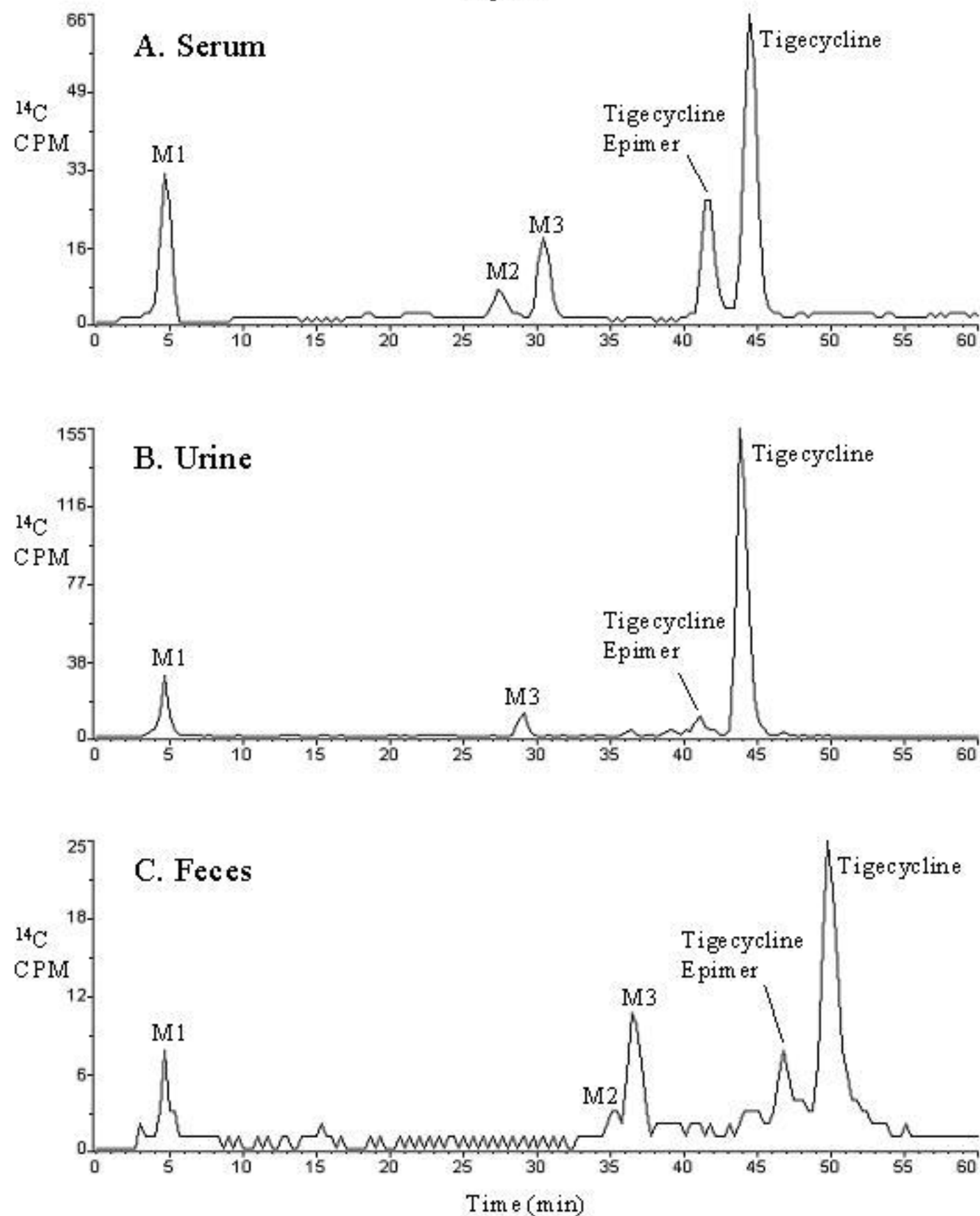
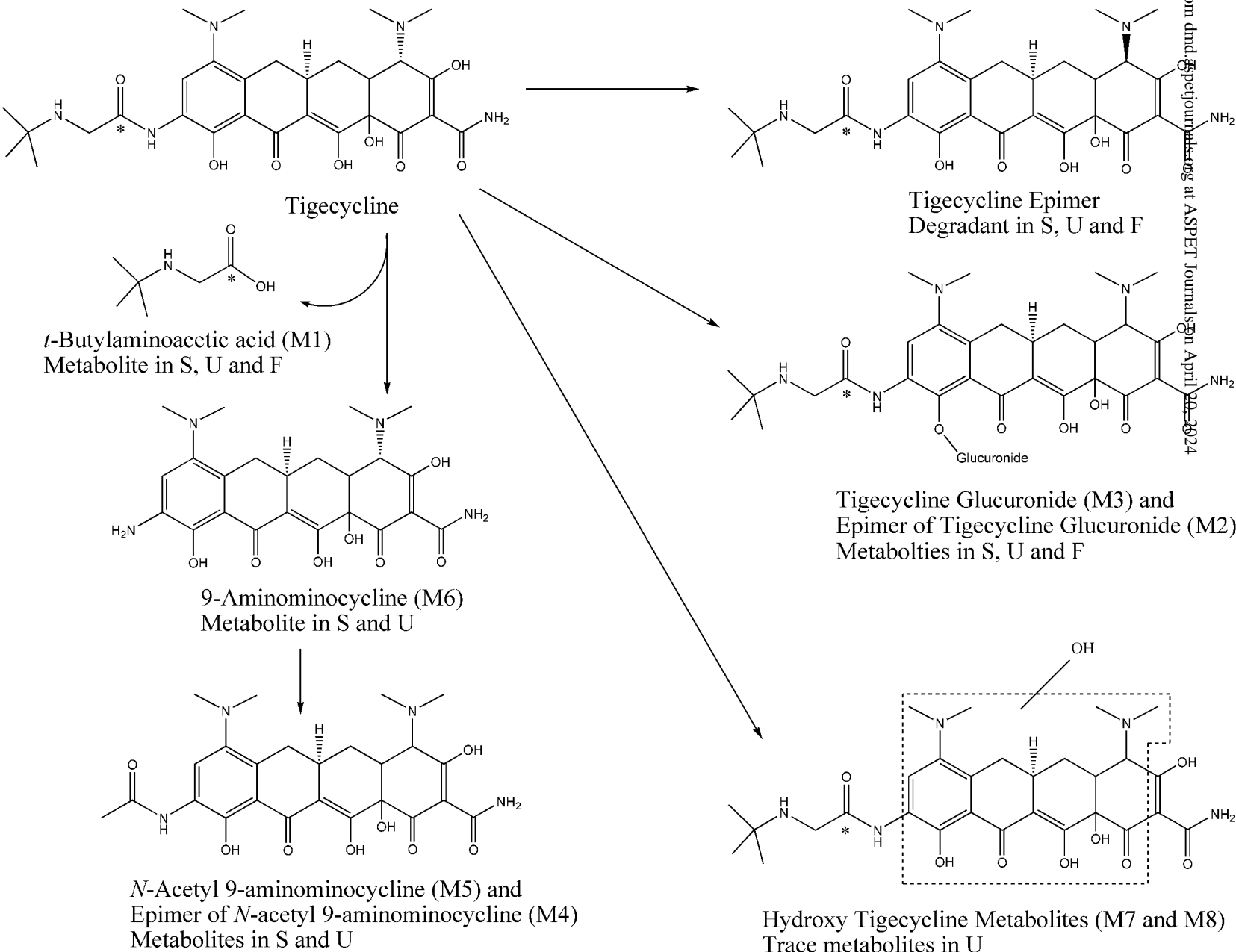


Figure 5



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Figure 6

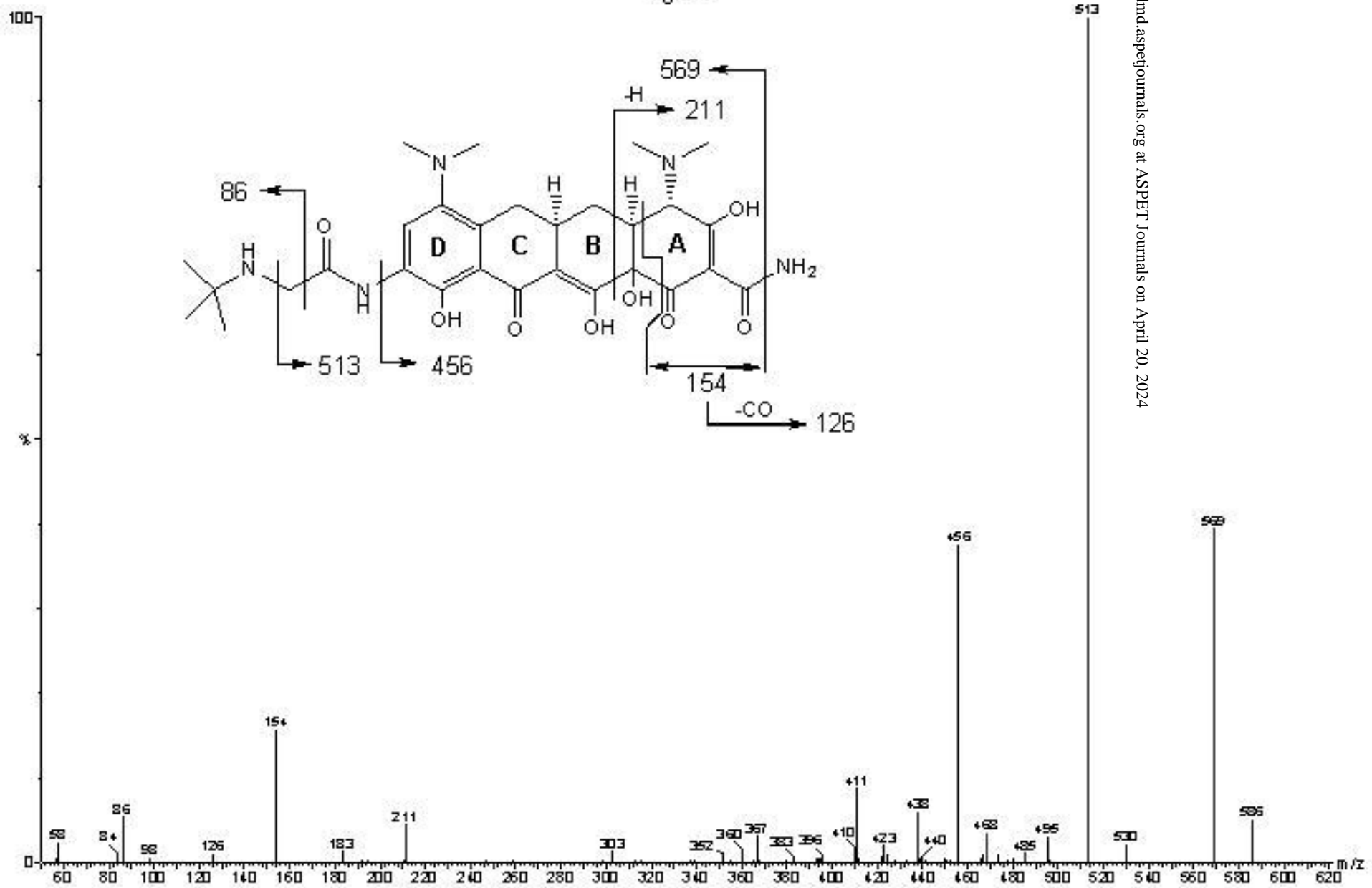


Figure 7

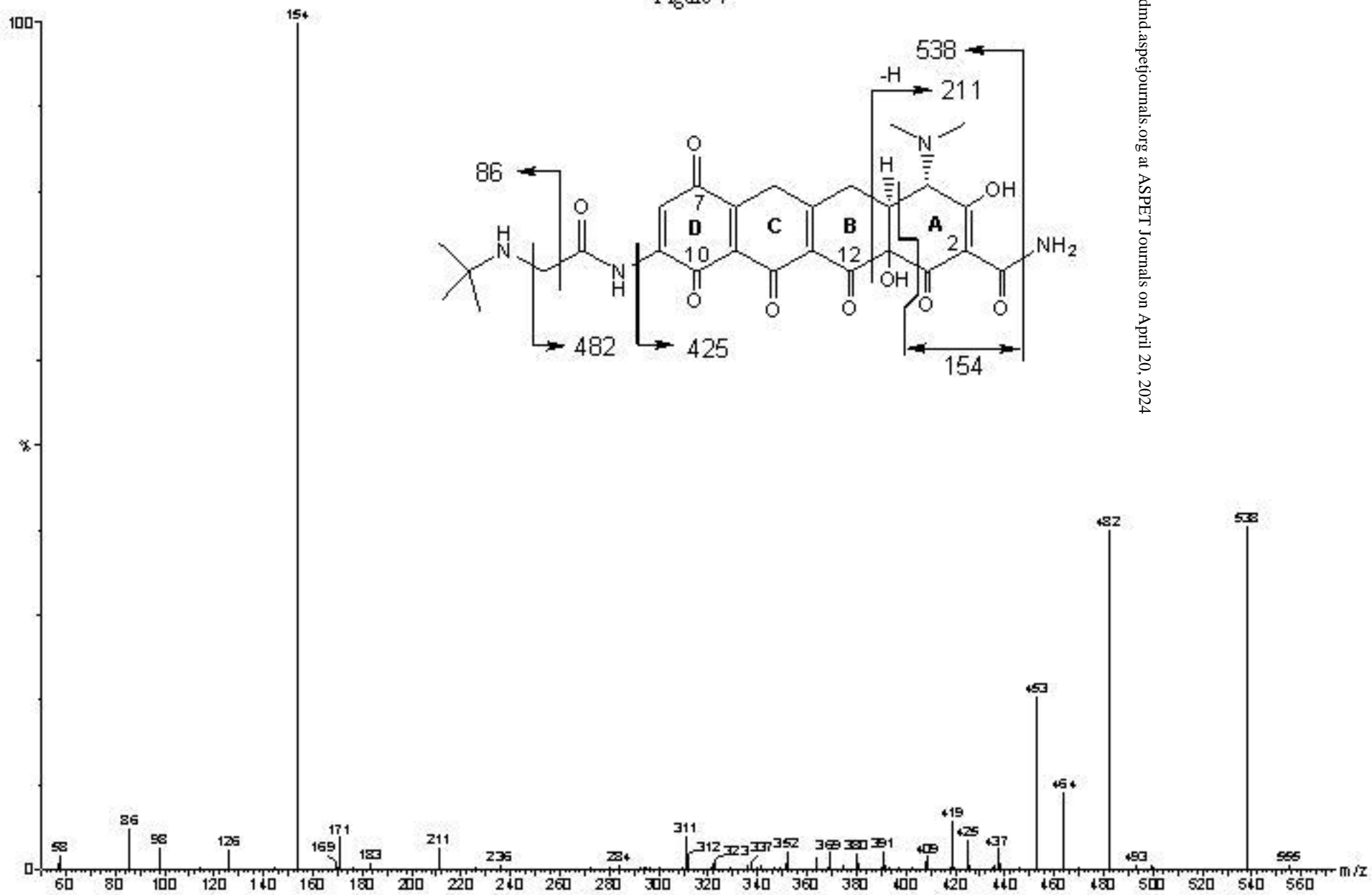
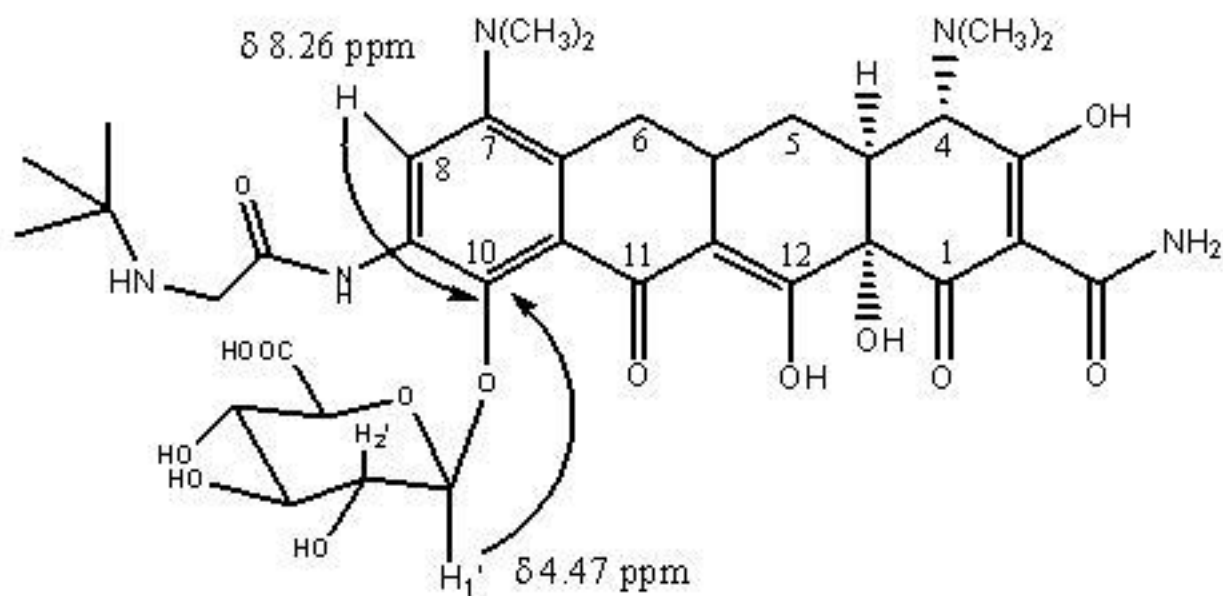


Figure 8



H8

H1'

