Synthesis and Characterization of Quaternary Ammonium-Linked Glucuronide Metabolites of Drugs with an Aliphatic Tertiary Amine Group

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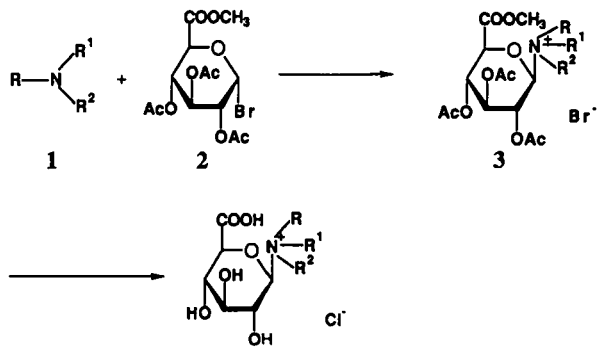
Abstract □ A synthetic approach was developed to make the quaternary ammonium-linked glucuronide metabolites of compounds with an aliphatic tertiary amine group. The key step involved quaternization of the compound with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate and sodium bicarbonate in a two-phase system of water and an organic solvent. The synthetic approach successfully yielded quaternary ammonium-linked glucuronides of 20 drugs and two of their phase I metabolites. The drugs were from various pharmacological classes: H_1 antihistamines, antipsychotic agents, and tricyclic antidepressants. Physical data such as HPLC retention times, and diagnostic fast-atom bombardment mass spectra and ^1H NMR spectra were obtained. These should aid in the characterization of compounds in samples isolated from biological media.



1 2 3 4

Scheme I

An aliphatic tertiary amine group is commonly present in the structures of many drugs from various pharmacological classes, including antiarrhythmic agents, H_1 antihistamines, antipsychotic agents, narcotic analgesics, and tricyclic antidepressants. However, a quaternary ammonium-linked glucuronide metabolite has been identified for only a few of the drugs in human urine. These drugs are amitriptyline and its hydroxylated metabolites,[1-3] chlorpromazine,[4] cyclobenzaprine,[5] cyproheptadine,[6-8] imipramine,[1] ketotifen,[9] and tripelennamine.[8,10] The slow development of research into the importance of the N^+-glucuronide metabolic pathway has been due mainly to unavailability of authentic synthetic reference standards and difficulties associated with the isolation and characterization of these polar and thermolabile metabolites. These two properties, coupled with the general low volatility of N^+-glucuronides, make them not readily amenable to identification by mass spectral (MS) analysis under conventional modes of ionization, such as electron impact and chemical ionization. The development of softer ionization techniques, such as fast-atom bombardment (FAB), has facilitated the study of N^+-glucuronides because the FAB mass spectra show molecular ions and other ions that are diagnostic of their structure.[1,4,8]

In a few cases, small quantities of reference standards have been isolated from human urine[2,3] or synthesized by a biochemical procedure.[1,4,8] To the best of our knowledge, however, there is no report on the chemical synthesis of N^+-glucuronide metabolites of drugs with aliphatic tertiary amines. The availability of a general synthetic procedure would greatly facilitate the identification of N^+-glucuronide metabolites in biological media. We developed such a general procedure for the organic synthesis of the quaternary ammonium-linked glucuronide metabolites of drugs with aliphatic tertiary amine (Scheme I, 4). The procedure was successfully applied to various drugs and their phase I metabolites (1) that were diverse in terms of both structure and pharmacological classification (Figure 1). The physical properties of the products were examined with regard to their usefulness in the confirmation of the identity of N^+-glucuronide metabolites isolated from biological extracts.

Experimental Section

Materials and Reagents—The following drugs were generously supplied as pure powders by the pharmaceutical company indicated in parentheses: azatadine maleate (Schering, Canada Inc., Pointe-Claire, Quebec, Canada), doxepin hydrochloride and *cis*-thiothixene hydrochloride (Pfizer, Inc., Groton, CT), fluphenazine dihydrochloride (Cord Laboratories, Broomfield, CO), loxapine succinate (Watson Laboratories, Libertyville, IL), mesoridazine besylate and sulforidazine (Sandoz Inc., East Hanover, NJ), thioridazine hydrochloride (Chelsea Laboratories Inc., Inwood, NY), and trifluoperazine dihydrochloride (Smith, Kline and French Laboratories, Philadelphia, PA). The following chemicals were purchased from the company shown in parentheses: chlorpheniramine maleate, cyclizine hydrochloride, diphenhydramine hydrochloride, doxylamine succinate, imipramine hydrochloride, pheniramine maleate, promethazine hydrochloride, pyrilamine maleate, tripelennamine hydrochloride, and XAD-2 ion-exchange resin (Sigma Chemical Company, St. Louis, MO); amitriptyline hydrochloride, chlorpromazine hydrochloride, and D-glucurono-6,3-lactone (Aldrich Chemical Company, Milwaukee, WI); and CD_3OD (MSD Isotopes, Montreal, Quebec, Canada). Chlorpromazine sulfoxide[11] and 7-tetrahydropyranyloxychlorpromazine[12,13] were synthesized in these laboratories according to literature procedures. All other reagents were purchased from either Aldrich or BDH Inc. (Toronto, Ontario, Canada). All drugs obtained as salts were converted to their respective free bases before use.

Instrumentation—Melting points were taken in open glass capillary tubes by using a Gallenkamp melting point apparatus and are reported uncorrected. The ^1H NMR spectra were taken on a Bruker AM-300 spectrometer in CD_3OD; chemical shifts were recorded in parts per million downfield from Me_4Si. Mass spectra were obtained in the positive-ion FAB mode, with argon as the source of fast atoms, at 7 kV and 1 mA current on a VG Micromass 7070HE instrument connected to a DEC PDP 11-250J data system. Either glycerol or 3-nitrobenzyl alcohol was used as sample matrix for MS analysis with a direct probe. The analysis by high-performance liquid chromatog-

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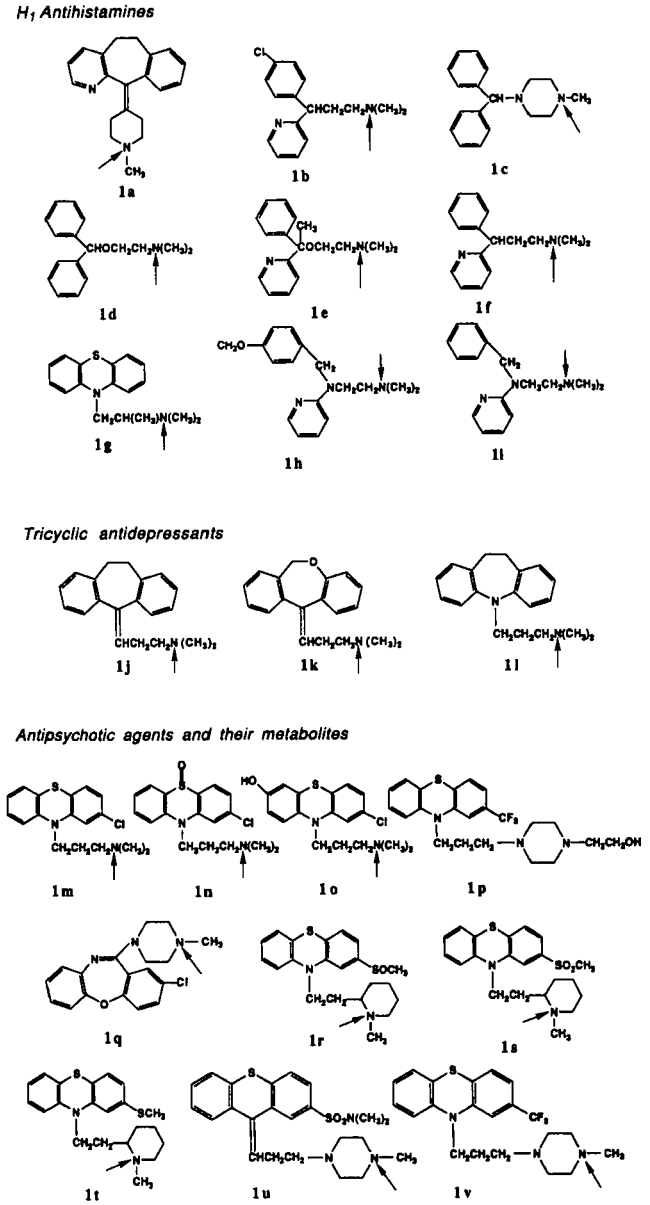


Figure 1—Structure, pharmacological classification, and site of N^+-glucuronidation (indicated by arrow) of the drugs and phase I metabolites used in the study. Key: (**1a**) azatadine; (**1b**) chlorpheniramine; (**1c**) cyclizine; (**1d**) diphenhydramine; (**1e**) doxylamine; (**1f**) pheniramine; (**1g**) promethazine; (**1h**) pyrilamine; (**1i**) tripelennamine; (**1j**) amitriptyline; (**1k**) doxepin; (**1l**) imipramine; (**1m**) chlorpromazine; (**1n**) chlorpromazine sulfoxide; (**1o**) 7-hydroxychlorpromazine; (**1p**) fluphenazine (site of quaternization not determined); (**1q**) loxapine; (**1r**) mesoridazine; (**1s**) sulforidazine; (**1t**) thoridazine; (**1u**) *cis*-thiothixene; (**1v**) trifluoperazine.

raphy (HPLC) was performed with a liquid chromatographic pump (Waters model M45) and a valve loop injector (Rheodyne model 7125) with a 200-μL sample loop connected to a variable-wavelength UV spectrophotometer (Waters model 480) set at a wavelength of 254 nm. The column (9.4 mm × 25 cm) was packed with 10-μm cyano packing (DuPont Instruments). The mobile phase (aqueous ethanol; 1:1, v/v) was deaerated before use by filtration (HVLP-type membrane filters, Millipore Canada Ltd.). The column was used at ambient temperature, with a flow rate of 3 mL/min. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer.

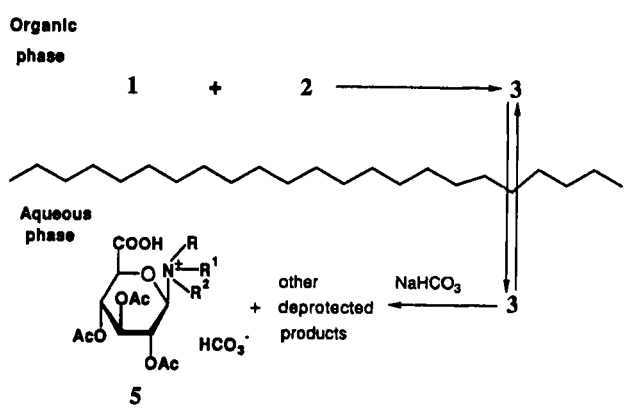
Synthetic reactions were monitored by thin-layer chromatography, with Whatman octadecylsilane reversed-phase plates and visualized with UV (254-nm) light. Lyophilization was performed with a Labconco Freeze Dryer-18.

Chlorpromazine N^+-Glucuronide Chloride (4m)—A solution containing 0.84 g (10 mmol) of $NaHCO_3$ in 20 mL of H_2O was added in one portion, with stirring in a nitrogen atmosphere, to a solution of **1m** (1.0 g, 3.1 mmol) and methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**2**; 1.0 g, 2.5 mmol) in 10 mL of benzene. (Compound **2** was prepared from D-glucurono-6,3-lactone.[14]) The resulting two-phase solution was stirred for 72 h at room temperature. However, after both 24 and 48 h, the aqueous phase was separated and replaced with a fresh solution of 0.84 g of $NaHCO_3$ in 20 mL of H_2O, and an additional 1.0 g of **2** was added. The benzene layer was discarded, and the combined aqueous layers were washed with diethyl ether (20 mL × 3) and then basified to pH 12.5 with 10% aqueous NaOH. After being washed again with diethyl ether (20 mL × 3), the aqueous solution was adjusted to pH 7.0 with 10% aqueous HCl, washed again with diethyl ether (20 mL × 3), and chromatographed on an ion-exchange column (XAD-2; 200 g). The XAD-2 ion-exchange resin was washed thoroughly prior to use by a previously described procedure.[15] The column was loaded and washed in turn with brine (200 mL), 0.1% aqueous HCl (100 mL), distilled H_2O (200 mL), and aqueous ethanol (7:3, v/v; 100 mL). Elution with aqueous ethanol (3:7, v/v) and collection and evaporation of the appropriate fraction gave a residue that was recrystallized from ethanol to give **4m** (0.71 g, 46%) as a white solid; ^{1}H NMR (300 MHz, CD_3OD): δ 7.32–6.92 (m, 7H, phenothiazine ring H), 4.58 (d, J = 8.8 Hz, 1H, C_1-H), 3.98 (t, J = 6.6 Hz, 2H, C_1-H_2), 3.81–3.66 (m, 3H, C_2-H, C_4-H, C_5-H), 3.51–3.38 (m, 3H, C_3-H_2, C_3-H), 3.11 and 3.13 [two s, 6H, $N(CH_3)_2$-], and 2.22 (m, 2H, C_2-H_2).

With only minor modifications, the method was used to prepare the other N^+-glucuronide metabolites of the previously mentioned drugs and two of their phase I metabolites. Quaternization occurred at the sites shown in Figure 1. The reaction conditions with respect to the organic solvent and the reaction time, the overall yield from **1**, and the physical data for each product **4** are given in Table I.

Isolation and Characterization of Intermediates (3m and 5m) in Synthesis of Chlorpromazine N^+-glucuronide (4m)—Upon completion of the reaction described for **4m**, a portion of the benzene layer was taken and directly injected into the HPLC system. The fraction of mobile phase with a retention time of 10.4 min was collected and lyophilized to dryness to give the triacetyl methyl ester derivative of chlorpromazine N^+-glucuronide (**3m**); FAB-MS: *m/z* (relative intensity, %) 635/637 (M^+, 5/3), 601 (3), 319/321 (11/3), 285 (8).

At the completion of the reaction described for **3m**, the combined aqueous layers were washed with diethyl ether (20 mL × 3) and then chromatographed on an ion-exchange (XAD-2) column (200 g). The column was washed with distilled water (200 mL), aqueous ethanol (7:3, v/v; 100 mL), and aqueous ethanol (1:1, v/v; 50 mL). The desired compound was eluted with absolute ethanol (200 mL), and the collected fraction was lyophilized to give the triacetyl derivative of chlorpromazine N^+-glucuronide (**5m**; Scheme II); ^{1}H NMR (300 MHz, CD_3OD): δ 7.31–6.94 (m, 7H, phenothiazine ring H), 5.51 (t, J = 8.1 Hz, 1H, C_3-H), 5.29 (t, J = 8.2 Hz, 1H, C_4-H), 5.24 (t, J = 8.8 Hz, 1H, C_2-H), 5.18 (d, J = 8.7 Hz, 1H, C_1-H), 4.01 (d, J = 8.3 Hz, 1H, C_5-H), 4.07–3.97 (m, 2H, C_1-H_2), 3.81–3.71 (m, 2H, C_3-H_2), 3.06 and 3.10 [two s, 6H, $N(CH_3)_2$], 2.28–2.20 (m, 2H, C_2-H_2), and 2.03–1.98 (three s, 9H, $COCH_3$); FAB-MS: *m/z* (relative intensity, %) 621/623 (M^+, 4/2), 587



Scheme II

Table I—Synthesis and Characterization of Quaternary Ammonium-Linked Glucuronides of Various Drugs or Their Phase I Metabolites

Drug or Phase I Metabolite	Reaction Conditions		Overall Yield, %	mp, °C[a]	Retention Time, Min	Formula of N^+-Glucuronide Salt[b]	Mass Spectral Data[c]				NMR Data for Anomeric Proton[d]	
	Organic Solvent	Reaction Time, h					M^+		$[XH]^+$		δ	J, Hz
1a	C_6H_6	96	16	141–145	6.2	—[e]	467	(9)	291	(26)	—	—
1b	$CHCl_3$	96	15	104–109	5.9	$C_{22}H_{28}Cl_2N_2O_6$	451/453	(5/2)	275/277	(5/1)	4.71	8.8
1c	$CHCl_3$	72	21	120–123	5.8	$C_{24}H_{31}ClN_2O_6$	443	(26)	267	(5)	4.83	8.9
1d	C_6H_6	72	32	102–103	5.9	$C_{23}H_{30}ClNO_7$	432	(25)	256	(12)	4.58	7.6
1e	C_6H_6	144	4	143–148	5.7	$C_{23}H_{31}ClN_2O_7$	447	(24)	271	(16)	4.77	9.0
1f	C_6H_6	144	13	105–110	5.7	$C_{22}H_{29}ClN_2O_6$	417	(6)	241	(29)	4.69	8.8
1g	C_6H_6	96	18	109–112	6.0	$C_{23}H_{29}ClN_2O_6S$	461	(18)	285	(18)	4.86	7.6
1h	$CHCl_3$	200	2	—	5.7	—	462	(1)	286	(10)	4.73	9.6
1i	$CHCl_3$	120	16	89–93	5.7	$C_{22}H_{30}ClN_3O_6$	432	(6)	256	(2)	4.73	8.8
1j	C_6H_6	72	36	119–122	6.4	$C_{26}H_{32}ClNO_6$	454	(16)	278	(8)	4.29	8.4
1k	C_6H_6	72	51	140–142	6.1	$C_{25}H_{30}ClNO_7$	456	(15)	280	(18)	4.44	6.6
1l	C_6H_6	72	55	115–117	5.9	$C_{25}H_{33}ClN_2O_6$	457	(19)	281	(6)	4.34	8.9
1m	C_6H_6	72	46	117–118	6.2	$C_{23}H_{28}Cl_2N_2O_6S$	495/497	(100/43)	319/321	(98/39)	4.58	8.8
1n	$CHCl_3$	96	6	129–131	5.9	$C_{23}H_{28}Cl_2N_2O_7S$	511/513	(2/1)	335/337	(9/4)	4.44	8.6
1o	CH_3CN	120	10	126–130	5.8	$C_{23}H_{28}Cl_2N_2O_7S$	511/513	(5/2)	335/337	(19/7)	4.59	8.8
1p	CH_3CN	144	5	99–101	6.1	$C_{28}H_{35}ClF_3N_3O_7S$	614	(1)	438	(20)	4.95	7.3
1q	$CHCl_3$	120	3	126–130	6.1	$C_{24}H_{27}Cl_2N_3O_7$	504/506	(4/2)	328/330	(21/8)	5.00	8.8
1r	C_6H_6	96	18	110–112	6.6	$C_{27}H_{35}ClN_2O_7S_2$	563	(5)	387	(29)	4.51	8.6
1s	C_6H_6	96	22	101–105	6.5	$C_{27}H_{35}ClN_2O_8S_2$	579	(10)	403	(28)	4.62	8.0
1t	C_6H_6	96	8	112–114	6.8	$C_{27}H_{35}ClN_2O_6S_2$	547	(16)	371	(24)	4.79	8.9
1u	$CHCl_3$	144	3	111–114	6.3	$C_{29}H_{38}ClN_3O_8S_2$	620	(18)	444	(44)	4.79	8.8
1v	C_6H_6	72	25	103–105	6.2	$C_{27}H_{33}ClF_3N_3O_6S$	584	(10)	408	(13)	4.72	8.7

[a] Melting occurred over the recorded range, but shrinking was observed over an ~10 °C range lower than this. A solid product was not obtained for **1h**. [b] N^+-Glucuronide salts with formula listed were analyzed for C, H, and N, and values obtained were within 0.5% of the calculated values. [c] All samples were run in the positive-ion FAB mode; M^+ and $[XH]^+$ refer to molecular cation (i.e., molecular formula minus chlorine) and pseudomolecular ion of the aglycone, respectively. Ions differing by two mass units were observed when chlorine (^{35}Cl and ^{37}Cl isotopes) was present in M^+ and $[XH]^+$ ions. Results show fragments (m/z) with relative abundances (%) in parentheses. [d] ^1H NMR assignments given for the anomeric proton; in the case of **1a**, an adequate amount of sample was not available. [e] —, Not determined.

(2), 319/321 (9/4), 285 (6).

Results and Discussion

We intended to synthesize the quaternary ammonium-linked glucuronide metabolites 4 by treatment of the drug or phase I metabolite containing an aliphatic tertiary amine group (compounds 1) with the quaternization reagent 2 followed by subsequent removal of the protecting groups from the resultant quaternary ammonium salt 3 by alkaline hydrolysis (Scheme I). In early work, however, when the quaternization reaction was carried out in an organic solvent, such as dichloromethane, and irrespective of whether or not a base catalyst (e.g., NaOH or $NaHCO_3$) was used, very low yields of the desired products were obtained. For example, for the phenothiazine antipsychotic agent **1m**, despite treatment for as long as 20 days with 2 in dichloromethane as solvent, the yield of **4m** was invariably <1%. Because it was likely that traces of **3m** once formed inhibited further reaction in the organic phase, synthetic approach to the desired compounds was developed in which the use of a two-phase system enabled removal of newly formed **3m** from the organic phase. The approach is hypothesized to proceed as depicted in Scheme II. Thus, briefly, the lipophilic reactants 1 and 2, dissolved in a suitable organic solvent, give the quaternary ammonium salt 3, which readily transfers to the aqueous solution of $NaHCO_3$ (pH 8.4). It is likely that partial hydrolysis of the protecting groups of 3, especially of the methoxycarbonyl group, occurs in the alkalinized aqueous medium; the resultant polar products are thereby trapped in the aqueous phase. To facilitate further the quaternization reaction, the aqueous layer was replaced with a fresh solution of $NaHCO_3$ and another portion of 2 was added every 24 h.

This hypothesis was substantiated by the isolation and characterization of the major product in each of the two phases of the reaction mixture. Thus, for the reaction that involved **1m**, FAB-MS analysis established that **3m** was the major product in the organic phase and **5m** was the major product in the aqueous phase.

Finally, the complete removal of the protecting groups was accomplished by NaOH treatment of the aqueous phase. Thus, isolation of the intermediates, such as 3 and 5, was not necessary. After NaOH treatment, subsequent purification (including chromatography on an XAD-2 column) enabled isolation of 4, which was characterized for each of the 22 aliphatic tertiary amines 1 examined. All but two of the products were analyzed for carbon, hydrogen, and nitrogen, and in each case, the data were consistent with the concept that compounds 4 were isolated as the chloride salts. The overall yield was low in many cases; for example, with 7 of the 22 compounds 1 examined, the overall yield of 4 was 2–10%. However, no attempt was made to optimize yields, except for **1m**. It was also possible, by using these general conditions, to recover a high proportion of the unreacted starting material. For example, for the synthesis of **4m**, 80% was accounted for either as the desired end product (46%) or **1m** (34%).

The pH of the aqueous layer of the two-phase system influenced both the yield and the purity of the desired products. Studies with **1m** in which the pH of the aqueous layer was varied (H_2O, pH 7.0; aqueous $NaHCO_3$ solution, pH 7.5, 8.0, 8.2, and 8.4; aqueous $NaHCO_3$–Na_2CO_3 solution, pH 9.0; and aqueous Na_2CO_3 solution, pH 10.0) demonstrated that the amount of **4m** product (and of water-soluble impurities) increased with an increase in pH. Thus, at pH values above 8.4, **4m** could not be isolated in pure form. Consequently, most syntheses were carried out with an aqueous

solution of $NaHCO_3$ at pH 8.4. However, for the quaternization reactions with **1h** and **1p**, only trace quantities of the desired products were obtained when the aqueous phase was at pH 8.4. In these cases, use of an aqueous solution at pH 9.0 resulted in significant improvement in the yields of the desired products **4h** and **4p**, which proved to be the most difficult of all the products to purify.

Some preliminary observations about the structural features important in the use of 2 as a quaternization reagent can be made in view of the diverse structural features of the amines 1 examined in the present work. First, even though seven of the drugs (**1a**, **1b**, **1e**, **1f**, **1h**, **1i**, and **1q**) contained both aliphatic and heteroaromatic tertiary amine functional groups, in no case was quaternization found to occur with the involvement of the heteroaromatic amine group, which is a less basic nitrogen center. Moreover, with each of these seven drugs with two ionizable groups, the overall yield (not optimized) of the isolated products was <20%. The low yields of these products can be explained in part on the basis that the 7:3 (v/v) aqueous ethanol used in the washing procedure resulted in greater loss of the product from the reversed-phase column. Consequently, in such cases we now use 9:1 (v/v) aqueous ethanol in the washing step to minimize the loss of product.

Steric factors also are important in the quaternization reaction, as indicated by the reaction of the two ethylenediamine compounds (**1h** and **1i**). For these compounds, quaternization occurred at the nitrogen atom with the dimethyl substituents rather than at the nitrogen atom with the bulky benzyl and 2-pyridyl substituents. Similarly, for the four compounds with a piperazine ring in the structure (**1c**, **1q**, **1u**, and **1v**), the resultant N^+–C linkage with the glucuronic acid moiety was at the piperazine nitrogen with a simple methyl substituent rather than the one with the bulky cyclic substituent.

The results demonstrate that N^+-glucuronides could be synthesized from a diverse range of drugs with aliphatic tertiary amine groups. The quaternized nitrogen is either part of piperidine (**4a**, **4r**, **4s**, and **4t**) and piperazine (**4c**, **4p**, **4q**, **4u**, and **4v**) rings or exocyclic (other cases). In addition, the synthetic approach can be applied even when other functional groups are present, such as the alcohol group of **1p**. However, the low yield of **4p** might be improved by use of an alcohol-protecting group, such as acetyl. In fact, for **1o**, the quaternization reaction was performed with the phenolic substituent protected by a tetrahydropyranyloxy group, which was subsequently removed by the aqueous HCl present during the regular work up procedure of the product. Therefore, the present synthetic approach may be applied to most parent drugs and their phase I metabolites with aliphatic tertiary amine groups.

The physical data for compounds 4 are given in Table I. The influence of the cationic charge on the partition of the molecule in the HPLC system was indicated by the fact that, despite diverse structural differences among the various aglycones, the HPLC retention times of all compounds 4 examined fell in the narrow range of 5.7–6.8 min. Thus, in our experience, the availability of any one of the synthetic samples of 4 greatly facilitates the isolation by HPLC of an unknown N^+-glucuronide from a biological sample.

The key role of FAB-MS in the identification of N^+-glucuronide metabolites is well recognized.[1,4,8] The characteristic ions include the molecular cation of the metabolite and the protonated aglycone that is formed from the cleavage of the glycosidic bond with transfer of a proton from the glucuronic acid moiety to the aglycone. For the 22 metabolites studied under the conditions examined in the present work, the relative intensities of these ions varied from 1–100% and 2–98%, respectively. In early work, the presence of ions in the FAB-MS spectra such as $[M - H + Na]^+$ and $[aglycone + Na]^+$ indicated that alkali metal contaminants were present in the examined samples so that, in part, the glucuronide moiety was present as the salts of such metals. Consequently, the purification procedure was modified to include sequential washing of the crude product adsorbed on a reversed-phase column with brine, 0.1% aqueous HCl, and H_2O so that the products were obtained as the chloride salts 4, as indicated by elemental analysis.

The value of definitive structural and stereochemical information from ^1H NMR spectra of organic molecules is very well established. However, ^1H NMR spectra for only a few N^+-glucuronides of drugs and phase I metabolites with aliphatic tertiary amine groups have been alluded to in the literature.[3,6,10] For all 22 compounds 4 examined in the present work, a doublet at δ 4.29–5.00 (J = 6.6–9.6 Hz) was seen on the NMR spectrum and was assigned to the anomeric proton (C_1–*H*) of the glucuronic acid moiety. Because coupling constants for α- and β-anomers of various types of glucuronides are reportedly 2–4 and 7–10 Hz, respectively,[16] the experimentally determined coupling constants indicate that 4 has a β-anomeric configuration.

The ^1H NMR spectra also were vital in establishing the site of N^+-glucuronidation for compounds in which the aglycone has more than one tertiary amine group in the molecule. First, the site of quaternization in 4 could be readily detected because, in comparison with the free base of 1, the protons vicinal to the site of quaternization are anticipated to be greatly shifted downfield. Furthermore, whereas two identical (either CH_3 or CH_2) and similarly substituted alkyl groups attached to the nitrogen atom in question have the same chemical shift in the free base of 1, this was not the case in the ^1H NMR spectrum of the analogous 4, because these alkyl groups can have two possible orientations about the quaternary nitrogen atom. Therefore, as noted in previous publications,[3,10] these groups appeared as two singlets instead of one in the ^1H NMR spectrum of the N^+-glucuronide metabolite. Consequently, by interpretation of ^1H NMR spectra, we established that the preferred site of quaternization is at an aliphatic tertiary amine group rather than at a heteroaromatic tertiary amine group in the same molecule (**4a**, **4b**, **4e**,

Table II—Chemical Shifts (ppm) for Protons Vicinal to Quaternization Site of Various N^+-Glucuronides: Comparison with Chemical Shifts for Analogous Protons of the Free Base and Salt of the Aliphatic Tertiary Amine

N^+-Glucuronide	Signal 1				Signal 2				Signal 3			
	Protons	Chemical Shift for:			Protons	Chemical Shift for:			Protons	Chemical Shift for:		
		4	1	1 Salt		4	1	1 Salt		4	1	1 Salt
4b	NCH_2	3.45	2.40	3.06	NCH_3	3.20	2.18	2.88	NCH_3	3.19	—	—
4c	NCH_3	3.17	2.22	2.88	NCH_2	2.83	2.43	2.99	NCH_2	2.66	—	2.32
4l	NCH_2	3.43	2.53	3.38	NCH_3	3.14	2.26	2.99	NCH_3	3.09	—	—
4q	NCH_3	3.33	2.46	3.52	NCH_2	3.58	2.46	3.52	NCH_2	3.47	—	3.40
4v	NCH_3	3.14	2.20	2.98	NCH_2	2.84	2.38	3.63	NCH_2	2.69	—	3.37

4f, 4h, 4i, and 4q) and that quaternization with an ethylenediamine group (4h and 4i) or a piperazine ring (4c, 4q, 4u, and 4v) occurs at the nitrogen atom of the aliphatic tertiary amine with the smaller substituent(s). To illustrate how such interpretation of ^{1}H NMR spectra was used to assign the site of quaternization when such choices were present, representative examples of each type are presented in Table II. Chemical shifts of the protons vicinal to the site of quaternization are given for 4b, 4c, 4i, 4q, and 4v and their analogous aglycone (free base and salt). However, in the case of the piperazine-ring-containing compound 4p, the ^{1}H NMR spectra of the N^+-glucuronide and its aglycone could not be adequately interpreted.

For doxepin, which has Z and E isomers, the ^{1}H NMR spectra of the starting material (1k) and product (4k) were examined to determine stereoselectivity in the quaternization reaction. Comparison of the integrals of the exocyclic alkene proton in 1k (Z: δ 5.65, t, J = 7.2 Hz; E: 5.98, t, J = 7.4 Hz) showed that the Z/E ratio was 14.4:85.6, values consistent with USP specifications (13.6–18.1% for Z and 81.4–88.2% for E[17]). However, a similar comparison for the product 4k (Z: 5.64, t, J = 7.4 Hz; E: 5.94, t, J = 7.4 Hz) gave a Z/E ratio of 25.6:74.4. The fact that the similarly calculated Z/E ratio of the intermediate 5k was 26.7:73.3 proved that the isolation and purification procedures were nonstereoselective. Therefore, there is stereoselectivity in favor of the Z isomer in the quaternization of 1 with 2.

The availability of synthetic samples of metabolites aids in the determination of the biological importance of the metabolic pathway involved. In previous work with seven drugs known to form N^+-glucuronides, it was established that such a metabolite was a major metabolite in humans for amitriptyline (1j) and its hydroxylated metabolites,[1–3] cyclobenzaprine,[5] cyproheptadine,[6,7] ketotifen,[9] and tripelennamine (1i).[10] In the present work, samples of 4i and 4j were synthesized. The availability of the synthetic samples of 4b–4i enabled us to identify each of these eight N^+-glucuronide metabolites of H_1 antihistamine drugs in the urine of healthy volunteers (n = 2) who were given small oral doses of the appropriate 1.[18] Furthermore, 4k was unequivocally identified as a metabolite of 1k in the urine of two patients under chronic oral treatment, and N^+-glucuronidation was found to be nonstereoselective for this drug, which is marketed as a mixture of geometrical isomers.[19] HPLC assays were developed for the quantitation of 4b–4i and 4k in urine, and it was shown that, in one or more of the volunteers examined, at least 5% of the administered dose of 4c, 4d, 4i, and 4k was excreted as the N^+-glucuronide metabolite.[18,19]

Conclusions

A simple approach was developed for the synthesis of the quaternary ammonium-linked glucuronide metabolites of drugs and the phase I metabolites with an aliphatic tertiary amine group. This approach yields N^+-glucuronides of structurally diverse compounds including alcohols, alkenes, ethers, heteroaromatic tertiary amines, sulfides, sulfones, and sulfoxides. Also, the method is applicable to phenols and primary or secondary amines, provided that the reactive hydroxyl and amino groups are protected during the quaternization reaction.

FAB-MS and ^{1}H NMR spectra for the 22 N^+-glucuronide metabolites synthesized were generated. These spectra should aid in the identification of such metabolites found in biological samples obtained in studies of the metabolism of the appropriate drug with an aliphatic tertiary amine group.

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