1,3,5-Trialkyl-2,4,6-triiodobenzenes: Novel X-ray Contrast Agents for Gastrointestinal Imaging[1]

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Examination of the gastrointestinal (GI) tract has been performed for decades using barium sulfate. Although this agent has many recognized limitations including extreme radiopacity, poor intrinsic affinity for the GI mucosa, and very high density, no alternative contrast agents have emerged which produce comparable or better contrast visualization. In fact, the various techniques of the GI radiologic examination (i.e., single contrast, double contrast, biphasic) were developed to compensate for its limitations. Each of these techniques requires complex patient manipulation to achieve adequate mucosal coating or compression to overcome the marked radiopacity of barium sulfate in order to obtain a diagnostically useful examination. A series of novel radiopaque oils, the 1,3,5-trialkyl-2,4,6-triiodobenzenes, was designed to improve the efficacy, stability, and safety of barium formulations. These substances were prepared in two steps from 1,3,5-trichlorobenzene. Compound **17** (1,3,5-tri-*n*-hexyl-2,4,6-triiodobenzene), formulated as an oil-in-water emulsion, was found to be well-tolerated in rodents (mice, hamsters, rats) following acute oral and/or intraperitoneal administrations at 4 times the anticipated human clinical dose. No metabolism of **17** was detected in rat, hamster, dog, monkey, or human hepatic microsomes, suggesting the lack of oral toxicity was a consequence of poor absorption. In imaging experiments in dogs, emulsions of **17** have demonstrated excellent mucosal coating and improved radiodensity relative to barium sulfate suspensions. On the basis of the preliminary imaging and toxicity data, compound **17** was selected as a potential development candidate.

Introduction

X-ray visualization of the gastrointestinal (GI) tract requires the use of a radiopaque contrast agent, as the differences in radiodensity between the bowel and surrounding soft tissues are too small to allow sufficient delineation. Suspensions of barium sulfate have been used for radiographic imaging of the GI tract since 1910 and have remained the most widely used radiocontrast media to detect pathologic conditions such as ulcers, inflammatory bowel disease, colonic polyps, and gastric cancer.[7] Despite the wide-spread use of barium sulfate for both upper and lower GI examinations, this agent has many recognized limitations. Its marked radiodensity and poor intrinsic affinity for the GI mucosa lead to inaccurate diagnoses resulting from poor coating of the contrast agent in the GI tract. Since the degree of opacification produced by a barium preparation is directly proportional to the density of the medium and the thickness of the coating, in certain GI examinations requiring a high-density barium preparation, the extremely radiopaque contrast medium may not permit X-ray penetration of the intestinal lumen, thereby interfering with the ability to discriminate lesions.[8] Suspensions of barium sulfate settle quickly due their high density, and the limited stability of the suspension in vivo gives rise to pooling and dispersion of the formulation, which also interferes with diagnostic accuracy. In fact, a number of techniques (single contrast, double contrast, etc.) of the radiologic GI exam were developed around the limitations of barium sulfate. Each technique required manipulation of the patient to achieve adequate mucosal coating and compensate for the high radiopacity of barium sulfate. Moreover, barium sulfate is highly toxic in the peritoneal cavity and is therefore contraindicated in patients with suspected bowel perforations or when postimaging surgery or CT examination is anticipated.[9] In such cases, soluble iodinated contrast media are the preferred agents.

Water-soluble iodinated organic compounds have been used extensively as contrast media due to the ability of iodine to attenuate X-rays.[10] None of these agents has been routinely applied to GI imaging. Aqueous solutions of water-soluble iodinated contrast agents have been used for opacification of the GI tract when barium is contraindicated and under certain conditions offer advantages over barium sulfate.[11] However, the hyperosmolarity of these parenteral formulations gives rise to a new set of complications regarding efficacy and safety. Further issues with respect to poor palatability, hypertonicity, low radiodensity made poorer by osmotic dilution, and poor intrinsic coating of the GI mucosa have prevented their general acceptance as viable alternatives to barium for routine GI examinations.

Emulsions of water-insoluble iodinated organic oils have also been studied in GI radiology, although no commercially viable agent has yet been identified. In 1964 Teplich and co-workers reported that an oil-in-

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water emulsion of Ethiodol, an iodinated poppy seed oil, produced mucosal coating of the small bowel superior to either barium sulfate or water-soluble contrast media.[12] However, between 50% and 70% of the iodinated component was absorbed from the GI tract of dogs, raising concerns regarding systemic exposure to large amounts of this substance. Oil-in-water emulsions of iophendylate (**1**), a radiopaque oil which was developed in the 1940s for myelography,[13] have also been studied by Pirkey and co-workers as contrast media for GI imaging.[14] However, they observed no advantage over conventional contrast agents. More recently, Rubin reported that emulsions of **1** gave small bowel images superior to that of conventional barium.[15] Although no adverse effects were observed following repeated oral administration of iophendylate formulations to dogs,[16] this emulsion was highly toxic to mice after acute oral dosing (vide infra).

As part of our research in novel X-ray contrast agents, we found the consistency and overall image quality obtained with emulsions of these iodinated oils were impressive and reproducible, and we sought to develop an iodinated organic molecule demonstrating acceptable acute safety, which when formulated as an oil-in-water emulsion would reproduce the continuous, uniform coating and fine detail observed with iophendylate formulations but without the associated toxicity. Herein we report the synthesis, formulation, safety evaluation, and imaging of a series of novel radiopaque compounds, the 1,3,5-trialkyl-2,4,6-triiodobenzenes, which were designed to overcome both the limitations of barium sulfate and the liabilities associated with existing iodinated oils as GI contrast agents.

Chemistry

Design Considerations. In designing an iodinated organic molecule to use as contrast media for GI radiographic examinations, a number of issues relating to chemical composition and synthetic feasibility of the agent required consideration. X-ray attenuation is directly proportional to the amount of iodine contained within a molecule, and doses of contrast media are calculated based on the weight percent of iodine present in the radiopaque component. Therefore, to minimize the quantity of a contrast agent administered during a GI imaging study, one primary objective was to maximize the number of iodine atoms contained within a given molecule. At the same time, to facilitate the formulation of these agents as stable emulsions, the melting points of viable compounds could not significantly exceed room temperature. Iodine which is bonded directly to a benzene ring has the highest degree of chemical stability and biological safety,[17] although this structural framework imparts a high degree of crystallinity to substances which should ideally be nonviscous oils. These two opposing properties, low melting point and high iodine content, added to the challenge of successful design.

Other critical factors such as toxicity also required consideration. Given the large quantities of contrast agent administered for routine GI examinations, the radiographic agent should ideally be nontoxic and pharmacologically inert. Chemical functionality associated with biological recognition was to be avoided. The safety of barium sulfate in an uncompromised bowel is attributed to extremely low aqueous solubility and minimal absorption from the GI tract. Although chemically inert and relatively nonabsorbed orally, extravasation of barium sulfate into the peritoneal cavity through either perforated colon, duodenum, or stomach may lead to serious reactions ranging from peritonitis, formation of dense adhesions, and even death. For these reasons, barium contrast is generally contraindicated in patients with known or suspected perforations and alternative contrast agents are required. Intravascular exposure of barium sulfate is rare but associated with serious or fatal complications including liver abscesses and pulmonary and cardiac embolizations.[7] Therefore to provide an advantage over barium sulfate, an iodinated contrast agent should not only demonstrate poor oral bioavailability but also be nontoxic upon systemic exposure, as in the case of a bowel perforation or accidental aspiration following oral administration.

Issues pertaining to synthetic feasibility also were considered in the design of a novel radiopaque oil. Lengthy synthetic processes would render the agent too costly for routine diagnostic use; thus sequences were limited to a maximum of three steps from relatively inexpensive starting materials. From a practical standpoint, the quantity of drug substance required for our preclinical screening protocol mandated that synthetic candidates be prepared readily on multigram scale.

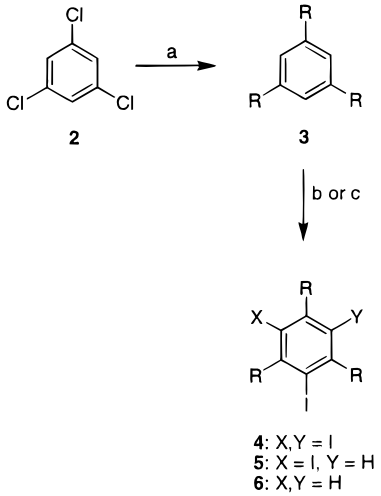
The physical properties of the products also presented unanticipated purification issues. Target compounds were noncrystalline substances with molecular weights > 700 amu. Distillation was difficult, as boiling points often exceeded 200 °C at <0.001 mmHg. Even if the compounds proved thermally stable at these temperatures, the vapors were often too dense to permit the use of conventional distillation procedures. Column chromatography was unacceptable for large-scale purification. Thus for a radiopaque oil to be considered a viable candidate, the synthetic process must afford the final product with >98% purity without the need for column chromatography or distillation.

The 1,3,5-trialkyl-2,4,6-triiodobenzenes meet all of the above criteria. They are structurally novel, free-flowing oils which appear to be chemically and pharmacologically inert. The flexibility of the long alkyl chains efficiently offsets the inherent crystallinity of a 1,3,5-triiodobenzene framework. They are prepared cleanly in two steps. Most importantly, when formulated as an oil-in-water emulsion, 1,3,5-tri-*n*-hexyl-2,4,6-triiodobenzene (**17**) provides excellent images of the GI tract with no apparent toxicity.

Chemical Synthesis. The 1,3,5-trialkyl-2,4,6-triiodobenzenes were synthesized according to the general, two-step procedure outlined in Scheme 1. In the initial step, the method of Tamborski[18a] was utilized to couple 1,3,5-trichlorobenzene (**2**) with alkyl Grignards under nickel catalysis. The resulting 1,3,5-trialkylbenzene intermediates **3** were then iodinated with *N*-iodosuccinimide (NIS) in the presence of catalytic amounts of trifluoromethanesulfonic acid (TfOH) to afford the desired products **4** (Table 1).

Several issues related to the success of this synthetic sequence warrant comment. The use of NIS/TfOH was critical to obtain the products with >98% purity, and

Scheme 1[a]



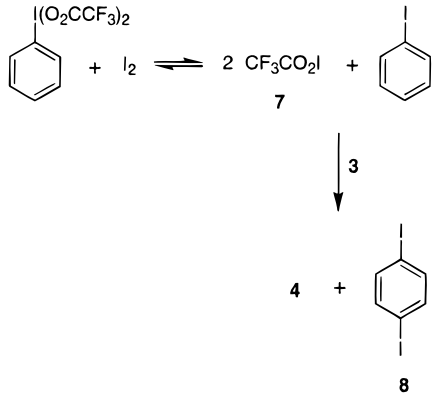
[a] (a) RMgBr, $NiCl_2$(dppp) (cat.), Et_2O; (b) method A: I_2, $PhI(O_2CCF_3)_2$, CCl_4; (c) method B: NIS, TfOH, $ClCH_2CH_2Cl$, reflux; dppp = 1,3-bis(diphenylphosphino)propane.

over 100 different iodination methods were explored before identifying these conditions.[18b] The only previously reported compound in the series is the trimethyl derivative **12**, which had been synthesized to exemplify methodologies for the iodination of aromatic rings.[19] These conditions included the treatment of mesitylene with iodine and mineral acids,[20] NIS and *p*-toluenesulfonic acid,[21] thallium(III) trifluoroacetate and trifluoroacetic acid,[22] benzyltrimethylammonium dichloroiodate and zinc chloride,[23] bis(pyridine)iodonium(I) tetrafluoroborate and TfOH,[24] and anodic oxidation of iodine.[25] However, none of these procedures was judged adequate for our purposes, due to incomplete iodination of the trialkylbenzene intermediates. The resulting diiodo and monoiodo impurities **5** and **6**, respectively, were extremely difficult to remove from the structurally similar triiodo products, especially on large scale. Thus any iodination conditions which gave incomplete conversion to **4** were not acceptable.

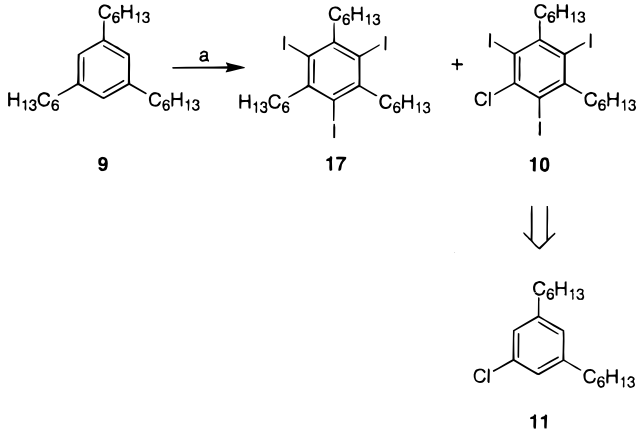
One method which routinely afforded complete triiodination involved the treatment of **3** with bis(trifluoroacetoxy)iodobenzene and iodine in carbon tetrachloride (method A).[26] While these conditions afforded **4** in high yields at room temperature, the products were contaminated with 1,4-diiodobenzene (**8**) which arose from the competing iodination of the iodobenzene generated during the course of the reaction (Scheme 2). While **8** could be separated via recrystallization from the products which were solids (i.e., **12**−**16**, **21**, **22**), **8** was readily soluble in analogues which were oils. Methods to physically remove this crystalline impurity from the desired oils via sublimation, steam distillation, or low-temperature crystallization were not successful. Chemical methods, such as selective reduction, were also unsuccessful. It was possible to obtain analytically pure oily analogues (**17**, **18**) via method A after repeated chromatography of the product mixture; however, this was not suitable for large-scale synthesis.

The complete conversion of **3** to **4** via method A was noteworthy and encouraging and was attributed to the reactive intermediate trifluoroacetyl hypoiodide (**7**). To avoid the formation of **8** or similar byproducts, alternative sources of **7** were sought. The direct treatment of **3** with NIS and trifluoroacetic acid was disappointing, as it gave the usual mixture of **4**−**6**. However, contrary to previous iodination attempts, this mixture was devoid of decomposition products and the reaction proceeded in very high yield. We were encouraged and reasoned that by increasing the reactivity of the hypoiodide intermediate, the iodination would proceed to completion. To that end, **3** was treated with NIS and catalytic TfOH (method B), conditions which consistently afforded the desired **4** in excellent yields.[27] The absence of iodinated contaminants permitted an abbreviated work-up, consisting of an aqueous wash and filtration through a short plug of silica gel.

Scheme 2



Scheme 3[a]



[a] (a) Method B.

The iodinated impurities **5** and **6** were not the only contaminants to impact the synthetic process used for the 1,3,5-trialkyl-2,4,6-triiodobenzenes. When method B was first applied to a 100-g batch of 1,3,5-tri-*n*-hexylbenzene (**9**; Scheme 3), HPLC analysis of the resulting **17** revealed unacceptable levels of another impurity (10%) which could not be separated from the desired product. This contaminant was subsequently identified as the chlorinated compound **10**, which ultimately originated from the incomplete coupling of **2** with *n*-hexylmagnesium bromide (Scheme 1). The amount of **11** formed in the coupling could be minimized by using excess Grignard; however, even the use of >10 equiv did not effect complete conversion of **2** to **9**. It was absolutely critical that the purity of **9** was at least 98% prior to the iodination step, as impurities could not be

removed from the product **17** (vide supra). Although the boiling points of **9** and **11** were similar, it was possible to separate them via fractional distillation.[28] Unfortunately, the increase in purity of **9** (88.6% of the crude mixture, increased to 98.7% after distillation) was achieved at the expense of chemical yield (>99% crude yield, decreased to 48% after distillation). However, once sufficient purity was attained, **9** (>98% pure) was readily converted to **17** (>98% pure) using method B. This iodination proceeded in 96% yield on a 2.5-kg scale.

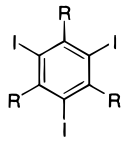
Several 1,3,5-trialkyl-2,4,6-triiodobenzenes were prepared in which the alkyl chains contained a terminal methyl branch (**21**−**23**). Attempts to triiodinate 1,3,5-triisopropylbenzene or 1,3,5-triisobutylbenzene via method B afforded mixtures with varying degrees of iodination. These reactions could not be driven to completion with excess reagents or increased temperature, presumably due to steric reasons. When the methyl branch was a minimum of three bonds removed from the benzene ring (**21**−**23**), complete triiodination could be achieved. Interestingly, it was necessary to increase the amount of NIS to over 4 equiv for this reaction to proceed to completion. Conditions which consistently afforded **17** gave only diiodinated product when applied to the isooctyl precursor of **23**. The source of this reactivity difference is unclear, but it appears to be common to branched analogues.

Results and Discussion

Physical Properties of 1,3,5-Trialkyl-2,4,6-triiodobenzenes. As noted in Table 1, the melting points of the 1,3,5-trialkyl-2,4,6-triiodobenzenes decrease as the length of the alkyl chain increases. The *n*-hexyl derivative **17** and analogues with longer *n*-alkyl chains (**18**−**20**) are free-flowing oils at room temperature, although the 5-methylhexyl analogue **22** is a solid. None of the 1,3,5-trialkyl-2,4,6-triiodobenzenes has measurable solubility in water, and the oily compounds are insoluble in alcohols or dipolar aprotic solvents. They have slight solubility in acetone and are readily soluble in nonpolar solvents such as hexanes and dichloroethane. The compound with the highest iodine content (weight percent) to remain an oil at room temperature was **17**. Thus, this was chosen as the prototypical analogue to examine in safety and imaging studies.

Rodent Safety Evaluation and Thyroid Toxicity. On the basis of considerations described above, the safety profile needed for a novel emulsion-based iodinated oral contrast agent to gain regulatory approval was expected to be high. While the ideal agent would be poorly absorbed, nontoxic, and pharmacologically inert, a rigorous screening program was established early in the discovery program to help guide synthetic efforts. Toxicity studies in the contrast media field are performed to determine drug-related effects that cannot be evaluated in standard pharmacology profiles or that may occur after repeat administration. This takes on particular significance given the large (multigram) quantities of contrast agent that are often administered in a radiographic procedure. A two-tiered approach was utilized to assess the acute toxicity of drug candidates. An initial characterization of gross oral toxicity was obtained through a 7-day, single administration, ascending dose test in mice. The novel radiopaque oils, which possessed acceptable physical properties (i.e., chemical stability, mp < 30 °C, emulsifiable) were formulated as light mineral oil-in-water emulsions at a concentration of 100−160 mg I/mL and were administered by oral gavage to mice at three volumes: 10, 20, and 40 mL/kg body weight, corresponding to ca. 1-, 2-, and 4-fold the anticipated clinical dosage (114 mg I/mL) for humans. The emulsion vehicle (without contrast agent) served as a control group and was dosed at 40 mL/kg, the maximum volume that could be reasonably administered orally. Animals were observed for 7 days, at which point the surviving mice were killed and a necropsy was performed. Compounds which produced mortality, adverse clinical symptoms (i.e., CNS effects), weight loss, or gross lesions at necropsy were removed from further consideration. The results of the acute murine toxicity screen for compounds **1**, **17**, and **24** are summarized in Table 2. The emulsion of iophendylate (**1**) caused death in all animals at all doses within 3 days. These results were surprising given the apparent lack of toxicity in dogs,[15] and the cause of this discrepancy is unclear. To probe the relationship between the formulation of this agent and toxicity, neat **1** was

Table 1. Syntheses and Physical Properties of 1,3,5-Trialkyl-2,4,6-triiodobenzenes



compd	R	method	% yield[a]	formula	% I	mp, °C
12	CH_3	B	80[b]	$C_9H_9I_3$	76.5	209−11[f]
13	C_2H_5	B	85[b,c]	$C_{12}H_{15}I_3$	70.6	195−96
14	*n*-C_3H_7	A	5[d]	$C_{15}H_{21}I_3$	65.5	95−96
15	*n*-C_4H_9	A	8[d]	$C_{18}H_{27}I_3$	61.1	92−93
16	*n*-C_5H_{11}	A	40[d]	$C_{21}H_{33}I_3$	57.2	71−72
17	*n*-C_6H_{13}	B	96[e]	$C_{24}H_{39}I_3$	53.8	oil
18	*n*-C_7H_{15}	A	35	$C_{27}H_{45}I_3$	50.8	oil
19	*n*-C_8H_{17}	B	70	$C_{30}H_{51}I_3$	48.1	oil
20	*n*-C_9H_{19}	B	88	$C_{33}H_{57}I_3$	45.7	oil
21	*i*-C_5H_{11}	A	5[d]	$C_{21}H_{33}I_3$	57.2	86−87
22	*i*-C_6H_{13}	A	31[d]	$C_{24}H_{39}I_3$	53.8	87−89
23	*i*-C_8H_{17}	B	81	$C_{30}H_{51}I_3$	48.1	oil

[a] Yields for solids based on first crop after recrystallization. [b] Recrystallized from xylenes. [c] Compound **13** obtained in 56% yield (method A) following recrystallization from cyclohexane. [d] Recrystallized from ethanol. [e] Compound **17** obtained in 55% yield using method A. [f] Lit.[20b] mp 208 °C.

Table 2. Murine Toxicity Following a Single Oral Dose

		dose[a,g]			mortality,
compd[b]	% I[c]	vol, mL/kg	g compd/kg	g I/kg	day 7[d]
1	30.5	10	5.34	1.63	3/3[e]
1 (neat)	30.5		2.80	0.853	1/3
1 (neat)	30.5		6.26	1.91	1/3
1 (neat)	30.5		12.5	3.81	3/3
17	53.7	40	8.49	4.56	0/3[f]
24	65.2	40	7.00	4.56	0/3[f]

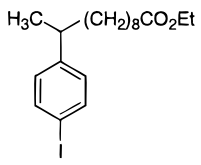
[a] Dosage volume and amount of iodinated compound contained therein. [b] Formulated as mineral oil-in-water emulsions, unless noted. [c] Weight percent iodine contained in compound. [d] Number of decedents over the total number of animals in each dosage group 7 days after oral administration. [e] 100% mortality was observed at 7 days in both the 20 mL/kg (3.27 g I/kg) and 40 mL/kg (6.53 g I/kg) dose groups. [f] No signs of toxicity were observed in either the low-dose (10 mL/kg; 1.14 g I/kg) or middose (20 mL/kg; 2.28 g I/kg) groups. [g] Emulsion vehicle, dosed at 40 mL/kg, served as the control group. No signs of toxicity were observed at this dose level.

Table 3. Hamster Thyroid Toxicity 48 h Following a Single Oral Dose

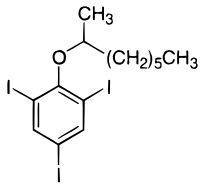
compd[a]	sex (*n*)	dose, g I/kg[b]	mitotic figures[c]	necrosis[d]	dilation[e]
vehicle	M (6)	0	0/6	0/6	0/6
vehicle	F (6)	0	0/6	0/6	0/6
17	M (3)	4.56	1/3[f]	0/3	1/3[f]
17	F (3)	4.56	0/3	0/6	0/3
23	M (5)	4.56	0/5	0/5	0/5
24	M (3)	4.56	3/3	2/3	3/3
24	F (3)	4.56	3/3	0/3	3/3

[a] Formulated as mineral oil-in-water emulsions at 114 mg I/mL (see the Experimental Section). [b] Dosage volume = 40 mL/kg. [c] Number of hamsters with follicular cell mitotic figures above control values over total number of animals in each dosage group. [d] Number of hamsters with follicular cell necrosis over total number of animals in each dosage. [e] Number of hamsters with follicular cell dilation over total number of animals in each dosage group. [f] Changes were minimal in severity.

examined in this assay. While toxicity was diminished compared to that of the emulsion, neat **1** induced mortality in one of the three mice tested at the lowest dose. These results were sufficient to suspend further evaluation of this agent. The remaining candidates (**17**, **24**) could not be readily differentiated in the mouse and were then evaluated in other acute rodent models for which the hamster became the second tier study.



1



24

Compound **24**[29] was initially identified as an alternative to **1** for GI imaging.[30] Although no signs of toxicity (Table 2) were evident in mice after a single oral administration of **24** at 4-fold the anticipated clinical dosage (4.56 g I/kg; see Preclinical Imaging Studies), **24** induced histomorphologic changes in the thyroids of hamsters 48 h after administration of this dose (Table 3). These changes included follicular cell mitosis, necrosis, and dilation. Thyroid gland changes also were evident in rats after a single administration of **24** at 1.14 g I/kg, although these effects were not as pronounced as in the hamster. To assess the possible specificity of thyroid toxicity for rodents, followup studies were performed with **24** in the dog. While no abnormalities were found after a single administration at 4-fold the clinical dosage, dogs receiving this dosage six times over a 2-week period showed increased thyroid weight accompanied by histomorphologic abnormalities. Therefore, **24** induced thyroid toxicity in all species tested, although the sensitivity greatly varied. Further development of **24** for GI imaging was therefore terminated. Despite the very similar profile of compounds **17** and **24** in the acute murine screen, thyroid toxicity became the principal hurdle to surmount for **17**.

Given the significance of thyroid toxicity with respect to advancement of an iodinated contrast agent, it became desirable to screen compounds for these effects at a very early stage in the discovery effort. Since hamsters had proven more sensitive to thyroid perturbations than rats, an acute hamster assay was devised to provide a quick indication of thyroid toxicity. Furthermore, the hamster was a preferred species for toxicity testing because it lacks a forestomach, unlike rats. In this assay, hamsters received a single dose of test article orally, and after 48 h, thyroid glands were removed and examined histomorphologically. In the screening protocol, compounds which passed the acute murine toxicity screen now proceeded directly into this assay for thyroid toxicity.

The acute hamster thyroid assay proved a significant hurdle for organic radiopaque oils to pass. Representative compounds from 10 different structural classes of iodinated benzenes were evaluated for thyroid toxicity in the hamster, including iodinated phenyl esters, phenyl ethers, benzoates, and isophthalates. While the radiopaque oils share some structural similarities to thyroxine (T_4) and related thyroid hormones, the mechanism by which **24** induced thyroid toxicity is unclear, and more importantly, this is not a property shared by barium sulfate. *The only molecules which did not induce thyroid toxicity were **17** and **23***, both of which belong to the class of 1,3,5-trialkyl-2,4,6-triiodobenzenes (Table 3). Compound **17** could now be differentiated from **24** in a rodent model of toxicity. Analysis of plasma samples drawn from hamsters treated orally with 4.56 g I/kg **17** or **23** failed to reveal significant levels of parent drug (minimum quantifiable level (MQL) = 0.19 μg/mL). The lack of acute toxicity of **17** and **23** therefore appeared due to low bioavailability, although it was not clear whether this was because of poor absorption, extensive first-pass metabolism, or a combination of the two.

To further address this issue, the rates of in vitro metabolism of **17** and **23** were determined in rat, hamster, and human liver microsomes. Since **24** had previously been shown to be extensively metabolized in all three species, [^{14}C]**24** was used as a positive control in this study. Compounds (substrate concentrations of 4 and 40 μM) were incubated for 60 min with microsomes, and aliquots were removed and then analyzed by isocratic reverse-phase HPLC. At the lower substrate concentration (4 μM), the rate of metabolism (half-life) of **24** in the hamster was 15 min compared to the rat (36 min) and human (132 min). At the higher substrate concentration, metabolism of **24** was generally less extensive with 73−100% of intact drug remaining after 60 min in the three test systems. In contrast, both **17** and **23** exhibited little or no metabolism in any of the microsomal preparations after a 60-min incubation at either substrate concentration. Intact drug accounted for between 99% and 111% of the control values (0 min), indicating low rates of metabolism across a range of species. These data indicate that **17** and **23** were poorly metabolized in the three microsome preparations and that the lack of thyroid toxicity observed in the hamster may be a result of poor absorption rather than rapid and extensive systemic clearance following metabolism.

While poor absorption is a desirable property for a GI contrast agent, the issue of toxicity upon systemic exposure remained for these compounds. An agent which is nontoxic upon leakage into the peritoneum would offer a distinct advantage over barium sulfate. To investigate this possibility with **17**, the hamster thyroid protocol was modified to mimic a bowel perfora-

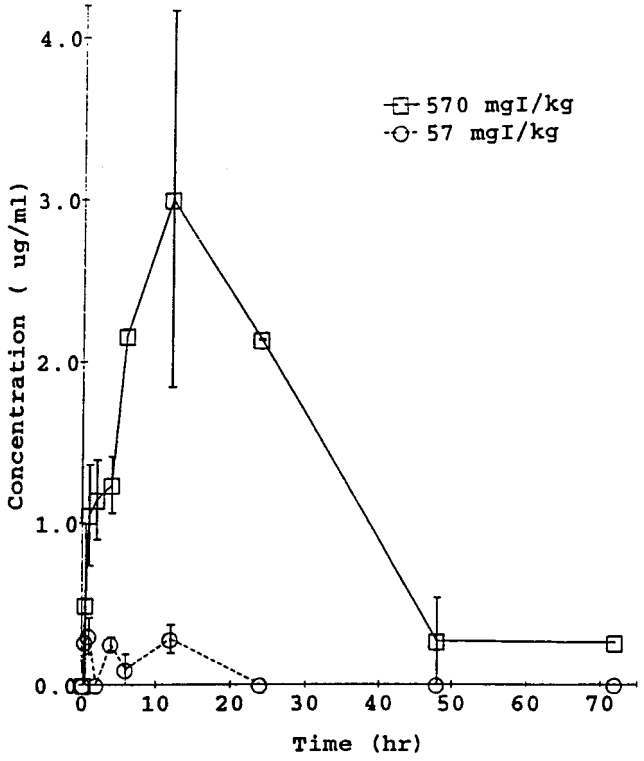


Figure 1. Plasma concentrations (μg/mL) after ip administration of **17** to hamsters. Bar indicates 1 SEM; the MQL for this study was 0.19 μg/mL.

tion. In this study, hamsters were dosed intraperitoneally (ip) with an emulsion of **17** at 57 and 570 mg I/kg and orally at 570 and 4560 mg I/kg for comparison. Plasma concentrations were monitored for 72 h, after which time the animals were examined for thyroid perturbations. As shown in Figure 1, increased systemic exposure of **17** was evident after ip dosing at 570 mg I/kg while plasma levels of **17** following oral dosing were < MQL (0.19 μg/mL) at the same dosage level. Absorption and/or elimination of drug was slow after ip dosing with detectable concentrations in plasma still present at 72 h in the high-dose group. Significantly, no changes in thyroid pathology were detected in animals treated with **17** compared to controls, while hamsters which received **24** ip exhibited thyroid toxicity similar to that observed after oral administration of that agent. There were no other indications of major organ toxicity in hamsters treated with **17**.

Preclinical Imaging Studies. Imaging studies were performed in rats and dogs to assess the radiographic properties of test formulations, designed to optimize both formulation parameters (i.e., emulsion stability) and imaging efficacy. Imaging efficacy was evaluated in the upper GI tract using the degree and distribution of radiopacity as a visual indicator of mucosal surface coating. Preliminary imaging studies were conducted in pentobarbital (125 mg/kg im) anesthetized rats. The contrast agent was formulated at a concentration of 114 mg I/mL (see below) and introduced via gastric intubation at a dose of 10 mL/kg. Manipulations of the animals were purposely avoided, thereby emphasizing the innate ability of the test formulation to adhere to the mucosal surface of the GI tract. The rodent radiographs provided a means to screen formulations based on uniformity, 50% or greater mucosal coating of the small intestine,

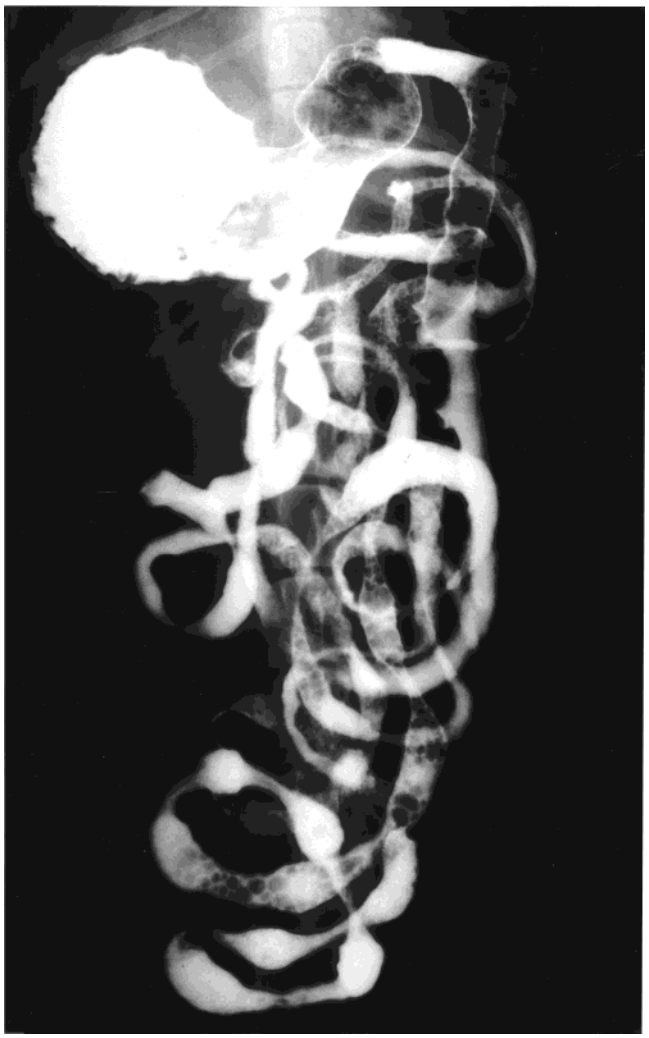


Figure 2. Representative upper GI canine radiograph following oral administration of a conventional barium sulfate suspension (Polibar, formulated at 70% w/v) taken 15 min postoral administration at a dose of 10 mL/kg in a 12−14-kg beagle. Air was administered just prior to the film.

and transradiation as evidenced by the ability to visualize loops of the bowel that overlap each other. Promising formulations were then prepared in larger volumes and evaluated in a canine model. Criteria of success in the dogs were based on (1) appearance of uniform mucosal coating in at least 50% of the small intestine, (2) persistence of mucosal coating for a minimum of 30 min postadministration, (3) appearance of mucosal detail, (4) multiple levels of transradiation, and (5) a high degree of reproducible images among dogs studied. The anticipated human clinical imaging dosage (iodine concentration) was derived from dose ranging studies (25−150 mg I/mL) in dogs comparing **24** (and **1** as a prototype) with barium sulfate and Gastrografin, a water-soluble, iodinated contrast agent. The optimal iodine concentration necessary to provide sufficiently opaque images was found to be 114 mg I/mL (i.e., 228 mg of compound containing 50 wt % iodine). The current formulation of **17** was found to satisfy all of the aforementioned criteria.

Representative canine radiographs which compare a conventional barium sulfate formulation with that of **17** are shown in Figures 2 and 3, respectively. The barium sulfate used was a commercial Polibar preparation,

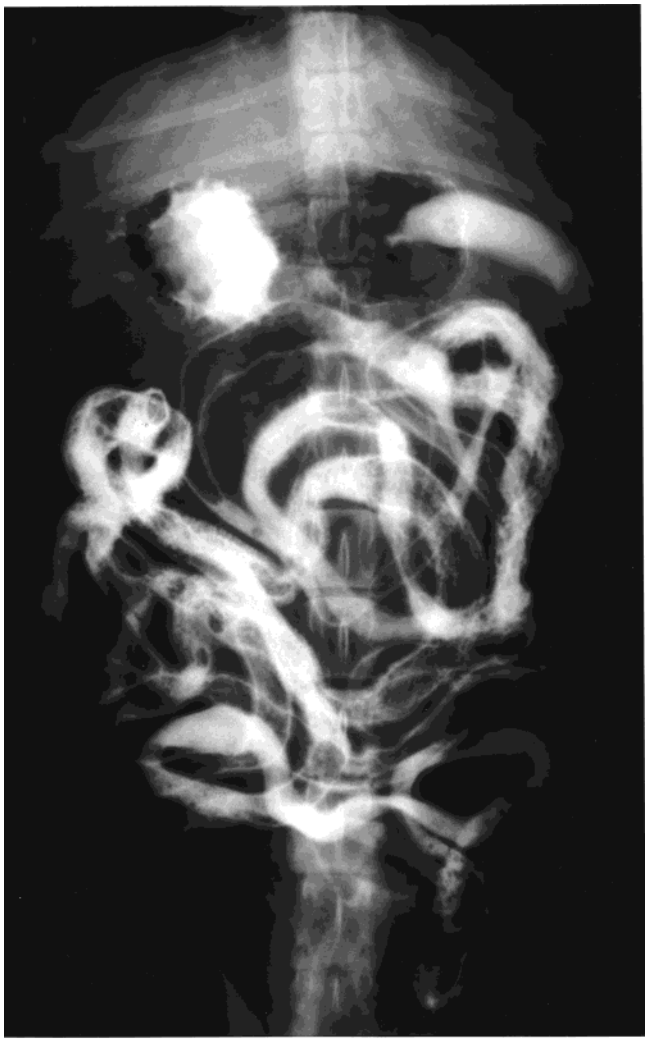


Figure 3. Representative upper GI canine radiograph following oral administration of an oil-in-water emulsion of compound **17** (formulated at 22.0% w/v oil, 118 mg I/mL) taken 30 min postoral administration at a dose of 10 mL/kg in a 12−14-kg beagle. Air was administered 15 min postadministration of the emulsion.

formulated as a 70% w/v suspension and administered at a dose of 10 mL/kg to a 12−14-kg beagle dog. Compound **17** was formulated as an oil-in-water emulsion (22.0% w/v oil, 118 mg I/mL) and administered at 10 mL/kg to a 12−14-kg beagle dog. In both studies, air was administered prior to contrast agent administration. These images are typical and highly reproducible for both agents. The emulsion formulation of **17** provided extensive, uniform, and persistent coating of the small intestines of both rats and dogs after oral administration. Transradiation was clearly evident in most of the animals studied, and mucosal detail was also evident in most animals. However, in contrast to the sharp granular appearance of the mucosal surface typically seen with barium formulations, the mucosal surfaces in the case of the emulsion were delineated by a smooth continuous zone of enhancement at the tangential margins of the lumen. Although the diagnostic implications of such a "halo" effect are unknown at the present, it is hypothesized that these new views may provide for significant advantages in the early diagnosis of brush-border abnormalities.

Conclusion

With the advent of managed health care, the role of routine radiological procedures is likely to depend on the efficiency and cost-effectiveness of these examinations. The labor-intensive techniques of GI examinations were developed almost entirely around the limitations of barium sulfate and involve manipulation of the patient to achieve adequate mucosal coating and/or abdominal compression to overcome the radiopacity of barium. A contrast agent which offers greater sensitivity for detecting subtle tissue abnormalities while increasing procedural ease and patient throughput would present advantages over current procedures.[31] We have identified a novel series of radiopaque oils which appear to fulfill these criteria. Oil-in-water emulsions of 1,3,5-trialkyl-2,4,6-triiodobenzenes have demonstrated excellent mucosal coating and optimal radiodensity without subject manipulation and offer improved physical stability in vivo when compared to barium sulfate suspensions. The *n*-hexyl analogue **17** was minimally absorbed from the GI tract, was resistant to metabolism, and appeared safe following oral and intraperitoneal administration. On the basis of these properties, **17** was selected for additional studies.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. Proton NMR (GE QE300), IR (Nicolet 10DX or 550 FT-IR spectrophotometer), and mass spectra (Kratos Concept or a Nermag R10-10C spectrometer) were consistent with the assigned structures. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet), and br (broad). Coupling constants are in hertz (Hz). Infrared spectra (IR) were measured as 1% KBr pellets or neat oils. Carbon, hydrogen, and iodine elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ, and were within ±0.4% of theoretical values. Nonaqueous reactions were generally carried out under a N_2 or Ar atmosphere.

The 1,3,5-trialkylbenzenes were purchased from Aldrich (1,3,5-trimethyl- and 1,3,5-triethylbenzene) or synthesized as outlined in Scheme 1.[32]

General Preparation of 1,3,5-Trialkylbenzenes 3. The following procedure illustrates the general method utilized for the preparation of all 1,3,5-trialkylbenzenes used to prepare **14**−**23.** The crude products were obtained as colorless to straw-colored, mobile oils and were used directly in the next step, following workup, filtration through a short pad of silica gel, and concentration in vacuo.

1,3,5-Tri-*n*-hexylbenzene. 1-Bromohexane (1 mL) was added to a stirred mixture of magnesium turnings (5.88 g, 242 mmol) in 25 mL of anhydrous diethyl ether at room temperature under an atmosphere of nitrogen. A crystal of iodine was added and the mixture was heated to reflux until the color faded. A solution of the remaining 1-bromohexane (33 mL, 234.8 mmol) in ether (100 mL) was added at a rate sufficient to maintain gentle reflux. After refluxing for 2 h, the solution was transferred by cannula to a solution of 1,3,5-trichlorobenzene (14.2 g, 77.4 mmol) in ether (200 mL) containing 0.32 g (0.25 mol %) of [1,3-bis(diphenylphosphino)propane]nickel(II) chloride ($NiCl_2$(dppp)). The solution was gently refluxed for several hours whereupon a solid began to precipitate from solution. The reaction mixture was carefully poured into 2000 mL of cold 1 N aqueous HCl and the layers were separated. The aqueous layer was extracted with additional ether (250 mL) and the ether extracts were combined. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 23 g of a brown oil. The oil was dissolved in hexane and filtered through a short pad of silica gel, eluting with hexane, and then

concentrated to dryness under vacuum to give 19.3 g (58.5 mmol, 75%) of 1,3,5-tri-*n*-hexylbenzene as a colorless, mobile oil ($C_{24}H_{42}$; M^+ 330): ^{1}H NMR ($CDCl_3$) δ 0.78 (m, 9H), 1.23 (m, 18H), 1.53 (m, 6H), 2.47 (m, 6H), 6.52 (s, 3H). The product was used in the subsequent step without additional purification.

1,3,5-Tri-*n*-hexyl-2,4,6-triiodobenzene (17). Method A. This procedure illustrates the general method for the preparation of **13**−**18**, **21** and **22**.[32] A mixture of 1,3,5-tri-*n*-hexylbenzene (81.8 g, 248 mmol), iodine (102.1 g, 402 mmol), and [bis(trifluoroacetoxy)iodo]benzene (213.3 g, 496 mmol) in 1.5 L of carbon tetrachloride was stirred at room temperature overnight. The solvent was evaporated, CH_2Cl_2 (400 mL) and H_2O (400 mL) were added and the layers were separated. The organic layer was washed with 400 mL of 10% aqueous sodium thiosulfate and then dried over magnesium sulfate. After concentration, the mixture of oil and solids was then subjected to flash chromatography over a silica gel plug (hexanes). The crude product was dissolved in hexanes (150 mL) and chilled; the precipitated solid (1,4-diiodobenzene) was removed. This crystallization procedure was repeated three times followed by repeated silica gel chromatography (heptane elution, 5 kg SiO_2 total) of the oily residue. After concentrating under vacuum, the mobile oil was dried with warming under high vacuum to afford 96.5 g (55%) of **17**.

For solids **13**−**16**, **21**, and **22**: The mixture of product and 1,4-diiodobenzene was dissolved in EtOH,[33] and the 1,3,5-trialkyl-2,4,6-triiodobenzene was precipitated. Recrystallization from EtOH afforded the crystalline products.

1,3,5-Tri-*n*-hexyl-2,4,6-triiodobenzene (17). Method B. This procedure illustrates the general method for the preparation of **12**, **13**, **19**, **20**, and **23**. A mixture of the 1,3,5-tri-*n*-hexylbenzene (1.4 kg, 3.64 mol) and *N*-iodosuccinimide (2.86 kg, 12.72 mol, freshly recrystallized from acetone/diethyl ether) was slurried in 20 L of 1,2-dichloroethane. Trifluoromethanesulfonic acid (0.55 kg, 3.64 mol) was added at a moderate rate and the reaction was heated under reflux for 2 h. Additional *N*-iodosuccinimide (0.41 kg, 1.82 mol) was added and reflux was maintained for 60 min. The solvent was evaporated and the residue was dissolved in 4 L hexanes and washed with 4 L water. The organic layer was separated, and then flash chromatographed over a silica gel plug (2 L of hexanes). The organics were washed with 6 L of 5% aqueous sodium thiosulfate. After concentrating under vacuum, the mobile oil was dried with warming (100 °C) under high vacuum to afford 2.45 kg (96%) of **17**: ^{1}H NMR ($CDCl_3$) δ 0.89 (m, 9H), 1.35 (m, 12H), 1.46 (m, 12H), 3.28 (m, 6H).

Biological Studies. The animal care, use of tissue, and in vivo experimentation conformed to the *NIH Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, 1985) and the *Animal Welfare Act* (P.L. 89-544, as amended). All research involving animals described in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division's (SWPRD) Policy on Animal Use and all national and federal legislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care International (AAALAC International).

Acute Murine Toxicity Assay. Male ICR mice (Harlan Sprague−Dawley) weighing 20−25 g received a single administration of the test articles via oral gavage. The contrast agents were formulated as oil-in-water emulsions at a concentration of 114 mg I/mL. Emulsions were prepared immediately prior to dosing by adding the appropriate % w/v of test article to a mixture of 12.5% w/v light mineral oil, 3.365% w/v Tween 80, 1.635% w/v Span 80, 0.5% w/v Avicel RC591 and water to 100 vol %. A control emulsion was prepared in the same manner by omitting the test article and adjusting the volume of water required. The mice were observed for clinical signs of toxicity and mortality several times on the day of dosing and once a day thereafter for a total of 7 days. Changes in body weight were assessed 24 h and 1 week after treatment. Seven days after dosing all mice were subjected to a gross necropsy of the abdominal and thoracic cavities.

Hamster Thyroid Toxicity Assay. Male golden Syrian hamsters (SASCO Inc., Madison, WI) weighing 90−120 g (6−8 weeks) were used for the oral administration study. The test articles were formulated as described above, and hamsters received either the control emulsion or test emulsion by oral gavage. The treatments were administered at dosage volumes of 40 mL/kg, which was the highest volume that could be reasonably administered, representing 4-fold the anticipated clinical dosage of 10 mL/kg. Animals received either a single administration of the test emulsion or four administrations over 8 days. The latter dosing schedule was selected as a surrogate for increasing drug exposure to the animals in a realistic manner, since volume restrictions limited the maximum dose that could be given by oral gavage. Body weights were recorded prior to dosing and necropsy. After an overnight fast, hamsters were killed by exsanguination following the administration of an intraperitoneal overdose of pentobarbitone. The study was thus terminated on 48 h after dosing for animals receiving a single dose and 1 week after dosing for animals receiving four doses. Plasma samples were collected, and all hamsters were subject to a gross necropsy. Thyroid glands were weighed following fixation. Stomach, thyroid glands and tissues containing macroscopic abnormalities were preserved in 10% neutral buffered formalin and examined histomorphologically using hematoxylin and eosin stained tissue sections.

The above hamster protocol was modified for single dose, intraperitoneal administration to mimic drug exposure as a result of a perforated bowel. Compound **24** was administered over the same dose range as the positive control, particularly for the thyroid. Male golden Syrian hamsters received a single intraperitoneal administration of the test article (day 1) formulated at 114 mg I/mL concentration. Control groups received 0.9% w/v sodium chloride at a dosage volume of 5.0 mL/kg or the emulsion vehicle (without iodinated agent) at 5.0 mL/kg. Hamsters were subjected to a full necropsy at the end of the study. Thyroid glands were examined histologically from all animals. In the absence of an overt effect on the thyroid, the following tissues were examined histologically from control hamsters and the high-dosage groups: adrenal glands, cecum, colon, duodenum, ileum, jejunum, heart, kidney, liver, lung, mesenteric lymph nodes, omentum, pancreas, rectum, and stomach.

In Vitro Metabolism. The in vitro metabolism of **17**, **23**, and [^{14}C]**24** (positive control) was investigated by HPLC following incubation with rat (male, Sprague−Dawley; Charles River (U.K.) Ltd.), hamster (male golden Syrian; Charles River (U.K.) Ltd., Manston Rd., Margate, Kent), and human liver microsomes at substrate concentrations of 4 and 40 μM. Aliquots (0.5 mL) from duplicate incubates were taken at 0, 15, 30 and 60 min, mixed with saturated zinc sulfate solution (0.5 mL), and extracted with propan-2-ol (0.7 mL). The resultant supernatants, including appropriate blank and control samples, were then analyzed by isocratic reversed-phase HPLC (IBSIL C8, 65% *i*-PrOH/water with 0.1% TFA). Recovery of drug in the microsomal extracts varied between 71−82% for the control, 71−89% for **17** and 65−105% for **23**. Results were normalized to 100% of the control values (0 min) in order to determine the half-life of each drug in the incubate ($n = 2$).

Canine Imaging Studies. A group of 12 beagle dogs (Marshall Farms, North Rose, NY) weighing 12−15 kg were assigned to this study. A Microfluidizer 110T (5000 psi) was used to prepare the contrast agent as an emulsion containing 22% **17** with 5% surfactants (0.8% Span 80, 0.2% Tween 80, 4% Pluronic F127), 1% methocel K4M as a viscosity enhancer, and 0.1% simethicone as an antifoaming agent. The dogs were radiographed using a Siemens Polyphos 50 at a maximum interval of once per week. This time period provided adequate time for the formulation to clear from the GI tract (=24 h) plus a minimum of 6 days of rest before dosing with the next formulation. Contrast agents were administered orally using a gavage tube placed into the distal portion of the esophagus. For the first few months, the dogs were lightly sedated with

acepromazine and placed in a ventral dorsal (VD) position on the radiographic table for a series of radiographs taken at 15, 30, 45, 60, 120, and 240 min. Air (100 mL) was administered immediately after the 15-min radiograph so as to distend the lumen and provide a double contrast examination for the subsequent radiographs. No movement or manipulation was performed on the dogs during the experiment. The dogs quickly became acclimated to this procedure such that the acepromazine sedation was unnecessary after 4−6 weeks.

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