Polyiodinated Triglyceride Analogs as Potential Computed Tomography Imaging Agents for the Liver†

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A series of glyceryl 2-oleoyl 1,3-bis[ω-(3-amino-2,4,6-triiodophenyl)] alkanoates was synthesized, radioiodinated with iodine-125, emulsified, and evaluated for their ability to selectively localize in the liver for potential use as hepatographic agents in computed tomography. All seven analogs displayed rapid liver uptake wherein between 65 and 78% of the injected dose accumulated in the liver by 30 min. Liver values ranged from 46 to 93% 3 h after injection which corresponded to liver to blood ratios ranging from 21 to 450. Moreover, subsequent elimination of radioactivity from the liver was nearly linear with respect to alkyl chain length. Analogs with longer alkyl chain length were eliminated from the liver more rapidly than their shorter chain counterparts. Because of their biochemical similarities to naturally occurring triglycerides, these novel analogs may prove useful not only for high-resolution anatomic imaging of focal liver lesions, but also for evaluating a variety of diffuse diseases known to affect hepatic function and biochemistry.

Introduction

Computed tomography (CT) using ionic and nonionic water-soluble, urographic contrast agents remains one of the most accurate, noninvasive radiologic exams for the detection of hepatic masses. Several limitations, however, including nonspecific biodistribution and short residence time in the liver, often result in the masking of small hypervascular lesions, and as a result, the use of urographic agents for liver CT is less than optimal. Accordingly, much effort has been devoted to the development of liver-specific contrast agents including radiopaque liposomes,[1] iodinated starch particles,[2] iodipamide ethyl ester,[3] and ethiodized oil emulsions (EOE).[4] The most promising of these agents, EOE-13, has been extensively studied in both animals and humans in the United States.[5] EOE-13, an emulsion of iodinated poppy seed oil (37% iodine by weight) in saline, offered considerable improvement in the detection of space-occupying lesions in the liver and spleen. Despite excellent clinical diagnostic efficacy, a relatively high incidence of adverse reactions coupled with problems relating to product sterilization have since precluded further studies with this agent.[6] All of these experimental approaches to liver-specific CT contrast involved particulate formulations with relatively large particle size, and therefore, liver uptake was predominantly mediated via sequestration by the reticuloendothelial (RE) system.

Our strategy for the site-specific delivery of imaging agents to the liver is based on a biochemical approach, whereby naturally occurring compounds known to be stored or metabolized in the liver serve as carriers for the radiologic moiety. Therefore, based on the known metabolic fate of lipids, both cholesteryl esters and triglycerides were viewed as appropriate liver-specific carrier molecules for the synthesis of hepatic imaging agents.

Lipids are transported in the plasma mainly in the form of free fatty acids, cholesteryl esters and triglycerides. Free fatty acids are transported as a complex with plasma albumin, whereas cholesteryl esters and triglycerides are transported in the lipophilic core of plasma lipoproteins.[7] The surface of lipoproteins is composed of a monolayer of phospholipid, cholesterol, and specific proteins known as apolipoproteins. Dietary sources of cholesterol and triglycerides are absorbed from the intestinal tract and incorporated into chylomicrons, large lipoproteins 80-500 nm in diameter, which are subsequently secreted into the lymphatic system and transported through the thoracic duct until they reach the circulation. This high-capacity system can process up to 100 g of triglyceride a day in an adult human.[8] Once in the circulation, the chylomicrons are acted upon by lipoprotein lipase in the capillary beds of peripheral tissues including adipose, muscle (heart), and lung.[9] Lipoprotein lipase hydrolyzes much of the core triglyceride to glycerol and free fatty acids, most of which are taken up by the tissues for storage or oxidation. The remaining triglyceride-depleted chylomicrons, called chylomicron remnants (30-100 nm in diameter), are then cleared very rapidly from the circulation by the liver via a receptor-mediated process. While lipoprotein lipase is the enzyme responsible for the hydrolysis of plasma triglycerides in extrahepatic tissues, hepatic triglyceride lipase (HL) is implicated in triglyceride hydrolysis and remnant uptake by hepatocytes.[10] This process is quite efficient in humans, as the half-time for the clearance of chylomicron remnants following a meal is about 15 min.[11] Moreover, hepatic clearance of chylomicron remnants from the circulation occurs mainly via the parenchymal (hepatocytes) rather than nonparenchymal (Kupffer) cell types.[11,12] Of par-

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ticular interest is the finding that remnant-incorporated cholesteryl esters sequestered by the liver are not taken up to any extent by the Kupffer or endothelial cells.[13] Chylomicron-remnant delivery thus offers an alternate mechanism for the delivery of radiologic agents to the liver,[14] differing from the phagocytic uptake of colloids, other larger particulates, and liposomes by Kupffer cells, the hepatobilliary uptake of iminodiacetate chelates in nuclear medicine, and the nonspecific, extra cellular association of water-soluble urographic agents currently used in CT.

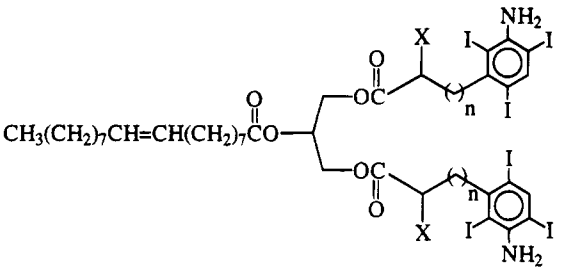
In order to exploit this natural lipid transport mechanism, we initially evaluated a series of radioiodinated cholesteryl esters for their ability to localize in the liver.[15,16] Preliminary radiotracer studies with a prototypic agent, cholesteryl iopanoate (CI), revealed a rapid liver uptake (30% administered dose at 30 min) in rats.[16] Prior incorporation of this agent into chylomicrons, however, afforded an almost 3-fold (87% administered dose) enhancement in liver uptake in the same time period.[17] Subsequent CT studies with CI in rabbits revealed high liver extraction, resulting in significant CT enhancement of the liver. This preferential accumulation in normal liver tissue subsequently made it possible to detect hepatic VX_2 tumors as small as 2 mm in diameter in rabbits.[18,19] A downfall of CI, however, was that it was so resistant to *in vivo* hydrolysis that it remained chemically unchanged in the liver 5 months after administration.[19] While this property proved to be desirable in subsequent atherosclerosis studies in rabbits,[20] such stability and organ retention were unsuitable for CT studies of the liver. Although other sterol esters have since been evaluated and have shown exceptional promise for scintigraphic functional imaging of the liver, formulation of these agents at doses appropriate for CT imaging has proven difficult.

In addition to cholesteryl esters, triglycerides represent the other major constituent of the lipophilic core of chylomicrons. Synthetic analogs of triglycerides, therefore, represented a natural extension of our studies. Preliminary results obtained with a series of radioiodinated 2-substituted, 1,3-disubstituted, and 1,2,3-trisubstituted polyiodinated triglyceride analogs confirmed our expectations and identified several excellent candidates for followup studies.[21,22] Most of these candidates, however, proved difficult to formulate at radiologic doses. The major purpose of this study was, therefore, to synthesize and evaluate a series of second generation 1,3-disubstituted polyiodinated triglyceride (ITG) analogs which would be more suitable to the formulation process while retaining a high degree of liver selectivity. It was also desirable for the agents to maintain a high liver concentration for a sustained time period (1–2 h) relative to current water-soluble urographic agents (1–2 min) and to be cleared from the liver within 24–48 h after administration. Moreover, due to the biochemical rationale behind the development and targeted delivery of these agents to the liver, it was expected that the effective CT dose would be substantially less than that of the non-tissue-specific water-soluble urographic agents used currently.

Chemistry

Several structural features were incorporated into the design of ITG analogs **24–30** shown in Table 1. The

Table 1. 2-Oleoylglycerol 1,3-Bis[ω(3-amino-2,4,6-triiodophenyl)alkanoates]



no.	*n*	X	yield, %	formula	analysis[a]
24	0	H	32	$C_{37}H_{48}O_6N_2I_6$	C, H
25	1	H	92	$C_{39}H_{52}O_6N_2I_6$	C, H
26	1	Et	53	$C_{43}H_{60}O_6N_2I_6$	C, H
27	2	H	58	$C_{41}H_{56}O_6N_2I_6$	C, H
28	3	H	71	$C_{43}H_{60}O_6N_2I_6$	C, H
29	4	H	81	$C_{45}H_{64}O_6N_2I_6$	C, H
30	5	H	79	$C_{47}H_{68}O_6N_2I_6$	C, H

[a] Elemental analyses were within ±0.4% for elements indicated.

amino 2,4,6-triiodophenyl moiety affords both sufficiently high iodine concentration for CT and enhanced chemical stability of the iodine toward deiodination relative to aliphatic iodides. Moreover, the alkyl chain length was varied in hopes of providing suitable liver uptake and clearance profiles. Finally, substitution at the 1- and 3-positions was utilized in order to avoid asymmetric center formation in the enzymatically relevant glycerol backbone of the molecule and also to allow for appropriate substitution in the 2-position in order to enhance formulation properties.

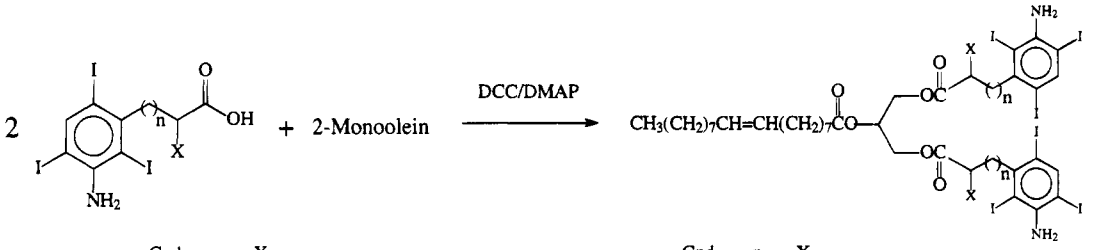
The syntheses of iodinated acids **1** and **2** were previously reported.[21] Iopanoic acid (**3**) was commercially available. (Iodophenyl)butyrate analog **4** was made from 3-nitrocinnamic acid via classical chain elongation methods as shown in Scheme 2. Accordingly, 1-chloro-3-(3-nitrophenyl)propane (**10**)[23] was converted to nitrile **11** in the presence of crown ether. Subsequent hydrolysis, catalytic hydrogenation, and iodination afforded the desired (triiodophenyl)butyric acid analog **4**.

The syntheses of phenylpentanoic acid (**5**) and phenylhexanoic acid (**6**) were accomplished using Knochel coupling chemistry as illustrated in Scheme 3.[24] This approach was based on highly functionalized copper reagents prepared by transmetalation of the alkylzinc iodide with the dilithio salt of CuCN and subsequent reaction with the appropriate alkyl halide. Following formation of nitrophenyl alkanoate (**14** and **15**), subsequent catalytic hydrogenation and iodination afforded triiodinated esters **18** and **19**. Saponification afforded triiodinated acids **5** and **6**, respectively.

Heptanoic acid analog **7** was synthesized via classical Wittig methodology as shown in Scheme 4. Treatment of (ω-carbethoxyhexylidine)triphenylphosphonium bromide (**20**) with LDA and subsequent reaction with 3-nitrobenzaldehyde afforded nitrophenyl alkenoate **21**, which was catalytically reduced to aminophenyl alkanoate **22** prior to triiodination with excess iodine monochloride. Saponification in ethanolic base afforded acid **7** in good overall yields.

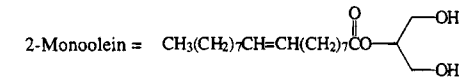
Target 1,3-bis(ω-(3-amino-2,4,6-triiodophenyl)alkyl)-2-oleates **24–30** were synthesized via DCC/DMAP coupling of 2-monoolein (1,2,3-trihydroxypropane 2-oleate) with 2 equiv of the corresponding ω-(3-amino-2,4,6-

Scheme 1. Synthesis of 2-Oleoylglycerol 1,3-Bis-[ω-(3-Amino-2,4,6-Triiodophenyl)alkanoates]

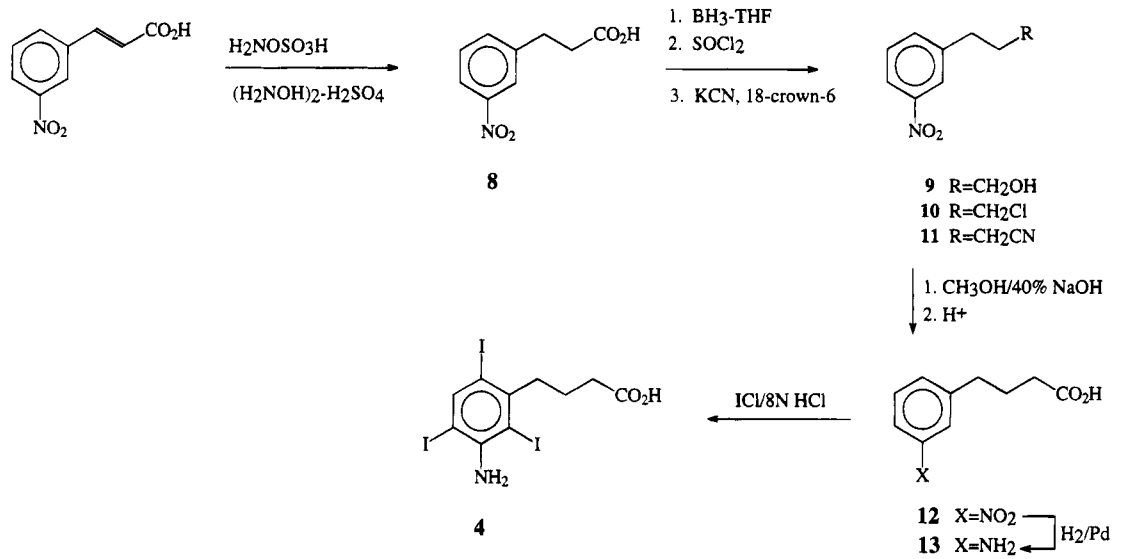


Cpd	n	X
1	0	H
2	1	H
3	1	Et
4	2	H
5	3	H
6	4	H
7	5	H

Cpd	n	X
24	0	H
25	1	H
26	1	Et
27	2	H
28	3	H
29	4	H
30	5	H



Scheme 2. Synthesis of 4-(3-Amino-2,4,6-triiodophenyl)butanoic Acid



triiodophenyl)alkanoic acid (**1**–**7**) as shown in Scheme 1. Isolated yields as well as some physical characteristics of the target triacylglycerol analogs are included in Table 1. Each target ITG was radioiodinated by isotope exchange with iodine-125 in a melt of pivalic acid[25] in order to evaluate its ability to selectively localize in the liver.

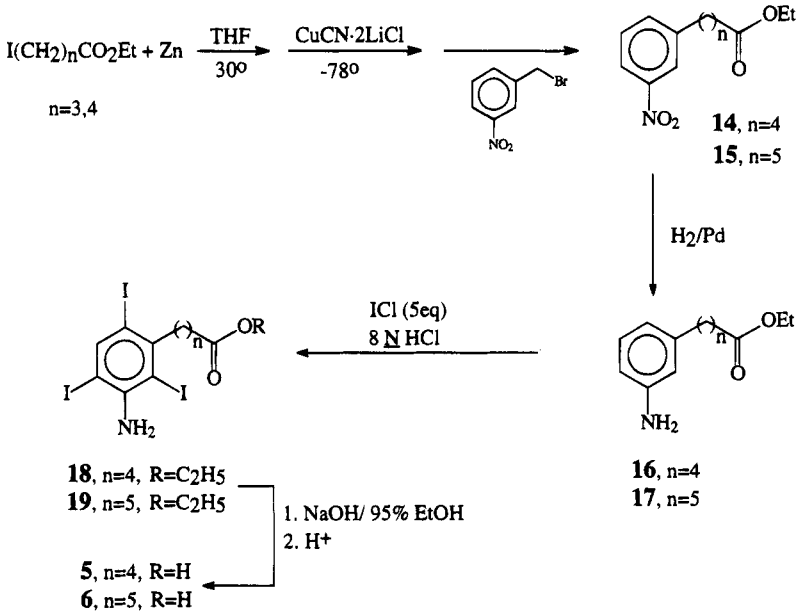
Emulsification

The iodinated triglycerides were formulated for intravenous injection in a lipid emulsion. Briefly, the (iodophenyl)triglyceride was combined with core triglycerides, phospholipid, cholesterol, and α-tocopherol in appropriate ratios. The lipid mixture was blended with anhydrous glycerol and water to form a crude oil in water emulsion which was processed further in a Microfluidizer (Microfluidics model 110S) to reduce mean particle size and provide homogeneity in the particle size distribution. Mean particle diameters ranged from 88 to 195 nm as determined by Nicomp number weighted calculations using photon correlation spectroscopy on a Nicomp-370 submicron particle analyzer (Nicomp Particle Sizing Systems, Santa Barbara, CA). Moreover, emulsion preparations were essentially devoid of any liposomal contamination as determined by negative stain transmission electron microscopy.

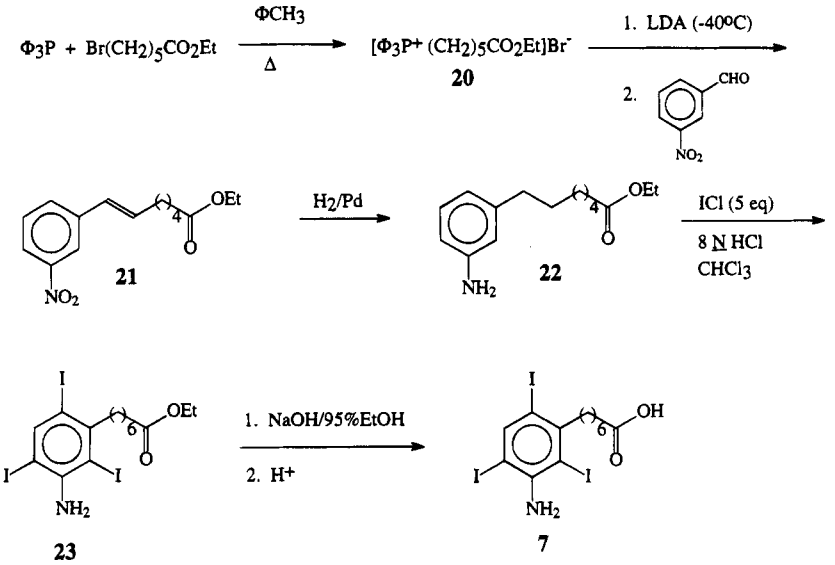
Biodistribution Studies

Once radioiodinated and emulsified, each of the target compounds was subjected to tissue distribution analysis in female Sprague–Dawley rats. The radiolabeled compound was administered intravenously to groups of animals which were then sacrificed at various times. Specific tissues (13 total) were removed and analyzed for radioactivity. Of these, the eight tissues containing the highest amounts of radioactivity are included in Table 2. The other tissues including muscle, ovary, adrenal cortex, fat, and bone marrow generally contained low levels of radioactivity and were therefore excluded from Table 2. Results obtained in liver, spleen, and blood were also calculated on a percent dose per total organ basis and are presented in Table 3. Whereas percent dose per gram of tissue illustrates radioactivity concentration levels, percent dose per organ values take into account the actual weight of the whole tissue and therefore more accurately represent the fraction of the

Scheme 3. Synthesis of ω-(3-Amino-2,4,6-triiodophenyl)alkanoic Acids (**5**, **6**)



Scheme 4. Synthesis of 7-(3-Amino-2,4,6-triiodophenyl)heptanoic Acid (**7**)



injected dose that actually resides in a given organ at any specific time.

Lipid extraction of both liver and plasma was performed in order to gain insight into the nature of the radioactive products in these tissues. Table 4 summarizes the percent of lipid-extractable material present in the liver at 30 min and 24 h and indicates the amount still present in the form of the parent compound.

Plasma samples were analyzed by polyacrylamide gel electrophoresis (PAGE) in order to define those macromolecules (lipoproteins, plasma proteins) involved in the transport of the agents to the tissues. The PAGE results shown in Table 5 provide a gross breakdown of the plasma proteins each compound is associated with while in the circulation. The stacking gel (SG) fraction contains among others, chylomicrons and very-low-density lipoproteins. The middle three fractions LDL, HDL, and ALB correspond to low-density and high-density lipoproteins and albumin, respectively. The below albumin (BA) fraction is associated with other proteins.

Results and Discussion

Tissue distribution analysis (Tables 2 and 3) obtained in rats indicated that all seven 2-oleoyl 1,3 disubstituted triglyceride analogs **24**–**30** displayed a high degree of liver selectivity, wherein between 65 and 78% of the injected dose resided in the liver 30 min after iv administration (Table 3). By 3 h, between 46 and 93% of the injected radioactivity remained in the liver. Liver-to-blood ratios at this time ranged from 21 to 450 based on injected dose per-gram-of-tissue calculations.

Although all compounds exhibited a high degree of liver selectivity, a lesser, but significant, amount of radioactivity became rapidly associated with the spleen when localization of radioactivity was evaluated on a concentration basis. When the total organ size is considered, however, the spleen typically contained from 8 to 14% of the injected dose 3 h after injection of ITG. Radioactivity in the other 11 tissues sampled generally contained low amounts of radioactivity at all time points.

Although 30 min tissue distribution data indicates

Table 2. Distribution of Radioactivity at 30 min, 3 h, and 24 h after iv Administration of ^{125}I-Labeled 2-Oleoylglycerol 1,3-Bis-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] in Rats[a]

tissue	24	25	26	27	28	29	30
				30 min			
blood	0.69 ± 0.22	0.49 ± 0.19	0.26 ± 0.07	0.58 ± 0.37	0.08 ± 0.03	0.38 ± 0.08	0.59 ± 0.28
heart	0.36 ± 0.11	0.51 ± 0.06	0.84 ± 0.19	0.66 ± 0.21	0.59 ± 0.09	0.23 ± 0.08	0.55 ± 0.13
kidney	0.10 ± 0.03	0.11 ± 0.02	0.07 ± 0.02	0.14 ± 0.05	0.05 ± 0.01	0.09 ± 0.01	0.20 ± 0.03
lung	0.72 ± 0.05	0.43 ± 0.04	0.34 ± 0.04	0.36 ± 0.09	0.54 ± 0.03	0.26 ± 0.19	0.66 ± 0.14
liver	7.90 ± 0.41	10.01 ± 0.56	9.67 ± 0.91	10.82 ± 0.24	7.37 ± 0.46	7.01 ± 0.03	7.23 ± 0.73
plasma	1.25 ± 0.46	0.46 ± 0.18	0.42 ± 0.12	0.79 ± 0.41	0.10 ± 0.02	0.65 ± 0.13	0.83 ± 0.50
spleen	23.42 ± 1.09	27.56 ± 2.34	24.82 ± 1.19	31.69 ± 3.88	17.94 ± 2.34	24.43 ± 1.16	21.80 ± 5.87
thyroid	0.16 ± 0.04	1.76 ± 0.21	2.88 ± 0.48	3.37 ± 1.02	0.54 ± 0.20	0.54 ± 0.16	0.31 ± 0.02
				3 h			
blood	0.04 ± 0.01	0.06 ± 0.00	0.02 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.20 ± 0.03	0.28 ± 0.03
heart	0.10 ± 0.02	0.31 ± 0.05	0.25 ± 0.08	0.15 ± 0.02	0.09 ± 0.01	0.20 ± 0.01	0.22 ± 0.04
kidney	0.04 ± 0.00	0.07 ± 0.01	0.03 ± 0.00	0.09 ± 0.01	0.09 ± 0.00	0.21 ± 0.02	0.18 ± 0.04
lung	0.38 ± 0.03	0.33 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	0.46 ± 0.03	0.21 ± 0.02	0.34 ± 0.04
liver	10.26 ± 0.53	10.87 ± 0.30	9.00 ± 0.83	13.81 ± 0.24	6.94 ± 0.53	8.74 ± 0.33	5.84 ± 0.58
plasma	0.07 ± 0.02	0.09 ± 0.01	0.03 ± 0.01	0.13 ± 0.00	0.09 ± 0.00	0.24 ± 0.04	0.29 ± 0.04
spleen	23.55 ± 2.11	30.03 ± 1.30	17.66 ± 2.37	23.80 ± 5.48	14.47 ± 0.88	23.48 ± 3.95	15.35 ± 2.85
thyroid	0.27 ± 0.12	3.29 ± 1.19	5.08 ± 0.23	3.77 ± 1.01	1.16 ± 0.08	0.91 ± 0.26	1.69 ± 0.11
				24 h			
blood	0.01 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.15 ± 0.00	0.14 ± 0.02	0.10 ± 0.01	0.22 ± 0.05
heart	0.03 ± 0.00	0.11 ± 0.02	0.06 ± 0.02	0.12 ± 0.05	0.07 ± 0.00	0.07 ± 0.00	0.10 ± 0.01
kidney	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.46 ± 0.01	0.20 ± 0.02	0.25 ± 0.03	0.22 ± 0.05
lung	0.52 ± 0.09	0.53 ± 0.02	0.62 ± 0.18	0.54 ± 0.05	0.59 ± 0.05	0.26 ± 0.04	0.29 ± 0.02
liver	9.09 ± 0.33	9.77 ± 0.08	10.25 ± 0.72	12.68 ± 1.02	4.53 ± 0.24	4.20 ± 0.44	1.10 ± 0.20
plasma	0.01 ± 0.00	0.05 ± 0.01	0.01 ± 0.00	0.24 ± 0.01	0.14 ± 0.02	0.14 ± 0.02	0.21 ± 0.04
spleen	24.23 ± 3.19	21.36 ± 3.87	21.70 ± 1.32	15.45 ± 0.29	13.65 ± 0.95	10.42 ± 0.96	1.43 ± 0.63
thyroid	2.58 ± 0.14	8.45 ± 1.78	17.31 ± 2.85	15.89 ± 2.81	6.74 ± 0.83	5.13 ± 1.54	6.53 ± 0.64

[a] Values expressed as percent administered dose per gram of tissue ± SEM; $n = 3$ for all cases.

Table 3. Liver, Spleen, and Blood Radioactivity After iv Administration of 1,3-Disubstituted Triacylglycerols to Rats[a]

tissue	24	25	26	27	28	29	30
				30 min			
liver	69.8 ± 7.1	66.2 ± 4.9	78.0 ± 2.5	75.4 ± 3.6	65.1 ± 2.2	66.8 ± 2.4	67.7 ± 6.5
spleen	12.0 ± 1.1	13.0 ± 0.2	14.4 ± 0.8	13.7 ± 1.1	9.5 ± 0.8	13.9 ± 0.5	10.5 ± 2.1
blood	7.6 ± 2.3	5.1 ± 2.0	2.6 ± 0.8	5.8 ± 3.5	0.9 ± 0.3	4.8 ± 1.2	6.6 ± 3.1
				3 h			
liver	79.0 ± 0.3	71.7 ± 0.6	80.0 ± 8.0	93.4 ± 4.3	60.7 ± 2.9	66.0 ± 1.9	45.8 ± 2.5
spleen	14.5 ± 1.2	12.9 ± 0.8	11.3 ± 1.4	11.7 ± 2.9	8.0 ± 0.4	11.3 ± 1.0	8.2 ± 1.0
blood	0.5 ± 0.1	0.7 ± 0.0	0.3 ± 0.0	0.9 ± 0.0	0.9 ± 0.7	2.2 ± 0.3	2.7 ± 0.3
				24 h			
liver	72.5 ± 0.3	69.8 ± 1.7	77.0 ± 3.4	74.3 ± 1.6	36.1 ± 1.6	34.9 ± 3.4	9.6 ± 1.3
spleen	12.2 ± 1.2	11.2 ± 2.2	12.5 ± 1.0	8.8 ± 0.5	6.4 ± 0.3	5.8 ± 0.7	0.8 ± 0.3
blood	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	1.5 ± 0.0	1.4 ± 0.1	1.1 ± 0.2	2.5 ± 0.6
				48 h			
liver	59.7 ± 2.6	65.1 ± 0.2	71.6 ± 3.3	58.2 ± 1.7	26.8 ± 2.6	22.4 ± 3.8	4.4 ± 0.3
spleen	16.6 ± 1.8	13.0 ± 0.9	9.8 ± 0.7	7.0 ± 0.4	3.7 ± 0.1	3.1 ± 0.9	0.3 ± 0.1
blood	0.1 ± 0.0	0.5 ± 0.2	0.1 ± 0.0	1.1 ± 0.4	0.7 ± 0.1	0.8 ± 0.3	0.4 ± 0.2

[a] Radioactivity is expressed as percent administered dose per organ ± SEM (based on actual organ weight for each animal), $n = 3$. Due to rounding down, SEM listed as 0.0 actually ranged from 0.01 to 0.049. At 5 days, liver, spleen, and blood values for compound **25** were 37.6, 8.13, and 0.7, respectively ($n = 1$).

consistent liver selectivity between all seven ITG analogs, two distinct pharmacokinetic profiles appear by 3 h. Liver retention between 30 min and 3 h was relatively consistent for all analogs except **30**, which appears to have undergone some degree of metabolism and subsequent elimination from the liver. The shorter chain length analogs **24**–**27** appear more resistant to metabolism and elimination from the liver as indicated by a relatively small decrease in liver radioactivity from 3 to 24 and 48 h. Analogs **28** and **29** displayed similar distribution profiles between 30 min and 3 h; however, by 24 h metabolism and elimination were apparent, albeit not to the same extent, as that observed for the longest chain analog, **30**. Elimination of liver radioactivity by 24 and even 48 h was minimal for short chain analogs, **24**–**26**, supporting their *in vivo* stability, whereas elimination of **27**–**30** demonstrated a direct correlation with increasing chain length. Specifically, the longer the chain length, the more rapid the apparent metabolism and elimination.

Although the 24 h data (Table 2) indicated that between 2.6 and 17.3% of the injected radioactivity resided in the thyroid on a per gram of tissue basis, this actually corresponds to less than 0.23% of the total injected radioactivity when considered on an injected dose per total organ weight basis. Thus, it appears that the relative amount of *in vivo* deiodination was minimal 24 h after administration.

Inspection of the liver and plasma extraction data obtained 30 min after administration of ITG analogs **24**–**30** (Table 4) revealed that 58–63% of the radioactivity present in the liver was organic soluble for analogs **24**, **25**, and **30**, whereas 91–97% of the liver radioactivity was organic soluble for analogs **26**–**29**. The fact that long-chain analog **30** has been observed to behave more like short-chain analogs **24** and **25** than

Table 4. Analysis of Lipid-Soluble Radioactivity Extracted from Rat Liver and Plasma after iv Administration of ^{125}I-Labeled 2-Oleoyl 1,3-Disubstituted Triacylglycerols[a]

		%$CHCl_3/CH_3OH$ extractable compd		% parent compound[b]	
compd	tissue	0.5 h	24 h	0.5 h	24 h
24	liver	62.6 ± 1.6	59.5 ± 0.9	85.1 ± 3.6	87.5 ± 1.7
	plasma	54.2 ± 0.6	24.4 ± 0.8	86.3 ± 11.3	IC
25	liver	58.3 ± 2.3	67.9 ± 2.1	93.5 ± 1.2	84.8 ± 0.9
	plasma	37.5 ± 5.4	26.6 ± 2.5	IC	IC
26	liver	97.5 ± 0.8	93.7 ± 1.0	78.6 ± 2.4	64.7 ± 9.2
	plasma	88.2 ± 2.8	83.0 ± 1.2	26.5 ± 26.5	IC
27	liver	93.7 ± 1.0	93.4 ± 1.0	84.5 ± 3.7	94.5 ± 0.5
	plasma	85.8 ± 2.8	30.0 ± 3.2	85.8 ± 3.9	IC
28	liver	91.1 ± 0.9	88.8 ± 1.0	98.9 ± 0.2	86.5 ± 1.2
	plasma	77.4 ± 2.2	54.5 ± 3.1	42.3 ± 21.1	IC
29	liver	91.3 ± 1.3	90.3 ± 0.9	96.8 ± 0.7	97.9 ± 0.3
	plasma	89.9 ± 1.3	80.6 ± 1.2	82.2 ± 3.4	IC
30	liver	56.0 ± 1.0	30.2 ± 5.0	91.9 ± 4.5	0.0 ± 0.0
	plasma	36.1 ± 9.8	15.3 ± 1.7	0.0 ± 0.0	IC

[a] Percent ± SEM; $n = 3$; IC = insufficient counts in sample at this time point. [b] By TLC with authentic ^{125}I-labeled standard.

Table 5. Polyacrylamide Gel Electrophoresis (PAGE) of Rat Plasma 30 min after iv Administration of ^{125}I-labeled 2-Oleoyl 1,3-Disubstituted Triacylglycerols[a]

compd	SG	LDL	HDL	ALB	BA
24	95.7 ± 1.3	0.6 ± 0.3	0.7 ± 0.1	1.8 ± 1.1	1.1 ± 0.6
25	67.3 ± 7.0	8.3 ± 3.8	4.1 ± 1.6	8.1 ± 1.4	12.4 ± 1.0
26	83.5 ± 4.9	7.4 ± 2.6	2.5 ± 1.4	4.9 ± 3.6	1.5 ± 0.6
27	80.9 ± 6.0	3.2 ± 1.3	1.8 ± 0.5	4.8 ± 1.5	9.4 ± 2.7
28	65.6 ± 2.9	8.1 ± 4.0	5.5 ± 0.6	20.7 ± 5.5	0.1 ± 0.1
29	72.6 ± 7.6	4.8 ± 3.0	7.0 ± 4.2	15.0 ± 3.9	0.6 ± 0.3
30	28.1 ± 24.0	0.2 ± 0.2	0.3 ± 0.3	66.3 ± 22.5	5.1 ± 2.5

[a] Values are expressed as percent of total radioactivity applied to gel ± SEM; $n = 3$; SG = stacking gel, LDL = low-density lipoprotein, HDL = high-density lipoprotein, ALB = albumin, BA = below albumin.

26–29 is interesting and is, perhaps, the result of more extensive enzymatic processing and extraction of secondary conjugates. A high degree of metabolism of **30** is also indicated upon comparison of the 30 min and 24 h lipid extraction results. By 30 min, 92% of the lipid-extractable radioactivity comigrated with authentic parent ITG by thin layer chromatographic analysis, whereas by 24 h, none of the corresponding radioactivity comigrated with parent ITG. All other analogs exhibited a much higher degree of *in vivo* stability at 24 h. Plasma extraction data generally followed similar trends, but caution must be exercised when interpreting these data due to the very low quantity of radioactivity associated with the plasma, especially at the 24 h time point.

Polyacrylamide gel electrophoresis data from plasma samples indicated a general relationship between chain length and association with both the stacking gel and albumin fractions (Table 5). In plasma samples taken 30 min after ITG administration between 28 and 96% of the radioactivity applied to the gel associated with the stacking gel, indicating a large difference in the plasma transport properties of these agents. Typically, greater than 90% of the radioactivity was associated with the stacking gel layer following application of the neat emulsion to the gel, thus suggesting a similarity to native chylomicrons and remnants. Moreover, short-chain analogs **24–27** were much more resistent to early (30 min) plasma hydrolysis than longer chain analogs **28–30** as indicated by comparison of radioactivity present in the albumin layer. A much higher relative amount of radioactivity was associated with the albumin layer for **28**, **29**, and **30**, thus indicating that these analogs were more susceptible to enzymatic hydrolysis than their shorter chain counterparts.

Upon incorporation into a chylomicron remnant-like vehicle, these ITG analogs exhibit a high degree of liver selectivity at radiologic doses. Subsequent metabolism and elimination from the liver appear to be somewhat dependent on the chain length of the iodoaryl aliphatic moiety. Moreover, unlike their saturated predecessors, these analogs were much more suitable for emulsification.

It is apparent that uptake of the ITG's into the liver is dependent in part on the nature of the delivery vehicle itself; however, the metabolism and subsequent elimination rate from the liver are influenced primarily by the chemical structure, including the alkyl chain length of the iodophenylacyl moieties present in the 1- and 3-positions of the ITG.

Current water-soluble urographic agents used for hepatic CT contrast enhancement pass rapidly through the liver prior to renal elimination. In fact, passage through the liver is so rapid that CT scans must be acquired within the first 2 min of injection while the bolus is traveling through the liver. Although spatial resolution is very good, the information obtained with these water-soluble urographic agents is purely anatomic in nature. Utilizing the biochemical rationale discussed herein, it is possible to design a CT agent specifically for the high-resolution evaluation of not only liver anatomy, but liver function as well. Accordingly, due to its biochemical nature, this approach may be useful in diagnosing and/or assessing liver patency in focal disease such as primary and metastatic cancer and hemangioma, and also in a variety of diffuse diseases known to affect liver function and biochemistry including alcoholic liver, diabetes, and cirrhosis.

Experimental Section

Infrared spectra (IR) were obtained on a Perkin-Elmer 1420 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on either a Varian EM 360L (60 MHz) or a Bruker WM 360 (360 MHz), 300 (300 MHz), or 270 (270 MHz) instrument, and all values are reported in parts per million (δ) from $(CH_3)_4Si$. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by either Midwest Microlabs, Ltd., Indianapolis, IN, or by the University of Michigan Department of Chemistry elemental analysis lab. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} polyethylene-backed or Analtech silica gel GHLF glass-backed plates and visualized by UV, iodine, or charring with 50% aqueous sulfuric acid or DNP in the case of aldehydes. Radio-TLC was performed on the Merck plastic-backed silica gel 60 plates and scanned for radioactivity with a Bioscan Model 200 Radio-TLC scanner. Column chromatography was performed on Davisil 62 silica gel (Grace-Davison Chemical, Baltimore, MD) or on Merck silica gel 60 (230–400 mesh). HPLC analyses were performed using a Gilson System-71 semi-preparative gradient system (Madison, WI) including both Holochrome variable-wavelength UV and Varex ELSD IIA evaporative light-scattering detectors. Columns included both normal phase (Whatman Partisil 5 analytical (25 cm) and reverse phase Partisil 10 ODS-3 (25 cm analytical and M20 preparative) columns.

THF was distilled from $LiAlH_4$ under nitrogen immediately before use, and CH_2Cl_2 was distilled from P_2O_5 and stored over molecular sieves under argon. Starting materials were generally purchased from Aldrich Chemical Co., Milwaukee, WI,

unless otherwise stated. Pure oleic acid was purchased from Nu-Chek Prep, Inc., Elysian, MN.

Preparation of ω-(3-Amino-2,4,6-triiodophenyl)alkanoic Acids (1–6). Synthesis of ω-(3-amino-2,4,6-triiodophenyl)acetic (**1**)[26] and propionic (**2**)[27] acids was accomplished by literature procedures. Iopanoic acid (**3**) was purchased from CTC Organics (Atlanta, GA) and used without further purification.

Preparation of 4-(3-Amino-2,4,6-triiodophenyl)butanoic acid (4). 3-(3-Nitrophenyl)propionic Acid (8). 3-Nitrocinnamic acid (20.0 g) was suspended in water (700 mL) and cooled to 10 °C. Hydroxylamine sulfate (26.6 g) and hydroxylamine-*O*-sulfonic acid (64.0 g) were added, and the pH was adjusted to 7.6 (pH meter) with concentrated NaOH. Additional base was added as needed over the next 6 h to maintain the pH at 7.6. The reaction mixture was filtered, acidified to pH 2 with 2 N H_2SO_4, and cooled to room temperature. The precipitate that formed was filtered and dissolved with heating in absolute ethanol (200 mL) and water (25 mL). Upon cooling, off-white crystals formed which were filtered and dried *in vacuo* to give desired propionic acid **8** (17.4 g, 86%): mp 115–117 °C (lit.[23] mp 115–116 °C).

3-(3-Nitrophenyl)-1-propanol (9). 3-(3-Nitrophenyl)propionic acid (**8**, 17.0 g, 87.1 mmol) was dissolved in anhydrous THF (50 mL) in a flame-dried round-bottom flask (250 mL) and cooled in an ice bath. A solution of 1 M borane in THF (113 mL, 113 mmol, Aldrich Chemical Co.) was added dropwise over a period of 1 h. The reaction mixture was stirred an additional 2 h at room temperature. Ice water was then slowly added followed by ether (300 mL). The ether layer was washed with water (2×), saturated $NaHCO_3$, and dried with Na_2SO_4. The solvent was removed *in vacuo* and the residue purified by chromatography on a silica gel column (10 × 15 cm) eluted with hexanes/EtOAc/$CHCl_3$ (7:2:1) to give **9** as a slightly yellow liquid (12.9 g, 82%). NMR and IR data were identical to those published by Strawn et al.[23]

3-(3-Nitrophenyl)-1-chloropropane (10). A solution of nitrophenyl alcohol **9** (12.0 g, 66.2 mmol) in dry benzene (20 mL) was added dropwise to a solution of $SOCl_2$ (8.7 g, 73 mmol) and pyridine (2 mL) in dry benzene (20 mL) under N_2 and maintained at room temperature. After stirring overnight, the reaction mixture was heated to reflux for 1 h, cooled in an ice bath, and then treated with ice water (20 mL). The aqueous layer was separated and washed twice with benzene. The combined benzene layers were washed with saturated aqueous $NaHCO_3$ and H_2O and dried ($MgSO_4$). Solvent was removed *in vacuo*, and the remaining yellow liquid residue was purified by column chromatography (silica gel, hexanes/EtOAc (15%) to give **10** (11.45 g, 87%) as a slightly yellow liquid. NMR and IR were as expected.

3-(3-Nitrophenyl)-1-propionitrile (11). Powdered KCN (7.4 g, 114 mmol) and 18-crown-6 (800 mg, 3 mmol, Aldrich Chemical Co.) were suspended in dry acetonitrile (50 mL) in a flame-dried round-bottom flask under N_2. A solution of nitrophenyl chloride **10** (11.35 g, 56.8 mmol) in dry acetonitrile (15 mL) was added dropwise via an addition funnel, whereupon the reaction mixture turned blue. The resulting reaction mixture was heated to reflux for 3 h, at which point additional KCN (1 g) and crown ether (100 mg) were added. After an additional 2 h (5 h total) the mixture was cooled, water was added, and the layers were separated. The water layer was washed twice with acetonitrile, and the organic layers were combined prior to solvent removal *in vacuo*. The residue which remained was purified by column chromatography on silica gel eluted with hexanes/EtOAc (5/2) to afford **11** as a light-orange liquid (8.11 g, 75%): IR (neat) 2920, 2850 (aliphatic CH), 2230 (CN), 1520, 1340; ^{1}H-NMR (60 MHz, $CDCl_3$) δ 8.10–7.60 (m, 4H, Aryl-H's), 2.92 (m, 2H, $PhCH_2$), 1.8–2.5 (m, 4H, $(CH_2)_2$). Anal. ($C_{10}H_{10}O_2N_2$) C, H.

4-(3-Nitrophenyl)butanoic Acid (12). Nitrile **11** (7.4 g, 38.9 mmol) was dissolved in methanol (45 mL). Aqueous 40% NaOH (25 mL) was added, and the resulting mixture was heated to reflux for 1 h, after which it was cooled and acidified with 8 N HCl to a pH of 2. The acidic solution was extracted twice with ether, and the ether layer was subsequently washed with H_2O and brine and dried ($MgSO_4$). Solvent removal *in vacuo* afforded an off-white solid residue which was crystallized from benzene/hexane (1:1) to give pure **12** (6.19 g, 76%): mp 87–90 °C. IR and NMR (60 MHz) were as expected. Anal. ($C_{10}H_{11}O_4N$) C, H.

4-(3-Aminophenyl)butanoic Acid (13). Compound **12** (7.75 g, 37 mmol) was dissolved in absolute ethanol (300 ml), 5% Pd on carbon (1.5 g) was added, and the resulting mixture was subjected to Parr hydrogenation conditions under H_2 at 50 psi. After 12 h, the catalyst was removed by filtration through Celite. Solvent was removed *in vacuo*, leaving 6.60 g (99%) of a viscous translucent liquid which was homogeneous by TLC. IR and NMR (60 MHz) were as expected.

4-(3-Amino-2,4,6-triiodophenyl)butanoic Acid (4). Iodine monocholoride (21.1 g, 133 mmol) was dissolved in concentrated HCl (70 mL) and added with vigorous mechanical stirring to a preheated (75 °C) solution of aminophenyl acid **13** (6.8 g, 37.9 mmol) in water (300 mL) and concentrated HCl (30 mL). After 2 h, additional ICl (2.0 g) in HCl (6 mL) was added. The resulting dark red reaction mixture was stirred at 75 °C for an additional 3 h, whereupon it was cooled in an ice bath and filtered. The brown filter cake was washed with cold water, suspended in water (300 mL), and heated to 70 °C. Sodium hydroxide (3 g) was added, and after 1 h, the resulting suspension was cooled in an ice bath and filtered. The sodium salt thus obtained was dissolved in aqueous 1% sodium bisulfite with the addition of 500 mg of additional NaOH while heating to 80 °C. The resulting yellow homogeneous solution was then acidified to pH 3 by dropwise addition of 8 N HCl. After the mixture was cooled, the precipitate that formed was filtered, washed with water, and dissolved in a solution of EtOH (300 mL) and water (30 mL) while heating on a steam bath. After the mixture was cooled to room temperature, the precipitate that formed was filtered to afford a cottony, cream-colored solid (9.3 g, 44%) which melted at 199–200 °C (decomposed at 230 °C): IR (KBr) 3300, 2930 (amine), 1690 (acid C=O), 1590, 1420, 1265 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.05 (s, 1H, aryl 5-H), 4.82 (s, 2H, NH_2), 3.10 (m, 2H, $PhCH_2$), 2.52 (t, 2H, CH_2CO_2), 1.87 (m, 2H, $CH_2CH_2CH_2$). Anal. ($C_{10}H_{10}O_2NI_3$) C, H.

Preparation of 5-(3-Amino-2,4,6-triiodophenyl)pentanoic Acid (5). Ethyl 5-(3-Nitrophenyl)pentanoate (14). Zinc (5.5 g, 84 mmol, Aldrich 99.99%) was suspended in a solution of 1,2-dibromoethane (1.1 g, 6 mmol) in anhydrous THF (5 mL), and the resulting mixture was heated to 65 °C for 1 min and allowed to cool to 30 °C. A solution of ethyl 4-iodobutyrate (16.0 g, 66 mmol) in anhydrous THF (15 mL) was added, and the resulting mixture was stirred under N_2 at 30 °C for 4 h. The resulting clear solution was added via cannula to a cooled (−78 °C) solution of CuCN (5.0 g, 55 mmol) and LiCl (5.0 g, 110 mmol) in anhydrous THF (40 mL). A solution of 3-nitrobenzyl bromide (10.8 g, 50 mmol) was then added, and the resulting reaction mixture was stirred at −25 °C for 3 h and then at room temperature overnight. The reaction mixture was quenched with aqueous NH_4Cl and extracted with ether. The ether extract was dried over $MgSO_4$ and placed *in vacuo* to remove the solvents. The resulting liquid residue was purified by column chromatography (silica gel 60) eluted with hexanes/EtOAc (50:1) to afford **14** as a light yellow oil which was used without further purification in the following reaction: yield 3.0 g (24%); IR ($CHCl_3$) 3010, 2970 (aryl CH), 2930, 2850 (aliphatic CH), 1735 (ester C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H, aryl 2,4-H), 7.44 (m, 2H, aryl 5,6-H), 4.12 (q, 2H, OCH_2), 2.74 (m, 2H, $PhCH_2$), 2.32 (m, 2H, CH_2CO_2), 1.66 (m, 4H, $(CH_2)_2$), 1.25 (t, 3H, CH_3).

Ethyl 5-(3-Aminophenyl)pentanoate (16). A solution of **14** (2.07 g, 8.2 mmol) in ethanol (50 mL) was hydrogenated at room temperature and 40 psi in the presence of 5% Pd–C (0.7 g) for 3 h. The catalyst was removed by filtration through a pad of Celite, and upon removal of solvent *in vacuo*, amino alkanoate **16** was isolated as a slightly yellow liquid which was homogeneous by TLC (silica gel, hexanes/EtOAc (5:2), R_f = 0.13): yield 1.64 g (91%); IR(neat), 3460, 3380 (amine), 2920, 2850 (aliphatic CH), 1730 (ester C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (m, 1H, aryl 5-H), 6.59 (d, 1H, aryl 6-H), 6.50 (m, 2H, aryl 2,4-H), 4.13 (q, 2H, OCH_2), 3.60 (s, 2H, NH_2), 2.50 (m, 2H, $PhCH_2$), 2.35 (t, 2H, CH_2CO_2), 1.74 (p, 2H,

$PhCH_2CH_2$), 1.53 (m, 2H, ($PhCH_2CH_2$), 1.26 (t, 3H, CH_3). Anal. ($C_{13}H_{19}O_2N$) C, H.

Ethyl 5-(3-Amino-2,4,6-triiodophenyl)pentanoate (18). A solution of ICl (5.87 g, 36 mmol) in 8 N HCl (35 mL) was diluted with $CHCl_3$ (40 mL) and heated to 60 °C in a three-necked round-bottom flask equipped with reflux condenser, addition funnel, and mechanical stirrer. A solution of amino ester **16** (1.6 g, 7.2 mmol) in $CHCl_3$ (30 mL) was then added dropwise over a period of 30 min. The resulting biphasic mixture was stirred rapidly at 60 °C for 5 h and was then allowed to cool to room temperature. The two layers that formed were separated, and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed successively with H_2O (2×), 10% aqueous $Na_2S_2O_3$, H_2O, and brine and dried ($MgSO_4$). The reddish residue (2.88 g) that remained was purified by column chromatography (silica gel, hexanes/EtOAc/$CHCl_3$ (5:2:1) (R_f = 0.39)). The appropriate fractions were combined and, upon solvent removal *in vacuo*, afforded **18** as a light yellow liquid which solidified upon standing: yield 2.50 g (58%); mp 111–113 °C; IR ($CHCl_3$) 3450, 3360 (amine), 2920, 2850 (aliphatic CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H, aryl 5-H), 4.78 (s, 2H, NH_2), 4.13 (q, 2H, OCH_2), 3.04 (ps t, 2H, $PhCH_2$), 2.40 (t, 2H, CH_2CO_2), 1.80 (pent, 2H, $PhCH_2CH_2$), 1.53 (m, 2H, $CH_2CH_2CO_2$), 1.26 (t, 3H, CH_3). Anal. ($C_{13}H_{16}O_2NI_3$) C, H.

5-(3-Amino-2,4,6-triiodophenyl)pentanoic Acid (5). Ester **18** (2.40, 4.0 mmol) was dissolved in refluxing 95% ethanol (75 mL) and treated with excess NaOH (640 mg, 16 mmol). The resulting mixture was refluxed for 1 h and then allowed to cool to room temperature. The sodium salt that formed was filtered and washed with cold (−25 °C) ethanol. The resulting white salt was dissolved in H_2O (100 mL) at 60 °C, and the resulting solution was acidified with 8 N HCl to a pH of 3–4 by litmus paper. The mixture was allowed to cool to room temperature, and the precipitate that formed was filtered, washed with cold ethanol, and dried *in vacuo* at 40 °C to afford **5** as a white solid: yield 1.80 g (79%); mp 145–147 °C; IR (KBr) 3500–2500 (broad OH), 3380, 3300 (amine), 2940, 2850 (aliphatic CH), 1690 (acid C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H, aryl 5-H), 4.80 (s, 2H, NH_2), 3.05 (m, 2H, $PhCH_2$), 2.47 (t, 2H, CH_2CO_2), 1.82 (pent, 2H, $PhCH_2CH_2$), 1.57 (m, 2H, $CH_2CH_2CO_2$). Anal. ($C_{11}H_{12}O_2NI_3$) C,H.

Preparation of 6-(3-Amino-2,4,6-triiodophenyl)hexanoic Acid (6). Ethyl 6-(3-nitrophenyl)hexanoate (15). Zinc (5.5 g, 84 mmol, Aldrich 99.99%) was suspended in a solution of 1,2-dibromoethane (1.1 g, 6 mmol) in anhydrous THF (5 mL), and the resulting mixture was heated to 65 °C for 1 min and allowed to cool to 30 °C. A solution of ethyl 5-iodopentanoate (17.0 g, 66 mmol) in anhydrous THF (15 mL) was added, and the resulting mixture was stirred under N_2 at 30 °C for 4 h. The resulting clear solution was added via cannula to a cooled (−78 °C) solution of CuCN (5.0 g, 55 mmol) and LiCl (5.0 g, 110 mmol) in anhydrous THF (40 mL). A solution of 3-nitrobenzyl bromide (10.8 g, 50 mmol) was then added, and the resulting reaction mixture was stirred at −25 °C for 2 h and then at room temperature overnight. The reaction mixture was quenched with aqueous NH_4Cl and extracted with ether. The ether extract was dried over $MgSO_4$ and placed *in vacuo* to remove the solvents. The resulting liquid residue was purified by column chromatography (silica gel 60) eluted with hexanes/EtOAc (50:1) to afford **15** as a light yellow oil which was used without further purification in the following reaction: yield 2.8 g (21%); IR ($CHCl_3$) 3010, 2970 (aryl CH), 2930, 2850 (aliphatic CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 2H, aryl 2,4-H), 7.46 (m, 2H, aryl 5,6-H), 4.12 (q, 2H, OCH_2), 2.72 (m, 2H, $PhCH_2$), 2.28 (m, 2H, CH_2CO_2), 1.67 (m, 4H, $PhCH_2CH_2$, $CH_2CH_2CO_2$), 1.41 (m, 2H, CH_2), 1.26 (t, 3H, CH_3).

Ethyl 6-(3-Aminophenyl)hexanoate (17). A solution of **15** (3.70 g, 13.9 mmol) in ethanol (75 mL) was hydrogenated at room temperature and 30 psi in the presence of 5% Pd–C (1.0 g) for 12 h. The catalyst was removed by filtration through a pad of Celite, and upon removal of solvent *in vacuo*, amino alkanoate **17** was isolated as a slightly yellow liquid which was homogeneous by TLC (silica gel, hexanes/EtOAc (5:2), R_f = 0.20): yield 2.68 g (82%); IR (neat) 3460, 3350 (amine), 2920, 2850 (aliphatic CH), 1730 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (m, 1H, aryl 5-H), 6.58 (d, 1H, aryl 6-H), 6.50 (m, 2H, aryl 2,4-H), 4.12 (q, 2H, OCH_2), 3.59 (s, 2H, NH_2), 2.50 (m, 2H, $PhCH_2$), 2.36 (t, 2H, CH_2CO_2), 1.73 (p, 2H, $PhCH_2CH_2$), 1.51 (m, 4H, $(CH_2)_2$), 1.25 (t, 3H, CH_3). Anal. ($C_{14}H_{21}O_2N$) C, H.

Ethyl 6-(3-Amino-2,4,6-triiodophenyl)hexanoate (19). A solution of ICl (9.16 g, 56 mmol) in 8 N HCl (50 mL) was diluted with $CHCl_3$ (40 mL) and heated to 60 °C in a three-necked round-bottom flask equipped with reflux condenser, addition funnel, and mechanical stirrer. A solution of amino ester **17** (2.5 g, 7.2 mmol) in $CHCl_3$ (40 mL) was then added dropwise over a period of 20 min. The resulting biphasic mixture was stirred rapidly at 60 °C for 5 h and was then allowed to cool to room temperature. The two layers that formed were separated, and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed successively with H_2O (2×), 10% aqueous $Na_2S_2O_3$, H_2O, and brine and dried ($MgSO_4$). The reddish residue (6.77 g) that remained was purified by column chromatography (silica gel, hexanes/EtOAc/$CHCl_3$ (4:1:1) (R_f = 0.39)). The appropriate fractions were combined and, upon solvent removal *in vacuo*, afforded **19** as a light yellow solid: yield 5.47 g (81%); IR ($CHCl_3$) 3460, 3360 (amine), 2920, 2840 (aliphatic CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H, aryl 5-H), 4.79 (s, 2H, NH_2), 4.13 (q, 2H, OCH_2), 3.02 (ps t, 2H, $PhCH_2$), 2.35 (t, 2H, CH_2CO_2), 1.72 (m, 2H, $PhCH_2CH_2$), 1.51 (m, 4H, $(CH_2)_2$), 1.26 (t, 3H, CH_3). Anal. ($C_{14}H_{18}O_2NI_3$) C, H.

6-(3-Amino-2,4,6-triiodophenyl)hexanoic Acid (6). Ester **19** (5.3 g, 8.6 mmol) was dissolved in refluxing 95% ethanol (150 mL) and treated with excess NaOH (1.42 g, 3.5 mmol). The resulting mixture was refluxed for 1 h and then allowed to cool to room temperature. The sodium salt that formed was filtered and washed with cold (−25 °C) ethanol. The resulting white salt (4.48 g, mp 250–260 °C w/decomp) was dissolved in H_2O (50 mL) at 80 °C, and the resulting solution was acidified with 8 N HCl to a pH of 3–4 by litmus paper. The mixture was allowed to cool to room temperature, and the precipitate that formed was filtered, washed with cold ethanol, and dried *in vacuo* at 40 °C to afford **6** as a white solid: yield 3.87 g (76%); mp 144–145 °C; IR (KBr) 3600–2800 (broad OH), 3420, 3330 (amine), 2910, 2830 (aliphatic CH), 1690 (acid C=O) cm^{-1}; ^1H NMR δ 8.03 (s, 1H, aryl 5-H), 4.80 (s, 2H, NH_2), 3.01 (bt, 2H, $PhCH_2$), 2.42 (t, 2H, CH_2CO_2), 1.73 (ps t, 2H, $PhCH_2CH_2$), 1.53 (m, 4H, $(CH_2)_2$). Anal. ($C_{12}H_{14}O_2NI_3$) C, H.

Preparation of 7-(3-Amino-2,4,6-triiodophenyl)heptanoic Acid (7). (ω-Carbethoxyheptylidene)triphenylphosphonium Bromide (20). Triphenylphosphine (106.9 g, 0.41 mol) was added to a solution of ethyl 5-bromopentanoate (100.0 g, 0.45 mol, Aldrich) in toluene (400 mL), and the resulting mixture was heated at reflux for 48 h under N_2. The majority of the solvent was removed by distillation, and acetonitrile (100 mL) was added to the residue. This solution was placed directly on a silica gel column (10 × 20 cm) and eluted first with ether (1.5 L) to remove excess triphenylphosphine, followed by acetonitrile to elute the phosphonium salt. The appropriate fractions were pooled and placed *in vacuo* to remove the solvent, thus affording salt **20** as a translucent amorphous solid which resisted crystallization. The phosphonium salt thus obtained was hygroscopic and was used without further purification: yield 154.0 g (78%).

Ethyl 7-(3-Nitrophenyl)-6-heptenoate (21). Phosphonium salt **20** was dissolved in anhydrous DMF (800 mL) in a flame-dried 1-L round-bottom flask under N_2 and cooled to −40 °C in an acetonitrile/dry ice bath. A solution of lithium diisopropylamide (158 mL, 0.32 mol, of a 2.0 M solution in THF/heptane/ethylbenzene, Aldrich) was added dropwise over a period of 1 h. The resulting reaction mixture was stirred for 30 min prior to the dropwise addition of a solution of 3-nitrobenzaldehyde (43.8 g, 0.29 mol) in dry DMF (100 mL) over a period of 30 min. The mixture was stirred an additional 4 h at −40 °C and was then allowed to warm to ambient temperature. After 36 h, the solvent was removed by rotary evaporation at 70 °C. The residue was triturated with ether and filtered. The resulting ethereal filtrate was placed *in*

vacuo to remove the solvent, and the liquid residue that remained was purified by column chromatography (silica gel, 10 × 25 cm), eluted initially with hexanes/CH_2Cl_2 (1:1) to remove a less polar impurity followed by hexanes/CH_2Cl_2/EtOAc (1:1:1) to elute the desired nitrophenyl alkenoate ester **21**: yield 51.0 g (63%): IR ($CHCl_3$) 2930, 2860 (aliphatic CH), 1735 (ester C=O) cm^{-1}; NMR (360 MHz, $CDCl_3$) δ 8.10 (m, 2H, aryl 2,4 H's), 7.55 (m, 2H, aryl 5,6 H's), 6.45 (m, 1.3H, trans C**H**=C**H** and cis PhC**H**=CH), 5.82 (dt, 0.7 H, cis CH=C**H**CH_2), 4.12 (q, 2H, OCH_2), 2.32 (m, 4H, =CHC**H**$_2$ and C**H**$_2CO_2$), 1.66–1.54 (m, 4H, $(CH_2)_2$), 1.24 (t, 3H, CH_3). Anal. ($C_{15}H_{19}O_4N$) C, H.

Ethyl 7-(3-Aminophenyl)heptanoate (22). A solution of **21** (50.25 g, 0.18 mol) in ethyl acetate (125 mL) was hydrogenated at room temperature and 30 psi in the presence of 5% Pd–C (3.0 g) for 12 h. The catalyst was removed by filtration through a pad of Celite, and upon removal of solvent *in vacuo*, amino alkane **22** was isolated as a colorless liquid which was homogeneous by TLC (silica gel, hexanes/EtOAc (5:2), R_f = 0.23): yield 45.0 g (99%); IR (neat) 3460, 3380 (amine), 2930, 2850 (aliphatic CH), 1730 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (m, 1H, aryl 5-H), 6.59 (d, 1H, aryl 6-H), 6.50 (m, 2H, aryl 2,4-H), 4.12 (q, 2H, OCH_2), 3.59 (s, 2H, NH_2), 2.50 (t, 2H, $PhCH_2$), 2.30 (t, 2H, CH_2CO_2), 1.67 (pent, 2H, $PhCH_2CH_2$), 1.47 (m, 6H, $(CH_2)_3$), 1.25 (t, 3H, CH_3). Anal. ($C_{15}H_{23}O_2N$) C, H.

Ethyl 7-(3-Amino-2,4,6-triiodophenyl)heptanoate (23). A solution of ICl (32.5 g, 0.20 mol) in 8 N HCl (175 mL) was diluted with $CHCl_3$ (200 mL) and heated to 60 °C in a three-necked round-bottom flask equipped with reflux condenser, addition funnel, and mechanical stirrer. A solution of amino ester **22** (10.0 g, 40 mmol) in $CHCl_3$ (100 mL) was then added dropwise over a period of 45 min. The resulting biphasic mixture was stirred rapidly at 60 °C for 5 h and was then allowed to cool to room temperature. The two layers that formed were separated, and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed successively with H_2O (2×), 10% aqueous $Na_2S_2O_3$, H_2O, and brine and dried ($MgSO_4$). TLC of this mixture (silica gel, hexanes/EtOAc (5:2)) indicated that the desired triiodinated product (R_f = 0.43) was accompanied by a more polar impurity (R_f = 0.30). Purification was performed on a silica gel column (10 × 30 cm) eluted with hexanes/$CHCl_3$ (1:1). The appropriate fractions were combined and, upon solvent removal *in vacuo*, afforded **23** as a light yellow liquid which solidified upon standing. Recrystallization from cold hexanes/EtOAc (5:2) gave off-white crystals. An analytical sample (30 mg) was obtained by semipreparative HPLC utilizing a Whatman M20 silica gel column eluted with hexanes/EtOAc/$CHCl_3$ (8:1:1): yield 17.6 g (70%); mp 70–71 °C; IR ($CHCl_3$) 3460, 3360 (amine), 2920, 2840 (aliphatic CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H, aryl 5-H), 4.78 (s, 2H, NH_2), 4.12 (q, 2H, OCH_2), 3.00 (ps t, 2H, $PhCH_2$), 2.30 (t, 2H, CH_2CO_2), 1.68 (pent, 2H, $PhCH_2CH_2$), 1.47 (m, 6H, $(CH_2)_3$), 1.25 (t, 3H, CH_3). Anal. ($C_{15}H_{20}O_2NI_3$) C, H.

7-(3-Amino-2,4,6-triiodophenyl)heptanoic Acid (7). Ester **23** (13.25 g, 21.1 mmol) was dissolved in refluxing 95% ethanol (600 mL) and treated with excess NaOH (3.4 g, 84 mmol). The resulting mixture was refluxed for 1 h and then allowed to cool to room temperature. The sodium salt that formed was filtered and washed with cold (−25 °C) ethanol. The resulting white salt (17.2 g) was dissolved in H_2O (300 mL) at 60 °C, and the resulting solution was acidified with 8 N HCl to a pH of 3–4 by litmus paper. The mixture was allowed to cool to room temperature, and the precipitate that formed was filtered and washed with cold ethanol to give **7** as a nearly white solid: yield 10.73 g (85%); mp 161–162 °C; IR (KBr) 3600–2800 (broad OH), 3400, 3300 (amine), 2920, 2850 (aliphatic CH), 1695 (acid C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H, aryl 5-H), 4.79 (s, 2H, NH_2), 3.01 (ps t, 2H, $PhCH_2$), 2.39 (t, 2H, CH_2CO_2), 1.70 (m, 2H, $PhCH_2CH_2$), 1.48 (m, 6H, $(CH_2)_3$). Anal. ($C_{13}H_{16}O_2NI_3$) C, H.

2-Oleoylglycerol 1,3-Bis[ω-(3-Amino-2,4,6-triiodophenyl)alkanoates] (24–30). General Procedure. A rapidly stirred suspension of 2-monoolein[28] (1.0 equiv), the carboxylic acid (2.05–2.1 equiv), and a catalytic amount of DMAP (0.1 equiv) in anhydrous CH_2Cl_2 (5 mL/mmol of alcohol) was treated with DCC (1.1 equiv to acid). The resulting mixture was stirred under N_2 overnight at room temperature, diluted with CH_2Cl_2, and filtered to remove precipitated DCU. The filtrate was washed with 0.5 N HCl, saturated aqueous $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the remaining residue was purified by column chromatography to afford the desired triacylglycerols.

2-Oleoylglycerol 1,3-Bis[2-(3-amino-2,4,6-triiodophenyl)acetate] (24). Treatment of a mixture of acid **1** (1.50 g, 2.8 mmol), 2-monoolein (481 mg, 1.35 mmol), and DMAP (37 mg) in anhydrous CH_2Cl_2 (20 mL) with DCC (635 mg, 3.1 mmol) according to the procedure described above gave a solid residue (1.56 g), which was purified by column chromatography on silica gel (4.5 × 30 cm) eluted with hexanes/EtOAc/$CHCl_3$ (35:10:5) to give a slightly yellow oil which resisted crystallization: yield 600 mg (32%); IR ($CHCl_3$) 3435, 3325 (amine), 2900, 2830 (aliphatic CH), 1730 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.05 (s,2H, aryl 5-H's), 5.4–5.2 (m,3H, CH=CH, glycerol 2-H), 4.80 (s, 4H, NH_2), 4.32 (dd, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.24 (s, 4H, $PhCH_2$'s), 4.15 (dd, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 2.30 (m, 6H, CH_2CO_2 and =CHC**H**$_2$'s), 2.0–1.0 (m, 22H, CH_2 envelope), 0.88 (t, 3H, CH_3). Anal. ($C_{37}H_{48}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[3-(3-amino-2,4,6-triiodophenyl)propionate] (25). Treatment of mixture of acid **2** (1.16 g, 2.1 mmol), 2-monoolein (356 mg, 1.0 mmol), and DMAP (24 mg) in anhydrous CH_2Cl_2 (15 mL) with DCC (444 mg, 2.15 mmol) according to the procedure described above gave a residue (1.50 g), which was purified by column chromatography on silica gel (4.5 × 30 cm) eluted with hexanes/EtOAc/$CHCl_3$ (16:2:1) to give a slightly yellow oil which resisted crystallization: yield 1.30 g (92%); IR ($CHCl_3$) 3440, 3330 (amine), 2905, 2830 (aliphatic CH), 1732 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.05 (s, 2H, aryl 5-H's), 5.4–5.2 (m, 3H, CH=CH, glycerol 2-H), 4.83 (s, 4H, NH_2's), 4.34 (dd, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.15 (dd, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.38 (dt, 4H, $PhCH_2$'s), 2.55 (dt, 4H, $OC(O)CH_2$'s), 2.35 (t, 6H, oleoyl CH_2CO_2 and =CHC**H**$_2$'s), 1.99 (pst, 4H, CH_2's), 1.29 (m, 22H, CH_2 envelope), 0.88 (t, 3H, CH_3). Anal. ($C_{39}H_{52}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[iopanoate] (26). Stirring a mixture of acid **3** (8.41 g, 14.7 mmol), 2-monoolein (2.50 g, 7.0 mmol), and DMAP (170 mg) in anhydrous CH_2Cl_2 (70 mL) with DCC (3.33 g, 16.0 mmol) according to the procedure described above for three days gave a residue (12.6 g), which was purified by column chromatography on silica gel (10 × 23 cm) eluted with hexanes/EtOAc/$CHCl_3$ (35:10:5) to give a nearly colorless oil which resisted crystallization: yield 5.37 g (53%); IR ($CHCl_3$) 3470, 3370 (amine), 2930, 2860 (aliphatic CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (270 MHz $CDCl_3$) δ 8.07 (d, 2H, aryl 5-H's), 5.32 (m, 2H, CH=CH), 5.19 (m, 1H, glycerol CH), 4.85 (s, 4H, NH_2), 4.30 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.10 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.32 (dq, 4H, $PhCH_2$'s), 2.78 (m, 2H, iopanoyl $CHCO_2$), 2.28 (t, 2H, oleoyl CH_2CO_2), 2.05 (m, 4H, allylic CH_2's), 1.85 (m, 2H, iopanoyl $CHCH_AH_BCH_3$),1.56 (m, 2H, iopanoyl $CHCH_AH_BCH_3$), 1.4–1.2 (m, 20H, CH_2 envelope), 0.88 (t, 9H, CH_3). Anal. ($C_{43}H_{60}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[4-(3-amino-2,4,6-triiodophenyl)butanoate] (27). Stirring a mixture of acid **4** (7.00 g, 12.6 mmol), 2-monoolein (2.19 g, 6.1 mmol), and DMAP (168 mg) in anhydrous CH_2Cl_2 (80 mL) with DCC (2.85 g, 13.8 mmol) according to the procedure described above for three days gave a residue (12.5 g), which was purified by column chromatography on silica gel (10 × 25 cm) eluted initially with 1 L of hexanes/EtOAc (5:1) and then with hexanes/EtOAc/$CHCl_3$ (75:15:10) to give a slightly yellow oil which resisted crystallization: yield 5.08 g (58%); IR ($CHCl_3$) 3470, 3370 (amine), 2930, 2860 (aliphatic CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.04 (s, 2H, aryl 5-H's), 5.33 (m, 3H, CH=CH, and glycerol 2-H), 4.81 (s, 4H, NH_2), 4.33 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.20 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.06 (m, 4H, $PhCH_2$'s), 2.48, (t, 4H, $)O_2CCH_2$'s), 2.33 (t, 2H oleate O_2CCH_2), 2.00 (m, 4H, allylic CH_2's), 1.84

(m, 4H, $PhCH_2CH_2$), 1.26 (d, CH_2 envelope), 0.89 (t, 3H, CH_3). Anal. ($C_{41}H_{56}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[5-(3-amino-2,4,6-triiodophenyl)pentanoate] (28). DCC (635 mg, 3.1 mmol) was added to a stirred suspension of acid **5** (1.60 g, 2.8 mmol), 2-monoolein (480 mg, 1.3 mmol), and DMAP (50 mg) in anhydrous CH_2Cl_2 (45 mL) according to the procedure described above for 36 h. Following workup, a residue (12.5 g) was obtained, which was purified by column chromatography on silica gel (4.6 × 42 cm) eluted with hexanes/EtOAc/$CHCl_3$ (80:15:5) to give a slightly yellow oil which resisted crystallization: yield 1.35 g (71%); IR ($CHCl_3$) 3475, 3375 (amine), 2940, 2850 (aliphatic CH), 1738 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.03 (s, 2H, aryl 5-H's), 5.26 (m, 3H, CH=CH, and glycerol 2-H), 4.79 (s, 4H, NH_2), 4.31 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.16 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.02 (m, 4H, $PhCH_2$'s), 2.43 (t, 4H, O_2CCH_2's), 2.30 (t, 2H, oleate O_2CCH_2), 2.01 (m, 4H, allylic CH_2's), 1.80 (m, 4H, $PhCH_2CH_2$'s), 1.62–1.24 (m, CH_2 envelope), 0.86 (t, 3H, CH_3). Anal. ($C_{43}H_{60}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[6-(3-amino-2,4,6-triiodophenyl)hexanoate] (29). DCC (801 mg, 3.9 mmol) was added to a stirred suspension of acid **6** (2.14 g, 3.7 mmol), 2-monoolein (620 mg, 1.7 mmol), and DMAP (70 mg) in anhydrous CH_2Cl_2 (45 mL) according to the procedure described above for 48 h. Following workup, a residue (3.26 g) was obtained, which was purified by column chromatography on silica gel (3 × 25 cm) eluted with hexanes/EtOAc/$CHCl_3$ (80:15:5) to give a slightly yellow oil which resisted crystallization: yield 2.04 g (81%); IR ($CHCl_3$) 3475, 3375 (amine), 2940, 2850 (aliphatic CH), 1738 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.03 (s, 2H, aryl 5-H's), 5.30 (m, 3H, CH=CH, and glycerol 2-H), 4.79 (s, 4H, NH_2), 4.31 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.16 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.01 (m, 4H, $PhCH_2$'s), 2.34 (m, 6H, O_2CCH_2's and oleate O_2CCH_2), 2.00 (m, 4H, allylic CH_2's), 1.72 (m, 4H, $PhCH_2CH_2$'s), 1.60, 1.50, 1.27 (m, CH_2 envelope), 0.87 (t, 3H, CH_3). Anal. ($C_{45}H_{64}O_6N_2I_6$) C, H.

2-Oleoylglycerol 1,3-Bis[6-(3-amino-2,4,6-triiodophenyl)heptanoate] (30). DCC (3.62 g, 17.5 mmol) was added to a stirred suspension of acid **7** (10.0 g, 16.7 mmol), 2-monoolein (2.83 g, 7.9 mmol), and DMAP (180 mg) in anhydrous CH_2Cl_2 (120 mL) according to the procedure described above for 24 h. Following workup, a residue (14.7 g) was obtained, which was purified by column chromatography on silica gel (10 × 25 cm) eluted with hexanes/EtOAc/$CHCl_3$ (80:15:5) to give a slightly yellow oil which resisted crystallization: yield 9.45 g (79%); IR ($CHCl_3$) 3450, 3359 (amine), 2915, 2840 (aliphatic CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.03 (s, 2H, aryl 5-H's), 5.30 (m, 3H, CH=CH, and glycerol 2-H), 4.79 (s, 4H, NH_2), 4.31 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 4.16 (m, 2H, glycerol $OCH_AH_BCH(O)CH_AH_BO$), 3.00 (m, 4H, $PhCH_2$'s), 2.32 (m, 6H, O_2CCH_2's and oleate O_2CCH_2), 2.00 (m, 4H, allylic CH_2's), 1.72 (m, 4H, $PhCH_2CH_2$'s), 1.61–1.26 (m, CH_2 envelope), 0.88 (t, 3H, CH_3). Anal. ($C_{47}H_{68}O_6N_2I_6$) C, H.

Radioiodide Exchange in Pivalic Acid Melt.[25] General Procedure. The compound to be radiolabeled (1 mg) was placed in a 300-μL V-vial fitted with Teflon-faced seal and screw cap. Freshly distilled THF (50 μL) was added via a microliter syringe followed by aqueous $Na^{125}I$ (2–10 μL no carrier-added in reductant free 0.1 N NaOH from Amersham Radiochemicals). The vial was gently swirled to dissolve the contents and ensure homogeneity. Inlet and outlet cannuli were inserted, and a gentle stream of nitrogen was applied to remove the solvents. Two successive in-line charcoal traps were placed on the outlet side in order to trap any volatile radioiodine present in the reaction vial. Once dry, solid pivalic acid (10 mg, Aldrich), previously dried by azeotropic removal of water with toluene and distilled under nitrogen, was added. The vial was sealed and heated at 155 °C in a preheated aluminum heating block containing 1 cm of sand in the bottom of the well. After 1.0 h, the reaction vial was removed from the heating block and allowed to cool to room temperature. THF (100 μL) was added via micro syringe followed by gentle agitation and subsequent removal of a TLC sample (1–2 μL). The entire contents of the reaction vial were then transferred to the top of a silica gel 60 column (1 × 10 cm) and eluted with an appropriate solvent system. Radiochemical purity of each fraction was monitored by TLC (γ and UV detection) and appropriate fractions were combined and the solvent removed with a gentle stream of nitrogen. HPLC analysis of the final compound confirmed both chemical (UV) and radiochemical (radioactivity) purity. Specific activity of injected compounds ranged from 300 to 1100 μCi/mg for iodinated triglycerides **24**–**30**. In all cases, radiochemical purity of final compounds exceeded 96%.

General Method for the Preparation of a 10% (w/v) Total Triglyceride Emulsion. The following example utilizing ITG **30** illustrates the general procedure for incorporating the ITG analogs into a chylomicron remnant-like emulsion. Briefly, analog **30** (0.5001 g of nonradioactive and 0.2 mg of ^{125}I-labeled), triolein (0.5028 g), cholesterol (0.0472 g), and α-tocopherol (0.0619 g) were weighed into a tared 29/42–50 mL tube. A solution of 1,2-dioleoylglycero-3-phosphocholine (0.2400 g) in 2.4 mL of C_2H_5OH was added to the tube along with 4.8 mL of EtOAc to dissolve the emulsion components. The tube was connected to a rotary evaporator to remove the solvents under vacuum at 40 °C over 1–2 h. The tube was detached from the rotavap and tared before addition of 0.5003 g of USP glycerol to the lipid mixture. This mixture was emulsified for 5 min under a blanket of N_2 below 50 °C at 12 500 rpm with a Polytron homogenizer. A 6.0 mL aliquot of sterile water was added to the lipid–glycerol homogenate with continuous mixing before 5 min of further emulsification at 25 000 rpm under the same conditions. The Polytron generator was rinsed into the tube with approximately 2.0 mL of sterile water, and the combined volume was then adjusted to 10.0 mL with additional sterile water. The rough emulsion was transferred to the Microfluidics Model 110-S Microfluidizer, where it was processed for 10 min at 14 700 psi between 34.2 and 35.6 °C. The emulsion was removed by syringe for sequential filtration through sterile 0.45 and 0.2 μm Gelman Acrodisc filters into a 10 mL sterile multidose vial. The final emulsion was stored at ambient temperature overnight before sizing by Photon Correlation Spectroscopy on a Nicomp Model 370 unit. Mean particle size of the final ITG emulsions ranged from 88–195 nm as determined by Nicomp number intensity calculations.

Tissue Distribution Studies. The radiolabeled emulsion was administered intravenously to adult female Sprague–Dawley rats (Harlen Sprague–Dawley, Hasslet, MI) weighing from 190–250 g. Groups of from three to five rats were used for each compound at each time period. Doses typically ranged from 4.5–6 μCi (10–45 μg) per animal. Overall ITG dose (radiolabeled and unlabeled) administered per rat was 30 mg of I/kg of body weight. The rats were euthanized by exsanguination under ether anesthesia at predetermined time points, and the desired organs were removed and blotted free of excess blood. Large organs were minced with scissors. Weighed tissue samples were placed in cellulose acetate capsules and analyzed for radioiodine content in a Searle-1195 well scintillation counter (84% efficiency).

Plasma and Tissue Extraction. Radioactivity was extracted from plasma by a modified Folch procedure described previously.[15] Liver samples were homogenized, extracted, and analyzed by TLC with hexanes/ethyl acetate (5:2) as eluent. Following development, the plates were air-dried and analyzed on a Bioscan Model 200 radio-TLC scanner (Washington, DC). In all cases, the unlabeled compound served as a UV reference standard. Results are expressed as a percentage of total radioactivity on each plate.

Plasma Electrophoresis. Polyacrylamide gel electrophoresis of plasma samples was performed according to the method previously described.[29] The amount of radioactivity associated with each lipoprotein class was determined by sectioning the gels and counting each section in a γ-counter. The radioactivity associated with each lipoprotein band is expressed as a percentage of the total radioactivity applied to the gel.

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References

(1) Seltzer, S. E.; Shulkin, P. M.; Adams, D. F.; Davis, M. A.; Hoey, G. B.; Hopkins, R. M.; Bosworth, M. E. Usefulness of liposomes carrying Iosefamate for CT opacification of liver and spleen. *Am. J. Roentgenol.* **1984**, *143*, 575–79.
(2) Cohen, Z.; Seltzer, S. E.; Davis, M. A.; Hanson, R. N. Iodinated starch particles: new contrast material for computed tomography of the liver. *J. Comput. Assis. Tomogr.* **1981**, *5*, 843–46.
(3) Violante, M. R.; Mare, K.; Fischer, H. W. Biodistribution of a particulate hepatographic CT contrast agent: a study of iodipamide ethyl ester in the rat. *Invest. Radiol.* **1981**, *16*, 40–45.
(4) Vermess M. New contrast material improves detection of liver and spleen metastases. *J. Am. Med. Assoc.* **1984**, *251* (6), 707–708.
(5) Sugarbaker, P. H.; Vermess, M.; Doppman, J. L.; Miller, D. L.; Simon, R. Improved detection of focal lesions with computerized tomographic examination of the liver using ethiodized oil emulsion (EOE-13) liver contrast. *Cancer* **1984**, *54* (8), 1489–95.
(6) Miller, D. L.; Vermess, M.; Doppman, J. L.; Simon, R. M.; Sugarbaker, P. H.; Oleary, T. J.; Grimes, G.; Chatterji, D. G.; Willis, M. CT of the liver and spleen with EOE-13: review of 225 examinations. *Am. J. Roentgenol.* **1984**, *143* (2), 235–43.
(7) Spector, A. A. Plasma lipid transport. *Clin. Physiol. Biochem.* **1984**, *2*, 123–34.
(8) Brown, M. S.; Kovanen, P. T.; Goldstein, J. L. Regulation of plasma cholesterol by lipoprotein receptors. *Science* **1981**, *212*, 628–35.
(9) Kinnunen, P. J.; Virtauen, J. A.; Vainio, P. Lipoprotein lipase and hepatic endothelial lipase: their roles in plasma lipoprotein metabolism. *Atherosclerosis Rev.* **1983**, *11*, 65.
(10) Cooper, A. D. Hepatic clearance of plasma chylomicron remnants. *Sem. Liver Disease* **1992**, *12* (4), 386–96.
(11) Floren, C. H.; Nilsson, A. Binding, internalization, and degradation of cholesteryl ester-labelled chylomicron remnant particles by rat hepatocyte monolayers. *J. Biochem.* **1977**, 168, 483–94.
(12) El Maghrabi, M. R.; Waite, M.; Rudel, L. C.; King, V. L. Metabolism of lipoprotein acylglycerols by liver parenchymal cells. *Biochem. Biophys. Acta* **1979**, *572*, 52–63.
(13) Lippiello, P. M.; Dijkstra, J.; Van Galen, M.; Scharphof, G.; Waite, M. The uptake and metabolism of chylomicron remnant lipids by nonparenchymal cells in perfused liver and by Kupffer cells in culture. *J. Biol. Chem.* **1981**, *256*, 7454–60.
(14) Counsell, R. E.; Pohland, R. C. Lipoproteins as potential site-specific delivery systems for diagnostic and therapeutic agents. *J. Med. Chem.* **1982**, *25*, 1115–20.
(15) Seevers, R. H.; Schwendner, S. W.; Swayze, S. L.; Counsell, R. E. Potential tumor- or organ imaging agents. 22. Acyl labeled esters of cholesterol. *J. Med Chem.* **1982**, 25, 618–21.
(16) Seevers, R. H.; Groziak, M. P.; Weichert, J. P.; Schwendner, S. W.; Longino, M. A.; Counsell, R. E. Potential tumor- or organ imaging agents. 23. Sterol esters of iopanoic acid. *J. Med. Chem.* **1982**, *25*, 1500–03.
(17) Damle, N. S.; Seevers, R. H.; Schwendner, S. W.; Counsell, R. E. Potential tumor- or organ imaging agents. 24. Chylomicrons as carriers for hepatographic agents. *J. Pharm. Sci.* **1983**, *72*, 898–901.
(18) Longino, M. A.; Weichert, J. P.; Schwendner, S. W.; Szabo, S. M.; Counsell, R. E.; Glazer, G. M. Biodistribution of new lipid-soluble CT contrast agents: Evaluation of cholesteryl iopanoate in the rabbit. *Invest. Radiol.* **1983**, *18*, 275–78.
(19) Glazer, G. M.; Longino, M. A.; Schwendner, S. W.; Counsell, R. E.; Weichert, J. P. Lipid soluble contrast agents for CT scanning of the liver: Results with cholesteryl iopanoate. *J. Comput. Assis. Tomogr.* **1983**, *7*, 775–79.
(20) DeGalan, M. R.; Schwendner, S. W.; Skinner, R. W.; Longino, M. A.; Gross, M.; Counsell, R. E. Iodine-125 cholesteryl iopanoate for measuring extent of atherosclerosis in rabbits. *J. Nucl. Med.* **1988**, *29* (4), 503–8.
(21) Weichert, J. P.; Longino, M. A.; Schwendner, S. W.; Counsell, R. E. Potential tumor- or organ imaging agents. 26. Polyiodinated 2-substituted triacylglycerols as hepatographic agents. *J. Med. Chem.* **1986**, *29*, 1674–82.
(22) Weichert , J. P.; Groziak, M. P.; Longino, M. A.; Schwendner, S. W; Counsell, R. E. Potential tumor- or organ imaging agents. 27. Polyiodinated 1,3-di- and 1,2,3-trisubstituted triacylglycerols. *J. Med. Chem.* **1986**, *29*, 2457–65.
(23) Strawn, L. M.; Martell, R. E.; Simpson, R. U.; Leach, K. L.; Counsell, R. E. Iodoaryl analogues of dioctanoylglycerol and 1-oleoyl-2-acetylglycerol as probes for protein kinase C. *J. Med. Chem.* **1989**, *32*, 2104–10.
(24) Knochel, P.; Yeh, M. C. P.; Berk, S. C.; Talbert, J. Synthesis and reactivity toward acyl chlorides and enones of the new highly functionalized copper reagents RCu(CN)ZnI. *J. Org. Chem.* **1988**, *53*, 2392–94.
(25) Weichert, J. P.; Van Dort, M. E.; Groziak, M. P.; Counsell, R. E. Radioiodination via isotope exchange in pivalic acid. *Int. J. Appl. Radiat. Isot.* **1986**, *37* (8), 907–913.
(26) Pitre, D.; Fumagalli, L. Methods of radiological contrast. IV. 2,4,6-Triiodo-3-pyridiyl-oxyacetic acid. *Farmaco (Pavia) Ed. Sci.* **1963**, *18* (7), 495–98.
(27) Cassebaum, H.; Dierback, K. Röntgenkontrastmittel auf der basis jierter phenoxyfettsäuren zimtsäuren und hydrozimtsäuren. *Die Pharmazie* **1961**, *16*, 389.
(28) Sederivich, C. Glyceride isomerizations in lipid chemistry. *J. Am. Oil Chem. Soc.* **1967**, *44*, 381.
(29) Counsell, R. E.; Schappa, L. W.; Korn, N.; Huler, R. J. Tissue distribution of high-density lipoprotein labeled with radioiodinated cholesterol. *J. Nucl. Med.* **1980**, *21*, 852–8.

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