Incubation was stopped by adding Dextran-coated charcoal. After centrifugation the radioactivity of a 100-μL supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion on a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and of test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth and the inhibition of the uterine growth stimulated by estrone, respectively, with immature NMRI mice as described previously.[20] Twenty-day-old female mice (weight 15.0 ± 2.5 g, mean ± SD) were randomly distributed into groups of 10 animals. They were subcutaneously (sc) injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

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Registry No. 1, 102808-03-7; 1a, 102808-10-6; 1b, 18228-46-1; 1d, 2040-20-2; 1e, 102808-11-7; 2, 102808-04-8; 2a, 86439-85-2; 2b, 6013-92-9; 3, 102808-05-9; 3a, 102808-12-8; 3b, 14248-29-4; 4, 102808-06-0; 4a, 102808-13-9; 4b, 100757-90-2; 5, 102808-07-1; 5a, 102808-14-0; 5b, 61751-33-5; 5d, 6026-75-1; 5e, 102808-15-1; 6, 102808-08-2; 6a, 102808-16-2; 6b, 32578-11-3; 6c, 102808-17-3; 7, 102808-09-3; 7a, 102808-18-4; 7b, 102808-19-5; 8, 78682-45-8; 8a, 78682-41-4; 8b, 78682-47-0; 2-iodobutane, 513-48-4; 4-methoxybenzaldehyde, 123-11-5; 3-methoxybenzaldehyde, 591-31-1; *tert*-butyl bromide, 507-19-7; isobutyl bromide, 78-77-3; 2-bromopropane, 75-26-3; 2-bromobutane, 78-76-2; 2-chloro-2-methylpropane, 507-20-0.

Supplementary Material Available: ^{1}H NMR data (Tables VI and VII) of compounds 1–8, 1a–8a, 1e, and 5e (5 pages). Ordering information is given on any current masthead page.

Potential Tumor- or Organ-Imaging Agents. 26. Polyiodinated 2-Substituted Triacylglycerols as Hepatographic Agents[1]

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A series of ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids and the corresponding 1,3-dipalmitoylglycerol 2-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] were synthesized, radioiodinated with iodine-125, and evaluated for their ability to selectively localize in the liver for potential use as hepatographic imaging agents. Acid analogues **1d** and **1e** afforded relatively high levels of radioactivity in the liver (45 and 49% injected dose) 5 min after intravenous administration to rats. These acids displayed a marked propensity to become bound to plasma albumin. In contrast, triacylglycerol analogues **10a** and **10c** did not become immediately associated with plasma albumin but instead rapidly became associated with plasma lipoproteins and showed a different tissue distribution profile than free acids **1a** and **1c**. Although long-chain triacylglycerol analogues **10d** and **10e** exhibited some capacity to accumulate in the liver at 5 and 30 min, respectively, analysis of the plasma revealed significant in vivo ester hydrolysis. It would thus appear that liver radioactivity following administration of **10d** and **10e** was due to uptake of the free acid and not the intact triacylglycerol. Triacylglycerol analogues **10a** and **10c**, on the other hand, were taken up intact and showed liver accumulations of 25 and 35% of the administered dose at 30 min.

Among the imaging modalities currently in widespread use, radiography and radioscintiscanning owe much of their success to the radiopaques (contrast agents) and radiopharmaceuticals used in conjunction with these procedures. A major pharmacological difference between radiopaques and radiopharmaceuticals is that large doses of the former are required for opacification of soft tissues, whereas radiopharmaceuticals are administered in tracer doses. In each case, the procedure generally depends on the ability of the agent to selectively accumulate in the organ under consideration.

The most widely used liver scintigraphy agent, [^{99m}Tc]sulfur colloid, is removed from the circulation by the phagocytic action of reticuloendothelial (Kupffer) cells. The static anatomic image thus obtained proves useful in the location of primary and secondary lesions as small as 2–3 cm in diameter, but has no utility in assessing hepatocyte function. Hepatocyte function can, however, be studied by monitoring the uptake and clearance of [^{131}I]-rose bengal, a dye which is extracted by hepatocytes and, under normal conditions, is quickly secreted into the bile.[2] Problems with this agent have recently been documented and mainly concern the slow rate of hepatic-to-intestinal output and the poor imaging characteristics and high radiation doses associated with iodine-131.[3] The need for a better hepatobiliary agent eventually led to the development of the ^{99m}Tc-labeled iminodiacetic acid (IDA) analogues. As a group, the [^{99m}Tc]Ida analogues improve hepatobiliary imaging, but they are less useful for the quantitative study of hepatocyte function because of uptake by extrahepatic tissues, variable urinary excretion, and presence of varying amounts of free ^{99m}Tc.[4] Furthermore, plasma clearance of these agents by the hepatocytes occurs via the anionic pathway and thus competes with serum bilirubin for uptake.[5] Therefore, as serum

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(1) Taken in part from the doctoral dissertation of JPW, and presented in part at the 186th National Meeting of the American Chemical Society, Washington, DC, 1983, and the Fourth International Symposium on Radiopharmacology, Banff, Alberta, Canada, 1985.

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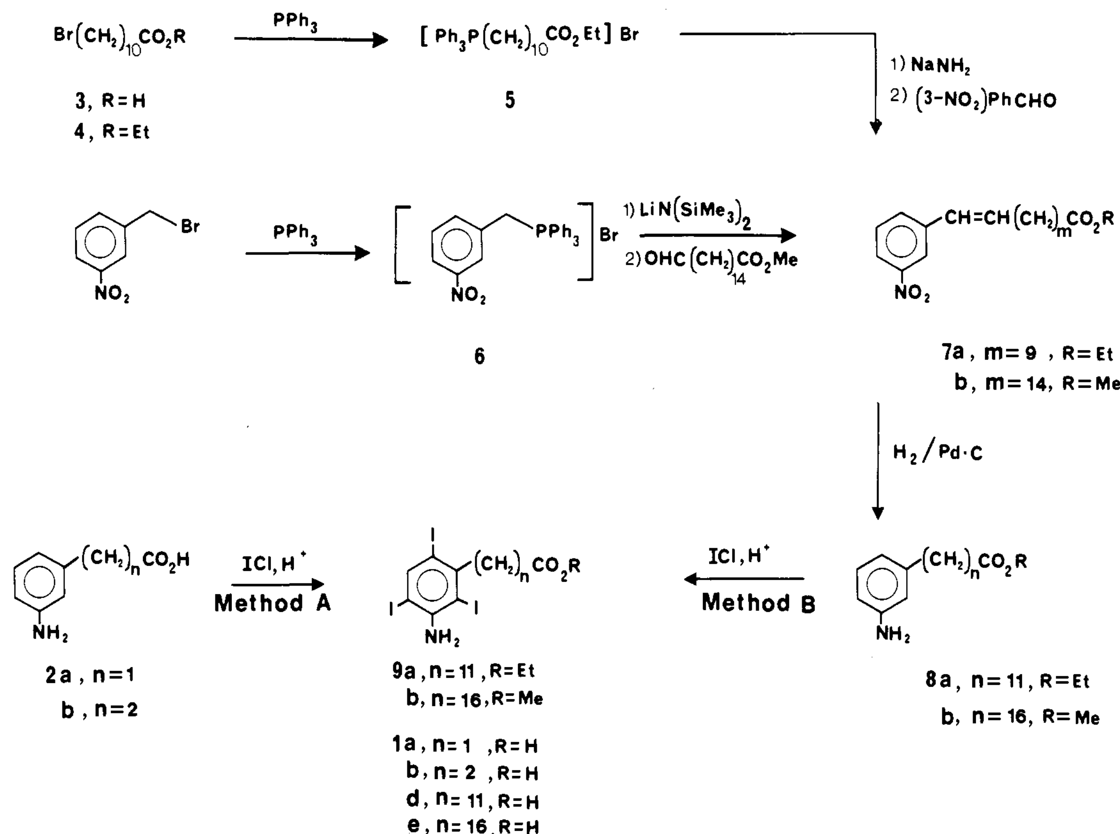
Scheme I. Synthesis of ω-(3-Amino-2,4,6-triiodophenyl)alkanoic Acids



bilirubin levels increase as observed in many hepatic disease states (e.g., jaundice), hepatocyte uptake of these agents decreases and renal clearance becomes more prevalent.[6,7]

Computed tomography (CT), on the other hand, has generally relied on water-soluble contrast agents for opacification of soft tissues and lesions.[8-10] These agents have been used because of their relative safety and availability. Problems with these agents, however, include short duration of contrast enhancement and lack of organ specificity. Contrast enhancement with water-insoluble agents such as radiopaque liposomes,[11] iodinated starch particles,[12] iodipamide ethyl ester,[13] and ethiodized oil emulsions[14-16] has achieved only limited success. Consequently, there remains a need for both a hepatocyte-specific scintigraphic agent and a liver-specific CT agent.

Our strategy for the site-specific delivery of radiologic agents to organs or tumors is based on a biochemical approach whereby naturally occurring compounds known to be metabolized and or synthesized in specific tissues serve as carriers for the radiologic probe. Accordingly, the aim of this research was to synthesize and evaluate various compounds for their ability to selectively localize in the liver. Cholesteryl esters and triacylglycerols were viewed as appropriate liver-specific carrier molecules, since these lipids are known to be transported in the plasma as constituents of lipoproteins, which in turn are sequestered and metabolized by the liver.

Previous papers have described various polyiodinated esters of cholesterol.[17,18] The best agent in this series was cholesteryl iopanoate (CI). Preliminary tracer studies with radioiodinated CI in the rat revealed that this agent was rapidly taken up by the liver (30% administered dose at 30 min).[18] Furthermore, prior incorporation of this agent into chylomicra revealed an almost 3-fold (87% of the administered dose) enhancement in liver uptake in the same time period.[19] Following CT studies with radiologic doses of CI in rabbits showed high liver extraction resulting in significant CT enhancement of the liver. Furthermore, its preferential accumulation in normal liver tissue subsequently made it possible to visualize hepatic tumors as small as 2 mm in diameter in rabbits.[20,21] A downfall of

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CI, however, was that it was so resistant to in vivo hydrolysis that it remained chemically unchanged in the liver for up to 5 months after administration.[21]

Based on these results, experiments were initiated to evaluate the potential of polyiodinated triacylglycerols as hepatographic agents. Triacylglycerols (TG) are primarily transported in the plasma as components of chylomicra (CM) and very low density lipoproteins (VLDL). These lipoproteins are metabolized by peripheral tissue lipoprotein lipase (LPL), which hydrolyzes the entrapped TG liberating free fatty acids for cellular metabolism.[22] Based on the finding that peripheral LPL is known to preferentially hydrolyze glyceryl esters at the 1- and 3-positions,[23] initial efforts focused on a series of polyiodinated triacylglycerols **10a–e** (Scheme II) modified in the 2-position. It was reasoned that if these modified triacylglycerols were also substrates for LPL, only the acyl groups in the 1- and 3-positions would be lost and the resulting radioiodinated 2-monoacylglycerol analogue would remain in the lipoprotein remnant to be rapidly sequestered by the liver.

Employing iopanoic acid as the prototype acid, additional polyiodinated phenylalkanoic acids shown in Scheme I were synthesized and subsequently esterified with 1,3-dipalmitoyl glycerol. Desethyliopanoic acid analogue **10b** was chosen for comparison with iopanoate **10c** in order to ascertain the importance of the α-ethyl group toward in vivo enzymatic hydrolysis. In addition, the chain length of the acids was varied, in order to gain insight into the relationship of this change to the relative rates of in vivo ester hydrolysis. It was viewed that the lability of these triacylglycerol analogues to hydrolysis could be an important determinant in the time of retention of such agents in the liver.

Chemistry. Synthesis of iodinated acids **1a,b,d,e** was accomplished by iodination of the appropriate (aminophenyl)alkanoic acid (method A) or the corresponding ester (method B) as shown in Scheme I. For example, synthesis of 3-amino-2,4,6-triiodophenylacetic (**1a**) and propanoic (**1b**) acids was accomplished by catalytic hydrogenation of the corresponding 3-(nitrophenyl)alkanoic acid followed by iodination with iodine monochloride in aqueous acid according to a procedure initially described by Lewis and Archer[24] and later modified by Wallingford and co-workers.[25]

Synthesis of extended-chain analogues **1d** and **1e** was accomplished by employing a Wittig reaction as a key step. As outlined in Scheme I, conversion of **3** to ethyl ester **4** and subsequent treatment with triphenylphosphine according to the method of Bergelson and Shemyakin[26] afforded (ω-carbethoxyundecylidine)triphenylphosphonium bromide (**5**) in nearly quantitative yield. Treatment of phosphonium salt **5** with sodium amide gave the corresponding (ω-carbethoxyundecylidene)triphenylphosphorane, which upon condensation with 3-nitrobenzaldehyde afforded (nitrophenyl)alkenoate **7a**. Although a variety of bases, including NaH, KH, NaOEt, and LDA, were effective for ylide formation on a small scale, only sodium amide gave acceptable yields of **7a** when conducted on a multigram scale.

When these findings were coupled with the general unavailability and/or high cost of similar types of starting materials, an alternate route of synthesis was sought for formation of heptadecenoate analogue **7b**. As shown in Scheme I, synthesis of **7b** involved the formation of (3-nitrobenzyl)triphenylphosphonium bromide (**6**) from 3-nitrobenzyl bromide and subsequent Wittig condensation of the corresponding phosphorane with methyl 16-oxohexadecanoate.

Preparation of methyl 16-oxohexadecanoate was achieved by a method similar to that recently reported by Waugh and Berlin,[27] whereby treatment of a solution of hexadecanoic acid lactone (dihydroambrettolide, Columbia Organics, Camden, SC) in methanol with a catalytic amount of p-toluenesulfonic acid under reflux conditions afforded methyl 16-hydroxyhexadecanoate. Oxidation of the alcohol with pyridinium chlorochromate according to the method of Corey and Suggs[28] afforded reasonably pure methyl 16-oxohexadecanoate. Any attempt to further purify the aldehyde by distillation or crystallization resulted in decomposition. Treatment of phosphonium salt **6** with lithium bis(trimethylsilyl)amide and treatment of the resulting ylide with methyl 16-oxohexadecanoate gave a good yield of (nitrophenyl)alkenoate **7b**. Catalytic hydrogenation of ω-(nitrophenyl)alkenoate esters **7a** and **7b** afforded high yields of ω-(3-aminophenyl)alkenoate esters **8a** and **8b**, respectively.

The subsequent iodination of long-chain analogues **8a** and **8b** was carried out on the esters according to Scheme I (method B). In contrast to method A which was conducted entirely in an aqueous acid medium, method B utilized chloroform as a cosolvent with the aqueous acid,[24] and therefore the iodination occurs in a biphasic medium. Preparation of iodinated dodecanoate **9a** under these conditions revealed after purification a minor impurity, which upon isolation and subsequent ^{1}H NMR analysis was found to be the diiodinated product, ethyl 12-(3-amino-4,6-diiodophenyl)dodecanoate. This type of impurity was not found upon formation of iodinated heptadecanoate **9b**, however, and in no case was competing hydrolysis of the ester detected. Hydrolysis of esters **9a** and **9b** with alcoholic NaOH in THF followed by acidification gave good yields of ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids **1d** and **1e** as crystalline solids.

The 1,3-dipalmitoylglycerol 2-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (**10a–e**) were synthesized via DCC/DMAP[29–31] coupling of dipalmitin (1,2,3–-trihydroxypropane 1,3-dipalmitate) with the corresponding ω-(3-amino-2,4,6-triiodophenyl)alkanoic acid as shown in Scheme II. Dipalmitin was prepared by diacylation of dihydroxyacetone with palmitoyl chloride followed by borohydride reduction of the resulting ketone according to the method of Bentley and McCrae.[32] Isolated yields as well as some physical characteristics of the target triacylglycerol analogues are included in Table I.

In order to evaluate their ability to selectively localize in the liver, esters **10a–e** as well as free acids **1a–e** were radioiodinated with iodine-125 by isotope exchange in a melt of pivalic acid.[33]

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Scheme II. Synthesis of 2-Monosubstituted Triacylglycerols

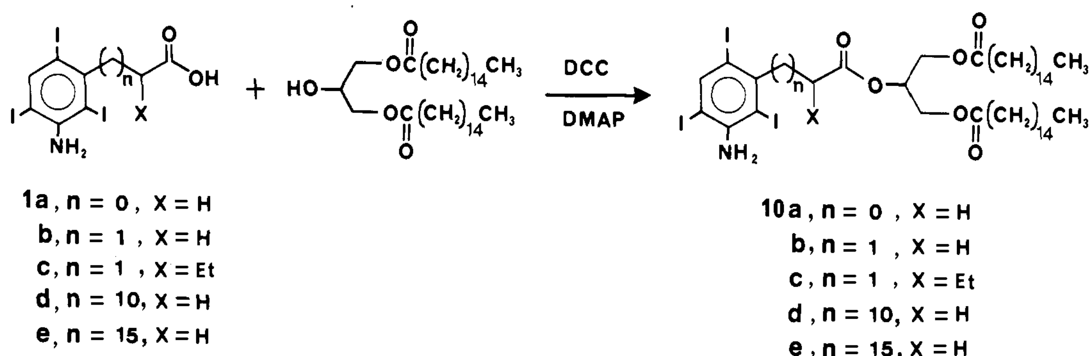

Table I. Yields and Physical Data of 1,3-Dipalmitoylglycerol 2-[ω-(3-Amino-2,4,6-triiodophenyl)alkanoates]

entry	n	X	yield, %	mp, °C	TLC[a]	formula	anal.[b]
10a	0	H	57	84–85.5	0.57	$C_{43}H_{72}I_3NO_6$	C, H, I
10b	1	H	70	74.5–75	0.50	$C_{44}H_{74}I_3NO_6$	C, H, I
10c	1	Et	75	55–57	0.63	$C_{46}H_{78}I_3NO_6$	C, H, I
10d	10	H	49	56–59	0.54	$C_{53}H_{92}I_3NO_6$	C, H, I
10e	15	H	52	65–67	0.57	$C_{58}H_{102}I_3NO_6$	C, H, I

[a] R_f given on silica gel with hexane/ethyl acetate (5:2). [b] Analysis within ±0.4% for elements listed.

Table II. Distribution of Radioactivity at 5 and 30 min after iv Administration of ^{125}I-Labeled ω-(3-Amino-2,4,6-triiodophenyl)alkanoic Acids to Female Rats[a]

tissue	compd 1a	1b	1c	1d	1e
			5 min		
blood	5.44 ± 0.37	2.52 ± 0.14	2.19 ± 0.10	1.21 ± 0.07	2.25 ± 0.10
heart	1.09 ± 0.08	4.00 ± 0.37	0.74 ± 0.03	1.15 ± 0.10	1.06 ± 0.10
kidney	4.62 ± 0.64	2.44 ± 0.09	0.71 ± 0.02	0.77 ± 0.04	0.48 ± 0.01
lung	1.49 ± 0.08	1.02 ± 0.06	0.81 ± 0.05	0.95 ± 0.07	1.11 ± 0.06
liver	0.87 ± 0.06	1.08 ± 0.11	1.64 ± 0.10	6.96 ± 0.31	6.30 ± 0.21
	(6.40 ± 0.47)[b]	(7.85 ± 0.54)	(13.43 ± 0.90)	(45.64 ± 1.38)	(49.40 ± 1.61)
			30 min		
blood	2.41 ± 0.08	1.15 ± 0.05	1.21 ± 0.08	1.06 ± 0.04	1.23 ± 0.07
heart	0.72 ± 0.04	4.14 ± 0.29	0.46 ± 0.03	0.73 ± 0.07	1.01 ± 0.05
kidney	5.82 ± 0.63	1.54 ± 0.10	0.52 ± 0.02	1.46 ± 0.09	1.01 ± 0.08
lung	1.07 ± 0.07	0.45 ± 0.03	0.61 ± 0.04	0.66 ± 0.06	0.84 ± 0.05
liver	0.50 ± 0.02	0.77 ± 0.03	1.46 ± 0.07	3.63 ± 0.59	4.37 ± 0.13
	(3.72 ± 0.09)	(5.67 ± 0.20)	(10.32 ± 0.59)	(26.64 ± 3.71)	(34.65 ± 0.91)

[a] Expressed as percent administered dose per gram of tissue ± SEM; n = 3–5. [b] Values in parentheses are expressed as percent administered dose per organ based on actual liver weight.

Biodistribution Studies. Once radioiodinated, each of the target compounds was subjected to tissue distribution analysis in female Sprague–Dawley rats. Following solubilization in normal saline with the aid of Tween 20, the radiolabeled compound was administered intravenously to groups of animals, which were then sacrificed at various times. Appropriate tissues were then removed and analyzed for radioactivity. Although 12 tissues were analyzed in this fashion, only 5 are included in Tables II and III. The other tissues, namely adrenal cortex, ovary, muscle, spleen, and fat, generally contained low levels of radioactivity. The significance of plasma and thyroid radioactivity is discussed separately.

The plasma was analyzed by polyacrylamide gel electrophoresis (PAGE) (Table IV) in order to define those macromolecules (lipoproteins, plasma proteins) involved in the transport of the agents to the tissues. The stacking gel (SG) fraction contains, among others, chylomicrons and VLDL's. The middle three fractions, LDL, HDL, and ALB, contain low-density lipoproteins, high-density lipoproteins, and albumin, respectively. The below albumin (BA) fraction is associated with other proteins.

Lipid extraction of both liver and plasma was performed in order to gain some insight into the nature of radioactive products in these tissues. Table V summarizes the percent of lipid-extractable material present in these tissues at 5 and 30 min and indicates the amount still present in the form of the parent compound.

The distribution of radioactivity in the tissues is presented in two ways: (1) as percent administered dose per gram of tissue and (2) for the liver as percent administered dose per organ (Tables II and III). The former describes the relative concentration of radioactivity in various tissues, while the latter takes into account the mass of the organ and thus represents more accurately the fraction of

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Table III. Distribution of Radioactivity at 5 and 30 min after iv Administration of ^{125}I-Labeled 1,3-Dipalmitoylglycerol 2-[ω-(3-Amino-2,4,6-triiodophenyl)alkanoates] to Female Rats[a]

tissue	10a	10b	10c	10d	10e
			5 min		
blood	6.23 ± 0.73	5.99 ± 0.34	6.04 ± 0.16	2.50 ± 0.17	2.50 ± 0.14
heart	0.63 ± 0.07	1.34 ± 0.18	0.60 ± 0.02	0.73 ± 0.06	0.49 ± 0.03
kidney	0.93 ± 0.09	1.18 ± 0.17	0.65 ± 0.04	0.59 ± 0.02	0.42 ± 0.03
lung	1.41 ± 0.14	1.20 ± 0.09	1.15 ± 0.13	1.73 ± 0.14	3.16 ± 1.15
liver	2.96 ± 0.40	1.74 ± 0.16	2.16 ± 0.01	6.62 ± 0.34	4.07 ± 0.32
	(21.86 ± 2.12)[b]	(12.07 ± 0.97)	(15.59 ± 0.60)	(48.73 ± 2.26)	(30.32 ± 0.99)
			30 min		
blood	6.50 ± 0.76	3.23 ± 0.23	3.73 ± 0.25	1.12 ± 0.03	1.42 ± 0.08
heart	0.68 ± 0.08	2.43 ± 0.08	1.92 ± 0.51	0.70 ± 0.04	0.74 ± 0.02
kidney	0.87 ± 0.06	1.65 ± 0.06	0.65 ± 0.02	1.21 ± 0.08	1.29 ± 0.09
lung	1.34 ± 0.25	0.93 ± 0.06	0.97 ± 0.16	1.84 ± 0.14	1.72 ± 0.13
liver	4.00 ± 0.35	1.22 ± 0.06	4.60 ± 0.76	5.22 ± 0.50	5.97 ± 0.39
	(24.57 ± 2.16)	(8.72 ± 0.19)	(34.96 ± 5.61)	(36.82 ± 3.64)	(44.97 ± 2.55)

[a] Expressed as percent administered dose per gram of tissue ± SEM; n = 3–5. [b] Values in parentheses are expressed as percent administered dose per organ based on actual liver weight.

Table IV. Polyacrylamide Gel Electrophoresis (PAGE) Analysis of Rat Plasma 5 and 30 min after iv Administration of ^{125}I-Labeled Acids and Triacylglycerols[a]

compd	SG	LDL	HDL	ALB	BA
			5 min		
1a	1.7 ± 0.3	1.0 ± 0.2	15.8 ± 7.0	76.0 ± 6.0	5.5 ± 1.5
1b	4.6 ± 1.2	2.4 ± 1.0	5.3 ± 1.9	83.6 ± 6.3	4.1 ± 1.0
1c	0.6 ± 0.1	0.5 ± 0.1	19.8 ± 7.1	77.5 ± 7.3	1.6 ± 0.2
1d	1.5 ± 0.3	1.0 ± 0.1	8.0 ± 0.5	84.6 ± 0.8	4.8 ± 0.3
1e	5.2 ± 0.8	4.7 ± 0.9	26.6 ± 2.0	60.0 ± 1.3	3.5 ± 0.5
10a	9.0 ± 3.8	34.0 ± 3.6	50.5 ± 3.3	3.3 ± 0.5	3.2 ± 0.7
10b	4.6 ± 0.6	20.2 ± 2.8	57.1 ± 0.6	14.6 ± 2.6	2.4 ± 0.4
10c	4.6 ± 1.2	35.1 ± 1.4	52.6 ± 3.7	4.4 ± 0.4	3.0 ± 1.4
10d	6.9 ± 0.5	26.0 ± 4.3	33.3 ± 2.3	30.8 ± 3.8	3.0 ± 0.6
10e	4.6 ± 0.8	12.6 ± 1.0	48.1 ± 0.8	32.1 ± 1.4	2.7 ± 0.2
			30 min		
1a	1.7 ± 1.1	0.4 ± 0.2	7.3 ± 2.5	83.6 ± 4.6	7.0 ± 1.1
1b	11.0 ± 2.9	6.9 ± 1.7	13.3 ± 2.6	51.5 ± 10.9	10.8 ± 3.4
1c	3.9 ± 2.4	2.2 ± 0.5	8.7 ± 1.5	78.4 ± 6.3	6.7 ± 2.1
1d	11.2 ± 1.8	3.8 ± 0.8	5.6 ± 0.3	54.4 ± 15.0	26.3 ± 15.5
1e	7.6 ± 2.1	3.2 ± 0.6	18.5 ± 1.0	60.6 ± 6.0	10.0 ± 3.5
10a	3.0 ± 1.4	41.2 ± 1.2	53.4 ± 0.7	1.9 ± 0.2	0.5 ± 0.1
10b	4.0 ± 1.0	15.7 ± 0.5	48.9 ± 2.5	27.0 ± 2.2	4.4 ± 0.6
10c	7.1 ± 1.9	27.5 ± 4.2	54.1 ± 1.1	5.4 ± 0.7	5.8 ± 2.6
10d	13.2 ± 2.6	9.7 ± 1.9	15.6 ± 1.5	48.9 ± 2.1	12.5 ± 2.2
10e	10.9 ± 2.8	8.6 ± 0.5	27.0 ± 4.8	44.6 ± 4.1	9.0 ± 0.9

[a] n = 3–5; ±SEM; SG, stacking gel; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALB, albumin; BA, below albumin.

Table V. Analysis of Lipid-Soluble Radioactivity Extracted from Rat Liver and Plasma after iv Administration of ^{125}I-Labeled 2-Substituted Triacylglycerols[a]

		% $CHCl_3/CH_3OH$ extractable compd		% parent compd by TLC	
compd	tissue	5 min	30 min	5 min	30 min
10a	liver	91.2 ± 0.8	83.1 ± 1.1	91.7 ± 1.3	89.0 ± 1.0
	plasma	89.4 ± 0.2	81.5 ± 1.0	97.7 ± 0.3	91.9 ± 2.3
10b	liver	68.4 ± 2.2	52.1 ± 1.8	72.5 ± 3.2	47.7 ± 3.2
	plasma	79.1 ± 1.4	69.4 ± 1.4	75.5 ± 11.3	86.1 ± 1.0
10c[b]	liver	84.4	79.7		
	plasma	85.7	92.2		
10d	liver	40.9 ± 2.1	61.2 ± 1.5	46.4 ± 2.7	42.9 ± 1.7
	plasma	81.8 ± 2.9	54.8 ± 2.0	43.2 ± 14.3	34.0 ± 3.0
10e	liver	44.3 ± 1.1	47.2 ± 4.1	24.0 ± 3.6	40.8 ± 17.7
	plasma	83.8 ± 2.6	71.3 ± 1.2	32.6 ± 2.9	23.8 ± 7.9

[a] Percent ± SEM; n = 3–5. [b] n = 1.

the injected dose that actually resides in a given organ at a particular time.

Results and Discussion

Data obtained for free acid analogues **1a–e** reveal several intersting characteristics, most notable of which was the vast increase in liver radioactivity with increasing alkyl chain length displayed by acids **1d** and **1e** (Table II). In fact, it appeared that compound **1a** was cleared for the most part by the kidneys and not the liver. As chain length and thus molecular weight increased, however, renal involvement became less important. Upon conversion of the values to % dose/organ, almost 50% of both **1d** and **1e** resided in the liver within 5 min. Compound **1d** was the most promising of the two, however, due to its more favorable liver-to-blood ratio of 5.75 as compared to 3.42 for

1d. Although not shown, the amount of radioactivity in the thyroid increased with time for all analogues. Accordingly, 24-h values for **1a–e** ranged from 192 to 567% dose/g of tissue. Because of the small weight of the thyroid, however, these values actually represent a very small percentage of the total dose. Nonetheless, accumulation of radioiodine in the thyroid does provide some indication of the stability of the tracer from in vivo deionidination.

Analysis of liver radioactivity following administration of the acids **1a,d,e** indicated that less than 50% of the liver radioactivity at either 5 or 30 min was lipid extractable, and of that, less than 50% generally comigrated with the parent compound.

Moreover, PAGE analysis (Table IV), in all cases, demonstrated the propensity of the acids to rapidly become associated with plasma albumin, which was anticipated in light of the fact that fatty acids are known to bind avidly to albumin.[34] In fact, albumin has been shown to have a specific iopanoic acid binding site.[35] This feature is very important, since it serves as a means for monitoring ester hydrolysis in the tissue distribution analysis of the triacylglycerols.

Comparison of **10a–c** with their corresponding free acids **1a–c** revealed the former to have increased liver uptake and delayed blood clearance (Table III). Although the liver values were also higher at both time points upon administration as the triacylglycerol (**10a–c**), concomitant high blood values somewhat nullified any advantage over administration as the free acid (**1a–c**). Triacylglycerols **10d** and **10e**, on the other hand, exhibited slightly less liver uptake at 5 min, but enhanced uptake at 30 min when compared to their free acid counterparts. Moreover, in contrast to the shorter chain analogues, there was no significant change in the blood clearance of either **10d** or **10e** upon comparison with the free acids. When calculated in % dose/organ, **10d** afforded the highest liver value at 5 min, while **10e** appeared most promising at 30 min. Further inspection of Table III, however, revealed a higher liver-to-blood ratio for **10d**, thereby supporting it as the most promising candidate in this series based on tissue distribution analysis. Although not shown, all of the 2-substituted triacylglycerols were essentially cleared from all tissues by 24 h except for the thyroid, where values ranged from 246 to 402% dose/g.

According to Table V, the percent of lipid-extractable radioactivity decreased with time in the plasma and liver for compounds **10a** and **10b** and also in the plasma for compounds **10d** and **10e**. In contrast, however, the latter two compounds gave rise to an increase in the percent of liver-extractable radioactivity with time. Analogue **10a** appeared to be most resistant to in vivo hydrolysis, while longer chain analogues **10d** and **10e** were rapidly hydrolyzed.

A prominent feature of the PAGE analysis results (Table IV) was the rapidity with which the triacylglycerols were distributed within the various plasma lipoprotein compartments following administration. Equally striking, was the rapid rate of hydrolysis of long-chain analogues **10d** and **10e**, as indicated by the high levels of radioactivity associated with albumin, especially at the early time points. In fact, the rate of hydrolysis appeared to increase with increasing chain length, except for the iopanoate analogue **10c**, which afforded low albumin levels at all time points. Direct comparison of iopanoate **10c** with its desethyl congener **10b** indicated that the α-ethyl group affords some degree of steric hindrance toward ester hydrolysis, at least in the plasma. Phenylacetate analogue **10a** also appeared equally resistant to hydrolysis at earlier time points, but was even more resistant than **10c** at 24 h. This 24-h value had little bearing on the overall evaluation of **10a**, however, because the amount of radioactivity present in the liver at that time was minimal (0.1% dose/g).

In summary, of the five radioiodinated acids studied, only the two long-chain analogues **1d** and **1e** afforded relatively high levels of radioactivity in the liver. PAGE analysis confirmed the propensity of the free acids to rapidly become associated with albumin following intravenous administration. Because albumin bound circulating acids are sequestered by the liver via an anionic pathway, these iodinated fatty acid analogues also have potential as liver scintigraphic agents. This is especially true for dodecanoic analogues **1d**, which displayed a very favorable liver-to-blood ratio of 5.75 at 5 min.

In contrast to the propensity of the free acids to become bound to albumin, 1,3-dipalmitoylglycerol 2-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (**10a–e**) rapidly became associated with plasma lipoproteins following administration. Although long-chain analogues **10d** and **10e** exhibited some capacity to accumulate in the liver at 5 and 30 min, respectively, PAGE analysis revealed that these esters underwent significant in vivo hydrolysis even at these early time periods. In would appear, therefore, that most of the radioactivity found in the liver following administration of **10d** and **10e** was due in part to uptake of the free acid and not the intact triacylglycerol. In contrast, it appears that triacylglycerol analogues **10a** and **10c** are sequestered by the liver intact. Moreover, the liver uptake values of 25% and 35% of the administered dose for **10a** and **10c**, respectively, are comparable to that of cholesteryl iopanoate.[12] A great advantage of the triacylglycerols, however, is that unlike CI they are cleared from the liver by 24 h.

A study aimed at ascertaining the hepatocyte specificity of **10c** is now in progress. In addition, these promising results have prompted further studies with di- and trisubstituted triacylglycerols.

Experimental Section

Infrared spectra (IR) were obtained on a Perkin-Elmer 281 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on either a Varian EM 360A or Bruker WM360 or WP270SY instrument; 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 Midwest Microlabs, Ltd., Indianapolis, IN. 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, and charring with 50% aqueous sulfuric acid or DNP in the case of aldehydes. Radio-TLC was performed on the Merck plates and scanned for radioactivity with a Vangard 930 auto scanner. Column chromatography was performed on Davisil 62 silica gel (Grace, Davison Chemical, Baltimore, MD), or in the case of radiolabeled compounds on Merck silica gel-60 (230–400 mesh). The high-performance liquid chromatography (HPLC) system consisted of an Altex 110A pump, Gilson 111 UV monitor (254 nm), and various columns including Whatman Partisil 10 ODS 3 (25 cm),Magnum 20 Partisil 10 ODS 3 (50 cm) reverse phase, and Partisil 10 (25 cm) and IBM (5 μm, 10 × 250 μ) silica gel columns. Radio-HPLC separations were performed on a Whatman Partisil 5 RAC column (9.4 × 100 mm) while simultaneously monitoring both UV (254 nm), as previously described, and radioactivity, with an in-line Bicron Analyst (Bicron Electronic Products, Newbury, OH) equipped with a model G1LE low-energy scintillation probe and strip chart output. All radioiodination reactions were conducted inside a plexiglass glove box, which was vented with a Model RIT-140 radioiodine trap (Hi Q Filter Products, La Jolla, CA).

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THF was distilled from $LiAlH_4$ under argon 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 Company, Milwaukee, WI, unless otherwise stated. Palmitic acid was purchased from Nu-Chek Prep, Inc., Elysian, MN. Iopanoic acid was purchased from CTC Organics, Inc., Atlanta, GA. Tween 20 (polyoxyethylene sorbitan monolaurate) was purchased from Sigma Chemical Co., St. Louis, MO.

Preparation of ω-(3-Aminophenyl)alkanoic Acids. Synthesis of (3-aminophenyl)acetic (**2a**)[36] and propionic (**2b**)[37] acids was accomplished by literature procedures. Ethyl 11-bromoundecanoate (**4**) was prepared from 11-bromoundecanoic acid according to the method of Chavare.[38]

(ω-Carbethoxyundecylidene)triphenylphosphonium Bromide (5). Triphenylphosphine (53.1 g, 203 mmol) was added to a solution of bromoester **4** (54.0 g, 184 mmol) in acetonitrile (150 mL), and the resulting mixture was heated at reflux for 36 h under N_2. The reaction mixture was cooled and placed in vacuo to remove the solvent. The remaining syrup was triturated with anhydrous ether until crystallization occurred. The solid salt was separated by filtration and washed thoroughly with anhydrous ether to remove any remaining triphenylphosphine. The salt thus obtained was hygroscopic and was used without further purification: yield, 91.04 g (90%); mp 85–87 °C; IR (KBr) 2940, 2860 (aliph. CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.90–7965 (m, 15 H, aryl H's), 4.09 (q, J = 7.2 Hz, 2 H, OCH_2), 3.85 (m, 2 H, PCH_2), 2.23 (t, J = 7.6 Hz, 2 H, CH_2CO_2), 1.56–1.20 (m, 16 H, $(CH_2)_8$).

Ethyl 12-(3-Nitrophenyl)-11-dodecenoate (7a). A suspension of $NaNH_2$ (4.11 g, 105 mmol) in anhydrous THF (20 mL) was cooled to 5 °C and treated with a solution of phosphonium salt **5** (53.0 g, 95 mmol) in dry THF (100 mL). After 30 min, a solution of 3-nitrobenzaldehyde (13.2 g, 87 mmol) in dry THF (20 mL) was added dropwise, and the resulting mixture was stirred under N_2 for 12 h at room temperature. The heterogeneous mixture was then filtered to remove NaBr, and the solvent was removed from the filtrate under reduced pressure. The residue was dissolved in an ether/H_2O (1:1) mixture; the layers were separated, and the organic layer was washed successively with H_2O, 2% aqueous sodium bisulfite (3×, until most of the unreacted aldehyde was removed according to TLC), H_2O and brine and dried ($MgSO_4$). The solvent was removed in vacuo and the residue triturated with anhydrous ether to precipitate any final traces of triphenylphosphine oxide. Filtration and concentration of the filtrate in vacuo afforded a yellow liquid, which was purified by column chromatography on silica gel (200 g) eluted with hexane/ether (3:2). The desired nitroalkene ester **7a** thus obtained decomposed upon attempted vacuum distillation and was therefore used as is in the subsequent hydrogenation reaction: yield, 11.56 g (38%); IR (neat) 2935, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.16 (m, 1 H, aryl 2-H), 8.05 (m, 1 H, aryl 4-H), 7.59 (m, 1 H, aryl 6-H), 7.46 (m, 1 H, aryl 5-H), 6.40 (m, 1.3 H, *trans*-Ar*CH*=*CH* and *cis*-Ar*CH*=CH), 5.82 (m, 0.7 H, *cis*-ArCH=*CH*), 4.11 (q, J = 7.1 Hz, 2 H, OCH_2), 2.27 (m, 4 H, =$CHCH_2$, CH_2CO_2), 1.70–1.20 (m, 17 H, $(CH_2)_7$, CH_3). Anal. Calcd for ($C_{20}H_{29}NO_4$): C, 69.14; H, 8.41. Found: C, 68.64; H, 8.14.

Methyl 17-(3-Nitrophenyl)-16-heptadecenoate (7b). A stirred suspension of (3-nitrobenzyl)triphenylphosphonium bromide (**6**)[39] (19.17 g, 40 mmol) in anhydrous THF (60 mL) under N_2 was cooled to 0 °C and treated dropwise with a solution of lithium bistrimethylsilylamide (44 mL of 1.0 M solution, 44 mmol) in anhydrous THF. After 20 min, a solution of methyl 16-oxohexadecanoate[27] (9.5 g, 33 mmol) in dry THF (50 mL) was added slowly via syringe over a period of 10 min. The reaction mixture was allowed to warm to room temperature and was stirred under N_2 for 13 h, at which time no aldehyde was present as indicated by TLC. Most of the solvent was removed in vacuo, and the remaining reaction mixture was diluted with a mixture of ether (150 mL) and H_2O (100 mL). The layers were separated, and the aqueous layer was extracted with ether (100 mL). The ether layers were combined and washed with 0.5 N HCl, 10% aqueous $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). The solvent was removed in vacuo to give a tan residue (20.54 g), which was dissolved in a small volume of ether and purified by column chromatography on silica gel (260 g) eluted in a gradient manner from hexane/ether (4:1) to hexane/ether (7:3). After combination of the appropriate fractions and removal of solvent by evaporation, a white solid residue remained, which was recrystallized from petroleum ether (bp 30–60 °C) to afford **7b** as white leaflets: yield, 9.73 g (72%); mp 68–69 °C; IR ($CHCl_3$) 2940, 2860 (aliph. CH), 1742 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.20–8.00 (m, 2 H, aryl 2,4-H), 7.60–7.40 (m, 2 H, aryl 5,6-H), 6.38 (m, 1.9 H, *trans*-Ar*CH*=*CH*, *cis*-Ar*CH*=CH), 5.83 (m, 0.4 H, *cis*-ArCH=*CH*), 3.65 (s, 3 H, CH_3), 2.25 (m, 4 H, =$CHCH_2$, CH_2CO_2), 1.70–1.2 (m, 24 H, $(CH_2)_{12}$). Anal. Calcd for $C_{24}H_{37}NO_4$: C, 71.43; H, 9.24. Found: C, 69.09; H, 9.32. This product was used without further purification in the following hydrogenation step.

Reduction of Nitroalkenoate Esters. Ethyl 12-(3-Aminophenyl)dodecanoate (8a). A solution of **7a** (11.2 g, 32 mmol) in absolute ethanol (150 mL) was hydrogenated at room temperature and 50 psi in the presence of 5% Pd/C (1.5 g) for 2 h. The catalyst was removed by filtration through a pad of celite, and upon removal of solvent in vacuo, a colorless liquid remained, which was homogeneous by TLC (silica gel, hexane/ether (3:2), R_f = 0.27): yield, 9.55 g (93%); IR (neat) 3460, 3380 (amine), 2930, 2855 (aliph. CH), 1730 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.05 (m, 1 H, aryl 5-H), 6.58 (d, J = 7.3 Hz, 1 H, aryl 6-H), 6.50 (m, 2 H, aryl 2,4-H), 4.12 (q, J = 7.1 Hz, 2 H, OCH_2), 3.59 (s, 2 H, NH_2), 2.50 (t, J = 7.6 Hz, 2 H, $ArCh_2$), 2.28 (t, J = 7.4 Hz, 2 H, CH_2CO_2), 1.7–1.2 (m, 18 H, $(CH_2)_9$). Anal. ($C_{20}H_{33}NO_2$) C, H.

Methyl 17-(3-Aminophenyl)heptadecanoate (8b). A solution of **7b** (7.8 g, 19 mmol) in methanol (100 mL) was hydrogenated at room temperature and 45 psi in the presence of 5% Pd/C (1.0 g) for 4 h. Removal of catalyst by filtration and subsequent removal of solvent in vacuo afforded a solid residue (7.0 g), which was dissolved in $CHCl_3$ and purified by column chromatography on silica gael (260 g) eluted in a stepwise gradient fashion with hexane/ether (9:1, 200 mL) followed by hexane/ether (1:1) until elution was complete. Combination of the appropriate fractions and removal of solvent in vacuo afforded a residue, which was recrystallized from petroleum ether (bp 30–60 °C)/ether to give **8b** as a white powder: yield, 5.77 g (80%); mp 68–69 °C; IR ($CHCl_3$) 3460, 3375 (amine), 2940, 2860 (aliph. CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.06 (m, 1 H, aryl 5-H), 6.59 (d, J = 7.8 Hz, 1 H, aryl 6-H), 6.51 (m, 2 H, aryl 2,4-H), 3.66 (s, 3 H, OCH_3), 3.59 (s, 2 H, NH_2), 2.50 (t, J = 7.4 Hz, 2 H, $ArCH_2$), 2.30 (t, J = 7.4 Hz, 2 H, CH_2CO_2), 1.7–1.2 (m, 28 H, $(CH_2)_{14}$). Anal. ($C_{24}H_{41}NO_2$) C, H.

Iodination of (Aminophenyl)alkanoates. Method A. (3-Amino-2,4,6-triiodophenyl)acetic Acid (1a). A solution of **2a** (3.8 g, 25 mmol) in H_2O (200 mL) and concentrated HCl (10 mL) was warmed to 75 °C in a three-necked, round-bottom flask (500 mL) equipped with condenser and mechanical stirrer it was treated with a solution of ICl (14.3 g, 88 mmol) in concentrated HCl (35 mL). The reaction mixture was stirred for 3 h, cooled to room temperature, and filtered. The brown filter cake was washed thoroughly with H_2O, transferred to an Erlenmeyer flask (1 L), and subsequently treated with excess NaOH (1.0 g) and heated to 80 °C on a steam bath. Additional H_2O (70 mL) was added to aid dissolution, and the resulting solution was heated for 30 min and cooled to room temperature. The sodium salt was filtered, washed with cold H_2O, and the dissolved in 1% aqueous sodium bisulfite (250 mL) with heating to 80 °C. After 15 min, the solution was acidified (pH 4–5 by litmus) with 8 N HCl and slowly cooled to 5 °C. The large off-white plates that formed were filtered, washed with cold H_2O, and dried under vacuum overnight at 100 °C: yield, 8.05 g (61%); mp 222–224 °C dec (lit.[36] mp 220 °C); IR (KBr) 3470, 3380 (amine), 2920 (aliph. CH), 1700 (acid C=O) cm^{-1}; ^1H NMR (60 MHz, Me_2SO-d_6) δ 8.10 (s, 1 H, aryl 5-H), 5.33 (s, 2 H, NH_2), 4.03 (s, 2 H, CH_2).

(3-Amino-2,4,6-triiodophenyl)propionic Acid (1b). Similar treatment of a solution of **2b** (6.0 g, 36 mmol) in H_2O (300 mL) and concentrated HCl (10 mL) with ICl (20.6 g, 127 mmol) in

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concentrated HCl (50 mL) for 3 h afforded crude **1b** which was converted to its sodium salt upon treatment with NaOH (1.5 g), crystallized, and precipitated from 1% aqueous sodium bisulfite in the manner described above. The desired acid was collected by filtration, washed with H_2O, and dried under vacuum at 100 °C overnight: yield, 12.7 g (64%); mp 232–235 °C dec (lit.[37] mp 233 °C); IR (KBr) 3460, 3360 (amine), 2930 (aliph. CH), 1705 (acid C=O) cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6) δ 8.01 (s, 1 H, aryl 5-H), 5.31 (s, 2 H, NH_2), 3.19 (br t, 2 H, $ArCH_2$), 2.32 (br t, 2 H, CH_2CO_2).

Method B. Ethyl 12-(3-Amino-2,4,6-triiodophenyl)dodecanoate (9a). A solution of ICl (16.2 g, 100 mmol) in $CHCl_3$ (100 mL) and 8 N HCl (50 mL) was heated to 70 °C in a three-necked, round-bottom flask equipped with reflux condenser, addition funnel, and mechanical stirrer. A solution of **8a** (8.0 g, 25 mmol) in $CHCl_3$ (30 mL) was added dropwise, and the resulting biphasic mixture was stirred for 3 h. The reaction mixture was allowed to cool to room temperature; the layers were separated, and the aqueous phase 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, brine, and dried ($MgSO_4$). TLC of this mixture (silica gel, hexane/ether (3:2)) indicated the presence of both the triiodinated product (R_f = 0.43) and a smaller amount of diiodinated (by NMR) product (R_f = 0.32). Upon removal of solvent in vacuo, a red liquid (18.6 g) remained, which solidified upon standing. Recrystallization from methanol/$CHCl_3$ gave **9a** as fine silvery needles: yield, 11.72 g (69%); mp 82–83.5 °C; IR ($CHCl_3$) 3470, 3380 (amine), 2940, 2860 (aliph. CH), 1742 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.01 (s, 1 H, aryl 5-H), 4.77 (s, 2 H, NH_2), 4.10 (q, J = 7.1 Hz, 2 H, OCH_2), 2.99 (ps t, 2 H, $ArCH_2$), 2.27 (t, J = 7.5 Hz, 2 H, CH_2CO_2), 1.70–1.26 (m, 18 H, $(CH_2)_9$), 1.24 (t, J = 7.2 Hz, 3 H, CH_3). Anal. ($C_{19}H_{28}I_3NO_2$) C, H, I, N.

Methyl 17-(3-Amino-2,4,6-triiodophenyl)heptadecanoate (9b). A solution of amino ester **8b** (1.0 g, 2.66 mmol) in $CHCl_3$ (10 mL) was added dropwise to a solution of ICl (2.16 g, 13.3 mmol) in 8 N HCl (10 mL) and $CHCl_3$ (15 mL) in the manner described above. Purification furnished a residue (1.95 g), which was recrystallized from methanol/$CHCl_3$ to afford **9b** as fine silvery needles: yield, 1.62 g (81%); mp 89–90 °C; IR ($CHCl_3$) 3480, 3380 (amine), 2930, 2860 (aliph. CH), 1732 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 1 H, aryl 5-H), 4.79 (s, 2 H, NH_2), 3.67 (s, 3 H, OCH_3), 3.01 (ps t, 2 H, $ArCH_2$), 2.30 (t, J = 7.4 Hz, 2 H, CH_2CO_2), 1.70–1.20 (m, 28 H, $(CH_2)_{14}$). Anal. ($C_{24}H_{38}I_3NO_2$) C, H, I.

Hydrolysis of Iodinated Esters. 12-(3-Amino-2,4,6-triiodophenyl)dodecanoic Acid (1d). Ester **9a** (9.7 g, 13.9 mmol) was dissolved in THF (40 mL) and 95% ethanol (100 mL) and treated with excess NaOH (1.5 g). The resulting mixture was refluxed for 3 h, acidified with 1 N H_2SO_4 (pH 4–5 by litmus paper), and dilued with $CHCl_3$ (200 mL). The layers were separated, and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined and washed with H_2O twice and brine and dried ($MgSO_4$). Solvent removal in vacuo afforded the crude acid (9.17 g), which was recrystallized from cold methanol/$CHCl_3$ to give **1d** as off-white needles: yield, 8.01 g (86%); mp 115–117 °C, IR ($CHCl_3$) 3480, 3380 (amine), 2930, 2860 (aliph. CH), 1710 (acid C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.01 (s, 1 H, aryl 5-H), 4.77 (s, 2 H, NH_2), 2.99 (ps t, 2 H, $ArCH_2$), 2.34 (t, J = 7.4 Hz, 2 H, CH_2CO_2), 1.70–1.20 (m, 18 H, $(CH_2)_9$). Anal. ($C_{18}H_{26}I_3NO_2$) C, H, I, N.

17-(3-Amino-2,4,6-triiodophenyl)heptadecanoic Acid (1e). Similar treatment of a solution of ester **9b** (1.51 g, 2.0 mmol) in a mixture of THF (10 mL) and 95% methanol (20 mL) with excess NaOH (0.7 g) gave a solid residue (1.45 g), which was crystallized from $CHCl_3$/petroleum ether (bp 30–60 °C) to give **1e** as off-white needles: yield, 1.35 g (91%); mp 106–107 °C; IR ($CHCl_3$), 3480, 3370 (amine), 2930, 2860 (aliph. CH), 1710 (acid C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 1 H, aryl 5-H), 4.79 (s, 2 H, NH_2), 3.01 (ps t, 2 H, $ArCH_2$), 2.35 (t, J = 7.4 Hz, 2 H, CH_2CO_2), 1.70–1.20 (m, 28 H, $(CH_2)_{14}$). Anal. ($C_{23}H_{36}I_3NO_2$) C, H, I.

1,3-Dipalmitoylglycerol 2-[ω-(3-Amino-2,4,6-triiodophenyl)alkanoates] (10a–e). General Procedure: A rapidly stirred suspension of 1,3-dipalmitin[32] (1.0 equiv), the acid (1.0–1.1 equiv), and a catalytic amount of DMAP (0.1 equiv) in anhydrous CH_2Cl_2 (5 mL/mmol of alcohol) was treated with DCC (1.1–1.2 equiv). The resulting mixture was stirred under N_2 for a specified period of time at room temperature, diluted with CH_2Cl_2, and filtered to remove precipitated DCU. The filtrate was washed with 0.5 N HCl (2×), saturated aqueous $NaHCO_3$ (2×), H_2O, and brine and dried ($MgSO_4$). The solvent was removed in vacuo, and the remaining residue was purified by column chromatography and/or crystallization to afford the desired triacylglycerols.

1,3-Dipalmitoylglycerol 2-[(3-Amino-2,4,6-triiodophenyl)acetate] (10a). Treatment of acid **1a** (264 mg, 0.5 mmol), and DMAP (7 mg) with DCC (113 mg, 0.55 mmol) according to the procedure described above gave a solid residue (580 mg), which was purified by column chromatography on silica gel (50 g) eluted with chloroform/hexanes (1:1). The product thus obtained was crystallized from acetone to give 305 mg of **10a** as white needles: IR ($CHCl_3$) 3480 (amine), 2932, 2860 (aliph. CH), 1742 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.08 (s, 1 H, aryl H), 5.34 (m, 1 H, glyceryl CH), 4.83 (s, 2 H, NH_2), 4.32 (dd, J = 11.9, 4.2 Hz, 2H, $OCH_AH_BCHCH_AH_BO$), 4.24 (s, 2 H, $ArCH_2$), 4.15 (dd, J = 11.9, 6.0 Hz, 2 H, $OCH_AH_BCHCH_AH_BO$), 2.30 (t, J = 7.4 Hz, 4 H, O_2CCH_2), 1.7–1.2 (m, 52 H, $(CH_2)_{13}$), 0.88 (t, J = 6.8 Hz, 6 H, CH_3).

1,3-Dipalmitoylglycerol 2-[(3-Amino-2,4,6-triiodophenyl)propionate] (10b). Treatment of acid **2a** (382 mg, 0.67 mmol), 1,3-dipalmitin (380 mg, 0.67 mmol), and DMAP (10 mg) with DCC (153 mg, 0.74 mmol) in a similar manner gave a residue (700 mg), which was purified by chromatography on a short silica gel column (50 g) eluted with hexanes/ethyl acetate (5:2). The product obtained was crystallized from acetone to give 527 mg of **10b** as a white powder: IR ($CHCl_3$) 3470, 3370 (amine), 2970, 2855 (aliph. CH), 1742 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.05 (s, 1 H, aryl H), 5.32 (m, 1 H, glyceryl CH), 4.83 (s, 2 H, NH_2), 4.34 (dd, J = 11.9, 4.4 Hz, 2H, $OCH_AH_BCHCH_AH_BO$), 4.18 (dd, J = 11.9, 5.9 Hz, 2 H, $OCH_AH_BCHCH_AH_BO$), 3.38 (m, 2 H, $ArCH_2$), 2.53 (m, 2 H, $ArCH_2CH_2$), 2.33 (t, J = 7.4 Hz, 4 H, O_2CCH_2), 1.7–1.0 (m, 52 H, $(CH_2)_{13}$), 0.88 (t, J = 6.6 Hz, 6 H, CH_3).

1,3-Dipalmitoylglycerol 2-Iopanoate (10c). Similar treatment of iopanoic acid (**1c**, 1.14 g, 2.0 mmol), 1,3-dipalmitin (1.14 g, 2.0 mmol), and DMAP (25 mg) with DCC (454 mg, 2.2 mmol) afforded a residue (1.89 g), which was purified by column chromatography on silica gel (150 g) eluted with hexanes/ethyl acetate (15:1). Following removal of solvents in vacuo, an off-white residue remained, which was recrystallized from acetone/methanol to give 1.68 g of **10c** as small white nuggets: IR ($CHCl_3$) 3470, 3370 (amine), 2940, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.07 (s, 1 H, aryl 5-H), 5.31 (m, 1 H, glyceryl CH), 4.85 (s, 2 H, NH_2), 4.22 (m, 4 H, glyceryl CH_2), 3.34 (m, 2 H, $ArCH_2$), 2.81 (m, 1 H, $CHCO_2$), 2.29 (t, J = 7.5 Hz, 4 H, O_2CCH_2), 1.85 (m, 1 H, $CHCH_AH_BCH_3$), 1.7–1.2 (m, 27 H, $(CH_2)_{13}$, $CHCH_AH_BCH_3$), 0.88 (m, 9 H, CH_3).

1,3-Dipalmitoylglycerol 2-[12-(3-Amino-2,4,6-triiodophenyl)dodecanoate] (10d). Substitution of acid **1d** (100 mg, 0.15 mmol), 1,3-dipalmitin (85 mg, 0.15 mmol), DMAP (2 mg), and DCC (334 mg, 0.17 mmol) in the above procedure gave a residue (125 mg), which was recrystallized from methanol/ether to afford 90 mg of **10d** as a pale-white solid: IR ($CHCl_3$) 3480, 3380 (amine), 2930, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.01 (s, 1 H, aryl H), 5.25 (m, 1 H, glyceryl CH), 4.78 (s, 2 H, NH_2), 4.28 (dd, J = 11.9, 4.3 Hz, 2 H, $OCH_AH_BCHCH_AH_BO$), 4.13 (dd, J = 11.9, 5.9 Hz, 2 H, $OCH_aH_BCHCH_AH_BO$), 2.99 (ps t, 2 H, $ArCH_2$), 2.30 (m, 6 H, O_2CCH_2), 1.6–1.1 (m, 70 H, $(CH_{213}CH_3$, $(CH_2)_9$), 0.87 (t, J = 6.6 Hz, 6 H, CH_3).

1,3-Dipalmitoylglycerol 2-[17-(3-Amino-2,4,6-triiodophenyl)heptadecanoate] (10e). Substitution of acid **1e** (160 mg, 0.21 mmol), 1,3-dipalmitin (115 mg, 0.20 mmol), DMAP (5 mg), and DCC (48 mg, 0.23 mmol) into the above procedure afforded a white residue (258 mg), which was purified by column chromatography on silica gel (85 g) eluted with hexanes/ethyl acetate (7:1). Removal of solvents in vacuo gave 135 mg of **10e** as a white powder: IR ($CHCl_3$) 3480, 3380 (amine), 2490, 2860 (aliph. CH), 1742 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 1 H, aryl H), 5.26 (m, 1 H, glyceryl CH), 4.79 (s, 2 H, NH_2), 4.29 (dd, J = 11.9, 4.3 Hz, 2 H, $OCH_AH_BCHCH_AH_BO$), 4.14 (dd, J = 11.9, 5.9 Hz, 2 H, $OCH_AH_BCHCH_AH_BO$), 3.01 (ps, t, 2 H, $ArCH_2$), 2.32 (t, J = 7.4 Hz, 6 H, O_2CCH_2), 1.6–1.1 (m, 80 H,

$(CH_2)_{14}$), 0.88 (t, J = 6.3 Hz, 6 H, CH_3).

Radioiodine Exchange in Pivalic Acid Melt.[33] **General Procedure.** The compound to be radiolabeled (1–5 mg) was placed in a 2-mL serum vial, which was then sealed with a Teflon-lined rubber septum and aluminum cap. Freshly distilled THF (100–200 μL) was added via a microliter syringe followed by aqueous $Na^{125}I$ (1–2.5 mCi, 10–50 μL no-carrier-added in reductant-free 0.1 N NaOH from New England Nuclear). The vial was swirled gently to dissolve the contents and ensure homogeneity. Inlet and outlet cannuli were inserted and a gentle stream of nitrogen applied to remove the solvents. The seal was then removed, and solid pivalic acid (5–20 mg), previously dried by azeotropic removal of water with toluene and distilled under nitrogen, was added. The vial was resealed as before and partially immersed in a preheated (155–160 °C) oil bath. When the reaction was complete (usually 1–2 h), the reaction vial was allowed to cool; distilled THF (200 μL) was added via a microliter syringe followed by gentle stirring and subsequent removal of a TLC sample (1–2 μL). The contents of the vial were then transferred to the top of a silica gel-60 chromatography column (1 × 10 cm) and eluted with the appropriate solvent system. Radiochemical purity of each fraction was monitored by TLC (radio and UV detection); 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 155 to 1109 μCi/mg for acids **1a–e**, and 139 to 581 μCi/mg for triacylglycerols **10a–e**. In all cases, radiochemical purity of final compounds exceeded 96%.

Tissue Distribution Studies. The radiolabeled compounds were dissolved in benzene, and Tween 20 was added. The solvent was evaporated with a stream of nitrogen. Physiological saline was added, and final traces of benzene were removed by passing nitrogen over the solution until no further traces of benzene were detected (2–3% of Tween). The radiolabeled compound, thus solubilized, was administered intravenously to adult female Sprague–Dawley rats weighing 200–250 g. Three or four rats were used for each compound at each time period, and the dose usually ranged between 5 and 30 μCi/animal. The rats were sacrificed 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 counted (84% efficiency) for radioactivity in a Searle 1185 well scintillation counter.

Plasma and Tissue Extraction. Radioactivity was extracted from plasma by a modified Folch procedure described previously.[17] Liver and adrenal cortex samples were homogenized, extracted, and analyzed by TLC with either hexane/ethyl acetate (5:2) or THF/hexane (1:1) as eluent for esters and acids, respectively. Following development, the plates were air dried and then cut into 1-cm strips. Each strip was placed in a counting tube and assayed for radioactivity. In all cases, the unlabeled compound served as a reference standard.

Plasma Electrophoresis. Polyacrylamide gel electrophoresis of plasma samples was performed according to the method previously described.[40] 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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Registry No. **1a**, 3119-17-3; **1a** (labeled), 102831-77-6; **1b**, 1206-91-3; **1b** (labeled), 102831-78-7; **1c**, 32636-96-7; **1c** (labeled), 102831-79-8; **1d**, 102831-71-0; **1d** (labeled), 102831-80-1; **1e** (labeled), 102831-81-2; **1e**, 102831-72-1; **2a**, 14338-36-4; **2b**, 1664-54-6; **4**, 6271-23-4; **5**, 102831-64-1; **6**, 1530-41-2; **7a**, 102831-65-2; **7b**, 102831-66-3; **8a**, 102831-67-4; **8b**, 102831-68-5; **9a**, 102831-69-6; **9b**, 102831-70-9; **10a**, 102851-37-6; **10a** (labeled), 102831-82-3; **10b**, 102831-73-2; **10b** (labeled), 102831-83-4; **10c**, 102831-74-3; **10c** (labeled), 102831-84-5; **10d**, 102831-75-4; **10d** (labeled), 102831-85-6; **10e**, 102831-76-5; **10e** (labeled), 102831-86-7; Ph_3P, 603-35-0; 3-$O_2NC_6H_4CHO$, 99-61-6; $OHC(CH_2)_{14}CO_2Me$, 45247-78-7; $H_3C(CH_2)_{14}CO_2CH_2CH(OH)CH_2O_2C(CH_2)_{14}CH_3$, 502-52-3.

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