tively. Poor binding molecules such as B26P (an ortho PCB), DIO8 (the octasubstituted dioxin), and B35Q distribute into low area contact (B26P, 7.6%) or high area contact (DIO8, 22%; B35Q; 19.6%). It is interesting that the ortho-substituted compound has too little contact; its low binding energy can be attributed to the fact that it has too few attractive (electrostatic) contributions to be a strong binder. On the other hand, the octachlorinated dioxin has too much contact with its additional chlorines riding up on the repulsive wall of the pocket.

The color display of the IRIS makes it especially easy to pick out these features, for instance to display only the "excluded" contact areas. For example, the stereopair energy-built complexes of TCDD and DIO8 are shown in Figure 3. The "excluded" contact areas for TCDD clearly reveal a good quality fit and draw attention to the importance of a rectangular shape. The "excluded" contact areas for DIO8, on the other hand, reveal a much poorer quality fit with colliding surfaces and considerable deviation from a rectangular shape. It is also interesting to note that DIO8 is positioned much deeper (than TCDD) in the binding pocket.

Conclusions

There are several avenues that should be explored to extend the work presented here. In most cases, only one initial orientation was minimized; a more prudent procedure would involve minimizing from many initial orientations with a thermal weighting of the final results. A related procedure would be to follow the trajectory of the system via molecular dynamics for a time long enough to realistically sample the various local minima for the ligand in the pocket volume. Free energies could then be assessed assuming a normal coordinate analysis would yield vibrational frequencies and thus entropies. The method of accounting for the solvation of the chlorinated analogues was empirical (with both the MODEL and the Hine procedures); experimental evaluation of the solvation energies would be difficult but very useful in this case. Only a relatively small fraction of the protein was included (see the Methods section); again a better procedure would be to include the entire protein dimer that forms the pocket or, perhaps, the entire tetramer since the binding of the second thyroxine is known to be anticooperative and thus at least some dimer–dimer interaction must be involved. The partial charges on the ligand molecules were somewhat qualitatively chosen; a procedure more consistent with the amino acid choices[20] would be to perform split valence level ab initio calculations on the ligand molecules to evaluate the electrostatic potential and then fit via least squares to find a set of partial charges that would also give the computed electrostatic potential. Finally, it would be of use and interest to employ another force field—perhaps the BIOSIM force field of Hagler et al.,[21] which includes cross terms and Morse type stretching—to determine how consistent binding results are with substantially different force fields.

In summary, the computations presented here suggest that molecular mechanics can be a useful tool for qualitatively predicting the relative binding to a protein by a series of molecules that contain the potential for varied noncovalent interactions. This work also provides theoretical confirmation of our previous experimental finding[5] that lateral chlorination is important for high binding activity with prealbumin but does not always guarantee it. Since lateral chlorination and a rectangular shape are also important for high toxicity and excess chlorination and deviation from a rectangular shape as in octachlorodibenzodioxin lower or eliminate toxicity, this theoretical model may be of use for estimating the toxic potential of PCBs and related compounds.

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Registry No. TCDD, 1746-01-6; TCDF, 51207-31-9; B35Q, 27728-29-6; DIO8, 3268-87-9; B354, 1137-59-3; B352, 5335-24-0; B35X, 67651-34-7; B35Z, 4400-06-0; B350, 13049-13-3; B345, 32774-16-6; B26P, 15968-05-5; B246, 14962-28-8; BBP, 93-52-4; T31R, 609-23-4.

Potential Tumor- or Organ-Imaging Agents. 27. Polyiodinated 1,3-Disubstituted and 1,2,3-Trisubstituted Triacylglycerols[1]

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A series of glyceryl 1,3-bis- and 1,2,3-tris[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. Of the nine target compounds synthesized and evaluated in rats, glyceryl 1,2,3-tris[3-(3-amino-2,4,6-triiodophenyl)propionate] (**5b**) displayed rapid and sustained liver specificity. This agent was found to accumulate in the liver in concentrations of 60, 75, and 86% of the administered dose at 5 min, 30 min, and 24 h, respectively. Moreover, the 24-h liver-to-blood ratio of 235 justifies further studies in higher animal species.

The liver is a common site for metastatic disease, particularly from gastrointestinal tract primary neoplasms. There is increasing evidence that early detection of liver metastases followed by prompt initiation of therapy results in an improved prognosis for survival of the patient.[2,3] X-ray computed tomography, employing water-soluble contrast agents, is currently the most accurate noninvasive radiologic procedure for visualizing hepatic masses, but

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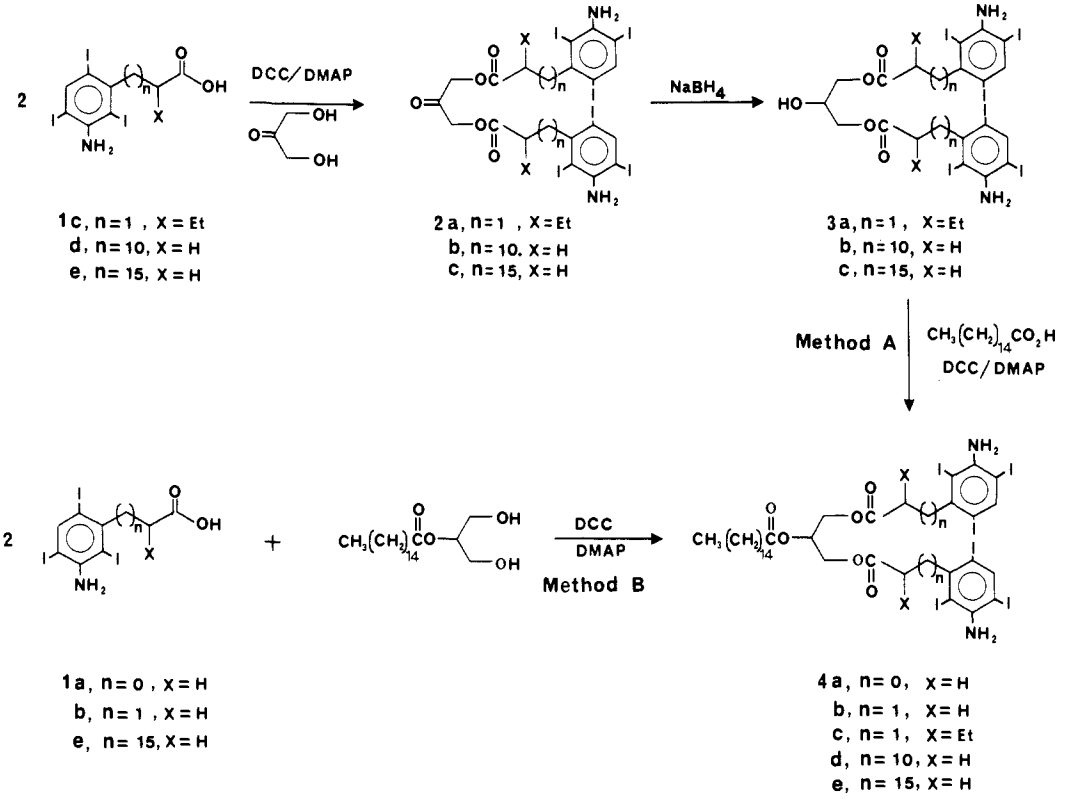
Scheme I. Synthesis of 1,3-Disubstituted Triacylglycerols



detection of lesions smaller than 2 cm is difficult.[4,5] For this reason, a number of investigators have attempted to develop contrast agents with more desirable properties than the water-soluble urographic agents.[6-10]

A major consideration in the development of a suitable hepatographic agent is the need for the agent to selectively localize in the liver shortly after administration. As outlined in the previous paper in this series,[11] our approach in the design of hepatic-specific contrast agents entails capitalizing upon the fact that the liver is the major site of lipid metabolism. For example, triacylglycerols are transported in the plasma as components of chylomicrons (CM) and very-low-density lipoproteins (VLDL). These lipoproteins are acted upon by peripheral lipoprotein lipase (LPL), whereby they lose some of their triglyceride content and become transformed into remnant vesicles. These remnants are then rapidly sequestered by the liver via a receptor-mediated process.[12] It was envisioned that appropriately modified triacylglycerols would be poor substrates for LPL, remain in the remnant particles, and thereby selectively localize in the liver. Accordingly, the previous paper in this series described the synthesis and biodistribution results obtained with a series of radioiodinated ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids and the corresponding 1,3-dipalmitoylglycerol 2-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates]. Two of these analogues, namely, 1,3-dipalmitoylglycerol 2-[2-(3-amino-2,4,6-triiodophenyl)acetate] and 1,3-dipalmitoylglycerol 2-iopanoate displayed respective liver accumulations of 25 and 35% of the administered dose 30 min after intravenous administration.[11] The current paper describes the synthesis and biodistribution of a number of 1,3-di- and 1,2,3-trisubstituted polyiodinated triacylglycerol analogues to further test this hypothesis and to achieve a suitable hepatospecific contrast agent.

Chemistry. The appropriately substituted arylalkanoic acids required for the acylation of glycerol and its derivatives were obtained by various means. For example, synthesis of the ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids **1a** and **1b** (Scheme I) was accomplished by literature procedures,[13,14] whereas iopanoic acid (**1c**) was commercially available. The long-chain acids **1d** and **1e** were prepared as described in the previous paper in this series.[11]

Two synthetic approaches were utilized in the synthesis of the 2-palmitoylglycerol 1,3-bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates) **4a–e** as shown in Scheme I. Method A, which is similar to an approach outlined earlier by Bentley and McCrae[15] for the synthesis of naturally occurring triacylglycerols, offered the advantage of providing the 1,3-diacylglycerol intermediates **3a–c** needed for the synthesis of 1,2,3-trisubstituted glycerol analogs **5b–e** (Scheme II). Diacylation of 1,3-dihydroxyacetone with the appropriate triiodinated acids (**1c–e**) in the presence of DCC and a catalytic amount of DMAP af-

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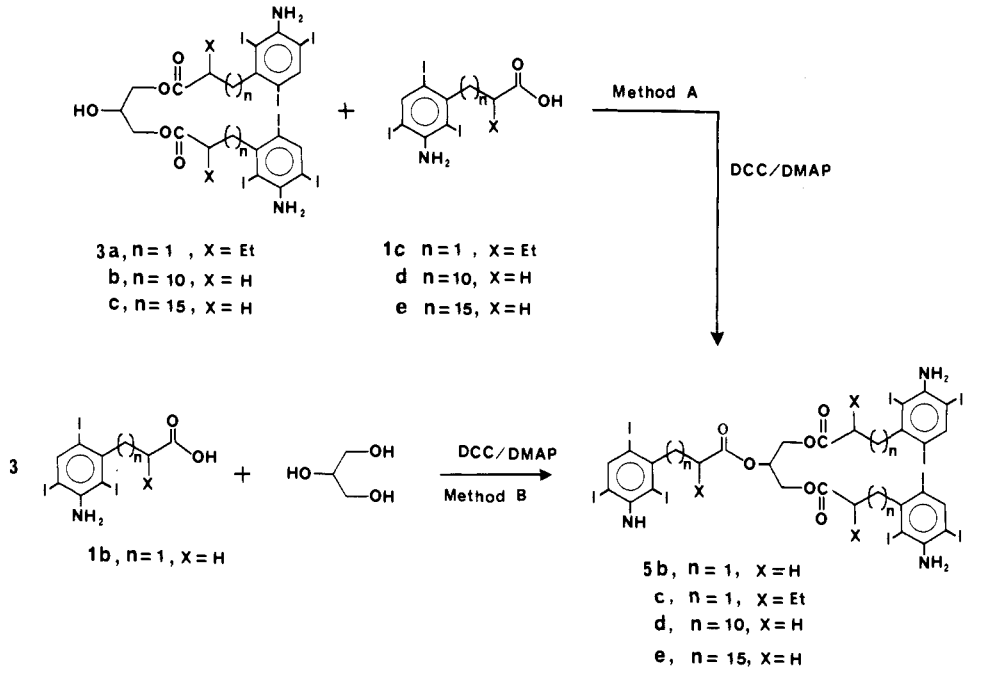
Scheme II. Synthesis of 1,2,3-Trisubstituted Triacylglycerols



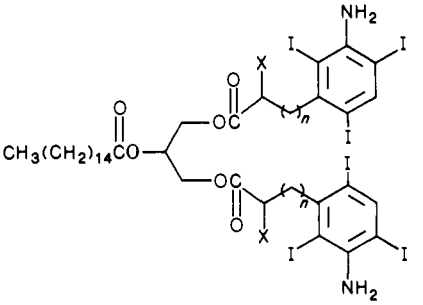
forded ketones **2a–c**. Reduction of the ketone with neutral sodium borohydride gave alcohols **3a–c**, which upon subsequent DCC/DMAP mediated acylation with palmitic acid gave 1,3-disubstituted triacylglycerols **4c–e**. Attempts to acylate dihydroxyacetone with acids **1a** and **1b** in a similar manner resulted in the recovery of unreacted starting materials, presumably due to both steric hindrance and/or a lack of solubility of both the acid and 1,3-dihydroxyacetone in the reaction medium. Synthesis of analogues **4a** and **4b** was possible, however, by the single-step reaction shown in method B of Scheme I. In this case, the desired 1,3-disubstituted triacylglycerol analogues **4a** and **4b** were obtained directly upon DCC/DMAP acylation of 2-palmitoyl glycerol[16,17] with 2 equiv of acid **1a** or **1b**.

Although analogue **4e** could also be prepared by method A, limited quantities of precursor **3c** necessitated the use of method B for the preparation of **4e**. Moreover, by comparison of the results obtained with heptadecanoate analogue **1e**, method B appears to be the method of choice for the preparation of these 1,3-disubstituted analogues when limited quantities of the polyiodinated acid analogues are available.

With 1,3-diacylglycerols **3a–c** in hand, subsequent treatment with acids **1c–e** in the presence of DCC and DMAP afforded the desired 1,2,3-trisubstituted triacylglycerols **5c–e** in yields ranging from 34 to 79% (Scheme II, method A). This method was not appropriate for target compounds **5a** (n = 0, X = H) and **5b**, however, because of the inability to form the necessary 1,3-diacylglycerol precursor. A sufficient quantity of trisubstituted triacylglycerol analogue **5b** was obtained, however, by direct acylation of anhydrous glycerol with excess desethyliopanoic acid (**1b**) (Scheme II, method B). Attempts to form trisubstituted phenylacetate analogue **5a** by either of these methods were unsuccessful presumably due to a lack of solubility of both components in the various reaction media.

Biodistribution Studies. In order to evaluate the ability of the triacylglycerol analogues to selectively localize

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



no.	n	X	method	mp, °C	formula	anal.[a]
4a	0	H	B	65–67	$C_{35}H_{46}I_6N_2O_6$	C, H, I
4b	1	H	B	93–95	$C_{37}H_{50}I_6N_2O_6$	C, H, I
4c	1	Et	A	*b*	$C_{41}H_{58}I_6N_2O_6$	C, H, I
4d	10	H	A	*b*	$C_{55}H_{86}I_6N_2O_6$	C, H, I
4e	15	H	B	78–79	$C_{65}H_{106}I_6N_2O_6$	C, H, I

[a] Elemental analysis within ±0.4% for elements indicated. [b] Amorphous solid.

in the liver, triacylglycerols **4a–e** and **5b–e** were radioiodinated with iodine-125 by isotope exchange in a melt of pivalic acid.[18] Once radioiodinated, each of the target triacylglycerols was subjected to tissue distribution analysis in female Sprague-Dawley rats. The radiolabeled compound was solubilized in normal saline with the aid of Tween-20. Following intravenous administration, groups of animals were sacrificed at various times, and the appropriate tissues were removed and analyzed for radioiodine content. Although 12 tissues were analyzed in this fashion, only 5 are included in Tables III and IV. The other tissues, namely adrenals, ovary, muscle, plasma, spleen, and fat generally contained lower quantities of radioactivity relative to those included in the tables. The significance of thyroid radioactivity is discussed when appropriate.

The distribution of radioactivity in the tissues is presented in two ways; (1) percent administered dose per gram

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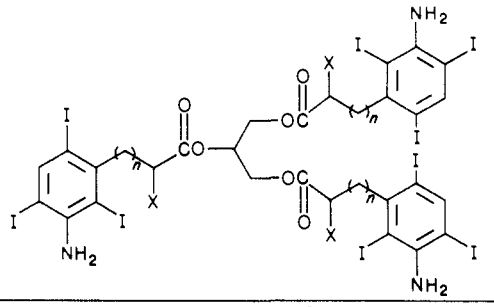
Table II. Glyceryl 1,2,3-Tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates]



no.	n	X	method	yield, %	mp, °C	formula	anal.[a]
5b	1	H	B	38	144–147	$C_{30}H_{26}I_9N_3O_6$	C, H, I
5c	1	Et	A	34	92–94	$C_{36}H_{38}I_9N_3O_6$	C, H, I
5d	10	H	A	79	60–63	$C_{57}H_{80}I_9N_3O_6$	C, H, I
5e	15	H	A	55	63–67	$C_{72}H_{110}I_9N_3O_6$	C, H, I

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

of tissue (Tables III and IV) and (2) percent administered dose per organ (Table V). 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 the injected dose that actually resides in a given organ at a particular time.

The plasma was analyzed by polyacrylamide gel electrophoresis (PAGE) in order to define those macromolecules (lipoproteins, plasma proteins) that are involved in the transport of the agents to the tissues. The PAGE results shown in Table VI provide a gross breakdown of the plasma proteins each compound is associated with in circulation. The stacking gel (SG) fraction contains, among others, chylomicra and very-low-density lipoproteins. The middle three fractions LDL, HDL, and ALB correspond to 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 VII summarizes the percent of lipid-extractable material present in the liver at 5 min, 30 min, and 24 h and indicates the amount still present in the form of the parent compound.

Results and Discussion

Tissue distribution analysis of 1,3-disubstituted analogues **4a–e** revealed three distinct pharmacokinetic profiles (Tables III and V). In the liver, both uptake and

Table III. Distribution of Radioactivity at 5 min, 30 min, and 24 h after iv Administration of ^{125}I-Labeled 2-Palmitoylglycerol 1,3-Bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] in Rats[a]

	compd				
tissue	**4a**	**4b**	**4c**	**4d**	**4e**
			5 min		
blood	4.75 ± 0.12	3.48 ± 0.10	7.44 ± 0.08	2.74 ± 0.19	4.78 ± 0.55
heart	0.64 ± 0.09	0.43 ± 0.02	0.87 ± 0.02	1.43 ± 0.23	0.87 ± 0.23
kidney	0.54 ± 0.03	0.50 ± 0.04	0.84 ± 0.10	0.64 ± 0.01	0.67 ± 0.12
lung	1.13 ± 0.09	0.75 ± 0.03	1.49 ± 0.05	1.38 ± 0.11	1.45 ± 0.15
liver	5.53 ± 0.19	6.19 ± 0.04	2.90 ± 0.11	5.84 ± 0.37	4.76 ± 0.30
			30 min		
blood	2.62 ± 0.18	4.25 ± 0.53	5.17 ± 0.32	1.49 ± 0.05	1.97 ± 0.15
heart	0.32 ± 0.03	0.56 ± 0.04	0.66 ± 0.10	1.06 ± 0.07	0.87 ± 0.02
kidney	0.23 ± 0.01	0.61 ± 0.06	0.63 ± 0.07	0.94 ± 0.08	1.00 ± 0.05
lung	0.73 ± 0.04	0.94 ± 0.06	1.10 ± 0.06	0.73 ± 0.07	1.03 ± 0.06
liver	7.62 ± 0.54	5.82 ± 0.61	4.54 ± 0.42	4.24 ± 0.20	4.20 ± 0.14
			24 h		
blood	0.12 ± 0.02	0.31 ± 0.03	0.54 ± 0.12	0.10 ± 0.02	0.07 ± 0.01
heart	0.05 ± 0.01	0.42 ± 0.02	0.21 ± 0.01	0.12 ± 0.01	0.03 ± 0.00
kidney	0.11 ± 0.00	0.29 ± 0.02	0.26 ± 0.04	0.14 ± 0.01	0.13 ± 0.01
lung	0.13 ± 0.01	0.21 ± 0.05	0.33 ± 0.03	0.12 ± 0.02	0.08 ± 0.11
liver	1.39 ± 0.15	2.19 ± 0.12	7.78 ± 0.34	1.06 ± 0.05	0.37 ± 0.03

[a] Expressed as percent administered dose per gram of tissue ± SEM; n = 3–5.

Table IV. Distribution of Radioactivity at 5 min, 30 min, and 24 h after iv Administration of ^{125}I-Labeled Glyceryl 1,2,3-Tris-[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] in Rats[a]

	compd			
tissue	**5b**	**5c**	**5d**	**5e**
		5 min		
blood	2.15 ± 0.14	6.95 ± 0.65	1.46 ± 0.17	4.14 ± 0.29
heart	1.93 ± 0.56	0.97 ± 0.21	2.67 ± 0.68	0.74 ± 0.08
kidney	0.44 ± 0.05	0.87 ± 0.13	0.60 ± 0.08	0.52 ± 0.08
lung	6.69 ± 1.66	1.49 ± 0.11	1.13 ± 0.14	1.08 ± 0.12
liver	8.02 ± 0.47	3.57 ± 0.39	6.94 ± 0.20	6.68 ± 0.27
		30 min		
blood	1.71 ± 0.12	3.31 ± 0.17	0.91 ± 0.02	2.10 ± 0.15
heart	0.82 ± 0.16	0.39 ± 0.04	0.65 ± 0.09	0.92 ± 0.04
kidney	0.33 ± 0.02	0.42 ± 0.03	0.69 ± 0.02	1.23 ± 0.11
lung	6.63 ± 0.67	0.67 ± 0.07	0.67 ± 0.03	0.94 ± 0.09
liver	10.38 ± 0.65	2.79 ± 0.15	5.24 ± 0.40	5.28 ± 0.05
		24 h		
blood	0.05 ± 0.01	0.14 ± 0.01	0.07	0.06 ± 0.00
heart	0.19 ± 0.05	0.10 ± 0.00	0.05	0.03 ± 0.00
kidney	0.09 ± 0.01	0.18 ± 0.01	0.10	0.14 ± 0.01
lung	2.64 ± 0.27	0.58 ± 0.20	0.09	0.07 ± 0.01
liver	11.78 ± 0.55	9.19 ± 1.34	0.71	0.44 ± 0.03

[a] Expressed as percent administered dose per gram of tissue ± SEM; n = 3–5 except for **5d** at 24 h where n = 1.

Table V. Liver Radioactivity after iv Administration of 1,3-Disubstituted and 1,2,3-Trisubstituted Triacylglycerols to Rats[a]

compd	5 min	30 min	24 h
4a	38.80 ± 1.06	57.22 ± 4.07	10.31 ± 1.29
4b	43.10 ± 2.77	38.42 ± 4.40	14.73 ± 0.71
4c	18.36 ± 0.73	30.25 ± 2.62	52.06 ± 2.85
4d	43.35 ± 1.21	30.66 ± 0.85	7.73 ± 0.49
4e	33.80 ± 2.59	32.70 ± 1.13	2.59 ± 0.09
5b	60.48 ± 3.54	75.54 ± 3.46	86.16 ± 4.49
5c	22.38 ± 1.31	26.04 ± 1.17	66.75 ± 4.54
5d	53.84 ± 2.88	45.72 ± 3.26	
5e	54.43 ± 2.36	38.79 ± 1.26	3.00 ± 0.08

[a] Radioactivity is expressed as percent administered dose per organ; n = 3–5 ± SEM, based on actual organ weight for each animal.

clearance were rapid with analogues **4b,d,e**. By comparison, however, uptake of phenylacetate analogue **4a** was slower but more efficient, with 57% of the administered dose residing in the liver by 30 min. Even more delayed was the uptake profile of diiopanoate **4c**, which required 24 h to achieve optimal liver concentration. At this time, over 50% of the radioactive dose resided in the liver and the liver-to-blood ratio was over 14. Although not shown, a subsequent decline in liver radioactivity to 25% of the administered dose by 48 h indicated a favorable degree of hydrolysis and/or metabolism of **4c**, much in contrast to the relative inertness previously observed with cholesteryl iopanoate.[19]

Inspection of the liver and plasma lipid extraction data obtained with 1,3-disubstituted triacylglycerols (**4a–e**) revealed the relative in vivo hydrolytic stability of short-chain analogues **4a–c** upon comparison to the long-chain analogues **4d** and **4e** (Table VII). For example, 5 min following administration, only 28 and 25% of the lipid-soluble radioactivity in the liver coincided with **4d** and **4e**, respectively, whereas from 82 to 94% of analogues **4a**, **4b**, and **4c** remained intact at the same time.

PAGE analysis of plasma samples supports the above findings. As shown in Table VI, there was an increase in the rate of ester hydrolysis with increasing chain length as indicated by the higher radioactivity levels found in the albumin fraction with analogues **4d** and **4e**. In addition, although not visible at the early time points, the retarding effect of the α-ethyl group on the rate of ester hydrolysis became evident at 24 h (i.e., comparison of **4b** with **4c**).

Perhaps the most striking results were obtained with the 1,2,3-trisubstituted triacylglycerol analogues (Tables IV and V). Administration of long-chain analogues **5d** and **5e** resulted in rapid accumulation of over 50% of the administered dose in the liver. This high uptake was coupled with liver-to-blood ratios of 4.8 and 5.8 at 5 and 30 min, respectively, for analogue **5d**. Another prominent feature was the relatively slow liver uptake of triiopanoate **5c**. By 24 h, 67% of the administered dose resided in the liver and the liver-to-blood ratio was 65. The most striking finding, however, was the rapid and sustained liver uptake of tridesethyliopanoate analogue (**5b**), which displayed liver concentrations of 60, 76, and 86% administered dose at 5 min, 30 min, and 24 h, respectively. The concentration of radioactivity in the liver at 24 h was found to be 235 times that in the blood.

Moreover, **5b** and **5c** were found to have substantial in vivo stability as indicated by their high lipid extraction efficiency and high relative percent ester values as listed in Table VII. This was in sharp contrast to long-chain analogues **5d** and **5e**, which were rapidly hydrolyzed.

The relative stability of short-chain analogues **5b** and **5c** toward in vivo hydrolysis was further illustrated by the minimal quantity of radioactivity found associated with albumin upon PAGE analysis at both 5 and 30 min as shown in Table VI. Moreover, the lower albumin radioactivity levels obtained for **5c** relative to **5b** (Table VI) demonstrate the stabilizing influence of the α-ethyl group toward in vivo hydrolysis. In agreement with the lipid extraction results noted above, long-chain analogues **5d** and **5e** were much more susceptible to hydrolysis than the short-chain analogues as indicated by the time-dependent elevation of albumin radioactivity levels.

In summary, in the series of 2-palmitoylglycerol 1,3-bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (**4a–e**), the long-chain analogues **4d** and **4e** were hydrolyzed more readily than the short-chain analogues (**4a–c**). Among the short-chain analogues, diiopanoate **4c** exhibited both an excellent liver uptake and a desirable liver-to-blood ratio at 24 h. Moreover, unlike cholesteryl iopanoate, which resisted in vivo hydrolysis for months,[19] **4c** was cleared much more readily as indicated by a 50% drop in liver radioactivity from 24 to 48 h. At earlier time periods, however, the most promising agent in the disubstituted series was the phenyl acetate analogue **4a**. The high liver values found for **4a** at 5 and 30 min make it an attractive candidate for labeling short-lived iodine-123 ($t_{1/2}$ = 13 h) for assessing its potential for liver imaging.

Despite the favorable liver specificity shown by many of the 1,3-disubstituted analogues, even higher liver uptake was observed for glyceryl 1,2,3-tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (**5b–e**). As expected, long-chain analogues **5d** and **5e** afforded respectable liver radioactivity levels, but because of very rapid in vivo hydrolysis, most of the uptake was in the form of the free acid. The most striking results were obtained with desethyliopanoate analogue **5b** and iopanoate **5c**. Uptake of **5c** was slow, but by 24 h, 67% of the administered dose was found in the liver, and the liver to blood ratio was 65. The most hepatospecific agent, however, was the tridesethyliopanoate **5b**. The total liver radioactivity levels of 60, 75, and 86% dose/organ found at 5 min, 30 min, and 24 h, coupled with liver-to-blood ratios of 3.7, 6.1, and 235, respectively, were the most impressive of any agent in the series. This very favorable distribution profile indicates that **5b** has excellent potential as a CT liver-imaging agent.

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 silia 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 mm) silica gel columns. Radio-HPLC separations were performed on a Whatman Partisil 5 RAC

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

compd	SG	LDL	HDL	ALB	BA
			5 min		
4a	18.7 ± 1.7	39.0 ± 0.8	34.7 ± 1.7	4.5 ± 0.4	3.2 ± 0.9
4b	14.2 ± 2.6	27.8 ± 4.9	52.8 ± 6.5	5.0 ± 0.9	0.2 ± 0.1
4c	6.2 ± 4.4	35.4 ± 2.2	55.1 ± 3.0	3.2 ± 0.8	0.1 ± 0.1
4d	61.6 ± 1.3	8.2 ± 0.3	12.0 ± 0.2	14.8 ± 0.6	3.4 ± 1.1
4e	2.5 ± 0.5	32.9 ± 5.5	34.4 ± 1.1	26.2 ± 3.2	4.0 ± 1.2
5b	18.3 ± 3.4	29.6 ± 2.5	38.4 ± 5.9	10.5 ± 1.5	3.2 ± 2.2
5c	15.2 ± 9.5	33.4 ± 0.2	48.2 ± 9.2	2.9 ± 0.5	0.3 ± 0.1
5d	24.4 ± 2.1	12.7 ± 2.1	27.2 ± 2.3	30.2 ± 2.0	5.5 ± 3.0
5e	6.4 ± 0.9	32.2 ± 3.4	32.7 ± 1.5	20.3 ± 1.5	7.1 ± 3.6
			30 min		
4a	16.6 ± 7.5	35.1 ± 4.9	42.0 ± 3.7	3.9 ± 0.5	2.4 ± 0.3
4b	19.0 ± 12.1	40.6 ± 9.0	36.3 ± 4.9	2.8 ± 0.4	1.2 ± 1.0
4c	6.8 ± 2.4	29.7 ± 1.9	59.7 ± 3.1	3.4 ± 0.8	0.3 ± 0.1
4d	8.6 ± 1.2	12.1 ± 0.7	25.7 ± 0.9	50.7 ± 2.2	3.1 ± 0.7
4e	9.7 ± 1.2	14.2 ± 2.2	24.8 ± 1.9	37.1 ± 7.6	14.3 ± 6.9
5b	20.2 ± 1.5	27.6 ± 1.4	34.5 ± 2.8	13.8 ± 1.0	4.1 ± 0.9
5c	9.9 ± 2.5	27.0 ± 2.2	55.8 ± 3.7	5.3 ± 1.1	1.9 ± 1.2
5d	5.8 ± 1.7	6.2 ± 0.4	33.6 ± 0.7	40.6 ± 2.0	12.6 ± 1.3
5e	3.8 ± 0.6	15.1 ± 1.3	28.1 ± 1.0	46.2 ± 2.9	6.8 ± 1.5

[a] Expressed as percent of total radioactivity in gel; n = 3–5 ± SEM; SG = stacking gel, LDL = low-density lipoprotein, HDL = high-density lipoprotein, ALB = albumin, BA = below albumin.

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

		% $CHCl_3/CH_3OH$ extractable compd		% parent compd as detd by TLC	
compd	tissue	0.5 h	24 h	0.5 h	24 h
4a	liver	90.5 ± 1.7	80.1 ± 4.3	89.5 ± 1.4	68.8 ± 9.1
	plasma	82.7 ± 0.9	67.0 ± 9.0	82.8 ± 2.3	60.8 ± 4.1
4b	liver	86.2 ± 2.6	83.1 ± 1.9	94.2 ± 0.8	88.0 ± 1.0
	plasma	62.9 ± 4.0	63.7 ± 3.1	90.6 ± 2.1	80.0 ± 1.4
4c	liver	88.4 ± 0.6	93.9 ± 0.8	86.0 ± 1.9	91.7 ± 0.5
	plasma	86.0 ± 0.3	82.1 ± 3.9	82.6 ± 2.1	77.4 ± 1.8
4d	liver	64.2 ± 2.9	81.7 ± 2.7	28.1 ± 4.2	44.9 ± 1.4
	plasma	64.6 ± 1.9	52.0 ± 10.8	11.3 ± 1.5	25.3 ± 3.4
4e	liver	42.3 ± 1.9	63.5 ± 5.2	25.3 ± 1.4	17.3 ± 3.8
	plasma	70.9 ± 2.1	44.1 ± 0.2	28.8 ± 3.8	18.0 ± 1.7
5b	liver	94.8 ± 0.5	91.1 ± 1.1	97.3 ± 0.6	96.5 ± 0.2
	plasma	89.0 ± 1.9	83.3 ± 3.5	94.5 ± 0.8	68.2 ± 7.0
5c	liver	89.0 ± 1.3	91.7 ± 2.3	92.9 ± 0.5	92.3 ± 1.4
	plasma	90.5 ± 1.8	83.4 ± 0.9	89.1 ± 1.2	75.1 ± 2.0
5d	liver	76.5 ± 1.2		68.8 ± 1.4	
	plasma	50.3 ± 2.5		24.1 ± 1.7	
5e	liver	50.7 ± 1.4	73.4 ± 1.5	15.1 ± 0.9	9.5 ± 0.6
	plasma	70.1 ± 1.1	54.2 ± 3.8	29.2 ± 3.0	20.2 ± 16.5

[a] Percent ± SEM; n = 3–5.

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). 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 (**1c**) was purchased from CTC Organics, Inc., Atlanta, GA.

Preparation of ω-(3-Amino-2,4,6-triiodophenyl)alkanoic Acids. Synthesis of ω-(3-amino-2,4,6-triiodophenyl)acetic (**1a**)[13] and propionic (**1b**)[14] acids was accomplished by literature procedures. Synthesis of ω-(3-amino-2,4,6-triiodophenyl)dodecanoic (**1d**) and heptadecanoic (**1e**) acids was described in the previous paper in this series.[11]

Preparation of 1,3-Dihydroxypropan-2-one 1,3-Bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (2a–c). 1,3-Dihydroxypropan-2-one 1,3-Diiopanoate (2a). General Procedure. DCC (9.8 g, 47.5 mmol) was added to a rapidly stirring suspension of dihydroxyacetone dimer (1.97 g, 21.9 mmol), iopanoic acid (**1c**; 25.0 g, 43.2 mmol), and a catalytic amount of DMAP (500 mg) in dry CH_2Cl_2 (120 mL). The reaction mixture was allowed to stir under N_2 for 66 h, was diluted with CH_2Cl_2 (200 mL), and was subsequently 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 to give a residue, which was triturated with anhydrous ether (100 mL) to precipitate any remaining traces of DCU. The DCU was removed by filtration and the filtrate placed in vacuo to remove the solvent. The resulting residue was recrystallized from acetone to give **2a** as an off-white powder: yield, 22.32 g (85%); mp 148–149 °C; IR (KBr) 3440, 3340 (amine), 2950 (aliph. CH), 1760–1720 (br C=O) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.08 (s, 2 H, aryl 5H), 4.85 (s, 4 H, NH_2), 4.69 (m, 4 H, CH_2O_2C), 3.44 (ddd, J = 14.1, 7.4, 1.8 Hz, 2 H, $ArCH_AH_B$), 3.31 (ddd, J = 14.1, 7.3, 1.6 Hz, 2 H, $ArCH_AH_B$), 2.89 (m, 2 H, CH), 1.91 (m, 2 H, $CH_AH_BCH_3$), 1.59 (m, 2 H, $CH_AH_BCH_3$), 0.98 (t, J = 7.3 Hz, 6 H, CH_3). Anal. ($C_{25}H_{26}I_6N_2O_5$) C, H, I.

1,3-Dihydroxypropan-2-one 1,3-Bis[12-(3-amino-2,4,6-triiodophenyl)dodecanoate] (2b). Similar treatment of dihydroxyacetone (180 mg, 2.0 mmol), acid **1d** (2.68 g, 4.0 mmol), and DMAP (55 mg) in anhydrous CH_2Cl_2 (30 mL) with DCC (908

mg, 4.4 mmol) for 18 h afforded 2.77 g of crude **11b**. The crude mixture was purified by column chromatography on silica gel (100 g) eluted with $CHCl_3$. The appropriate fractions were combined and placed in vacuo to remove the solvent. The white residue that remained was recrystallized from acetone/CH_2Cl_2 to afford **2b** as a white powder: yield, 1.64 g (59%); mp 104–106 °C; IR ($CHCl_3$) 3470, 3380 (amine), 2960, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.03 (s, 2 H, aryl 5H), 4.79 (s, 4 H, NH_2), 4.75 (s, 4 H, CH_2O_2C), 3.00 (ps t, 4 H, $ArCH_2$), 2.42 (t, J = 7.5 Hz, 4 H, O_2CCH_2), 1.8–1.1 (m, 36 H, $(CH_2)_9$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.23). Anal. ($C_{39}H_{54}I_6N_2O_5$) C, H, I.

1,3-Dihydroxypropan-2-one 1,3-Bis[17-(3-amino-2,4,6-triiodophenyl)heptadecanoate] (2c). Treatment of dihydroxyacetone (24 mg, 0.26 mmol), acid **1e** (400 mg, 0.53 mmol), and DMAP (10 mg) in anhydrous CH_2Cl_2 (5 mL) with DCC (121 mg, 0.58 mmol) in a similar manner for 48 h afforded a tannish residue (470 mg), which was purified by column chromatography on silica gel (100 g) eluted with $CHCl_3$/ethyl acetate (9:1). The compound thus obtained was crystallized from acetone/CH_2Cl_2 to afford **2c** as a white powder: yield, 187 mg (46%); mp 84–86 °C; IR ($CHCl_3$) 3480, 3380 (amine), 2935, 2860 (aliph. CH), 1740 (C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 2 H, aryl 5H), 4.79 (s, 4 H, NH_2), 4.75 (s, 4 H, CH_2O_2C), 3.00 (ps t, J = 6.2 Hz, 4 H, $ArCH_2$), 2.42 (t, J = 6.4 Hz, 4 H, O_2CCH_2), 1.8–1.1 (m, 56 H, $(CH_2)_{14}$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.38); HPLC (IBM 5-μm silica, hexane/$CHCl_3$ (1:1), K_1' = 0.38). Anal. ($C_{49}H_{74}I_6N_2O_5$) C, H, I.

Preparation of Glyceryl 1,3-Bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (3a–c). Glyceryl 1,3-Diiopanoate (3a). A stirred suspension of ketone **2a** (4.3 g, 3.6 mmol) in a mixture of THF (30 mL), benzene (8 mL), and water (2 mL) was cooled to 5 °C in an ice bath and treated with neutral $NaBH_4$ (204 mg, 5.4 mmol). The reaction mixture was stirred an additional 30 min at 5 °C and treated with glacial acetic acid (0.3 mL) to destroy excess borohydride. The resulting solution was diluted with CH_2Cl_2 (250 mL) and extracted with saturated aqueous NH_4Cl (2×), H_2O, and brine and dried ($MgSO_4$). Removal of solvent in vacuo afforded semipure **3a** (4.24 g), which was further purified by column chromatography on silica gel (150 g) eluted with $CHCl_3$/hexane/ethyl acetate (5:3:2). Combination of the appropriate fractions and removal of solvents in vacuo afforded alcohol **3a** as a yellow amorphous solid, which resisted crystallization: yield, 3.24 g (75%); mp 75–80 °C; IR ($CHCl_3$) 3580 (OH), 3480, 3380 (amine), 2870 (aliph. CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.08 (s, 2 H, aryl 5H), 4.85 (s, 4 H, NH_2), 4.10 (m, 4 H, glyceryl CH_2), 3.75 (m, 1 H, glyceryl CH), 3.40 (dd, J = 14.0, 7.7 Hz, 2 H, $ArCH_AH_B$), 3.27 (dd, J = 14.0, 7.2 Hz, 2 H, $ArCH_AH_B$), 2.80 (m, 1 H, $CHCO_2$), 1.87 (m, 2 H, $CHCH_ACH_BCH_3$), 1.60 (m, 2 H, $CHCH_AH_BCH_3$), 0.93 (t, J = 7.3 Hz, 6 H, CH_3). Anal. ($C_{25}H_{28}I_6N_2O_5$) C, H, I.

Glyceryl 1,3-Bis[12-(3-amino-2,4,6-triiodophenyl)dodecanoate] (3b). Treatment of ketone **11b** (1.45 g, 1.04 mmol) in THF (15 mL), benzene (3 mL), and H_2O (0.5 mL) with neutral $NaBH_4$ (59 mg, 1.56 mmol) in the same manner gave an off-white semisolid (1.03 g), which was recrystallized from acetone to give **12b** as colorless flakes: yield, 1.27 g (88%); mp 70–72 °C; IR ($CHCl_3$) 3530 (OH), 3480, 3380 (amine), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.02 (s, 2 H, aryl 5H), 4.79 (s, 4 H, NH_2), 4.15 (m, 4 H, glyceryl CH_2), 3.76 (m, 1 H, glyceryl CH), 3.00 (ps t, 4 H, $ArCH_2$), 2.45 (d, J = 4.7 Hz, 1 H, OH, D_2O exch.), 2.35 (t, J = 7.4 Hz, 4 H, 0_2CCH_2), 1.7–1.2 (m, 36 H, $(CH_2)_9$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.14); HPLC (Whatman 10-μm silica, hexane/$CHCl_3$ (1:1), K_1' = 0.47). Anal. ($C_{39}H_{56}I_6N_2O_5$) C, H, I.

Glyceryl 1,3-Bis[17-(3-amino-2,4,6-triiodophenyl)heptadecanoate] (3c). Similar treatment of ketone **2c** (268 mg, 0.17 mmol) in THF (3 mL), benzene (0.5 mL), and H_2O (0.05 mL) with neutral $NaBH_4$ (10 mg, 0.26 mmol) afforded a tan solid (245 mg), which was purified by column chromatography on silica gel (15 g) eluted with hexane/ethyl acetate (4:1). The compound thus obtained (230 mg) was crystallized from acetone to give **3c** as an off-white powder: yield, 226 mg (84%); mp 76–78 °C; IR ($CDCl_3$) 3550 (OH), 3470, 3370 (amine), 2960, 2860 (aliph. CH), 1735 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 2 H, aryl 5H), 4.79 (s, 4 H, NH_2), 4.14 (m, 4 H, glyceryl CH_2), 3.66 (m, 1 H, glyceryl CH), 3.01 (ps t, 4 H, $ArCH_2$), 2.43 (d, J = 4.5 Hz, 1 H, OH, D_2O exch.), 2.35 (t, J = 7.4 Hz, 4 H, O_2CCH_2), 1.7–1.2 (m, 56 H, $(CH_2)_{14}$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.21); HPLC (IBM 5-μm silica, hexane/$CHCl_3$ (1:1), K_1' = 0.27). Anal. ($C_{49}H_{76}I_6N_2O_5$) C, H, I.

Preparation of 2-Palmitoylglycerol 1,3-Bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (4c,d). Method A. 2-Palmitoylglycerol 1,3-Diiopanoate (4c). DCC (379 mg, 1.84 mmol) was added to a rapidly stirring solution of alcohol **3a** (2.0 g, 1.67 mmol), palmitic acid (428 mg, 1.67 mmol), and a catalytic amount of DMAP (20 mg) in anhydrous CH_2Cl_2 (80 mL) under N_2. The reaction mixture was stirred for 66 h, diluted with CH_2Cl_2 (200 mL), and filtered to remove precipitated DCU. The filtrate was washed successively with 0.5 N HCl (2×), saturated aqueous $NaHCO_3$ (2×), H_2O, and brine and dried ($MgSO_4$). Removal of solvent in vacuo afforded a yellow gum (2.11 g), which was purified by column chromatography on silica gel (100 g) eluted with hexane/ethyl acetate (4:1) to give **4c** as a slightly yellow gum that resisted crystallization (Table I): yield, 1.80 g (75%); IR ($CHCl_3$) 3480, 3380 (amine), 2930, 2860 (aliph. CH), 1740 (ester C=O (cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.07 (d, J = 1.5 Hz, 2 H, aryl 5H), 5.19 (m, 1 H, glyceryl CH), 4.85 (s, 4 H, NH_2), 4.30 (m, 2 H, glyceryl $CHCH_AH_BO$), 4.11 (m, 2 H, glyceryl $CHCH_AH_BO$), 3.37 (dd, J = 14.0, 7.0 Hz, 2 H, $ArCH_AH_B$), 3.25 (dd, J = 14.0, 8.0 Hz, 2 H, $ArCH_AH_B$), 2.77 (m, 2 H, CHO_2C), 2.27 (t, J = 7.4 Hz, 2 H, O_2CCH_2), 1.84 (m, 2 H, $CHCH_AH_BCH_3$), 1.54 (m, 2 H, $CHCH_AH_BCH_3$), 1.4–1.2 (m, 26 H, $(CH_2)_{13}$), 0.86 (m, 9 H, CH_3). Anal. ($C_{41}H_{58}I_6N_2O_6$) C, H, I.

2-Palmitoylglycerol 1,3-Bis[12-(3-amino-2,4,6-triiodophenyl)dodecanoate] (4d). Treatment of alcohol **3b** (200 mg, 0.14 mmol), palmitic acid (55 mg, 0.21 mmol), and DMAP (5 mg) with DCC (49 mg, 0.24 mmol) for 18 h in the manner described above yielded crude **4d** (222 mg). Purification by column chromatography on silica gel (20 g) eluted with hexane/ethyl acetate (4:1) afforded pure **4d** as an amorphous solid (Table I): yield, 160 mg (68%); IR ($CHCl_3$) 3470, 3370 (amine), 2950, 2850 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.03 (s, 2 H, aryl 5H), 5.25 (m, 1 H, glyceryl CH), 4.79 (s, 4 H, NH_2), 4.29 (dd, J = 11.9, 4.3 Hz, 2 H, glyceryl $CHCH_AH_BO$), 4.15 (dd, J = 11.9, 5.9 Hz, 2 H, glyceryl $CHCH_AH_BO$), 3.00 (ps t, J = 6.8 Hz, 4 H, $ArCH_2$, 2.31 (t, J = 7.6 Hz, 6 H, O_2CCH_2), 1.8–1.2 (m, 62 H, CH_2 envelope), 0.87 (t, J = 6.4 Hz, 3 H, CH_3); HPLC (Whatman 10-μm silica gel, hexane, K_1' = (0.36); TLC (hexane/ethyl acetate (5:2), R_f = 0.60). Anal. ($C_{55}H_{86}I_6N_2O_6$) C, H, I.

Preparation of 2-Palmitoylglycerol 1,3-Bis[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (4a,b,e). Method B. Glyceryl 2-Monopalmitate. Glyceryl 2-monopalmitate was synthesized by acylation of 1,3-benzylidine glycerol with palmitoyl chloride according to the method of Sedarivich[16] followed by benzylidine removal with trimethyl borate/boric acid as reported by Stimmel and King.[17]

2-Palmitoylglycerol 1,3-Bis[2-(3-amino-2,4,6-triiodophenyl)acetate] (4a). Treatment of acid **1a** (1.15 g, 2.2 mmol) and glyceryl 2-monopalmitate (351 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (30 mL) with DCC (500 mg, 2.4 mmol) and a catalytic amount of DMAP (30 mg) for 72 h in the fashion described above gave crude **4a** as an off-white powder. Recrystallization from hexane/acetone at −26 °C afforded **4a** as a white powder (Table I): yield, 811 mg (60%); mp 65–67 °C; IR ($CHCl_3$) 3480, 3380 (amine), 2938, 2860 (aliph. CH), 1747 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.08 (s, 2 H, aryl 5H), 5.23 (m, 1 H, glyceryl CH), 4.83 (s, 4 H, NH_2), 4.38 (dd, J = 11.8, 4.5 Hz, 2 H, glyceryl $CHCH_AH_BO$), 4.23 (s, 4 H, $ArCH_2$), 4.19 (dd, J = 11.8, 5.5 Hz, glyceryl $CHCH_AH_BO$), 2.28 (t, J = 7.4 Hz, 2 H, O_2CCH_2), 1.7–1.1 (m, 26 H, $(CH_2)_{13}$), 0.88 (t, J = 7.0 Hz, 3 H, CH_3); HPLC (IBM 5-μm silica, hexane/$CHCl_3$/ethyl acetate (6:3:1), K_1' = 0.18); TLC (silica, hexane/ethyl acetate (5:2), R_f = 0.45). Anal. ($C_{35}H_{46}I_6N_2O_6$) C, H, I.

2-Palmitoylglycerol 1,3-Bis[3-(3-amino-2,4,6-triiodophenyl)propionate] (4b). Similar treatment of acid **1b** (1.14 g, 2.1 mmol), 2-monopalmitin (351 mg, 1.0 mmol), and DMAP (30 mg) with DCC (477 mg, 2.3 mmol) for 56 h gave an oil (1.44 g), which was purified by column chromatography on silica gel (100 g) eluted with hexane/ethyl acetate (8:1). The white residue thus obtained was recrystallized from hexane/ethyl acetate (10:1) to give **4b** as a white powder (Table I): yield, 650 mg (47%); mp

93–95 °C; IR ($CHCl_3$) 3480, 3380 (amine), 2938, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.04 (s, 2 H, aryl 5H), 5.35 (m, 1 H, glyceryl CH), 4.82 (s, 4 H, NH_2), 4.39 (dd, J = 11.8, 4.4 Hz, 2 H, glyceryl CHCH_AH_BO), 4.25 (dd, J = 11.8, 5.9 Hz, 2 H, glyceryl CHCH_AH_BO), 3.38 (ps t, 4 H, ArCH_2CH_2), 2.54 (ps t, 4 H, ArCH_2CH_2), 2.35 (t, J = 7.4 Hz, 2 H, O_2CCH_2), 1.7–1.1 (m, 26 H, $(CH_2)_{13}$), 0.88 (t, J = 6.9 Hz, 3 H, CH_3); HPLC (IBM 5-μm silica, hexane/$CHCl_3$/ethyl acetate (6:3:1), K_1' = 0.36); TLC (silica, hexane/ethyl acetate (5:2), R_f = 0.48). Anal. ($C_{37}H_{50}I_6N_2O_6$) C, H, I.

2-Palmitoylglycerol 1,3-Bis[17-(3-amino-2,4,6-triiodophenyl)heptadecanoate] (4e). Similar treatment of acid **1e** (300 mg, 0.41 mmol), 2-monopalmitin (64 mg, 0.19 mmol), and DMAP (6 mg) with DCC (92 mg, 0.45 mmol) for 18 h gave a residue, which was purified by column chromatography on silica gel (40 g) eluted initially with hexane/ethyl acetate (9:1) and then with hexane/ethyl acetate (1:1). The compound thus obtained was crystallized from hexane/ethyl acetate to afford **4e** as a white solid (Table I): yield, 255 mg (75%); mp 78–79 °C; IR ($CHCl_3$) 3475, 3375 (amine), 2935, 2860 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 2 H, aryl 5H), 5.23 (m, 1 H glyceryl CH), 4.79 (s, 4 H, NH_2), 4.29 (dd, J = 11.9, 4.3 Hz, 2 H, glyceryl CHCH_AH_BO), 4.14 (dd, J = 11.9, 5.9 Hz, 2 H glyceryl CHCH_AH_BO), 2.99 (ps t, 4 H, ArCH_2), 2.31 (m, 6 H, O_2CCH_2), 1.63–1.25 (m, 82 H, $(CH_2)_{13}$), 0.88 (t, J = 6.6 Hz, 3 H, CH_3); TLC (hexane/ethyl acetate (5:2), R_f = 0.41). Anal. ($C_{65}H_{106}I_6N_2O_6$) C, H, I.

Preparation of Glyceryl 1,2,3-Tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] (5b–e). Method A. A rapidly stirred suspension of diacylglycerol (**3a–c**; 1.0 equiv), acid (**1c–e**; 1.0–1.1 equiv), and a catalytic amount of DMAP (0.1 equiv) in anhydrous CH_2Cl_2 5 mL/mmol alcohol was treated with DCC (1.1–1.2 equiv). 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 (2×), saturated aqueous $NaHCO_3$ (2×), H_2O, and brine, and the remaining residue was purified by column chromatography and/or crystallization to afford the desired triacylglycerols (**5c–e**, Table II).

Glyceryl 1,2,3-Triiopanoate (5c). Treatment of alcohol **3a** (240 mg, 0.2 mmol), iopanoic acid (**1c**; 125 mg, 0.22 mmol), and DMAP (5 mg) with DCC (50 mg, 0.24 mmol) in the manner described above afforded crude **5c** (310 mg), which was purified by column chroamtography on silica gel (30 g) with $CHCl_3$/ethyl acetate (4:1) as eluant. Removal of solvent in vacuo afforded an off-white solid, which was recrystallized from acetone/CH_2Cl_2 to give 124 mg of **5c**: IR (film) 3480, 3380 (amine), 2960 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.07 (s, 3 H, aryl 5H), 5.20 (m, 1 H, glyceryl CH), 4.84 (s, 6 H, NH_2), 4.40–4.10 (m, 4 H, glyceryl CH_2), 3.32 (m, 6 H, ArCH_2), 2.76 (m, 3 H, O_2CCH), 1.85 (m, 3 H, CH_AH_BCH_3), 1.53 (m, 3 H, CH_AH_BCH_3), 0.89 (m, 9 H, CH_3); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.20).

Glyceryl 1,2,3-Tris[12-(3-amino-2,4,6-triiodophenyl)dodecanoate] (5d). Treatment of alcohol **3b** (200 mg, 0.14 mmol), acid **1d** (105 mg, 0.16 mmol), DMAP (5 mg), and DCC (36 mg, 0.17 mmol) in a similar manner gave crude **5d** (300 mg), which was dissolved in a small volume of $CHCl_3$ and purified by column chromatography on silica gel (30 g) eluted with $CHCl_3$. Removal of solvent in vacuo gave an amorphous solid (255 mg), which was crystallized from hexane/CH_2Cl_2 to afford 230 mg of **5d** as a white powder: IR ($CHCl_3$) 3480, 3380 (amine), 2960, 2860 (aliph. CH), 1738 (ester C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.02 (s, 3 H, aryl 5H), 5.26 (m, 1 H, glyceryl CH), 4.78 (s, 6 H, NH_2), 4.29 (dd, J = 12.0, 4.3 Hz, 2 H, glyceryl CH_AH_BO), 4.15 (dd, J = 11.9, 6.0 Hz, 2 H, glyceryl CH_AH_BO), 3.00 (ps t, 6 H, ArCH_2), 2.31 (dt, J = 7.5, 2.2 Hz, 6 H, O_2CCH_2), 1.7–1.2 (m, 54 H, $(CH_2)_9$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.20); HPLC (Whatman 50-cm M9, 10-μm silica gel, $CHCl_3$/hexane (1:1), K_1' = 0.42).

Glyceryl 1,2,3-Tris[17-(3-amino-2,4,6-triiodophenyl)heptadecanoate] (5e). Similar treatment of alcohol **3c** (200 mg, 0.13 mmol), acid **1e** (103 mg, 0.14 mmol), and DMAP (3 mg) with DCC (31 mg, 0.15 mmol) afforded a tan residue (290 mg), which was purified by column chromatography on silica gel (40 g) eluted with $CHCl_3$. The solvent was removed in vacuo to yield a white residue (178 mg), which was recrystallized from acetone/CH_2Cl_2 to afforded 160 mg of **5e** as a white powder: IR ($CHCl_3$) 3480, 3380 (amine), 2970, 2865 (aliph. CH), 1740 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.03 (s, 3 H, aryl 5H), 5.26 (m, 1 H, glyceryl CH), 4.79 (s, 6 H, NH_2), 4.29 (dd, J = 11.9, 4.3 Hz, 2 H, glyceryl CH_AH_BO), 4.14 (dd, J = 11.9, 5.9 Hz, 2 H, glyceryl CH_AH_BO), 3.00 (ps t, 3 H, ArCH_2), 2.31 (t, J = 7.4 Hz, 6 H, O_2CCH_2), 1.7–1.2 (m, 84 H, $(CH_2)_{14}$); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.35); HPLC (IBM 5-μm silica gel, hexane/$CHCl_3$ (1:1), K_1' = 0.23).

Preparation of Glyceryl 1,2,3-Tris[3-(3-amino-2,4,6-triiodophenyl)propionate] (5b). Method B. DCC (397 mg, 1.9 mmol) was added to a rapidly stirred suspension of glycerol (46 mg, 0.5 mmol), acid **1b** (951 mg, 1.75 mmol), and a catalytic amount of DMAP (24 mg) in anhydrous CH_2Cl (10 mL). The reaction mixture was stirred under N_2 for 96 h, diluted with CH_2Cl_2 (20 mL), and filtered to remove DCU and a yellow precipitate, which was less polar than the DCC–acid adduct, but more polar than the free acid by TLC. The filter cake was dissolved in anhydrous Me_2SO with heating to 100 °C. The yellow solution was then allowed to cool, and the white needles that formed (DCU) were removed by filtration. The filtrate thus obtained was placed under high vacuum at 100 °C to remove the solvent. The yellow residue that remained was dissolved in a small amount of THF and purified by column chromatography on silica gel (70 g) eluted with hexane/ethyl acetate (1:1). Subsequent removal of solvents in vacuo and recrystallization of the residue from acetone/CH_2Cl_2 gave 320 mg of **5b** as a yellow solid (Table II): IR ($CHCl_3$) 3480, 3380 (amin), 1745 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.04 (d, J = 3.7 Hz, 3 H, aryl 5H), 5.40 (m, 1 H, glyceryl CH), 4.80 (s, 6 H, NH_2), 4.44 (dd, J = 11.9, 4.3 Hz, 2 H, glyceryl CH_AH_BO), 4.27 (dd, J = 11.9, 6.1 Hz, 2 H, glyceryl CH_AH_BO), 3.38 (m, 6 H, ArCH_2), 2.55 (m, 6 H, O_2CCH_2); TLC (silica gel, hexane/ethyl acetate (5:2), R_f = 0.26); HPLC (IBM 5-μm silica gel, hexane/ethyl acetate (4:1), K_1' = 1.65).

Radioiodine Exchange in Pivalic Acid Melt.[18] **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 0.5–2.5 mCi of aqueous $Na^{125}I$ (10–50 μL, no-carrier-added in reductant-free 0.1 N NaOH from DuPont, NEN Research Products). 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 and 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. In all cases, radiochemical purity of final compounds exceeded 96%. Reaction times, radiochemical yields, and radio-TLC results are included in Table VIII.

Tissue Distribution Studies. The radiolabeled compounds were dissolved in benzene and Tween-20 (Sigma Chemical Company, St. Louis, MO) 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 it became clear (2–3% Tween). The radiolabeled compound, thus solubilized, was administered intravenously to adult female Sprague-Dawley rats (Harlan Sprague-Dawley, Hasslett, MI) weighing 200–250 g. From three to five rats werre used for each compound at each time period, and the dose usually ranged between 5 and 30 μCi (9–83 μg)/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

Table VIII. Radioiodination of Glyceryl 1,3-Bis- and 1,2,3-Tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] in Pivalic Acid[a]

[125I]compd	time, h	radiochemical yield,[b] %	R_f (solvent system)[c]
4a	2	83	0.36 (A)
4b	2	96	0.34 (A)
4c	3	94	0.39 (A)
4d	1.5	91	0.46 (A)
4e	2	90	0.43 (A)
5b	2	92	0.68 (B)
5c	6	70	0.41 (A)
5d	1.5	93	0.39 (C)
5e	2	80	0.44 (A)

[a] Temperature = 155–160 °C. [b] Based on TLC on reaction mixture. Isolated yields ranged from 5 to 20% less than that shown. [c] Solvent systems employed were as follows: A (hexane/EtOAc, 5:2), B (benzene/EtOAc, 1:1), C (chloroform).

capsules and counted (84% efficiency) for radioiodine content in a Searle 1185 well scintillation counter.

Plasma and Tissue Extraction. Radioactivity was extracted from plasma by a modified Folch procedure described previously.[20] Liver samples were homogenized, extracted, and analyzed by TLC with hexane/ethyl acetate (5:2) as eluent. 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. 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.[21] 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; **1b**, 1206-91-3; **1c**, 96-83-3; **1d**, 102831-71-0; **1e**, 102831-72-1; **2a**, 103959-66-6; **2b**, 103959-67-7; **2c**, 103959-68-8; **3a**, 103980-81-0; **3b**, 103959-69-9; **3c**, 103959-70-2; **4a**, 103959-71-3; **4b**, 103959-72-4; **4c**, 103959-73-5; **4d**, 103959-74-6; **4e**, 104013-73-2; **5b**, 103959-75-7; **5c**, 103959-76-8; **5d**, 103959-77-9; **5e**, 103980-82-1; $CO(CH_2OH)_2$, 96-26-4; $CH_3(CH_2)_{14}COOCH(CH_2OH)_2$, 23470-00-0; $CH_3(CH_2)_{14}CO_2H$, 57-10-3.

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Multisubstrate Inhibitors of Dopamine β-Hydroxylase. 1. Some 1-Phenyl and 1-Phenyl-Bridged Derivatives of Imidazole-2-thione

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The synthesis and characterization of some 1-(phenylalkyl)imidazole-2-thiones as a novel class of "multisubstrate" inhibitors of dopamine β-hydroxylase (DBH) are described. These inhibitors incorporate structural features that resemble both tyramine and oxygen substrates, and as evidenced by steady-state kinetics, they appear to bind both the phenethylamine binding site and the active site copper atom(s) in DBH. A series of structural congeners that incorporate different bridging chain lengths between the phenyl ring (dopamine mimic) and the imidazole-2-thione group (oxygen mimic) define the optimum distance for inhibitory potency and the likely intersite distance in the DBH active site. Additional bridging analogues were prepared to determine the active site bulk tolerance and the effects of heteroatom replacement.

The sympathetic nervous system and its neurotransmitter norepinephrine have long been implicated in the regulation of vascular tone and the pathophysiology of hypertension.[1-5] Indeed, some studies in hypertensive patients have correlated increased plasma levels of norepinephrine with elevated resting recumbent blood pressure.[2,6] Several investigations in animals[7-10] also correlate increases in circulating norepinephrine with high blood pressure. An increased level of circulating catecholamines has similarly been noted in patients with congestive heart failure.[11] Consequently, it is plausible that interference with the biosynthesis of norepinephrine might provide a means for treating cardiovascular disorders such as hypertension and congestive heart failure.

As an approach to such potential therapeutic agents, a search was undertaken[12] for inhibitors of the enzyme dopamine β-hydroxylase (EC 1.14.17.1, DBH), a copper-containing monooxygenase, localized in the adrenal me-

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