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Both total chain length and position of dimethyl-branching effect the myocardial uptake and retention of radioiodinated analogues of 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP)

F.F. KNAPP, Jr.,* M.M. GOODMAN,*,[1] G. KIRSCH,*,[2] S.N. RESKE,**,[3] J. KROPP,**,[4] H.-J. BIERSACK,** K.R. AMBROSE,* C.R. LAMBERT* and A. GOUDONNET*

*Nuclear Medicine Group, Health Sciences Research Division, Oak Ridge National Laboratory (ORNL), USA
**Clinic for Nuclear Medicine, University of Bonn, Germany
[1]Department of Radiology, Emory University Hospital, Atlanta, Georgia
[2]Department of Chemistry, University of Metz, Metz, France
[3]Department of Nuclear Medicine, University Hospital, Ulm, Germany
[4]Clinic and Polyclinic for Nuclear Medicine, Technical University, Dresden, Germany

Introduction of geminal dimethyl-branching into the 3-position of 15-(p-iodophenyl)pentadecanoic acid (IPPA) significantly delays myocardial clearance in rats and dogs following intravenous administration. Several new analogues of DMIPP have been synthesized and evaluated in fasted rats. The effects of both the position of dimethyl-branching and the total chain-length of 3,3-dimethyl analogues on heart uptake and clearance kinetics have been studied. In the first series of compounds, two methyl groups were introduced into the 3-, 4-, 6-, or 9-position. Tissue distribution studies of the 15-(p-[I-125]iodophenyl)-analogues demonstrated that the position of dimethyl-branching is an important factor affecting both myocardial specificity and retention. The [I-125]labeled 3,3- and 4,4-DMIPP analogues showed higher myocardial uptake and faster blood clearance than the 6,6- and 9,9-DMIPP analogues [heart, % dose/gm (heart : blood), 30 min: 3,3-DMIPP = 5.06 (12 : 1); 4,4-DMIPP = 8.03 (16.7 : 1); 6,6-DMIPP = 2.26 (3.1 : 1); 9,9-DMIPP = 3.06 (2.77)]. In the second series, the effects of total fatty acid chain length were evaluated with 3,3-dimethyl-substituted analogues with C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, and C_{19} chain lengths. The C_{14} and C_{15} chain length analogues showed the best properties [global heart uptake (heart : blood ratios): 30 min: C_{11}, 0.70 (0.82); C_{12}, 1.25 (0.68); C_{13}, 0.47 (0.90); C_{14}, 1.63 (3.54); C_{15}, 5.06 (12); C_{19}, 1.29 (0.82). These detailed studies have demonstrated that both total chain length and the position of geminal dimethyl-branching are important structural parameters which affect myocardial specificity and retention of ω-(p-iodophenyl)-substituted fatty acid analogues and that 3,3-DMIPP and 4,4-DMIPP are the best candidates with optimal properties for further study.

Key words: cardiac SPECT, cardiodine, DMIPP, iodine-123, methyl-branched fatty acids

INTRODUCTION

THE AVAILABILITY of iodine-123 labeled fatty acid analogues for the evaluation of myocardial fatty acid energy substrate uptake and metabolism with single photon emission computed tomography (SPECT) has continued to stimulate widespread interest.[1–6] Our development of 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP; Fig. 1; R,S-notation indicates a racemic mixture) involved introduction of a methyl group into the 3-position of the 15-(p-iodophenyl)pentadecanoic acid (IPPA). The iodine-123-BMIPP is now commercially available as "Cardiodine" from Nihon Medi-Physics, Inc., an approved cardiac imaging agent. Radioiodinated BMIPP shows excellent myocardial localization and considerably longer retention in rats[7,8] and dogs[9] and this agent has

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For reprint contact: F.F. (Russ) Knapp, Jr., Ph.D., Group Leader, Nuclear Medicine, Health Sciences Research Division, Oak Ridge National Laboratory (ORNL), P.O. Box 2008, Oak Ridge, TN, 37831-6229, USA.
E-mail: JKP@ornl. gov

proven to be an important tool in cardiac research. Examples include the use of radioiodinated BMIPP for the evaluation of aberrations in regional fatty acid uptake in rat hearts using autoradiography,[10,11] evaluation of uptake and washout in an occlusion-reperfusion canine model[12] and investigation of the effects of high energy phosphate (ATP) levels on myocardial fatty acid uptake in mice using the 2,4-dinitrophenol (DNP) uncoupler.[13,14] BMIPP analogues have also been used by several groups for the evaluation of fatty acid uptake and metabolism by both working and non-working Langendorff isolated rat heart models.[15–18] Subsequent to our studies, an analogue of BMIPP in which the racemic methyl-substitution has been introduced into the 9-position has been synthesized[19] and evaluated in an infarction model in dogs[20] and more recently in patients with exercise-induced ischemia.[21]

Clinical use of iodine-123-labeled "modified" fatty acids analogues which show delayed myocardial clearance for myocardial single photon computerized tomographic (SPECT) imaging results from differences between regional myocardial fatty acid uptake patterns and flow tracer distribution which are often observed in various myocardial disorders.[1–6] The use of Cardiodine in conjunction with flow tracers may represent a unique opportunity to correlate energy substrate metabolism with regional myocardial viability using SPECT. Although the physiological basis is not completely understood, differences between regional fatty acid and flow tracer distribution may reflect alterations in important parameters of metabolism which can be useful for planning patient management and therapy.

The role of Cardiodine in nuclear cardiology is rapidly being defined and a variety of clinical studies have shown the usefulness of this agent with dual radioisotope studies in conjunction with flow tracers for detection and characterization of patients with hypertrophic cardiomyopathy.[22–25] The data clearly demonstrate that reduced regional accumulation of BMIPP in comparison with thallium is often observed in hypertrophic myocardium and that Cardiodine SPECT in conjunction with flow tracer assessment is thus a useful new technique to assess functional integrity of the myocardium. In spite of the fact that the physiological factors affecting such distribution patterns is not well understood, the significant differences often observed between flow tracer distribution and BMIPP uptake are felt to represent abnormalities of myocardial fatty acid metabolism in cardiomyopathic myocardium, reflecting an "intrinsic" impairment of myocardial free fatty acid utilization.

In patients with myocardial infarction and ischemia, the regional uptake and clearance of Cardiodine has also been evaluated in a number of studies.[22,26–28] "Mismatch" between BMIPP uptake and perfusion tracer distribution (i.e. Thallium-201 > BMIPP) is often observed in areas which had suffered an acute myocardial infarction and areas which were supplied by revascularized compared with nonrevascularized areas. The regional myocardial distribution of Cardiodine at rest has also been compared with [Tc-99m]-Sestamibi (MIBI).[29] The important finding from these combined studies is that lower BMIPP uptake is consistently more often observed in segments which exhibited wall motion scores lower than perfusion scores in comparison to segments showing a concordant decrease in both wall motion and perfusion scores.[29–31]

Most importantly, Cardiodine SPECT may represent a new clinical tool which could be used routinely where PET is not available for the differentiation of viable myocardium from scar following thrombolysis for acute myocardial infarction. In this regard, comparison of Cardiodine uptake, ventricular function and [^{18}F]-labeled-2-FDG has evaluated the factors which result in deceased fatty extraction in myocardial segments that have adequate perfusion but demonstrate significantly decreased contractile function. The regional uptake and clearance kinetics of Cardiodine and [1-^{11}C]palmitate with successive SPECT and PET studies have been recently evaluated in the same patients by Tamaki, et al.[32] Comparison of PET FDG results with Cardiodine SPECT clearly suggests that Cardiodine/thallium-201 mis-match is significant and can be corroborated by PET data.[32,33]

Based on the expected interference of the C-3 monomethyl group on subsequent catabolism of the fatty acid chain, we envisaged that introduction of two methyl groups (i.e. dimethyl-substitution) at the 3-position may impart complete inhibition of carbon-carbon bond cleavage and result in essentially irreversible myocardial retention.[34–36] Initial studies with the 3,3-dimethyl-branchedanalogue, 15-(p-iodophenyl)-3,3-dimethyl-pentadecanoic acid ("DMIPP" the dimethyl analogue of "Cardiodine"; Fig. 1), have demonstrated rapid and pronounced myocardial uptake in rats and dogs with essentially irreversible retention over 60 min following i.v. administration.[34,37]

The goals of the present study were to further evaluate the important effects of dimethyl-substitution by synthesizing and systematically testing myocardial uptake and retention in rats several new analogues in which we varied both the position of dimethyl-branching and the total chain length. Nine new iodine-125-labeled dimethyl-substituted terminal p-iodophenyl fatty acid analogues have been synthesized and evaluated. The four analogues in the first series have the same 15-carbon aliphatic chain length as Cardiodine with dimethyl-branching introduced at the C-3, C-4, C-6, and C-9 positions. The second series consists of five 3,3-dimethyl-branched analogues with total carbon chain lengths of C_{11}, C_{12}, C_{13}, C_{14}, C_{15} (dimethyl analogue of Cardiodine) and C_{19}. The availability of these nine analogues has now permitted the first systematic evaluation of the effects of both chain length and dimethyl substitution on myocardial uptake, retention, and heart : blood ratios. A preliminary report of the effects of the position of dimethyl-branching in IPPA ana-

logues on heart uptake in rats has been discussed.[36]

MATERIALS AND METHODS

General

All solvents, chemicals and reagents were analytical grade and were used without further purification unless otherwise indicated. The melting points were determined on a Thomas-Hoover apparatus in open capillary tubes and are uncorrected. The proton nuclear magnetic resonance spectra (NMR) was determined at 60 MHz using a Varian 360-A insrument in the solvents indicated and resonances (ppm) are reported as downfield (δ) from an internal tetramethylsilane standard. The thin-layer chromatographic (TLC) analyses were performed using precoated 250 nm layers of SiO_2 glass plates (Analtech, Inc.). The mass spectral analyses (MS) were determined by the Organic Spectroscopy Group, Analytical Chemistry Division at ORNL, by laser ionization FT mass spectrometry. All final compounds [**9,24,28e–28f**] were chromatographically pure and exhibited mass spectral and nuclear magnetic resonance (^1H) spectral properties which were consistent with the assigned structures.

Friedal-Crafts Acylation of Thiophenes—General Procedure

This procedure was used for the acylation reactions as described earlier.[7] For acylation of thiophenes containing carboxyl substituent, the carboxyl was converted to the methyl ester by initial conversion to the acid chloride by refluxing with a 10% excess of $SOCl_2$, and the resulting acyl chloride was then slowly added to MeOH. After evaporation of the MeOH, the product was dissolved in ether and filtered through a short SiO_2 column and the solvent evaporated to provide the substrate for the second acylation step. The thiophene substrate was combined with a 10% excess of the acid chloride acylating agent in 100 mL of CH_2Cl_2 at 0°C. A two molar excess of $SnCl_2$ was added dropwise and the solution then warmed to room temperature and allowed to stir overnight. Excess 6 M HCl was slowly added and the organic layer washed thoroughly with 10% HCl, H_2O, 10% NaOH and then dried over anhydrous Na_2SO_4 or $MgSO_4$. Evaporation of the CH_2Cl_2 then provided the substituted thiophene product.

Wolff-Kishner Reduction of Thiophene Keto Esters—General Procedure

The initial part of this procedure consisted of conversion of the acid to the methyl ester by refluxing with excess $SOCl_2$ and subsequent treatment with MeOH as described above. For reduction, the ketone was refluxed with 30% excess of 85% hydrazine hydrate and a ten-fold excess of KOH in 100 m*l* diethylene glycol for 1–1.5 h. Water was removed by distillation and the mixture then refluxed 3–4 h. The reaction was cooled to room temperature, diluted with a large excess of water and acidified with 1 M HCl to pH 2–3. The product was extracted with ether and the organic layer washed thoroughly with H_2O, dried and the solvent removed *in vacuo* to provide the deoxygenated product.

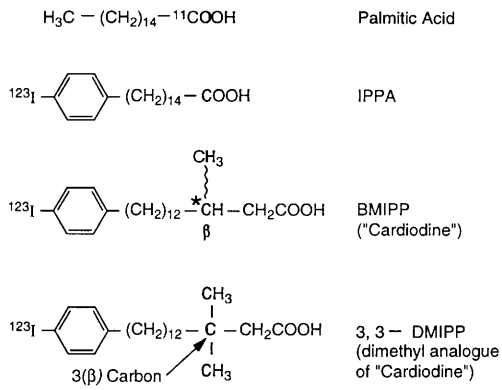


Fig. 1 Comparison of the structures of radiolabeled fatty acids for myocardial imaging. 1-[^{11}C]-Palmitic acid is compared with iodine-123-labeled fatty acids IPPA, BMIPP ("Cardiodine") and 3,3-DMIPP. In Cardiodine, position C-3 is an asymmetric center (*) and consists of a mixture of the R- and S-isomers. In 3,3-DMIPP and the other analogues with geminal dimethyl-branching, this center is not asymmetric and only one isomer exists.

Preparation of (p-Iodophenyl)dimethyl-substituted Fatty Acids—General Procedure

The acid (0.5 mmol) and thallium(III)trifluoroacetate (405 mg, 0.75 mmol) in 3 m*l* of trifluoroacetic acid were stirred at room temperature under red lights for 5 d. Potassium iodide (415 mg, 2.5 mmol) in 2 m*l* of H_2O was then added and the mixture stirred for 15 min. Sodium thiosulfate (0.5 g) was then added and the mixture stirred an additional 15 min, poured into 50 mL of H_2O, and extracted several times with Et_2O. The combined ether extracts were thoroughly washed with H_2O and dried over anhydrous Na_2SO_4, and the solvent removed *in vacuo*. The crude product was applied to a silicic acid (30 g) column slurried in C_6H_6. Fractions (20 m*l*) 1–10 were eluted with C_6H_6 followed by elution with 20 mL fractions of CH_2Cl_2. Fractions 13–20 were combined and the solvent was removed *in vacuo* to afford iodinated products **9**, **18**, and **24** (Fig. 2), and **28a–28f** (See Fig. 3). The chemical purity was confirmed by TLC (SiO_2-GF) in $CHCl_3$-CH_3OH 96 : 4, $R_f = 0.5$.

Synthesis of 15-(p-iodophenyl)-4,4-dimethylpentadecanoic Acid (4,4-DMIPP: Scheme I)

2-(3,3-Dimethyl-5-hydroxypentyl)thiophene (2). The acid 1 (1.84 g, 8.5 mmol) was added dropwise over a 15-min period to a stirred suspension of $LiAlH_4$ (1.5 g, 0.04 Mol) in 50 mL of Et_2O under an argon atmosphere at room temperature. The resulting mixture was heated under reflux for 4 h and cooled. The unreacted $LiAlH_4$ was

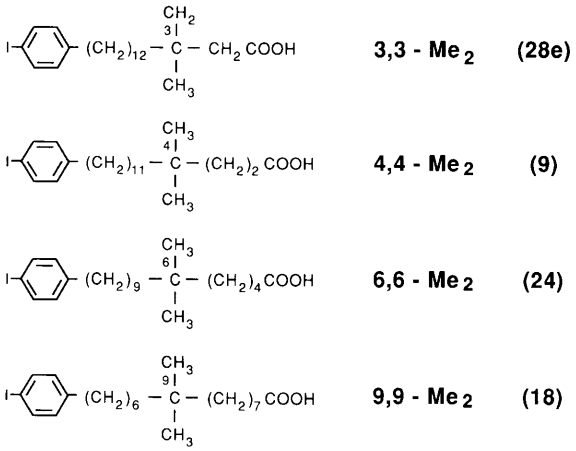
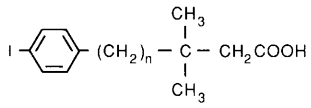


Fig. 2 Structures of 3,3-dimethyl (**28e**), 4,4-dimethyl (**9**), 6,6-dimethyl (**24**), and 9,9dimethyl (**18**) analogues of 15-(p-iodophenyl)pentadecanoic acid (IPPA).

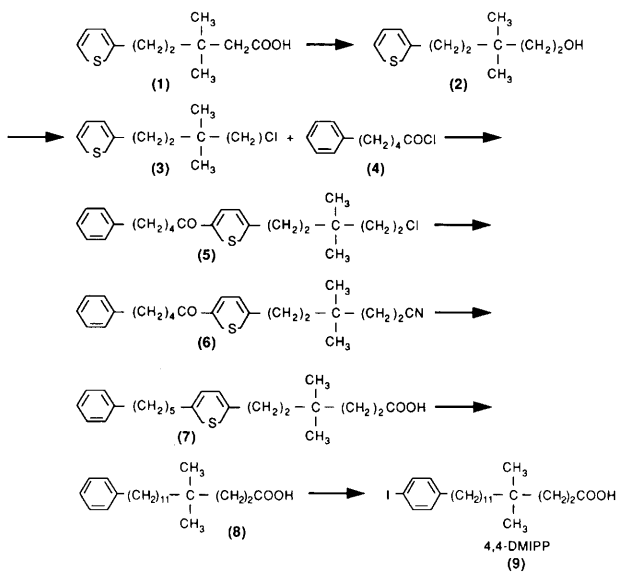


3,3 - DIMETHYL ANALOGUE	n	DESIGNATION
28a	8	C-11
28b	9	C-12
28c	10	C-13
28d	11	C-14
28e	12	C-15 (3,3 - DMIPP)
28f	16	C-19

Fig. 3 Structures of analogues in the 3,3-dimethyl series: **28a** (C_{11}), **28b** (C_{12}), **28c** (C_{13}), **28d** (C_{14}), **28e** (C_{15}, "3,3-DMIPP"), and **28f** (C_{19}).

decomposed by a dropwise addition of ethyl acetate (5 m*l*) and H_2O (5 m*l*). The resulting reaction mixture was carefully poured into ice-H_2O (100 m*l*), acidified with 10% H_2SO_4, and extracted thoroughly with Et_2O. The combined Et_2O extracts were washed thoroughly with H_2O and dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The crude product was purified on a column of silica gel (30 g). The product was eluted with benzene to give 1.4 g (85%) of **2** as a light yellow oil; NMR ($CDCl_3$) δ 1.05 (s, 6H, $(CH_3)_2$), 1.7 (m, 2H, thienyl-CH_2-CH_2-), 1.7 (m, CH_2OH-CH_2-), 2.85 (m, 2H, thienyl-CH_2), 3.5 (t, 3 = 6 Hz, 2H, CH_2OH), 6.9–7.25 (m, 3H, aromatic).

2-(3,3-Dimethyl-5-chloropentyl)thiophene (3). Thionyl chloride (3.6 g, 0.03 mol) in 10 m*l* of $CHCl_3$ was added dropwise over a 30 min period to a solution of **2** (3.9 g, 0.02 mol) and dimethylaniline (3.6 g, 0.02 mol) in 10 m*l* of $CHCl_3$ vigorously stirred at 0–5°C. The solution was stirred an additional 10 min at 0–5°C and then heated on a steam bath for 60 min and poured into 100 m*l* of ice-cold 1 M HCl. The aqueous phase was separated and extracted thoroughly with $CHCl_3$. The $CHCl_3$ fractions were combined and washed with 1 M HCl, 5% $NaHCO_3$, and H_2O and dried over anhydrous $MgSO_4$, and $CHCl_3$ was removed *in vacuo*. The crude material was dissolved in petroleum ether and applied to a column containing silica gel slurried in petroleum ether (100 g). Fractions (20 m*l* in volume) were eluted with petroleum ether (1–10) followed by fractions (20 m*l* in volume) with petroleum ether-benzene 3 : 1 (11–25). Fractions 8–19 were combined to give 3 (2.34 g, 53%) as a light yellow oil; NMR ($CDCl_3$) δ 1.25 (s, 6H, $(CH_3)_2$), 1.7 (m, 2H, thienyl-CH_2-CH_2-), 1.7 (m, CH_2Cl-CH_2-), 2.85 (m, 2H, thienyl-CH_2), 3.5 (t, J = 7 Hz, 2H, CH_2Cl), 6.9–7.25 (m, 3H, aromatic).



Scheme I

2-(3,3-Dimethyl-5-chloropentyl)-5-(1-oxo-5-phenylpentyl)thiophene (5). The acid chloride **4** (1.5 g, 7.6 mmol) was added to a solution of thiophene **3** (1.65 g, 7.6 mmol) in 50 m*l* of CH_2Cl_2. The resulting mixture was cooled to 0°C and anhydrous $SnCl_4$ (2.08 g, 8 mmol) was added dropwise. The solution was stirred at 0°C for 30 min then allowed to warm to room temperature and stirred an additional 2 h. The resulting purple-colored solution was then treated with 6 M HCl until an amber solution was obtained. The CH_2Cl_2 layer was separated and thoroughly washed stepwise with 10% HCl, H_2O and 10% NaOH, and dried over anhydrous $MgSO_4$. Evaporation of the CH_2Cl_2 *in vacuo* yielded 2.2 g (77%) of **5** as an oil; NMR ($CDCl_2$) δ 1.05 (s, 6H, $(CH_3)_2$), 1.5–1.9 (m, 10H, CH_2), 2.5–3.0 (m, 6H, thienyl-CH_2-, and Ph-CH_2-), and thienyl-C = O-CH_2-), 3.5 (t, J = 7 Hz, 2H, CH_2Cl), 6.9 (d, 1H, J = 4 Hz, aromatic), 7.27 (s, 5H, aromatic), 7.53 (d, 1H, J = 4 Hz, aromatic).

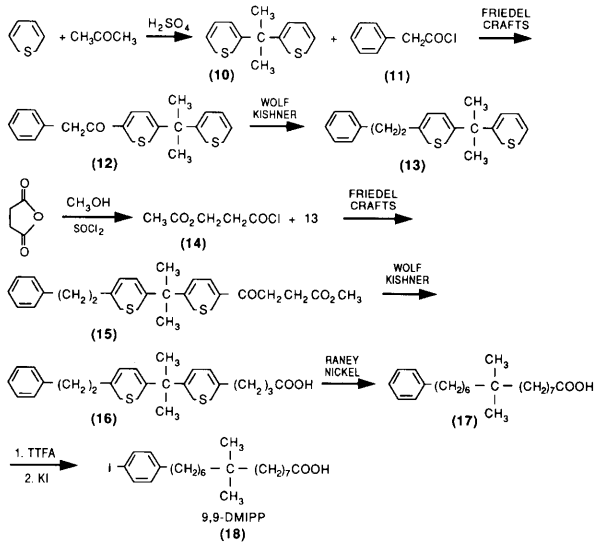
2-(3,3-Dimethyl-5-cyanopentyl)-5-(1-oxo-5-phenylpentyl)thiophene (6). A mixture of **5** (2.0 g, 5.3 mmol), NaI (0.15 g, 1 mmol), and sodium cyanide (0.4 g, 7.95 mmol) was stirred at 60°C for 6 h in 25 m*l* of dimethyl sulfoxide (DMSO). The mixture was cooled to room temperature poured into 250 m*l* of H_2O and extracted

several times with CH_2Cl_2. The combined CH_2Cl_2 extracts were thoroughly washed with H_2O and dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The crude product was dissolved in benzene and applied to a column containing silica gel (30 g) slurried in benzene. Fractions (5 m*l* in volume) were eluted with benzene. Fractions 16–24 were combined to give 0.66 g of starting material, **5**, and fractions 26–40 were combined to give 2(3,3-dimethyl-5-cyanopentyl)-5-(1-oxo-5-phenylpentyl)thiophene (6; 0.80 g, 40%) as a colorless oil; NMR ($CDCl_3$) δ 1.05 (s, 6H, $(CH_3)_2$), 1.5–1.9 (m, 10H, CH_2); 2.21 (d, 2H, J = 5 Hz, $CH_2C \equiv N$), 2.5–3.0 (m, 6H, thienyl-CH_2-, Ph-CH_2-, thienyl-C=O-CH_2-), 6.9 (d, 1H, J = 4 Hz, aromatic), 7.27 (s, 5H, aromatic), 7.53 (d, 1H, J = 4 Hz, aromatic).

2-(3,3-Dimethyl-6-hydroxyhexanoyl)-5-(5-phenylpentyl)thiophene (7). The nitrile (0.8 g, 2.18 mmoles) was added to 20 m*l* of diethylene glycol containing KOH (1.25 g, 22 mmol) and 85% hydrazine hydrate (256 mg, 4 mmol) and the mixture was refluxed for 1 h. The mixture was distilled until the solution reached a temperature of 210°C and then heated under reflux for 3 h. After cooling to 27°C the reaction mixture was poured into 100 m*l* of H_2O, acidified to pH 3 with 12 M HCl, and extracted several times with Et_2O. The combined Et_2O extracts were washed thoroughly with H_2O and dried over anhydrous $MgSO_4$, and the Et_2O was removed *in vacuo* to afford 2(3,3-dimethyl-6-hydroxyhexanoyl)-5-(5-phenylpentyl)thiophene (**7**; 742 mg, 92%) as a pale yellow oil; NMR ($CDCl_3$) δ 1.05 (s, 6H, $(CH_3)_2$), 1.5–1.9 (m, 10H, CH_2), 2.3–3.0 (m, 8H, thienyl-CH_2-, Ph-CH_2-, CH_2COOH), 6.6 (s, 2H, thienyl), and 7.27 (s, 5H, phenyl).

15-Phenyl-4,4-dimethylpentadecanoic acid (8). Raney nickel (5 g) and the thienyl acid **7** (0.6 g, 1.6 mmol) were vigorously stirred and refluxed in 100 m*l* of 10% Na_2CO_3 for 4 h. The hot solution was filtered through Celite, and the cooled filtrate carefully acidified to pH = 3 with 12 M HCl and extracted thoroughly with Et_2O. The combined Et_2O extracts were washed several times with H_2O, and dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo* to give an oil. The crude product was applied to a silicic acid (30 g) column slurried in C_6H_6. Fractions (20 m*l*) 1–10 were eluted with C_6H_6 followed by elution with 20 m*l* fractions of CH_2Cl_2. Fractions 11–20 were combined to give 15-phenyl–4,4′-dimethylpentadecanoic acid (**8**; 520 mg, 95%) as a colorless oil; NMR ($CDCl_3$) δ 1.05 (s, 6H, $(CH_3)_2$), 1.27 (s, 22H, CH_2), 2.3 (m, 2H, CH_2COOH), 2.6 (m, 2H, $PhCH_2$), and 7.3 (s, 5H, aromatic); MS, M^+ = m/z 346.

15-(p-Iodophenyl)-4,4-dimethylpentadecanoic acid (9). The acid **8** was treated with thallium(III)trifluoroacetate followed by KI as described previously (*vide ante*) to provide the para-iodo-substituted product **9**, 4,4-DMIPP; NMR ($CDCl_3$) δ0.82 (s, 6H, $(CH_3)_2$), 1.24 (m, ~22H, CH_2), 2.30 (t, 2H, -CH_2CO_2-), 2.59 (t, 2H, $PhCH_2$), and 7.24 (A_2B_2, 4H, J = 18 Hz); and MS, M^+ = m/z 472.



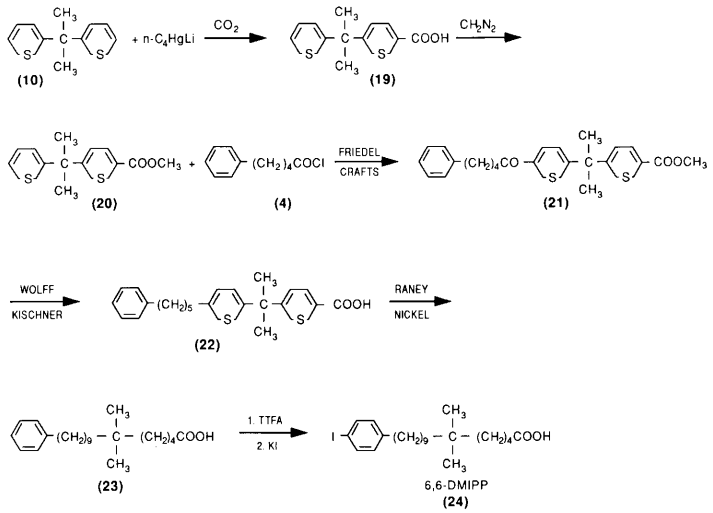
Scheme II

Synthesis of 15-(p-iodophenyl)-9,9-dimethylpentadecanoic Acid (9,9-DMIPP; Scheme II)

2-(2′-Thienyl)-2-(5′-2-phenyl-1-oxo-ethyl-2′-thienyl)-propane (12). Phenacyl chloride (**11**; 4.06 g, 36 mmol) and 2,2-di(2′-thienyl)propane (**10**; 6.24 g, 30 mmol) in 125 m*l* of CH_2Cl_2 and anhydrous $SnCl_4$ (10.4 g, 40 mmol) was reacted as described for **5**. The resulting purple-colored solution was then treated with 6 M HCl until an amber-colored solution was obtained. The CH_2Cl_2 layer was separated and thoroughly washed stepwise with 10% HCl, H_2O, and 10% NaOH, and dried over anhydrous $MgSO_4$. Evaporation of the CH_2Cl_2 *in vacuo* afforded **12** as an orange-colored oil. The crude product was applied to a silica gel (200 g) column slurried in C_6H_6. Fractions (20 m*l*) 1–40 were eluted with C_6H_6. Fractions 19–31 were combined to give 5.73 g of **12** (63%) as light yellow-colored oil; NMR ($CDCl_3$) δ1.8 (s, 6H, $(CH_3)_2$), 3.3 (s, 2H, $PhCH_2C$=O), 6.4–7.6 (m, 5H, thienyl), 7.4 (s, 5N, aromatic).

2-(2′-Thienyl)-2-(5′-2-phenylethyl-2′-thienyl)propane (13). The ketone **12** (4.5 g, 15 mmol) was added to 40 m*l* of diethylene glycol containing KOH (6 g, 107 mmol) and 85% hydrazine hydrate (3 g, 48 mmol) and the mixture was reacted as described for **6**. After cooling to 27°C the reaction mixture was poured into 300 m*l* of H_2O and extracted several times with Et_2O. The combined Et_2O extracts were washed thoroughly with H_2O and dried over anhydrous $MgSO_4$, and the Et_2O was removed *in vacuo* to give **13** as a yellow-colored oil. The crude product was applied to column containing silica gel (100 g) slurried in petroleum ether. Fractions (20 m*l*) 1–40 were eluted with petroleum ether. Fractions 35–60 were combined to give 2-(2′-thienyl)-2-(5′-2-phenylethyl-2′-thienyl)propane (**13**; 2.51 g, 60%) as a colorless oil; NMR ($CDCl_3$) δ1.8 (s, 6H, $(CH_3)_2$), 3.0 (s, 4H, $C\underline{H}_2$-$\underline{C}H_2$), 6.4–7.2 (m, 5H, thienyl).

2-(2′-1-Oxo-4-carbomethoxybutanoyl-2′-thienyl)-2-(5′-2-phenylethyl-2′-thienyl)propane (15). 4-Carbo-



Scheme III

methoxypropionyl chloride (**14**; 2.0 g,12 mmol) and 2-(2′-thienyl)-2-(5′-2-phenylethyl-2′-thienyl)propane (**13**; 2.2 g, 8 mmol) in 80 mL of CH_2Cl_2 and anhydrous $SnCl_4$ (6 g, 24 mmol) was reacted as described for **5**. The resulting purple-colored solution was then treated with 6 N HCl until an amber solution was obtained. The CH_2Cl_2 layer was separated and thoroughly washed with 10% HCl, H_2O, 10% NaOH and dried over anhydrous $MgSO_4$. Evaporation of the CH_2Cl_2 *in vacuo* afforded **12** as an orange-colored oil. The crude product was applied to a silica gel (150 g) column slurried in C_6H_6. Fractions (20 m*l*) 1–40 were eluted with C_6H_6. Fractions 25–38 were combined to give 1.08 g of **15** (27%) as light yellow oil; NMR ($CDCl_3$) δ 1.8 (s, 6H, $(CH_3)_2$), 2.7–3.4 (m, 8H, Ph-$\underline{CH_2CH_2}$, C=O-$C\underline{H_2}$-$\underline{CH_2}$), 3.67 (s, 3H, $COOCH_3$), 6.4–7.6 (m, 4H, thienyl), 7.4 (s, 5H, aromatic).

2-(2′-4-Hydroxybutyl-2′-thienyl)-2-(5′-2-phenylethyl-2′-thienyl)propane (16). The ketoester **15** (1.00 g, 2.5 mmol) was added to 30 mL of diethylene glycol containing KOH (750 mg, 13.5 mmol) and 85% hydrazine hydrate (500 mg, 8 mmol) and the mixture was reacted as described for **6**. After cooling to 27°C the reaction mixture was poured into 400 m*l* of H_2O acidified to pH 3 with 12 M HCl, and extracted several times with Et_2O. The combined Et_2O extracts were washed thoroughly with H_2O and dried over anhydrous $MgSO_4$, and the Et_2O was removed *in vacuo* to give **16** as a yellow-colored oil. The crude product was applied to a silica gel (30 g) column slurried in CH_2Cl_2. Fractions (20 m*l*) 1–20 were eluted with CH_2Cl_2. Fractions 4–10 were combined to give acid 16 (435 mg, 45%) as a colorless oil; NMR ($CDCl_3$) δ 1.8 (s, 6H, $(CH_3)_2$), 1.5–1.9 (m, 2H, $\underline{CH_2}$-CH_2COON), 2.5–2.9 (m, 8H, Ph $\underline{CH_2CH_2}$, thienyl-CH_2-, -CH_2COOH), 6.6 (d, 2H, thienyl), 6.9 (d, 2H, thienyl), 7.4 (s, 5H, aromatic).

15-Phenyl-9,9-dimethylpentadecanoic acid (17). Raney nickel (35 g) and the thienyl acid (**16**; 390 mg, 1 mmol) were reacted in 300 mL of 10% Na_2CO_3 containing 30 m*l* ethanol as described for **8**. The filtered Raney nickel was cooled and carefully acidified to pH 3 with 12 M HCl and extracted thoroughly with Et_2O. The filtered Raney nickel was dissolved in 12 M HCl and the resulting green solution was extracted with Et_2O as described above. The combined Et_2O extracts were washed several times with H_2O, and dried over anhydrous $MgSO_4$ and the solvent was evaporated *in vacuo* to give an oil. The crude product was applied to a silicic acid (30 g) column slurried in C_6H_6. Fractions (20 m*l*) 1–10 were eluted with C_6H_6 followed by elution with 20 m*l* fractions of CH_2Cl_2. Fractions 9–16 were combined to give 15-phenyl-9,9-dimethylpentadecanoic acid (**17**; 200 mg, 60%) as a colorless oil; NMR ($CDCl_3$) δ 0.8 (s, 6H, $(CH_3)_2$), 1.0–1.8 (brs, 22H, CH_2), 2.34 (t, 2H, J = 4 Hz, CH_2COOH), 2.65 (t, 2H, J = 7 Hz, Ph-CH_2), 7.3 (s, 5H, aromatic); MS, M^+ = m/z 346.

15-(p-Iodophenyl-9,9-dimethylpentadecanoic acid (18). The acid substrate **17** was converted to the para-iodo product 18 in the usual manner (*vide ante*); NMR ($CDCl_3$) δ0.799 (s, 6H, $(CH_3)_2$), 1.23 (m, 22H, -CH_2) and 7.24 (A_2B_2, 4H, J = 18 Hz); MS, M^+ = m/z 472.

Synthesis of 15-(p-iodophenyl)-6,6-dimethyl-pentadecanoic Acid (6,6-DMIPP: Scheme III)

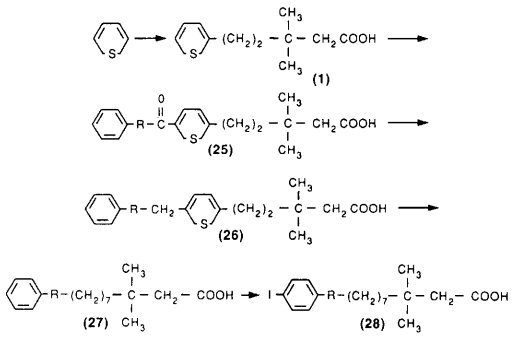
2-(2′-thienyl)-2-(5′-carboxy-2′-thienyl)propane (19). The 2,2-di(2′-thienyl)propane (**10**; 10.4 g, 0.05 mol) was stirred in 100 m*l* of dry diethyl ether under nitrogen at ambient temperature and 40 m*l* of a 1.55 M n-butyllithium solution was added with a syringe through a rubber septum. The resulting mixture was stirred at ambient temperature for 15 min and poured onto a mixture of diethyl ether and crushed dry ice. The resulting solution was poured into 100 m*l* of H_2O and extracted several times with diethyl ether. The diethyl ether extracts were washed with 50 m*l* of H_2O and the combined H_2O phases were acidified to pH 3 with 12 M HCl and extracted several times with diethyl ether. The combined diethyl ether

extracts were washed several times with water, dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo* to yield 14.8 g of crude 2-(2′-thienyl)-2-(5′-carboxy-2′-thienyl)propane (**19**); NMR ($CDCl_3$) δ 1.9 (s, 6H, $(CH_3)_2$), 6.8–7.6 (m, 5H, thienyl).

2-(2′-Thienyl)-2-(5′-carbomethoxy-2′-thienyl)propane (20). The acid **19** (14.8 g, 0.059 mol) was added to an ether solution (100 m*l*) containing excess CH_2N_2, prepared from N-methyl-N′-nitro-N-nitrosoguanidine (MNNG; 7.5 g). The mixture was stirred at 0°C under red lights for 12 h and the Et_2O solution dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo* to yield an oil. The crude product was applied to a silica gel (100 g, Davidson) column slurried in petroleum ether (30–60°C boiling range). Fractions (20 m*l*) were eluted with 1 : 1 petroleum ether (30–60°C boiling range): benzene. Fractions 20–50 were combined to give 2-(2′-thienyl)-2-(5′-carbomethoxy-2′-thienyl)propane (**20**; 4.73 g, 37%) as a pale yellow oil; NMR ($CDCl_3$) δ 1.9 (s, 6H, $(CH_3)_2$), 3.65 (s, 3H, $-COOCH_3$), 6.8–7.6 (m, 5H, thienyl).

2-(5′-5-Phenyl-1-oxo-pentyl-2′-thienyl)-2-(5′-carbomethoxy-2′-thienyl)propane (21). Anhydrous $SnCl_4$ (5.2 g, 20 mmol) was added dropwise to a solution of 5-phenylpentanoyl chloride (**4**; 2.0 g, 10 mmol) and 2-(2′-thienyl)-2-(5′-carbomethoxy-2′-thienyl)propane (**20**; 2.66 g, 10 mmol) in CH_2Cl_2 (100 m*l*) stirred at 0°C. The resulting purple solution was stirred at 0°C for 30 min and then at room temperature for 2 h and treated with 150 m*l* of 6 M HCl at 0–5°C until a yellow-colored solution was obtained. The CH_2Cl_2 layer was washed (4 × 100 m*l*) with 1 M HCl, with H_2O (3 × 100 mL), dried over anhydrous Na_2SO_4 and the CH_2Cl_2 was removed *in vacuo* to afford 4.31 g of **21** as a yellow oil; NMR ($CDCl_3$), 1.5–1.9 (m, 4H, Ph-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-C=O), 1.9 (s, 6H, $(CH_3)_2$), 2.4–2.9 (m, 4H, Ph-CH_2-, CH_2-C=O), 3.65 (s, 3H, $COOCH_3$), 6.85 (d, 2H, J = 4 Hz, thienyl), 7.2 (s, 5H, aromatic), 7.45 (d, 1H, J = 4 Hz, thienyl), 7.6 (d, 1H).

2-(5′-5-Phenylpentyl-2′-thienyl)-2-(5′-carboxy-2′-thienyl)propane (22). The acid **21** (3.2 g, 7.5 mmoles) was added to 60 m*l* of diethylene glycol containing KOH (1.5 g, 27 mmol) and 85% hydrazine hydrate (1 g, 30 mmol) and the mixture refluxed for 1 h. The mixture was distilled until the solution reached a temperature of 210°C and then heated under reflux for 3 h. After cooling to 27°C the reaction mixture was poured into 300 m*l* of H_2O, acidified to pH 3 with 12 M HCl, and extracted several times with Et_2O. The combined Et_2O extracts were washed thoroughly with H_2O and dried over anhydrous Na_2SO_4, and the Et_2O removed *in vacuo* to afford 2-(5′-5-phenylpentyl-2′-thienyl)-2-(5′-carboxy-2-thienyl)propane (**22**; 2.84 g, 94%) as a pale yellow oil; NMR ($CDCl_3$) δ 1.4–1.7 (m, 6H, Ph-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_2}$), 1.85 (s, 6H, $(CH_3)_2$), 2.5–2.9 (m, 4H, Ph-CH_2-, thienyl-CH_2-), 6.55 (d, 1H, J = 4 Hz, thienyl), 6.7 (d, 1H, J = 4 Hz, thienyl), 6.85 (d, 1H, J = 4 Hz, thienyl), 7.2 (s, 5H, aromatic), 7.8 (d, 1H, J = 4 Hz, thienyl).



Scheme VI

15-Phenyl-6,6-dimethylpentadecanoic acid (23). Raney nickel (80 g) and the thienyl acid **22** (2.0 g, 5 mmol) were vigorously stirred and refluxed in 300 m*l* of 10% Na_2CO_3 for 10 h. The hot solution was filtered through Celite, and the cooled filtrate carefully acidified to pH 3 with 12 M HCl and extracted thoroughly with Et_2O. The combined Et_2O extracts were washed several times with H_2O, and dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo* to give 600 mg of an oil which crystallized on standing. The filtered Raney nickel was dissolved in 12 M HCl and the resulting green-colored solution extracted with Et_2O as described above to give an additional 425 mg of 15-phenyl-6,6-dimethylpentadecanoic acid (**23**), 59% yield as a clear yellow-colored oil; NMR ($CDCl_3$) δ 0.8 (s, 6H, $(CH_3)_2$), 1.0–1.8 (s, 22H, CH_2), 2.3–2.8 (m, 4H, Ph-CH_2, CH_2COOH), 7.2 (s, 5H, aromatic); MS, M^+ = m/z 472.

15-(p-Iodophenyl)-6,6-dimethylpentadecanoic acid (24). The acid **23** was converted to 24 in the usual manner (*vide infra*); NMR ($CDCl_3$) δ 0.811 (s, 6H, $(CH_3)_2$), 1.24 (m, 22H, $-CH_2$), 2.35 (t, 2H, $-CH_2CO_2$-), 2.51 (t, 2H, Ph-CH_2), and 7.24 (A_2B_2, 4H, J = 18 Hz); MS, M^+ = m/z 472.

Preparation of 2-(3,3)-Dimethyl-1-hydroxypentanoyl)-Thiophene (1) Substrate and Preparation of 3,3-Dimethyl Analoques 28a–28f

This substrate was used for the synthesis of the 3,3-dimethyl-substituted analogues **28a–28f** and was prepared as described earlier by the acylation of thiophene with the monomethyl ester of 3,3-dimethylglutaric acid followed by Wolff-Kishner reduction (Knapp et al., 1986a).

Synthesls of 15-(p-iodophenyl)-3,3-dimethylpentadecanoic Acid (3,3-DMIPP, 28e)

The 3,3-dimethyl analogue (Fig. l) was prepared as described earlier*, m.p. 37–39°C; MS, M^+ = m/z 472.

Synthesis of 3,3-Dimethyl Analogues of 15-phenylpentadecanoic Acid (28a–28f, Scheme IV)

These analogues (Scheme IV) were synthesized by Freidal-Craft acylation of the thiophene template (**1**) with the appropriate terminal phenyl-substituted acyl chloride followed by Wolf-Kishner reduction and para-iodination, as

Table 1 Data for Friedal-Crafts and Wolff-Kishner intermediates for synthesis of 3,3-dimethyl-ω-(p-iodophenyl) fatty acid analogues. The synthetic sequence is outlined in scheme IV[1,2]

Friedal Crafts product[3,4]			Wolff-Kishner products[3]	
Compound	Chain length	% Yield	Compound	% Yield
25a	C_{11}	65%	26a	61%
25b	C_{12}	75%	26b	43%
25c	C_{13}	88%	26c	75%
25b	C_{14}	—	26d	—
25e	C_{15}	91%	26e	89%
25f	C_{19}	95%	26f	86%

[1] All products were pure by TLC, and proton NMR and mass spectra were consistent with proposed structures.
[2] The synthesis and properties of **25e**, **26e**, **27e**, and **28e** have been reported earier (Ref. #34).
[3] Intermediates **25** and **26** were used directly for the next step of the reaction sequence.
[4] The Friedel-Craft products were generally oils at room temperature.

described in Table 1. The acyl chlorides, prepared from the commercially available ω-phenylalkanoic acids, used for the various analogues were as follows: **28a**, phenylacetic acid; **28b**, 3-phenylpropionic acid; **28c**, 4-phenylbutyric acid; **28d**, 5-phenylpentanoic acid; **28e**, 6-phenylhexanoic acid; 28f, 10-phenyldecanoic acid.

Preparation and Formulation of Iodine-125-labeled Fatty Acids

Iodine-125 was introduced into the *para*-position of the terminal phenyl-substituted analogues in the usual manner by potassium iodide treatment of the thallated intermediate prepared by reaction of the substrate with 2 equivalents of thallium(III)trifluoroacetate in trifluoroacetic acid in the dark overnight. All radioiodinated fatty acid analogues had similar specific activity values of 2.4–4 Ci/mmole. The fatty acid was dissolved in a minimal amount of warm ethanol (~100 μl) which was added slowly with stirring to a 6% solution of bovine serum albumin at 45–50°C. The solution was filtered through a Millipore filter before injection.

Tissue Distribution Studies

The distribution of radioactivity was determined in tissues of 6–8 week-old female Fischer 344 rats (120–130 gm) after i.v. administration of the radioiodinated fatty acids. Food was removed from the rats 18 h prior to initiation of the experiment, but the animals were allowed water *ad libitum* prior to and during the course of the experiment. The radioiodinated fatty acids were dissolved in absolute ethanol (~100 μl) which was added dropwise to a stirred solution of 6% bovine serum albumin at ~40°C. The final ethanol concentration was less than 10%. The solution was filtered through a 0.22-μm Millipore filter and injected through a lateral tail vein into the diethyl ether-anesthetized animals. After the times indicated, the animals were killed and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline, and blotted dry to remove residual blood. The organs were weighed and counted in a NaI autogamma counter. For the dual-labeling experiments, the ^{123}I (159 keV) photopeak was counted and the samples then stored in the cold until the ^{123}I contribution to the ^{125}I x-ray photopeak region was < 4–5%. The samples were then counted again to determine the distribution of ^{125}I. Samples of the injected radioactive solutions were also assayed at both counting periods as standards for decay corrections and to calculate the percent injected dose per gm of tissue values. The thyroid glands were not weighed directly. The weight of the thyroid glands was calculated in the usual manner by multiplying the animal weight by 7.5 mg/100 gm.

RESULTS

Chemical Syntheses of New Analogues

Four dimethyl-branched analogues of Cardiodine were synthesized (Fig. 2). The 4,4-DMIPP analogue was synthesized in a 7-step reaction sequence (Scheme I) from a key synthetic intermediate, 2-(3,3-dimethyl-5-hydroxypentanoyl)thiophene (**1**), which was also utilized earlier for introduction of dimethyl-branching into the alkanoic chain for preparation of 3,3-DMIPP[34] and 3,3-dimethyl-19-E-iodononadecanoic acid (3,3-DMIVN).[35] In this synthetic approach, 2-(3,3-dimethyl-5-hydroxypentanoyl)-thiophene (**1**) was reduced with $LiAlH_4$ to afford 2-(3,3-dimethyl-5-hydroxypentyl)thiophene (**2**). The thienyl alcohol (**2**) was converted to the alkyl chloride (**3**) by treatment with N,N′-dimethylaniline and thionyl chloride. The phenylalkyl moiety was then introduced into the 5′-thienyl position by coupling of the acid chloride of 5-phenylpentanoic acid (**4**) with chloride (**3**) catalyzed by treatment with tin(IV) chloride in methylene chloride. The chloroketone (**5**) was then converted to the nitrile (**6**) by treatment with NaCN in DMSO. The keto function of compound (**6**) was reduced by the Wolff-Kishner (Huang-Minlon) method which concomitantly hydrolyzed the nitrile function to give 2-(4,4-dimethyl-6-hydroxyhexanoyl)-5-(5-phenylpentyl)thiophene (**7**). Raney Nickel desulfurization of the thienyl acid (**7**) to compound (**8**)

Table 2 Data for Raney Nickel and iodination reactions for synthesis of 3,3-dimethyl-ω-(p-iodophenyl) fatty acid analogues. The synthetic sequence is outlined in scheme IV[1,2]

Raney Nickel product			Final iodinated product	
Compound (% yield)	mp, °C	Low resolution M^+, m/z (%)	Compound	mp, °C
27a (95%)	oil	290 (20%)	28a	oil
27b (51%)	oil	304 (22%)	28b	—
27c (55%)	oil	318 (11%)	28c	—
27d (52%)	wax	332 (10%)	28d	—
27e (51 %)	39–40	346 (20%)	28e	37
27f	liquid	402 (10%)	28f	—

[1] All products were pure by TLC, and proton NMR and mass spectra were consistent with proposed structures.
[2] The synthesis and properties of **25e**, **26e**, **27e**, and **28e** have benn reported earlier (Ref. #34).

followed by subsequent treatment with thallium(III)-trifluoroacetate and potassium iodide gave 4,4-DMIPP (**9**).

For synthesis of the 6,6- and 9,9-dimethyl analogues, dimethyl-branching was introduced utilizing *bis*(thienyl)-propane (**10**, Scheme II) with subsequent chain elongation via fabrication of the 5,5′-disubstituted thiophene system by successive Friedal-Crafts and Wolff-Kishner sequences. By selection of the substituents introduced into the 2- and 2′-positions of the thiophene ring of *bis*-(thienyl)propane (**10**), a variety of dimethyl-branched fatty acids with branching at positions 6 through 10 can be prepared. In the synthetic approach for preparation of these analogues, *bis*(thienyl)propane (**10**) was prepared by treatment of thiophene and acetone with 75% sulfuric acid. The synthesis of the analogue 9,9-DMIPP (**18**) was accomplished in 9-steps as shown in Scheme II. Utilizing this approach, commercially available phenylacetyl chloride (**11**) was coupled with *bis*(thienyl)propane (**10**) by treatment with tin(IV) chloride in dichloromethane which gave 2-(2′-thienyl)-2-(5′-2-phenyl-1-oxo-ethyl-2′-thienyl)propane (**12**). Wolff-Kishner reduction of 12 gave 2-(2′-thienyl)-2-(5′-2-phenylethyl-2-thienyl)propane (**13**). The carboxylic acid moiety was introduced into the 2′-thienyl position by acylation of the half ester acid chloride of succinic anhydride. The 3-carbomethoxypropionyl chloride (14) was prepared from commercially available succinic anhydride by treatment with methanol followed by thionyl chloride. The thienyl derivative (**13**) was subjected to Friedel-Crafts condensation with the acyl chloride (**14**) to afford 2-(2′-1-oxo-4-carbomethoxy-butanoyl-2′-thienyl)-2-(5′-2-phenylethyl-2′-thienyl)-propane (**15**). Wolff-Kishner reduction of the keto ester (**15**) gave 2-(2′-4-hydroxybutyl-2′-thienyl)-2-(5′-2-phenyl-ethyl-2′-thienyl)propane (**16**). The pivotal step in the synthesis involved the Raney nickel desulfurization of **16** to provide **17**, followed by subsequent treatment with the thallium(III)trifluoroacetate and potassium iodide to give 9,9-DMIPP (**18**).

The preparation of 15-(p-iodophenyl)-6,6-dimethyl-pentadecanoic acid (6,6-DMIPP) was achieved in a manner analogous to the 9,9-analogue as shown in Scheme III. The 2,2-di(2′-thienyl)propane (**10**) was converted to the 2-lithio derivative using n-butyllithium and then reacted with dry ice to give 2-(2′-thienyl)-2-(5′-carboxy-2′-thienyl)propane (**19**). Compound **19** was then treated with diazomethane to give the corresponding methyl ester (**20**). The resulting ester and 5-phenylpentanoyl chloride (**4**) were subjected to Friedel-Crafts acylation to afford 2-(5′-5-phenyl-1-oxo-pentyl-2′-thienyl)-2-(5′-carbo-methoxy-2′-thienyl)propane (**21**). Wolff-Kishner reduction of ketone (**21**) gave 2-(5′-5-phenylpentyl-2′-thienyl)-2-(5′-carboxy-2′-thienyl)propane (**22**). Raney nickel desulfurization of the thienyl acid (**22**) and subsequent iodination using thallium(III)trifluoroacetate and potassium iodide afforded 6,6-DMIPP (**24**).

The 3,3-dimethyl analogues 28a–28f (Fig. 3) were synthesized by acylation of 2-(3,3-dimethyl-5-hydroxy-pentyl)thiophene (1, Scheme IV). Five 3,3-dimethyl analogues (Fig. 1) with C-11, C-12, C-13, C-14, C-15 (the original 3,3-DMIPP analogue reported earlier; Knapp et al., ref. #34) and C-19 carbon chain lengths were synthesized from the common 2-(3,3-dimethyl-1-hydroxy-pentanoyl)-thiophene intermediate (1, Scheme IV). By choice of the appropriate ω-phenylalkanoyl chloride, the various analogues were readily prepared by acylation of (1) followed by Wolff-Kishner deoxygenation of the disubstituted thiophene product (**25**) and sulfur extrusion of (**26**) with Raney Nickel. Iodine was then introduced into the para-position of (**27**) by the usual thallation-potassium iodide route to provide the final product (**28**). The iodine-125-labeled analogues were prepared for tissue distribution studies in fasted rats in the same manner using iodine-125.

Biological Studies—Effects of Position of Dimethyl Branching on 15-Carbon Chain Length Analogues

The 3,3-, 4,4-, 6,6-, and 9,9-DMIPP analogues were radioiodinated with [iodine-125] sodium iodide and the radioiodinated agents evaluated in fasted female Fisher rats. The results of the biological evaluation are summarized in Table 2. The level of accumulation of

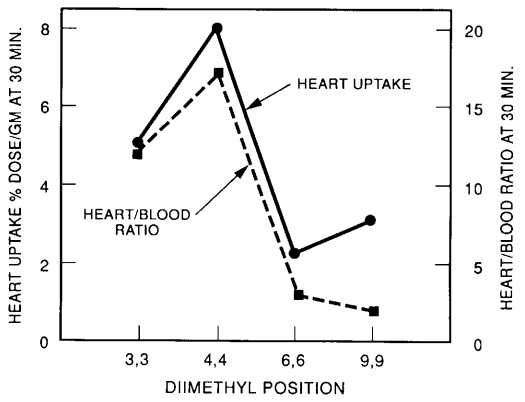


Fig. 4 Comparison of myocardial uptake (% injected dose/gram) and heart : blood ratios of the four dimethyl analogues with various chain lengths 30 min after administration to fasted rats.

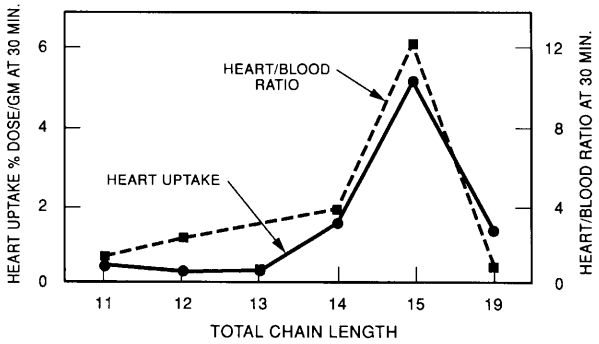


Fig. 5 Comparison of myocardial uptake and heart : blood ratios of the six 3,3-dimethyl carbon-chain analogues 30 min after administration to fasted rats.

radioactivity in the myocardium after injection of 6,6- and 9,9-DMIPP was only moderate. The blood levels of activity were relatively high resulting in modest heart: blood ratios (< 3 : 1). These analogues thus appear to show lower heart uptake, more rapid myocardial washout, and higher blood levels than for the 3,3-DMIPP analogue. Since these analogues all have the same C_{15} chain length, the results indicate that the position of dimethyl-branching is an important structural feature. In contrast, both the 3,3-DMIPP and 4,4-DMIPP analogues exhibit excellent myocardial retention (> 95%) 30 min following intravenous injection, which decreases to < 65% after 60 min (Fig. 4).

Enects of Chain Length of 3,3-Dimethyl Analogues

In a similar manner to the comparative evaluation of the 3,3-, 4,4-, 6,6-, and 9,9-dimethyl analogues of 15-(p-iodophenyl)-pentadecanoic acid, the six 3,3-dimethyl analogues with chain lengths varying from 11–19 carbon atoms (**28a–28f**) were also evaluated in fasted rats (Table 4). This series of experiments demonstrated that the total chain length is also an important feature, with the 15-carbon chain length 3,3-dimethyl analogue (**28e**) showing the highest myocardial extraction and resulting in the highest heart: blood ratios (Fig. 5). Consistent with the higher *in vivo* uptake of the 4,4-DMIPP analogue (**9**) observed in rats in comparison with 3,3-DMIPP (**28e**), other studies with the traditional non-working Langendorff-perfused isolated rat heart preparation have demonstrated good uptake *in vitro* and nearly irreversible retention with only very low subsequent loss of activity in the outflow (unpublished data).

DISCUSSION

The presence of dimethyl-branching in the 3-position of radioiodinated DMIPP[34] has been shown to result in significantly increased retention of radioactivity after intravenous administration to rats (3,3-DMIPP; Table 2). The results of the individual studies summarized in Table 2 appear to indicate that the 4,4-DMIPP analogue has greater heart uptake and higher heart: blood ratios than the model 3,3-DMIPP analogue. In addition, radioiodinated 3,3-DMIPP also shows much longer retention in the canine heart in comparison with the unbranched IPPA and 3-(R,S)-monomethyl-branched BMIPP analogues.[1–6,34] In addition to the studies described above, the subcellular and lipid pool distribution of radioiodinated 3,3-DMIPP have also been evaluated in detail *in vivo* following administration to Fischer rats[8] and in the endogenous lipids of Langendorff perfused rat hearts. The high myocardial extraction and longer retention of radioiodinated DMIPP in comparison with Cardiodine determined in these studies appears to be related to the slow conversion to triacylglyceride storage products. More recently, the high myocardial uptake and prolonged retention was further confirmed in the normoxic hearts of a canine model and the incorporation into endogenous lipids has also been reported,[37] clearly demonstrating that geminal 3,3-dimethyl substitution does not interfere with targeting of selected long chain fatty acids to the myocardium. Both 3,3-DMIPP and 4,4-DMIPP are thus good candidates for further evaluation.

Although the exact mechanism of retention has not yet been elucidated, analogues which show retention such as 3,3- and 4,4-DMIPP are presumed to be incorporated into myocardial triglyceride storage products, as has been demonstrated in earlier studies with 3-R,S-BMIPP and 3,3-DMIPP.[1–5,8,16] In addition, the mechanisms involved in myocardial uptake of fatty acids and the involvement of fatty acid binding proteins on the transfer from the interstitial space and through the myocyte membrane are not well understood. The various analogues also show varying degrees of deiodination expressed as the per cent injected dose per gram of tissue values (Tables 3 and 4). Although the reasons for these differences are not well understood, when expressed as per cent injected dose values, the deiodination in all cases is very low.

The results of studies reported by other investigators

Table 3 Effects of position of dimethyl substitution—comparison of tissue distribution results (mean percent injected dose/gm) in fasted Fischer rats with the 3,3-, 4,4-, 6,6-, and 9,9-DMIPP analogues

Tissue	Time (min) after injection	Mean values of dimethyl (DMIPP) analogues of 15-(p-iodophenyl)pentadecanoic acid (compound number)			
		3,3-Me_2 (**28e**)	4,4-Me_2 (**9**)	6,6-Me_2 (**24**)	9,9-Me_2 (**18**)
Heart	5	4.67 ± 0.26	7.33 ± 0.07	2.36 ± 0.09	3.13 ± 0.54
	30	5.06 ± 0.34	8.03 ± 0.11	2.26 ± 0.25	3.06 ± 0.79
	60	4.49 ± 0.39	8.86 ± 0.30	1.83 ± 0.13	1.90 ± 0.26
Blood	5	1.48 ± 0.62	0.81 ± 0.08	1.40 ± 0.13	1.20 ± 0.09
	30	0.42 ± 0.02	0.48 ± 0.03	0.73 ± 0.06	1.09 ± 0.17
	60	0.36 ± 0.02	0.50 ± 0.08	0.55 ± 0.06	0.90 ± 0.05
Liver	5	7.73 ± 0.21	8.94 ± 0.05	9.40 ± 0.24	8.51 ± 0.57
	30	7.30 ± 0.28	8.74 ± 0.73	9.22 ± 0.80	6.54 ± 0.81
	60	6.02 ± 0.29	7.09 ± 0.42	6.24 ± 0.45	5.00 ± 0.16
Lungs	5	2.15 ± 0.06	2.02 ± 0.04	1.78 ± 0.12	1.81 ± 0.14
	30	1.42 ± 0.18	1.83 ± 0.11	1.26 ± 0.10	1.28 ± 0.23
	60	1.17 ± 0.06	1.85 ± 0.01	0.92 ± 0.07	1.17 ± 0.09
Thyroid	5	16.65 ± 3.01	13.40 ± 0.32	9.01 ± 2.04	11.54 ± 2.97
	30	18.46 ± 3.72	15.88 ± 2.09	9.61 ± 2.26	12.52 ± 3.08
	60	15.77 ± 7.81	20.37 ± 1.92	13.21 ± 1.79	14.72 ± 1.09
Ratio of mean Heart : Blood	5	3	9	1.7	2.6
	30	12	16.7	3.1	2.7
	60	12.5	17.4	3.3	2.1

Mean values for five fasted female Fischer rats. Each rat received 0.5 m*l* of a 6% BSA solution containing the following levels of each dimethyl analogue: 3,3-Me_2 (**28e**), 16.9 μCi; 4,4-Me_2 (**9**), 20.2 μCi; 6,6-Me_2 (**24**), 7.4 μCi; 9,9-Me_2 (**18**), 34.0 μCi.

with 1-[^{11}C]-3,3-dimethylheptadecanoic acid (DMHDA) have suggested that 3,3-dimethyl branching decreases myocardial uptake and retention in comparison with the analogues mono-branched analogues.[38] In fasted rats (n = 4) the DMHDA analogue showed relatively low myocardial uptake and relatively rapid washout (mean % injected dose/gm heart, heart : blood = H/B ratio): 5 min, 0.63, H/B 1.19 : 1; 15 min, 0.46, H/B 0.94; 30 min, 0.42, H/B 1.08.[38] In another study, [I-125]-14-iodo-3,3-dimethyltetradecanoic acid (IDTDA), also showed decreased myocardial uptake and retention, although no systematic results were reported to evaluate the effects of chain length.[39] In comparison with our results with 3,3-DMIPP and several other analogues described in the present work, the behavior of DMHDA and IDTDA is unexpected, but indicates that other structural features in addition to dimethyl-branching must affect myocardial extraction and release kinetics. These results demonstrate that both DMHDA (PET) and IDTDA (SPECT) would not be expected to be good candidates for tomography because of the low heart uptake, low target/non-target ratios and relatively rapid myocardial washout.

Our systematic evaluation with the nine new dimethyl-branched terminal p-iodophenyl-substituted analogues in comparison with 3,3-DMIPP has clearly shown that geminal 3,3-dimethyl substitution in itself does not necessarily decrease either myocardial specificity or retention and demonstrate that both chain length and the position of dimethyl branching are important structural features. There are a variety of factors which may affect the unusual behavior of DMHDA which may in fact include an oxidative decarboxylation process, since the possibility of expired $^{11}CO_2$ was evidently not measured by these investigators. In addition, fatty acid transport of DMHDA may be uniquely impaired by a combination of structural features, i.e. total chain length in conjunction with 3,3-dimethyl substitution. Geminal 3,3-dimethyl substitution alone does not reduce the myocardial specificity of 3,3-dimethyl substituted long chain fatty acids. These agents, if appropriately designed, can result in very high heart uptake and quite prolonged retention, as evidenced by both 3,3-DMIPP and 4,4-DMIPP in the current studies.

The current study focused on detailed structure-activity studies with ten dimethyl-branched fatty acid analogues in animals. Both 3,3-DMIPP (**28c**) and 4,4-DMIPP (**9**) analogues show the best myocardial uptake and retention. These combined results conclusively demonstrate that dimethyl-substitution can be an effective structural modification which can significantly prolong myocardial residence and that iodine-123-labeled 3,3-DMIPP (**28e**) and 4,4-DMIPP (**9**) are candidates for more detailed future SPECT studies in humans.

Table 4 Effects of chain length. Comparison of tissue distribution data from fasted female Fischer rats following intravenous abministration of iodine-125-labeled 3,3-dimethyl terminal (p-iodophenyl)-substituted fatty acid analogues with various chain lengths

Tissue	Time (min) after injection	Mean percent injected dose values ± SD					
		Chain length (compound number)					
		C_{11} **28a**	C_{12} **28b**	C_{13} **28c**	C_{14} **28d**	C_{15} **28e**	C_{19} **28f**
Heart	5	1.65 ± 0.16	1.78 ± 0.07	1.15 ± 0.06	2.38 ± 0.19	4.67 ± 0.26	1.91 ± 0.13
	30	0.70 ± 0.08	1.25 ± 0.12	0.47 ± 0.04	1.63 ± 0.29	5.06 ± 0.34	1.29 ± 0.11
	60	0.47 ± 0.02	0.86 ± 0.06	0.42 ± 0.03	1.33 ± 0.24	4.49 ± 0.39	0.88 ± 10.07
Blood	5	2.01 ± 0.18	4.64 ± 0.12	3.30 ± 0.22	1.17 ± 0.09	1.48 ± 0.62	2.08 ± 0.06
	30	0.70 ± 0.06	1.82 ± 0.14	0.52 ± 0.07	0.46 ± 0.07	0.42 ± 0.02	1.57 ± 0.03
	60	0.59 ± 0.04	1.16 ± 0.07	0.35 ± 0.03	0.34 ± 0.06	0.26 ± 0.02	1.10 ± 0.16
Liver	5	9.64 ± 0.65	5.97 ± 0.17	6.96 ± 0.21	5.43 ± 0.37	7.73 ± 0.21	10.21 ± 0.33
	30	8.38 ± 0.67	5.53 ± 0.36	4.21 ± 0.28	4.71 ± 0.65	7.36 ± 0.28	8.82 ± 0.32
	60	6.49 ± 0.22	4.62 ± 0.35	3.43 ± 0.21	3.57 ± 0.39	6.02 ± 0.29	7.95 ± 0.91
Lungs	5	1.80 ± 0.11	2.22 ± 0.11	1.91 ± 0.08	1.64 ± 0.12	2.15 ± 0.06	1.31 ± 0.08
	30	0.75 ± 0.08	1.92 ± 0.06	0.62 ± 0.13	0.74 ± 0.10	1.42 ± 0.18	1.19 ± 0.08
	60	0.54 ± 0.04	0.87 ± 0.04	0.44 ± 0.06	0.62 ± 0.09	1.17 ± 0.06	0.87 ± 0.10
Thyroid	5	7.04 ± 2.16	5.38 ± 1.25	7.92 ± 2.27	9.99 ± 1.19	16.60 ± 3.01	8.30 ± 1.57
	30	11.37 ± 0.82	11.61 ± 3.44	6.04 ± 1.66	5.64 ± 1.48	18.50 ± 3.72	20.56 ± 4.86
	60	37.27 ± 9.92	17.10 ± 4.52	14.05 ± 3.21	5.64 ± 1.02	15.80 ± 7.81	54.08 ± 13.93
Mean Heart : Blood	5	0.82	0.38	0.35	2.03	3.00	0.92
	30	1.00	0.68	0.90	3.54	12.0	0.82
	60	0.79	0.74	1.20	3.91	12.5	0.80

Mean values for five fasted rats for each time point. Rats received 0.50 m*l* of a 6% BSA solution containing the following levels of the analogues: C-11 (**28a**), 5.0 μCi; C-12 (**28b**), 13.5 μCi; C-13 (**28c**), 10.0 μCi; C-14 (**28d**), 8.6 μCi; C-15 (**28e**), 16.9 μCi; C-19 (**28f**), 6.33 μCi.

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