The resultant suspension was heated at 50 °C for 6 h. The solvents were removed in vacuo, and the brown residue obtained was extracted with CCl_4. Evaporation of the extract gave crude **27**, which was recrystallized from 2-PrOH, giving **27** as a pale yellow solid (0.50 g, 35.2%), mp 172–174 °C.

Cardiotonic activity of the substituted quinazolines was determined according to the following general procedure.

Adult mongrel dogs are anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and artificially respired. Mean arterial pressure (MAP) is recorded from a cannulated femoral artery and drugs are infused into a cannulated femoral vein. The arterial pressure pulse is used to trigger a cardiotachometer for determination of heart rate (HR). Left ventricular pressure is measured with a Miller catheter and dP/dt_{max} is derived. A right thoracotomy is performed and myocardial contractile force (CF) is measured with a Walton Brodie strain gauge sutured to the right ventricle. A lead II EKG is also recorded. A standard dose of dopamine is administered to determine myocardial responsiveness.

Test compounds were solubilized in a small volume of DMF diluted to a final concentration of 10% in physiological saline. Alternatively, where possible, a soluble hydrochloride salt was prepared by addition of 0.1 N HCl diluted in physiological saline. Vehicles were tested in appropriate volumes and found to exert less than a 5% effect on contractile force. Compounds were administered by infusion pump (one drug per animal) at rates of 0.58–2.2 mL/min in three to four stepwise increasing doses. Each dose was infused over 5 min immediately after the effect of the previous dose peaked. MAP, HR, and CF responses were continuously monitored on a Beckman or Gould recorder and expressed as a percent change from predrug control values vs. the cumulative dose of drug administered. Quantitation of the inotropic potency was obtained by calculation of the contractile force 50% effective dose (CF ED_{50}). This is defined as the dose of compound that produces a 50% increase in myocardial contractile foce and is a means of comparing potencies among compounds. The value was obtained from three to four point dose–response curves using either graphic estimation (where $n = 1, 2$) or linear regression analysis (where $n \geq 3$).

For oral evaluations, a Konigsberg transducer was implanted into the left ventricle of dogs as well as arterial and venous catheters, using sterile technique. MAP, HR, and the peak of the first derivative of left ventricular pressure (LV dP/dt_{max}) was obtained as an index of contractility. ORF 16600 was administered as a hydrochloride salt solution in distilled H_2O by gavage tube.

Dog Heart Nucleotide Phosphodiesterase Fraction III (PDE-III). The PDE-III enzyme preparation was obtained from dog heart by the method described by Thompson.[9]

Briefly, crude supernatent is chromatographed on a DEAE-cellulose column equilibrated with a NaOAc buffer (pH 6.5) containing 30% ethylene glycol. The third fraction eluted from the column is isolated by a 70–1000 mM NaOAc gradient. The enzyme is dialyzed and stored, until use, at −20 °C in 70 mM NaOAc buffer (pH 6.5) containing 30% ethylene glycol. Potential inhibitors are tested at several concentrations in the presence of cyclic AMP (0.25 μM, containing 0.2 μCi of [^{3}H]cyclic AMP), enzyme, and 0.05 M Tris·HCl buffer (pH 7.4) containing 5 mM $MgCl_2$. The reaction is stopped by heating at 100 °C for 1 min. After cooling, 0.10 mL of a solution containing snake venom (1 mg/mL) is added to convert the enzymatic product, 5′-AMP, to adenosine. After 30 min, 1.0 mL of 33% Dowex AGIX8 slurry is added to remove unreacted excess cyclic AMP. An aliquot (0.2 mL) is removed from the resulting supernatant and [^{3}H]adenosine is quantitated by liquid scintillation spectrometry.

Activity of the inhibitor is expressed as the IC_{50} or the micromolar concentration of compound required to inhibit 50% of the PDE-III activity. The IC_{50} values are the result of at least two separate determinations at inhibitor concentrations ranging from 0.01 to 1000 μM and are reported with a precision of ±15% (SEM) or better.

Acknowledgment. We thank Dr. M. L. Cotter's staff for spectral measurements and elemental analyses.

gem-Diphosphonate and *gem*-Phosphonate–Phosphate Compounds with Specific High Density Lipoprotein Inducing Activity

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New diphosphonate compounds and related derivatives[1] were synthesized and investigated for their activity in specifically inducing plasma high density lipoproteins (HDL) and high density lipoprotein cholesterol (HDL-C) in normal rats. The screening of numerous compounds has permitted the determination of the structural variations leading to optimal plasma lipid altering activity, indicating antiatherosclerotic potential. Among the compounds observed to be the most active, dimethyl α-(dimethoxyphosphinyl)-*p*-chlorobenzyl phosphate (**20**, SR-202, mifobate) was selected for further pharmacological and subsequent clinical development.

In spite of the well-established relationship between high levels of plasma cholesterol and coronary heart disease, the treatment of hyperlipoproteinemia has remained a controversial issue, mainly because of a W.H.O. study[2] where clofibrate failed to produce the evidence for an overall beneficial effect. More recently however, the Lipid Research Clinics Program[3] provided conclusive results demonstrating the efficacy and safety of cholestyramine, a nonabsorbable bile acid sequestering resin, in reducing mortality and morbidity due to coronary heart disease. The study also clearly established the direct correlation between the increase in plasma high density lipoprotein cholesterol (HDL-C) concentration due to treatment and the protection from coronary heart disease.

Screening programs have been established by research groups with the purpose of identifying compounds that would increase HDL levels in humans.[4] This activity, however, has been evaluated by using cholesterol-fed rats in which it is well known that the HDL-C (α-cholesterol) levels are markedly depressed and are inversely correlated with dietary cholesterol intake.[3a] Under these conditions of cholesterol feeding, either a drug-induced reduction in cholesterol absorption or a decrease in plasma low and very

(1) Bentzen, C. L.; Nguyen, L. M.; Niesor, E.; Symphar, S. A. U.S. Patent 4 309 364, 1982; *Chem. Abstr.* **1981**, *94*, 84297*d*.
(2) Committee of Principal Investigators *Lancet* **1980**, *2*, 379.
(3) (a) Lipid Research Clinics Program *J. Am. Med. Assoc.* **1984**, *251*, 361. (b) Lasser, N. L.; Roheim, P. S.; Edelstein, D.; Eder, H. A. *J. Lipid Res.* **1973**, *14*, 1.
(4) (a) Sircar, I.; Hoefle, M.; Maxwell, R. E. *J. Med. Chem.* **1983**, *26*, 1020. (b) D'Atri, G.; Gomarasca, P.; Resnati, G.; Tronconi, G.; Scolastico, C.; Sirtori, C. R. *J. Med. Chem.* **1984**, *27*, 1621.

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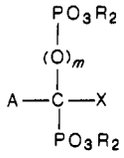
low density lipoprotein cholesterol levels (LDL-C and VLDL-C, β-cholesterol) might prevent the HDL-C depression caused by cholesterol feeding. It should be stressed, however, that in this cholesterol-fed animal model the absolute concentration of HDL-C after drug treatment never exceeds the level measured in control rats receiving a normal diet. For these reasons this type of model is not considered suitable for the identification of compounds that act specifically and directly to induce HDL production.

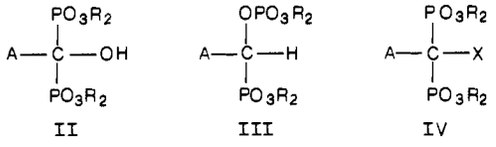
During the development of new animal models for the screening of lipid altering drugs we observed that cholestyramine increases HDL-C levels in rats fed a normal diet. Since it has been shown that this drug prevents tissue cholesterol deposition in relevant animal models,[5] and since its efficacy has also been clinically proven in humans,[3] the increase in HDL and α-/β-cholesterol ratio in rats fed a normal diet was used as a primary screening end point for testing new compounds synthesized in our laboratories.

In view of the known importance of phospholipid interactions with other lipid classes, various diphosphonate derivatives having the general structure I were prepared as a part of a research program for the discovery of new compounds with the potential therapeutic use as antiatherosclerotic drugs. The extensive screening of compounds I in a series of pharmacological tests revealed that changes in lipid metabolism and HDL levels were the most remarkable activities associated with this class of products.



I: A = aryl or arylalkyl; R = Me or Et; X = OH, H, or Cl; m = 0 or 1

This paper reports the synthesis and lipid altering activities of new diphosphonate derivatives represented by the general formula I. This general series of compounds I can be divided into three types: *gem*-hydroxydiphosphonates (II), *gem*-phosphonate–phosphates (III), and *gem*-diphosphonates (IV).



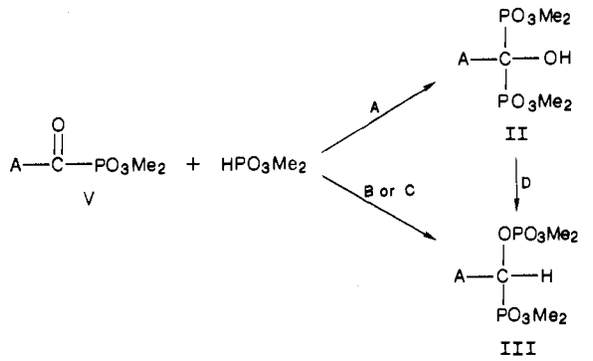
II III IV

Chemistry

Starting with the general reaction conditions of Nicholson[6] for the preparation of *gem*-hydroxy-diphosphonates II we have found that, by increasing the quantity of base, the isomers *gem*-phosphonate–phosphates III could be obtained as well. Compounds II and III were readily prepared from the same starting materials by reacting the dimethyl acylphosphonates V with dimethyl phosphite in the presence of various amounts of a dialkylamine in ether at 0 °C (Scheme I). A catalytic amount (about 5 mol %) of base, e.g., diethylamine or di-*n*-butylamine, gave hydroxy diphosphonate compounds II as reported[6] (method A) while the use of about 80–100 mol % of the same base yielded the corresponding phosphonate–phosphates III (method B).

The advantage of the process consists in that, while the reactants (acylphosphonates, dimethyl phosphite, and amine) are soluble in cold ether, the products II or III are not and therefore can be conveniently removed from the reaction mixture after precipitation. This allows an easy recrystallization of the isolated compounds to analytical purity. Compounds III that could not be conveniently obtained by method B were prepared by heating a mixture of V and dimethyl phosphite (method C) or isomerization by heat or base of the corresponding hydroxy diphosphonates II (method D).[7] Purification of compounds III that are oils were carried out by column chromatography.

Scheme I[a]

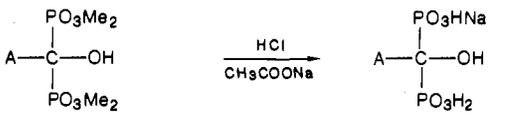


[a] (A) 5% Et_2NH or Bu_2NH in ether, 0 °C; (B) 80–100% Et_2NH or Bu_2NH in ether, 0 °C; (C) neat mixture heated to 80 °C; (D) EtOH, 78 °C.

Compounds II and III were definitively identified by MS, IR, and NMR spectroscopy. The MS spectra of both series showed a characteristic pattern: a molecular ion M^+ of significant intensity (5–30%) and a base peak (100%) corresponding to the loss of a phosphonate ester group $(M - PO_3Me_2)^+$. An absorption at 3260–3300 cm^{-1} (OH) was observed for all compounds II, this band being absent in IR spectra of compounds III. Both series presented strong phosphonate absorptions around 1260 cm^{-1} (P=O group) and 1060 cm^{-1} (P—O—C). The ^1H NMR spectra of the hydroxy diphosphonate compounds II all displayed the characteristic resonances of a hydroxyl group: a sharp singlet or a triplet at $\delta \sim 4$ that was removed through exchange with deuterium oxide. As for the phosphonate–phosphates III, the characteristic absorption of the methine hydrogen was a double doublet at $\delta \sim 5$ ($J = 10$ and 12 Hz) due to the coupling of the H with two different phosphorus atoms.

Compounds II and III can be readily distinguished by thin-layer chromatography (SiO_2) using the solvent system $CHCl_3$/MeOH (from 95/5 to 9/1, v/v) where they are clearly separated: R_f (II) 0.3 and R_f (III) 0.4. This is consistent with the fact that the hydroxy diphosphonates are more polar than their phosphonate–phosphate isomers.

The structure of the hydroxy diphosphonate esters II was further confirmed by acid hydrolysis of ester compounds **6** and **11**. For ease of purification, the corresponding hydroxy diphosphonic acids obtained were isolated under their monosodium salt forms.[8]



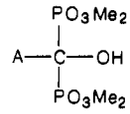
II

6: A = 4-ClC_6H_4
11: A = 4-[4-$ClC_6H_4C(O)]C_6H_4$

64: A = 4-ClC_6H_4

(5) De Palma, R. G.; Bellon, E. M.; Koletsky, S.; Schneider, D. L. *Exp. Mol. Pathol.* **1979**, *31*, 423.
(6) Nicholson, D. A.; Vaughn, H. *J. Org. Chem.* **1971**, *36*, 3843.
(7) Fitch, S.; Moedritzer, K. *J. Am. Chem. Soc.* **1962**, *84*, 1876.
(8) Pflaumer, P. F.; Filcik, J. P. *Belg. Patent* 712 159, 1968.

Table I. Tetramethyl *gem*-Hydroxy-Diphosphonates[a] II

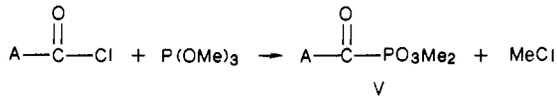


II

no.	A	mp, °C	yield,[b] %	formula[c,d]	plasma lipids (% controls) triglycerides	phospholipids	α/β-chol ratio	HDL-C
1	n-C_5H_{11}	62–64	66	$C_{10}H_{24}O_7P_2$	−21	+10	−17	−16
2	n-$C_{15}H_{31}$	57–58	58	$C_{20}H_{44}O_7P_2$	+14	+4	−38	−3
3	$(CH_3)_3C$	108–110	82	*e*	+24	−43	+32	0
4	C_6H_5	129–131	75	*e*	+4	+57	+80	+13
5	4-$CH_3C_6H_4$	110–113	75	$C_{12}H_{20}O_7P_2$	+43	+19	+68	+19
6	4-ClC_6H_4	119–123	80	$C_{11}H_{17}ClO_7P_2$	+15	+19	+130	+44
7	4-FC_6H_4	118–122	75	$C_{11}H_{17}FO_7P_2$	+27	+3	+64	+23
8	3,4,5-$(CH_3O)_3C_6H_2$	120–123	65	$C_{14}H_{24}O_{10}P_2$[f]				
9	4-$ClC_6H_4OC(CH_3)_2$	137–138	66	$C_{14}H_{23}ClO_8P_2$	−33	+26	−8	+8
10	4-$ClC_6H_4CH_2C(CH_3)_2$	128–131	51	$C_{15}H_{25}ClO_7P_2$				
11	4-[4-$ClC_6H_4C(O)$]C_6H_4	150–153	53	$C_{18}H_{21}ClO_8P_2$[g]	−16	+39	+46	+22
12	2-naphthyl	109–115	36	$C_{15}H_{20}O_7P_2$[g]	+24	+25	+51	+35
13	4-biphenylyl	136–142	33	$C_{17}H_{22}O_7P_2$[g]	+5	+1	+5	+74

[a] Preparation (method A) as described in the text. [b] Yields refer to purified materials and were not optimized. [c] General recrystallization solvents: acetone/Et_2O (1/3) at room temperature. [d] Compounds were analyzed for C, H, P and results were within ±0.4% of theoretical values. [e] Compounds synthesized by Nicholson,[6] included here for completeness of biological results. [f] Recrystallized from CH_2Cl_2. [g] Recrystallized from CH_2Cl_2/Et_2O.

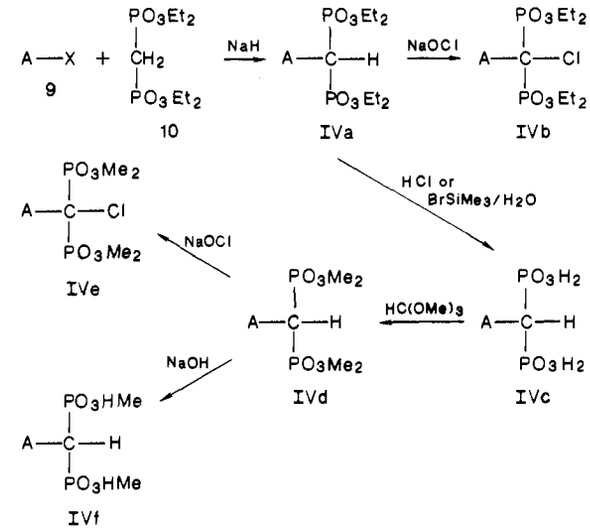
The starting compounds dimethyl acylphosphonates V were prepared in high yield (75–100%) by an Arbuzov reaction between acid chlorides and trimethyl phosphite. Most of the acylphosphonates V prepared in this project are new compounds that were purified by vacuum distillation and identified by IR. A few known derivatives were previously published by Berlin[9,10] and Marmor.[11]



V

The synthetic sequence followed for the preparation of *gem*-diphosphonates IV is shown in Scheme II. Substituted tetraethyl diphosphonates IVa were obtained by reacting appropriate halides **9** with tetraethyl methylenebis(phosphonate) (**10**) with sodium hydride in toluene.[12] Hydrolysis of IVa in aqueous hydrochloric acid or with trimethylsilyl bromide followed by treatment with water gave the diphosphonic acids IVc. These were esterified with trimethyl orthoformate[13] to give tetramethyl diphosphonates IVd. The diphosphonates IVa and IVd were chlorinated in the α position with a sodium hypochlorite solution buffered with sodium bicarbonate in following the Quimby's procedure[14] as modified by Seyferth.[15] Partial hydrolysis of the tetramethyl esters IVd with sodium hydroxide gave the dimethyl esters IVf.[16a]

Scheme II



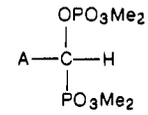
Biological Results and Discussion

In preliminary experiments representative compounds I at a dose of 200 mg/kg per day for 4 days were given orally to Wistar rats fed a normal diet in order to determine their activity on plasma lipids and more specifically on plasma HDL-C concentration. From Table IV it can be seen that the absolute concentration of HDL-C, measured after precipitation of VLDL and LDL with heparin and $CaCl_2$, was increased by treatment. This diphosphonate-induced elevation in HDL-C was associated with an increase in plasma phospholipids and in α-/β-cholesterol ratio. Lipoprotein fractions isolated after ultracentrifugation according to Havel[16b] were analyzed and the results confirmed a specific increase in HDL components identified as HDL cholesterol and HDL phospholipids.

On the basis of these results, other diphosphonates I were screened to establish the structural requirements for optimal primary activity on increasing the plasma α-/β-cholesterol ratio and phospholipids. Plasma triglyceride levels were also measured. Biological results are listed in Tables I–III.

(9) Berlin, K. D.; Taylor, H. A. *J. Am. Chem. Soc.* **1964**, *86*, 3862.
(10) Berlin, K. D.; Hellwege, D. M.; Nagabhushanam, M. *J. Org. Chem.* **1965**, *30*, 1265.
(11) Marmor, R. S.; Seyferth, D. *J. Org. Chem.* **1971**, *36*, 128.
(12) Hays, H. R.; Logan, T. J. *J. Org. Chem.* **1966**, *31*, 3391.
(13) Nicholson, D. A.; Cilley, W. A.; Quimby, O. T. *J. Org. Chem.* **1970**, *35*, 3149.
(14) Quimby, O. T.; Curry, J. D.; Nicholson, D. A.; Prentice, J. B.; Roy, C. H. *J. Organometal. Chem.* **1968**, *13*, 199.
(15) Seyferth, D.; Marmor, R. S. *J. Organometal. Chem.* **1973**, *59*, 237.
(16) (a) Rabinowitz, R. *J. Am. Chem. Soc.* **1960**, *82*, 4564. (b) Havel, R. J.; Eder, H. A.; Bragdon, H. J. *J. Clin. Invest.* **1975**, *34*, 1345.
(17) Davidson, R. S.; Sheldon, R. A.; Tripett, S. *J. Chem. Soc. C* **1967**, 1547.

Table II. Tetramethyl *gem*-Phosphonate–Phosphates III



III

no.	A	mp,[a] °C	yield,[b] %	formula[c,d]	method	plasma lipids (% controls) triglycerides	phospholipids	α-/β-chol ratio	HDL-C
14	$(CH_3)_2CH$	oil	50	$C_8H_{20}O_7P_2$	C	−26	+7	+38	+15
15	$(CH_3)_3C-CH_2$	oil	72	$C_{10}H_{24}O_7P_2$	C	−9	+18	+44	+47
16	cyclohexyl	oil	58	$C_{11}H_{24}O_7P_2$	C	−46	0	−20	+5
17	C_6H_5	oil	66	$C_{11}H_{18}O_7P_2$[e]	C	+32	+21	+76	+4
18	2-ClC_6H_4	oil	44	$C_{11}H_{17}ClO_7P_2$	C	−42	+22	+34	
19	3-ClC_6H_4	oil	46	$C_{11}H_{17}ClO_7P_2$	C	−37	+14	+69	+56
20	4-ClC_6H_4	81.5–83	85	$C_{11}H_{17}ClO_7P_2$	B	0	+26	+180	+157
21	2,4-$Cl_2C_6H_3$	oil	24	$C_{11}H_{16}Cl_2O_7P_2$	C	+13	+28	+39	+51
22	4-FC_6H_4	48–49	75	$C_{11}H_{17}FO_7P_2$	B	−9	+2	+81	+40
23	3-BrC_6H_4	oil	80	$C_{11}H_{17}BrO_7P_2$	D	+14	+3	+71	+28
24	4-$CH_3C_6H_4$	62–63	70	$C_{12}H_{20}O_7P_2$	B	−33	−8	+47	+4
25	2,5-$(CH_3)_2C_6H_3$	oil	30	$C_{13}H_{22}O_7P_2$	C	−3	−16	+15	+10
26	4-$(CH_3O)C_6H_4$	48–49	65	$C_{12}H_{20}O_8P_2$	B	+41	−3	+2	0
27	3,4,5-$(CH_3O)_3C_6H_2$	58–59	75	$C_{14}H_{24}O_{10}P_2$	D				
28	4-$CF_3C_6H_4$	59–60	55	$C_{12}H_{17}F_3O_7P_2$	B	+4	+3	+29	+22
29	$C_6H_5C(CH_3)_2$	47–48	38	$C_{14}H_{24}O_7P_2$	B	+36	+15	+25	+33
30	4-$ClC_6H_4C(CH_3)_2$	62–63	73	$C_{14}H_{23}ClO_7P_2$	B	−7	+37	+59	+56
31	4-$ClC_6H_4CH_2C(CH_3)_2$	45–46	38	$C_{15}H_{25}ClO_7P_2$	D	−19	−27	−38	−30
32	4-[4-$ClC_6H_4C(O)$]C_6H_4	113–114	80	$C_{18}H_{21}ClO_8P_2$	D	0	+53	+55	+33
33	2-naphthyl	60–62	59	$C_{15}H_{20}O_7P_2$	D	+28	+27	+134	+47
34	4-biphenylyl	67–68	65	$C_{17}H_{22}O_7P_2$	D	0	+18	+40	+24

[a] Compounds were purified by recrystallization or column chromatography (SiO_2, $CHCl_3$/MeOH, 95/5). [b] Yields refer to purified materials and were not optimized. [c] General recrystallization solvents: CH_2Cl_2/Et_2O (1/3). [d] Analysis C, H, P within ±0.4% theoretical values. [e] Compound previously published in the literature.[17]

Although compounds of each subclass II, III, or IV display significant activity, the effect of increasing plasma α-/β-cholesterol ratio is associated most consistently with the phosphonate–phosphate compounds of structure III (Table II). In particular, the hydroxy diphosphonate derivatives II are less potent than their phosphonate–phosphate isomers III. For instance, compounds **6**, **7**, **12**, and **13** are less active than compounds **20**, **22**, **33**, and **34**, respectively. Compounds having a phenyl moiety in general displayed good activity. Substitution of the phenyl ring by a monohalogen results in active compounds with a maximum of potency when the substituent is a *p*-Cl group (**20**). Compounds **29** and **30**, which have in addition a *gem*-dimethyl group, are moderately active. Various aryl structures other than phenyl, in particular a naphthyl group (**12**, **33**), are also active. It is also of interest to note that compound **9**, having the (*p*-chlorophenoxy)isopropyl moiety of the clofibrate molecule, is inactive.

With regard to the *gem*-diphosphonate series IV (Table III), their respective acids and hemiesters are all inactive (**46–51**, **62–63**). The ester analogues display good activity with the methyl derivatives being slightly more potent than their ethyl counterparts (**52–58** vs. **37–43**). The *p*-chlorobenzyl and phenethyl structures are the most potent while phenoxyalkyl analogues are less active. The fatty acid like substituent, *p*-tetradecyloxy group reported to possess hypolipidemic properties,[18] provides only marginal activity (**58**). Finally, substitution of a chlorine atom for the methine hydrogen decreases the α-/β-cholesterol ratio, with the exception of compound **59**.

Analogues of compound **20** with different ester groups (Et, Pr, *i*-Pr, and Bu, **65**, **66**, **67**, and **68**) and the corresponding hydroxy diphosphonic acid **64** were tested to evaluate the importance of the ester functional group (Table V). The results demonstrate that none of these analogues of compound **20** produced a favorable change in α-/β-cholesterol ratio or in phospholipids. This finding parallels the observation made with the *p*-chlorobenzyl *gem*-diphosphonates **35**, **36**, **38**, **47**, and **53** where the methyl ester **53** is the most potent derivative.

In a comparative experiment, some representative hypolipidemic drugs and the most active diphosphonate derivative (**20**) were tested in rats fed a normal diet (Table VI). The classical hypolipidemic agents clofibrate, fenofibrate, and estradiol were found to decrease markedly the HDL-C level, leading to a lower α-/β-cholesterol ratio (−53%, −80%, and −47%). These results are in agreement with those published by Day[19] and Muller[20] demonstrating that clofibrate decreases HDL-C in rats. On the contrary, with cholestyramine and compound **20** (both 100 mg/kg per day) the α-/β-cholesterol ratio was increased by 95% and 52%, respectively. A direct correlation between HDL-C levels, α-/β-cholesterol ratios, and plasma phospholipids was also observed in this experiment, thus validating the protocol and parameters used for the screening of compounds I.

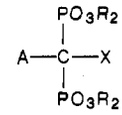
The mechanism of action by which these compounds specifically increase HDL is unknown but appears to be related to direct effects on the liver involving lipid synthesis and/or metabolism, such as an inhibition of hepatic lipase. In these primary screening studies in rat as well as secondary screening models in rabbit and monkey performed with selected compounds, liver cholesterol concentrations have been consistently found to be diminished, thus indicating that these compounds are most likely

(18) Parker, R. A.; Kariya, T.; Grisar, J. M.; Petrow, V. *J. Med. Chem.* **1977**, *20*, 781.

(19) Day, C. E.; Phillips, W. A.; Schurr, P. E. *Artery* **1979**, *5*, 90.

(20) Muller, K. R.; Cortesi, R. G. *Artery* **1978**, *4*, 564.

Table III. Tetraalkyl *gem*-Diphosphonates IV



IV

no.	A	R	X	mp or bp (mmHg), °C	yield,[a] %	formula[b]	plasma lipids (% controls) tri-glycerides	phospho-lipids	α-/β-chol ratio	HDL-C
35	4-$ClC_6H_4CH_2$	Bu[c]	H	190–195 (0.05)[d]	40	$C_{24}H_{43}ClO_6P_2$	+1	+6	+2	0
36	4-$ClC_6H_4CH_2$	*i*-Pr[c]	H	180–185 (0.05)[d]	35	$C_{20}H_{35}ClO_6P_2$	+19	+13	−1	+12
37	$C_6H_5CH_2$	Et	H	135–138 (0.05)	58	$C_{16}H_{28}O_6P_2$	−47	+5	+28	+5
38	4-$ClC_6H_4CH_2$	Et	H	153–158 (0.05)	59	$C_{16}H_{27}ClO_6P_2$	+8	+23	+22	+26
39	$C_6H_5(CH_2)_2$	Et	H	137–140 (0.05)	45	$C_{17}H_{30}O_6P_2$	+11	+16	+74	+41
40	$C_6H_5(CH_2)_3$	Et	H	140–143 (0.05)	49	$C_{18}H_{32}O_6P_2$	+46	+17	+33	+62
41	$C_6H_5O(CH_2)_2$	Et	H	160–165 (0.05)	46	$C_{17}H_{30}O_7P_2$	−38	+25	+26	+29
42	$C_6H_5O(CH_2)_3$	Et	H	170–175 (0.05)	49	$C_{18}H_{32}O_7P_2$	0	0	+37	+16
43	4-(n-$C_{14}H_{29}O$)$C_6H_4CH_2$	Et	H		37	$C_{30}H_{56}O_7P_2$	−27	−4	−28	+27
44	$C_6H_5(CH_2)_3$	Et	Cl		24	$C_{18}H_{31}ClO_6P_2$	−4	+27	−23	−2
45	$C_6H_5O(CH_2)_3$	Et	Cl		32	$C_{18}H_{31}ClO_7P_2$				
46	$C_6H_5CH_2$	H	H	210–212[e]	95	*f*	−11	+3	−10	−8
47	4-$ClC_6H_4CH_2$	H	H	237–239	95	*f*	−146	0	−40	−14
48	$C_6H_5(CH_2)_2$	H	H	167–170	45	*f*	−17	−16	−27	−24
49	$C_6H_5(CH_2)_3$	H	H	190–192	99	*f*	+11	+12	+14	+3
50	$C_6H_5O(CH_2)_2$	H	H		40	*f*				
51	$C_6H_5O(CH_2)_3$	H	H	188–192	95	*f*	−7	−6	−57	−18
52	$C_6H_5CH_2$	Me	H	130–132 (0.05)	66	$C_{12}H_{20}O_6P_2$	−3	+25	+22	+32
53	4-$ClC_6H_4CH_2$	Me	H	141–144 (0.05)	66	$C_{12}H_{19}ClO_6P_2$	+62	+51	+75	+38
54	$C_6H_5(CH_2)_2$	Me	H	132–135 (0.05)	86	$C_{13}H_{22}O_6P_2$	+22	+16	+82	+40
55	$C_6H_5(CH_2)_3$	Me	H	135–138 (0.05)	58	$C_{14}H_{24}O_6P_2$	+42	+33	+49	+52
56	$C_6H_5O(CH_2)_2$	Me	H	160–162 (0.05)	63	$C_{13}H_{22}O_7P_2$				
57	$C_6H_5O(CH_2)_3$	Me	H	160–165 (0.05)	72	$C_{14}H_{24}O_7P_2$	+2	−11	−13	+6
58	4-(n-$C_{14}H_{29}O$)$C_6H_4CH_2$	Me	H	43–45	44	$C_{26}H_{48}O_7P_2$	+4	+10	+14	+20
59	4-$ClC_6H_4CH_2$	Me	Cl	150–152 (0.05)	90	$C_{12}H_{18}Cl_2O_6P_2$	+11	+7	+81	+10
60	$C_6H_5(CH_2)_3$	Me	Cl	153–155 (0.05)	57	$C_{14}H_{23}ClO_6P_2$	−36	+24	−9	+26
61	$C_6H_5O(CH_2)_3$	Me	Cl	52–54	31	$C_{14}H_{23}ClO_7P_2$	−3	+15	−42	+57
62	4-$ClC_6H_4CH_2$	Me, H	H	146–149	60	$C_{10}H_{15}ClO_6P_2$	−30	+6	−20	+27
63	$C_6H_5(CH_2)_3$	Me, H	H	93–95	54	$C_{12}H_{20}O_8P_2$	+9	+11	−23	−9

[a] Yields are provided for each last step of the synthetic sequence and refer to purified materials. [b] Compounds were analyzed for C, H, P and results were within ±0.4% of theoretical values. [c] Prepared by reaction of the respective tetraalkyl methylenebis(phosphonate) with *p*-chlorobenzyl chloride according to the procedure described for compound **40**. [d] Kugelrohr distillation (oven temperature noted). [e] British Patent 1 026 366. [f] Acid compounds IVc were not microanalyzed but were characterized by IR, NaOH titration, and the analysis of their subsequent reaction products IVd.

Table IV. Effect of Selected Diphosphonates on HDL Concentrations and Plasma Lipids

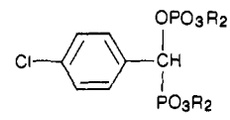
compd	plasma lipids (% controls) HDL cholesterol	phospholipids	α-/β-chol ratio
28	+22	+3	+9
39	+41	+16	+74
53	+38	+51	+75
58	+20	+10	+14

increasing cholesterol turnover. Additional investigations with selected compounds are being performed in order to better delineate the mechanism of action.

Conclusions

gem-Diphosphonates I were found to specifically increase plasma HDL-C levels and to augment the α-/β-cholesterol ratio of rats receiving a normal diet as observed for cholestyramine-treated animals. In contrast, the clofibrate derivatives decrease the α-/β-cholesterol ratio in this animal model. The results of the structure–activity study of the three subclasses II, III, and IV demonstrated that maximal pharmacological activity is displayed by the

Table V. Effect of Acid and Ester Analogues of Compound **20** on Plasma Lipids



no.	R	mp or bp[a] (mmHg), °C	yield, %	formula	method	plasma lipids (% controls) triglycerides	phospholipids	α-/β-chol ratio	HDL-C
64[b]			60	$C_7H_8ClNaO_7P_2$		−35	+4	+17	+6
20	Me	81.5–83	83	$C_{11}H_{17}ClO_7P_2$	B	+17	+35	+208	+153
65	Et	150–155 (0.05)	75	$C_{15}H_{25}ClO_7P_2$	C	+8	+16	−6	+15
66	Pr	165–170 (0.05)	65	$C_{19}H_{33}ClO_7P_2$	C	−17	+4	+4	−6
67	*i*-Pr	155–160 (0.05)	60	$C_{19}H_{33}ClO_7P_2$	C	−37	−20	−46	−34
68	Bu	175–180 (0.05)	60	$C_{23}H_{41}ClO_7P_2$	C	−11	+13	−53	−10

[a] Kugelrohr distillation (oven temperature noted). [b] [Hydroxy(*p*-chlorophenyl)methylene]bis(phosphonic acid) monosodium salt (see Experimental Section).

Table VI. Effect of Reference Hypolipidemic Drugs and Compound 20 on Plasma Lipids in Rats Fed a Normal Diet

compound	dose, mg/kg per day	plasma lipids (% controls)			
		triglycerides	phospholipids	α-/β-cholesterol ratio	HDL cholesterol
clofibrate[a]	100 po	+28	−22	−53	−34
fenofibrate[a]	100 po	+12	−19	−80	−45
estradiol[a]	5 sc	−51	−77	−47	−83
cholestyramine[a]	1000 po	−25	+2	+95	+34
20	100 po	0	+17	+52	+26

[a] Reference drugs were commercially available.

phosphonate–phosphates III.

Compound **20** was selected among the most active diphosphonates for further pharmacological development. Clinical studies performed with compound **20** in humans[21] confirmed that plasma HDL-C, cholesterol turnover (fractional catabolic rate), and tissue cholesterol pools were favorably modified by treatment. Therefore these results are in agreement with the hypothesis that the *gem*-diphosphonates I are potentially antiatherosclerotic agents acting by specifically increasing HDL which is the lipoprotein primarily involved in cholesterol turnover and removal from peripheral tissues to the liver for elimination.

Experimental Section

Chemistry. Melting points were taken on a Büchi 510 apparatus; all melting points and boiling points listed are uncorrected. IR spectra were recorded on a Beckman Acculab 9 spectrophotometer as KBr for solids and neat for liquids. MS spectra were recorded on a Varian SM1 spectrometer and NMR were measured on a Varian XL 100 spectrometer at 100 MHz with Me_4Si as internal standard. Chromatographic separations were performed on a silica gel column (Kieselgel 60, 0.063–0.200 mm, Merck). Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values.

Preparation of Dimethyl Acylphosphonates V. The general procedure followed was the simplified method reported by Nicholson,[6] with the exception of the starting compound for **11** and **32**, which is specially described. Trimethyl phosphite was added dropwise to an equimolar amount of acid chloride cooled to 0 °C. After 3–5 h at room temperature the mixture was vacuum distilled: dimethyl acylphosphonates V were obtained pure in excellent yield (80–90%). These compounds are hygroscopic and prolonged contact with air resulted in partial cleavage of the P–C bond and formation of the starting carboxylic acids.[8,9]

The acid chlorides were prepared by refluxing in thionyl chloride the corresponding acids that were either commercially available or synthesized according to the literature. The preparation of those synthesized are briefly noted here. α-(*p*-Chlorophenoxy)isobutyric acid (starting compound for **9**) was obtained from saponification of ethyl α-(*p*-chlorophenoxy)isobutyrate (clofibrate) (Fluka). α-(*p*-Chlorobenzyl)isobutyric acid (starting compound for **10** and **31**) was prepared according to the Creger's procedure.[22] 4-(4-Chlorobenzoyl)benzoic acid (starting compound for **11** and **32**) was the product of oxidation of 4-chloro-4′-methylbenzophenone.[23,24] α-Phenylisobutyric acid (starting compound for **29**) was prepared by a Friedel–Crafts reaction of α-bromoisobutyric acid and benzene.[25] α-(*p*-Chlorophenyl)isobutyric acid (starting compound for **30**) was the product of hydrolysis of the nitrile obtained by dimethylation of *p*-chlorophenylacetonitrile.[26]

Tetramethyl [(*p*-Chlorophenyl)hydroxymethylene]bis(phosphonate) (6). Method A. Dimethyl phosphite (4.40 g, 40 mmol) and Et_2NH (0.15 g, 2 mmol) were dissolved in 90 mL of Et_2O, and the resulting solution was cooled to 0 °C. Dimethyl *p*-chlorobenzoylphosphonate (9.96 g, 40 mmol) was added dropwise with rapid stirring. A white solid separated out almost immediately. The mixture was stirred for 1 h at 0 °C and filtration yielded 13.0 g of **6**. Recrystallization at room temperature from an acetone/Et_2O mixture gave 7.9 g (55%) of white crystals: mp 119–123 °C; IR (KBr) 3260, 1260, 1060 cm^{-1}; MS, *m/e* 360, 358, 251, 249 (100); NMR ($CDCl_3$) δ 7.90–7.20 (m, 4 H, phenyl), 4.10 (t, J = 7 Hz, removed by D_2O, OH), 3.90–3.60 (m, 12 H, OCH_3). Anal. ($C_{11}H_{17}ClO_7P_2$) C, H, P.

As verification of its structure, compound **6** was transformed into the mono sodium salt of the corresponding hyroxy diphosphonic acid **64**. A mixture of **6** (3.59 g, 10 mmol) and 15 g of 37% HCl was refluxed for 3 h. Evaporation of HCl and H_2O left 3.2 g (100% crude yield) of white solid; mp 192–194 °C (crude). For purpose of purification the [hydroxy(*p*-chlorophenyl)-methylene]bis(phosphonic acid) obtained was transformed into its monosodium salt according to the method adapted from Pflaumer and Filcik.[8] The solid obtained as described above was dissolved in a mixture of 4.8 g (80 mmol) of CH_3COOH and 0.7 g (39 mmol) of H_2O at 95 °C. $CH_3COONa{\cdot}3H_2O$ (1.36 g, 10 mmol) was then added gradually. A voluminous precipitate appeared almost instantly. It was filtered and washed copiously with ether until the smell of CH_3COOH disappeared. The rinsed precipitate was recrystallized in an EtOH/H_2O (20/80) mixture to give 1.94 g (60%) of a white powder of [hydroxy(*p*-chlorophenyl)-methylene]bis(phosphonic acid) monosodium salt (**64**). Anal. ($C_7H_8ClNaO_7P_2$) C, H, P.

Tetramethyl [[4-(4-Chlorobenzoyl)phenyl]hydroxymethylene]bis(phosphonate) (11). Method A. Trimethyl phosphite (10.9 g, 88 mmol) was added dropwise to 4-(4-chlorobenzoyl)benzoyl chloride (22.4 g, 80 mmol), which was heated to just below the melting point (about 100 °C). The reaction was exothermic, and the white crystals of acid chloride turned into a brown oil with considerable foaming. The reaction mixture was stirred at 100 °C for 30 min. Upon standing and cooling, the oily material transformed into an orange solid. Recrystallization from a 50/50 $CHCl_3$/petroleum ether solution gave 20 g (71%) of pure dimethyl 4-(4-chlorobenzoyl)benzoylphosphonate: mp 95–97 °C (yellow powder); IR (KBr) 1665, 1650, 1590, 1260, 1040 cm^{-1}. A filtered solution of the above compound (7.04 g, 20 mmol) in 40 mL of CH_2Cl_2 was introduced dropwise into a cooled (0 °C) mixture of dimethyl phosphite (2.20 g, 20 mmol) and Bu_2NH (0.14 g, 1.10 mmol) in 40 mL of Et_2O. A white solid soon separated out of the yellow solution. After 1 h at 0 °C the precipitate was filtered and the mother liquor was concentrated to give an additional amount. The combined fractions were recrystallized with slight warming (40 °C) from acetone, yielding 4.9 g (53%) of white crystals of **11**: mp 149–153 °C; IR (KBr) 3280, 1670, 1250, 1040 cm^{-1}; MS, *m/e* 464, 462, 355, 353 (100%); NMR ($CDCl_3$) δ 7.95–7.40 (m, 8 H, phenyl groups), 4.4 (t, J = 7 Hz, 1 H, removed by D_2O, OH), 3.95–3.70 (m, 12 H, OCH_3). Anal. ($C_{18}H_{21}ClO_8P_2$) C, H, P. Compound **11** was transformed into the monosodium salt of the corresponding hydroxydiphosphonic acid, by following exactly the procedure used for the hydrolysis of **6**. Anal. ($C_{14}H_{12}ClO_8P_2Na$) Calcd: P, 14.45. Found: P, 14.01.

Dimethyl α-(Dimethoxyphosphinyl)-*p*-chlorobenzyl Phosphate (20). Method B. Dimethyl *p*-chlorobenzoylphosphonate (9.96 g, 40 mmol) was introduced dropwise with rapid stirring into a well-cooled (0 °C) solution of dimethyl phosphite (4.40 g, 40 mmol) and Et_2NH (2.63 g, 36 mmol) in 80 mL of Et_2O. A white precipitate soon appeared; stirring was continued for 1

(21) Schwartz, C. C.; Van Denbroek, J.; Niesor, E.; Bentzen, C. L. *Proceedings of the 7th International Symposium on Atherosclerosis*, Nestel, P. S., Ed.; International Atherosclerosis Society, 1985; p 143.
(22) Creger, P. L. *Org. Synth.* **1970**, *50*, 58.
(23) Robinson, G. E.; Vernon, J. M. *J. Chem. Soc. C* **1970**, 2586.
(24) Wertheim, E. *J. Am. Chem.* **1933**, *55*, 2540.
(25) Campaigne, E.; Maulding, D. R. *J. Org. Chem.* **1963**, *28*, 1391.
(26) Cope, A. C.; Foster, T. T.; Towle, P. H. *J. Am. Chem. Soc.* **1949**, *71*, 3929.
(27) Burnstein, M.; Scholnick, H. R.; Morfin, R. J. *J. Lipid Res.* **1970**, *11*, 583.

h at 0 °C and then the solid was filtered. Recrystallization from a 1/3 CH_2Cl_2/Et_2O mixture gave 12.2 g (85%) of white crystals of **20**: mp 81.5–83 °C; IR (KBr) 1500, 1280, 1050 cm^{-1}; MS, *m/e* 360, 358, 251, 249 (100); NMR ($CDCl_3$) δ 7.5–7.3 (m, 4 H, phenyl), 5.6 (dd, *J* = 11 and 13 Hz, 1 H, methine group, *not* removed by D_2O exchange), 3.95–3.50 (m, 12 H, OCH_3). Anal. ($C_{11}H_{17}ClO_7P_2$) C, H, Cl, P.

Dimethyl (Dimethoxyphosphinyl)cyclohexylmethyl Phosphate (16). Method C. A mixture of dimethyl (cyclohexylcarbonyl)phosphonate[11] (5 g, 23 mmol) and dimethyl phosphite (3 g, 27 mmol) was heated at 90 °C overnight. The excess of reactants were distilled, and the residue was fractionated in a Kugelrohr apparatus (150 °C/0.05 mmHg) to yield 4.4 g (58%) of **16** as a white oil: IR (film) 1280, 1050 cm^{-1}; NMR ($CDCl_3$) δ 4.7–4.4 (m, 1 H, methine H), 3.9–3.7 (m, 12 H, OCH_3 group), 2.0–1.50 and 1.50–1.0 (m, total 11 H, cyclohexyl H). Anal. ($C_{11}H_{24}O_7P_2$) C, H, P.

Dimethyl α-(Dimethoxyphosphinyl)-4-(4-chlorobenzoyl)benzyl Phosphate (32). Method D. A solution of 4.90 g (10.6 mmol) of compound **11** in 450 mL of EtOH was refluxed for 6 h whereupon a TLC control indicated that the isomerization reaction was complete (SiO_2, 9/1 $CHCl_3$/MeOH, R_f (**11**) 0.4; R_f (**32**) 0.5). The solvent was then evaporated and the residue was recrystallized from a CH_2Cl_2/Et_2O mixture. An amount of 3.9 g (80%) of white crystals of **32** was obtained: mp 113–114 °C; IR (KBr) 1660, 1290, 1270, 1040 cm^{-1}; MS, *m/e* 464, 462, 355, 353 (100); NMR ($CDCl_3$) δ 7.85–7.45 (m, 8 H, phenyl groups), 5.65 (dd, *J* = 11 and 13 Hz, 1 H, methine H), 3.80–3.60 (m, 12 H, OCH_3). Anal. ($C_{18}H_{21}ClO_8P_2$) C, H, P.

Tetraethyl (4-Phenylbutylidene)bis(phosphonate) (40). Tetraethyl methylenebis(phosphonate) (28.8 g, 100 mmol) was added dropwise to a dispersion of sodium hydride (80% in mineral oil, 3.0 g, 100 mmol) in 25 mL of toluene. When the evolution of H_2 ceased, 3-phenylpropyl bromide (20.9 g, 105 mmol) was added and the mixture was warmed to 60 °C for 16 h. After removal of toluene under vacuum, the residue was dissolved in $CHCl_3$, washed (saturated NaCl solution), and dried ($MgSO_4$). Distillation under reduced pressure gave, after a small forerun of starting materials, a colorless oil boiling between 138 and 145 °C (0.05 mm). A careful redistillation yielded 19.9 g (49%) of **40** (colorless oil): bp 140–143 °C (0.05 mm); IR (film) 2980, 1500, 1260, 1170 (P–O–C_2H_5), 1050 cm^{-1}; MS, *m/e* 406, 269; NMR ($CDCl_3$) δ 7.3–7.2 (m, 5 H, phenyl group), 4.2 (qt, 8 H, *J* = 7 Hz, OCH_2CH_3), 2.66 (t, *J* = 7 Hz, 2 H, benzyl CH_2), 2.40–1.80 (m, 5 H, 2 side chain CH_2 and methine H), 1.40 (t, 12 H, *J* = 7 Hz, OCH_2CH_3). Anal. ($C_{18}H_{32}O_6P_2$) C, H, P.

(4-Phenylbutylidene)bis(phosphonic acid) (49). A mixture of 13.9 g (34 mmol) of **40** and 75 mL of 37% HCl was refluxed for 15 h. Evaporation of the acid solution left a white solid weighing 9.9 g (crude yield: 99%). For analytical purposes, a small amount of compound **49** was recrystallized from a mixture of acetone/Et_2O/hexane (6/3/1): mp 190–192 °C; IR (KBr) 3400, 1500, 1230, 1040 cm^{-1}; neutral equivalent, calcd 294, found 296.

Tetramethyl (4-Phenylbutylidene)bis(phosphonate) (55). A suspension of 8.9 g (30 mmol) of **49** in 32 g (300 mmol) of trimethyl orthoformate was refluxed with rapid stirring for 2 h. The MeOH and HCOOMe produced in the process were removed by distillation, thereby allowing the reaction temperature to rise. When the boiling point of trimethyl orthoformate (100 °C) was reached, distillation was interrupted, fresh reagent was added (17 g, 160 mmol), and the reaction mixture was further refluxed for 2 h. After removal of the reagent, the brown residue was distilled under vacuum to give 8.2 g (77%) of **55** (colorles oil): bp 135–138 °C (0.05 mm); IR (film) 2980, 1500, 1270, 1195 (P–O–CH_3), 1050 cm^{-1}; NMR ($CDCl_3$) δ 7.3–7.2 (m, 5 H, phenyl), 3.9–3.7 (splitted d, 12 H, OCH_3), 2.64 (t, *J* = 7 Hz, 2 H, benzyl CH_2), 2.4–1.8 (m, 5 H, 2 side-chain CH_2 and methine H). Anal. ($C_{14}H_{24}O_6P_2$) C, H, P.

Tetramethyl (1-Chloro-4-phenylbutylidene)bis(phosphonate) (60). A suspension of 3.6 g (43 mmol) of $NaHCO_3$ in 4.6 g of a 14% NaOCl solution (0.64 g, 8.6 mmol) was cooled to 0 °C, and compound **55** (2.1 g, 6 mmol) was introduced dropwise. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The reaction mixture was extracted by $CHCl_3$ and the organic phase was washed (saturated NaCl solution) and dried over $MgSO_4$. Vacuum distillation gave 1.7 g (74%) of **60** (colorless viscous oil): bp 155 °C (0.05 mm); NMR ($CDCl_3$) δ 7.3–7.2 (m, 5 H, phenyl group), 3.96–3.84 (splitted d, 12 H, OCH_3), 2.66 (t, *J* = 7 Hz, 2 H, benzyl CH_2), 2.5–1.9 (m, side chain CH_2, 4 H). Anal. ($C_{14}H_{23}ClO_6P_2$) C, H, P.

Bis(methyl, hydrogen) (4-Phenylbutylidene)bis(phosphonate) (63). A suspension of 2.10 g (60 mmol) of **55** in 60 mL of 20% NaOH was refluxed for 2 h whereupon a white precipitate appeared. After cooling, the solid was filtered and resuspended in 10 mL of 2 N HCl solution. The acid solution was evaporated to dryness and the residue was extracted with EtOH. The filtered EtOH solution was evaporated to give a white oil that slowly solidified. Recrystallization from a acetone/Et_2O mixture gave 1.05 g (54%) of **63**: mp 93–95 °C; IR (KBr) 2900 + 2300 (br), 1240, 1030 cm^{-1}; NMR ($CDCl_3$) δ 8.5 (sharp peak, removed by D_2O extraction, OH), 7.3–7.15 (m, 5 H, phenyl), 3.74 (d, *J* = 11 Hz, 6 H, $POCH_3$), 2.64 (t, *J* = 7 Hz, 2 H, benzyl CH_2), 2.4–1.8 (m, 5 H, 2 side-chain CH_2 and methine H); neutral equivalent, calcd 322, found 322. Anal. ($C_{12}H_{20}O_6P_2$) C, H, P.

Tetraethyl [2-[4-(*n*-Tetradecyloxy)phenyl]ethylidene]bis(phosphonate) (43). Phosphorus tribromide (27 g, 100 mmol) was added at 0 °C to 4-(*n*-tetradecyloxy)benzyl alcohol[18] (64 g, 200 mmol) and the mixture was heated to 100 °C for 2 h. Upon cooling a white solid was formed that was partitioned between $CHCl_3$ and H_2O. Recrystallization of the organic phase residue from acetone gave 20.4 g (26%) of a white solid; mp 49–53 °C. The bromide obtained (12 g, 31 mmol) was reacted at 50 °C for 15 h with a solution obtained by reacting tetraethyl methylenebis(phosphonate) (9.1 g, 31 mmol) with an equimolar amount of sodium hydride in toluene. After workup, column chromatography (SiO_2, $CHCl_3$) gave 6.8 g (37%) of **43** as a white oil: IR (film) 2980, 1260, 1170, 720 cm^{-1}; NMR ($CDCl_3$) δ 7.25–6.75 (m, 4 H, phenyl group), 4.1 (qt, *J* = 7 Hz, 8 H, $POCH_2CH_3$), 3.94 (t, *J* = 7 Hz, 2 H, $OCH_2C_{13}H_{27}$), 3.2 (dxt, *J* = 7, 16 Hz, 2 H, benzyl CH_2), 2.6 (txt, *J* = 7, 24 Hz, 1 H, methine H), 1.9–1.6 (m, 2 H, $OCH_2CH_2C_{12}H_{25}$), 1.4–1.25 (m, 34 H, $C_{11}H_{22}CH_3$ + $POCH_2CH_3$), 0.9 (t, 3 H, *J* = 7 Hz, $O(CH_2)_{13}CH_3$). Anal. ($C_{30}H_{56}O_7P_2$) C, H, P.

Tetramethyl [2-[4-(*n*-Tetradecyloxy)phenyl]ethylidene]bis(phosphonate) (58). To 10 mL of a $CHCl_3$ solution of **43** (5.7 g, 10 mmol) were added 7.5 g (50 mmol) of trimethylbromosilane at 0 °C, and the mixture was left to react overnight at room temperature. Hydrolysis was carried out by additon of 5 mL of H_2O, whereupon a white precipitate was formed. The mixture was concentrated to dryness and the residue was triturated with acetone to yield 4.1 g (86%) of crude [2-[4-(*n*-tetradecyloxy)phenyl]ethylidene]bis(phosphonic acid); mp 185–195 °C.

The above mentioned (4.1 g, 8.6 mmol) was reacted with trimethyl orthoformate as described for the preparation of **55**. Upon removal of excess reactant, a solid residue was obtained which gave 2.0 g (44%) of **58** after recrystallization from petroleum ether: mp 43–45 °C; IR 2980, 1270, 1195, 720 cm^{-1}; NMR ($CDCl_3$) δ 7.25–6.78 (m, 4 H, phenyl group), 3.92 (t, *J* = 7 Hz, 2 H, $OCH_2C_{13}H_{27}$), 3.74 (d, *J* = 11 Hz, 12 H, $POCH_3$), 3.2 (dxt, *J* = 7, 16 Hz, 2 H, benzyl CH_2), 2.64 (txt, *J* = 7, 24 Hz, 1 H, methine H), 1.9–1.6 (m, 2 H, $OCH_2CH_2C_{12}H_{25}$), 1.5–1.1 (m, 22 H, $C_{11}H_{22}CH_3$), 0.9 (t, *J* = 7 Hz, 3 H, $O(CH_2)_{13}CH_3$). Anal. ($C_{26}H_{48}O_7P_2$) C, H, P.

Biological Methods. Groups of five male Wistar rats weighing 175–250 g were treated with diphosphonates or reference compounds for 4 days by oral gavage. The animals were fed a standard rat diet (A03 manufactured by U.A.R., Villemoisson-sur-Orge, France). In each set of experiments the animals were divided into a control group receiving the vehicle alone and four additional treatment groups, one receiving compound **20** (as an internal standard) and the three other groups receiving the diphosphonates to be tested. Diphosphonates were dissolved in water or suspended in 1% (carboxymethyl)cellulose and were administered at the dose of 200 mg/kg per day in a volume of 4 mL/kg body weight. On the fifth day the animals were sacrified under ether anesthesia after overnight fasting. Blood samples were obtained by decapitation and collected in tubes containing EDTA as anticoagulant.

The following analyses were performed on plasma samples: (1) Total cholesterol was determined by using the enzymatic method (Ames kit). Agarose gel electrophoresis was routinely performed to obtain a qualitative evaluation of the changes of lipoprotein

profile. (2) β-Lipoprotein cholesterol was measured after heparin-$CaCl_2$ precipitation according to Burnstein.[27] (3) Total phospholipids were measured chemically by using the molybdate/vanadate reaction (Boehringer kit) or the enzymatic method phospholipid B test (Wako kit). (4) Total triglycerides were measured by the enzymatic method (Boehringer kit or Ames kit). Applying these methods, the typical range of control values obtained with a set of over 30 experiments are as follows (mean ± SD): triglycerides 44 ± 12 mg/100 mL, HDL phospholipids 124 ± 31 mg/100 mL, HDL cholesterol 53 ± 12 mg/100 mL, β-cholesterol 9 ± 11 mg/100 mL, α-/β-cholesterol ratio 5.9 ± 2.3.

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Pyrroloisoquinoline Antidepressants. 2. In-Depth Exploration of Structure-Activity Relationships†

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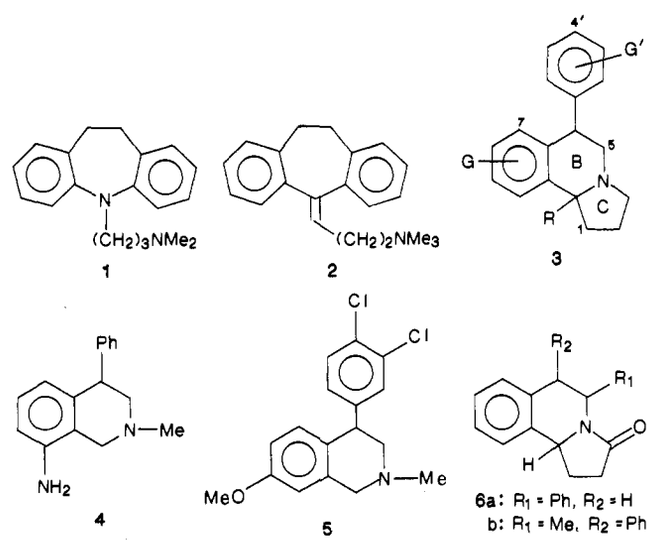
A series of pyrrolo[2,1-*a*]isoquinolines, and related compounds, were examined for antidepressant-like activity, by virtue of their antagonism of tetrabenazine-induced ptosis and sedation, and inhibition of biogenic amine uptake. Thus, we have identified some of the most potent antagonists of TBZ-induced ptosis and some of the most potent inhibitors of the uptake of dopamine, norepinephrine, and serotonin (in rat brain synaptosomes) ever reported. Compounds of particular note, in this regard, are **52b**, **29b**, **22b**, and **48b**, respectively. Biological activity was chiefly manifested by the trans isomeric class. Also, through resolution of four compounds, **7b**, **24b**, **37b**, and **48b**, biological activity was found to be associated with the (+) enantiomer subgroup (salts measured at 589 nm in MeOH), corresponding to the 6*S*,10b*R* absolute configuration for **7b**, **37b**, and **48b**, and the 6*R*,10b*R* configuration for **24b**. An X-ray determination on (+)-**24b**·HBr established its absolute configuration; configurations for the other compounds were verified by enantiospecific synthesis starting with (+)-(*R*)-2-phenylpyrrolidine. Regarding the pendant phenyl ring, diverse substitution patterns were investigated. Those substitutions that were particularly unfavorable were 3′,4′,5′-trimethoxy (**20b**), 2′,3′,4′,5′,6′-pentafluoro (**34b**), 2′-trifluoromethyl (**38b**), 3′,5′-bis(trifluoromethyl) (**42b**), 4′-*n*-butyl (**44b**), 2′-cyano (**47b**), 4′-methylsulfonyl (**50b**), and 2′-carboxy (**58b**). Exceedingly potent compounds, in one way or another, were **10b–12b**, **22b**, **23b**, **25b**, **28b**, **29b**, **33b**, **45b**, **48b**, **51b–53b**. The pattern of aromatic substitution had a strong impact on selectivity in the uptake tests (NE vs. DA vs. 5-HT). Activity was significantly diminished by methyl substitution of **7b** at the 5 (**65**, **66**), 6 (**61b**), or 10b (**60b**) position, by changing the phenyl group of **7b** to cyclohexyl (**67b**), benzyl (**68b**), or H (**72**), by moving the phenyl group of **7b** to the 5 (**69**) or 10b (**70**) position, by expansion of ring B to an azepine (**78b**), and by modification of ring C to an azetidine (**77b**), piperidine (**75b**), or azepine (**74b**). The interaction of selected analogues with various CNS receptors is reported. Little affinity was shown for the muscarinic cholinergic receptor, suggesting a lack of anticholinergic side effects. Interestingly, **24b** and **33b** displayed a high affinity for the serotonin-2 receptor, analogous to mianserin and clomipramine. After the body of data was reviewed, derivatives **24b** and **48b** were chosen for advanced development.

Because of the shortcomings of classical antidepressant drugs,[1] such as imipramine (**1**) and amitriptyline (**2**), the search for novel agents continues apace. In the past decade, much emphasis has been placed on the discovery of molecular series lacking the tricyclic, "butterfly-like" structure that characterizes the classical antidepressants. These newer "nontricyclic" compounds have been referred to as "nonclassical" antidepressants.

For several years, we have been conducting research in this direction.[2,3] Our CNS testing program has uncovered a new series of compounds, possessing a hexahydropyrrolo[2,1-*a*]isoquinoline structure (viz. **3**), which exhibit significant activity in pharmacological and biochemical assays indicative of their potential in the treatment of depression. Recently, we published a preliminary account to disclose, in particular, representative compounds that are the most potent uptake inhibitors presently known for norepinephrine (NE), dopamine (DA), and serotonin (5-HT).[3] These compounds are also potent antagonists of tetrabenazine-induced ptosis. Another salient aspect of this series is the high enantioselectivity demonstrated in the pharmacological and biochemical tests. Herein we



describe the details of our work, with an emphasis on structure–activity relationships (SAR).

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(1) For reviews on antidepressant agents, including their side effects and deficiencies, see: (a) Kaiser, C.; Setler, P. E. In *Burger's Medicinal Chemistry*, 4th ed.; Wolff, M. E., Ed.; Wiley-Interscience: New York, 1981; Part III, Chapter 58. (b) Csaky, T. Z. *Cutting's Handbook of Pharmacology*, 6th ed.; Appleton-Century-Crofts: New York, 1979; pp 630–637.

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