The jacketed cell was pretreated with an aqueous Na_2EDTA solution. All runs were carried out in xylenes (Aldrich) as the solvent. The initial dioxetane concentration of a run was kept at $\sim 10^{-4}$ M to avoid induced decomposition of the dioxetane. Runs carried out without added fluorescer, with DPA, and with DBA were of the first order for at least 3 half-livers and showed no dependence on type or amount of added fluorescer. The yields of excited states produced upon dioxetane thermolysis was determined at 50.0 °C by variation of the concentration of fluorescer at constant dioxetane concentration (DBA/DPA method). The method of calculation has been discussed in detail.[2a] The value of ϕ_{ET} for energy transfer of triplet carbonyls to DBA was assumed to be 0.2 for all five cases. The apparatus was calibrated by taking the yield of triplet excited products from thermolysis of trimethyl-1,2-dioxetane determined by the DBA method, as 0.15.[15]

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Registry No. 1, 86954-68-9; 2, 86954-69-0; 3, 86954-70-3; 4, 86954-71-4; 5, 86954-72-5; 1-bromo-2-hydroperoxy-2-methylbutane, 86954-73-6.

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Inert Carbon Free Radicals. 4. Spin Labeling of Amino Acids and Peptides

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The first examples of spin labeling with inert free-radical reagents are presented. Those here described involve tagging of amino acids and peptides, the labeling reagents being tetradecachloro-4-(chlorocarbonyl)triphenylmethyl (**2**), 4-aminotetradecachlorotriphenylmethyl (**18**), and tetradecachloro-4-hydroxytriphenylmethyl (**25**) radicals, which give rise to derivatives of the so-called C-, N-, and O-link series, respectively. The labeled amino acids and peptides are as follows: from glycine, **4** and **9** (C link), **19** and **20** (N link); from alanine, **5**, **8**, and **10** (C link), **30** and **36** (O link); from phenylalanine, **6** and **11** (C link), **31** and **37** (O link); from leucine, **7** and **12** (C link); from valine, **32** and **38** (O link); from proline, **33** and **39** (O link); from alanylglycine, **23** and **24** (N link); from glycylglycylphenylalanine, **15** and **16** (C link). In this connection, inert free radicals **3** and **22** have also been obtained. The IR, UV–vis, and ESR spectra of the above-mentioned derivatives have been recorded. The hyperfine (ESR) spectra of the compounds of the N-link series show, in addition to the ^{13}C couplings, those with ^{1}H and ^{14}N. Some of the radicals here described display abnormal variations of the magnetic susceptibility with temperature.

The EPR spectrum of an organic free radical may afford information on its structure and on that of its immediate environment. The relevant parameters are the *g* values (Lande factor), the line width, and the hyperfine structure: the number of lines and their relative intensities and the coupling constants with the spin-active nuclei in the molecule.

When a free radical is attached somehow to a nonradical molecule, the EPR spectrum of the resulting species also affords structural information on the added molecule. This technique, called "spin labeling", has been employed extensively in the domains of chemistry, biochemistry, molecular biology (proteins,[1-3] nucleotides,[2] enzymes,[1,3] nucleic acids,[1] lipids and membranes,[1-3] immunology,[1,2] drug detection,[1,2] etc.), and industrial research (polymers,[2,4] detergents,[2] liquid crystals,[2] etc.). Practically, all the work done so far has been performed with conveniently stable aminyl oxide (nitroxide) radicals.

With the discovery of the "inert free radicals" (IFR),[5-7] some extremely stable trivalent carbon species, new prospects within the spin-labeling technique have emerged. In fact, such radicals withstand extremely aggressive chemicals, as well as temperatures up to 300 °C,[5] and in those containing functional substituents, their substituents usually react without impairment of the radical character,[6,7] some features that might be of significance in certain spin-labeling applications. (Recall the well-known lability of aminyl oxide radicals in aqueous acidic media.)

The size of the triphenylmethyl system might pose nearly unsurmontable problems in some applications, although it might as well be a desirable feature in others. The possibility of an anchoring of the inert spin label by a long "umbilical cord" might afford additional prospects.

In this connection it was decided to attempt the spin labeling of some amino acids and peptides[8] with functionalized inert free radicals, mainly to seek information on the relevant synthetic aspects. This aim has been achieved, and the results obtained are presented here.[10]

According to the spin-label reagent employed, the products reported next are grouped in three classes: C-link, N-link, and O-link series.

Results and Discussion

The inert spin labels used here are the radicals tetradecachloro-4-(chlorocarbonyl)triphenylmethyl (**2**), 4-

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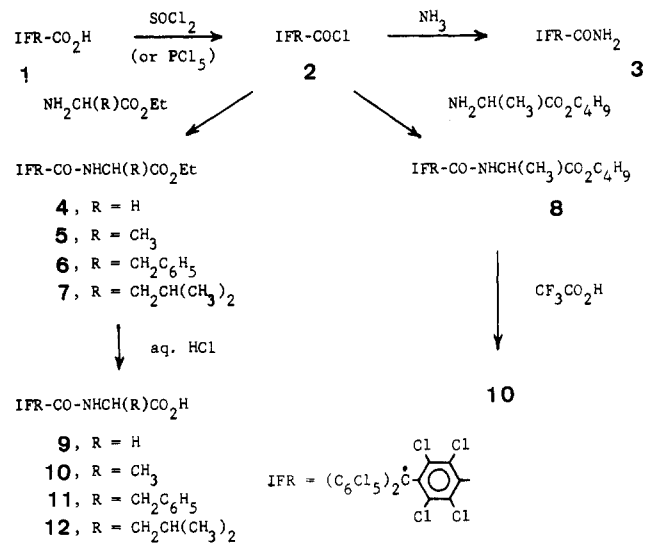
(7) Ballester, M.; Riera, J.; Castañer, J.; Rodríguez, A.; Veciana, J.; Rovira, C. *J. Org. Chem.* **1982**, *47*, 4498.

(8) A few amino acids labeled with aminyl oxides have been reported so far.[9]
(9) Hideg, K.; Lex, L.; Hankowszky, H. O.; Tigyi, J. *Synthesis* **1978**, 914.
(10) Short notices have been advanced on this subject.[11-13]
(11) Ballester, M.; Riera, J.; Rodríguez, A.; Rovira, C. *Tetrahedron Lett.* **1977**, 2355.
(12) Ballester, M.; Castañer, J.; Riera, J.; Veciana, J. *Tetrahedron Lett.* **1978**, 479.
(13) Ballester, M.; Riera, J.; Castañer, J.; Onrubia, C.; Juliá, L.; Oliver, J. *An. Quim.* **1981**, *77C*, 220.

0022-3263/83/1948-3716$01.50/0

Scheme I



aminotetradecachlorotriphenylmethyl (18), and tetradecachloro-4-hydroxytriphenylmethyl (25). The syntheses of 18 and 25 have been described previously.[7] That of 2 has been effected from 4-carboxytetradecachlorotriphenylmethyl radical (1)[6] with $SOCl_2$ (97%) or PCl_5. In connection with the spin tagging at the amino group, the reaction of chlorocarbonyl radical 2 with NH_3 at room temperature to give 4-carbamoyltetradecachlorotriphenylmethyl radical (3) has been performed (95%).

C-Link Series. In this series the inert free radical fragment is attached to the amino group of the amino acids through a carbonyl (C link) group. Before labeling, the amino acids are protected at the carbonyl by esterification.

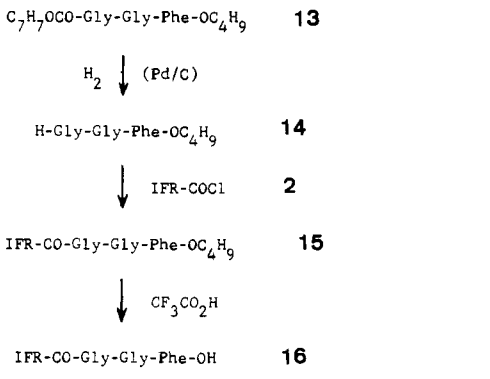
The amino acid ester hydrochlorides are treated at room temperature with chlorocarbonyl radical 2 in benzene–water containing (excess) triethylamine to give the spin-labeled amino acid esters (Scheme I). Starting from glycine, L-alanine, L-phenylalanine, and L-leucine, we obtained the labeled esters tetradecachloro-4-(glycinocarbonyl)triphenylmethyl (4), 4-(alaninocarbonyl)tetradecachlorotriphenylmethyl (5), tetradecachloro-4-(phenylalaninocarbonyl)triphenylmethyl (6), and tetradecachloro-4-(leucinocarbonyl)triphenylmethyl (7) radical ethyl ester, respectively. 4-(Alaninocarbonyl)tetradecachlorotriphenylmethyl radical *tert*-butyl ester 8 was also obtained.

The removal of the protecting ethyl group to give the corresponding spin-labeled amino acids tetradecachloro-4-(glycinocarbonyl)triphenylmethyl (9), 4-(alaninocarbonyl)tetradecachlorotriphenylmethyl (10), tetradecachloro-4-(phenylalaninocarbonyl)triphenylmethyl (11), and tetradecachloro-4-(leucinocarbonyl)triphenylmethyl (12) radicals was performed with an aqueous HCl–dioxane mixture. The removal of the *tert*-butyl group of 8 was effected with trifluoroacetic acid in methylene chloride.

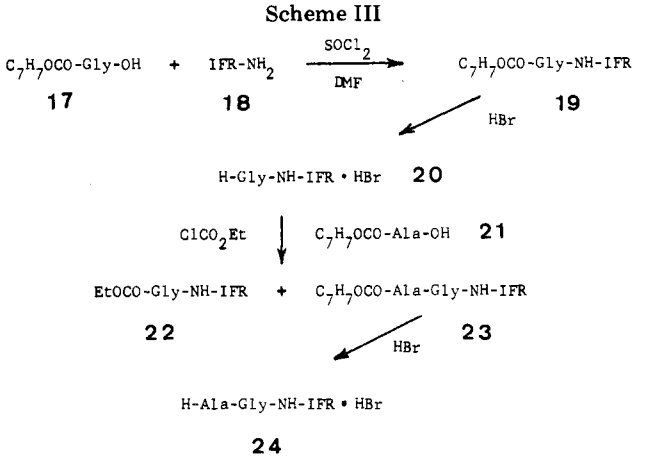
This general procedure was extended to a tripeptide: the glycylglycylphenylalanine *tert*-butyl ester (14). The starting material was the corresponding *N*-benzyloxycarbonyl *tert*-butyl ester derivative 13, the amino group of which was deprotected by catalytic hydrogenation to give 14. The spin labeling gave tetradecachloro-4-(phenylalaninoglycinoglycinocarbonyl)triphenylmethyl radical *tert*-butyl ester (15), and the corresponding acid radical 16 (Scheme II).

N-Link Series. In this series the carboxyl group of the substrate (the amino acid) forms an amide link with the amino group of the spin label. Since the selected spin label

Scheme II



Scheme III



(amino radical 18) is relatively unreactive at the amino group, proper activation of the amino acid at the carboxyl is ensured by Palomo's reagent,[14] the necessary protection of the amino group being attained by benzyloxycarbonylation.

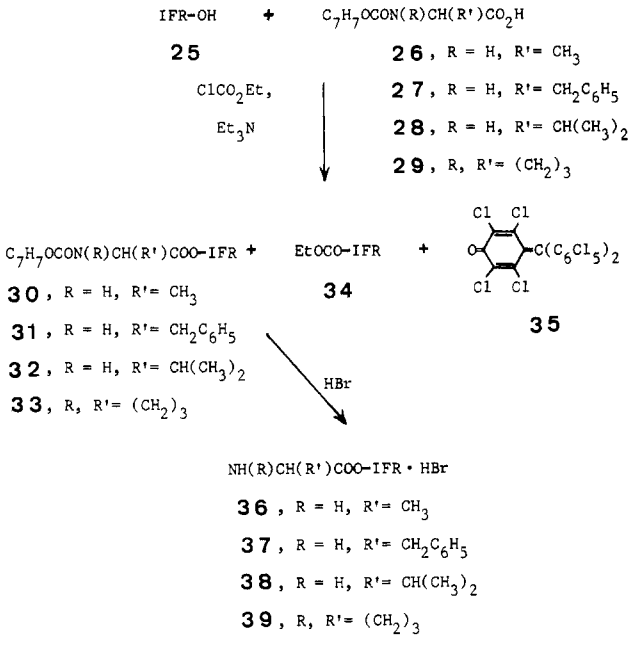
N-(Benzyloxycarbonyl)glycine (17) was activated with a mixture of $SOCl_2$ and DMF, and the product was treated next with spin label 18 to give 4-[[*N*-(benzyloxycarbonyl)glycyl]amino]tetradecachlorotriphenylmethyl radical (19, Scheme III). The removal of the protecting group was carried out with HBr in dioxane, giving tetradecachloro-4-(glycylamino)triphenylmethyl radical hydrobromide (20). The reaction of the labeled glycine 20 with *N*-(benzyloxycarbonyl)-L-alanine (21), activated with ethyl chloroformate–triethylamine, gave 4-[[*N*-(benzyloxycarbonyl)alanylglycyl]amino]tetradecachlorotriphenylmethyl radical (23) and some 4-[[*N*-(ethoxycarbonyl)glycyl]amino]tetradecachlorotriphenylmethyl radical (22). Labeled dipeptide 23 was deprotected as above, giving 4-[(alanylglycyl)amino]tetradecachlorotriphenylmethyl radical hydrobromide (24). All these reactions were performed at room temperature.

O-Link Series. In this series the carboxyl group of the amino acids is made to react with the hydroxyl group of the spin label 25, forming an ester link. As in the N-link series, activation of the amino acid at the carboxyl group is required, the amino group being protected as in that series.

The *N*-benzyloxycarbonyl (Z) amino acids were activated with ethyl chloroformate–triethylamine, and the product was treated next with hydroxy radical 25 to give the corresponding labeled derivatives (Scheme IV). Starting from

(14) Palomo, A. L. *An. R. Soc. Esp. Fis. Quim.* **1969**, *65*, 12.
(15) Attempts using HCl instead failed, giving perchlorofuchsone[7] (35; ~90% yield).

Scheme IV



N-(benzyloxycarbonyl)alanine (**26**), *N*-(benzyloxycarbonyl)-L-phenylalanine (**27**), *N*-(benzyloxycarbonyl)-L-valine (**28**), and *N*-(benzyloxycarbonyl)-L-proline (**29**), the following products were obtained: 4-[[*N*-(benzyloxycarbonyl)alanyl]oxy]tetradecachlorotriphenylmethyl (**30**), 4-[[*N*-(benzyloxycarbonyl)phenylalanyl]oxy]tetradecachlorotriphenylmethyl (**31**), 4-[[*N*-(benzyloxycarbonyl)valyl]oxy]tetradecachlorotriphenylmethyl (**32**), and 4-[[*N*-(benzyloxycarbonyl)prolyl]oxy]tetradecachlorotriphenylmethyl (**33**) radicals, respectively. As byproducts, tetradecachloro-4-[(ethoxycarbonyl)oxy]triphenylmethyl radical[7] (**34**) and perchlorofuchsone[7] (**35**) were isolated. The removal of the protecting Z group was performed as in the N-link series, giving 4-(alanyloxy)tetradecachlorotriphenylmethyl (**36**), tetradecachloro-4-[(phenylalanyl)oxy]triphenylmethyl (**37**), tetradecachloro-4-(valyloxy)triphenylmethyl (**38**), and tetradecachloro-4-(prolyloxy)triphenylmethyl (**39**) radical hydrobromides, respectively.

Ultraviolet–Visible Spectra. The spectra of all radicals here studied were taken in nonpolar solvents (C_6H_{12} or $CHCl_3$), except those of the hydrobromides which were recorded in polar solvents (dioxane or acetonitrile). They present the same spectral features as those described previously,[5-7] i.e., two benzenoid bands (about 220 and 280 nm) and two radical bands (around 385 and 500–565 nm).

Electron Spin Resonance Spectra. The spectral data are summarized in Table I of the supplementary material and include 25 radicals. The Lande *g* values are normal.[5] The spectra of the C- and O-link series radicals consist of a single main line and the three pairs of ^{13}C satellites, as normally observed.[5-7]

As far as the N-link series goes, the radicals show either a distorted single main line (in C_2Cl_4) or two distorted main lines (in dioxane), as occurs in the closely related tetradecachloro-4-[(chloroacetyl)amino]triphenylmethyl and 4-[(bromoacetyl)amino]tetradecachlorotriphenylmethyl radicals.[7] These results are also due to an increase in the coupling constants, mainly with the N proton, and a decrease in the line width[7] (2.14 to 2.6 MHz). The ^1H and ^{14}N couplings range from 3.5 to 4.2 and from 1.18 to 1.26 MHz, respectively.

Magnetic Susceptibilities. Table II of the supplementary material shows some magnetic susceptibility and related data on all 25 of the radicals here reported. Least-squares correlation of their Curie–Weiss plot (from 77 K to room temperature) often gives low, unacceptable values for the specific diamagnetic susceptibility (χ_{dia}), showing an abnormal magnetic behavior. Consequently, χ_{dia} values were calculated from Pascal systematics[7] and were included as a data in that correlation, which affords the radical purities (spins/mole), listed in the last column, above 90%, most of them nearly 100%.

Conclusion

The preceding results indicate that general spin labeling with inert free radicals (IFR) can be effected by simple, well-established methods and under mild conditions, in spite of the steric shielding of the spin-label reacting group afforded by its two ortho chlorines. Such a labeling is persued further with other spin labels designed for complex structural studies.

Experimental Section

General Methods. The IR, UV–vis, and ESR spectra have been recorded with Perkin-Elmer 457, Perkin-Elmer 350, and Varian E4 spectrometers, respectively. The magnetic susceptibilities have been measured in helium with a Varian 4-in. magnet with constant-force caps and a Cahn RG electrobalance.

The handling of radicals in solution was performed in the dark.

Since the locations of the IR peaks of perchloroorganic compounds differ markedly from those of their nonchlorinated counter parts, it is regarded as useful to include them in this section.

The ESR and magnetic susceptibility data for all the radicals and spin-labeled compounds described below are given as supplementary material.

Tetradecachloro-4-(chlorocarbonyl)triphenylmethyl Radical (2). (a) With $SOCl_2$. A solution of 4-carboxytetradecachlorotriphenylmethyl radical[6] (**1**, 0.203 g) in $SOCl_2$ (20 mL) was refluxed (24 h). Elimination of the solvent gave a residue which was passed through silica gel in $CHCl_3$, giving chloroformyl radical **2**: 0.201 g (97%); red crystals; mp 304–306 °C dec; IR (KBr) 1785, 1500, 1335, 1320, 1258, 1154, 1046, 938, 858, 810, 762, 725, 700, 665, 635, 540, 514 cm^{-1}; UV–vis ($CHCl_3$) 290, 335 (sh), 370 (sh), 385, 480, 510, 565 nm (ϵ 7400, 6140, 20 200, 39 100, 1280, 1260, 1190). Anal. Calcd for $C_{20}Cl_{15}O$: C, 30.5; Cl, 67.5 Found: C, 30.5; Cl, 67.5.

(b) With PCl_5. A mixture of carboxy radical **1** (0.050 g), PCl_5 (0.136 g), and $POCl_3$ (2 mL) was refluxed (24 h). Distillation of the solvent gave a residue which was treated with cold water and extracted with $CHCl_3$. Elimination of the solvent afforded a residue which was treated as before, yielding chloroformyl radical **2** (0.036 g, 70%).

4-Carbamoyltetradecachlorotriphenylmethyl Radical (3). A stream of dry NH_3 was passed through a solution of chloroformyl radical **2** (0.066 g) in $CHCl_3$ (55 mL). The reaction mixture was washed with water, dried, and evaporated. The residue (0.066 g) was recrystallized from $CHCl_3$–hexane to give the carbamoyl radical **3**: 0.061 g (95%); red crystals; influsible up to 320 °C dec; IR (KBr) 3660–3040, 1658, 1590, 1505, 1335, 1320, 1258, 815, 735, 715, 700, 670, 650 cm^{-1}; UV–vis ($CHCl_3$) 287, 337 (sh), 368 (sh), 382, 480 (sh), 506, 560 nm (ϵ 6400, 6370, 19 700, 38 500, 1190, 1215, 1160). Anal. Calcd for $C_{20}H_2Cl_{14}NO$: C, 31.3; H, 0.3; N, 1.8; Cl, 64.6. Found: C, 31.5; H, 0.2; N, 1.7; Cl, 64.6.

Spin-Labeled Amino Acid Esters by N-Acylation (C-Link Series). (1) Synthesis: General Procedure. A mixture of chlorocarbonyl radical **2** (0.200 g, 0.254 mmol), amino acid ester hydrochloride (0.329–0.345 mmol), triethylamine (0.180 g, 1.78 mmol), benzene (10 mL), and water (3 mL) was shaken vigorously (24 h) at room temperature. The resulting mixture was poured into aqueous HCl and extracted with ether. The ethereal layer was washed with water, dried, and evaporated. The residue was digested with *n*-pentane and purified by TLC (silica gel, $CHCl_3$).

Products. (a) Tetradecachloro-4-(glycinocarbonyl)triphenylmethyl radical ethyl ester (4): 91%; red needles; mp 221–224 °C dec; IR (KBr) 3600–3120, 2970, 2920, 1740, 1660, 1540, 1505, 1335, 1320, 1255, 1200, 1022, 815, 730, 712, 655 cm^{-1}; UV–vis ($CHCl_3$) 287, 335 (sh), 368 (sh), 382, 483 (sh), 507, 562 nm (ϵ 5850,

6240, 20000, 39700, 1190, 1230, 1170). Anal. Calcd for $C_{24}H_8Cl_{14}NO_3$: C, 33.7; H, 0.9; N,1.6; Cl, 58.1. Found: C, 33.8; H, 1.0; N, 1.5; Cl, 58.4.

(b) 4-(Alaninocarbonyl)tetradecachlorotriphenylmethyl radical ethyl ester (5): 91%; red crystals; mp 154–158 °C; IR (KBr) 3560–3140, 2960, 2920, 1735, 1685, 1500, 1450, 1335, 1320, 1258, 1200, 1128, 810, 728, 708, 660 cm^{-1}; UV–vis (C_6H_{12}) 222, 286 (sh), 335 (sh), 365 (sh), 382, 482 (sh), 507, 560 nm (ϵ 88500, 6200, 6500, 19400, 39600, 1190, 1240, 1160). Anal. Calcd for $C_{25}H_{10}Cl_{14}NO_3$: C, 34.5; H, 1.2; N, 1.6; Cl, 57.1. Found: C, 34.3; H, 1.4; N, 1.6; Cl, 57.0.

(c) Tetradecachloro-4-(phenylalaninocarbonyl)triphenylmethyl radical ethyl ester (6): 95%; red powder; mp 127–133 °C; IR (KBr) 3560–3160, 3010, 2970, 2920, 1740, 1685, 1500, 1335, 1320, 1255, 1210, 1030, 812, 730, 710, 690, 665 cm^{-1}; UV–vis (C_6H_{12}) 222, 287, 336 (sh), 368 (sh), 382, 472 (sh), 505, 560 nm (ϵ 93400, 7120, 7310, 20750, 40300, 1200, 1240, 1160). Anal. Calcd for $C_{31}H_{14}Cl_{14}NO_3$: C, 39.4; H, 1.5; N, 1.5; Cl, 52.5. Found: C, 39.4; H, 1.6; N, 1.6; Cl, 52.4.

(d) Tetradecachloro-4-(leucinocarbonyl)triphenylmethyl radical ethyl ester (7): 95%; red powder; mp 145 °C; IR (KBr) 3540–3160, 2950, 2920, 2890, 1740, 1685, 1500, 1335, 1320, 1260, 1195, 1150, 1030, 810, 730, 710, 660, 515 cm^{-1}; UV–vis (C_6H_{12}) 221, 285, 335 (sh), 362 (sh), 382, 484 (sh), 506, 562 nm (ϵ 90000, 6620, 6690, 19800, 39000, 1160, 1190, 1130). Anal. Calcd for $C_{28}H_{16}Cl_{14}NO_3$: C, 36.9; H, 1.8; N, 1.5; Cl, 54.5. Found: C, 37.2; H, 1.8; N, 1.3; Cl, 54.6.

(e) 4-(Alaninocarbonyl)tetradecachlorotriphenylmethyl radical *tert*-butyl ester (8): 97%; red powder; mp 214–218 °C; IR (KBr) 3640–3120, 2980, 2930, 1730, 1675, 1542, 1500, 1450, 1335, 1321, 1255, 1154, 848, 820, 735, 715, 670 cm^{-1}; UV–vis ($CHCl_3$) 285, 335 (sh), 365 (sh), 382, 480 (sh), 506, 560 nm (ϵ 7200, 7740, 22500, 41600, 1215, 1270, 1220). Anal. Calcd for $C_{27}H_{14}Cl_{14}NO_3$: C, 36.2; H, 1.6; N, 1.6; Cl, 55.4. Found: C, 36.2; H, 1.4; N, 1.8; Cl, 55.3.

(2) Hydrolysis General Procedure. A mixture of N-labeled amino acid ethyl ester (0.100 g), 12 N aqueous HCl (5 mL), and dioxane (15 mL) was refluxed (48 h). The resulting mixture was poured into water and extracted with ether. The ethereal layer was washed with water, extracted with aqueous $NaHCO_3$, dried, and evaporated, giving back some starting material (8–10%, IR). The aqueous layer was acidified with aqueous HCl and extracted with ether. This ethereal extract was washed with water, dried, and evaporated. The residue was recrystallized from ether–hexane.

Products. (a) Tetradecachloro-4-(glycinocarbonyl)triphenylmethyl radical (9): 94%; red powder; mp ~215 °C; IR (KBr) 3660–2320, 1722, 1655, 1540, 1330, 1320, 1250, 1038, 815, 730, 712, 692, 664 cm^{-1}; UV–vis ($CHCl_3$) 288, 338 (sh), 368 (sh), 382, 480 (sh), 507, 560 nm (ϵ 5700, 5850, 17600, 34700, 1040, 1080, 1040). Anal. Calcd for $C_{22}H_4Cl_{14}NO_3$: C, 32.0; H, 0.5; N, 1.7; Cl, 60.1. Found: C, 32.1; H, 0.5; N, 1.7; Cl, 60.3.

(b) 4-(Alaninocarbonyl)tetradecachlorotriphenylmethyl radical (10): 94%; red crystals; mp 221–224 °C; IR (KBr) 3680–2300, 1725, 1680, 1650, 1535, 1500, 1450, 1335, 1325, 1255, 1115, 1030, 810, 722, 645, 510 cm^{-1}; UV–vis ($CHCl_3$) 288, 338 (sh), 368 (sh), 382, 482 (sh), 508, 560 nm (ϵ 6600, 6650, 20200, 39600, 1180, 1230, 1170). Anal. Calcd for $C_{23}H_6Cl_{14}NO_3$: C, 32.9; H, 0.7; N, 1.7; Cl, 59.0. Found: C, 33.0; H, 0.9; N, 1.7; Cl, 59.0.

(c) Tetradecachloro-4-(phenylalaninocarbonyl)triphenylmethyl radical (11): 80%; red crystals; mp 185–187 °C; IR (KBr) 3640–2300, 1720, 1675, 1540, 1495, 1335, 1322, 1255, 810, 728, 708, 690, 665 cm^{-1}; UV–vis ($CHCl_3$) 285, 335 (sh), 365 (sh), 382, 485 (sh), 507, 560 nm (ϵ 6220, 6300, 19300, 37500, 1100, 1140, 1090). Anal. Calcd for $C_{29}H_{10}Cl_{14}NO_3$: C, 38.0; H, 1.1; N, 1.5; Cl, 54.1. Found: C, 38.3; H, 1.5; N, 1.5; Cl, 54.1.

(3) Hydrolysis of 4-(Alaninocarbonyl)tetradecachlorotriphenylmethyl Radical *tert*-Butyl Ester (8). Trifluoroacetic acid (2 mL) was added to a solution of *tert*-butyl ester radical **10** (0.059 g) in CH_2Cl_2 (8 mL), and the mass was left undisturbed (5 h) at room temperature. The resulting solution was evaporated in vacuo to dryness, and the residue was dissolved in ether. The ethereal solution was extracted with aqueous $NaHCO_3$, dried, and evaporated, giving back some 8 (0.003 g, 5%). The aqueous extract was acidified with aqueous HCl and extracted with ether. The ethereal extract was washed with water, dried, and evaporated to give 4-(alaninocarbonyl)tetradecachlorotriphenylmethyl radical (**10**; 0.053 g, 96%), which was identified by elemental analysis and IR and UV–vis spectra.

(4) Synthesis of Tetradecachloro-4-(phenylalaninoglycinoglycinocarbonyl)triphenylmethyl Radical *tert*-Butyl Ester (15). *N*-(Benzyloxycarbonyl)glycylglycylphenylalanine *tert*-butyl ester (**13**, 0.105 g) was hydrogenated with H_2 over Pd/C (0.101 g) in methanol (45 mL)/water (5 mL) at room temperature (5 h). The catalyst was filtered off, and the resulting solution was evaporated to dryness in vacuo, giving a thick oil (0.076 g) which, when mixed with triethylamine (0.115 g), was added to a solution of 4-(chlorocarbonyl)tetradecachlorotriphenylmethyl radical (**2**, 0.180 g) in CH_2Cl_2 (30 mL) and left undisturbed (29 h) at room temperature. The resulting solution was evaporated to dryness, and the residue, dissolved in ether, was washed with water, dried, and evaporated. The new residue (0.258 g) was purified (TLC; silica gel, $CHCl_3$–methanol) and recrystallized from ether–hexane to give *tert*-butyl ester radical **15**: 0.109 g (45%); red powder; mp 204–206 °C; IR (KBr) 3740–3100, 3070, 2980, 2930, 1730, 1660, 1520, 1370, 1335, 1325, 1255, 1155, 820, 735, 720, 700, 670 cm^{-1}; UV–vis ($CHCl_3$) 287, 336 (sh), 368 (sh), 384, 480 (sh), 510, 560 nm (ϵ 6270, 6380, 19400, 37900, 1140, 1170, 1115). Anal. Calcd for $C_{37}H_{24}Cl_{14}N_3O_5$: C, 40.9; H, 2.2; N, 3.9; Cl, 45.7. Found: C, 41.1; H, 2.1; N, 3.8; Cl, 45.7.

(5) Hydrolysis of *tert*-Butyl Ester Radical 15. This hydrolysis was carried out as in that of 4-(alaninocarbonyl)-tetradecachlorotriphenylmethyl radical *tert*-butyl ester (**8**). The starting materials were *tert*-butyl ester radical **15** (0.046 g), trifluoroacetic acid (2 mL) and CH_2Cl_2 (10 mL). The product was purified by recrystallization in ether-pentane affording pure tetradecachloro-4-(phenylalanino-glycino-glycinoformyl)triphenylmethyl radical (**16**) (0.037 g; 61%), red powder mp 178-81°; IR (KBr) 3700-2700, 1730, 1660, 1520, 1333, 1320, 1255, 1120, 815, 730, 715, 695, 665, 610, 527 cm^{-1}; UV-vis ($CHCl_3$) 288, 336 (sh), 366 (sh) 384, 510 (sh), 562 nm (ϵ 5100, 4800, 14200, 26900, 936, 810). Anal. Calcd for $C_{33}H_{16}Cl_{14}N_3O_5$: C, 38.4; H, 1.5; N, 4.1; Cl, 48.2. Found: C, 38.3; H, 1.6; N, 4.1; Cl, 48.2.

Spin-Labeled Amino Acid by Amidation (N-Link Series). (a) 4-[[*N*-(Benzyloxycarbonyl)glycyl]amino]tetradecachlorotriphenylmethyl Radical (19). A solution of *N*,*N*-dimethylformamide (1.75 mL, 0.022 mol) and $SOCl_2$ (1.65 mL, 0.022 mmol) in anhydrous benzene (30 mL) was shaken at room temperature (Palomo's reagent[14]). After a few minutes, the oily precipitate formed was added to a stirred suspension of *N*-(benzyloxycarbonyl)glycine (**17**; 4.7 g, 0.022 mol) in anhydrous $CHCl_3$ (100 mL) at 0 °C and in a dry atmosphere. The resulting mixture was left at room temperature (30 min) and then evaporated in vacuo. To the resulting oily residue, a solution of 4-aminotetradecachlorotriphenylmethyl radical (**18**) (0.400 g) in $CHCl_3$ (3 mL) was added, the mass was left undisturbed (4 days) at room temperature in a dry atmosphere, and then it was submitted to column chromatography (silica gel, $CHCl_3$). The first fraction after recrystallization (ether) gave unreacted amino radical **18** (0.264 g, 62% recovery), identified by IR. The second fraction after recrystallization (ether) gave the spin-labeled amino acid **19**: 0.148 g (30% yield, 76% conversion); red needles; mp 231–233 °C; IR (KBr) 3450–2900, 1690, 1650, 1510, 1460, 1330, 1260, 1210, 1040, 975, 860, 765, 710, 650 cm^{-1}; UV–vis (C_6H_{12}) 229, 286, 336 (sh), 364 (sh), 383, 480 (sh), 510, 564 nm (ϵ 82100, 8200, 6480, 17800, 38100, 1170, 1280, 1360). Anal. Calcd for $C_{29}H_{11}Cl_{14}N_2O_3$: C, 37.4; H, 1.2; N, 3.0; Cl, 53.3. Found: C, 37.2; H, 1.2; N, 3.0; Cl, 53.1.

(b) 4-[[*N*-(Benzyloxycarbonyl)alanylglycyl]amino]-tetradecachlorotriphenylmethyl Radical (23). Triethylamine (0.080 mL, 0.566 mmol) and ethyl chloroformate (0.055 mL, 0.566 mmol) were added to a cooled (0 °C), stirred solution of *N*-(benzyloxycarbonyl)-L-alanine (**21**; 0.175 g, 0.792 mmol) in anhydrous $CHCl_3$. A while later, (10 min), solid tetradecachloro-4-(glycylamino)triphenylmethyl radical hydrobromide (**20**; 0.085 g, 0.102 mmol) was added and then a solution of triethylamine (0.015 g, 0.102 mmol) in $CHCl_3$ (0.57 mL). The resulting mixture was kept (3 h) at low temperature (0–10 °C) and then left at room temperature (1.5 h). The reaction mass was evaporated to dryness, and the residue was submitted to TLC (silica gel, CH_2Cl_2–acetone). The first fraction after recrystallization (ether–hexane) afforded 4-[[*N*-(ethoxycarbonyl)glycyl]amino]tetradecachlorotriphenyl-

methyl radical (**22**): 0.007 g (8%); red crystals; mp 277–279 °C; IR (KBr) 3550–3300, 2980, 2930, 1715, 1680, 1510, 1410, 1340, 1330, 1260, 1050, 870, 820, 735, 710, 530 cm^{-1}; UV–vis (C_6H_{12}) 222, 280 (sh), 330 (sh), 366, 382, 510, 560 nm (ε 64 000, 5600, 5800, 18 500, 31 800, 1250, 1290). Anal. Calcd for $C_{24}H_9Cl_{14}N_2O_3$: C, 33.1; H, 1.0; N, 3.2; Cl, 57.0. Found: C, 33.3; H, 1.1; N, 2.9; Cl, 57.1. The second fraction after recrystallization (ether–hexane) gave the spin-labeled dipeptide **23**: 0.065 g (64%); red needles; mp 218–221 °C; IR (KBr) 3440–3100, 3065, 3035, 2980, 2930, 1690, 1655, 1510, 1330, 1260, 1060, 820, 735, 710, 650 cm^{-1}; UV–vis (C_6H_{12}) 230, 284, 334 (sh), 365 (sh), 383, 510, 563 nm (ε 73 000, 6150, 6200, 18 600, 36 000, 1290, 1320). Anal. Calcd for $C_{32}H_{16}Cl_{14}N_3O_4$: C, 38.3; H, 1.6; N, 4.2; Cl, 49.5. Found: C, 38.4; H, 1.3; N, 4.1; Cl, 49.4.

Spin-Labeled Amino Acids by Esterification (O-Link Series). Synthesis: General Procedure. Ethyl chloroformate (1.00 mmol) was added with vigorous stirring to a cooled solution (0 °C) of the *N*-benzyloxycarbonyl amino acid (1.00 mmol) and triethylamine (1.00 mmol) in $CHCl_3$ (3.5 mL). After a while (8 min), tetradecachloro-4-hydroxytriphenylmethyl radical (**25**, 0.50 mmol) was added, next the mixture was stirred (30 min), and finally it was refluxed (15 min). The resulting mass was evaporated to dryness, and the residue was submitted to column chromatography (silica gel). The first fraction (CCl_4–$CHCl_3$ 2:1) was recrystallized (ether–pentane), affording pure tetradecachloro-4-[(ethoxycarbonyl)oxy]triphenylmethyl radical (**34**): 11–27%; red crystals; mp 156–158 °C; identified by mixture melting point and IR. The second fraction (CCl_4–$CHCl_3$ 2:1) was perchlorofuchsone (**35**, 4–18%), identified by mixture melting point and IR.[7] The third fraction ($CHCl_3$) was recrystallized (ether–hexane), yielding the corresponding labeled *N*-benzyloxycarbonyl amino acid.

Products. (a) 4-[[*N*-(Benzyloxycarbonyl)alanyl]oxy]-tetradecachlorotriphenylmethyl radical (30): 80%; red crystals; mp 128–130 °C; IR (KBr) 3430–3310, 3060, 3020, 2980, 2940, 1785, 1720, 1510, 1450, 1390, 1330, 1320, 1280, 1250, 1100, 1050, 950, 860, 810, 730, 710, 690, 650 cm^{-1}; UV–vis (C_6H_{12}) 221, 272 (sh), 336 (sh), 365 (sh), 381, 482 (sh), 500, 554 nm (ε 86 800, 5750, 6800, 19 300, 34 000, 1080, 1020, 990). Anal. Calcd for $C_{30}H_{12}Cl_{14}NO_4$: C, 38.1; H, 1.3; N, 1.5; Cl, 52.4. Found: C, 38.0; H, 1.6; N, 1.3; Cl, 52.3.

(b) 4-[[*N*-(Benzyloxycarbonyl)phenylalanyl]oxy]-tetradecachlorotriphenylmethyl radical (31): 76%; red crystals; mp 109–111 °C; IR (KBr) 3430–3340, 3060, 3040, 2960, 2920, 1785, 1730, 1500, 1450, 1390, 1340, 1325, 1280, 1260, 1110, 1080, 1050, 820, 750, 740, 710, 700, 600 cm^{-1}; UV–vis (C_6H_{12}) 218, 274 (sh), 334 (sh), 365 (sh), 380, 480 (sh), 500, 556 nm (ε 88 000, 5930, 6560, 18 700, 35 400, 1100, 1120, 1090). Anal. Calcd for $C_{36}H_{16}Cl_{14}NO_4$: C, 42.3; H, 1.6; N, 1.4; Cl, 48.5. Found: C, 42.4; H, 1.5; N, 1.5; Cl, 48.7.

(c) 4-[[*N*-(Benzyloxycarbonyl)valyl]oxy]tetradecachlorotriphenyl methyl radical (32): 59%; red crystals; mp 176–178 °C; IR (KBr) 3430–3320, 3060, 3020, 2960, 2860, 1785, 1730, 1500, 1460, 1390, 1340, 1320, 1260, 1220, 1080, 960, 890, 810, 730, 710, 690, 650 cm^{-1}; UV–vis (C_6H_{12}) 218, 276 (sh), 334 (sh), 364 (sh), 378, 480 (sh), 500, 554 nm (ε 88 300, 5180, 6370, 18 500, 35 000, 1090, 1120, 1085). Anal. Calcd for $C_{32}H_{16}Cl_{14}NO_4$: C, 39.4; H, 1.7; N, 1.4; Cl, 50.9. Found: C, 39.2; H, 1.8; N, 1.7; Cl, 51.2.

(d) 4-[[*N*-(Benzyloxycarbonyl)-L-prolyl]oxy]tetradecachlorotriphenylmethyl radical (33): 53%; red crystals; mp 222–223 °C; IR (KBr) 2960, 2880, 1790, 1715, 1455, 1410, 1340, 1330, 1280, 1260, 1110, 1070, 1030, 810, 760, 730, 710, 690, 650, 530 cm^{-1}; UV–vis (C_6H_{12}) 218, 276 (sh), 338 (sh), 367 (sh), 378, 478 (sh), 500 , 555 nm (ε 90 800, 6040, 6640, 18 100, 34 300, 1080, 1130, 1095). Anal. Calcd for $C_{32}H_{14}Cl_{14}NO_4$: C, 39.5; H, 1.5; N, 1.4; Cl, 51.0. Found: C, 39.5; H, 1.7; N, 1.7; Cl, 50.8.

Removal of the Benzyloxycarbonyl Protecting Group. General Procedures with HBr.[15] (1) In Acetic Acid. The spin-labeled *N*-benzyloxycarbonyl amino acid (~0.12 g) was added to a solution of dry HBr in anhydrous acetic acid (20 mL), and the resulting solution was left undisturbed (1 h) at room temperature. Ether was added next (~60 mL), and the insoluble spin-labeled amino acid hydrobromide was filtered off and dried.

(2) In Dioxane. The spin-labeled *N*-benzyloxycarbonyl amino acid (~0.120 g) was added to a saturated solution of dry HBr in anhydrous dioxane (20 mL), and the mass was left undisturbed (1 h) at room temperature. The resulting solution was evaporated in vacuo, and the residue was digested with ether at room temperature, leaving the spin-labeled amino acid hydrobromide as a solid residue.

Products: (a) Tetradecachloro-4-(glycylamino)triphenylmethyl radical hydrobromide (20): procedure 2; 99.8%; red powder; mp 226 °C dec; IR (KBr) 3500–2650, 1715, 1535, 1485, 1340, 1330, 1260, 820, 735, 710, 650, 530 cm^{-1}; UV–vis (dioxane) 232, 284 (sh), 365 (sh), 383, 510, 563 nm (ε 58 000, 6560, 17 800, 32 000, 965, 1020). Anal. Calcd for $C_{21}H_6BrCl_{14}N_2O$: C, 28.7; H, 0.7; N, 3.2; Br, 9.1; Cl, 56.5. Found: C, 28.9; H, 1.0; N, 3.0; Br, 9.1; Cl, 56.4.

(b) 4-[(Alanylglycyl)amino]tetradecachlorotriphenylmethyl radical hydrobromide (24): procedure 2; 99.8%; red powder; mp 215 °C dec; IR (KBr) 3500–2900, 1670, 1510, 1470, 1330, 1320, 1255, 810, 730, 710, 650 cm^{-1}; UV–vis (dioxane) 226, 285 (sh), 367 (sh), 385, 511, 566 nm (ε 64 200, 5730, 18 500, 34 500, 1230, 1250). Anal. Calcd for $C_{24}H_{11}BrCl_{14}N_3O_2$: C, 30.4; H, 1.2; N, 4.4; Br, 8.4; Cl, 52.3. Found: C, 30.2; H, 1.2; N, 4.1; Br, 8.3; Cl, 52.5.

(c) 4-(Alanyloxy)tetradecachlorotriphenylmethyl radical hydrobromide (36): procedure 1, 99%; procedure 2, 98%; red powder; mp 271 °C dec; IR (KBr) 3560–2380, 1780, 1670, 1515, 1455, 1380, 1330, 1320, 1270, 1250, 1150, 1100, 860, 810, 730, 710, 645, 530 cm^{-1}; UV–vis (acetonitrile) 219, 270 (sh), 336 (sh), 366 (sh), 381, 510, 564 nm (ε 90 900, 5240, 5670, 16 000, 28 000, 1000, 1050). Anal. Calcd for $C_{22}H_7BrCl_{14}NO_2$: C, 29.6; H, 0.8; N, 1.6; Br, 8.9; Cl, 55.6. Found: C, 29.8; H, 0.8; N, 1.7; Br, 8.7; Cl, 55.3.

(d) Tetradecachloro-4-[(phenylalanyl)oxy]triphenylmethyl radical hydrobromide (37): procedure 2; 91%; red powder; mp 162–164 °C dec; IR (KBr) 3660–2400, 1785, 1580, 1500, 1385, 1340, 1330, 1280, 1260, 1150, 1070, 1050, 860, 810, 730, 710, 655, 520 cm^{-1}; UV–vis (acetonitrile) 218, 273 (sh), 336 (sh), 366 (sh), 382, 512, 564 nm (ε 100 500, 5840, 5870, 15 400, 27 400, 995, 1005). Anal. Calcd for $C_{28}H_{11}BrCl_{14}NO_2$: C, 34.7; H, 1.1; N, 1.4; Br, 8.2; Cl, 51.2. Found: C, 34.5; H, 1.1; N, 1.6; Br, 8.4; Cl, 51.0.

(e) Tetradecachloro-4-(valyloxy)triphenylmethyl radical hydrobromide (38): procedure 2; 93%; red powder; mp 184–185 °C dec; IR (KBr) 3590–2340, 1790, 1580, 1510, 1470, 1390, 1340, 1320, 1300, 1280, 1260, 1180, 1140, 1120, 820, 740, 710, 650, 530 cm^{-1}; UV–vis (acetonitrile) 218, 272 (sh), 336 (sh), 366 (sh), 380, 506, 556 nm (ε 99 700, 5450, 5580, 16 200, 29 700, 1000, 1025). Anal. Calcd for $C_{24}H_{11}BrCl_{14}NO_2$: C, 31.3; H, 1.2; N, 1.5; Br, 8.7; Cl, 53.9. Found; C, 31.5; H, 1.2; N, 1.8; Br, 8.4; Cl, 53.7.

(f) Tetradecachloro-4-(prolyloxy)triphenylmethyl radical hydrobromide (39): procedure 2; 91%; red powder; mp 181–183 °C dec; IR (KBr) 3600–2200, 1785, 1740, 1660, 1510, 1390, 1340, 1325, 1300, 1280, 1260, 1150, 1090, 1040, 980, 860, 810, 730, 710, 650, 530 cm^{-1}; UV–vis (acetonitrile) decomposes slowly. Anal. Calcd for $C_{24}H_9BrCl_{14}NO_2$: C, 31.4; H, 0.9; N, 1.5; Br, 8.7; Cl, 54.1. Found: C, 31.5; H, 1.1; N, 1.5; Br, 8.8; Cl, 53.9.

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Registry No. **1**, 77212-86-3; **2**, 77225-19-5; **3**, 86822-11-9; **4**, 82818-58-4; **5**, 82818-59-5; **6**, 82818-60-8; **7**, 86803-86-3; **8**, 82825-38-5; **9**, 82818-61-9; **10**, 82825-36-3; **11**, 82825-36-3; **13**, 82825-39-6; **14**, 82825-40-9; **15**, 82825-41-0; **16**, 82825-42-1; **17**, 1138-80-3; **18**, 64269-29-0; **19**, 65639-03-4; **20**, 65639-05-6; **21**, 1142-20-7; **22**, 65638-92-8; **23**, 65639-04-5; **24**, 65639-02-3; **25**, 67173-39-1; **27**, 1161-13-3; **28**, 1149-26-4; **29**, 1148-11-4; **30**, 67494-38-6; **31**, 67173-41-5; **32**, 67173-42-6; **33**, 67173-43-7; **34**, 86803-87-4; **35**, 29972-98-3; **36**, 67173-44-8; **37**, 67173-45-9; **38**, 67173-46-0; **39**, 71963-88-7; $NH_2CH(H)CO_2Et$, 459-73-4; $NH_2CH(CH_3)CO_2Et$, 3082-75-5; $NH_2CH(CH_2Ph)CO_2Et$, 3081-24-1; $NH_2CH(CH_2CHMe_2)CO_2Et$, 2743-60-4; $NH_2CH(CH_3)CO_2C_4H_9$-*t*, 21691-50-9.

Supplementary Material Available: Tables I and II containing ESR and magnetic susceptibility data for all the radicals and spin-labeled compounds described (3 pages). Ordering information is given on any current masthead page.